# Chromosome Analysis Suite (ChAS) v4.5 USER GUIDE

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# Introduction



The Chromosome Analysis Suite (ChAS) software for cytogenetic analysis enables you to view and summarize chromosomal aberrations across the genome.

Chromosomal aberrations may include copy number gain or loss, mosaicism, or loss of heterozygosity (LOH).

ChAS provides tools to:

- Perform single sample analysis of CEL files from CytoScan<sup>™</sup> Arrays and OncoScan Arrays.
- Automatic workflow from scanner to results file with no manual intervention required.
- Seamless integration with Franklin<sup>™</sup> by Genoox<sup>™</sup>.
- Analyze segment data at different levels of resolution.
- View results data (CYCHP, XNCHP, and OSCHP files) that summarize chromosomal aberrations in table and graphical formats.
- Display CNCHP data from Genome-Wide Human SNP 6.0 Array.
- Customize and load your own annotations and regions for focused analysis.
- Display ReproSeq Aneuploidy data from Ion Reporter<sup>™</sup>.
- Apply separate filters to the entire genome and user-specified regions of interest to remove irrelevant information such as segments in areas that are not of interest.
- Perform detailed comparisons between different samples.
- Directly access external databases such as UCSC Genome Browser, NCBI, DECIPHER, ClinVar, and ClinGen.
- Export user-selected data in graphical and tabular formats.
- Store and query segment data for streamlined analysis.
- Check for Mendelian Inheritance errors across related samples.
- Combine gene expression and copy number data in both tabular and graphical formats.
- Export data for viewing in Integrative Genome Viewer (IGV).
- Directly access the TaqMan website for follow up analysis.

**IMPORTANT!** The results from ChAS are for Research Use Only and not for use in diagnostic procedures.

Chromosome Analysis Suite is not a secondary analysis package. However, it does create CYCHP, OSCHP, XNCHP, and tab-delimited text files required for secondary analysis packages.

### Features in v4.5

- Automatic upload of CytoScan and OncoScan data files from scanner to ChAS and Franklin<sup>™</sup> by Genoox<sup>™</sup>.
- Support Auto CEL Analysis for CytoScan HD Accel array.
- Enhanced cybersecurity protection with ChAS DB administrator password change.
- Option to display OMIM Gene with Phenotype map key of 3 only.
- Easier scrolling within the Detail View.

### About this user guide

This user guide provides step-by-step instructions for performing the procedures required to use ChAS and can also be accessed from the software by clicking on the top menu bar's **Help** drop-down.

The steps outlining procedures through out this User Guide are frequently supplemented with screen captures to further illustrate the instructions.

**Note:** The screens that were captured for this User Guide may not exactly match the windows displayed on your screen.

### **Customer support**

Visit thermofisher.com/support for the latest in service and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols

# **Installing ChAS**



ChAS is a stand-alone application that supports the analysis and/or visualization of the following results data files:

- CytoScan (CYCHP)
- OncoScan (OSCHP)
- CytoScan XON (XNCHP)
- Genome-Wide Human SNP 6.0 (CNCHP)
- ReproSeq Aneuploidy (.zip)
- BED/AED files
- VCF files

**IMPORTANT!** Due to the amount of memory that ChAS requires to operate, Thermo Fisher Scientific VERY STRONGLY recommends that you DO NOT install the ChAS software on instrumentation computers being used for scanning and operating fluidics systems.

### **Recommended and minimum requirements**

**Note:** The full Database and Browser software must be installed on at least one or more 64-bit analysis workstations to create results data files.

| Table 1 | Software |
|---------|----------|
|---------|----------|

| System Properties  | Recommended System Requirements                   | Minimum System Requirements                       |
|--|---|---|
| Processor  | 3 GHz (or greater) Pentium Quad<br>Core Processor | 3 GHz (or greater) Pentium Dual Core<br>Processor |
| 64-bit Windows <sup>®</sup> Operating System and Web Browser | Windows 10/11<br>Windows Edge                     | Windows 10/11<br>Windows Edge                     |
| Available Disk Space   | 250 GB HD + data storage                          | 150 GB HD + data storage                          |
| Free Disk Space Required at Installation                     | ≥ 5 GB  | ≥ 5 GB  |
| RAM  | 32 GB   | 16 GB   |



#### Table 2 Server

| System Properties                        | Recommended System Requirements     | Minimum System Requirements         |
|--|-------------------------------------|-------------------------------------|
| Processor                                | 3.1 or 3.3 GHz Quad Core Processor  | 2.7GHz Quad core processor          |
| Windows Operating System                 | Windows Server 2022 Standard 64-bit | Windows Server 2022 Standard 64-bit |
| Available Disk Space                     | 1 TB HD + data storage              | 512 GB HD + data storage            |
| Free Disk Space Required at Installation | ≥ 5 GB                              | ≥ 5 GB                              |
| RAM                                      | <u>≥</u> 24 GB                      | 16 GB                               |

### Requirements and prerequisites for arrays

**IMPORTANT!** A Windows 64-bit Operating System is required for all array types.

**IMPORTANT!** Chromosome Analysis Suite requires AGCC 4.3/GCC 6.1 or higher to produce CytoScan/Oncoscan CEL files.

**IMPORTANT!** The larger file sizes associated with the CytoScan HD Array should be taken into account when calculating the necessary free space requirement. A CytoScan HD Array CYCHP file is ~155 MB. A CytoScan XON Array XNCHP files is ~174MB.

**IMPORTANT!** The ChAS software has been verified for use on a Windows 64-bit Operating System. ChAS may work on other Windows Operating Systems, but only the 64-bit version has been verified.

### Zip file contents

Go to thermofisher.com ChAS product page to downloaded the zip files.

Before performing an analysis, you must download the appropriate zip file package(s) listed in the table below.

| Array name           | hg19   | hg38   |
|----------------------|--|--|
| CytoScan HD          | CytoScanHD_Array_Analysis_Files_hg19_NA33.r10.zip      | CytoScanHD_Array_Analysis_Files_hg38_NA36.r7.zip       |
| CytoScan 750K        | CytoScan750K_Array_Analysis_Files_hg19_NA33.10.zip     | CytoScan750K_Array_Analysis_Fileshg38_NA36.r7.zip      |
| CytoScan HD<br>Accel | CytoScanHD_Accel_Array_Analysis_Files_hg19_NA33.r1.zip | CytoScanHD_Accel_Array_Analysis_Files_hg38_NA36.r1.zip |

 Table 3
 Available Library file packages

| ∿∿∆  |  |
|------|--|
| SK B |  |
|      |  |
|      |  |
|      |  |
|      |  |

| Array name           | hg19   | hg38   |
|----------------------|--|--|
| CytoScan<br>Optima   | CytoScanOptima_Array_Analysis_Files_hg_19_NA33.r10.zip   | CytoScanOptima_Array_Analysis_Files_hg_38_NA36.r7.zip  |
| CytoScan XON         | CytoScan_XON_Array_Analysis_files_hg19_NA33.r8-Part1.zip<br>CytoScan_XON_Array_Analysis_files_hg19_NA33.r8-Part2.zip | CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part1.zip<br>CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part2.zip |
| OncoScan CNV<br>Plus | OncoScan_Array_Analysis_Files_hg_19_NA33.r9.zip  | OncoScan_Array_Analysis_Files_hg38_NA36.r7.zip   |
| OncoScan CNV         | OncoScan_CNV_Analysis_Files_hg_19_NA33.r7.zip  | OncoScan_CNV_Analysis_Files_hg38_NA36.r7.zip   |
| CytoScan<br>HTCMA    | CytoScan_HTCMA_96_ChAS_Files_hg19.r3.zip   | CytoScan_HTCMA_96_ChAS_Files_hg38.r3.zip   |

### Table 3 Available Library file packages

After downloading and extracting the **Chromosome\_Analysis\_Suite\_4.5zip** file, a **Chromosome Analysis Suite 4.5** folder appears containing the following files:

- ChAS4.5\_setup.exe
- ChAS\_4.5\_Manual.pdf
- ChAS\_4.5\_Release\_Notes.pdf
- ChASDB\_adgv\_hg19\_Gold.backup
- ChASDB\_adgv\_hg38\_Gold.backup
- ChASDB\_adgv\_hg19\_Gold\_HD.backup
- ChASDB\_adgv\_hg38\_Gold\_HD.backup
- ChASDB\_adgv\_hg19\_Gold\_XON.backup
- ChASDB\_adgv\_hg38\_Gold\_XON.backup

### **Installing ChAS**

**Note:** The installation process also installs additional required components, which includes Java components and Visual C++ runtime.

New Installation 1. Double click on the ChAS4.5\_setup.exe file from the "Chromosome Analysis Suite 4.5" folder.

The Install Shield Wizard for Chromosome Analysis Suite begins.

2. At the Welcome window, click Next.

The License Agreement window appears.

3. Please read the license agreement carefully, click the "I accept the terms of the license agreement" radio button, then click **Next**.

The Setup Type window appears.

4. Click the appropriate drive's check box, then click **Next**.

The PostgreSQL Database Server Installation window appears.

**Note:** Make sure you select a drive with the most available space. Consider 1 GB of space is required for every 1000 samples you add to the database.



The installer auto-detects and displays a default **Port** number. It is recommended that you do not change this number.

5. Click Next.

The Start Copying Files window appears.

- 6. Click **Next**, then follow the on-screen instructions to complete the installation.
- 7. After all software installation is complete, you must download the new Analysis files from the NetAffx site or copy them into your ChAS Library folder. If you are unable to connect to the Internet, refer to "Copying analysis files" on page 25.

**IMPORTANT!** If your Windows Firewall is enabled during the installation of ChAS and you want to Backup the ChAS Database and Restore it to your local ChAS DB (see "Using a shared ChAS database while off-line" on page 452) a message may appear indicating that you cannot connect to the shared folder.

If this message appears, contact your IT department for help in allowing file sharing through the Windows Firewall.

# Upgrade installation

**IMPORTANT!** The ChAS 4.5 Installer does NOT support upgrade from previous versions. Due to an updated version of the ChAS DB, previous versions of ChAS must be uninstalled using add/ remove programs prior to running the ChAS 4.5 installer. Be sure to make a backup of your ChAS DB prior to uninstalling your previous version of ChAS.

To keep current preferences, see "Exporting and importing preferences" on page 439.

| Copying analysis files | The CytoScan Analysis Library Files zip package download contains the Analysis Files required to process their respective CytoScan Array CEL files into CYCHP files.         |
|------------------------|--|
|                        | The OncoScan Analysis Library Files.zip package download contains the Analysis Files required to process their respective OncoScan Array CEL files into OSCHP files.         |
|                        | The CytoScan XON Analysis Library Files.zip package download contains the Analysis Files required to process their respective CytoScan XON Array CEL files into XNCHP files. |

Also included in the CytoScan HD Analysis Library Files. zip are the files for GenomewideSNP\_6 files (required in ChAS to view GenomeWideSNP\_6 CNCHP files).

If you are unable to download library files through the software, you can download the zipped library files from the ChAS product page at www.thermofisher.com, then extract (unzip) the files into the following location: C:\Affymetrix\ChAS\Library

Analysis file locations in Windows 10

- Library: C:\Affymetrix\ChAS\Library
- Preference file: C:\ProgramData\Affymetrix\ChAS\preferences.xml
- All other user profile related preference files and saved settings: C:\ProgramData\Affymetrix\ChAS\users

### **Viewing Hidden Files and Folders**

The ChAS preference files may be placed in folders and files that are normally hidden from the user in Windows.

### Displaying hidden files and folders in Windows 10

1. At the Windows 10 Desktop, move your mouse to the bottom right of the Task bar (right of the clock).

Five large icons appear.

- 2. Click on the **Settings** icon.
- 3. Click Control Panel.

The Control Panel window opens.

- 4. Click **Appearance and Personalization** in Control Panel. Under Folder Option, click "Show hidden files and folders".
- 5. In the Folder Options window that appears, click the View tab. Under Hidden files and folders, click **Show hidden files and folders**.

Hidden files and folders are dimmed to indicate they are not typical items. If you know the name of a hidden file or folder, you can search for it.

- 6. Click OK.
- 7. Close all open windows.

# Analysis file download

When you start ChAS for the first time, you will be prompted to:

1. Create a user profile. (See "Creating and using user profiles" on page 435)

**Note:** To process the CytoScan Arrays in GCC, you must install the appropriate library files for GCC on the GCC workstation (see the specific array product page at www.thermofisher.com for details).

You can download the ChAS analysis files from NetAffx using either the ChAS Browser or the Analysis Workflow. The files will be saved into the same folder whether downloading through the ChAS Browser or the Analysis Workflow.

# Downloading ChAS analysis files from NetAffx for use with the ChAS Browser

1. Start ChAS.

If no annotations are installed, a Download Annotations notice appears. (Figure 1)

| Figure 1 Download Annotations notice  |
|---|
| Download Annotations X  |
| Chromosome Analysis Suite cannot launch without annotations. Would you like to download the latest annotation database now? |
| OK Cancel   |

2. Click **OK**.

The Library File Download Service window opens.

**Note:** You can also open the Library File Download Service window by selecting **Update Library and Annotation Files** from the Help menu.

- 3. From the Library File Download Service window, click **OK** to view available Library Files for download.
- 4. Select the library and annotation files you want to download.
- 5. Click **Download**.

The Download Progress window displays the progress of the downloading and unpacking of the files.

- 6. Click **OK** when the download is complete.
- 7. The NetAffx Authentication window remains open, click **Close** when finished downloading the library files.

### Downloading ChAS analysis files from NetAffx using the Analysis Workflow

- 1. Select Analysis  $\rightarrow$  Perform Analysis Setup.
- 2. Select Utility Actions → Download library Files.
- 3. From the Library File Download Service window, click **OK** to view available Library Files for download.
- Select the array type check box(es) for the analysis files that you want to download, then click **Download**.

### Updating NetAffx Genomic Annotation files

The publicly available annotations in the NetAffx Genomic Annotation file are updated quarterly. When launching the ChAS Browser or Analysis Workflow, the software will check for the availability of new NetAffx Genomic Annotation files.

If more current files are available, you will be prompted to download the files from NetAffx using the instructions from either of the previous two sections.

If you are unable to download from NetAffx through the software, please contact Technical Support for access to the current NetAffx Genomic Annotation file.

### Setting up proxy server access

**Note:** If you do not know what the proxy settings are, contact your IT department.

Follow the steps below if your system has to pass through a Proxy Server before it can access the NetAffx server.

To change from a Local Disk (C: or D:) Database to a dedicated remote Database Server, see "Access to a remote ChAS database server from the ChAS browser" on page 29.

### Access to NetAffx from the analysis workflow

- 1. Launch the Analysis Workflow by selecting Analysis  $\rightarrow$  Perform Analysis Set Up in the ChAS Browser.
- 2. Click Utility Actions  $\rightarrow$  Download Library Files.

The Library File Download Service window appears.

- 3. Click the **Proxy Settings** tab. (Figure 2)
- 4. Click the Enable Custom Proxy Server check box.
- 5. Enter the Host Address, Port (if not listed), User and Password.

**IMPORTANT!** This proxy user ID and password is NOT the same ID and password used to connect to NetAffx.

| Figure 2      | Proxy Settings window         |
|---------------|-------------------------------|
| 👔 NetAffx Do  | wnload Service                |
| NetAffx Accou | nt Information Proxy Settings |
| 🔽 Enable (    | Lustom Proxy Server           |
| Address:      |                               |
| Port:         | 3128                          |
| User:         |                               |
| Password:     |                               |
|               | Save Cancel                   |
|               |                               |

6. Click Save.

### Access to NetAffx from the ChAS browser

- From the Help drop-down menu, click Update Library and Annotation Files... The Library File Download Service window appears.
- 2. Click the Proxy Settings tab. (Figure 2)
- 3. Click the Enable Custom Proxy Server check box.
- 4. Enter the Host Address and Port information, then enter your user name and password.

**IMPORTANT!** This proxy user ID and password is NOT the same ID and password used to connect to NetAffx.

5. Click Save.

Access to a remote ChAS database server from the ChAS browser

1. From the Preferences drop-down menu, click Edit Application Configuration...

The Configuration window appears. (Figure 3)

| Figure 3 Proxy Settings window       |   |
|--------------------------------------|---|
| Application Configuration            | × |
| Connection ChAS DB Server            |   |
| <ul> <li>Use system proxy</li> </ul> |   |
| Use custom proxy                     |   |
| Host:                                | 1 |
| Port:                                |   |
|                                      |   |
|                                      |   |
|                                      |   |
|                                      |   |
| OK Cancel                            |   |
|                                      |   |

- 2. Click the **Use custom proxy** button.
- 3. Contact your IT department for help with entering the Host and Port information.
- 4. After you have completed the appropriate fields, click **OK**.

### Uninstalling

**IMPORTANT!** It is strongly recommended you backup your database BEFORE uninstalling ChAS.

- 1. From the Windows Start Menu, navigate to the Windows Control Panel,
- 2. Select Uninstall or change a program.
- 3. Locate the **Chromosome Analysis Suite** application, then perform the uninstall as you normally would.
- 4. Click **OK** to acknowledge the message box that warns the ChAS application must be closed (before removing it).





# Starting ChAS

1. Double-click on the Desktop ChAS 🔏 icon.

The Select User window appears. (Figure 4)

| Figure 4 Select User window |
|-----------------------------|
| X Select User X             |
| Pete Create New             |
| Manual connection           |
| OK Cancel                   |

2. Use the drop-down button to select a user or click **Create New** to create a new user profile. For more information, see "Creating and using user profiles" on page 435.

ChAS

- 3. Optional: Click the Manual connection check box. For information on manual connections, see "Manual or automatic connection mode" on page 403.
- 4. Click OK.

The Chromosome Analysis Suite application opens. as shown in Figure 5 on page 31 after logging into the ChAS DB. To login, see "Logging into the ChAS database" (below).

**Note:** A message may appear indicating a more current version of the NetAffx Genomic Annotation file is available for download. To download the newer version of the file, see "Analysis file download" on page 26. If you are unable to download the files via the NetAffx dialog, please contact Technical Support for alternative downloading options.

# Logging into the ChAS database

See Chapter 21, "Database tools" on page 442 for steps on how to log into the ChAS Database.

**IMPORTANT!** After installation and before you can access the ChAS database, the default **admin** password must be changed from **admin** to a more secure password. To do this and to add additional user profiles, see "Administration" on page 451.

**Note:** Your login is retained throughout a working session, however if you close the ChAS browser window, then re-open it, you must login again.



The ChAS browser window has the following components:

- Menu Bar Provides access to the functions of the software.
- Tool Bar Provides quick access to commonly used functions. Note: Some features that were previously in the Tool bar (such as Dark/Light Schema) have been removed, but continue to be available under the View menu item.
- Files List Shows the data and annotation files that are available for display. See "Files list" on page 141.
- Data Types List Displays the type of data available in the files. See "Data types list" on page 143.
- Named Settings Displays a list of the previously saved display settings for ChAS. See "Named settings" on page 144.

- Status Bar Displays information on the status of the software, the ChAS Browser NetAffx Genomic Annotation file version, the hg version, information about the annotation or probe that the mouse pointer is nearest to in the Detail View, and the user profile name. See "Status bar" on page 144.
- **Display Area** Displays the following data in graphical and table formats:
  - CYCHP, CNCHP, XNCHP, OSCHP, RHCHP, and/or ReproSeq Aneuploidy data
  - Detected segments
  - Region information file data
  - Histogram data (representing segments uploaded to the database)
  - Reference annotations

For more display area information, see "Viewing data" on page 139.

## Analysis workflow module

The Analysis Workflow generates xxCHP files from CEL files and tracks ongoing ChAS analysis tasks. You can access the Analysis Workflow at any time by clicking on its experimentation in the second s

**First time setup** After installation, you must configure your data paths.

The Analysis Workflow requires the following steps:

1. From the Analysis menu, select Perform Analysis Setup. (Figure 6)



The Analysis Workflow Configuration window tab appears. (Figure 7)

### If it does not appear, click **Utility Actions** $\rightarrow$ **Configuration**.

| nalysis setup V     | Workflow dashboard Q | C results | Configuration |  |      |  |  |      |      |      | Utility Ac |
|---------------------|----------------------|-----------|---------------|--|------|--|--|------|------|------|------------|
| nput sample pati    | h(s)                 |           |               |  |      |  |  |      |      |      |            |
| Please select input | t sample path        |           |               |  |      |  |  |      |      |      |            |
|                     |                      |           |               |  |      |  |  |      |      |      |            |
|                     |                      |           |               |  |      |  |  |      |      |      | Add.       |
|                     |                      |           |               |  |      |  |  |      |      |      | Derror     |
|                     |                      |           |               |  |      |  |  |      |      |      | Remo       |
|                     |                      |           |               |  |      |  |  |      |      |      |            |
| Jutput results pa   |                      |           |               |  |      |  |  |      |      |      |            |
| Please select outpi | ut results path      |           |               |  |      |  |  |      |      |      |            |
|                     |                      |           |               |  | <br> |  |  | <br> | <br> | <br> |            |
|                     |                      |           |               |  |      |  |  |      |      |      |            |
| Central QC histor   | y path               |           |               |  |      |  |  |      |      |      |            |
| Please select centr | ral QC history path  |           |               |  |      |  |  |      |      |      |            |
| C:\QC History OC    |                      |           |               |  |      |  |  |      |      |      |            |
| ibrary file nath    |                      |           |               |  |      |  |  |      |      |      |            |
|                     |                      |           |               |  |      |  |  |      |      |      |            |
| C:\Affymetrix\ChA   | AS\Library           |           |               |  |      |  |  |      |      |      |            |
|                     |                      |           |               |  |      |  |  |      |      |      |            |

### Assigning an Input sample path(s)

A minimum of two sample paths is recommended.

1. Click Add.

The Add Input sample files window appears. (Figure 8)

| Figure 8 Add Input         | sample files window |              |
|----------------------------|---------------------|--------------|
|                            |                     | v.           |
| Current Directory C:\Users |                     | Up one level |
| Folder                     | Date Modified       |              |
| 🗀 Administrator            | 2011-01-19 09:10:37 |              |
| 🗀 alew                     | 2012-01-18 14:34:34 |              |
| 🗀 All Users                | 2009-07-13 22:08:56 |              |
| 🗀 alucia                   | 2012-04-17 08:27:51 |              |
| 🚞 brhom                    | 2012-09-28 09:51:30 |              |
| anguy                      | 2011-01-28 08:36:08 |              |
| 🗀 Default                  | 2009-07-13 20:20:08 |              |
| 🗀 Default User             | 2009-07-13 22:08:56 |              |
| 🗀 jburri                   | 2012-07-10 13:34:56 |              |
| 🗀 ppavic                   | 2012-10-01 09:41:03 |              |
| Dublic Public              | 2009-07-13 20:20:08 |              |
| 🚞 rallso                   | 2011-01-21 09:37:40 |              |
|                            |                     |              |
|                            |                     |              |
|                            |                     |              |
| Create New Folder          | Folder Name:        | OK Cancel    |
|                            |                     |              |

2. Click the **Up one level** button to navigate to the recommended C:\Users directory, then click the **Create new folder** button to label a new input sample folder.

(Example: C:\Users\YourUserName\CytoScan Data)

3. Repeat **Step 2** to create a second input folder in an easy to access area. (Example: C:\Users\YourUserName\Collaborator\_OncoScan Data)

| Assigning an              | 1. Click the <b>Browse</b> button.   |
|---------------------------|--|
| Output results<br>path    | A good practice is to navigate to your current ChAS data folder location, then click <b>Create New Folder</b> to create an output results path folder <u>inside</u> this folder. (Example: C:\Users\YourUserName\CytoScan Data\CytoScan_Results_Files) |
|                           | 2. Click <b>Save</b> .   |
| Assigning a<br>Central QC | <ol> <li>Click the <b>Browse</b> button, then navigate to a folder in which to store the QC<br/>history file. (Example: C:\Cytoscan_data\)</li> </ol>  |
| history path              | <ol> <li>Click Create New Folder to create a central QC history path folder. (Example:<br/>QC_History)</li> </ol>  |

## File types and data organization in ChAS

To fully use the capabilities of ChAS, you need to understand the ChAS file types and data organization in ChAS.

### ChAS file types ChAS uses the following types of files:

- Data files
- Region Information files
- Support files

### File Types Supported in ChAS

Some data files that ChAS uses are generated by other Thermo Fisher Scientific software, as shown in Table 4.

| File Type   | Created In | ChAS   |
|---|------------|--|
| Sample file (ARR)   | GCC        | Uses this information to associate sample attribute information with CEL and xxCHP or CNCHP files.   |
| Intensity Data file (CEL)   | GCC        | Analyzes the intensity data in the CEL file, then generates a xxCHP files.<br>Note: A 64-bit system is required to analyze intensity data. |
| Analysis Results (CYCHP)<br>CytoScan array: CYCHP<br>contains copy number, LOH,<br>mosaicism, and genotype call<br>information                                      | ChAS       | Displays results in graphical and tabular formats.   |
| Analysis Results (RHCHP from<br>HTCMA) contains copy<br>number, LOH, Carrier Variant,<br>and SMN results  | RHAS       | Displays results in graphical and tabular formats.   |
| Analysis Results (OSCHP)<br>OncoScan FFPE and<br>OncoScan_CNV arrays,<br>OSCHP contains copy<br>number/LOH and somatic<br>mutation information (FFPE<br>Assay only) | ChAS       | Displays the probe-level analysis, segment level data, and somatic mutation data.  |

 Table 4
 Supported file types



#### Table 4Supported file types

| File Type  | Created In                    | ChAS  |
|--|-------------------------------|---|
| Analysis Results (XNCHP)<br>CytoScan XON array: XNCHP<br>contains LOH, Exon Region,<br>and Genotype information. | ChAS                          | Displays probe-level analysis, segment level data in graphical and tabular formats.   |
| Analysis Results (CNCHP)<br>GenomeWide SNP 6.0 array<br>contains copy number and<br>LOH segments                 | GTC                           | Displays probe-level analysis data and generates segment data on-the-fly.   |
| CHP Change Archive<br>(CHPCAR)   | ChAS                          | Stores user-annotated segment and sample annotations as well as modifications made to the segment data.   |
| Analysis Results (.zip)<br>ReproSeq Aneuploidy<br>contains copy number and<br>tiling information                 | ION Reporter                  | Displays copy number segment data and sequence tile information.  |
| Region Information File (BED or AED)   | ChAS or Text<br>Editor        | Allows users to display their own custom data and optionally use the information to define CytoRegions or an Overlap Map. ChAS can export data in BED format for use with the UCSC Browser and other programs which understand this format. |
| Tab-separated values (TSV,<br>TXT, and DOCX)   | ChAS                          | Exports data in this format for use in a spreadsheet program or other user-<br>defined uses. This format is for export only. ChAS does not import TSV or<br>TXT files.  |
| VCF Files  | Other<br>software<br>packages | Allows users to view genotype and indel data in the Detail View.  |

### **Region information files**

The region information files in Browser Extensible Data (BED) and Affymetrix Extensible Data (AED) format provide lists of regions in the genome with position information and other annotations. To open a BED or AED file, click the position or select **File**  $\rightarrow$  **Open** on the menu bar. All BED or AED files that are opened during a session will reload when you start a new session with the same user profile.

**Note:** You can use the reference annotations to provide region information or use the Export feature to export data in existing BED files to an AED file. See "Exporting information in AED or BED format" on page 314.

# Analysis and visualization library files

**IMPORTANT!** Every feature in ChAS requires support files.

- Array-type specific Library file sets with files for running Copy Number/LOH/Mosaicism analysis and Reference Creation workflows (Analysis files)
- Files for visualizing and exporting data from xxCHP results data files.
- Reference Annotation files
  - Browser Annotation files are named using the following format: <NetAffxGenomicAnnotations.Homo\_sapiens.hgXX.naYYYYMMDD.db>

Data organization in ChAS

ChAS enables you to keep your CEL and Analysis Results files in any folder on your computer. As long as you know where the files are, you can load them from anywhere and move them around at your convenience.

**IMPORTANT!** It is recommended that you perform analysis operations with all analysis files stored on a local disk drive.

### Basic workflow for cytogenetics analysis

Note: xxCHP is used when referring to CYCHP, CNCHP, XNCHP, and OSCHP files.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

ChAS can be used to:

- Perform probe-level analysis of CEL file data for CytoScan and OncoScan Arrays.
- Display probe-level analysis data (xxCHP) from:
  - CytoScan arrays (CYCHP)
  - Genome-Wide Human SNP array 6.0 (CNCHP)
  - OncoScan arrays (OSCHP)
  - CytoScan XON arrays (XNCHP)
  - CytoScan HTCMA arrays (RHCHP)

**Note:** There are some differences in the way the ChAS handles these different types of arrays and how it treats the data from these four types of files. The basic cytogenetic analysis workflow includes the following steps:

- "Array processing workflow (using instrument control software)" on page 37.
- "Probe-level Analysis of CEL file data" on page 37.
  - For CytoScan arrays, this analysis is performed in CHAS and produces CYCHP or XNCHP files depending on array type. See "CN/LOH/Mosaicism analysis" on page 44.


- For CytoScan HTCMA arrays, analysis is performed in the RHAS and produces RHCHP files.
- For the Genome-Wide Human SNP Array 6.0, this analysis is performed in Genotyping Console (GTC) software and produces CNCHP files. For more details, please refer to the GTC User Guide.
- For the OncoScan files, this analysis can be performed in ChAS and produces OSCHP files.
- Viewing data and features of interest using the ChAS display controls" on page 41.

## Array processing workflow (using instrument control software)

Array processing is performed in AGCC 4.1.2/GCC 5.0 or higher for the CytoScan Arrays, OncoScan Arrays, and Genome-Wide Human SNP 6.0 Array.

**Note:** You need to have the appropriate library files installed on the instrument control workstation to perform these analyses for the different array types.

The array processing includes the following steps:

- 1. Registering samples and arrays.
- 2. Washing and staining the arrays.
- 3. Scanning arrays and generating intensity (CEL) file data.

The following file types are produced:

- Sample (ARR files)
- DAT Files
- CEL Files
- Audit
- JPG

See the Instrument Control Console Users Guide for more information.

## Probe-level Analysis of CEL file data

Copy number data is handled differently from genome-wide genotyping data in this step.

**Note:** You need to have the appropriate ChAS library files installed to perform these analyses for different array types. A 64-bit system is required to analyze CytoScan CEL files.

- For CytoScan arrays, this analysis is performed in ChAS and produces CYCHP or XNCHP files (depending on array type) and contain the data shown in Table 5. See "CN/LOH/Mosaicism analysis" on page 44.
- For CytoScan HTCMA arrays, the analysis is performed in the RHAS which produces a RHCHP file for viewing in ChAS.
- Genome-Wide Human SNP Array 6.0 Data: The probe level analysis on CEL file data is performed in GTC and produces the CNCHP file data types shown in Table 5. See the GTC User Guide for more information.

For OncoScan Data: The probe level analysis on CEL file data is performed in ChAS and produces the OSCHP file data types show in Table 5.

#### Table 5

|  | Analysi                        | s Results *  |                        |                              |
|--|--------------------------------|--|------------------------|------------------------------|
|  | CytoScan<br>Array <sup>1</sup> | Genome-Wide<br>Human SNP<br>Array 6.0 <sup>2</sup> | OncoScan <sup>3</sup>  | CytoScan<br>XON <sup>4</sup> |
| Graph Data for the individual CN and SNP probes  | •                              | •  |                        |                              |
| Copy Number State  | Yes                            | Yes  | Yes                    | No                           |
| Log2 Ratio   | Yes                            | Yes  | Yes                    | Yes                          |
| Weighted Log2 Ratio  | Yes                            | No   | Yes                    | Yes                          |
| LOH  | Yes                            | Yes  | Yes                    | Yes                          |
| Allele Difference  | Yes                            | Yes  | Yes                    | Yes                          |
| Genotype Calls   | Yes                            | No   | No                     | Yes                          |
| Smooth Signal  | Yes                            | Yes  | Yes                    | Yes                          |
| Variant Data   | No                             | No   | Yes<br>(CNV Plus only) | No                           |
| B-allele Frequency   | Yes                            | No   | Yes                    | Yes                          |
| Segment data   | 1                              |  |                        |                              |
| Gain and Loss segments based on runs of aberrant<br>Copy Number State data               | Yes                            | Yes  | Yes                    | Yes                          |
| Mosaic Gain and Loss segments of non-integer Copy<br>Number States between CN=1 and CN=3 | Yes                            | No   | No                     | No                           |
| LOH (Loss of Heterozygosity) based on runs of SNPs where heterozygote calls are absent   | Yes                            | Yes  | Yes                    | Yes                          |
| Exon Region Gain and Loss segments   | No                             | No   | No                     | Yes                          |

\* CYCHP for CytoScan, CNCHP for Genome-Wide Human SNP 6.0 Array, XNCHP for CytoScan XON Array, and OSCHP for OncoScan Array.

1) For more details on CytoScan Array data, see Table 14 on page 171.

2) For more details on Genome-Wide SNP Array 6.0 data, see Table 17 on page 173.

3) For more details on OncoScan FFPE Assay Data, see Table 18 on page 174.

4) For more details on CytoScan XON array data, see Table 16 on page 173.

**Note:** Segment types drawn with flat ends (Gain and Loss) are the result of algorithms which can ascertain precise marker-to-marker breakpoints. Segment Types drawn with rounded ends (LOH, GainMosaic, LossMosaic) are the output of algorithms which closely approximate breakpoints based on the data.)

# Loading data into ChAS for display

You perform the same steps for the different types of analysis data (CYCHP, CNCHP, XNCHP, OSCHP and .zip (ReproSeq Aneuploidy), but ChAS handles these types of data differently.

# CytoScan array (CYCHP files)

When loading CYCHP files into ChAS for viewing, the software:

- 1. Selects the segments in the CYCHP file to display as segments.
- 2. Applies any smoothing or joining that would alter the length and other properties of segments.
- 3. Displays the segments and graph data:
  - Segment Data
    - Copy Number Gain/Loss
    - Loss of Heterozygosity (LOH)
    - Mosaic Gain/Loss
  - Graph Data
    - Copy Number State
    - Log2 Ratio
    - Weighted Log2 Ratio
    - Smooth Signal
    - Loss of Heterozygosity (LOH)
    - Allele Difference
    - B-allele Frequency
    - Genotype calls

## CytoScan XON array (XNCHP files)

When loading XNCHP files into ChAS for viewing the software:

- 1. Selects the segments in the XNCHP file to display as segments.
- 2. Displays the segments and graph data:
  - Segment Data
    - Loss of Heterozygosity
    - XON Region Gain/Loss
    - Whole Genome Gain/Loss Segments
  - Graph Data
    - Log2 Ratio
    - Weighted Log 2 Ratio
    - Smooth Signal
    - Loss of Heterozygosity (LOH)
    - Allele Difference
    - B-allele Frequency (BAF)
    - Genotype Calls

#### CytoScan HTCMA array (RHCHP files)

When loading RHCHP files into ChAS for viewing, the software:

- 1. Selects the segments in the RHCHP file to display as segments.
- 2. Displays the segments and graph data:
- Segment Data
  - Copy Number Gain/Loss
  - Loss of Heterozygosity (LOH)
- Graph Data
  - Copy Number State
  - Log2 Ratio
  - Smooth Signal
  - Loss of Heterozygosity (LOH)
  - Allele Difference
  - B-allele Frequency
  - Variant Data

#### Genome-wide SNP array 6.0 (CNCHP files)

When loading CNCHP files into ChAS for viewing, the software:

- 1. Performs segment generation by analyzing the CN and LOH graph data in the CNCHP file.
- 2. Applies any smoothing or joining that would alter the length and other properties of Copy Number segments.

In GTC software, these steps were performed in the Segment Reporting Tool.

- 3. Displays the segments and graph data:
  - Segment data
    - Copy Number Gain/Loss
    - Loss of Heterozygosity (LOH)
  - Graph Data
    - Copy Number State
    - Log2 Ratio
    - Allele Difference
    - SmoothSignal
    - Loss of Heterozygosity (LOH)

#### **OncoScan array (OSCHP files)**

When loading OSCHP files into ChAS for viewing, the software:

- 1. Selects the segments in the OSCHP file to display as segments.
- 2. Displays the segments and graph data:
  - Segment data
    - Copy Number Gain/Loss
    - Loss of Heterozygosity

- Graph Data
  - Copy Number State
  - Log2 Ratio
  - Weighted Log2 Ratio
  - Allele Difference
  - B-allele frequency
  - Smooth Signal
  - Loss of Heterozygosity
  - Variants/Somatic Mutations (OncoScan CNV Plus only)

# ReproSeq aneuploidy (zip files)

When loading zip files from Ion Reporter into ChAS for viewing, the software displays the following segments and graph data:

- Segment data
  - Copy Number Gain/Loss
- Graph Data
  - Copy Number State (sequence tiles)

Viewing data and features of interest using the ChAS display controls ChAS provides the following options for viewing and studying your loaded analysis results data:

Graphic Displays

See "Displaying data in graphic views" on page 148.

Tables

See "Displaying data in table views" on page 323.

After the data is loaded, you can:

- Filter the segments by Segment Parameters to hide segments that do not meet your requirements for significance. mSee "Filtering segments" on page 213.
- Select a region information file for use as a CytoRegion file and:
- Perform differential filtering for segments in CytoRegions and in the rest of the genome. See "Using CytoRegions" on page 263.
- Display only segments that appear in CytoRegions using Restricted Mode.
- Query segments from a loaded sample against segments previously uploaded to a ChAS Database. See "Querying a segment from the segment table" on page 387.
  - See which samples had segments similar to the current sample.
  - View the Calls and Interpretations of previous segments to help in the analysis of the current sample.
- Select a region information file for use as an Overlap Map and use the Overlap filter to identify or conceal segments that appear in the Overlap Map regions. See "Using the overlap map and filter" on page 275.
- Add selected features of the genome to new or existing Region (AED) files, and edit annotation data on existing annotations. (To open a BED or AED file, click the provide the provided the prov

- View genotype data from Next Generation Sequencing data via VCF files. See "VCF files" on page 318.
- Prepare reports on your findings by exporting graphics and table data in PDF and other formats. See "Exporting results" on page 410.
- Save setups of ChAS for different tasks in user profiles and named settings. See "User profiles and named settings" on page 433.

# Working with ChAS

# Accessing functions in ChAS

Commands in ChAS can be accessed in the following ways:

- Main menus
- Tool bar
- Right-click menu options in:
  - Files List
  - Data Types List
  - Karyoview
  - Selected Chromosome View
  - Detail View
  - Table Headers

# Changing pane sizes

Do one of the following to change the size of the panes in the ChAS window, as shown in Figure 9 on page 43.

- Click and drag the dividers between panes.
- Click the arrows in the dividers (\* or 5) to hide or maximize an entire pane.





# Opening panes in separate windows

You can display a pane in a separate window by clicking the spicon on the tab. To close the window and return the information to the tab panel, click the spicon in the window.



# **CN/LOH/Mosaicism analysis**

ChAS analyzes the intensity data (CEL file) from both CytoScan and OncoScan Arrays. The software performs a single sample analysis which compares the data in a CEL file to a previously created reference file, using analysis parameters specified in the **.chasparam** file. The analysis generates a CYCHP/XNCHP/OSCHP data file that you load and view in ChAS. The analysis detects segments that exhibit are as follows:

- Copy Number State Gain or Loss: Regions of integer copy number gain or integer copy number loss.
- Mosaic Gain and Loss (CytoScan HD, CytoScan 750k, CytoScan Optima, and CytoScan HD Accel only): Regions of non-integer copy number gain or loss (CN states between 1 and 3).
- XON Region Gain and Loss (CytoScan XON only): Regions of gain or loss at the exon level.

**Note:** The mosaicism segmentation analysis is currently only available for CytoScan Array CEL files. However, xxCHP files for the other array types contain the SmoothSignal data type which displays non-integer copy number changes.

Loss of Heterozygosity (LOH): Regions where the preponderance of SNPs do not display heterozygosity.

For more details on loading and viewing CHP data, see "Loading data" on page 114.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

**Note:** Reference files are provided as part of the complete Library file packages. You can also create your own reference file using ChAS.

Load Genome-Wide Human SNP Array 6.0 CNCHP into ChAS to display and detect Copy Number and Loss of Heterozygosity segments. See "Loading data" on page 114.

Load CytoScan HTCMA Array RHCHP into ChAS to display and detect Copy Number segments, Loss of Heterozygosity segments, variant data, and SMN data. See "Loading data" on page 114.

**IMPORTANT!** It is recommended to perform analysis operations with all associated analysis files in a locally stored folder(s).



# Single sample analysis

Single Sample Analysis compares the values in one or more user-selected CEL files with the values in a reference file that is created from a set of sample files. You can use the included default reference file or create your own (For more details, see "Creating a reference file" on page 86)

# ChAS analysis file Table 6 lists the compatibility between ChAS Analysis file versions for the CytoScan Arrays.

**Note:** ChAS automatically prevents you from selecting an incompatible analysis file version for analysis or when viewing analysis results.

| CytoScan Array<br>Analysis File Set<br>Version | ChAS<br>v4.5 | ChAS<br>v4.4 | ChAS<br>v4.3 | ChAS<br>v4.2.1 | ChAS<br>v4.2 | ChAS<br>v4.1 | ChAS<br>v4.0 |
|--|--------------|--------------|--------------|----------------|--------------|--------------|--------------|
| NA36 (hg38)                                    | Yes          | Yes          | Yes          | Yes            | Yes          | Yes          | Yes          |
| NA36 (hg19)                                    | Yes          | Yes          | Yes          | Yes            | Yes          | Yes          | Yes          |
| NA33(hg19)                                     | Yes          | Yes          | Yes          | Yes            | Yes          | Yes          | Yes          |

Table 6 Compatibility table

**Note:** Refer to the ChAS Release Notes for data equivalency information between the ChAS software and the Library file versions used to create CHP files.

#### Single sample analysis requires:

- ChAS analysis files for the array. See "Analysis file download" on page 26.
- A previously created reference model file

**Note:** You can use the included reference model file or create one using your own CEL file data and the Reference File creation function. The Reference Model file in the CytoScan Array set includes 380 microarrays which were run as part of a larger set of microarrays by nine operators processing ~48 unique samples in two rounds each, with randomization of the placement of sample DNAs across the PCR plates and randomization of the reagents and instruments used. The source DNA includes:

- 284 HapMap samples including at least one replicate of each of 270 HapMap samples: 90 from each of the Yoruban, Asian, and Caucasian ethnic groups, from cell-line derived DNAs from the Coriell Institute of Medical Research.
- 96 DNA samples from blood of phenotypically healthy male and female individuals obtained from BioServe Biotechnologies.
- CEL file data

During the analysis, ChAS generates CYCHP files with:

- Graph Data
  - Copy Number State
  - Log2 Ratio

# Introduction to single sample analysis



- Weighted Log2 Ratio
- LOH
- Allele Difference
- Smooth Signal
- Genotype Calls
- B-allele Frequency
- Segment Data
  - Copy Number Gain/Loss
  - Mosaicism Gain/Loss
  - Loss of Heterozygosity (LOH)

The CYCHP files can be loaded into ChAS for viewing and study.

Figure 10 on page 47 shows an overview of single sample analysis for the CytoScan Array.





#### Copy number segments on the X and Y chromosomes

The expected copy number state on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes, and the fact that they share extensive homology with each other only in the Pseudo Autosomal Regions (PARs) that they each have at either end. PAR1 is at the top of the p-arm and PAR2 at the bottom of the q-arm.

Markers occurring in the PAR regions are mapped exclusively to the X Chromosome. Therefore, in normal males the PAR regions of the X are expected to be CN=2 (probes on the X and Y contribute to the signal), while the rest of the Chr X is expected CN=1 for normal males. As a result, we treat the two X PARs in males as independent units (CN=2 expected) from the rest of the X chromosome (CN=1 in males) when generating Copy Number Segments.

Aberrant segments that cross PAR/non-PAR boundaries may be normalized into one segment if they have equivalent type (Gain or Loss) and CN State. During this normalization process, ChAS will not combine an aberrant (Gain or Loss) segment with a normal segment across PAR/non-PAR boundaries, even if they have the same CN State. If smoothing is subsequently applied, aberrant segments with different copy number state may be combined. If joining is subsequently applied, aberrant segments separated by a non-aberrant segment may be combined.

Because only Y-specific probes are mapped to the Y chromosome, the expected state of the entire Y chromosome is 1 for males and is 0 for females.

#### Mosaic copy number segments on the X chromosome

The expected copy number state on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes and the fact that they share extensive homology with each other only in the Pseudo Autosomal Regions (PARs) that they each have at either end. PAR1 is at the top of the p-arm and PAR2 at the bottom of the q-arm.

Markers occurring in the PAR regions are mapped exclusively to the X Chromosome. Therefore, in normal males the PAR regions of the X are expected to be CN=2 (probes on the X and Y contribute to the signal), while the rest of the Chr X is expected CN=1 for normal males.

Mosaic Segments whose boundaries start and end entirely in one of the PAR regions will use CN=2 as normal to determine the type (GainMosaic or LossMosaic) of Mosaic segment to draw.

Because the Mosaicism algorithm can generate segments which cross the PAR boundaries, Mosaic Segments that touch the non-PAR region of the X chromosome use the gender call of the sample to determine the Type of Mosaic segment to draw.

Because only Y-specific probes are mapped to the Y chromosome, the expected state of the entire Y chromosome is 1 for males and is 0 for females.



# LOH segments on X and Y chromosomes

#### CytoScan arrays

For normal XY male samples, the X chromosome will have single-copy based LOH calls (CN = 1). Male samples with more than one X chromosome (for example, XXY) may have LOH calls on the X chromosome, depending on the constitution of the X chromosomes' origins.

The tables below briefly describe how the array-specific algorithms call LOH segments for the X or Y chromosome.

| ·  | -  |  |
|--|--|--|
| LOH Segments   | X Chromosome   | Y Chromosome   |
| Normal male sample (XY)                                    | LOH calls that are single copy-based LOH call (CN = 1).  |  |
| Male sample with multiple X chromosomes (for example, XXY) | LOH calls are possible, depending on the constitution of the X chromosomes' origins.   | No LOH calls are made for the Y                                    |
| Normal female sample (XX)                                  | LOH calls are possible, depending on the constitution of the X chromosomes' origins.   | chromosome. Genotype calling is not performed on the Y chromosome. |
| Female sample with a single X chromosome (X0)              | LOH calls on X regions which have only a<br>single copy. Heterozygous SNP genotypes<br>are possible, but are due to the low inherent<br>Heterozygote call error rate noise, not the<br>true presence of two alleles. |  |

| Table 7 | Expected LOH calls on the X and Y chromosomes for the CytoSca | n arrays |
|---------|---|----------|
|---------|---|----------|

| <b>Table 8</b> Expected LOH calls on the X and Y chromosomes for the Genome-Wide Human SNP Arr |
|--|
|--|

| LOH Segments   | X Chromosome   | Y Chromosome                          |
|--|--|---------------------------------------|
| Normal male sample (XY)                                    | LOH calls on the non-PAR region of the X chromosome resulting from "forced" homozygote-only calls due to the presence of the Y chromosome. |                                       |
|  | Heterozygous calls are ignored on the X chromosome in males.   | LOH calls that are due to single copy |
| Male sample with multiple X chromosomes (for example, XXY) | LOH calls are possible, depending on the constitution of the X chromosomes' origins.   | genotyping calls (CN = 1).            |
|  | SNP genotypes are not constrained to homozygous calls.   |                                       |
|  | Heterozygous calls are ignored on the X chromosome in males.   |                                       |

| LOH Segments                                  | X Chromosome   | Y Chromosome  |
|---|--|---|
| Normal female sample (XX)                     | LOH calls are possible, depending on the constitution of the X chromosomes' origins.   | LOH analysis is not performed on the Y  |
| Female sample with a single X chromosome (X0) | LOH calls on X regions with only a single<br>copy. Heterozygous SNP genotypes are<br>possible, but are due to the low inherent<br>Heterozygote call error rate noise, not the<br>true presence of two alleles. | chromosome since it is assumed that<br>there not substantial Y chromosomal<br>Female sample with a single X material. |

#### Table 9 Expected LOH calls on the X and Y chromosomes for the OncoScan arrays

| LOH Segments  | X Chromosome  | Y Chromosome                    |
|---|---|---------------------------------|
| Normal male sample (XY)                                       | LOH calls that are single copy-based LOH call (CN = 1).   |                                 |
| Male sample with multiple X<br>chromosomes (for example, XXY) | LOH calls are possible where there is either loss or low heterozygosity.  | No LOH calls are made for the Y |
| Normal female sample (XX)                                     | LOH calls are possible depending on the constitution of the X origins or in regions of either loss or low heterozygosity. | chromosome.                     |
| Female sample with a single X chromosome (X0)                 | LOH regions on X which have only a single<br>copy. Will be called LOH where there is<br>single copy X.                    | -                               |

# Performing a single sample analysis

The following procedure is for setting up a manual analysis. To setup an automatic analysis see "Automatic CEL file analysis" on page 501.

You only need to perform the following steps once, as the data and selections you input (throughout this section) are retained for your convenience with future single sample analysis runs.

## Setting up and running a single sample analysis

**Note:** If you want to setup and run an OncoScan Analysis, see "Setting up and running an OncoScan single sample analysis" on page 67. If your samples are cancer samples and you suspect aberrations for at least 50% of the genome, then running a Normal Diploid Analysis is recommended. For more information, see "Setting up and running a normal diploid analysis" on page 65.

1. From the Analysis menu, select Perform Analysis Setup. (Figure 11)

| Figure 11         | Analysis drop-down menu           |
|-------------------|-----------------------------------|
| appliedbiosyst    | ems                               |
| Eile View Exports | Analysis ChAS DB Preferences Help |
| 📓 🚺 🕅 💅           | Perform Analysis Setup            |

4

#### The Analysis Setup window tab opens. (Figure 12)

| igure in allowed the  | rkflow          |  |                         |                       |                                    |                                     |
|---|-----------------|--|-------------------------|-----------------------|------------------------------------|-------------------------------------|
| pplied biosystems   |                 |  |                         |                       |                                    | 9                                   |
| Analysis setup Workflow dashboard   | QC results      |  |                         |                       |                                    | Utility Action                      |
| rray type: CytoScanHD_Array   | Genome Version: | hg19 • Analysis workflow:  | CytoScanHD_Array Single | Sample Analysis: NA33 | <ul> <li>Workflow name:</li> </ul> | Workflow                            |
| Array information   |                 |  |                         |                       |                                    |                                     |
| Select the reference model file for the a   | inalysis        |  |                         |                       |                                    |                                     |
| CytoScanHD_Array.na33.r3.REF_MODE   | L               |  |                         |                       |                                    | -                                   |
| Annotation to be used for analysis  |                 |  |                         |                       |                                    |                                     |
| CytoScanHD_Array.na33.annot.db  |                 |  |                         |                       |                                    |                                     |
| Select the intensity (CEL) file(s) to an  | alyze           |  |                         |                       | т                                  | otal records: 0                     |
|   |                 |  |                         |                       |                                    |                                     |
|   |                 |  |                         |                       |                                    |                                     |
| Output result information   |                 |  |                         |                       | Add                                | Remove                              |
| Output result information<br>Please select output file folder   |                 |  |                         | Select a s            | Add                                | Remove<br>analysis results          |
| Output result information<br>Please select output file folder   |                 |  |                         | Select a s            | Add                                | Remove<br>analysis results          |
| Output result information Please select output file folder Optional   |                 |  |                         | Select a s            | Add                                | Remove<br>analysis results          |
| Output result information         Please select output file folder  | Generate        | e Results Summary File   | Report format: PDF      | Select a s            | Add                                | Remove<br>analysis results          |
| Output result information         Please select output file folder         Optional         Set Gender Manually         Analyze all CEL files as male   | Generate        | e Results Summary File<br>Select CytoRegions File:                             | Report format: PDF      | Select a s            | Add<br>suffix to append to the     | Remove<br>analysis results          |
| Output result information         Please select output file folder         Optional         Set Gender Manually         Analyze all CEL files as male         Analyze all CEL files as female | Generate        | e Results Summary File<br>Select CytoRegions File:<br>Select Overlap Map File: | Report format: PDF      | Select a s            | Add suffix to append to the        | Remove analysis results Clear Clear |

2. From the **Select array type** drop-down list, click to select CytoScan array type. (Example: CytoScanHD\_Array)

**Note:** Once you select the array type, analysis workflow, and reference model file, then the annotation file will be auto selected for you based on your earlier selections. The Select array type drop-down list includes only the array types for which library (analysis) files have been downloaded from NetAffx or copied from the Library package provided with the installation.

- 3. Choose a Genome Build. (Example: hg38)
- 4. From the **Select analysis workflow** drop-down list, click to select an analysis workflow. (Example CytoScanHD\_Array Single Sample Analysis: NA33 or higher)
- 5. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.
- From the Select the reference model file for the analysis drop-down list, click to select a reference model file for the analysis. (Example: CytoScanHD\_Array.na33.r2.REF\_MODEL or higher).

**Note:** For Single Sample Analysis, the **Annotation to be used for analysis** field is auto-populated and set with the annotation filename used when the reference model file was generated.

#### 7. At the Select the intensity (CEL) file(s) to analyze pane, click Add.

**Note:** The Workflow Analysis window retains the drop-down selections used in your last submitted analysis. However, it does not display a previously set workflow name, CEL files to analyze list, or any suffix used to append your last analysis results. These three fields must be completed again.

| romosome Analysis S | uite |                  |                    |                          |               |                   |             |
|---------------------|------|------------------|--------------------|--------------------------|---------------|-------------------|-------------|
| Current directo     | ory  | C:\CytoScan\Data | ta\Batch 1         |                          |               | \$                | Up one lev  |
|                     |      | Name             |                    | Date Modified            | Size          | Туре              | Array ID    |
|                     |      | Sample_01.0      | CEL                | 8/8/2011 7:15 AM         | 66 MB         | AGCC CEL File     | 9e5653c3-de |
| C:\Users\cgates     |      | X Sample_02.0    | CEL                | 10/14/2011 6:23 AM       | 66 MB         | AGCC CEL File     | 12f20ec2-03 |
| (beskiep            |      | X Sample_03.0    | CEL                | 7/31/2013 4:41 PM        | 66 MB         | AGCC CEL File     | 9e565f0a-98 |
|                     |      | 🐹 Sample_04.0    | CEL                | 5/17/2013 8:19 AM        | 66 MB         | AGCC CEL File     | cb115160-64 |
| C) Users) and the   |      | Sample_06.0      | CEL                | 5/28/2013 11:10 AM       | 66 MB         | AGCC CEL File     | 8efa3b98-48 |
| \Documents          |      | Sample_07.0      | CEL                | 5/28/2013 11:11 AM       | 66 MB         | AGCC CEL File     | 664b82e7-d  |
|                     |      | Sample_08.0      | CEL                | 11/23/2011 11:11 AM      | 66 MB         | AGCC CEL File     | de2455c4-a  |
| C(                  |      |                  |                    |                          |               |                   |             |
| C:\Cytoscan         |      |                  |                    |                          |               |                   |             |
| Data                |      |                  |                    |                          |               |                   |             |
|                     | •    | File Name: Sar   | mple_01.CEL;Sample | _02.CEL;Sample_03.CEL;Sa | ample_04.CEL; | Sample_06.CEL;San | nple Ope    |

The following window appears: (Figure 13)

- 8. If your CEL files are located somewhere other than your input path location, navigate to the desired folder. Single click, Ctrl click, Shift click or Ctrl a (to select multiple CEL files).
- 9. Click Open.

The **Select the intensity (CEL) file(s) to analyze** pane is now populated with your CEL files, as shown in Figure 14.

**Note:** You can load several CEL files at a time for a Single Sample Analysis.

| Figure 14 Select the intens   | ity (CEL) file(s) to analyze  | pane                                     |  |
|---|---|--|--|
| applied biosystems  |   |  | 0  |
| Analysis setup Workflow dashboard Q<br>Array type: CytoScanHD_Array Gen   | C results<br>ome Version: hg19 - Analysis workflow:                                     | CytoScanHD_Array Single Sample Analysis: | Utility Actions NA33 • Workflow name: Workflow   |
| Array information Select the reference model file for the analys CytoScanHD_Array.na33.r3.REF_MODEL Apportation to be used for analysis | is  |  | •  |
| CytoScanHD_Array.na33.annot.db  |   |  |  |
| Sample_01.CEL<br>Sample_02.CEL<br>Sample_03.CEL<br>Sample_04.CEL<br>Sample_05.CEL   |   |  | Add Remove                                       |
| Output result information Please select output file folder C:\ChAS 4.0 Optional   |   | S  | elect a suffix to append to the analysis results |
| <ul> <li>Analyze all CEL files as male</li> <li>Analyze all CEL files as female</li> </ul>  | ✓ Generate Results Summary File<br>Select CytoRegions File:<br>Select Overlap Map File: | Report format: DOCX                      | Clear<br>Clear<br>Submit Reset                   |

To remove a CEL file from this list, click to highlight it, then click **Remove**.

10. At the **Output result information** pane, confirm the path shown for your output file folder. To change the current path/folder, click <sup>•••</sup> button to select a different output path/folder.

**Note:** To better organize your output results, you can add sub-folders to your assigned output result path/folder.

#### Adding sub-folders to your assigned result path/folder

- Click the "button to return to your assigned output path and/or folder.
- Click Create New Folder.
- Enter a sub-folder name.
- Click **OK**. Repeat the above steps to add more sub-folders.



The newly created sub-folders now appear in the output result information window. (Figure 15)

| Figure 15 Outp                       | out result informat                | ion window with sub- | folders | example     |           |  |  |  |
|--------------------------------------|------------------------------------|----------------------|---------|-------------|-----------|--|--|--|
| Chromosome Analysis Suit             | e                                  |                      |         |             | ×         |  |  |  |
| Current directory                    | Current directory C:\CytoScan\Data |                      |         |             |           |  |  |  |
|                                      | Name                               | Date Modified        | Size    | Туре        |           |  |  |  |
|                                      | 퉬 Batch 1                          | 3/12/2014 5:04 PM    |         | File Folder |           |  |  |  |
| C:\Users\cgates                      | 퉬 Batch 2                          | 3/12/2014 5:04 PM    |         | File Folder |           |  |  |  |
| C:\Users\cgates<br>\Documents<br>C:\ |                                    |                      |         |             |           |  |  |  |
| C:\Cytoscan<br>Data                  |                                    |                      |         |             |           |  |  |  |
| Create New Folder                    | Folder Name: Batch 2               |                      |         |             | OK Cancel |  |  |  |

11. If you are using a previously analyzed CEL file(s) to verify new CHP data (against CHP data generated from previous versions of ChAS and Library files), you may want to use a suffix to append the new resulting CHP file(s). To do this, click inside the **Select a suffix to append to the analysis results** field to enter an appending file suffix. (Figure 16)

**IMPORTANT!** If you are saving the same .CYCHP file into the same output file folder that contains your originally run CYCHP file, a "1" is automatically added into the filename (in addition to any suffix you may add) to differentiate the two runs of identical CEL file names. Example: na33(1).cyhd.cychp

| gure 16      | Adding a suffix                       |
|--------------|---------------------------------------|
| Optional     |                                       |
|              |                                       |
| Select a suf | fix to append to the analysis results |

12. Optional: If you have a CEL file(s) in which the Y chromosome is partially/fully deleted and therefore determined to be female by the gender calling algorithm, go to the Analysis Setup's Optional pane (Figure 17), click the **Set Gender Manually** check box, then click to select the appropriate radio button.



| Figure 17 Set Gender option     |
|---------------------------------|
| Optional                        |
| Set Gender Manually             |
| Analyze all CEL files as male   |
| Analyze all CEL files as female |
|                                 |

13. Optional: If you want to have an automatic export of the Karyoview, Segments Table, and Detail View for Copy Number and LOH Segments in the CHP file, click the check box **Generate a Results Summary File**, then and select the output format of either PDF or DOCX. (Figure 18)

**Note:** You can assign a CytoRegion and Overlap Map region file that will highlight these regions in the export. The export is placed in the same folder as the CYCHP file. This automatic export feature is only available for CytoScan arrays.

| Figure 18 Results Summary F   | ile                 |       |
|---|---------------------|-------|
| 🔽 Generate Results Summary File   | Report format: DocX |       |
| Select CytoRegions File:  |                     | Clear |
| Image: Properties       Report format:       DocX         Image: Select CytoRegions File:       Image: Select Overlap Map File:       Image: Select Overlap Map File:         Image: Select Overlap Map File:       Image: Select Overlap Map File:       Image: Select Overlap Map File:         Image: Select Overlap Map File:       Image: Select Overlap Map File:       Image: Select Overlap Map File:         Image: Select Overlap Map File:       Image: Select Overlap Map File:       Image: Select Overlap Map File: |                     |       |
|   | Submit              | Reset |

#### 14. Click Submit.

If the following warning message appears (Figure 19), acknowledge it, then click **OK**.

| Figu   | re 19 CEL warning message   |   |
|--------|---|---|
| Chromo | osome Analysis Suite  | ×   |
| A      | The CEL files(s) listed below have been previously analy<br>or currently in process. Press OK to name the result file<br>below. Press Cancel to return to the Analysis Setup. | zed and/or were selected for workflows that are pending<br>(s) generated by this workflow to the ones suggested |
|        | CEL File Name   | New Result File Name  |
|        | Sample_01.CEL   | Sample_01(1)  |
|        | •   | •   |
|        |   | OK Cancel   |

The Workflow dashboard window appears and your annotation files begin to load. (Figure 20).



The Analysis Workflow Dashboard tracks ongoing analysis tasks for ChAS. It also delivers the results of the analyses and can restart the Browser (if it was shut down to free up memory for the analysis).

| Figure 20 CEL files loading inside t                 | he Workflow dashboard            |                 |
|--|----------------------------------|-----------------|
| Analysis setup Workflow dashboard QC results Co      | nfiguration                      | Utility Actions |
| Workflows are available on the dashboard for 7 days. |                                  |                 |
| Workflow 03/12/2014 17:09:48                         |                                  | ×               |
| CytoScanHD_Array Single Sample Analysis: NA33        |                                  |                 |
| CytoScanHD_Array                                     | Running Copy Number Cyto Engine. |                 |
| Pause  |                                  |                 |

After loading is complete, a Workflow completed successfully message appears. (Figure 21)

| Figure 21 Workflow  | ashboard example with multiple Single Samples loaded     |                  |
|---|--|------------------|
| Reall for an enderson   |  |                  |
| lysis setup Workflow dashboard QC results                                       |  | Utility Actions  |
| rkflows are available on the dashboard for 7 days.                              |  |                  |
| orkflow 11/29/2016 06:45:48   | View Re  | ×<br>esults List |
| toScanHD_Array Single Sample Analysis: NA33<br>toScanHD_Array<br>1. Source File | Current workflow status: Workflow completed successfully | r Logs           |
| orkflow 11/23/2016 04:08:41   | . View Re  | ×<br>esults List |
| toScanHD_Array Single Sample Analysis: NA33<br>toScanHD_Array<br>1 Source File  | Current workflow status: Workflow completed successfully | (Logs            |

**Note:** The View Logs button will access the algorithm pipeline logs which may be useful if you have a Workflow that fails to complete.

15. Click to choose the analysis you want to view, then click View Results List.

The QC Results tab window appears showing the Basic View QC settings. A Detail View QC setting, which provides more columns of data, is also available in the QC Settings drop-down list. (Figure 22)

**Note:** QC parameters can also be viewed in the ChAS Browser see setting QC parameters in ChAS Browser.

| Figu     | Figure 22 QC Results window tab                                      |                  |              |                 |                         |          |               |  |  |
|----------|--|------------------|--------------|-----------------|-------------------------|----------|---------------|--|--|
| applied  | dbiosystems  |                  |              |                 |                         |          |               |  |  |
| Analysis | setup Workflow dashboard QC results                                  |                  |              |                 |                         |          |               |  |  |
| Array Ty | pe: CytoScanHD_Array  QC Settings: CytoScanHDMetrics-Basic View.r1   | - Edit or Create | QC Settings  |                 |                         |          |               |  |  |
| Resu     | Ilt Files  |                  |              |                 |                         |          |               |  |  |
| Add F    | iles Remove Selected File(s) Select All Clear Selection Export QC Ta | ble Generate Re  | eport 🔻 View | In Browser Impo | ort Attributes Export   | to IGV ( | QC Analysis 🔻 |  |  |
| F        | Filename   | Threshold Test   | MAPD ≤ 0.25  | SNPQC ≥ 15.00   | Waviness SD $\leq 0.12$ | Gender   | NA Version    |  |  |
| 0        | 9-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.ChAS3.cyhd.cychp  | Within Bounds    | 0.2049336    | 24.3833         | 0.08295833              | female   | 33            |  |  |
| 1        | 1-0810_LC_ONC13B_A6_PoP#2_CytoScan-PS_20110511.ChAS3.cyhd.cychp      | Within Bounds    | 0.1800587    | 18.46222        | 0.07722669              | male     | 33            |  |  |
| 1        | 1-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.ChAS3.cyhd.cychp    | Within Bounds    | 0.1618529    | 19.62777        | 0.09670192              | male     | 33            |  |  |
| 1        | 1-0816_LC_ONC41B_A12_PoP#2_CytoScan-PS_20110511.ChAS3.cyhd.cychp     | Within Bounds    | 0.1573046    | 26.37237        | 0.09822541              | male     | 33            |  |  |
| 1        | 1-1311_6210_B6_PoP_CytoScan_20160713.cyhd.cychp                      | Outside Bounds   | 0.1523745    | 14.88578        | 0.1865632               | male     | 33            |  |  |
|          | A2_0646_FH105810.ChAS3.cyhd.cychp                                    | Within Bounds    | 0.152364     | 21.75827        | 0.07173241              | male     | 33            |  |  |
|          | ARUP18_B02_CytoScanHD_ARUP_Beta1_LR_06012011.ChAS3.cyhd.cychp        | Within Bounds    | 0.1918176    | 21.0435         | 0.09861065              | male     | 33            |  |  |
|          |  |                  |              |                 |                         |          |               |  |  |

16. Click each sample's check box or click the 'Select All' button to select all samples.

#### Creating your own custom QC setting

1. Click on the Edit or Create QC Settings button.

The New QC Setting window appears. (Figure 23)

| Fig  | gure 23 New (                            | QC Setting win   | dow             |               |             |  |  |  |  |  |
|------|--|------------------|-----------------|---------------|-------------|--|--|--|--|--|
| New  | New QC Setting                           |                  |                 |               |             |  |  |  |  |  |
| Exis | Existing QC Settings: My Custom Settings |                  |                 |               |             |  |  |  |  |  |
| Т    | hresholds: Add Thre                      | eshold           |                 |               |             |  |  |  |  |  |
|      | Threshold                                | Threshold Option | Threshold Value | Error Message |             |  |  |  |  |  |
| ×    | MAPD -                                   | ≤ •              | 0.25            |               |             |  |  |  |  |  |
| ×    | SNPQC -                                  | <u>&gt;</u> •    | 15.00           |               |             |  |  |  |  |  |
| ×    | Waviness SD 🔹                            | <u>≤</u> •       | 0.12            |               |             |  |  |  |  |  |
| ×    | Gender •                                 | None 👻           |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               |             |  |  |  |  |  |
|      |  |                  |                 |               | ]           |  |  |  |  |  |
| QC   | Setting File Name: My                    | Custom Settings  |                 |               | Save Cancel |  |  |  |  |  |

- 2. Click Add Threshold (Figure 23) to create a new row.
- 3. Select the threshold you want to view in your custom QC Setting.

Note: The threshold metric you select is not required to have a threshold value.



4. Name your custom QC settings. (Example: My Custom Settings) (Figure 23), then click **Save**.

Your custom QC Setting is now available from the QC Settings drop down menu, as shown in Figure 24.

| Fig   | Figure 24 New QC Settings menu item   |                  |                  |                    |                    |        |            |  |  |
|-------|---|------------------|------------------|--------------------|--------------------|--------|------------|--|--|
| Analy | Analysis setup Workflow dashboard QC results Utility Actions                              |                  |                  |                    |                    |        |            |  |  |
| Array | Array Type: CytoScanHD_Array  QC Settings: My Custom Settings  Edit or Create QC Settings |                  |                  |                    |                    |        |            |  |  |
| Res   | ult Files   |                  | My Custom Settir | ngs                |                    |        |            |  |  |
| Ad    | d Files Remove Selected   | File(s) Select A | CytoScanHDMetr   | ics-Basic View.r1  | Generate Report    | ▼ View | In Browser |  |  |
|       | Filename  | Threshold Test   | CytoScanHDMetr   | ics-Detail View.r1 | Waviness SD ≤ 0.12 | Gender | NA Version |  |  |
|       | Sample_01(1).cyhd.cychp   | Within Bounds    | 0.1501459        | 24.92837           | 0.09934235         | female | 33         |  |  |
|       | Sample_02.cyhd.cychp  | Outside Bounds   | 0.1501282        | 14.87112           | 0.1873557          | male   | 33         |  |  |
|       | Sample_03.cyhd.cychp  | Within Bounds    | 0.1522362        | 25.01636           | 0.07105686         | female | 33         |  |  |
|       | Sample_06.cyhd.cychp  | Within Bounds    | 0.1764182        | 19.11598           | 0.07544088         | female | 33         |  |  |
|       | Sample_07.cyhd.cychp  | Within Bounds    | 0.1650564        | 21.62358           | 0.06506537         | female | 33         |  |  |
|       | Sample_08.cyhd.cychp  | Within Bounds    | 0.1686128        | 22.20452           | 0.1069141          | male   | 33         |  |  |

#### Viewing results in the browser

At the QC Results window, click the **View in Browser** button or the **View in MSV** button. For more MSV information, see the RHAS User Guide.

If the following warning message appears (Figure 25), acknowledge it, then click **OK**.

| Figu | re 25 Recommended maximum exceeded message   |   |
|------|--|---|
| 🐴 Re | ecommended maximum exceeded  | < |
|      | The recommended maximum number of analysis results files to be loaded at a time is 3.<br>Loading more files may impact the performance of the software.<br>Are you sure you want to load them? |   |

If the following warning message appears (Figure 26), acknowledge it, then click **OK**.



If the following warning message appears (Figure 27), click **Yes** to acknowledge it.

| Figure 27 NetAffx versions message  |   |
|---|---|
| NetAffx Versions  | × |
| NetAffx version of one or more files does not match loaded NetAffxGenomicAnnotations Browser annotation database file version 32. Are you sure you want to load these files? <ul> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\09-1420_B2_Phase4CustomerPanel_CytoScan_P(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0810_LC_ONC13B_A6_PoP#2_CytoScan-PS_(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS_(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS_(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS_(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS_(32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS_(32.3)</li> </ul> |   |

A progress bar appears. (Figure 28)

| Figure 28 Progr     | ess bar  |
|---------------------|----------|
| Please wait         | ×        |
| Opening file 2 of 6 | X Cancel |

**Note:** The ChAS Browser allows for loading of xxCHP files analyzed from different versions of ChAS. However, xxCHP files analyzed from different genome versions (hg18, hg19, hg38) cannot be loaded at the same time.

After a few moments, the ChAS browser featuring your selected samples appears. (Figure 29)





Recentering CytoScan HD, 750K, HD Accel, and Optima, arrays

Due to the complexity and low diploid count in a small fraction of cancer samples, there may be a need to manually assign the diploid region of the sample or recenter it.

In Figure 30, Chromosome 1 is called as a mosaic copy number loss, the log 2 ratio data is shifted downward, the smooth signal averages 1.75 copies, but the Allele Difference (AD) and B- Allele Frequency (BAF) Graphs are displaying three tracks.

**Note:** Since it is unlikely to have three tracks in AD/BAF data in a region of loss (unless the loss is CN=0), this sample needs to be re-centered.





If the region that is true diploid is a whole chromosome, use **Method 1**. If the region that is true diploid is part of a chromosome, use **Method 2**.

# Method 1

# Determining the median Log 2 ratio for the region in the sample that is truly diploid

- 1. Open the ChAS Browser.
- 2. Click on the **Chromosome Summary Data** tab, then click the drop-down to select **MedianSignal**. (Figure 31)

|   | Figure 3 <sup>-</sup><br>example  | Chromo           | some Sum      | mary tab - | median I    | og2 ratio val   | ue for Chro  | moso   | ome 1   | 16    |
|---|---|------------------|---------------|------------|-------------|-----------------|--------------|--------|---------|-------|
|   | Chromosome Summary Data Type: Median Signal (Median log2 ratio value found in the chromosome) |                  |               |            |             |                 |              |        |         |       |
|   | 0   | 11               | 12            | 13         | 14          | 15              | 16           | 17     |         | 18    |
|   | 0.16470781  | 0.2531421        | 0.2511932     | 0.30106622 | 0.08378549  | 0.16174491      | -0.060710527 | 0.2571 | 597     | 0.2   |
|   | Calculated Pro  | operties. LOH di | sabled.       | Create     | 2           | Modified        | Autosome %   | LOH    | Total A | utosa |
|   | <b>Ω Φ</b> σ <sup>*</sup> στι   | 20130819_CN0     | 37P21_240K_A  |            | 6-11T10:3   | 2015-06-11T15:3 |              |        |         |       |
| Ĩ | 🚥 Detail Vie  | w 🤊 🖫 QC         | and Sample In | fo 🏹 🚊 Cł  | romosome Si | ummary Data 🄊   |              |        |         |       |

# Method 2

# Determining the median Log 2 ratio for the region in the sample that is truly diploid

- 1. Open the ChAS Browser.
- 2. In the Detail View (Figure 30), zoom into the region determined to be diploid.
- 3. Go to the **Graphs** tab, then click **i** to include only the selected view.
- 4. Highlight the Log2 Ratio column, then right-click to select Sum, mean and median. (Figure 32)

4

| Figure 32 Graphs tab - Log 2 Ratio column                    |  |
|--|--|
| Preferences Help   |  |
| 🖲 🚓 } 📽 🖉 🛄 🔲 👯 💷 💷 } 🍢 🔼 🙋 🚥 🕞                              | 🕴 🤨 🖉 chr16: 50,167,373 - 74,187,531                             |
| 💲 🏭 Karyoview ষ 🔛 Segments ষ 🕌 Cytor 🔽 ons 🔊 🖉 🖉 Overlap Mag | ap 🎙 🖾 Graphs 🎙 📴 Som Mut 🎙 🖼 Query Samples 🎙 🖼 Query Segments 🎙 |
| 🖩 🗟 🖀 > Σ > 🖩 🖡 🚺 🖪  | 1,724 results  |
| Chromosome Position In Cytoregion   Markers O   BAF: CTL     | <mark>Q Log2 Ratio:</mark>                                       |
| 16 74.029,004 A Stag1297 0.004000048                         | 8 -U.31140084<br>92 0.21012146                                   |
| 16 74,044,550 X S-tag1101 0.001420110.                       | -0.09681079  |
| 16 74,062,203 X S-tag1909 0.47065228                         | -0.14623095  |
| 16 74,086,300 X S-tag1339 0.5423078                          | -0.35421756  |
| 16 74,098,974 X S-tag2157 0.490758                           | 0.26729825   |
| 16 74,107,201 X <u>S-tag1544</u> 0                           | -0.6092055   |
| 16 74,123,785 X <u>S-tag1041</u> 0.5027519                   | 0.08870722   |
| 16 74,148,932 X <u>S-tag0959</u> 0.48566595                  | 0.02667547   |
| 16 74,159,147 X <u>S-tag1737</u> 0.00043977                  | 0.025229357  |
| 16 74,169,321 X <u>S-tag1511</u> 0.9980694                   | -0.04669896  |
| 16 74,186,767 X <u>S-tag2043</u> 0.005392160                 | 07 -0.32854828 S Supersonal anadian                              |
|  | Z Sum, mean and median   |
|  |  |

A Sum, mean and median window appears. (Figure 33)

| Figure 3 | <b>33</b> Sum, mean and median message  |
|----------|---|
|          | Sum: -381.5232014879366<br>Mean: -0.1510982976189848<br><mark>Median: -0.14455513656139374</mark> |
|          | ок  |

- 5. Acknowledge the message, then click **OK**.
- 6. In the Analysis Workflow, set up the CEL file in the Single Sample Workflow, as described in "Performing a single sample analysis" on page 50.
- Check the Use Manual Recentering check box to enable the parameter fields, then enter the value of the median Log2 (determined by the browser) into the Adjust this log 2 to 0 text field. (Optional) Enter a suffix. Note: A suffix is recommended in order to differentiate the re-centered CYCHP file from the original.

| Figure 34 Use Manual Recentering check box |
|--|
|  |
| Use Manual Recentering                     |
| Adjust this Log2 to 0                      |
|  |
|  |



**Note:** By entering a median Log2 Ratio value (for the region you have determined to be diploid, The Recentering Algorithm has re-centered the log2 ratio data (for the region determined to be diploid) around 0 and there is no longer a mosaic loss segment called in this region, as shown in the Chromosome 1 example below. (Figure 35)



# No gender single sample analysis

The No Gender Single Sample Analysis (Figure 36) is the same analysis as described previously for Single Sample Analysis with the exception that no gender information is displayed in ChAS. The gender will not be reported and no segment or probe level data from X or Y chromosomes are displayed.

The metric, **Sex Chromosomes Aberrated** can be added to the QC table and reports either a Yes or No.

- Yes: Indicates that the sample does have segments meeting the following default thresholds: 50 Markers/200kb for copy number and 50 Markers/ 10,000kb for LOH segments.
- No: Indicates no copy number or LOH segments meet the previously defined thresholds.



| Figure 36          | Jo gender workflow                                      |
|--------------------|---|
|                    | C + C   |
| Analysis workflow: | CytoscanhD_Array single sample Analysis: NA33 V Workfl  |
|                    | CytoScanHD_Array Single Sample Analysis: NA33           |
|                    | CytoScanHD_Array No Gender Single Sample Analysis: NA33 |
|                    | CytoScanHD_Array Normal Diploid Analysis: NA33          |
|                    | CytoScanHD_Array Mendelian Error Check: hg19            |
|                    | CytoScanHD_Array Reference Model Creation: NA33         |

Setting up and running a normal diploid analysis

The Normal Diploid Analysis for CytoScan is recommended for cancer samples in which >50% of the genome is likely to be rearranged. This analysis will automatically determine the normal diploid regions and normalize the rest of the sample based on those regions resulting in properly centered data.

Note: Normal diploid analysis is <u>not</u> supported for CytoScan HD Accel arrays.

A Normal Diploid Analysis has the identical setup steps as "Setting up and running a single sample analysis" on page 50. The only difference is you must select **Normal Diploid Analysis** from the **Select analysis workflow** drop-down menu, as shown in Figure 37.

| Figure 37 Norm            | nal Diploid Analysis                           |
|---------------------------|--|
| Select analysis workflow: | CytoScanHD_Array Normal Diploid Analysis: NA33 |
|                           | CytoScanHD_Array Single Sample Analysis: NA33  |
|                           | CytoScanHD_Array Normal Diploid Analysis: NA33 |
|                           | CytoScanHD_Array Mendelian Error Check         |



The data shown above is from a cancer sample in which >50% of the genome was non-diploid. The top graph (purple) shows the sample run through the traditional single sample analysis. There are no Copy Number Segments called, the weighted log2 is centered around 0, but there are 4 allele difference tracks indicating more than two copies of this chromosome. In the bottom graph (pink), this same sample is run through the Normal Diploid normalization algorithm. The Copy Number Gain segment is called, the weighted log2 is shifted above the 0 line which is in agreement with the four allele difference tracks.

#### **Recommended QC metrics for Normal Diploid Analysis**

- ndSNPQC
- MAPD
- ndwavinessSD
- SNPQC
- wavinessSD

#### Viewing the recommended QC metrics (listed above)

**Note:** For samples run through the Normal Diploid Analysis, the following QC metrics are recommended:

- MAPD < 0.25
- SNPQC or ndSNPQC >= 15
- wavinessSD or ndwavinessSD < 0.12</p>
- 1. From the Analysis Workflow, click the **QC Results** window tab.
- 2. Click the **Settings** drop-down menu, then select **NDN View.r1**, as shown in Figure 39.

| Figur     | re 39 NDN View.r1 from             | the Setting d                               | rop-down    | menu          |                         |                  |                         |  |  |
|-----------|------------------------------------|---|-------------|---------------|-------------------------|------------------|-------------------------|--|--|
| QC re     | sults                              |   |             |               |                         |                  | Utility A               |  |  |
| Settings: | CytoScanHDMetrics-NDN View.r1 *    | Edit or Create QC S                         | ettings     |               |                         |                  |                         |  |  |
|           | CytoScanHDMetrics-Basic View.r1    | 1   |             |               |                         |                  |                         |  |  |
| Select A  | CytoScanHDMetrics-Detail View.r1   | "iew.r1 e Generate Report ▼ View In Browser |             |               |                         |                  |                         |  |  |
|           | CytoScanHDMetrics-NDN View.r1      | Threshold Test                              | MAPD ≤ 0.25 | SNPQC ≥ 15.00 | Waviness SD $\leq 0.12$ | ND SNPQC ≥ 15.00 | ND Waviness SD ≤ 0.12 N |  |  |
| CytoScan  | PS_20110706.Oct30APT2.cyhd.ND.cych | Within Bounds                               | 0.1518024   | 22.38932      | 0.06252074              | 22.38932         | 0.06252074              |  |  |

Setting up and running an OncoScan single sample analysis 1. From the Analysis menu, select Perform Analysis Setup. (Figure 40)



The Analysis Setup window tab opens. (Figure 41)



|   | Analysis Wo  | orkflow             |                      |               |                    |                     |                     |                  |                 |
|---|--|---------------------|----------------------|---------------|--------------------|---------------------|---------------------|------------------|-----------------|
| Chromosome Ar   | nalysis Suite - Analysis                                       | Workflow [For Resea | rch Use Only. Not fo | or use in dia | agnostic procedure | s.]                 |                     |                  |                 |
| <b>pplied</b> biosys                                  | stems  |                     |                      |               |                    |                     |                     |                  | (               |
| Analysis setup  | Workflow dashboard   | d QC results        |                      |               |                    |                     |                     |                  | Utility Acti    |
| Array type: Onco                                      | oScan  | Genome Version:     | hg19 • Analysis      | workflow:     | FFPE Analysis: NAS | 33                  |                     | • Workflow name: | Workflow        |
| Array informat  | ion  |                     |                      |               |                    |                     |                     |                  |                 |
| Copy number re  | eference model file  |                     |                      |               | Annotation to be   | used for a          | nalysis             |                  |                 |
| OncoScan.FFPE   | E.na33.r1.REF_MODEL  |                     |                      | •             | OncoScan.na33.r1   | Lannot.db           | )                   |                  |                 |
| Somatic mutation                                      | on reference model file  | e                   |                      |               | Somatic mutation   | threshold           | file                |                  |                 |
| OncoScan.FFPE   | E.na33.r1.SOM_REF_M  | ODEL                |                      | •             | OncoScan.Som1.0    | 0.r2.Som_t          | hresh.txt           |                  |                 |
| Calant the inter                                      |  |                     |                      |               |                    |                     |                     | -                |                 |
| Select the inter                                      | Isity (CEL) file(s) to a                                       | naiyze              |                      | las s l       |                    |                     |                     | •                | otal records: ( |
| Undo Redo   | Add CEL Files 🔻  | Import Batch File   | Export Batch File    | Display:      | File Name 🔹        | Sort All            | Result File Names 🔻 |                  |                 |
| AT Channel  |  |                     | GC Channel           |               |                    | R                   | esult File Name     |                  |                 |
|   |  | No CEL files s      | elected. Click on '  |               |                    |                     |                     |                  |                 |
|   |  |                     |                      | "Add CEL      | Files" to import   | the CEL t           | files for analyze.  |                  |                 |
| Output result in                                      | nformation   |                     |                      | "Add CEL      | Files" to import   | the CEL f           | files for analyze.  |                  |                 |
| Output result in<br>Please select ou                  | nformation<br>Itput file folder                                |                     |                      | "Add CEL      | Files" to import   | Optiona<br>Select a | files for analyze.  | nalysis results  |                 |
| Output result in<br>Please select ou<br>Manual Recent | nformation<br>Itput file folder<br>ering                       |                     |                      | "Add CEL      | Files" to import   | Optiona<br>Select a | files for analyze.  | nalysis results  |                 |
| Output result in<br>Please select ou<br>Manual Recent | nformation<br>Itput file folder<br>ering<br>Recentering TuScar | Log2Ratio Adj.      | Adjust this          | "Add CEL      | Files" to import   | Optiona<br>Select a | files for analyze.  | nalysis results  |                 |

2. From the Select array type drop-down list, click to select OncoScan.

**Note:** Once you select the array type, analysis workflow, and reference model file, then the annotation file will be auto selected for you based on your earlier selections.

**IMPORTANT!** The Select array type drop-down list includes only the array types for which library (analysis) files have been downloaded from NetAffx or copied from the Library package provided with the installation.

- 3. Select the appropriate Genome Build.
- 4. From the **Select analysis workflow** drop-down list, click to select an appropriate analysis workflow.

**IMPORTANT!** For FFPE samples use the FFPE Analysis NAXX workflow. For Control DNA use the Control DNA Analysis.

5. By default, the **Set workflow name** is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.



- 6. Click the **Copy number reference model file** from the drop-down list, then click to select an appropriate file.
- 7. Click the **Somatic mutation reference model file** from the drop-down list, then click to select an appropriate file.
- 8. At the Select the intensity (CEL) file(s) to analyze pane, click the Add CEL Files drop-down, then click to select AT Channel.

An Explorer Window appears.

- 9. Highlight the CEL file(s) using Ctrl click or Shift click, then click **Open**.
- 10. At the Select the intensity (CEL) file(s) to analyze pane, click the Add CEL Files drop-down, then click to select GC Channel.

An Explorer Window appears.

11. Highlight the CEL file(s) using Ctrl click or Shift click, then click **Open**.

The **Select the intensity (CEL) file(s) to analyze** pane is now populated with AT and GC Channel CEL files.

**IMPORTANT!** After loading the CEL files, check that the AT lines up with the matching GC CEL file.

12. Click the **Result File Names** drop-down menu to enable ChAS to automatically generate Output Names.

**Note:** Output file names are only auto-generated if the two CEL files have the same root name. It is recommended to use an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "\_AS\_05A.CEL" is an AT Channel file, while "\_AS\_05C.CEL" is a GC Channel file.

You can also clear this (populated) column by clicking Clear Column.

13. OPTIONAL: To choose a different output folder from the saved output path that is displayed, click the Output result information's **Browse** button.

An Explorer window appears.

14. Navigate to an output folder location, then click **OK**.

**Note:** To better organize your output results, you can add sub-folders to your assigned output result path/folder.

#### Adding sub-folders to your assigned Output Results folder

- 1. Click the "" button to return to your assigned output path and/or folder.
- 2. Click Create New Folder.
- 3. Enter a sub-folder name.
- 4. Click **OK**. Repeat the above steps to add more sub-folders.

The newly created sub-folders now appear in the output result information window.



5. If you are using a previously analyzed CEL file(s) to verify new CHP data (against CHP data generated from previous versions of ChAS and Library files), you may want to use a suffix to append the new resulting CHP file(s). To do this, click inside the Select a suffix to append to the analysis results field to enter an appending file suffix. (Figure 42)

**IMPORTANT!** If you are saving the same OSCHP file into the same output file folder that contains your originally run OSCHP file, a "1" is automatically added into the filename (in addition to any suffix you may add) to differentiate the two runs of identical CEL file names. Example: na33(1).oschp

| Figure 42 Adding a suffix                         |
|---|
| Optional  |
| Select a suffix to append to the analysis results |
| _NA32.3   |
|   |

6. Optional: If you have a CEL file(s) in which the Y chromosome is partially/fully deleted and therefore determined to be female by the gender calling algorithm, go to the Analysis Setup's Optional pane (Figure 43), click the Set Gender Manually check box, then click to select the appropriate radio button.

| Figure 43 Set Gender option     |
|---------------------------------|
| Optional                        |
| Set Gender Manually             |
| Analyze all CEL files as male   |
| Analyze all CEL files as female |
|                                 |

7. Click Submit.

If the following warning message appears (Figure 44), acknowledge it, then click **OK**.

| Figu   | re 44 CEL warning message  |                      |  |  |  |  |  |  |  |
|--------|--|----------------------|--|--|--|--|--|--|--|
| Chromo | osome Analysis Suite   | ×                    |  |  |  |  |  |  |  |
| A      | The CEL files(s) listed below have been previously analyzed and/or were selected for workflows that are pending<br>or currently in process. Press OK to name the result file(s) generated by this workflow to the ones suggested<br>below. Press Cancel to return to the Analysis Setup. |                      |  |  |  |  |  |  |  |
| 1      | CEL File Name  | New Result File Name |  |  |  |  |  |  |  |
|        | Sample_01.CEL  | Sample_01(1)         |  |  |  |  |  |  |  |
|        |  | •                    |  |  |  |  |  |  |  |
|        |  | OK Cancel            |  |  |  |  |  |  |  |

The Workflow dashboard window appears and your annotation files begin to load. (Figure 45).

The Analysis Workflow Dashboard tracks ongoing analysis tasks for ChAS. It also delivers the results of the analyses and can restart the Browser (if it was shut down to free up memory for the analysis).

| Figure 45 CEL files loading inside the Workflow dashboard |   |                 |  |  |  |  |  |  |
|---|---|-----------------|--|--|--|--|--|--|
| Analysis setup Workflow dashboard QC results              |   | Utility Actions |  |  |  |  |  |  |
| Workflows are available on the dashboard for 7 days.      |   |                 |  |  |  |  |  |  |
| Workflow 08/29/2014 13:57:16                              |   | ×               |  |  |  |  |  |  |
| FFPE Analysis: NA33                                       |   |                 |  |  |  |  |  |  |
| OncoScan Pause 8 Source Files                             | CopynumberOncoNodeNormalDiploidDetection::doRun() start |                 |  |  |  |  |  |  |

After loading is complete, a Workflow completed successfully message appears. (Figure 46)

| Figure 46 Workflow Dashboard with Single Samples loaded |  |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|--|
| applied biosystems                                      |  |  |  |  |  |  |  |  |
| Analysis setup Workflow dashboard QC results            |  |  |  |  |  |  |  |  |
| Workflows are available on the dashboard for 7 days.    |  |  |  |  |  |  |  |  |
| Workflow 07/06/2017 12:34:05                            | 0  |  |  |  |  |  |  |  |
| Non-FFPE Analysis: NA33                                 |  |  |  |  |  |  |  |  |
| OncoScan<br>2 Source Files                              | Current workflow status: Workflow completed successfully |  |  |  |  |  |  |  |

**Note:** The View Logs button will access the algorithm pipeline logs which may be useful if you have a Workflow that fails to complete.

8. Click to choose the analysis you want to view, then click View Results List.

The QC Results tab window appears showing the Basic View QC settings. (Figure 47) A Detail View QC setting (which provides more columns of data) is also available in the QC settings drop down list.

**Note:** QC parameters can also be viewed in the ChAS Browser see "Setting QC parameters in the ChAS browser" on page 129.

| Figure 47 QC Results window tab  |                |            |              |             |                                 |              |                  |                |                |  |  |
|--|----------------|------------|--------------|-------------|---------------------------------|--------------|------------------|----------------|----------------|--|--|
| appliedbiosystems  |                |            |              |             |                                 |              |                  |                |                |  |  |
| Analysis setup Workflow das  | hboard QC res  | ults       |              |             |                                 |              |                  |                | Utility Action |  |  |
| Array Type: OncoScan • QC Settings: OncoScanMetrics-Basic View.1 • Edit or Create QC Settings  |                |            |              |             |                                 |              |                  |                |                |  |  |
| Result Files   |                |            |              |             |                                 |              |                  |                |                |  |  |
| Add Files Remove Selected File(s) Select All Clear Selection Export QC Table Generate Report V View In Browser Import Attributes Export to IGV QC Analysis V |                |            |              |             |                                 |              |                  |                |                |  |  |
| Filename   | Threshold Test | MAPD ≤ 0.3 | ndSNPQC ≥ 26 | SNP QC Type | CelPairCheck Status Equals Pass | ndWavinessSD | Low Diploid Flag | % Aberr. Cells | TuScan Ploidy  |  |  |
| Normal01OSCHP  | Within Bounds  | 0.156494   | 58.758807    | ND          | Pass                            | 0.04980727   | No               | homogeneous    | 2              |  |  |
| Normal02OSCHP  | Within Bounds  | 0.145891   | 69.901331    | ND          | Pass                            | 0.04333016   | No               | homogeneous    | 2              |  |  |
| Normal03OSCHP  | Within Bounds  | 0.16881    | 42.842395    | ND          | Pass                            | 0.04173682   | No               | homogeneous    | 2              |  |  |
| Normal04OSCHP  | Within Bounds  | 0.155762   | 46.764626    | ND          | Pass                            | 0.06148678   | No               | homogeneous    | 2              |  |  |

9. Click each sample's check box or click the **Sample File** check box to select ALL samples.

#### Creating your own custom QC settings

See "Creating your own custom QC setting" on page 57

#### Viewing results in the browser

1. At the QC Results window, click View In Browser.

If the following warning message appears (Figure 48), click **Yes** to acknowledge it.


4

If the following warning message appears (Figure 49), acknowledge it, then click **OK**.



If the following warning message appears (Figure 50), click **Yes** to acknowledge it.

| Figure 50 NetAffx versions message   |
|--|
| NetAffx Versions X   |
| NetAffx version of one or more files does not match loaded NetAffxGenomicAnnotations Browser annotation database file version 32. Are you sure you want to load these files?         • C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\09-1420_B2_Phase4CustomerPanel_CytoScan_P (32.3)         • C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0810_LC_ONC13B_A6_PoP#2_CytoScan_PS (32.3)         • C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3) |

A progress bar appears. (Figure 51)

| Figure 51 Progress bar |   |
|------------------------|---|
| N Please wait          | × |
| Opening file 2 of 6    |   |
| × <u>C</u> ancel       |   |

**Note:** The ChAS Browser allows loading files analyzed using different NetAffx version at the same time (as long as the versions are all from all the same reference and genome builds). If NetAffx versions are from different builds of the genome (for example Hg18 and Hg19), The ChAS Browser does not load the files.

After a few moments, the ChAS browser featuring your selected samples appears.



## Recentering OncoScan CNV and OncoScan CNV Plus arrays

Due to the complexity and low diploid count in a small fraction of cancer samples, there may be a need to manually assign the diploid region of the sample or "recenter" it.

In Figure 52, Chromosome 16q is called as a loss, the log 2 ratio data is shifted downward, but the Allele Difference Graph is displaying three tracks representing AA, AB, BB calls. Having an Allele Difference graph with three tracks means this region must have at least two copies. Since you cannot have three Allele Difference tracks in a region of loss, this sample needs to be recentered. For more information, see "Manual re-centering algorithm (OncoScan)" on page 494.



If the region that is true diploid is a whole chromosome, use **Method 1**. If the region that is true diploid is part of a chromosome, use **Method 2**.

## Method 1 Determining the median Log 2 ratio for the region in the sample that is truly diploid

- 1. Open the ChAS Browser.
- 2. Click on the **Chromosome Summary Data** tab, then click the drop-down to select **MedianSignal**. (Figure 53)

| example          |                 |                 |        |          |                |              |           |              |       |         |       |
|------------------|-----------------|-----------------|--------|----------|----------------|--------------|-----------|--------------|-------|---------|-------|
| Chromosome       | Summary Data    | Type: Media     | nSigna | I (Media | n log2 ratio v | alue found / | in the ch | nromosome)   |       |         |       |
| 0                | 11              | 12              | 13     |          | 14             | 15           |           | 16           | 17    |         | 18    |
| 0.16470781       | 0.2531421       | 0.2511932       | 0.3010 | 6622     | 0.08378549     | 9 0.16174    | 491       | -0.060710527 | 0.257 | 1597    | 0.2   |
|                  |                 |                 |        |          |                |              |           |              |       |         |       |
| Calculated Pr    | onerties IOH di | isahlad         |        |          |                |              |           |              | _     |         |       |
| File             | 0001003.20110   | ioubiou.        |        | Created  |                | Modified     |           | Autosome %   | LOH   | Total A | utosc |
| Га<br>∎О́ О́ сті | .20130819 CN0   | 37P21_240K      | A10    | 2015-06  | -11T10:3       | 2015-06-11   | T15:3.    |              |       |         |       |
|                  |                 |                 | F      |          |                |              |           | ·            |       |         |       |
| caaco Detail Vi  | ew 🔻 📳 Q (      | C and Sample Ir | nfo 🔻  | 🚆 Chr    | omosome S      | ummary Dat   | a 🔻       |              |       |         |       |

## Method 2 Determining the median Log 2 ratio for the region in the sample that is truly diploid

- 1. Open the ChAS Browser.
- 2. In the Detail View (Figure 52), zoom into the region determined to be diploid.
- 3. Go to the **Graphs** tab, then click **1** to include only the selected view.
- 4. Highlight the Log2 Ratio column, then right-click to select Sum, mean and median. (Figure 54)

| Figure 54         Graphs tab - Log 2 Ratio column  |              |  |  |  |  |  |  |
|--|--------------|--|--|--|--|--|--|
| Preferences Help   |              |  |  |  |  |  |  |
| 🖻 🛤 🛿 🕼 📑 👫 💷 💷 🏂 🖬 🕡 💷 🚱 🌵 🎯 🔤 chr16: 50,167,373 - 74,187,531   |              |  |  |  |  |  |  |
| 💲 🏭 Karyoview 🎙 🖼 Segments 🎙 🕌 Cytor 🔊 ons 🎙 🥒 Overlap Map 🎙 🖾 Graphs 🎙 🕀 Som Mut 🎙 🖼 Query Samples 🎙 🖽 Query Segments 🎙 |              |  |  |  |  |  |  |
|  | ,724 results |  |  |  |  |  |  |
| Chromosome Position In Cytoregion • Markers O • BAF: CTL • Log2 Ratio:   |              |  |  |  |  |  |  |
| 16 74,044,996 × S-taq1161 0.0014261183 -0.31013146   |              |  |  |  |  |  |  |
| 16 74,058,446 × S-tag1122 0.99794906 -0.09681079   |              |  |  |  |  |  |  |
| 16 74,062,203 × <u>S-tag1909</u> 0.47065228 -0.14623095  |              |  |  |  |  |  |  |
| 16 74,086,300 × <u>S-tag1339</u> 0.5423078 -0.35421756   |              |  |  |  |  |  |  |
| 16 74,098,974 X <u>S-tag2157</u> 0.490758 0.26729825   |              |  |  |  |  |  |  |
| 16 74,107,201 X <u>S-tag1544</u> 0 -0.6092055  |              |  |  |  |  |  |  |
| 16 74,123,785 X S-tag1041 0.5027519 0.08870722   |              |  |  |  |  |  |  |
| 16 74,148,932 × <u>S-tag0959</u> 0.48566595 0.02667547   |              |  |  |  |  |  |  |
| 16 74,159,147 X <u>S-tag1737</u> 0.00043977 0.025229357  |              |  |  |  |  |  |  |
| 16 74,169,321 X S-tag1511 0.9980694 -0.04669896  |              |  |  |  |  |  |  |
| 16 74,186,767 × S-tag2043 0.0053921607 -0.32854828   |              |  |  |  |  |  |  |
| 2 Sum, mean and median   |              |  |  |  |  |  |  |

A Sum, mean and median window appears. (Figure 55)



- 5. Acknowledge the message, then click **OK**.
- 6. Open the **ChAS Analysis Workflow**, then click on the **QC Results** tab. (Figure 56)

| Figure 56 ChAS Analysis Workflow - QC Results window tab   |  |  |  |  |  |  |  |  |  |
|--|--|--|--|--|--|--|--|--|--|
| a Chromosome Analysis Suite - Analysis Workflow [For Research Use Only. Not for use in diagnostic procedures.]   |  |  |  |  |  |  |  |  |  |
| appliedbiosystems  |  |  |  |  |  |  |  |  |  |
| Analysis setup Workflow dashboard QC results Utility Actions Array Type: OncoScan  QC Settings OncoScanMetrics-Recenter View.r1 Edit or Create QC Settings |  |  |  |  |  |  |  |  |  |
| Result Files   |  |  |  |  |  |  |  |  |  |
| Add Files Remove Selected File(s) Select All Clear Selection Export QC Table Generate Report View In Browser Import Attributes Export to IGV QC Analysis - |  |  |  |  |  |  |  |  |  |
| Filename Threshold Test MAPD ≤ 0.3 ndSNPQC ≥ 26 ndWavinessSD TuScan L2R Adj Adjusted Log2 ratio ACDC % Aberr. Cells TuScan Ploidy Gender 1                 |  |  |  |  |  |  |  |  |  |
| CTL20130819_CN037P21_240K Within Bounds 0.259455 37.326906 0.113567 0 0 No NA NA male  |  |  |  |  |  |  |  |  |  |

- Load the OSCHP file into the QC Results tab by clicking on the Add Files button. (Figure 56)
- 8. From the **QC Settings** drop-down menu, select **Recenter View**, then make a note of the **TuScan L2R Adj** value. (Figure 56)
- 9. Click on the Analysis setup tab. (Figure 57)

|   | systems   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|---|---|-------------------------------------|----------|--------------------|----------------|---------------------|-------------------------------|------------|-------------------|----------------|-------------------------|
| alysis setup                                | Workflow dashboar   | d QC results                        |          |                    |                |                     |                               |            |                   |                | Utility A               |
| ay type: O                                  | IncoScan  | <ul> <li>Genome Version:</li> </ul> | hg19 🔹   | Analysis workflow: | FFPE Analysis: | NA33                |                               | -          | Workflow name:    | Workflow       |                         |
| rray inform                                 | nation  |                                     |          |                    |                |                     |                               |            |                   |                |                         |
| Copy numbe                                  | er reference model file                                     |                                     |          |                    |                | Annotation to be us | sed for analy                 | sis        |                   |                |                         |
| OncoScan.F                                  | FPE.na33.r1.REF_MODEL                                       | -                                   |          |                    | •              | OncoScan.na33.r1.a  | annot.db                      |            |                   |                |                         |
| omatic mut                                  | tation reference model fi                                   | le                                  |          |                    |                | Somatic mutation t  | hreshold file                 |            |                   |                |                         |
| OncoScan.F                                  | FPE.na33.r1.SOM_REF_N                                       | IODEL                               |          |                    | •              | OncoScan.Som1.0.    | r2.Som_thres                  | h.txt      |                   |                |                         |
| elect the in                                | atensity (CEL) file(s) to                                   | analyze                             |          |                    |                |                     |                               |            |                   |                | Total records           |
| Undo Rec                                    | do Add CEL Files 🔻  | Import Batch File                   | Export E | Batch File Display | : File Name    | Sort All            | Result File N                 | ames 🔻     | ]                 |                | Total records           |
| AT Char                                     | anal  |                                     |          | GC Channel         |                | • L                 |                               | Popult     | File Name         |                |                         |
| x 2013081                                   | 9 CN037P21 240K Jan   | oma CHTNWD WW 1                     | 0A CEL   | 20130819 CN037     | P21 240K lan   | oma CHTNWD WW       | 10C CEL                       | 201308     | 19 CN037P21 24    | DK A um Melano | oma CHTNWD WW 10        |
| 2013001                                     | 5_01057721_240101811  |                                     | OAICEE   | 20130015_01057     | P21_240KIdit   |                     | _100.010                      | 201500     | 15_01057721_24    | ok_Aum_weand   | 5///a_c/////wb_/////_10 |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
| •   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          | 1                  |                |                     |                               |            |                   |                |                         |
|   |   |                                     |          |                    |                |                     |                               |            |                   |                |                         |
| utput resu                                  | It information  |                                     |          |                    |                |                     | Optional                      |            |                   |                |                         |
| utput resul                                 | It information  |                                     |          |                    |                |                     | <b>Optional</b><br>Select a s | uffix to a | ppend to the anal | ysis results   |                         |
| utput resu<br>lease select<br>:\Clinical Sj | It information<br>coutput file folder<br>pecialist Training |                                     |          |                    |                |                     | Optional<br>Select a s        | uffix to a | opend to the anal | ysis results   |                         |

- 10. Select the FFPE or non-FFPE analysis based on the sample type.
- 11. Load in the two CEL files into the appropriate channel.
- 12. Check the **Manual Recenter** check box to enable the parameter fields. (Figure 57)
- 13. Enter the TuScan Log2 Adjustment value you noted earlier.
- 14. Enter the value of the **median Log2** determined from the browser into the **Adjust this log 2 to 0** field.
- 15. Enter a suffix if desired.

**Note:** An RC will automatically be appended onto any OSCHP file that goes through Manual Recentering for an RC.OSCHP extension.

Figure 58 shows the original OSCHP file (pink data) and the manually recentered RC.OSCHP (green data).

By inputting both the TuScan Log2 Ratio value (derived from the algorithm) and the median Log2 Ratio value (for the region you have determined to be diploid, Chromosome 16q for our example), the Recentering Algorithm has recentered the log2 ratio data (for the region determined to be diploid) around 0 and there is no longer a loss segment called in this region.





Setting up and running an OncoScan matched normal analysis

As long as your library file folder contains the necessary analysis files for the array, your configuration paths are established (Figure 59) your Array Information fields will auto-populate. (Figure 60)



| Figure 60          | Figure 60 Matched Normal Analysis Configuration                     |         |                           |         |                                       |   |                 |                 |  |
|--------------------|---|---------|---------------------------|---------|---------------------------------------|---|-----------------|-----------------|--|
| Analysis setup     | Workflow dashboard  | QC resu | ults Configuration        |         |                                       |   |                 | Utility Actions |  |
| Select array type: | OncoScan  | -       | Select analysis workflow: | FFPE Ar | alysis including Matched Normal: NA33 | - | Set workflow na | me: Workfle     |  |
| Array informat     | Array information   |         |                           |         |                                       |   |                 |                 |  |
| Copy number r      | Copy number reference model file Annotation to be used for analysis |         |                           |         |                                       |   |                 |                 |  |
| OncoScan.FFP       | E.na33.r1.REF_MODEL   |         |                           | -       | OncoScan.na33.r1.annot.db             |   |                 |                 |  |
| Somatic mutati     | on reference model file   |         |                           |         | Somatic mutation threshold file       |   |                 |                 |  |
| OncoScan.FFP       | E.na33.r1.SOM_REF_MOD   | EL      |                           | •       | OncoScan.Som1.0.r1.Som_thresh.txt     |   |                 |                 |  |

1. From the Select array type drop-down list, select OncoScan.

**Note:** The Select array type drop-down list includes only the array types from the library (analysis) files that have been downloaded from NetAffx or copied from the Library package provided in the OncoScan installation package.

**IMPORTANT!** After adding new library files to the library file folder, always close and relaunch OncoScan Console to ensure the newly added files are recognized by the software.

2. From the **Select analysis workflow** drop-down list, click to select **FFPE Analysis including Matched Normal NAXX**.

Other available Analysis Workflow options are:

Control DNA Analysis NAXX - Use this workflow for the Control DNA in the OncoScan Kit.

**Non-FFPE Analysis NAXX** - Use this workflow with cell line DNA.

- **FFPE Analysis NAXX** Use this workflow for a standard analysis.
- Enter a Workflow name (optional). By default, the Set workflow name is Workflow. Click Workflow (upper right) to enter a different workflow name.



**Note:** Customizing a Workflow name can be a useful tool in keeping track of analysis workflows as all the related output files (outside of the OSCHP file) are pre-fixed with this workflow name.

The Annotation file is automatically selected for you and is based on your selected reference model file. (Example: **OncoScan.na33.v1.annot.db**)

**Note:** The Annotation to be used for analysis field is auto-populated based on your Ref Model file selection. The analysis is not be permitted to run if the appropriate annotation file is not available in your Library folder.

- Select a Somatic mutation reference model file. By default, it is set to OncoScan.na33.v1.SOM\_REF\_MODEL. If you created your own reference model file, click the drop-down list to select your .SOM\_REF\_MODEL.
- Confirm the displayed Somatic mutation threshold file to be used is correct. If you need to change it, click the **Browse** button, navigate to the appropriate threshold TXT file, then click **OK**.

**IMPORTANT!** If the Reference Model File and Somatic mutation Reference Model File were created independently of each other, a warning message appears after you click Submit (to start the Workflow Analysis process). Click OK to acknowledge the message.

# Adding CEL files to analyze

You can manually add CEL files or import them as a tab-delimited text file.

### Manually adding CEL files to analyze

- 1. At the **Select the intensity (CEL) file(s) to analyze** pane, click the **Add CEL files** drop-down.
- 2. Click Tumor AT Channel.

The CEL file window appears. (Figure 61)

| Figure 61 CEL file folder example |  |  |  |  |  |  |  |  |  |
|-----------------------------------|--|--|--|--|--|--|--|--|--|
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |
|                                   |  |  |  |  |  |  |  |  |  |

3. Click any header to sort your files or click the **Files of type** drop-down to filter your CEL files by AT Channel, as shown in Figure 62.

| Figure 62 Files of type drop-down list |                           |  |  |  |  |  |  |  |
|--|---------------------------|--|--|--|--|--|--|--|
| File Name:                             |                           |  |  |  |  |  |  |  |
| Files of type:                         | Intensity (CEL) File(s) - |  |  |  |  |  |  |  |
|  | Intensity (CEL) File(s)   |  |  |  |  |  |  |  |
|  | AT Channel Files (*a.cel) |  |  |  |  |  |  |  |
|  | GC Channel Files (*c.cel) |  |  |  |  |  |  |  |

4. Single click, Ctrl click, or Shift click (to select multiple Tumor AT Channel files).

**IMPORTANT!** It is recommended to use an "A" or "C" as the last character to designate the channel in the CEL file naming convention. Example: "\_AS\_05A.CEL" is an AT Channel file, while "\_AS\_05C.CEL" is a GC Channel file. See Figure 61.

5. Click Open.

The Tumor AT Channel fields are now populated. (Figure 63)

| F  | Figure 63 Tumor AT Channel file list                        |   |                  |  |  |  |  |  |  |  |
|----|---|---|------------------|--|--|--|--|--|--|--|
| Se | Select the intensity (CEL) file(s) to analyze               |   |                  |  |  |  |  |  |  |  |
| U  | ndo Redo Add CEL Files  Import Batch File Export Batch File | Display: File Name   Sort All Result File Names |                  |  |  |  |  |  |  |  |
|    | AT Channel  | GC Channel                                      | Result File Name |  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_A01_1227701A7_Std_AS_01A.CEL              |   |                  |  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_A02_1221447B1_Std_AS_02A.CEL              |   |                  |  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_A03_1214010E3_Std_AS_03A.CEL              |   |                  |  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_A05_128596A1_Std_AS_05A.CEL               |   |                  |  |  |  |  |  |  |  |
| ×  | 20130327 CN037P01 A06 123183A2 Std AS 06A.CEL               |   |                  |  |  |  |  |  |  |  |

- 6. Click the Add CEL files drop-down.
- 7. Click Tumor GC Channel. The CEL file window appears. (Figure 61 on page 80)
- 8. Single click, Ctrl click, or Shift click (to select multiple Tumor GC Channel files).
- 9. Click Open.

The Tumor GC Channel fields are now populated. (Figure 64)

| Figure 64 Tumor GC Channel file list          |          |                                |                   |                   |                  |  |  |  |  |
|---|----------|--------------------------------|-------------------|-------------------|------------------|--|--|--|--|
|   |          |                                |                   |                   |                  |  |  |  |  |
| Select the intensity (CEL) file(s) to analyze |          |                                |                   |                   |                  |  |  |  |  |
| Tumor AT Channel                              | Impor    | Tumor GC Channel               | Normal AT Channel | Normal GC Channel | Result File Name |  |  |  |  |
| × 20130327_CN037P01_222K_A                    | 01_12277 | 20130327_CN037P01_222K_A01_122 | 77                |                   |                  |  |  |  |  |
| × 20130327_CN037P01_222K_A                    | 02_12214 | 20130327_CN037P01_222K_A02_122 | 14                |                   |                  |  |  |  |  |
| × 20130327_CN037P01_222K_A                    | 03_12140 | 20130327_CN037P01_222K_A03_121 | 40                |                   |                  |  |  |  |  |
| × 20130327_CN037P01_222K_A                    | 04_WD66  | 20130327_CN037P01_222K_A04_WD  | 66                |                   |                  |  |  |  |  |
|   |          | · ·                            |                   | ·                 |                  |  |  |  |  |

10. Click the **Add CEL files** drop-down.

11. Click Normal AT Channel. The CEL file window appears. (Figure 61)



12. Single click, Ctrl click, or Shift click (to select multiple Normal AT Channel files).

#### 13. Click Open.

The Normal AT Channel fields are now populated. (Figure 65)

| F | Figure 65 Normal AT Channel file list         |                                  |                                  |                   |                  |  |  |  |  |  |
|---|---|----------------------------------|----------------------------------|-------------------|------------------|--|--|--|--|--|
|   |   |                                  |                                  |                   |                  |  |  |  |  |  |
|   | Select the intensity (CEL) file(s) to analyze |                                  |                                  |                   |                  |  |  |  |  |  |
|   | Tumor AT Channel                              | Tumor GC Channel                 | Normal AT Channel                | Normal GC Channel | Result File Name |  |  |  |  |  |
| > | 20130327_CN037P01_222K_A01_12277              | 20130327_CN037P01_222K_A01_12277 | 20130327_CN037P01_222K_A01_12277 |                   |                  |  |  |  |  |  |
| > | 20130327_CN037P01_222K_A02_12214              | 20130327_CN037P01_222K_A02_12214 | 20130327_CN037P01_222K_A02_12214 |                   |                  |  |  |  |  |  |
| > | 20130327_CN037P01_222K_A03_12140              | 20130327_CN037P01_222K_A03_12140 | 20130327_CN037P01_222K_A03_12140 |                   |                  |  |  |  |  |  |
| > | 20130327_CN037P01_222K_A04_WD66               | 20130327_CN037P01_222K_A04_WD66  | 20130327_CN037P01_222K_A04_WD66  |                   |                  |  |  |  |  |  |

#### 14. Click the Add CEL files drop-down.

- 15. Click Normal GC Channel. The CEL file window appears. (Figure 61)
- 16. Single click, Ctrl click, or Shift click (to select multiple Normal GC Channel files).
- 17. Click Open.

