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[™] Arrays and OncoScan Arrays.
- Automatic workflow from scanner to results file with no manual intervention required.
- Seamless integration with Franklin[™] by Genoox[™].
- 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[™].
- 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[™] by Genoox[™].
- 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 Core Processor	3 GHz (or greater) Pentium Dual Core Processor
64-bit Windows [®] Operating System and Web Browser	Windows 10/11 Windows Edge	Windows 10/11 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 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 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 CytoScan_XON_Array_Analysis_files_hg19_NA33.r8-Part2.zip	CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part1.zip CytoScan_XON_Array_Analysis_Files_hg38_NA36.r8-Part2.zip
OncoScan CNV 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 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	
 Use system proxy 	
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										
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 Browse button.
Output results path	A good practice is to navigate to your current ChAS data folder location, then click Create New Folder to create an output results path folder <u>inside</u> this folder. (Example: C:\Users\YourUserName\CytoScan Data\CytoScan_Results_Files)
	2. Click Save .
Assigning a Central QC	 Click the Browse button, then navigate to a folder in which to store the QC history file. (Example: C:\Cytoscan_data\)
history path	 Click Create New Folder to create a central QC history path folder. (Example: QC_History)

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. Note: A 64-bit system is required to analyze intensity data.
Analysis Results (CYCHP) CytoScan array: CYCHP contains copy number, LOH, mosaicism, and genotype call information	ChAS	Displays results in graphical and tabular formats.
Analysis Results (RHCHP from HTCMA) contains copy number, LOH, Carrier Variant, and SMN results	RHAS	Displays results in graphical and tabular formats.
Analysis Results (OSCHP) OncoScan FFPE and OncoScan_CNV arrays, OSCHP contains copy number/LOH and somatic mutation information (FFPE 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) CytoScan XON array: XNCHP contains LOH, Exon Region, and Genotype information.	ChAS	Displays probe-level analysis, segment level data in graphical and tabular formats.
Analysis Results (CNCHP) GenomeWide SNP 6.0 array contains copy number and LOH segments	GTC	Displays probe-level analysis data and generates segment data on-the-fly.
CHP Change Archive (CHPCAR)	ChAS	Stores user-annotated segment and sample annotations as well as modifications made to the segment data.
Analysis Results (.zip) ReproSeq Aneuploidy contains copy number and tiling information	ION Reporter	Displays copy number segment data and sequence tile information.
Region Information File (BED or AED)	ChAS or Text 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, TXT, and DOCX)	ChAS	Exports data in this format for use in a spreadsheet program or other user- defined uses. This format is for export only. ChAS does not import TSV or TXT files.
VCF Files	Other software 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 Array ¹	Genome-Wide Human SNP Array 6.0 ²	OncoScan ³	CytoScan XON ⁴
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 (CNV Plus only)	No
B-allele Frequency	Yes	No	Yes	Yes
Segment data	1			
Gain and Loss segments based on runs of aberrant Copy Number State data	Yes	Yes	Yes	Yes
Mosaic Gain and Loss segments of non-integer Copy 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 Analysis File Set Version	ChAS v4.5	ChAS v4.4	ChAS v4.3	ChAS v4.2.1	ChAS v4.2	ChAS v4.1	ChAS 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 single copy. Heterozygous SNP genotypes are possible, but are due to the low inherent Heterozygote call error rate noise, not the true presence of two alleles.	

Table 7	Expected LOH calls on the X and Y chromosomes for the CytoSca	n arrays
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Table 8 Expected LOH calls on the X and Y chromosomes for the Genome-Wide Human SNP Arr
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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 copy. Heterozygous SNP genotypes are possible, but are due