The Normal GC Channel fields are now populated. (Figure 66)

| F  | Figure 66 Normal GC Channel file list   |                                  |                                  |                                  |                  |  |  |  |  |  |  |
|----|---|----------------------------------|----------------------------------|----------------------------------|------------------|--|--|--|--|--|--|
| Se | Select the intensity (CEL) file(s) to analyze   |                                  |                                  |                                  |                  |  |  |  |  |  |  |
| U  | Undo Redo Add CEL Files  Import Batch File Export Batch File Display: File Name  Sort All Result File Names |                                  |                                  |                                  |                  |  |  |  |  |  |  |
|    | Tumor AT Channel  | Tumor GC Channel                 | Normal AT Channel                | Normal GC Channel                | Result File Name |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_222K_A01_12277  | 20130327_CN037P01_222K_A01_12277 | 20130327_CN037P01_222K_A01_12277 | 20130327_CN037P01_222K_A01_12277 |                  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_222K_A02_12214  | 20130327_CN037P01_222K_A02_12214 | 20130327_CN037P01_222K_A02_12214 | 20130327_CN037P01_222K_A02_12214 |                  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_222K_A03_12140  | 20130327_CN037P01_222K_A03_12140 | 20130327_CN037P01_222K_A03_12140 | 20130327_CN037P01_222K_A03_12140 |                  |  |  |  |  |  |  |
| ×  | 20130327_CN037P01_222K_A04_WD66   | 20130327_CN037P01_222K_A04_WD66  | 20130327_CN037P01_222K_A04_WD66  | 20130327_CN037P01_222K_A04_WD66  |                  |  |  |  |  |  |  |

### CEL file displaying options (optional)

The File Name drop-down list (Figure 67) is dynamically populated and based on what attributes are populated in the ARR file.

#### To use this display option, you must:

- 1. Provide the appropriate attributes at the time of sample registration in AGCC.
- 2. The ARR files must reside in the same folder as the CEL files.

| <b>Figure 6</b><br>Display ( | 7 EXAMPLE: File<br>Choices | Name |
|------------------------------|----------------------------|------|
|                              | File Name 🔹                |      |
|                              |                            |      |
|                              | File Name                  |      |
|                              | channel                    |      |

To see "channel" (as an option in the drop down), you must use a template (or the

4

OncoScan template provided in the AGCC library files) that contains a "channel" attribute. The resulting ARR file must also reside in the same folder as the CEL files you are analyzing.

You can display one of the attributes from the ARR file in the table. For example, "Channel" can be chosen (Figure 67) to confirm the assignment of a CEL file to its appropriate channel.

## Selecting a File Name display attribute

1. Click the **File Name** drop-down button, then click to select the attribute you want displayed along with your CEL file names.

The two examples (Figure 68 and Figure 69) show how the table appears with the display set to Filename, then to Channel.

| Figure 68 Table with Filename displayed  |                          |                         |                          |                           |  |  |  |  |  |  |
|--|--------------------------|-------------------------|--------------------------|---------------------------|--|--|--|--|--|--|
|  |                          |                         |                          |                           |  |  |  |  |  |  |
| ect the intensity (CEL) file(s) to a   | analyze                  |                         |                          |                           |  |  |  |  |  |  |
| ido Redo   Add CEL Files 🔻 Import Batch File Export Batch File Display: File Name 🔹 Sort All   Result File Names 🖛 |                          |                         |                          |                           |  |  |  |  |  |  |
| Tumor AT Channel   | Tumor GC Channel         | Normal AT Channel       | Normal GC Channel        | Result File Name          |  |  |  |  |  |  |
| 20130327_CN0o_AS_01A.CEL   | 20130327_CN0o_AS_01C.CEL | 20130327_CNAS_04A.CEL   | 20130327_CNo_AS_06C.CEL  | 20130327_CN0antiago_AS_01 |  |  |  |  |  |  |
| 20130327_CN0o_AS_02A.CEL   | 20130327_CN0o_AS_02C.CEL | 20130327_CNo_AS_05A.CEL | 20130327_CN0o_AS_07C.CEL | 20130327_CN0antiago_AS_02 |  |  |  |  |  |  |
| 20130327_CN0o_AS_03A.CEL   | 20130327_CN0o_AS_03C.CEL | 20130327_CNo_AS_06A.CEL | 20130327_CN0o_AS_08C.CEL | 20130327_CN0antiago_AS_03 |  |  |  |  |  |  |
|  |                          |                         |                          |                           |  |  |  |  |  |  |

| bel   | ect the intensity (CEL) file(s | ;) to a | analyze               |    |                      |    |                      |    |                          |
|---|--------------------------------|---------|-----------------------|----|----------------------|----|----------------------|----|--------------------------|
| Undo Redo   Add CEL Files 🔻 Import Batch File Export Batch File Display: Channel 🔹 Sort All Result File Names 💌 |                                |         |                       |    |                      |    |                      |    |                          |
|   | Tumor AT Channel               | _       | Tumor GC Channel      |    | Normal AT Channel    |    | Normal GC Channel    |    | Result File Name         |
| ×   | 20130327_CAS_01A.CEL           | AT      | 20130327_CAS_01C.CEL  | GC | 20130327AS_04A.CEL   | AT | 20130327_CAS_06C.CEL | GC | 20130327_CN0antiago_AS_0 |
| ×   | 20130327_CAS_02A.CEL           | AT      | 20130327_CAS_02C.CEL  | GC | 20130327_CAS_05A.CEL | AT | 20130327_CAS_07C.CEL | GC | 20130327_CN0antiago_AS_0 |
| ×   | 20130327 C AS 03A.CEL          | AT      | 20130327 C AS 03C.CEL | GC | 20130327 CAS 06A.CEL | AT | 20130327_CAS_08C.CEL | GC | 20130327 CN0antiago AS 0 |

## Importing CEL files using batch import

The batch file must be saved as a text (Tab-delimited) format and include the full directory path for your CEL files, as shown in Figure 70.

**Note:** The resulting OSCHP files are saved to your output path location, therefore it is not necessary to include a path under RESULT. Simply enter the desired results filename in this column.

The format for this tab-delimited file is 5 columns (A,B, C, D, and E) with the headers:

- ATCHANNELCEL
- GCCHANNELCEL
- ATChannelMatchedNormalCel
- GCChannelMatchedNormalCel
- RESULT

You must provide the full path to the CEL files for each Channel column.

### (Example: C:\Desktop\OncoScan\Data\Sample1.cel)

| F | Figure 70 List from Windows Excel |                                   |                                   |                                   |                           |  |  |  |  |  |  |  |  |
|---|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|---------------------------|--|--|--|--|--|--|--|--|
|   | А                                 | В                                 | С                                 | D                                 | E                         |  |  |  |  |  |  |  |  |
| 1 | ATCHANNELCEL                      | GCCHANNELCEL                      | ATChannelMatchedNormalCel         | GCChannelMatchedNormalCel         | RESULT                    |  |  |  |  |  |  |  |  |
| 2 | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | 20130327_CN037P01_222K_A0 |  |  |  |  |  |  |  |  |
| 3 | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | 20130327_CN037P01_222K_A0 |  |  |  |  |  |  |  |  |
| 4 | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | L:\OncoScan\AnalysisWorkflow\Onco | 20130327_CN037P01_222K_A0 |  |  |  |  |  |  |  |  |

#### 1. Click Import Batch File

A File window appears.

2. Navigate to your text (tab delimited) file location, then click on the file you want to import.

#### **IMPORTANT!** Excel must be closed before you import.

3. Click **Open**.

The Tumor AT, Tumor GC, Normal AT, Normal GC and Result File Name fields are now populated. (Figure 71)

| Fi | Figure 71 Tab-delimited text file imported into OncoScan Console  |                          |                         |                          |                           |  |  |  |  |  |  |  |
|----|---|--------------------------|-------------------------|--------------------------|---------------------------|--|--|--|--|--|--|--|
| Se | Select the intensity (CEL) file(s) to analyze   |                          |                         |                          |                           |  |  |  |  |  |  |  |
| U  | Undo Redo Add CEL Files  Import Batch File Export Batch File Display: File Name  Sort All Result File Names |                          |                         |                          |                           |  |  |  |  |  |  |  |
|    | Tumor AT Channel  | Tumor GC Channel         | Normal AT Channel       | Normal GC Channel        | Result File Name          |  |  |  |  |  |  |  |
| ×  | 20130327_CN0o_AS_01A.CEL  | 20130327_CN0o_AS_01C.CEL | 20130327_CNAS_04A.CEL   | 20130327_CNo_AS_06C.CEL  | 20130327_CN0antiago_AS_01 |  |  |  |  |  |  |  |
| ×  | 20130327_CN0o_AS_02A.CEL  | 20130327_CN0o_AS_02C.CEL | 20130327_CNo_AS_05A.CEL | 20130327_CN0o_AS_07C.CEL | 20130327_CN0antiago_AS_02 |  |  |  |  |  |  |  |
| ×  | 20130327_CN0o_AS_03A.CEL  | 20130327_CN0o_AS_03C.CEL | 20130327_CNo_AS_06A.CEL | 20130327_CN0o_AS_08C.CEL | 20130327_CN0antiago_AS_03 |  |  |  |  |  |  |  |



## **Reference files**

This section explains how to create a reference file which is required to perform single sample analysis in ChAS. The software analyzes a sample file by comparing it to a reference file. You can use the reference file provided with ChAS, or you can create a reference file using your own sample data.

See Figure 72 for an overview of the analyses involved in creating a reference file for the CytoScan Arrays.





## Creating a reference file

**IMPORTANT!** When creating a CytoScan reference file, it is recommended that you use a minimum of 44 CEL files. These CEL files must include at least 20 male and 20 female samples.

1. From the Analysis menu, select Perform Analysis Setup. (Figure 73)

| Figure 73         | Analysis drop-down m              | enu        |
|-------------------|-----------------------------------|------------|
| appliedbiosyst    | ems                               | Chromosome |
| Eile View Exports | Analysis ChAS DB Preferences Help |            |
| 💕 🔀 🛃 💔           | Perform Analysis Setup            | 📕 i 击 💷    |
| Files             | Analysis Dashboard 🔻 🚼 Segments   | CytoRegion |

The Analysis Workflow window tab opens. (Figure 74)

| Figure 74         | Analysis Worl               | kflow           |            |                     |                               |                      |                |                 |
|-------------------|-----------------------------|-----------------|------------|---------------------|-------------------------------|----------------------|----------------|-----------------|
| applied biosyst   | tems                        |                 |            |                     |                               |                      |                | 0               |
| Analysis setup    | Workflow dashboard          | QC results C    | onfigurati | ion                 |                               |                      |                | Utility Action  |
| Array type: CytoS | ScanHD_Array •              | Genome Version: | hg19 🔻     | Analysis workflow:  | CytoScanHD_Array Reference Mo | del Creation: NA33 🝷 | Workflow name: | Workflow        |
| Array information | on                          |                 |            |                     |                               |                      |                |                 |
| Select assay prep | paration type used          |                 |            |                     |                               |                      |                |                 |
| Manual            |                             |                 |            |                     |                               |                      |                | •               |
| Select the annota | ation file for this analysi | S               |            |                     |                               |                      |                |                 |
| CytoScanHD_Ar     | rray.na33.annot.db          |                 |            |                     |                               |                      |                | •               |
| Select the intens | sity (CEL) files to analy   | ze; minimum 44. | with 20 n  | nale samples and 20 | female samples recommended    |                      | Т              | otal records: 0 |
|                   |                             |                 |            |                     |                               |                      |                |                 |
| Output result in  | formation                   |                 |            |                     |                               |                      | Add            | Remove          |
| Input the name o  | of the reference model f    | file            |            |                     |                               |                      |                |                 |
|                   |                             |                 |            |                     |                               |                      | Submit         | Reset           |

2. From the **Select array type** drop-down list, click to select an array type (Example: CytoScanHD\_Array.

**Note:** The Select array type drop-down list includes only the array types for which library (analysis) files have been downloaded from NetAffx or copied from the Library package provided with the installation.

3. Select a Genome Build. (Example: hg19)

- 4. From the **Select analysis workflow** drop-down list, click to select an analysis workflow. (Example: CytoScanHD\_Array Reference Model Creation:NA33)
- 5. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.
- 6. Click the **Assay Preparation type used** drop-down button, then select one of the following:
  - Manual (CEL files whose assays were performed by hand)
  - Automation (CEL files whose assays were assisted by a robot)
  - Automation, Manual (a mix of CEL files whose assays were performed either by hand or with the assistance of a robot.

**Note:** The Annotation file for analysis is auto-selected once the array type, analysis workflow and assay preparation type used fields are selected.

- 7. From the **Select the annotation file for this analysis** drop-down list, verify the selection of the annotation file. (Example, CytoScanHD\_Array.na.33.annot.db)
- 8. At the Select the intensity (CEL) file(s) to analyze pane, click Add.

**IMPORTANT!** The same annotation file you used to create a Reference Model File MUST also be used with future Single Sample Analyses runs that utilize your created Reference Model File.

| r .                           | [        |                          |                     |       |               |              |
|-------------------------------|----------|--------------------------|---------------------|-------|---------------|--------------|
| Current direct                | ory      | C:\CytoScan\Data\Batch 1 |                     |       | <i>¥</i>      | Up one leve  |
|                               | <b>A</b> | Name                     | Date Modified       | Size  | Туре          | Array ID     |
|                               |          | 🐹 Sample_01.CEL          | 8/8/2011 7:15 AM    | 66 MB | AGCC CEL File | 9e5653c3-de8 |
| C:\Users\cgates<br>\Desktop   |          | Sample_02.CEL            | 10/14/2011 6:23 AM  | 66 MB | AGCC CEL File | 12f20ec2-034 |
| ·                             | ≣        | Sample_03.CEL            | 7/31/2013 4:41 PM   | 66 MB | AGCC CEL File | 9e565f0a-989 |
| 1                             |          | 🐹 Sample_04.CEL          | 5/17/2013 8:19 AM   | 66 MB | AGCC CEL File | cb115160-647 |
| C:\Users\cgates<br>\Documents |          | 🐹 Sample_06.CEL          | 5/28/2013 11:10 AM  | 66 MB | AGCC CEL File | 8efa3b98-481 |
|                               |          | Sample_07.CEL            | 5/28/2013 11:11 AM  | 66 MB | AGCC CEL File | 664b82e7-d43 |
|                               |          | 💥 Sample_08.CEL          | 11/23/2011 11:11 AM | 66 MB | AGCC CEL File | de2455c4-a8a |
| CA                            |          |                          |                     |       |               |              |
| C:\Cytoscan                   |          | 4                        |                     |       |               |              |
| Udld                          |          | •                        | 1111                |       |               |              |

The following window appears: (Figure 75)

 Single click, Ctrl click, or Shift click (to select multiple CEL files), then click Open. The Select the intensity (CEL) file(s) to analyze pane is now populated with your CEL files. (Figure 76)

| nalysis setup Workflow dashboard QC re           | sults   |   | Ut                                    | ility Actio |
|--|---|---|---------------------------------------|-------------|
| elect array type: CytoScanHD_Array               | <ul> <li>Select analysis workflow:</li> </ul> | CytoScanHD_Array Reference Model Creation: NA33 | <ul> <li>Set workflow name</li> </ul> | :: Workf    |
| Array information                                |   |   |                                       |             |
| Select assay preparation type used               |   |   |                                       |             |
| Manual   |   |   |                                       | •           |
| Select the annotation file for this analysis     |   |   |                                       |             |
| CytoScanHD_Array.na33.annot.db                   |   |   |                                       | •           |
| Select the intensity (CEL) files to analyze; min | imum 44, with 20 male san                     | nples and 20 female samples recommended         | Total re                              | cords: 14   |
| Sample_03.CEL                                    |   |   |                                       |             |
| Sample_04.CEL                                    |   |   |                                       |             |
| Sample_06.CEL                                    |   |   |                                       |             |
| Sample_07.CEL                                    |   |   |                                       |             |
| Sample_08.CEL                                    |   |   |                                       |             |
| Sample_09.CEL                                    |   |   |                                       | =           |
| Sample_10.CEL                                    |   |   |                                       |             |
| Sample_11.CEL                                    |   |   |                                       |             |
| Sample_12.CEL                                    |   |   |                                       |             |
| Sample_13.CEL                                    |   |   |                                       |             |
| Sample_15.CEL                                    |   |   |                                       | -           |
|  |   |   | Add R                                 | emove       |
| Output result information                        |   |   |                                       |             |
| Input the name of the reference model file       |   |   |                                       |             |

To remove a CEL file from this list, click to highlight it, then click **Remove**.

10. At the **Output result information** pane, enter a name for your reference model file. (Figure 77)



11. Click Submit.



## Analysis workflow exports and QC tools

## Displaying and exporting data from the analysis workflow

# Adding files to the QC results table

1. Click the **QC results** tab. (Figure 78)

The QC results window tab appears.

2. Click Add Files.

A Chromosome Analysis Suite window appears.

- 3. Navigate to your folder's location, then select the xxCHP files you want to add.
- 4. Click Open.

Your selected files appear in the Export QC Table window tab, as shown in Figure 78.

# Exporting QC table information

- Check the adjacent check box next to the file(s) you want to export or click the Select All button (atop the check boxes) to auto-check all the displayed files.
- 2. Click on the **Export QC Table** tab.

| Fig   | Figure 78 Workflow Dashboard loaded  |                 |                 |               |                     |              |              |                 |  |  |  |  |
|-------|--|-----------------|-----------------|---------------|---------------------|--------------|--------------|-----------------|--|--|--|--|
| appl  | applied biosystems   |                 |                 |               |                     |              |              |                 |  |  |  |  |
| Analy | Analysis setup Workflow dashboard QC results   |                 |                 |               |                     |              |              |                 |  |  |  |  |
| Array | Array Type: CytoScanHD_Array  QC Settings: CytoScanHDMetrics-Basic View.r1  Edit or Create QC Settings |                 |                 |               |                     |              |              |                 |  |  |  |  |
| R     | Result Files   |                 |                 |               |                     |              |              |                 |  |  |  |  |
| Ad    | d Files Remove Selected File(s)  | Select All Clea | ar Selection Ex | port QC Table | Generate Report 🔻 🕔 | 'iew In Brov | vser View In | MSV Attributes  |  |  |  |  |
|       | Filename   | Threshold Test  | MAPD ≤ 0.25     | SNPQC ≥ 15.00 | Waviness SD ≤ 0.12  | Gender       | NA Version   | Genomic Version |  |  |  |  |
|       | Sample_01.cyhd.cychp   | Within Bounds   | 0.1494849       | 20.56247      | 0.09027517          | male         | 33           | hg19            |  |  |  |  |
|       | Sample_02.cyhd.ND.cychp  | Within Bounds   | 0.1711026       | 23.7153       | 0.0644021           | male         | 33           | hg19            |  |  |  |  |
|       | Sample_03.cyhd.cychp Within Bound  |                 | 0.1618743       | 19.62793      | 0.09671989          | male         | 33           | hg19            |  |  |  |  |
|       | Sample_04.CHAS_4_2.cyhd.cychp  | Within Bounds   | 0.1443655       | 23.29296      | 0.06793946          | female       | 33           | hg19            |  |  |  |  |
| -     | Sample_05.cychp  | Within Bounds   | 0.1831227       | 22.61078      | 0.08421145          | female       | 33           | hg19            |  |  |  |  |

An Explorer window appears.

- 3. Navigate to the export location you want, then enter a name for your QC file.
- 4. Click Save.



## Exporting probelevel data

- 1. Check the adjacent box next to the file(s) you want to open in the ChAS Browser.
- Click the View in Browser button to open the files in the ChAS Browser or click the View in MSV button to open the files in the Multi-Sample Viewer. For more information on the MSV see, the RHAS User Guide.

## Exporting CytoScan Probe-Level Data

- In the QC Results tab, click the check box adjacent to the Results File(s) you want to generate a report for or click the Select All button (atop the check boxes), as shown in Figure 79.
- 2. To export probe-level data, click the Generate Report drop-down. (Figure 79)

| Figure 79 Generate Report drop-dowr   | n meni       | L           |                             |                  |               |            |               |                 |
|---|--------------|-------------|-----------------------------|------------------|---------------|------------|---------------|-----------------|
| Chromosome Analysis Suite - Analysis Workflow [For Research Use Only. Not for u   | se in diagno | tic procedu | res.]                       | ****             |               | _          |               |                 |
| applied biosystems  |              |             |                             |                  |               |            |               | •               |
| Analysis setup Workflow dashboard QC results  |              |             |                             |                  |               |            |               | Utility Actio   |
| Array Type: CytoScanHD_Array QC Settings: CytoScanHDMetrics-Basic View.r1   | Edit or      | Create QC S | ettings                     |                  |               |            |               |                 |
| Result Files           Add Files         Remove Selected File(s)         Select All         Clear Selection         Export QC T | able Gene    | rate Report | <ul> <li>View In</li> </ul> | Browser Import A | ttributes Exp | ort to IGV | QC Analysis 💌 |                 |
| Filename  | Thre         | Export Gen  | otype Data                  |                  | ness SD ≤ 0.1 | 2 Gender   | NA Version    | Genomic Version |
| A2_0646_FH105810.ChAS3.cyhd.cychp   | With         | Export Prob | e level Data                |                  | 0.0717324     | 1 male     | 33            | hg19            |
| 11-0810_LC_ONC138_A6_PoP#2_CytoScan-PS_20110511.ChAS3.cyhd.cychp  | With         |             |                             |                  | 0.0772266     | 9 male     | 33            | hg19            |
| I1-0816_LC_ONC418_A12_PoP#2_CytoScan-PS_20110511.ChAS3.cyhd.cychp   | With         | Copy Numb   | er Expresssio               | n Overlap Report | 0.0982254     | 1 male     | 33            | hg19            |
| C474_A8_CytoScanHD_LabCorp_BetaTest-1_C8_06022011.ChAS3.cyhd.cychp  | With         | Export Gene | e Report                    |                  | 0.0876115     | 9 male     | 33            | hg19            |
| ARUP18_B02_CytoScanHD_ARUP_Beta1_LR_06012011.ChAS3.cyhd.cychp   | Within Box   | inds (      | 0.1918176                   | 21.0435          | 0.0986106     | 5 male     | 33            | hg19            |
| 11-1311_6210_86_PoP_CytoScan_20160713.cyhd.cychp  | Outside Bo   | unds        | 0.1523745                   | 14.88578         | 0.186563      | 2 male     | 33            | hg19            |
| 99-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.ChAS3.cyhd.cychp  | Within Box   | unds (      | 0.2049336                   | 24.3833          | 0.0829583     | 3 female   | 33            | hg19            |
| 11-0816 LC ONC1348 B10 PoP#2 CytoScan-PS 20110511.ChAS3.cyhd.cychp  | Within Box   | inds        | 0.1618529                   | 19.62777         | 0.0967019     | 2 male     | 33            | hg19            |

The following export reporting options appear: (Figure 79)

## 3. Click to select Export Probe Level Data. (Figure 79)

Your previously assigned Output folder file window appears.

**Note:** The default root filename is Result. Click inside the File Name field to enter a different root filename.

4. Enter a File Name for your text (tab-delimited) reporting file, then click **Save**, or navigate to different save location.

## Exporting OncoScan probe-level data

- In the QC Results tab, click each check box next to the Results File(s) you want to generate a report for. Click the Select All button (atop of the check boxes) (Figure 80) to auto-check all the displayed files.
- 2. To export probe-level data, click the Generate Report drop-down.

The following export reporting options appear: (Figure 80)

| Figure 80 Generate Report drop-down menu   |                    |                |              |                    |                 |                    |                  |                         |               |                |               |        |            |  |  |
|--|--------------------|----------------|--------------|--------------------|-----------------|--------------------|------------------|-------------------------|---------------|----------------|---------------|--------|------------|--|--|
| Chromosome Analysis Suite - Analysis Workflow (For Research Use Only. Not for use in diagnostic procedures.) |                    |                |              |                    |                 |                    |                  |                         |               |                |               |        |            |  |  |
| appl   | appliedbiosystems  |                |              |                    |                 |                    |                  |                         |               |                |               |        |            |  |  |
| Analysis setup Workflow dashboard QC results Utility Actions   |                    |                |              |                    |                 |                    |                  |                         |               |                |               |        |            |  |  |
| Array  | Type: OncoScan     | QC Settings:   | OncoScanMetr | rics-Basic View.r1 | Edit or         | Crea               | te QC Settings   |                         |               |                |               |        |            |  |  |
| R  | esult Files        |                |              |                    |                 |                    |                  |                         |               |                |               |        |            |  |  |
| Add Files Remove Selected File(s) Select All Clear Selection Export QC Table                                 |                    |                |              | Ger                | nerate Report 🔻 | View In Browser Im | port Attrib      | Export to               | IGV QC Analys | sis 💌          |               |        |            |  |  |
|  | Filename           | Threshold Test | MAPD ≤ 0.3   | ndSNPQC ≥ 26       | SNP QC T        |                    | Export All Probe | e Level Data            | - 1           | % Aberr. Cells | TuScan Ploidy | Gender | NA Version |  |  |
|  | Normal01OSCHP      | Within Bounds  | 0.156494     | 58.758807          |                 | 1                  | Export CelPairC  | heck Report             |               | homogeneous    | 2             | male   | 33         |  |  |
|  | Normal02_OSCHP     | Within Bounds  | 0.145891     | 69.901331          |                 |                    | Export Probe le  | vel Data                |               | homogeneous    | 2             | male   | 33         |  |  |
|  | Normal03OSCHP      | Within Bounds  | 0.16881      | 42.842395          |                 |                    | Export Segmen    | t Data                  |               | homogeneous    | 2             | female | 33         |  |  |
|  | Normal04OSCHP      | Within Bounds  | 0.155762     | 46.764626          |                 |                    | Export Somatic   | Mutation Data           |               | homogeneous    | 2             | female | 33         |  |  |
|  | RenalCell_01.OSCHP | Within Bounds  | 0.165205     | 67.689381          |                 |                    |                  |                         | NA            | NA             | male          | 33     |            |  |  |
|  | RenalCell_02.OSCHP | Within Bounds  | 0.173248     | 68.885982          |                 |                    | Copy Number 8    | expresssion Overlap Rep | ort           | 55             | 2             | male   | 33         |  |  |
|  | RenalCell_03.OSCHP | Within Bounds  | 0.196893     | 41.014252          |                 |                    | Export Gene Re   | port                    |               | 65             | 2             | female | 33         |  |  |
|  | RenalCell_04.OSCHP | Within Bounds  | 0.199442     | 53.992391          |                 | ND                 | Pass             | 0.124813                | No            | 95             | 2             | female | 33         |  |  |

 If you want to export all 4 available reports at once, click to select Export All Probe Level Data. (Figure 80) Otherwise, click to select the specific report(s) you want export.

The appropriate (previously assigned) folder file window appears.

**Note:** The default root filename is Result. Click inside the File Name field to enter a different root filename.

4. Enter a File Name for your reporting file, then click **Save** or navigate to a different save location.

This report summarizes the copy number segments that overlap user defined regions of interest (e.g., Genes) as defined in the selected BED file.

1. From the **QC Results** tab, click the **Generate Report** drop-down menu and select **Export Gene Report**. (Figure 81)

| CvtoScan arrav report                  | OncoScan array report  |  |  |  |  |  |  |
|--|--|--|--|--|--|--|--|
| Generate Report 🔻 View In Browser      | Generate Report  View In Browser                             |  |  |  |  |  |  |
| Export All Probe Level Data            | Export Genotype Data   |  |  |  |  |  |  |
| Export CelPairCheck Report             | Export Probe level Data                                      |  |  |  |  |  |  |
| Export Probe level Data                |  |  |  |  |  |  |  |
| Export Segment Data                    | Copy Number Expresssion Overlap Report<br>Export Gene Report |  |  |  |  |  |  |
| Export Somatic Mutation Data           |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| Copy Number Expresssion Overlap Report |  |  |  |  |  |  |  |
| Export Gene Report                     |  |  |  |  |  |  |  |

The following window appears. (Figure 82)

Exporting a gene report (CytoScan or OncoScan arrays)

| Figure 82 Sele                | ect the BEI        | D file for the Gene      | e Report window   | V     |          |   |              |
|-------------------------------|--------------------|--------------------------|-------------------|-------|----------|---|--------------|
| OncoScan: Select the BE       | D file for the Ger | e Report                 |                   |       |          |   |              |
| Current directo               | ry C:\Users\ppa    | vic\Desktop\OncoScan_1.1 | _Library_Files    |       |          | 2 | Up one level |
|                               | Name               |                          | Date Modified     | Size  | Туре     |   |              |
|                               | OncoSci            | an.na33.r1.bed           | 2/24/2014 3:52 PM | 8 MB  | BED File |   |              |
| C:\Users\ppavic<br>\Desktop   | OncoSci            | anGeneBoundaries.r1.bed  | 5/5/2014 3:16 PM  | 27 KB | BED File |   |              |
| C:\Users\ppavic<br>\Documents |                    |                          |                   |       |          |   |              |
| €<br>D:\                      |                    |                          |                   |       |          |   |              |
|                               | , File Name:       | OncoScanGeneBoundaries   | .r1.bed           |       |          |   | Open         |
|                               | Files of type:     | .bed                     |                   |       |          |   | • Cancel     |

2. Click to select the appropriate BED file, then click **Open**.

**Note:** Any BED file can be used to generate the Gene Report on any regions of interest contained within the BED file.

Your previously assigned Output folder file window appears. (Figure 83)

| Figure 83 Onco  | Scan Out                     | tput folder wi          | ndow          |      |      |   |                |
|---|------------------------------|-------------------------|---------------|------|------|---|----------------|
| OncoScan  |                              |                         |               |      |      |   | ×              |
| Current directory   | C:\Users\ppa                 | wic\Desktop\OncoSc      | an_Output     |      |      | 2 | Up one level   |
| C:\Users\ppavic<br>\Desktop<br>C:\Users\ppavic<br>\Documents<br>C:\ | Name                         |                         | Date Modified | Size | Туре |   |                |
| D:\   |                              |                         |               |      |      |   | 1              |
| Create New Folder   | File Name:<br>Files of type: | Result *.genereport.txt |               |      |      | • | Save<br>Cancel |

3. The default root filename is Result. Click inside the File Name field to enter a different root filename, then click **Save**.

A progress bar appears while your report generates, followed by a message window. (Figure 84)

| Figure 84 Gene Report finished successfully |  |        |  |  |  |  |  |  |  |  |
|---|--|--------|--|--|--|--|--|--|--|--|
| OncoScan                                    |  | ×      |  |  |  |  |  |  |  |  |
| Export Gene Report finished successfully.   |  |        |  |  |  |  |  |  |  |  |
| Do you want to view the result?             |  |        |  |  |  |  |  |  |  |  |
|   |  | Yes No |  |  |  |  |  |  |  |  |

4. Click Yes.

The Results Output folder window appears.

5. Locate the Gene Report text file, then open it in Microsoft Excel.

The following window appears. (Figure 85)

| <b>J</b> • • |              | •              |              |        |                |                |                                |                   |             |          |   |
|--------------|--------------|----------------|--------------|--------|----------------|----------------|--------------------------------|-------------------|-------------|----------|---|
| Filename     | Chromosome   | Start Position | End Position | Genes  | Threshold Test | % Aberr. Cells | TuScan Ploidy Low Diploid Flag | Median Log2 Ratio | Median BAF  | State LC | ᆘ |
| Normal02     | 1            | 59236462       | 59259785     | JUN    | Within Bounds  | homogeneous    | 2 No                           | 0.007             | 0.484       | 2 -      |   |
| Normal02     | 1            | 156020966      | 156050295    | RAB25  | Within Bounds  | homogeneous    | 2 No                           | 0.011             | 0.485       | 2 -      |   |
| Normal02     | 2            | 16070682       | 16097129     | MYCN   | Within Bounds  | homogeneous    | 2 No                           | 0.013             | 0.486       | 2 -      |   |
| Normal02     | 2            | 61098751       | 61160178     | REL    | Within Bounds  | homogeneous    | 2 No                           | 0.013             | 0.486       | 2 -      |   |
| Normal02     | 2            | 99051320       | 99208284     | INPP4A | Within Bounds  | homogeneous    | 2 No                           | 0.011             | 0.485       | 2 -      |   |
| Normal02     | 3            | 10173318       | 10205354     | VHL    | Within Bounds  | homogeneous    | 2 No                           | 0.009             | 0.486       | 2 -      |   |
| Normal02     | 3            | 69778585       | 70027488     | MITE   | Within Bounds  | homogeneous    | 2 No                           | 0.009             | 0.486       | 2 -      |   |
| Normal02     | 7            | 55076724       | 55234644     | EGFR   | Within Bounds  | homogeneous    | 2 No                           | 0.009             | 0.484       | 2 -      |   |
| Normal02     | 7            | 116302458      | 116448440    | MET    | Within Bounds  | homogeneous    | 2 No                           | 0.009             | 0.484       | 2 -      |   |
| Normal02     | 8            | 128738314      | 128763680    | MYC    | Within Bounds  | homogeneous    | 2 No                           | -0.177            | NaN         | 1.5 -    |   |
| Normal02     | 9            | 21957750       | 21984826     | CDKN2A | Within Bounds  | homogeneous    | 2 No                           | 0.006             | 0.485       | 2 -      |   |
| Normal02     | 9            | 21992901       | 22019312     | CDKN2B | Within Bounds  | homogeneous    | 2 No                           | 0.006             | 0.485       | 2 -      |   |
| Normal02     | 10           | 89613194       | 89738532     | PTEN   | Within Bounds  | homogeneous    | 2 No                           | -0.002            | 0.486       | 2 -      |   |
| Normal02     | 11           | 69445872       | 69479242     | CCND1  | Within Bounds  | homogeneous    | 2 No                           | 0.002,-0.432      | 0.485,NaN   | 2,1 -    |   |
| Normal02     | 12           | 58131509       | 58156230     | CDK4   | Within Bounds  | homogeneous    | 2 No                           | -0.003            | 0.485       | 2 -      |   |
| Normal02     | 12           | 69191970       | 69249212     | MDM2   | Within Bounds  | homogeneous    | 2 No                           | -0.003            | 0.485       | 2 -      |   |
| Normal02     | 13           | 32879616       | 32983809     | BRCA2  | Within Bounds  | homogeneous    | 2 No                           | 0.016             | 0.485       | 2 -      |   |
| Normal02     | 13           | 48867882       | 49066026     | RB1    | Within Bounds  | homogeneous    | 2 No                           | 0.016             | 0.485       | 2 -      |   |
| Normal02     | 17           | 7561719        | 7588811      | TP53   | Within Bounds  | homogeneous    | 2 No                           | -0.016            | 0.486       | 2 -      |   |
| Normal02     | 17           | 37834392       | 37894915     | ERBB2  | Within Bounds  | homogeneous    | 2 No                           | 0.021             | 0.488       | 2 -      |   |
| Normal02     | 17           | 41186311       | 41286132     | BRCA1  | Within Bounds  | homogeneous    | 2 No                           | -0.097            | 0.488       | 1.5 -    |   |
| Normal02     | 17           | 48702217       | 48755288     | ABCC3  | Within Bounds  | homogeneous    | 2 No                           | -0.027            | 0.485       | 2 -      |   |
| Normal02     | 19           | 1195797        | 1238434      | STK11  | Within Bounds  | homogeneous    | 2 No                           | 0.407             | 0.484       | 2.5 -    |   |
| Normal02     | 19           | 40726223       | 40801302     | AKT2   | Within Bounds  | homogeneous    | 2 No                           | -0.046            | 0.486       | 2 -      |   |
| Normal02     | х            | 66753873       | 66960461     | AR     | Within Bounds  | homogeneous    | 2 No                           | -0.851            | NaN         | 1 LC     | 가 |
| Normal02     | 1            | 3559128        | 3662765      | TP73   | Within Bounds  | homogeneous    | 2 No                           | 0.328,-0.038      | 0.487,0.485 | 2.5,2 -  |   |
| Normal02     | 1            | 3763844        | 3811993      | DFFB   | Within Bounds  | homogeneous    | 2 No                           | -0.038            | 0.485       | 2 -      |   |
| Normal02     | 1            | 9701789        | 9799172      | PIK3CD | Within Bounds  | homogeneous    | 2 No                           | -0.038            | 0.485       | 2 -      |   |
| Normal02     | 1            | 11156587       | 11332608     | MTOR   | Within Bounds  | homogeneous    | 2 No                           | -0.038            | 0.485       | 2 -      |   |
|              | Result.gener | eport 🦓        |              |        |                |                |                                |                   |             |          |   |
| Ready        |              |                |              |        |                |                |                                | ⊞ □ □ 1009        |             | 2        | 6 |

5

Figure 85 Gene Report

| Gene Report<br>Column             | Description   |
|-----------------------------------|---|
| Filename                          | Name of the xxCHP file containing the data.   |
| Chromosome                        | Chromosome on which the probeset is located.  |
| Start Position                    | Start position of gene or region as defined in the bed file.  |
| End Position                      | End position of gene or region as defined in the bed file.  |
| Genes                             | This column is populated from the name column of the bed file. In most cases, it will contain gene names.   |
| Threshold Test                    | Displays Outside Bounds if any of the QC metrics fail to meet a threshold test. For more information on thresholds, see "Creating your own custom QC setting" on page 57.   |
| % Aberr.Cells<br>(OSCHP only)     | If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor.<br>If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.  |
| TuScan Ploidy<br>(OSCHP bnly)     | TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred.<br>TuScan Ploidy is assigned the median CN state of all markers, provided that %AC could be<br>determined and integer copy numbers are returned. If %AC cannot be determined, NA (Not Available)<br>is reported for both ploidy and %AC.   |
| Low Diploid Flag<br>(OSCHP only)  | An essential part of the algorithm is the identification of "normal diploid" markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that "normal diploid markers" result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of "normal diploid" markers and no "normal diploid calibration occurs. This event triggers "low diploid flag" = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary. |
| Median Log2 Ratio<br>(OSCHP only) | Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.  |
| Median BAF<br>(OSCHP only)        | B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is reported for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported,  |
| State                             | This is a comma separated list of the copy number state of the segments that overlap the gene or region.  |
| LOH                               | Flag to indicate whether the gene or region is in a Loss of Heterozygosity region (0=No, 1=Yes).  |

## Exporting a XON region report (CytoScan XON only)

Note: This export provides XON Region gains/loses for a defined list of genes.

- 1. From the QC Results tab, select the check box for the sample file(s) for which you would like to generate an XON Region report.
- 2. Click on the Generate Report drop down menu and select Export XON Region Report.
- 3. In the browse window, click to select the appropriate aed/bed file, then click **Open**.
- 4. Click the browse button to assign an output path.

Note that the default root filename is **Result**.

- 5. Optional: Click inside the Filename field to enter a difference root filename, then click **Save**.
- 6. Click the appropriate radio button (for each XNCHP file) to either export all data into a single text file or into individual text files.
- 7. Locate the XON Region Report text file, then open it in Microsoft Excel.

| XON Region<br>Report Column  | Description   |
|------------------------------|---|
| Filename                     | Name of the XNCHP file containing the data  |
| Gene                         | This column is populated from the name column of the bed file. In most cases, it will contain gene names. |
| Chromosome                   | Chromosome on which the probeset is located.  |
| Gene Start<br>Position       | Start position of the gene or region as defined in the bed file.  |
| Gene End Position            | End position of gene or region as defined in the bed file.  |
| XON Region Start<br>Position | Start position of the Exon Region segment as defined in the XNCHP file.                                   |
| XON Region Stop<br>Position  | End position of the Exon Region segment as defined in the XNCHP file.                                     |
| XON Region Type              | Gain or Loss  |
| Size (bp)                    | Size of the Exon Region Segment.  |
| Marker Count                 | Number of markers in the Exon Region Segment.   |

## Exporting a copy number expression overlap report

This report summarizes the copy number segments with the fold change from expression data that overlap user defined regions of interest (e.g. Genes) as defined in the selected AED or BED file.

1. In the QC Results tab, click on the Generate Report drop-down menu, then select Copy Number Expression Overlap Report. (Figure 86)

| Figure 86 Generate Report drop-down menu |   |  |  |  |  |  |  |  |  |
|--|---|--|--|--|--|--|--|--|--|
| Generate Report ▼                        |   |  |  |  |  |  |  |  |  |
| Export Genotype Data                     |   |  |  |  |  |  |  |  |  |
| Export Probe level Data                  |   |  |  |  |  |  |  |  |  |
|  |   |  |  |  |  |  |  |  |  |
| Copy Number Expresssion Overlap Report   | - |  |  |  |  |  |  |  |  |
| Export Gene Report                       |   |  |  |  |  |  |  |  |  |
|  |   |  |  |  |  |  |  |  |  |

The following window appears: (Figure 87)

| Figure 87 Select files for the Overlap Report window           | w      |
|--|--------|
| Copy Number Expression Overlap Report Selections               | ×      |
| Select the files for the Copy Number Expression Overlap Report |        |
| Select the region file:  |        |
|  |        |
| Select the AED file (TAC output):                              |        |
|  |        |
| Select the output file name:                                   |        |
|  |        |
|  |        |
| OK   | Cancel |
|  |        |

2. Click the Select the region file **Browse** button to navigate to and select the appropriate file.

**Note:** For the Regions file, you can use the default bed files provided in the in the library files for use with the Copy Number Expression Overlap report. You may also create your own AED/BED file containing your custom regions of interest.

- 3. Click the Select the AED file (TAC output) **Browse** button to navigate to and select the appropriate file. Refer to the Transcriptome Analysis Console (TAC) 4.0 User Manual for analyzing and exporting expression data as an AED file.
- 4. Click the Select output file name **Browse** button to navigate to an existing report location, or click inside the text field to enter a different root filename (other than the default Results filename), then click **OK**.

A progress bar appears while your report generates.

5. Locate the **cnexoverlapreport.txt** file, then double-click on it to open it. It is recommended to open the tab delimited file with Excel for easier viewing.

## The following window appears. (Figure 88)

|--|

|                  | GexCN9yearFFPELungRep   | ort20141006 cne                     | expoverlaprep                | oort - Notepad  | -  |                              |                                     |                              |                                    |   |  |                                  |                            |                     |
|------------------|---|-------------------------------------|------------------------------|---|--|------------------------------|-------------------------------------|------------------------------|------------------------------------|---|--|----------------------------------|----------------------------|---------------------|
| F                | ile Edit Format View  | Help                                |                              |   |  |                              |                                     |                              |                                    |   |  |                                  |                            |                     |
| F<br>S<br>B<br>O | ilename Gene<br>egment End Positior<br>AF TCID Fold<br>009457_B04.OSCHP | chromos<br>Segment<br>Change<br>JUN | some<br>t Size (b<br>TCID St | Gene Start Posi<br>p) Percent<br>art Position<br>59246462 | tion<br>Gene Ove<br>TCID End<br>59249785 | Gene End<br>erlap<br>Positio | d Positic<br>% Aberr.<br>on<br>3324 | on<br>Cells<br>TCID P-'<br>2 | Gene Bo<br>TuScan I<br>Value<br>59 | undary S<br>Ploidy<br>TCID Ge<br>754192 | ize (bp)<br>Low Dip<br>ne Symbo<br>1101959 | CN State<br>loid Flag<br>l<br>01 | e<br>g<br>1094417:         | Segm<br>LOH<br>10   |
| 0                | -0.004 0.48<br>009457_B04.05CHP   | RAB25                               | 1 1                          | -2.26 5924646<br>156030966                                | 0<br>15604029                            | 59249999<br>)5               | 9330                                | 2                            | 50N<br>61                          | 1102463                                 | 59   | 2076560                          | 50                         | 9740                |
| 0                | 0.00<br>009457_B04.05CHP  | MYCN                                | 2                            | 16080682  | 16087129                                 |                              | 6448                                | 2                            | 64                                 | 21494                                   | 6195371                                    | 8                                | 6193222                    | 5                   |
| 0                | 009457_B04.05CHP<br>0.002 0.48  | REL                                 | 2                            | 61108751  | 61150178                                 | -                            | 41428                               | 2                            | 64                                 | 21494                                   | 6195371                                    | 8                                | 6193222                    | 5                   |
| 0<br>N           | 009457_B04.OSCHP<br>00.0  | INPP4A<br>001 0.487                 | 2                            | 99061320  | 99198284<br>-                            | -                            | 136965                              | 2                            | 66                                 | 6235017                                 | 5  | 2430523                          | 31                         | 1807                |
|                  | 009457_B04.OSCHP<br>ОН -0.387 0.31                                      | VHL<br>16 –                         | 3                            | 10183318  | 10195354                                 | -                            | 12037                               | 1                            | 67                                 | 63411                                   | 1978525                                    | 64                               | 1977891                    | 54                  |
|                  | 009457_в04.05СНР<br>ОН -0.387 0.31                                      | MITF<br>L6 TC03000                  | 3<br>0419.hg.1               | 69788585<br>3.85 6978858                                  | 70017488<br>6                            | 70017488                     | 228904<br>3                         | 1 0                          | 67<br>MITF                         | 63411                                   | 1978525                                    | 64                               | 1977891                    | 54                  |
| 0<br>N<br>0      | 009457_B04.0SCHP<br>0 - 0.28<br>009457_B04.0SCHP                        | EGFR<br>3 0.396<br>MET              | 7<br>TC07000<br>7            | 55086724<br>328.hg.1//тс0700<br>116312458                 | 55224644<br>2321.hg.1<br>11643844        | 0                            | 137921<br>-2.36//-<br>125983        | 3<br>-2.21<br>3              | 96<br>5508671<br>96                | 1659291<br>4//55224<br>1659291          | 5<br>300<br>5                              | 15911844<br>55324313<br>15911844 | 43<br>3//552389<br>43      | 1425<br>904<br>1425 |
| 0<br>N           | 0 - 0.26<br>009457_B04.0SCHP<br>0 - 0.28<br>009457_B04.0SCHP            | MYC<br>0.399<br>CDKN2A              | -<br>8<br>TC08000<br>9       | 128748314<br>749.hg.1 2.32<br>21967750                    | -<br>12875368<br>12874768<br>21974826    | 0                            | -<br>5367<br>12875368<br>7077       | 3<br>30<br>1                 | 101<br>0<br>102                    | 1135870<br>MYC<br>204738                | 68<br>1410547                              | 14629273<br>61                   | 34<br>1408500              | 3270<br>74          |
| L                | он -0.393 0.31<br>009457_в04.05СНР                                      | L5 –<br>CDKN2B                      | 9                            | 22002901  | 22009312                                 | -                            | 6412                                | 1                            | 102                                | 204738                                  | 1410547                                    | 61                               | 1408500                    | 24                  |
|                  | он -0.393 0.31<br>009457_в04.05СНР                                      | PTEN                                | 10                           | 89623194  | -<br>89728532                            | -                            | 105339                              | 1                            | 103                                | 126070                                  | 1354343                                    | 03                               | 1353082                    | 34                  |
| 0                | 0H -0.396 0.33<br>009457_B04.05CHP                                      | L6 -<br>CCND1                       | -<br>11                      | -<br>69455872   | _<br>                                    | -                            | 13371                               | 2                            | 104                                | 192764                                  | 8892962                                    | 4                                | 8873686                    | 1                   |
| 0                | -0.009 0.48<br>009457_B04.0SCHP   | CDK4                                | 12<br>-                      | 58141509  | 58146230                                 | 09409242<br>)<br>-           | 4722                                | 1                            | 109                                | 3790298                                 | 8  | 1338181                          | 15                         | 9591                |
| ON               | 009457_B04.05CHP  | MDM2                                | 12<br>TC12001                | 69201970<br>704. hg. 1 -2. 17                             | 69239212<br>69235068                     |                              | 37243<br>69237017                   | , <b>1</b>                   | 109<br>0.00012                     | 3790298                                 | 8  | 13381811                         | 15                         | 9591                |
| 0<br>N           | 009457_B04.OSCHP<br>00.0  | BRCA2                               | 13                           | 32889616  | 32973809                                 | _                            | 84194                               | 2                            | 110                                | 1908482                                 | 3  | 1111353                          | 53                         | 9205                |
| 0<br>N           | 009457_B04.OSCHP<br>00.0  | RB1<br>008 0.487                    | 13                           | 48877882  | 49056026                                 | -                            | 178145<br>-                         | 2                            | 110                                | 1908482                                 | 3  | 1111353                          | 53                         | 9205                |
| 0000             | 009457_B04.0SCHP<br>.244 TC17001094.1<br>009457_B04.0SCHP               | TP53<br>ng.1//TC1700<br>ERBB2       | 17<br>02468.hg.<br>17        | 7571719 7578811<br>1 2.21//2<br>37844392                  | 7093<br>.63<br>37884915                  | 2<br>7565097/                | 124<br>//7571720<br>40524           | 400959<br>)<br>2             | 8026342<br>7590868,<br>124         | 7<br>//759086<br>400959                 | 7986246<br>3<br>8026342                    | 9<br>0//0<br>7                   | 0.01<br>TP53//-<br>7986246 | 55<br>9             |
| ō                | 009457_B04.05CHP  | BRCA1                               | 17                           | 41196311  | 41276132                                 | -                            | 79822                               | 2                            | 124                                | 400959                                  | 8026342                                    | 7                                | 7986246                    | 9                   |
| Ō                | 009457_B04.05CHP<br>0H -0.006 0.24                                      | ABCC3                               | 17                           | 48712217  | 48745288                                 | -                            | 33072                               | 2                            | 124                                | 400959                                  | 8026342                                    | 7                                | 7986246                    | 9                   |
| Ō                | 009457_B04.OSCHP  | STK11                               | 19<br>-                      | 1205797 1228434   | 22638                                    | 2                            | 126                                 | 247232                       | 2012477                            | 1765246                                 | 1.28                                       | 55                               | 2                          | NO                  |
| 0                | 009457_B04.0SCHP<br>-0.021 0.48   | АКТ2<br>37 -                        | 19<br>-                      | 40736223  | 40791302                                 | -                            | 55080                               | 2                            | 128                                | 2303276                                 | 5909323                                    | 9                                | 5678996                    | 4                   |
|                  | 009457_B04.OSCHP<br>OH -0.808 NaN                                       | AR<br>-                             | ×                            | 66763873  | 66950461                                 | -                            | 186589                              | 1                            | 147                                | 2813287                                 | 1549294                                    | 12                               | 1521161                    | 26                  |
| 0                | 009457_B04.OSCHP<br>.486  | TP73<br>-                           | 1                            | 3569128 3652765   | 83638                                    | 2                            | 59                                  | 754192                       | 1101959                            | 01                                      | 1094417                                    | 10                               | 0.08                       | 55                  |
| 0                | 009457_B04.OSCHP<br>.486  | DFFB<br>-                           | 1<br>-                       | 3773844 3801993   | 28150                                    | 2                            | 59                                  | 754192                       | 1101959                            | 01                                      | 1094417                                    | 10                               | 0.03                       | 55                  |

| Expression Gene<br>Report Column | Description   |
|----------------------------------|---|
| Filename                         | Name of the OSCHP file containing the data  |
| Gene                             | This column is populated from the name column of the bed file. In most cases, it will contain gene names. |
| Chromosome                       | Chromosome on which the probeset is located.  |
| Gene Start<br>Position           | Start position of gene or region as defined in the bed file.  |
| Gene End Position                | End position of gene or region as defined in the bed file.  |
| CN State                         | This is a comma separated list of the copy number state of the segments that overlap the gene or region.  |

| Expression Gene<br>Report Column     | Description   |
|--------------------------------------|---|
| Segment ID                           | The unique identifier for the copy number segment.  |
| Segment Start<br>Position            | Start position of the overlapping copy number segment in the xxCHP file.  |
| Segment End<br>Position              | End position of the overlapping copy number segment in the xxCHP file.  |
| Segment Size (bp)                    | The Segment Stop Position minus The Segment Start Position.   |
| Percent Gene<br>Overlap              | Gene Boundary Size/Segment Size.  |
| % Aberr.Cells<br>(OncoScan only)     | If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.   |
| TuScan Ploidy<br>(OncoScan only)     | TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred.<br>Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before<br>%AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to<br>correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be<br>determined NA (Not Available) is reported.  |
| Low Diploid Flag<br>(OncoScan only)  | An essential part of the algorithm is the identification of "normal diploid" markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that "normal diploid markers" result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of "normal diploid" markers and no "normal diploid calibration occurs. This event triggers "low diploid flag" = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary. |
| Median Log2 Ratio<br>(OncoScan only) | Log2 Ratio is the log2 ratio of the normalized intensity of the sample over the normalized intensity of a reference with further correction for sample specific variation. The Median Log2 Ratio is computed for each segment.  |
| Median BAF<br>(OncoScan only)        | B-allele frequency (BAF) is (Signal (B)/{Signal(A) + Signal(B), where signal (A) is the signal from the AT chip and signal (B) is the signal from the G/C chip. Median BAF is reported for each segment and is the median BAF of the markers identified as heterozygous, after mirroring any marker BAFs above 0.5 to the equivalent value below 0.5. If the number of heterozygous markers in the segment is below 10 or the percent of homozygous markers is above 85% no value is reported,  |
| LOH                                  | Flag to indicate whether the gene or region is in a Loss of Heterozygosity region (0=No, 1=Yes).  |
| Fold Change                          | The level of fold change as determined from the TAC software.   |
| TCID                                 | The Transcript Cluster ID overlapping the Gene or Region defined in the bed file.   |
| TCID Start Position                  | Start Position: Start position of the overlapping TCID(s).  |
| TCID End Position                    | End Position of the overlapping TCID(s).  |
| TCID P-Value                         | ANOVA p-value (Condition1 vs Condition2)  |
| TCID GeneSymbol                      | Gene Symbol: the Gene Symbol assigned to the TCIS(s) based on the TAC analysis.   |

## 5

## Exporting genotype data using the analysis workflow

**Note:** For exporting genotypes from an entire sample or multiple samples at one time, exporting genotypes from the Analysis Workflow is generally faster than from the ChAS browser.

1. In the **QC Results** tab, click on the **Generate Report** drop-down menu, then select **Export Genotyping Data**. (Figure 89)

| Figure 89 Generate Report drop-down menu |  |  |  |  |  |  |  |
|--|--|--|--|--|--|--|--|
| Generate Report 🔻                        |  |  |  |  |  |  |  |
| Export Genotype Data                     |  |  |  |  |  |  |  |
| Export Probe level Data                  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| Copy Number Expresssion Overlap Report   |  |  |  |  |  |  |  |
| Export Gene Report                       |  |  |  |  |  |  |  |

The following window appears: (Figure 90)

| Figure 90           | Genotype Export Selections window                     |
|---------------------|---|
| Genotype Export Sel | ections ×   |
| Genotype Export     | Array Information                                     |
| Array Type          | CytoScanHD_Array                                      |
| Annotation File     | CytoScanHD_Array.na33.annot.db 🔹                      |
| Export Options      |   |
| All Chromosom       | es  |
| SNP List            |   |
| Chromosome          | Select RegionStart:                                   |
|                     | Stop:   |
| Select Output Pat   | th and File Name                                      |
| Output Path         | C:\Users\Public\Documents                             |
| File Name           |   |
| Multiple File Out   | put Options   |
| Separate File fo    | r each Chromosome 🛛 🗹 Separate File for each CHP File |
| L                   | OK Cancel   |

**Exporting options** Note: The default export is All Chromosomes.

## **Exporting a SNP List**

- 1. Click the Annotation File drop-down menu (Figure 90), then click to select which array annotation file you want to use for exporting SNP information (along with the genotypes).
- 2. Click the SNP List radio button, then click the SNP List Browse button.

The Select SNP List window appears. An example SNP List can be seen in Figure 385 on page 344.

- 3. Navigate to, then click to select the SNP List you want to export.
- 4. Click Open.

Your SNP List is now set for exporting.

#### Exporting a specific chromosome

- 1. Click the Chromosome radio button.
- 2. Click the **Chromosome** drop-down menu, then click to select the specific number or chromosome type you want to export.
- 3. Optional: Click to check the **Select Region** check box, then enter a **Start** and **Stop** value in the provided text fields.

## Selecting an output path and filename

1. Click the Output Path Browse button.

The Select Output Path for the Genotype Export Report appears.

- 2. Navigate to an existing report location, or click inside the text field to enter a different root filename, then click **OK**.
- 3. Click inside the File Name text field to enter a report name.

### Multiple file output options

- 1. Click to check the **Separate File for each Chromosome** and/or **Separate File for each CHP File** check box.
- 2. Click **OK**.

A Please Wait...Exporting Genotype Data progress bar appears.

3. After a few moments, the **Export Genotype Data finished successfully** window appears. Click **Yes** to view the report, click **No** to view the report later.

Clicking **Yes** opens your previously assigned Output folder window. Locate the newly exported file, then double-click on it to open it. It is recommended to open the tab-delimited text file with Excel for easier viewing.

| Saving and<br>importing<br>attributes |             | Sample attributes can be added to the Results table for use in the PCA and Export IGV functions. A tab-delimited text file containing sample attributes can also be added to the Results table, however, column A must contain the name of the xxCHP file. Subsequent columns can contain other sample attributes. Alternatively, sample attributes listed in an ARR file generated in AGCC will also be automatically displayed in the Results table (as long as the ARR and xxCHP files are located in the same directory). |
|---------------------------------------|-------------|---|
|                                       | 1.          | In the ChAS Analysis Workflow, select the QC Results tab.   |
|                                       | 2.          | Click Add Files to navigate to and load your CHP files.   |
|                                       |             | The <b>Attributes</b> button is enabled.  |
|                                       | 3.          | Click on the <b>Attributes</b> drop down, select <b>Import</b> , then navigate to the corresponding TXT file.   |
|                                       | 4.          | Click <b>Open</b> .   |
|                                       |             | The attributes now appear in the table.   |
|                                       | 5.          | Optional: To save an individual text file of sample attributes for each loaded chp file, click the <b>Attributes</b> drop-down, then select <b>Save</b> .   |
| Exporting to<br>Integrative           | The<br>IGV. | ChAS Analysis Workflow enables you to export a variety of graphs for viewing in . To access this viewer, go to: http://software.broadinstitute.org/software/igv/  |
| Genomics Viewer                       | 1.          | In the ChAS Analysis Workflow, click the QC Results tab.  |
| (IGV)                                 | 2.          | Load results files by clicking on <b>Add Files</b> , then navigate to and highlight the CHP files.  |
|                                       | 3.          | Click <b>Open</b> .   |
|                                       | 4.          | Select files by either clicking the <b>Select All</b> , or checking each filename's adjacent check box.   |
|                                       | 5.          | Click Export to IGV.  |
|                                       |             | The IGV Exporter window appears. (Figure 91)  |
|                                       | 6.          | Click the Browse button to assign an output folder.   |
|                                       | 7.          | From the IGV Exporter window, click the check box(es) adjacent to the data you want to export, as shown in Figure 91.   |
|                                       | 8.          | Click <b>OK</b> .   |
|                                       |             | <b>Note:</b> To include sample attribute information in the IGV Export, click on <b>Attribute</b> $\rightarrow$ <b>Save</b> prior to running the IGV Export.  |
|                                       |             |   |

5

**Note:** XON Region segments are exported as CN segments when working with CytoScan XON arrays.

| Figure 91 IGV Exporter window |
|-------------------------------|
| IGV Exporter                  |
| Export Output Folder:         |
| C:\Users\cgates\Desktop       |
| LOH Segment                   |
| CN Segment                    |
| C Log2Ratio                   |
| Weighted Log2Ratio            |
| Smooth Signal                 |
| Allele Difference             |
| ☑ B Allele Frequency          |
| Sample Attributes             |
|                               |
|                               |
| OK Cancel                     |

**Note:** The export process may take several minutes to complete, as it is dependent on your sample(s) and data type(s).

If the export was successful, an IGV Export Complete message appears. (Figure 92) Acknowledge the message, then click **OK**.

| Figure 92     | IGV Export Complete message        | ÷ |
|---------------|------------------------------------|---|
| Chromosome An | alysis Suite                       | × |
| IGV Expo      | rt Complete: 00:00:11 (hr:min:sec) |   |
|               | 0                                  | К |

Your data is now ready to be imported into IGV's browser.

For more information on importing and viewing your data in IGV, go to:

http://software.broadinstitute.org/software/igv/UserGuide

Principle component analysis ChAS Analysis Workflow enables you to perform Principle Component Analysis (PCA) on signal (CHP) data. PCA identifies a new set of variables (PCA1, PCA2, and PCA3) that account for a majority of the variance in the original data set. The first principal component (PCA1) captures as much variability in the data as possible. PCA2 captures as much of the remaining variability (not accounted for by PCA1) as possible. PCA3 captures as much of the remaining variability (not accounted for by PCA2) as possible.

### PCA plot generation

1. In the ChAS Analysis Workflow, select the QC Results tab. Load results files by clicking on Add Files, and navigating to xxCHP files, then click **Open**.

When files are loaded, the Import Attributes, Export to IGV and QC Analysis buttons will be enabled.

- 2. Select files, by either clicking **Select All** button or selecting files individually by ticking box to left of file.
- 3. Click on the QC Analysis button and in the resulting drop down menu, select PCA.

The 3-dimensional PCA graph(s) appears. The graph axes represent the top three variables (PCA1, PCA2, and PCA3) that account for the majority of the variability among the samples.

#### Sample display options

By default, the table is set to a vertical view. Click to change the view to a horizontal view.

1. The file can be identified by clicking on an icon in the plot.

The corresponding file in the table is highlighted, as shown in Figure 93.

| Figu        | Ire 93 PCA                | Display         |                  |                   |                 |                          |            |                 |        |                              |                   |             |                                   |
|-------------|---------------------------|-----------------|------------------|-------------------|-----------------|--------------------------|------------|-----------------|--------|------------------------------|-------------------|-------------|-----------------------------------|
| appliedb    | piosystems                |                 |                  |                   |                 |                          |            |                 |        |                              |                   |             |                                   |
| Analysis se | tup Workflow dashboard    | QC results      |                  |                   |                 |                          |            |                 |        |                              |                   |             | Utility                           |
| Array Type: | CytoScanOptima_Array •    | QC Settings: Cy | /toScanOptimaMet | rics-Basic View.r | Edit or Cre     | ate QC Se                | ttings     |                 |        |                              |                   |             |                                   |
| Result      | Files                     |                 |                  |                   |                 |                          |            |                 | Anal   | ysis Results                 |                   |             |                                   |
| Add Files   | s Remove Selected File(s) | Select All Clea | ar Selection Exp | ort QC Table      | Generate Report | <ul> <li>View</li> </ul> | In Browser | View In MSV     | Export | Image                        |                   |             |                                   |
| Import A    | Attributes Export to IGV  | QC Analysis 🔻   |                  |                   |                 |                          |            |                 | PCA    | Concordance Refresh Needed 🥠 |                   |             |                                   |
| File        | name                      |                 | Threshold Test   | MAPD ≤ 0.29       | SNPQC ≥ 8.5     | Gender                   | NA Version | Genomic Version |        |                              | Manning 02 52%    |             |                                   |
| 201         | 41015_132422_004_APL01732 | 2_A0003367_P0   | Within Bounds    | 0.1845187         | 19.76187        | male                     | 33         | hg19            |        | ru -                         | A mapping 55.5576 |             | Color By Attribute                |
| 201         | 41015_132425_008_APL01732 | 2_A0007934_P0   | Within Bounds    | 0.1580607         | 22.78714        | male                     | 33         | hg19            |        |                              |                   |             | Shape By Attribut                 |
| 201         | 41015_132427_010_APL01732 | 2_A0008268_P0   | Within Bounds    | 0.171226          | 18.62315        | female                   | 33         | hg19            |        |                              | PCA3 7.06%        |             | <select></select>                 |
| 201         | 41015_132428_011_APL01732 | 2_A0002867_P0   | Within Bounds    | 0.171234          | 19.55705        | female                   | 33         | hg19            |        |                              | 1                 |             | Colored by <sele< th=""></sele<>  |
| 201         | 41015_135329_002_APL01732 | 2_A0002867_P0   | Within Bounds    | 0.1707999         | 21.28073        | female                   | 33         | hg19            |        |                              |                   |             | Shaped by <selec< th=""></selec<> |
| 201         | 41015_135330_006_APL01732 | 2_A0008268_P0   | Within Bounds    | 0.1637085         | 21.21414        | female                   | 33         | hg19            |        |                              |                   |             |                                   |
| 201         | 41015_135332_009_APL01732 | 2_A0003367_P0   | Within Bounds    | 0.1676489         | 17.04203        | male                     | 33         | hg19            |        |                              |                   |             |                                   |
| 201         | 41015_135339_024_APL01732 | 2_A0007934_P0   | Within Bounds    | 0.1625992         | 18.72401        | male                     | 33         | hg19            |        |                              |                   | <b>•</b>    |                                   |
| 201         | 41015_165517_006_APL01732 | 2_A0003367_P0   | Within Bounds    | 0.1759291         | 20.35406        | male                     | 33         | hg19            |        |                              |                   | <b>.</b> .  |                                   |
| 201         | 41015_165519_008_APL01732 | 2_A0007934_P0   | Within Bounds    | 0.1753269         | 19.91465        | male                     | 33         | hg19            |        | -                            | - I 💼 🛡           |             |                                   |
| ✓ 201       | 41015_165533_020_APL01732 | 2_A0002867_P0   | Within Bounds    | 0.1843393         | 18.35457        | female                   | 33         | hg19            |        |                              | -                 |             |                                   |
| 201         | 41015_165534_021_APL01732 | 2_A0008268_P0   | Within Bounds    | 0.1556032         | 23.98967        | female                   | 33         | hg19            |        | •                            |                   |             |                                   |
|             |                           |                 |                  |                   |                 |                          |            |                 |        | PCA1 71.73%                  | •                 | PCA2 14.74% |                                   |

2. Icons can be lassoed by left-clicking and drawing circle around sample(s) of interest, as shown in Figure 94. This action also highlights the corresponding files in the table.



3. Points on the plot can also be hidden by right-clicking, then selecting the appropriate option.

Note: The selected samples are only hidden from the plot.

4. Use the drop-down menus (Figure 95) to select attributes for display by **Color By Attribute** and by **Shape By Attribute**.

| Figure 95 Sample Display   |
|--|
| Color By Attribute<br>Gender<br>Shape By Attribute<br>Threshold Test                               |
| Colored by Gender<br>female<br>male<br>Shaped by Threshold Test<br>Within Bounds<br>Outside Bounds |

### Additional PCA graph display options

- To rotate the graph, right-click, then drag the graph to change its view perspective.
- Click **Export Image** to save the displayed plot as a PNG graphics file.

#### Concordance The ChAS Analysis Workflow enables you to perform pairwise comparison concordance checks on genotype calls for all selected samples. checks

The concordance between all pairwise comparisons for the samples in the results table are reported.

A reference sample can be selected. Once selected, concordances are displayed.

Compare to reference allows you to compare every sample to a single reference file.

### Performing a concordance check

- 1. In the ChAS Analysis Workflow, select the QC Results tab.
- 2. Load results files by clicking on Add Files, then navigate to xxCHP files.

After the files are loaded Import Attributes, Export to IGV, and QC Analysis buttons are enabled.

- Select files, by either clicking Select All button or by clicking each file's (left) check box.
- 4. Click on the QC Analysis button.

A drop-down menu appears.

5. Click Concordance.

The concordance table with sample, reference and percent concordance is generated, as shown in Figure 96.

| Figure     | 96 Conce     | ordance t   | able e | xample | )    |      |                    |           |     |  |       |       |   |
|------------|--------------|-------------|--------|--------|------|------|--------------------|-----------|-----|--|-------|-------|---|
| Analysis F | Results      |             |        |        |      |      |                    |           |     |  |       |       |   |
| xport Text | Export Image | Export Pdf  |        |        |      |      |                    |           |     |  |       |       |   |
| PCA Refre  | sh Needed    | Concordance | ,      |        |      |      |                    |           |     |  |       |       | × |
|            |              | concordance |        |        |      |      |                    |           |     |  |       |       |   |
| 80         | 82 84        | 86          | 88     | 90     | 92   | 94   | 96                 | 98        | 100 | <all comparisons="" pairwise=""></all> |       |       | ~ |
|            |              | × 95        |        |        | ✓ 98 |      |                    | ~         | 100 | Table                                  |       |       | ~ |
|            | •            |             |        |        |      |      |                    | _         |     |  |       |       |   |
| In 🔺       | Sample       |             |        |        |      | Refe | erence             |           |     |  | %     | Pass  |   |
| T          |              |             |        |        |      |      |                    |           |     |  |       |       |   |
| 1          | DD_15_0153_0 | 3Mar15      |        |        |      | DD_  | 15_0153_03         | Mar15_re  | do  |  | 98.71 | Pass  | - |
| 2          | DD_15_0153_0 | 3Mar15_redo |        |        |      | DD_  | DD_15_0153_03Mar15 |           |     |  |       | Pass  |   |
| 3          | DD_15_0154_1 | 3May13      |        |        |      | DD_  | 15_0154_13         | May13_re  | edo |  | 98.43 | Pass  |   |
| 4          | DD_15_0154_1 | 3May13_redo |        |        |      | DD_  | DD_15_0154_13May13 |           |     |  |       | Pass  |   |
| 5          | DD_CytoRef10 | 3_062515    |        |        |      | DD_  | CytoRef103         | 080415    |     |  | 98.32 | Pass  |   |
| 6          | DD_CytoRef10 | 3_080415    |        |        |      | DD_  | CytoRef103         | 062515    |     |  | 98.32 | Pass  |   |
| 7          | DD_15_0155_1 | 4Jan15      |        |        |      | DD_  | 15_0155_14         | Jan15_red | do  |  | 97.68 | Margi |   |
| 8          | DD_15_0155_1 | 4Jan15_redo |        |        |      | DD_  | 15_0155_14         | Jan15     |     |  | 97.68 | Margi |   |
| 9          | DD_15_0309_2 | 4Jul15      |        |        |      | DD_  | CytoRef103         | 080415    |     |  | 61.71 | Fail  |   |
| 10         | DD_CytoRef10 | 3_080415    |        |        |      | DD_  | 15_0309_24         | Jul15     |     |  | 61.71 | Fail  |   |
| 11         | DD_15_0309_2 | 4Jul15      |        |        |      | DD_  | CytoRef103         | 062515    |     |  | 61.48 | Fail  |   |
| 12         | DD_CytoRef10 | 3_062515    |        |        |      | DD_  | 15_0309_24         | Jul15     |     |  | 61.48 | Fail  |   |
| 13         | DD_15_0153_0 | 3Mar15_redo |        |        |      | DD_  | 15_0309_24         | Jul15     |     |  | 61.45 | Fail  |   |
| 14         | DD 15 0309 2 | 410115      |        |        |      | חח   | 15 0153 03         | Mar15 re  | do  |  | 61.45 | Fail  |   |

### Concordance table filter and display options

To compare to reference sample, select the sample from drop down-menu.

 $\sim$ 

<all pairwise comparisons>

- The default values are set at >98%, 95-98%, and below 95%.
- The three categories of QC are passing, failing, and marginal.
- Each QC category is represented by its own unique color, as shown in Figure 97.

| Figure | <b>97</b> Dis | splayed of | concorda | ance QC | exampl | е    |      |      |    |     |
|--------|---------------|------------|----------|---------|--------|------|------|------|----|-----|
| 80     | 82            | 84         | 86       | 88      | 90     | 92   | 94   | 96   | 98 | 100 |
|        |               | ~          | 95       |         |        | ♥ 98 |      |      | ~  | 100 |
| Fai    | ling          |            | 1        | Margin  | al     |      | Pass | sing |    |     |

Colors in the table can be changed by clicking on the color drop-down (Figure 98), then choosing a different color from the pallet.



- The default values can be changed by either moving the hash marks on the line bar, or typing in a number in the designated category (Figure 98).
- The data can also be viewed in matrix format by selecting Matrix in the drop-down menu, as shown in Figure 99.

| Figure 99  | Table and Matrix options |   |
|--|--------------------------|---|
|  |                          |   |
| <all pairwise<="" td=""><td>e comparisons&gt;</td><td>~</td></all> | e comparisons>           | ~ |
| Table  |                          | ~ |
| Table  |                          |   |
| Matrix   | -                        |   |
|  | 11 1                     |   |

## Filtering

1. At the table, place your mouse cursor over a Sample or Reference cell.

A filter icon appears. (Figure 100)

| Figure | 100 Filter icon |             |   |
|--------|-----------------|-------------|---|
| Index  | Sample 9        | A Reference | % |
| T      |                 |             |   |

2. Right-click on the Filter icon.

A drop-down list appears. (Figure 101)

| Fig | ure 101 Drop-down list        |           |                                       |   |
|-----|-------------------------------|-----------|---------------------------------------|---|
| dex | Sample                        | Reference | P 94<br>DD_15_0153_03Mar15.cyhd.cychp |   |
|     | DD 15 0152 02Mad5 add auto    | 00.15.015 | DD_15_0153_03Mar15_redo.cyhd.cychp    |   |
| 10  | DD_15_0153_03Mar15.cynd.cychp | 00_15_015 | DD_15_0154_13May13.cyhd.cychp         |   |
| 15  | DD_15_0153_03Mar15.cynd.cychp | 00_15_030 | DD_15_0154_13May13_redo.cyhd.cychp    |   |
| 43  | DD_15_0153_03Mar15.cyhd.cychp | DD_Cytoke | DD_15_0155_14Jan15.cyhd.cychp         |   |
| 45  | DD_15_0153_03Mar15.cyhd.cychp | DD_15_015 | DD_15_0155_14Jan15_redo.cyhd.cychp    |   |
| 59  | DD_15_0153_03Mar15.cyhd.cychp | DD_CytoRe | DD_15_0195_14May15.cyhd.cychp         |   |
| 69  | DD_15_0153_03Mar15.cyhd.cychp | DD_15_015 | DD_15_0196_14May15.cyhd.cychp         |   |
| 79  | DD_15_0153_03Mar15.cyhd.cychp | DD_15_029 | DD_15_0291_06May13.cyhd.cychp         | = |
| 83  | DD_15_0153_03Mar15.cyhd.cychp | DD_15_019 | DD_15_0296_14Feb10.cyhd.cychp         |   |
| 89  | DD_15_0153_03Mar15.cyhd.cychp | DD_15_019 | DD_15_0308_29Jul15.cyhd.cychp         |   |

3. Click to select the samples you want to filter from the drop-down list.

### Changing the view and/or order of sample and reference columns

1. Left-click the Filter icon.

A menu appears. (Figure 102)

| <b>Figu</b><br>icon m | Figure 102 Left-click Filter con menu |  |  |  |  |  |
|-----------------------|---------------------------------------|--|--|--|--|--|
| Ź↓                    | Sort Ascending                        |  |  |  |  |  |
| Z↓                    | Sort Descending                       |  |  |  |  |  |
| 2¥                    | Clear Sorting                         |  |  |  |  |  |
|                       | Best Fit                              |  |  |  |  |  |
|                       | Best Fit (all columns)                |  |  |  |  |  |
| ۲ <mark>x</mark>      | Clear Filter                          |  |  |  |  |  |
| 0,                    | Show Search Panel                     |  |  |  |  |  |

2. Use the menu selections to customize your sample and reference columns.

### Exporting the currently displayed table

1. Click the appropriate, Export Text Export Image Export Pdf button, then export the file as you normally would.

## Mendelian error checking

Note: The Mendelian Error Checking feature is for the CytoScan family of arrays only.

# Running an error checking analysis

1. From the Analysis menu, select Perform Analysis Setup.

The Analysis Workflow window opens. (Figure 103)

| alysis setup Workflow dashboard QC results   |                                    | Utility Actio |
|--|------------------------------------|---------------|
| ct array type: CytoScanHD_Array   Select analysis workflow: CytoScanHD_Array Mendelian Error Check | <ul> <li>Set workflow r</li> </ul> | name: Workf   |
| Results files (CYCHP) to analyze   |                                    |               |
| Select the results file for the child  |                                    |               |
|  |                                    | Clear         |
| Select the results file for the mother   |                                    |               |
|  |                                    | Clear         |
| Select the results file for the father   |                                    |               |
|  |                                    | Clear         |
| Illele Frequency File  |                                    |               |
| Select the allele frequency file   |                                    |               |
| D:\chAS_Data\Mendelian\CytoScanHD_Array.AlleleFrequency.r1.txt                                     |                                    |               |
| inalysis Output File   |                                    |               |
| Select the analysis output file name   |                                    |               |
| D:\ChAS_3.0_Output\MendelianErrorCheckResults.txt  |                                    |               |
|  |                                    |               |
|  |                                    |               |
|  |                                    |               |
|  |                                    |               |
|  |                                    |               |

2. From the **Select array type** drop-down list, click to select an array type (Example: **CytoScanHD\_Array**.

**Note:** The **Select array type** drop-down list includes only the array types for which library (analysis) files have been downloaded from NetAffx or copied from the Library package provided with the installation.

- From the Select analysis workflow drop down, click to select the appropriate Mendelian Error Check Workflow. (Example: CytoScanHD\_Array Mendelian Error Check)
- 4. By default, the Set workflow name is **Workflow**. Click inside the Workflow's (upper right) text box to enter a different workflow name.


5. Click each of the three Results files (CYCHP or XNCHP) to analyze **Browse** buttons to navigate to and select the appropriate Results File for **Child**, **Mother**, and **Father**.

You can also run a Mendelian Error Check using two Results files: **Child** and **Mother** or **Child** and **Father**.

6. Click the Allele Frequency File **Browse** button to navigate to and select the appropriate Allele text file.

**Note:** An Allele Frequency file is provided in the library file package or you can create your own custom Allele Frequency File for use in this analysis.

- 7. Click the Analysis Output File **Browse** button to navigate to and enter a name (tab-delimited text filename) for your Mendelian Error Check output result. By default, the Output name is based on the CYCHP/XNCHP filename used for the Child.
- 8. Click Submit.

An error appears if a male is assigned as mother or a female is assigned as father or if the gender in the actual cychp file is wrong.

The Workflow Dashboard window appears. (Figure 104).

|   | Figure 104 .Files loading inside th                  | ne Workflow Dashboard                                |                 |
|---|--|--|-----------------|
| 1 | Analysis setup Workflow dashboard QC results         |  | Utility Actions |
|   | Workflows are available on the dashboard for 7 days. |  |                 |
|   | Workflow 10/07/2014 11:13:02                         |  | ×               |
| ł | CytoScanHD_Array Mendelian Error Check               |  |                 |
|   | CytoScanHD_Array Pause 3 Source Files                | Mendelian Error Check started: 10/7/2014 11:13:04 AM |                 |

After loading is complete, a Workflow completed successfully message appears. (Figure 105)

| Figure 105 Workflow Dashboard lo   | oaded  |                        |
|--|--|------------------------|
| Analysis setup Workflow dashboard QC results                                 |  | Utility Actions        |
| Workflows are available on the dashboard for 7 days.                         |  |                        |
| Workflow 10/07/2014 11:13:02   | ٥  | ×<br>View Results List |
| CytoScanHD_Array Mendelian Error Check<br>CytoScanHD_Array<br>3 Source Files | Current workflow status: Workflow completed successfully | View Logs              |

9. Click View Results List.

The Output folder window (you assigned earlier) appears.

- 10. Locate the newly created **\*.cyhd.txt/cyex.txt** (or **\*.cychp.ND.txt**) file, then double-click it to open it in MS Notepad to view it as tab-delimited text file, as you normally would.
- 11. Optional: If you want to view the Workflow's Log File, click **View Logs**.

The C:\ProgramData\Affymetrix\ChAS\Log folder window appears.

Locate the newly created Log.txt file, then double-click it to open it in MS Notepad to view it as tab-delimited text file, as you normally would.

The Mendelian Error Check analysis provides two key points of information:

- 1. Are the input samples related?
- Mother-Child
- Father-Child

If the samples are related, the **Role Validity** equals 1. If the samples are not related, **Role Validity** equals 0. (Figure 106) The output also indicates which CYCHP/XNCHP file is assigned as the Mother, Father and Child (Index). The analysis also can be run as a DUO analysis. (Mother-Child or Father-Child).

| Fig | gure 106 Trio | Output Tool Va  | lidity examples    |              |                |
|-----|---------------|-----------------|--------------------|--------------|----------------|
|     |               |                 |                    |              |                |
| 1   | #%GroupName=F | Familial        |                    |              |                |
| 2   | #%SetName=Sam | nples           |                    |              |                |
| 3   | #%Columns=4   |                 |                    |              |                |
| 4   | #%Rows=3      |                 |                    |              |                |
| 5   | SampleKey     | CHPFilename     | CHPID              | Role         |                |
| 6   | 0             | A03_CytoScanHD_ | 00001316-386a-4744 | index        |                |
| 7   | 1             | A01_CytoScanHD_ | 00006534-6a66-4722 | mother       |                |
| 8   | 2             | A02_CytoScanHD_ | 00006996-3b6b-4e53 | father       |                |
| 9   | #%SetName=Rel | atednessTest    |                    |              |                |
| 10  | #%Columns=5   |                 |                    |              |                |
| 11  | #%Rows=3      |                 |                    |              |                |
| 12  | AnalysisType  | ReferenceSample | FamilialSampleKey  | RoleValidity | RoleIndexScore |
| 13  | 0             | 0               | 2                  | 1            | 80375.99       |
| 14  | 1             | 0               | 1                  | 1            | 65454.53       |
| 15  | 2             | 0               | 2                  | 1            | 48592.95       |
| 16  | #%SetName=MIE |                 |                    |              |                |

2. Do any chromosomes have an elevated occurrence of Mendelian Errors?

In Figure 107, chromosome 15 has a higher error rate (7.2%) compared to the rest of the chromosomes in this trio. In this example, the mother has 0% errors on chromosome 15, whereas the father has 7%, indicating that both chromosome 15 (or some portion of) alleles were inherited from the mother. It is recommended to compare the genotypes on chromosome 15 for these samples using the Graphs tab. For more information, see "Graphs table" on page 341.

#### Interpreting an error checking analysis

| 19 | Chromosome | Display |    | MarkerCount | MIE-Trio | MIE-Mat | MIE-Pat | Percent-T | Percent-N | Percent-Pa |
|----|------------|---------|----|-------------|----------|---------|---------|-----------|-----------|------------|
| 20 | 1          |         | 1  | 56482       | 119      | 21      | 38      | 0.21      | 0.04      | 0.07       |
| 21 | 2          |         | 2  | 62283       | 134      | 32      | 35      | 0.22      | 0.05      | 0.06       |
| 22 | 3          |         | 3  | 52106       | 108      | 14      | 39      | 0.21      | 0.03      | 0.07       |
| 23 | 4          |         | 4  | 49517       | 101      | 37      | 25      | 0.2       | 0.07      | 0.05       |
| 24 | 5          |         | 5  | 46205       | 108      | 25      | 36      | 0.23      | 0.05      | 0.08       |
| 25 | 6          |         | 6  | 51944       | 128      | 29      | 32      | 0.25      | 0.06      | 0.06       |
| 26 | 7          |         | 7  | 46413       | 131      | 39      | 31      | 0.28      | 0.08      | 0.07       |
| 27 | 8          |         | 8  | 38796       | 84       | 20      | 29      | 0.22      | 0.05      | 0.07       |
| 28 | 9          |         | 9  | 30622       | 72       | 24      | 20      | 0.24      | 0.08      | 0.07       |
| 29 | 10         |         | 10 | 35472       | 97       | 23      | 35      | 0.27      | 0.06      | 0.1        |
| 30 | 11         |         | 11 | 38846       | 105      | 37      | 28      | 0.27      | 0.1       | 0.07       |
| 31 | 12         |         | 12 | 33424       | 65       | 12      | 20      | 0.19      | 0.04      | 0.06       |
| 32 | 13         |         | 13 | 27733       | 45       | 12      | 17      | 0.16      | 0.04      | 0.06       |
| 33 | 14         |         | 14 | 26983       | 75       | 20      | 32      | 0.28      | 0.07      | 0.12       |
| 34 | 15         |         | 15 | 24981       | 1799     | 1       | 1752    | 7.2       | 0         | 7.01       |
| 35 | 16         |         | 16 | 20915       | 48       | 14      | 19      | 0.23      | 0.07      | 0.09       |
| 36 | 17         |         | 17 | 17465       | 44       | 13      | 10      | 0.25      | 0.07      | 0.06       |

| Mendelian Errors<br>Report Column | Description  |
|-----------------------------------|--|
| Analysis Type                     | Provides the samples being run through the analysis based on the sample key.<br>0 = Proband, $1 = Mother$ , $2 = Father$ .   |
| Familial Sample Key               | Tells you the parent for which relatedness is being tested.<br>1= Mother only used in the analysis.<br>2= Father used in the analysis (may be father only (duo) or trio).  |
| Role Validity                     | A logical value with 0 being False and 1 being True. If the Role Index Score is $> 1000$ then the Role Validity = 1 (likely related). If the Role Index Score is $< 1000$ then Role Validity = 0 (likely unrelated).   |
| Role Index Score                  | Role Index Score: This score is basically a Log Odds score that computes the probabilities of the observed genotype calls for the trio while accounting for potential genotyping error. Assume the following hypothesis: H1 – alleged father is true H2 – alleged father is random male. For each marker, compute a likelihood ratio of H1 vs H2. Sum all markers. In theory a value of zero means equally likely probability for either hypothesis. Positive means more likely Paternity related. Negative means more likely unrelated. |
| Chromosome/<br>Display            | Chromosome number  |
| Marker Count                      | Number of genotypeable SNPs on the chromosome.   |
| MIE Trio                          | Mendelian Inheritance Error for the Trio. (Ex 119 errors of 56482 SNPs).   |
| MIE Mat                           | Number of errors for Mom.  |
| MIE Pat                           | Number of errors for Dad.  |
| Percentage MIE<br>Trio            | Number of raw error turned into a percent 119/56482 *100.  |
| Percentage MIE<br>Mat             | Percent of errors for Mom.   |
| Percentage MIE Pat                | Percent of errors for Dad.   |

# Analysis workflow troubleshooting

- 1. Click the Utility Actions button (top right of the Analysis Workflow window)
- 2. Click to select Log Collection.

The following dialog window appears. (Figure 108)

| Figure       | • 108 Log Co     | ollection di   | alog window                             |  |                               |                         |                                 |                   |              |                 |
|--------------|------------------|----------------|---|--|-------------------------------|-------------------------|---------------------------------|-------------------|--------------|-----------------|
| Analysis set | up Workflow da   | ashboard Q     | C results                               |  |                               |                         |                                 |                   | -            | Utility Actions |
| Array Type:  | CytoScanHD_Array | y 🝷 QC Settin  | gs: CytoScanHDMe                        | trics-Basic View.r1 🝷  | Edit or Creat                 | e QC Sett               | tings                           |                   |              |                 |
| Result F     | iles             |                |   |  |                               |                         |                                 |                   |              |                 |
| Add Files    | Remove Selected  | d File(s) Sele | ct All Clear Selection                  | on Export QC Table   | Generate R                    | eport 🔻                 | View In Browser                 | Import Attributes | Export to IG | /               |
| QC Analys    | sis 🔻            |                |   |  |                               |                         |                                 |                   |              |                 |
| Filename     | Threshold Test   | MAPD ≤ 0.25    | SNPQC ≥ 15.00                           | Waviness SD $\leq 0.12$  | NA Version                    |                         |                                 |                   |              |                 |
|              |                  |                |   |  |                               | 1                       |                                 |                   |              |                 |
|              |                  |                |   |  |                               |                         |                                 |                   |              |                 |
|              |                  |                | Chromosom                               | e Analysis Suite   |                               |                         | ×                               |                   |              |                 |
|              |                  |                | Cog | Collection has comple<br>S 4.0\LogCollection2<br>s folder: C:\ChAS 4.0 | eted successfu<br>01610150357 | lly. The :<br>54.zip ca | zip file C:<br>n be found<br>OK |                   |              |                 |

- 3. Make note of the assigned zip folder filename and its location.
- 4. Use Windows Explorer to navigate to the location. Example: C:\ProgramData\Affymetrix\ChAS\log
- 5. Locate the zip folder you noted earlier, then double-click on it to open it.

The folder opens.

### Log rollover

When the software determines that the log file for the Analysis Workflow (C:\ProgramData\Affymetrix\ChAS\log\AnalysisWorkflow.log) has reached a defined size (approximately 4MB), the following steps will be completed:

- A sub-folder will be created in C:\ProgramData\Affymetrix\ChAS\log called 'Log\*' (the \* denotes the current date and time).
- A zip file called RolledLogFile\*.zip is created in that folder. The '\*' is the same date and time used for the folder name. The files in the C:\ProgramData\Affymetrix\ChAS\log folder and all files found in the currently selected QC History Log folder will be included in this zip file.
- The Analysis Workflow files that are associated with analysis workflows that are no longer active on the Dashboard will be deleted from C:\ProgramData\Affymetrix\ChAS\log
- A new AnalysisWorkflow.log file will be created in C:\ProgramData\Affymetrix\ChAS\log



Log collection When the Log Collection option is selected from the Utility Actions menu, a file called LogCollection\*.zip (the \* denotes the current date and time) is created in the folder C:\ProgramData\Affymetrix\ChAS\log.

This created file contains the full contents of the folder C:\ProgramData\Affymetrix\ChAS\log, including the log file for the browser (ChAS\_RUO.log).

If available, the sub-folders of C:\ProgramData\Affymetrix\ChAS\log and all files found in the currently selected QC History Log folder.

**Note:** All log files for the ChAS database, ChAS Browser and Analysis Workflow can be found in: **\ProgramData\Affymetrix\ChAS\Log** 



# Loading data

ChAS can display data from:

- CytoScan Array CYCHP/XNCHP files, generated in ChAS.
- CytoScan HTCMA files, generated in the RHAS.
- Genome-Wide Human SNP Array 6.0 CNCHP files, generated in Genotyping Console (GTC).
- OncoScan OSCHP files, generated in OncoScan Console or ChAS.
- ReproSeq Aneuploidy results (.zip) from Ion Reporter.

**Note:** When referring to steps that apply to both CytoScan CYCHP and SNP 6.0 CNCHP data files, the CHP files are described as CxCHP files. When referring to steps that apply to CytoScan CYCHP, CytoScan XNCHP, CytoScan HTCMA RHCHP, SNP 6.0 CNCHP data, and OncoScan files, the resultant files are described as xxCHP files.

## Introduction to loading data

The same steps are used to load results xxCHP files from CytoScan arrays, Genome-Wide Human SNP 6.0 Array, or OncoScan Arrays.

When loading CYCHP files into ChAS for viewing, the software:

- 1. Loads the run-length encoded segments in the CYCHP file to display as segments.
- 2. Applies any smoothing or joining that would alter the length and other properties of segments.

**IMPORTANT!** In a new user profile, smoothing and joining are turned on by default for CytoScan 750K and CytoScan HD arrays. Smoothing and joining are disabled for CytoScan Optima arrays. Smoothing and joining are OFF by default for OncoScan and CytoScan HTCMA arrays. The smoothing and joining settings are specific for each array type (for more details on smoothing and joining, see "Copy number segment smoothing and joining (optional)" on page 123).

- 3. Displays the segments and graph data:
  - Segment Data
    - Copy Number Gain/Loss
    - Mosaicism Copy Number Gain and Loss
    - Loss of Heterozygosity (LOH)
  - Graph Data
    - Copy Number State
    - Log2 Ratio
    - Weighted Log2 Ratio
    - LOH
    - Smooth Signal

- Allele Difference
- B-allele Frequency
- Genotype Calls

When loading XNCHP files into ChAS for viewing from CytoScan XON arrays, the software:

- 1. Selects the segments in the XNCHP file to display as segments.
- 2. Displays the segments and graph data:
  - Segment Data
    - Loss of Heterozygosity
    - Exon Region Gain/Loss
  - Graph Data
    - Log2 Ratio
    - Weighted Log 2 Ratio
    - Smooth Signal
    - Loss of Heterozygosity (LOH)
    - Allele Difference
    - B-allele Frequency (BAF)
    - Genotype Calls

When loading RHCHP files into ChAS for viewing, the software:

- 1. Selects the segments in the RHCHP file to displays as segments.
- 2. Displays the segments and graph data:
  - Segments Data
    - Copy Number Gain/Loss
    - Loss of Heterozygosity
  - Graph Data
    - Copy Number State
    - Log 2 Ratio
    - Smooth Signal
    - LOH
    - Allele Difference
    - B-allele Frequency

When loading SNP6 CNCHP files into ChAS for viewing, the software does the following:

1. Performs segment detection by analyzing the CN and LOH graph data in the CNCHP file.

**Note:** When running the Segment Reporting Tool in GTC on SNP 6 data, the software sets the end coordinate such that the segment ends at the base position of the last marker in the segment. When loading SNP 6 data into ChAS, the segment detection sets the end coordinate for a segment such that the segment ends one base after the last marker in the segment. This may result in a discrepancy between the end position for segments when comparing data analyzed in both GTC and ChAS.



2. Applies any smoothing, removing, or joining that would alter the length and other properties of segments.

Smoothing is similar to the process applied when running the Segment Reporting Tool in GTC.

**Note:** ChAS uses the median of the aberrant markers' CNStates, as the recalculated CNState of the smoothed (and/or joined) segment.

**IMPORTANT!** For CNCHP files from the SNP 6.0 Array, smoothing but not joining is turned on by default in a new user profile.

Displays the Segments and Graph Data:

- Segment data
  - Copy Number Gain/Loss
  - Loss of Heterozygosity (LOH)

**Note:** The expected Copy Number State on the X chromosome of normal males is not constant over its entire length. This is due to the structure of the sex chromosomes. For more information see "Copy number segments on the X and Y chromosomes" on page 48.

- Graph Data
  - Copy Number State
  - Log2 Ratio
  - Allele Difference
  - SmoothSignal
  - LOH

When loading OncoScan OSCHP files for viewing, the software does the following:

 Displays segments in the OSCHP created by the TuScan Copy number algorithm. For details on this algorithm, please refer to the OncoScan Console User Guide (P/N 703195) or Appendix F.

**IMPORTANT!** Smoothing and Joining are OFF by default for OSCHP files.

- 2. Displays the segments and graph data:
  - Segment Data
    - Copy Number Gain/Loss
    - Loss of Heterozygosity
  - Graph Data
    - Copy Number State
    - Log2 Ratio
    - Weighted Log2 Ratio
    - Allele Difference
    - B-allele Frequency
    - LOH

- Smooth Signal
- Variant/Somatic Mutation
- Somatic Mutation (OncoScan FFPE Assay only)

When loading ReproSeq Aneuploidy data for viewing, the software does the following:

- 1. Displays segments from the Ion Reporter software. For details, refer to the Ion Reporter User Guide: https://ionreporter.thermofisher.com/ir/
- 2. Displays the whole genome sequencing tiles on the Copy Number State graph.

## Loading files

Loading xxCHP data for viewing in ChAS involves the following steps:

1. **Optional:** Before loading: Select Segment Smoothing and Segment Joining parameters for processing the CN Gain and Loss Segment data for CxCHP files.

**IMPORTANT!** Smoothing and Joining are ON by default for CytoScan 750K, HD, and HD Accel arrays. Both Smoothing and Joining are OFF by default for OncoScan, CytoScan HTCMA, and GenomeWide Human SNP 6.0 arrays. and is disabled for CytoScan Optima arrays and ReproSeq Aneuploidy data.

XON Segment Merging is turned ON by default for CytoScan XON arrays. For details, see "XON segment merging" on page 128.

- 2. Select analysis results from:
  - CytoScan Arrays (generated by analyzing CEL files in ChAS)
  - CytoScan HTCMA arrays (generated from CEL files in the RHAS.
  - Genome-Wide Human SNP 6.0 Arrays (generated by analyzing CEL files in GTC)
  - OncoScan Arrays (generated by analyzing in ChAS)
  - ReproSeq Aneuploidy .zip files (generated in Ion Reporter)

You can also select region information files in AED and BED format for loading. Use the Open window (click the button) to load xxCHP data files, Affymetrix Extensible Data (AED), or Browser Extensible Data (BED) annotation files. The AED and BED files that you open will be automatically loaded when a new session is started with the same user profile. **Note:** You may want to edit smoothing and joining parameters. This can be done before or after loading the CxCHP data. See "Copy number segment smoothing and joining (optional)" on page 123 for more information.

 Select File → Open on the menu bar. Alternatively, click the File Open button.

The Open window appears. (Figure 109)



4. To view information about results, select one or more files, then click **Sample** Info.

| The Sample Info window opens | . (Figure 110) |
|------------------------------|----------------|
|------------------------------|----------------|

| 🐴 Sample Ir | ifo           |               | × |
|-------------|---------------|---------------|---|
| Name        | Date          | Array Type    |   |
| 3 08-0989_A | 2013-01-04T11 | CytoScan HD A | ^ |
| 3 09-1420_B | 2013-01-04T11 | CytoScan HD A |   |
| 3 09-1420_B | 2011-07-15T16 | CytoScan HD A |   |
| 11-0810_L   | 2013-01-04T11 | CytoScan HD A |   |
| P 11-0810_L | 2011-07-15T16 | CytoScan HD A |   |
| 11-0816_L   | 2013-01-04T11 | CytoScan HD A |   |
| P 11-0816_L | 2011-07-15T15 | CytoScan HD A |   |
| 11-0816_L   | 2013-01-04T11 | CytoScan HD A |   |
| 11-0816_L   | 2011-07-15T16 | CytoScan HD A |   |
| 20120730    | 2013-01-04T11 | CytoScan HD A |   |
| 20120730    | 2013-01-04T11 | CytoScan HD A |   |
| 20120730    | 2013-01-21T20 | CytoScan HD A |   |
| 20120730    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120830    | 2013-01-04T11 | CytoScan HD A |   |
| 20120913    | 2013-01-04T11 | CytoScan HD A | ^ |
| 20120913    | 2013-01-04T12 | CytoScan HD A | ~ |

**Note:** If the xxCHP and ARR files are located in the same folder, the Sample Info window shows information about both the sample and the results. To load files from the **Sample Info** window, select the files, then click **Open Selected Files**.

# Using the search feature

- Click Close Dialog (Figure 110) to close the Sample Info window. The Open window appears.
- 2. Navigate to the folder with the files that you want to search for and load.
- 3. Enter a text string with an asterisk (\*) **before** and **after** the search term in the **File Name** field. See example in Figure 111 on page 119.
- 4. Select a file type from the drop-down list. See example in Figure 111 on page 119.
- 5. Click Open.

Files with names that include the search term are displayed in the **Open** window. See example in Figure 112 on page 120.

| Figure 111                     | Enter a file name search term (*text string*) and select a file type  |
|--------------------------------|---|
| 🖏 Open                         | ×   |
| Look In                        | 🔋 chAS_Data 🛛 🖌 🏠 🐯 🗸   |
| Recent<br>Desktop<br>Documents | <ul> <li>ChAS_2.0_files</li> <li>New_CYCHP_files</li> <li>08-0989_A3_2.cyhd.cychp</li> <li>09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228(2)_2.cyhd.cychp</li> <li>09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.cyhd.cychp</li> <li>11-0810_LC_ONC13B_A6_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC13B_A6_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC41B_A12_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC41B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC41B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC134B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp</li> <li>11-0816_LC_ONC134B_B10_POP#2_CytoScan-PS_20110511(2)_c.cyhd.cychp</li> <li>20120730_181709_001_FFPE 3_2.cyhd.cychp</li> <li>20120730_181709_002_FFPE 6_2.cyhd.cychp</li> </ul> |
| Notwork                        | File Name: *b1*   |
| Network                        | Files of Type: CYCHP Cancel CYCHP CNCHP Affymetrix Extensible Data (AED) Browser Extensible Data (BED) All known file types.  |

| Figure 112 | The Open window shows files with names that include your search terr  |
|------------|---|
| 🖏 Open     | x   |
| Look In:   | 🔋 chAS_Data 🛛 🖌 🏠 🖓   |
| <u></u>    | ChAS_2.0_files  New_CYCHP_files  1.0.9316 L.C. ONC134E R10 PoP#2 OutoScap_PS 20110511(2), 2 outof cyclop  |
| Recent     | ■         11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.CEL           ■         11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.cytd.chpcar-prototy           ■         11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.cytd.chpcar-prototy           ■         11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.cytd.chpcar-prototy |
| Desktop    | 11-0816_LC_ONC134B_B10_P0#2_CytoScan-PS_20110511.cyhd.cychp     11-0816_LC_ONC134B_B10_P0P#2_CytoScan-PS_20110511.cyhd.cychp     11-0816_LC_ONC134B_B10_P0P#2_CytoScan-PS_20110511.cyhd.cychp.chpcar  |
| Documents  | Nijm10_B10_Nijmegen Alpha_Cytoscan_MB_20110203_2.cyhd.cychp     Nijm10_B10_Nijmegen Alpha_Cytoscan_MB_20110203.CEL     YU0844608l307_cousins_B10_CytoScanHD_MS_20110512_2.cyhd.cychp  |
|            | YU0844608l307_cousins_B10_CytoScanHD_MS_20110512.CEL           YU0844952l335_cousins_B11_CytoScanHD_MS_20110512_2.cyhd.cychp           YU0844952l335_cousins_B11_CytoScanHD_MS_20110512.CEL   |
|            | File Name:         hp" "YU0844952I335_cousins_B11_CytoScanHD_MS_20110512.CEL"         Open  |
| Network    | Files of Type: *b1* Cancel  |

6. Select the files (you can use Shift click or CTRL click to select multiple files)

#### 7. Click Open.

If any of the files fail the QC checks, a warning notice appears (Figure 113).

You can click **Yes** to continue to load the files.

| Figu  | re 113 Warning Notice for QC failure  |   |
|-------|---|---|
| 🐴 Fil | e Failed QC   | < |
|       | One or more files failed QC or had insufficient information to<br>determine QC. Are you sure you want to load these files?<br>• C:\Users\ppavic\Desktop\chAS_Data\20120913_17532<br>(Failed QC) |   |
|       | Yes <u>N</u> o Cancel   |   |

It is recommended you load no more than 3 files at one time.

If the following warning message appears (Figure 114), click **OK** to acknowledge it or click **Cancel** to reselect a maximum of 3 files.

| Figu | re 114 Recommended maximum exceeded message   |   |
|------|---|---|
| 🖏 Re | commended maximum exceeded  | × |
|      | The recommended maximum number of analysis results files to be loaded at a time is 3<br>Loading more files may impact the performance of the software.<br>Are you sure you want to load them? |   |
|      | Yes   |   |

If the following warning message appears (Figure 115), acknowledge it, then click **OK**.



If the following warning message appears (Figure 116), click **Yes** to acknowledge it.

| Figure 116 NetAffx versions message  |
|--|
| NetAffx Versions X   |
| NetAffx version of one or more files does not match loaded NetAffxGenomicAnnotations Browser annotation database file version 32. Are you sure you want to load these files? <ul> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\09-1420_B2_Phase4CustomerPanel_CytoScan_P (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC13B_A6_PoP#2_CytoScan_PS (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3)</li> <li>C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_PoP#2_CytoScan_PS (32.3)</li> </ul> |

**Note:** The ChAS Browser allows loading different NetAffx versions at the same time (as long as the versions are all from all the same reference and genome builds). If NetAffx versions are from different builds of the genome (for example Hg18 and Hg19), The ChAS Browser does not load the files.

A progress bar appears (Figure 117)

| Figure 117 Progress bar |
|-------------------------|
| Please wait X           |
| Opening file 2 of 6     |
| Cancel                  |

After a few moments, the ChAS browser featuring your selected samples appears.

The loaded files appear in the Files list pane. (Figure 118).



# Copy number segment smoothing and joining (optional)

"Smoothing" and "Joining" are non-destructive processes that affect the display of Copy Number segments. Smoothing and joining are performed on the Copy Number State data during the loading process, based on settings that are specified before loading. Any data filtering is applied after smoothing and joining.

**IMPORTANT!** Smoothing and joining are turned on by default in a new user profile for CytoScan 750k, HD, and HD Accel arrays.

Smoothing and Joining are specified per array type. The processes do not affect the marker data in the CNCHP or CYCHP file. If these settings are turned off, the Copy Number segment data is displayed without smoothing or joining.

**IMPORTANT!** Smoothing and Joining affect only data loaded from CNCHP and CYCHP files. This ONLY applies to copy number data, NOT LOH or Mosaic types. Smoothing and Joining is OFF by default for CytoScan Optima, OncoScan, and ReproSeq Aneuploidy files.

Segments which have been smoothed and/or joined are indicated by a blue check mark in the Smoothed/Joined column of the Segments table (Figure 119). The segment ID name indicates whether smoothing and/or joining has occurred. A red "X" indicates no smoothing or joining has been applied.

#### Viewing what segments were smoothed/joined

- 1. Click the Segments tab.
- 2. Use the horizontal scroll bar to move the window to the far right.

The Smoothed/Joined column appears. (Figure 119)

For more information:

- See "About smoothing" on page 126.
- See "About joining" on page 127.

| Figure 119 Example Segments table with smooth/joined segments |              |               |                   |              |  |            |    |
|---|--------------|---------------|-------------------|--------------|--|------------|----|
| Karyoview   | Segments     | V LytoRegio   | ons 🎙 🏓 Overlap I | Map 🎙 🔀 (    | Graphs 🔻   |            |    |
| 🔠 👼 🗎   | Σ            | 🛊   🔏 🗹 🔲     |                   |              |  | 52 results | 00 |
| BACs  | OMIM ® Genes | OMIM ® Phenot | Segmental Dup S   | moothed/Joir | ned Microarray Nomenclature                        |            |    |
| RP11-161019,  | N/A          | N/A           | chr1:82549932,    | X            | arr[hg19] 8q23.2q23.3(110,551,033-114,048,383)x3   |            | ^  |
| CTD-2534I17,  | N/A          | N/A           |                   | ~            | arr[hg19] 8q23.3(114,065,943-115,632,043)x3        |            |    |
| RP11-113A21,  | N/A          | N/A           | L                 | X            | arr[hg19] 8q23.3(115,650,683-116,541,758)x3        |            |    |
| RP11-595J20,  | N/A          | N/A           | chr4:15928053     | ×            | arr[hg19] 8q23.3q24.12(116,594,211-120,882,163)x3  |            |    |
| RP11-992I7, C   | N/A          | N/A           |                   | ×            | arr[hg19] 8q24.12(120,885,308-121,317,090)x3       |            |    |
| RP11-585L3, R   | N/A          | N/A           | chr1:19221808     | ×            | arr[hg19] 8q24.12q24.13(121,490,686-124,103,255)x3 |            |    |
| CTC-497I19, R   | N/A          | N/A           | chr8:50630701,    | ×            | arr[hg19] 8q24.13q24.21(124,437,925-129,442,671)x3 |            |    |
| RP11-299H6,   | N/A          | N/A           | chr20:6290485     | ×            | arr[hg19] 8q24.21q24.3(129,458,472-140,761,415)x3  |            |    |
| RP11-765I17,  | N/A          | N/A           | chr8:14120600     | ×            | arr[hg19] 8q24.3(140,809,247-141,802,431)x3        |            |    |
| RP11-1021M1   | N/A          | N/A           | chr5:79654182,    | ×            | arr[hg19] 8q24.3(142,224,672-146,295,771)x3        |            |    |
| RP11-794B16,  | N/A          | N/A           | chr15:3569328     | ~            | arr[hg19] 10q11.22(46,966,533-48,298,893)x3        |            |    |
| RP11-596C13,  | N/A          | N/A           | chr11:1120569.    | X            | arr[hg19] 11q23.1(110,945,179-111,874,060)x1       |            |    |
| CTD-2348C13,  | . N/A        | N/A           | chr2:20818151     | ~            | arr[hg19] 14q32.33(104,410,114-107,053,817)x3      |            |    |
| CTD-2191D7,   | N/A          | N/A           | chr17:4516771     | Х            | arr[hg19] 17q21.31(44,212,823-44,784,639)x3        |            |    |
| CTD-2191D7,   | N/A          | N/A           | chr17:4516771     | ×            | arr[hg19] 17q21.31(44,212,823-44,784,639)x3        |            |    |
| CTD-2557A13,  | N/A          | N/A           | chr6:114181918    | ×            | arr[hg19] 21q21.3(27,447,808-28,032,671)x3         |            |    |
| CTD-2542C17,  | . N/A        | N/A           |                   | ×            | arr[hg19] 21q21.3q22.11(31,424,401-31,852,180)x3   |            |    |
| CTD-3148D21,  | . N/A        | N/A           | chr22:1893775     | ×            | arr[hg19] 22q11.21(20,184,050-20,761,384)x3        |            |    |
| CTD-2644P19   | N/A          | N/A           | chr22:2275688     | ×            | ambn19122n1122(22673638-23258438)x3                |            | ~  |

Ô

#### Setting smoothing and joining parameters

1. Click Preferences  $\rightarrow$  Edit User Configuration.

The User Configuration window appears.

- 2. Click the Segment Data tab.
- 3. Click the **Choose Array Type** drop-down menu to select the array type to view or edit its Smoothing and Joining settings. (Figure 120 on page 124)

**Note:** If you change the smoothing or joining parameters, the new rules are applied to the original, raw segments of CxCHP files which have not had modifications, Calls, Interpretations, or Inheritance made. CxCHP files which have had segments modified or had Calls, Interpretations or Inheritance made will have had their smoothing and joining parameters fixed and will not adopt changes made to the array type's Segment Data smoothing and joining settings.

| User Configuration  |     |  |  |  |
|---|-----|--|--|--|
| Sogmant Data OC Thrashelde Calar Pulas Miss Vassbularias DP Quany Eiltered DP Quany Experts   | -   |  |  |  |
|   |     |  |  |  |
| CytoScan HD Array   |     |  |  |  |
| For CHP files of each array type, edit the configuration of Smoothing, Joining or Merging to create Copy Number Segments.<br>This processing happens at file loading and at configuration saving (always before any Segment Filtering). |     |  |  |  |
| Reset customized to defaults  |     |  |  |  |
| Enable/disable and configure Copy Number State data processing for generating Copy Number Segments  |     |  |  |  |
| Smoothing [merges only contiguous aberrations of the same type (Gain, or Loss)]   |     |  |  |  |
| Smooth Gain or Loss CNState runs to the most common marker state value  |     |  |  |  |
| Smoothing maximum jump limit [adjacent data points farther apart in CNState will not be smoothed together]  |     |  |  |  |
| Limit smoothing of CNState data to not smooth aberrant segments more distant than this number of CNStates   | 2   |  |  |  |
| Joining [merges aberrations of the same type (Gain, or Loss) which are separated by some normal state data]   |     |  |  |  |
| Join Gain or Loss CNState runs separated by no more than this number of markers of normal state data  | 50  |  |  |  |
|   |     |  |  |  |
| 0   | 5   |  |  |  |
| Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than this distance measured in kbp   | 200 |  |  |  |
|   |     |  |  |  |
| 0   | 20  |  |  |  |
| Joining maximum jump limit [aberrant normal-data-flanking data which is farther apart in CNState will not be joined together]   |     |  |  |  |
| Limit the joining of CNState data (which flanks normal state data) to not join aberrant segments more distant than this number of CNStates  |     |  |  |  |
|   |     |  |  |  |
|   |     |  |  |  |
| OK Cancel   |     |  |  |  |
| on Cancer   |     |  |  |  |

#### Segment data tab options and descriptions

| Option   | Description   |
|--|---|
| Use default segment data rules configuration   | <ul> <li>For the CytoScan 750K and HD Arrays, the default smoothing and joining rules are: <ul> <li>Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.</li> <li>Join any "split" CNState runs separated by no more than 50 normal-state markers.</li> <li>Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp</li> <li>Skip segments from the smoothing operation for all arrays that have a CN &lt; 1.</li> </ul> </li> <li>For SNP 6 arrays, the default smoothing rule: <ul> <li>Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.</li> </ul> </li> </ul> |
| Smooth Gain or Loss CNState runs to the most common marker value   | Smoothing to the most common marker state value is only applied to contiguous CNState runs of the same type (gain or loss).   |
| Limit smoothing of CNState data to not smooth<br>aberrant segments more distant than this number<br>of CNStates  | If this option is chosen, CNState runs which are farther apart than the "smoothing maximum jump limit" will not be smoothed. For example, if the smoothing maximum jump limit is set at 1, then adjacent segments with CNState 3 and 5 will not be smoothed.  |
| Join Gain or Loss CNState runs separated by no<br>more than this number of markers of normal state<br>data   | If this option is chosen, only Gain or Loss CNState Runs which are<br>separated by less than a threshold number of markers of normal state<br>data will be joined. For example, if the marker threshold is set at 50,<br>then CNState runs separated by more than 50 markers of normal<br>state data will not be joined.  |
| Join Gain or Loss CNState runs interrupted by<br>normal state data which are separated from each<br>other by no more than this distance measured in<br>kbp | <ul> <li>For the CytoScan 750K and HD Arrays, the default smoothing and joining rules are: <ul> <li>Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.</li> <li>Join any "split" CNState runs separated by no more than 50 normal-state markers.</li> <li>Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp</li> </ul> </li> <li>For SNP 6 arrays, the default smoothing rule: <ul> <li>Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.</li> </ul> </li> </ul>   |
| Limit the joining of CNState data (which flanks<br>normal state data) to not join aberrant segments<br>more distant than this number of CNStates           | Smoothing to the most common marker state value is only applied to contiguous CNState runs of the same type (gain or loss).   |

**IMPORTANT!** If multiple smoothing and/or joining check boxes are selected, all criteria must be met to smooth and/or join the segments.

After smoothing and joining, the marker count, mean marker distance and confidence values get recalculated. For more information:

- See "About smoothing" on page 126.
- See "About joining" on page 127.

About smoothing Note: The examples shown below are for a case where the expected copy number is 2. Similar calculations take place for the X and Y chromosomes where the expected copy number may be 0, 1 or 2, depending on gender and whether the segment is located within or outside of the PAR region.

If you have a contiguous set of segments with gain values (for instance, of CN State values of three and four), with no markers of copy number 2 or lower, **without smoothing** they will be treated as a series of individual gain segments. The same rules apply to a set of segments with loss values of 0 or 1.

If you have a contiguous set of markers with gain values of three and four, with no intervening markers of copy number 2 or lower, **with smoothing** they will be consolidated into a single gain segment. (Figure 121)



If you have a contiguous set of markers with loss values of zero and one, with no intervening markers of copy number 2 or higher, after smoothing, they will be consolidated into a single loss segment.

The smoothing process is the same as the process automatically performed by the Segment Reporting Tool in GTC. Different methods are used to assign the CN state value and perform the confidence calculations, as described below. See the GTC User Manual for more information.

#### Copy Number State for Smoothed Segments

The median CNState of all the markers in the Segment is assigned as the Copy Number State value for the new smoothed segment. The median will thus always be either an integer or a half integer (like 3.5).

For all the half-integer cases:

- Gains are rounded up to the next full integer (3.5 goes to 4)
- Losses are rounded down to the next full integer (0.5 goes to 0, 1.5 goes to 1).

#### About joining

The joining options enable you to join segments with the same type (gain or loss) aberrant CNState that are separated by no more than a specified number of normal-state markers or by no more than a specified distance of normal-state data (Figure 122).



The equivalence of CNState of the segments to be joined could have happened as a result of smoothing, or been from "raw" unsmoothed segments with the same CNState.

## **XON** segment merging

**Note:** Implementation of the new Whole Genome Copy Number segmentation algorithm may make XON merging no longer necessary in your workflow.

XON Merging is on by default. XON Merging combines consecutive XON segment calls of the same Type (XON Region Gains or XON Region Loss) into a larger segment. Once an XON segment of a different Type (normal state or gain/loss) is encountered, the XON Merge is terminated. A Merged XON segment is represented as the larger segment in the Segments Table.

**Note:** The individual XON segments can still be viewed within the rectangle, provided the appropriate Levels are selected in the Filter Settings. In addition, the breakpoints of the Merged XON Segment will stop with an XON segment and may not line up exactly with the probe level data.

**Note:** A merged XON segment is assigned to the lowest XON annotation number within that merged segment. For example, if a merged XON segment overlaps XON Regions annotations of Level 1 and Level 3, the whole merged XON segment will be considered Level 1.

The Merged XON segment is represented by the transparent rectangle. (Figure 123) If you want to turn off this feature see "Turning off XON merging".



# Turning off XON merging

 To turn off XON Merging, go to Preferences → User Configuration. The User Configuration window appears. (Figure 124)

| Figure 124 User Configuration window, Segment Data tab  |   |  |  |  |  |
|---|---|--|--|--|--|
| User Configuration  | × |  |  |  |  |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports   | _ |  |  |  |  |
| CytoScan XON 🗸  | - |  |  |  |  |
| For CHP files of each array type, edit the configuration of Smoothing, Joining or Merging to create Copy Number Segments.<br>This processing happens at file loading and at configuration saving (always before any Segment Filtering). |   |  |  |  |  |
| Reset customized to defaults 🗹 Use default segment data rules configuration   |   |  |  |  |  |
| XON Merging [merges XON segments of the same type (Gain, or Loss) which not separated by any normal state data]   |   |  |  |  |  |

- 2. Click the Segment Data tab, then select CytoScan XON from the array dropdown.
- 3. Uncheck the Use default segment data rules configuration check box
- 4. Uncheck the XON Merging check box.
- 5. Close the ChAS browser, then reopen it.

XON Merging is now off/disabled.

## Setting QC parameters in the ChAS browser

ChAS checks the analysis results files for certain QC values. The software notifies you if the QC parameters do not meet the thresholds.

**Note:** Custom QC metrics can be viewed in both the Analysis Workflow and the Browser. However, any custom settings you wish to use, have to be entered separately in both the Analysis Workflow and the Browser. To create custom QC settings in the Browser see below. To create custom QC settings in the Analysis Workflow, see "Creating your own custom QC setting" on page 57.

You can adjust the QC threshold values or select different QC metrics.

#### **IMPORTANT!** Selecting different QC thresholds is not recommended.

**Note:** When using custom QC thresholds for CytoScan HTCMA in RHAS, these custom thresholds will also need to be updated in ChAS to reflect the desired QC thresholds.

Viewing QC thresholds

1. Click **Preferences**  $\rightarrow$  **Edit User Configuration**.

The User Configuration window appears.

- 2. Click the QC Thresholds tab. (Figure 125)
- 3. Select an array type from the drop-down list.

**Note:** QC parameters are specified per array type.

| Figure 125 CytoScan HD array QC Thresholds default settings.  |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|
| Segment Data       QC Thresholds       Color Rules       Misc       Text       DB Query         CytoScan HD Array |  |  |  |  |  |  |  |
| 🕂 😑 🔝 💟 Use default (   | 🕂 😑 💽 Use default QC configuration. Reset Custom to Defaults |  |  |  |  |  |  |
| Property Name   | Property Name Type Operator Value                            |  |  |  |  |  |  |
| mapd (CHP Summary)  | mapd (CHP Summary) Decimal Number ≤ 0.25                     |  |  |  |  |  |  |
| snpQC (CHP Summary) Decimal Number ≥ 15   |  |  |  |  |  |  |  |
| wavinessSd (CHP Summary) Decimal Number ≤ 0.12  |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |

#### QC Thresholds tab options and descriptions

| Option        | Description   |
|---------------|---|
| Property Name | SNPQC is a QC metric for SNP probes that is derived from polymorphic (SNP) probes   |
|               | <b>MAPD</b> is a QC metric for all probes used to determine copy number that is derived from both polymorphic (SNP) and non-polymorphic (CN) probes   |
|               | <b>Waviness SD</b> is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation.  |
|               | nd SNP QC is a QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions.  |
|               | nd Waviness SD is the same measure as Waviness SD, but only calculates in those regions that are identified as normal diploid.  |
|               | <b>Cel Pair Check</b> is a test that inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel. (OSCHP only)                   |
|               | <b>DQC (DishQC)</b> measures the amount of overlap between two homozygous peaks created by non-polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad.           |
|               | <b>QC Call Rate</b> is the percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step).   |
|               | <b>SMN MAPD</b> is a QC metric for all probes used to determine copy number that is derived from both polymorphic (SNP) and non-polymorphic (CN) probes calculated during SMN analysis.                         |
|               | <b>SMN WavinessSD</b> is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation calculated during SMN analysis.                    |
|               | <b>Note:</b> The property names are from the header information of the xxCHP file. QC thresholds are not established for Reproseq Aneuploidy files, but QC metrics are displayed in the QC and Sample Info tab. |
| Туре          | Value or algorithm used for that type of QC.  |
| Operator      | The type of comparison performed.   |
| Value         | Value assigned to the threshold.  |



| Array Type                             | QC Parameter     |                  |                  |                  |                  |
|--|------------------|------------------|------------------|------------------|------------------|
|  | MAPD             | snpQC            | Waviness SD      | ndsnpQC          | ndWavinessSD     |
| CytoScan 750K, HD, and HD Accel arrays | <u>&lt;</u> 0.25 | <u>≥</u> 15.0    | <u>&lt;</u> 0.12 | -                | -                |
| CytoScan XON Arrays                    | <u>&lt;</u> 0.20 | <u>≥</u> 10.0    | <u>&lt;</u> 0.08 | -                | -                |
| CytoScan (Normal Diploid Analysis)     | <u>&lt;</u> 0.25 | <u>&gt;</u> 15.0 | <u>&lt;</u> 0.12 | <u>&gt;</u> 15.0 | <u>&lt;</u> 0.12 |
| CytoScan HTCMA                         | =< 0.28          | >= 10            | = 0.07           | = -              | = -              |
| CytoScan Optima Array                  | <u>&lt;</u> 0.29 | <u>&gt;</u> 8.5  | -                | -                | -                |
| Genome-Wide Human Array SNP 6.0        | <u>&lt;</u> 0.35 | -                | -                | -                | -                |

Table 11 Default Genotyping QC Thresholds

| Array Type            | QC Parameter |              |  |
|-----------------------|--------------|--------------|--|
|                       | DQC          | QC Call Rate |  |
| CytoScan HTCMA arrays | > = 0.88     | 98.5%        |  |

 Table 12
 Default SMN QC Thresholds

| Array Type            | QC Parameter |                |  |
|-----------------------|--------------|----------------|--|
|                       | SMN MAPD     | SMN WavinessSD |  |
| CytoScan HTCMA arrays | = < 0.35     | = < 0.1        |  |

Table 13 Default QC metrics for OncoScan Arrays

| Array Type      | QC Parameter    |                |                    |  |
|-----------------|-----------------|----------------|--------------------|--|
|                 | MAPD            | ndSNPQC        | CelPairCheckStatus |  |
| OncoScan arrays | <u>&lt;</u> 0.3 | <u>&gt;</u> 26 | Pass               |  |

**Note:** The waviness SD metric is applicable to blood and cell line data. The waviness SD metric is not intended for alternative sample types such as solid tumor or FFPE samples in which the results may vary as a result of the biological complexity. For these sample types, it is recommended using **nd Waviness SD**.

Effect of SNPQC value (for a Single Sample Analysis) on the Allele Difference Track. (Figure 126)



SNPQC is one of the CytoScan within-array QC metrics which provides insight into the overall level of data quality from a SNP perspective. When evaluating the SNPQC values, the key consideration is to ensure that the threshold is exceeded. The quality of the SNP allele data is compromised, from an interpretation perspective, when the SNPQC values are below the recommended acceptance threshold as illustrated by the two left most graphs representing the two and three copy allelic state.

For the CytoScan HD array, when the SNPQC value is below 15, (as illustrated by the data in the two graphs above), the noise within the array is higher than expected. This in turn, compromises the overall data quality and clarity of the results. However, when the SNPQC value is above 15, the consideration is whether the SNPQC value is above or below the threshold value and not the absolute magnitude.

As long as the SNPQC value exceeds the threshold, there is a retention in the data quality as illustrated by the graphs which demonstrate clear allelic data across a broad range of SNPQC values that exceed the recommended threshold. The threshold was determined from thousands of arrays processed across multiple reagent lots, operators, and sample aberration types. SNPQC is one of the metrics used to assess array quality and should be helpful in determining which experimental data sets are of satisfactory quality to continue with subsequent interpretation.

# Adding a QC property

1. Click **Preferences**  $\rightarrow$  **Edit User Configuration**.

The User Configuration window appears.

2. Click the QC Thresholds tab. (Figure 127)

| Figure 127 Adding a new row to the QC Thresholds table  |                                   |                                |       |  |
|---|-----------------------------------|--------------------------------|-------|--|
| User Configuration X  |                                   |                                |       |  |
| Segment Data QC Thresholds C  | olor Rules Misc Vocabularies DB Q | uery Filtered DB Query Exports |       |  |
| CytoScan HD Array   | CytoScan HD Array                 |                                |       |  |
| For each array type, enter the threshold value for each property. Property names are normalized as listed in the documentation, not necessarily the exact names in the files. If a file does not contain a property, that threshold will be ignored and will not cause the file to fail QC. |                                   |                                |       |  |
| Property Name   | Туре                              | Operator                       | Value |  |
| MAPD  | Decimal Number                    | ≤                              | 0.25  |  |
| SNP QC  | Decimal Number                    | 2                              | 15    |  |
| Waviness SD   | Decimal Number                    | ≤                              | 0.12  |  |
| New Row   |                                   |                                |       |  |

- 3. Select an array type from the drop-down list.
- 4. In the QC Thresholds tab, uncheck the **Use default QC configuration** check box.
- 5. Click the Add button 🕂

A new row appears in the table.

To delete a property row, select the row, then click the **Remove** = button.

- 6. Click the **Property Name** field, then enter a new QC property name.
- 7. Click the Type field to select Decimal Number or Whole Number.
- 8. Click the **Operator** field to select an operator from the drop-down list.
- 9. Double-click the **Value** field to enter the threshold value for the newly added QC property.
- 10. To add another QC property, repeat steps 5-9.

11. Click **OK** to apply the newly added QC threshold(s).

#### 1. Click on an existing **Property Name**, then edit its QC property name.

#### 2. Click the Type field to edit its Decimal Number or Whole Number.

- 3. Click the **Operator** field to choose a different operator from the drop-down list.
- 4. Double-click the **Value** field to edit the current threshold value.
- 5. To edit another existing QC property, repeat steps 1-4.
- 6. Click **OK** to apply the newly edited QC threshold(s).

#### Editing an existing QC threshold

# Histogram data

#### Loading histogram data

**IMPORTANT!** You must be logged into the ChASDB to view Histogram data.

The histograms load by default. If they are not currently displayed, click **ChAS DB**  $\rightarrow$  **Refresh ChAS DB data** to view them in the ChAS Browser.

**Note:** The histograms are only available for NetAffx Genomic annotation files for genome build Hg19. The Browser produces an error message, if you try to load Hg19-based histograms while a hg18 or hg38 based NetAffxGenomeAnnotation is currently displayed.

# Changing the default histogram filters

- 1. From the File tree (upper left column), locate the Default Histogram entry.
- 2. Right click on **Default Histogram**.

A menu appears.

3. Click View/Edit Properties.

The File Properties window appears. (Figure 128)

| Figure 128 File Properties window and Default Histogram Filters tab |
|---|
| File Properties   |
| Basic Extended Histogram Filters Histogram Colors                   |
| Default Histogram   |
| Graph Style   |
| Categories and Filters  |
| Parameters:<br>Categories: LOH, Loss, Gain                          |
| Change Filter Parameters  |
| OK Cancel   |

- 4. Click the **Histogram Filters** window tab, then use its check box(es), selections, and radio buttons to modify the default Histogram's factory settings.
- 5. Click on the Change Filter Parameters button to select new filter settings.

 Optional: To change filter parameters, click Change Filter Parameters. The Set Filter Parameters window appears. (Figure 129)

| Figure 129 Set Filter parameters window   |   |
|---|---|
| Set Filter Parameters   | ×   |
| Categories       Image: Gain       Image: Loss       LOH         Image: Detected       Image: Undetected       Undetected         Image: Gain (XON Region)       Image: Loss (XON Region)         XON Region Levels       Level 1       Level 2       Level 3 | Sample Types <ul> <li>Not Filtered</li> </ul> <li>No Value</li> |
| (Gain and Loss) Published D D   | Array Types   |
| Sample Interpretation Segment Interpretation Phenotyne  |   |
| Publisher Sample ID   | Calls   Not Filtered No Value                                   |
| File       Sexes     Female       Male     Unknown       Not Reported       Reset       OK  |   |

- 7. Use this window's check boxes, pre-populated entries, and/or the provided text fields (to enter the filter parameters you want).
- 8. Click **OK** to save your changes.

**Note:** If using the aDGV containing segments from both HD and XON arrays, use the Categories filters to limit the histogram data to only HD (check Gain and Loss) or only XON (Gain XON Region and Loss XON region)

- 1. From the File tree (upper left column), locate the Default Histogram entry.
- 2. Right click on **Default Histogram**.

A menu appears.

3. Click View/Edit Properties.

The File Properties window appears.

4. Click on the Histogram Colors tab. (Figure 130)

# Changing the default histogram colors

| Figure 130 File Properties window and Histogram Colors tab |   |
|--|---|
| % File Properties  | < |
| Basic Extended Histogram Filters Histogram Colors          |   |
| Select custom colors                                       |   |
| Gain   |   |
| Loss   |   |
| LOH  |   |
| Gain (XON Region)  |   |
| Loss (XON Region)  |   |
|  |   |
|  |   |
|  |   |
|  |   |
|  |   |
|  |   |
|  |   |
|  |   |
| Reset To Defaults  |   |
| OK Cancel  |   |

- 5. Click on the color square representing the histogram track you want to change. A change color window appears.
- 6. Select a new color, then click **OK**.
- 7. Repeat steps 5-6 as needed.
- 8. Click **OK** to save your color changes or click **Reset to Defaults** to return to the default color settings.

# Adding filtered histogram data

You can add additional histograms filtered on certain properties for viewing in the Detail View.

#### 1. Go to **ChASDB** $\rightarrow$ **Add Histogram**

The Add a Histogram window appears. (Figure 131)

| Add a histogram  | >        |
|--|----------|
| Histogram Filters Histogram Colors                                   |          |
| abel   |          |
| Graph Style  |          |
| Categories and Filters<br>Parameters:<br>Categories: LOH, Loss, Gain |          |
| Change Filter Parameters   | <u> </u> |

- 2. In the Label text box, enter a name for the histogram.
- 3. In the **Graph Style** pane, click the appropriate check box(es) and/or radio button(s) to define your filtered Histogram's graphic style.
- 4. Use this window's check boxes and radio buttons to create the desired graph style. Click on the Change Filter Parameters button to select filters to be applied to your new histogram. Click on the Histogram Colors tab to select colors for your new histogram tracks. See "Changing the default histogram colors" on page 135 for details.
- 5. Click **OK**.

The histogram is added to the bottom of the File tree list. The histogram can be moved to a different position in the Detail View by clicking on the name of the histogram, then dragging it to the desired location within the File tree.

You can access what segments are in a particular bin of the histograms by rightclicking on the histograms and selecting, **Show Histograms items in bin**. For more details, see "Segment intersections" on page 388.

**Note:** When right clicking to Show Histograms in bin, the percentages for DB Coverage Count and DB Overlap Count columns in the Segment Intersections window represent the percentage intersection with the query region (the light blue vertical bars) shown in the Segment Intersections window. For more details on this Segment Intersection window, see Figure 428 on page 389.

# Removing a histogram

- 1. Locate the Histogram you want to remove in the Files window pane. (Figure 132)
- 2. Right-click on it, then click **Close**.

**Note:** Closing a Histogram removes the Histogram from the Detail View. In order to view this Histogram again, you must recreate it using the Add Histogram steps listed above. See "Adding filtered histogram data" on page 137.

| Figure 132 Right-click on a Histogram to remove it.   |  |
|---|--|
| Histogram to remove it.   |  |
| <ul> <li>Disorder-Causing OMIM ® Genes</li> <li>OMIM ® Phenotype Loci</li> <li>OMIM ® Region Phenotype Loci</li> <li>Protein Coding Ensembl Genes</li> <li>Protein Coding Genes</li> <li>Recurrent/Curated Regions</li> <li>Segmental Duplications</li> <li>Triplosensitivity</li> <li>III sno/miRNA</li> <li>Markers</li> <li>Cytobands</li> </ul> |  |



# **Viewing data**

# Displaying options of analysis results data

Graphic Displays

See "Displaying data in graphic views" on page 148.

Tables

See "Displaying data in table views" on page 323.

After the data is loaded, you can:

- Filter the segments by Segment Parameters to hide segments that do not meet your requirements for significance. See "Filtering segments" on page 213.
- Select a region information file for use as a CytoRegion file and:
  - Perform differential filtering for segments in CytoRegions and in the rest of the genome.
  - Display only segments that appear in CytoRegions using Restricted Mode. See "Using CytoRegions" on page 263.
- Select a region information file for use as an Overlap Map and use the Overlap filter to conceal segments that overlap with the Overlap Map items. This functionality may be helpful for tracking or filtering out benign copy number change regions. See "Using the overlap map and filter" on page 275.
- Add selected features of the genome to new or existing Region (AED) files, and edit annotation data on existing annotations. See "Creating and editing AED files" on page 283.
- Prepare reports on your findings by exporting graphics and table data in PDF and other formats. See "Exporting results" on page 410.
- Save setups of ChAS for different tasks in user profiles and named settings.See "User profiles and named settings" on page 433.

# **Overview of ChAS window components**



The ChAS window components (Figure 133) include:

- Menu Bar: Access to the functions of the software.
- **Tool Bar**: Quick access to commonly used functions.
- Files List: Displays data and annotation files that can be displayed. (page 141)
- Data Types List: Displays the type of data available in the files. (page 143)
- Named Settings: Displays a list of the previously saved display settings for ChAS. (page 144)
- Status Bar (page 144): Displays:
  - Software status
  - ChAS Browser's NetAffx Genomic Annotation file version.
  - hg version
  - The annotation or probe that your mouse pointer is nearest (in the Detail View).
- **Display Area** (page 145): Displays the following data in graphical and table formats:
  - Analysis results graph data.
  - Detected Segments
  - Histograms
  - Regions
  - Reference Annotations

## **Files list**

The Files list (Figure 134) displays the different sources of data and annotations that are loaded in the Chromosome Analysis Suite. Files are grouped by type in the Files list.

| Figure 134 Files list                              |  |  |
|--|--|--|
| _ Files  |  |  |
| Sectional_Blood_01_CytoScanHD.cyhd.cychp           |  |  |
| Image: Sample data     Sample data                 |  |  |
| ☑ 🕄 🖓 🔥 ♀ CancerSample_01_CytoScanHD.cyhd.cychp    |  |  |
| ☑ Hard ScanDDG2PGeneList.r1.aed                    |  |  |
| ✓  |  |  |
| Demo_AmpliSeq_Exome_CNV_case.vcf                   |  |  |
| DGV  |  |  |
| Default Histogram                                  |  |  |
| 🗹 🗟 Ensembl Genes                                  |  |  |
| ■ Seference annotations <sup>*</sup> and Histogram |  |  |
| Some Series  |  |  |
| 🔲 💈 OMIM ® Phenotype Loci                          |  |  |
| Segmental Duplications                             |  |  |
| 🔲 🖽 sno/miRNA                                      |  |  |
| Markers Markers and Cytobands                      |  |  |
| ✓ ∞ Cytobands                                      |  |  |
|  |  |  |

\* Reference annotations displayed may be different than shown above depending on the NetAffx Genomic Annotation file loaded.



Displayed files grouped by type:

Sample Data

Colored nibs  $\bigcirc$  display the color used for the data lanes for that sample in the Karyoview, Selected Chromosome View, and Detail View. The appropriate gender symbol is displayed to the right of the colored nib.

If a loaded file has a QC parameter that is out of range, an alert symbol  $\cancel{1}$  appears next to the file name. (Figure 135)

| Figure 135 QC alert for Sample Data files  |
|--|
|  |
| Mathematicell_02.0SCHP   |
| Image: Second Secon |
|  |

- Region Information Files
- Icons indicate the file type (AED or BED) and whether the loaded files have been selected as a CytoRegion file III or Overlap Map III.
- Reference Annotations (loaded during software start-up). Note: Icons indicate the annotation type.
- Histogram information (loaded during software start up if connected to ChAS DB).
- Cytobands (Separated from other reference annotations because cytobands cannot be moved in the displays)

See "Selecting data for display" on page 186. for information on using the Files list to select loaded data and region information files, and reference annotation for display.

You can export the feature information in these files to a new AED region information file. See "Exporting information in AED or BED format" on page 314..

## Data types list

The Data Types list (Figure 136) shows the types of data available for display in the Karyoview, Selected Chromosome View, and Detail View. The available data types may vary, depending upon the type of sample data available. See "Introduction to loading data" on page 114.

| Figure 136 Data Types list   |
|------------------------------|
| Data Types                   |
| Copy Number State            |
| 🗹 🔺 🍸 Gain                   |
| ✓ ▼ ∠Loss                    |
| Mosaic Copy Number State     |
| 🗹 🔺 🍸 GainMosaic             |
| ✓ ▼ LossMosaic               |
| XON Region Copy Number State |
| 🗹 🕂 🛛 Gain (XON Region)      |
| 🗹 🕂 🍸 Loss (XON Region)      |
| 🗹 🖾 Log2 Ratio               |
| 🗹 🖾 Weighted Log2 Ratio      |
| 🗹 🖂 SmoothSignal             |
| 🗹 🖾 Allele Difference        |
| 🗹 🖾 BAF                      |
| 🗹 🕱 🔽 LOH                    |
| 🗹 🖾 LOH                      |
| 🗹 👬 Genotype Calls           |
| 🗹 🖾 Copy Number State        |
| 🗹 🖾 Summarized Log2Ratio     |
| Variants                     |
| Detected                     |
| ✓ • Undetected               |

The Data Types list enables you to select from Segments data and Graph data.

The Segments data is displayed graphically in:

- Karyoview
- Selected Chromosome View
- Detail View

If filtering is applied to a segment type, a funnel icon  $\mathbb{Y}$  appears next to the segment symbol in the list.

Graph data, indicated by the *icon*, is displayed only in the Detail View. See "Detail view" on page 166 for more information.

Unselected data is also concealed from the different tables and graphs.

See "Selecting data types for display" on page 188. for information on using the Data Types list to select different data types for display.

### Named settings

The Named Settings drop-down list (Figure 137) enables you to apply a previously created setting for ChAS. The settings may include things like:

- Segment Filter and Overlap Map Filter settings
- Types of data to be displayed
- Restricted Mode ON/OFF status. See "Using restricted mode" on page 271.

| Figure 137   | Named Settings      |
|--|---------------------|
| A u<br>Named Settin  |                     |
| Named Setur  | ly -                |
| 酱 Standard   | <b>v</b>            |
| Contraction Contractica Contra | al Gains and Losses |
| 鵗 High Res   | olution             |
| ڏ LOH only   | (3Mb and 50 SNPs)   |
| ڏ LOH only   | (5Mb and 50 SNPs)   |
| ConcoSca   | n Default           |
| 酱 Standard   |                     |
| 🚜 XON-Lev  | el 1                |

**Note:** Default Named Settings (indicated by the **i**con) should not be deleted from the system because they are shared by all user profiles.

See Chapter 20, "User profiles and named settings" on page 433 for information on creating and using Named Settings.

**Note:** The OncoScan Default Named Setting has no filters applied so all segments called by the TuScan algorithm can be viewed. Users can create their own appropriate custom filter Named Setting for their data, see "User profiles and named settings" on page 433.

## Status bar

The status bar (Figure 138) (very bottom of browser) displays information on:

- NetAffx Genomic Annotation database and its hg version
- Restricted Mode status (See "Using restricted mode" on page 271.)
- Edit Mode status (See "Using edit mode" on page 220.)
- Cursor (Mouse Over) Position
- User Profile ID


| Figure 138 Status Bar  |   |   |   |
|--|---|---|---|
| ChAS Browser: NetAffx 32 (hg19) Restricted Mode: Off<br>NetAffx Genomic Annotation<br>database and its hg version<br>currently loaded in the ChAS<br>Browser (the database is not<br>array-specific) | SmoothSignal: 11-0810_LC_ONC13B_A<br>Restricted Mode<br>Indicator | 6_PoP#2_CytoScan-PS_20110511.cyhd.cychp, Nearest chromosome positi<br>Cursor Position information | user Pete<br>User Profile<br>USer Profile |

## **Display area**

The Display Area (Figure 139) is divided into three panes:

- "Upper panes" on page 146
- "Selected chromosome view" on page 146
- "Lower panes" on page 146

| Figure 139 Display Area showing Segments Table and Detail View  |                  |               |
|---|------------------|---------------|
| 111 Karyoview 🏾 🏜 Segments 🎙 🛄 CytoRegions 🎙 🖉 Overlap Map 🎙 🖾 Graphs 🎙 🕀 Variants 🎙 🏛 Query Samples 🎙 💷 Query Segments 🎙 | p13.3            |               |
|   | p13.2            | 3             |
|   | p12              | 5             |
|   |                  | 7             |
|   | pilii            | 9<br>10       |
|   | q11.1            | 11<br>12      |
|   |                  | △ 13          |
|   | q12              | 15<br>16      |
| CancerSample_01_CytoScanHD cythd ND.cychp: Copy Number State (regments)   | q21.31           | ▲<br>18<br>19 |
| CancerSample_01_Oyb/ScanHD_syndh D.syndh. LOH (segments)  | q21.32<br>q21.33 | 20            |
| Details View  | g22              | △ 22<br>X     |
| HD Default LOH  | <b>633</b> 3     | Y             |
|   | ne               |               |
|   | <mark>st</mark>  |               |
|   | q25.1            |               |
| Detail View      Q C and Sample Into     Z Chromosome Summary Data  | q25.3            |               |

The tabs in the upper and lower panes display different types of data, in both graphical and table formats. Data from the same sample files is displayed in all three panes.

You can display a pane in a separate window by clicking the Nicon on the tab.

To close the window and return the information to the tab panel, click the **b** icon in the window.

|      |    | $\sim$ |   |
|------|----|--------|---|
| - 4  |    | $\geq$ |   |
| - 6  |    | 17     | a |
|      |    | r 🖌    |   |
| - 12 | 84 | Æ      |   |
| - 12 |    |        |   |
| - 8  |    |        |   |

| Upper panes                    | The Upper pane displays the following data in graphics and table formats:   |
|--------------------------------|---|
|                                | "Karyoview" on page 149: Displays selected segment types for selected sample<br>files for all chromosomes.  |
|                                | "Segments table" on page 333: Displays a list of the detected segments in the selected sample files.  |
|                                | "CytoRegions table" on page 268: Displays a list of the Regions in the AED/BED<br>file selected as the CytoRegion file. Includes information on detected segments<br>which lie in CytoRegions.  |
|                                | "Viewing the overlap map table" on page 279: Displays a list of the Regions in the AED/BED/Reference Annotation file selected as the Overlap Map. Includes information on detected segments that are overlapped by Overlap Map Items. |
|                                | <ul> <li>"Graphs table" on page 341: Displays marker data for the loaded and selected<br/>xxCHP files.</li> </ul>   |
|                                | <ul> <li>"Variants table" on page 346: Displays somatic mutation data from OncoScan CNV<br/>Plus and Carrier Variant data from CytoScan HTCMA arrays.</li> </ul>  |
|                                | <ul> <li>Query Samples Table: Displays sample level results from searches performed on<br/>the ChAS DB.</li> </ul>  |
|                                | <ul> <li>Query Segments Table: Displays segment level results from searches performed<br/>on the ChAS DB.</li> </ul>  |
| Selected<br>chromosome<br>view | The Chromosome View displays detected segments in selected sample files for the chromosome selected in the Karyoview, while the Chromosome Selection List (far right column) displays its number.                                     |
|                                | See "Selected chromosome view" on page 164 for more information.  |
| Lower panes                    | The Lower Pane displays:  |
|                                | "Detail view" on page 166: Displays the selected section of the chromosome displayed in Selected Chromosome View and includes:  |
|                                | Detected segments, variants, and graph data in selected xxCHP files.  |
|                                | Histograms, AED/BED file regions and annotations.   |
|                                | Reference annotation files  |
|                                | "QC and sample info tab" on page 350: Displays QC metric information as well as<br>information about the loaded Sample and Region (AED) files.  |
|                                | Chromosome summary data" on page 357:   |
|                                | Summarizes particular data across each chromosome in the loaded sample<br>data files (for example, proportion of each chromosome found to be in the state<br>of LOH).   |
|                                | Calculated Properties: Calculates the Percent Autosome LOH in the sample based on the LOH filter setting.   |



## Changing the NetAffx genomic annotation file version

- 1. At the top menu bar, click File  $\rightarrow$  ChAS browser NetAffx Genomic Annotation file version.
- 2. From the **Select NetAffx Database** drop-down menu (Figure 140), click to select the NetAffx Build version you want, then click **OK**.

**Note:** If there are loaded xxCHP files with hg version different from the selected NetAffx database, a message appears stating that these data files will be closed (in both the ChAS browser and the MSV) <u>before</u> the NetAffx annotations are loaded.

**Note:** More current NetAffx Genome Annotation files have the date the file was generated imbedded into the file name. To load the most current file from your library folder, select the file with the most recent date.



For more information on what types of data is in the database files and how this information varies in different versions, see "ChAS browser NetAffx Genomic Annotations" on page 474.



# **Displaying data in graphic views**

ChAS provides multiple graphic views for detected segments and other data. Use these graphic views to:

- Get an overview of the detected segments.
- Get an overview of segments stored in the ChAS DB.
- Compare segments between samples.
- View sample data for the whole genome.
- Drill down to examine areas of interest in more detail.
- View the graph and marker information used to generate the detected segments.
- Take advantage of reference annotations and external web sites.
- Create your own Affymetrix Extensible Data (AED) files with regions of interest and annotations.

### **Graphic views**

Data can be displayed in a Karyoview, Whole Genome View, Selected Chromosome View, and a Detailed View, as shown in Figure 141.



| 🗱 Karyoview 🌂 🕍 Segments 🌂 🕌 CytoRegions 🌂 🏓 Overlap Map 🌂 🖾 Graphs 🌂 🔛 Som Mut 🌂   |             |
|---|-------------|
|   |             |
| i ân ân ann ann ann ann ann ann ann ann a   | = *         |
| O       BenalCell 04 OSCHP: Copy Number State (segments)  |             |
| Sample_07.cyhd.cychp: Copy Number State (segments)  RenalCell_04.0SCHP: Copy Number State  Selecter  Chrome   | 1<br>Some   |
| Sample_07.cyhd.cychp: Copy Number State   |             |
| RenalCell_04.OSCHP: Log2 Ratio<br>1.5<br>Sample_07.cyhd.cychp: Log2 Ratio   | q12         |
| Sample_07.cyhd.cychp: Mosaic Copy Number State (segments)   | <b>_</b> \$ |
| Sample_07.cyhd cychp: LOH (segments)  | =.          |
|   | = ,         |
| 0 50000kb 100000kb 200000kb × 12  | q41         |
| Image: Part of the second s | q43         |

Data from the same sample files is displayed in all the views at different scales.

If an item in any of the views is selected, the icon for that item is enlarged or highlighted in the views.

#### Karyoview

The Karyoview (Figure 142) displays a genome-wide view of the detected segments and other data.

In the Karyoview:

- Click a chromosome in the Karyoview to select it.
- Press Ctrl + Left/Right Arrow keys to move between chromosomes.
- To jump to chromosome 1, press Ctrl+Home
- To jump to chromosome Y (last chromosome in the Karyoview), press the Ctrl+End.





Using the mouse, click and drag on a selected chromosome to select an area for display in the Detail View. This area is highlighted in the Selected Chromosome View. (Figure 143)



**Note:** To easily remove the blue highlight surrounding the selected chromosome for image captures, go to **View**  $\rightarrow$  **Hide Karyoview Highlights**.

Use the Stretch Slider and Vertical Scroll Bar to zoom in on a section of the Karyoview. You can also use the mouse wheel as detailed below:

- Alt + mouse wheel stretches the display.
- Mouse wheel scrolls up and down.

The following information is displayed for each chromosome: (Figure 144)

- Chromosome number
- Cytobands
- Segment Data Uses separate lanes for each sample file and each displayed segment type. Each sample is assigned a unique color in the display that is used for the lane. You can:
  - Change the grouping of samples and segment types. See "Changing the grouping of samples and data types" on page 189.
  - Change the position of samples. See "When the lanes are grouped by sample, the different segment types for each sample are kept together in the Karyoview and Selected Chromosome View (Figure 186) and in the Detail View." on page 190 and "Lanes grouped by sample" on page 190.
- Variant Data (only available for OSCHP and RHCHP)
- CytoRegions in selected CytoRegion File.
- Items in selected Overlap Map file.





Click on a chromosome in the Karyoview to select it for display in the Selected Chromosome View and the Detail View. The selected Chromosome is highlighted in the Karyoview.

The Stretch Slider and the vertical scroll bar controls the vertical stretch of the Display area.

At higher magnifications, more details of the Cytobands are displayed in the Karyoview. You can see Cytoband labels if the display has room.

The portion of the chromosome selected in the Selected Chromosome View and displayed in the Detail View is highlighted in the Karyoview.



If you have selected a CytoRegions file, the CytoRegions are displayed as gray bands that stretch across the entire chromosome cell, from right to left of the Cytobands.

If you assigned an Overlap Map file, the overlap map items are displayed as small rectangles to the left of the cytobands. Its color is the same color used in the Details View.

**Note:** You can mouse over a feature in the Karyoview, Selected Chromosome View, or Detail View to display a pop-up with information about the feature. Also, you can right-click on a feature in the Karyoview or Selected Chromosome View to open a shortcut menu of options, as shown in Figure 145.

| Figure 145 Karyoview shortcut menu   |                                 |            |  |  |
|--|---------------------------------|------------|--|--|
| 4  | One Item Selected               |            |  |  |
| Ð  | Zoom to selection               | Ctrl+Space |  |  |
| te de la companya de | Selection Details               | Ctrl+D     |  |  |
|  | Add to a File                   |            |  |  |
|  | View/Edit Annotation Properties |            |  |  |
| 16   | Query ChAS DB                   |            |  |  |

#### Whole genome view

The Whole Genome View (Figure 146) displays the following data in a single display from Chromosome 1 to Y for:

- CYCHP: Log2 Ratio, Weighted Log2 Ratio, Copy Number State, Smooth Signal, LOH Allele Difference, and B-allele Frequency.
- XNCHP: Log2 Ratio, Weighted Log2 Ratio, Smooth Signal, LOH, Allele Difference, and B-allele Frequency.
- **CNCHP:** Log2 Ratio, Copy Number State, Smooth Signal, Allele Difference
- RHCHP: Log2Ratio, Smooth Signal, Copy Number State, LOH, Allele Difference, and B-allele Frequency.
- OSCHP: Log2 Ratio, Weighted Log2 Ratio, Copy Number State, Smoothed Signal, Allele Difference, and B-allele Frequency.
- ReproSeq Aneuploidy .zip: Copy number state data provides the sequencing tile data.

**Note:** When opening ReproSeq for the first in the Whole Genome View, the graphs may appear empty. Use **Choose Data** to select the Copy Number Graph type to view the data.

**Note:** The Y axis on the left represents the data points in that graph (Log2/Weighted Log2 Ratio). The Y axis on the right represents the line graph data (Smooth Signal/Copy Number).

#### Displaying the Whole Genome View for a sample(s)

1. Right-click on the Sample name in the File Tree (Figure 146), then select **Show WGV**.



2. The Whole Genome View for the selected file(s) opens. (Figure 147)



- 3. To select a different sample(s) to display:
  - Select File  $\rightarrow$  Choose File.
  - Click on a different file name, then click **OK**.

#### Changing graph types

1. Click on **View**  $\rightarrow$  **Choose Data**.

#### The Choose Data window appears. (Figure 148)

| Figure 148 Choose Data windo  | ow   |   |           |                          |           |           |             |
|---|--|---|-----------|--------------------------|-----------|-----------|-------------|
| Choose Data   | ę  |   |           |                          |           |           |             |
| 1.5 - Save as def Choose Data Colors and Categories<br>Save WGV state | noose Data   |   | ×         | 1                        |           |           | S           |
| 1 – Load a WGV state<br>Delete a WGV state Choo                       | ose Colors   |   |           | Part                     | 126.1     |           |             |
| 0.5 - Show all chromosomes Bac  | Single Color   | Foreground Lines                              | in second | - <mark>hunderl</mark> e | La Clark  |           | and a start |
|   | Alternating Colors                                     |   |           |                          |           |           |             |
| -1- To  | ose Data   | Top Row Line                                  |           | ·                        |           |           |             |
| -1.5  | Weighted Log2 Ratio                                    | SmoothSignal                                  | <b>v</b>  | . <b>.</b>               |           |           |             |
| 1.5-<br>1-  | in: -1.5 Max: 1.5                                      | Min: 0 Max: 4 Show gridlines Show zero lin    | ne Will   |                          |           | New W     |             |
| 0.5 - M   | liddle Row Dots  | Middle Row Line                               | <b>_</b>  |                          |           |           |             |
|   | Allele Difference                                      | <no line=""></no>                             | ~         | in the second            |           |           |             |
|   | in: -1.8 Max: 1.8<br>✔ Show gridlines ✔ Show zero line | Min: 0 Max: 4<br>Show gridlines Show zero lin | ne        |                          |           |           |             |
| + BAF:01 Be   | ottom Row Dots   | Bottom Row Line                               |           |                          |           |           |             |
| 1- American Second Second Second Second                               | BAF  | <no line=""></no>                             | ▼         |                          |           | le in ter |             |
| 0.8- M  |  | Min: 0 Max: 4                                 |           | 2444                     |           |           |             |
| 0.6-  | eset To Defaulte                                       | Show grownes Show zero in                     |           | . Perse.                 |           |           |             |
|   | OK   | Cancel  |           |                          | NOTE NOTE |           |             |
|   |  | المتناتير أفقر الغارينية للمدار               |           |                          | 1000 1000 |           |             |
| 1 2 3   | 4 5 6  | 7 8 9 10                                      | 11 12     | 13                       | 14 15     | 16 17 18  | 19 20 21 2  |

- Up to three rows of data can be viewed simultaneously for each sample.
- Use the Row Data and/or Row Line to assign which data tracks to view.
- Select <no Dots> or < no Line> to disable the display (of any data) in the row. Figure 149 is an example of no data being assigned to the Bottom Row, therefore only two rows of data are displayed in the Whole Genome View.

8



| Figure 149 Choose Data wir   | ndow  |   |                                       |
|--|---|---|---------------------------------------|
| Whole Genome View - Sample_01.cyh  | d.cychp   |   | _ 🗆 X                                 |
| File View  |   |   |                                       |
| ∎♥ or Sample_01.cyhd.cychp   | 🖏 Choose Data   | x   |                                       |
| + Weighted Log2 Ratio: -1.5 1.5  | Choose Colors   |   | SmoothSignal: 0 4 +                   |
| 1=   | Background Dots Single Color Alternating Colors                               | Foreground Lines                            | -3.5                                  |
| 0.5  | Choose Data   |   | -3                                    |
| 0 – <mark>Million Historia (1997) (</mark> | Weighted Log2 Ratio   | I op Row Line                               | -2                                    |
| -0.5 -   | Min: -1.5 Max: 1.5  | Min: 0 Max: 4 Show gridlines Show zero line | dat Ubbal                             |
|  | Middle Row Dots   | Middle Row Line                             | -0.5                                  |
| -1.5 -   | Allele Difference   | <no line=""></no>                           |                                       |
| + Allele Difference: -1.8 1.8  | Min: -1.8 Max: 1.8  | Min: 0 Max: 4                               |                                       |
| 1.5  | Show gridlines 🗹 Show zero line   | Show gridlines Show zero line               | -1.5                                  |
| 1-   | Bottom Row Dots   | Bottom Row Line                             | · · · · · · · · · · · · · · · · · · · |
| 0.5  | <no dots=""></no>   | <no line=""></no>                           | -0.5                                  |
|  | Min:     0     Max:     1       ✓     Show gridlines     ✓     Show zero line | Min: 0 Max: 4 Show gridlines Show zero line |                                       |
| -0.5   | Reset To Defaults   | Cancel                                      |                                       |

**Note:** The Y axis settings for the Whole Genome View are initially determined from the Y axis settings set in the Detail View Graph Settings. The Y axis on the left of the WGV pertains to the Row Data. The Y axis on the right of the WGV pertains to the Line data. Also, not all graphs are available for a given xxCHP file. If a graph type is selected in which data is not available, the graph will appear with no data points.

#### Changing colors of the data points or line data

Graphs can be viewed in a single color or alternating colors every three chromosomes.

1. Click **View**  $\rightarrow$  **Choose Data**. (Figure 150)

| Figure 150 Choose C  | Colors pane      |   |
|--|------------------|---|
| 🍾 Choose Data  |                  | x |
| Choose Colors<br>Background Dots<br>Single Color<br>Alternating Colors | Foreground Lines |   |

2. Select the radio button to change to either 1 color data points or alternating 3 color data points.



- 3. Click on the colored square(s) to open a Color Selection palette. Select new colors for the Whole Genome View.
- 4. Click OK to return to the Whole Genome View with your new selection

**Note:** Changing Data display and/or Colors affects the current sample only. To save these settings as default setting (whenever a file is opened), save these settings as the Default WGV State. (See "Setting a default WGV state display and colors".)

#### Setting a default WGV state display and colors

Creating WGV

states

- Select the Data and Colors to be displayed as your Default settings when opening files in the WGV, as described in "Changing colors of the data points or line data" on page 156.
- 2. Select View  $\rightarrow$  Save as default WGV State. (Figure 151)

| Fig                  | Figure 151 Save as default WGV State |  |  |  |  |
|----------------------|--------------------------------------|--|--|--|--|
| " <sub>GV</sub> /    | Whole Genome View - Ca               | ancerSample_01_CytoScanHD.cyhd.N   |  |  |  |
| File                 | View                                 |  |  |  |  |
| <b>₿₽</b> ₽ <u>⊿</u> | Choose Data                          | canHD.cyhd.ND.cychp  |  |  |  |
|                      | Apply default WGV State              | 1.5  |  |  |  |
| 1.5 —                | Save as default WGV State            |  |  |  |  |
| 1 —                  | Save WGV state                       | Saves current state as the default WGV State   |  |  |  |
|                      | Load a WGV state                     | Although the second |  |  |  |
| 0.5 —                | Delete a WGV state                   |  |  |  |  |
|                      | Show all chromosomes                 | anne an air an   |  |  |  |

A confirmation message appears.

3. Acknowledge the message, then click **OK** to save these settings as the Default WGV display.

**Note:** If changes have been made to colors, data or Y axis values, you can return to this WGV Default State by clicking **View**  $\rightarrow$  **Apply default WGV State** or click **Load a WGV State**  $\rightarrow$  **Default**.

Multiple WGV States can be created, then saved for a quick selection of different graph/ color settings.

- 1. Select the Data and Colors to be displayed as your Default settings when opening files in the WGV.
- 2. To save these selections as a WGV State, click on  $\textbf{View} \rightarrow \textbf{Save WGV State}.$
- 3. Enter a name in the dialog box, then click **OK** to save this new WGV State.

| Applying a saved<br>WGV state(s) | 1. Select View $\rightarrow$ Load a WGV State.  |
|----------------------------------|---|
|                                  | 2. Choose a saved WGV state from the drop-down list.  |
|                                  | 3. Click <b>OK</b> to apply the WGV State (or click <b>Cancel</b> to keep the current display).               |
| Deleting a saved                 | 1. Click on <b>View</b> $\rightarrow$ <b>Delete a WGV State</b> .   |
| WGV state                        | <ol> <li>Select the saved WGV state you want to delete from the drop-down list, then<br/>click OK.</li> </ol> |
|                                  | A message appears asking if you are sure you want to delete it.   |
|                                  | 3. Click <b>OK</b> or click <b>Cancel</b> to keep the WGV State.  |

## Using the WGV zoom feature

1. Click on the chromosome number that appears along the bottom row, as shown in Figure 152.



2. The following "zoomed in" WGV window appears. (Figure 153)



- 3. Click **Previous** or **Next** to view adjacent chromosomes in this window.
- 4. To return to the Whole Genome View (Figure 152), click on the chromosome number again or click View→ Show all chromosomes.

## Adding a reference line to the WGV

1. Click on the + (above the panel) to add a reference line to that panel.

**Note:** When a row of data has both Dots and Line data, click the + sign next to the data type to which you would like to add the Reference Line.

| Figure 154 Add a reference line   |                     |
|---|---------------------|
| Note Genome View - Cancer_ONC134BCytoScan_20110511.cychp  | _ 0                 |
| File View   |                     |
| P a Cancer_ONC134BCytoScan_20110511.cychp   | ~                   |
| Veighted Log2 Ratio: -1.5 1.5   | SmoothSignal: 0 4 + |
| 1.5 Adds a reference line<br>1.5 Adds a reference line<br>0.5 - X Add Reference Line X  | -                   |
| 0 -       Weighted Log2 Ratio         -0.5 -       Weighted Log2 Ratio         Choose color and position for a reference line.         Line color       Coordinate:         0.4 |                     |
| -1.5 - + Allele Peaks: -1.8 1.8   |                     |
| 1.5-  | -                   |

- 2. Click on the Line color button to select a color for the Reference Line.
- In the Coordinate text field, enter an approximate coordinate (based on the Y axis) where you want the Reference Line placed.

**Note:** The Reference Line can be dragged and dropped to a different location once placed onto the graph.

- 4. Click OK.
- 5. Repeat steps 2-4 to place additional Reference Lines onto the graph.

**Note:** Additional Reference Lines can also be added by right-clicking in the graph and selecting **Add Reference Line for...** (Figure 155)

- Delete a single Reference Line by right-clicking on the Line and selecting Delete Selected Line. Remove all Reference lines by right-clicking in the graph and selecting Delete All Lines. (Figure 155)
- 7. To change the color or coordinate of a Reference Line, right-click on the selected Reference Line, then select **Edit Selected Line**. (Figure 155)





# Adding a comparison file

Use this feature to view two samples in the same WGV window.

**Note:** Before using this feature, both analysis files MUST BE loaded and available in the ChAS Browser.

- 1. Open the first file in the Whole Genome View, as you normally would.
- 2. Click File  $\rightarrow$  Add Comparison File.

A Comparison File window opens with available analysis results files loaded into ChAS Browser.

3. Click to highlight the file to be viewed with the first file (already open in the WGV), then click **OK**.

The data for the files are loaded in the same window (Figure 156) displaying the following alternating tracks:

- Track 1: Weighted Log 2 and Smooth Signal for File 1
- Track 2: Weighted Log 2 and Smooth Signal for File 2
- Track 3: Allele Difference for File 1
- Track 4: Allele Difference for File 2
- Track 5: B-allele Frequency for File 1
- Track 6: B-allele Frequency for File 2





Note: To remove a comparison file, click File  $\rightarrow$  Remove Comparison File.

# Selecting a new comparison file

- 1. Click on the second of the filenames to open the file selection window
- 2. Highlight a new file from the **Choose a comparison file** window (Figure 157), then click **OK**.

| Figure 157 Selectin           | ng a new comparison file               |               |   |
|-------------------------------|--|---------------|---|
| 🔖 Whole Genome View - Onco    | ScanFFPEAssaySample_01.OSCHP           |               |   |
| File View                     |  |               |   |
| € OncoScanFFPEAssaySample_    | 1.OSCHP                                |               |   |
| € a RenalCellCarcinoma.OSCHP  |  |               |   |
| + Weighted Log2 Ratio: -1.5 1 | 5                                      |               |   |
| 1.5 -                         |  |               |   |
|                               | Choose a comparison file               | ×             | <u>ى بەر مەرىكە مەرىمە مەرىمە مەرمەر مەرمەر</u> |
| -0.5 –                        | Cancer ONC134B CytoScan 20110511.cychp | A Select None | and Based - Looking and Party   |
| -1.5                          |  | Select None   |   |
| 1.5                           | OncoScanFFPEAssaySample_02.OSCHP       |               |   |
| 0.5                           | Q OncoScanFFPEAssaySample_03.OSCHP     | ^             | Los and raises  |
| -0.5                          | OncoScanFFPEAssaySample_04.OSCHP       | ~             |   |
| -1.5 -                        |  | < >           |   |
| + Allele Difference: -1.8 1.8 | OK Cancel                              |               |   |
| 1.5 -                         |  |               | ليزانية أمنانية المتعن الشمال   |
|                               |  |               |   |

The newly selected file and its data is displayed.

#### Exporting a WGV

1. Click File  $\rightarrow$  Export window PNG.



### Selected chromosome view

The Selected Chromosome View (Figure 158) is similar to the Karyoview, but it displays a single selected chromosome at higher magnification. Click, then drag in the Chromosome View to select an area for display in the Detail View.



Use the Stretch Slider and Vertical Scroll Bar (Figure 158) or press the **Alt** key and turn the mouse wheel to zoom in on a section of the **Selected Chromosome View**.

If you have selected a CytoRegions file, the CytoRegions are displayed as gray bands that stretch across the entire chromosome cell (from right to left of the Cytobands).



If you have selected an Overlap Map file, the overlap map items are displayed as small rectangles (Figure 158) to the left of the Cytobands. Its color is the same color used in the Details View.

The Position Indicator is a dashed horizontal blue line. Click in the Selected Chromosome View to set the position of the indicator. The position is highlighted in the graphs table and used as the center point when zooming.

The marker positions are displayed to the right of the Cytobands. When zoomed out, they appear as green ribbons. When zoomed in, the markers and their positions can be seen, as shown in Figure 159.



- SNP markers are displayed in the light green band nearest the cytobands. The SNP marker/probe names in the CytoScan start with the letter 'S'.
- There is one marker track for every distinct array type that is loaded.
- Copy Number markers are displayed in the dark green band nearest the detected segments. The non-polymorphic copy number probe names on the CytoScan start with the letter 'C'.
- You can mouse over a marker to learn more about it.
- Segments selected in any view are highlighted in the Selected Chromosome View.
- For information on the other features of the Selected Chromosome View, see "Selected chromosome view" on page 164.



### **Detail view**

Figure 160 Detail View - Detail Vie 🦉 | 🔈 Θ FGC-15.cyhd.cychp: Copy Number State (segments) Position Indicator hp: Weighted Log2 Ratio FGC Data - -0.5 - -1 FGC-15.cvhd.cvchp: Allele Peaks - 1 - 0.5 - 0 - -0.5 and a manage of the last states of the management of the the d -1.5 (s. 1.5 ) Region Files **Histograms** -istogram: Blood Samples: Los 0 Reference Annotations . .... Chromosome Info Details ------. . . . (see below) 20000kt p13.3 p13.32 p13.1 p12.2 p11.21 12<sup>p1</sup> Chromosome Number Chromosome Info Details Markers CytoScan HD Array dbSnp: 135 NetAffx: 33 Markers Data 20000kb 40000kb 60000kb 80000kb 🖉 q11.2 q14.3 q15 5

The Detail View (Figure 160) enables you to look in detail at the detected segments, marker data, regions, and reference annotations for the loaded files.

#### Viewing CytoScan XON region segments in detail view

CytoScan XON generates both whole genome segments and XON region segments. Whole genome segments capture the larger copy number events while XON region segments capture copy number aberrations at the exon level.

CytoScan XON regions segments and probe level data are categorized into four different levels. **Note:** The four levels do not apply to Whole Genome segmentation for CytoScan XON.

- Level 1: Includes genes with the highest level of evidence: developmental delay, epilepsy, ASD, XLID, Metabolic disorders, hereditary cancer OMIM<sup>™</sup> Morbid genes.
- Level 2: ClinVar genes not covered in Level 1.
- Level 3: Other OMIM genes.
- Level 4: Other Ref Seq, UCSC, Ensembl genes, LOVD.

In the Filters tab, when only Level 1 is selected and Levels 2-4 remain unchecked (as shown in Figure 161 on page 168) then:

XON Region segments that overlap regions of the genome designated as Level 1 is visible in the XON Region Segment Track and in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 1 regions is colored the same color as the color nib of the sample. XON Region segments that overlap regions of the genome designated as Levels 2-4 are not visible on the XON Region segment track or in the Segments Track. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 2-4 regions is colored the same data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 2-4 regions is colored gray.

In the Filters tab, when Levels 1 and 2 are selected and Levels 3 and 4 remain unchecked (as shown in Figure 162 on page 168) then:

XON Region segments that overlap regions of the genome designated as Level 1 or Level 2 will be visible in the XON Region Segment Track and in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, Ballele Frequency) contained within Levels 1 or 2 regions will be colored the same color as the color nib of the sample. XON Region segments that overlap regions of the genome designated as Levels 3 or 4 will not be visible on the XON Region segment track or in the Segments Table. All remaining data (log2 ratio, weighted log2 ratio, smooth signal, allele difference, B-allele Frequency) contained within Level 3 and 4 regions are colored gray.

If selected Level 3, then XON Region Segment calls are displayed on the XON Region Segment track and the marker level data are colored the same color as the color of the color nib of the sample.

When all four Levels are selected in the Filters Tab, all XON Region segment calls for the sample are displayed and all marker level data is colored the same color as the color of the color nib of the sample.







#### Viewing CytoScan XON whole genome segments in the detail view

The CytoScan XON array offers whole genome segmentation for larger duplications and deletions. These segments appear on the Copy Number State Gain and Loss track. The track can be turned on/off using the Copy Number State Gain/Loss check boxes in the Data Types File tree.

Viewing CytoScan XON whole genome segments and xon region segments simultaneously in the Detail View.

Whole genome segments and xon region segments maybe be viewed at the same time in the Detail View by turning on both the Copy Number State and XON Regions tracks. When both tracks are turned on, any xon region segment that overlaps a larger whole genome segment will appear grayed out and will not be displayed in the Segments Table or published to the ChAS Db. Using the whole genome segment to represent the copy number event eliminates redundant segment calls in the Segment Table. To view only the xon region segment calls, turn off the Copy Number State Gain/Loss track.

XON merging can still be used on the XON region segments but is no longer necessary due to the more robust whole genome segmentation algorithm. Any merged XON regions that overlap the whole genome segments in the Copy Number State track will appear grayed out and will not appear in the Segment Table. To enable grayed out XON region segments, turn off the Copy Number Gain/Loss Segment Track.

XON regions that overlap a larger Whole Genome Copy Number segment that is below the filter thresholds will be enabled and will appear in the Segment table.

Grayed out segments will not appear in the Segments Table as the loss is already represented by the larger loss in the Copy Number State Track, as shown in Figure 163.





## Annotation color codes

In the Detail View, DGV annotations are color-coded to indicate association with gain or loss.



- Purple Gain and loss are associated with the region
- Red Only loss is associated with the region
- Blue Only gain is associated with the region
- Gray Copy number variation is associated with the region, but information regarding the number of times gains or losses were observed is not present in the annotation record in the DGV database

## Annotation OMIM color codes

In Browser annotation files version NA32.3 and higher, the following OMIM colored gene entries were generated by genome.ucsc.edu and are based on the associated OMIM phenotype map key. For more information on OMIM display conventions, go to: www.genome.ucsc.edu

- Lighter Green for phenotype map key 1 OMIM records the disorder has been placed on the map based on its association with a gene, but the underlying defect is not known.
- Light Green for phenotype map key 2 OMIM records the disorder has been placed on the map by linkage; no mutation has been found.
- Dark Green for phenotype map key 3 OMIM records the molecular basis for the disorder is known; a mutation has been found in the gene.
- Purple for phenotype map key 4 OMIM records a contiguous gene deletion or duplication syndrome; multiple genes are deleted or duplicated causing the phenotype.
- Light Gray for Others no associated OMIM phenotype map key info available.

#### Changing an annotation color

1. Right-click an annotation type in the Files window pane and select **Set Custom Color** on the shortcut menu, as shown in Figure 164.



| Figure 164    | Open the color palette   |   |
|---------------|--|---|
| Figure 164    | Open the color palette   | Set Custom Color X  |
|               | Search in selected file Ctrl+F<br>View and Edit annotations in this file<br>CytoRegions for Targeted XON analysis<br>Include in CytoRegions<br>Set File as Overlap Map<br>View/Edit Properties<br>Set Custom Color<br>Clear Custom Color | OK       Cancel       Reset         Current color of Genes annotations in the Detail View |
| 🔲 🎩 Proteir 🔚 | Open case in Franklin  |   |

2. Specify a color for the selected annotation type using the color controls in the color palette (Figure 164), then click **OK**.

The new color is applied to the annotations in the Details View.

3. To return to the default annotation color, right-click the annotation in the Files windowpane, and select **Clear Custom Color** on the shortcut menu.

#### Data in the detail view

The Detail View displays the following types of data for CytoScan (CYCHP):

| Data Types                                 | Definition   |  |  |
|--|--|--|--|
|  | Detected Segment Types   |  |  |
| GainMosaic                                 | Non-integer amplifications or duplications   |  |  |
| LossMosaic                                 | Non-integer hemizygous or homozygous deletions   |  |  |
| Gain                                       | Amplifications or duplications   |  |  |
| Loss                                       | Hemizygous or homozygous deletions   |  |  |
| LOH  | Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple)<br><b>Note:</b> In Dark Scheme (page 191), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple. |  |  |
| Probe array data (displayed as graph data) |  |  |  |
| Copy Number<br>State                       | HMM-derived integer Copy Number State  |  |  |



| Data Types             | Definition   |
|------------------------|--|
| Log2 Ratio             | Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.  |
| Weighted Log2<br>Ratio | Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM.   |
| LOH                    | Loss of Heterozygosity   |
| Allele Difference      | Filtered and smoothed values for individual markers. Nonparametric estimation is used to understand possible regional peak structure towards which the data is smoothed. The amount of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information. |
| Genotype Calls         | SNP genotype calls (single sample, BRLMM-P-plus algorithm)   |
| Smooth Signal          | Gaussian Smoothed Calibrated Copy Number Estimate  |
| B-allele Frequency     | Number of B alleles/number of A+B alleles used to show allelic imbalances.   |

#### Table 14 Data for CytoScan array

The Detail View displays the following types of data for a CytoScan HTCMA array:

| Table 15 | Data for C | ytoScan | HTCMA | array |
|----------|------------|---------|-------|-------|
|----------|------------|---------|-------|-------|

| Data Types                                 | Definition   |  |
|--|--|--|
|  | Detected Segment Types   |  |
| Gain                                       | Amplifications or duplications   |  |
| Loss                                       | Hemizygous or homozygous deletions   |  |
| LOH  | Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple)<br><b>Note:</b> In Dark Scheme (page 191), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.   |  |
| Probe array data (displayed as graph data) |  |  |
| Copy Number State                          | HMM-derived integer Copy Number State  |  |
| Log2 Ratio                                 | Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.  |  |
| LOH  | Loss of Heterozygosity   |  |
| Allele Difference                          | Filtered and smoothed values for individual markers. Nonparametric estimation is used to<br>understand possible regional peak structure towards which the data is smoothed. The amount<br>of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is<br>computed based on differencing A signal and B signal, then standardizing based on reference<br>file information. |  |
| Genotype Calls                             | SNP genotype calls (single sample, BRLMM-P-plus algorithm)   |  |
| Smooth Signal                              | Gaussian Smoothed Calibrated Copy Number Estimate  |  |
| B-allele Frequency                         | Number of B alleles/number of A+B alleles used to show allelic imbalances.   |  |
| Variants                                   | Location and detection of variants.  |  |

**Note:** There is a subset of ~55,000 SNP probes which are used for allelic information analysis but which are not used for Copy Number analysis (on the CytoScan HD Array).

For these SNP probes, LOH and Allele Peaks data will be displayed, but these SNP probes will not have Log2 Ratio, Weighted Log2 Ratio, SmoothSignal, or Copy Number State data displayed, nor will they be used for ascertainment of Mosaicism.

The calculation of Segment data for all the various Segment types takes this into account. All non-polymorphic (copy number) and the vast majority of SNP probes are NOT affected by this change, and will continue to display all graphs and their data points from the CytoScan HD Array CYCHP files.

| Data Types             | Definition   |
|------------------------|--|
|                        | Detected Segment Types   |
| XON Region Gain        | Amplifications in XON regions.   |
| XON Region Loss        | Hemizygous or homozygous deletions in XON regions.   |
| Gain                   | Amplifications or duplications.  |
| Loss                   | Hemizygous or homozygous deletions.  |
| LOH                    | Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple)<br><b>Note:</b> In Dark Scheme (page 191), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple.   |
|                        | Probe array data (displayed as graph data)   |
| Log2 Ratio             | Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.  |
| Weighted Log2<br>Ratio | Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM.   |
| LOH                    | Loss of Heterozygosity   |
| Allele Difference      | Filtered and smoothed values for individual markers. Nonparametric estimation is used to understand possible regional peak structure towards which the data is smoothed. The amount of filtration and smoothing is dynamically adapted based on sample quality. Allele difference is computed based on differencing A signal and B signal, then standardizing based on reference file information. |
| Genotype Calls         | SNP genotype calls (single sample, BRLMM-P-plus algorithm)   |
| Smooth Signal          | Gaussian Smoothed Calibrated Copy Number Estimate  |
| B-allele Frequency     | Number of B alleles/number of A+B alleles used to show allelic imbalances.   |

 Table 16
 Data for CytoScan XON Array

The Detail View displays the following kind of data for Genome-Wide SNP Array 6.0 Array data (CNCHP):

| Data Types             | Definition                         |  |
|------------------------|------------------------------------|--|
| Detected Segment Types |                                    |  |
| Gain                   | Amplifications or duplication      |  |
| Loss                   | Hemizygous or homozygous deletions |  |

| Data Types                                 | Definition   |  |
|--|--|--|
| LOH  | Loss of Heterozygosity (CN <2 LOH = light purple, CN 2 (or higher) LOH = dark purple)<br><b>Note:</b> In Dark Scheme (page 191), CN <2 LOH = dark purple, CN 2 (or higher) LOH = light purple. |  |
| Probe array data (displayed as graph data) |  |  |
| Copy Number State                          | HMM-derived integer Copy Number State  |  |
| Log2 Ratio                                 | Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.  |  |
| LOH  | Loss of Heterozygosity   |  |
| Allele Difference                          | Difference of A signal and B signal, each standardized with respect to their median values in the reference.   |  |
| Smooth Signal                              | Smoothed Calibrated Copy Number Estimate   |  |

#### Table 17 Data for Genome-Wide SNP Array 6.0 (CNCHP)

#### Table 18 Data for OncoScan Array (OSCHP)

| Data Types                                 | Definition   |  |
|--|--|--|
|  | Detected Segment Types   |  |
| Gain                                       | Amplifications or duplication  |  |
| Loss                                       | Hemizygous or homozygous deletions   |  |
| LOH  | Loss of Heterozygosity   |  |
| Probe array data (displayed as graph data) |  |  |
| Copy Number State                          | TuScan derived Copy Number State.  |  |
| Log2 Ratio                                 | Per marker Log2 Ratio of normalized intensity with respect to a reference, with further correction for sample specific variation.              |  |
| Weighted Log2 Ratio                        | Contains the Log2 Ratios processed through a Bayes wavelet shrinkage estimator. These processed values are input to the CNState algorithm HMM. |  |
| LOH  | Loss of Heterozygosity.  |  |
| Allele Difference                          | Difference of A signal and B signal, each standardized with respect to their median values in the reference.                                   |  |
| Smooth Signal                              | Smoothed Calibrated Copy Number Estimate.  |  |
| Variants                                   | Location and detection of Somatic Mutation. (OncoScan FFPE Assay only)   |  |
| B-allele Frequency                         | Number of B alleles/ number of A+B alleles, used to show allelic imbalances.   |  |

#### Table 19 ReproSeq Aneuploidy data

| Data Types             | Definition                         |  |
|------------------------|------------------------------------|--|
| Detected Segment Types |                                    |  |
| Gain                   | Amplifications                     |  |
| Loss                   | Hemizygous or Homozygous deletions |  |

#### Table 19 ReproSeq Aneuploidy data

| Data Types        | Definition                              |  |
|-------------------|---|--|
| Graph Data        |   |  |
| Copy Number State | The copy number state of sequence tiles |  |

See "Changing graph appearance" on page 192 for more information about controlling the display of graph data.

In addition, the Detail View displays:

- Regions: Features in the various region files loaded into ChAS, including CytoRegions and Overlap Map items.
- Annotations: Indicate the known or suspected locations of features, such as mRNAs, exons, structural variants, and so forth.

You can expand or contract the annotations. See "Expanding and contracting annotations" on page 191.

- Database Display
  - Default Histograms: displays all segments in the database.
  - Filtered Histograms: displays all segments meeting the filter criteria set by the user. For information on how to create a filtered histogram, see "Adding filtered histogram data" on page 137.
- Chromosome info, with:
  - Coordinate scale
  - Marker position information
  - Chromosome number
  - Cytoband information

Selected segments are displayed with enlarged icons; selected regions or annotations are outlined and highlighted. (Figure 165)

| Figure 165 Selected Segment                             |  |
|---|--|
| Beta10_F_01_Cyto_VH.cychp: Copy Number State (segments) |  |
|   |  |
|   |  |
|   |  |



#### Navigation controls in detail view



| Control                         | Function  |
|---------------------------------|---|
| Chromosome<br>Coordinates scale | Shows the position along the genome.  |
| Zoom Slider                     | Controls the horizontal zoom and the area of the chromosome displayed.  |
| 3x Zoom In                      | Press Ctrl + Plus to view up to three preset Zoom In settings.  |
| 3x Zoom Out                     | Press Ctrl + Minus to view up to three preset Zoom Out settings.  |
| Zoom in Using<br>Click and Drag | Place your mouse cursor over a point of interest. Press Shift while holding down the left mouse button. Drag the mouse cursor to frame/zoom in on your point of interest.                                 |
| Stretch Slider                  | Controls the vertical stretch of the Display area.  |
| Scroll bars                     | Used to select the area displayed after zooming or stretching the vertical or horizontal scale.   |
| Position Indicator              | Dashed vertical blue line. Click in the view to set the position of the indicator<br>The position that is highlighted in the graphs table.<br>The position that is used as the center point when zooming. |

Use Stretch Slider and Vertical Scroll Bar to zoom in on a section of the Detail. You can also use the mouse wheel as shown below:

- Alt + mouse wheel stretches the display
- Ctrl + mouse wheel zooms in on the horizontal scale
- Mouse wheel scrolls up and down



#### Obtaining summary metrics for a zoomed in region

You can obtain summary metrics from the data tracks displayed in the Detail View once you have zoomed in to a region of interest.

- 1. In the Detail View, zoom in to a region of interest using any of the techniques described above.
- 2. Hover the mouse over a data point on the data track for which you would like summary metrics. For example, log 2 ratio, weighted log 2 ratio, Allele Difference, etc.

A pop-up appears and displays the following metrics for markers currently displayed in the Detail View:

- Region Size
- Number of Markers
- Min Marker Value
- Max Marker Value
- Mean Value
- Standard Deviation
- Median Value

#### Selecting a chromosome section for display

Data from the same sample files is displayed in all 3 views, at different scales.

You can select a particular chromosome, or a section of the chromosome, for detailed study using:

- "Karyoview and selected chromosome view"
- "Coordinate range box" on page 178
- "Zooming to a selected item" on page 179
- "Navigation controls in detail view" on page 176

You can also double-click on an item in a table to zoom to the region of the chromosome where that item is located.

Karyoview and selected chromosome view

#### Selecting a chromosome for detailed examination

1. Click a chromosome in the Karyoview.

The chromosome is displayed in the Selected Chromosome View and the Detail view.

#### Examining a section of the chromosome

1. Click and drag on the section in the Karyoview or the Selected Chromosome View.

The selected section is displayed in the Detail View. (Figure 167)



# Coordinate range box

The Coordinate Range box (Figure 168) is located in the ChAS main tool bar. It shows the selected chromosome and the start and stop positions displayed in the Detail View. You can enter coordinates in the box to update the Detail View.



#### Going to a specific coordinate or coordinate range

- Enter the desired location in any one of these formats then press the <Enter> key:
  - "chromosome number: start end": sets the view to the given start and end coordinates on the given chromosome.
  - "start: end" or "start end": sets the view to the given start and end coordinates of the current chromosome.

#### Returning to a previous location

To return to previous genomic coordinates, click on the < > buttons above the chromosome number field (far right), as shown in Figure 169.



#### Zooming to a selected item

There are several ways to zoom in on a feature.

- Click a segment in the Segment table, CytoRegions table, or Overlap Map. Clicking on items auto-zooms to your configured zoom buffer (15% of item size is default), To edit the zoom buffer, see "Zoom buffer" on page 182.
- Double-click a feature in the Details View, Karyoview or Selected Chromosome View.
- Press Ctrl+Space to zoom all the way in to the selected item's start and end coordinates, with no buffer.
- In the Karyoview, Selected Chromosome View, or Details View, you can use the Zoom to selection option in the feature right-click menu (Figure 170) to go to the start and stop coordinates of the selected feature.



#### Setting a vertical highlight

Use this feature to set a vertical highlight on a selected segment(s) or annotation(s) to view its breakpoints across other data types and/or annotations. This highlighting option can be useful when aligning a segment/annotation of interest with other samples or annotations.

Right-click on a selected segment(s) or annotation(s), then click Set highlight region from selection to apply a vertical yellow highlight through the Detail View, as shown in Figure 171.



### Edit configurations in the misc tab

The Misc tab contains:

- Autosave
- Coordinate Box Format
- Zoom Buffer
- Chromosome Sorting Order
- CHP File Colors
- Remapping Segment patterns
- Microarray Nomenclature configuration

## Accessing the Misc tab

1. Click **Preferences**  $\rightarrow$  **Edit User Configurations** or click P on the upper tool bar.

The User Configuration window appears.

2. Click the Misc tab. (Figure 172)
| igure 172 User Configuration window - Misc tab window  | ,   |
|--|---|
| Jser Configuration   | ×   |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query  | Filtered DB Query Exports   |
| Files  | Remapped Segment Patterns<br>With no added/removed markers:<br>Diagonal Pattern<br>With added/removed markers:<br>Diamond Pattern<br>Pestore Default  |
| ISCN order: (X, Y, 1-22)      Zoom Buffer      When zooming to a segment, optionally add a buffer on both the left and right sides.  | Microarray Nomenclature<br>Coordinates:   1000000   1,000,000<br>Genome Names:  GRCh37/GRCh38  hg19/hg38<br>Bance Separator:  100,200  100-200  |
| <ul> <li>No buffer</li> <li>Number of bases: 10000</li> <li>Percent of segment length</li> <li> <sup>1</sup> <sup>1</sup></li></ul> | Mosaic Separator:  (a) x2-3 ((x2-3)) x2-3 ( |
| CHP File Colors  |   |
| OK   | Jancei  |

#### **Autosave**

Click to check the Autosave check box to automatically save your files as they are edited. Uncheck to disable auto-save. (Figure 173).



# Coordinated box format

 Click the appropriate radio button to choose the format of your displayed data. (Figure 174)



## Chromosome sorting order

 Click the appropriate radio button to sort the segments in the Segment Table. (Figure 175)



#### Zoom buffer

By default, the zoom percentage is set to 15% in new user profiles.

The Zoom Buffer feature offers 3 settings:

- **No Buffer**: Click this radio button to turn off the Zoom Buffer feature.
- Number of bases: Click this radio button, then manually enter the number of bases you want.
- Percent of segment length: Click, then drag the slider bar (Figure 176) to the zoom percentage you want.

| Figure 176 Zoom Buffer   |
|--|
| Coom Buffer<br>When zooming to a segment, optionally add a buffer on both the left and right sides.<br>O No buffer<br>Number of bases: 10000 |
| Percent of segment length     10     50     100     150  |

#### **CHP** file colors



There are five preset CHP file colors assigned to your CHP data (Figure 177), but each default color, can be changed to a different color.

#### Changing a CHP file color

1. Click on the colored icon you want to change.

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| Figure 178 Pick a Color window |
|--------------------------------|
| Pick a Color X                 |
|                                |
|                                |
|                                |
| OK Cancel Reset                |

- Use the color wheel to locate the specific color you want or click on one of the several coloring options 
   Image: Im
- 3. Click OK.
- 4. Repeat steps 1-3 to change additional default colors.

At anytime, click **Reset to Default** to return the 5 CHP file colors back to their default colors. (Figure 179)

| Figure 179 CHP File | Colors            |
|---------------------|-------------------|
| CHP File Colors     | Reset to defaults |

To return a single CHP file color back to its default color, click on the CHP file color, then click the Color Wheel's **Reset** button.

5. Click the User Configuration window's **OK** button to save your changes and exit.

**Note:** Samples that are currently loaded while a color change is made, may not reflect your new color scheme. To remedy this, close, then re-open ChAS to ensure your new color choices are reflected throughout all your samples.



### Remapped segment patterns

Choose how segments (remapped from an hg19 ChAS db to an hg38 ChAS db) are represented. See "Additional segment intersection information" on page 392 for information on default patterns.

- 1. Click the **With no added/removed markers:** drop-down to select a pattern to represent segments in an hg38 ChAS db that were mapped from an hg19 ChAS DB, in which all the markers in the original segment mapped to the new genome version.
- Click the With added/removed markers: drop-down to select a pattern to represent segments in an hg38 ChAS db that were mapped from an hg19 ChAS DB, in which one or more markers in the original segment no longer map to the new genome version.

Microarray nomenclature configuration Define how the ISCN Microarray nomenclature will be represented in the segments table and exports. Any format from ISCN 2013, 2016 and 2020 can be configured

- Coordinates: choose the radio button to display genomic coordinates with or without commas.
- Genome names: choose the radio button to display genome versions using either GRCh or hg.
- Range separator: choose the radio button to display either a dash (-) or an underscore (\_) between genomic positions.
- Mosaic separator: choose the radio button to display either a dash (-) or a tilde (~) between copy number values on mosaic segments.
- (Optional) Select the check box to automatically have information in the Inheritance field appended to the Microarray nomenclature field.

### Controlling the display of data

ChAS (Figure 180) provides controls for:

- "Selecting data for display" on page 186
- "Selecting data types for display" on page 188
- "Changing the grouping of samples and data types" on page 189
- "Selecting display schemes" on page 191
- "Expanding and contracting annotations" on page 191
- "Changing graph appearance" on page 192

Later chapters explain other options for filtering data, how to specify certain regions for extra attention or ignoring, and how to create Region files with region information and annotations.



8



### Selecting data for display

The Files list (Figure 181) enables you to select sample data, region files, histograms, and reference annotations for display in the graphic view.

| Figure 181 Files list  |  |
|--|--|
| Files<br>♥ Î₽♥♂ Sample01.cyhd.cychp<br>♥ Î₽♥♂ Sample02.cyhd.cychp<br>♥ Ĩ₽♥♂ GeneList bed<br>♥ Ĩ₽↓ Regions.bed<br>♥ Ŷ DGV   | Sample Data<br>AED and BED annotation and region files |
| GV-GS Gain     GV-GS Loss  | Indicates the file is selected as an Overlap Map file  |
|  | Reference Annotations                                  |
| SOMIM ® Region Phenotype Loci     Reprotein Coding Ensembl Genes     Frotein Coding Genes     Recurrent/Curated Regions     Segmental Duplications     Triplosensitivity | Histogram Data from the ChAS Database                  |
| <ul> <li>□ ii sno/miRNA</li> <li>✓ Markers</li> <li>✓ ∞∞ Cytobands</li> </ul>  | Cytobands  |

The Files list displays the files grouped by:

#### Sample Data

- Colored nibs of display the color used for the data lanes for that sample in the Karyoview, Selected Chromosome View, and Detail View.
- The appropriate gender symbol gi is displayed to the right of the colored nib. GenomeWide SNP 6.0 CNCHP file data may also contain a "?" if the gender was determined to be "unknown".
- If a loaded file has a QC parameter that is out of range, an alert symbol appears next to the file name.
- Region Data Files

Icons indicate the file type (Hard AED or Hard BED) and whether the loaded files have been selected as a CytoRegions file Hard or Overlap Map file —. VCF files are also loaded here.

- Histograms: Displays Gains, Loss, and LOH segments stored in ChAS DB. The display is loaded in the Detail View upon software start up or by selecting ChAS DB → Load Histograms (when connected to the ChAS DB).
- Reference Annotations: Loaded during software installation and startup. Only displayed in Detail View.
- Cytobands: Separated from other reference annotations because they cannot be moved in the displays, and because they are also displayed in the Karyoview and Selected Chromosome View.

#### Selecting and deselecting files for display

1. Click in the check box next to the file name.

The order in the Files list determines the order of display of the lanes in the Karyoview, Chromosome view, and Detail View.

#### Changing the order of the Sample lanes or reference annotations

1. In the Files list, click a file name and drag it to a new position.

#### Viewing data properties

1. Right-click a file and select View/Edit Properties on the shortcut menu.

The **Properties** window appears.

(Optional) Use the Filter "Property Name" search text field (Figure 182) to quickly locate a property of interest.

| Figure 182 View data properties - Filter "Property Name" field |                |  |      |               |                           |                        |                                       |
|--|----------------|--|------|---------------|---------------------------|------------------------|---------------------------------------|
| appliedbiosystems  |                |  |      |               | File Properties           |                        |                                       |
| <u>File View Exports An</u>                                    | alys           | sis <u>C</u> hAS DB <u>P</u> references <u>H</u> elp |      |               | Basic Sample Properties   | Extended               |                                       |
| 📔 🕅 🗟 💔 😒  | 1.1            | T   Y 📧 🎟 🚰   💽 🗚   🎭 🧣                              |      | _             | Property Name             | Туре                   | Value                                 |
| Files  |                |  |      |               | Algorithm Name            | Text                   | CYTO2                                 |
| Tiles  |                |  |      |               | Algorithm Version         | Text                   | 2.2.0                                 |
| 🔲 🗐 🖓 🗗 CytoScan_HTC   | CM/            | _96F_P01_GGA_GGA-96_GT5_H12.rhchp                    |      | ſ             | All Probeset RLE Mean     | Decimal Number         | 0.17560375                            |
|  |                |  | _    |               | Annotation File           | Text                   | C:\Affymetrix\ChAS\Library\CytoScanHD |
| ♥ ∎ ♥ o 11-1400JL_A4   |                | Close  | - 1  |               | Antigenomic Ratio         | Decimal Number         | 0.122673035                           |
| □ ■ Go Father A0012  |                | -  | -1   |               | aptOptTextOutput (CN Ref  | Text                   | false                                 |
|  |                | Save   | - 1  |               | aptStateLog2ratioTextOutp | Text                   | false                                 |
| XON Region Levels  | <b>B</b>       | Export File as AED                                   | - 1  |               | aptStateReferenceTextOut  | Text                   | false                                 |
|  | HED            | Export file as ALD                                   | - 1  |               | Autonomo LOH              | Decimal Number         | 0.021010429                           |
|  | <b>A</b> A     | Search in selected file Ctrl+F                       | 1    |               | Barcode                   | Text                   | @52082500746312063012414100331232     |
| 🔲 📘 🔽 Default Histogra   |                |  | - 1  |               | Call Rate                 | Decimal Number         | 0.9958195                             |
|  |                | view and Edit annotations in this file               |      |               | CFL File                  | Text                   | 11-1466.JL A4 RC 8-4-11 CMA-172.CFI   |
| Ensembl Genes  |                | Discard Changes                                      |      |               | CHP File Date             | Text                   | Tue Nov 15 07:42:39 2016              |
| 🗹 🚎 Genes  | <u> </u>       | Biocard Ondrigeo                                     | -1   |               | CHP UUID                  | UUID                   | 00000065-4c12-4fdc-36fc-005554003d93  |
|  |                | View Reference Model Parameters                      | - 1  |               | Created                   | DateTime               | Nov 15, 2016 7:42:36 AM               |
| 📃 🚦 OMIM 🖲 Genes   |                |  | - 1  |               | dbSNP Version             | Text                   | 147                                   |
|  | (A)            | View Process Pipeline                                |      |               | Frequency Heterozygous C  | Decimal Number         | 0.26100597                            |
|  | 1              | View/Edit Properties                                 |      | l             | Frequency Homozygous Call | Decimal Number         | 0.738994                              |
| 🔲 🚟 Segmental Duplica  |                | View Edit i Topernes.                                | 4    |               | Gender Call Y-gender      | Text                   | male                                  |
|  |                | Set Custom Color                                     |      |               | Genome LOH                | Decimal Number         | 0.08076882                            |
| XON_DGV  |                | View and edit (if editable) propertie                | es c | of the select | Genome Version            | Text                   | hg38                                  |
|  |                | Clear Custorn  |      |               | Low Diploid Flag          | Text                   | Unknown                               |
| ^×   |                | View In Multi-Sample Viewer                          | - 1  |               | Manufacturer              | Text<br>Decised Number | Anymetrix                             |
| Named Setting  |                | tiett in mail oumple tietter                         | - 1  |               |                           | Liacimal allimbar      |                                       |
|  |                | Publish File(s) to Database                          | -1   | 1             | Filter "Property Name"    |                        |                                       |
| <u> </u>   | W <sub>c</sub> | Show WCV for colocted file(c)                        | ÷    |               |                           |                        | OK Cancel                             |
| Data Types   | v              | SHOW WOVIDI SElected IIIe(S)                         | H    |               |                           |                        |                                       |
| Copy Number State  |                | Clear highlight region                               |      | C             | • • • • •                 |                        |                                       |
| copy rumber ofaic  | -              |  | _    |               | -2                        |                        |                                       |
|  |                |  |      |               |                           |                        |                                       |

#### Closing a file

- 1. Right-click on the file you want to close.
- 2. Select Close from the menu. (Figure 183)

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The file is removed from the Files list and the data is no longer displayed.

# Selecting data types for display

The Data Types list (Figure 184) shows the data types that can be displayed in ChAS.



Figure 184 displays a list of data that can be displayed in the Karyoview, Selected Chromosome View, Detail View, and tables. The available data types may vary, depending upon the type of sample data available.

It enables you to select from Segments data and CN/LOH discrete graph data.

The Segments data is displayed in:

- Karyoview
- Selected Chromosome View
- Detail View

If segment parameter filters have been applied to a segment type, a funnel symbol  $\gamma$  appears next to the segment type name, as shown in Figure 185.

| <b>Figure 185</b> The funnel graphic denotes filter have been applied. | ′S |
|--|----|
| Data Types<br>Copy Number State  |    |

The Graph data is displayed only in the Detail View.

#### Selecting and deselecting data types for display

Click in the check box next to the Data Type name.

#### Changing the order of the data types:

In the Data Types list, click a file name and drag it to a new position.

**Note:** The selections made here can be saved with a Named Setting (see "Named settings" on page 434).

#### Turning the symbols used for segments on or off:

From the View menu, select or de-select Segment Symbols.

Changing the A grouping of S samples and data E types

A unique color is assigned to each sample and used for the lanes in the Karyoview, Selected Chromosome View, and Detail View.

Each segment type is assigned to its own lane and has its own symbol.

#### Changing the grouping

1. From the View menu, select Group by Sample or Group by Type or in the tool bar, click the Group by Sample in or Group by Type in button.

This enables you to perform different types of comparisons between samples and segment types.

#### Lanes grouped by sample

When the lanes are grouped by sample, the different segment types for each sample are kept together in the Karyoview and Selected Chromosome View (Figure 186) and in the Detail View.



When the lanes are grouped by data type, the lanes for different samples are kept together for each segment or graph type in the Karyoview and Selected Chromosome View and in the Detail View.

**Note:** You can change the order of samples and Data types in the views by clicking and dragging in the Files and Data Types list.

## Selecting display schemes

You can switch to a dark background.

1. From the View menu, select Switch Color Scheme.

The color scheme changes, as shown in Figure 187.



# Expanding and contracting annotations

For tracks containing multiple rows of annotations, collapsing tracks consolidates all rows within a track into two rows. Any annotations after the first one will be placed in the second row (Figure 188).

When there are multiple annotations of one type at the same coordinate, the separate annotations will be shown on separate rows.

Collapsing tracks is useful if you don't need to see all the details. However, be aware that in collapsed tracks larger annotations may obscure smaller ones; annotations with introns may be obscured by annotations that don't show the intron.

To expand or collapse just a single annotation track, right-click on the track name in the Files tree and choose Expand (or Collapse) Annotation track. The Annotation track check box must be checked in order to see this option in the right-click menu.

**Note:** The maximum number of tracks that can be displayed for any reference annotation is 25.



#### Toggling between collapsed and expanded display of annotations:

From the View menu, select Expand/Collapse Annotations

OR

Click the **Expand/Collapse** button on the main tool bar.

### Changing graph appearance

You can modify many properties of the graphs in the Detail View. ChAS provides options for:

- "Selecting different graph styles" on page 193
- "Changing graph attributes" on page 197
- "Changing scale" on page 198

Settings and adjustments that are specific for graphs can be made using the **Graph Settings** window.

#### **Opening the Graph Settings window**

1. From the **View** Menu, select **Graph Settings** or click on the **Graph Settings** button in the Graphs Tab tool bar.

The Graph Settings window opens. (Figure 189)

| Figure 189 Graph Sett   | ings window                 |   |
|-------------------------|-----------------------------|---|
|                         | ×                           | : |
| Types                   |                             | ٦ |
| Mallele Difference      |                             |   |
| 🔟 🖾 Allele Peaks        |                             |   |
| 🗹 🖾 BAF                 |                             |   |
| 🔟 🖾 Copy Number State   |                             |   |
| 🔟 🖾 Log2 Ratio          |                             |   |
| 🗹 🖾 LOH                 |                             |   |
| 🗹 🖾 SmoothSignal        |                             |   |
| 📓 🖾 Weighted Log2 Ratio |                             |   |
| <u>  </u>               | Reset to Default            |   |
| Style                   | Graph Attributes            |   |
| 🔘 Bar 🛛 🔘 Line          | 🗹 Values 🔲 Zero Line 🔛 Grid |   |
| Points                  | Range                       | - |
| Big Dots Stairstep      | Min: -1.8                   |   |
| Auto-Size Dots          | Max: 1.8                    |   |
| Threshold (bases)       | Automatic Dynamic Range     |   |
| 10,000,000              | Always Include Zero         |   |
| O Heat Map              | - Height                    |   |
| ×                       | noight                      | 1 |
|                         |                             |   |

The Types box displays the graph data types being displayed in the Detail View.

#### Changing the settings for a graph type

- 1. Click on a data type to change the settings for that type.
- 2. Make changes to the graph settings by typing in new values or by operating sliders in the Graph Adjuster panel. For details, see:
- "Selecting different graph styles" on page 193
- "Changing graph attributes" on page 197
- "Changing scale" on page 198

**Note:** Any changes you make to the values in the Graph Settings window will apply to all currently selected graph types.

#### Selecting different graph styles

Graphs can be shown in various representational styles. The type of graph that is most appropriate depends on the type of question being asked about the data. For example, when comparing trends and patterns, it is very useful to use the line graph display method. The user is encouraged to experiment with the different display types to find out which method works best for specific purposes and at specific zoom in magnifications.

#### Changing the graph style

1. In the Style section choose one of the options. (Figure 190)

| Figure 190 Graph Sett  | ngs window       |
|--|------------------|
| 🐁 Graph Settings   | x                |
| Types<br>Types<br>Allele Difference<br>Allele Peaks<br>Allele Peaks<br>Allele Peaks<br>Allele Peaks<br>Copy Number State<br>Copy Number State | Reset to Default |
| Style<br>Bar Line<br>Points Min/Max/Avg<br>Big Dots Stairstep<br>Auto-Size Dots<br>Threshold (bases)<br>10,000,000<br>Heat Map   | Graph Attributes |

**Note:** Line, Min/Max/Avg, and Stairstep are not available for CytoScan XON arrays due to the differential coloring based on Level assignment.

 Bar – Individual values are shown as vertical bars that are one base wide for position graphs. (Figure 191)



 Line – Subsequent values are linked with a line. Even if the input file was not sorted, the values will be connected in order along the genomic coordinate axis. (Figure 192)



Points – Shows a single dot for each data value. (Figure 193)



Big dots – Shows a single big dot for each data value. (Figure 194)



Min/Max/Avg – This style is especially useful for showing very densely populated graphs with data points for large numbers of positions. (Figure 195) Note: This data style is not available for CytoScan XON arrays due to regions annotated by Levels.



When Detail View is zoomed all the way in, the display is equivalent to the Line style. When zooming out, ChAS starts to summarize values. When the scale of the display reaches the point where individual x-values are associated with multiple score values, ChAS picks the maximum and minimum values and draws a vertical bar between them. In addition, ChAS draws lines through the average of all the data points represented at each x value.

Stairstep – Similar to the bar graph style, except that bar widths along the horizontal axis are stair-stepped. (Figure 196) Note: This data style is not available for CytoScan XON arrays due to regions annotated by Levels.



For example, if position 100 has a value of 50 and position 200 has a value of 75 and there are no values in between, then ChAS will draw a bar of height 50 that starts at position 100 and stops at position 200. Then, at position 200, ChAS will draw a new bar of height 75 that terminates at the next location with a value.

 Auto-Size Dots - Transition from Points to Big Dots when zooming in the Detail View. You can select the window size (in base pairs) in which the transition occurs. (Figure 197)





Heat map – Instead of showing relative intensity via the height of the line at each pixel or coordinate as in most other graph styles, a heat map shows expression levels via color or brightness of the line at each pixel or coordinate (Figure 198). This graph style is useful if you want areas of unusual values to jump out at you. If a graph does not render or is hard to see, adjust the visible bounds of the graph until features are readily visible. Several heat map color schemes are available to choose from.



There are now two Red/Gray/Blue heat maps.

One is designed to look good for copy number data scaled from 0 to 4 (or 1 to 3), with 2 = normal, and the other is designed to look good for copy number data scaled from 0 to 5 with 2 = normal.

The user must be careful to test that the heat map scaling is appropriate for the data.

#### Changing graph attributes

You can display graphs with:

- Value scale
- Zero Line
- Grid

#### Changing graph attributes

Select the attributes you want to display in the Detail View. (Figure 199)

| Figure 199 Graph Setti   | ngs window   |
|--|--|
| Sraph Settings   | x  |
| Types<br>Types<br>Allele Difference<br>Allele Peaks<br>E BAF<br>Copy Number State<br>E Log2 Ratio<br>E LOH<br>E LOH<br>E SmoothSignal<br>E Weighted Log2 Ratio |  |
| Style<br>Bar Line<br>Points Min/Max/Avg<br>Big Dots Stairstep<br>Auto-Size Dots<br>Threshold (bases)<br>10,000,000<br>Heat Map                                 | Reset to Default     Graph Attributes   Values   Zero Line   Grid   Range   Min:   1.8   Max:   1.8   Automatic Dynamic Range   Always Include Zero   Height |

#### **Changing scale**

Changing the visible bounds involves changing the scale of the graph by setting the maximum and minimum values to be displayed.

To set these visible bounds, use the **Range** section of the **Graph Properties** dialog.

| Rang  | je —               |              |  |  |  |
|-------|--------------------|--------------|--|--|--|
| Min:  | -1.5               | 0            |  |  |  |
| Max:  | 1.5                |              |  |  |  |
| 🗹 A   | utomatic D         | ynamic Range |  |  |  |
| A 🕑 🖌 | Mways Include Zero |              |  |  |  |

#### Setting specific minimum and maximum values

Use the sliders, or type in values to the boxes.

These values will be applied to each selected graph. You are free to set maximum and minimum values that cover a range smaller or larger than the actual range of your data.

- Click the Automatic Dynamic Range check box to auto-set the Y-axis min/ max range.
- Click the Always include Zero check box to include a 0 point of reference on the Y axis.

**Note:** The algorithm detects CN state up to 4 for Genome-Wide Human SNP Array 6.0 CNCHP files and CytoScan Array CYCHP files. The TuScan algorithm detects CN State > 50 for OncoScan FFPE Assay OSCHP files. In the Graphs Settings, the Copy Number State range can be set to what is appropriate to the data.

#### Changing the vertical height of a graph

Use the Height Slider to stretch all the graph type in the vertical direction.

| -Height — |  |
|-----------|--|
| -         |  |
|           |  |
|           |  |

The graph height slider is used to increase or decrease the size of a given graph type. The size is specified in a relative manner. The final graph size will depend on the number of other graphs and annotations being displayed.

### Learning more about features

You can use the following tools to learn more about features in the different views:

- "Pop-ups"
- Right-click menu options" on page 202
- "Selection details table" on page 204
- "Linking to external websites" on page 209

#### Pop-ups

You can mouse over a feature in any of the views to display a popup box with information on the feature. The information provided depends on the type of data that the mouse arrow is on.

Pop-ups are available for:

- Cytobands
- Detected segments
- Graph data
- Marker position indicators
- Histograms
- Reference annotations

**Note:** You should expand the reference annotations before selecting one to avoid selecting multiple annotations. See "Expanding and contracting annotations" on page 191.

- Displayed Region files, including
  - Overlap Map Regions
  - CytoRegions

The information displayed differs depending upon the type of feature selected, as

shown in the samples below.

For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 475).

You can learn more about the terms used in the pop ups in "Selection details table" on page 204.

#### Turning pop-ups on or off

1. From the View menu, select Mouse-over Pop-ups.

The information (Figure 200, Figure 201, Figure 203, and Figure 204) can include custom properties created by a user (see "Viewing and editing annotations" on page 293 for more information).

| Figure 200 Pop-up for CN State Graph |                      |  |  |  |  |
|--------------------------------------|----------------------|--|--|--|--|
| Sample_01.cyt                        | Sample_01.cyhd.cychp |  |  |  |  |
| Array Type Cyte                      | oScanHD_Array        |  |  |  |  |
| Copy Number                          | State                |  |  |  |  |
| Туре                                 | Copy Number State    |  |  |  |  |
| NetAffx Version                      | 33                   |  |  |  |  |
| Array Type                           | CytoScan HD Array    |  |  |  |  |
| Nearest Coord                        | 16,501,954           |  |  |  |  |
| Nearest Value                        | 2                    |  |  |  |  |

| Figure 201 Pop-up for a Gain Segment |                             |  |  |  |  |  |
|--------------------------------------|-----------------------------|--|--|--|--|--|
| Sample_01.cyhd                       | Sample_01.cyhd.cychp        |  |  |  |  |  |
| Array Type CytoS                     | canHD_Array                 |  |  |  |  |  |
| Gain 11: 20,519,                     | 743 - 21,166,430            |  |  |  |  |  |
| Туре                                 | Type Gain                   |  |  |  |  |  |
| Location                             | 11: 20,519,743 - 21,166,430 |  |  |  |  |  |
| Size (kbp)                           | 647                         |  |  |  |  |  |
| CN State 3.00                        |                             |  |  |  |  |  |
| Marker Count 958                     |                             |  |  |  |  |  |
| Median Log2Ratio                     | 0.207                       |  |  |  |  |  |

| Figure 202Pop-up for a XON Region GainSegment  |                              |  |  |  |  |
|--|------------------------------|--|--|--|--|
| CytoScanXON_Sampl                              | e.xnchp                      |  |  |  |  |
| Array Type CytoScan X                          | ON                           |  |  |  |  |
| Gain (XON Region) 1: 196,715,959 - 196,717,057 |                              |  |  |  |  |
| Туре   | Gain (XON Region)            |  |  |  |  |
| Location                                       | 1: 196,715,959 - 196,717,057 |  |  |  |  |
| Size (kbp)                                     | 1                            |  |  |  |  |
| XON Region Level                               | 1                            |  |  |  |  |
| Marker Count                                   | 17                           |  |  |  |  |
| Median Log2Ratio                               | 0.147                        |  |  |  |  |
| Summarized Log2Ratio                           | 0.404                        |  |  |  |  |

| Figure 203 Pop-up for a Gene |                             |  |  |  |
|------------------------------|-----------------------------|--|--|--|
| AP4E1                        | •                           |  |  |  |
| Туре                         | Protein Coding Genes        |  |  |  |
| Location                     | 15: 51,200,880 - 51,298,092 |  |  |  |
| Size (kbp)                   | 97                          |  |  |  |
| Accession Number             | NM_007347                   |  |  |  |
| CDS Min                      | 51,200,975                  |  |  |  |
| CDS Max                      | 51,294,859                  |  |  |  |
| %HI                          | 43.26                       |  |  |  |
| pLl                          | 0.00                        |  |  |  |
| CI                           | Not Available               |  |  |  |
| pHaplo                       | 0.459                       |  |  |  |
| pTriplo                      | 0.266                       |  |  |  |
| Exon/Intron                  | Intron 3/20 →               |  |  |  |



# Right-click menu options

You can right-click on any of the following types of features to open a menu with options for learning more about the feature:

- Detected segments (Figure 205)
- Histograms (Figure 206)
- Reference annotations, including cytobands (Figure 207)

**Note:** You should expand the reference annotations before selecting one to avoid selecting multiple annotations. See "Expanding and contracting annotations" on page 191.

- Displayed Region files, including (Figure 208):
  - Overlap Regions
  - CytoRegions

**Note:** Not all options are available for the different feature types. Also, In the Detail View you can select multiple items of different types. The available options will differ, depending upon the number and types you have selected.

Some menu options are common to the different types of features:

- Number of items selected (if more than one item is selected, the options available may differ, depending upon the type and number of items).
- **Zoom to selection:** See "Zooming to a selected item" on page 179.
- Selection Details: Opens the Selection Details box with information about the feature. See "Selection details table" on page 204.
- Add to a file: Add the selected segment, annotation, or region to a region file. See "Adding regions to a new or existing AED file" on page 288.
- View/Edit Annotation Properties: Displays the Annotations Properties window for the selected feature. You may or may not be able to edit the properties. See "Viewing and editing annotations" on page 293.
- Query ChAS DB: Displays the segments in the database that match the user defined Overlap and Coverage threshold settings. See "Setting up a ChAS DB query" on page 385.

| Fig          | gure 205 Segment right-click menu |            |
|--------------|-----------------------------------|------------|
|              | One Item Selected                 |            |
| Ð            | Zoom to selection                 | Ctrl+Space |
|              | Selection Details                 | Ctrl+D     |
| $\mathbf{A}$ | Add to a File                     |            |
|              | View/Edit Annotation Properties   |            |
|              | Query ChAS DB                     |            |



Special options for annotations include:

Link to remote web site for more information. (Figure 207)

| Fig | ure 207 Annotation right-click menu                         |            |
|-----|---|------------|
|     | One Item Selected   |            |
| Ð   | Zoom to selection   | Ctrl+Space |
| Ħ   | Selection Details   | Ctrl+D     |
| 2   | Link to www.ncbi.nlm.nih.gov for Accession Number NM_001835 |            |
| 2   | Link to www.ncbi.nlm.nih.gov for Gene Name CLTCL1           |            |
| ☆   | Add to a File   |            |
| ø   | View/Edit Annotation Properties                             |            |
|     | Query ChAS DB   |            |

Custom functions for regions include (Figure 208):

- New User Annotation: Opens the New User Annotation window, which enables you to add user annotation to a region. For more information, see "New user annotations" on page 300.
- Increment Counter: Increments the counter in the annotation properties, allowing you to track the number of times a feature has been seen. For more information about the annotation properties, see "Viewing and editing annotations" on page 293.

| Fi            | gure 208 Region r        | ight-click menu |
|---------------|--------------------------|-----------------|
|               | One Item Selected        |                 |
| Ð             | Zoom to selection        | Ctrl+Space      |
|               | Selection Details        | Ctrl+D          |
|               | New User Annotation      |                 |
| UNIC .        | Link to omim.org for OM  | IM ID 609242    |
| $\Rightarrow$ | Add to a File            |                 |
| ÷             | Increment Counter (0 ->  | 1)              |
| *             | Delete Annotation        | _               |
| M.            | View/Edit Annotation Pro | operties        |
|               | Query ChAS DB            |                 |

# Selection details table

The Selection Details table (Figure 209) displays information available for items selected in the graphic display views (Karyoview, Chromosome View, and Detail View). It is accessed by right-clicking on an item in one of the views and selecting **Selection Details**.

The information is presented in two tables:

- Upper table shows one item per row Provides summation feature and PDF or tabseparated text file export capabilities.
- Lower table shows one item per column Provides ability to export the table to a tab-separated text file.

To reorder the columns in the upper table, drag a column header left or right. The corresponding row in the lower table is automatically moved to the new location in the table.

| Figure 209 Example: Selection Details table |              |                   |           |            |    |      |         |       |             |                                 |           |             |    |
|---|--------------|-------------------|-----------|------------|----|------|---------|-------|-------------|---------------------------------|-----------|-------------|----|
| I Selection Details X                       |              |                   |           |            |    |      |         |       |             |                                 |           |             |    |
| E Deper table: One item per row             |              |                   |           |            |    |      | 232 res | sults |             |                                 |           |             |    |
| Label                                       | Chromosome   | Min N             | lax       | Size (kbp) | Ту | pe   | Gain    | Loss  | Frequency   | Method                          |           | Total Gain/ | Lc |
| Variation 71249                             | 11           | 4,364,906 4       | ,367,233  | 2          | V  | DGV  | 1       | 0     | NA          | NimbleGen custom 42M oli        | go arr    |             | ^  |
| Variation 112920                            | 11           | 4,509,001 4       | ,562,286  | 53         | V  | DGV  | 0       | 1     |             | Custom Agilent 24M oligon       | ucleoti   |             |    |
| Variation 38231                             | 11           | 4,510,137 4       | ,562,394  | 52         | V  | DGV  | 0       | 2     | 0.004761905 | Affymetrix SNP 6.0 Array        |           |             |    |
| Variation 9172                              | 11           | 4,510,284 4       | ,562,395  | 52         | V  | DGV  |         | 2     |             | Affymetrix 500K SNP Mappi       | ng Arr    |             |    |
| Variation 2899                              | 11           | 4,510,285 4       | ,562,459  | 52         | V  | DGV  | 0       | 2     |             | Affymetrix 500K EA SNP Mapping  |           |             |    |
| Variation 9173                              | 11           | 4,535,321 4       | ,578,715  | 43         | V  | DGV  |         | 1     |             | Affymetrix 500K SNP Mapping Arr |           |             |    |
| Variation 31801                             | 11           | 4,542,530 4       | ,559,145  | 17         | V  | DGV  | 0       | 1     |             | Agilent Custom CGH Arrays       | 6         |             | ^  |
| Variation 65845                             | 11           | 4,542,816 4       | ,544,039  | 1          | V  | DGV  | 0       | 31    | 0.139325843 | Agilent custom oligo CGH a      | rray      |             | ~  |
| <   |              |                   |           |            |    |      |         | _     | 1           |                                 |           | < >         | •  |
|   |              |                   |           |            |    | •••• | •       |       |             |                                 |           |             |    |
|   |              |                   | _         |            |    |      |         |       | Lower ta    | ole: One item per col           | umn       |             |    |
| Click an arrow to h                         | ide or show  | <mark>,</mark> 20 | Variation | n_38231    |    | 1    | Variati | on_91 | 172         | Variation_2899                  | Variation | 1_9173      |    |
| one of the tables                           |              | <u>920</u>        | Variatio  | on 38231   |    | 1    | Varia   | tion  | <u>9172</u> | Variation 2899                  | Variatio  | on 9173     | ^  |
| one of the tables.                          | rour display | 11                |           |            |    | 11   |         |       | 11          | 11                              |           |             |    |
| choice is retained t                        | he next time | 4,509,001         |           | 4,5        | 10 | ,137 |         |       | 4,510,284   | 4,510,285                       |           | 4,5         | 3  |
| ChAS is started.                            |              | 4,562,286         | 6         | 4,5        | 62 | 394  |         |       | 4,562,395   | 4,562,459                       |           | 4,5         | 7  |
|   | 2            | 53                |           |            |    | 52   |         |       | 52          | 52                              |           |             |    |
|   |              |                   | -         |            |    | _    | -       |       |             |                                 |           |             |    |

#### The Selection Details table may include the following columns:

| Column     | Description  |  |  |  |  |  |
|------------|--|--|--|--|--|--|
|            | Common   |  |  |  |  |  |
| Label      | Identifier for the item.   |  |  |  |  |  |
| Chromosome | hromosome on which the item is located.  |  |  |  |  |  |
| Min        | Zero-based index position of the first base pair in the sequence.  |  |  |  |  |  |
| Мах        | Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that the length of any (hypothetical) segment containing a single marker would be one, and ensures that the coordinates match the coordinate system used in BED files. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 475). |  |  |  |  |  |
| Size (kbp) | Size of the item.  |  |  |  |  |  |
| Туре       | Type of segment (Gain, Loss, GainMosaic, LossMosaic LOH) or annotation.  |  |  |  |  |  |

8

| 1            | $\sim$ | $\sim$ | ч. |  |
|--------------|--------|--------|----|--|
| $\mathbf{T}$ | ٢l     | ×      | T  |  |
| $\sim$       | K      | 48     | )) |  |
|              | 57     | à      | 1  |  |
| $\sim$       | 4      | 5      | 2) |  |
|              |        | 83     | 1  |  |
|              |        |        |    |  |

| Column   | Description  |  |  |  |  |
|--|--|--|--|--|--|
|  | Segments   |  |  |  |  |
| File   | File the segment was detected in.  |  |  |  |  |
| CN State                                       | Copy Number State (not displayed for LOH segment types).<br>The expected Copy Number State on the X chromosome in normal males is not constant over<br>its entire length. This is due to the structure of the sex chromosomes. See "LOH segments on X<br>and Y chromosomes" on page 49 for more information. |  |  |  |  |
| Mean Marker Distance                           | ength of the segment in base pairs divided by the number of markers in the segment.  |  |  |  |  |
| Interpretation                                 | User-editable field for free-text interpretation on the segment  |  |  |  |  |
| Call   | User-editable field populated by a user-configurable drop list of Calls.   |  |  |  |  |
| Inheritance                                    | User-editable field populated by a user-configurable drop list of Inheritance.   |  |  |  |  |
| Curation By                                    | The current computer Operating System login ID and ChAS user profile name at the time that the Call or Interpretation field was last edited.   |  |  |  |  |
| Curation Time                                  | The time and date when the Call or Interpretation field was last edited.   |  |  |  |  |
| Materially Modified<br>Segment                 | Indication that segment was previously merged, deleted, or had its start or end boundary, type, or state altered by a ChAS user. (ChAS-based processes of Smoothing and Joining are not "Modifications", nor are making Calls or Interpretations, in this context).  |  |  |  |  |
| Materially Modified By                         | The current computer Operating System login ID and ChAS user profile name at the time that the segment was last materially modified.   |  |  |  |  |
| Materially Modified Time                       | The time and date when the Segment was last materially modified.   |  |  |  |  |
| Max % Overlap                                  | The highest percentage by which some item(s) in the Overlap Map overlaps the segment.<br>Segments completely overlapped by an Overlap Map item are 100% overlapped. This number<br>is used for Filtering Segments out by "Overlap".  |  |  |  |  |
| Overlap Map Items (% of<br>Segment overlapped) | Item(s) in the Overlap Map which overlap the segment, followed by the percentage by which the segment is overlapped by that Item.  |  |  |  |  |
| CytoRegions                                    | Names of the CytoRegions with which the segment shares coordinates.  |  |  |  |  |
| Use in Report                                  | Allows manual selection of Segments for export to a Segments Table PDF, DOCX, or Text rather than all segments in the table.   |  |  |  |  |
| Marker Count                                   | Number of markers in the segment.  |  |  |  |  |
| Cytoband Start                                 | Cytoband in which the segment begins.  |  |  |  |  |
| Cytoband End                                   | Cytoband in which the segment ends.  |  |  |  |  |
| Genes  | List of RefSeq genes from the Genes track that share coordinates with the segment. Identic named gene isoforms are NOT repeated.   |  |  |  |  |
| Gene Count                                     | A count of the gene names listed in the Genes column   |  |  |  |  |
| DGV  | List of DGV variations that share coordinates with the segment.  |  |  |  |  |
| sno/miRNA                                      | List of sno/miRNA features that share coordinates with the segment.  |  |  |  |  |
| OMIM Genes                                     | List of OMIM Genes that share coordinates with the segment.  |  |  |  |  |
| Disorder Causing OMIM<br>Genes                 | List of OMIM Genes designated with a Phenotype Map Key of 3  |  |  |  |  |

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| Column                                       | Description   |
|--|---|
| Disorder Causing OMIM<br>Genes Count         | A count of the Disorder Causing OMIM Gene names listed in the Disorder Causing OMIM Genes Count.  |
| OMIM Gene Count                              | A count of the OMIM Gene names listed in the OMIM Genes column.   |
| OMIM Phenotype Loci                          | List of OMIM Phenotype Loci that share coordinates with the segment.  |
| OMIM Region<br>Phenotype Loci                | List of OMIM Phenotype associated with a genomic region. The OMIM Morbidity information is displayed when using all three OMIM tracks. (OMIM Genes, OMIM Phenotype Loci and OMIM Region Phenotype Loci) |
| Segmental Duplications                       | List of Segmental Duplications that share coordinates with the segment.   |
| Smoothed/Joined                              | Indication that segment was created by smoothing or joining two or more segments in the initial segment detection.  |
| Segment Label                                | A label comprised of the segment's Type, State, and Filename.   |
| Segment Name/ID                              | File-specific identifier assigned to the detected segment.  |
| Start Marker                                 | The array marker name which marks the beginning of the segment.   |
| End Marker                                   | The array marker name which marks the end of the segment.   |
| Preceding Marker                             | The array marker just above the segment in the data track used as input for the segment. Note: This column is only applicable to CNState Gain and Loss segments.  |
| Preceding Marker<br>Location                 | The coordinate location of the array marker just above the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments.          |
| Following Marker                             | The array marker just below the segment in the data track used as input for the segment. <b>Note:</b> This column is only applicable to CNState Gain and Loss segments.                                 |
| Following Marker<br>Location                 | The coordinate location of the array marker just below the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments.          |
| Mean Log2 Ratio                              | The mean of all the Log2 Ratio values contained in the segment.   |
| Mean Weighted Log2<br>Ratio                  | The mean of all the Weighted Log2 Ratio values contained in the segment.  |
| Microarray<br>Nomenclature                   | An ISCN-based description of the segment.   |
| Sample UUID                                  | Unique identifier for the CHP file.   |
| Max % Coverage                               | The highest percentage by which a segment covers some item(s) in the Overlap Map.   |
| Number of Overlap Map<br>Items               | Number of Overlap Map items which share genomic coordinates with the segment.   |
| % of Overlaps Map Item<br>covered by Segment | Overlap Map Item and the percentage by which it is covered by the segment.  |
| Full Location                                | Chromosome Start and Stop in a user-friendly format for use in external databases.  |
| Median log2                                  | The median of all the Log2 Ratio values contained in the segment.   |
| DB Count Both                                | Number of segments in the database meeting both the user defined thresholds of minimum Percent Overlap Count and Coverage Count.  |

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| Column  | Description  |  |  |  |
|---|--|--|--|--|
| DB Coverage Count                               | Number of segments in the database meeting the minimum Percent Coverage Count.   |  |  |  |
| DB Overlap Count                                | Number of segments in the database meeting the minimum Percent Overlap Count.  |  |  |  |
| XON Region Level                                | The annotation Level assigned to this region of the genome.  |  |  |  |
| Summarized Log 2 Ratio                          | The median of the LR, after transformation to adjust for individual marker responsiveness.   |  |  |  |
|   | Genes  |  |  |  |
| Chromosome                                      | Chromosome on which the item is located.   |  |  |  |
| Min   | Zero-based index position of the first base pair in the sequence.  |  |  |  |
| Мах   | Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that the length of any (hypothetical) segment containing a single marker would be one, and ensures that the coordinates match the coordinate system used in BED files. For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 475). |  |  |  |
| Size (kbp)                                      | Size of the item.  |  |  |  |
| Туре  | Type of segment (Gain, Loss, GainMosaic, LossMosaic LOH) or annotation.  |  |  |  |
| Accession Number                                | Unique identifier assigned to the sequence in GenBank.   |  |  |  |
| CDS Min   | Minimum position of the coding sequence (BED-style coordinates).   |  |  |  |
| CDS Max   | Maximum position of the coding sequence (BED-style coordinates).   |  |  |  |
| Strand         The sequence strand of the item. |  |  |  |  |
| %Hi   | Dosage sensitivity indicator derived from DECIPHER (https://decipher.sanger.ac.uk/). The lower the percentage, the more likely the gene is to be dosage sensitive.   |  |  |  |
| pLl   | Probability of loss intolerance. Genes with higher numbers are more likely to be dosage sensitive. Derived from gnomAD (https://gnomad.broadinstitute.org/).   |  |  |  |
| CI  | Dosage sensitivity indicator derived from gnomAD<br>(https://gnomad.broadinstitute.org/) for hg38 only. Not yet implemented by gnomAD.   |  |  |  |
| рНарІо  | Probability of Haploinsufficiency): The higher the value (0-1), the more likely to be dosage sensitive. Collins et al. A cross-disorder dosage sensitivity map of the human genome. 2022. PMID: 35917817   |  |  |  |
| pTriplo   | Probability of Triplosensitivity): The higher the value (0-1), the more likely to be dosage sensitive.<br>Collins et al. A cross-disorder dosage sensitivity map of the human genome. 2022. PMID: 35917817   |  |  |  |
| Ensembl Genes                                   |  |  |  |  |
| CDS Min   | Minimum position of the coding sequence (BED-style coordinates).   |  |  |  |
| CDS Max   | Maximum position of the coding sequence (BED-style coordinates).   |  |  |  |
| Strand  | The sequence strand of the item.   |  |  |  |

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| Column                              | Imn Description  |  |  |  |  |
|-------------------------------------|--|--|--|--|--|
|                                     | ОМІМ   |  |  |  |  |
| OMIM Gene Title                     | The title of the gene associated with the OMIM entry.  |  |  |  |  |
| OMIM Gene Symbol List               | A list of genes associated with the OMIM entry.  |  |  |  |  |
| OMIM Disorder                       | Disorder associated with the OMIM entry.   |  |  |  |  |
| OMIM Phenotype Key                  | Indicates how this phenotype was placed on the map.  |  |  |  |  |
| OMIM Gene Symbol                    | Symbol of the gene based on gene title.  |  |  |  |  |
|                                     | OMIM Phenotype Loci  |  |  |  |  |
| OMIM Phenotype ID                   | Unique identifier to an OMIM phenotype.  |  |  |  |  |
| OMIM Phenotype Map<br>Key           | Indicates how this phenotype was placed on the map.  |  |  |  |  |
| OMIM Phenotype Locus<br>Description | Describes the phenotype or disorder associated at the OMIM Phenotype Loci.   |  |  |  |  |
|                                     | OMIM Region Phenotype Loci   |  |  |  |  |
| OMIM ID                             | Unique identifier to an OMIM phenotype.  |  |  |  |  |
| Cytoband                            | Location of the region.  |  |  |  |  |
| Min                                 | Start location of Genome.  |  |  |  |  |
| Мах                                 | End location of Genome.  |  |  |  |  |
| Gene Symbol                         | Symbol of the gene (based on gene title).  |  |  |  |  |
| Gene Title                          | The title of the region associated with the OMIM entry.  |  |  |  |  |
| Comment                             | Additional information for the entry from OMIM.  |  |  |  |  |
| Gene-Phenotype Loci<br>Data         | Information of Gene-Phenotype relationships for the region.  |  |  |  |  |
| Sentimental Duplications            |  |  |  |  |  |
| Score                               | Score based on the raw BLAST alignment score. The score for segmental duplications is set to zero in NetAffx annotation 31 and higher. |  |  |  |  |
| FracMatch                           | The fraction of matching bases.  |  |  |  |  |
| FracMatchIndel                      | The fraction of matching bases with Indels.  |  |  |  |  |
| Strand                              | The sequence strand of the item.   |  |  |  |  |

**Note:** Thermo Fisher Scientific does not generate or verify the information for genes, FISH clones, Segmental Duplications, sno/miRNAs, DGV annotations, or OMIM data. Segmental Duplication and sno/miRNA annotations do not have any unique terms; but sno/miRNA annotations use the "type" field to indicate subtypes like "cdBOX" and "HAcaBOX".

Some information may not be displayed, depending upon the feature type. The information can include custom properties created by a user (see "Viewing and editing annotations" on page 293).

You can export data from the table using the standard table export tools (see "Exporting table data" on page 417).

You can perform multi-column sorts. See "Sorting by columns" on page 324.

### Linking to external websites

You can view a selected area within the Detail View at one of the following public sites:



1. In the Detail View, zoom and scroll to the area of interest. (Figure 210)

#### Viewing a selected area at a public site



2. From the View menu, select **View Region at [site name]** or click the appropriate site's tool bar button.

A browser opens, displaying the selected area of the chromosome.



### Linking to TaqMan copy number and genotyping assays

Viewing and ordering TaqMan assays for CN

TaqMan assays can easily be accessed from within ChAS. These assays can be used for confirmation of copy number aberrations. TaqMan assays can only be ordered based on hg38 genome coordinates.

Do the following for the region(s) you would like to view and order TaqMan assays for Copy Number:

- 1. Locate the region containing the aberration in the Detail View.
- 2. Select the TaqMan shortcut in the tool bar (Figure 211) to view which TaqMan assays are available within the genomic coordinates populated in the text box at the top center of the browser.

The TaqMan assays load in a track in the Detail view.

**Note:** This track is only available for the genomic coordinates for the current query. To view TaqMan assays in another region of the genome, repeat steps 1 and 2.



3. Right-click on the TaqMan assays you want to order, then click **Order TaqMan assay** to link out to the website, as shown in Figure 212.



Viewing and Ordering TaqMan assays for genotyping TaqMan assays for genotyping can be ordered from VCF files that contain dbSNP IDs.

**Note:** The VCF file must contain an rsID for the SNP to directly access the TaqMan website for that SNP. Also, TaqMan assays can only be ordered based on hg38 genome coordinates.

- 1. Load a VCF file clicking **File**  $\rightarrow$  **Open**.
- 2. Right-click on an SNP for which you would like to view and order a TaqMan assay.

A menu appears. (Figure 213)



3. Click on the provided "Link to" link.

You will be directed to the TaqMan website for details about the assay.

| Figure 214 Assay details  | example                  |                                 |                               |  |                                   |  |
|---|--------------------------|---------------------------------|-------------------------------|--|-----------------------------------|--|
| Your search for "rs8017" for Human returned <b>1</b> TaqMan <sup>®</sup> SNP Genotyping Assay |                          |                                 |                               |  | Support documents                 |  |
| Home > TaqMan® SNP Genotyping As  | says › Search Tool › All | Results > Search                | Results                       |  |                                   | Change Size & Dye for All V  |
| Change Your Search  | 🖶 🖾 Email                | ▲ Export                        |                               |  |                                   |  |
| Your Results  | Assay ID                 | 5_10                            |                               |  |                                   | 🇯 View Assay on Map  |
| Species<br>[Hs] Human<br>Gene<br>TCEB2<br>Assay Type ?  | SNP ID<br>rs8017         | Gene Loca<br>TCEB2 Chr.<br>on C | tion<br>16: 2771572<br>SRCh38 | SNP Type<br>Transition<br>Substitution,<br>Intron,<br>Mis-sense<br>Mutation,<br>UTR 3,<br>Intragenic | Assay Type<br>Functionally Tested | Availability Catalog #<br>Made to Order 4351379<br>S: 300 rxns ▼<br>Price (USD): 259.00<br>Check your price ><br>Add To Cart |
| Functionally Tested   | O View Details           | ✓ Allele Freq                   | uency 🔻                       |  |                                   |  |
| SNP Type Intragenic Intron Mis-sense Mutation   |                          |                                 |                               |  |                                   |  |



# **Filtering segments**

ChAS enables you to filter the detected segments using different segment parameters, concealing segments that do not meet requirements for significance for:

- Marker Count
- Length
- XON Level Assignment (XNCHP only)

You can apply these filters to different segment types, using different parameters for each type. The filtering is done on the fly, with changes to the parameters reflected in the different views as they are made.

A segment must pass all filter requirements for the segment type to be displayed.

You can apply different filter values for areas inside CytoRegions and areas outside the CytoRegions (genomewide). See "Using filters with CytoRegions" on page 271.

The Overlap Map filter is described in "Using the overlap map and filter" on page 275.

Filter settings are saved when a Named Setting is created and can be reapplied. See "User profiles and named settings" on page 433.

**IMPORTANT!** The Filters set in the browser are NOT linked to the filters for the MSV. The same filter settings should be set in both the ChAS browser and the MSV separately. The MSV does have a flag to indicate when filter settings do not match. For more information, see the RHAS User Guide.

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### Applying segment parameter filters

#### Opening the Segment Filters window

- 1. Select  $\textbf{View} \rightarrow \textbf{Segment Filters}$  on the menu bar
- 2. Click the **Segment Filters** | Y tool bar button
- 3. Right-click on a segment type in the Data Types list, then select **Filters...** from the right-click menu. (Figure 215)



**Note:** If you use the right-click menu option, only the filter settings for the selected segment type are displayed.

The Segments Filters window opens. (Figure 216)

| Figure 216 Segment Filt                 | ers window  |
|---|---|
| Genome and CytoRegions                  | s tabs are displayed only when a cytoregions file<br>is selected. |
|   | Segments Filters X  |
|   | XON Region Levels (Gain and Loss)                                 |
|   | V Level 1 V Level 2 Level 3 Level 4                               |
|   | Gain (XON Region)   |
|   | Marker Count  |
|   | Size (kbp) 0 20000  |
|   | Overlap 100 0   |
|   | + Loss (XON Region)   |
|   | Marker Count 0 5000   |
|   | Size (kbp)  |
| Different                               | Overlap 100 0   |
| Types                                   | X LOH   |
| .,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, |   |
|   | ✓ Size (kbp) 3000 0 20000   |
|   | Overlap 100 0   |
|   | LOH with Copy Number Loss   |

**Note:** The Overlap Map filtering parameter is set using the same window. The Overlap Map function is described in "Using the overlap map and filter" on page 275.

For XON, check the Level check box(es) (Figure 217) to reveal any XON region segment calls in regions assigned to the Level(s) you selected.

|   | Figure 217 | Level sel | ections     |         |   |
|---|------------|-----------|-------------|---------|---|
| Ш |            |           | 100         |         | U |
|   | XON Regior | Levels (G | ain and Los | s)      |   |
|   | 🗹 Level 1  | 🗹 Level 2 | Level 3     | Level 4 |   |
|   |            |           |             |         |   |

| Segment Filter<br>Option            | Function   |
|-------------------------------------|--|
| Hide All Segments<br>in this Region | Hides all the segments. This is particularly useful when using a CytoRegions file for CytoScan XON arrays. Check the Hide All Segments in this Region check box on the Genome Tab so that ONLY segments overlapping CytoRegions will be shown. <b>Note:</b> This option is only available with a CytoRegions file is assigned.   |
| Hide LOH where<br>median CN < 2     | Selecting this option will hide LOH segments that are assigned a median copy number less than 2. LOH with median copy number of 2 or higher will still be displayed.   |
| Marker Count                        | The number of markers the segment encompasses from start to finish. A segment must have at least as many markers as you specify to be displayed. Each marker represents a probe which represents a sequence along the genome at a particular spot. Markers are probe sequences of DNA, each sized from 12-50 base pairs long, depending on the type of array data. The 12-50 bp sequence is unique to that one spot on the genome it represents.   |
| Size                                | Based on the start and end markers of a segment. Because each segment represents a single place in the genome, you can measure from start to end, in DNA base pairs, and by filtering, demand a segment be at least that long to be visualized.  |
| XON Segment<br>Level                | <ul> <li>Based on the Level Assignment to the region in the genome.</li> <li>Level 1: Medical Research exome and cancer</li> <li>Level 2: ClinVar genes not covered in Level 1</li> <li>Level 3: Other OMIM genes</li> <li>Level 4: Opportunistic regions from Refseq/UCSC/Enseble/LOVD.</li> </ul> The XON Segment Filters can be used to narrow down the number of XON segments to review based on their annotation level assignment. Those regions assigned as Level 1 contain genes/regions that have been identified as part of the Medical Research Exome along with regions associated with cancer. By selecting only Level 1 in the filter settings, only XON segment calls in regions assigned as Level 1 will be displayed. XON segments for all other Levels will be hidden from view as well as hidden in the Segments Table. To expose XON segment calls in other regions simply check the box for those Levels in the Filters Windows. For more details on CytoScan XON analysis workflow recommendations, Appendix G, "Recommended CytoScan XON array workflows" on page 498. |
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# Using segment parameter filters

1. Click the check box next to the parameters you want to use as filters. (Figure 218)

| Figure 218 Gain Segment filter settings  |  |  |  |  |  |  |  |
|--|--|--|--|--|--|--|--|
| Segments Filters X   |  |  |  |  |  |  |  |
| ▲ Gain<br>Marker Count 50<br>5000<br>5000  |  |  |  |  |  |  |  |
| Size (kbp)       400         0       20000         Overlap       100         100       0 |  |  |  |  |  |  |  |

 Use the slider to set the value for the parameter or enter a value in the provided text field. (Figure 218) Note: As you move the slider from left to right, more segments are removed.

Your filtered results are displayed instantly in all tables and graphs, as shown in Figure 219.



For information about using the Overlap setting, see "Using the overlap map and filter" on page 275. For information on using different filtering settings in CytoRegions, see "Using filters with CytoRegions" on page 271.

## **Bypassing segment filters**

A specific segment can be assigned to ignore the current filter settings. The segment will still displays even though its marker or size thresholds are not met. To use this feature:

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click on the segment you want to bypass the segments filters with.

A menu appears.

2. Click View/Edit Annotation Properties.

The Annotation Properties window appears. (Figure 220)

| Figure 220 Annotation Properties wir                        | ldow            |                   |
|---|-----------------|-------------------|
| Annotation Properties                                       |                 |                   |
| General Structure Additional Curation                       |                 |                   |
| Call  | Inheritance     | Oncomine Reporter |
| <b>~</b>  | ✓               |                   |
| Call Approval   | Use In Export   | Ignore Filters    |
| Median Copy Number 3 ISCN Copy Number Te                    | ext             |                   |
| Microarray Nomenclature: arr[GRCh37] 17q12(348224           | 466_36404138)x3 |                   |
| Segment Interpretation (Right-click to add preconfigured sn | ippets)         |                   |
|   |                 |                   |
|   |                 |                   |

- 3. Click the **Curation** tab.
- 4. Click the **Ignore Filters** check box.
- 5. Click **OK**.

The segment you wanted to bypass your filter settings with is now displayed.



# **Segment modification**

# Editing segment data overview

- ChAS enables you to edit data segments; merge, delete, draw *de novo*, adjust boundaries, and change the Copy Number State of CHP data segments, or undo your changes.
- The instant a file is modified by editing of one or more segments, having a segment interpreted, or called, ChAS auto-generates a "sidecar" or CHP Change Archive (CHPCAR) file which saves these modifications. ChAS then uses this CHPCAR file for further user-defined edits and modifications, while your original (native) xxCHP file (CYCHP, CNCHP, or OSCHP) is safeguarded (remains un-touched).
- The smoothing, joining and XON merging settings for each CHP file are locked at the time of CHPCAR file creation, while unmodified CHP files without CHPCAR files will still respond to array type-specific changes in smoothing and joining settings.
- In order for the changes stored in the CHPCAR file to show up in the CHP file data displayed in the ChAS Browser, the CHP file and CHPCAR file must be stored in the same directory/folder (the xxCHP file is still the only file that is required to be "loaded" into the browser, the CHPCAR file's edits, calls and interpretations will load when the CHP file is loaded).
- CHPCAR files are named using the entire CHP file's name, and contain the extension: \*.chpcar
- Changing the name of either file and not the other to match will disrupt the ability for the files to be recognized as associated with each other in the ChAS Browser.
- CHP files which have associated CHPCAR files detected (and in use), display as a special "CHP" icon in the File → Open dialog window, and in the Files tree of the Browser.
- Please move the CHP and CHPCAR files together when moving or archiving data.
- Two people using CHAS on different systems should NOT attempt to edit a CHP file at the same time.
- Modification made to segments in the ChAS browser will also be updated in the MSV.
- Files downloaded from ChAS DB can only use the delete segment option for editing segments.

Note: Segment modification does not apply to ReproSeq Aneuploidy data.



## Using edit mode

**IMPORTANT!** Make sure the Edit Mode feature is turned on BEFORE you start editing segments. Segments can not be published to ChAS DB while in Edit Mode. Edit Mode must be turned off to enable the Publish function.

Edit Mode is accessible in three places:

1. The Browser's icon row (top).



2. The Detail View's icon row (top right)



3. Click View  $\rightarrow$  Edit Mode

By default, Edit Mode is OFF. Click *(located on the Browser's top icon row or above the Detail View)* to turn Edit Mode ON.

- Click 1 to turn Edit Mode OFF and remove all visual indications of your segment changes.
  - When Edit Mode is **ON**, deleted segments are visible, and edited segments appear distinct from non-edited segments.
  - When Edit Mode is ON, a track on a dotted axis line will appear showing the original calls made by the software for comparison with the manual modifications on the segment track.
  - When Edit Mode is OFF, deleted segments are invisible, and edited segments look identical to non-edited segments.

**IMPORTANT!** Turn Edit Mode OFF, before exporting a report of your data. Also, Edit Mode must be OFF, before publishing to the database.

| Figure 221 Edit Mode ON/OFF Deleted Segment example                             |
|---|
| Edit Mode ON  |
| 11-0810_LC_ONC13B_A6_PoP#2_CytoScan-PS_20110511.cyhd.cychp: Weighted Log2 Ratio |
| Edit Mode OFF   |
|   |
| 11-0810_LC_ONC13B_A6_PoP#2_CytoScan-PS_20110511.cyhd.cychp: Weighted Log2 Ratio |



# Types of segment editing

- "Tracking original calls" on page 222
- "Merging all segments types" on page 222
- "Deleting all segments types" on page 228
- "Editing the start/end Coordinates of all segment types" on page 231
- "De Novo segment drawing" on page 233
- "Changing all copy number segment types" on page 237
- "Promoting mosaic segments" on page 239
- "Editing the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields" on page 242

**Note:** Before you start any segment editing, make sure the Detail View tab is selected, as shown in Figure 222.

| Figure 222 Detail tab (bottom left)                          |
|--|
| 1.5<br>08-0989_A3na32.3.cyhd.cychp: Copy Number State<br>5 4 |
| <  |
| 📼 Detail View 🍡 🖼 QC and Sample Info 🎙  🗟 Chromosome Sun     |
|  |



# **Tracking original calls**

Original calls can be tracked to view original segment calls made by the software.

- This track is only visible when Edit Mode is ON.
- This track is only for visualization in the Detail View and is not populated elsewhere in the software.
- The Original Calls track will disappear when the Edit Mode is OFF.

| Figure 223 Original Call Track in Ec   | lit Mode example  |  |
|--|---|--|
| Cancer_ONC134BCytoScan_20110511.cyhd.cychp: Copy Numl  | per State (segments)  |  |
| - W TANE W T- W W W W W - T - ANGAN  |   |  |
|  | og2 Ratio   |  |
| -0.5   | and Anderstein und La bonne ander La strannen det stade in processes ser et en ser en ser en ser en ser en bonn<br>An en stransport programme fragment in ser en se<br>An en stransport programme fragment in ser en se | rene men en en de la companya per esta de la companya de la companya de la companya de la companya de la compa<br>Anticipa de la companya de la company<br>Anticipa de la companya de la company |
| <ul> <li>-1.5</li> <li>Cancer_ONC134B_CytoScan_20110511.cyhd.cychp: Allele Differ</li> <li>-1.5</li> </ul> | ence  | and the state of the state of the second   |
|  |   |  |
| 1.5<br>Genes   |   | a na faran ya kata na k  |

# Merging all segments types

There are two ways to merge segments:

- "Merging segment groups"
- "Segment to segment merge" on page 225

# Merging segment groups

**Note:** Merging segment groups together, cancels out any previously assigned Calls. However, un-doing the group of merged segments (page 228) reinstates their original Calls.

1. Click File  $\rightarrow$  Open.

Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

The file appears in the ChAS browser's Detail view.

3. Left-click, hold, then move the mouse to frame the segments you want to merge together. (Figure 224)





4. Release the mouse button.

Your selected segments, including their Loss symbols are now highlighted in **blue**, as shown in the example below. (Figure 225)



5. Right-click on a highlighted segment.

The following menu appears. (Figure 226)



6. Click Merge together selected segments.

A message regarding your planned merge may appear. (Figure 227)



| Figure 227 Merge All? message   |                  |
|---|------------------|
| Merge all?  | ×                |
| Additional segments in-between the selected segments will also be merged. Some of the<br>not currently be visible due to any current filtering rules. | ose segments may |
| OK Cancel   |                  |

7. Click **OK** to acknowledge it.

The Pick a state window appears. (Figure 228)

| Figure 228 Pick a state window |  |  |  |  |  |  |
|--------------------------------|--|--|--|--|--|--|
| 🐴 Pick a state X               |  |  |  |  |  |  |
| Pick a state 0.0 or larger.    |  |  |  |  |  |  |
| Category: Copy Number State    |  |  |  |  |  |  |
| chr5: 38,138 - 46,401,271      |  |  |  |  |  |  |
| State: (Normal = 2)            |  |  |  |  |  |  |
| 🛦 Gain 💙                       |  |  |  |  |  |  |
| ▲ Gain                         |  |  |  |  |  |  |
| ▼ Loss                         |  |  |  |  |  |  |

8. In the State field, enter a Copy Number, then use the drop-down menu to select a Type (Gain/Loss) for the new segment. (Figure 228)

**Note:** For CytoScan XON arrays (XNCHP files), it is not required to enter a copy number state value.

9. Click **OK**.

In cases where the segment to be merged into a group <u>contains</u> a **Call or Interpretation**, the following message appears: (Figure 229)

| Figure 229 Merge Warning message  |   |
|---|---|
| Continue Merge?   | < |
| Segments being merged have Call(s) or Interpretation(s).<br>This information will not be applied to the merged segment<br>Continue Merge? |   |

10. Acknowledge the message, then click **OK**.

# 11. Your selected segments are now merged together and appear as shown. (Figure 237)



# Segment to segment merge

**IMPORTANT!** When merging segments during the editing of the start or end of one particular segment, only the segment whose start or end you are editing has the option of having its Call or Interpretation saved (or not).

Segments which are being engulfed by the edit start/end procedure being performed will not have the option of having their Call or Interpretations placed on the resulting segment. However, un-doing the edit resulting in this type of merging (page 228) will reinstate previous Call and Interpretation information for all the original segments involved.

1. Click **File**  $\rightarrow$  **Open**.

Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

The file appears in the ChAS browser's Detail view.

3. Click to select the segment you want to merge with another. (Figure 231)



4. Click the **Start Editing** button.

### 5. A shaded area over your selected segment appears. (Figure 232)



### 6. Click, then drag the shaded area over the segment you want to merge it with. Make sure you overlap your target segment just slightly, as shown. (Figure 233)



7. Click the **Start/End** button.

The following message appears: Figure 234



8. Acknowledge the message, then click **OK.** 

If the segment whose Start or End you are editing <u>contains</u> **Call or Interpretation** information, the following message appears: Figure 235

| Figure 235 Keep Call or Interpretation message           |      |  |  |  |  |  |  |  |
|--|------|--|--|--|--|--|--|--|
| 🐐 Keep Call or Interpretation                            | n? X |  |  |  |  |  |  |  |
| The segment being modified has a Call or Interpretation. |      |  |  |  |  |  |  |  |
| Yes No Cancel  |      |  |  |  |  |  |  |  |

9. Click the appropriate button.

Your two segments are now merged together. While Edit Mode is **ON**, the segment appears in a distinct color. (Figure 236). (When Edit Mode is **OFF**, the edited segments look identical to non-edited segments.)





### **Un-Merging**

| Fi | gure 237 Merged seg     | me             | nts   |                      |     |      |        |     |      |    |   |   |   |   |          |   |   |              |   |    |   |
|----|-------------------------|----------------|---|----------------------|-----|------|--------|-----|------|----|---|---|---|---|----------|---|---|--------------|---|----|---|
| l  |                         |                |   |                      |     |      |        |     |      |    |   |   |   |   |          |   |   |              |   |    |   |
| ŀ  |                         |                | One Item Selected                                 | Δ                    |     | 7.   | Δ      | Į   | Δ    | 22 | Δ | Δ | Δ | Δ | <u> </u> | Δ | 4 | <u>م</u> م م | Δ | 44 | 2 |
|    | 11-0816_LC_ONC134B_B10_ | <b>€</b>       | Zoom to selection<br>Selection Details            | Ctrl+Space<br>Ctrl+D | eig | phte | d Log: | 2 R | atio |    |   |   |   |   |          |   |   |              |   |    |   |
|    | 4                       | <mark>☆</mark> | Add to a File<br>Un-do Merge                      |                      |     |      |        |     |      |    |   | • |   |   |          |   |   | ÷            |   |    | ł |
|    |                         | *              | Delete Segment<br>View/Edit Annotation Properties |                      |     |      |        |     |      |    |   |   |   |   |          |   |   |              |   |    |   |
|    |                         | ⇔<br>₽         | Edit start/end<br>Change copy number              |                      |     |      |        |     |      |    |   |   |   |   |          |   |   |              |   |    |   |

### Merged Segments

1. Right-click on the newly merged segments (Figure 237), then click **Un-do Merge**.

The following message appears. (Figure 238)



2. Acknowledge the message, then click **OK**.

Your previously merged segments return to their original states, including any Calls or Interpretations made on the original segments (prior to their merging).

## Deleting all segments types

1. Click File  $\rightarrow$  Open.

Your Sample File data folder window appears.

2. Click to select the file you want to edit, then click **Open**.

The file appears in the ChAS browser.

# 3. Left-click, hold, then move the mouse to frame the segments you want to delete. (Figure 239)



4. Release the mouse button.

Your selected segments, including their Loss symbols are now highlighted, as shown in the example below. (Figure 240)



5. Right-click on a highlighted segment.

The following menu appears. (Figure 241)



6. Click **Delete Segments**.

A message regarding your planned deletion appears. (Figure 242)



7. Click OK to acknowledge it.

Your selected segments are deleted and graphically represented as "ghosts" while Edit mode is **ON**. (Figure 243). When Edit Mode is OFF, deleted segments are invisible and NOT displayed in any view or table.



**Note:** Even though your selected segments are deleted, ChAS preserves their data for future reference. See "Right-click menu options" on page 202 for information on how to view and edit segment properties and view segment details.

Un-deleting a deleted segment(s)

1. Right-click on the deleted segment(s), then click **Un-do Delete**.

The following message appears. (Figure 244)

| Figur | e 244 Confirm deletion   |   |
|-------|--|---|
| - M   | essage >   | < |
|       | Please verify the Calls & Interpretations of the reverted segments |   |
|       | ОК   |   |

2. Acknowledge the message, then click **OK**.

Your previously deleted segment(s) return to their original states, including any Calls or Interpretations made on the original segments (prior to their deletion).



# Editing the start/end Coordinates of all segment types

**IMPORTANT!** Only the boundaries of 1 segment, can be adjusted/modified at one time.

**Note:** Segment start or end boundaries can be moved left and/or right. The Adjusting/ Modifying Segment Boundaries example that follows, shows how to move a segment end boundary farther to the right.

- 1. Using the zoom tools and scroll bars (if needed), identify the segment whose start or end (or both) you want to modify
- Figure 245 Selected segment
- 2. Single-click on the segment to highlight it. (Figure 245)

3. Click the Start editing... button.

The following appears: (Figure 246 on page 231)

4. Place the mouse cursor on the very-right edge of the current segment boundary. (Figure 246)



5. Click, then drag the segment's right-edge boundary to the right, then stop at an appropriate point. (Figure 247) You will ONLY be allowed to set the Start or End position to match the position of a marker probeset.





### 6. Click the Set start/end... button. (Figure 248)

The newly adjusted/modified segment boundary appears as shown. (Figure 248)



7. ChAS auto-calculates various properties of the newly edited segment. Mouse over the segment to view its new details. (Figure 249)

| Figure 249     | Selected segment                                      | - Start editir  | ıg   |
|----------------|---|---|--|
| *** ? 61 5 1)[ | · - 5 <sup>01</sup> 797 - 5988 38 - 499 <sup>84</sup> | Sample_07.cyhd<br>Array Type Cytos<br>Loss 6: 257,064<br>Type<br>Location<br>Size (kbp)<br>CN State<br>Marker Count | Ccychp           icanHD_Array           - 405,900           Loss           6: 257,064 - 405,900           149           1.00           270 |
| omere          |   | Median Log2Ratio  | -0.471   |

Un-doing the edited start/end coordinates of a segment

 Right-click on the previously adjusted/modified segment, then click Un-do Edit. The segment returns to its previous state, including, previous boundaries, calls, and interpretations.



# De Novo segment drawing

- 1. Within an existing segment track, locate an empty space where you want to draw in a brand new segment.
- 2. Right-click inside this empty space.

A **Create a Copy Number State segment around XX,XXX,XXX** ribbon appears (Figure 250)

| Figure 250 Selected segment - Change Copy Number - Pick a state window          |
|---|
| · · · · · · · · · · · · · · · · · · ·   |
|   |
|   |
| Create a Copy Number State segment around 35,498,785.                           |
| -1  |
|   |
| 1<br>15   |
| 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.cyhd.cychp: Copy Number State |
| -3.   |
|   |
| -1  |
| -0.   |
|   |

3. Click on the ribbon, as shown in Figure 250.

The Pick a state window appears. (Figure 251)

| Figure 251 Drawing a new segment Pick a state window |
|--|
| 🐴 Pick a state 🛛 🗙                                   |
| Pick a state 0.0 or larger.                          |
| Category: Copy Number State                          |
| chr7: 44,817,005 - 97,385,627                        |
| State: (Normal = 2)                                  |
| 🛦 Gain 💌   |
| ▲ Gain   |
| ▼ Loss   |

- 4. Enter a **Copy Number** and **Type** for the new segment.
- 5. Click OK.



### Your newly drawn segment appears. (Figure 252)

**IMPORTANT!** Newly drawn segments will stop when they encounter another segment, whether or not that segment is currently drawn or whether it is currently filtered out; see Figure 252 for an example in the CN State data track which is used to draw Copy Number Segments of Gain and Loss.

New segments will also stop when they encounter the last appropriate marker on the chromosome. Because of this, new segments drawn will vary in their initial size.

6. Move the mouse cursor over the newly drawn segment to reveal its properties. (Figure 253)

| Figure 253 Newly drawn segment -                        | Mouse ove  | r to see its proper   | ties                          |
|---|--|---|-------------------------------|
| 0 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_             | 20110511.cyhd.c  | ychp: Copy Number State   | (segments)                    |
|   |  | 4   | Δ                             |
| 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_<br>1.5<br>0.5 | 20110511.cyhd.c<br>11-0816_LC_O<br>Array Type Cyto<br>Loss 8: 35,021 | ychp: Weighted Log2 Ratic<br>NC134B_B10_PoP#2_Cyl<br>ScanHD_Array<br>410 - 36,455,932 | toScan-PS_20110511.cyhd.cychp |
| 1.5<br>11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_        | Type<br>Location   | Loss<br>8: 35,021,410 - 36,455,932  |                               |
| Ħ   | Size (kbp)   | 1,435   | -                             |
| Markers CytoScan HD Array dbSnp: 132 NetAffx: 32.       | CN State   | 1.00  |                               |
|   | Median Log2Ratio   | -0.211  |                               |

### Un-doing a segment De Novo drawing

- 1. Right-click on the newly drawn segment.
  - The following menu appears. (Figure 254)

| O  | ppy Number State (segments)           |
|--|---------------------------------------|
|  | ∆                                     |
|  | One Item Selected                     |
| 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.cyhd.cychp: W<br>- 1   | I Zoom to selection Ctrl+Space        |
| 1  | Election Details Ctrl+D               |
| 05   | Add to a File                         |
| - U.D f  | 🗅 Un-do Create 🚽 🗕                    |
| and the second of the second   | * Delete Segment                      |
| 012 to the product of the state of the sta | View/Edit Annotation Properties       |
| 1  | ⇐⇒ Edit start/end                     |
| 1.5  | Change copy number                    |
| -4   | opy Number State                      |
|  |                                       |
| -3   |                                       |
|  | l<br>I                                |
|  | · · · · · · · · · · · · · · · · · · · |
| 1  | 1                                     |
|  |                                       |
| 0  |                                       |

2. Click Un-do Create.

The previously drawn segment is removed, along with any Calls or Interpretations it had been given. (Figure 255)







## Changing all copy number segment types

**Note:** For CytoScan XON arrays only, you are not required to assign a copy number to the segment.

- 1. Right-click on the segment that contains the copy number you want to change
- 2. Click Change copy number. (Figure 256)



The Pick a state window appears. (Figure 257)

| Figure 257 Selected segment -<br>Change Copy Number - Pick a state<br>window |
|--|
| 🐴 Pick a state 🛛 🗙   |
| Pick a state 0.0 or larger.  |
| Category: Copy Number State  |
| chr8: 36,596,983 - 38,556,532  |
| State: (Normal = 2)  |
| 🔺 Gain 🔍   |
| ▲ Gain   |
| ▼ Loss   |

3. Enter a new Copy Number State value and Type (Gain/Loss).

**Note:** For CytoScan XON arrays (XNCHP files), it is not required to enter a copy number state value.

4. Click OK.

The segment now reflects your revised Copy Number State and Type.



### Un-doing a copy number change

- 1. Right-click on the segment you performed a Copy Number State change on, then click **Un-do Edit**.
- 2. The following message appears. (Figure 258)



3. Acknowledge the message, then click **OK**.

The segment's original Copy State Number is reinstated.



# Promoting mosaic segments

**IMPORTANT!** Segments in the Mosaic Segment Track are NOT uploaded to the database.

To capture the information for a mosaic segment in the database, that segment must be "promoted" to the Copy Number State track. This is done to reduce redundancy in those regions in which segments were called by both the copy number algorithm and the mosaic detection algorithm. (Figure 259)

| Figure 259 Promoting Mosaic Seg   | ments - Example  |   |
|---|--|---|
| 0<br>11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F<br>11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F   | PS_20110511.cyhd.cychp: Copy Number State (segments<br>PS_20110511.cyhd.cychp: Mosaic Copy Number State (se  | >><br>>><br>>><br>>><br>>><br>>><br>>><br>>><br>>><br>>>                                    |
| 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-<br>- 1.5<br>- 0.5<br>- 0.5<br>- 1.5<br>- 1.5<br>- 1.5<br>11-0816_LC_ONC134B_B10_PoP#2_CytoScan-<br>- 1.5<br>- 1.5 | One Item Selected         Image: Selection Details         Ctrl+Space         Image: Selection Details         Ctrl+D         Add to a File         Delete Segment         Image: View/Edit Annotation Properties         Query ChAS DB         Edit start/end         Change copy number         Promote to copy number segment | 2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2<br>2 |
| 1.5<br>11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F<br>  | Promot<br>PS_20110511.cyhd.cychp: SmoothSignal   | e a mosaic segment to a copy number gain or loss segment.                                   |

Promoted mosaic segments maintain their non-integer copy number state, marker count, median log2 ratio, genome coordinates and size when they are promoted to the copy number state track. (Figure 260)

The mosaic gain segments will have the same blue/red used for integer copy number Gains/Losses, however, they maintain their non-integer copy number state to indicate they are a mosaic.





## Modified segments in the segments table

**IMPORTANT!** When exporting a Segments table to text (TXT), please note that deleted segments will be part of the export when Edit Mode is On, and will NOT be part of the export when Edit Mode is Off.

In PDF reports, deleted segments are never shown in graphical views, nor are they listed in the Segments Table, as Edit Mode is required to be OFF to generate a PDF report.

4 Editing mode on When the Edit Mode is ON , the Modified segments appear differently within the

- Segments table, as shown in Figure 261.
- Deleted Segments are represented with a red X and a strike-through line. Deleted segments do NOT show up in PDF reports because PDF reports cannot be created while Edit Mode is **ON**.
- Materially Modified Segments (including segments that have been merged, boundaries edited, or had their Copy Number States changed) are represented with italicized text.

| Figure 2   | <b>61</b> Segments table with Edit i    | node ON  |                   |                 | _                 |                   |                  |
|--|---|----------|-------------------|-----------------|-------------------|-------------------|------------------|
| Karyoview 🎙 🔝 Segments 🍡 🛄 CytoRegions 🎙 🍠 Overlap Map 🎙 🖾 Graphs 🎙 🖉 Segments 🖉 🦊 Strike-through text example |   |          |                   |                 |                   |                   |                  |
| POF C  | Σ   🏢 🚺 🛊   🚮 🗹 🗆                       |          |                   |                 |                   | 7 results         | from chr 5 📗     |
| In Report  | ▲ File                                  | CN State | Туре              | Chromosome      | Cytoband Start    | Size (kbp)        | Marker Count     |
|  | [₽♥ 0" 11-0816_LC_ONC134B_B             | 2.61     | A GainMo          | 5               | p15.33            | 38,092            | 36,2             |
|  | [₽♥] 0" 11-0816_LC_ONC134B_B            | 3.00     | ▲ Gain            | 5               | p15.33            | 8,739             | 10,1             |
| ×  | ĨŶ♥ <mark>♂ 11 0816_LC_ONC134B_B</mark> | 3.00     | ▲ Gain            | 6               | <del>p15.31</del> | <del>8,304</del>  | <del>10,0</del>  |
| ×  |   | 3.00     | ▲ <del>Cain</del> | 5               | <del>p15.1</del>  | 1,949             | - <del>1,1</del> |
| ×  |   | 3.00     | ▲ <del>Cain</del> | 6               | p14.3             | <del>15,225</del> | <del>10,7</del>  |
|  |   | 1.00     | ▼ Loss            | E               | p13.2             | 2,314             | 2,4              |
|  | P 0 11-0816_LC_ONC134B_B                | 3.00     | ▲ Gain            | 5               | p13.2             | 493               | 5                |
|  |   |          |                   | Italicized text | example           |                   |                  |



### Editing mode off Turning the Edit Mode 🔐 OFF, displays the Segments table as follows: (Figure 262)

**Note:** The three deleted segment rows which were in strike-through text are removed from the table when Edit Mode is OFF. In addition to when Edit Mode is OFF, the rows which indicated Modified segments are no longer italicized.

| Figure 26   | 2 Segments table with Edit r | mode OFF |          |            |                |            |              |
|---|------------------------------|----------|----------|------------|----------------|------------|--------------|
| Karyoview ষ 📷 Segments 🔊 🕌 CytoRegions ষ 🥔 Overlap Map ষ 🖾 Graphs ষ |                              |          |          |            |                |            |              |
|   | Σ 🔢 🕕 🛊 🚮 🔍 🗆                |          |          |            |                | 4 results  | from chr 5   |
| In Report   | ▲ File                       | CN State | Туре     | Chromosome | Cytoband Start | Size (kbp) | Marker Count |
|   | ST-0816_LC_ONC134B_B         | 2.61     | A GainMo | 5          | p15.33         | 38,092     | 36,2         |
|   | 🚱 🖓 🖓 11-0816_LC_ONC134B_B   | 3.00     | 🛦 Gain   | 5          | p15.33         | 8,739      | 10,1         |
|   | 🚱 🖓 🖓 11-0816_LC_ONC134B_B   | 1.00     | V Loss   | 5          | p13.2          | 2,314      | 2,4          |
|   | Sector 11-0816_LC_ONC134B_B  | 3.00     | 🔺 Gain   | 5          | p13.2          | 493        | 5            |

## Removing all edits made to a sample

1. Right-click on the File name in the Files list, then select **Discard Changes**. (Figure 263)



The Discard Changes window appears and displays the following options:

Purge All Edits: Reverts all edits to segments and removes all calls and interpretations.

**Note:** The log of the edits that have been performed on the sample can still be viewed.



- Clear Edit Log: Purges all edits and clear the log of any edits performed on the sample.
- Discard all changes: Purges all edits, clears the edit log, and deletes the CHCAR file.
  - 2. Check the appropriate box, then click Yes. (Figure 264)

| Figure 264 Discard Changes Confirmation wind               | ow |
|--|----|
| 📸 Discard Changes  | ×  |
| Are you sure you want to discard changes made to this file | ?  |
| 🗹 Purge all edits.   |    |
| Clear edit log.  |    |
| Discard all changes. (Danger! Deletes CHPCAR!)             |    |
| Yes <u>N</u> o   |    |

# Editing the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields

The View/Edit Annotation Properties Curation tab enables you to update the copy number nomenclature in the Microarray Nomenclature (ISCN 2013) and Microarray Nomenclature fields.

**Example**: For Mosaic segments, the default name will show a range of copy number such as x2-3. If you have the percent mosaicism information, this part of the Microarray Nomenclature can be updated to reflect the percent mosaicism by typing x3[0.6]. The field will be updated as follows:

**Default name:** arr[GRCh37] 5p15.33p13.2(113576\_38205477)x2-3

Updated name: arr[GRCh37] 5p15.33p13.2(113576\_38205477)x3[0.6]

1. Right-click on the Microarray Nomenclature cell for the segment you would like to update, then click **View/Edit Annotation Properties**, as shown in Figure 265.

| Figur | e 265    | Discarc                  | d Changes   |             |         |                        |           |
|-------|----------|--------------------------|---|-------------|---------|------------------------|-----------|
| E P   | 🔱 😡 🛛    | chr5:0-4                 | 3,919,262<br>Som Mut ♥ III Query Samples ♥ III Query Segments ♥ |             |         |                        |           |
|       |          | . T                      |   | <b>F</b> -4 |         | DD Court Dath          | Cine (In  |
|       | CN State | <ul> <li>Type</li> </ul> | Microarray Nomenciature (ISCN 2016)                             | Full        | Loca    | DB Count Both          | Size (K   |
|       | 2.61     | A Gain                   | arr[GRCh37] 8p11.22q24.3(38556627_146295771)x2-3                | chr8        | 3:38    | 7                      |           |
|       | 2.61     | A Gain                   | arr[GRCh37] 5p15.33p13.2(113576_38205477)x2-3                   |             |         |                        |           |
|       | 2.43     | A Gain                   | arr[GRCh37] 21g21.1g22.12(20605960_37047544)x2-3                | $\Sigma$ s  | Sum, m  | ean and median         |           |
|       | 2.43     | A Gain                   | arr[GRCh37] 22q11.1q13.33(16888899_51197838)x2-3                |             | /iew/Ed | lit Annotation Propert | ies       |
|       | 2.31     | A Gain                   | arr[GRCh37] 6p25.3q12(330740_65524866)x2-3                      | -           |         |                        |           |
|       | 2.27     | A Gain                   | arr[GRCh37] 14q32.31q32.33(102931332_107285437)x2-3             |             | Query C | View or edit propertie | es of the |
|       | 2.24     | A Gain                   | arr[GRCh37] 17g21.32g22(45010182_57572273)x2-3                  | Tchr1       | 7:4     | 3                      |           |
|       | 2.24     | A Gain                   | arr[GRCh37] 17q24.1q25.1(63940809_72905641)x2-3                 | chr1        | 17:6    | 3                      | 3         |

### The Annotation Properties window appears. (Figure 266)

| Figure 266 Discard Cha            | nges                              |     |
|-----------------------------------|-----------------------------------|-----|
| Annotation Properties             |                                   | ×   |
| General Structure Additional      | Curation                          |     |
| Call                              | Inheritance                       | OKR |
|                                   |                                   | ✓   |
| Call Approval                     | Use In Export                     |     |
| Median Copy Number                | Ch37I 1g44(248753184, 248795277)y | x1  |
| Segment Interpretation (Right-cli | k to add preconfigured snippets)  |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
|                                   |                                   |     |
| Curation Time                     | Curation By                       |     |
|                                   |                                   |     |
|                                   | OK Canc                           | cel |

- 2. Click the **Curation** tab.
- 3. At the **ISCN Copy Number Text** field (Figure 266), type in the copy number nomenclature text you want, then click **OK**. **Note:** This field can be edited for LOH segments to designate copy number in the Microarray Nomenclature field for LOH segments as well.
- 4. Optional: To reset the text to the original default format, click the **Reset ISCN** button.

**Note:** To automatically add the Inheritance field to the Microarray Nomenclature ISCN 2016 field, please see "Adding or removing inheritance calls" on page 255.



# Sample and segment annotations

# Sample annotations

Sample file level annotations such as Sample-type, Phenotype, and Sample Interpretation can be added to each sample.

Adding, removing, and changing the order of sample type text

1. Click **Preferences**  $\rightarrow$  **Edit User Configurations** or click  $\bigcirc$  on the upper tool bar.

The User Configuration window appears.

2. Click the Vocabularies tab, then click the Sample Type tab. (Figure 267)

### Figure 267 Sample Type window tab

| User Configuration  | ×   |
|---|-----|
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports |     |
| Call Interpretation Inheritance Sample Type Phenotype                                       |     |
| Text snippets   |     |
| Add or remove short texts. Drag with the mouse to change the order.                         |     |
| POC   |     |
| Amnio/CVS (cultured)  |     |
| Amnio/CVS (direct)  |     |
| Buccal Swab   |     |
| Blood (constitutional)  |     |
| Blood (cancer)  |     |
| Bolie Marrow  |     |
| Suite funite  |     |
| Fixed Pellet  |     |
| Blood   |     |
| Cell-line   |     |
| Unknown   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   | Add |
| Remove Restore Defaults Default list contains 10 items                                      |     |
|   |     |
| OK  |     |
|   |     |

### Adding a Sample Type

- 1. Click inside the Add Sample Type field, then enter your new Sample Type.
- 2. Click Add.



### **Deleting a Sample Type**

- 1. Click to highlight the Sample Type you want to delete.
- 2. Click Remove.

### Re-arranging the order currently displayed Sample Types

- 1. Click to highlight the Sample Type you want to move.
- 2. Drag and drop it to its new location (order).
- 3. If needed, repeat steps 1-2 to re-arrange additional Sample Types.

### Restoring the factory default Sample Types

1. Click Restore Defaults.

The factory default Sample Types are restored.

Adding, removing, and changing the order of phenotype text Click Preferences → Edit User Configurations or click on the upper tool bar.

The User Configuration window appears.

Click the Vocabularies tab, then click the Phenotype tab.
 The following window tab appears: (Figure 268)

### Figure 268 Phenotype window tab

| User Configuration       X         Segment Data       QC Thresholds       Color Rules       Misc       Vocabularies       DB Query       Filtered DB Query       Exports         Call       Interpretation       Inheritance       Sample Type       Phenotype         Text snippets       Add or remove short texts. Drag with the mouse to change the order.       Pervasive Developmental Delay (behavioral/psychiatric)         Attention Deficit Hyperactivity Disorder (behavioral/psychiatric)       Attention Deficit Hyperactivity Disorder (behavioral/psychiatric)         Behavioral/Psychiatric Abnormality (behavioral/psychiatric)       Attention Deficit (cardiac)       Ventricular Septial Defect (cardiac)         Coarcitation of the aotta (cardiac)       Cardiac Abnormality (cardiac)       Ventricular Septial Defect (cardiac)         Cardiac Abnormality (cardiac)       Structural Hace Infect (cardiac)       Ventricular Septial Defect (cardiac)         Cleft lip (craniofacial)       Cardiac Abnormality (cardiac)       Ventricular Septial Defect (cardiac)         Cleft lip (craniofacial)       Macrocephaly (craniofacial)       Ventricular Septial Defect (cardiac)         Cleft lip (craniofacial)       Macrocephaly (craniofacial)       Ventricular Septial Defect (cardiac)         Cleft lip (craniofacial)       Macrocephaly (craniofacial)       Ventricular Septial Defect (cardiac)         Cleft lip (craniofacial)       Septia   |  |      |
|---|--|------|
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports Cal Interpretation Inheritance Sample Type Phenotype Text snippets Add or remove short texts. Drag with the mouse to change the order. Pervasive Developmental Delay (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Attention Deficit Hyperactivity Olisorder (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Attention Deficit (cardiac) Cardiac Abnormality (cardiac) Cardiac Abnormality (cardiac) Cardiac Abnormality (cardiac) Structural Heart Defect (cardiac) Cieft ig (caniofacial) Dysmorphic facial features (caniofacial) Ear maformations (craniofacial) Learning Disability (Cognitive/developmental) Intellectual Disability (Cognitive/developmental) Fine Motor Delay (cognitive/developmental) Goss Motor Delay (cognitive/developmental) Hyperjogmentation (cutaneous) Gastroschisis (gastrointestinal) And atresia (gastrointestinal) And atresia (gastrointestinal) Tracheoesophageal fistual (gastrointestina)   | User Configuration   | ×    |
| Segment Data Oc. Intrestious Coor Rules Misc.       Vocaduaries       Dis Ouery Printered Dis Ouery Expons         Call Interpretation Inheritance Sample Type Phenotype       Interpretation Inheritance Sample Type Phenotype         First snippets       Add or remove short texts. Drag with the mouse to change the order.         Pervasive Developmental Delay (behavioral/psychiatric)         Attention Deficit Hyperactivity Disorder (behavioral/psychiatric)         Behavioral/Psychiatric Abnormality (behavioral/psychiatric)         Ventricular Septal Defect (cardiac)         Coarctation of the aorta (cardiac)         Cardiac Abnormality (cardiac)         Structural Heart Defect (cardiac)         Celft palate (cardiacial)         Dysmorphic facial features (craniofacial)         Macrocephaly (craniofacial)         Macrocephaly (craniofacial)         Intelectual Distilty/MR (coopinitive/developmental)         Fine Motor Delay (cognitive/developmental)         Fine Motor Delay (cognitive/developmental)         Hyperpigmentation (cutaneous)         Hyperpigmentation (cutaneous)   | Comment Party OC Therebuildy Only Party March (March March 1990 Overs) Ethered DB Overs    |      |
| Call Interpretation Inheritance Sample Type Phenotype         Text snippets         Add or remove short texts. Drag with the mouse to change the order.         Pervasive Developmental Delay (behavioral/psychiatric)         Attention Deficit Hyperativity Disorder (behavioral/psychiatric)         Attention Deficit Hyperativity Disorder (behavioral/psychiatric)         Aria Septal Defect (cardiac)         Coacretation of the aorta (cardiac)         Coacretation of the aorta (cardiac)         Coacretation of the aorta (cardiac)         Cardiac Abnormality (cardiac)         Dysmorphic facial features (craniofacial)         Dysmorphic facial features (craniofacial)         Macrocephaly (craniofacial)         Learing Disability (Cognitive/developmental)         Intellectual Disability/MR (cognitive/developmental)         Speech Delay (cognitive/developmental)         Speech Delay (cognitive/developmental)         Speech Delay (cognitive/developmental)         Hyperpigmentation (cutaneous)         Hyperpigmentation (cutaneous)         Gastroschissi (gastrointestinal)   | Segment Data QC Inresholds Color Rules Misc Vocabulanes DB Query Filtered DB Query Exports |      |
| Text snippets Add or remove short texts. Drag with the mouse to change the order. Pervasive Developmental Delay (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Behavioral/Psychiatric Abnormality (behavioral/psychiatric) Ventricular Septal Defect (cardiac) Ventricular Septal Defect (cardiac) Coardiac Abnormality (cardiac) Structural Heart Defect (cardiac) Ceft tiple (cardiac) Dysmorphic facial features (craniofacial) Dysmorphic facial features (cardiac) Microcephaly (craniofacial) Microcephaly (craniofacial) Hielectual Disability/MR (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Hyperpigmentation (cutaneous) Hyperpigmentation (cutaneous) Hyperpigmentation (cutaneous) Atta stepial Defection) Center Defet tiple a fact the data data  | Call Interpretation Inheritance Sample Type Phenotype                                      |      |
| Add or remove short texts. Drag with the mouse to change the order. Pervasive Developmental Delay (behavioral/psychiatric) Attenion Derlich Hyperactivity Disorder (behavioral/psychiatric) Behavioral/Psychiatric Ahoromality (behavioral/psychiatric) Atria Septal Defect (cardiac) Coarctation of the aorta (cardiac) Tetralogy of Fallot (cardiac) Cardiac Ahoromality (cardiac) Cieft lip (craniofacial) Cieft taptate (cardiac) Cieft lip (craniofacial) Dysmorphic facial features (craniofacial) Bernations (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Intellectual Disability/MR (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Anal atresia (gastrointestinal) Anal atresia (gastrointestinal)  | Text snippets  |      |
| Pervasive Developmental Delay (behavioral/psychiatric) Attention Deficit Hyperactivity Disorder (behavioral/psychiatric) Behavioral/Psychiatric Ahnormality (behavioral/psychiatric) Atria Septal Defect (cardiac) Ventricular Septal Defect (cardiac) Cardiac (Cardiac) Cardiac Ahnormality (cardiac) Cardiac Ahnormality (cardiac) Cleft lip (caranidacia) Cleft palate (cranidacial) Cleft palate (cranidacial) Dysmophic facial features (craniofacial) Barmalomations (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) The Motor Delay (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Hypergigmentation (cutaneous) Hypopigmentation (cutaneous) Hypopigmentation (cutaneous) Anal atresia (gastrointestinal) Anal atresia (gastrointestinal)  | Add or remove short texts. Drag with the mouse to change the order.                        |      |
| Attention Deficit Hyperactivity Disorder (behavioral/psychiatric)<br>Behavioral/Psychiatric Abnormality (behavioral/psychiatric)<br>Atria Septal Defect (cardiac)<br>Ventricular Septal Defect (cardiac)<br>Coarctation of the aota (cardiac)<br>Tetralogy of Fallot (cardiac)<br>Cardiac Abnormality (cardiac)<br>Structural Heart Defect (cardiac)<br>Cleft plate (cardiaca)<br>Cleft plate (cardiacai)<br>Cleft plate (cardiacaia)<br>Dysmorphic facial features (craniofacial)<br>Macrocephaly (craniofacial)<br>Macrocephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hypeprigmentation (cutaneous)<br>Approprimentation (cutaneous)<br>Hypopogianetation (cutaneous)<br>Anal artesia (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal artesia (gastrointestinal)  | Pervasive Developmental Delay (behavioral/psychiatric)                                     |      |
| Behavoral/Psychiatric Abnormality (behavioral/psychiatric)<br>Atria Septal Defect (cardiac)<br>Coarctation of the aorta (cardiac)<br>Coarctation of the aorta (cardiac)<br>Cardiac Abnormality (cardiac)<br>Structural Heart Defect (cardiac)<br>Cleft tip (craniofacial)<br>Cleft tip (craniofacial)<br>Dysmorphic fcalia features (craniofacial)<br>Ear maformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Intellectual Disability (cognitive/developmental)<br>Intellectual Disability (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperjigmentation (cutaneous)<br>Hypopigmentation (cutaneous)<br>Castroschiski (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Anal atresia (Dastrointestinal)   | Attention Deficit Hyperactivity Disorder (behavioral/psychiatric)                          |      |
| Atria Septal Defect (cardiac) Ventricular Septal Defect (cardiac) Coarctation of the aota (cardiac) Tetralogy of Fallot (cardiac) Cardiac Abnormality (cardiac) Cardiac Abnormality (cardiac) Cardiac Abnormality (cardiac) Cleft ig (carniofacial) Cleft plate (carniofacial) Dysmophic facial features (craniofacial) Bar maformations (craniofacial) Microcephaly (carniofacial) Microcephaly (carniofacial) Microcephaly (carniofacial) Learning Disability (cognitive/developmental) Intellectual Disability (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Gross Motor Delay (cognitive/developmental) Hyperpigmentation (cutaneous) Gastroschists (gastrointestinal) Anal atresia (gastrointestinal) Anal atresia (gastrointestinal)   | Behavioral/Psychiatric Abnormality (behavioral/psychiatric)                                |      |
| Ventricular Septal Defect (cardiac)<br>Caarctation of the aorta (cardiac)<br>Tetralogy of Fallot (cardiac)<br>Cardiac Abnormality (cardiac)<br>Structural Heart Defect (cardiac)<br>Cleft lip (craniofacial)<br>Cleft plate (craniofacial)<br>Dysmorphic facial features (craniofacial)<br>Ear mafformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Macrocephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/INK (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tercheoresophageal fistuk (gastrointestinal)<br>Macrocephals (cardintestinal)<br>Anal atresia (gastrointestinal)  | Atria Septal Defect (cardiac)  |      |
| Coarciation of the aorta (cardiac)<br>Tetralogy of Fallot (cardiac)<br>Cardiac Abnormality (cardiac)<br>Structural Heart Defect (cardiac)<br>Cleft plate (carainofacial)<br>Dysmorphic facial features (craniofacial)<br>Ear maformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Macrocephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hypoprigmentation (cutaneous)<br>Hypoprigmentation (cutaneous)<br>Hypoprigmentation (cutaneous)<br>Haresia (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Anal atresia (Dastrointestinal)<br>Anal atresia (Dastrointestinal)<br>Anal atresia (Dastrointestinal)  | Ventricular Septal Defect (cardiac)  |      |
| Tetralogy of Fallot (cardiac) Cardiac Abnormality (cardiac) Structural Heart Defect (cardiac) Cleft lip (craniofacial) Dysmorphic facial features (craniofacial) Ear mafformations (craniofacial) Macrocephaly (craniofacial) Microcephaly (craniofacial) Learning Disability (Osgnitive/developmental) Intellectual Disability/MR (cognitive/developmental) Intellectual Disability/MR (cognitive/developmental) Speech Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Hypergigmentation (cutaneous) Hypopigmentation (cutaneous) Hypopigmentation (cutaneous) Anal atresia (gastrointestinal)   | Coarctation of the aorta (cardiac)   |      |
| Cardiac Abnormality (cardiac)<br>Structural Heart Defect (cardiac)<br>Cleft ipi (craniofacial)<br>Dysmorphic facial features (craniofacial)<br>Ear maformations (craniofacial)<br>Microcephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability (Cognitive/developmental)<br>Intellectual Disability (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyporgimentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Terchecesophageal fistuk (gastrointestinal)   | Tetralogy of Fallot (cardiac)  |      |
| Structural Heart Defect (cardiac)<br>Cleft lip (craniofacial)<br>Dysmorphic facial features (craniofacial)<br>Ear maformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistual (gastrointestinal)   | Cardiac Abnormality (cardiac)  |      |
| Cleft Ipi (craniofacial)<br>Cleft palate (craniofacial)<br>Dysmorphic facial features (craniofacial)<br>Ear malformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability (MR (cognitive/developmental)<br>Intellectual Disability MR (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hypopigmentation (cutaneous)<br>Hypopigmentation (cutaneous)<br>Gastroschiski (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Macroschistical (astrointestinal)  | Structural Heart Defect (cardiac)  |      |
| Cleft palate (craniofacial) Dysmorphic facial features (craniofacial) Ear maformations (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Microcephaly (craniofacial) Intellectual Disability (Cognitive/developmental) Intellectual Disability (Cognitive/developmental) Gross Motor Delay (cognitive/developmental) Gross Motor Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Hyporgimentation (cutaneous) Gastroschists (gastrointestinal) Anal atresia (gastrointestinal) Tracheoesophageal fistula (gastrointestinal)   | Cleft lip (craniofacial)   |      |
| Dysmophic facial features (craniofacial)<br>Ear maformations (craniofacial)<br>Microcephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability (Cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Trachecesophageal fistula (gastrointestinal)<br>Add  | Cleft palate (craniofacial)  |      |
| Ear mafformations (craniofacial)<br>Macrocephaly (craniofacial)<br>Microcephaly (craniofacial)<br>Learning Disability/icognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Add  | Dysmorphic facial features (craniofacial)  |      |
| Macrocephaly (craniofaciai)<br>Microcephaly (craniofaciai)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/MR (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperjigmentation (cutaneous)<br>Hypopigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Atd   | Ear malformations (craniofacial)   |      |
| Microcophaly (cranidacia)<br>Learning Disability (cognitive/developmental)<br>Intellectual Disability/MC (cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Add  | Macrocephaly (craniofacial)  | 0    |
| Learning Disability (cognitive/developmental)<br>Intellectual Disability (Cognitive/developmental)<br>Fine Motor Delay (cognitive/developmental)<br>Gross Motor Delay (cognitive/developmental)<br>Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)   | Microcephaly (craniofacial)  |      |
| Intellectual Disability/MR (cognitive/developmental) Fine Motor Delay (cognitive/developmental) Gross Motor Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Hypergigmentation (cutaneous) Gastroschisis (gastrointestinal) Omphalocele (gastrointestinal) Anal atresia (gastrointestinal) Tracheoesophageal fistula (gastrointestinal)   | Learning Disability (cognitive/developmental)  |      |
| Fine Motor Delay (cognitive/developmental) Gross Motor Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Hypopigmentation (cutaneous) Hypopigmentation (cutaneous) Gastroschisis (gastrointestinal) Omphalocele (gastrointestinal) Tracheoesophageal fistula (gastrointestinal)  | Intellectual Disability/MR (cognitive/developmental)                                       |      |
| Cross Motor Delay (cognitive/developmental) Speech Delay (cognitive/developmental) Hypopigmentation (cutaneous) Hypopigmentation (cutaneous) Castroschisis (gastrointestinal) Omphalocele (gastrointestinal) Tracheoesophageal fistula (gastrointestinal) Tracheoesophageal fistula (gastrointestinal)  | Fine Motor Delay (cognitive/developmental)   |      |
| Speech Delay (cognitive/developmental)<br>Hyperpigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Add   | Gross Motor Delay (cognitive/developmental)  |      |
| Hyperjigmentation (cutaneous)<br>Hypopigmentation (cutaneous)<br>Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)<br>Add   | Speech Delay (cognitive/developmental)   |      |
| Hypopigmentation (cutaneous) Gastroschisis (gastrointestinal) Omphalocele (gastrointestinal) Anal atresia (gastrointestinal) Tracheoesophageal fistula (gastrointestinal) Add   | Hyperpigmentation (cutaneous)  |      |
| Gastroschisis (gastrointestinal)<br>Omphalocele (gastrointestinal)<br>Anal atresia (gastrointestinal)<br>Tracheoesophageal fistula (gastrointestinal)   | Hypopigmentation (cutaneous)   |      |
| Omphalocle (gastrointestinal)         Anal atresia (gastrointestinal)         Tracheoesophageal fistula (gastrointestinal)         Image: State of the state | Gastroschisis (gastrointestinal)   |      |
| Anal atresia (gastrointestinal) Tracheoesophageal fistula (gastrointestinal) Add Add  | Omphalocele (gastrointestinal)   |      |
| Tracheoesophageal fistula (gastrointestina) Add   | Anal atresia (gastrointestinal)  |      |
| Add   | Tracheoesophageal fistula (gastrointestinal)   |      |
|   |  | Add  |
| Destroy Defaults D. C. William and CO.  |  | , au |
| Remove Restore Defaults Default list contains 53 items.   | Remove Restore Defaults Default list contains 53 items.                                    |      |
| OK Carcel   |  |      |
|   | Or Canor   |      |

# 11

### Adding a short Phenotype text

- 1. Click inside the Add Phenotype Type field, then enter your new Phenotype.
- 2. Click Add.

### Deleting a short Phenotype text

- 1. Click to highlight the Phenotype you want to delete.
- 2. Click Remove.

### Re-arranging the currently displayed Phenotypes order

- 1. Click to highlight the Phenotype you want to move.
- 2. Drag and drop it to its new location (order).
- 3. If needed, repeat steps 1-2 to re-arrange additional Phenotypes.

### **Restoring the factory default Phenotypes**

1. Click Restore Defaults.

The factory default Phenotypes are now restored.

- Adding annotations at the sample (xxCHP) file level
- Right-click on a File name you want to add a Sample level annotation to. A menu appears.
- 2. Click View/Edit Properties.

The File Properties window appears. (Figure 269)

| File Properties                       |                        |
|---------------------------------------|------------------------|
| Basic Sample Properties Extended      |                        |
| Protect File                          |                        |
| File                                  |                        |
| Constutional_Blood_01_CytoScanHD.cyho | .cychp                 |
| Genome                                | NetAffx Build          |
| hg19                                  | 33                     |
| Array                                 | Modified               |
| CytoScanHD_Array                      | Jan 3, 2015 9:26:46 AM |
| oytoodan ho_yaray                     |                        |
|                                       |                        |
|                                       |                        |
|                                       |                        |
|                                       |                        |
|                                       |                        |
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|                                       |                        |
|                                       |                        |
|                                       |                        |
|                                       |                        |

3. Click on the Sample Properties tab. (Figure 270)

| Fig | ure 270 Sample Properties window |
|-----|----------------------------------|
| -   | File Properties X                |
|     | Basic Extended Sample Properties |
|     | Sample ID                        |
|     |                                  |
|     | sample Type                      |
|     | Phenotype                        |
|     |                                  |
|     |                                  |
|     |                                  |
|     |                                  |
|     |                                  |
|     |                                  |
|     | Interpretation                   |
|     |                                  |
|     |                                  |
|     |                                  |
|     |                                  |
|     |                                  |
|     | OK Cancel                        |

- 4. Use this window to Add/Enter Sample ID(s), choose a Sample Type(s), enter Phenotype(s), and Sample Interpretation(s). **Note:** The Sample ID defaults to the File Name, but you can edit/change the Sample ID name if you want.
- 5. Click **OK**.



# Segment annotations

Segment level annotations such as Call, Interpretation and Inheritance can be added to segment data.

# Setting up the calls feature

**Note:** If you are using a user profile from a previous version of ChAS, your default set of Calls will NOT appear in the Calls drop-down list. To restore them, click on Edit User Configurations  $\rightarrow$  Vocabularies  $\rightarrow$  Calls, then click the Restore Defaults button, as shown in Figure 271 on page 248.

### Adding and removing calls

1. Click **Preferences**  $\rightarrow$  **Edit User Configurations** or click  $\bigcirc$  on the upper tool bar.

The User Configuration window appears.

2. Click the **Vocabularies** tab.

The Calls window tab appears. (Figure 271)

| Figure 271 User Configuration window - Vocabularies tab window                              |
|---|
| User Configuration X  |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports |
| Call Interpretation Inheritance Sample Type Phenotype                                       |
| Add or remove short texts. Drag with the mouse to change the order.                         |
| Pathogenic<br>Likely Pathogenic<br>Unknown Significance<br>Likely Benign<br>Benign          |
| Add   |
| Remove         Restore Defaults         Default list contains 5 items.                      |
| OK Cancel   |



### Adding, deleting, and re-arranging calls

The Calls window enables you to Add, Delete and Re-arrange current Calls.

### Adding calls to the Call drop-down list

- 1. Click inside the Add Call field, then enter your new Call.
- 2. Click Add.

Your newly added Call now appears in the Call drop-down-list.

### Deleting calls from the Call drop-down list

- 1. Click to highlight the Call you want to delete.
- 2. Click Remove Call.

Your newly deleted Call is removed from the Call drop-down-list.

### Re-arranging the order of Calls in the Call drop-down list

- 1. Click to highlight the Call you want to move.
- 2. Drag and drop it to its new location (order).

Your Call is now in its revised position/order within the Call drop-down-list.

3. If needed, repeat steps 1-2 to re-arrange additional Calls.

### Restoring the factory default Calls

1. Click Restore Defaults.

The factory default Call(s) are now populated in the Call drop-down-list.

Using the calls feature

### Method 1: At the segments table

- 1. Click the Browser's Segments tab.
- 2. Scroll the Segment table to the right until you see the Call column.
- 3. Locate the appropriate row.
- 4. Single-click inside the field.

A blue drop-down bar appears.

 Click on the drop-down to reveal the list of available Calls, as shown in Figure 272

| Figure  | 272 Calls drop                | -down menu bar  |              |             |                       |
|---------|-------------------------------|-----------------|--------------|-------------|-----------------------|
| <b></b> | 🗟 🗎 Σ 🔢 🗄 🏚 🐼 🔲 157 results 🚺 |                 |              |             |                       |
| (kbp)   | Marker Count                  | Genes           | OMIM ® Genes | CytoRegions | Call                  |
| 38,092  | 36,223                        | PLEKHG4B, LRR   | N/A          |             | <u>^</u>              |
| 8,739   | 10,198                        | AHRR, C5orf55,  | N/A          |             |                       |
| 8,304   | 10,048                        | SEMA5A, SNOR    | N/A          |             | Pathogenic            |
| 1,949   | 1,176                         |                 | N/A          |             | Likely Pathogenic     |
| 15,225  | 10,752                        | CDH18, GUSBP1   | N/A          |             | Linknown Significance |
| 207     | 168                           | RAI14, TTC23L,  | N/A          |             | Likoly Popign         |
| 2.026   | 2.188                         | PRLR. SPEF2. IL | N/A          |             | Likely benign         |



- 6. Click to select the appropriate Call.
- 7. Click outside the field or press **Enter** on the keyboard.
- 8. Your Call is entered. Note that the **Curation By** column is populated with the user's Windows login ID (left) and ChAS User Profile ID (right). (Figure 273)

| Figure 27 | Figure 273 Call is entered and Curation By field is populated         |             |               |                |                          |    |
|-----------|---|-------------|---------------|----------------|--------------------------|----|
| Karyovie  | 👭 Karyoview ষ 🔝 Segments 🔊 🕌 CytoRegions 🔊 🧔 Overlap Map ষ 🖾 Graphs 🥄 |             |               |                |                          |    |
|           | 🗎 Σ 🔢 🗄 🛊 🚮 🗹 🗆   |             |               |                | 157 results              | 11 |
|           | OMIM ® Genes  | CytoRegions | Call          | Interpretation | Call & Interpretation By |    |
| IG4B, LRR | N/A   |             | Likely Benign |                | ppavic:Pete_11           | ^  |
| C5orf55,  | N/A   |             |               |                |                          |    |
| 5A, SNOR  | N/A   |             |               |                |                          |    |
|           | N/A   |             |               |                |                          |    |
| 8, GUSBP1 | N/A   |             |               |                |                          |    |
| TTC23L,   | N/A   |             |               |                |                          |    |
| SPEF2, IL | N/A   |             |               |                |                          |    |

A Call can be assigned to multiple segments at the same time. To do this:

- 1. Shift-click or Ctrl-click on the Calls fields for the segments you want to assign to the same call to.
- 2. Right-click on the highlighted area, then click Set Value.
- 3. Select the Call from the drop-down, then click **OK** to assign that call to all the selected segments.

### Method 2: At the View/Edit Annotation Properties Window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click on the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

The Annotation Properties window appears.

2. Click the **Curation** tab.

The Interpretation window tab appears: (Figure 274)



| Figure 274 Annotatio           | on Properties window - Interpretation windo         | w          |
|--------------------------------|---|------------|
| Annotation Properties          |   | ×          |
| General Additional Curation    |   |            |
| Call                           | Inheritance   | OKR        |
|                                | vv  | ~          |
| Call Approval                  | ✓ Use In Export                                     |            |
| ISCN Copy Number Text          |   | Reset ISCN |
| Microarray Nomenclature 20     | 16: arr[GRCh37] 8q24.21q24.3(129458472_140761415)x3 |            |
| Segment Interpretation (Right- | click to add preconfigured snippets)                |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
|                                |   |            |
| Curation Time                  | Curation By   |            |
|                                |   |            |
|                                | OK Cancel   |            |
|                                | Calicer   |            |

 Click the Call drop-down menu, then select your appropriate interpretation Call. (Figure 275)

| Figure 275 Call drop-down menu |  |  |  |
|--------------------------------|--|--|--|
| General Additional Call &      |  |  |  |
| Call                           |  |  |  |
| ×                              |  |  |  |
| 1                              |  |  |  |
| Pathogenic                     |  |  |  |
| Likely Pathogenic              |  |  |  |
| Unknown Significance           |  |  |  |
| Likely Benign                  |  |  |  |
| Benign                         |  |  |  |



### Adding or removing interpretation snippets

The Snippets feature can be used in conjunction with the free-typing Interpretation field (below the Calls drop-down). It allows for a convenient "shortcut" when common words or phrases are used often in an interpretation.

1. Click **Preferences** → **Edit User Configurations** or click bar.

The User Configuration window appears.

2. Click the Vocabularies tab, then click the Interpretation tab.

The Interpretations window tab appears. (Figure 276)

| Figure 276 User Configuration window - Interpretation tab window                            |   |
|---|---|
| User Configuration  | × |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports | _ |
| Call Interpretation Inheritance Sample Type Phenotype                                       |   |
| Add or remove short texts. Drag with the mouse to change the order.                         |   |
| The CNV in this region does not overlap any genes of known function                         |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
|   |   |
| Region found in common population (DGV)   | ן |
| Remove All Default list contains 0 items.   | - |
| OK Cancel   | _ |

- 3. At the Interpretation window, click inside the field shown (Figure 276), then enter the snippet you want to use with your interpretation(s).
- 4. Click Add.

The snippet now appears and is saved in the Interpretations List pane.

- 5. Repeat steps 3 and 4 to add additional snippets.
- 6. Click **OK**.

### Removing a saved snippet

- 1. Click to highlight the snippet you want to remove.
- 2. Click Remove.
# 11

#### Removing multiple saved snippets

- 1. Shift click or Ctrl click to highlight each snippet you want to remove.
- 2. Click Remove All.

# Using the interpretation snippets feature

#### Method 1: At the segments table

- 1. Click the Browser's Segments tab.
- 2. Scroll the Segment table to the right until you see the Interpretation column.
- 3. Locate the appropriate row.
- 4. Click inside the field.
- 5. Right-click on the flashing cursor. If the field has existing text, place the flashing cursor to the point where you want to add a snippet, then right-click.
  - A floating drop-down menu bar appears. (Figure 277)

| Figure 277 Floating Snip | pets drop-down menu     | bar              |                 |                          |              |
|--------------------------|-------------------------|------------------|-----------------|--------------------------|--------------|
|                          |                         |                  |                 |                          |              |
| oview ষ 🔠 Segments 🔻 🕌 C | CytoRegions 🎙 🥊 Overlap | Map 🎙 🖾 Graphs 🎙 |                 |                          | [            |
| ) 🛎   Σ 💷 🎚 🛊   🚮        | <b>V</b>                |                  |                 |                          | 132 results  |
| nes                      | CytoRegions             | Call             | Interpretation  | Call & Interpretation By | Materially M |
|                          |                         |                  | Click to select |                          |              |
|                          |                         |                  |                 |                          |              |
|                          |                         |                  |                 |                          |              |

# 6. Click the snippet bar's drop-down to display the available snippets, then click on the appropriate snippet. (Figure 277)

| Figure 278 Snippets drop  | o-down menu             |                  |                      |                               |                   |
|---------------------------|-------------------------|------------------|----------------------|-------------------------------|-------------------|
| yoview ষ 🔝 Segments 🔊 🔐 ( | CytoRegions ষ 🧲 Overlap | Map 🎙 🔀 Graphs 🎙 |                      |                               |                   |
| ] 🛎   Σ 🔢 🛚 🕸 🚮           |                         |                  |                      | [                             | 132 results       |
| anes                      | CytoRegions             | Call             | Interpretation       | Call & Interpretation By      | Materially M      |
|                           |                         |                  |                      |                               | >^                |
|                           |                         |                  | Close                |                               | ~                 |
|                           |                         |                  | Close                |                               |                   |
|                           |                         |                  | The CNV in this re   | gion does not impact any gene | es with known fur |
|                           |                         |                  | Gene's function is i | impacted.                     |                   |
|                           |                         |                  | Save for later. Do r | not discard!                  |                   |

The snippet appears in the appropriate Segment Interpretation row, as shown in Figure 279.



| Figure 279 Snipp      | et appears i     | n field               |             |                     |       |                    |  |     |
|-----------------------|------------------|-----------------------|-------------|---------------------|-------|--------------------|--|-----|
|                       |                  |                       |             |                     |       |                    |  |     |
| Segments              | Regions 🎙 🏼 🏓 Ov | erlap Map 🎙 📈 Graphs  | s 🎙 📑 Varia | nts 🎙 💷 Query Sar   | nples | Query Segments     | , <b>1</b>                                 |     |
| Σ Π Φ                 |                  |                       |             |                     |       |                    | 8 resu                                     | lts |
| ount OMIM ® Genes     | DB Count Both    | CytoRegions           | Call        | Segment Interpretat | ion   | Curation By        | Microarray Nomenclature (ISCN 2016)        | M   |
| 1 COL24A1 (610025)    |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] 1p22.3(86225156_86399016)x1    | -   |
| 0                     |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] 10q23.1(85645599_85701417)x3   |     |
| 0                     |                  | 1 CytoRegions Not Set | Pathogenic  | no genes            |       | casey.gates:cgates | arr[GRCh37] 11q11(55402801_55452996)x0     |     |
| 0                     |                  | 1 CytoRegions Not Set | Benign      | intronic            |       | casey.gates:cgates | GRCh37] 14q32.33(106079823_10632907        |     |
| 1 FAM30A (616623)     |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] 14q32.33(106329184_10677733    |     |
| 4 KANSL1 (612452), LR |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] 17q21.31(44187492_44784639)x4  | ŧ   |
| 0                     |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] Xp22.33 or Yp11.32(433595_494. |     |
| 2 SHOX (312865), SHO  |                  | 1 CytoRegions Not Set |             |                     |       |                    | arr[GRCh37] Xp22.33 or Yp11.32(513590_729. |     |
|                       |                  |                       |             |                     | _     |                    |  |     |
|                       |                  |                       |             |                     |       |                    |  |     |
|                       |                  |                       |             |                     |       |                    |  | •   |

7. Click outside the field.

Your snippet is entered. **Note:** The **Curation By** column is populated with the user's Windows login ID (left) and ChAS User Profile ID (right), as shown in Figure 279.

#### Method 2: At the View/Edit annotation properties window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click on the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

The Annotation Properties window appears.

- 2. Click the Curation tab.
- 3. Type in your interpretation. If at any point you want to insert your preset snippet(s), right-click inside the interpretation window.

A drop-down bar graphic appears. (Figure 280)

| Figure 280    | Drop-down menu bar |   |
|---------------|--------------------|---|
|               |                    |   |
| Click to sele | oct                | ~ |
|               |                    |   |

4. Click to select the appropriate snippet or click Close to exit. (Figure 281)

| Figure 281 Snippet drop-down                                    |
|---|
| Close   |
| Close   |
| The CNV in this region does not impact any genes with known fur |
| Gene's function is impacted.                                    |
| Save for later. Do not discard!                                 |



Your snippet (preset word, sentence, or phrase) now appears "in line" with your typed text. (Figure 282)

| Figure 282 Snippet in the Interpretation field pane                   |  |
|---|--|
| Interpretation  |  |
| The CNV in this region does not impact any genes with known function. |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |
|   |  |

| Adding or         | 1. | Click Preferences $\rightarrow \rightarrow$ Edit User Configurations or click $[]$ | on the upper |
|-------------------|----|--|--------------|
| removing          |    | tool bar.  |              |
| inheritance calls |    | The User Configuration window appears.   |              |

The User Configuration window appears.

2. Click the Vocabularies tab, then click then Inheritance tab.

The Inheritance window tab appears. (Figure 283) The Inheritance window enables you to Add, Delete and Re-arrange current Inheritance calls.



| Figure 283 User Configuration window - Inheritance window tab                               |     |
|---|-----|
| User Configuration  | ×   |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports |     |
| Call Interpretation Inheritance Sample Type Phenotype                                       |     |
| Text snippets Add or remove short texts. Drag with the mouse to change the order.           |     |
| dn  |     |
| pat   |     |
| unknown   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   |     |
|   | Add |
| Remove Restore Defaults Default list contains 4 items.                                      |     |
| OK Cancel   |     |
|   |     |

 Check the Include Inheritance in Microarray Nomenclature check box to have Inheritance column entries automatically appended to the Microarray Nomenclature field.

Example: If the dn is selected in the Inheritance column then the Microarray Nomenclature field would be updated to read: arr[GRCh37] 22q13.31(44582928\_44851090)x3 dn

#### Adding an Inheritance call to the Inheritance drop-down list

- 1. Click inside the Add Inheritance Call text field (Figure 283), then enter your new call.
- 2. Click Add.

#### Deleting a call from the Inheritance drop-down list

- 1. Click to highlight the call you want to delete.
- 2. Click Remove.

#### Re-arranging the order of calls displayed in the Inheritance drop-down list

1. Click to highlight the Inheritance you want to move.



2. Drag and drop it to its new location (order).

Your call is now in its revised position/order within the Inheritance drop-downlist.

3. If needed, repeat steps 1-2 to re-arrange additional Inheritance.

# Using the inheritance feature

#### Method 1

- 1. Click the Browser's Segments tab.
- 2. Scroll the Segment table to the right until you see the Inheritance column.
- 3. Single-click inside the field.

A blue drop-down bar appears.

4. Click on the drop-down to reveal the list of available Inheritance Calls. (Figure 284)

| Fi  | gure 284 C | alls drop-dow   | n menu bar         |  |                       |             |
|-----|------------|-----------------|--------------------|--|-----------------------|-------------|
|     | PF 😢 🗎     | Σ 🔢 🕴 🛊         | <b>M</b>           |  |                       | 103 results |
|     | Gene Count | Genes           | OMIM ® Genes Count | OMIM ® Genes                               | CytoRegions           | ▼ Inheritan |
| 733 | 6          | LINC00487, CMP  | 2                  | CMPK2 (611787), RSAD2 (607810)             | Cytoregions Not Set   | <u>^</u>    |
| 344 | 2          | MIR4432, BCL11A | 1                  | BCL11A (606557)                            | Cytoregions Not Set   |             |
| 216 | 67         | DNAH1, BAP1, P  | 39                 | DNAH1 (603332), BAP1 (603089), TNNC1 (1    | Cytoregions Not Set   | dn          |
| 408 | 23         | PTPRG, LOC100   | 8                  | PTPRG (176886), FEZF2 (607414), CADPS (.   | . Cytoregions Not Set | mat         |
| 223 | 139        | PLEKHG4B, LRR   | 76                 | SDHA (600857), PDCD6 (601057), AHRR (6.    | . Cytoregions Not Set | nat         |
| 198 | 54         | AHRR, C5orf55,  | 27                 | AHRR (606517), EXOC3 (608186), SLC9A3 .    | . Cytoregions Not Set | unknown     |
| 048 | 29         | SEMA5A, LOC10   | 17                 | SEMA5A (609297), TAS2R1 (604796), CCT5.    | Cytoregions Not Set   | unknown     |
| 176 | 0          |                 | 0                  |  | Cytoregions Not Set   |             |
| 752 | 29         | CDH18, GUSBP1   | 20                 | CDH18 (603019), CDH12 (600562), PMCHL.     | . Cytoregions Not Set |             |
| 188 | 15         | PRLR, SPEF2, IL | 7                  | PRLR (176761), SPEF2 (610172), IL7R (146.  | . Cytoregions Not Set |             |
| 536 | 2          | WDR70, GDNF     | 1                  | GDNF (600837)                              | Cytoregions Not Set   |             |
| 808 | 61         | EMB, PARP8, LO  | 35                 | ISL1 (600366), PELO (605757), ITGA1 (1929. | Cytoregions Not Set   | ^           |
| 808 | 61         | EMB, PARP8, LO  | 35                 | ISL1 (600366), PELO (605757), ITGA1 (1929. | . Cytoregions Not Set | v           |
| 277 | 054        | LIDAEX CENTRO   | 100                | UDAEX (\$10054) OF of 20 (\$00740) TIEAD ( | Outorogiona Not Cot   |             |
| <   |            |                 |                    |  |                       | < >         |

- 5. Click to select the appropriate Inheritance.
- 6. Click outside the field or press Enter on the keyboard.

Your Inheritance is entered.

#### Method 2: From the View/Edit annotation properties window

1. From any graphical view (Karyoview, Selected Chromosome View, or Details View), right-click on the segment you want to add an interpretation to, then click the menu selection **View/Edit Annotation Properties**.

The Annotation Properties window appears.

- 2. Click the Curation tab.
- 3. Select the Inheritance call from the drop down.



# **Adding Oncomine Reporter annotations**

You may add annotations based on approved Oncomine Reporter search terms. Once your segments have been annotated with Oncomine Reporter term(s), the Segments Table can be exported as a txt file and directly uploaded to Oncomine Reporter for literature searches based on the assigned annotation.

The annotation options for use with Oncomine Reporter are a controlled vocabulary.

**Note:** The options available in the drop-down list for the Oncomine Reporter column are compatible nomenclature with Oncomine Reporter application.

- 1. Select to view the Oncomine Reporter column in the Segments Table. See "Selecting columns to display or hide" on page 325.
- 2. Click in the Oncomine Reporter column for a given segment to assign the appropriate annotation for that segment. Segments on a given chromosome will only see relevant Oncomine Reporter terms for that chromosome. For example, if there is a gain of Chromosome 8q, you will only see Oncomine Reporter terms for Chromosome 8. All other terms for other chromosomes are hidden for that segment.

| Figure 285                       | Oncomine R | eporter column exa             | mple  |                             |
|----------------------------------|------------|--------------------------------|---|-----------------------------|
| CytoRegions                      | Overlap    | ∾ 🖸 💟 💽 🖓 Map 🎙 🕅 🕅 Graphs 🎙 🏾 | ▼ ♥  <br>↓ Variants ▼ III Query Samples ▼ III Query | / Segments 🎙                |
| ‡   ☑ □   ∎                      | CN State   | Туре                           | Full Location                                       | Oncomine Reporter           |
| 34C2_0N_100T_W                   | 4.00       | ▲ Gain                         | chr8:108295136-110598198                            |                             |
| 34C2_0N_100T_W                   | 1.00       | V Loss                         | chr11:88087357-134938847                            |                             |
| 34C2_0N_100T_W<br>34C2_0N_100T_W | 3.00       | ▲ Gain<br>▲ Gain               | chr12:189400-1104608<br>chr12:1216632-50589836      |                             |
| 34C2_0N_100T_W<br>34C2_0N_100T_W | 3.00       | ▲ Gain<br>▼ Loss               | chr12:50895901-133818115<br>chr13:19084823-83475931 |                             |
| 34C2_0N_100T_W                   | 3.00       | ▲ Gain                         | chr15:94993371-97760294                             |                             |
| 34C2_0N_1001_W<br>34C2_0N_100T_W | 1.00       | ▼ Loss<br>▼ Loss               | chr17:400959-27580756                               | Deletion 13<br>Deletion 13q |
| 34C2_0N_100T_W<br>34C2_0N_100T_W | 3.00       | ▲ Gain<br>▲ Gain               | chr17:27600648-35975176<br>chr17:36165624-37832222  | Chromosomal abnormality     |
| 34C2_0N_100T_W                   | 4.00       | ▲ Gain<br>▲ Gain               | chr17:37898475-38563079<br>chr17:38570168-80263427  |                             |
|                                  | 0.00       |                                |   |                             |

For information on Oncomine Reporter Table State, "Saved table states" on page 328 on Table States.



# Tracking and reviewing the log file

1. Right-click on the file, then click View Process Pipeline. (Figure 286)



#### The Process Pipeline window appears. (Figure 287)



2. Click Smooth and Join for its description and summary. (Figure 287)

3. Click **Edits** for its description and summary of the file's past user edits. (Figure 288)



4. Click Details.

A Edit Details window appears featuring an Edit and Log tab. (Figure 289)



| Figure  | 89 Process Pipeline - Edit tab                             |          |
|---------|--|----------|
| Edit De | hils   | ×        |
|         | Edits Log Modified Properties Removed Properties           |          |
| U       | Order Operation Label                                      |          |
|         | 1 interpret Gain2.0_Constutional_Blood_01_CytoScanHD.cy    | ħ        |
|         | 2 interpret Loss0.0_Constutional_Blood_01_CytoScanHD.cy    | rh       |
|         | 3 interpret Loss1.0_Constutional_Blood_01_CytoScanHD.cy    | rh       |
|         | 4 interpret Loss1.0_Constutional_Blood_01_CytoScanHD.cy    | h        |
|         | 5 interpret Loss0.0_Constutional_Blood_01_CytoScanHD.cy    | <u>h</u> |
|         | / interpret Gain3.0_Constutional_Blood_01_CytoScanHD.cy    | h        |
|         | 9 Interpret Loss1.0_Constitutional_Blood_01_CytoScanHD.cy  | li<br>dh |
|         | 11 interpret Loss1.0_Constitutional_Blood_01_CytoScanHD.cy | h<br>h   |
|         |  |          |
|         |  |          |

The **Edit** tab lists only recent edits that were NOT reverted. Use the horizontal scroll bar to reveal additional edit information. For a complete historic account of all edits made (including reversions of some edits), click on the **Log** tab.

5. Click **OK** to return to the **Process Pipeline** window or click the **Log** tab.

The **Log** tab displays an extensive summary of the file's edited history to date. Use the horizontal scroll bar to reveal additional log information. (Figure 290)

|     | 不   |   |    |
|-----|-----|---|----|
| 1.5 | 18  | 2 | 1  |
| Y   | 12  |   |    |
|     | i k |   |    |
|     | 1.5 |   | 10 |
|     |     |   |    |

| 01001 | Operation     | Label     | Chromoso | Min         | Max         |
|-------|---------------|-----------|----------|-------------|-------------|
|       | merne         | seg165-m  | chr8     | 2 095 440   | 29 153 037  |
| 1     | revert-mer    | seq165-m  | chr8     | 2 095 440   | 29 153 037  |
| 2     | merae         | seq165-m  | chr8     | 2.095.440   | 29.153.037  |
| 3     | revert-mer    | seg165-m  | chr8     | 2,095,440   | 29,153,037  |
| 4     | delete        | seg302-de | chr8     | 43,308,594  | 57,006,476  |
| 5     | delete        | seg241-de | chr8     | 20,871,989  | 21,381,652  |
| 6     | delete        | seg195-de | chr8     | 9,799,952   | 10,428,420  |
| 7     | delete        | joined551 | chr8     | 5,515,516   | 8,657,463   |
| 8     | delete        | seg165-de | chr8     | 2,095,440   | 3,238,755   |
| 9     | delete        | seg235-de | chr8     | 18,632,189  | 19,150,880  |
| 10    | delete        | seg243-de | chr8     | 21,453,224  | 22,037,634  |
| 11    | delete        | seg269-de | chr8     | 28,608,428  | 29,153,037  |
| 12    | delete        | seg245-de | chr8     | 22,489,199  | 23,142,234  |
| 13    | revert-delete | seg195-de | chr8     | 9,799,952   | 10,428,420  |
| 14    | delete        | seg466-de | chr11    | 101,950,388 | 102,575,754 |
| 15    | delete        | seg466-de | chr11    | 101,950,388 | 102,575,754 |

6. Click OK to return to the Process Pipeline window. (Figure 291)



- 7. Click Calculated Properties for its description and summary.
- 8. Click **OK** to return to the ChAS browser.



# **Using CytoRegions**

# CytoRegions overview

The CytoRegions feature enables you to define parts of the genome that are of special interest to you.

**Note:** The CytoRegions feature is designed for use with up to a few thousand regions. Larger numbers of regions can be used, but will impact performance. A reference annotation file, such as Genes, is not recommended for use as a CytoRegions file due to the large number of reference annotations.

To use CytoRegions, you need to select a file(s) with position information for regions of the genome as the CytoRegions file.

- Select Region information files in AED or BED format.
- Use existing Region file(s), or create a new one in AED format in ChAS, then add regions to it by selecting segments, annotations, or regions in other loaded files.
- Add annotations to regions to help you track the information. See "Creating and editing AED files" on page 283.

After selecting a CytoRegions file, you can:

- Use the Restricted Mode to display only Segments and graph data that appear in those regions. While in this mode, annotations are not hidden by CytoRegions or by the application of Restricted Mode.
- Use differential filtering options for these regions and for the rest of the genome.
- Protect a CytoRegions file. See "Protecting an AED file" on page 304.



# Selecting a CytoRegions information file

Select the CytoRegions file from the available region information files. See "Loading files" on page 117. The software automatically checks the hg version of an AED or BED file before loading. See Figure 292 for an example BED file. The file will not be loaded if the hg version does not match what is loaded in the ChAS Browser. If an hg version is not found for the AED or BED file, a warning message appears.

| Figure 292 Example BED file  |                         |
|--|-------------------------|
| Sample BED.txt - Notepad   |                         |
| # Sample BED file. # UCSC Browser and ChAS should both accept this file. # Lines beginning with "#" are ignored.   |                         |
| # This sample data comes from UCSC<br># for dbSNP 129 database on hg18 on chr6<br># near the gene SLC22A1 (chosen at random)<br># The columns ave:                         |                         |
| # Sequence Min Max Name<br># The "browser" line is ignored by ChAS, used by UCSC Browser.<br># The "track" line is used by both ChAS and UCSC Browser.                     |                         |
| # ChAS ignores all parameters except "db".<br># UCSC Browser also uses "name", "description" and others.<br>#<br># Specify genome version on track line as: db="hg18"<br># |                         |
| browser position chr6:160460899-160501368<br>track_db="hg18" pame="SNPs near SLC22A1" description="test"<br>chr6 150457853 160462854 re41262793                            |                         |
| chr6 160462997 160462998 rs34447885<br>chr6 160463023 160463024 rs34570655<br># This comment is ignored  | <sup>•</sup> hg version |
| chr6 160463069 160463070 rs35888596<br>chr6 160463079 160463080 rs2297373<br># This comment is ignored   |                         |

Do either of the following to select a CytoRegions file:

- In the files list, right-click a file and select Include as CytoRegion on the shortcut menu, as shown in Figure 293.
- Multiple files can be selected to be included in the CytoRegions. To do this, rightclick on each AED/BED file to be included, then select Include in CytoRegions.

Note: All files (included in CytoRegions) are treated as they are one file.



| Figure 293 Select          | a CytoRegions file from the file list                    |          |
|----------------------------|--|----------|
|                            |  |          |
| <u>File View Exports A</u> | nalysis <u>C</u> hAS DB <u>P</u> references <u>H</u> elp |          |
| 📔 🕅 🖉 V S                  | T 7 🗷 🎟 🖀 💽 🗚 😪 🖌  |          |
| Files                      | 🗧 🏭 Karvoview 🎙  🔚 Sec                                   | ments    |
| 🗹 🛐 ♂ 11-2243_B11_         | 0737_PS_POP#31.c   |          |
| Constutional E             | Blood 01 CytoScanH                                       |          |
|                            | VIIM ® Genes Count OMI                                   | M ® Ge   |
|                            | Close  | 4A1 (6   |
| 🔲 🎷 DGV                    | Save   | -5 (611- |
| Default H                  |  | (10 (6   |
|                            | Expand Annotation Track                                  | IAP2 (   |
| 🔲 🛃 Ensembl G              | Export File as AED                                       | 12       |
| 🗹 🍀 Genes 👔                | Search in selected file Ctrl+F                           | (6016    |
| 🔲 🚦 OMIM ® G               | View and Edit annotations in this file                   | -        |
| 🔲 💈 OMIM 🛽 Pł              | CuteRegion for Targeted XON applying                     |          |
| Segmental                  | CytoRegion for Targeted XON analysis                     |          |
|                            | Include in CytoRegions                                   | H        |
|                            | Set File as Overlap Map Select/unselect the file to in   | clude ir |
|                            | View/Edit Properties                                     | to Coor  |
| Named Setting              | Set Custom Color   | noscar   |
|                            | Clear Custom Color                                       | OP#31    |
|                            |  | _        |
| Data Turan                 | Marrie Multi Cample Marrier                              | toScar   |

### Or

■ From the menu bar, click View → Select as CytoRegions file(s).

Alternatively, in the CytoRegions Tab of the Upper display, click the **Select to include in CytoRegions** witton.

The Select CytoRegions File(s) window opens. (Figure 294)

| Figure 294 Select CytoRegions File(s) window  |             |
|---|-------------|
| Select CytoRegions File(s)  | ×           |
| Image: Decipher_DDG2P_with_genomic_coordinates_20161010.aed         Image: My Lab Cases.aed         Image: OncoScan_Impact_Genes.aed         Image: Orgonal Concestance         Image: Orgonal Concestance< | Select None |

Select a regions information file(s) to use for CytoRegions, then click **OK**.

**Note:** You may select more than one Region file (AED/BED) to include as CytoRegions. ChAS handles multiple selected files as single (larger) CytoRegion file.

The **Create New** feature is described in "Creating an AED file of annotations" on page 283.



**Note:** If a region file was selected for the CytoRegion file before shutting off the software, that file is automatically loaded as the CytoRegion file after the software is opened the next time with the same user profile. To clear a CytoRegions file map from ChAS, click **Select None** (Figure 294). Alternatively, right-click the file in the Files list, then deselect **Include in CytoRegions** on the shortcut menu to toggle the check mark off.

# **Viewing CytoRegions**

After selecting a CytoRegions file, it can be displayed in either a graphic view or table.

- "CytoRegions in the graphic views" on page 266
- "CytoRegions table" on page 268

**Note:** CytoRegions that share genomic coordinates with a detected segment are listed in the "CytoRegions" column of the Segments table. See "Segments table" on page 334.

# CytoRegions in the graphic views

Regions specified in the CytoRegions file are displayed as dark gray stripes in the Karyoview and Chromosome Display (Figure 295) and Detail View (Figure 296).







You can turn off highlights by un-checking the View menu's **Hide CytoRegion Background Highlights** check box. (Figure 297) This action keeps the CytoRegions in the Table View, however they are no longer highlighted in the Graph Views.

| Figure 297 View menu - Hide CytoRegion check box |                                       |             |  |
|--|---------------------------------------|-------------|--|
| L 📟  |                                       |             |  |
| 2  | Zoom to selection                     | Ctrl+Space  |  |
| Q  | Zoom out × 3                          | Ctrl+Minus  |  |
| •  | Zoom in × 3                           | Ctrl+Equals |  |
|  | Auto-zoom to table selection          |             |  |
| 🗆 📓  | E <u>d</u> it Mode                    |             |  |
|  | Restrict to CytoRegion                | Ctrl+R      |  |
|  | Selected Chromosome View              | Ctrl+L      |  |
| 🗹 <u>+1</u>                                      | Expand/Collapse Annotations           |             |  |
| 🗹 🛣  | Segment Symbols                       |             |  |
|  | Mouse-over Pop-ups                    |             |  |
|  | Hide CytoRegion background highlights |             |  |
|  | Hide Karyoview Highlights             |             |  |
| 🔲 😵  | Switch Color Scheme                   |             |  |
| ۱ ا  | Group Data by File                    |             |  |
| 0 III  | Group Data by Type                    |             |  |



# CytoRegions table

The CytoRegions table (Figure 298) shows the intersections between regions in the designated CytoRegions file and the segments in the Segments table. Every region in the CytoRegions file will be listed on at least one line of the CytoRegions table, even if it does not intersect any segments. For those regions which intersect one or more segments, there will be one table row for each intersection. Depending on the columns which have been used to sort the CytoRegions table, these rows may or may not be near each other. A segment that intersects more than one region in the CytoRegions file appears multiple times in the CytoRegions table, one row for each intersection.

In the CytoRegions table, the "CN State" value corresponds to the state of copynumber and mosaicism segments that intersect the CytoRegion. There is no CN State value for other types of segments that do not correspond to a copy-number call.

#### Figure 298 CytoRegions Table 🟥 Karyoview 🎙 🛤 Segments 🎙 📕 CytoRegions 🖣 🏓 Overlap Map 🎙 📨 Graphs 🎙 🔛 Variants 🎙 💷 Query Samples 🎙 💷 Query Segments 🎙 🔜 🔐 🗎 Σ 🔚 🖡 🐥 Tool bar 2,180 results CytoRegion Temerinte Dear-Belind Fit Portgimentation Stinuronie PERIPHERAL DEMYELINATING NEUROPATHY, CENTRAL DYSMY.. WAARDENBURG SYNDROME TYPE 2E WAARDENBURG SYNDROME TYPE 4C NEURODEGENERATION WITH BRAIN IRON ACCUMULATION 2B INFANTILE NEUROAXONAL DYSTROPHY 1 ADEMY 051 ICCINASE DEFICIENCY CytoRegion Type Min CytoRegion File Max Chromo.. 22 22 22 Default User Annotation 38,368,3.. 38, DECIPHER\_DDG2P\_with\_genomic\_coordinates\_20161010.aec 22 38,368,3... 22 38,368,3... 22 38,368,3... 22 38,507,5... 23 8,507,5... 22 40,742,5... 22 41,253,0... 22 41,488,6... 22 41,488,6... 22 41,484,6... 22 42,54,2 DECIPHER\_DDG2P\_with\_genomic\_coordinates\_20161010.aed DECIPHER DDG2P with genomic coordinates 20161010.aeg Default User Annotation 38. 38,3 38,5 38,5 40,7 Default User Annotation Default User Annotation Default User Annotation EPHROPATHY TYPE 1 Default User Annotation ADENYLOSUCCINASE DEFICIENCY 40, 41, 41, 41, Two different files within the rows of the CytoRegions File column can be displayed. INAL DEGENERATION 22 42,454,3. 22 42,454,3. 22 43,013,8. 42,4 SCHINDLER DISEASE METHEMOGLOBINEMIA DUE TO DEFICIENCY OF METHEMOGLO... Default User Annotation GALACTOSEMIA SYNPOLYDACTYLY, 3/3-PRIME/4, ASSOCIATED WITH METACARP.. CONGENITAL DISORDER OF GLYCOSYLATION TYPE 1G LEUKOENCEPHALOPATHY MEGALENCEPHALIC WITH SUBCORT.. 22 43,088,1.. 22 43,088,1.. 22 45,898,7.. 22 50,296,8.. 22 50,497,8.. 43 45,9 Default User Annotation 50. MICROCEPHALY AND CHORIORETINOPATHY WITH OR WITHOU... ARYLSULFATASE A DEFICIENCY PHELAN-MCDERMID SYNDROME 22 50,656,1. 22 51,061,1. 22 51,113,0. Default User Annotation Default User Annotation 50,6 51,0 Default User Annotation My Lab Cases.aed gain Default User Annotation Gain2.0\_Sample\_01.cyhd.cychp Y 25,848,0.. 26,3 test Loss (Exon Region)\_20170426\_152508\_007\_XONAlpha\_CMH\_CMH7... Loss1.0 Constutional Blood 01 CvtoScanHD.cvhd.cvchp 4 122,617,... 122 22 18,626,1... 21,5 loss Table Filtering field ilter "CvtoRegion" column: Clear Filter mapd (CHP Summary) snpQC (CHP Sum... wavinessSd (CHP Sum... antigenomicRatio (CHP S... medianf arrayType (Algorithm) QC 111 Store CytoscanHD\_Group2\_2.cyhd.cychp 0.23 0.089 0.14958863 CytoScanHD\_Array 19.66 943.0 modified (General) Oct 10, 2016 1:07:35 PM Apr 13, 2018 7:16:42 AM Feb 5, 2018 4:52:36 AM File ucscGenom... created (General) Oct 10, 2016 12:59:07 PM Jan 21, 2016 10:51:59 AM Oct 2, 2017 8:09:43 AM DECIPHER\_DDG2P\_with\_ge... hg19 # J. DECIPHER\_DDG2P\_with\_ge... hg19 # J. My Lab Cases.aed hg19 OncoScan\_Impact\_Genes.aed hg19

--- Detail View 🎙 🕲 QC and Sample Info 🎙 🗟 Chromosome Summary Data 🎙

#### Highlighting regions in the CytoRegions table and details view

If the Details View displays the CytoRegions file (the CytoRegions file is check marked in the Files list), you can conveniently find and view items.

Click a row in the CytoRegions table to select the corresponding annotation from the CytoRegions file. All of the lines for that region are highlighted in the table. The Details View zooms to the currently selected region. Note: If the Details View does not automatically zoom to the selected region, confirm that the Auto-zoom option is selected (click View → Auto-zoom to table selection from the menu bar.)



- In the Detail View, click a region or select multiple regions of the CytoRegion file to highlight all of the corresponding rows in the CytoRegions table. The CytoRegions table automatically scrolls to show at least one of the highlighted rows.
- To quickly find a particular Segment in the CytoRegions table, first double-click that segment in any of the views or in the Segments table (the current region will be set to that segment), then press the tool bar button in the CytoRegions table to show only the cytoregions in the current region.

### CytoRegions tool bar

The tool bar (Figure 299) provides quick access to table functions. The standard functions are described in "Standard tool bar controls" on page 324.

| Figure 299 CytoRegions table tool bar      |                            |
|--|----------------------------|
| 🔢 Karyoview 🎙 🔛 Segments 🎙 🛄 CytoRegions 🤻 | 🥊 Overlap Map 🎙 🖾 Graphs 🎙 |
| 🔠 📠 🗀   Σ   🏢 🎚 🜵   🔉                      | 12 results 📗               |

The tool bar has one specialized button.

Select CytoRegions file (See "Selecting a CytoRegions information file" on page 264 )

| CytoRegion Table<br>Column | Description  |
|----------------------------|--|
| CytoRegion File            | Name of the AED/BED file that contains the region.   |
| CytoRegion Type            | Type of file or element from which the CytoRegion is derived. Default User Annotations are annotations derived from AED or BED file. |
| CytoRegion                 | Identifier for region.   |
| Chromosome                 | Chromosome in which the region is located.   |
| Min                        | Starting position of the region.   |
| Мах                        | Ending position of the region.   |
| Size (kbp)                 | Size of the region.  |
| Segment Label              | Label assigned to the detected segment by ChAS.  |
| Segment Name/ID            | Name/ID of the Segment.  |
| Segment File               | Sample File that the segment was detected in.  |
| Segment Type               | Type of segment:<br>– CN loss or gain<br>– XON loss or gain<br>– Mosaic loss or gain<br>– LOH  |
| Segment Min                | Starting position of segment.  |



| CytoRegion Table<br>Column             | Description   |
|--|---|
| Segment Max                            | Ending position of segment.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic<br>position coordinates" on page 475). |
| Segment Size (kbp)                     | Size of the segment.  |
| CN State                               | Copy Number State (Displayed for Gain, Loss, and Mosaicism segment types).  |
| Shared Size                            | Size of the contact between segment and cytoregion.   |
| % of CytoRegion touching Segment       | How much of the CytoRegion is contacted by the Segment.   |
| % of Segment<br>touching<br>CytoRegion | How much of the detected segment is contacted by the CytoRegion.  |

You can:

- Use the common table functions to control the display of data in the table. See "Common table operations" on page 323.
- Export data in various formats. See "Exporting table data" on page 417..
- Search CytoRegions to display only regions of interest (see below).

# Searching CytoRegions

The Search CytoRegions feature enables you to search the "CytoRegion" column for text strings that match a search string.

- 1. Enter the search string in the Search CytoRegions box at the bottom of the CytoRegions table.
- 2. Press Enter.

The table displays only annotations that match the search string.

3. To restore the table, click **Clear Search Field**.



# Using filters with CytoRegions

If you have CytoRegions information loaded into ChAS, you have the option to apply different segment filtering parameters to the parts of the genome that are defined as CytoRegions and the parts of the genome that are not within these regions (Genome). For segment filtering to function as described below, the CytoRegion segment filters should always be set identical to or less stringent than the counterpart Genome segment filter settings.

The filter settings for CytoRegions and for the rest of the genome interact in different ways, depending upon whether:

- A cytoregion information file is selected
- Restricted Mode is selected

If a cytoregion information file is selected, but Restricted Mode is not selected:

- Segments wholly within a cytoregion are filtered using the CytoRegions filter settings.
- Segments that don't touch a cytoregion are filtered using the Whole Genome settings.
- A segment that touches both Genome and CytoRegions must pass both the CytoRegions filter thresholds and Whole Genome filter thresholds, otherwise it will not be shown.

If Restricted Mode is selected:

 Segments in or straddling cytoregion boundaries are filtered using the CytoRegions filter settings.

These rules also apply when a region information file has been selected as an Overlap Map and the Overlap filters are used.

### Using restricted mode

The Restricted mode enables you to view detected segments and graph data only in regions you have defined in advanced in the CytoRegions file.

**Note:** Restricted mode is not available unless a region information file or one of the Reference Annotations is selected for the CytoRegions function.

#### Selecting/De-selecting Restricted Mode

From the **View** menu, select **Restrict to CytoRegion**.

Or

Click the **Restricted Mode** button **He** from the main tool bar.

**Note:** Segments and graph data that are not in the defined CytoRegions are concealed in the display. Segments in or straddling CytoRegions boundaries are filtered using the CytoRegions filter settings. (Figure 300, Figure 301)









When you deselect Restricted Mode, an Exit Restricted Mode window appears. (Figure 302)

| Figure 302 E>   | kit Restricted Mode window               |
|-----------------|--|
| Exit Restricted | 1ode X                                   |
| Are you su      | re you want to turn off Restricted Mode? |
|                 | Yes <u>N</u> o                           |

Click **Yes** to exit Restricted Mode.

# Assigning a CytoRegion for targeted XON analysis

This option is designed for use with CytoScan XON array data. Assigning a CytoRegion for a Targeted XON analysis combines these two steps:

- 1. Assigns the file as the CytoRegion.
- 2. Automatically sets the appropriate filters for Targeted XON analysis.
  - Hides all segments in the Genome region and colors all the data gray.
  - Turns on all levels in CytoRegions to reveal any XON Region segment call and colors the data the assigned sample color.

## Creating an AED File from a gene list

To create an AED file from a Gene List for use with the CytoRegion for Targeted XON analysis or other region file (CytoRegion or Overlap Map) do the following:

- 1. Create a text tab delimited file containing your list of Genes for which you would like to generate an AED file. The gene coordinates will be pulled from a selected NetAffx Genomic Annotation file. The Gene List should just be a list of gene names with no header and saved as a text tab delimited file.
- 2. Click Exports  $\rightarrow$  Create and Export an AED file from a Gene List.

The following window appears: (Figure 303)



| Figure 303 Create Panel (AED) File from Gene List window                                      |             |
|---|-------------|
| ✗ Create Panel (Aed) File from Gene List  | ×           |
| Select NetAffx Genomic Annotations NetAffx Genomic Annotations File NetAffx Build 33.2 (hg19) |             |
|   | Select File |
| Select Output Panel (AED) File  | Select File |
|   | OK Cancel   |

- 3. Select the NetAffx Genomic Annotation file from the drop down list. The annotation database files visible are available files from the Library file folder. If you do not see a NetAffx Genomic Annotation file that you want to use, please copy the file into C:\Affymetrix\ChAS\Library and restart the ChAS browser.
- 4. Select an Input Gene List File: Click on Select File button and browse to the location of your TXT tab-delimited Gene List file.
- 5. Select an Output AED File: Click on the Select File button, select the location to save the output file, name the AED file, click **Save**.
- 6. Click OK to generate the AED file. (Figure 304)

| Fig | gure 304 Export Panel File confirmation window   |
|-----|--|
| ×.  | Export Panel File X  |
|     | 55 gene symbols found.<br>Successfully created "C:\CHAS 3.3\Charcot_Marie_Panel_File.aed". |
|     | 1 gene symbol(s) cannot be found:<br>ZNF673  |
|     | ОК   |

**Note:** If entries in the Gene List are not found in the NetAffx Genomic Annotation file, they will be listed in a message box. Any skipped entries can be added to the AED file. See "Creating and editing AED files" on page 283 and "Adding annotations to an AED file" on page 285.



# Using the overlap map and filter

# Overlap map and filter overview

- The Overlap Map enables you to show or hide segments in areas of the genome that you are not interested in. For example, in regions of Benign Copy Number Polymorphism, you can:
  - Specify regions of the genome in an Overlap Map File.
  - Optionally filter out detected segments that are overlapped by these regions. You can also specify the percentage of the segment (between 1 and 100%) that must be overlapped by the Overlap Map items before being filtered from display.
- The Overlap Map filter operates separately from the CytoRegions features, but you can apply different overlap map filtering parameters to CytoRegions and to areas outside of CytoRegions.
- Protect an Overlap Map file. See "Protecting an AED file" on page 304..

## Using the overlap filter

- 1. Select a region information file for the Overlap Map. For supported file types, see "Selecting the overlap map file" (below).
- 2. Set Percent Overlap value for each segment type you want to filter in the Segment Filters window. See "Using the overlap filter" on page 282.
- 3. Choose how you want to view the Overlap Map regions.
  - "Viewing overlap map regions in the graphic displays" on page 278
  - "Viewing the overlap map table" on page 279



# Selecting the overlap map file

Use the following file types for the Overlap Map:

- Region information files in AED and BED format.
- Position information from Reference annotation files.

The software automatically checks the hg version of an AED or BED file before loading (see Figure 305 for an example BED file). The file will not be loaded if the hg version does not match what is loaded in the ChAS Browser. If an hg version is not found for the AED or BED file, a warning message appears.

| Figure 305 Example BED file   |
|---|
| File Edit Format View Help  |
| # Sample BED File.<br>#   |
| # UCSC Genome Browser and ChAS should both accept this file.                            |
| # Lines beginning with "#" are ignored.<br>#  |
| # The UCSC genome version is hg38<br>#  |
| # The columns are:  |
| # Chromsome Min Max Name<br>#   |
| <pre># The "browser" line is ignored by ChAS, used by UCSC Genome Browser #</pre>       |
| # The "track" line is used by both ChAS and UCSC Genome Browser.                        |
| # UCSC Genome Browser also uses "name", "description" and others.                       |
| #<br># Specify genome version on the track line as: db="hg38"<br># hg version           |
| browser position chr6:160460899-160501368   |
| track db=("hg38") name="MyBEDFILE" description="IMAGE306 2022-06-07T11:55:07.782-07:00" |
| chr1 818811 3685376 Gain3.0_H22000776r3.hg38.OSCHP                                      |
| chr1 6579786 7622177 Gain3.0_H22000776r3.hg38.0SCHP                                     |
| chr1 10330637 12770000 Gain2.5_Sample1.hg38.0SCHP                                       |
| chr1 83550636 121852400 Gain4.0_Sample1.hg38.0SCHP                                      |
| chr1 143549777 248918679 Gain6.0_Sample1.hg38.0SCHP                                     |
| chr2 21493 10668078 Gain6.0_Sample1.hg38.0SCHP  |
| chr2 106/8600 17653487 Gain4.0_Sample1.hg38.OSCHP                                       |

Do either of the following to select an Overlap Map:

1. In the files list, right-click the file and select **Set File as Overlap Map** on the shortcut menu. (Figure 306)



| Figure 306 Select an Overlap Map from the files list                                |   |  |  |  |
|---|---|--|--|--|
| Eile View Expo<br>EXP<br>Files<br>✓ Start of Cytosc<br>✓ Bull DECIPH<br>✓ B* UNY La | Analysis       ChAS DB       Preferences       Help         ✓       S       ↓       ▼       ✓       ✓       ▲         anHD_Group2_2       ↓       ★       ★       ★       ★       ★         anHD_Group2_2       ↓       ★       ★       ★       ★       ★       ★       ★         b       Cases.aed       ↓       ★       ↓       ★       ★       ↓       ★       ↓ |  |  |  |
| XON Reg   | Close<br>Save<br>≟ Expand Annotation Track<br>ಔ Export File as AED  |  |  |  |
| Some     Some   | Search in selected file Ctrl+F     View and Edit annotations in this file     CytoRegion for Targeted XON analysis     Include in CytoRegions   |  |  |  |
| Named Settin  | Set File as Overlap Map View/Edit Properties Set Custom Color Clear Custom Color  |  |  |  |

#### OR

#### 1. On the menu bar, click Select View $\rightarrow$ Overlap Map.

Alternatively, in the Overlap Map tab in the upper display, click the Select Overlap Map Map tool bar button.

The Select Overlap Map window appears. (Figure 307)

| Figure 307 Select Overlap Map window  |            |
|---|------------|
| Select Overlap Map  | ×          |
| <ul> <li>✓ DGV</li> <li>DGV-GS Gain</li> <li>DGV-GS Loss</li> <li>☑ Ensembl Genes</li> <li>☑ Genes</li> <li>I Banloinsufficiency</li> </ul> | Create New |
| S OMIM ® Genes OK Cancel  |            |

The window displays a list of the region and annotation files you can select for an overlap map.

2. Select the file you want to use, then click **OK**.

The region file loads and its regions are displayed with overlap information in the **Overlap Map** tab.



The Overlap Map icon 🕊 appears next to your selected file within the **File List**, as shown below.



**Note:** To clear an Overlap map, click **Select None**. (Figure 307) Alternatively, rightclick the file in the files list, then select **Set File as Overlap Map** from the shortcut menu.

# Viewing overlap regions

You can view the Overlap Map regions:

- In the graphic display views. See "Viewing overlap map regions in the graphic displays" on page 278.
- In the Overlap Map window tab. See "Viewing the overlap map table" on page 279.

**Note:** Overlap Map items that are covered by a detected segment are listed in a column of the Segments table. "Segments table" on page 334.

Viewing overlap map regions in the graphic displays

In the Karyoview and Selected Chromosome View, regions specified in the Overlap Map file are displayed as short rectangles to the immediate left of the Cytobands. (Figure 308)





In the Detail View, the overlap map regions are displayed as rectangles below the Cytobands (Figure 309). The default color is yellow, but you may select different colors for displaying regions in a region information file.

You can change the colors used to display items by using:

- Specifying color when adding the item to an AED file. See "Entering general information" on page 295.
- Using the Color Rules. See "AED/BED color rules" on page 306.



# Viewing the overlap map table

The Overlap Map table (Figure 310 on page 280) displays a list of overlapping items from the overlap map file and the Segments table. Each region in the Overlap Map file will be listed on at least one row of the table, even if it does not overlap any segments. For those regions which overlap one or more segments, there will be one for each overlap. Depending on the columns which have been used to sort the Overlap Map table, these rows may or may not be near each other. A segment that overlaps more than one region in the Overlap Map file will appear multiple times in the Overlap Map table, one row for each overlap.

#### Highlighting overlap regions in the overlap map table and details view

If the Details View displays the Overlap Map file (the Overlap Map file is check marked in the Files list), you can conveniently find and view items.

Click a row in the Overlap Map table to select the corresponding annotation from the Overlap Map file. All of the rows for that region are highlighted in the table. The Details View zooms to the currently selected region.

**Note:** If the Details View does not automatically zoom to the selected region, confirm that the Auto-zoom option is selected (click **View**  $\rightarrow$  **Auto-zoom to table selection** from the menu bar.)



- In the Detail View, click a region or select multiple regions from the Overlap Map file to highlight all of the corresponding rows in the Overlap Map table. The Overlap Map table automatically scrolls to show at least one of the highlighted rows.
- To quickly find a particular Segment in the Overlap Map table, first double-click that segment in any of the views or in the Segments table (the current region will be set to that segment), then press the is tool bar button in the Overlap Map table to show only the overlaps in the current region.

| Figure 310       | Overlap   | Map Tab     | le          |            |            |                           |              |              |
|------------------|---|-------------|-------------|------------|------------|---------------------------|--------------|--------------|
| 🔠 Karyoview 🔒    | 👭 Karyoview 🔚 Segments 🕌 CytoRegions 🔎 Overlap Map 🛛 🖾 Graphs |             |             |            |            |                           |              |              |
| 🗄 🐻 🗎 📗          |   | <b>~</b>    |             |            |            |                           |              | 81 results 📋 |
| Overlap Map Item | Chromosome  | Min         | Max         | Size (kbp) | Segment ID | ▼ Segment File            | Segment Type | Segment Min  |
| Variation_31239  | 5   | 69,000,764  | 69,578,673  | 577        | seg2209    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 69,502,982   |
| Variation_31241  | 5   | 69,876,869  | 70,578,999  | 702        | seg2217    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 70,232,999   |
| Variation_31240  | 5   | 69,580,505  | 69,875,037  | 294        | seg2211    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 69,802,877   |
| Variation_0283   | 6   | 68,604,421  | 70,408,242  | 1,803      | seg2211    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 69,802,877   |
| Variation_0283   | 5   | 68,604,421  | 70,408,242  | 1,803      | seg2217    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 70,232,999   |
| Variation_0283   | 5   | 68,604,421  | 70,408,242  | 1,803      | seg2209    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 69,502,982   |
| Variation_0283   | 5   | 68,604,421  | 70,408,242  | 1,803      | seg2195    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 68,766,135   |
| RPL35            | 9   | 126,659,978 | 126,664,061 | 4          | seg3898    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 126,586,233  |
| RP11-348K2       | 9   | 126,439,465 | 126,643,093 | 203        | seg3898    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 126,586,233  |
| RP11-62A6        | 9   | 126,439,614 | 126,592,579 | 152        | seg3898    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 126,586,233  |
| RP11-174P6       | 9   | 126,048,268 | 126,895,604 | 847        | seg3898    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 126,586,233  |
| Variation_7787   | ×   | 40,635,684  | 42,901,712  | 2,266      | seg7580    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 41,099,363   |
| Variation_7787   | ×   | 40,635,684  | 42,901,712  | 2,266      | seg7578    | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain       | 41,022,335   |
| <                |   |             |             |            |            |                           |              | >            |

### Overlap map tool bar

The tool bar provides quick access to table functions. The standard functions are described in "Standard tool bar controls" on page 324.





Select Overlap Map file (see "Selecting the overlap map file" on page 276).

| Overlap Map Table<br>Column | Description  |
|-----------------------------|--|
| Map Item Type               | Source of the position information (CN Gain or Loss segment, reference annotation, etc.) |
| Overlap Map Item            | Identifier for the item.   |
| Chromosome                  | Chromosome in which the item is located.   |
| Min                         | Starting position of the item.   |
| Max                         | Ending position of the item.   |
| Size (kbp)                  | Size of the item.  |
| Segment Label               | Label assigned to the detected segment by ChAS.  |



| Overlap Map Table<br>Column | Description   |
|-----------------------------|---|
| Segment Name/ID             | Name/ID of the Segment.   |
| Segment File                | Sample File that the segment was detected in.   |
| Segment Type                | Type of segment:<br>CN loss or gain<br>Mosaicism<br>LOH   |
| Segment Min                 | Starting position of segment.   |
| Segment Max                 | Ending position of segment.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic<br>position coordinates" on page 475). |
| Segment Size                | Size of the segment.  |
| CN State                    | Copy Number State (Not displayed for other segment types).  |
| % Overlap                   | How much of the detected segment is covered by the Overlap Map item. A Segment which has 20% of its length somehow encompassed within the boundaries of an Overlap Map item has an Overlap value of 20%.<br>This percentage value is used to filter segments out of the displays and tables when filtering segments by "Overlap" in the filter slider dialogs.                  |
| % Coverage                  | How much of the Overlap Map item is covered by the Segment.   |
| Shared Size                 | Size of the overlap between segment and Overlap Map item. Coverage values are not presently used in filtration of Segments from the displays or tables.   |



### Using the overlap filter

After selecting an Overlap Map Region file, you have the option to set Overlap filters for the different segment types.

- 1. Select a region information or Reference Annotation file for the Overlap Map.
- 2. Click the Y button to open the **Segment Filters** window. (Figure 311)

| Figure 311 LOH filter settings, Overlap selected |     |
|--|-----|
| X LOH  |     |
| Marker Count 50                                  |     |
| Q  | -   |
| 0 50   | 000 |
| Size (kbp) 3000                                  |     |
| 0  |     |
| 0 200  | 000 |
| Vverlap 100                                      |     |
| Q  | _   |
| 100  | 0   |
|  |     |

- 3. Select the Overlap check box(es) for the segment types you want to filter.
- 4. Use the slider to set the parameter's value or enter a value in the adjacent text box.
- As you move the slider farther to the right (or enter smaller values in the box) more and more of the Overlapped segments are removed from the display.
- The detected segments must share at least the specified percentage of their length with the Overlap Map region to be filtered out and hidden from display. A Segment which has 20% of its length somehow encompassed within the boundaries of an Overlap Map item has an Overlap value of 20%.
- The minimum value of a setting is 1%.
- The results of filtering are seen instantly in all tables and graphs.
- See "Using filters with CytoRegions" on page 271. for information about the interactions of the Overlap Map filter with the CytoRegions.



# **Creating and editing AED files**

# Creating an AED file of annotations

You can create AED files to include:

- User-selected annotations. The annotations in an AED annotation file can be edited in ChAS.
- Position information on regions of the chromosome, as well as additional annotation information on the regions. Note: AED region information files can be used for CytoRegions or Overlap Map functions.
- Third party reference annotations converted to AED file format.
- User-generated AED region information files can be used for CytoRegions or Overlap Map functions. Note: You can add a region to a new or previously created AED file by selecting these feature types:
  - Detected Segments
  - Reference Annotations
  - Regions in previously loaded files
  - 1. In the Detail View, select the non-AED annotation(s) for the AED file using one of the following methods:
  - Right-click an annotation and select Add to a File on the shortcut menu. (Figure 312)

Or

- Draw a box around multiple annotations, right-click the selection, and select Add to a File on the shortcut menu.
- 2. In the window that appears (Figure 312), click Create New.





3. In the **Create** window (Figure 313), click to select a folder, then enter a file name.

| Figure 31  | 3 Create window                                   |               |
|--|---|---------------|
| Create<br>Look in:<br>My Docum<br>My Compu<br>My Netwo | Desktop<br>ients<br>der<br>rk Places              |               |
| File <u>N</u> ame:<br>Files of <u>T</u> ype:           | MyAnnotations<br>Affymetrix Extensible Data (AED) | Create Cancel |

4. Click Create.

The **Select Destination File** window appears and displays the name of your new AED file. (Figure 314)



| Figure 314 | Select Destination | File window |
|------------|--------------------|-------------|
| Select D   | estination File    | ×           |
|            | MyAnnotations.aed  | Create New  |

5. Click OK.

The Details View shows the new annotation (AED).

**Note:** The AED file is automatically assigned the same genome assembly version (i.e., "hg18", "hg19", etc.) as the currently loaded NetAffx annotations.

- 1. In the Detail View, use one of the following methods to select the annotation(s) you want to add to an AED file:
  - Right-click an annotation and select **Add to a File** on the shortcut menu

#### OR

- Draw a box around multiple annotations, right-click the selection, and select Add to a File on the shortcut menu.
- 2. In the Select Destination File window, select an AED file, then click OK.

**Note:** Adding annotations to an AED file does not modify the genome assembly version. If the AED file does not specify a genome assembly version, none is automatically assigned to the AED file when annotations are added.

### Adding annotations to an AED file



# AED file of regions

**Note:** You may want to incorporate new segments of features from a set of samples in a new Regions file. You can also use the Export feature to export data in existing files to an AED file. See "Exporting information in AED or BED format" on page 314.

### Creating a new CytoRegions file in AED file format

1. From the View menu, choose Select CytoRegions file or Select Overlap Map.

The appropriate Select File window appears. (Figure 315)

| Figure 315 Select CytoRegions file window  |             |
|--|-------------|
| Select CytoRegions File(s)   | ×           |
| V DGV DGV-GS Gain DGV-GS Loss C Ensembl Genes Genes Haploinsufficiency OK Cancel OK Cancel | Select None |

Note: You can also create a new AED file when adding a region to an AED file.

| Figure 316 Select Destination File window |                              |  |  |  |
|---|------------------------------|--|--|--|
| Select I                                  | Destination File X           |  |  |  |
|   | Regions_03_02.aed Create New |  |  |  |
|   | OK Cancel                    |  |  |  |

2. Click Create New in the Select File window.

The Create window appears. (Figure 317)



| Create                              |                        |                       |
|-------------------------------------|------------------------|-----------------------|
| Look In: 📋 My Documents             | ,                      | - 🔥 🖻 📄 🗖             |
| 🚞 ePublisher Express Projects       | 🛅 My PSP Files         | 🛗 CNPs from canary-   |
| 🚞 ePublisher Pro Projects           | 🚞 New Folder           | 🚟 File_03_24.aed      |
| 🚞 ePublisher Pro User Formats       | 🚞 Personal             | 🚟 Regions_02_25.aed   |
| 🚞 ePublisher Stationery             | 🚞 preference_RA        | 🚟 Regions_03_02.aed   |
| 🚞 eval_docs                         | 🚞 preferences          | 🛗 Regions_03_10.aed   |
| 🚞 Exchange                          | 🚞 Preferences_Test     | 🛗 Regions Genes Of In |
| 付 My Music                          | 🚞 Snagit Catalog       |                       |
| 💾 My Pictures                       | 🛗 CNPs from canary-v1a | .aed                  |
| <                                   |                        |                       |
| File <u>N</u> ame:                  |                        |                       |
| Files of Type: Affymetrix Extensite | le Data (AED)          |                       |

- 3. Use the window controls to browse to a folder for the AED file.
- 4. Enter a file name.
- 5. Click Create in the Create window.

The Select File window appears with the new file selected. (Figure 318)

**Note:** The AED file is automatically assigned the same genome assembly version (i.e. "hg18", "hg19", etc.) as the currently loaded NetAffx annotations.

| Figure 318 Select Destination File  |   |                           |  |  |
|---|---|---------------------------|--|--|
| Select CytoRegions File   |   | ×                         |  |  |
| WLA_8192.aed BACs DGV FISH Clones Genes OMIM © Segmental Duplications III sno/miRNA OK Canced | < | Select None<br>Create New |  |  |

You can select the new file or add regions to it, depending upon what function you were performing initially.

**Note:** To open an AED file, click the  $\geq$  button or select **File**  $\rightarrow$  **Open** on the menu bar.

Chapter 14 Creating and editing AED files AED file of regions



### Adding regions to a new or existing AED file

You can add a new region to either an existing AED file or by creating a new AED file by selecting the following features in the ChAS graphic views:

- Detected Segments
- Reference Annotations
- Regions in previously loaded files.

**Note:** You can edit the color of annotations that have been added to an AED file by creating a color rule (see "Creating a color rule" on page 308). Alternatively, you can edit the color of a particular DGV annotation added to an AED file, in the Detail View (see "Entering general information" on page 295).

### Adding a section to a new region (AED) file

- 1. Right-click on any of the following feature types in the graphic displays and select **Add to a Copy to a File...** from the right-click menu (Figure 319):
- Segment
- Reference Annotation (including Cytobands) Note: You should expand the reference annotations before selecting one to add to an AED file to avoid selecting multiple annotations (see "Expanding and contracting annotations" on page 191).
- Region

| Converted out with a watery Converting to a State (converted) |
|---|
| Sample_01.cynd.cycnp: Copy Number State (segments)            |
| Sample One Item Selected                                      |
| -2 Zoom to selection Ctrl+Space                               |
| Selection Details Ctrl+D                                      |
| Add a Copy to a File  |
| View/Edit Annotation Properties                               |
| Sample Query ChAS DB  |
| Set highlight region from selections                          |

The Select Destination File window appears. (Figure 320)

**Note:** Some options may not be available, depending upon the number of type of items you have selected.


| Figure 320 Select Destination File window |            |
|---|------------|
| Select Destination File                   | ×          |
| Decipher_66_CNV_Syndromes.aed             | Create New |
|   |            |
| OK Cancel                                 |            |

The Select Destination File window displays a list of the currently existing AED files to which you may add the segment.

- 2. Select the region file you want to use, then click **OK**.
- (Optional) To start a new AED file, click on the Create New button (Figure 320), then enter a name, choose a destination to save the new AED file, then click Create.

**Note:** The **Annotation Properties** window opens if you have selected a single item (Figure 321). If you have selected multiple items, the **Annotation Properties** window does not open.

| Figure 321 Annotation Properties window             | W   |   |
|---|---|---|
| Annotation Properties                               |   | × |
| General Structure Additional Curation               |   |   |
| Loss1.0_Constutional_Blood_01_CytoScanHD.cyhd.cychp |   |   |
| Name/ID<br>seg85                                    | Category<br>copynumber/loss   |   |
| Strand Chromosome Min                               | Max State   |   |
| chr7 148,012,817                                    | 148,013,077 1   |   |
| Materially Modified                                 | Materially Modified By  |   |
|   |   | 2 |
| Counter 0   Modified                                | Color       Image: Clear Color       Image: Clear Color       Modified By |   |
| ОК  | Cancel  |   |



See "Viewing and editing annotations" on page 293 for information on editing the annotations.

4. After editing the annotation properties, click **OK**.

The section is saved in the Region file.

### Deleting regions from an AED file

1. Right-click on a region in a region file and select **Delete Annotations(s)** (Figure 322).

| One Item Selected          Image: Construction of the selection of th |
|--|
| Increment Counter (0 -> 1)         Delete Annotation(s)         Yiew/Edit Annotation Properties  |

The region is deleted from the Region (AED) file.

### **Viewing or Editing AED File Properties**

In ChAS, you can:

- View AED or BED file properties
- Edit AED file properties (modify, add, or delete properties)

**Note:** BED files cannot be edited in ChAS. For more information on editing BED files, see "Editing BED files" on page 477.

### Viewing file properties

1. Right-click on a file, then click to select View/Edit Properties.

The File Properties window appears.

2. Click on the **Extended** tab.

A table with the file properties appears. (Figure 323 on page 291)



| Figure 323 View AED or BED file properties |         |   |                             |                |                                      |
|--|---------|---|-----------------------------|----------------|--------------------------------------|
|  |         |   |                             |                |                                      |
| 🐰 Chromosome Analysis Su                   | iite. [ | For Research Use Only. Not for use in diagnostic proced | File Properties             |                |                                      |
| <u>File View Exports Analysis</u>          | is (    | ChAS DB Preferences Help                                | Basic Sample Properties Ex  | tended         |                                      |
| 🐸 🖸 🗴 🖾                                    | ຮ       | 💅 ኛ   T   Y 🗷 🎟 😁   💽 👭   🤅                             | 4 <b>–</b>                  |                |                                      |
| Files                                      |         | 🕻 💷 Karyoview 🔊 📷 Se                                    | Property Name               | Туре           | Value                                |
| Sample_01.cyhd.c                           | cych    |   | Algorithm Name              | Text           | CYT02                                |
| Sample 03.cvhd.c                           | cvch    |   | Algorithm Version           | Text           | 1.16.0                               |
|  | oveh    |   | All Probeset RLE Mean       | Decimal Number | 0.16365719                           |
| Sample_06.cynd.c                           | cycn    |   | Annotation File             | Text           | CytoScanHD_Array.na33.annot.db       |
| Sample_07.cyhd.c                           | cych    | p 🗍 💭   | Antigenomic Ratio           | Decimal Number | 0.116083294                          |
| ✓ # Q Sample_01.aed                        |         |   | Application                 | Text           | Chromosome Analysis Suite 4.5.0.20   |
|  |         | Close   | Array Annotation File       | Text           | CytoScanHD_Array.na33.annot.db       |
|  |         | Cavo  | Array Name                  | Text           |                                      |
| DGV-GS Gain                                | 1       | Save  | Autosome LOH                | Decimal Number | 0.013084932                          |
| DGV-GS Loss                                | ÷.      | Expand Annotation Track                                 | Barcode                     | Text           | @52082500912945040112413391009020    |
| Default Histogram                          | <b></b> | Export File as AED                                      | Call Rate                   | Decimal Number | 0.9949463                            |
|  | 44      | Search in selected file Ctrl+F                          | CEL_File                    | Text           | Sample_01.CEL                        |
|  |         | View and Edit appatetions in the file                   | CHP File Date               | Text           | Wed Mar 12 17:24:44 2014             |
| Genes                                      |         | view and Edit annotations in this file                  | CHP UUID                    |                | 0000478d-7d67-44db-54e9-000d2e001804 |
| Haploinsufficiency                         |         | New User Annotation in "Szmple_01.aed"                  | Chr X Probe List            | Text           | CytoScanHD_Array.chrXprobes          |
| MIM ® Genes                                |         | CytoRegions for Targeter XON analysis                   | Chr Y Probe List            | lext           | CytoScanHD_Array.chrYprobes          |
|  | 121     | Include in Cute Decional                                | Contrast QC Random          | Decimal Number | 2.465                                |
| Disorder-Causing (                         | (tilit) | Include In CytoRegions                                  | Created                     | Date I Ime     | Aug 15, 2023, 2:24:26 PM             |
| 🔲 💈 OMIM ® Phenotype 🔾                     |         | Set File as Overlap Map                                 | dDSNP Version               | Text           | 135                                  |
| 🔲 💈 OMIM ® Region Ph                       | 1       | View/Edit Properties                                    | Frequency Heterozygous Call | Decimal Number | 0.3052963                            |
| ~ ×  |         | Set Custom Color  | Conder Call XX Patio        | Decimal Number | 0.6947037                            |
| Named Setting                              |         | Set Custom Color  |                             | Desimal Museum |                                      |
|  |         | Clear Custom Color                                      | Filter "Property Name"      |                |                                      |
| Data Turan                                 |         | Open Sample(s) in MSV                                   |                             | OK             | Cancel                               |
| Data Types                                 | h       | Open case in Franklin                                   |                             | (•             |                                      |

# Viewing the genome assembly version

The assigned genome assembly version of a loaded file can be viewed in the Properties box (Figure 323). The property, if it has been set for the file, is shown as "ucscGenomeVersion(Affx)". An AED file that is created within ChAS is automatically assigned the same genome assembly version as the loaded NetAffx annotations (for example, "hg19"). If you add annotations to an existing AED file, its genome assembly version will not be modified; and if no version is specified for the AED file, no version will be assigned to it.

**Note:** When you save a file as AED or BED, the current value of the genome assembly version property, if present, will be saved in the file. If two or more files are merged into an AED or BED file, the current value of the genome assembly version, if present in at least one of the files, will be saved in the merged file.

If an AED file does not include a genome assembly version, you can manually set it. To do this, in the Properties window:

- 1. Click + to add a new property row, as shown in Figure 324 on page 292.
- 2. Select the Property Name ucscGenomeVersion(Affx) from the drop-down list.
- 3. Select **Text** under the Type drop-down list.
- 4. In the **Value** column, enter the genome assembly version (for example, "Constitutional").

**Note:** You can manually set the genome version of an AED file by editing the "ucscGenomeVersion(Affx)" property. For more details on editing a property value, see "Editing a property value" on page 293.

#### Adding a property

- 1. In the **Properties** window, click the + symbol to create a blank row (Figure 324).
- 2. In the **Property Name** field, enter a property name or make a selection from the Property Name drop-down list. (Figure 324)

| Figure 324 Spe     | Figure 324 Specify a property name and type |             |                          |                |             |
|--------------------|---|-------------|--------------------------|----------------|-------------|
| Click h            | ere to add a                                | blank row   | or remove a selected rov | N              |             |
| File Properties    |   |             | File Properties          |                |             |
| Basic Sample Pro   | Basic Sample Properties Extended            |             | Basic Sample Properties  | Extended       |             |
| + -                |   |             | 4 <b>–</b>               |                |             |
| Property Name      | Туре  | Value       | Property Name            | Туре           | Value       |
| Application        | Text  | ChAS Brows  | Application              | Text           | ChAS Brows  |
| Created            | DateTime                                    | May 28, 201 | Created                  | DateTime       | May 28, 201 |
| ıeVersion (Affx) ∨ | Text  |             | NCBI Genome Version      | Text 🗸         |             |
| median Civ State   |   |             |                          | Text           |             |
| Median Raw Inter   |   |             |                          | Whole Number   |             |
| Modified By        |   |             |                          | Decimal Number |             |
| Name               |   |             |                          | DateTime       |             |
| NCBI Genome V€     |   |             |                          | Boolean        |             |
| nd Normalization   |   |             |                          |                | 1           |
| nd SNP QC          |   |             |                          |                |             |
| nd waviness SD     |   |             |                          |                |             |
|                    | 1   |             |                          |                |             |
|                    |   |             |                          |                |             |
|                    |   |             |                          |                |             |
|                    |   |             |                          |                |             |
| 1                  |   |             |                          |                |             |

- 3. Click **Type**, then make a selection from the drop-down list.
- 4. Click Value

A cursor appears inside the Value field. (Figure 325)

| Figure 325 | Value field |  |
|------------|-------------|--|
| Text       | OK Cancel   |  |

5. Enter a value, then click **OK**. For significantly longer values, click (Figure 325) to open a Value editing window. Enter your (longer) value in this window, then click **OK**.

The new value is entered in the File Properties table.

6. Click **OK**.



### Removing a property

### Editing a property value

- 1. In the Properties window, select the row that you want to delete.
- 2. Click the  $\_$  icon.
- Right-click the file and select View/Edit Properties on the shortcut menu. The File Properties window appears. (Figure 326)
  - 2. Click on a row to select it.

| Figure 326 Select a property to edit |                               |                                |  |  |  |
|--------------------------------------|-------------------------------|--------------------------------|--|--|--|
| File Properties                      | File Properties               |                                |  |  |  |
| Basic Sample Pro                     | perties Extend                | led                            |  |  |  |
|                                      |                               |                                |  |  |  |
| Property Name                        | Туре                          | Value                          |  |  |  |
| Application                          | Text                          | ChAS Browser 4.1.0.78 (r29370) |  |  |  |
| Collection Method                    | Collection Method Text Buccal |                                |  |  |  |
| Created                              | DateTime                      | May 28, 2018 9:08:39 AM        |  |  |  |
|                                      |                               |                                |  |  |  |
| 1                                    |                               |                                |  |  |  |

For example, click the Value field. (Figure 327).

| Figure 327 Set a | new property value |  |
|------------------|--------------------|--|
|------------------|--------------------|--|

| File Properties    |                |                                |   |
|--------------------|----------------|--------------------------------|---|
| Basic Sample Prope | erties Extende | b                              |   |
| ÷ =                |                |                                |   |
| Property Name      | Туре           | Value                          |   |
| Application        | Text           | ChAS Browser 4.1.0.78 (r29370) |   |
| Collection Method  | Text           | Blood                          | ] |
| Created            | DateTime       | May 28, 2018 9:08:39 AM        | ] |
|                    |                |                                |   |

3. Enter a value, then click **OK**. For significantly longer values, click <u>under the value significantly longer</u> to open a Value editing window. Enter your (longer) value in this window, then click **OK**.

The new value is entered in the File Properties table.

4. Click **OK**.

### Viewing and editing annotations

The Annotation Properties window opens:

- When adding a region to a Region (AED) file
- When you select View/Edit Annotation Properties from the right-click menu (Figure 328) for the following types of features:
  - Detected Segments



- Reference Annotations (view annotations only)
- AED Annotations (view and edit annotations)

| <b>Figure 328</b> Right-click menu for View/Edit Annotation Properties in the Detail View |                                 |        |       |  |  |  |  |
|---|---------------------------------|--------|-------|--|--|--|--|
| noScan-PS 20110511.cyhd.cychp.aed<br>≷ai⊂1.0 -11-0816 LC ONC134B B10 PoP≴                 |                                 |        |       |  |  |  |  |
|   | One Item Selected               |        |       |  |  |  |  |
| Ð   | Zoom to selection               | Ctrl+S | pace  |  |  |  |  |
| III   | Selection Details               | C      | trl+D |  |  |  |  |
| ₽   | New User Annotation             |        |       |  |  |  |  |
|   | Add to a File                   |        |       |  |  |  |  |
| ÷   | Increment Counter (0 -> 1)      |        |       |  |  |  |  |
|   | View/Edit Annotation Properties | ←      |       |  |  |  |  |

**Note:** The **View/Edit Annotation Properties** menu option is not available if you have more than one feature selected in the Detail View.

The Annotation Properties window has three tabs:

- General (See "Entering general information" on page 295)
- Additional (See "Adding Properties" on page 296)
- Curation (See "Adding a curation (Optional)" on page 299)

You can also create new user annotations if you select an element. This feature enables you to create a region that is not based on a segment or reference annotation. See "New user annotations" on page 300.



## Entering general information

| Figure 329 Annotation prope           | rties window of CHP | Segment exported to | AED |
|---------------------------------------|---------------------|---------------------|-----|
| Annotation Properties                 |                     |                     | ×   |
| General Structure Additional Curation |                     |                     |     |
| Label                                 |                     |                     |     |
| CNTNAP2                               |                     |                     |     |
| Name/ID                               | Category            |                     |     |
| CNTNAP2                               | refseq              |                     |     |
| Strand Chromosome Min                 | Мах                 | State               |     |
| Forward (+) V chr7 146,47             | 1,363 148,118,088   | 8                   |     |
| Materially Modified                   | Materially          | Modified By         |     |
|                                       |                     |                     |     |
| Note                                  |                     |                     |     |
|                                       |                     |                     |     |
|                                       |                     |                     |     |
|                                       |                     |                     |     |
|                                       |                     |                     |     |
|                                       |                     |                     |     |
| S Reference                           |                     |                     |     |
|                                       |                     |                     |     |
| Counter                               | Color               |                     |     |
|                                       | 0 🗘 🛛 🖾 Pick        | a Color Clear Color |     |
| Modified                              | Modified F          | Ву                  |     |
|                                       |                     |                     |     |
| L                                     | OK Cancel           |                     |     |
|                                       |                     |                     |     |

#### Editing the General tab properties in AED annotations

| 1.  | In the General window tab | (Figure 329). | enter, edit, or selec  |
|-----|---------------------------|---------------|------------------------|
| ••• |                           |               | onicon, ounc, or obroo |

| Annotation               | Description   |
|--------------------------|---|
| Label                    | Label given to the AED, CHP, or other annotation. For annotations originating from CHP segments, can be the Type, State, and Filename of the CHP file. The Label is not editable.     |
| Name/ID                  | Name/ID assigned to the annotation in the AED, CHP or other file.   |
| Category                 | Information on the source of the region. If the region was added by selecting a CHP segment, the segment type is saved.   |
| Strand                   | The Sequence Strand of the item.  |
| Chromosome               | Cannot be edited in Annotation Properties box. See "New user annotations" on page 300.  |
| Min                      | The smallest of the annotation's chromosomal coordinates.   |
| Max                      | The largest of the annotation's chromosomal coordinates.  |
| Materially Modified Time | Time stamp of when the start or end coordinate, type, or state of the segment or BED/AED annotation was last altered.   |
| Materially Modified By   | The Operating System user and ChAS User IDs of the Modifier who last changed a Material property (start or end coordinate, type, or state) of the segment or annotation.              |
| Note                     | Information and comments about the region.<br>Note: Always use alphanumeric characters and underscores. Avoid the use of odd characters.<br>(Examples: & + () [] { } ~ ^ and commas.) |



| Annotation  | Description   |
|-------------|---|
| Reference   | URL/web address associated with the annotation.   |
|             | Click Click Click directly to the Reference from the Annotation Properties window. Internet connection is required. |
| Modified    | The time stamp of the last modification of the annotation.  |
| Modified By | The Operating System user and ChAS User IDs of the Modifier who last changed the annotation.                        |
| Counter     | enables you to track the number of times something has been seen.   |
| Color       | Allows assignment of a hard-coded color to the Annotation in ChAS's graphical views.                                |

## Customizing properties

#### **Adding Properties**

In the **Additional** tab you can enter new annotation information for an AED annotation. The information will be displayed in the:

- Tool tip when you position the mouse arrow over an annotation in the Details View
- Selection Details window

#### Adding custom properties

1. In the Annotation Properties window, click the **Additional** tab. (Figure 330 on page 296)

| Figure 330 Additional Annotation Properties tab   |                |                         |  |
|---|----------------|-------------------------|--|
| Annotation Properties                             |                |                         |  |
| General Additional Curation                       |                |                         |  |
| ÷ –   |                |                         |  |
| Annotation Property Name                          | Туре           | Value                   |  |
| End Marker  | Text           | C-6IYHT                 |  |
| Following Marker                                  | Text           | C-6QJZM                 |  |
| Following Marker Location                         | Whole Number   | 21,916,217              |  |
| Full Location                                     | Text           | chr22:18626108-21915509 |  |
| Marker Count Whole Number 3,856                   |                | 3,856                   |  |
| Mean Log2Ratio Decimal Number -0.6218365309868057 |                |                         |  |
| Mean Marker Distance Whole Number 853             |                | 853                     |  |
| Mean Weighted Log2Ratio                           | Decimal Number | -0.6218369371870955     |  |
| Median Log2Ratio                                  | Decimal Number | -0.6297943592071533     |  |

| Additional Annotations   | Description                     |
|--------------------------|---------------------------------|
| Annotation Property Name | Name assigned to the property.  |
| Туре                     | Type assigned to the property.  |
| Value                    | Value assigned to the property. |

**Note:** Different default properties may already be assigned to the annotations added from reference annotations.

2. Click the + button at the top of the table.

A new row appears in the table. (Figure 331)

If needed, click on a column's edge, then drag it (right) to make it wider.

You can delete a property by selecting a row, then clicking the - button.

| Figure 331 New p          | roperty row ad | ded                     |   |
|---------------------------|----------------|-------------------------|---|
| Annotation Properties     |                |                         | × |
| General Additional Cura   | tion           |                         |   |
| ÷ -                       |                |                         |   |
| Annotation Property Name  | Туре           | Value                   |   |
| End Marker                | Text           | C-6IYHT                 |   |
| Following Marker          | Text           | C-6QJZM                 |   |
| Following Marker Location | Whole Number   | 21,916,217              |   |
| Full Location             | Text           | chr22:18626108-21915509 |   |
| Marker Count              | Whole Number   | 3,856                   |   |
| Mean Log2Ratio            | Decimal Number | -0.6218365309868057     |   |
| Mean Marker Distance      | Whole Number   | 853                     |   |
| Mean Weighted Log2Ratio   | Decimal Number | -0.6218369371870955     |   |
| Median Log2Ratio          | Decimal Number | -0.6297943592071533     |   |
| Preceding Marker          | Text           | C-6TZDX                 |   |
| Preceding Marker Location | Whole Number   | 18,625,533              |   |
| Start Marker              | Text           | C-7ACHV                 |   |
|                           |                |                         |   |
|                           |                |                         | - |

3. Click in the row under the Property Name column and enter a name for the property. Note that your new entry is followed by the text "(custom)". (Figure 332)

| Туре                      |                  |                         |  |
|---------------------------|------------------|-------------------------|--|
| Annotation Properties     |                  |                         |  |
| General Additional Cura   | ation            |                         |  |
| 4 -                       |                  |                         |  |
| Annotation Property Name  | Туре             | Value                   |  |
| End Marker                | Text             | C-6IYHT                 |  |
| Following Marker          | Text             | C-6QJZM                 |  |
| Following Marker Location | Whole Number     | 21,916,217              |  |
| Full Location             | Text             | chr22:18626108-21915509 |  |
| Marker Count              | Whole Number     | 3,856                   |  |
| Mean Log2Ratio            | Decimal Number   | -0.6218365309868057     |  |
| Mean Marker Distance      | Whole Number     | 853                     |  |
| Mean Weighted Log2Ratio   | Decimal Number   | -0.6218369371870955     |  |
| Median Log2Ratio          | Decimal Number   | -0.6297943592071533     |  |
| Preceding Marker          | Text             | C-6TZDX                 |  |
| Preceding Marker Location | Whole Number     | 18,625,533              |  |
| Start Marker              | Text             | C-7ACHV                 |  |
| Collaborator (Custom)     |                  |                         |  |
|                           | Text             |                         |  |
|                           | Whole Number     |                         |  |
|                           | Decimal Num Text |                         |  |
|                           | DateTime         |                         |  |
|                           | Daterine         |                         |  |
|                           | Boolean          |                         |  |

4. Click in the row under Type and select a property type from the drop-down list (Figure 333).

---



Choose from:

- Text
- Whole Number
- Decimal Number
- Date Time
- Boolean
- 5. Click in the row under Value and enter the value (Figure 333).

| Figure 333 Enter a value for the property type |                       |                         |  |  |
|--|-----------------------|-------------------------|--|--|
| Annotation Properties                          | Annotation Properties |                         |  |  |
| General Additional Cura                        | tion                  |                         |  |  |
| + -  |                       |                         |  |  |
| Annotation Property Name                       | Туре                  | Value                   |  |  |
| End Marker                                     | Text                  | C-6IYHT                 |  |  |
| Following Marker                               | Text                  | C-6QJZM                 |  |  |
| Following Marker Location                      | Whole Number          | 21,916,217              |  |  |
| Full Location                                  | Text                  | chr22:18626108-21915509 |  |  |
| Marker Count                                   | Whole Number          | 3,856                   |  |  |
| Mean Log2Ratio                                 | Decimal Number        | -0.6218365309868057     |  |  |
| Mean Marker Distance                           | Whole Number          | 853                     |  |  |
| Mean Weighted Log2Ratio                        | Decimal Number        | -0.6218369371870955     |  |  |
| Median Log2Ratio                               | Decimal Number        | -0.6297943592071533     |  |  |
| Preceding Marker                               | Text                  | C-6TZDX                 |  |  |
| Preceding Marker Location                      | Whole Number          | 18,625,533              |  |  |
| Start Marker                                   | Text                  | C-7ACHV                 |  |  |
| Collaborator (Custom)                          | Text                  | Pavich                  |  |  |

Alternatively, click the **Browse** button (Figure 333), then in the **Value** window (Figure 334) that appears, enter the property value, then click **OK**.

| Figure 334 Value win | ndow |        |
|----------------------|------|--------|
| Value                |      | ×      |
| Pavich               |      |        |
|                      | ОК   | Cancel |



The property entry is completed. (Figure 335)

| Figure 335 Property entry completed |                |                         |
|-------------------------------------|----------------|-------------------------|
| Annotation Properties               |                |                         |
| General Additional Curation         |                |                         |
| + -                                 |                |                         |
| Annotation Property Name            | Туре           | Value                   |
| Collaborator (Custom)               | Text           | Pavich                  |
| End Marker                          | Text           | C-6IYHT                 |
| Following Marker                    | Text           | C-6QJZM                 |
| Following Marker Location           | Whole Number   | 21,916,217              |
| Full Location                       | Text           | chr22:18626108-21915509 |
| Marker Count                        | Whole Number   | 3,856                   |
| Mean Log2Ratio                      | Decimal Number | -0.6218365309868057     |
| Mean Marker Distance                | Whole Number   | 853                     |
| Mean Weighted Log2Ratio             | Decimal Number | -0.6218369371870955     |
| Median Log2Ratio                    | Decimal Number | -0.6297943592071533     |
| Preceding Marker                    | Text           | C-6TZDX                 |
| Preceding Marker Location           | Whole Number   | 18,625,533              |
| Start Marker                        | Text           | C-7ACHV                 |

6. Click **OK** in the Annotation Properties window.

#### Adding a curation (Optional)

1. Click the Curation tab. (Figure 336)

| Figure 336 Curation tab                         |                     |          |   |
|---|---------------------|----------|---|
| Annotation Properties                           |                     |          | × |
| General Additional Curation                     |                     |          |   |
| Call  | Inheritance         | OKR      |   |
| Pathogenic V                                    | <b>v</b>            | <b>v</b> |   |
| Segment Interpretation (Right-click to add prec | onfigured snippets) |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
|   |                     |          |   |
| Curation Time                                   | Curation By         |          |   |
|   |                     |          |   |
|   | OK Cancel           |          |   |



2. Click the Call drop-down to select a Call. (Figure 337)



- 3. Click to select an appropriate Call.
- 4. If needed, (in the Interpretation field) type in your interpretation or right-click to add a pre-configured snippet.
- 5. Click the Inheritance drop-down menu to select an Inheritance mode.
- 6. Click on the **OKR** drop-down menu to select an OKR annotation.
- 7. Click **OK**.

### New user annotations

You can create a new annotation from an AED annotation.

1. In the Details view, right-click an AED annotation and select New User Annotation on the shortcut menu. (Figure 338)



 In the window that appears, enter the annotation information (Figure 339). For more details, see "Entering general information" on page 295 and "Customizing properties" on page 296.



| Figure 339 Enter information for the new a | nnotation   |
|--|---|
| New User Annotation                        | ×   |
| General Additional Curation                |   |
| Label                                      |   |
| Name/ID                                    | Category  |
| Strand Chromosome Min                      | Max State   |
| Materially Modified                        | Materially Modified By                            |
| ■ Note                                     |   |
|  |   |
| S Reference                                |   |
| Counter                                    | Color       Image: Color       Image: Clear Color |
| Modified                                   | Modified By                                       |
| ОК   | Cancel  |

3. Click **OK**.

The new user annotation is created and saved in the AED file.

**Note:** The default New User Annotation information includes only the chromosome number. It does not include any information or properties associated with the AED annotation.



### Viewing and batch editing AED file contents

The AED Editor enables you to view multiple AED annotations and their properties in a table format and edit the values of properties easily - for single annotations or to edit a property for multiple annotations in a batch - to a new value.

1. At the Files pane (top left), right-click on the AED file you want to view/edit.

A pop-up menu appears. (Figure 340)

| Figure 340 AED file - F                     | light-click menu                                     |
|---|--|
| 🎖 Chromosome Analysis Suite                 | e. [For Research Use Only. Not for use in diagnostic |
| <u>File View Exports Analysis</u>           | ChAS DB Preferences Help                             |
| 🐸 🙆 🍳 🖾                                     | 1 🕫 🥰 🔳 🍸 🖾 🐨 🔁 🤅                                    |
| Files                                       | S III Karyoview                                      |
| Sample_01.cyhd.cy                           | chp 9  |
| Sample_03.cynd.cy                           | cnp  |
| Sample_00.cynd.cy                           | chp  |
| ✓ ■♥ ¥ Sample_07.cynd.cy ✓ ■♥ Sample_01.aed |  |
|   | Close  |
| 🔲 🚡 DGV-GS Gain                             | Save   |
| 🔲 笔 DGV-GS Loss                             | Expand Annotation Track                              |
| 🔲 📘 Default Histogram                       | Export File as AED                                   |
| 🔲 🖉 Ensembl Genes 🛛 🕯                       | Search in selected file                              |
| 🔲 🎞 Genes                                   | View and Edit annotations in this file               |
| Haploinsufficiency                          | New User Annotation in "Sample_01.aed"               |
| 🔲 💈 OMIM ® Genes                            | CytoRegions for Targeted XON analysis                |
| 🔲 💈 Disorder-Causing 🔍 🛱                    | Include in CytoRegions                               |
| 🔲 💈 OMIM ® Phenotype 🗆 🌢                    | Set File as Overlap Map                              |
|   | A Record Barris Brown after                          |

#### 2. Click View and Edit annotations in this file.

The AED File Editor table window appears. (Figure 341)

| 🐐 AED File Editor                  |                |             |            |            | ×                                |
|------------------------------------|----------------|-------------|------------|------------|----------------------------------|
| File                               |                |             |            |            |                                  |
| File: DiseaseRegionSamples.aed     |                |             |            |            |                                  |
| 🖩 🖬 🖺 Σ 🔢 🛙                        | ф              |             |            |            | 7 results                        |
| Label                              | Chromosome     | Min         | Max        | Size (kbp) | Name/ID                          |
| supercalifragilisticexpialidocious | 2              | 666,666     | 666,666    | < 0.5 kbp  | supercalifragilisticexpialidocio |
| Prader-Willi NA1132                | 15             | 21,090,916  | 26,834,826 | 5,744      | Prader-Willi NA1132              |
| Miller-Dieker LIS1                 | 17             | 2,436,548   | 2,537,677  | 101        | Miller-Dieker LIS1               |
| Smith-Magenis                      | 17             | 16,500,000  | 20,500,000 | 4,000      | Smith-Magenis                    |
| 18q NA16447                        | 18             | 62,091,988  | 76,061,246 | 13,969     | 18q NA16447                      |
| DiGeorge2 Big                      | 22             | 16,300,000  | 22,800,000 | 6,500      | DiGeorge2 Big                    |
| regionY1                           | Y              | 20,000,000  | 20,500,000 | 500        | regionY1                         |
|                                    |                |             |            |            |                                  |
| Note: Each r                       | ow in this tal | ole represe | ents a spe | cific Anno | otation, while each              |

**Note:** The AED File Editor table displays ALL properties and tabs of every annotation contained in an AED file.



Use the scroll bar to move the table to the right to see more column entries and/ or click, then drag the AED File Editor table window to make it wider.

To locate your table data faster, click on any appropriate column header to sort in a descending or ascending order. Also, the number of columns displayed in the Select Columns window, varies with each AED file.

- 3. Locate the group of editable annotations (rows) from a column (property) you want to batch edit (combine together) as a single annotation.
- 4. Shift click or Ctrl click to select (highlight) each annotation (row) entry. (Figure 342).

| Figure 342 Selection of multi | ple annotations    |
|-------------------------------|--------------------|
| collaborator names (custom)   | Property (Column)  |
| Emerson                       | X .                |
| Paul Jacobs                   | $\mathbf{X}$       |
| P. Pavich                     | Annotations (Rows) |
| Alexander Cole                | Annotations (nows) |
| Lucas Ryan                    |                    |
| N. Pear                       |                    |

5. Right-click on a newly selected annotation.

The following menu appears: (Figure 343)

| Figure 34<br>menu) | 43 AED table field (right-click |
|--------------------|---------------------------------|
| collaborat         | or names (custom)               |
| Plant D            | Sum, mean and median            |
| Jame 😭             | View/Edit Annotation Properties |
| Lifesc             | Edit single property            |
| Pear               | Clear single property values    |

**IMPORTANT!** Only editable fields can be edited. If a field is non-editable, the Annotations Property Window pops-up. This window displays which fields are not editable (grayed out) versus those that are editable. Also, not all user-editable AED file fields may currently be edited from within the AED Editor. Some basic values (start, stop, type) cannot be edited in the AED Editor table directly. You MUST use "View/Edit Annotation Properties..." for editing the particular field in the annotation of interest.

6. Click Edit single property.



7. The following window appears: (Figure 344)

| Figure 344 Edit Text Value                          |      |
|---|------|
| 🖏 Edit Text value                                   | ×    |
| Edit value of "endMarker (Biology)" on 3 annotation | ons? |
| Jacobs_Cole_Ryan                                    |      |
| OK Cancel   |      |

- 8. Enter an appropriate label to distinguish your new batch annotation entry, then click **OK**.
- 9. Your batch (group) of annotations appear as follows: (Figure 345)

| Figure 345 Batch edit results | - Before and After          |
|-------------------------------|-----------------------------|
| Before Batch Editing          | After Batch Editing         |
| collaborator names (custom)   | collaborator names (custom) |
| Emerson                       | Emerson                     |
| Paul Jacobs                   | Jacobs_Cole_Ryan            |
| P. Pavich                     | P. Pavich                   |
| Alexander Cole                | Jacobs_Cole_Ryan            |
| Lucas Ryan                    | Jacobs_Cole_Ryan            |
| N. Pear                       | N. Pear                     |
| Thorberg, Southkey            | Thorberg, Southkey          |

### Protecting an AED file

Protecting an AED file provides a warning prior to adding an annotation to an AED file. This is an optional field and is intended to double-check the intention of adding an annotation to the selected AED file.

- 1. Right click on the AED file in the File Tree, select View/Edit Properties.
- 2. In the **Basic** tab window, click the **Protect File** check box, as shown in Figure 346.



| Figure 346 Basic window tab - Protect Fi | ile option                          |
|--|-------------------------------------|
| 😨 File Properties                        | ×                                   |
| Basic Sample Properties Extended         |                                     |
| Vrotect File                             |                                     |
| File<br>CytoScanDDG2PGeneList.r1.aed     |                                     |
| Genome                                   | NetAffx Build                       |
|  |                                     |
| Array                                    | Modified<br>Jul 11, 2016 5:26:23 AM |
|  |                                     |
|  |                                     |
|  |                                     |
|  |                                     |
|  |                                     |
|  |                                     |
|  |                                     |
|  |                                     |
| ОК                                       | Cancel                              |

When adding annotations to a Protected AED file, the following warning message appears. (Figure 347)

| Figure 347 Protected File message  |    |
|--|----|
| Rotected File  | ×  |
| The file is protected, do you want to add the region to CytoScanDDG2PGeneList.r1.aed | 1? |
| <u>Y</u> es <u>N</u> o   |    |

3. Click **Yes** to acknowledge the message, then click **OK** to add the annotation to the AED file.

Click  $\ensuremath{\text{No}}$  to return to the browser window without adding the annotation to the AED file.

Note: Protected AED files are noted with an asterisk, as shown in Figure 348.

| Figure 348 Protected AED file |  |
|-------------------------------|--|
|                               |  |
| # HearingLossPanel.aed        |  |
|                               |  |
| CytoScanDDG2PGeneList.r1.aed  |  |
|                               |  |



### **AED/BED** color rules

The Color Rules feature enables you to set display colors for annotations in AED files by various annotation properties, depending upon the original source of the region (detected segment, reference annotation, etc.). You can color annotations using the properties of the annotations, including:

- name
- category
- markerCount
- confidence

Using the color rules, you can assign a different color to annotations with different properties, making it easier to track the different types of segment data stored in AED files. For example, you could assign different colors to different categories:

- GainMosaic
- LossMosaic
- Gain
- Loss
- LOH

You can also perform comparisons for numerical values, coloring only values above or below a certain level.

By default, ChAS displays the regions in an AED or BED file in a single color. Do one of the following to edit annotation color:

- Select a new default color for all AED or BED files (page 306).
- Create a color rule that specifies a color for annotations which meet user-specified requirements. See "Creating a color rule" on page 308.
- In the Detail View, choose a color for a particular annotation in the Annotation Properties window. See "Changing an annotation color" on page 313.

**Note:** An AED annotation color set in the Annotation Properties window (accessed in the Detail View) takes precedence over a color rule and the default AED/BED file color. A color rule can overwrite the AED or BED file default color.

Selecting a new default color for loaded AED or BED files

- 1. Open the User Configuration window (click the  $\bigcirc$  button or select **Preferences**  $\rightarrow$  Edit User Configuration on the menu bar).
- 2. In the Color Rules tab (Figure 349), click the AED/BED Annotation Color button.
- 3. In the window that appears, choose a color swatch or use the color controls to specify a color.
- 4. Click **OK** in the AED/BED Annotation Color window.







### Creating a color rule

- Click the button or select Preferences → Edit User Configuration on the menu bar.
- 2. The User Configuration window appears. (Figure 350)
- 3. Click the **Color Rules** tab.

| Figure 350     | User Config       | guration w  | /indo | v - Color F  | Rules tab | )                 |         |       |
|----------------|-------------------|-------------|-------|--------------|-----------|-------------------|---------|-------|
| User Configura | ation             |             |       |              |           |                   |         | ×     |
| Segment Data   | QC Thresholds     | Color Rules | Misc  | Vocabularies | DB Query  | Filtered DB Query | Exports |       |
|                | prototion Propert | v Nomo      |       | Turpo        | _         | Operator          | Voluo   | Color |
| AED/BED P      | unotation Propert | yname       |       | Type         |           | Operator          | Value   | COIOI |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                |                   |             |       |              |           |                   |         |       |
|                | Annatation Color  |             |       |              |           |                   |         |       |
| AED/RED A      | vinotation Color  | Clear Colo  |       |              | OK        | Cancel            |         |       |
|                |                   |             |       |              |           | Cancer            |         |       |

The Color Rules tab has five columns:

| Column                              | Description  |
|-------------------------------------|--|
| AED/BED Annotation<br>Property Name | Name assigned to the property.                     |
| Туре                                | Type assigned to the property (for example, text). |
| Operator                            | Type of comparison with value performed.           |
| Value                               | Value assigned to the property.                    |
| Color                               | Color assigned to the region property and value.   |

4. Click the Add 🕂 button at the top of the table (Figure 351).



| Segment Data                       | OO THE HILL        |             | -    |              |          |                   |         |       |
|------------------------------------|--------------------|-------------|------|--------------|----------|-------------------|---------|-------|
|                                    | QC Inresholds      | Color Rules | Misc | Vocabularies | DB Query | Filtered DB Query | Exports |       |
| + - 🖬 🛛                            | ↓                  |             |      |              |          |                   |         |       |
| AED/BED A                          | nnotation Property | y Name      |      | Туре         |          | Operator          | Value   | Color |
| name (General)                     |                    | ~           | Text |              |          |                   |         |       |
| nutScore (Biology                  | y)                 |             |      |              |          |                   |         |       |
| nut_syntax_aa (C                   | COSMIC)            |             |      |              |          |                   |         |       |
| mut_syntax_cds (                   | (COSMIC)           |             |      |              |          |                   |         |       |
| name (General)                     |                    |             |      |              |          |                   |         |       |
| tote (General)                     |                    | 0           |      |              |          |                   |         |       |
| neneSymbol (OM                     | IMD                |             |      |              |          |                   |         |       |
| peneSymbol (stri                   | OMIM)              |             |      |              |          |                   |         |       |
| eneSymbol (OMI<br>eneSymbolList (( | IM)<br>OMIM)       |             |      |              |          |                   |         |       |
|                                    |                    |             |      |              |          |                   |         |       |

A new row appears in the table.

5. Click in the row under the Property Name column and enter a name for the property or select a property from the drop-down list (Figure 352).

To delete a property, select the appropriate row, then click the **Remove** button.



| Segment Data       QC Thresholds       Color Rules       Misc       Vocabularies       DB Query       Filtered DB Query       Exports         + - • • • •       •       •       •       •       •       •       Color         AED/BED Annotation Property Name       Type       Operator       Value       Color         name (General)       •       Text       •       •       •       •         nutScore (Biology)       • <td< th=""><th></th><th></th><th></th><th></th><th></th><th></th><th></th></td<>  |  |      |              |          |                   |         |       |
|---|--|------|--------------|----------|-------------------|---------|-------|
| AED/BED Annotation Property Name       Type       Operator       Value       Color         name (General) <ul> <li>Text</li> <li>Text</li></ul> | Segment Data QC Thresholds Color Rules | Misc | Vocabularies | DB Query | Filtered DB Query | Exports |       |
| AED/BED Annotation Property Name     Type     Operator     Value     Color       name (General) <ul> <li>Text</li> <li>Text</li></ul>   |  |      | -            |          |                   | N L SA  | 0.1   |
| name (General) Viext Iext Iext Iext Iext Iext Iext Iext I   | AED/BED Annotation Property Name       | ]    | Туре         |          | Operator          | Value   | Color |
| nut_sontax_aa (COSMIC)<br>nut_sontax_cds (COSMIC)<br>ame (General)<br>ote (General)<br>isorder (OMIM)<br>eneSymbol (OMIM)<br>eneSymbol List (OMIM)  | name (General)                         | Text |              |          |                   |         |       |
| nut_syntax_aa (COSMIC)<br>ame (General)<br>ote (General)<br>isorder (OMIM)<br>eneSymbol (OMIM)<br>eneSymbolList (OMIM)  | nutScore (Biology)                     |      |              |          |                   |         |       |
| ame (General)<br>ote (General)<br>isorder (OMIM)<br>eneSymbol (OMIM)<br>eneSymbolList (OMIM)  | nut_syntax_aa (COSMIC)                 |      |              |          |                   |         |       |
| ote (General)<br>isorder (OMIM)<br>eneSymbol (OMIM)<br>eneSymbolList (OMIM)   | name (General)                         |      |              |          |                   |         |       |
| iisorder (OMIM)<br>eneSymbol (OMIM)<br>eneSymbolList (OMIM)   | note (General)                         |      |              |          |                   |         |       |
| eneSymbolList (OMIM)  | disorder (OMIM)                        |      |              |          |                   |         |       |
| eneSymbolList (OMIM)  | geneSymbol (OMIM)                      |      |              |          |                   |         |       |
|   |  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  | ]    |              |          |                   |         |       |
|   | jeneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | jeneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |
|   | geneSymbolList (OMIM)                  |      |              |          |                   |         |       |

- 6. Click in the row under **Type**, then select a property type from the drop-down list.
- 7. Click in the row under **Operator**, then select an appropriate operator from the drop-down list. (Figure 353)



| Figure 353 Selecting property         | type and operator     | for the comparison             |       |       |
|---------------------------------------|-----------------------|--------------------------------|-------|-------|
| User Configuration                    |                       |                                |       |       |
| Segment Data QC Thresholds Color Rule | s Misc Vocabularies [ | DB Query Filtered DB Query Exp | orts  |       |
| + - • •                               |                       |                                |       |       |
| AED/BED Annotation Property Name      | Туре                  | Operator                       | Value | Color |
| ame (General)                         | Text                  | contains 🗸                     |       |       |
|                                       |                       | =                              |       |       |
|                                       |                       | ŧ                              |       |       |
|                                       |                       | <                              |       |       |
|                                       |                       | ≤                              |       |       |
|                                       |                       | >                              |       |       |
|                                       |                       | contains                       |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
|                                       |                       |                                |       |       |
| AED/BED Annotation Color Clear Co     | lor                   |                                |       |       |
|                                       |                       | OK Cancel                      |       |       |
|                                       |                       | Cancer                         |       |       |

- 8. Click in the row under Value, then enter a value for the property.
- 9. Click in the row under **Color**.

The Pick a Color window appears. (Figure 354)

10. Select a color, then click **OK**.



| Figure 354 Property entry comp                              | pleted   |
|---|--|
| User Configuration  | ×  |
| Segment Data QC Thresholds Color Rules                      | Misc Vocabularies DB Query Filtered DB Query Exports   |
| + – C<br>AED/BED Annotation Property Name<br>name (General) | Type       Operation       X       Color         Text       Contains       Image: Color       Image: Color         Image: Color       Image: Color       Image: Color       Image: |
| AED/BED Annotation Color Clear Col                          | OK Cancel Reset  |

Your property entry is completed.

11. Click **OK** to close the **Color Rules** window.

Regions that satisfy the property comparison are displayed in your selected color (for all views), as shown in Figure 355 on page 312.

| Figure 355 Regions displayed i | n color |
|--------------------------------|---------|
| File_03_24.aed                 |         |
|                                |         |
| BACs                           |         |



### Changing an annotation color

1. Click Choose Color.

The Pick a Color window appears. (Figure 356)



- 2. Choose a color in the Swatches tab, or click the HSB or RGB tab to define a color.
- 3. Click OK.

**Note:** The color set in the Annotation Property window overrides the colors specified by a Color Rule created in the User Configuration window. For more details, see "AED/BED color rules" on page 306.



### Exporting information in AED or BED format

You can export position data for the different features to AED or BED file format. The exported BED file contains only the names and locations of the detected segments. The exported AED file contains additional information, such as header information, feature ID, and hg version (which is the same as the NetAffx Genomic Annotations Database file loaded in the Browser at export).

For more information on the AED file format, see page 469.

**Note:** AED or BED files created in ChAS 1.0, 1.0.1, or 1.1 do not automatically include the hg version.

| Position Data  | Export to AED File             | Export to BED File |
|--|--------------------------------|--------------------|
| Detected Segments for xxCHP files                    | Regions, names, and properties | Regions and names  |
| Annotation Features in Reference<br>Annotation files | Regions, names, and properties | Regions and names  |

Exporting position data as an AED or BED file

1. From the File menu, select Export as AED...

Alternatively, right-click the file in the Files list and select **Export File as AED** on the menu. (Figure 357)



The Export window appears. (Figure 358)



| Figure 358 Export window, AED file format sele  | cted                     |
|---|--------------------------|
|   |                          |
| Export  | ×                        |
| Look In: 🛅 Project Files 🗸 🗸                    |                          |
| HLEM_153.aed                                    | Filter Exported Segments |
| S_178.cy2wg.cychp.aed                           |                          |
| 🛗 WLA_8192.aed                                  |                          |
|   |                          |
|   |                          |
|   |                          |
|   |                          |
|   |                          |
|   |                          |
| File Name:                                      |                          |
| Files of Type: Affymetrix Extensible Data (AED) | ~                        |
|   |                          |
|   | Export Cancel            |
|   |                          |

 Click the Filter Exported Segments check box to restrict the export to the contents of the Segments table. If filters have been applied to the data, only the retained segments will be exported. Graph data and Chromosome Summary data will not be exported.

**Note:** If this option is not selected, all segments which were loaded with the CxCHP file will be exported along with header information, regardless of whether filters are applied. The export includes all segment data, regardless of check mark status (ON or OFF) in the Files window pane.

- 3. Select a folder location for the file, as you normally would.
- 4. To export to AED file format, enter a name for the file.

To export to BED file format (Figure 359), enter a name for the file, then select **Browser Extensible Data (BED)** from the **Files of Type** drop-down list.

| Figure 35              | 9 Export window; BED file format selected    |  |  |  |
|------------------------|--|--|--|--|
| Export                 | ×  |  |  |  |
| Look <u>I</u> n: 🛅     | Project Files                                |  |  |  |
| 🛗 New Mark             | New Markers.bed     Filter Exported Segments |  |  |  |
|                        |  |  |  |  |
|                        |  |  |  |  |
|                        |  |  |  |  |
|                        |  |  |  |  |
|                        |  |  |  |  |
| File <u>N</u> ame:     |  |  |  |  |
| Files of <u>T</u> ype: | Browser Extensible Data (BED)                |  |  |  |
|                        | Affymetrix Extensible Data (AED)             |  |  |  |
|                        | Browser Extensible Data (BED)                |  |  |  |



- 5. Optional: Select the Filter Exported Segments window to export only segments that meet filter criteria.
- 6. Click **Export** in the Export window.

The AED file is exported and can be loaded as a region information file or sent to another user.

You can also merge feature position data from multiple different files.

#### Merging and exporting feature position information for multiple files

- 1. Select files in the File List by clicking on them while pressing the CTRL key.
- 2. Right-click the selected files, then click Merge Files to AED. (Figure 360)

| Figure 360 outputs  | Files selecte                            | ed for merging AED                        |
|---|--|---|
| <ul> <li>✓ □ ● Bet</li> <li>✓ □ ● ▲</li> <li>✓ □ ● ▲</li> <li>✓ □ ● ▲</li> <li>✓ □ ● ▲</li> </ul> | a13_F_01_Cyt<br>Beta15_M A<br>s from car | o_VH.cychp<br>Close<br>Merge Files to AED |

The Export window appears. (Figure 361)

| Figure 361 Export window                                |               |
|---|---------------|
| Export  | ×             |
| Look In: 🕒 My Documents                                 |               |
| 🛅 ePublisher Express Projects                           | 🛅 SnagIt Ca   |
| 🛅 ePublisher Pro Projects                               | 🗷 Angelman    |
| 🛅 ePublisher Pro User Formats                           | 🗷 HapMap-A    |
| 🛅 ePublisher Stationery                                 | 🗷 HapMap-A    |
| 🛅 eval_docs   | 🗷 HapMap-A    |
| 🛅 Exchange  | 🗷 merge3.a    |
| 📸 My Music  | 🗷 merged.aa   |
| 😬 My Pictures   | 🗷 merged2.a   |
| C My PSP Files  | 🗷 New_Regi    |
| <   | >             |
| File <u>N</u> ame:                                      |               |
| Files of <u>Type</u> : Affymetrix Extensible Data (AED) | ~             |
|   | Export Cancel |

- 3. Use the navigation controls to select a folder for the merged AED file and enter a file name for the file.
- 4. Click Export.

The file with the merged AED region information is created and can be used as a region information file in ChAS.



**IMPORTANT!** After two AED files are merged, the original metadata in the header is not retained. Also, when two or more files are merged into AED/BED format, the current value of the genome assembly version property, if present in at least one of the files, will be saved in the merged file.

#### Expression analysis AED file generation

AED files containing Gene Expression information and data can be created using Thermo Fisher Scientific Human Gene Expression arrays analyzed in the Transcription Analysis Console (TAC) 3.0 or higher.

For details on how to create an AED file containing Gene Expression/miRNA data, please see the Gene Expression Copy Number Analysis Quick Reference Card.

#### Viewing a Gene Expression AED file in ChAS

1. Load the AED file exported from TAC using **File**  $\rightarrow$  **Open**.

The AED is now visible in the Detail View. (Figure 362)

|   |                              |   |                      |   |   |  | 🖉   📘 👄                                  | 😹 🔾 🗨  |
|---|------------------------------|---|----------------------|---|---|--|--|--|
| 0009457_B04                             | 4.OSCHP: Copy N              | umber State (segme                      | ents)                |   |   |  |  |  |
|   |                              |   |                      | V   |   |  |  |  |
| 0009457_B04                             | 1.0SCHP: Log2 R              | atio                                    |                      |   |   |  |  |  |
| - 1.3<br>- 0                            |                              |   |                      |   |   |  |  |  |
| -1.5                                    | a subsection of the          | 10000                                   | Second Second Second | a de la   |   | a da se da se se de se de s  |  | a service a service serv |
| 0009457_B04                             | 4.0SCHP: BAF                 |   |                      |   |   |  |  |  |
| E                                       | 1 I. J. J.                   | 10 C 10 C 1                             |                      | ;   |   | 11. I.                                       | 2 - L V.                                 | - 12 - 14 - 14 - 14 - 14 - 14 - 14 - 14  |
| -0.4                                    |                              | 1 C 1 C 1 C 1 C 1 C 1 C 1 C 1 C 1 C 1 C | 18 C 19 C 19 C       |   |   | and the second second  | 1. | 1.1.1  |
| Lung_9yr_Tur                            | morivs Lung_9yr_             | Normal_20140930.a                       | aed                  | in de la compañía                                     |   |  |  |  |
| Lung_9yr_Tur                            | mor vs Lung_9yr_             | Normal_20140930.a                       | aed                  |   |   |  |  |  |
| Lung_9yr_Tur<br>Genes                   | mor vs Lung_9yr_             | Normal_20140930.a                       | aed                  |   | TC06000935.hg.  | 1  |  |  |
| Lung_9yr_Tur<br>Genes                   | mor vs Lung_9yr_             | Normal_20140930.a                       | aed                  | CEES  | <b>ТС06000935.hg</b> .<br>Туре  | 1<br>DownRegulated   |  |  |
| Lung_9yr_Tur                            | nor vs Lung_9yr_             | Normal_20140930.a                       | aed                  | CEES  | TC06000935.hg.<br>Type<br>Location  | 1<br>DownRegulated<br>6: 118,869,442 - 118,881,893                               |  | <br>₩1Δ1←  |
| Lung_9yr_Tur<br>Genes                   | mor vs Lung_9yr_             | SLC35F1→                                | aed                  |   | TC06000935.hg.<br>Type<br>Location<br>Size (kbp)  | 1<br>DownRegulated<br>6: 118,869,442 - 118,881,893<br>12                         | -<br>+ <br>                              | N1A1←<br>+   |
| Genes                                   | nor vs Lung_9yr_<br>118200kb | SLC35F1-+<br>118400kb                   | aed                  | CEES<br>HHIIIII<br>CE<br>HHIIIIIIIIIIIIIIIIIIIIIIIIII | TC06000935.hg.<br>Type<br>Location<br>Size (kbp)<br>TAC Fold Change                                   | 1<br>DownRegulated<br>6: 118,869,442 - 118,881,893<br>12<br>-4.390               |  | NIAI←<br>↓↓↓↓↓↓↓<br>119600kb   |
| Lung_9yr_Tur<br>Genes<br>+#<br>118000kb | 118200kb                     | SLC35F1+<br>118400kb                    | aed                  |   | TC06000935.hg.<br>Type<br>Location<br>Size (kbp)<br>TAC Fold Change<br>TAC P-Value                    | 1<br>DownRegulated<br>6: 118,869,442 - 118,881,893<br>12<br>-4.390<br>0.0        |  | NIAI←<br>↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓   |
| Lung_9yr_Tur<br>Genes<br>               | nor vs Lung_9yr_<br>118200kb | SLC35F1-+<br>118400kb<br>922.2          | aed                  |   | TC06000935.hg.<br>Type<br>Location<br>Size (kbp)<br>TAC Fold Change<br>TAC P-Value<br>TAC Gene Symbol | 1<br>DownRegulated<br>6: 118,869,442 - 118,881,893<br>12<br>-4.390<br>0.0<br>PLN |  | NIA1←<br>+   |

You can simultaneously view fold change from your Gene Expression dataset with copy number data from xxCHP files. Positive gene expression fold changes are represented by blue transcript cluster IDs. Negative fold changes are represented by red transcript cluster IDs. The deeper the color (blue or red) the larger the fold change.

### **VCF** files



### Loading VCF files

VCF files containing genotyping, copy number and structural variants can be visualized in the Detail View in conjunction with CHP file data.

VCF files are a flexible format, therefore you must use the following guidelines when formatting VCF files for use in ChAS.

- Each VCF file must contain a single sample only.
- The VCF file will display genotype/indel/copy number/LOH and Structural Variation data.
- The VCF file must contain only 10 columns.
- The chromosome ID should use the format chr1, chr2 or 1, 2.
- Only chromosomes 1-22, X and Y are supported. Information from other sequences or variant chromosome assemblies will be ignored.
- The DATA column (column 10) can not be left blank/empty.
- A binary compressed VCF is not supported.
- The vcf.gz format can also be viewed in the ChAS browser.
- VCF entries representing structural variations (CNV, DEL, DUP, etc.) do not require GT values.
- Other VCF entries must contain GT in the FORMAT column, preferably in the first position.
- The length of structural variation segments should be indicated with a SVLEN value. If missing, the software auto-checks for LEN and END.

**IMPORTANT!** If your VCF file(s) do not strictly adhere to the above guidelines, the file(s) will not be compatible with, nor load into ChAS.

- 1. From your software, export your VCF file (based on the above guidelines).
- 2. Click File  $\rightarrow$  Open.

An Explorer window appears.

3. Navigate to your VCF file location, click to highlight it, then click **Open**.

A message appears indicating that the Genome Version could not be detected. If you are certain that the genome build for the VCF file matches the Genome Version loaded in the ChAS Browser, click **OK** to acknowledge the message. The VCF file loads, then appears inside the Detail View. Copy number and structural variants are displayed in a upper track, while genotype calls are display on the lower track, as shown in Figure 363.



| Genotype Data (Lower track) |                                 |         |  |  |
|-----------------------------|---------------------------------|---------|--|--|
| Data Type                   | Description                     | Color   |  |  |
| GT                          | Heterozygous Genotype           | Cyan    |  |  |
| GT                          | Homozygous Alternate Genotype   | Magenta |  |  |
| GT                          | Homozygous Reference Genotypes  | Gray    |  |  |
| Segment Data (Upper track)  |                                 |         |  |  |
| Data Type                   | Description                     | Color   |  |  |
| CNV                         | Alt allele = DUP                | Blue    |  |  |
| CNV*                        | Copy Number Value >2 (>1 on Y)  | Blue    |  |  |
| CNV                         | Alt allele = DEL                | Red     |  |  |
| CNV*                        | Copy Number Value < 2 (<1 on Y) | Red     |  |  |
| LOH                         | Alt allele = LOH                | Purple  |  |  |
| INS                         | Insertion                       | Gray    |  |  |
| INV                         | Inversion                       | Gray    |  |  |
| DUP:Tandem                  | Tandem Duplication              | Gray    |  |  |

\* CNV segments on the X and Y chromosomes may appear gray if the gender of the file cannot be determined which in turn, prevents the gain/loss status from being determined.

5

4. Optional: Mouse over a segment or a genotype in the VCF track to display more detail, as shown in the genotype example. (Figure 364)

| Figure 364 VCF file mouse over details |                              |  |
|--|------------------------------|--|
|  |                              |  |
| Туре                                   | VCF                          |  |
| Location                               | 5: 180,374,484 - 180,374,485 |  |
| Reference                              | С                            |  |
| Alternate                              | A                            |  |
| GT                                     | 0/1                          |  |
| Quality                                | 233.25                       |  |
| Zygosity                               | Heterozygous                 |  |
| GT DNA                                 | C/A                          |  |

| Mouse-ove | Mouse-over table definitions                            |  |
|-----------|---|--|
| Туре      | File Type   |  |
| Location  | Genome location for the SNP (or Indel)                  |  |
| Reference | Reference Base  |  |
| Alternate | Variant base  |  |
| GT        | Genotype Call, 0 = Reference Base, 1 = Variant Base     |  |
| Quality   | Quality Score assigned from the assay platform          |  |
| Zygosity  | Homozygous or Heterozygous Call                         |  |
| GT DNA    | Call in genotyping format (applies to segment data too) |  |

To link to TaqMan assays from the VCF file, see "Viewing and Ordering TaqMan assays for genotyping" on page 211. **Note:** Linking feature applies to hg38 analyses only.

### **Exporting VCF files**

**IMPORTANT!** Before you can export VCF files, you must install RHAS on the same workstation.

**Exporting CN** and/or variant data as a VCF file (for use in 3rd party browsers)

#### 1. Click Exports $\rightarrow$ Export CHP files to VCF. (Figure 365)

| Figure 365 Exports menu                   |                                   |                                  |  |  |
|---|-----------------------------------|----------------------------------|--|--|
| Exports Analysis ChAS DB Preferences Help |                                   |                                  |  |  |
| POF                                       | Export - PDF Format               | I 😭 🖻 👫 🧕 🛄                      |  |  |
| Ê   | Export Genotype Results Text File | /oview ষ  📷 Segments 🔊           |  |  |
|   | Export CHP files to VCF           |                                  |  |  |
|   | Export AED file from Gene List    |                                  |  |  |
|   | ClinVar Export Ex                 | port samples to VCF format files |  |  |
| <b>P</b> 2                                | Export - Word (docx) Format       | 1.00 V Loss                      |  |  |
| PNG                                       | Export window PNG                 | 3.00 🛦 Gain                      |  |  |
| PNG                                       | Export Karyoview PNG              | 1.00 V Loss                      |  |  |

The Export to VCF window appears. (Figure 366)

| Figure 366 Export to VCF window   |   |  |  |
|---|---|--|--|
| Export to VCF   | × |  |  |
| Select files to export to VCF format.         Files         Image: Select files of FGC-20.cyhd.cychp (NA33)         Image: Select files of FGC-20.cyhd.cychp (NA33)         Image: Select files of FGC-04.cyhd.cychp (NA33)         Image: Select files of FGC-02.cyhd.cychp (NA33)         Image: Select files of FGC-02.cyhd.cychp (NA33) | 0 |  |  |
| Genome<br>Gain. Marker Count ≥ 50, Size (kbp) ≥ 50.<br>Loss. Marker Count ≥ 25, Size (kbp) ≥ 25.<br>LOH. Type turned off. Will be skipped.  |   |  |  |
| These types cannot be exported to VCF: GainMosaic; LossMosaic. Path Select Output Directory   |   |  |  |
| Options Include Genotyping Best and Recommended Probesets Only OK Cancel  |   |  |  |

2. Select the files to be exported in VCF format by clicking on the check box next to the file name.

- Review the Genome filters to make sure the correct data will be exported into the VCF format. If not, click Cancel, update the filters accordingly, then repeat Step 1.
- 4. Click the Path **Browse** button.

A Explorer window appears.

5. Navigate to the location where you want to save your exported VCF, then click **Save**.

Your selected output directory appears.

- Optional: Click the Include Genotyping check box to export the copy number segments and genotype data together. For HTCMA arrays, check Best and Recommended Probesets Only to export genotypes for those probesets the algorithm selected as the best and recommended.
- 7. Click **OK**.



## **Displaying data in table views**

### **Display overview**

The data in the xxCHP files can be displayed and exported in tabular format, as well as the graphic representation in the Karyoview, Selected Chromosome View, and Detail View.

- "Selection details table"
- "CytoRegions table"
- "Viewing the overlap map table"

#### **Common table operations**

The controls that are common to all tables are described in this section and include:

- "Standard tool bar controls"
- "Sorting by columns"
- "Changing the order of table columns"
- "Selecting columns to display or hide"
- Sum, mean, and median calculator"



### Standard tool bar controls

The tool bar provides quick access to table options.

| Left side   |  |
|-------------|--|
| TEV         | Export as TXT file. See "Exporting tables as TXT file" on page 421.  |
| PDF         | Export as PDF Report. See "Exporting table data into a PDF" on page 417.   |
| PO<br>CX    | Export as MS Word DOCX file. See "Exporting as Word (DOCX) format" on page 415.                                      |
|             | Copy selected cells to clipboard. See "Exporting a segments table with modified segments to a TXT file" on page 423. |
| Σ           | Calculate the sum, median, and mean of the selected values from a numeric column.                                    |
|             | Display results for entire genome.   |
|             | Display results for selected chromosome.   |
|             | Display results for portion of chromosome displayed in Detail View.  |
| Far Right   |  |
| 372 results | The number of rows in the table.   |
|             | Opens the Select Columns window that enables you to choose the column headers to show or hide.                       |

The Export functions are described in "Exporting table data" on page 417.

### Sorting by columns

You can sort a table by a single column's values, or by the values in up to three columns. **Note:** You may sort on any column except, for reasons of efficiency, the marker name column in the graphs table.

Sorting on certain columns can cause a noticeable decrease in performance. For example, it is recommended that you do not sort a table using the columns in the Segments table that show the overlapping RefSeq, FISHClones, or other items. The data for these table cells is calculated only on an as-needed basis when it needs to be displayed. Using such a column for sorting would force the calculation of the data for all such cells. Since the sorting would be alphabetical, it is unlikely to be useful. Similarly, for reasons of efficiency, sorting based on the marker name column in the Graphs table is not allowed.

#### Sorting a table by a single column

1. Click in the header of the column to sort the table by that column's values.

A triangle appears in the header.

- Up triangle Min = ascending order.
- Down triangle ▼ Min = descending order.
- 2. Click the header to toggle between Ascending, Descending, or no sort order.

**Note:** The **Type** column in the Segments table sorts segments based on the order that they appear in the **Data Types** window pane, not in an ascending/descending alphabetical order.
#### Performing a multi-column sort

- 1. Click in the header of the first column you want to sort on.
- 2. Click in the header of the second column.
- 3. Click in the header of the third column, if desired.

The last selected column has sort priority.

Sort priority is indicated by the size of the triangle in the header (Figure 367).

| Figure 367 Tabl                 | le sorted by | / descendir | ng order of | Segment II  | D and asce   | ending orde  | r of Size                 |         |
|---------------------------------|--------------|-------------|-------------|-------------|--------------|--------------|---------------------------|---------|
| CytoRegion Type                 | CytoRegion   | Chromosome  | Min         | Max         | 🔺 Size (kbp) | ▼ Segment ID | ▼ Segment File            | Segment |
| Default User Annotation         | CNP505       | 3           | 163,995,351 | 164.106.575 | 111          | seq684       | Beta10 F 01 Cvto VH.cvchp | ▲ Gain  |
| Default User Annotation         | CNP2269      | 17          | 41,521,619  | 41,719,991  | 198          | seg6392      | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP12506     | 17          | 26,040,482  | 26,043,732  | 3            | seg6312      | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP2197      | 16          | 72,953,795  | 73,009,537  | 55           | seg6151      | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP2156      | 16          | 21,422,575  | 21,498,841  | 76           | seg5977      | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP147       | 1           | 194,997,658 | 195,068,695 | 71           | seg587       | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP148       | 1           | 195,089,940 | 195,168,372 | 78           | seg587       | Beta13_F_01_Cyto_VH.cychp | 🔺 Gain  |
| Default User Annotation         | CNP933       | 6           | 32,539,530  | 32,681,749  | 142          | seg572       | Beta15_M_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP901       | 5           | 180,311,316 | 180,350,709 | 39           | seg537       | Beta15_M_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP12104     | 13          | 69,640,329  | 69,670,896  | 30           | seg5218      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP1952      | 13          | 68,149,981  | 68,166,243  | 16           | seg5216      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP12099     | 13          | 66,945,140  | 66,954,900  | 9            | seg5214      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP12094     | 13          | 65,094,528  | 65,103,708  | 9            | seg5210      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP1946      | 13          | 63,122,789  | 63,134,693  | 11           | seg5208      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP12093     | 13          | 64,198,454  | 64,209,619  | 11           | seg5208      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| Default User Annotation         | CNP12091     | 13          | 63,227,094  | 63,303,323  | 76           | seg5208      | Beta13_F_01_Cyto_VH.cychp | V Loss  |
| · · · · · · · · · · · · · · · · |              |             |             |             |              |              | <u></u>                   |         |

1. Click and drag in the column header to move the column to a new location.

# Changing the order of table columns

You can select columns using these two methods:

- Select Columns window.
- Right click on a column header, then choose **Select columns**.
  - 1. Click the Select Columns tool bar button . The Select Columns window opens. (Figure 368)

**Note:** Specific items may vary, as they are dependent upon the type of table and data being displayed.

Selecting columns to display or hide



| Figure 368 Select Columns window  |   |
|---|---|
| 📆 Select Columns  | ×   |
| Choose columns to display by dragging between 'Availat<br>Chosen<br>Use In Export<br>File<br>Name/ID<br>CN State<br>Type<br>Full Location<br>DB Count Both<br>Score (Exon Region)<br>Size (kbp)<br>Marker Count<br>CytoRegions<br>Call<br>Table Sorting<br>Optionally select one or more columns for sorting data i<br>Sort by: CN State<br>Then by: Chromosome | Available<br>% of Overlap Map Item covered by Segment<br>BACs<br>Call Approval<br>Chromosome<br>Curation By<br>Curation Time<br>Cytoband End<br>Cytoband Start<br>DB Coverage Count<br>DB Overlap Count<br>DGV<br>End Marker<br>New York Start<br>DESCENDING V<br>ASCENDING V |
| Then by: Type   | Cancel  |

The columns in the left side pane are displayed in the table (in their default order).

- To hide columns within the table, move the column entry from the left (Chosen) pane into the right (Available) pane.
- To view different columns in the table, click and drag entries from the right pane to the left pane.
- Column order can be determined by clicking onto a column entry, then dragging it into its desired location (inside the left pane).
- Use the **Table Sorting** drop-down menus to sort your columns.

**Note:** These choices are auto-saved between sessions.

#### Using the rightclick menu

Right-click on a column header to perform the following:

- Hide the selected column.
- Show all columns, including hidden ones.
- Expand selected column to display complete heading.
- Auto-size all columns.
- Restore the default selection of columns.
- Select other columns to hide or show.
- Create, select, save, and delete saved table states.

6

#### Using a column header's right-click menu

1. Right-click on the appropriate column header.

A menu appears. (Figure 369)

**Note:** Right-click menu items vary, as they are dependent upon the type of table and data that is displayed.

| Figure 369 Column heading right-click menu   |  |  |            |     |  |       |
|--|--|--|------------|-----|--|-------|
| <ul> <li>Image: Segments Segments CytoRegions Cy</li></ul> |  |  |            |     |  |       |
| File<br>[■♥ ♂ 11-0810_L<br>■♥ ♂ 11-0810_L<br>■♥ ♂ 11-0810_L<br>■♥ ♂ 11-0810_L  | ▼ CN State<br>3.00<br>3.00<br>3.00<br>3.00 | Type<br>▲ Gain<br>▲ Gain<br>▲ Gain<br>▲ Gain | Chromosome | 000 | Hide "Chromosome" 6,<br>Show all columns 5,<br>Expand "Chromosome" 8,<br>Auto-size all columns 4,<br>Restore defaults<br>Select columns<br>Save table state<br>Apply table state ><br>Delete a table state | 6 8 6 |

2. Click to select the option you want.

### Sum, mean, and median calculator

Use this calculator to calculate the sum, mean, and median of the selected or multiple numeric fields.

Calculating multiple numeric values in a column

- 1. Ctrl click or Shift click to highlight multiple numeric fields (MUST BE in the same column).
- 2. Click  $\sum$ .

Your multiple numeric values are calculated and summarized, as shown in. Figure 370.

3. Click OK



| Figure 370 Calculator                                    |              |          |                 |                |            |                    |              |
|--|--------------|----------|-----------------|----------------|------------|--------------------|--------------|
| 👫 Karyoview ষ 🔝 Segments 🔊                               | 🛄 CytoRegio  | ns 🄊 🍊 C | overlap Map 🎙 🔀 | Graphs 🔻       |            |                    |              |
| 🖩 📾 📮 Σ 🔢 🖡 💠  | M 🗹 🗆        |          |                 |                |            | 11                 | results      |
| ▲ File   | CN State     | Туре     | Chromosome      | Cytoband Start | Size (kbp) | Marker Count       | Genes        |
| ∎ Ø o 11-0810_LC_ONC13B_A6                               | 3.00         | 🛦 Gain   | 2               | p16.1          | 488        | 344                | BCL11A       |
| ∎ O 0 11-0810_LC_ONC13B_A6                               | 3.00         | 🛦 Gain   | 2               | p16.1          | 330        | 228                | BCL11A, PAP  |
| ∎ O 0 11-0810_LC_ONC13B_A6                               | 3.00         | 🛦 Gain   | 2               | p16.1          | 161        | 344                | KIAA1841, LO |
| <b>■0</b> 11-0810_LC_ONC13B_A6                           | 3.00         | 🛦 Gain   | 2               | p15            | 159        | 212                | USP34, XPO1  |
| <b>∎</b> ♥♂ 11-0810_LC_ONC13B_A6                         | 3.00         | 🛦 Gain   | 7               | q36.2          | 604        | 440                |              |
| ∎ O 0 11-0810_LC_ONC13B_A6                               | 1.00         | Loss     | 8               | q24.23         | 185        | 120                |              |
| <b>ີ 0</b> d <sup>*</sup> 11-0810_LC_ONC13B_A6           | 3.00         | 🛦 Gain   | 10              | q11.22         | 1,332      | 276                | SYT15, GPRIN |
| <b>■0</b> <sup>*</sup> 11-0810_LC_ONC13B_A6              | 3.00         | 🛦 Gain   | 14              | q32.33         | 248        | 128                |              |
| ∎ O 11-0810_LC_ONC13B_A6                                 | 3.00         | 🛦 Gain   | 14              | q32.33         | 260        | 113                | NCRNA00226   |
| <b>■0</b> <sup>*</sup> 11-0810_LC_ONC13B_A6              | 3.00         | 🔺 Gain   | 17              | q21.31         | 572        | 72                 | KIAA1267, LO |
| <b>○ 0</b> 11-0810_LC_ONC13B_A6                          | 3.00         | 🛦 Gain   | 19              | q13.41         | 202        | 216                | ZNF350, ZNF6 |
|  |              | * S      | um, mean and me | edian X        |            | •                  |              |
| Sum: 4,541 (<br>Mean: 412.829 kbp<br>Median: 259.952 kbp |              |          |                 |                |            | <b>∢ &gt;</b><br>₀ |              |
| 11-0810_LC_ONC13B_A6_PoP#                                | 5_20<br>5_20 | ОК       |                 | ts)            |            | ^                  |              |

### Saved table states

Saving multiple customized tables is a very useful tool, as it enables you to use a large number of columns in a table for interpreting the biology of a particular sample. On the other hand, saving a table state with a smaller subset of columns in a different order - may be more appropriate for including in a report.

In either case, having various Saved Table States enables you to switch back and forth between multiple sets of tables/columns without having to painstakingly recreate interpreting and reporting columns for each of your sample selections.

In the Segments Table, there are six pre-loaded Table States available for use in ChAS:

- Cytogenetic
- Default
- Oncology
- Oncomine Reporter
- ReproSeq
- ClinVar

There are two pre-loaded Table States available for use in the Variants table. See "Variants table" on page 346 for details.

- CytoScan HTCMA
- OncoScan

There are six pre-loaded Table States in the QC & Sample Info table based on relevant metrics for the array type loaded.

CytoScan QC View



- Default
- CytoScan HTCMA QC View
- OncoScan QC View
- ReproSeq QC View
- SMN Sample Info View

**IMPORTANT!** You can restore your default settings at any time by right-clicking on a table column header and selecting Restore Defaults from the menu or selecting the Default Table State under Apply Table State.

Saving the current segment table state to its default

1. Right-click on any column header.

A menu appears.

2. Click Save table state.

The following window appears: (Figure 371)

| Figure 371 Save Table St<br>- Enter a name     | ate |
|--|-----|
| 🖏 Enter a name                                 | ×   |
| Enter a name for the saved table state Default |     |
| OK Cancel                                      |     |

- 3. Type Default.
- 4. Click **OK**.

The table is now saved (as Default) to your User Profile for future use and/or reference.

## Adding columns to table states

1. Click the Select Columns iii icon. (Top right of Segments table)

A list of available columns appears. (Figure 372)

| Figure 372 Save Table State - Availa  | able Columns  |
|---|---|
| Select Columns  | ×   |
| Choose columns to display by dragging between 'Availa<br>Chosen<br>Use In Export<br>File<br>Name/ID<br>CN State<br>Type<br>Full Location<br>DB Count Both<br>Score (Exon Region)<br>Size (kbp)<br>Marker Count<br>CytoRegions<br>Call | able' and 'Chosen'. Re-order entries in 'Chosen' by dragging.<br>Available<br>% of Overlap Map Item covered by Segment<br>BACs<br>Call Approval<br>Chromosome<br>Curation By<br>Curation Time<br>Cytoband End<br>Cytoband Start<br>DB Coverlap Count<br>DB Overlap Count<br>DGV<br>End Marker |
| Table Sorting<br>Optionally select one or more columns for sorting data<br>Sort by: CN State  | in the table.   |
| Then by: Type   | ASCENDING     ASCENDING     Cancel  |

- 2. Move the scroll bar downward to reveal more available columns.
- 3. Click and drag the column header inside the right pane into the desired location inside the left pane.
- 4. Click OK.

The additional column(s) is now added to your the left pane location. If the column is not in the position you want, click on the column, then drag and drop it into its correct spot.

1. Right-click on a column header you want to remove (hide) from the table.

The following menu appears: (Figure 373)

| Figure 373 Save Table State - Remove (Hide) Column |  |     |                               |  |  |  |  |  |
|--|--|-----|-------------------------------|--|--|--|--|--|
| III Karyov   | 👫 Karyoview 🎙 🚰 Segments 🎙 🛄 CytoRegions 🤊 🏉 Overlap N |     |                               |  |  |  |  |  |
|  | 🗎 Σ 🔢  | 1   | 🚮 🗹 🔲                         |  |  |  |  |  |
| te (kbp)   | Mean Marker Dist                                       | anc | Hide "Mean Marker Distance"   |  |  |  |  |  |
| 604  |  |     | Show all columns              |  |  |  |  |  |
| 1,332  |  | 4   | Expand "Mean Marker Distance" |  |  |  |  |  |
| 572  |  | 8   | Auto-size all columns         |  |  |  |  |  |
|  |  |     | Restore defaults              |  |  |  |  |  |
|  |  | 011 | Select columns                |  |  |  |  |  |
|  |  |     | Save table state              |  |  |  |  |  |
|  |  |     | Apply table state             |  |  |  |  |  |
|  |  |     | Delete a table state          |  |  |  |  |  |

2. Click to select Hide "Column Name".

#### Removing (hiding) columns in a table (for report use)



The column is now removed (hidden) from your table.

3. Repeat steps 1-2 to remove (hide) additional columns that are not required for a report.

#### 1. Right-click on any column header.

A menu appears.

#### 2. Click Save table state.

A pop-up window appears. (Figure 371)

| 👭 Karyoview 🎙 🚹  | Segments                     | CytoRe | egions 🎙 🤳 Ove | erlap Map ষ 🔀  | Graphs     |           |               |             |
|--|------------------------------|--------|----------------|----------------|------------|-----------|---------------|-------------|
| 🗄 📠 🗎 Σ  | 🛄 🛛 🕸                        | u 🚮 🗹  |                |                |            |           |               | 4 results   |
| File   | <ul> <li>CN State</li> </ul> | Туре   | Chromosome     | Cytoband Start | Size (kbp) | ▲ Gene Co | ▲ Genes       | OMIM ® Gene |
| ≝♥♂ 11-0810_L  | 3.00                         | 🛦 Gain | 7              | q36.2          | 604        | 0         |               | N/A         |
| ∎♥0 11-0810_L  | 3.00                         | ▲ Gain | 2              | p16.1          | 488        | 1         | BCL11A        | N/A         |
| ∎♥0' 11-0810_L   | 3.00                         | ▲ Gain | 17             | q21.31         | 572        | 8         | KIAA1267, LOC | N/A         |
| ∎ ♥ 0' 11-0810_L   | 3.00                         | 🔺 Gain | 10             | q11.22         | 1,332      | 17        | SYT15, GPRIN  | N/A         |
| Enter a name for the saved table state Reporting_brief OK Cancel |                              |        |                |                |            |           |               |             |
|  |                              |        |                |                |            |           |               |             |

3. Type a name for your new table state. (Example: Reporting\_brief)

The saved table state remembers the columns that you selected, their order, their widths, and which ones were used for sorting.

4. Click **OK**.

The edited table is now saved to your User Profile for future use/reference.

There are six Table States that are created automatically in ChAS.

- Default: Commonly used columns in any xxCHP file analysis
- Oncomine Reporter: Simplified Table State for export and use with Oncomine Reporter Software.
- **Cytogenetic**: Commonly used columns when analyzing constitutional samples.
- **Oncology**: Commonly used columns when analyzing cancer samples.
- **ReproSeq**: Standard columns for use with analyzing ReproSeq samples.
- **ClinVar**: All required columns for using the ClinVar export.

#### Saving a newly edited segment table state

Applying previously saved table states



1. Right-click on any column header.

A menu appears.

2. Click Apply table state.

A sub-menu appears displaying your saved tables, as shown in Figure 375.

| Fig | ure 375 Apply Table State        | - Saved tables list |
|-----|----------------------------------|---------------------|
|     | 0                                | 20 results          |
|     | onclaturo                        |                     |
| 3   | Hide "Microarray Nomenclature"   |                     |
| 3   | Show all columns                 |                     |
| 31  | Expand "Microarray Nomenclature" |                     |
| 3   | Auto-size all columns            | 586,352_1,920       |
| 3   | Restore defaults                 | 92,294_581,22       |
|     | Select columns                   |                     |
| 3   | Save table state                 |                     |
| 3   | Apply table state                | ClinVar             |
| 31  | Delete a table state             | Cytogenetic         |
| _   |                                  | Default             |
|     |                                  | Oncology            |
|     |                                  | Oncomine Reporter   |
|     |                                  | ReproSeq            |

3. Click on the table you want to display.

The table is now displayed.

4. (Optional) Repeat steps 1-3 to apply (open) other previously saved tables.

#### Deleting previously saved table states

1. Right-click on any column header.

A menu appears.

2. Click Delete table state.

The following dialog window appears: (Figure 376)

| Figure 376 Apply Table State - Deleting saved tables |
|--|
| Select State to Delete X                             |
| Default -  |
| OK Cancel  |
| 🖏 Select State to Delete X                           |
| Default  |
| Default  |
| Interpretation_columns_set                           |
| Reporting_brief                                      |

- 3. Click the drop-down button to reveal your saved tables.
- 4. Click to select the table you want to delete.



- 5. Click OK
- 6. (Optional) Repeat steps 1-5 to delete other saved tables.

### Segments table

The Segments table (Figure 377) displays a list of the detected segments in the loaded sample data files.

| Fig  | jure 377 | Segmer       | nts Table   |            |             |             |            |            |            |             |             |              |   |
|------|----------|--------------|-------------|------------|-------------|-------------|------------|------------|------------|-------------|-------------|--------------|---|
|      | <b>p</b> | Σ            | 1 🛊 👪       | <b>V</b>   |             |             |            |            |            |             | 4           | 02 results   | ] |
| File |          | 🛡 CN State 👘 | ▼ Туре      | Chromosome | Min         | Max         | Size (kbp) | Mean Marke | Max % Over | Overlap Map | CytoRegions | Use in Repor |   |
|      | ) s_111  | 4.0          | 🔺 Gain      | 8          | 13,405,640  | 14,703,847  | 1,298      | 822        |            |             |             |              | ^ |
|      | ) S_111  | 3.0          | 🔺 Gain      | ×          | 58,186,054  | 62,269,568  | 4,084      | 19,261     |            |             |             |              |   |
|      | ) S_111  | 3.0          | 🔺 Gain      | ×          | 1,034,120   | 1,620,357   | 586        | 4,844      |            |             | PAR1        |              |   |
|      | ) S_111  | 3.0          | 🔺 Gain      | X          | 2,274,261   | 2,325,565   | 51         | 841        |            |             | PAR1        |              |   |
|      | S_113    | 3.0          | 🔺 Gain      | X          | 1,289,655   | 1,586,449   | 297        | 4,946      |            |             | PAR1        |              |   |
|      | ) S_111  | 2.466        | 🔷 Mosaicism | 8          | 10,389,935  | 17,167,028  | 6,777      | 1,165      |            |             |             |              |   |
|      | ) S_111  | 2.326        | 🔷 Mosaicism | ×          | 50,129,131  | 92,389,241  | 42,260     | 1,912      |            |             |             |              |   |
|      | ) S_111  | 2.228        | 🔷 Mosaicism | X          | 99,724,529  | 107,568,304 | 7,844      | 1,342      |            |             |             |              |   |
|      | ) S_111  | 2.22         | 🔷 Mosaicism | X          | 125,958     | 3,337,170   | 3,211      | 1,775      |            |             | PAR1        |              |   |
|      | ) S_111  | 2.212        | 🔷 Mosaicism | X          | 108,081,093 | 109,525,168 | 1,444      | 1,342      |            |             |             |              |   |
|      | S_111    | 2.203        | 🔷 Mosaicism | X          | 140,637,962 | 140,994,866 | 357        | 1,230      |            |             |             |              |   |
|      | S_111    | 2.203        | 🔷 Mosaicism | X          | 107,604,073 | 107,688,354 | 84         | 1,204      |            |             |             |              |   |
|      | S_111    | 2.203        | 🔷 Mosaicism | X          | 123,823,789 | 123,907,942 | 84         | 914        |            |             |             | <b>v</b> ,   | ~ |
| <    |          |              |             |            |             |             |            |            |            |             |             | >            |   |

Segment Table components:

- "Segments table tool bar" on page 334
- "Segments table" on page 334

There are certain columns in the Segments table which dynamically compute intersections of reference annotations with the segments. The data in these columns is computed on an as-needed basis for each cell. You may see text such as "<Working...>" in these cells while the data is being calculated. The results for all cells will be calculated when exporting to PDF or TXT, or copying to the clipboard. Hiding columns that are not needed may improve performance, particularly during export operations.

**Note:** Sorting the table based on the dynamically computed columns may be slow.

To highlight segments in the views or the table:

- Double-click in a row of the table to zoom to the segment in the Karyoview, Selected Chromosome and Detail Views.
- Click on a segment in the Karyoview, Selected Chromosome or Detail View to highlight the segment in the Segments table.



## Segments table tool bar

The tool bar (Figure 378) provides quick access to table functions. The standard functions are described in "Standard tool bar controls" on page 324.



Use the **Use in Export** check boxes to Select all or select all or select all or select all segments displayed in the table.

**Note:** If the **Use in Export** column is hidden from the Segments table, then all rows are exported.

#### Segments table

In the Segments table, "N/A" can mean that the information (for example, FISH Clones or sno/mRNA) is not available in the NetAffx database because the information has not yet been mapped. For example, FISH Clones or sno/mRNA files will not appear in the Files list for the NA31 (hg19) ChAS Browser NetAffx Genomic Annotation file. "N/A" can also mean that a column which has been persisted to appear from a previous user profile, no longer has data in the current NetAffx Genomic Annotation file that is loaded.

Annotations which share genomic coordinates with a segment are listed in order of start coordinate value, smallest values first (i.e. from left to right in the Details View). For annotations with the same start coordinate (for example, isoforms of a single gene), the one with the smallest end coordinate is listed before others with larger stop coordinates.

If a column in the Segments table contains more than 10 items, "..." is displayed after the 10<sup>th</sup> item to indicate that some data are not displayed in order to save calculation time. For example, "..." will follow the 10<sup>th</sup> name in the Genes column. However, a complete list of the genes will be included when the information is copied to the system clipboard or exported to reports. For gene isoforms with identical names, only one instance of the gene locus will be listed in the Segment table to reduce duplicate gene names.

The table can display each segment with the following information (the default set of columns in a new user profile may include only a subset of the total columns listed. For instructions on how to switch quickly between table column sets for a particular table, see "Saved table states" on page 328.

Materially Modified Segments (merged, created *de novo*, segments with edited start or end coordinates) and deleted segment have a different appearance in the Segments Table and export to TXT differently depending on the software settings. For more information, see "Exporting a segments table with modified segments to a TXT file" on page 423.



## Segment table columns

| Column  | Description  |
|---|--|
| % of Overlaps Map<br>Item covered by<br>Segment | Overlap Map Item and the percentage by which it is covered by the segment.   |
| Call  | User-editable field populated by a user-configurable drop list of Calls  |
| Call Approval                                   | Can be used as a bookmark for segments that have been reviewed.  |
| Call From<br>Prioritization                     | The Call term assigned based on Tier or Score Classification. For more information, see "Viewing segment prioritization in the segments table" on page 375.  |
| Chromosome                                      | Chromosome on which the segment was found.   |
| CN State  | Copy Number State (not displayed for LOH segment types).<br>The expected Copy Number State on the X chromosome in normal males is not constant over its entire length. This is due to the structure of the sex chromosomes. See "LOH segments on X and Y chromosomes" on page 49 for more information.   |
| Confidence<br>(ReproSeq)                        | The log likelihood that the called copy number state is not normal ploidy, example 2 on autosomes (reflects the likelihood of the region's ploidy number being different than the normal ploidy 2).  |
| Curation By                                     | The current computer Operating System login ID and ChAS user profile name at the time that the Curation field was last edited.   |
| Curation Time                                   | The time and date when the Curation field was last edited.   |
| Cytoband End                                    | Cytoband in which the segment ends.  |
| Cytoband Start                                  | Cytoband in which the segment begins.  |
| CytoRegions                                     | Names of the CytoRegions with which the segment shares coordinates.  |
| DB Both   | The number of segments in the database meeting BOTH the Minimum Percent Overlap and the Minimum Percent Coverage. This number can change depending on whether the "match only same gain/loss type" box is checked. Right-click on the a row in the Segment Table. From the menu, click DB Count Both. See "Querying a segment from the segment table" on page 387. |
| DB Coverage                                     | The number of segments in the database meeting the Minimum Percent Coverage. This number can change depending on whether the "match only same gain/loss type" box is checked. Right-click on the a row in the Segment Table. From the menu, click DB Coverage Count. See "Setting up a ChAS DB query" on page 385.   |
| DB Overlap                                      | The number of segments in the database meeting the Minimum Percent Overlap. This number can change depending on whether the "match only same gain/loss type" box is checked. Right-click on the a row in the Segment Table. From the menu, click DB Overlap Count. See "Setting up a ChAS DB query" on page 385.   |
| DGV   | List of DGV variations that share coordinates with the segment.  |
| DGV-GS  | List of curated Database of Genomic Variants considered "Gold Standard" that share coordinates with the segment.   |
| End Marker                                      | The array marker name which marks the end of the segment.  |
| Evidence  | Provides information on which annotations the segment overlapped. For more information, see "Viewing segment prioritization in the segments table" on page 375.  |



| Column                         | Description  |
|--------------------------------|--|
| File                           | File the segment was detected in.  |
| Filtered DB Both               | The number of segments in the database meeting the Minimum Percent Overlap and Minimum Percent Coverage and the selected Filter Criteria.  |
| Filtered DB<br>Coverage        | The number of segments in the database meeting the Minimum Percent Coverage and the selected Filter Criteria.  |
| Filtered DB<br>Overlap         | The number of segments in the database meeting the Minimum Percent Overlap and the selected Filter Criteria.   |
| Following Marker               | The array marker just below the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments.  |
| Following Marker<br>Location   | The coordinate location of the array marker just below the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments.   |
| Full Location                  | Chromosome Start and Stop in a user-friendly format for use in external databases.   |
| Gene Count                     | A count of the gene names listed in the Genes column   |
| Genes                          | List of RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.   |
| Inheritance                    | User-editable field populated by a user-configurable drop list of Inheritance.   |
| Marker Count                   | Number of markers in the segment.  |
| Materially Modified<br>By      | The current computer Operating System login ID and ChAS user profile name at the time that the segment was last materially modified.   |
| Materially Modified<br>Segment | Indication that segment was previously merged, deleted, or had its start or end boundary, type, or state altered by a ChAS user. (ChAS-based processes of Smoothing and Joining are not "Modifications", nor are making Calls or Interpretations, in this context).  |
| Materially Modified<br>Time    | The time and date when the Segment was last materially modified.   |
| Мах                            | End position of segment.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic<br>position coordinates" on page 475). |
| Max % Coverage                 | The highest percentage by which a segment covers some item(s) in the Overlap Map.  |
| Max % Overlap                  | The highest percentage by which some item(s) in the Overlap Map overlaps the segment. Segments completely overlapped by an Overlap Map item are 100% overlapped. This number is used for Filtering Segments out by "Overlap".  |
| Mean Log2 Ratio                | The mean of all the Log2 Ratio values contained in the segment.  |
| Mean Marker<br>Distance        | Length of the segment in base pairs divided by the number of markers in the segment.   |
| Mean Weighted<br>Log2 Ratio    | The mean of all the Weighted Log2 Ratio values contained in the segment.   |
| Median Log2 Ratio              | The median of all the Log2 Ratio values contained in the segment.  |



| Column  | Description  |
|---|--|
| Microarray<br>Nomenclature                        | An ISCN-based description of the segment.  |
| Microarray<br>Nomenclature<br>ISCN 2013           | ISCN 2013 based description of the segment.  |
| Min   | Start position of segment.   |
| Number of Overlap<br>Map Items                    | Number of Overlap Map items which share genomic coordinates with the segment.  |
| OMIM Gene Count                                   | A count of the OMIM Gene names listed in the OMIM Genes column.  |
| OMIM Genes  | List of OMIM Genes that share coordinates with the segment.  |
| OMIM Phenotype<br>Loci                            | List of OMIM Phenotype Loci that share coordinates with the segment.   |
| OMIM Region<br>Phenotype                          | Lists the Gene Title of any region that overlaps with a copy number or LOH segment.  |
| Oncomine Report                                   | A drop-down list of annotations compatible with the Oncomine Reporter Software. For details on this application, go to: https://www.thermofisher.com/order/catalog/product/A34298              |
| Overlap Map Items<br>(% of Segment<br>overlapped) | Item(s) in the Overlap Map which overlap the segment, followed by the percentage by which the segment is overlapped by that Item.  |
| Preceding Marker                                  | The array marker just above the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments.                            |
| Preceding Marker<br>Location                      | The coordinate location of the array marker just above the segment in the data track used as input for the segment.<br>Note: This column is only applicable to CNState Gain and Loss segments. |
| Precision<br>(ReproSeq)                           | The log likelihood that the called copy number state is different than next closest copy number state (reflects the likelihood that the precise ploidy number is correct).                     |
| Protein Coding<br>Genes                           | List of protein coding RefSeq genes from the Genes track that share coordinates with the segment.<br>Identically named gene isoforms are NOT repeated.   |
| Protein Coding<br>Genes Count                     | Number of Protein Coding RefSeq genes that shapre coordinates with the segment.  |
| Protein Coding<br>Ensembl Genes                   | List of protein coding Ensembl Genes annotations that share coordinates with the segment. Identically named gene isoforms are NOT repeated.  |
| Protein Coding<br>Ensembl Genes<br>Count          | Number of Protein Coding Ensembl Genes that share coordinates with the segment.  |
| Sample UUID                                       | Unique identifier for the CHP file.  |
| Segment<br>Interpretation                         | User-editable field for free-text interpretation on the segment  |
| Segment Label                                     | A label comprised of the segment's Type, State, and Filename.  |
| Segment Name/ID                                   | File-specific identifier assigned to the detected segment.   |



| Column                         | Description   |
|--------------------------------|---|
| Segmental<br>Duplications      | List of Segmental Duplications that share coordinates with the segment.   |
| Size (kbp)                     | Size of the segment.  |
| Smoothed/Joined/<br>XON merged | Indication that the segment was created by smoothing, joining or merging two or more segments in the initial segment detection.   |
| sno/miRNA                      | List of sno/miRNA features that share coordinates with the segment.   |
| Start Marker                   | The array marker name which marks the beginning of the segment.   |
| Summarized Log 2<br>Ratio      | The median of the LR, after transformation to adjust for individual marker responsiveness.  |
| Tier or Score                  | The assigned Tier or Score value based on the Segment Prioritization method selected. When using Tier based, the column will display the assigned Tier. When using Score based, the column will display the score value based on the annotations the segment overlaps.For more information, see "Viewing segment prioritization in the segments table" on page 375. |
| Туре                           | Type of segment, for example, LOH. When sorting by this column, the segments of a particular sample are listed in the same order that they appear in the Data Types window pane.  |
| Use in Report                  | Allows manual selection of Segments for export to a Segments Table PDF, DOCX, or Text rather than all segments in the table.  |
| XON Region Level               | The annotation Level assigned to this region of the genome.   |

#### Obtaining all annotations associated with a segment

 Select a segment in the Segments table, Karyoview, Detail View, or Selected Chromosome View, then press Ctrl+Space or right-click on the segment to select Zoom to selection from the menu.

The exact length of the selected segment fills the entire width of the Detail View (Figure 379).



2. Click the 🔝 tool bar button to expand all annotation tracks.







- 3. Using the mouse, draw a box around all of the genes and annotations of interest. When you release the mouse button, a blue box outlines the selected items in the Detail View. (Figure 380)
- 4. Click the 🛄 tool bar button.

The Selection Details table appears (Figure 381). It includes all of the items selected in the Detail View. For more details on the table, see "Selection details table" on page 204.

| Figure 381 Selection Details table |                     |                |            |             |            |           |            |              |            |                |            |      |
|------------------------------------|---------------------|----------------|------------|-------------|------------|-----------|------------|--------------|------------|----------------|------------|------|
|                                    | Selection Details X |                |            |             |            |           |            |              |            |                |            |      |
| Click a button                     |                     | Σ              |            |             |            |           |            |              |            |                | 220 resu   | itts |
| to export the                      | Annotation          | Chromosome     | Min        | Max         | Size (kbp) | Туре      | Strand     | DGV ID       | Gain       | Loss           | Frequency  | Ē    |
| information                        | Variation 37        | 7              | 57,701,877 | 57,723,959  | 22         | 🗸 dgv     |            | Variation 37 | 3          | 0              | 0.00478468 | ^    |
|                                    | q11.1               | 7              | 59,100,000 | 61,100,000  | 2,000      | 🚥 Cytoban |            |              |            |                |            |      |
|                                    | p11.1               | 7              | 57,400,000 | 59,100,000  | 1,700      | 🚥 Cytoban |            |              |            |                |            |      |
|                                    | ZNF138              | 7              | 63,892,205 | 63,931,458  | 39         | 📮 Genes   | FORWARD    |              |            |                |            |      |
|                                    | Variation 34        | 7              | 61,718,176 | 62,343,622  | 625        | 🗸 DGV     |            | Variation 34 | 2          | 0              |            |      |
|                                    | Variation 36        | 7              | 64,593,557 | 64,594,836  | 1          | 🗸 DGV     |            | Variation 36 | 1 1        | 0              |            |      |
|                                    | Variation 23        | 7              | 63,953,259 | 63,955,511  | 2          | V DGV     | 1          | Variation 23 | 0          | 1              |            | ~    |
|                                    | <                   |                |            |             |            |           |            |              |            |                | >          |      |
|                                    |                     |                |            |             |            |           |            |              |            |                |            |      |
|                                    | Property            | Variation_3775 | 7          | q11.1       |            | p11.1     |            | ZNF138       |            | Variation_3444 | 4          |      |
|                                    | Annotation          | Variation 37   | 757        | q11.1       |            | p11.1     |            | ZNF138       |            | Variation 34   | 444        | ^    |
|                                    | Chromosome          |                | 7          |             | 7          |           | 7          |              | 7          |                |            |      |
|                                    | Min                 |                | 67,701,877 |             | 59,100,000 |           | 57,400,000 |              | 63,892,205 |                | 61,718,1   |      |
|                                    | Max                 |                | 57,723,959 |             | 61,100,000 |           | 59,100,000 |              | 63,931,458 |                | 62,343,6   |      |
|                                    | Size (kbp)          |                | 22         |             | 2,000      |           | 1,700      |              | 39         |                | e          |      |
|                                    | Туре                | 🗸 dgv          |            | 🚥 Cytobands |            | Cytobands | 3          | 📜 Genes      |            | 🗸 dgv          |            |      |
|                                    | Strand              |                |            |             |            |           |            | FORWARD      |            |                |            |      |
|                                    | DGV ID              | Variation 37   | 757        |             |            |           |            |              |            | Variation 34   | 444        |      |



For details on exporting from the Segment table, see "Exporting table data" on page 417.

### **Graphs table**

**IMPORTANT!** The results from ChAS are for <u>Research Use Only</u>. Not for use in diagnostic procedures.

The Graphs table displays the marker data used to create the graphs in the Detail view. Markers that are not used for the graphs currently displayed do not appear in this table. As in the Detail View, only markers from a single chromosome are displayed.

The column headings are colored according to the tracks used for the Karyoview, Selected Chromosome View, and Details View.

The Graphs table includes genotype SNP calls for CytoScan array results (Figure 382).

| Figure 3  | Figure 382 Example Graphs table with Genotype Calls for CytoScan HD results |             |                |           |            |          |           |           |               |      |
|-----------|---|-------------|----------------|-----------|------------|----------|-----------|-----------|---------------|------|
| Karyoviev | 🏥 Karyoview ষ 📩 Segments ষ 🕌 CytoRegions ষ 🧈 Overlap Map ষ 🖂 Graphs 🔊       |             |                |           |            |          |           |           |               |      |
|           | Σ   | I  l        | 25 🔒           |           |            |          |           | 65133 re: | sults from ch | ir 3 |
| Chromoso  | Position  | In Cytoregi | Markers        | 🔷 Genotyp | OAllele Pe | OLOH: NA | 🔷 Genotyp | Allele Pe | 🔷 LOH: NA     |      |
| 3         | 98,950,964  | ×           | <u>S-3ZZMW</u> | RR        | -0.86      | Ŭ        | RR        | -1.12     | Ŭ             | ~    |
| 3         | 98,951,834  | ×           | S-40ICY        | AA        |            | 0        | AA        | 0.84      | 0             |      |
| 3         | 98,952,443  | ×           | S-3IWYV        | BB        | -1.44      | 0        | AB        | 0.19      | 0             |      |
| 3         | 98,964,877  | ×           | S-4CHOC        | AA        | 0.73       | 0        | AA        | 0.67      | 0             |      |
| 3         | 98,969,437  | ×           | S-4HXZU        | BB        | -0.90      | 0        | AB        | 0.00      | 0             | 1    |
| 3         | 98,971,751  | ×           | S-3UIQV        | AA        | 0.79       | 0        | AA        | 0.94      | 0             | 1    |
| 3         | 98,976,066  | ×           | S-4PLOX        | AB        | -0.01      | 0        | AB        | -0.04     | 0             | 1    |
| 3         | 98,987,381  | ×           | S-4RZBP        |           | -0.16      | 0        |           | -0.11     | 0             | Н    |
| 3         | 98,987,607  | ×           | S-3NFFM        | AB        | -0.18      | 0        | AB        | -0.19     | 0             | Н    |
| 3         | 98,988,001  | X           | S-3BEAX        | AB        | 0.02       | 0        | AB        | 0.11      | 0             | 1    |
| 3         | 98,988,161  | X           | S-3EGSO        | BB        | -1.44      | 0        | BB        | -1.43     | 0             | 1    |
| 3         | 98,990,993  | X           | S-4NDAG        | AB        | 0.01       | 0        | AB        | -0.06     | 0             |      |
| 3         | 99,013,299  | X           | S-4BPQE        | BB        | -0.98      | 0        | BB        | -1.07     | 0             |      |
| 3         | 99,013,781  | X           | S-3XSEU        | AA        | 0.79       | 0        | AA        | 0.85      | 0             |      |
| 3         | 99.013.835  | ×           | S-3KYKV        | BB        | -0.76      | 0        | BB        | -1.12     | 0             | ^    |
| 3         | 99.013.936  | ×           | S-3ASGB        |           | -0.95      | 0        |           | -0.95     | 0             | ~    |
| <         |   |             |                |           |            |          |           |           |               |      |
|           |   |             |                |           |            |          |           |           |               |      |

The table displays each data point in the displayed graphs.

To highlight markers in the views or the table:

- Click in a row of the table to place the cursor on the marker in the Chromosome and Detail Views.
- Click on a marker in the Selected Chromosome or Detail View to highlight the marker in the Graphs table.

Graphs table properties

The Graphs columns display:



| Column           | Description   |
|------------------|---|
| In CytoRegion    | <ul> <li>Whether marker is located in a CytoRegion or not:</li> <li>✓ In CytoRegion</li> <li>➤ Not in CytoRegion.</li> <li>See "Using CytoRegions" on page 263 for more information.</li> </ul> |
| Markers          | Marker ID. Right-click to link to NetAffx information about the marker.<br>Note: For efficiency reasons, it is not possible to sort the table on this column.                                   |
| Genotype         | The SNP genotype call.  |
| Graph Data types | Log2Ratio: NA18526_C09_01.cychp The Column header displays: Color Nib Data Type Name of sample file The table cell displays the value for the marker.   |

## Graph Table Tool bar

| Figure 383 Graphs tool bar   |                           |  |  |
|------------------------------|---------------------------|--|--|
| 🖩 🗟 😢 🗂 \Sigma   🎟 🚺 🛊   🗷 諸 | 218228 results from chr 1 |  |  |

The tool bar (Figure 383) enables you to:

- Export data in TXT format only. See "Exporting tables as TXT file" on page 421
- View data for chromosome and selected chromosome region only. You cannot display data from the whole genome in Graphs tab. You can export this data for the whole genome using the "Displaying and exporting data from the analysis workflow" on page 89.
- Export genotype results

The Graphs Settings button in opens the Graph Settings panel, enabling you to change the style of graph, scale, and other features for the data graphs. See "Changing graph appearance" on page 192.

#### Note: Exporting genotypes is not available for OncoScan or ReproSeq data.

- 1. Click the tool bar button. Alternatively, select **Reports**  $\rightarrow$  **Export Genotype Results Text File** from the menu bar.
- 2. In the window that appears (Figure 384 on page 343), select the array type, results file(s) (CYCHP), and annotation database to use for the export.

## Exporting genotype calls



| Figure 384 Export Genotype Results  |
|---|
| Export Genotype Results Text File X   |
| Select Array Type   |
| CytoScan HD Array   |
| Files<br>▼ № ♀ 09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.hg38.<br>□ № ♂ 11-1466JL_A4_RC_8-4-11_CMA-172.hg38.cyhd.cychp (NA36)   |
| Select Annotation Database  |
| CytoScanHD_Array.na36.annot.db  |
| Region to Export         Image: SNP List         Image: Selected Region (chr22: 1 - 50,818,468)         Image: Selected Chromosome (22)         Image: Image: Selected Chromosome (22)         Image: Image: Selected Chromosome (22) |
| Select Output   |
| Path:     C:\ChAS 4.1       Name:     Sample_GenotypeExport   |
| Multiple File Output  |
| Separate File for each Chromosome Separate File for each CHP File   |
| OK Cancel   |

- 3. Specify a region to export:
- **SNP List**: SNPs specified in a user-created SNP list (.txt).
- Selected Region: SNPs included in the chromosome region selected in the Karyoview.
- Selected Chromosomes: SNPs on the chromosome selected in the Karyoview.
- All Chromosomes: SNPs on all 24 chromosomes.

**Note:** The SNP list should include one column header named Probe Set ID or ProbeSet\_ID and one probe set name per row, as shown in Figure 385 on page 344.

The export will not proceed without one of these column headers.



| Figure 385 Example SNP list |              |  |  |
|-----------------------------|--------------|--|--|
|                             | А            |  |  |
| 1                           | Probe Set ID |  |  |
| 2                           | S-3FHVO      |  |  |
| 3                           | S-3PWJF      |  |  |
| 4                           | S-3HSFV      |  |  |
| 5                           | S-3IYFW      |  |  |
| 6                           | S-4CTUJ      |  |  |
| 7                           | S-3APZN      |  |  |
| 8                           | S-3UDWU      |  |  |

- 4. Enter the path name or click the **Browse** button to select a folder for the output.
- 5. Enter a file name prefix. If only one output file is created (see below), this will be the file name. If multiple files are created, a suffix will be added to this string to create the file name. Do not include the file extension here.
- 6. Select a Multiple File Output option which determines if a separate file will be created for each chromosome and/or CYCHP file.

| Selected Output Option(s)   | Files Created  |
|---|--|
| None  | One output file will be exported that contains all chromosome and all CYCHP file data.<br>There will be separate data columns for each CYCHP file in the exported file.                                  |
| Separate File for each<br>Chromosome  | Creates a separate file for each chromosome in the output data. If all chromosomes are selected, 24 files will be created. There will be separate data columns for each CYCHP file in the exported file. |
| Separate File for each CYCHP File   | Creates one text file per CYCHP file. Each file contains genotype calls for all chromosomes.   |
| Separate File for each<br>Chromosome and Separate File for<br>each CYCHP File | Create a separate file for each CYCHP file and for each chromosome. If three CYCHP files are selected and all chromosomes are reported on, this will create 72 files.                                    |

#### 7. Click **OK**.

**Note:** Exporting of Genotypes may take several minutes to complete, as this process is dependent on the total number of SNPs selected for export.



| Figure 386 Example of exported genotypes from a                                 | selected region of chromosome X                   |             |
|---|---|-------------|
| Genotypes_10624_NA00682_B1_MS_plate3A_CytoScan_VH_20101206.CytoScanHD_Array - N | Notepad   |             |
| File Edit Format View Help  |   |             |
| # Apportation DB Used: C:\ProgramData\Affymetrix\Chromosome Apa                 | lysis Suite\Library\CytoScanHD_Array_na31_annot_d | h .         |
| # Array Type Name: CytoScan HD Array  |   | -           |
| # Array Type Internal Name: CytoScanHD_Array                                    |   |             |
| # Export GUID: 57825efe-111e-4c1f-b246-0ac30e90a955                             |   |             |
| # Array Annotation Database NetAffx Build: 31                                   |   |             |
| # UCSC Genomic Version: hg19  |   |             |
| # dbsnp version: 121  |   |             |
| # CHP File: C:\Users\Public\Documents\Pesults\NA00682 B1 MS nl                  | ate34 CytoScan VH 20101206 CytoScanHD Array cychn | (NA31)      |
| # Input Chromosome: X   | acesA_eyeosean_m_zororzooreyeoseanno_Array.eyenp  | (MADI)      |
| # Input Region: 32467670 to 39977200  |   |             |
| # output chromosome: All  |   |             |
| Probe Set ID Call Codes Confidence Signal A                                     | Signal B Forward Strand Base Calls                | dbSNP RS ID |
| S-3FHVO AB 2.6645353E-15 1439.5713 1465.9479                                    | AG rs5972570 X 32467970                           |             |
| S-3PWJF BB 3.164554E-4 1819.0908 3027.3113                                      | CC rs6631589 X 32474756                           |             |
| 5-3H5FV AA 3.0839404E-14 2010.413 033.0292                                      | TT nc591338/2 X 324/4/93                          |             |
| S-ACTUL BR 1 1014025E-11 587 60876 2515 3006                                    | G rs73610003 X 32474003                           |             |
| 5-54PZN AA 2.220446E-16 1630.4667 325.15146                                     | CC rs7886431 X 32477868                           |             |
| S-3UDWU AB 0.0 1046.0934 973.08466 GA   | rs228392 X 32487337                               |             |
| 5-4IMJH BB 0.0 205.25491 1140.2894 GG   | rs1033462 X 32491061                              |             |
| 5-4QILH AB 4.6629367E-15 343.2968 343.11258                                     | CT rs228397 X 32491351                            |             |
| S-3RURA BB 3.2804226E-10 1042.2152 2928.8074                                    | CC rs16998310 X 32491564                          |             |
| S-3XUYV AB 1.26565425E-14 1885.5472 2282.4426                                   | TC rs228403 X 32499010                            |             |
| S-45GGR AA 8.881784E-16 1264.3896 256.46527                                     | CC rs5927077 X 32499987                           |             |
| 5-4EVRJ BB 0.0 203.82622 902.2788 CC  | r5/884521 X 32508989                              |             |
| S-3650W AB 6 6613385-16 855 1064 085 78174                                      | AC rs228314 Y 32511002                            |             |
| 5-460EV AA 0.0 1065,4402 196,95076 CC   | rs2023557 x 32515117                              |             |
| 5-4NK5K BB 0.0 542.1877 2601.7148 TT  | rs41500547 X 32518960                             |             |
| 5-3HOWD AB 0.0 1236.4248 1168.713 AG  | rs228333 X 32536452                               |             |
| S-3WWFU AB 6.1460135E-9 1957.5522 2141.443                                      | AT rs228337 X 32539807                            |             |
| 5-3HYJZ AB 8.245182E-12 1427.3093 1196.1893                                     | GA rs228338 X 32540070                            |             |
| S-3ZAGU AA 0.0 2544.556 436.40463 AA  | rs/3453/49 X 32540396                             |             |
| 5-3QCVZ AB 2.032050/E-10 931.81/0 014.83085                                     | CI CS119301 X 32543241                            |             |
| S-Shuno AA 0.0 3115 7256 554 60006 cc   | rs170606 ¥ 32544026                               |             |
| 5-4JAXT AA 5.4622307E-11 1249.4127 410.03027                                    | TT rs5972592 X 32544255                           |             |
| 5-4NXQF BB 2.220446E-15 370.76147 1828.4547                                     | CC rs228347 X 32548066                            |             |
| 5-4FZYD AA 6.340586E-5 3566.6406 1767.4355                                      | TT rs17318147 X 32552118                          |             |
| 5-4AZFO BB 7.19913E-12 526.51654 1727.5386                                      | GG rs7888911 X 32561313                           |             |
|   |   | *           |
|   |   | ► a         |

The exported text file (Figure 386) includes information about the analysis (for example, array type, NetAffx annotation database, hg version, and chromosome).

**Note:** If the option "Separate File for each CYCHP File" was not selected, many of the headers will be repeated for each CYCHP file. The header titles will be appended with the CYCHP file name to indicate which file the column belongs to.

The column headers report the following information:

| Column                    | Description                                     |
|---------------------------|---|
| Probe Set ID              | Probe set identifier                            |
| Call Codes                | Genotype call for the SNP.                      |
| Confidence                | Confidence value for the call.                  |
| Signal A                  | Raw signal value for Signal A on the probe set. |
| Signal B                  | Raw signal value for Signal B on the probe set. |
| Forward Strand Base Calls | Base calls for the forward strand.              |
| dbSNP RS ID               | dbSNP RS ID value                               |
| Chromosome                | Chromosome associated with the probe sets.      |
| Chromosomal Position      | Chromosome position of the SNP.                 |



### Variants table

For OncoScan FFPE and CytoScan HTCMA arrays only. The Variants table (Figure 387) displays the somatic mutation information from OncoScan FFPE arrays and/or the variant information from CytoScan HTCMA arrays.

| Figure 387 Variants          | table           |                          |                    |             |                 |          |             |         |
|------------------------------|-----------------|--------------------------|--------------------|-------------|-----------------|----------|-------------|---------|
| 👫 Karyoview 🎙 🛛 🙀 Segments 🎙 | 🕌 CytoRegions 🎙 | 루 Overlap Map 🎙 🛛 🖾 Graj | ohs 🎙 🕞 Variants 🎙 | 💷 Query Sar | mples 🎙 💷 Query | Segments |             |         |
| 🖩 👪 🖺 Σ 🛄                    | ] 🕸             |                          |                    |             |                 |          | 1,831 re    | sults 🔟 |
| File                         | ProbeSet Type   | ▲ Affx SNP ID            | Name/ID            | Marker T    | . Common Name   | ▲ Gene   | c.name      | Туре    |
| Scan_HTCMA_96F_P             |                 | Affx-149264330           | AX-169280280       | GT          |                 | VPS53    | c.1556+5G>A | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-142712903       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-142704177       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169328150       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| OcytoScan_HTCMA_96F_P        |                 | Affx-149264393           | AX-169315566       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169255309       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan HTCMA 96F P         |                 | Affx-149264393           | AX-169261313       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169312899       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169286092       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169252293       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169300412       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169261418       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| CytoScan_HTCMA_96F_P         |                 | Affx-149264393           | AX-169294699       | GT          |                 | VPS53    | c.2084A>G   | • Ui    |
| ■ ♥ o Output10.OSCHP         | SOM             |                          | som-93107501A      |             | NRAS:p.Q61L:c   |          |             | • Ui    |
| Output10.OSCHP               | SOM             |                          | som-93107513C      |             | NRAS:p.Q61R:    |          |             | • Ui    |
| ■♥ o Output10.OSCHP          | SOM             |                          | som-93107510A      |             | NRAS:p.Q61K:c   |          |             | • Ui    |
| ■♥ o Output10.OSCHP          | SOM             |                          | som-93107498A      |             | NRAS:p.G12V:c   |          |             | • Ui    |
| ■♥ o Output10.OSCHP          | SOM             |                          | som-93107496A      |             | NRAS:p.G12D:    |          |             | • Ui    |
| Output10.OSCHP               | SOM             |                          | som-93107497A      |             | NRAS:p.G12S/    |          |             | • Ui    |
| ■♥ o Output10.OSCHP          | SOM             |                          | som-93107483A      |             | IDH1:p.R132H:c  |          |             | • Ui    |
| Contraction Output 10. OSCHP | SOM             |                          | som-93107511A      |             | PIK3CA:p.E542   |          |             | • Ui    |
| Output10.OSCHP               | SOM             |                          | som-93107500A      |             | PIK3CA:p.E545   |          |             | • Ui    |
| ■♥ o Output10.OSCHP          | SOM             |                          | som-93107515C      |             | PIK3CA:p.Q546   |          |             | • UIC   |
| Go Output10.OSCHP            | SOM             |                          | som-93107512C      |             | PIK3CA:p.H104   |          |             | • Ui    |

The Variants table components include:

- Tool bar (below)
- Variants table on page 346

To highlight segments in the views or in the table:

- Click the row of the table to zoom to the somatic mutation in the Karyoview, Selected Chromosome View and Detail View.
- Click on the somatic mutation in the Karyoview, Selected Chromosome View or Detail View to highlight the somatic mutation in the Somatic Mutations Table.
  - Detected: Large, bright green circles in the graphic views denote somatic mutations of high confidence (OncoScan) or variants called het, hom, NoCall, NRP (anything that isn't a major homozygous call)
  - Undetected: Small, dark gray dots in the graphic views denote somatic mutations and variants in which the wild type or major homozygous genotype was called.

**Note:** Calls can be removed from the table (and graph view) by de-selecting the Undetected or Detected check box(es). in the Data Types window.



## **Tool bar** The Tool bar (Figure 388) provides quick access to table functions. Its standard functions are described in "Standard tool bar controls" on page 324.

| Figure 388 Tool bar |            |
|---------------------|------------|
|                     | 64 results |

# Variants table The table can display each mutation with the following information (the default set of columns in a new user profile may include only a subset of the total columns listed below).

#### CytoScan HTCMA

| Column                  | Description   |  |
|-------------------------|---|--|
| Affx SNP ID             | Unique Thermo Fisher Scientific generated identifier for the SNP.   |  |
| Alt Allele              | The call for the first alternate allele associated with a non-normal phenotype.                               |  |
| Alt Code                | The Call Code (A,B,C,D,) of the Alternate Allele.   |  |
| Alternate Name          | Displays the alternative names for the variant.   |  |
| Associated Phenotype    | Displays the Phenotype that is associated with the variant.   |  |
| c.name                  | Displays standard variant nomenclature based on coding DNA reference sequences.                               |  |
| Chromosome              | The chromosomal location of the variant.  |  |
| File                    | Name of the sample file.  |  |
| g.name                  | Displays the genomic coordinates for the variant.   |  |
| Gene                    | Name of heritable genetic sequence that encodes proteins.   |  |
| Inheritance Pattern     | Method of Inheritance (Example: AR (Autosomal Recessive), XLR (X-linked Recessive).                           |  |
| Marker Type             | The type of marker (Indel, SNP, CN).  |  |
| Мах                     | Ending genomic position for the variant.  |  |
| Min                     | Starting genomic position for the variant.  |  |
| Name/ID                 | Thermo Fisher Scientific identifier for the probeset.   |  |
| p.name                  | Displays the change in protein translation for the variant.   |  |
| Recommended<br>Probeset | A quality control metric determined by SNP Polisher algorithm that chooses the best probesets querying a SNP. |  |
| Ref Allele              | The call for the reference allele associated with a normal phenotype.   |  |
| Ref Code                | The Call Code (A,B,C) of the Reference Allele.  |  |
| RSID                    | dbSNP ID  |  |



| Column                    | Description  |
|---------------------------|--|
| Size                      | Size in bp of the variant.   |
| Transcript                | RefSeq transcript ID associated with the c.name.   |
| Туре                      | Undetected/Detected. Detected are those mutations with any call other than the wild-type or major homozygous genotype. |
| Variant Status            | Status of the variant based on the genotype (i.e. Not Detected, Het, Hom, NoCall, NRP).                                |
| Variant Status Alt Allele | Severity status for the variant mapped to Alt Allele.  |
| Variation ID              | ClinVar ID   |

#### **OncoScan CNV Plus**

| Column           | Description   |
|------------------|---|
| accession number | RefSeq Gene accession number.   |
| Channel          | CEL file from which the signal is measured. "A" is the AT CEL, "C" is the GC CEL.   |
| Chromosome       | Chromosome on which the somatic mutation is found.  |
| Common Name      | Abbreviated description of the mutations to which this ProbeSet is known to respond. The name has the form [Gene]:[amino acid change for mutation]:[cDNA change for mutation]. In the event that the ProbeSet cannot differentiate among multiple mutations to which it can respond, the slash (/) delimits the multiple known mutations. |
| COSMIC ID        | The identifier of the mutation as listed in the COSMIC database, which is a catalogue of somatic mutations in cancer. More information on these mutations can be found at: http://cancer.sanger.ac.uk   |
| Event            | Describes if the probeset is a point mutation, deletion, insertion or sequence variant.   |
| Event Type       | Describes if the event is missense, frame-shift, in-frame insertion or deletion.  |
| File             | Name of the OSCHP file the somatic mutation is in.  |
| Fwd 3' flank     | Sequence flanking the mutation at the 3' end.   |
| Fwd 5' flank     | Sequence flanking the mutation at the 5' end.   |
| Genes            | RefSeq gene that shares coordinates with the somatic mutation.  |
| High Threshold   | High confidence MutScore threshold. Measurements equal to or greater than this threshold are called "High confidence," describing the likelihood that the mutation is present.  |
| Low Threshold    | Lower confidence MutScore threshold. Measurements with a MutScore below this value are called "Undetected". Measurements equal to or greater than this threshold but less than the High Threshold are called "Lower confidence," describing the likelihood that the mutation is present.  |
| Max              | Stop position of the somatic mutation.  |
| Min              | Start position of the somatic mutation.   |
| Mutation (aa)    | Wild type > mutant amino acid change on the coding strand.  |
| Mutation (nt)    | Wild type > mutant nucleotide change on the coding strand.  |



| Column                | Description  |
|-----------------------|--|
| Mutation Syntax (aa)  | Encoding of which nucleotide was changed and its location in the CDS.  |
| Mutation Syntax (cds) | Encoding of which amino acid was changed and the location of the codon.  |
| MutScore              | Measures somatic mutation probeset response. The stronger the response, the more likely it is that the somatic mutation is present. The MutScore calculation depends on the algorithm version. The newer MutScore calculation also corrects for sample-specific effects, and thereby reduces false positive calls, which were sample specific.   |
|                       | For algorithm versions 1.0 - 1.2 (ChAS 3.0 and earlier, OncoScan Console 1.2 and earlier):<br>MutScore.old = (measured quantile normalized signal - median signal for this marker in the<br>reference model file) / (95th percentile signal for this marker in the reference model file - median<br>signal for this marker in the reference model file).   |
|                       | For algorithm versions 1.3 and newer (ChAS 3.1 and newer, releases of OncoScan Console after 1.2):<br>MutScore.new = (MutScore.old - median MutScore.old for this sample) / standard deviation of MutScore.old for this sample (where standard deviation is calculated for all but the num-out-std strongest MutScore.old for this sample, median is calculated for all but the num-out-med strongest MutScore.old for this sample, and the used median is the maximum of zero and the measured median). |
| Name/ID               | Thermo Fisher Scientific identifier for the marker.  |
| ProbeSet Type         | Probeset Type is Somatic Mutation.   |
| Size (bp)             | The size of the Somatic mutation in base pairs.  |
| Source DB             | Cosmic database version.   |
| Strand                | Coding strand of the associated gene (Forward or Reverse).   |
| Туре                  | HighConfidence, LowerConfidence or Undetected call as to the presence of the mutation.   |

**Note:** Changes made to an OSCHP file in the Somatic Mutation Viewer Application requires the OSCHP file to be reloaded into the ChAS Browser to reflect the change made to the sample.



## QC and sample info tab

The QC and Sample Info tab (Figure 389) in the lower pane displays information about the loaded Data and Region files.

| Status       | Restricted Mode: Off   |             |  |   |                  |                     |     |
|--------------|------------------------|-------------|--|---|------------------|---------------------|-----|
|              | (ChAS)                 |             |  |   |                  |                     |     |
|              | C 400 au Ouran au alam | qc (ChAS)   | snpQC (CHP Summary)                                  | mapd (CHP Summ                              | name (Algorithm) | version (Algorithm) | ))) |
| Data Files   | S_178.cy2wg.cychp      | ~           | 2.932  | 0.186                                       | СҮТО2<br>СҮТО2   | 1.0.0               |     |
| Region Files | e (ChAS)<br>           | c<br>S<br>S | reated (General) modifie<br>at Jul 03 07:56:1 Sat Ju | sd (General)<br>103 13:56:5<br>Summary Data |                  | <u>&gt;</u>         |     |

The top section displays Status for Restricted Mode (see "Using restricted mode" on page 271)

The tables display information on:

- Loaded data files. "QC and sample information table" on page 350
- Loaded region files. "Loaded AED/BED files table" on page 356

## QC and sample information table

The QC table has six pre-loaded Table States allowing you to quickly toggle to the relevant information based on array type. For detailed information, see "Saved table states" on page 328.

| File       QC       Gender       MAPD       SNP QC       Waviness SD       Program Name       Program Name         Image: International control of the state of the st   | Figure 390 Data Files table  |                          |       |        |             |                           |             |
|---|--|--------------------------|-------|--------|-------------|---------------------------|-------------|
| File     QC     Gender     MAPD     SNP QC     Waviness SD     Program Name     Program       Image: International Control of the state of the st   |  |                          |       |        |             |                           | 1           |
| Image: Second state         Male         0.18         18.462         0.077         Chromosome Analysis Suite         3.1.1           Image: Second state         0.400         0.400         0.400         0.400         0.401         0.411  | File   | QC Gender N              | MAPD  | SNP QC | Waviness SD | Program Name              | Program Ver |
|   | Section 11-0810_LC_ONC1  | LC_ONC1 🗸 male           | 0.18  | 18.462 | 0.077       | Chromosome Analysis Suite | 3.1.1       |
| ■V of 11-0816_LC_ONC1 V male 0.162 19.628 0.097 Chromosome Analysis Suite 3.1.1   | ■ 🗣 🗗 11-0816_LC_ONC1  | LC_ONC1 🗸 male           | 0.162 | 19.628 | 0.097       | Chromosome Analysis Suite | 3.1.1       |
| ■ 🗊 🗣 or Sample_01.cyhd.cy 🗸 male 0.149 20.562 0.09 Chromosome Analysis Suite 3.2   | Sample_01.cyhd.cy  | 01.cyhd.cy 🗸 male        | 0.149 | 20.562 | 0.09        | Chromosome Analysis Suite | 3.2         |
| 🔋 🗣 🛷 Sample_02.cyhd.cy 🗸 male 0.153 23.715 0.065 Chromosome Analysis Suite 3.2   | Sample_02.cyhd.cy  | _02.cyhd.cy 🗸 male       | 0.153 | 23.715 | 0.065       | Chromosome Analysis Suite | 3.2         |
| Image: Provide a state         Male         0.162         19.628         0.097         Chromosome Analysis Suite         3.2  | Sample_03.cyhd.cy  | _03.cyhd.cy 🗸 male       | 0.162 | 19.628 | 0.097       | Chromosome Analysis Suite | 3.2         |
| Sample_04.cyhd.cy ✓ female 0.144 23.293 0.068 Chromosome Analysis Suite 3.2   | 🔋 🖓 o Sample_04.cyhd.cy  | _04.cyhd.cy 🗸 female     | 0.144 | 23.293 | 0.068       | Chromosome Analysis Suite | 3.2         |
| Image: Point of the state of the | Sample_05.cyhd.cy  | 05.cyhd.cy 🗸 female      | 0.183 | 22.611 | 0.084       | Chromosome Analysis Suite | 3.2         |
| 🗈 👫 🕈 POC_WGTriploid 🔥 male 0.203 7.215 0.196 Chromosome Analysis Suite 3.1.1   | Sector Alignment of the sector | WGTriploid <u>A</u> male | 0.203 | 7.215  | 0.196       | Chromosome Analysis Suite | 3.1.1       |



The six pre-loaded QC Table States and their column descriptions are shown below:

#### CytoScan QC view

| Column                    | Description   |
|---------------------------|---|
| Antigenomic Ratio         | Ratio of median intensity antigenomic control probes/median intensity all copy number probes.   |
| File                      | File Name   |
| Genome Version            | Build of the genome (i.e. hg19, hg38)   |
| MAPD                      | Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| Median Raw Intensity      | Pre-processed Median signal of the array.   |
| QC                        | In or Out of QC bounds.   |
| Sex                       | Gender call for the sample. See "Gender call algorithms" on page 355).  |
| SNPQC                     | SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| Waviness SD               | A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information. |
| Waviness Segment<br>Count | Number of raw segments without any post-processing.   |

#### **Default QC view**

| Column            | Description   |
|-------------------|---|
| Algorithm Name    | Name of the algorithm used in processing the array.   |
| Algorithm Version | Version of the algorithm used in processing the array.  |
| Annotation File   | Name of the Annotation file used to create the xxCHP file.  |
| Array Type        | Type of array used in the analysis.   |
| Autosome LOH      | The proportion of LOH on chromosome 1 to 22.  |
| Cel Pair Check    | Inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel. |
| Created           | Date the xxCHP file was created.  |
| File              | File Name   |



| Column           | Description   |
|------------------|---|
| Low Diploid flag | An essential part of the algorithm is the identification of "normal diploid" markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that "normal diploid markers" result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of "normal diploid" markers and no "normal diploid calibration occurs. This event triggers "low diploid flag" = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary. |
| MAPD             | Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| Modified         | Date the xxCHP file was last modified.  |
| ndSNPQC          | QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions.   |
| ndwavinessSD     | Measure of variation of probes in normal diploid regions that are insensitive to short-range variation and focus on long-range variation.   |
| Parameter File   | Name of the chasparam file used to create the xxCHP file.   |
| QC               | In or Out of QC bounds.   |
| Reference        | Reference Model file used in the single sample analysis.  |
| Sex              | Gender call for the sample. See "Gender call algorithms" on page 355).  |
| SNPQC            | SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| SNPQC Type       | ND or non-ND  |
| TuScan %AC       | If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample.   |
| TuScan Ploidy    | The most likely ploidy state of the tumor before additional aberrations occurred. TuScan Ploidy is assigned the median CN state of all markers, provided that %AC could be determined and integer copy numbers are returned. If %AC cannot be determined, NA (Not Available) is reported for both ploidy and %AC.   |
| Waviness SD      | A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information.   |

### CytoScan HTCMA QC view

| Column       | Description   |
|--------------|---|
| DishQC (DCQ) | Measures the amount of overlap between two homozygous peaks created by non polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad. |
| File         | File Name   |
| MAPD         | Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |



| Column         | Description   |
|----------------|---|
| QC             | In or Out of QC bounds.   |
| QC Call Rate   | Percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step).  |
| QC Het Rate    | Percentage of SNPs called AB (i.e. the heterozygosity) for autosomal SNPs (measured at the Sample QC step).   |
| Sex            | Gender call for the sample. See "Gender call algorithms" on page 355).  |
| SMN MAPD       | Median Absolute Pairwise Difference value calculated from the CNVMix algorithm for the SMN pipeline.  |
| SMN WavinessSD | A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation from the CNVMix algorithm for the SMN pipeline. |
| SNPQC          | SNP QC value. Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| Waviness SD    | A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. See Appendix F for detailed information.       |

#### OncoScan QC view

| Column                        | Description   |  |  |  |  |
|-------------------------------|---|--|--|--|--|
| Cel Pair Check                | Inspects each pair of intensity (*.cel) files to determine whether the files have been properly paired and assigned to the correct channel.   |  |  |  |  |
| Cel Pair Check<br>Concordance | Percentage of SNPs that match between the AT and GC arrays.   |  |  |  |  |
| File                          | File Name   |  |  |  |  |
| Genome Version                | Build of the genome (i.e. hg19, hg38)   |  |  |  |  |
| Low Diploid flag              | An essential part of the algorithm is the identification of "normal diploid" markers in the cancer samples. This is particularly important in highly aberrated samples. The normal diploid markers are used to calibrate the signals so that "normal diploid markers" result in a log2 ratio of 0 (e.g. copy number 2). The algorithm might later determine that the "normal diploid" markers identified really correspond to (for example) CN=4. In this case the log2 ratio gets readjusted and TuScan ploidy will report 4. Occasionally (in about 2% of samples) the algorithm cannot identify a sufficient number of "normal diploid" markers and no "normal diploid calibration occurs. This event triggers "low diploid flag" = YES. In this case the user needs to carefully examine the log2 ratios and verify if re-centering is necessary. |  |  |  |  |
| MAPD                          | Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |  |  |  |  |
| ndSNPQC                       | QC metric for SNP probes that is derived from polymorphic SNP probes in normal diploid regions.   |  |  |  |  |
| ndWaviness SD                 | Measure of variation of probes in normal diploid regions that are insensitive to short-range variation and focus on long-range variation.   |  |  |  |  |
| Number nd                     | Number of probes called normal diploid by the algorithm.  |  |  |  |  |
| Percentage nd                 | Percentage of probes called normal diploid by the algorithm   |  |  |  |  |



| Column                           | Description   |
|----------------------------------|---|
| QC                               | In or Out of QC bounds.   |
| Sex                              | Gender call for the sample. See "Gender call algorithms" on page 355).  |
| TuScan %AC                       | If % AC = 100%, we return "homogeneous" because it could be 100% normal or 100% tumor. If % AC =NA, the percent aberrant cells could not be determined and TuScan returns non-integer CN calls. This metric is an algorithmically determined estimate of the % of aberrant cells in the sample. |
| TuScan Log 2 Ratio<br>adjustment | Log 2 ratio determined from TuScan algorithm needed to "center" the diploid region of the sample (around Log $2 = 0$ ).   |

### ReproSeq QC view

| Column                 | Description   |  |  |  |  |  |
|------------------------|---|--|--|--|--|--|
| Application (ReproSeq) | Name of Application from the ReproSeq assay.  |  |  |  |  |  |
| Batch File (ReproSeq)  | Jame of the batch file downloaded from Ion Reporter.                                |  |  |  |  |  |
| Chr MA Ratio           | latio of Mitochondrial/Autosome.  |  |  |  |  |  |
| File                   | File Name   |  |  |  |  |  |
| MAPD                   | Median Absolute Pairwise Difference value. See Appendix F for detailed information. |  |  |  |  |  |
| Sex                    | Gender call for the sample. See "Gender call algorithms" on page 355).              |  |  |  |  |  |
| Single File (ReproSeq) | Name of the current file from a Batch File downloaded from Ion Reporter.            |  |  |  |  |  |
| Workflow (ReproSeq)    | Name of the workflow run in Ion Reporter.   |  |  |  |  |  |

#### **SMN Sample Info view**

| Column       | Description   |
|--------------|---|
| DishQC (DCQ) | Measures the amount of overlap between two homozygous peaks created by non polymorphic probes. DQC of 1 is no overlap, which is good. DQC of 0 is complete overlap, which is bad. |
| File         | File Name   |
| MAPD         | Median Absolute Pairwise Difference value. See Appendix F for detailed information.   |
| QC           | In or Out of QC Bounds.   |
| QC Call Rate | Percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step).  |
| QC Het Rate  | Percentage of SNPs called AB (i.e. the heterozygosity) for autosomal SNPs (measured at the Sample QC step).   |
| Sex          | Gender call for the sample. See "Gender call algorithms" on page 355).  |
| SMN MAPD     | Median Absolute Pairwise Difference value calculated from the CNVMix algorithm for the SMN pipeline.  |



| Column         | Description   |
|----------------|---|
| SMN WavinessSD | A global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation from the CNVMix algorithm for the SMN pipeline. |
| SMN1 (variant) | Genotype call for SMN variant(s) as defined in the SMN.SNP.list<br>For more details, see the RHAS User Guide.   |
| SMN1 CN        | Copy number for SMN1.   |
| SMN1 Exon7     | Copy number for Exon 7 of SMN1.   |
| SMN1 Exon8     | Copy number for Exon 8 of SMN1.   |
| SMN2 CN        | Copy number for SMN2.   |

Other data from the header of the Sample Data file or the ARR file can also be selected for display in the Select Columns window.

You can only hide or display columns by using the Column Select window, at the right of the table.

**Note:** or samples run through the Normal Diploid Analysis for CytoScan, the ndSNPQC and ndwavinessSD metrics can be viewed in the QC Information Tab, but will not flag a sample as pass/fail.

**Gender call** The table below explains which algorithm is used to make the gender calls for the different arrays.

The CytoScan Array uses the call "Y-gender" which gives a male/female call.

Depending on the version they were created under, various GTC 2.x and 3.x SNP6 CNCHP files use other gender calls present in their CNCHP file header.

These calls used from the CNCHP file header are NOT the same gender calls used for those files in GTC, since the GTC-displayed gender calls were stored in GQC or CN\_SEGMENTS files which are not supported in ChAS.

**Note:** For more details how the array-specific algorithms call LOH segments for the X or Y chromosome, see "LOH segments on X and Y chromosomes" on page 49.

| Software/Array Type                               | Gender Call<br>Algorithm          | Call                | Gender Call<br>Confidence |
|---|-----------------------------------|---------------------|---------------------------|
| ChAS 1.X, 2.X and 3.X/4.X<br>CytoScan Arrays      | Y-gender                          | male/female         | Yes                       |
| GTC 3.0 to GTC 4.1/ Genome-<br>Wide SNP Array 6.0 | affymetrix-<br>chipsummary-Gender | male/female/unknown | No                        |
| GTC 2.1/ Genome-Wide SNP<br>Array 6.0             | affymetrix-<br>chipsummary-hasY   | male/female         | No                        |
| GTC 2.0/ Genome-Wide SNP<br>Array 6.0             | affymetrix-<br>chipsummary-hasY   | male/female         | No                        |



## Loaded AED/BED files table

This table (Figure 391) displays information about the loaded Region (AED or BED) files.

| Figure 391 AED/BED Files information |            |              |    |
|--------------------------------------|------------|--------------|----|
| File                                 | oracted    | modified     |    |
| Chille from concru ut ob cod         | Wod Eab 19 | Man Mar 20   | UU |
| Eile 02.04 and                       | Tue Mer 24 | Fri Mar 37.4 |    |
|                                      |            |              |    |
|                                      |            |              |    |

The Region information files section displays information on:

| File     | File Name with Icons displayed if selected as Overlap File or CytoRegions File). |
|----------|--|
| Created  | Date and time file was created.  |
| Modified | Date and time file was last modified.  |

You can only hide or display columns by using the Column Select window, at the right of the table.



## Chromosome summary data

The Chromosome Summary Data tab has two components:

- Chromosome Summary Data
- Auto-Generated Autosome LOH Percentage

The Chromosome Summary Data table (Figure 392) summarizes particular data across each chromosome in the loaded sample data files.

The available data types are:

Note: ReproSeq Anueploidy data is not supported within this tab.

- Min Signal minimum Log2 Ratio value found in the chromosome
- Median Signal median Log2 Ratio value found in the chromosome
- Max Signal maximum Log2 Ratio value found in the chromosome
- Median CN State median calibrated Log2 Ratio
- Mosaicism median mosaicism mixture value

**Note:** The mosaicism CN state value is not an integer due to cell populations with different CN state values. In the Chromosome Summary Data table, the mosaicism value indicates how much the median CN state value is above or below two, the normal CN state value for autosomes and X in females. For example, a median mosaicism mixture CN state value of 2.48 is displayed as 0.48 in the Chromosome Summary table.

**Note:** Mosaicism (median mosaicism mixture value) for normal males is -1.0 for chromosome X and -1.0 for chromosome Y. Mosaicism for normal females is 0.0 for chromosome X and -2.0 for chromosome Y. A mosaic XO female is treated the same as a mosaic autosomal monosomy (i.e., the mosaicism level of chromosome X will be between -1.0 and 0). A mosaic XXY male is also given a mosaicism level between -1 and 0 for chromosome X.

LOH – proportion of genomic distance of LOH calls per chromosome

| $^{\star}$ | $\circ$ | ĸ         |   |   |
|------------|---------|-----------|---|---|
| 1          |         | Sk        | 6 |   |
| 1          | 4       | $\square$ | 6 | 2 |
|            | ĭ       |           | e | 2 |
| $\sim$     |         |           |   |   |

| Figure 392 Chromosome Summary Data, Min Signal in each chromosome |               |               |                |                |               |            |            |      |
|---|---------------|---------------|----------------|----------------|---------------|------------|------------|------|
| 📳 Karyoview 🎙 🙀 Seg   | iments 🄊 📙    | CytoRegion    | s 🄊 🌗 Over     | rlap Map 🄊 [   | 🔀 Graphs 🎙    |            |            |      |
| 📰 📑 🗎 Σ 🛄   | ] 🛛 💠   1     | <b>M</b> 🗹 🗆  |                |                |               | 4          | 44 results | ]]]] |
| File  | CN State      | 🛦 Туре        | Chromoso       | Min            | Max           | Size (kbp) | Mean Mark  |      |
| [ 🗐 🔷 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 61,742,422     | 61,742,502    | < 0.5 kbp  | 4          | ^    |
| 🚺 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 152,632,636    | 152,633,559   | 1          | 40         |      |
| 🔋 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 123,017,753    | 123,018,369   | 1          | 61         |      |
| 🚺 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 129,375,795    | 129,376,465   | 1          | 27         | -    |
| 🚺 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 115,751,358    | 115,751,501   | < 0.5 kbp  | 12         |      |
| 🚺 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 98,378,843     | 98,400,772    | 22         | 3,132      |      |
| 🚺 🗘 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 118,653,619    | 118,658,459   | 5          | 179        |      |
| 🔋 🔷 08-0989_A3_Phase  | 2.0           | 🔺 Gain        | X              | 6,633,722      | 6,633,894     | < 0.5 kbp  | 11         |      |
| 🔋 🔷 08-0989_A3_Phase  | 3.0           | 🔺 Gain        | 1              | 185,096,575    | 185,131,493   | 35         | 997        |      |
| 08-0989_A3_Phase  | 3.0           | 🔺 Gain        | 1              | 161,501,915    | 161,618,019   | 116        | 2,276      |      |
| 08-0989_A3_Phase  | 3.0           | 🔺 Gain        | 1              | 153,673,661    | 153,688,275   | 15         | 1,124      |      |
| 08-0989_A3_Phase  | 3.0           | 🔺 Gain        | 1              | 104,205,810    | 104,261,193   | 55         | 2,051      |      |
| 😧 🗘 08-0989_A3_Phase  | 3.0           | 🔺 Gain        | 1              | 216,378,897    | 216,398,102   | 19         | 872        |      |
| 08-0989 A3 Phase  | 3.0           | 🔺 Gain        | 11             | 34,941,138     | 34,943,229    | 2          | 122        |      |
| CO RE-RORD AR Phace   | 30,           | A Gain        | 11             | 121 597 445    | 121 634 608   | 37         | 2 4 7 7    | ~    |
| <   |               |               |                |                |               |            | < >        |      |
| ^ u   |               |               |                |                |               |            |            |      |
| Chromosome Summary Da   | ata Type: Mir | nSignal (Mini | imum log2 rati | io value found | in the chromo | osome)     | ~          |      |
| File 1  | 2             | 3             | 4              | 5              | 6             | 7          | 8          |      |
| ■ 08-092.789493   | -3.5788167    | -3.410571     | -3.7624521     | -4.4898906     | -3.926444     | -3.0583477 | -3.6873658 |      |
|   |               |               |                |                |               |            |            | POF  |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
|   |               |               |                |                |               |            |            |      |
| <   |               |               |                |                |               |            | < >        |      |
|   | Sand Canada   | luán 🔊 😑 /    |                | 0              |               |            |            |      |
| Come Detail View 📍 🖓 🛄 QU   | 2 and Sample  | into , 🚍 (    | Infomosome     | Summary Dat    | a             |            |            |      |

To choose the data type, make a selection from the drop-down list. (Figure 393)

| Figure 393 Selecting the data type for the Chromosome Summary table  |   |  |  |  |  |
|--|---|--|--|--|--|
| Chromosome Summary Data Type:  | Min Signal (Minimum log2 ratio value found in the chromosome) |  |  |  |  |
| File       1       Min Signal (Minimum log2 ratio value found in the chromosome)         Image: S_113.CN5.cychp       Max Signal (Maximum log2 ratio value found in the chromosome)         Image: S_125.CN5.cychp       Median Cn State (Median calibrated log2 ratio)         Image: Mosaicism (Median mosaicism mixture value)       Image: Chromosome)         Image: LOH (Proportion of genomic distance of LOH calls per chromosome) |   |  |  |  |  |
| Detail View     QC and Sample Info     Chromosome Summary Data   |   |  |  |  |  |



## Auto-generated Autosome LOH percentage

The percentage LOH displayed is calculated for the Autosome based on the filter size set for LOH in the Filters Window. (Figure 394)

The Covered Autosome Length is the base pairs of the Autosome covered by probes.

| Figure 394 Custom Autosor   | mal Genome    | LOH Perce       | ntage        |                         |     |
|---|---------------|-----------------|--------------|-------------------------|-----|
| X LOH           Marker Count           0           50           Size (kbp)           0      <   |               |                 |              |                         |     |
| File  | Created       | Modified        | Autosome % L | Covered Autosome Length | 111 |
| Scheric Content of the second | 2013-12-19T16 | 2013-12-19T16   | 12.59        | 2,781,797,045           |     |
| □ Detail View ♥ 🕄 🔁 QC and Sample Info  | Chromos       | some Summary Da | ata <b>N</b> |                         |     |

**IMPORTANT!** You must check the LOH Segment Data type to view the sample's percent LOH.



### Searching results

The Search function enables you to search:

- Detected Segments
- Reference Annotations
- Loaded Region Information Files

The search can find:

- Names of Reference Annotations
- BED and AED file elements, including those in files designated as CytoRegions or Overlap Maps
- Loaded and displayed segments

You can search by:

- File (select the files to be searched)
- ID Label
- Туре
- From the View menu, select **Search** or click the upper tool bar's a icon. The Search window opens. (Figure 395)

|   | Figu | re 395 Search window   |   |
|---|------|--|---|
|   | Sear | rch  | × |
|   | A    | Find By File All Files Find By ID (Name/ID or Label) Find By Type Find By Text | < |
| - |      | Reset Search Cancel  |   |

2. Search all files in the File List.

Alternatively, click the All Files drop-down to select the file you want to search.

3. In the Find By ID text field, enter the ID/Name you want to search.

A wildcard (\*) is not required when performing searches, however the "\*" can be used to narrow searches. For example, performing the search *HOX* returns SHOX and RHOXF1 findings, while using a "\*" (\*HOX), returns SHOX.


4. Optional: Use the **Find By Type** and/or **Find By Text** fields to enter a type name (not case sensitive) for the search.

You can enter:

- Names for types of reference annotation features (Genes, DGV, etc.)
- Names for types of segments (Loss, Gain, etc.)
  - 5. Click **Search...** to begin the search.

If no results are found, the following notice appears. (Figure 396)

| Figure | 396 No results notice  |
|--------|--|
| No res | sults ×  |
|        | There were no results for your query.<br>Results may change if you modify the filter settings. |
|        | OK   |

If the search takes more than a few seconds, a **Searching...** window appears. (Figure 397)

If results are found, the Search Results table opens. (Figure 398)



| Figure 398 | Search Resul | ts          |             |            |    |       |             |   |
|------------|--------------|-------------|-------------|------------|----|-------|-------------|---|
| Search Res | sults        |             |             |            |    |       |             | × |
| 🔠 📠 🗎      |              | þ           |             |            |    |       | 855 results |   |
| Name/ID    | Chromosome   | Min         | Max         | Size (kbp) | Ty | pe    |             |   |
| KIAA1804   | 1            | 231,530,136 | 231,587,517 | 57         | ÷  | Genes |             | ^ |
| KCNJ9      | 1            | 158,317,983 | 158,325,836 | 7          | 4  | Genes |             |   |
| KLHDC9     | 1            | 159,334,777 | 159,336,760 | 1          | 1  | Genes |             |   |
| KPRP       | 1            | 150,997,129 | 151,001,153 | 4          | 1  | Genes |             |   |
| KIAA0859   | 1            | 170,017,383 | 170,033,479 | 16         | į. | Genes |             |   |
| KISS1      | 1            | 202,426,091 | 202,432,242 | 6          | 1  | Genes |             |   |
| KCNK2      | 1            | 213,245,507 | 213,477,059 | 231        | 1  | Genes |             |   |
| KLF17      | 1            | 44,357,108  | 44,373,396  | 16         | 1  | Genes |             |   |
| KIAA1383   | 1            | 231,007,260 | 231,012,715 | 5          | 4  | Genes |             |   |
| KCNA3      | 1            | 111,015,832 | 111,019,178 | 3          | 4  | Genes |             |   |
| KCNK2      | 1            | 213,323,182 | 213,477,059 | 153        | 4  | Genes |             |   |
| KT0.01751  | 1            | 1 874 611   | 1 025 136   | 50         | H  | Cenec |             | ~ |

# Searching within a selected file

1. Right-click on a selected (checked) file inside the Files list pane.



2. Click to select Search in selected file... (Figure 399)

The Search window opens. (Figure 400)



3. Click the appropriate radio button(s), enter your search criteria, then click **Search**.

| Figure 400 Search window  |   |
|---|---|
| Search  | × |
| Here       Find By File         All Files       Image: Constraint of the second s |   |

If no results are found, the following notice appears. (Figure 401)

| Figure     | 401 No results notice  |
|------------|--|
| No res     | sults X  |
| $\bigcirc$ | There were no results for your query.<br>Results may change if you modify the filter settings. |
|            | OK   |

If the search takes more than a few seconds, an In Progress notice appears. (Figure 402)

| Figure 402 Searching notice |
|-----------------------------|
| Searching. X                |
| Searching, Please wait      |
|                             |
|                             |
|                             |

If results are found, the Search Results window/table opens. (Figure 403)



| Figure 403 | Search Resul | ts window/tab | ble         |            |         |             |
|------------|--------------|---------------|-------------|------------|---------|-------------|
| Search Re  | sults        |               |             |            |         | ×           |
| 🔠 📠 🖺      | I 🔢 I (      | þ             |             |            |         | 855 results |
| Name/ID    | Chromosome   | Min           | Max         | Size (kbp) | Туре    |             |
| KIAA1804   | 1            | 231,530,136   | 231,587,517 | 57         | a Gene: | s ^         |
| KCNJ9      | 1            | 158,317,983   | 158,325,836 | 7          | 📒 Gene: | s           |
| KLHDC9     | 1            | 159,334,777   | 159,336,760 | 1          | 📒 Gene: | 5           |
| KPRP       | 1            | 150,997,129   | 151,001,153 | 4          | a Gene: | 5           |
| KIAA0859   | 1            | 170,017,383   | 170,033,479 | 16         | 📒 Gene: | s           |
| KISS1      | 1            | 202,426,091   | 202,432,242 | 6          | 📒 Gene: | s           |
| KCNK2      | 1            | 213,245,507   | 213,477,059 | 231        | 📒 Gene: | s           |
| KLF17      | 1            | 44,357,108    | 44,373,396  | 16         | 📒 Gene: | s           |
| KIAA1383   | 1            | 231,007,260   | 231,012,715 | 5          | 📒 Gene: | s           |
| KCNA3      | 1            | 111,015,832   | 111,019,178 | 3          | 📒 Gene: | s           |
| KCNK2      | 1            | 213,323,182   | 213,477,059 | 153        | a Gene: | s           |
| KT001751   | 1            | 1 874 611     | 1 025 136   | 50         | 🗢 Gener | ×           |

To highlight features in the views or the table:

- Double-click in a row of the table to zoom to the feature in the Selected Chromosome and Detail Views.
- Click on a feature in the Selected Chromosome or Detail View to highlight the feature in the Search Results table (the feature must be listed in the table to be highlighted).

You can perform the common table operations in the Search Results table (see "Common table operations" on page 323).

The Search Results table displays the following information:

| Column     | Description   |
|------------|---|
| Chromosome | Chromosome the items are located in.  |
| Label      | Name or ID of the item.   |
| Max        | Ending position of the item.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for the starting probe of the segment as reported in the graphs table while the end coordinate matches the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic position coordinates" on page 475). |
| Min        | Starting position of the item.  |
| Size (kbp) | Size of the item.   |
| Туре       | Type of item.   |



## **Finding intersections**

The **Find Intersection** feature enables you to find segments and regions that overlap for different:

- Detected Segments
- Reference Annotations
- Loaded Region Information Files
- 1. From the View menu, select Find Intersections...

The Find Intersection window opens. (Figure 404)

| Figure 40   | 4 Find Intersection window                  |   |
|-------------|---|---|
| Find Inter  | section                                     | < |
| P<br>F<br>L | ïle A:<br>✓<br>ïle B:<br>✓<br><u>R</u> eset |   |
| Find Ir     | ntersection                                 |   |

2. Select the first file for the comparison from the File A drop-down list. (Figure 405)

| Figure 405 | 5 Drop-down list  |   |
|------------|---|---|
| Find Inte  | ersection   | × |
| Fine       | File A:<br>Beta10_F_01_Cyto_VH.cychp<br>Beta15_M_01_Cyto_VH.cychp<br>Beta13_F_01_Cyto_VH.cychp<br>V DGV<br>Genes<br>PAR | ~ |

The list shows the available Sample files, Region Information Files, and Reference Annotations.

**Note:** Only files that are check marked in the Files List appear in the Match File drop-down list.

- 3. Select the second file from the File B drop-down list.
- 4. Click Find Intersection...



The Finding Intersection notice opens. (Figure 406)

| Figure 406 Finding Intersection notice |   |
|--|---|
| Finding intersection.                  | × |
| Finding Intersection. Please wait      |   |
|  |   |
|  |   |

When the comparison is finished, the Intersection Results table opens. (Figure 407)

|         | tion Results    |            |             |             |              |            |   |       |          |             | ,       |
|---------|-----------------|------------|-------------|-------------|--------------|------------|---|-------|----------|-------------|---------|
|         | 15 M 04 0.4- 34 | Lauraha    |             |             |              |            |   |       |          |             |         |
| A: Beta | 15_M_01_Cyto_VF | 1.cycnp    |             |             |              |            |   |       |          |             |         |
| B: Gene | 35              |            |             |             |              |            |   |       |          |             |         |
|         |                 | <b>P</b>   |             |             |              |            |   |       |          | 152 re:     | sults 📋 |
| А Туре  | A               | Chromosome | A Min       | A Max       | A Size (kbp) | A CN State | В | Туре  | В        | B Min       | B Max   |
| V Loss  | smoothed25      | 1          | 25,467,089  | 25,543,873  | 76           | 1          | - | Genes | RHD      | 25,471,567  | 25,52   |
| 🔻 Loss  | smoothed25      | 1          | 25,467,089  | 25,543,873  | 76           | 1          | - | Genes | RHD      | 25,471,567  | 25,62   |
| V Loss  | smoothed25      | 1          | 25,467,089  | 25,543,873  | 76           | 1          | - | Genes | TMEM50A  | 25,537,397  | 25,56   |
| 🔺 Gain  | seg16           | 1          | 12,960,917  | 13,028,391  | 67           | 3          | 1 | Genes | PRAMEF22 | 12,958,129  | 12,96   |
| 🔺 Gain  | seg114          | 1          | 194,989,498 | 195,064,665 | 75           | 3          | 1 | Genes | CFHR3    | 195,010,552 | 195,02  |
| 🔺 Gain  | seg114          | 1          | 194,989,498 | 195,064,665 | 75           | 3          | 1 | Genes | CFHR1    | 195,055,483 | 195,06  |
| 🔺 Gain  | seg1014         | 10         | 46,383,964  | 46,453,447  | 69           | 3          | 1 | Genes | GPRIN2   | 46,413,551  | 46,42   |
| 🔺 Gain  | seg1014         | 10         | 46,383,964  | 46,453,447  | 69           | 3          | - | Genes | SYT15    | 46,378,532  | 46,39   |
| 🔺 Gain  | seg1014         | 10         | 46,383,964  | 46,453,447  | 69           | 3          | 1 | Genes | SYT15    | 46,375,450  | 46,39   |
| 🔺 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | 1 | Genes | PTPN20A  | 48,357,047  | 48,44   |
| 🔺 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | - | Genes | PTPN20A  | 48,357,047  | 48,44   |
| 🔺 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | - | Genes | PTPN20B  | 48,357,047  | 48,44   |
| 🛦 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | - | Genes | PTPN20A  | 48,357,047  | 48,44   |
| 🔺 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | - | Genes | PTPN20B  | 48,357,047  | 48,44   |
| 🔺 Gain  | seg1040         | 10         | 48,373,168  | 48,454,852  | 81           | 3          | - | Genes | PTPN20B  | 48,357,047  | 48,44   |
|         | 1010            |            | 40.070.400  | 10 151 050  |              |            | - |       | DTDNOOD  | 40.057.047  | 10.10   |

The table displays the names of the A and B files above the tool bar.

To highlight features in the views or the table:

- Double-click in a row of the table to zoom to the feature for File A in the Selected Chromosome and Detail Views.
- Click on a feature in the Selected Chromosome or Detail View to highlight the feature in the Intersection Results table (the feature must be listed in the table to be highlighted).

You can perform the common table operations in the Intersection Results table (see "Common table operations" on page 323).



#### The Intersection Results table displays the following information:

| Column            | Description  |
|-------------------|--|
| % A Touching B    | How much of the A item is covered by the B item.   |
| % B Touching A    | How much of the B item is covered by the A item.   |
| Α                 | Identifier used for item in A file.  |
| A CN State        | Copy number of the segment in file A.  |
| A Max             | Ending position of the A item.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic<br>position coordinates" on page 475). |
| A Min             | Starting position of the A item.   |
| A Size (kbp)      | Size of the A item.  |
| А Туре            | Type of item in A file with overlap.   |
| В                 | Identifier used for item in B file.  |
| B CN State        | Copy number of the segment in file B.  |
| B Max             | Ending position of the B item.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table (see Appendix D, "Genomic<br>position coordinates" on page 475)  |
| B Min             | Starting position of the B item.   |
| B Size (kpb)      | Size of the B item.  |
| В Туре            | Type of Item in B file with overlap.   |
| Chromosome        | Chromosome the items are located in.   |
| Shared Size (kbp) | Size of the overlap.   |



# **Prioritizing segments**

Segment Prioritization enables the sorting of copy number segments based on userdefined relevance with overlapping annotations.

Thermo Fisher Scientific offers multiple ways to help quickly annotate segments in order to reduce interpretation time for each sample.

Segment prioritization options:

- "ChAS Professional version of Franklin by Genoox"
- "Tier-based prioritization in ChAS" on page 371
- Score-based prioritization in ChAS<sup>®</sup> on page 376

**Note:** The following NetAffx Genomic Annotation files are required for full use of all the parameter in Segment Prioritization:

- NetAffxGenomicAnnotations.Homo\_sapiens.hg19.na20230601/(or more current)
- NetAffxGenomicAnnotations.Homo\_sapiens.hg38.na20230601 (or more current) for optimal segment prioritization results.

**IMPORTANT!** All data results from the Segment Prioritization process should be manually reviewed.

### ChAS Professional version of Franklin by Genoox

Sample data can be uploaded to your Franklin account automatically using ChAS AIR (Automated Interpretation and Report) tokens. Use these tokens to take advantage of Franklin's ACMG classification. phenotype to gene relationships, literation searching, and access community and historical data. For more information on ChAS AIR tokens, contact your local sales representative.

# **ChAS AIR tokens** Contact your local sales representative to purchase AIR Tokens in quantities of 24, 96 or 384 samples.

- Your sales representative will provide a link for setting up an account in Franklin (required for new accounts only).
- The ChAS AIR Tokens will be deposited into your Franklin account.
- One token is deducted from your AIR token balance for each CHP file you upload to Franklin (1 CHP file = 1 AIR token).

#### Uploading your sample(s) to Franklin

- 1. Load the CHP files into the ChAS Browser
- 2. Review the QC metrics to ensure the sample quality prior to interpretation.
- 3. Review the breakpoints of each segment using the Detail View to compare the segment and the probe level data. Make any necessary adjustments/edits, as described throughout Chapter 10, "Segment modification" on page 219.

**Note:** Segments in the mosaic segment track will not be published. For these segments to be included in the upload to Franklin, they must first be promoted to the Gain/Loss Copy Number track See "Promoting mosaic segments" on page 239. Promoting is done to mitigate redundancy of Whole Integer Copy Number segments and Mosaic copy number segments in the same region.

4. In the File window, right-click on the sample filename(s) you want to upload to Franklin, then click **Send File(s) to Franklin...** (Figure 408)

| Figure 408 Send File(s) to Franklin |  |  |
|-------------------------------------|--|--|
| Files                               |  |  |
| ClinSpecTra                         | Close                                  |  |
| XON Region Levels                   | Save                                   |  |
|                                     | Export File as AED                     |  |
| DGV-GS Gain                         | Search in selected file Ctrl+F         |  |
|                                     | View and Edit annotations in this file |  |
| Default Histogram                   | Discard Changes                        |  |
| Ensembli Genes                      | View Reference Model Parameters        |  |
| V - Genes                           | View Process Pipeline                  |  |
|                                     | View/Edit Properties                   |  |
| S OMIM ® Genes                      | Set Custom Color                       |  |
| SOMIM ® Phenoty                     | Clear Custom Color                     |  |
|                                     | Open Sample(s) in MSV                  |  |
| Protein Coding E                    | Publish File(s) to Database            |  |
| Protein Coding G                    | Send File(s) to Franklin               |  |
|                                     | Open case in Franklin                  |  |
|                                     | Show WCV for selected file(s)          |  |

A window appears confirming what data type and filters are going to be uploaded to Franklin. (Figure 409)



- Click **Cancel** to make adjustments to the segment type and/or filter criteria.
- Click **OK** to keep the displayed settings.

After clicking **OK**, an Enter credentials window appears prompting you to log into your Franklin account. If you do not have a Franklin account or do not know your account credentials, contact **support@genoox.com**.

5. After entering your Username and Password, click OK.

**Note:** Your Franklin credentials are saved until ChAS is closed. This eliminates the need to log in for each additional CHP file you want to upload.

A message appears confirming your files have been successfully sent. If ChAS was unable to connect to Franklin, an Error message will appear. If it does, check your Internet connection to the genoox.com website.

6. After Franklin processes your sample(s), go to genoox.com to view their analysis.

**Note:** If edits were made to a file already uploaded to Franklin, you will not be charged an additional AIR token for uploading and reanalyzing the file again.

**IMPORTANT!** Do not delete the open case in Franklin, simply re-upload the edited file(s) to Franklin to overwrite the existing data.

#### Returning to an open case in Franklin from ChAS

1. To access a previously uploaded file in Franklin from ChAS, go to the File window, right-click on the file(s), then click **Open Case in Franklin**. (Figure 410)



The Franklin web page opens displaying the details of your open case.

**Note:** Before you can review a segment from the Franklin website in ChAS, the **HTTP** service in ChAS must be enabled. From the ChAS Browser, click **Preferences**  $\rightarrow$  **Configure HTTP Service**. Confirm the **Enable** box is checked and **Port 8348** is displayed. Also make sure Port 8348 is not blocked by your firewall.



## **Tier-based prioritization in ChAS**

The tier-based prioritization assigns Tiers 1-5 to copy number segments that overlap defined annotations. Copy number segments that overlap an annotation with an assigned tier will get have that tier assignment. If a copy number segment is assigned multiple tiers, the lowest tier number will be assigned to the copy number segment. The exception to this general rule are annotations for DGV-GS and ChAS DB Count both. If a segment is meets the criteria for either of these annotation, the tier assignment will take priority over lower number tiers.

# Configuring the tier-based option

1. From the Segment Table tool bar, click the 🔛 button.

The Segment Prioritization Options window appears. (Figure 411)

| Figure 411 Segment Prioritizatio  | n Options window   |
|---|--|
| Segment Prioritization Options  | ×  |
| Ose Tier-Based Rules  | Use Score-Based Rules  |
| View/Edit Tier-Based Rules  | View/Edit Score-Based Rules For Gain Segments  |
|   | View/Edit Score-Based Rules For Loss Segments  |
| Tier to Call<br>Optional: Associate some or all tiers with calls.<br>Tier 1 v<br>Tier 2 v<br>Tier 3 v<br>Tier 4 v<br>Tier 5 v | Score to Call         Optional: Assign calls from score thresholds.         Score $\geq$ 0.99         Score $\geq$ 0.9         Other score $\checkmark$ Score $\leq$ -0.9         Score $\leq$ -0.9         Score $\leq$ -0.99 |
| Restore Defaults  | Restore Defaults   |
| ОК  | Cancel   |

2. Click the View/Edit Tier-Based Rules button.

The Select Tier Rules window appears. (Figure 412)

| Figure 412 Select Tier Rules window  |
|--|
| Select Tier Rules X  |
| Select tiers to associate with rules.  |
| Tier Rule  |
| 5 V DGV-GS: Completely inside a same-type Gain/Loss DGV-GS with '% Frequency' ≥ 10   |
| 5 <b>DB-B:</b> Segment has a 'DB Count Both' ≥     0   |
| ✓         DB-F: Segment has a 'Filtered DB Count Both' ≥         0   |
| <b>OM-3</b> : Segment overlaps an OMIM annotation with OMIM Phenotype Map Key = 3  |
| OM: Segment overlaps an OMIM annotation with OMIM Phenotype Map Key ≠ 3  |
| CytoR: Segment overlaps a CytoRegion   |
| TS: Gain segment overlaps a Triplosensitivity region with TS Score = 3   |
| HI: Loss segment overlaps a Haploinsufficiency region with HI Score = 3  |
| 2 RS: Segment overlaps any Protein Coding Genes  |
| 4 EN: Segment overlaps any Protein Coding Ensembl Genes  |
| 5 P-HI: Overlaps any RS or EN where '%HI' ≤ 10 and 'pLI' ≥ 0.9   |
| NoGene: Not RS and not EN  |
| The highest tier (lowest number) will be applied for segments matching more than one rule with different tier assignments.   |
| When a segment matches the rules for either DGV-GS or DB-B, the assigned tier (if any) will override any other rules.<br>When a segment matches both the DGV-GS and DB-B rules, the DGV-GS assigned tier will be reported. |
|  |
|  |
|  |

 To prioritize your copy number segments in a tier-based order, assign a tier value to the rule(s) you want to use. To do this, click the drop-down arrow adjacent to the rule, then click the tier value (1-5) you want to assign to it. A Tier 1 assignment denotes the highest rule priority, while Tier 5 is the lowest.
 Note: Not every Rule requires an assigned tier. See the table below for Rule definitions.

**IMPORTANT!** For copy number segments that meet rule(s) with different tier assignments, the highest tier (lowest number) will be assigned to the segment. **Example:** A copy number segment overlaps a rule assigned as Tier 1 and also a rule that is Tier 3, the segment will be assigned as a Tier 1 since that is the higher of the 2 tiers. Two Rules are exceptions: DGV-GS and DB-B, if either of these rules are met, the assigned tier overrides any other tiers. If both of these rules are met, the tier assignment for DGV-GS is assigned.



| Tier-based<br>rule/evidence | Description   |
|-----------------------------|---|
| DGV-GS                      | Database of Genomic Variants - Gold Standard. The copy number segment is completely contained within an entry of like type (Gain or Loss) from the Gold Standard DGV track and meeting a defined frequency. Default frequency is >=1%. This rule WILL override higher ranked tiers based on the tier selected in this rule.   |
| DB-B                        | ChAS DB Both. Compare the copy number segment to the ChAS DB Both column data. If the number of entries in this column exceeds the defined threshold, then the copy number segment will be assigned the tier associated with this rule (unless the DGV-GS rule is also met). This rule WILL override rules with higher ranked tiers (with the exception of DGV-GS). |
| DB-F                        | ChAS DB - Filtered. Compare the copy number segment to the Filtered ChAS DB Both column. Example: Set the Filtered ChAS DB query to filter on segments in the database with the Call 'unknown significance'. If the copy number segment overlaps enough segments in your ChAS DB called 'unknown significance'', then the selected Tier will be assigned.           |
| OM-3                        | Any OMIM Genes annotation that is dark green in color. Dark Green is assigned for phenotype map key 3 OMIM records indicating the molecular basis is known; a mutation has been found in the gene.  |
| ОМ                          | Any OMIM Genes annotation that is NOT dark green in color. See OM-3 (above).  |
| Cyto-R                      | CytoRegions file. The segment overlaps any region in the assigned CytoRegion file. For more information on CytoRegions, see "Using CytoRegions" on page 263.  |
| TS                          | Triplosensitivity. The copy number segment overlaps an entry in the Triplosensitivity track which has an assigned TS_score of 3.  |
| HI                          | Haploinsufficiency. The copy number segment overlaps an entry in the Haploinsufficiency track which has an assigned HI_score of 3.  |
| RS                          | RefSeq. The copy number segment overlaps an entry in the Protein Coding Genes Track.  |
| EN                          | User-editable field populated by a user-configurable drop list of Calls   |
| P-HI                        | The copy number segment overlaps a Protein Coding Gene or Protein Coding Ensembl Gene with predicted haploinsufficiency values meeting the defined thresholds. pLI derived from gnomAD (https://gnomad.broadinstitute.org/) and %HI derived from DECIPHER (https://decipher.sanger.ac.uk/).   |
| NoGene                      | The copy number segment does not overlap any known Protein Coding Gene or Protein Coding Ensembl.   |

4. Once all desired rules have an assigned tier, click **OK** to save the selections and return to the Segment Prioritization Options window.

Click **Cancel** to return to the Segment Prioritization Options window without saving any tier assignment changes.

Click **Restore Defaults** to return to the installation settings.

Tier to call<br/>settingsYou can assign a Call to represent each tier. The contents of the drop-down list was<br/>generated from the Calls Vocabulary list. There are a set of default 'Calls", but this list<br/>can be customized, as detailed in "Using the calls feature" on page 249.

1. Click on the drop-down list adjacent to the Tier(s) you want to assign a Call to, then click on a selection, as show in Figure 413.

| Figure 413 Tier to Call drop-dov                  | wn lists                                      |
|---|---|
| Segment Prioritization Options                    | ×   |
| Ose Tier-Based Rules                              | Use Score-Based Rules                         |
| View/Edit Tier-Based Rules                        | View/Edit Score-Based Rules For Gain Segments |
|   | View/Edit Score-Based Rules For Loss Segments |
| Tier to Call                                      | Score to Call                                 |
| Optional: Associate some or all tiers with calls. | Optional: Assign calls from score thresholds. |
| Tier 1 Level 1                                    | Score ≥ 0.99                                  |
| Tier 2 Level 2                                    | Score ≥ 0.9                                   |
| Tier 3 Level 3                                    | Other score                                   |
| Tier 4 Probably nothing                           | Score ≤ _0.9                                  |
| Tier 5 Review Last                                | Score ≤ -0.99                                 |
| Restore [Level 1<br>Level 2                       | Restore Defaults                              |
| Level 3   |   |
| Level 4 O OF                                      | Cancel  |
| VOUS  |   |
| Probably nothing                                  |   |
| Review Last                                       |   |
| <ul> <li>Review Last</li> </ul>                   |   |

**Note:** Tiers are not required to have a Call assigned. Unassigned Tiers will appear blank in the Segment Table's **Calls From Prioritization** column.

2. After your Tier to Call assignments are complete, click OK.



#### Viewing segment prioritization in the segments table

Three new segment prioritization columns now appear in the Segment Table, as shown in Figure 414.

| Figure 414 New Segment Table columns  |             |                          |                       |                 |
|---|-------------|--------------------------|-----------------------|-----------------|
|   |             |                          |                       |                 |
| Microarray Nomenclature   | Call        | Call From Prioritization | Evidence              | ▲ Tier or Score |
| arr[GRCh37] 22q11.21(18626109_21915509)x1                                   |             | Level 1                  | 1: OM-3 2: P-HI 3: RS | 1               |
| arr[GRCh37] 20q11.23(36895218_36987660)x1 Probably nothing                  |             | Level 3                  | 3: RS                 | 3               |
| arr[GRCh37] Xq28(154912942_154946888)x0                                     |             | Level 3                  | 3: RS                 | 3               |
| arr[GRCh37] 1q44(248753184_248795277)x1                                     |             | Review Last              | *5* DGV-GS 3: RS      | 5               |
| arr[GRCh37] 5q35.3(180378754_180430789)x1                                   | Review Last | *5* DGV-GS 3: RS         | 5                     |                 |
| arr[GRCh37] 8p11.22(39247098_39386952)x1                                    |             | Review Last              | *5* DGV-GS 5: NoGene  | 5               |
| arr[GRCh37] 11q11(55374019_55452996)x0                                      |             | Review Last              | *5* DGV-GS 3: RS      | 5               |
| arr[GRCh37] 16q12.2(55796376_55822431)x1 Review Last *5* DGV-GS 5: NoGene 5 |             | 5                        |                       |                 |
| arr[GRCh37] Xq21.31(86337774_86693619)x2 Review Last 5: NoGene 5            |             |                          |                       |                 |

- Call From Prioritization: Displays the Call associated with the Tier assigned to the copy number segment.
- .Evidence: Displays the abbreviation representing the rules met based on which annotations the copy number segment overlaps. See the Tier-based rule/ evidence table of definitions above for more details.

**Note:** A copy number segment that does not overlap any rules with an assigned tier will display *No rules match*.

Tier or Score: This will be a number from 1-5 representing the Tier that was assigned to the copy number segment based on the user-defined Tier-Based rules selected.

If the Call from Prioritization assignments are correct, they can be accepted as the Calls for each segment. To do this:

1. Click the **I** button.

The Call from Prioritization assignments will be copied into blank cells in the Call column. **Note:** Any Calls manually assigned will remain in the Call column and not be overwritten. Segments hidden by the filters will not have calls copied from the Calls from Prioritization column.

A confirmation message appears (Figure 415) summarizing the Call from Prioritization assignments are to be copied into the Call column.

| Figure 415 Confirm copy into Call column  |
|---|
| Confirm Copy Calm Prioritization X  |
| Copy 8 values from Call From Prioritization to Call?<br>6: Review Last<br>2: Unknown Significance<br>Yes No |

2. Click **Yes** to acknowledge the message.

**Note:** Any Call can be manually adjusted by clicking on the Call cell and selecting a new option from the drop-down list, as detailed in "Using the calls feature" on page 249.

## Score-based prioritization in ChAS

The Score-Based prioritization is a research-based adaptation that is similar, but not identical to, the guidelines proposed in the Riggs et al. 2019 paper: *Genetics in MEDICINE*, Published online: 06 November 2019.

This segment prioritization method assigns numeric values (i.e. scores) based on the overlap of a copy number segment with public and/or private annotations. Default score assignments are based on the aforementioned paper.

- A copy number segment's final score is summed based on the rules the segment matches.
- The score assigned to a copy number segment is then associated with a call based on user defined thresholds.
- Overlap between copy number segments and annotations are performed on all transcripts for a given gene that are >=90% of the size of the gene coordinates in the Triplosensitivty and Haploinsufficiency tracks and have identical gene symbols.
- Segment prioritization applies to the following segment types: Gains, Mosaic Gains, XON Region Gains, Loss, Mosaic Loss, and XON Regions Loss.
  - 1. From the Segment Table tool bar, click the 🔛 button.

The Segment Prioritization Options window appears. (Figure 416)

#### Figure 416 Segment Prioritization Options window

| Segment Prioritization Options                                    | ×  |  |  |
|---|--|--|--|
| O Use Tier-Based Rules  | Use Score-Based Rules  |  |  |
| View/Edit Tier-Based Rules  | View/Edit Score-Based Rules For Gain Segments                  |  |  |
|   | View/Edit Score-Based Rules For Loss Segments                  |  |  |
| Tier to Call<br>Optional: Associate some or all tiers with calls. | Score to Call<br>Optional: Assign calls from score thresholds. |  |  |
| Tier 1  | Score ≥ 0.99   |  |  |
| Tier 2  | Score ≥ 0.9  |  |  |
| Tier 3  | Other score  |  |  |
| Tier 4  | Score ≤0.9   |  |  |
| Tier 5  | Score ≤0.99  |  |  |
| Restore Defaults  | Restore Defaults   |  |  |
| ОК  | Cancel   |  |  |

# Configuring the score-based option



2. Click on the Use Score-Based radio button.

#### View/Edit Score-Based Rules for Gain Segments

1. Click the View/Edit Score-Based Rules for Gain Segments button.

The Select Score Options for Gain window appears. (Figure 417)

| Figure 417 Select Score Options for Gain window                            |   |
|--|---|
| Select Score Options for Gain  | × |
| Select scores to associate with rules.                                     |   |
| Score Rule   |   |
| 0 1A: Overlaps a Protein Coding Genes gene                                 |   |
| -0.6 1B: Does not overlap a Protein Coding Genes gene                      |   |
| 1 2A: Completely overlaps an Established TS gene or region                 |   |
| 0 2B: Partially overlaps an Established TS gene or region                  |   |
| -1 2C/2F: Contains same gene content as Established Benign CNV region      |   |
| -1 2D/2E: Completely contained in an Established Benign CNV gene or region |   |
| 0 2H: Completely overlaps an Established HI gene or region                 |   |
| 0.3 21: Completely contained within an Established HI gene or region       |   |
| 0.9 <b>2I+:</b> and also covers a percentage of that region ≥ 90           |   |
| 0 2J/2K: Partially overlaps an Established HI gene or region               |   |
| 0 3A: Overlaps one or more Protein Coding Genes gene(s)                    |   |
| 0.45 3B: and overlaps at least this many: 35                               |   |
| 0.9 3C: and overlaps at least this many: 50                                |   |
| -1 <b>40-DB-B</b> : Has 'DB Count Both' ≥ 400                              |   |
| -1 40-DGV-GS: Contained within a DGV-GS region with frequency % ≥ 1        |   |
| 0 CY: Overlaps a CytoRegion  |   |
| Restore Defaults   |   |
| OK Cancel  |   |

2. Use the text field adjacent to the Rule to enter a new numerical. Click the **Restore Defaults** button to return to the factory values. See the table below for Score-based rule/evidence, descriptions, and default value information.



| Score-based rule/evidence | Description  | Default<br>value |
|---------------------------|--|------------------|
| 1A                        | The Gain copy number segment fully or partially overlaps at least 1 annotation in the Protein Coding Genes track.  | 0                |
| 1B                        | The Gain copy number segment does not fully or partially overlap any annotation in the Protein Coding Genes track.   | -0.6             |
| 2A                        | The Gain copy number segment completely overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3.   | 1                |
| 2B                        | The Gain copy number segment partially overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3. Partial overlap indicates one breakpoint of the Gain segment is located within the TS_Score = 3 gene/region.   | 0                |
| 2C/2F                     | The Gain copy number segment contains the same gene content as a Triplosensitivity or Recurrent/Curated Regions annotation with a TS Score = 40. The copy number Gain segment might be larger than the gene/region, but contains the same gene content as listed in the Triplosensitivity or Recurrent/Curated Regions tracks. | -1               |
| 2D/2E                     | Both breakpoints of the Gain copy number segment are contained within an annotation having a TS Score = 40 in either the Triplosensitivity or Recurrent/Curated Regions.   | -1               |
| 2H                        | The Gain copy number segment completely overlaps an annotation in either the Haploinsufficiency or Recurrent/Curated Regions track with a HI Score = 3.  | 0                |
| 21                        | Both breakpoints of the Gain copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency or Recurrent/Curated Regions. The copy number Gain segments is smaller than the user defined threshold (Default >=90%).  | 0.3              |
| 21+                       | Both breakpoints of the Gain copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency or Recurrent/Curated Regions. The copy number Gain segments is larger than the user defined threshold (Default >=90%).   | 0.9              |
| 2J/2K                     | The Gain copy number segment partially overlaps an annotation in either the Haploinsufficiency or Recurrent/Curated Regions track with a HI Score = 3. Partial overlap indicates one breakpoint of the Gain segment is located within the HI_Score = 3 gene/region.  | 0                |
| 3A                        | The Gain copy number segment (partially or completely) overlaps at least 1 Protein Coding Gene annotation. Default is 1-34 genes.  | 0                |
| 3В                        | The Gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A. Default is 35-49.   | 0.45             |
| 3C                        | The Gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A or 3B. Default is $> =50$ .  | 0.9              |
| 40-DB-B                   | The Gain copy number segment overlaps/covers a defined number of segments in your ChAS database (DB Count Both column). Default is 400 segments. Configuration of DB Count Both parameters can be found in "Querying a segment from the segment table" on page 387.  | -1.0             |
| 40-DGV-GS                 | Both breakpoints of the Gain copy number segment are contained within an annotation in the DGV-GS Gain (blue). The DGV-GS annotation must have an NR frequency greater than track defined. Default NR frequency is 1%.   | -1.0             |
| СҮ                        | The Gain copy number segment overlaps an annotation in the customer supplied CytoRegions File(s). For more information on CytoRegion files, see "Using CytoRegions" on page 263.   | 0                |

17

3. After your score assignments are complete, click **OK**.

#### View/Edit Score-Based Rules for Loss Segments

1. Click the View/Edit Score-Based Rules for Loss Segments button.

The Select Score Options for Loss window appears. (Figure 417)

| Figure 418 Select Score Options for Loss window |   |  |
|---|---|--|
| Select S  | Score Options for Loss X  |  |
| Select sco                                      | pres to associate with rules.   |  |
| Score   | Rule  |  |
| 0   | 1A: Overlaps a Protein Coding Genes gene  |  |
| -0.6  | 1B: Does not overlap a Protein Coding Genes gene                                |  |
| 1   | 2A: Completely overlaps an Established HI gene or region                        |  |
| 0.45  | 2B-r: Partially overlaps an Established HI region                               |  |
| 0.0   | 2B-g: Partially overlaps an Established HI gene                                 |  |
| 1   | 2C-1: 5'UTR and CDS   |  |
| 0.45  | 2C-2: 5'UTR but no CDS  |  |
| 0   | 2D-1: 3'UTR only  |  |
| 0.3   | 2D-2/3: last 3' coding exon only  |  |
| 0.9   | 2D-4: multiple 3' coding exons  |  |
| 0.3   | 2E: Completely contained in an Established HI gene or region                    |  |
| 0.9   | <b>2E+:</b> and also covers a percentage of that region $\ge$ 90                |  |
| -1  | $\mathbf{2F}:$ Completely contained in an Established Benign CNV gene or region |  |
| 0.15  | <b>2H</b> : Overlaps any RS or EN where '%HI' $\leq$ 10 and 'pLI' $\geq$ 0.9    |  |
| 0   | 3A: Overlaps one or more Protein Coding Genes gene(s)                           |  |
| 0.45  | <b>3B</b> : and overlaps at least this many: 25                                 |  |
| 0.9   | <b>3C:</b> and overlaps at least this many: 35                                  |  |
| -1  | <b>4O-DB-B</b> : Has 'DB Count Both' ≥ 400                                      |  |
| -1  | <b>4O-DGV-GS</b> : Contained within a DGV-GS region with frequency % ≥ 1        |  |
| 0   | CY: Overlaps a CytoRegion   |  |

2. Use the text field adjacent to the Rule to enter a new value. Click the **Restore Defaults** button to return to the factory values. See the table below for Scorebased rule/evidence, descriptions, and default value information.



| Score-based rule/evidence | Description   | Default<br>value  |  |  |
|---------------------------|---|---|--|--|
| 1A                        | The Loss copy number segment fully or partially overlaps at least 1 annotation in the Protein Coding Genes track.   |   |  |  |
| 1B                        | The Loss copy number segment does not fully or partially overlap any annotation in the Protein Coding Genes track.  | -0.6  |  |  |
| 2A                        | The Loss copy number segment completely overlaps an annotation in either the Triplosensitivity or Recurrent/Curated Regions track with a TS Score = 3.  | 1   |  |  |
| 2B-r                      | The Loss copy number segment partially overlaps an annotation in the Recurrent/Curated Regions track with a HI Score = 3. Partial overlap indicates one breakpoint of the Loss segment is located within the HI Score = 3 region.   |   |  |  |
| 2B-g                      | The Loss copy number segment partially overlaps an annotation in the Haploinsufficiency track with a HI Score = 3. Partial overlap indicates one breakpoint of the Loss segment is located within the HI Score = 3 gene. If 2B-g is met, then move on to 2C - 2E to assess a value based on location of the partial overlap.  | 0<br>(static<br>value,<br>further<br>assessmen<br>t required) |  |  |
| 2C-1                      | The Loss copy number segment overlaps the 5'UTR and some CDS of a gene with HI score<br>= 3 in the Haploinsufficiency track.<br>TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the<br>Structure tab to view the exons and CDS coordinates.<br>Note: All transcripts for a gene are assessed as long as the transcript is =< 90% of the<br>length of the gene as defined in the Haploinsufficiency track and have identical gene<br>symbols.                            | 0.9   |  |  |
| 2C-2                      | The Loss copy number segment overlaps the 5'UTR, but no CDS of a gene with HI score<br>= 3 in the Haploinsufficiency track.<br>TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the<br>Structure tab to view the exons and CDS coordinates.<br>Note: All transcripts for a gene are assessed as long as the transcript is =< 90% of the<br>length of the gene as defined in the Haploinsufficiency track and have identical gene<br>symbols.                             | 0   |  |  |
| 2D-1                      | The Loss copy number segment overlaps the 3'UTR only, no CDS is involved for a gene with HI score = 3 in the Haploinsufficiency track.<br>TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates.<br>Note: All transcripts for a gene are assessed as long as the transcript is =< 90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols.                           | 0   |  |  |
| 2D2/2D3                   | The Loss copy number segment overlaps the 3'UTR AND the last exon in the coding region<br>for a gene with HI_score = 3 in the Haploinsufficiency track.<br>TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the<br>Structure tab to view the exons and CDS coordinates.<br>Note: All transcripts for a gene are assessed as long as the transcript is =< 90% of the<br>length of the gene as defined in the Haploinsufficiency track and have identical gene<br>symbols. | 0.3   |  |  |



| Score-based rule/evidence | Description  | Default<br>value |  |  |
|---------------------------|--|------------------|--|--|
| 2D-4                      | The Loss copy number segment overlaps the 3'UTR AND multiple exons in the coding region for a gene with HI_score = 3 in the Haploinsufficiency track.<br>TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates.<br>Note: All transcripts for a gene are assessed as long as the transcript is =< 90% of the length of the gene as defined in the Haploinsufficiency track and have identical gene symbols. |                  |  |  |
| 2E                        | Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_Score = 3 in either the Haploinsufficiency track or Recurrent/Curated Regions track. The copy number Loss segment is smaller than the annotation in the track by less than the user defined threshold (Default >=90%).   | 0.3              |  |  |
| 2E+                       | Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_ Score = 3 in either the Haploinsufficiency track or Recurrent/Curated Regions track. The copy number Loss segment is larger than the annotation in the track by less than the user defined threshold (Default >=90%).   | 0.9              |  |  |
| 2F                        | Both breakpoints of the Loss copy number segment are contained within an annotation having a HI_ Score = 40 in either the Haploinsufficiency track or Recurrent/Curated Regions track.   | -1               |  |  |
| 2H                        | The Loss copy number segment overlaps a Protein Coding Gene or Protein Coding Ensembl Gene with predicted haploinsufficiency values meeting the defined thresholds. pLI derived from gnomAD (https://gnomad.broadinstitute.org/) and %HI derived from DECIPHER (https://decipher.sanger.ac.uk/).   | 0.15             |  |  |
| ЗА                        | The Loss copy number segment (partially or completely) overlaps at least 1 Protein Coding Gene annotation. Default is 1-24 genes.  | 0                |  |  |
| 3В                        | The Loss copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A. Default is 25-34.   | 0.45             |  |  |
| 3C                        | The gain copy number segment (partially or completely) overlaps more Protein Coding Gene annotations than in 3A or 3B. Default is $> =35$ .  | 0.9              |  |  |
| 40-DB-B                   | The Loss copy number segment overlaps/covers a defined number of segments in your ChAS database (DB Count Both column). Default is 400 segments. Configuration of DB Count Both parameters can be found in "Querying a segment from the segment table" on page 387.  | -0.9             |  |  |
| 40-DGV-GS                 | Both breakpoints of the Loss copy number segment are contained within an annotation in the DGV-GS gain (red). The DGV-GS annotation must have an NR frequency greater than track defined. Default NR frequency is 1%.  | -0.9             |  |  |
| СҮ                        | The Gain copy number segment overlaps an annotation in the customer supplied CytoRegions File(s). For more information on CytoRegion files, see "Using CytoRegions" on page 263.   | 0                |  |  |

3. After your score assignments are complete, click **OK**.

# Configuring the score-based option

1. From the Segment Prioritization Options window, go to the Score to Call pane. (Figure 419)

| Figure 419 Score to Call pane  |   |  |  |  |
|--|---|--|--|--|
| Segment Prioritization Options X   |   |  |  |  |
| O Use Tier-Based Rules   | Use Score-Based Rules                         |  |  |  |
| View/Edit Tier-Based Rules   | View/Edit Score-Based Rules For Gain Segments |  |  |  |
|  | View/Edit Score-Based Rules For Loss Segments |  |  |  |
| - Score to Call - Score to Call - Score to Call - Score thresholds Score threshold |   |  |  |  |
| Tier 1   | Score ≥ 0.99                                  |  |  |  |
| Tier 2   | Score ≥ 0.9                                   |  |  |  |
| Tier 3   | Other score                                   |  |  |  |
| Tier 4   | Score ≤ _0.9                                  |  |  |  |
| Tier 5   | Score ≤ -0.99                                 |  |  |  |
| Restore Defaults   | Restore Defaults                              |  |  |  |
| OK Cancel  |   |  |  |  |

- Define the Score Thresholds: In the appropriate text field, enter a Call based on the segment score as defined above. Your entered threshold values for each Call will be populated in the Segment Table's Call from Prioritization column.
- Select Calls: Use the drop-downs adjacent to each threshold to assign a Call that will be associated with a range of scores.

**Note:** Calls in the drop-down lists can be customized by adding to the Calls Vocabulary window in the User Configuration.

In the example below (Figure 420), a copy number segment with a Score of 1.3 would have a Call from Prioritization assignment of "Level 1". A copy number segment with a score of -0.96 would have a Call from Prioritization assignment of "Probably nothing".



| Figure 420 Score to Call drop-down lists                          |   |  |  |  |  |
|---|---|--|--|--|--|
| Segment Prioritization Options X                                  |   |  |  |  |  |
| O Use Tier-Based Rules  | Use Score-Based Rules                                       |  |  |  |  |
| View/Edit Tier-Based Rules  | View/Edit Score-Based Rules For Gain Segments               |  |  |  |  |
|   | View/Edit Score-Based Rules For Loss Segments               |  |  |  |  |
| Tier to Call<br>Optional: Associate some or all tiers with calls. | Score to Call Optional: Assign calls from score thresholds. |  |  |  |  |
| Tier 1  | Score ≥ 0.99 Level 1 ✓                                      |  |  |  |  |
| Tier 2  | Score ≥ 0.9 Level 2 ✓                                       |  |  |  |  |
| Tier 3  | Other score Unknown Significance 🗸                          |  |  |  |  |
| Tier 4  | Score ≤ -0.9 Probably nothing ✓                             |  |  |  |  |
| Tier 5  | Score ≤ _0.99   |  |  |  |  |
| Restore Defaults  | Restore Defaults  |  |  |  |  |
| OK  | Cancel Level 3<br>Level 4                                   |  |  |  |  |
|   | VOUS<br>Probably nothing<br>Review Last                     |  |  |  |  |

2. Click **OK** to accept the Score thresholds and Calls or click Cancel to return to the ChAS Browser without saving any new assignments. Click the **Restore Defaults** button to return to the factory values.

#### Viewing segment prioritization in the segments table

Three new segment prioritization columns now appear in the Segment Table. (Figure 414).

| Figure 421 New Segment Table colum               | ins    |                          |   |                   |
|--|--------|--------------------------|---|-------------------|
| 🔠 👼 😢 🗎   Σ [ 🏢 ] ] 🜵   🗹 🗆   🔣                  |        |                          |   |                   |
| Microarray Nomenclature                          | Call   | Call From Prioritization | Evidence                                | ▼ Tier or Score D |
| arr[GRCh37] 22q11.1q11.21(16888900_22141230)x1-2 |        | Level 1                  | 1A 2A ("22q11.2_recurrent_(DGS/VCFS)_re | 1.9               |
| arr[GRCh37] 22q11.21(18626109_21915509)x1        |        | Level 1                  | 1A 2A ("22q11.2_recurrent_(DGS/VCFS)_re | 1.9               |
| arr[GRCh37] 20q11.23(36895218_36987660)x1        |        | Unknown Significance     | 1A 3A (2)                               | 0                 |
| arr[GRCh37] Xq28(154912942_154946888)x0          |        | Unknown Significance     | 1A 3A (1)                               | 0                 |
| arr[GRCh37] Xq21.31(86337774_86693619)x2         | Benign | Unknown Significance     | 1B                                      | -0.6              |
| arr[GRCh37] 14q32.33(106160630_106736911)x3      |        | Review Last              | 1B 4O-DB-B (3217)                       | -1.5              |
| arr[GRCh37] 1q44(248753184_248795277)x1          |        | Review Last              | 1A 3A (2) 4O-DB-B (794) 4O-DGV-GS (gssv | -1.8              |
| arr[GRCh37] 5q35.3(180378754_180430789)x1        |        | Review Last              | 1A 3A (1) 40-DB-B (531) 40-DGV-GS (gssv | -1.8              |
| arr[GRCh37] 11q11(55374019_55452996)x0           |        | Review Last              | 1A 3A (3) 4O-DB-B (1366) 4O-DGV-GS (gss | -1.8              |
| arr[GRCh37] 8p11.22(39247098_39386952)x1         |        | Review Last              | 1B 40-DB-B (2106) 40-DGV-GS (gssvL124   | -2.4              |
| arr[GRCh37] 16q12.2(55796376_55822431)x1         |        | Review Last              | 1B 40-DB-B (557) 40-DGV-GS (gssvL4460   | -2.4              |

 Call From Prioritization: Displays the "Call" associated with the score threshold ranges.



- Evidence: Displays the rules met based on which annotations the copy number segment overlaps.
- Tier or Score: This will be a numeric value representing the score generated and assigned to the copy number segment based on the user-defined Score-Based rules selected.

If the Call from Prioritization assignments are correct, they can be accepted as the Calls for each segment. To do this:

1. Click the 🚺 button.

The Call from Prioritization assignments will be copied into blank cells in the Call column.

**Note:** Any Calls manually assigned will remain in the Call column and not be overwritten. Segments hidden by the filters will not have calls copied from the Calls from Prioritization column.

A confirmation message appears (Figure 422) summarizing the Call from Prioritization assignments are to be copied into the Call column.

| Figure 422         Confirm copy into Call column  |   |  |  |  |
|---|---|--|--|--|
| Confirm Copy Callom Prioritization  | × |  |  |  |
| Copy 11 values from Call From Prioritization to Call<br>2: Level 1<br>6: Review Last<br>3: Unknown Significance<br><u>Y</u> es <u>N</u> o | ? |  |  |  |

2. Click **Yes** to acknowledge the message.



# Interacting with the ChAS database

A segment can be queried against the ChAS Database for intersecting segments from previously published samples. Using both the Overlap Threshold and Coverage Threshold can focus the query results to segments that are of approximately the same size as the segment in the current sample.

**Note:** ReproSeq Aneuploidy data can not be published into the ChAS DB.

# Setting up a ChAS DB query

- 1. From the ChAS Browser, click **Preferences**  $\rightarrow$  **Edit User Configuration**.
- 2. Click the **DB Query** window tab. (Figure 423)

| Figure 423 User Configuration - DB Query window/tab   |
|---|
| User Configuration X  |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports                                 |
| Copy Number Query Parameters  |
| Minimum % CN Coverage 50  |
| Minimum % CN Overlap 50   |
| 🗹 Match only same gain/loss type 问 Include LOH 问 Include XON Regions  |
| LOH Query Parameters  |
| Minimum % LOH Coverage 50   |
| Minimum % LOH Overlap 50  |
| XON Regions Query Parameters  |
| Minimum % Coverage 50   |
| Minimum % Overlap 50 50   |
| 🗹 Match only same gain/loss type 🔲 Include Copy Number Segments   |
| Minimum % Coverage: The minimum percentage that the current segment is covered by other similar segments from the database. |
| Minimum % Overlap: The minimum percentage that the current segment overlaps similar segments in the database.               |
| Parameters:<br>Call: = "pathogenic"   |
| Restore Defaults     Change Filter Parameters       OK     Cancel   |



#### Setting up query parameters for a copy number search

**Note:** When querying on a copy number segment in the Browser, the values set in the Copy Number Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 424) **Note:** The default values are set to 50%.

| Figure 424 Copy       | Number Query Parameters  |
|-----------------------|--|
| Copy Number Query Pa  | Irameters  |
| Minimum % CN Coverage | 50   |
| Minimum % CN Overlap  | 50   |
|                       | 🗹 Match only same gain/loss type 📄 Include LOH 📄 Include XON Regions |

- 2. Check the **Match only same gain/loss type** check box if you want to query the database for only similar copy number types (gains only or losses only). Uncheck this check box if you want to query all copy number segment types.
- 3. Check the **Include Exon Regions** check box if you want to include Exon Region Segments in your query.
- 4. Check the Include LOH box to include LOH segments in your query.
- 5. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

**Note:** When querying on an LOH segment in the Browser, the values set in the LOH Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 425) **Note:** The default values are set to 50%.

| Figure 425 LOH Query Parameters |
|---------------------------------|
| LOH Query Parameters            |
| Minimum % LOH Coverage 50       |
| Minimum % LOH Overlap 50        |

2. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

Setting up query parameters for an XON region segment search **Note:** When querying on an XON Region segment in the Browser, the values set in the Exon Region Query Parameters section are used.

1. Enter minimum percentage values for both Overlap and Coverage using the text boxes or click and drag the appropriate slider bar. (Figure 426) The default values are set to 50%.

#### Setting up query parameters for an LOH segment search



| Figure 426 XON Regions Query Parameters |   |  |  |  |
|---|---|--|--|--|
| XON Regions Query F                     | Parameters  |  |  |  |
| Minimum % Coverage                      | 50  |  |  |  |
| Minimum % Overlap                       | 50  |  |  |  |
|   | 🗹 Match only same gain/loss type 🔲 Include Copy Number Segments |  |  |  |

- 2. Check the **Match only same gain/loss type** check box if you want to query the database for only similar XON Region segment types (gains only or losses only). Uncheck this check box if you want to query all XON Region segment types.
- 3. Check the **Include Copy Number Segments** check box to include Copy Number Segments in your query.
- 4. Click **OK** to save your changes or click **Restore Defaults** to return the parameter settings back to their default settings.

## Querying a segment from the segment table

To view what segments in the database intersect with the currently loaded segment, you must first make the **DB Count Both** column visible. The DB Count Both column displays the number of segments in the database that meet the criteria set in the DB Query tab.

1. Right-click in the DB Count Both cell for the segment(s) you want to view, then click **Query ChAS DB for "DB Count Both"**. (Figure 427)

**Note:** Segments matching ONLY the Coverage OR Overlap thresholds can also be returned in the Segment Intersections window by right clicking in either the DB Coverage Count or DB Overlap Count columns respectively. You can also right click on the segment in the Detail View and choose Query ChAS DB to return segments meeting either the Coverage OR the Overlap thresholds. See "Filtering DB count columns" on page 396. If you want to add column(s) to your Segment table, see "Selecting columns to display or hide" on page 325.



| Figure 427 Query CHAS DE for DE Count Both  | Figure 427 Query ChAS DB for "DB Count Both" |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|
| 🖽 📾   🛰 🚛 🔹 🛲 📑 👬 💷 💷 🐂 🖓 💽 🖬   🕎 🖓 🖤   011/22, 18,034,002 - 21,847,987   |  |  |  |  |  |  |  |
| 🖞 👭 Karvoview 🥄 🔛 Segments 🕄 🛄 CytoRegions 🌂 🛑 Overlap Map 🔍 🖾 Graphs 🕄 🗰 Som Mut 🕄   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
|   | 10 results                                   |  |  |  |  |  |  |
| Full Location Call Interpretation Inheritance DB Count DB Overlap Microa  | array Nomenclature                           |  |  |  |  |  |  |
| chr1:248753183-248795277 Benign 4 38 4 arr[hg1  | 19] 1q44(248,753,183-248,795,2               |  |  |  |  |  |  |
| chrb:1803/8/53-180430/89 Benign 3 38 3 arr[h]   | 19] 5q35.3(180,378,753-180,43)               |  |  |  |  |  |  |
| cnr8.3924/09/-39380952 Benign 13 45 13 arring 1 6 9 9 9 9 13 14 15 15 15 15 15 15 15 15 15 15 15 15 15  | 19] 8p11.22(39,247,097-39,386,               |  |  |  |  |  |  |
| CITIT 1.063/4018-06402990 Berligit 6 30 6 attribute 6 30 6 attribute 7 30 5 attribute 7 30 | 19 110 1100,374,018-00,402,98                |  |  |  |  |  |  |
| children 10 00029*100730511 Denigin 11 43 10 altitut<br>children 155706275.55927421 Denigin 11 43 10 altitut  | 101 16a12 2(55 706 275 55 222                |  |  |  |  |  |  |
| chr0:36895217-36987660 0 29 0 arthur  | 19] 20g11 23(36 895 217-36 987               |  |  |  |  |  |  |
| chr2:18916827-21465662 2 31 6 artho   | 19122011.21(18.916.827-21.465                |  |  |  |  |  |  |
| chrX:86337773-86693619  | 191 Xg21.31(86.337.773-86.693.               |  |  |  |  |  |  |
| chrX:154912941-154946888  | 19] Xq28(154,912,941-154,946,8               |  |  |  |  |  |  |
| Query Châs DB for "DB Count Poth"   |  |  |  |  |  |  |  |
|   | < >  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
| 15 DC 40.044 subdisuber Comp Number State (assessments)   |  |  |  |  |  |  |  |
| As_rs_russ-rr.cynu.cycnp. Copy Number State (segments)  | ~  |  |  |  |  |  |  |
| $\nabla$  |  |  |  |  |  |  |  |
| A 15 DS 40 0 44 who who Whitehold Land Date   |  |  |  |  |  |  |  |
| A5 - F5 - 1.5   |  |  |  |  |  |  |  |
| 0.5   |  |  |  |  |  |  |  |
|   | - h. t                                       |  |  |  |  |  |  |
| 1. F. das 1. S. Maller and Market Market (B. Market Market & Stater & Parket And Astronomy (Barket & Stater & S   |  |  |  |  |  |  |  |
| -1.5  |  |  |  |  |  |  |  |
| A6_PS_10-3-11.cyhd.cychp: Allele Peaks  |  |  |  |  |  |  |  |
| +1.5  |  |  |  |  |  |  |  |
| - 0.6 in a set and all an Ar Artistic and it a state at a   | Charles and a second                         |  |  |  |  |  |  |
| -0.5 A subscription of the subscription of    | - N.H. 20 (204                               |  |  |  |  |  |  |
| -1.5  |  |  |  |  |  |  |  |
| DDG2P_20121101.aed  |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
| Histogram: Blood Samples: Gain  |  |  |  |  |  |  |  |
| Histogram: Blood Samples: Loss  |  |  |  |  |  |  |  |
|   |  |  |  |  |  |  |  |
| Genes   |  |  |  |  |  |  |  |

# Segment intersections

The **Segment Intersections** view appears with the results from the query. (Figure 428)

The Segment Intersections view shows samples in the database that contain segments that meet the criteria set in the DB Query.

The middle portion of this view contains table information about the samples in the database including any Call, Interpretation or Inheritance information assigned to the segments for the samples shown in the example above. To display or hide columns within this table, click []] (upper right corner).

The lower portion of the view provides the same external annotations available in the Detail View. To display an annotation track, go to the ChAS Browser's Files Menu and check the box. The annotation track will then be displayed in both the Detail View and the Segment Intersections View. (Figure 428)

You can return segments from the database based on either DB Overlap or DB Coverage. These segments meet either one of the overlap or coverage threshold criteria.



**Note:** Segment Intersection search results are limited to 1000 intersecting segments. When querying on a copy number segment in the Browser, the Copy Number Query Parameter Thresholds are used for all segment types. When querying on an LOH segment in the Browser, the LOH Query Parameter Thresholds are used for all segment types.

Example: You have a gain segment in the ChAS Browser and run a query to retrieve intersecting segments. Both Gain and LOH segments counts appear in DB Count Both, the LOH segment being returned are using the Copy Number Query Parameter Thresholds since the original segment is a GAIN.

| Figure 428 Query C   | hAS DB - Segment In                          | tersections window                      |                                      |  |  |
|--|--|---|--------------------------------------|--|--|
|  |  |   |                                      |  |  |
| Segment Intersections  |  |   |                                      |  | _ C                                    |
| Search View  |  |   |                                      |  |  |
| chr22: 18,626,108 - 21,915,509 (Gain, Loss, Gai                              | in (XON Region), Loss (XON Region)); Coverag | e >= 50% AND Overlap >= 50%             |                                      |  |  |
| 🔛 🔜 🔛 🖀 Σ  |  |   |                                      |  | 7 results                              |
| Label File   | Type CN State Chromo Min                     | Max Size (kbp) 🗾 Call                   | Segment Interpretation               | E Inheritance DB Overlap                     | DB Coverage Sex Genes                  |
| smooth Constutional_Blood_01_CytoScan  | . ▼Loss 1.00 22 18,626,                      | 108 21,915,509 3,289 Pathogenic         | 22q11.1 microdeletion                | 100%   | 100% male USP18, L0                    |
| seg112 CyHD_0425121_35357_400049<br>seg132 CyHD_021312T_SS135_401763S        | . A Gain 3.00 22 18,648,                     | 21,465,659 2,817 polymorphic (ADGV)     | Cogmont Intercept                    | iono Toblo <sup>100%</sup>                   | 85% male USP18, L0                     |
| seg406 20140222B_02908_P0000505_Cyt  | ▲ Gain 3.00 22 18,648,                       | 866 21,465,659 2,817 polymorphic (ADGV) | Segment intersect                    |  | 85% female USP18, L0                   |
| seg100 20130530A_04090_P00007327_Cy.<br>seg110 20111018_IR72_IR-72_CytoScanH | A Gain 3.00 22 18,648,                       | 842 21,465,659 2,549 polymorphic (ADGV) |                                      | 100%   | 77% male PRODH, I                      |
| seg92 N_Blood_control103.cyhd.cychp  | ▲ Gain 3.00 22 18,916,                       | 842 21,465,659 2,549 polymorphic (ADGV) |                                      | 100%   | 77% female PRODH, I                    |
| This segment   | t example is represent                       | ed as a loss (in the sampl              | e currently loaded in th             | e hrowser)                                   |  |
|  |  |   |                                      |  |  |
|  | ~  |   |                                      |  |  |
|  |  |   |                                      |  |  |
| 18200kb 18400kb 18600kb 18   | 1900kb 19000kb 19200kb 19400kb               | 19600kb 19800kb 20000kb 20200kb 2040    | 0.kb 20600.kb 20800.kb 21000.kb 2120 | 0kb 21400kb 21600kb                          | 21800kb 22000kb 22200kb                |
|  |  |   |                                      |  | -                                      |
|  |  | ▼                                       |                                      |  |  |
|  |  | Δ                                       |                                      |  |  |
|  |  |   | ointe of the Segment                 |  |  |
|  |  | de lines represent break p              | onts of the Segment                  |  |  |
| Segment Intersection   | ons  | Δ                                       |                                      |  |  |
| Graph  |  | Δ                                       |                                      |  |  |
| Giapii   |  | Δ                                       |                                      |  |  |
| 22   |  | q11.21                                  |                                      |  | q11.22                                 |
| A Y  |  |   |                                      |  |  |
| O GETTER   |  |   |                                      |  |  |
|  |  |   | HE DIC-                              | 1000 C 1                                     |  |
| MICAL3-  | pacez- CLTCL1+ HIRA+-                        | LOC                                     | 101927859 VEDIS- PI4KA               | 0072-  | MAPK1-                                 |
|  |  |   |                                      | H-141 AB | ······································ |
| OHIM @ Genes   |  | External An                             | notations                            |  |  |
|  |  |   |                                      |  |  |
|  |  |   |                                      |  |  |
| MICAL3 (608862)  |  |   | P184C4.(600228)                      |  |  |
| ·  |  |   |                                      |  |  |
|  |  |   |                                      |  |  |

Click the Side-by-Side icon (upper right) to split the Segment Intersections window, as shown in Figure 429 on page 390.

**Note:** Columns with a pad and pencil *icon* represent a segment field that can be edited. All edits are stored directly to the database.



| Figure 429 Query ChAS DB - Segment Intersections window (Side-by-Side)       |     |              |                               |                   |                               |  |  |  |
|--|-----|--------------|-------------------------------|-------------------|-------------------------------|--|--|--|
| Segment Intersections  |     |              |                               |                   |                               |  |  |  |
| arch View  |     |              |                               |                   |                               |  |  |  |
| 22: 18,626,108 - 21,915,509 (Gain, Loss); Coverage >= 50% AND Overlap >= 50% | ٠   |              |                               |                   |                               |  |  |  |
| 1 🔐 😢 🗎 } Σ 🚺 11 🖿   | >   | o 0 😰        | Ŭ                             |                   |                               |  |  |  |
| File Type CN State Chromo Min Max  | S   |              |                               | T<br>V            |                               |  |  |  |
| Constutional_Blood_01_CytoScan ▼Loss 1.00 22 18,626,108 21,915,509           |     | l0kb         | 19000kb                       | 20000kb           | 21000kb                       |  |  |  |
| CVHD_0425121_SS337_400649 ▲ Gain 3.00 22 18,640,729 21,465,659               | -   |              |                               |                   |                               |  |  |  |
| 20140222B_02908_P0000505_Cyt   | - ' |              |                               |                   |                               |  |  |  |
| N_Blood_control103.cyhd.cychp ▲ Gain 3.00 22 18,946,866 21,796,907           |     |              |                               |                   |                               |  |  |  |
| 20111018_IR72_IR-72_CytoScanH  ▲ Gain 3.00 22 18,916,842 21,465,659          |     |              |                               | Δ                 |                               |  |  |  |
|  |     |              |                               |                   |                               |  |  |  |
|  |     |              |                               | Δ                 |                               |  |  |  |
|  |     |              |                               | ۵                 |                               |  |  |  |
|  |     |              |                               | Δ                 |                               |  |  |  |
|  | :   |              |                               |                   |                               |  |  |  |
|  | :   |              |                               | Δ                 |                               |  |  |  |
|  |     |              |                               | q11.21            |                               |  |  |  |
|  |     | DGV          |                               |                   | •                             |  |  |  |
|  |     |              | BN                            |                   |                               |  |  |  |
|  |     |              | nevi34                        | 127 nsv828938     | maaria nsv688293 nsv588305 ns |  |  |  |
|  |     | Genes        |                               |                   |                               |  |  |  |
|  |     | HHH HI I II  | C                             | THE CHARMENTER OF | 0 ( • 1899-98 ( OII )         |  |  |  |
|  |     |              | (* ** **) # **** (*### H# ### | LONG AND HAND THE |                               |  |  |  |
|  |     | OMIN @ Genes |                               |                   |                               |  |  |  |
|  |     |              |                               |                   |                               |  |  |  |
|  |     |              |                               |                   |                               |  |  |  |
| <  |     | <            |                               |                   |                               |  |  |  |

The Segment Intersection View table has the following columns:

Note: Columns listed with an adjacent 🧾 icon denote the column is user-editable.

| Column                                  | Description   |
|---|---|
| 🗾 Call                                  | User-editable field populated by a user-configurable drop list of Calls.                                      |
| Inheritance                             | User-editable field populated by user-configurable drop-down list of Inheritance.                             |
| Oncomine<br>Reporter                    | Displays the Oncomine Reporter terminology assigned to the segment.   |
| Segment Interpretation                  | Displays Segment Interpretation assigned to this sample.  |
| % of Overlap Item<br>covered by Segment | The percentage of the Overlap Map Item covered by the segment.  |
| Call from<br>Prioritization (Stored)    | The Call term assigned based on Tier or Score Classification at the time the sample was published to ChAS DB. |



| Column                    | Description   |
|---------------------------|---|
| Chromosome                | Chromosome on which the item is located.  |
| CN State                  | Copy Number State.  |
| CytoRegions               | Names of the CytoRegions with which the segment shares coordinates.   |
| DB Coverage               | Number of segments in the database meeting the minimum Percent Coverage Count.  |
| DB Overlap                | Number of segments in the database meeting the minimum Percent Overlap Count.   |
| DGV                       | List of DGV variations that share coordinates with the segment.   |
| Evidence (Stored)         | Provides information on which annotations the segment overlapped at the time the sample was published to ChAS DB.   |
| Genes                     | List of RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.  |
| Interpretation            | User-editable field for free-text interpretation on the segment.  |
| Label                     | Identifier for the item.  |
| Max                       | Zero-based index position of the last base pair in the sequence, plus one. Adding one ensures that<br>the length of any (hypothetical) segment containing a single marker would be one, and ensures that<br>the coordinates match the coordinate system used in BED files.<br>For all segments, the segment start coordinates are always lower by one bp from the coordinate for<br>the starting probe of the segment as reported in the graphs table while the end coordinate matches<br>the coordinate for the ending probe as reported in the graphs table |
| Min                       | Zero-based index position of the first base pair in the sequence.   |
| OMIM Genes                | List of OMIM Genes that share coordinates with the segment.   |
| Overlap Map               | Item(s) in the Overlap Map which overlap the segment.   |
| Overlap Map Items         | The percentage of the segment that is overlapped by the Overlap Map Item.   |
| Phenotype                 | Displays Phenotype annotation assigned to this sample.  |
| Published                 | Displays the date and time of your query.   |
| Publisher                 | Displays Publisher's name.  |
| Sample DB ID              | A xxCHP file ID automatically assigned when the xxCHP file is published to the database.  |
| Sample Type               | Displays the Sample Type assigned to this xxCHP file.   |
| Sample UUID               | Unique identifier for the CHP file.   |
| Segment DB ID             | An ID automatically assigned to each segment when the xxCHP file is published to the database   |
| Segment ID                | The unique identifier for the copy number segment.  |
| Segmental<br>Duplications | List of Segmental Duplications that share coordinates with the segment.   |
| Sex                       | Displays Male or Female.  |
| Size (kpb)                | Size of the item.   |



| Column                 | Description  |
|------------------------|--|
| Tier or Score (Stored) | The assigned Tier or Score value based on the Segment Prioritization method selected at the time the sample was published to ChAS DB. When using Tier based, the column will display the assigned Tier. When using Score based, the column will display the score value based on the annotations the segment overlaps. |
| Туре                   | Type of segment (Gain, Loss, GainMosaic, LossMosaic LOH) or annotation.  |

# Additional segment intersection information

Additional Segment Intersection information becomes available after querying a ChAS DB that has been remapped from a previous genome build that includes additional columns that are populated in the Segment Intersection Table. These remapped segments, are also represented in different graphical patterns, as shown in Figure 430 and detailed in Figure 431.

| Figure 430                           | Additiona            | ıl Segment      | t Interacti                               | on Inform     | nation examp | le   |           |                    |           |                   |             |        |   |
|--------------------------------------|----------------------|-----------------|---|---------------|--------------|------|-----------|--------------------|-----------|-------------------|-------------|--------|---|
| 🤰 Segment Interse                    | ctions               |                 |   |               |              |      |           |                    |           |                   |             | _ 🗆    | x |
| Search View                          |                      | 500/ 41         |   |               |              |      |           |                    |           |                   |             |        |   |
| chrX: 6,727,671 - 6,728,8<br>□ Σ □ Σ | 24 (Gain); Cove<br>; | arage >= 50% AN | ID Overlap >= 5                           | 0%            |              |      |           |                    |           | 1                 | 13 results  | n II : | _ |
| ment Interpretation                  | Inheritance          | DB Overlap      | DB Coverage                               | Genes         |              | N    | Markers I | Markers in remap A | dded m Re | move Original loc | cation      | Segmer | r |
|                                      |                      | 86%             | 57%                                       |               |              |      | 40        | 43                 | 3         | 0 chrX:6645       | 611-6646376 |        | ^ |
|                                      | _                    | 91%             | 57%                                       |               |              |      | 32        | 32                 | 0         | 0 chrX:6645       | 647-6646376 |        | ^ |
| <                                    |                      | 91%             | 57%                                       |               |              |      | 30        | 30                 | 0         | 0 chrX:6645       | 650-6646376 |        |   |
| ^ v                                  |                      |                 |   |               | •••••        |      |           |                    |           |                   |             |        | _ |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               | Á            |      |           |                    |           |                   | 1           |        |   |
| 6727600                              |                      | 6727800         |   | 6728kb        | 6728200      |      | 6728      | 3400               | 6728600   | 672               | 8800        |        | _ |
| 0                                    |                      |                 | Δ   |               |              |      |           |                    |           |                   |             |        | ^ |
|                                      |                      |                 | Δ   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 | 1000000000000<br>100000000000000000000000 |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               | A            |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   | Δ             |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               |              |      |           |                    |           |                   |             |        | ^ |
| U                                    |                      |                 |   | <del>  </del> |              |      |           |                    |           |                   |             |        | v |
|                                      |                      |                 |   |               | p22.31       |      |           |                    |           |                   |             |        |   |
| DGV                                  |                      |                 |   |               |              |      |           |                    |           |                   |             |        | ^ |
|                                      |                      |                 |   |               | esv2752      | 2331 |           |                    |           |                   |             |        |   |
|                                      |                      |                 |   |               | eev/2422     | 2345 |           |                    |           |                   |             |        | ^ |
|                                      |                      |                 |   |               | 6502422      | .945 |           |                    |           |                   |             |        | ~ |
| <                                    |                      |                 |   |               |              |      |           |                    |           |                   |             | <      | > |



| Figure 431 Additional Segment Interaction Legend   |
|--|
| = Gain segment added to the database from a xxCHP file analyzed using the current genome build (hg38).   |
| = Gain segment remapped from hg19, at least one marker added<br>and/or removed.                          |
| = Gain segment remapped from hg19, no markers were added or removed.                                     |
| = Loss segment added to the database from a xxCHP file analyzed using the current genome build (hg38).   |
| = Loss segment remapped from hg19, at least one marker added and/or removed.                             |
| = Loss segment remapped from hg19, no markers were added or removed.                                     |
| = LOH segment added to the database from a xxCHP file analyzed<br>using the current genome build (hg38). |
| = LOH segment remapped from hg19, at least one marker added and/or removed.                              |
| = LOH segment remapped from hg19, no markers were added or removed.                                      |



The additional Segment Intersection table columns and their definitions, are as follows:

| Column                      | Description  |
|-----------------------------|--|
| Original location           | Chr:start-stop genome location of the original genome build for the segment. |
| Markers in original segment | Total number of markers in the original segment.                             |
| Added markers               | Number of markers to the segment added by remapping.                         |
| Removed markers             | Number of original markers removed from the segment from remapping.          |
| Segment length difference   | Size difference in the segment (Original - Remapped).                        |
| Original Genome Build       | Genome Build from the original analysis prior to remapping.                  |
| Markers in remapped segment | Total number of markers in the remapped segment.                             |

## Downloading segments from a sample file in ChAS DB

From the Segment Intersection window, all the segments for a selected sample can be downloaded and viewed in the Segments Table and Detail View. Only the segment data and annotations from the sample are displayed.

Note: Files downloaded from ChAS DB can not be opened in the MSV.

#### Downloading and viewing Segments from a sample(s) stored in ChAS DB

- 1. From the Segment Intersections window, right-click on the sample(s) in either the table or the graphical view.
- 2. Select Download file(s) from ChAS DB. (Figure 432)



| Figure      | 432 Segment Inte                  | rsections -   | Downloa      | ad file(s) fr | om ChA   | AS DB                   |              |         |
|-------------|-----------------------------------|---------------|--------------|---------------|----------|-------------------------|--------------|---------|
| 🝃 Segr      | ment Intersections                |               |              |               |          |                         |              |         |
| Search      | View                              |               |              |               |          |                         |              |         |
| chrX: 86,33 | 37,773 - 86,693,619 (Gai<br>👷 🗎 Σ | n, Loss, Gain | (XON Regio   | on), Loss (X  | ON Regio | n)); Coverage >= 50%    | AND Over     | ap >= { |
| Label       | File                              |               | Туре         | CytoReg       | 🗾 Oncor  | mine Knowledgebase      | . Original I | Overla  |
| seg264      | Constutional_Blood_01             | _CytoScan     | ▲ Gain       |               |          |                         |              |         |
| seg197      | 20121228_102529_005               | _242. Σ Sur   | n, mean an   | d median      |          | ]                       |              |         |
|             |                                   |               | wnload file( | s) from ChA   | SDB .    |                         |              |         |
|             |                                   |               | ata Casmaa   |               |          |                         |              |         |
|             |                                   | T Del         | ete Segme    | nt(s) from C  |          | ownload a copy of file( | s) from ChA  | S DB    |
|             |                                   |               |              |               |          |                         |              |         |
| $\frown$    |                                   |               |              |               |          |                         |              |         |
| ^ ¥         |                                   |               |              |               |          |                         | 0            |         |
|             | 0.00                              |               |              |               |          |                         |              |         |
| 9           | × 🕅                               |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |
| · · · · ·   | 86300kb                           | 86350kb       | )            |               | 6400kb   | 86450                   | Okb          |         |
| 0           |                                   |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |
|             |                                   |               |              |               |          |                         |              |         |

**Note:** As shown in Figure 433, sample files downloaded from ChAS DB are listed in the Files Tree with a database symbol. Segments from samples files downloaded from ChAS DB are listed in the Segments Table with a database symbol in the File column.





**Note:** Downloaded segments can be deleted when in Edit Mode, but no other segment modifications are enabled as the underlying data is unavailable.

## Updating downloaded segment annotations

Segment annotations in the ChAS DB can be updated using the following methods:

- Right-clicking on the segment and selecting View/Edit Annotation Properties → Curation Tab.
- Clicking on a column/field pad and pencil licon to edit the segment annotation(s).
- Right-clicking on the Filename in the Files tree, select View/Edit Properties → Sample Properties.

### Filtering DB count columns

Filtered DB count columns are available in the Segment Table and reflect the number of segments in the database matching the filtered criteria.

**Note:** The DB count columns reflect the number of segments in the database meeting the Minimum Percent Overlap/Coverage criteria only. The Filtered DB count columns allow you to display segments that not only meet the Minimum Percent Overlap/Coverage criteria, but also additional filters such as Call or Gender.

1. Click Edit User Configuration, then click on the Filtered DB Query tab. (Figure 434)

| Figure 434 User Configuration - Filtered DB Query window/tab   |   |
|--|---|
| User Configuration   | × |
| Segment Data QC Thresholds Color Rules Misc Vocabularies DB Query Filtered DB Query Exports  |   |
| Copy Number Query Parameters   |   |
| Minimum % CN Coverage 50   |   |
| Minimum % CN Overlap 50  |   |
| ✓ Match only same gain/loss type □ Include LOH ✓ Include XON Regions   |   |
| LOH Query Parameters   |   |
| Minimum % LOH Coverage 50  |   |
| Minimum % LOH Overlap 50   |   |
| XON Regions Query Parameters   |   |
| Minimum % Coverage 50  |   |
| Minimum % Overlap 50   |   |
| Match only same gain/loss type 🔲 Include Copy Number Segments  |   |
| Minimum % Coverage: The minimum percentage that the current segment is covered by other similar segments from the database.              |   |
| Minimum % Overlap: The minimum percentage that the current segment overlaps similar segments in the database.                            |   |
| Parameters:         Sample Type: has no value         OR = "blood (constitutional)"         OR = "blood marrow"         OR = "cell line" |   |
| Restore Defaults     Change Filter Parameters       OK     Cancel  |   |

2. Refer to "Setting up a ChAS DB query" on page 385 for how to set the Percent Minimum Overlap/Coverage Thresholds.
- 3. Return to the Filtered DB Query window tab.
- 4. The Filtered DB Query window tab displays the current filter setting. To change it, click **Change Filter Parameters**.

A Set Filter Parameters window appears. (Figure 435)

| Figure 435 Set Filter Parameters window |                                      |                      |  |  |  |  |
|---|--------------------------------------|----------------------|--|--|--|--|
| 🞇 Set Filter Parameters                 | 3                                    | ×                    |  |  |  |  |
| Sexes Female                            | Sexes Female Male Unknown            |                      |  |  |  |  |
| Sample Types  Not Filte                 | ered Array Types  Not Filtered       | Calls O Not Filtered |  |  |  |  |
| No Value                                | <ul> <li>cytoscanhd_array</li> </ul> | No Value             |  |  |  |  |
| blood (constitutional)                  | oncoscan                             | benign               |  |  |  |  |
| bone marrow                             |                                      | likely benign        |  |  |  |  |
| breast tumor                            | ×                                    | pathogenic 🗸         |  |  |  |  |
|   | OK Cancel                            |                      |  |  |  |  |

- 5. Use the windows check boxes, radio buttons, and selections to change your filter parameters.
- 6. Click **OK**.

Your new query parameters are saved and displayed at the bottom of the Filtered DB Query window tab, as shown in Figure 436.

| Figure 436 Displayed Filter Parameters example  |          |
|---|----------|
| Parameters:         Sample Type: = "blood (constitutional)"         Call: = "pathogenic"         Restore Defaults         Change Filter Parameters         OK | <b>^</b> |

To reset the Filtered DB Query Parameters back to default settings, click **Restore Defaults**. For more information see, "Filtering DB count columns" on page 396.

Figure 437 shows the **DB Count Both** and **Filtered DB Count Both** columns. It illustrates that DB Count Both queries the database for all segments matching the Minimum Percent Overlap/Coverage and gain/loss/LOH parameters. The Filter DB column reflects the additional Filter Criteria.

| Figure 437 Displayed Filter Parameters example           |                           |               |                        |            |    |  |  |
|--|---------------------------|---------------|------------------------|------------|----|--|--|
|  |                           |               |                        |            |    |  |  |
| Microarray Nomenclature (ISCN 2016)                      | Full Location             | DB Count Both | Filtered DB Count Both | Size (kbp) | Ma |  |  |
| arr[GRCh37] 14q32.33(105864870_107283202)x2-3            | chr14:105864870-107283202 | 163           | 27                     | 1,418      |    |  |  |
| arr[GRCh37] Xq28 or Yq12(154941868_155233731 or 59044874 | chrX:154941868-155233731  | 7             | 3                      | 292        |    |  |  |
| arr[GRCh37] 8p11.22q24.3(38556627_146295771)x2-3         | chr8:38556627-146295771   | 7             | 0                      | 107,739    |    |  |  |
| arr[GRCh37] 5p15.33p13.2(113576_38205477)x2-3            | chr5:113576-38205477      | 5             | 0                      | 38,092     |    |  |  |
| arr[GRCh37] Xp22.33(168546_2703986)x3-4                  | chrX:168546-2703986       | 3             | 0                      | 2,535      |    |  |  |
| arr[GRCh37] 21q21.1q22.12(20605960_37047544)x2-3         | chr21:20605960-37047544   | 3             | 0                      | 16,442     |    |  |  |
| arr[GRCh37] 22q11.1q13.33(16888899_51197838)x2-3         | chr22:16888899-51197838   | 3             | 0                      | 34,309     |    |  |  |
| arr[GRCh37] 17q21.32q22(45010182_57572273)x2-3           | chr17:45010182-57572273   | 3             | 0                      | 12,562     |    |  |  |
| arr[GRCh37] 17q24.1q25.1(63940809_72905641)x2-3          | chr17:63940809-72905641   | 3             | 0                      | 8,965      |    |  |  |





## Querying overlapped segments

- 1. Right-click on a Segment of interest within the Detail View. (Figure 438)
- 2. Click Query ChAS DB.

| Figure          | Figure 438 Query ChAS DB from Detail View              |   |         |  |  |  |  |
|-----------------|--|---|---------|--|--|--|--|
|                 |  | ∅ ⊲ ⊲ [] 📓  | 20<br>X |  |  |  |  |
| 0.5<br>1<br>1.5 | an an training and a second                            | ender die der Angeleichen die eine Bereichen die gester die       | ^       |  |  |  |  |
| 09-14           | 20_B2_Phase4CustomerPanel_CytoScan_PS_20110228(2)2.cyh | yhd.cychp: Copy Number State (segments)   |         |  |  |  |  |
|                 |  | One Item Selected   |         |  |  |  |  |
| 09-14           | 20_B2_Phase4CustomerPanel_CytoScan_PS_20110228(2)2.cyh | yh         Zoom to selection         Ctrl+Space           Im         Selection Details         Ctrl+D |         |  |  |  |  |
|                 |  |   |         |  |  |  |  |
| 09-14           | 20_B2_Phase4CustomerPanel_CytoScan_PS_20110228(2)2.cyh | Query ChAS DB<br>yhd.cychp: Weighted Log2 Ratio   |         |  |  |  |  |
| - 1.5           |  | Snow items in ChAS DB which intersect this segment.   |         |  |  |  |  |

The results returned when querying a segment in the detail view will contain segments that meet the DB Coverage filter or the DB Overlap filter set up previously. (Figure 423 on page 385)

## 18

## Changing or refining the DB query criteria

| = Sea    | ment Intersections  |          |          |          |          | - 0     |    |
|----------|---|----------|----------|----------|----------|---------|----|
| Search   |   |          |          |          |          |         |    |
| Soarch a | anin 1439 (LOH, Loss, Gain)   |          |          |          |          |         |    |
| Searcha  | gain<br>Σ.  |          |          |          | 84       | results | 1  |
|          |   | _        |          | _        |          | TCOULD  |    |
| abel     | File Name   | Туре     | CN State | Chromoso | Min      | Ma      | X  |
| eg218    | 20120613D_2886_P9242_CytoScanHD_Ar  | V Loss   | 1.00     | X        | 168      | 546     | _  |
| eg260    | 20120904C_01467_P7846_CytoScanHD  | V Loss   | 1.00     | X        | 168      | ,546    | _  |
| eg160    | 20111006_IR13_IR-13_CytoScanHD_Array  | V Loss   | 1.00     | X        | 168      | ,546    |    |
| eg307    | 5d720742-d4ba-4de3-975d-d8469d29c61   | V Loss   | 1.00     | X        | 168      | ,546    | _  |
| eg93     | PN12-2544.cyhd.cychp  | V Loss   | 1.00     | X        | 168      | ,546    |    |
| eg169    | 20130525C_06031_P005243_CytoScanH   | ▲ Gain   | 4.00     | X        | 168      | 3,546   |    |
| eg201    | AFFY-65.cyhd.ND.cychp   | ▲ Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg330    | 20121213A_1701_P0006985_CytoScanH   | ▲ Gain   | 4.00     | X        | 168      | \$,546  |    |
| eg299    | AFFY-116.cyhd.ND.cychp  | ▲ Gain   | 3.00     | X        | 168      | \$,546  |    |
| eg140    | 20120805D_10_P322_CytoScanHD_Array  | ▲ Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg366    | AFFY-79.cyhd.ND.cychp   | ▲ Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg332    | 20120830C_09562_P00763_CytoScanHD   | ▲ Gain   | 3.00     | X        | 168      | \$,546  |    |
| eg280    | AFFY-96.cyhd.ND.cychp   | ▲ Gain   | 3.00     | X        | 168      | \$,546  |    |
| eg88     | CyHD_100112G_SS18-19_401437MJ.cyh   | ▲ Gain   | 3.00     | X        | 168      | \$,546  |    |
| eg119    | Nijm42_A6_Nijmegen_Alpha_Cytoscan_M   | V Loss   | 1.00     | Х        | 168      | 3,546   |    |
| eg153    | 20111109C_IR145_IR145_CytoScanHD_A  | V Loss   | 1.00     | X        | 168      | 3,546   |    |
| eg299    | 20140201B_008295_P000455_CytoScanH  | V Loss   | 1.00     | X        | 168      | 3,546   |    |
| eg144    | 20130701B_05125_P0004464_CytoScanH  | 🔺 Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg862    | 20121017C_9640_P01853_CytoScanHD  | ▲ Gain   | 3.00     | Х        | 168      | 3,546   |    |
| eg344    | 20130523C_04836_P6446_CytoScanHD  | ▲ Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg225    | 20121223B_04823_P000002023_CytoSca  | ▲ Gain   | 3.00     | X        | 168      | 3,546   |    |
| eg116    | PN12-1747.cyhd.cychp  | ▲ Gain   | 3.00     | Х        | 168      | 3,546   |    |
|          |   |          |          |          |          |         | >  |
|          |   |          |          |          |          |         | -  |
|          | 0   |          |          |          |          | • P     | 9  |
|          | -   | eev27600 |          |          |          |         |    |
|          |   | 0000     |          |          |          |         |    |
| 50 CON   | 140kb 160kb 18  | Okb 📊    | 200kb    |          | 220kb    | 897 M28 | 24 |
|          | · · _ ·   |          |          |          |          |         |    |
| R        | n in the second s | - i -    |          |          |          |         |    |
|          |   |          |          |          |          |         |    |
|          |   | - X      |          |          |          |         |    |
|          |   | Å        |          |          |          |         |    |
|          |   |          |          |          |          |         |    |
|          |   | - X      | <u>.</u> |          |          |         |    |
|          |   |          | ÷        |          | <u> </u> |         | _  |
|          |   |          |          |          |          |         |    |
|          |   |          |          |          |          |         |    |
|          |   | -        | <u> </u> |          |          |         |    |
|          |   |          | A        |          |          |         |    |
|          |   |          |          |          |          |         |    |
| U        |   |          |          |          |          |         |    |

1. From the upper left corner, click **Search**  $\rightarrow$  **Search Again...** (Figure 439)

The Search parameters window appears. (Figure 440)



| Figure 440 Search Parameter window  |   |              |
|---|---|--------------|
| Detail View Region () chr2: 114,392,537 - 114,393,438         Custom Region () chr2: 114,392,641 - 114,393,334         Chromosome () chr2         Minimum % Coverage 50 | Sample Types<br><i>No Value</i><br>blood (constitutional)<br>bone marrow<br>cell line   | Not Filtered |
| AND OR Minimum % Overlap 50 Categories Gain Loss LOH Detected Undetected Gain (XON Region) Loss (XON Region) XON Region Level 2 Level 3 Level 4                         | Array Types<br>cytoscan_xon_array<br>cytoscanhd_array   | Not Filtered |
| Published   |   |              |
| Segment Interpretation         Phenotype         Publisher         Sample ID         File         Sexes       Female         Male       Unknown         Not Reported    | Calls<br>No Value<br>benign<br>likely benign<br>likely pathogenic<br>pathogenic<br>polymorphic (adgv)<br>unknown significance | Not Filterea |
| Reset OK Cancel   | vous  |              |

2. Use the provided radio buttons, check boxes, and text fields to customize your search, then click **OK**.

**Note:** Altering these parameters only affects the current segment query. The following fields, **Sample Types**, **Array Types** and **Calls** are populated based on what has been published by the user into the ChAS database If the ChAS Browser is unable to contact the database, these fields are populated based on the library file and vocabularies entries.



### Publishing data to the database

Once sample and its segments have been analyzed, curated and annotated, it can be added to a database by a process called publishing.

**IMPORTANT!** You MUST have Manager or Admin Role permissions to publish data to the database. For details, see "Administration" on page 451.

#### Publishing data or multiple data to the database

#### Method 1

1. In the File tree, right-click on a file name, then click **Publish File(s) to Database...** (Figure 441)



#### Method 2

1. Click to highlight the sample name(s), then click the tool bar's Publish to Database icon .

| Figure 442 Publish? window                    |  |  |  |  |  |
|---|--|--|--|--|--|
| 🐐 Publish? X                                  |  |  |  |  |  |
| Gain is enabled.                              |  |  |  |  |  |
| Loss is enabled.                              |  |  |  |  |  |
| LOH is not enabled and will not be published. |  |  |  |  |  |
| These types cannot be published.              |  |  |  |  |  |
| GainMosaic is not publishable.                |  |  |  |  |  |
| LossMosaic is not publishable.                |  |  |  |  |  |
| Publish?                                      |  |  |  |  |  |

A summary of uploaded segments/Publish? appears. (Figure 442)

2. Acknowledge the message, then click **OK**.



**Note:** Only segments in the copy number, XON region and LOH segment tracks can be uploaded. Segments uploaded are reflective of the current filters and settings applied.

**Note:** Only segments listed as enabled and filtered will be published. If a segment category is listed as not enabled, click Cancel and check the check box in the Data Types File tree and then right click on the sample filename(s) to start the publishing again.

Note: Publishing time is dependent on the number of segments in the sample.

**Note:** Segments in the Mosaic track are not uploaded to the database. Mosaic segment must be promoted to the copy number state track in order to be published to the database. See "Promoting mosaic segments" on page 239.

**Note:** If a xxCHP file has been previously Published to the database, you will receive a warning indicating this sample already exists in the database. You can choose to overwrite the existing information or cancel to keep the existing information.

**Note:** The Segment Table Columns DB Count and Filtered DB Count are automatically updated.

**Note:** If ChAS is set to manual mode, the histogram need to be manually updated to include recently published samples. To update the histograms, click **ChAS DB**  $\rightarrow$  **Refresh ChAS DB data**.

## Publishing to database

#### Important rules and restrictions

- A sample cannot be published if it is in Edit Mode. See "Using edit mode" on page 220.
- Only samples that are highlighted (not checked) are published to the database.
- Samples published using hg18 cannot be published to the database.
- Multiple filenames can be highlighted and published at the same time.
- Segments from xxCHP files analyzed using hg19 analysis files cannot be loaded into a ChAS Database genome version hg38. The opposite is also true.
- Mosaic Segments can not be published.
  - To promote mosaicism in the database, the Mosaic Segment should be assigned a copy number value and promoted to Copy Number segments using the edit function. For more details, see "Promoting mosaic segments" on page 239.
- You must be logged in as an administrator or manager (with manager role permissions) to publish data.
- Previously published samples can be edited and published again, however publishing a second time overwrites the original database entry.



## Manual or automatic connection mode

By default, ChAS starts in automatic connection mode.

While in automatic connection mode, the DB count columns and histograms are refreshed/updated whenever a file is published.

If you do not want to wait for the data to be refreshed/updated each time a file is published, choose manual connection mode. In manual mode, the DB count columns and histograms are only be updated when you click **ChAS DB**  $\rightarrow$  **Refresh ChAS DB data** or click the [] icon.

There are two ways to switch from automatic to manual connection mode.

At start up, select a user (as you normally would), click on the **Manual connection** check box, then click **OK**. (Figure 443)

| Figure 443 Select User window |   |  |  |  |
|-------------------------------|---|--|--|--|
| X Select User                 | × |  |  |  |
| Pete Create New               | N |  |  |  |
| Manual connection             | _ |  |  |  |
| OK Cancel                     |   |  |  |  |

During a ChAS session, go to the upper bar of icons and click on etc. for automatic connection or click on etc. for manual connection.

Or

■ Click ChAS DB → Auto-Connect, then click on the check box to toggle between connection modes, as shown in Figure 444.

| Figure 444 ChAS DB drop-down menu |                               |  |  |  |  |
|-----------------------------------|-------------------------------|--|--|--|--|
| ChAS DB                           | Preferences Help              |  |  |  |  |
| <ul><li>✓</li></ul>               | Auto-Connect                  |  |  |  |  |
|                                   | Show/Change Login Credentials |  |  |  |  |
| Ū                                 | Refresh ChAS DB Data          |  |  |  |  |
|                                   | Add Histogram                 |  |  |  |  |



### Querying samples in the ChAS database

 Click ChAS DB → Query Samples or click the Query Samples tab, then click the Query Samples button.

The Query Samples window appears. (Figure 445)

| Figure 445 Que   | ery Samples window  |  |  |  |  |
|--|---|--|--|--|--|
| Query Samples  |   | ×  |  |  |  |
| Detail View Region     Image: Constraint of the constraint | chrX: 620,972 - 650,195<br>chrX: 620,972 - 650,195<br>chrX 🗸  | Sample Types  Not Filtered No Value blood blood (constitutional) cell-line |  |  |  |
| Categories 🗹   | Gain     Image: Second s | unknown  |  |  |  |
| XON Region Levels  | Gain (XON Region)       Loss (XON Region)         Level 1       Level 2       Level 3       Level 4   | Array Types   Not Filtered cytoscan_xon_array cytoscanhd array             |  |  |  |
| Published  | -,,   |  |  |  |  |
| Sample Interpretation<br>Segment Interpretation  |   |  |  |  |  |
| Phenotype<br>Publisher   |   | Calls   Not Filtered No Value  |  |  |  |
| Sample ID  |   |  |  |  |  |
| File   |   |  |  |  |  |
| Sexes Female Male Unknown Not Reported   |   |  |  |  |  |
| Reset  | OK Cancel   |  |  |  |  |

2. Use the provided radio buttons, check boxes, and text fields to customize your query, then click **OK**.

The Query Samples table populates with your filtered search results. (Figure 446)

The contents for columns in which the headers have an Edit Icon can be modified. Changes will apply directly to the ChAS database.

Columns with a chip/pencil 🗾 icon represent sample properties that can be edited.

- Editing column contents
- 1. Double click on a cell to be edited.

An Edit Value window appears. (Figure 446)

- 2. Enter or change the value in the window.
- 3. Click **OK**.

Your changes are now saved to the ChAS Database.

#### Editing multiple cells with the same value

- 1. Highlight the cells you want to edit.
- 2. Right-click, then select Edit Property Values.

An Edit Value window appears. (Figure 446)

- 3. Enter a new value or edit the existing displayed value.
- 4. Click **OK**.

Your changes (for the multiple cells you selected) are now saved to the ChAS Database.

| Figure 446 Qu           | ery Samples wir         | ndow tab table    | - Filtered sea        | rch results                                  |              |                    |                       |               |     |
|-------------------------|-------------------------|-------------------|-----------------------|--|--------------|--------------------|-----------------------|---------------|-----|
| 👭 Karyoview 🎙 🛛 🙀 Segm  | nents 🎙 🛄 CytoRegions 🎙 | 🧲 Overlap Map 🎙 🛛 | 🖾 Graphs 🎙 🛛 🚹 Variar | ts <sup>▼</sup> I Query Samples <sup>▼</sup> | 💷 Query Seg  | ments <b>N</b>     |                       |               | (   |
| 🔠 🔜 😰 🗎 Σ               | Query Samples           |                   |                       |  |              |                    |                       | 1,218 results | s 🔟 |
| ▼ File                  | Sample Type             | Sample ID         | Sample Interpret      | C Phenotype                                  | Published    | Sex                | Publisher             |               |     |
| PN13-1959.cyhd.cychp    | Blood (constitutional)  | ADMX_PN13-1959    | Normal                | Normal-ADGV                                  | May 1, 2015  | female             |                       |               | 0   |
| PN13-1868.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1868     | Normal                | Normal-ADGV                                  | Apr 30, 2015 | female             |                       |               | -   |
| PN13-1773.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1773     | Normal                | Normal-ADGV                                  | Apr 30, 2015 | female             |                       |               |     |
| PN13-1649.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1649     | Normal                | Normal-ADGV                                  | Apr 30, 2015 | female             |                       |               |     |
| PN13-1639.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1639     | Normal                | Normal-ADGV                                  | May 1, 2015  | female             |                       |               |     |
| PN13-1621.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1621     | Normal                | Normal-ADGV                                  | Apr 30, 201  | <b>E</b> -14.) (-1 |                       | ~             |     |
| PN13-1620.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1620     | Normal                | Normal-ADGV                                  | Apr 30, 201  | Edit Value         |                       | ^             |     |
| PN13-1570.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1570     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-1555.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1555     | Normal                | Normal-ADGV                                  | Apr 30, 201  | Change value       | of "Phenotype" on 1 a | nnotations?   |     |
| PN13-1492.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1492     | Normal                | Normal-ADGV                                  | Apr 30, 201  | Normal-ADC         | /                     |               |     |
| PN13-1473.cyhd.cychp    | Blood (constitutional)  | ADMX PN13-1473    | Normal                | Normal-ADGV                                  | Apr 30, 201  | Norman ADON        |                       |               |     |
| PN13-1457.cvhd.cvchp    | Blood (constitutional)  | CEU PN13-1457     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1423.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1423     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-1355.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1355     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1333.cvhd.cvchp    | Blood (constitutional)  | CEU PN13-1333     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1320.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1320     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1290 01.cyhd.cychp | Blood (constitutional)  | CEU PN13-1290 01  | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1288.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1288     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-1258.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1258     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1191.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1191     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-1175.cyhd.cychp    | Blood (constitutional)  | CEU_PN13-1175     | Normal                | Normal-ADGV                                  | May 1, 2015  |                    |                       |               |     |
| PN13-1163.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1163     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1152.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1152     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1119.cvhd.cvchp    | Blood (constitutional)  | CEU PN13-1119     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1081.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1081     | Normal                | Normal-ADGV                                  | Apr 30, 201  |                    |                       |               |     |
| PN13-1061.cyhd.cychp    | Blood (constitutional)  | CEU PN13-1061     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-1049.cvhd.cvchp    | Blood (constitutional)  | CEU PN13-1049     | Normal                | Normal-ADGV                                  | May 1, 2015  |                    |                       | - I           |     |
| PN13-0985.cyhd.cychp    | Blood (constitutional)  | CEU PN13-0985     | Normal                | Normal-ADGV                                  | May 1, 201   |                    | OK Cancel             |               |     |
| PN13-0928.cyhd.cychp    | Blood (constitutional)  | CEU PN13-0928     | Normal                | Normal-ADGV                                  | May 1, 201   |                    |                       |               |     |
| PN13-0891.cyhd.cychp    | Blood (constitutional)  | ADMX_PN13-0891    | Normal                | Normal-ADGV                                  | Apr 30, 2015 | Temale             |                       |               |     |
| PN13-0790.cyhd.cychp    | Blood (constitutional)  | CEU PN13-0790     | Normal                | Normal-ADGV                                  | Apr 30, 2015 | female             |                       |               |     |
| PN13-0766 cybd cychn    | Blood (constitutional)  | CELL PN13-0766    | Normal                | Normal-ADGV                                  | May 1 2015   | female             |                       |               |     |

For instructions on how to use the table's features, see "Common table operations" on page 323.

**Note:** The following fields, **Sample Types**, **Array Types** and **Calls** are populated based on what has been published by the user into the ChAS database. If the ChAS Browser is unable to contact the database, these fields are populated based on the library file and vocabularies entries. Queries are not automatically refreshed when publishing to or deleting from the ChAS DB. Queries must be re-run to reflect changes to the database made after the initial query.



### Removing a sample from the query window

You can remove a sample from the query window, however this action does NOT remove the sample from the database.

- 1. Highlight the sample(s) you would like to remove from the results display.
- 2. Right-click, then click Remove Query Results. (Figure 447)

A warning message appears asking you to confirm the removal of the file(s) from the results window.

3. Click **OK** to remove the files from the results. Click **Cancel** to return to the main screen.

| Figure 447 Remove Query Results                    |                                |  |  |  |  |
|--|--------------------------------|--|--|--|--|
| 🔡 🔓 🞇 🐸 🗵 Query Samples                            |                                |  |  |  |  |
| File   | Sample Type Publis             |  |  |  |  |
| 20130315A_3192_P0007910_Cy                         | Bone Marrow                    |  |  |  |  |
| 20130217B_0914_P03663_Cyto                         | Bone Marrow                    |  |  |  |  |
| 20140118B_1891_P3594_CytoS                         | Bone Marrow                    |  |  |  |  |
| 20121024C_09200_P009086_C                          | Bone Marrow                    |  |  |  |  |
| 20130930A_05537_P000003806                         | Blood (constitutional)         |  |  |  |  |
| CyHD_110612G_SS21-24_4001                          | Blood (constitutional)         |  |  |  |  |
| 20121219D_5950_P02822_Cyto                         | Bone Marrow                    |  |  |  |  |
| 20130714B_2951_P00530_Cyto                         | Bone Marrow                    |  |  |  |  |
| CyHD_030712T_SS253_400688                          | Blood (constitutional)         |  |  |  |  |
| 20121220A_3922_P109_CytoSc                         | Blood (constitutional)         |  |  |  |  |
| 20120524A_07996_P2992_Cyto                         | Bone Marrow                    |  |  |  |  |
| CyHD_050712T_SS371_401736                          | Blood (constitutional)         |  |  |  |  |
| 20130325C_02015_P56<br>20111115C_IR149_IR14 ∑ Sum, | mean and median                |  |  |  |  |
| • • Remo   | ove File(s) from Query Results |  |  |  |  |
| Delet  | e File(s)                      |  |  |  |  |

## Deleting sample(s) from the ChAS database

**WARNING!** You must have manager or admin permissions for the ChAS database to delete samples.

Sample(s) deleted from the ChAS Database are permanently deleted and cannot be retrieved. There is no undo delete feature.

## Deleting a single sample

To remove a single sample in a database, use the Query Samples window to locate the file to be deleted.

1. In the Query Samples window (Figure 445 on page 404), enter the file's **Filename** or **Sample ID**, then click **OK**.

The sample appears in the table. (Figure 448)



| Figure 448 Query Samples window tab tab              | le - Deleti    | ng a sam   | ple      |            |               |              |            |
|--|----------------|------------|----------|------------|---------------|--------------|------------|
| 👫 Karyoview ষ 🕅 🙀 Segments ষ 🛄 CytoRegions 🔊 🖉 🛹 Ove | rlap Map 🔊     | 🖾 Graphs 🔻 | 👍 Som Mu | it 🎙 🔝 Que | ery Samples 🔻 | Query        | Segments 🔊 |
| 🖩 📴 😫 🗎 🗴 Query Samples                              |                |            |          |            |               | [            | 1 results  |
| ▲ Name/ID ▼ Sample ID                                | Sample Ty      | Array Type | Gender   | Phenotype  | Publisher     | Publish Date | UUID Ir    |
| 01fafd20-7 ASI_01fafd20-7339-4db7-921f-cf0e8a8de1ad  | Plood (con     | CutoScon   | male     | Normal-AD  |               | May 5, 2015  | 000003d8 M |
| 2  | Sum, mean ar   | nd median  |          |            |               |              |            |
|  | Delete File(s) |            |          |            |               |              |            |

2. Right-click on the sample, then click **Delete**.

When a sample is deleted from a database, the reason for the deletion is required. Enter the reason in the **Enter delete reason** window. (Figure 449)

**Note:** This reasoning you enter is captured in the ChAS DB and it can be exported. For details, see "Exporting a deletion log" on page 432.

| Figure 449 Enter deletion reason window  |
|--|
| 🕅 Enter delete reason X  |
| Please provide a reason for deleting this data.<br>This information will be tracked for all highlighted samples.<br>To capture separate information for each file, please delete individually. |
| OK Cancel  |

3. Click **OK** to delete. Click **Cancel** to return to the query window.

The sample is removed.

## Deleting multiple samples

 Multiple samples can be highlighted to delete. They can be selected using the following keyboard and mouse combinations: Ctrl click, Shift click or Ctrl a.. (Figure 450)

| Figure 4   | 50 Query Samples window tab tab          | ole - Deletir | ng multipl   | e samp | oles      |             |           |   |
|------------|--|---------------|--------------|--------|-----------|-------------|-----------|---|
|            |  |               |              |        |           |             |           |   |
| 0138c046   | ASI_0138c046-24df-4f64-b45c-a0ced1f0a87e | Blood (con    | CytoScan     | female | Normal-AD | May 5, 2015 | 0000565b  | ^ |
| 01fafd20-7 | ASI_01fafd20-7339-4db7-921f-cf0e8a8de1ad | Blood (con    | CytoScan     | male   | Normal-AD | May 5, 2015 | 000003d8  |   |
| 04a6b213   | ASI_04a6b213-d6b2-408e-892d-19eee644290d | Blood (con    | CytoScan     | female | Normal-AD | May 5, 2015 | 00000ded  |   |
| 07a0d066   | ASI_07a0d066-d0cd-4b71-ac04-28d4c63286ae | Blood (con    | CytoScan     | female | Normal-AD | May 5, 2015 | 00000cd   |   |
| 093417b2   | ASI_093417b2-1eef-4713-8cfb-ba438f46d57e | Blood (con    | CytoScan     | female | Normal-AD | May 5, 2015 | 00005123  |   |
| 0a172519   | ASI_0a172519-da3c-4bd4-b207-38a8ed4c2a96 | Blood (con    | CytoScan     | male   | Normal-AD | May 5, 2015 | 00000f02  |   |
| 0e644cee   | ASI_0e644cee-80e9-405a-b16a-5c2fcd0f49c3 | Blood (con    | CytoScan     | male   | Normal-AD | May 5, 2015 | 000000b6  |   |
| 123d724e   | ASI_123d724e-5e53-4e79-93ab-c115961efbb5 | Blood (con    | CytoScan     | female | Normal-AD | May 5, 2015 | 0000681b  |   |
| 142410f2   | ASI_142410f2-34c2-458d-bdd6-a966e8e83215 | Dlood (oon    | CutoCoop     | male   | Normal-AD | May 5, 2015 | 00004358  |   |
| 15a629e7   | ASI_15a629e7-e1d5-4546-aaf5-3e65f3a3fb90 | Σ Sum, mear   | n and median | hale   | Normal-AD | May 5, 2015 | 000006eb  |   |
| 19ff384c-8 | ASI_19ff384c-8ec5-47c5-a74a-90b87f988d61 | Delete File   | (S)          | hale   | Normal-AD | May 5, 2015 | 00003273  |   |
| 1aabh3h8-  | ASI 1aabb3b8-8a57-4d01-9519-20b196860500 | Blood (con    | CytoScan     | male   | Normal-AD | May 5, 2015 | 0000193c- |   |

2. Right-click on the highlighted area, then click **Delete**.

The samples are removed.



### Querying segments to the ChAS database

1. Click ChAS DB  $\rightarrow$  Query Segments or click the Query Segments tab, then click the Query Segments button.

The Query Segments window appears. (Figure 451)

| Figure 451         Query Segments window   |  |
|--|--|
| Query Segments   | ×  |
| Detail View Region ()       chrX: 620,972 - 650,195         Custom Region ()       chrX: 620,972 - 650,195         Chromosome ()       chrX ()         All Regions ()       ()         Minimum % Coverage 50 ()       () | Sample Types  Not Filtered No Value blood blood (constitutional) cell-line unknown |
| Image: AND Image: OR         Image: OR           Minimum % Overlap         50  | Array Types   Not Filtered   |
| Categories <table-cell> Gain <table-cell> Loss <table-cell> LOH<br/>Solution Detected Undetected<br/>Gain (XON Region) Loss (XON Region)</table-cell></table-cell></table-cell>  | cytoscan_xon_array<br>cytoscanhd_array   |
| XON Region Levels Level 1 Level 2 Level 3 Level 4 (Gain and Loss)  |  |
| Published  |  |
| Sample Interpretation Segment Interpretation   | Calls      Not Filtered No Value   |
| Phenotype  |  |
| Sample ID  |  |
| File   |  |
| Sexes Female Male Unknown Not Reported   |  |
| Reset OK Cancel  |  |

2. Use the provided radio buttons, check boxes, and text fields to customize your query, then click **OK**.

The Query Segments table populates with your filtered search results. (Figure 452)

The contents for columns in which the headers have an Edit Icon can be modified. Changes will apply directly to the ChAS database. Editable columns are: Call, Segment Interpretation, Inheritance, Oncomine Reporter.

1. Double click on a cell to be edited.

An Edit Value window appears. (Figure 452)

- 2. Enter or change the value in the window.
- 3. Click OK.

Your changes are now saved to the ChAS Database.

## Editing column contents

#### Editing multiple cells with the same value

- 1. Highlight the cells you want to edit.
- 2. Right-click, then select Edit Property Values.

An Edit Value window appears. (Figure 452)

- 3. Enter a new value or edit the existing displayed value.
- 4. Click **OK**.

Your changes (for the multiple cells selected) are now saved to the ChAS Database.

| Figure 452 Query Se               | gments window tal             | b table - Filtered     | search results          |                       |                        |             |                  |
|-----------------------------------|-------------------------------|------------------------|-------------------------|-----------------------|------------------------|-------------|------------------|
| 👭 Karyoview 🎙 🛯 😹 Segments 🎙 🖕    | 🜡 CytoRegions 🎙 🛛 🎜 Overlap N | Map 🎙 🖾 Graphs 🎙 📳     | Variants 🎙 🔛 Query Samp | oles 🎙 🗌 Query Segmen | ts 🔻                   |             |                  |
| 🔝 🔜 🐹 🖀 Σ Query Se                | gments                        |                        |                         |                       |                        |             | 64 results       |
| File                              | Full Location                 | Sample Type            | 🗾 Call                  | 🗾 Segment Interpre 🔻  | Sample Interpretation  | Sex         | CN State Size    |
| 20121204B_07329_P2472_CytoScan    | . chr21:15006457-15222860     | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 3.00             |
| 20130105B_536_P0000001889_Cyto    | . chr21:15168392-15255326     | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 1.00             |
| PN12-0244.cyhd.cychp              | chr21:15909027-15933011       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 4.00             |
| 20111115C_IR147_IR147_CytoScan    | chr21:17260593-17432845       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 3.00             |
| CyHD_101612G_SS19-40_401962B      | chr21:18252327-18293364       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 1.00             |
| CyHD_021312T_SS168_401019MP       | chr21:18808979-19081476       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 3.00             |
| 20130201A_07174_P0000000074_C     | chr21:18853646-18880914       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 1.00             |
| 20121224_154428_003_18.cyhd.cychp | chr21:18993202-19505046       | Blood (constitutional) | polymorphic (ADGV)      | N                     | ormal                  | female      | 3.00             |
| PN12-4503.cyhd.cychp              | chr21:20943207-21031022       | Blood (constitutional) | polymorphic (ADGV)      | N                     | Edit Value             |             | ×                |
| CyHD_012712K_SS1121_400564SN      | . chr21:21266215-21448325     | Blood (constitutional) | polymorphic (ADGV)      | N                     |                        |             | ^                |
| 20130820B_04773_P000000009618     | . chr21:21800804-21835609     | Blood (constitutional) | polymorphic (ADGV)      | N                     | Characteristics of Wat |             |                  |
| 201110012 IR44 IR-44 CytoScanH    | chr21:22813621-22836474       | Blood (constitutional) | polymorphic (ADGV)      | N                     | Change value of "Int   | erpretation | on Tannotations? |
| PN13-0570.cyhd.cychp              | chr21:22883403-48084820       | Blood (constitutional) | polymorphic (ADGV)      | N                     | Common Variant         |             |                  |
| CyHD_061112T_SS553_401817MC       | chr21:23603281-23710385       | Blood (constitutional) | polymorphic (ADGV)      | N                     | 0                      |             |                  |
| CyHD 100112G SS18-12 401677M      | . chr21:23667120-24120841     | Blood (constitutional) | polymorphic (ADGV)      | N                     | 4                      |             |                  |
| 20130607B 2173 P02212 CytoScan    | . chr21:23769752-23944028     | Blood (constitutional) | polymorphic (ADGV)      | N                     | 4                      |             |                  |
| CvHD 052912T SS471 401965TG       | chr21:24161828-24218094       | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| 20111109C IR137 IR137 CvtoScan    | chr21:24176862-24207625       | Blood (constitutional) | polymorphic (ADGV)      | N                     | <b>a</b>               |             |                  |
| 20111006 IR19 IR-19 CytoScanHD    | chr21:24176862-24213659       | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| CvHD 112812G SS24-27 400218W      | . chr21:25218961-25324875     | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| CvHD 022112T SS216 400839MM       | . chr21:25278620-25324875     | Blood (constitutional) | polymorphic (ADGV)      | N                     |                        |             |                  |
| CvHD 052912T SS473 400274TL.c.,   | . chr21:26753980-26814544     | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| 20130614B 3821 P00000005160 C     | chr21:27175766-27252173       | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| CvHD 061112T SS549 401175FA.c.,   | chr21:27299633-27307419       | Blood (constitutional) | polymorphic (ADGV)      | N                     | 4                      |             |                  |
| CvHD 011712T SS14 400737GC.c.,    | chr21:29225315-29359939       | Blood (constitutional) | polymorphic (ADGV)      | N                     |                        |             |                  |
| CvHD 112812G SS24-35 400683E      | chr21:29514717-33285300       | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| PN12-3379.cvhd.cvchp              | chr21:31913231-32002762       | Blood (constitutional) | polymorphic (ADGV)      | N                     | d                      |             |                  |
| 20111109C IR139 IR139 CvtoScan    | chr21:32210608-32300112       | Blood (constitutional) | polymorphic (ADGV)      | N                     | 4                      |             |                  |
| AFFY-69.cvhd.ND.cvchp             | chr21:34003503-34106011       | Blood (constitutional) | , ,                     | N                     | d                      |             |                  |
| PN12-2960.cvhd.cvchp              | chr21:35726225-35901512       | Blood (constitutional) | polymorphic (ADGV)      | N                     |                        |             |                  |
| CULD 040010T SCOLE 400600DM       | chr01-06706006 06006060       | Plood (constitutional) | polymorphic (ADC\A      | N                     | OK                     | C           | ancel            |
| (                                 |                               |                        |                         |                       |                        |             |                  |
| ~ *                               |                               |                        |                         |                       | L                      |             |                  |

For instructions on how to use the table's features, see "Common table operations" on page 323.

**Note:** To delete sample files from the Query Segments tab, follow the same instructions outlined in "Deleting sample(s) from the ChAS database" on page 406. This procedure deletes the entire sample file and all file information associated with it.



## **Exporting results**

**IMPORTANT!** Edit Mode must be **OFF** before exporting from ChAS.

Chromosome Analysis Suite includes the following tools for reporting results:

- Export the Karyoview, Selected Chromosome View, and Detail View as a DOCX, PDF or PNG file. See "Exporting graphic views".
- Export to a DOCX file. See "Creating signature and background profiles".
- Export table data as a DOCX, PDF, TXT file, or copy selected data onto your clipboard. See "Exporting table data".
- Combine PDF reports. See "Combining PDFs into a single PDF"
- Use a ClinVar export template. See "Exporting with ClinVar".
- Export copy number and variant data as VCF. See "Exporting VCF files".

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

**Note:** If you have trouble displaying non-English characters on screen or in exported PDF files, make sure that the font "Arial Unicode MS" is installed on the machine. Open the "C:\Windows\Fonts" folder and search for a **ARIALUNI.TTF** file.



### Exporting graphic views

You can export the Karyoview, Selected Chromosome View, and Detail View in the following formats:

- PDF "Exporting as a PDF"
- MS Word (DOCX) "Exporting as Word (DOCX) format" on page 415
- PNG graphic file "Exporting as PNG" on page 417

Exporting as aChAS provides a variety of options for exporting graphic views as PDFs. The PDFPDFReport displays the graphic with basic information about data files and settings.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

- 1. From the **Exports** menu, select the PDF option you want to use.
  - Export application window PDF Creates PDF with entire software screen and information about data files
  - Export Karyoview PDF Creates PDF with Karyoview and information about data files.
  - Export Selected Chromosome PDF Creates PDF with Selected Chromosome View and information about data files.
  - Export Detail View PDF Creates PDF with selected Detail View and information about data files.
  - Export Segments Table PDF Creates PDF with Segment Table
  - Export QC and Sample Info PDF Creates PDF with the QC and Sample Information
  - Export Chromosome Summary Data PDF Creates PDF with the Chromosome Summary Data

An Export Details window appears. (Figure 453)



| Figure 453 Export Details window |                                     |
|----------------------------------|-------------------------------------|
| Sector Details                   | x                                   |
| - Interpretation                 |                                     |
| Page Numbers                     |                                     |
| Add To Existing Export           |                                     |
| Select File                      | Select File                         |
|                                  | Mauto Launch Export OK Reset Cancel |

- 2. Optional: If you have added a Sample Interpretation in the View/Edit Sample Properties window, the information will be populated into the Interpretation dialog box. You can also type free text into the Interpretation box.
- 3. Optional: Select the option for adding page numbers.
- 4. Optional: Select the option for adding to an existing report, if desired. See "Combining PDFs into a single PDF" on page 425.
- 5. Click Select File.

The Save window opens.

6. Select a folder location for the PDF using the navigation tools.

This folder location is automatically selected when exporting other PDFs during a session.

7. Enter a name for your PDF file.

If you are adding the graphic to an existing PDF, select the PDF file.

8. Click Save.

You are returned to the Export Details window.

- 9. Optional: Leave the Auto Launch Export check box checked, if you want your newly saved PDF to open automatically after clicking **OK**.
- 10. Click **OK**.

A PDF is created with your selected export details saved.



## Creating signature and background profiles

# Signature profiles Signature Profiles, including a logo, address, reviewer name and credentials, can be added to a DOCX export. Use saved signature profiles for quick recalls with any DOCX export

- 1. Click on the Preference Menu, then select Edit User Configuration.
- 2. Click the **Exports** Tab.
- 3. Click the Summary Exports tab, then click the Summary Export tab. (Figure 454)

| Figure 454 Summary Expo  | ort window - Signatu      | res      |                   |         |   |
|--|---------------------------|----------|-------------------|---------|---|
| User Configuration   |                           |          |                   |         | × |
| Segment Data QC Thresholds Colo<br>ClinVar Summary Export<br>Signatures<br>Thermo Fisher | r Rules Misc Vocabularies | DB Query | Filtered DB Query | Exports |   |
| New<br>Edit  | New Edit                  |          |                   |         |   |
| Delete   | Delete                    |          |                   |         |   |
|  |                           | ОК       | Cancel            |         |   |

#### Creating a new signature

- 1. Click the New button.
- 2. Complete the text fields.
- 3. Optional: Click on the Upload Logo button to add your organization's logo.
- 4. Click OK to save.

Your saved signature name will be the same as your organization name.

5. Optional: Repeat steps 1-4 to create additional signatures.



#### Editing a saved Signature

- 1. Highlight the Signature name you would like to modify.
- 2. Click the Edit button.
- 3. Modify the appropriate fields.
- 4. Click **OK** to save your updated signature.

#### **Deleting a signature**

- 1. Highlight the Signature name you would like to delete.
- 2. Click the **Delete** button.
- 3. Click **OK** to permanently remove the signature.

## Background profiles

Background profiles (Figure 455) provide saved text(s) that can be added to each DOCX export. For example, a noteworthy background about the assay profiles you want to save.

| Figure 455 Summary Expo          | rt window - Backgrounds          |                             |
|----------------------------------|----------------------------------|-----------------------------|
| User Configuration               |                                  | ×                           |
| Segment Data QC Thresholds Color | Rules Misc Vocabularies DB Query | r Filtered DB Query Exports |
| ClinVar Summary Export           |                                  |                             |
| Signatures<br>Thermo Fisher      | Backgrounds                      |                             |
| mannoriana                       | Gytobean                         |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
| New                              | New                              |                             |
| Edit                             | Edit                             |                             |
| Delete                           | Delete                           |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  |                                  |                             |
|                                  | ОК                               | Cancel                      |

#### Creating a new background

- 1. Click on the **New** button.
- 2. Name the Background using the Title field.



- 3. Enter the text for the background information that you want to appear on the export.
- 4. Click **OK** to save.
- 5. Optional: Repeat steps 1-4 to create additional Backgrounds.

#### Editing a saved background

- 1. Highlight the Background name you want to modify.
- 2. Click the Edit button.
- 3. Modify the text.
- 4. Click **OK** to save your updated background.

#### Deleting a background

- 1. Highlight the Background name you would like to delete.
- 2. Click the **Delete** button.
- 3. Click **OK** to permanently remove the Background.

#### Exporting as Word (DOCX) format

| 1. | From the Exports menu, | select Export - Word (docx) Format. |
|----|------------------------|-------------------------------------|
|    |                        |                                     |

The Export Details window opens. (Figure 456)

|   | ils  |   |              |                              |      |            |
|---|--|---|--------------|------------------------------|------|------------|
| Select the exp  | orts to include. Exports   | will be added in the  | e order show | n in the panel on the right. |      |            |
|   | Available Exports  |   |              | Selected Exports             |      |            |
|   | Detail View  |   |              | Sample Analysis Information  |      |            |
|   | Karyoview  |   | 0            |                              |      |            |
|   | Sample and QC Inform   | nation  | >>           |                              | Up   |            |
|   | Segment Intersections  |   |              |                              | Down |            |
|   | Segments Table   |   |              |                              | Domi |            |
|   | Selected Chromosom   | e   |              |                              |      |            |
|   | Whole Genome View  |   | U            |                              |      |            |
| Select Signatu  | ure (Organization and Re   | viewer) </td <td>e Signature&gt;</td> <td></td> <td></td> <td></td> | e Signature> |                              |      |            |
| Select Backou   | round Paragraph CytoSc   |   | - 3          |                              |      |            |
| Jatamatatian  | ound raragraph Cytose  | an  |              |                              |      | -          |
| This comple   |  |   |              |                              |      |            |
| mis sample.   |  |   |              |                              |      |            |
|   | I  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
|   |  |   |              |                              |      |            |
| Existing Exp  | ort  |   |              |                              |      |            |
| Existing Exp  | ort  |   |              |                              |      |            |
| Existing Exp  | ort<br>disting Export  |   |              |                              |      |            |
| Existing Exp  | ort<br>disting Export  |   |              |                              |      |            |
| Existing Exp<br>Add to Exp<br>Table Data<br>Convert t   | ort  | ments Table   |              |                              |      |            |
| Existing Exp<br>Add to Ex<br>Table Data   | ort<br>disting Export<br>o paragraph style for Seg<br>ow Numbers                                     | ments Table   |              |                              |      |            |
| Existing Exp<br>Add to Ex<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror                             | ort<br>disting Export<br>o paragraph style for Seg<br>ow Numbers<br>nosome                           | ments Table   |              |                              |      |            |
| Existing Expr<br>Add to Ex<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror<br>Hide the                | ort<br>disting Export<br>o paragraph style for Seg<br>ow Numbers<br>nosome<br>Y Chromosome in the Ka | ments Table   |              |                              |      |            |
| Existing Exp<br>Add to Ex-<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror<br>Hide the                | ort<br>disting Export<br>o paragraph style for Seg<br>ow Numbers<br>nosome<br>Y Chromosome in the Ka | ments Table   |              |                              |      |            |
| Existing Exp.<br>Add to E2<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror<br>Hide the<br>Select File | ort<br>o paragraph style for Seg<br>ow Numbers<br>nosome<br>Y Chromosome in the Ka                   | ryoview   |              |                              |      |            |
| Existing Exp<br>Add to Ex<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror<br>Hide the<br>Select File  | ort<br>o paragraph style for Seg<br>ow Numbers<br>nosome<br>Y Chromosome in the Ka                   | ryoview   |              |                              |      | elect File |
| Existing Exp<br>Add to Ex<br>Table Data<br>Convert t<br>Include R<br>Hide Y Chror<br>Hide the<br>Select File  | ort<br>o paragraph style for Seg<br>.ow Numbers<br>nosome<br>Y Chromosome in the Ka                  | ryoview   |              |                              |      | elect File |



**Note:** In order to export a graph (Karyoview, Whole Genome View, Detail View, Selected Chromosome View), the graph must be visible in the ChAS Browser before export.

- Single-click, Ctrl-click, or Shift-click on the Available Exports (left pane), then click the right double arrow button (Figure 456) to add them to the Selected Exports (right pane). Use the Up and Down buttons to define each report's order within the master report.
- 3. Optional: Select a saved Signature and/or Background from the drop-down lists to add them to your DOCX export.

If you previously entered Sample Level Interpretations, they will appear in the Interpretation field.

**Note:** Before adding an entry in the Interpretation field, the Selected Exports pane must first display **Sample Analysis Information**, as shown in Figure 456.

- Optional: Click the Add to Existing Export check box to add this new report to an existing one. After checking this check box, click on the Select File button to navigate to and select an existing DOCX file.
- 5. Optional: Click the **Convert to paragraph style for Segments Table** check box if you want your Segments Table data translated into a paragraph-style format, as show in Figure 457.



- 6. Optional: Click the **Include Row Number** check box to add row numbers to the Segment Table.
- 7. Optional: Click the **Hide Y Chromosome** check box to export the Karyoview without the Y chromosome ideogram for female samples.



8. Click the **Select File** button to navigate to a saved report location, enter a filename, then click **Save**.

The name of the file defaults to the name of the filename.

- 9. By default, the report automatically opens in MS Word after it is generated. Uncheck the **Auto Launch Export** check box to disable this feature.
- 10. Click **OK** to generate the DOCX report. Click the **Reset** button to return the Report Details window back to its factory defaults.
- **Exporting as PNG** You can also create a PNG screen shot of the entire software screen.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

1. From the Exports menu, select **Export application window PNG**.

A Save As window opens.

- 2. Select a folder location for the file using the navigation tools.
  - This folder location is automatically selected when exporting other screen shots during a session.
- 3. Enter a name for the PNG file in the File Name box.
- 4. Click the Save button.

The PNG file screen shot is saved in the selected location.

The PNG file can be cropped in a graphics program like Paint and inserted into a word processing document if desired.

### Exporting table data

ChAS provides several options for exporting table data:

- "Exporting table data into a PDF"
- "Exporting views and tables as a DOCX file"
- "Exporting tables as TXT file"
- "Exporting a segments table with modified segments to a TXT file"

#### **Exporting table** data into a PDF For information on how to choose and export preset column content (from previously saved table states). See "Saved table states" on page 328.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

**Note:** You can export data from the Segment Table by selecting Export Segment Table PDF from the Exports menu, but you cannot export graph table data in a PDF format.

In order to track which of your segments have been modified (merged, created de novo, deleted, had their start or end coordinates edited, or had their type or state



changed), you **MUST** perform the following **BEFORE** exporting the Segments Table to PDF. The only PDF format capable of tracking which segments have been modified is the Segments Table PDF, all other PDFs show NO visual or textual distinction between modified and non-modified segments.

**BEFORE** exporting table data into a PDF file:

1. Click **[]]** (top right of Segments table).

The Select Columns window appears.

- 2. Scroll down, locate, then click to check the Modified Segment check box.
- 3. Click **OK**.

The Modified Segment column is now added to your Segments table.

#### Exporting table data into a PDF file

1. At the Segments table upper tool bar, click the PDF button

The Select Columns and Files window appears. (Figure 458)

| Select Columns and File             |           |                         |          |                |             |
|-------------------------------------|-----------|-------------------------|----------|----------------|-------------|
| ample Properties                    |           |                         |          |                | <br>        |
| clude Sample Properties             |           |                         |          |                |             |
| terpretation                        |           |                         |          |                | <br>        |
|                                     |           |                         |          |                |             |
|                                     |           |                         |          |                |             |
|                                     |           |                         |          |                |             |
| Column Selection and Preferences    |           |                         |          |                |             |
| olumn Name                          | Add Colum | n Column Truncation Cho | oices Tr | uncated Length |             |
| se In Export                        | Add       | NO_TRUNCATION           | ~        | 100            |             |
| le                                  | 🗹 Add     | NO_TRUNCATION           | ~        | 100            |             |
| ame/ID                              | 🗹 Add     | NO_TRUNCATION           | ~        | 100            |             |
| N State                             | bbA 🔽     |                         | ~        | 100            |             |
| N Otale                             |           |                         | •        | 100            |             |
| уре                                 | Add Add   |                         | ~        |                |             |
| licroarray Nomenclature (ISCN 2016) | M Add     | NO_TRUNCATION           | ~        | 100            |             |
| low Numbers                         |           |                         |          |                |             |
| dd Row Numbers                      |           |                         |          |                |             |
| age Numbers                         |           |                         |          |                |             |
| dd Page Numbers 🗹                   |           |                         |          |                |             |
| victing Export                      |           |                         |          |                |             |
| dd To Evioting Evport               |           |                         |          |                |             |
|                                     |           |                         |          |                |             |
| elect File                          |           |                         |          |                |             |
|                                     |           |                         |          |                | Select File |

If you have added a Sample Interpretation in the View/Edit Sample Properties window, the information will be populated into the Interpretation dialog box. You can also type free text into the Interpretation box. You must check the **Include Sample Properties** check box in order to enable the Interpretation field

2. Enter the appropriate text in the Interpretation field.

To add the other Sample Properties, check the **Include Sample Properties** check box. If these fields have been populated, they will be exported in the PDF.

- 3. Select the columns to be displayed and the columns truncation rules. The Column Selection and Preferences window (Figure 459), include:
  - **Column Name**: Header of the column in the table.
  - Add Column: Click the check box to display the column in the PDF report.
  - Column Truncation Options:

**No\_Truncation** - Field is exported as is, using wrap-around if necessary.

**Truncate\_Beginning** - Truncates content at the beginning of the field, leaving as many characters as specified in Truncated Length box.

**Truncate\_Middle** - Truncates content in the middle of the field, leaving characters at the beginning and end, with ellipses (...) to mark the truncated characters.

**Truncate\_End** - Truncates content at the end of the field, leaving as many characters as specified in Truncated Length box.

**Truncated Length**: Number of characters displayed after truncating the data.

| Figure 459           | Column Selection and Preferences                        |    |
|----------------------|---|----|
| Column Selection and | l Preferences   | -1 |
| Column Name          | Add Column Column Truncation Choices Truncated Length 🔼 |    |
| Segment ID           | Add NO_TRUNCATION V 100                                 |    |
| File                 | Add NO_TRUNCATION V 100                                 |    |
| CN State             | Add NO_TRUNCATION V 100                                 |    |
| Туре                 | Add NO_TRUNCATION V 100                                 |    |
| Chromosome           | Add NO_TRUNCATION V 100                                 |    |
| Min                  | Add NO_TRUNCATION V 100                                 |    |
| Max                  | Add NO_TRUNCATION V 100                                 |    |
|                      |   |    |

- 4. Select the option for adding page and row numbers, if desired.
- 5. Select the option for adding to an existing report, if desired. See "Combining PDFs into a single PDF" on page 425.
- 6. Click the Select File button.

Chromosome Analysis Suite (ChAS) User Guide



The Save As window opens. (Figure 460)

| Figure 460 Save As for Segment Table PDF  | :         |
|---|-----------|
|   |           |
| Save As   | ×         |
| Save In: 📋 My Documents   |           |
| ePublisher Express Projects     ePublisher Pro Projects     ePublisher Pro Vuser Formats     ePublisher Stationery     ePublisher Stationery     eval_docs     eval_docs     Exchange     Exchange     My Music     My Prictures     My PSP Files |           |
| File Name:  |           |
| Files of Type: PDF Files  | ~         |
| Sa  | ve Cancel |

- 7. Select a folder location for the file using the navigation tools.
- 8. Enter a name for the PDF file, or select a file for the information to be appended to.
- 9. Click **Save** in the Save As window.
- 10. Click **OK** in the **Select Columns and File** window.

A PDF file is created with the selected data type saved.

The PDF report displays:

- Table type
- Information on chromosome and genome region
- Interpretation
- Data files
- Genome or CytoRegion Segment Filters used
- Settings for Data Processing
- Microarray Nomenclature
- Details of the table data

#### Exporting tables tips

Follow the tips below to improve the export of table data in a PDF file:

- Use truncation.
- Select only columns you need or select columns from a saved table state. See "Saved table states" on page 328.
- Use filtering options (Segment filters, displaying only results for a chromosome or area in the detail view, etc.) to limit the number of values being exported.

#### Exporting views and tables as a DOCX file

You can save the currently viewed table in a new or existing DOCX report.

1. While in a desired View or Table, click the DOCX button 22.

A Save window appears.

Do one of the following:

- New Reports: Enter a filename to create a new DOCX file, then click Save.
- Adding to an existing report: Click to select an existing DOCX file. Click Save. The message window, You will be adding contents to an existing file. Continue? appears. Click Yes to append the currently displayed table contents onto an existing file.

**IMPORTANT!** Before adding a file, it must be closed.

**Exporting tables** The TXT file format enables you to transfer data to other software for analysis. as **TXT file** 

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

#### Exporting table information as a text file

|                    | egments     | table wi  | th data filt  | ered       |             |             |            |            |           |              |
|--------------------|-------------|-----------|---------------|------------|-------------|-------------|------------|------------|-----------|--------------|
| 🔢 Karyoview 🛛 📷 Se | gments 🛄 Cy | toRegions | 🟓 Overlap Map | 📈 Graphs   |             |             |            |            |           |              |
| 🔠 📑 💼 🛄            | 1 + 🔏 (     | <b>V</b>  |               |            |             |             |            |            | 18 result | s from chr ( |
| Segment ID         | File        | CN State  | Туре          | Chromosome | Min         | Max         | Size (kbp) | Mean Marke | Max % Cov | Max % Ov     |
| seg2024            | Sample 0    |           | 3 🛦 Gain      | 3          | 131,253,287 | 131,321,447 | 68         | 1,286      |           |              |
| seg2100            | • Sample_0  |           | 0 🔻 Loss      | 3          | 164,007,533 | 164,019,098 | 11         | 1,051      |           |              |
| seg2014            | Sample_0    |           | 3 🔺 Gain      | 3          | 127,155,074 | 127,159,387 | 4          | 862        |           |              |
| smoothed164019930  | Ó Sample_0  |           | 5 🔺 Gain      | 3          | 164,019,930 | 164,100,879 | 80         | 1,245      |           |              |
| seg1830            | 🔆 Sample_0  |           | 3 🔺 Gain      | 3          | 75,531,222  | 75,600,607  | 69         | 1,176      |           |              |
| smoothed163887385  | Sample_0    |           | 3 🔺 Gain      | 3          | 163,887,385 | 164,006,897 | 119        | 1,475      |           |              |
| seg1832            | 🔆 Sample_0  |           | 3 🔺 Gain      | 3          | 75,624,861  | 75,637,504  | 12         | 1,053      |           |              |
| seg2096            | Sample_0    |           | 3 🔺 Gain      | 3          | 75,838,844  | 75,962,472  | 123        | 1,188      |           |              |
| seg2515            | Sample_0    |           | 1 🔻 Loss      | 3          | 190,842,432 | 190,853,556 | 11         | 855        |           |              |
| seg2204            | Sample_0    |           | 3 🔺 Gain      | 3          | 116,133,749 | 116,146,833 | 13         | 817        |           |              |
| seg2557            | Sample_0    |           | 3 🔺 Gain      | 3          | 196,822,294 | 196,914,807 | 92         | 1,492      |           |              |
| seg2252            | Sample_0    |           | 3 🔺 Gain      | 3          | 126,917,179 | 126,991,856 | 74         | 1,037      |           |              |
| smoothed75549695   | Sample_0    |           | 3 🔺 Gain      | 3          | 75,549,695  | 75,633,780  | 84         | 1,356      |           |              |
| seg2094            | Sample_0    |           | 3 🔺 Gain      | 3          | 75,716,050  | 75,800,870  | 84         | 1,101      |           |              |
| seg2088            | Sample_0    |           | 3 🔺 Gain      | 3          | 75,459,878  | 75,536,466  | 76         | 1,160      |           |              |
| seg2010            | Sample_0    |           | 3 🔺 Gain      | 3          | 47,804,033  | 47,876,603  | 72         | 1,133      |           |              |
| seg2264            | Sample_0    |           | 4 🔺 Gain      | 3          | 129,883,492 | 129,904,220 | 20         | 1,727      |           |              |
| smoothed164002633  | Sample_0    |           | 1 🔻 Loss      | 3          | 164,002,633 | 164,091,128 | 88         | 1,229      |           |              |

1. Perform pre-filtering on the data in the table. (Figure 461)

2. In the Table tool bar, click the Export **TXT** button.

The Save as TXT window opens. (Figure 462)

.. Nu



| Figure 462 Save as TXT window   |   |             |
|---|---|-------------|
| Save  |   | ×           |
| Save In: 📋 My Documents   | ~ |             |
| Publisher Express Projects     New Folder     Publisher Pro Projects     Publisher Pro User Formats     Preferences     Publisher Stationery     Preferences_Test     val_docs     Exchange     My Music     My Pictures     My PSP Files |   |             |
| File Name:           Files of Iype:           TSV Files (*.tsv)   |   | ✓           |
|   |   | Save Cancel |

- 3. Select a folder location for the file using the navigation tools.
- 4. Enter a name for the TXT file.
- 5. Click Save.

The TXT file is saved in the selected location (Figure 463).

It can be opened using a text editing or spreadsheet program, or in other software designed to use Tab Separated Value TXT format.

|           | А            | В                     | С        | D    | E          | F         | G         | Н          | I                    |   |
|-----------|--------------|-----------------------|----------|------|------------|-----------|-----------|------------|----------------------|---|
| 1 Segme   | nt ID        | File                  | CN State | Туре | Chromosome | Min       | Max       | Size (kbp) | Mean Marker Distance | Μ |
| 2 seg202  | 4            | Sample_01.cyto2.cychp | 3        | Gain | 3          | 131253287 | 131321447 | 68         | 1286                 |   |
| 3 seg210  | 0            | Sample_01.cyto2.cychp | 0        | Loss | 3          | 164007533 | 164019098 | 11         | 1051                 |   |
| 4 seg201  | 4            | Sample_01.cyto2.cychp | 3        | Gain | 3          | 127155074 | 127159387 | 4          | 862                  |   |
| 5 smoot   | ned164019930 | Sample_01.cyto2.cychp | 5        | Gain | 3          | 164019930 | 164100879 | 80         | 1245                 |   |
| 6 seg183  | 0            | Sample_01.cyto2.cychp | 3        | Gain | 3          | 75531222  | 75600607  | 69         | 1176                 | 1 |
| 7 smoot   | ned163887385 | Sample_01.cyto2.cychp | 3        | Gain | 3          | 163887385 | 164006897 | 119        | 1475                 |   |
| 8 seg183  | 2            | Sample_01.cyto2.cychp | 3        | Gain | 3          | 75624861  | 75637504  | 12         | 1053                 |   |
| 9 seg209  | 6            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 75838844  | 75962472  | 123        | 1188                 |   |
| 10 seg251 | 5            | Sample_03.cyto2.cychp | 1        | Loss | 3          | 190842432 | 190853556 | 11         | 855                  |   |
| 11 seg220 | 4            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 116133749 | 116146833 | 13         | 817                  |   |
| 12 seg255 | 7            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 196822294 | 196914807 | 92         | 1492                 |   |
| 13 seg225 | 2            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 126917179 | 126991856 | 74         | 1037                 |   |
| 14 smoot  | ned75549695  | Sample_03.cyto2.cychp | 3        | Gain | 3          | 75549695  | 75633780  | 84         | 1356                 |   |
| 15 seg209 | 4            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 75716050  | 75800870  | 84         | 1101                 |   |
| 16 seg208 | 8            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 75459878  | 75536466  | 76         | 1160                 |   |
| 17 seg201 | 0            | Sample_03.cyto2.cychp | 3        | Gain | 3          | 47804033  | 47876603  | 72         | 1133                 |   |
| 18 seg226 | 4            | Sample_03.cyto2.cychp | 4        | Gain | 3          | 129883492 | 129904220 | 20         | 1727                 |   |
| 19 smoot  | ned164002633 | Sample_03.cyto2.cychp | 1        | Loss | 3          | 164002633 | 164091128 | 88         | 1229                 |   |
| 20        |              |                       |          |      |            |           |           |            |                      |   |
| 21        |              |                       |          |      |            |           |           |            |                      |   |



#### Exporting a segments table with modified segments to a TXT file

In the Segments Table:

- Materially Modified segments are shown in italic text when Edit Mode is ON, but not italicized when Edit Mode is OFF.
- Calls and Interpretations don't cause a segment's row text to be italicized in Edit Mode.
- Deleted segments are shown in strike-through text when Edit Mode is ON, and are not present in the table when Edit Mode is OFF.
- In the TXT Exports of the Segments Table table, please note that Deleted segments will be part of the export when Edit Mode is ON, and will not be part of the export if Edit Mode is OFF

Figure 464 is an example of a Segment Table with Edit Mode **ON**. Note its italicized text representing materially modified segments and the strike-through text showing deletions.

| Karyoview     | ٦ 🚹   | Segments  | Cyto     | Regions <b>1</b> | P Ove | erlap Map 🎙 🔀  | Graphs 🔻             |                        |                         |                          |  |
|---------------|-------|-----------|----------|------------------|-------|----------------|----------------------|------------------------|-------------------------|--------------------------|--|
| 🔠 📠 🗎         | Σ     |           | - 🚮 🛛    |                  |       |                |                      |                        | 4 results from chrX: 11 | 17,340,551 - 136,127,416 |  |
| Use In Report | File  |           | CN State | Туре             | Chr   | Cytoband Start | Call                 | Materially Modified Se | Materially Modified By  | Call & Interpretation By |  |
|               |       | NA00857   | 1.44     | V LossMosaic     | X     | q26.3          | Likely Benign        | ~                      | cdowds:18               | cdowds:lalala            |  |
|               | [ P ? | NA00857   | 1.00     | V Loss           | X     | q23            |                      | ~                      | cdowds:lalala           |                          |  |
| ×             | [ P . | NA00867   | 1.00     | V Loss           | ×     | <del>q25</del> |                      | ~                      | cdowds:lalala           |                          |  |
|               | S 99  | NA00857   | 1.00     | V Loss           | Х     | q24            | Unknown Significance | X                      |                         | cdowds:lalala            |  |
|               | ∎r∨ ⊊ | 2 NAU0857 | 1.00     | LOSS             | X     | q24            | Unknown Significance | ~                      |                         | cdowds:lalala            |  |

Figure 465 is an example of a Segment table that has been exported with the Edit Mode **ON**. Note the 4th row of the Use in Report column. In the case of this segment, it reads FALSE, because this segment was deleted.

| Fig | gure 4     | <b>65</b> S | egmen      | t table | • ТХ | (T EXP   | ORT - Edit M       | ode ON                    |                        |                          |
|-----|------------|-------------|------------|---------|------|----------|--------------------|---------------------------|------------------------|--------------------------|
| R   | SegTableTX | TexportEdit | ModeON tyt |         |      |          |                    |                           |                        | = X                      |
|     | A          | B           | C          | D       | E    | F        | G                  | н                         | T                      |                          |
| 1   | Use In Re  | File        | CN State   | Туре    | Chr  | Cytoband | Call               | Materially Modified Segme | Materially Modified By | Call & Interpretation By |
| 2   | TRUE       | NA00857     | 1.44       | LossMos | aiX  | q26.3    | Likely Benign      | TRUE                      | cdowds:18              | cdowds:lalala            |
| 3   | TRUE       | NA00857     | 1          | Loss    | x    | q23      |                    | TRUE                      | cdowds:lalala          |                          |
| 4   | FALSE      | NA00857     | 1          | Loss    | Х    | q25      |                    | TRUE                      | cdowds:lalala          |                          |
| 5   | TRUE       | 00857       | 1          | Loss    | х    | q24      | Unknown Significan | FALSE                     |                        | cdowds:lalala            |
| 6   |            | ~           |            |         |      |          |                    |                           |                        |                          |
| H   | + + H Se   | gTableTXT   | exportEdit | ModeON  | 2    |          |                    | [] 4 [                    | l III                  |                          |

Figure 466 is an example of a Segment Table with Edit Mode **OFF**. The italicized text representing materially modified segments is no longer present. The deleted segment and strike-through text showing a deletion (shown in Figure 464 and Figure 465) also do not appear.



| Fi | <b>gure 4</b> | 66<br><b>1</b> 7 | Segme     | ent tab  | le - Edit    | Mo<br>• ov | de OFF<br>rerlap Map 🎙 🔀 | Graphs 🎙             |                        |                         |                            |
|----|---------------|------------------|-----------|----------|--------------|------------|--------------------------|----------------------|------------------------|-------------------------|----------------------------|
| E  | 🗎 📠 🗎         | Σ                |           | • 🔏 🛙    | <b>V</b>     |            |                          |                      |                        | 3 results from chrX: 11 | 17,340,551 - 136,127,416 🔢 |
| Us | se in Report  | File             |           | CN State | Туре         | Chr        | Cytoband Start           | Call                 | Materially Modified Se | Materially Modified By  | Call & Interpretation By   |
|    |               |                  | Q NA00857 | 1.44     | V LossMosaic | X          | ( q26.3                  | Likely Benign        | 1                      | cdowds:18               | cdowds:lalala              |
|    |               |                  | Q NA00857 | 1.00     | V Loss       | X          | ( q23                    |                      | ~                      | cdowds:lalala           |                            |
|    |               |                  | Q NA00857 | 1.00     | V Loss       | Х          | ( q24                    | Unknown Significance | X                      |                         | cdowds:lalala              |
| <  |               |                  |           |          |              |            |                          |                      |                        |                         | < >                        |

Figure 467 is an example of how an exported TXT table appears with Edit Mode **OFF**. Note the deleted segment shown in Figure 464 and Figure 465 is not present.

| Fi | gure 4     | <b>67</b> Se | egmen        | t table  | ТΧ  | T EXP    | ORT - Edit M       | lode OFF                  |                        |                          |
|----|------------|--------------|--------------|----------|-----|----------|--------------------|---------------------------|------------------------|--------------------------|
| 8  | SegTableTX | TexportEditN | /lodeOFF.txt |          |     |          |                    |                           |                        | - = ×                    |
|    | А          | В            | С            | D        | E   | F        | G                  | Н                         | I.                     | J                        |
| 1  | Use In Re  | File         | CN State     | Туре     | Chr | Cytoband | Call               | Materially Modified Segme | Materially Modified By | Call & Interpretation By |
| 2  | TRUE       | NA00857      | 1.44         | LossMosa | X   | q26.3    | Likely Benign      | TRUE                      | cdowds:18              | cdowds:lalala            |
| 3  | TRUE       | NA00857      | 1            | Loss     | х   | q23      |                    | TRUE                      | cdowds:lalala          |                          |
| 4  | TRUE       | NA00857      | 1            | Loss     | х   | q24      | Unknown Significan | FALSE                     |                        | cdowds:lalala            |
| 5  |            |              |              |          |     |          |                    |                           |                        |                          |
| 6  |            |              |              |          |     |          |                    |                           |                        |                          |
| H. | I ► ► Se   | gTableTXT    | exportEdit   | ModeOFF  | 1   | /        |                    | 14                        | i<br>                  | ► <b>I</b>               |

## Transfer to clipboard

You can copy data from selected cells to the clipboard for pasting into a text or spreadsheet file.

1. Select the cells you want to copy in the table. (Figure 468)

| Figure 468 Se     | egment ta | able with | cells sele | ected      |             |             |            |            |            |            |     |
|-------------------|-----------|-----------|------------|------------|-------------|-------------|------------|------------|------------|------------|-----|
|                   | J W   🖮 U | <u>×</u>  |            |            |             |             |            |            | 18 results | rrom cnr 3 |     |
| Segment ID        | File      | CN State  | Туре       | Chromosome | Min         | Max         | Size (kbp) | Mean Marke | Max % Cov  | Max % Ove  | Nui |
| seg2024           | Sample_0  | 3         | 🛦 Gain     | 3          | 131,253,287 | 131,321,447 | 68         | 1,286      |            |            |     |
| seg2100           | Sample_0  | 0         | ▼ Loss     | 3          | 164,007,533 | 164,019,098 | 11         | 1,051      |            |            |     |
| seg2014 (         | Sample_0  | 3         | 🔺 Gain     | 3          | 127,155,074 | 127,159,387 | 4          | 862        |            |            |     |
| smoothed164019930 | Sample_0  | 5         | 🛦 Gain     | 3          | 164,019,930 | 164,100,879 | 80         | 1,245      |            |            |     |
| seg1830 (         | Sample_0  | 3         | 🛦 Gain     | 3          | 75,531,222  | 75,600,607  | 69         | 1,176      |            |            |     |
| smoothed163887385 | Sample_0  | 3         | 🛦 Gain     | 3          | 163,887,385 | 164,006,897 | 119        | 1,475      |            |            |     |
| seg1832           | Sample_0  | 3         | 🛦 Gain     | 3          | 75,624,861  | 75,637,504  | 12         | 1,053      |            |            |     |
| seg2096           | Sample_0  | 3         | 🛦 Gain     | 3          | 75,838,844  | 75,962,472  | 123        | 1,188      |            |            |     |
| seg2515           | Sample_0  | 1         | ▼ Loss     | 3          | 190,842,432 | 190,853,556 | 11         | 855        |            |            |     |
| seg2204           | Sample_0  | 3         | 🔺 Gain     | 3          | 116,133,749 | 116,146,833 | 13         | 817        |            |            |     |
| seg2557           | Sample_0  | 3         | 🔺 Gain     | 3          | 196,822,294 | 196,914,807 | 92         | 1,492      |            |            |     |
| seg2252           | Sample_0  | 3         | 🛦 Gain     | 3          | 126,917,179 | 126,991,856 | 74         | 1,037      |            |            |     |
| smoothed75549695  | Sample_0  | 3         | 🔺 Gain     | 3          | 75,549,695  | 75,633,780  | 84         | 1,356      |            |            |     |
| seg2094           | Sample_0  | 3         | 🔺 Gain     | 3          | 75,716,050  | 75,800,870  | 84         | 1,101      |            |            |     |
| seg2088           | Sample_0  | 3         | 🔺 Gain     | 3          | 75,459,878  | 75,536,466  | 76         | 1,160      |            |            |     |
| seg2010           | Sample_0  | 3         | 🔺 Gain     | 3          | 47,804,033  | 47,876,603  | 72         | 1,133      |            |            |     |
| seg2264           | Sample_0  | 4         | 🔺 Gain     | 3          | 129,883,492 | 129,904,220 | 20         | 1,727      |            |            |     |
| smoothed164002633 | Sample_0  | 1         | ▼ Loss     | 3          | 164,002,633 | 164,091,128 | 88         | 1,229      |            |            |     |
| <                 |           |           |            |            |             |             |            |            |            |            | >   |
| A 14              |           |           |            |            |             |             |            |            |            |            |     |

2. Click the **Copy to Clipboard** button in the table tool bar.

The selected data is copied to the clipboard.

You can paste the data on the clipboard into a text or spreadsheet file (Figure 469).

| Figure 469 Data pasted int    | o text file     |     |           |           |           |     |      |
|-------------------------------|-----------------|-----|-----------|-----------|-----------|-----|------|
| smoothed164019930 Sample      | 01 cvto2 cvcho  | 5.0 | Cain 3    | 164019930 | 164100879 | 80  | 1245 |
| seq1830 Sample_01.cyto2.cychp | 3.0 Gain        | 3   | 75531222  | 75600607  | 69 1176   | 00  | 1245 |
| smõothed163887385 Sample_     | _01.cyto2.cychp | 3.0 | Gain 3    | 163887385 | 164006897 | 119 | 1475 |
| seg1832 Sample_01.cyto2.cychp | 3.0 Gain        | 3   | 75624861  | 75637504  | 12 1053   |     |      |
| seg2096 Sample_03.cyto2.cychp | 3.0 Gain        | 3   | 75838844  | 75962472  | 123 1188  |     |      |
| seg2515 Sample_03.cyto2.cychp | 1.0 Loss        | 3   | 190842432 | 190853556 | 11 855    |     |      |
| seg2204 Sample_03.cyto2.cychp | 3.0 Gain        | 3   | 116133749 | 116146833 | 13 817    |     |      |
| seg2557 Sample_03.cyto2.cychp | 3.0 Gain        | 3   | 196822294 | 196914807 | 92 1492   |     |      |
| seg2252 sample_03.cyto2.cychp | 3.0 Gain        | 3   | 126917179 | 126991856 | 74 1037   |     |      |

## **Combining PDFs into a single PDF**

You can combine different PDFs into a single PDF with multiple pages and content.

You can do this by:

- Adding new data to an existing PDF file.
- Merging two or more existing PDF files.

**IMPORTANT!** The results from ChAS are for Research Use Only. Not for use in diagnostic procedures.

#### Adding a new PDF export to an existing PDF file

1. When exporting a table or graph as a PDF, click the **Add to Existing Export** check box in the **Export Details** window. (Figure 470)

| Export Details  |      |      |  |
|-----------------|------|------|--|
| -Interpretation |      |      |  |
| - Page Numbers  | <br> | <br> |  |
| Existing Export |      |      |  |
|                 |      | <br> |  |
| Select File     |      |      |  |



2. Click Select File and select an existing PDF file for the data to be added to.

The Save As window opens. (Figure 471)

| Figure 471 Save As               | window             |                             |
|----------------------------------|--------------------|-----------------------------|
| Save As                          |                    | X                           |
| Save In: 📋 My Documents          |                    |                             |
| 🛅 ePublisher Express Projects  📔 | New Folder         | 📩 Segments_Table_Export.pdf |
| 🛅 ePublisher Pro Projects 🛛 📔    | preference_RA      | 📩 Whole Screen.pdf          |
| 📄 ePublisher Pro User Formats 📄  | preferences        | 🔂 Whole_Screen.pdf          |
| 📄 ePublisher Stationery 🛛 📔      | Preferences_Test   |                             |
| 📄 eval_docs 🛛 📔                  | 🕽 SnagIt Catalog   |                             |
| 🔁 Exchange 🛛 📅                   | Chromosome_03.pdf  |                             |
| 📑 My Music 🛛 💆                   | Detail_Screen.pdf  |                             |
| 💾 My Pictures 🛛 💆                | Segment_Table.pdf  |                             |
| 🗀 My PSP Files 🛛 💆               | Segments_Table.pdf |                             |
| <                                |                    | >                           |
| File Name: Chromosome_03.pd      | df                 |                             |
| Files of Type: PDF Files         |                    | ~                           |
|                                  |                    | Save Cancel                 |

3. Select a PDF file, then click **Save** in the Save As window.

A confirmation message appears, asking if you want to overwrite or add to the data (Figure 472).

| Figure 472 Confirmation message  |         |   |  |  |
|--|---------|---|--|--|
| File e   | xists > | < |  |  |
| The file already exists;<br>you will be adding to it or overwriting it.<br>Continue? |         |   |  |  |

- 4. Click Yes in the Confirm Rewrite notice to append the data in the selected file.
- 5. Click **OK** in the **Select Columns and File** or **Export Details** window.

The new report data is combined with the existing report.

Combining two existing PDF files

1. Click **Exports**  $\rightarrow$  **Combine PDFs**.

The Combine PDF Exports window opens. (Figure 473)

| ×      |        |     |
|--------|--------|-----|
| T L Y  | 1      | Χ.  |
|        | 10     | 21  |
| 114    | Γ.     | ч.  |
| $\sim$ |        | S 1 |
| $\sim$ | <br>L. | - 4 |
| 1000   |        |     |

| Figure 473 Combine PDF Exports window |                       |        |
|---------------------------------------|-----------------------|--------|
| 🐴 Combine F                           | PDF Exports           | ×      |
|                                       | Select Input Files    |        |
|                                       |                       |        |
|                                       |                       |        |
|                                       |                       |        |
|                                       |                       |        |
| - Select Output F                     | Add Files Remove File |        |
|                                       |                       |        |
|                                       | Auto Launch Export OK | Cancel |

2. Click Add Files....

The Select PDF Files to combine window opens. (Figure 474)

 Select the PDF files to combine, then click **Open** in the Select PDF Files window. The selected files are displayed in the Select Input Files list.

You can use the Remove File button to remove a selected input file.

Click and drag on a file in the list to change the order of data in the Combined PDF.

4. Click the Select Output File button.



The Save As window opens. (Figure 475)

| Figure 475 Save As window  |             |
|--|-------------|
| Save As  | ×           |
| Save In: 📋 My Documents  |             |
| Publisher Express Projects New Folder     Publisher Pro Projects Preference_RA     ePublisher Pro User Formats Proferences     ePublisher Stationery SnagIt Catalog     eval_docs Statutor |             |
| File Name: CH14_Detail_Segments  |             |
|  | Save Cancel |

5. Enter a file name for the combined PDF file, then click **Save** in the **Save As** window.

You are returned to the **Combine PDF Exports** window.

6. Click **OK**.

Your selected PDFs are combined.



### Exporting with ClinVar

The ClinVar export enables exporting of copy number data using the ClinVar Full Template for easy submission directly to ClinVar. A ClinVar Submission Profile must be created to use this Export. For details on the template, go to: www.ncbi.nlm.nih.gov/clinvar/docs/submit/

**IMPORTANT!** ChAS is a research use only application and any submission to ClinVar is the responsibility of the submitter.

All fields exported into the ClinVar submission template are selected and defined by the user. Standard ClinVar nomenclature is provided for required submission fields and can be customized as shown in "Adding vocabulary content" on page 430.

Creating a ClinVar submission profile

#### 1. Click **Preferences** $\rightarrow$ **Edit User Configuration**.

The User Configuration window appears.

2. Select the Exports tab, then select the ClinVar tab. (Figure 476)

| User Configuration   |  |  |  |  |
|--|--|--|--|--|
| Segment Data       QC Thresholds       Color Rules       Misc       Vocabularies       DB Query       Exports         ClinVar       Summary Export       ClinVar Submission Info       Image: ClinVar Vocabulary       Affected Status       Image: ClinVar Vocabulary       Image: ClinVa |  |  |  |  |
| L Add  |  |  |  |  |
| Remove     Restore Defaults     Default list contains 2 items.       OK     Cancel   |  |  |  |  |

3. In ClinVar Submission Info pane, click the **New** button to create a new submission profile.

An Edit Profile window appears.

- 4. Complete all the appropriate fields. Fields with an \* are required by ClinVar.
- 5. Click **OK** to save the profile.
- 6. Optional: To create additional ClinVar profiles, repeat steps 3-5

Chapter 19 Exporting results Exporting with ClinVar



| Editing an<br>existing ClinVar | <ol> <li>Highlight the profile name in the ClinVar Submission Info list box that is to be<br/>modified.</li> </ol>   |
|--------------------------------|--|
| profile                        | 2. In ClinVar Submission Info pane, click the Edit button.   |
|                                | 3. Edit the appropriate fields.  |
|                                | 4. Click <b>OK</b> to save your changes.   |
| Deleting a profile             | <ol> <li>Highlight the profile name in the ClinVar Submission Info list box you want to<br/>delete.</li> </ol>   |
|                                | 2. Click the <b>Delete</b> button.   |
|                                | 3. Click <b>OK</b> to confirm the profile deletion, or click <b>Cancel</b> to keep the profile.  |
| Adding<br>vocabulary           | By default, recommended ClinVar vocabularies are stored for certain required fields, but additional terms can be added to any field.   |
| content                        | 1. Click Preferences $\rightarrow$ Edit User Configuration.  |
|                                | The User Configuration window appears.   |
|                                | 2. Click the <b>Exports</b> tab, then click the <b>ClinVar</b> tab.  |
|                                | 3. Select a category you want to add a term(s) to, then use the text field (Figure 476) to enter the additional term(s).   |
|                                | <ol> <li>From the ClinVar vocabularies drop-down, select the category that you want to<br/>add a term(s) to.</li> </ol>  |
|                                | 5. Use the text field (at the bottom) to enter the additional term(s).   |
|                                | 6. Click the <b>Add</b> button to add the term to the category's list.   |
| Removing<br>vocabulary         | 1. From the ClinVar vocabularies drop-down list, select a category that contains the term you want to remove.  |
| content                        | 2. Highlight the term, then click <b>Remove</b> .  |
| Exporting in<br>ClinVar format | There are certain fields that are required before uploading to ClinVar. It is recommended you use the <b>ClinVar Table State</b> in the Segments Table to expose all required fields. To use the ClinVar Table State, refer to "Saved table states" on page 328. |
|                                | 1. In the Segments Table, apply the ClinVar Table State.   |
|                                | 2. Select the segments to be exported using the <b>Use in Export</b> check box.  |
|                                | 3. Fill in all columns for the selected segments, as all columns in the ClinVar Table State are required before you can upload to Clinvar.   |
|                                | 4. In the Exports Menu, select ClinVar Export.   |
|                                | A browse window appears.   |
|                                | 5. Select a location to save the export, then use the File Name text box to name the export, then click <b>OK</b> .  |
|                                | A Submission Info/Segment Data window appears.   |
|                                | 6. Select the ClinVar Profile you want to use for this export.   |



- 7. Optional: If you want to add any additional comments to the export, click on the Segment Data tab to enter them within this tab.
- 8. Click **OK** to export.

**Note:** You can open the ClinVar export in Excel to add information to the optional fields. Opening the ClinVar export from ChAS, auto-populates all currently required ClinVar fields.

 Table 20
 Variant tab: Auto-populated columns into the ClinVar submission template (all other optional columns are blank upon export).

| Auto-populated column   | Description  |  |  |
|---|--|--|--|
| Chromosome  | Populated from Segment Table (Chromosome)  |  |  |
| Variant type  | Populated from Segment Table (Gain/Loss)   |  |  |
| Variant length  | Populated by ClinVar upon submission   |  |  |
| Copy Number   | Populated from Segment Table (Gain/Loss)   |  |  |
| Variation identifiers   | Populated using OMIM track   |  |  |
| Condition category  | Populated from ClinVar Vocabulary  |  |  |
| Clinical significance   | Populated from Segment Table (Call)  |  |  |
| Date last evaluated   | Uses date of export unless otherwise specified on the Submission Info window before exporting. |  |  |
| Comment on clinical significance                                | Populated from Segment Table (Segment Interpretation)  |  |  |
| Collection method   | Populated from ClinVar Vocabulary  |  |  |
| Allele origin   | Populated from ClinVar Vocabulary  |  |  |
| Affected status   | Populated from ClinVar Vocabulary  |  |  |
| Structural variant method/<br>analysis type                     | SNP Array  |  |  |
| Platform type   | Microarray   |  |  |
| Platform name   | Populated based on the Microarray used   |  |  |
| Software name and version         Chromosome Analysis Suite 4.5 |  |  |  |

 Table 21
 Case Data tab: Auto-populated columns into the ClinVar submission template (all other optional columns are blank upon export).

| Auto-populated column | Description                                  |
|-----------------------|--|
| ##Linking ID          | Populated from Segment Table (Full Location) |
| Collection method     | Populated from ClinVar Vocabulary            |
| Allele origin         | Populated from ClinVar Vocabulary            |
| Affected status       | Populated from ClinVar Vocabulary            |



 Table 21
 Case Data tab: Auto-populated columns into the ClinVar submission template (all other optional columns are blank upon export).

| Auto-populated column  | Description                                    |  |
|--|--|--|
| Structural variant method/<br>analysis type                  | SNP Array                                      |  |
| Clinical Features  | Populated from Sample Properties (Phenotype)   |  |
| Tissue   | Populated from Sample Properties (Sample Type) |  |
| Sex  | Populated from Gender determination            |  |
| Platform type  | Microarray                                     |  |
| Platform name         Populated based on the Microarray used |  |  |
| Software name and version                                    | Chromosome Analysis Suite 4.5                  |  |

## Exporting a deletion log

The user, time, filename and reason why a file is deleted or republished to a ChAS database can be exported.

1. Click ChAS DB  $\rightarrow$  Download Deletion Log (Figure 477)



Your default Internet browser opens.

- 2. Enter your ChAS Database credentials, as you normally would.
- 3. Depending on your Internet browser, you may be prompted to either download the file or save it. To save it, click **File**  $\rightarrow$  **Save As.**
- 4. To view your exported log file, open it in Excel. (Figure 478)

|   | Fig | ure 478 File viewed in MS Excel example |            |        |                               |   |
|---|-----|---|------------|--------|-------------------------------|---|
| 1 | C12 |   | • (=       | $f_x$  |                               |   |
|   | 1   | A                                       |            | B      | C                             | D   |
|   | 1   | Filename                                |            | User   | Timestamp                     | Comment   |
|   | 2   | 20120622_1                              | 111026_005 | cgates | 2015-10-15T05:56:10.851-07:00 | Added Calls to Segments no calls were made in original file |
| 2 |     |   |            |        |                               |   |

Deletion Logs can also be exported from the ChAS DB Tools Maintenance Page. See "Downloading deletion logs" on page 449.


# User profiles and named settings

ChAS provides many options for customizing the display of data and annotations.

The User Profiles and Named Settings functions enable you to save your analysis and display settings.

### **Types of settings**

ChAS provides two ways to store setup information. User profiles and Named settings. Each way works differently and performs different functions.

#### **User profiles**

A ChAS Browser User Profile stores your selections for various display settings as they were when the software was last shut down while using that user profile.

A new user profile can be created or selected only when starting the software.

The user profile saves the following display settings:

- Screen size, displayed tabs, and sizing of display areas
- The views displayed in ChAS, and the size of the display panes
- Available Named Settings: Different users can have different lists of named settings to choose from
- Name of the currently selected named setting
- Copies of the user's custom (not shared) named settings
- Data Display Configurations
- Region information files selected for CytoRegions and Overlap Map
- Which types of graph and segment data are turned on or off
- Display options for graph data (height, grid, values, etc.)
- Chromosome and area displayed.
- Selected Reference Annotation database (ChAS Browser NetAffx Genomic Annotations file)
- Loaded AED and BED files
- The files and Reference Annotations (Genes, DGV, etc.) that are checked or unchecked
- Custom color rules



#### Named settings A Named Setting stores the user's choices for:

- Which types of graph and segment data are turned on or off
- Segment Filter Settings
- Restricted Mode on/off

The Named Setting doesn't save a particular CytoRegion file, but does inform you if no file is selected when you select a setting with restricted mode on.

It is possible to apply a Named Setting with restricted mode using a different CytoRegion file than was selected for the initial creation of the setting.

You can switch between different Named Settings in the same user profile to look at different types of data.

ChAS provides pre-configured (shared) Named Settings indicated by the kinetic icon as described in the table below. These named settings cannot be deleted.

| Named Setting                 | Genome Segment<br>Filters   | CytoRegion<br>Segment Filters  | Data Types  |
|-------------------------------|---|--|---|
| Standard                      | Gain:<br>Marker Count = 50<br>Size (kbp) = 400;<br>Loss:<br>Marker Count = 50<br>Size (kbp) = 400 | Gain:<br>Marker Count = 50<br>Size (kbp) = 400<br>Loss:<br>Marker Count = 50<br>Size (kbp) = 400 | Gain<br>Loss<br>GainMosaic<br>LossMosaic<br>Copy Number State<br>Weighted Log2 Ratio<br>Allele Peaks<br>Allele Difference |
| High Resolution               | Gain:<br>Marker Count = 50<br>Size (kbp) = 100;<br>Loss:<br>Marker Count = 50<br>Size (kbp) = 100 | Gain:<br>Marker Count = 25<br>Size (kbp) = 50<br>Loss:<br>Marker Count = 25<br>Size (kbp) = 50   | Gain<br>Loss<br>GainMosaic<br>LossMosaic<br>Copy Number State<br>Weighted Log2 Ratio                                      |
| LOH only<br>(3Mb and 50 SNPs) | LOH:<br>Marker Count = 50<br>Size (kbp) = 3000  | LOH:<br>Marker Count = 50<br>Size (kbp) = 3000   | LOH<br>Genotype Calls<br>Allele Peaks<br>Allele Difference  |
| Differential Gains and Losses | Gain:<br>Marker Count = 50<br>Size (kbp) = 400;<br>Loss:<br>Marker Count = 50<br>Size (kbp) = 100 | Gain:<br>Marker Count = 25<br>Size (kbp) = 50<br>Loss:<br>Marker Count = 25<br>Size (kbp) = 50   | Gain<br>Loss<br>GainMosaic<br>LossMosaic<br>Copy Number State<br>Weighted Log2 Ratio                                      |



| Named Setting     | Genome Segment<br>Filters   | CytoRegion<br>Segment Filters | Data Types  |
|-------------------|---|-------------------------------|---|
| OncoScan Defaults | 0   | 0                             | 0   |
| XON-Level 1       | XON Level:<br>Level 1 = On<br>Level 2-4 = Off<br>XON Gain/Loss Marker:<br>Count = 0<br>Size (kbp) = 0 | 0                             | XON Region Gain,<br>XON Region Loss,<br>LOH Segments, Log2<br>Ratio, Smooth Signal,<br>Allelic Difference |

### Creating and using user profiles

You can only select or create user profiles upon starting ChAS.

- 1. Double-click on the ChAS icon on the desktop; or
  - From the Windows Start Menu, select Programs  $\rightarrow$  Thermo Fisher Scientific  $\rightarrow$  Chromosome Analysis Suite  $\rightarrow$  Chromosome Analysis Suite.

The ChAS Splash Screen and the Select User window open. (Figure 479)

| Figure 4 | 79 Select User window |
|----------|-----------------------|
| Select   | User X                |
| 0        | ra 🗸 Create New       |
|          | OK Cancel             |

2. Click Create New in the Select User window.

The Create New User window opens. (Figure 480)

| Figure 480 Create New User window |   |
|-----------------------------------|---|
| Create New User                   | × |
| UserID UserID                     |   |
| OK Cancel                         |   |

- 3. Enter a name for the new profile in the User ID field.
- 4. Click **OK** in the Create New User window.



The new user appears in the drop-down User list in the **Select User** window. (Figure 481)

| Figure 48<br>profile | Select User V | window v | vith new user |
|----------------------|---------------|----------|---------------|
| Select I             | Jser          |          | ×             |
|                      | RA2           | ~        | Create New    |
|                      | ra            |          |               |
|                      | RA2           |          |               |
|                      |               |          |               |
|                      |               |          |               |

5. Select the new user, then click **OK**.

Any changes you make to the setup of the software that is listed in "User profiles" on page 433 will be saved when you shut down the software and used the next time the software is opened with this user profile.

#### Go to the Windows folder where the user profiles are stored and delete the folder with the profile name you want to delete.

You can see the location of the folder in the About window, as described in "Analysis file locations in Windows 10" on page 25.

#### Creating and using named settings

Deleting a user

profile

You can save a snapshot of your favorite settings as a Named Setting. To apply a particular Named Setting to the active data (check marked in the Files List), make a selection from the Named Setting drop-down list. Some pre-configured Shared Named Settings may be available for use by all users. Only an administrator can add or remove Shared Named Settings, but any user can apply them to their data.

| Saving a named | 1. Set the display data settings as desired.        |
|----------------|---|
| setting        | These can include:                                  |
|                | Which graphs and segment types are turned on or off |
|                | Segment Filter Settings                             |

- Restricted mode on/off
- 2. From the Preferences menu, select Save Named Setting.



The Setting Name window opens. (Figure 482)

| Figure 482 Named Setting List         |   |
|---------------------------------------|---|
| Setting Name                          | × |
| Enter Setting Name<br>NS_4Mb_150 SNPs |   |
| OK Cancel                             |   |

3. Enter a name for the setting you want to create, then click **OK**.

The setting is saved and appears in the Named Setting drop-down list. (Figure 483)

| Figure 483 Named Setting drop | -down list  |
|-------------------------------|-------------|
| _Named Setting                |             |
| NS_4Mb_150 SNPs               | ~           |
| Differential Gains and Losses | ,           |
| 📲 High Resolution             |             |
| LOH only (3Mb and 50 SNPs)    |             |
| 📲 Standard (400kbp and 50 ma  | rkers, Gain |
| NS_4Mb_150 SNPs               |             |

**Note:** The Named Setting saves the settings at the time it was created. Subsequent changes to the settings will not be saved in the Named Setting.

# Selecting a named setting

1. From the Named Setting drop-down list, select the setting. (Figure 484)

| Figure 484<br>down list | Named Setting drop-  |
|-------------------------|----------------------|
| Named Sett              | ing                  |
|                         |                      |
| Different               | ial Gains and Losses |
| 🚵 High Re               | solution             |
| 👪 LOH onl               | y (3Mb and 50 SNPs)  |
| 🛛 🏜 LOH onl             | y (5Mb and 50 SNPs)  |
| CncoSca                 | an Default           |
| 🏜 Standard              | d                    |

Alternatively, from the Preference menu, select Apply Named Setting...

20

The Select Named Setting window opens. (Figure 485)

| Figure 485 Select Named Setting window |   |
|--|---|
| Select Named Setting                   | × |
| High Resolution     OK Cancel          | ~ |

2. Select the Named Setting from the drop-down list, then click OK.

The selected setting is applied. **Note:** A Named Setting is not modified by any changes that you make to the settings in ChAS. If you want to keep a copy of your new settings, you will need to save them as a new Named Setting.

## Deleting a named setting

1. From the Preferences menu, select **Delete Named Setting.** 

The **Delete Setting** window opens. (Figure 486) **Note:** Shared Named Settings (the **i** icon in the Named Setting list) do not appear in the Delete Setting dropdown list. Users cannot delete or modify a shared Named Setting.

| Figure 4 | <b>186</b> Delete Setting window |   |
|----------|----------------------------------|---|
| Selec    | t Setting to Delete              | × |
| ?        | NS_4Mb_150 SNPs                  | ~ |
|          | OK Cancel                        |   |

 Select the setting you want to delete from the drop-down list, then click OK. The setting is deleted.



### Exporting and importing preferences

Preferences functions enable you to transfer most of the settings in a User Profile between one system and another.

**Note:** If you import "exported" preferences that reference a Shared Named Setting which no longer exists, such as a Shared Named Setting from ChAS 1.0.1 or ChAS 1.1, the profile will be changed to point to the default Shared Name Setting.

# Exporting preferences

1. From the Preferences menu, select Export Preferences...

The Select Directory to export preferences to window opens. (Figure 487)

| Figure 487 Select Directory to export preferences to |   |
|--|---|
| Select Directory to export preferences to            | × |
| Save In: 🔰 Public 🔹 💽 💼 💷                            |   |
| 🎍 Analysis 🛛 🤑 Libraries                             |   |
| 🎍 ChAS Parameters 🎍 Markers                          |   |
| 🍌 CytoScanHD CELs 🌽 Music                            |   |
| 🎍 Desktop 🛛 🤑 Pictures                               |   |
| \mu Documents 🛛 🕛 Profile_0643                       |   |
| 🔒 Downloads 🛛 🎍 Recorded TV                          |   |
| 🔒 Favorites 🛛 🔒 Videos                               |   |
| 🔒 Genotypes  |   |
|  |   |
| File Name: C:\Users\Public\Profile_0643              |   |
| Files of Type: All Files                             | ~ |
|  |   |
| Select Directory Cance                               | I |

2. Use the navigation features of the window to select or create a directory for the preferences. **Note:** The software creates a folder named "preferences" in the directory you select or create. If you select a directory that already contains a "preferences" folder, it will be overwritten. When you want to import the preferences, select the directory that contains the "preferences" folder that is indicated by the icon.

#### 3. Click Select Directory.

If the directory already contains a "preferences" folder, the Overwrite notice appears. (Figure 488)



| Figure 4 | 88 Overwrite notice        |   |
|----------|----------------------------|---|
| Overw    | rite                       | × |
| ?        | Overwrite/delete contents? |   |
|          | <u>Y</u> es <u>N</u> o     |   |

- Click **Yes** to export the preference files to the directory that you selected. You can then transfer the preferences to another user profile or system.
- 1. From the Preferences menu, select Import Preferences...

The Select Folder to import preferences from window opens. (Figure 489)

| Figure 489 Select Folder to import preferences from   | 1   |
|---|---|
| Select Folder to import preferences from  | ×   |
| Look In: 🔒 Public   |   |
| <ul> <li>Analysis</li> <li>Libraries</li> <li>ChAS Parameters</li> <li>Markers</li> <li>CytoScanHD CELs</li> <li>Music</li> <li>Desktop</li> <li>Pictures</li> <li>Documents</li> <li>Profile_0643</li> <li>Downloads</li> <li>Recorded TV</li> <li>Favorites</li> <li>Genotypes</li> </ul> | Directory that contains the<br>"preferences" folder |
| File Name: Profile_0643   |   |
| Files of Type: All Files  | *   |
|   | Select Profile Cancel                               |

- 2. Use the navigation features of the window to select the directory that the preferences were exported to (directories that contain a "preferences" folder are indicated by the 📥 icon.)
- 3. Click Open to import the preference files.

If you selected a directory that doesn't contain the "preferences" folder, the following notice appears. (Figure 490)

## Importing preferences





Click **OK** and repeat steps 1 through 3, selecting the correct folder.

The Restart notice appears. (Figure 491)

| Figure 4 | 91 Restart notice  |
|----------|--|
| Notice   | ×  |
|          | Please restart application to apply imported preferences |
|          | ОК   |

Note: The imported preferences will not be applied until you restart ChAS.

| Importing<br>hyperlinks         | External websites may update their links from time to time. To remedy this, a feature as been added to update an outdated link(s) within ChAS.  |  |  |
|---------------------------------|---|--|--|
|                                 | 1. Click Preferences $\rightarrow$ Import Hyperlinks.   |  |  |
|                                 | The Load Hyperlinks Configuration window opens.   |  |  |
|                                 | 2. Navigate to your updated hyperlinks ( <b>.chaslink</b> ) file, click to highlight it, then click <b>Select File</b> .  |  |  |
|                                 | 3. Restart the ChAS Browser to apply the link update(s).  |  |  |
|                                 | Note: For .chaslink file help, contact Technical Support.   |  |  |
| Configuring the<br>HTTP service | The HTTP service may enable external applications to interact with the ChAS Browser.<br>By default, this service is off. Please contact Technical Support before activating this feature. |  |  |
|                                 | 1. Click Preferences $\rightarrow$ Configure HTTP Service.  |  |  |
|                                 | The Configure HTTP Service window opens.  |  |  |
|                                 | <ol><li>Click the check box to enable the service, then enter the designated Port<br/>number.</li></ol>   |  |  |
|                                 | 3. Close the window.  |  |  |
|                                 | The service and Port are now activated.   |  |  |

## **Database tools**



### Connecting to a remote ChAS DB server

NOTE: The ChAS v4.5 browser cannot access previous ChAS database versions.

You must upgrade any older version of the ChAS database to ChAS v4.5 before the ChAS v4.5 browser can access it. To do this, see "Backing up a database" on page 446 and "Restoring a database" on page 446.

1. From the **Preferences** drop-down menu, click **Edit Application Configuration...** 

The **Configuration** window appears.

2. Click the Server tab.

The Server window/tab appears. (Figure 492)

 Type in the name or IP address of the computer/server name that you would like to connect to in the Hostname or IP Address text field or contact your IT Department for help completing this form.

Note: Up to three Hostnames/IP Addresses can be saved/stored.

| Figure 492    | Server window/tab      |         |
|---------------|------------------------|---------|
| 🖏 Configurat  | tion                   | ×       |
| Connection    | Server                 |         |
| Scheme        | Hostname or IP Address | Port    |
| http          | localhost              | 8099    |
| Base API F    | Path                   |         |
| /api/         |                        | Default |
| Full API UF   | રા                     |         |
| http://localh | nost:8099/api/         |         |
| Full Web U    | IRL                    |         |
| http://localh | nost:8099/             |         |
|               | OK                     |         |

4. Click OK.



If a connection to the ChAS DB cannot be established or the server/computer containing ChAS DB is not turned on, the following message appears (Figure 493). Please check the name/IP address and make sure the server/ computer is turned on.

| Figure | re 493 Error message  |
|--------|---|
| 🐴 Er   | rror X  |
| •      | Could not connect to server page http://aruwin:8099/api/.<br>You may have entered an invalid server URI, invalid username and password, or it may be offline. |

### Accessing the ChAS DB server tools

**Note:** The screen captures used in this chapter may vary depending on which Browser you are using.

 From the Chas DB drop-down menu, click to select **Database Tools**. (Figure 495)

| Figure 494 Select User            | window  |
|-----------------------------------|---|
| 🖏 Chromosome Analysis             | Suite. [For Research Use Only. Not for use in diag          |
| <u>File View Reports</u> Analysis | ChAS DB Preferences Help                                    |
| 🎽 🕺 🖏 🗡                           | 📊 Load Histograms 🖡 🖉 💷 📑 👫 💷 💷                             |
| Files                             | Add Histogram ments 🔻 🛄 CytoRegions 🎙 🥊                     |
| 🗹 🗓 ♥♂ 08-0989_/ ^                | Database Tools  |
|                                   | Inter Open the database configuration page in a web browser |

The following web page appears. (Figure 495)

**IMPORTANT!** The ChAS Server Home Page requires an active Internet connection, requires a browser (Chrome and Internet Explorer v11 are recommended). Also, if you are using the local ChAS DB, an active Internet connection is not required.



| Figure 495 Sign in to ChAS window                  |                |
|--|----------------|
| ChAS Server: Sign In to Ch 🗙                       |                |
| ← → C □ localhost:8099/wicket/bookmarkable/com     | m.affyme 🖣 🏠 🚍 |
| CHAS   |                |
| Status   |                |
| Maintenance  |                |
| Administration                                     |                |
|  |                |
| Sign In to ChAS                                    | 🕒 Sign out     |
| Sign In to ChAS<br>Username:                       | D Sign out     |
| Sign In to ChAS<br>Username:<br>admin<br>Password: | G Sign out     |

2. Log in using the installer's factory default Username: **admin** and Password: **admin**. After logging in, it is recommended new users go to "Administration" on page 451 to create a New User(s) and/or edit User(s) roles.

**Note:** Make sure you look in the URL field to identify which ChAS database the ChAS Database Tools is accessing.

The ChAS DB Home Page appears. (Figure 496)

| Figure 496 ChAS DB Home Page  |                 |
|---|-----------------|
| ChAS Server: Home ×   | _ <b>D</b> X    |
| ← → C [ localhost:8099/?10  | ☆ =             |
| CHAS  |                 |
| Status  |                 |
| Maintenance   |                 |
| Administration  |                 |
| ChAS Database   | ເ⊯ Sign out     |
| The status page provides summary information about the database and   | d its contents. |
| The maintenance page contains tools:  |                 |
| <ul> <li>to enable batch uploading of CHP and CHPCAR files.</li> <li>to backup and restore the database.</li> <li>run cleanup scripts.</li> </ul> |                 |
| The administration page contains the tools to add, edit, and assign role  | es.             |



#### Status page

Use this page to view how many samples and segments are in the Database. (Figure 497)

| Figure 497 ChAS DB Status Page |              |       |                |              |
|--------------------------------|--------------|-------|----------------|--------------|
| ChAS Server: Status            |              | 4     |                |              |
|                                | SOBA/MICKET/ | DOOKM | arkable/com.at | rymetrix 🔀 = |
| CHAS                           |              |       |                |              |
| Status                         |              |       |                |              |
| Maintenance                    |              |       |                |              |
| Administration                 |              |       |                |              |
| Status                         |              |       |                | 🕒 Sign out   |
| ChAS Database 3.0.0.27 (r79    | 13)          |       |                |              |
| <b>O Uptime</b> 6:34:5         |              |       |                |              |
| Information                    |              |       |                |              |
| Property                       | Туре         | Value |                |              |
| aed:version                    | aed:String   | 1     |                |              |
| affx:ucscGenomeVersion         | aed:String   | hg19  |                |              |
| Data                           |              |       |                |              |
| Samples0Segments0              |              |       |                |              |

### Maintenance

Use this page to perform a backup, restore, and database clean up. (Figure 498)



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## Backing up a database

1. Click Backup Database, then click the Backup button.

A backup is automatically generated and is stored in the Affymetrix directory shown in Figure 499.

(\Affymetrix\ChAS\PostgreSQL\Backups\ChASDB\_yyMMdd\_HHmmss.backup) **Note:** A backup is automatically done whenever a Restore operation is performed.

**IMPORTANT!** It is strongly recommended that you perform scheduled routine backups of the database.



**Note:** The ChAS installer creates a disabled Windows Task that automatically backs up the ChASDB database on a weekly basis once it is enabled.

# Restoring a database

**Note:** Restoring a backup file created in ChAS 3.0/3.1/3.2/3.3/4.0 automatically upgrades it to ChAS 4.1.

1. Click Restore Database, then click the Choose File button. (Figure 500)

An Explorer window opens.

2. Navigate to the location where your ChAS DB was last backed up, then click Open.

By default, a backup of your current database is stored/resides here: \\Affymetrix\ChAS\PostgreSQL\Backups

3. Click **Restore** to start the restore process.

**IMPORTANT!** Do not leave this page once choosing the Restore button until you see the message that the database has been successfully restored. Also, once the restore process has successfully completed, you must click ChAS DB  $\rightarrow$  Refresh ChAS DB Data to view the data in the database using the ChAS Browser.



| Figure 500 ChAS DB Home Page   |
|--|
| ChAS Server: Maintenance × □<br>← → C □ localhost:8099/wicket/bookmarkable/com.affymetris ☆ ≡  |
| CHAS   |
| Status   |
| Maintenance  |
| Administration   |
| Maintenance: Restore<br>Database   |
| Choose File No file chosen   |
| Restores the selected database backup file, overwriting the current database.<br>Do not leave this page until the restore operation is complete. |

**IMPORTANT!** After restoring the database, you must click ChAS DB  $\rightarrow$  Refresh ChAS DB data to view the newly restored data from the database.

#### Merging ChAS databases

When merging the contents of two ChAS databases into a single database, one database should be restored into ChAS and the other database must exist as a **backup.db** file.

When merging the segments from two databases, if a duplicate entry is found then the merge keeps the entry for the database currently active in ChAS. The duplicate from the **backup.db** is skipped.

#### Merging two ChAS databases

**IMPORTANT!** The two ChAS databases to be merged, must be from the same version of ChAS. Also, the library files for CytoScan HD and OncoScan CNV Plus must be present in your Library folder before merging the two databases.

Make sure one of the ChAS databases is restored into ChAS (for details on how to Restore a ChAS DB, see page "Restoring a database" on page 446). Since any duplicate segments between the databases will keep the copy from the actively restored database, make sure the database with the more complete content is the one that is actively restored in ChAS.

1. From the ChAS browser, go to **ChAS DB**  $\rightarrow$  **Database Tools**.

If prompted, log into the ChAS database as you normally would.



- 2. Click on the Maintenance link.
- 3. Click on the Merge database link.
- 4. Click the **Browse** button to navigate to the **backup.db** file you want to merge with the current database.
- 5. Click Merge.

#### Merging an older ChAS database

If you want to merge a database (from an older version of ChAS) with a current ChAS 4.0 database, perform these steps:

- 1. Backup your current ChAS 4.0 database.
- Restore the database from (e.g.) ChAS 3.1. See "Restoring a database" on page 446. During the restore process, the older ChAS database is automatically upgraded and is now compatible with ChAS 4.0.
- 3. Backup the 3.1 database you just restored.
- 4. Restore the ChAS 4.0 database you backed up in Step 1.

**Note:** If the databases to be merged contain duplicate entries, the copy that is in the currently restored database will be kept. The entry from the backup.db that is being merged will be skipped.

5. From the ChAS browser, go to **ChAS DB**  $\rightarrow$  **Database Tools**.

If prompted, log into the ChAS database as you normally would.

- 6. Click on the Maintenance link.
- 7. Click on the Merge database link.
- 8. Click the **Browse** button to navigate to the **backup.db** file you want to merge with the current database.
- 9. Click Merge.

## Cleaning up a database

ChAS DB will automatically run re-indexing scripts to maintain optimal performance. You can also run these scripts manually if desired.

Note: You must have a Manager or an Admin role to clean up a database.

1. Click the **Clean up database** button (Figure 501) to run the Vacuum Analyze and Reindex Database optimization process.



| Figure 501 ChAS DB - Cleanup Database                           |              |
|---|--------------|
| Figure 501 CIIAS DB - Cleanup Database                          |              |
|   |              |
| ChAS Server: Maintenance ×                                      |              |
| ← → C [] localhost:8099/wicket/bookmarkable/com.af              | fymetri> 😭 🔳 |
| CHAS  |              |
| Status  |              |
| Maintenance   |              |
| Administration  |              |
|   | 🕩 Sign out   |
| Maintenance: Clean Up   |              |
| Database  |              |
|   |              |
|   |              |
| Runs VACUUM ANALYZE and REINDEX DATABASE operations on the data | base.        |
|   |              |

### **Downloading deletion logs**

Use this feature to export a list of filenames that were deleted from ChAS DB.

- 1. Click the Download Log of Deleted Files
- 2. If asked, provide your ChAS DB Username and Password.
- 3. Save the genome-model log file.
- 4. Open with Microsoft Excel for easier viewing.

#### Creating a blank ChAS DB

Use this feature to create a blank hg19 or hg38 ChAS DB. Make sure you backup your previous database prior to creating an empty ChAS DB, as the database will be erased and recreated.

Note: xxCHP files are only compatible with a ChAS DB of the same genome version.

- 1. Back up the current ChAS DB.
- 2. Click on the Reset to Empty (hg19 or hg38) ChAS DB
- Use the drop-down to select the genome version for your new database. (Figure 502)



| Figure 502 ChAS DB - Cleanup Database   |
|---|
| Maintenance: Reset to empty hg19 or hg38 database   |
| Genome version for the new database hg19 V  |
| Automatically save a backup first? It is recommended that you leave this checked true.  |
| Delete all data and reset database  |
| Reset to empty hg19 or hg38 database. This will optionally first save a backup of the current database and will then empty all data from the database and set the chosen genome v |
|   |

- 4. Check the box to have a backup of the current ChAS DB before the database is deleted and an empty DB is created.
- 5. Click Delete all data and reset database.

#### **Deidentifying Files**

Deidentifying files will remove potentially sensitive information from the ChAS DB. By running Deidentification, the file names stored in the ChAS DB will be replaced by an alpha numeric ID.

**Note:** If you have included sensitive information in custom database fields, this process will not remove that information.

- 1. Click on **De-identify Files**.
- 2. (Optional) Create a backup of the database to preserve the original content, then click the **Start De-identification** button to replace the file names in your ChAS Database.



### Administration

**IMPORTANT!** The default **admin** password assigned at installation must be changed before you can access the ChAS database. This applies to new ChAS installations and restored ChAS databases that still use the default **admin** password.

When logging into the ChAS DB for the first time, a message appears prompting you to change the default **admin/admin** password.

- 1. Click ChAS DB  $\rightarrow$  Database Tools.
- A window appears.
- 2. Log in using the **admin/admin** for the username and password.
  - The **Profile** option is enabled.
- 3. Click **Profile**.

The Profile window appears

- 4. Go to the **Password** field and enter a secure password at least 8 characters in length, then enter it again in the **Password Confirmation** field.
- 5. Click Accept

Your new password is set and the ChAS database is now accessible. **Note:** Make sure you write down and store your new password in a safe place, as it cannot be reset.

**Note:** You must have an Admin role to perform Administration functions. Log files for the ChAS database can be found in: \ProgramData\Affymetrix\ChAS\Log

#### 1. Click Administration.

The following window appears: (Figure 503)

| Figure 503 Ch    | AS DB - Admi     | nistration  |              |               |
|------------------|------------------|-------------|--------------|---------------|
| ChAS Server: Adm | inistrati ×      | -           |              |               |
|                  | calhost:8099/wic | ket/bookmar | kable/com.at | ffymetri> 🏠 🔳 |
| CHAS             |                  |             |              |               |
| Status           |                  |             |              |               |
| Maintenance      |                  |             |              |               |
| Administration   | ←                |             |              |               |
| Adminis          | tration: E       | dit Use     | ers          | ເ⊯ Sign out   |
| Login Username   | Email First Name | Last Name   |              |               |
| admin 🛓          |                  |             | C Edit       | × Delete      |
| + Add User       |                  |             |              |               |

2. Click Edit Users.

Using the Administration feature

Changing the

default admin

password

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The following window appears: (Figure 504)

| Figure 504 Ch                 | AS D                 | B - Admir  | nistration ( | Edit Users)  | I            |
|-------------------------------|----------------------|------------|--------------|--------------|--------------|
| ChAS Server: Admi             | inistrati<br>calhost | ×          | et/bookmar   | kable/com.af | fymetrix 🛣 🔳 |
| CHAS<br>Status                |                      |            |              |              |              |
| Maintenance<br>Administration |                      |            |              |              |              |
| Administ                      | trat                 | ion: E     | dit Use      | ers          | 🗭 Sign out   |
| Login Username                | Email                | First Name | Last Name    |              |              |
| 🛔 admin                       |                      |            |              | C Edit       | × Delete     |
| + Add User                    |                      |            |              |              |              |
| << < 1 > >>                   |                      |            |              |              |              |

Do one of the following:

- Click the **Edit** button to edit a current Username.
- Click Add User to add (grant privileges to) a new ChAS DB user.

#### **Permission Guidelines**

- User Role has permission to query the database, edit segments, add segment and sample annotations, but does not have permission to upload data to the database, run backup/restore, maintenance or edit users.
- Manager Role has permission to query the database, edit segments, add segment and sample annotations, upload data to the database, run backup/restore, maintenance, but does not have permission to edit users.
- Admin Role has permissions to run all functions in ChAS Browser and ChAS DB.

#### Using a shared ChAS database while off-line

**IMPORTANT!** If your Windows Firewall is enabled during the installation of ChAS and you want to Backup the ChAS Database and Restore it to your local ChAS DB, a message may appear indicating that you cannot connect to the shared folder. If this message appears, contact your IT department for help in allowing file sharing through the Windows Firewall.

#### Working off-line using a ChAS database that resides on a shared server

 Connect to the shared ChAS DB server click Preferences → Edit Application Configuration, ChAS DB server tab. For details on connecting to another ChAS DB, see "Connecting to a remote ChAS DB server" on page 442.



- 2. Follow the procedure to perform a back up, locate the back up, then copy it to your local computer.
- 3. Return to **Preferences** → **Edit Application Configuration**, **ChAS DB server** tab, then click on the default to redirect to your local ChAS DB server.

See "Restoring a database" on page 446 for instructions on how to restore the back up database - you just copied from the server.

**IMPORTANT!** You must have a Manager or Admin role and make sure you log back into the local host before restoring your computer. While performing a restore from a backup of a shared server, the roles associated with the shared server are displayed, therefore any roles that were created on the local server are replaced by those used on the shared server (until local host ChAS DB is restored again).

Publishing data you have analyzed in off-line mode to the shared ChAS DB server

- Log back into the shared ChAS DB server. To do this. click Preferences → Edit Application Configuration, ChAS DB server tab, then enter the server name/IP address.
- 2. Click OK.

**Note:** After you are logged into the shared ChAS DB server, publish the samples to the database as you normally would. See "Publishing data to the database" on page 401. If a xxCHP file has been previously Published to the database, you will receive a warning indicating this sample already exists in the database. You can choose to overwrite the existing information or cancel to keep the existing information.



### Remapping a hg19 ChAS DB to hg38

A ChAS DB populated with data from hg19 analyses can be remapped to hg38 coordinates using the ChAS DB Remapper feature.

The ChAS DB Remapper maps the segments from your hg19 ChAS DB to the hg38 genome. All segments in ChAS DB start and end with a marker currently mapped in hg19. The Remapper takes the probe locations for all markers in the segment from hg19 and remaps them to hg38. It locates the best representation of the remapped segment in hg38 by taking into account the number of additional markers in the remapped segment, as well as the number of markers removed from the original segment.

Remap confidence = (originalMarkerCount - addedCount - 2\*removedCount)/ originalMarkerCount

For remapped segment definitions in a ChAS DB, see the table on page 390.

Segments with a Remap confidence >0.75 are remapped to the hg38 ChAS DB. The Remapper makes a copy of the database prior to remapping. However, always make a backup or copy of the ChAS DB that you would like to remap for safe keeping.

- 2. Click the **Browse** button (Figure 505) to select a ChAS db.backup to be remapped to hg38.

| Figure 505 ChAS DB Remapper            |                         |        |
|--|-------------------------|--------|
| applied biosystems                     | ChAS DB Remapper        | _      |
| DB File: C:\Affymetrix\DBBackup\ChASDB | _20150924_045120.backup | Browse |

3. Click Start.

**Note:** Depending on the size of the ChAS DB.backup to be remapped, this process may take several minutes.

The ChAS Remapper window appears. (Figure 506)



| applied biosystems      | ChAS DB Remapper                       | _ [                    |      |
|-------------------------|--|------------------------|------|
| B File: C:\Affymetrix\D | BBackup\ChASDB_20150924_052901.backup  | Bro                    | owse |
|                         |  | Target Genome Version: | hg38 |
| Time                    | Message                                |                        |      |
| 7/24/2017 6:02:16 AM    | Loading Segments                       |                        |      |
| 7/24/2017 6:02:18 AM    | 135319 segments found                  |                        |      |
| 7/24/2017 6:02:18 AM    | Updating Segments (hg19 to hg38)       |                        |      |
| 7/24/2017 6:02:19 AM    | Reading CytoScanHD_Array.na33.annot.db |                        |      |
| 7/24/2017 6:02:41 AM    | Reading CytoScanHD_Array.na36.annot.db |                        |      |
| 7/24/2017 6:03:50 AM    | 7.39 % completed                       |                        |      |
| 7/24/2017 6:04:46 AM    | 14.78 % completed                      |                        |      |
| 7/24/2017 6:05:46 AM    | 22.17 % completed                      |                        |      |
| 7/24/2017 6:06:47 AM    | 29.56 % completed                      |                        |      |
| 7/24/2017 6:07:50 AM    | 36.95 % completed                      |                        |      |
| 7/24/2017 6:08:56 AM    | 44.34 % completed                      |                        |      |
| 7/24/2017 6:10:02 AM    | 51.73 % completed                      |                        |      |
| 7/24/2017 6:11:09 AM    | 59.12 % completed                      |                        |      |
| 7/24/2017 6:12:18 AM    | 66.51 % completed                      |                        |      |
| 7/24/2017 6:13:30 AM    | 73.90 % completed                      |                        |      |
| 7/24/2017 6:14:47 AM    | 81.29 % completed                      |                        |      |
| 7/24/2017 6:16:04 AM    | 88.68 % completed                      |                        |      |
| 7/24/2017 6:17:23 AM    | 96.07 % completed                      |                        |      |
| 7/24/2017 6:18:12 AM    | 100.00 % completed                     |                        |      |
| 7/24/2017 6:18:12 AM    | Failed Segments: 7012                  |                        |      |
| 7/24/2017 6:18:12 AM    | Updating Genomodels                    |                        |      |
| 7/24/2017 6:18:19 AM    | Creating database file                 |                        |      |

The following files are generated in the same folder as your original ChAS DB select to remap:

- ChAS DB.hg38.backup this backup can be restored as the active ChAS DB for querying within the Browser.
- A TXT file listing all original segments provides a text file of the original segment information and the remapped segment information along with success or fail criteria.
- A TXT file listing the segments that failed to remap to hg38 provides s text file of the original segments that did not remap to the new genome build.

For more information on viewing a remapped database, see "Additional segment intersection information" on page 392.



# **ChAS Database Loader (CDL)**

CDL is now part of the ChAS Browser. CDL enables uploading of xxCHP files from any previous (including the current) version of ChAS. You may upload up to 500 xxCHP files at one time (as long as they are all analyzed from the same genome build).

CDL supports the following array types:

- Genomewide SNP 6
- CytoScan 750K
- CytoScan HD
- CytoScan HD Accel
- CytoScan Optima
- CytoScan XON
- CytoScan HTCMA
- OncoScan CNV Plus
- OncoScan CNV
- Cytogenetics 2.7M



### Starting CDL

#### 1. Click $ChASDB \rightarrow ChAS Database Loader$

#### The ChAS Database Loader window appears. (Figure 507)

| Figure 50              | 07 Main CDI  | _ window                  |                   |                |                           |           |           |
|------------------------|--|---------------------------|-------------------|----------------|---------------------------|-----------|-----------|
| ChAS Data              | abase Loader   |                           |                   |                |                           |           | ×         |
| <u>File T</u> ools     |  |                           |                   |                |                           |           |           |
| 7 🗹 🛦 🤆                | Gain <b>I ▼ L</b> oss (                                | 🔲 🗙 LOH 🛛 🗹 🔵 Dete        | cted 🗹 • Undetect | ed 🗹 🗖 Gain (X | CON Region) 🗹 🗖 Loss (XON | N Region) |           |
|                        | Genome   |                           | ]                 |                |                           |           |           |
| Gain. Not filt         | ered. All will be use                                  | d.                        |                   |                |                           |           |           |
| Loss. Not filt         | ered. All will be use                                  | d.                        |                   |                |                           |           |           |
| Detected. N            | lot filtered. All will be<br>I. Not filtered. All will | e used.<br>I be used.     |                   |                |                           |           |           |
| LOH. Type              | turned off. Will be                                    | e skipped.                |                   |                |                           |           |           |
| XON Regio<br>Gain (XON | n Levels. These le<br>Region) No filters               | vels will be used: 1      |                   |                |                           |           |           |
| Loss (XON              | Region). No filters                                    | other than level filters. |                   |                |                           |           |           |
| Add Files              | Export file list                                       | Import file list          | 1                 |                | <u> </u>                  |           |           |
| CHP File               |  |                           |                   | Status         | Status Message            |           |           |
|                        |  |                           |                   |                |                           |           |           |
| Publish                | Pause  |                           |                   |                |                           |           | Close CDL |



### Adding files to CDL

1. From the main CDL window, click **Files**  $\rightarrow$  **Add Files** (Figure 508)

| Figure 508 Add Files   |   |
|--|---|
| ChAS Database Loader   | × |
| <u>Elle</u> <u>Tools</u>   |   |
| Add Files Ctrl+A X LOH I Detected I ODetected I Gain (XON Region) I Loss (XON Region)                                  |   |
| Y     Segment Filters     Ctrl+F       Close CDL     Ctrl+W     sed.       Undetected. Not filtered. All will be used. |   |

An Explorer window appears. (Figure 509)

| Figure 5                  | 09 Add Fi  | es  |              |    |
|---------------------------|--|---|--------------|----|
| Open                      |  |   |              | ×  |
| Look In                   | n: <u>]</u> Result   | × 1   | 0.0.<br>0.0. |    |
| Recent<br>Desktop<br>Docu | <ul> <li>HTCMAV2_</li> </ul> | P40_A0000076_GT6_F01.rhchp i HTCMAv2_P40_A0012100_GT6_<br>P40_A0000091_GT6_F06.rhchp i HTCMAv2_P40_A0012103_GT6_<br>P40_A0000623_GT6_F12.rhchp i HTCMAv2_P40_A0012164_GT6_<br>P40_A0000819_GT6_F03.rhchp i HTCMAv2_P40_A0012165_GT6_<br>P40_A0000819_GT6_F04.rhchp i HTCMAv2_P40_A0012169_GT6_<br>P40_A0000943_GT6_F05.rhchp i HTCMAv2_P40_A0012174_GT6_<br>P40_A0000988_GT6_F02.rhchp i HTCMAv2_P40_A0012175_GT6_<br>P40_A0001076_GT6_F07.rhchp i HTCMAv2_P40_A0012178_GT6_<br>P40_A00012064_GT6_A07.rhchp i HTCMAv2_P40_A0012184_GT6_<br>P40_A0012097_GT6_D07.rhchp i HTCMAv2_P40_A0012185_GT6_<br>P40_A0012099_GT6_D03.rhchp i HTCMAv2_P40_A0012185_GT6_<br>P40_A0012099_GT6_D03.rhchp i HTCMAv2_P40_A0012185_GT6_ | Sample Info  |    |
| Comp                      |  |   |              |    |
|                           | File Name:   | D:\RHAS Data\CytoScan_H1CMA_P40_Default_20191024\Result   | Ope          | n  |
| Network                   | Files of Type:   | All Supported Types   | Canc         | el |
|                           |  | CYCHP<br>CYCHP<br>OSCHP<br>RHCHP<br>XNCHP<br>All Supported Types  |              |    |
|                           |  | C   | Close CDL    |    |

By default, the **Files of Type** is set to All Supported Types. (Figure 508) If you want to view a specific supported file type, click the drop-down, then select the file extension you want to display.

- 2. Single click, Ctrl click, Shift click or Ctrl a (to select multiple files).
- 3. Click Open.

Your selected files now appear in CDL's main window. (Figure 511)

Repeat steps **1-3** if you want to add files (up to 500) from different saved locations.



## Sample info 1. If you want to view the properties of the files displayed in the Explorer window, click Edit Configuration.

The Sample Info window appears. (Figure 510)

| Sample Info           |                         |                   |  |
|-----------------------|-------------------------|-------------------|--|
| Name                  | Date                    | Array Type        |  |
| HTCMAv2_P12_96F_CCL_5 | Feb 20, 2020 9:52:54 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_CCL_6 | Feb 20, 2020 9:52:55 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_CD000 | Feb 20, 2020 9:52:56 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_CD000 | Feb 20, 2020 9:52:58 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_CD000 | Feb 20, 2020 9:52:59 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA000 | Feb 20, 2020 9:53:01 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA000 | Feb 20, 2020 9:53:02 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA002 | Feb 20, 2020 9:53:04 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA004 | Feb 20, 2020 9:53:05 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA006 | Feb 20, 2020 9:53:07 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA008 | Feb 20, 2020 9:53:08 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA008 | Feb 20, 2020 9:53:09 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA008 | Feb 20, 2020 9:53:11 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA009 | Feb 20, 2020 9:53:12 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA015 | Feb 20, 2020 9:53:14 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA016 | Feb 20, 2020 9:53:15 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA017 | Feb 20, 2020 9:53:17 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA018 | Feb 20, 2020 9:53:18 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA019 | Feb 20, 2020 9:53:20 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA024 | Feb 20, 2020 9:53:21 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA025 | Feb 20, 2020 9:53:22 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA026 | Feb 20, 2020 9:53:24 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA027 | Feb 20, 2020 9:53:25 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA027 | Feb 20, 2020 9:53:27 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA031 | Feb 20, 2020 9:53:28 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA032 | Feb 20, 2020 9:53:30 AM | CytoScan HTCMA 96 |  |
| HTCMAv2_P12_96F_NA034 | Feb 20, 2020 9:53:31 AM | CytoScan HTCMA 96 |  |

- 2. Single click, Ctrl click, Shift click or Ctrl a (to select multiple files).
- 3. Click Open Selected Files.

Your selected files now appear in CDL's main window.

**Note:** A special icon is used to indicate a CHPCAR or "sidecar" file, as shown in Figure 511. For more information on sidecar files, "Editing segment data overview" on page 219.



| Figure 511 Main CDL window populated                                   |                 |                                |           |
|--|-----------------|--------------------------------|-----------|
| ChAS Database Loader   |                 |                                | ×         |
| <u>File T</u> ools   |                 |                                |           |
| ✓ Ø ▲ Gain Ø ▼ Loss ■ X LOH Ø ● Detected Ø • Undetect                  | ted 🗹 🗖 Gain () | KON Region) 🗹 🗖 Loss (XOI      | N Region) |
|  |                 |                                |           |
| Genome   |                 |                                |           |
| Gain. Not filtered. All will be used.                                  |                 |                                |           |
| Loss. Not filtered. All will be used.                                  |                 |                                |           |
| Undetected. Not filtered. All will be used.                            |                 |                                |           |
| LOH. Type turned off. Will be skipped.                                 |                 |                                |           |
| XON Region Levels. These levels will be used: 1                        |                 |                                |           |
| Gain (XON Region). No filters other than level filters.                |                 |                                |           |
| Loss (XON Region). No filters other than level filters.                |                 |                                |           |
| Add Files Export file list Import file list                            |                 | <mark>∢</mark> <u>C</u> lear ▼ |           |
| CHP File   | Status          | Status Message                 |           |
| HTCMAv2_P40_A0012100_GT6_G11.rhchp                                     |                 |                                |           |
| HTCMAv2_P40_A0012103_GT6_D02.rhchp                                     |                 |                                |           |
| HTCMAv2_P40_A0012164_GT6_E11.rhchp                                     |                 |                                |           |
| HTCMAv2_P40_A0012165_GT6_E10.mchp                                      |                 |                                |           |
| HTCMAv2_P40_A0012169_GT6_E12.rhchp                                     |                 |                                |           |
| HTCMAv2_F40_A0012174_GT6_C10.rhchp                                     |                 |                                |           |
| - THCMAV2_P40_A0012175_G16_D01.rhchp                                   |                 |                                |           |
| HICMAV2_P40_A0012178_G16_F09.mcnp                                      |                 |                                |           |
| HTCMAy2_F40_A0012185_GT6_D08.thchp                                     |                 |                                |           |
| HTCMAv2_P40_A0012187_GT6_C09 rbchp                                     |                 |                                |           |
| 99-1420 B2 Phase4CustomerPanel CytoScan PS 20110228.hg38.cvh           |                 |                                |           |
|  |                 |                                |           |
| 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_20110511.hg38.cyh             |                 |                                |           |
| Paralleline 11-0816_LC_ONC41B_A12_PoP#2_CytoScan-PS_20110511.hg38.cyhd |                 |                                |           |
| 26 files in list. 0 successfully published. 0 failed. 0 skipped.       |                 |                                |           |
| Pause  |                 |                                | Close CDL |

**IMPORTANT!** File level properties are optional fields that are available to CHP files analyzed in ChAS 3.0 or higher. Any file level properties entered are stored in the CHPCAR file, these properties will not populate in the CDL window. However, if those properties were entered for a CHP file and are contained in the CHPCAR file, they will be published to the database. Entries in a CHPCAR file supersede entries in the text file. File level properties for your xxCHP files can be added directly to the database after publishing has completed. For more details, see "Interacting with the ChAS database" on page 385.

#### Adding files to be published using a text file

Files can be loaded into CDL using a tab-delimited text file. Place the file names, including their paths in the first column, then label the first column header **CHP File** as shown in Figure 512.

| F  | igure 512 Tab-delimited text file Header 1 example   |
|----|--|
|    | А  |
| 1  | CHP File   |
| 2  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000076_GT6_F01.rhchp |
| 3  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000091_GT6_F06.rhchp |
| 4  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000623_GT6_F12.rhchp |
| 5  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000770_GT6_F03.rhchp |
| 6  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000819_GT6_F04.rhchp |
| 7  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000943_GT6_F05.rhchp |
| 8  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0000988_GT6_F02.rhchp |
| 9  | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0001076_GT6_F07.rhchp |
| 10 | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012064_GT6_A07.rhchp |
| 11 | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012097_GT6_D07.rhchp |
| 12 | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012099_GT6_D03.rhchp |
| 13 | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012100_GT6_G11.rhchp |
| 14 | D:\RHAS Data\CytoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012103_GT6_D02.rhchp |
| 15 | D:\RHAS Data\CvtoScan_HTCMA_P40_Default_20191024\Result\HTCMAv2_P40_A0012164_GT6_F11.rhchp |

1. Click on Import File List or click Files  $\rightarrow$  Import file list.

An Explorer window appears.

2. Navigate to, then select the tab-delimited text file containing path to the xxCHP files to be loaded into CDL.

### Publishing to the ChAS database

**IMPORTANT!** Before you use CDL to publish your files, it is highly recommended you backup your ChAS database first. For instructions on how to access and backup your database, refer to Chapter 21, "Database tools" on page 442. Also, xxCHP files can ONLY be published to a ChAS DB of the same genome version assignment.

| Testing your                   | Before publishing, you may want to test your ChAS database connection. To do this:   |
|--------------------------------|--|
| connection                     | 1. Click ChAS DB $\rightarrow$ ChAS Database Loader  |
| (optional)                     | 2. Click <b>Tools</b> $\rightarrow$ <b>Test Connection</b>   |
|                                | A message appears if there is a successful connection to the ChAS database.  |
| Verifying the<br>ChAS database | <ol> <li>From the ChAS Browser, click the <b>Preferences</b> drop-down menu, then select<br/>Edit Application Configuration</li> </ol> |
|                                | The <b>Configuration</b> window appears.   |
|                                | 2. Click the <b>Server</b> tab.  |
|                                | The Server window/tab appears. (Figure 513)  |
|                                |  |



| Fig | gure 513                      | Server window/tab |         |
|-----|-------------------------------|-------------------|---------|
| -   | Configurati                   | ion               | ×       |
|     | Connection                    | Server            |         |
|     | Scheme Hostname or IP Address |                   | Port    |
|     | http                          | localhost         | 8099    |
|     | Base API Pa                   | ath               |         |
|     | /api/                         |                   | Default |
|     | Full API UR                   | L                 |         |
|     | http://localhost:8099/api/    |                   |         |
|     | Full Web URL                  |                   |         |
|     | http://localho                | ost:8099/         |         |
|     |                               | OK Cancel         |         |

3. Verify the ChAS DB you are publishing to is correct, then click OK.

# Before publishing files

Before publishing files, you must check the Genome dialog box (Figure 514) to make sure your desired filter settings and data types are enabled.



**Note:** QC thresholds and Smooth/Joining settings in the ChAS Browser will be used when publishing xxCHP files using CDL. To use different QC thresholds and/or Smoothing and Joining settings, see "Setting QC parameters in the ChAS browser" on page 129.

Changing segment filters (optional)

- 1. Click on the Filter icon (or click Files  $\rightarrow$  Segments Filters).
  - The Segments Filters window opens. (Figure 515)



| Figure 515          | Segments Filters window | v     |
|---------------------|-------------------------|-------|
| Segments Filter     | s                       | >     |
| ✓ Genome            |                         |       |
| Whole Region        |                         | ſ     |
| Hide All Segm       | ents In This Region     |       |
| <b>▲</b> Gain       |                         |       |
| Marker Count        | 50                      |       |
| Size (kbp)          | 50 0                    |       |
|                     | 0                       | 2000( |
| Overlap             | 100                     | 0     |
| ▼ Loss              |                         |       |
| Marker Count        | 25 0                    | 500(  |
| Size (kbp)          | 25 0                    |       |
| Overlap             | 100                     |       |
| XON Region Lev      | els (Gain and Loss)     |       |
| Level 1 Level 1     | evel 2 Level 3 Level 4  |       |
| -<br>→ Gain (XON Re | egion)                  |       |
| Marker Count        | 0                       | 500(  |
| Size (kbp)          |                         | 2000( |
| Overlap             | 100                     | 0     |
| + Loss (XON Re      | egion)                  |       |
| Marker Count        | 0                       | 500(  |
| Size (kbp)          |                         | 20001 |
| Overlap             | 100                     |       |
| XI OH               | 100                     | 0     |
| Marker Count        |                         |       |
|                     | 0                       | 5001  |

- 2. Update the appropriate filters using the provided check boxes, text boxes and sliders.
- 3. Click **X** to save your changes and close the window.

### 1. Use the Segments Filters window (Figure 515) to click the check box of the data type(s) you want to publish.

- 2. Click X to save your changes and close the window
- 3. Review the Genome dialog window (Figure 514) again to make sure your data types to be published are displayed.

#### Managing data types (optional)



## Publishing your files

 Check your table before publishing, as all displayed files are published. Note: If there are specific files you do not want published, single click, Ctrl click, or Shift Click to highlight them, click the Clear drop-down menu, then click Clear Selected. (Figure 516)

| Figure 516 Selecting files not to publish  |        |                          |                         |           |  |  |
|--|--------|--------------------------|-------------------------|-----------|--|--|
| ChAS Database Loader   |        |                          |                         | ×         |  |  |
| File Tools   |        |                          |                         |           |  |  |
| Image: Second secon |        |                          |                         |           |  |  |
| Genome   | Genome |                          |                         |           |  |  |
| Gain. Marker Count ≥ 50, Size (kbp) ≥ 50.         Loss. Marker Count ≥ 25, Size (kbp) ≥ 25.         Detected. Not filtered. All will be used.         Undetected. Type turned off. Will be skipped.         LOH. Type turned off. Will be skipped.         XON Region Levels. These levels will be used: 1         Gain (XON Region). No filters other than level filters.         Loss (XON Region). No filters other than level filters.   |        |                          |                         |           |  |  |
| Add Files Export file list Import file list  |        | <u>×</u> <u>C</u> lear ▼ |                         |           |  |  |
| CHP File   | Status | Clear All                | ssage                   |           |  |  |
| HTCMAv2 P40 A0000076 GT6 F01.rhchp   |        |                          | 5                       | $\cap$    |  |  |
| HTCMAv2 P40 A0000091 GT6 F06.rhchp   |        | Clear Selected           |                         |           |  |  |
| HTCMAv2 P40 A0000623 GT6 F12.rhchp   |        | Clear Published Cl       | ear selected files from | m table   |  |  |
| PHTCMAv2 P40 A0000770 GT6 F03.rhchp  |        |                          |                         |           |  |  |
| PHTCMAv2 P40 A0000819 GT6 F04.rhchp  |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0000943 GT6 F05.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0000988 GT6 F02.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0001076 GT6 F07.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0012064 GT6 A07.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0012097 GT6 D07.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2_P40_A0012099_GT6_D03.rhchp   |        |                          |                         | U         |  |  |
| HTCMAv2 P40 A0012100 GT6 G11.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0012103 GT6 D02.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2 P40 A0012164 GT6 E11.rhchp   |        |                          |                         |           |  |  |
| HTCMAv2_P40_A0012165_GT6_E10.rhchp   |        |                          |                         |           |  |  |
| 22 files in list. 0 successfully published. 0 failed. 0 skipped. <b>Publish</b>  |        |                          |                         | Close CDL |  |  |

**IMPORTANT!** You must have ChAS DB Manager or Admin privileges before you can publish. For information on setting up ChAS DB role assignments, see "Administration" on page 451.

2. Click **Publish**.

A Publish? window appears. (Figure 517)



| Figure 517 Publish? window |  |  |  |  |  |
|----------------------------|--|--|--|--|--|
| Publish                    | Publish?   |  |  |  |  |
|                            | Before uploading new sample data to the database, create a Backup of the current database using the Database Tools option in ChAS Browser. |  |  |  |  |
|                            | Please check that the Data Processing, QC thresholds and Segment Filters are set as desired.   |  |  |  |  |
|                            | Do not close, modify or uninstall the ChAS Database Loader during the publishing process.  |  |  |  |  |
|                            | I have created a backup of my database, checked my settings and am ready to proceed.   |  |  |  |  |
|                            | OK Cancel  |  |  |  |  |

- 3. Acknowledge the message, click to check its check box, then click **OK**.
- 4. An **Overwrite?** message may appear. (Figure 518) Click the appropriate button to continue.

| ? message   |                               |  |  |  |  |
|---|-------------------------------|--|--|--|--|
|   | ×                             |  |  |  |  |
| Overwrite previously published files in the database? |                               |  |  |  |  |
|   | usly published files in the d |  |  |  |  |

The publishing process begins. (Figure 519)



| Figure 519 Publishing in progress example                          |                   |                       |           |
|--|-------------------|-----------------------|-----------|
| ChAS Database Loader   |                   |                       | ×         |
| <u>File Tools</u>  |                   |                       |           |
| ▼  | Gain (XON Region) | Loss (XON Region)     |           |
|  |                   |                       |           |
| Genome   |                   |                       |           |
| Gain. Marker Count ≥ 50, Size (kbp) ≥ 50.                          |                   |                       |           |
| Loss. Marker Count $\geq$ 25, Size (kbp) $\geq$ 25.                |                   |                       |           |
| Detected. Not filtered. All will be used.                          |                   |                       |           |
| Undetected. Type turned off. Will be skipped.                      |                   |                       |           |
| XON Region Levels. These levels will be used: 1                    |                   |                       |           |
| Gain (XON Region). No filters other than level filters.            |                   |                       |           |
| Loss (XON Region). No filters other than level filters.            |                   |                       |           |
| Add Files Export file list Import file list                        | Clea              | ar 🔻                  |           |
|  | Status            | Status Massaga        |           |
| Chi File   |                   | 25 segments published | 0         |
| HTCMAy2_140_A0000070_GT0_101.inclip                                |                   | 35 segments published |           |
| C HTCMAy2 P40_A0000623_GT6_E12 rbchp                               |                   | OC test failed        |           |
| PHTCMAv2_P40_A0000770_GT6_E03.rbchp                                |                   | 23 segments published |           |
| TCMAv2 P40 A0000819 GT6 F04.rhchp                                  | ✓ PUBLISHED       | 35 segments published |           |
| HTCMAv2 P40 A0000943 GT6 F05.rhchp                                 | <b>PUBLISHING</b> |                       |           |
| HTCMAv2_P40_A0000988_GT6_F02.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0001076_GT6_F07.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012064_GT6_A07.rhchp                                 |                   |                       | U         |
| HTCMAv2_P40_A0012097_GT6_D07.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012099_GT6_D03.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012100_GT6_G11.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012103_GT6_D02.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012164_GT6_E11.rhchp                                 |                   |                       |           |
| HTCMAv2_P40_A0012165_GT6_E10.rhchp                                 |                   |                       |           |
| * 26 files in list. 4 successfully published. 0 failed. 1 skipped. |                   |                       |           |
| Publish Pau <u>s</u> e   |                   |                       | Close CDL |

To pause the publishing process, click **Pause**. While in pause mode, you can add more files to the table, as described in "Adding files to CDL" on page 458.

After the publishing process is complete, each **Status** column is marked with a result icon.

- The file was successfully published to the ChAS database.
- The file was skipped over and not published, because it was already found in the database or it did not meet the assigned QC thresholds.
- **•**  $\mathbf{X}$  = The file failed and was not published.

**Note:** Refer to the table's Status Message column (Figure 519) for details regarding a skipped or failed file.



### **Clearing Table Data**

After clicking **Clear Published**, files with a Skipped or Failed status remain in the table. Click **Export properties...** to export these files for further investigation.

 $\label{eq:ClearAll} Click \ \ Clear \ \ All \ to \ remove \ all \ files \ from \ the \ table.$ 



### **Closing CDL**

1. Click Close CDL or click File  $\rightarrow$  Close CDL.



# **Analysis parameters**

### Analysis parameters for single sample analysis

copynumber-cyto.html

For information on CytoScan algorithm parameters and their values, go to: http://media.affymetrix.com/support/developer/powertools/changelog/aptcopynumber-cyto.html

#### Reference model file creation

Reference Model File Creation is done with fixed parameters in the Reference Creation workflow.

It is essential that the input for Reference Model Creation include at least 44 total CEL files and at least 20 males and 20 females.

There are no user-adjustable parameters for the Reference Model File Creation

For information on CytoScan algorithm parameters and their values, go to: http://media.affymetrix.com/support/developer/powertools/changelog/apt-
# **AED** file format



Affymetrix Extensible Data (AED) files contain data that annotate positions on a genome. AED allows custom, typed fields, can be edited in the ChAS Browser's AED Editor feature ("Viewing and batch editing AED file contents" on page 302), and supports internationalization.

This appendix covers the formatting and use of Affymetrix Extensible Data (AED) files with ChAS.

- "AED file description"
- "Compatibility"
- "References"

AED files can be created by Chromosome Analysis Suite (ChAS) and loaded into ChAS as region information files to:

- Define CytoRegion and Overlap Map regions
- Record information of interest about features in the genome

AED files can be produced and edited in:

- ChAS (Recommended)
- Text-editing software (Not Recommended)
- Spreadsheet software such as Microsoft Excel (Not Recommended)

#### **AED** file description

An AED file contains a list of annotations, descriptions of features on a biological sequence such as a chromosome. This description is comprised of several properties—either properties defined by this specification, such as the annotation start and stop positions; or properties defined by users or third parties.

An AED file may also provide **metadata** which describe the particular group of annotations in the file as a whole, such as the author of the file or the genome assembly for which the annotations were produced.

Properties and metadata have certain types which define the semantics and constrain the range of values they may have. Properties should begin with a lowercase letter, while types should begin with an uppercase letter.

The AED file format uses a tab-delimited text format with the **\*.aed** file extension. It uses Unicode character sets and has the following components: (Figure 521)

| Figure 521  | AED file in Excel w        | ith required he        | ader fields fo       | or properties and r         | netadata   |
|-------------|----------------------------|------------------------|----------------------|-----------------------------|--|
|             | Regions_03_02.aed          |                        |                      |                             | - = X  |
|             | A                          | В                      | С                    | D                           | E  |
| Header Row  | 1 bio:sequence(aed:String) | bio:start(aed:Integer) | bio:end(aed:Integer) | aed:name(aed:String)        | aed:value(aed:String)                              |
|             | 2                          |                        |                      | aed:application(aed:String) | Chromosome Analysis Suite CytoB-N1.0.0.284 (r1764) |
|             | 3                          | Metadat                | а                    | aed:created(aed:DateTime)   | 2009-03-02T11:06:40.517-08:00                      |
|             | 4                          |                        |                      | aed:modified(aed:DateTime)  | 2009-03-02T11:06:40.517-08:00                      |
|             | 5 chr1                     | 110034485              | 110041690            | seg10                       |  |
|             | 6 chr1                     | 112497197              | 112507652            | seg12                       |  |
| Annotations | 7 chr4                     | 66574165               | 66587983             | seg126                      |  |
|             | 8 chr4                     | 69102606               | 69592855             | seg130                      |  |
|             | 9 chr4                     | 68970721               | 68970726             | seg128                      |  |
|             | 10 chr4                    | 70177951               | 70276607             | seg132                      |  |
|             | 11 chr5                    | 102163811              | 102287067            | test_variation              |  |
|             | 12 chr5                    | 102622340              | 102642260            | New_Gene                    |  |
|             | 13                         |                        |                      |                             |  |
|             | 14<br>                     |                        |                      |                             | n n n n n n n n n n n n n n n n n n n              |

**IMPORTANT!** AED supports only Unicode, which can be stored in one of various encodings (charsets such as UTF-8, UTF-16LE, and UTF-16BE). The AED file indicates the charset with an initial Byte Order mark (BOM). An AED file with no initial BOM is not recommended. An AED file that does not begin with a BOM will be interpreted as containing only the ASCII subset of Unicode, resulting in an error if any characters lie outside the range of ASCII. (With no indication of a charset, it is not possible to determine which non-ASCII characters were intended. File formats such as BED that make assumptions about non-ASCII characters have the potential to corrupt data when transported between systems.)

- "Header row": Names the properties that can be used in the annotations
- "Metadata records" on page 471 Optional: Provides information about the AED file itself and the group of annotations it contains.
- "Annotations Rows" on page 472: The annotation row displays values for the properties listed in the header rows (for each feature that is annotated).

## **Header row** The header row of an AED file is a tab-delimited list of the properties that can be used to describe a region of the genome.

Each AED file header represents a property. Normal records in the file represent annotations, and the record fields represent annotation properties. Special metadata records represent metadata properties for the file as a whole, rather than for a particular annotation.

A property name has the following format:

namespacePrefix:propertyIdentifier(namespacePrefix:TypeIdentifier)

- namespacePrefix
- A namespacePrefix is optional. It assigns the property or type to a vocabulary grouping called a namespace. The lack of a namespace prefix indicates that the property has been created by a user and is not part of the formal AED specification.

The lack of a namespacePrefix indicates that the property is in the default/ custom namespace; this namespace enables users to add properties to an annotation just by adding new columns, such as foo(aed:String) or bar(aed:Integer).

- propertyldentifier
  - The propertyldentifier names the property that the values in the column are for.

Each annotation can be assigned an unlimited number of properties. Each property has a certain meaning, and this meaning is usually defined by the documentation for the property namespace. The purpose of the AED file is to indicate values for certain properties for each annotation. For example, by use of the aed:name(aed:String) column, the AED file indicates a string value to be used as the name each annotation).

- Typeldentifier
  - The TypeIdentifier (always capitalized) specifies the data type of the value to be used for the property in the AED file. Examples include:
    - bio:sequence(aed:String)
    - aed:value(aed:String)
    - medianMarkerDistance(aed:Integer)
- Required Fields
  - Fields may appear in any order, except that the following predefined fields must always appear in the following order at the beginning of the header:
    - bio:sequence(aed:String)
    - bio:start(aed:Integer)
    - bio:end(aed:Integer)
    - aed:name(aed:String)
    - aed:value(aed:String) Optional: You must use this property if you are including metadata information in the file.

**IMPORTANT!** ChAS verifies the property types when importing an AED file. If a file header specifies a known property, but includes an incorrect data type for the property, the file will not be loaded. For example, "fish:score" is a known property with "whole number" data type. An AED file header that specifies "fish:score(aed:String)" would be treated as an error.

**Metadata records** Some records, instead of providing annotation about a location on a genome assembly, provide metadata information about the AED file itself (Figure 522). These metadata records are identified by the presence of an empty string in the **bio:sequence** field. The **bio:start** and **bio:end** fields must also be left blank for metadata records. If there are metadata records present, the **aed:value** field is required.

In a metadata record, the value in the **aed:name** field is interpreted as the name of the metadata property, with type identification rules identical to those of the header fields. The value in the **aed:value** field is interpreted as the value of the metadata property, and the characters that make up its string value must follow the lexical and semantic rules specified by the type indicated in the **aed:name** field.

| Figure 522 Metadata entries  | Metadata property names     | Metadata property values                            |  |
|--|-----------------------------|---|--|
|  | Metadata property names     | Metadata property values                            |  |
| bio:sequence(aed:String) bio:start(aed:Integer) bio:end(aed:Integer) | eger) aed:name(aed:String)  | aed:value(aed:String)                               |  |
| Blanks for bio:sequence and other properties                         | aed:application(aed:String) | Chromosome Analysis Suite CytoB-N1.0.0.284 (r1764)  |  |
|  | aed:created(aed:DateTime)   | created(aed:DateTime) 2009-03-02T11:06:40.517-08:00 |  |
|  | aed:modified(aed:DateTime)  | 2009-03-02T11:06:40.517-08:00                       |  |

All other metadata record fields should be blank.

| Annotations<br>Rows | The rows below the Metadata properties are the annotations. Each row is a tab-<br>delimited list of values. Each value must have the correct data type, as described in<br>the property name for that value |
|---------------------|---|
|                     | the property name for that value.   |

### Compatibility

#### UCSC Browser Extensible Data (BED)

The BED file format, developed at UCSC, is widely used for transfer of simple region coordinates. However, the format has been interpreted and implemented in multiple ways by various software within and outside of UCSC. Some implementations require a TAB delimited format, others require a space-delimited format, and still others accept both. Characters outside of the ASCII character set are not well supported. We created the AED format with very strict and explicit definitions so as to avoid some of these compatibility issues.

Although the AED format is preferred, ChAS supports both the import and export of data in BED format. When exporting data in BED format, ChAS exports only the basic 4-column tab-delimited BED format containing the position and name of each item. If the names of any of your items contain spaces or non-ASCII characters, there is no guarantee that all programs will be able to interpret those names correctly.

When importing data in BED format, ChAS supports the reading of BED files with anywhere from 4 to 12 columns.

- The file must be TAB delimited
- Only ASCII characters should be used
- The values for thickStart and thickEnd will be ignored for display purposes
- The value for itemRgb will be honored for display purposes
- The values for blockCount, blockSizes and blockStarts can be used to import and display data with intron/exon structure, such as genes.
- Formatting rules in the BED header are ignored
- BED files containing multiple tracks are not supported; use a separate BED file for each track.

The UCSC Browser, as well as ChAS, uses the strict definition of BED where chromStart is not allowed to be greater than chromEnd. ChAS will accept import of BED files even if this convention is violated, but will auto-correct and export BED files properly with chromStart  $\leq$  chromEnd.

AED has been structured to facilitate as much as possible migration of data rows to and from BED. Starting with existing AED and BED files, data records from AED may be transferred to BED by using:

- The "Export" function from inside ChAS (recommended)
- A text editor (not recommended) if the AED files are first prepared in the following manner:
- Remove all fields except for bio:sequence, bio:start, bio:end, and aed:name.
- Ensure that no non-ASCII characters are included. (The treatment of non-ASCII characters by a BED processor is undefined.)
- Ensure that no name contains whitespace characters.
- Data rows from the first four columns of a BED file can be transferred to AED with no constraints as long as the columns are delimited by TAB.

| Microsoft Excel<br>and other<br>spreadsheet<br>applications | Though not recommended, an AED file may be edited using most spreadsheet<br>programs that support tab-separated value (TSV) files and that recognize a byte order<br>mark (BOM). An AED file can be edited in Microsoft Excel, for example, using the<br>following rules:<br>When loading an AED file into Microsoft Excel as a TSV file, make sure that the<br>Unicode code page for the correct encoding is selected (preferred), or accept the<br>default "Windows (ANSI)" code page (which should still recognize Unicode characters<br>if the correct BOM is present in the file).<br>When saving an AED file from Microsoft Excel, make sure the "Unicode Text" type is<br>selected. This will result in a file encoded in UTF-16LE, which is still a valid AED file as<br>it begins with the appropriate BOM. |
|---|--|
| Microsoft<br>Notepad and<br>other Text editors              | Though not recommended, an AED file may be edited by any text editor that supports<br>Unicode and that uses a byte order mark (BOM) to indicate the charset. The version<br>of Microsoft Notepad in Windows XP, for example, will both correctly read text files<br>marked with a BOM and save text files using the appropriate BOM if the following rules<br>are followed:<br>When saving an AED file from Microsoft Notepad, make sure the encoding is set to<br>"UTE-8" or "Unicode".   |
|   | For other text editors, make sure the correct preferences are set both to recognize and write BOMs for files.  |
|   | Text Editors   |
|   | EmEditor <http: www.emeditor.com=""></http:> is a commercial text editor that has extremely good Unicode and BOM support, and is able to open up gigantic text files.  |
|   | PSPad <http: www.pspad.com=""></http:> is a free text editor that has particularly extensive Unicode and BOM support and is available in many localizations.   |
|   | UniPad <http: www.unipad.org=""></http:> is a shareware text editor that correctly handles<br>Unicode and BOM, and provides a wide range of built-in glyphs for representing<br>Unicode code points that cannot be viewed on most other text editors.  |
| References  |  |
|   | <b>ISO 8601:</b> ISO 8601:2004(E): <i>Data elements and interchange formats — Information interchange — Representation of dates and times.</i> International Organization for Standardization, 2004-12-01.   |
|   | Microsoft Byte Order Mark: http://msdn.microsoft.com/en-us/library/<br>ms776429(VS.85).aspx  |
|   | <b>RFC 3986:</b> <i>RFC 3986: Uniform Resource Identifier (URI): Generic Syntax.</i> T. Berners-Lee, R. Fielding, and L. Masinter. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc3986   |
|   | <b>RFC 4122:</b> <i>RFC 4122: A Universally Unique IDentifier (UUID) URN Namespace</i> . P. Leach, M. Mealling, and R. Salz. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc4122   |
|   | <b>RFC 4180:</b> <i>RFC 4180: Common Format and MIME Type for Comma-Separated Values</i> ( <i>CSV</i> ) <i>Files.</i> Y. Shafranovich. Internet Engineering Task Force, 2005. http://tools.ietf.org/html/rfc4180   |

Unicode Byte Order Mark FAQ: http://unicode.org/faq/utf\_bom.html



# ChAS browser NetAffx Genomic Annotations

## Homo Sapiens database files

NetAffx Genomic Annotation files

#### Source of content

- NetAffx Genomic Annotation files are used by the ChAS Browser to display recent snapshots of genomic annotations downloaded from public databases.
- The UCSC Genome Browser is the source of the data that populates the following Browser tracks: Genes, Ensembl, Segmental Duplications, sno/miRNA, and Cytobands. UCSC was also the source of OMIM data for ChAS Browser file versions NA31-NA33.1 and 32.1. NA33.2 and NA36 no longer contain annotation information for BACs and FISH Clones.
- The Database of Genomic Variants is the source of the data displayed in the DGV track.
- The ClinGen Resource is the source of the data displayed in the Triplosensitivity, Haploinsufficiency, and Recurrent/Curated Regions tracks.
- The OMIM database (with curation and processing assistance from UCSC and NCBI) is the source of the data displayed in the OMIM Genes, OMIM Phenotype Loci, and OMIM Region Phenotype Loci tracks.

**IMPORTANT!** Starting with ChAS v3.3, the naming convention of the NetAffx Genomic Annotation database file will change. This file will now include a date (as opposed to a specific NetAffx build number) and will be updated more regularly than its current schedule of being updated with each software release. The new naming convention is as follows: **NetAffxGenomicAnnotations.Homo\_sapiens.hg38.naYYYYMMDD.db** 

For optimal use of the Segment Prioritization methods described in Chapter 17, "Prioritizing segments" on page 368, you must download the NetAffx Genomic Annotation files released with ChAS v4.5 or use more current ones when available.

**IMPORTANT!** It is VERY highly recommended to confirm findings obtained using the ChAS Browser's NetAffx Genomic Annotations file contents by linking out to external databases using the ChAS software coordinates for the most current annotation information. See "Linking to external websites" on page 209.



# Genomic position coordinates

There are multiple conventions and file formats to describe locations in chromosomal DNA sequences. This appendix describes a few issues that relate to ChAS.

#### Genome assemblies

First, it is important to know which set of DNA sequences is being used as the reference. For the human genome, the reference assembly is available for download from public sources such as UCSC and Ensembl. Those two sites currently use identical genome assemblies, but refer to them by different names. UCSC uses names such as "hg18", "hg19" and "hg38". The identical genome assemblies are known as "NCBI36", "GRCh37"and "GRCh37"at Ensembl. Assemblies at NCBI can have a decimal point as well, for example, "36.3" or "37.1". For positions on the chromosomes 1-22, X and Y, there is no difference between assemblies "36.1", "36.2" and "36.3" and we expect the same will be true for future "point" releases.

#### SNP and marker positions

When referring to individual positions on a chromosome, such as the positions of SNPs, it is sufficient to give a single coordinate. There are different conventions about whether to consider the first DNA base pair on the chromosome as position 0 or position 1.

For SNP marker positions, all of the following consistently use a 1-based index position coordinate: CYCHP files, CNCHP files, NetAffx detail pages for SNP markers, NCBI pages for SNP positions of dbSNP entries, and the Graphs Table in ChAS.

Consider the (randomly-chosen) SNP marker"S-3SRJC" from the CytoScan HD array. This marker is designed to correspond to the SNP with ID "rs4376202" in the dbSNP database. The NCBI website reports the position as chr4:1822637 on GRCh38. On the NetAffx website, the identical coordinate is also given for this SNP. The same coordinate value is given in CYCHP files and in the ChAS graphs table. Refer to http://www.ncbi.nlm.nih.gov/SNP/snp\_ref.cgi?type=rs&rs=7641618 for this particular example.

For copy number markers which are not based on SNP positions, we continue to use a 1-based index position. For these markers, we continue to use a 1-based index position. Unlike the case for SNPs, there is no particular single base pair that the marker corresponds to. The convention in CYCHP files is to use the position of the first DNA pair corresponding to the position where the marker hybridizes with the DNA. When two or more markers have the same start position on a chromosome, the coordinate of one of them will be shifted by one or occasionally a few more bases such that each marker is reported at a unique position.



## Segment positions

BED and AED file formats are used for storing and sharing region files between software. The BED format was created by UCSC for use with their genome browser, and is also used in other software. The AED format was created by Thermo Fisher Scientific for use with ChAS and possible future software, but used the BED format as a starting point.

The BED file format is explicitly defined to use a 0-based coordinate system where the second column (chromStart) in the file is the position of the first base-pair and the third column (chromEnd) is the position of the last base-pair plus one. Another way of saying this is that the start index is inclusive and the end index is exclusive. As an example, to refer to the first 100 based on the chromosome, you would use chromStart=0 and chromEnd=100. The length of any region is always given simply by chromEnd minus chromStart.

The UCSC browser strictly requires that chromStart not be larger than chromEnd. In order to support file outputs from non-conforming programs, ChAS will accept BED files where chromStart > chromEnd. It will simply switch those two coordinates and act as if the coordinates were given in the correct order.

Since a SNP has, by definition, a length of one base-pair, the proper way to represent a SNP position is with chromEnd = chromStart + 1. The UCSC browser does allow chromStart to be equal to chromEnd. But this is used for representing insertion points, and is not used to represent SNP positions. Because the AED format was intended to be compatible with BED format, we use the same coordinate system.

For example, suppose there are three markers with the following positions on a chromosome given in the CYCHP file: Marker A at 1000, Marker B at 2000, Marker C at 3000. Marker positions in the CYCHP file are 1-based index positions. To represent these in a BED file, we would need a file like this:

- Chr3 999 1000 markerA
- Chr3 1999 2000 markerB
- Chr3 2999 3000 markerC

If there were a segment starting at markerA and ending at markerC, we would need to represent it in a BED or AED file as:

Chr3 999 3000 segment\_1

# **Editing BED files**



A BED file is essentially a tab-delimited text file, as shown in Figure 523.

#### Figure 523 Example BED file File Edit Format View Help # Sample BED File. # # UCSC Genome Browser and ChAS should both accept this file. # Lines beginning with "#" are ignored. # # The UCSC genome version is hg38 # The columns are: # Chromsome Min Max Name # # The "browser" line is ignored by ChAS, used by UCSC Genome Browser # # The "track" line is used by both ChAS and UCSC Genome Browser. # ChAS ignores all parameters except "db". # UCSC Genome Browser also uses "name", "description" and others. # Specify genome version on the track line as: db="hg38" hg version # browser position chr6:160460899-160501368 track db ("hg38") name="MyBEDFILE" description="IMAGE306 2022-06-07T11:55:07.782-07:00" 818811 3685376 Gain3.0\_H22000776r3.hg38.OSCHP chr1 6579786 7622177 Gain3.0\_H22000776r3.hg38.0SCHP chr1 10330637 12770000 Gain2.5\_Sample1.hg38.OSCHP chr1 121852400 83550636 Gain4.0\_Sample1.hg38.OSCHP chr1 chr1 143549777 248918679 Gain6.0 Sample1.hg38.OSCHP chr2 21493 10668078 Gain6.0\_Sample1.hg38.OSCHP 10678600 17653487 Gain4.0\_Sample1.hg38.OSCHP chr2

Using a text editor such as MS WordPad and MS NotePad (not a spreadsheet application like Microsoft Excel) to edit BED files is recommended and work well.

Editing a BED file using a spreadsheet application such as Excel is not recommended because these programs may not preserve the correct BED file format. For example, when exporting data from Excel into tab-delimited text, Excel may add quotation marks around some text, which would cause the file to be invalid and unusable with ChAS or other applications.

There is no easy way to prevent Excel from adding extra quotation marks which corrupt the output. Advanced Excel users can use macro programming to create special output formats. Other options include:

Do not use Excel to edit BED files. You may use a text editor, but be certain to separate the columns with TAB characters and do not use non-ASCII characters. The BED format was not designed with such characters in mind; therefore, problems may occur when you try to share these files with others. ChAS will reject BED files containing non-ASCII characters, and will never export non-ASCII characters into a BED file.

- After exporting a BED file from Excel, edit the file in another application to remove extra quotation marks.
- Use AED format for the data, then use ChAS to export to BED format if needed.
- Be careful to create a BED file that does not cause Excel to add quotation marks. Do not include the itemRgb column, quotation marks, or special characters in a track line. For example, the following is an acceptable track line: Track db=hg19 name=My\_Track description=This\_is\_my\_data



# CytoScan algorithms and QC metrics

## Algorithm overview

|  | This section provides a high level overview of how copy number calls are generated within the software. The copy number workflow starts with the intensities on the array, include normalization and scaling, reference set ratios, Log2 transformation, CN state segmentation, and how CN segment calls are made.<br><b>Note:</b> For CytoScan HTCMA algorithm and QC, see the RHAS User Guide.  |
|--|---|
| Feature<br>identification and<br>signal extraction | GeneChip Cartridge Microarrays are scanned on the GeneChip Scanner and<br>processed by the GCC scanner software package. GCC aligns a grid on the DAT file<br>(the original scanned image) to identify each microarray feature and calculates the<br>signal from each feature. This process uses the DAT file, containing the raw signal, and<br>creates a CEL file, which contains a single signal intensity for each feature. The CEL<br>file is used for all downstream analyses.  |
| Single sample<br>CytoScan<br>workflow              | Beginning with the raw signal data in the CEL file, the Single Sample CytoScan<br>Workflow implements a series of steps that perform probe set summarization,<br>normalization, removal of variation caused by known properties and residual variation,<br>and completing with calling genotypes, copy number segments and LOH segments.<br>A brief overview of each step performed by the CytoScan Workflow is shown in<br>Figure 525 on page 481. In addition, a rough sketch of Analysis Pipeline (for Single<br>Sample Analysis) is demonstrated in Figure 525 on page 481. |

XE









The first level of covariate adjustors operate on the raw signal.

#### Fragment adapter covariate adjustor

After the Nsp I restriction digest, an Nsp I-specific adaptor is ligated onto the cohesive end termini. Since Nsp I is a 6-nucleotide cutter with degenerate sites, meaning that they contain one or more base pairs that are not specifically defined, these ends are of various sequences and the ligated adaptors are a variety of sequences. The exact sequences of the cut site and ligation adaptor have an effect on the overall efficiency of ligation and subsequent PCR amplification. The Adaptor Covariate Adjustor corrects for these differences by normalizing the signals for each adaptor/cut site sequence class to an overall median.

#### Fragment length covariate adjustor

The length of each Nsp I fragment impacts the efficiency of PCR amplification and therefore the signal. Fragments of 300-500 bp are amplified with the highest efficiency and the degree of amplification tapers off as the fragments get longer. The Length Covariate Adjustor corrects for these differences by normalizing the signals for a series of fragment size bins to an overall median.

#### Dual quantile normalization

Dual quantile normalization is simply a two-phase process where probes used for copy number detection and probes used for SNP genotype detection are normalized separately. In both cases, a normalization sketch is built using the autosomal probes in the reference set. The normalization sketch is the prototype distribution of probe intensities that defines what this distribution looks like for all arrays. The single sample autosomal probes are fit to the sketch and the X and Y probes are interpolated into the distribution.

Quantile normalization makes the assumption that the distribution of probes on the array is fairly consistent from array to array. Since the X-chromosome is one of the largest chromosomes (155Mbp, ~5% of the genome), differences between males and females would stretch this assumption. That is why the quantile normalization focuses on creating an autosomal sketch and normalizing the autosome to it. The X and Y chromosome probes are then handled in a special way. Each of them is matched to the closest pre-normalization signal value. Based on that match, their normalized signal should be close to the signal for the very same autosomal probe. So the normalized values for X and Y probes are simply "looked-up" in the pre-normalization autosomal sketch, and transformed to the post-normalization value.



## Copy number workflow

| Log2 ratio<br>calculation                     | Log2 Ratios for each marker are calculated relative to the reference signal profile. The Log2 Ratio is simply Log2(sample <sub>m</sub> ) – Log2(reference <sub>m</sub> ), for each marker, "m".  |
|---|--|
| High pass filter<br>image correction          | Since most probes map to genomic markers associated with a normal copy number,<br>most Log2 Ratios should be centered at a value of zero. Also, since markers from any<br>genomic region are scattered across the surface of the microarray, regions of altered<br>copy number will not appear as regional changes on the microarray image.  |
|   | Some samples do reveal spatial trends away from zero that are gradual and this spatial bias when scattered back across the genome exhibits itself as added noise in the Log2 Ratios. The High Pass Filter Image Correction identifies these gradual spatial trends and adjusts Log2 Ratios to remove the spatial bias and lower the level of noise.Log2 Ratio-Level Covariate Adjustors  |
| Log2 ratio-level                              | Super GC covariate adjustor  |
| covariate<br>adjustors                        | The GC content of genomic DNA sequence impacts probe signal dose-response and therefore probe Log2 Ratios. The sequence GC content of the microarray probe impacts hybridization kinetics. In addition, the genomic GC content of the Nsp I fragment and the 500 kbp surrounding the probe (local GC) all impact the efficiency of target preparation in the genomic region of each probe. The super GC covariate adjustor combines the probe GC content, the fragment GC content and the local GC content into one covariate that corrects for Log2 Ratio differences based on the combination of GC contents associated with each probe. |
|   | Reference intensity covariate adjustor   |
|   | Probes in different intensity categories have different dose responses in Log2 Ratio space. Using the Reference Set probes to define bins based on probe intensity, the single sample probes are binned and the median of the distribution of Log2 Ratios within each bin is adjusted to the median Log2 Ratio of the corresponding bin from the reference set.  |
|   | Marker Type Covariate Adjustor   |
|   | Polymorphic probes designed for SNP detection and non-polymorphic probes designed for copy number detection have different properties and different dose responses. The Marker Type Covariate Adjustor normalizes the median Log2 Ratios of SNP and CN markers to account for differences in Log2 Ratios between the two groups.   |
| Median<br>Autosome<br>normalization           | This final level of normalization simply shifts the median Log2 Ratio of the autosomes to a copy-number state equal to 2, i.e. a Log2 Ratio of 0.  |
| Systematic<br>residual variability<br>removal | Even after all of the Covariate Adjustors, there is some residual variation with unknown origins. During product development we have introduced variation into the protocol in an attempt to capture other forms of unanticipated variation. The Systematic Residual Variability Removal step matches sample variability to the residual variability of the reference set, and when matched, corrects the data to remove the residual.   |



Segmentation Copy Number Calls for each Marker based on Log2 Ratios

For CytoScan arrays, markers are individually assigned a copy number call by a Hidden Markov model (HMM). The sample specific inputs to the HMM are the Weighted Log2 Ratios generated by the Signal Restoration module.

The weighted Log2 Ratios are centered on copy number (CN) = 2. In theory, when Log2 Ratio = 0 then CN = 2, when Log2 Ratio = -1 the CN = 1, etc. In truth, microarrays, or any hybridization-based technology, exhibit Log2 Ratio compression due to many factors, so the Log2 Ratios never exhibit the amplitude expected by the math. The following table shows theoretical and actual Log2 Ratios for different Copy Number States.

| Copy Number Truth | Theoretical Log2 Ratio | Actual Log2 Ratio |
|-------------------|------------------------|-------------------|
| 1                 | -1                     | -0.45             |
| 2                 | 0                      | 0                 |
| 3                 | 0.58                   | 0.3               |

 Table 22
 Copy number states and Log2 Ratios

The actual Log2 Ratios observed are best derived from a very large data set with wellcharacterized copy number changes. To this end, we have analyzed over 1400 samples that have copy number changes across 75% of the genome and have established stable empirical values for these expected Log2 Ratios. These values, as well as the dispersion characteristics of the Log2 Ratio data, are used as inputs to the HMM along with the weighted Log2 Ratios of the sample data.

The HMM uses these inputs to convert observed Log2 Ratios into a CN state for each marker. It uses a table of transition probabilities that express the probability of changing from any CN state to another. As can be seen in the following example (Figure 526), there are many potential paths through the possible CN states of a set of markers.



The HMM uses the Viterbi algorithm to calculate the most probable path through the set of markers using the transition probabilities between each pair of CN states. Essentially, the graph of potential CN states is the "hidden" layer of the HMM, and the measure Log2 Ratios are the observed layer. The HMM algorithm finds the most probably CN states given the observed Log2 Ratios.

#### **Segment Formation**

Once markers are assigned Copy Number States by the HMM, contiguous stretches of adjacent markers ordered by chromosome position having the same state are aggregated into segments. These segments are described in a segment table within the resulting CYCHP file that provides for each segment, the common Copy Number State, the number of markers in the segment, the genomic marker position that initiates the segment and the genomic marker position that terminates the segment.

#### **Enforce Minimal Segment Length**

Default parameters enforce a minimum segment length of 5 markers. This is a subjective choice of parameter that implicitly states that the user is not interested in segments with fewer markers than the minimum. The algorithm that enforces minimum segment size distributes markers from any segment with fewer than the minimum to its larger neighboring segments by changing the copy number call on the modified markers to conform with those of the neighbors.

#### **Smoothing & Joining**

To stabilize the calling of copy number gains or losses, the ChAS software implements a smoothing step. Smoothing will combine adjacent segments that are both gains, even if they are not the same Copy Number State. For example, smoothing will combine a set of adjacent segments of Copy Number State 3 and 4 into one segment and assign it the most prevalent Copy Number State of the markers in the original segments, (rounding up for gains and rounding done for losses in case of a tie). Smoothing will also combine Copy Number States 0 and 1. But smoothing will not combine gains with losses or either with normal segments.

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Joining combines segments of gains or losses if they are separated by small spans of normal copy number segments. The default value defining "small spans" in ChAS is ≤50 markers and ≤200 kbp. Small segments of less than 50 markers/200kbp of normal copy number are removed, and the adjacent gain segments are joined. Likewise for flanking loss segments. This is a dynamic process in ChAS, in that smoothing and joining can be turned on and off, and parameters altered, resulting in modifications of the displayed segments, but not altering the underlying CNState graph.

Segment table output The final result of the copy number pipeline is a table of segments identified in the sample. The table in the CYCHP file includes segments of normal and non-normal copy number. Segments called on the X- and Y-chromosomes are characterized as normal or non-normal using gender information and adjusting for the Pseudo Autosomal Regions (PAR) that are present on the X and Y. In ChAS, the segment table display only shows segments of non-normal copy number.

Mosaicism<br/>segment<br/>algorithmThe algorithm for detection of copy number aberrations in the presence of mosaicism<br/>considers single copy deletions and gains. The algorithm is tuned to be most accurate<br/>when the normal/expected Copy Number State is two. The algorithm targets detection<br/>of changes of approximately 3MB or more in size (for CytoScan HD). Copy number<br/>change events less than this size may be detected; however, sensitivity and specificity<br/>will be reduced.

Limitations in mosaicism segmentations

- The algorithm is designed to detect only mosaicism between approximately15%-70% mosaicism for copy numbers between 1 and 3 for regions on the order of 3MB in size or larger for autosomes and chrX.
- The algorithm is designed to detect only mosaicism between approximately 15%-70% mosaicism for copy numbers between 0 and 2 for regions on the order of 3MB in size or larger for chrY.
- The algorithm will not call events below 1MB in size irrespective of number of markers.
- The algorithm will not call events below 10% mosaicism irrespective of genomic size or number of markers.
- The algorithm does not use allelic difference or BAF data for segmentation purposes. It is recommended that allelic difference and/or BAF data tracks be examined in conjunction with the reported mosaicism segmentation.



## SNP marker workflow

| Signal summarization                | CytoScan arrays contain six probes for each SNP probe set, three targeting each allele. The first step of the SNP-specific workflow is to summarize the previously-normalized probe intensities for the A and B alleles, yielding allelic signal values.   |
|-------------------------------------|--|
| Allelic signal computation          | For each marker, the Allelic Difference is calculated as the difference between the summarized signal of the A allele minus B allele, standardized such that an A-allele genotype is scaled to a positive value, and the B allele is scaled to a negative value. The standardization is determined based on median values for this difference under different genotype configurations determined by the reference set. In this way a homozygous AA maps to approximately +1, and a homozygous BB allele maps to approximately -1, with the heterozygote mapping to approximately 0. Additionally, single A and B allele signals will map to 0.5 and -0.5, respectively. This scaling provides a useful way of discerning two copies of an A allele from a single copy, enabling detection of regions of copy-neutral LOH (e.g. IBD) from hemizygous LOH. |
| Genotyping                          | Genotyping for CytoScan arrays is accomplished using the BRLMM-P algorithm described in the White Paper: BRLMM-P: A Genotype Calling Method for the SNP Array 5.0 (2007).  |
| Allelic difference<br>GC correction | Systematic changes in Allelic Differences can be related to differences in GC content.<br>For instance, on a given sample Allelic Differences representing AA and BB genotype<br>markers might get progressively closer or further from each other as the GC content<br>changes. It is assumed that such changes represent unwanted variability. The Allelic<br>Difference GC correction determines differences in the structure of the allelic<br>differences associated with GC and then removes these differences. For CytoScan<br>HD the super GC covariate is used. For CytoScan 750K the Local GC covariate is<br>used.  |
| Detection of LOH                    | The LOH algorithm frames the problem in terms of a statistical hypothesis test. Given a specific region containing $N$ SNP markers with heterozygous and homozygous genotype calls, decide between the following two hypotheses:   |
|                                     | Null Hypothesis: Region is LOH<br>Alternative Hypothesis: Region is non-LOH  |
|                                     | To decide between the two hypotheses the number of heterozygous calls is compared with a critical value that is computed for each sample. When the number of heterozygous calls is above the critical value, then the alternative hypothesis is favored, i.e. region is not LOH. If there are not a sufficient number of heterozygous calls then the decision is made in favor of LOH. The algorithm moves the region of <i>N</i> markers along the genome to determine LOH events. Further details are provided in the While Paper: The Loss of Heterozygosity (LOH) Algorithm in Genotyping Console  |

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#### Array data QC metrics

This section provides a high level overview of the key QC metrics used with the CytoScan arrays.

Median of the<br/>Absolute values<br/>of all Pairwise<br/>Differences<br/>(MAPD)MAPD is a global measure of the variation of all microarray probes across the genome.<br/>It represents the median of the distribution of changes in Log2 Ratio between adjacent<br/>probes. Since it measures differences between adjacent probes, it is a measure of<br/>short-range noise in the microarray data. Based on an empirical testing dataset, we<br/>have determined that array data with MAPD > 0.25 (for CytoScan 750K and HD, MAPD<br/>> 0.29 for CytoScan Optima) has too much noise to provide reliable copy number<br/>calls.

- Waviness SD Waviness-SD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. Based on an empirical testing dataset, we have determined that array data with Waviness-SD > 0.12 has either sample or processing batch effects that will reduce the quality of the copy number calls. Elevated Waviness-SD is not always an indication of too much noise. Elevated Waviness with good MAPD and SNPQC metrics can occur in samples with many copy number changes or very large regions of change. It is therefore advised to check the data when observing elevated Waviness with good MAPD and SNPQC.
- SNPQCSNPQC is a measure of how well genotype alleles are resolved in the microarray data.<br/>Based on an empirical testing dataset, we have determined that array data with<br/>SNPQC < 15 (for CytoScan 750K and HD, SNP QC < 8.5 for CytoScan Optima) is of<br/>poorer quality than is required to meet genotyping QC standards.

## ndSNPQC (SNP Quality Control of Normal Diploid Markers)

The metric, SNPQC is a measure of how well genotype alleles are resolved in the microarray data. ndSNPQC is the same metric but only applied to normal diploid markers (that is those that have been determined to have Copy Number =2 in the sample). Larger ndSNPQC values are better.

## ndWavinessSD (Normal Diploid Waviness Standard Deviation)

ndWavinessSD is a global measure of variation of microarray probes that is insensitive to short-range variation and focuses on long-range variation. ndWavinessSD is computed on normal diploid markers.

## MAPD – Detailed Description

For quality assessment purposes, we define metrics that assess whether the microarray data is useful for copy number (CN) analysis. One of these metrics is Median of the Absolute values of all Pairwise Differences (MAPD).

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between Log2 Ratios for a given chip. Each pair is defined as adjacent in terms of genomic distance, with SNP markers and CN markers being treated equally. Hence, any two markers that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every marker belongs to two pairs (Figure 527).



Formally, if xi: is the Log2 Ratio for marker i:

MAPD = median( $|x_{i-1} - x_i|$ , with *i* ordered by genomic position)

MAPD is a per-microarray estimate of variability, like standard deviation (SD) or interquartile range (IQR). If the Log2 Ratios are distributed normally with a constant SD, then MAPD/0.96 is equal to SD and MAPD\*1.41 is equal to IQR. However, unlike SD or IQR, using MAPD is robust against high biological variability in Log2 Ratios induced by conditions such as cancer.

Variability in Log2 Ratios in a microarray arises from two distinct sources:

- Intrinsic variability in the starting material, hybridization cocktail preparation, microarray or scanner
- Apparent variability induced by the fact that the reference may have systematic differences from this microarray

Regardless of the source of the variability, increased variability decreases the quality of CN calls.

#### Effect of MAPD on functional performance

As a measure of performance, we measured copy number gain and loss using samples with large chromosome aberrations that spanned approximately 70% of the genome. With this dataset of nearly 1500 microarrays we measured the sensitivity for detecting regions of copy number change across all of these regions. The sensitivity of detecting an aberration on each array was binned into groups of varying sensitivities, and plotted versus MAPD for each array in the following graph. (Figure 528)



The bins of detection sensitivity are displayed as coordinates along the x-axis, with 0% detection at the left and 100% at the right. The number of arrays is listed above each box plot. The majority of the arrays had sensitivities above 90%. Based on this analysis, we established a QC cutoff for MAPD of 0.25. Arrays with MAPD above 0.25 cannot be reliably used to determine copy number.

## Waviness-SD – Detailed Description

For quality assessment purposes, we define metrics that assess whether the microarray data is useful for copy number (CN) analysis. In addition to MAPD (above) we define an alternate form of measurement of variance in the array data that is called Waviness-SD, where SD stands for Standard Deviation.

Waviness refers to an effect seen in all genomic microarrays (see Maroni et al. (2007) Genome Biology **8**:R228) where long-range variation is observed, often associated with regional genomic differences like local GC-content changes.

Waviness-SD is a QC metric that focuses on measuring these long-range effects. As described separately, MAPD is a metric that measures short-range variation, the variation of adjacent probes. The long-range variation measurement is accomplished by calculating the variation in Log2 Ratios across the whole genome and subtracting out the short-range variation, specifically, for autosomal probes:

Define:

 $X_i$  as the Log2 Ratios of autosomal probes

And Z<sub>i</sub> as the variance between adjacent probes:

$$Z_i = X_{2i+1} - X_{2i}$$

Waviness-SD is the total variance (X<sub>i</sub>) minus the local variance (Z<sub>i</sub>):

Waviness-SD =  $sqrt(Var(X_i)-Var(Z_i)/2)$ 

While this metric is useful in most cases, it does make the assumption that most of the genome is of normal copy number. This assumption may not be reasonable for some types of cancer samples with large amounts of genomic copy number variations, or for multiple-chromosome constitutive trisomies, where a considerable fraction of the genome is duplicated.

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For most samples, a Waviness-SD value below 0.12 for CytoScan arrays indicates that the long-range variation is within levels that can be accommodated by the CytoScan algorithms. But a high Waviness-SD measure on a sample with good MAPD and SNPQC metric values should be checked for the presence of large regions of copy number change to assess whether it is a sample effect or a QC failure.

Waviness-SD can be a good indicator of process drift since it measures long-range variation relative to the CytoScan HD or CytoScan 750K reference profile. A general rise of Waviness-SD for all samples coming from your laboratory may be an indication of a change of protocol, technique or reagents.

## **SNPQC – Detailed Description**

SNPQC is a metric that estimates the distributions of homozygous AA, heterozygous AB and homozygous BB alleles and calculates the distance between them. The better the separation of these distributions, the better the ability to identify a genotype based on its cluster position (Figure 529).



SNPQC correlates well with genotype performance, as measured by Call Rate and Concordance to published HapMap genotypes. To establish this relationship, we scored 380 microarrays from the Reference Set by calculating SNPQC, Call Rate and Concordance. The following graphs show the relationships between SNPQC and the other two metrics (Figure 530).



The left panel shows that when SNPQC > 15, Call Rate is above 98%. The right panel shows that when SNPQC > 15, Concordance is above 99%. This functional mapping of SNPQC has allowed us to set a functional threshold for this QC metric at 15. Microarrays with SNPQC > 15 are considered of high quality and interpretation of the data is possible.

## Effect of SNPQC on Functional Performance

SNPQC provides insight into the overall level of data quality from a SNP perspective. The key consideration when evaluating the SNPQC value is to ensure the threshold is exceeded. The quality of the SNP allele data is compromised, and is noisier and more difficult to interpret when the SNPQC values are below the recommended acceptance threshold as illustrated in the figure below. When the SNPQC value is below 15, the noise within the array is higher than normal which compromises the overall data quality and clarity of results. However, when the SNPQC value is above 15, the data is of excellent quality and can be relied upon as robust with regard to performance (Figure 531).

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**Figure 531** Examples of Allele Track data quality at various levels of SNPQC. The lower row of figures show data for a CN=2 and the upper for CN=3 regions. The panels from left-to-right represent increasing SNPQC quality. The functional threshold for SNPQC is 15, so all values above 15 show excellent data quality.



The key consideration is whether the SNPQC value is above or below the threshold value and not the absolute magnitude. As long as the SNPQC value exceeds the threshold there is a retention in the data quality as illustrated by the graphs to the right which demonstrate clear allelic data across a broad range of SNPQC values which exceed the recommended threshold. SNPQC is one of the metrics used to assess array quality and should be helpful towards determining which experimental data sets are of satisfactory quality to continue with subsequent interpretation.

**Note:** For detailed information on algorithms and QC metrics for the OncoScan array, refer to the **OncoScan Console User Manual** (**P/N 703195**).



### **TuScan algorithm**

The TuScan algorithm uses B-allele frequencies (BAFs) and log2 ratios to estimate the ploidy and percentage of aberrant cells in the sample (%AC) which in turn are used to calculate copy number calls (CN). The BAFs and log2 ratios contribute equally to CN determination. TuScan first uses the BAFs and log2 ratio data to identify segments of equal CN. Next TuScan uses the BAFs, log2ratios and segment data to find the combination of %AC and ploidy that best fits the data. When TuScan can successfully determine %AC, the algorithm assigns each aberrant segment an integer copy number representing the copy number in the tumor portion of the sample. This is possible because CN is well approximated by an integer when the tumor is nearly homogeneous. If the tumor is highly heterogeneous (i.e., lacks a dominant clone), or contains a large amount of "normal" cells %AC cannot be determined. In other words, if the percentage of aberrant cells contributing to the various aberrations in the sample varies across all aberrations, %AC and ploidy cannot be determined. When %AC cannot be determined, the segmentation algorithm will still identify segments of equal CN, but the CN in just the aberrant cells cannot be determined. In this case, TuScan bins the copy numbers and returns fractional CN values in 1/3 increments (e.g., 2, 2.33, 2.66, 3 etc.). This fractional copy number is derived from the normal contamination as well as the heterogeneous population of tumor cells; therefore, the fractional CN calls represent the average CN observed for that segment. Users should look at the value of %AC to determine whether the CN value represents the CN in the tumor (%AC= number) or the average CN in the sample (%AC=NA). Tumor heterogeneity also affects the interpretation of the CN number calls when %AC cannot be determined. For example, a TuScan call of 2.33 can result from 40% of the aberrant cells having 3 copies, 10% of aberrant cells having 5 copies, or a more complex heterogeneous mixture of copy numbers. Since nearly every tumor sample will have some amount of normal contamination combined with tumor heterogeneity it is not possible to predict how often TuScan will be able to determine the %AC, it will vary depending on the sample.

## Manual re-centering algorithm (OncoScan)

TuScan identifies normal diploid markers in a sample of interest, determines the copy number for these markers (2, 4 or 6) and ensures that markers with CN=2 have a log ratio of 0. This is referred to as "centering" the sample.

When no or an insufficient number of normal diploid markers are found, the automatic recentering does not occur. In addition, occasionally the automatic recentering misses the true CN =2 markers and does not correctly center the sample. In these cases, it is advised to center the sample manually to get correct CN calls. Manual recentering is now available through the CHAS software and the recentered sample is re-run through TuScan (described above) to provide integer copy number.

The new RC.OSCHP files can be viewed in ChAS or the BioDiscovery software, Nexus.

To manually recenter samples, an offset (median log2 ratio) is provided that tells the algorithm how much a sample should be pushed up (positive value) or pushed down (negative value) so that this region resides at the log 2 ratio = 0, indicative of normal diploid.

In the example below (Figure 532) the sample should be centered at chromosome 4q. The median log ratio on 4q is -0.17, therefore the manual recentering adjustment would be given this offset value, resulting in an increment adjustment of 0.17 for all log ratios.



#### Copy number effect on somatic mutations

Somatic mutation probesets in the OncoScan FFPE Assay are designed to selectively respond to the presence of mutation sequences. However, large copy number amplifications spanning the somatic mutation targets can sometimes lead to falsely reporting the presence of mutations in amplified regions.

If the copy number state is greater than  $\sim$ 15, you may observe false positive somatic mutation calls. The only region for which we have observed this problem is the EGFR gene, which is prone to very high copy number in certain cancer types.

In the example below (Figure 533), the predicted copy number state for the EGFR gene is greater than 30, which affects the somatic mutation score. Another side effect shown in the example below is that three mutations are called in the high Copy Number region, a contradictory event for at least two of these mutations.

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| Figure 533   |                      |
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## CytoScan XON region calling algorithm

For CytoScan XON arrays a region focused calling algorithm was developed to distinguish between neutral, gain and loss copy number. Regions are defined by genomically contiguous stretches of closely spaced markers. Each region is assessed independently.

A three state conditional random field (CRF) model is used to segment each region. The CRF model uses the log2 ratio measurements corresponding to that marker and four markers on either side, plus the state of the previous marker, to assess whether a given marker is in one of the three states. Before using the CRF model the log2 ratio is independently adjusted for every marker to account for expected differences in responsiveness to copy number.

For resistance to outliers, the t-distribution is used for computation of emission probabilities. The mean parameter for the emission distributions are fixed for each state across all markers, but the standard deviation parameter includes a component that differs for each marker, based on expected marker variability. Adjacent regions with the same copy number determination are not joined into larger segments so large copy number aberration events may be represented by multiple region based calls.



# Recommended CytoScan XON array workflows

**IMPORTANT!** The Segment Prioritization feature outlined in Chapter 17, "Prioritizing segments" on page 368 can be used in conjunction with the recommended steps below to quickly prioritize XON regions.

#### Whole exome analysis

The markers on the CytoScan XON array have been categorized into these four levels (based on the annotation in the region of the genome).

- Level 1: Includes genes with the highest level of evidence: developmental delay, epilepsy, ASD, XLID, Metabolic disorders, hereditary cancer OMIM Morbid genes.
- Level 2: ClinVar genes not covered in Level 1.
- **Level 3:** Other OMIM genes not identified as Level 1.
- **Level 4:** Other Ref Seq, UCSC, Ensembl genes, LOVD.

Recommended workflow for analyzing the whole exome

#### Loading XNCHP file(s)

- 1. Click File  $\rightarrow$  Open.
- 2. Select the XON-Level 1 Named Setting.

The XON Region Segment Calls in genomic regions assigned as Level 1 are shown.

3. Turn off any filters that are based on markers or size.

The XON gain/loss segments that are contained in/or overlap with Level 1 regions will be exposed on the XON Region Segment Track. The data in the Level 1 regions (Log2 Ratio, Weighted Log2 Ratio, Smooth Signal, Allele Difference, B-allele Frequency) will show in color (based on the sample color assignments in the User Configuration). The data in the remaining Level 2-4 regions will remain gray and no XON segments will be revealed.

| Figure 534 Example: Level           | 1 check only  |                                       |
|-------------------------------------|---|---------------------------------------|
| xnchp: Copy Number State (segments) |   |                                       |
| xnchp: XON Region Copy Number Star  | e (segments)  |                                       |
| nchp: Weighted Log2 Ratio           |   |                                       |
| -+ 1316.4 5                         | + <del>5 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + </del> | -                                     |
| xnchp: SmoothSignal                 |   |                                       |
|                                     |   | : : : : : : : : : : : : : : : : : : : |
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- 4. Add the XON Region Level and Summarized Log 2 Ratio columns to the segment table, then use the Segments Table to review the XON Region segment calls.
- 5. Optional: Annotate the XON Region segments as you normally would using the Call and Interpretation columns, as described in "Adding annotations at the sample (xxCHP) file level" on page 246.
- 6. Optional: Exon Region segment calls from other Levels can be displayed by selecting the check box(es) in the Filters Tab.

| Figure 535 Example: Level           | 1 and 2 checked                         |         |
|-------------------------------------|---|---------|
| xnchp: Copy Number State (segments) |   |         |
| knchn: XON Region Conv Number Stat  | e (segments)                            |         |
|                                     |   |         |
| knchp: Weighted Log2 Ratio          |   |         |
| - 1 1210 M - 2                      | + 3 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 | Statut. |
| .xnchp: SmoothSignal                |   |         |
|                                     |   |         |
|                                     |   | i hanal |
| n Levels                            |   |         |
|                                     |   |         |

7. Optional: Publish the XNCHP file to the ChAS DB, as described in "Publishing data to the database" on page 401.

**Targeted analysis** When you have a specific set of genes that you want to search for XON Region segment calls, a targeted analysis can be performed using CytoRegions for Targeted XON analysis. Details on this feature can be found in "Assigning a CytoRegion for targeted XON analysis" on page 273.

- 1. Create an AED file from your list of genes. See "Creating an AED File from a gene list" on page 273. If you already have an AED/BED file containing your genes of interest, go to step 2.
- 2. Click **File**  $\rightarrow$  **Open** to load the AED/BED file.
- 3. Locate the file, then click **Open**.
- 4. Right-click on the loaded file, then select Set File as CytoRegion for Targeted XON Analysis.

This assigns the file as a CytoRegions File, as well as automatically sets the appropriate filters for Targeted XON analysis. The filters hide all segments in the Genome region and colors all the data gray and turns on all levels in CytoRegions to expose any XON Region segment call and colors the data the assigned sample color.

**Note:** Only XON Regions calls within the CytoRegions are displayed in the views and tables.

- 5. Optional: Annotate the XON segments as you normally would, as described in "Adding annotations at the sample (xxCHP) file level" on page 246.
- 6. Optional: Publish the XNCHP file to the ChAS DB, as described in "Publishing data to the database" on page 401.



# Automatic CEL file analysis

**ChASCelUploader** is installed on the workstation of the GCS3000 Scanner. This application monitors a user specified folder for CEL files and then copies the CEL files to a user specified network share drive for analysis by the **Automatic CEL File Analysis Tool**. This tool is located in the ChAS Analysis Workflow module. It converts CEL files to CHP files and includes an option for automatically upload the CHP files to your Genoox Franklin account using ChAS AIR tokens.

### **ChASCelUploader**

ChASCelUploader monitors a user specified folder for CEL files generated by the GCS3000 Scanner, then automatically uploads them to a user specified network drive.

Installing and configuring the uploader

- 1. Locate the **ChASCelUploader\_Setup.exe** file in the CHAS zip file and doubleclick on it. Follow the on-screen prompts to install it.
- 2. After the installation is successful, navigate to: C:\ProgramData\ChASCelUploader
- Locate the ChASCelUploader.config file, then open it in a text editor such as MS Notepad. (Figure 536)



- 4. At the **DestDir** line, change the drive letter to the network drive letter and folder (directory) you want your data automatically uploaded to. Note: By default the ChASCelUploader monitors the GCC default directory for CEL files. You can change this default by updating the DataDir path to a different folder on the GCC Workstation. If you are using GCDC workstation, please update the DataDir path to: D:\Command\_Console\Data\Default
- 5. Save and close the Notepad window.
- To start the tool, click on the ChASCelUploader Desktop Shortcut to start it or click the Windows Start button and navigate to Thermo Fisher Scientific → ChASCelUploader.

The ChASCelUploader icon appears in your system tray to show it is running. To stop it, right-click on the icon and select **Exit**. **Note:** After the Uploader starts, only newly added CEL files are uploaded.

Existing CEL files are not.

#### Automatic CEL file analysis tool

The **Automatic CEL File Analysis** tool in ChAS converts CEL files into ChAS Browser compatible CHP files automatically.

# Converting CEL files to CHP files

#### How it works

The Automatic CEL File Analysis tool continually scans up to five designated input folders for new CEL files to analyze. Once a CEL file is detected, it is analyzed resulting in a CHP file (which is auto-saved to a designated output folder). Once a CEL file is processed, the ARR and CEL files are moved to the designated Archive folder.

Supported array types

- CytoScan HD (Single Sample Analysis or Normal Diploid Analysis only)
- CytoScan 750K (Single Sample Analysis or Normal Diploid Analysis only)
- CytoScan HD Accel (Single Sample Analysis only)
- CytoScan XON (Single Sample Analysis only)
- CytoScan Optima (Single Sample Analysis only)
- OncoScan CNV (FFPE Analysis only)
- OncoScan CNV Plus (FFPE Analysis only)

**Note:** In order to properly match up CEL files when running OncoScan CNV or OncoScan CNV Plus arrays, the CEL files from the "AT" and "GC" channels must have the exact same root name and include the array type. Each CEL file must indicate which channel they are using a concluding A or C. For example, the following CEL files will be paired properly for OncoScan FFPE analysis:

- OncoScan\_Sample1\_(OncoScanCNV\_Array)A.cel
- OncoScan\_Sample1\_(OncoScanCNV\_Array)C.cel

The following CEL files will NOT be paired properly for OncoScan FFPE analysis because the root names do not match:

- OncoScan\_Sample1\_(OncoScanCNV\_Array)A.cel
- OncoScan\_Sample2\_(OncoScanCNV\_Array)C.cel

The following CEL files will NOT be paired properly for OncoScan FFPE analysis because the array names are not included:

- OncoScan\_Sample1\_A.cel
- OncoScan\_Sample1\_C.cel

# Launching the tool

1. Click the Utility Actions button (top right of the Analysis Workflow window)

#### 2. Click Automatic CEL File Analysis.

The window opens. (Figure 537)

|                      |                                     |  |                                       | _                               |
|----------------------|-------------------------------------|--|---------------------------------------|---------------------------------|
| applied biosystems   | AutoCelAnalysis                     |  | -                                     |                                 |
| Input folder 1 C:\Au | itoCELAnalysis\Director1_CEL_files  | Br   | owse                                  | Clear                           |
| Output folder 1 C:\/ | AutoCELAnalysis\Director1_CHP_files | Br   | owse                                  | Clear                           |
| Input folder 2 C:\Au | itoCELAnalysis\Director2_CEL_files  | Br   | owse                                  | Clear                           |
| Output folder 2 C:\/ | AutoCELAnalysis\Director2_CHP_files | Br   | owse                                  | Clear                           |
| Input folder 3 C:\Au | itoCELAnalysis\Director3_CEL_files  | Br   | owse                                  | Clear                           |
| Output folder 3 C:\/ | AutoCELAnalysis\Director3_CHP_files | Br   | owse                                  | Clear                           |
| Input folder 4       |                                     | Br   | owse                                  | Clear                           |
| Output folder 4      |                                     | Br   | owse                                  | Clear                           |
| Archive folder C:\A  | utoCELAnalysis\Archive              | Br   | owse                                  | Clear                           |
| QC History output fo | Ider C:\AutoCELAnalysis\QC History  | Br   | owse                                  | Clear                           |
|                      | T<br>Use Normal Diploid a           | arget Genor<br>malysis for C<br>Upload to Fr | ne Versio<br>ytoScan H<br>ranklin (Ge | n: hg19<br>D/750K:<br>enoox): [ |
| Time                 | Message                             |  |                                       |                                 |
|                      |                                     |  |                                       |                                 |
|                      |                                     |  |                                       |                                 |

#### Setting up the tool

**IMPORTANT!** Input, Output, and Archive folder names and their assigned paths should not contain spaces. If a space is needed, use the underscore symbol [\_].

Any non-ARR and non-CEL files detected in your assigned input folder will remain in this folder. Any detected ARR and CEL files will be processed and moved into your assigned Archive folder.

#### Assigning your input folder(s)

The Input folder is where your CEL files reside.

1. Click the **Input folder 1** the " button.

An Explorer window appears.

- 2. Navigate to your CEL file folder.
- 3. Click on the folder to highlight it, then click Select Folder.

Your CEL file folder path is displayed.

4. Optional: Once the first Input folder is assigned, an option to designate a second Input folder appears (Input folder 2). Repeat steps above to assign a second Input folder. Up to five Input folders can be assigned.

**Note:** Make sure you assign the Input folder using the folder path defined in **DestDir** (Figure 536) when you want to upload the data from the GCS3000 to Franklin. Just one input folder can be assigned for this work upload process.

Assigning your output folder(s)

After the tool produces a CHP file, it is placed in an Output folder.

1. Click the **Output folder 1** the " button.

An Explorer window appears.

- 2. Navigate to an existing folder where you want your newly converted CHP files saved or click **New Folder** to create a new Output folder.
- 3. Click Select Folder.

Your Output folder 1 path is displayed.

4. Optional: Once the first Output folder is assigned, an option to designate a second Output folder appears (Input folder 2). Repeat steps above to assign a second Output folder. Up to five Output folders can be assigned.

Assigning an archive folder

Copies of the processed CEL files are placed in the Archive folder.

1. Click the **Archive folder** the <sup>...</sup> button.

An Explorer window appears.

- 2. Navigate to the folder where you want the CEL files saved or click **New Folder** to create a new Archive folder.
- 3. Click Select Folder.

Your Archive folder path is displayed.

Assigning a QC History file folder

1. Click the **QC History folder** button.

An Explorer window appears.
- Navigate to the folder where you want the CEL files saved or click New Folder to create a new QC History folder.
- 3. Click Select Folder.

Your QC History folder path is displayed.

Selecting a target genome version

1. By default, **hg19** is selected. If needed, click the **Target Genome Version** dropdown to select **hg38**, a shown in Figure 538.

| Figure 538  | Version drop-down |        |  |  |  |
|-------------|-------------------|--------|--|--|--|
| Target Geno | me Version:       | hg19 ~ |  |  |  |
|             |                   | hg19   |  |  |  |
|             |                   | hg38   |  |  |  |

Normal diploid analysis check box

|--|

1. Click the check box to use the Normal Diploid algorithm. Leave unchecked to run the default single sample analysis.

Uploading to Gennox Franklin

ChAS can upload segment data automatically to Franklin after AutoCelAnalysis.

- 1. Click the Upload to Franklin (Gennox) check box. (Figure 539)
- Enter your Franklin credentials, email and password, then choose your ChAS profile in the expanded window to automatically upload your segment data to Franklin.

| Figure 53                                | 9 Franklin credentials         |  |  |  |
|--|--------------------------------|--|--|--|
| потна прои аназы от сусозсан под гоок. 🗌 |                                |  |  |  |
|  | Upload to Franklin (Genoox): 🗹 |  |  |  |
| Email:                                   | yong.yu@thermofisher.com       |  |  |  |
| Password:                                | •••••                          |  |  |  |
| ChAS Profile:                            | Yong ~                         |  |  |  |
|  |                                |  |  |  |

**Note:** All Segment Filter settings, Smoothing and Joining rules in the selected ChAS profile will be applied to the segment data that is uploaded to Franklin. Data that has failed QC will not be uploaded to Franklin.

## Running the tool

1. Click Start

The CEL file analysis begins and the **Time** and **Message** pane populates with information, as shown in Figure 540.

Click **Cancel** to stop an analysis in progress. **Note:** The time for CEL file analysis to complete should be comparable to analysis times when manually setting up CEL files in the Analysis Workflow.

**Note:** During the analysis process, the tool detects the array type, then autogenerates an appropriately named array staging folder. As each CEL file analysis completes, the tool moves the newly created CHP file from its staging folder to the output folder you assigned earlier.

**Note:** If the Automatic CEL File Analysis stops for any reason (such as losing connection to the input folder or is inadvertently canceled) a log file of processed CEL files can be found in the designated Archive folder.

| applied biosystems                           |   | AutoCelAnalysis   |  | -   |                          |  |  |  |  |
|--|---|---|--|---|--------------------------|--|--|--|--|
| Input folder 1 Q:\ChAS\U                     | ser\Director  | 1\Autouploader  |  | Browse  | Clear                    |  |  |  |  |
| Output folder 1 C:\Users\Director1\CHP files |   |   |  | Browse  | Clear                    |  |  |  |  |
| Input folder 2                               |   |   |  | Browse  | Clear                    |  |  |  |  |
| Output folder 2                              |   |   |  | Browse  | Clear                    |  |  |  |  |
| Archive folder C:\Users\D                    | irector1\Arch   | ive   |  | Browse  | Clear                    |  |  |  |  |
| QC History output folder                     | C:\Users\Dir  | ector1\QC   |  | Browse  | Clear                    |  |  |  |  |
|  |   |   | Target G                                   | enome Version                                       | : hg38                   |  |  |  |  |
|  | U   | lse Normal Diplo  | id analysis f                              | or CytoScan HI                                      | )/750K:                  |  |  |  |  |
|  |   |   | Upload                                     | to Franklin (Ge                                     | noox):                   |  |  |  |  |
|  |   | Email:  | yong:@the                                  | ng@thermofisher.com                                 |                          |  |  |  |  |
|  |   | Password:   | •••••                                      |   |                          |  |  |  |  |
|  |   | ChAS Profile:   | Yong                                       |   |                          |  |  |  |  |
| Time   | Message   |   |  |   |                          |  |  |  |  |
| 8/11/2023 6:26:48 PM                         | Successfull   | y uploaded '21-3  | 351.cychp' t                               | o Franklin.   |                          |  |  |  |  |
| 8/11/2023 6:26:48 PM                         | Copying file: C:\ProgramData\Affymetrix\ChAS<br>\AutoCelAnalysis\CytoHD\21-3351.CEL to archive folder   |   |  |   |                          |  |  |  |  |
| 8/11/2023 6:26:48 PM                         | Copying fil<br>Scientific\E<br>\CHP\2023<br>archive fold  | e: C:\Users\yong:<br>Documents\Proje<br>-08-12T01-25-252<br>der | xin.yu\OneE<br>cts\ChAS 4.5<br>Z.cyhd.Copy | )rive - Thermo F<br>5\Autouploader<br>NumberReport. | isher<br>_Test<br>txt to |  |  |  |  |
| 8/11/2023 6:26:50 PM                         | CytoHD file   | CytoHD file type detected.                                      |  |   |                          |  |  |  |  |
| 8/11/2023 6:26:50 PM                         | Copying fil   | Copying file: 21-3912.CEL to staging folder                     |  |   |                          |  |  |  |  |
| 8/11/2023 6:27:15 PM                         | Running apt-copynumber-cyto-ssa.exe: 21-3912.CEL  |   |  |   |                          |  |  |  |  |
| 8/11/2023 10:13:16 PM                        | apt-copynu  | apt-copynumber-cyto-ssa.exe Finished (03:46:01)                 |  |   |                          |  |  |  |  |
| 8/11/2023 10:13:16 PM                        | Running ap  | ot-copynumber-f   | rag-qc.exe:                                | 21-3912.CEL   |                          |  |  |  |  |
| 8/11/2023 10:14:05 PM                        | apt-copynu  | umber-frag-qc.ex  | e Finished (                               | 00:00:48)   |                          |  |  |  |  |
| 8/11/2023 10:14:05 PM                        | QC history file path: C:\Users\yongxin.yu\OneDrive - Thermo<br>Fisher Scientific\Documents\Projects\ChAS 4.5<br>\Autounloader Test\CHP\CvtoScanHD_Atrav(CCHistory 10) tyt |   |  |   |                          |  |  |  |  |

Opening the newly generated CHP file(s)

1. From the ChAS Browser window, click **File**  $\rightarrow$  **Open**.

An Explorer window appears.

2. Navigate to your assigned Automatic CEL File Analysis output folder(s). See "Assigning your output folder(s)" on page 504.

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3. Click or Ctrl click to highlight the CHP files you want to open in the ChAS Browser, then click **Open**.

The file(s) appear in the ChAS Browser.

## Viewing cases in Genoox Franklin

If using the automatic upload to Franklin features:

- 1. Go to https://franklin.genoox.com/clinical-db/home and sign in.
- 2. Click the **My Case** tab to view your case(s).

The All Cases window appears. (Figure 541)

| Figure 541 Genoox Franklin - All Cases wind  | dow example  |         |             |          |          |         |              |       |
|--|--|---------|-------------|----------|----------|---------|--------------|-------|
| All Cases  | 165 Total 158 Active 22 Cases created 0 Cases resolved in the last 28 days |         |             | ys       | New Case |         |              |       |
| Q Search analyses  |  |         | Assig       | ned to 🔹 | Status   | •       | Assay type   | •     |
| 165 cases  |  |         |             |          | Sor      | t by: I | Date Created | •   F |
| CASE DETAILS   |  | SAMPLES | ASSIGNED TO |          | STATUS   |         |              |       |
| 21-3912.cychp (00003388-56e0-4a7<br>Created: Aug 11, 2023   Assay: CytoScan HD HG38  | 72-7f5b-00187600   | 1       | unassigned  |          | Active   | )       |              | :     |
| 21-3351.cychp (00004f80-29be-47fe<br>Created: Aug 11, 2023   Assay: CytoScan HD HG38 | e-24c5-000c8f002c  | 1       | unassigned  |          | Active   | •       |              | :     |

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com** thermofisher.com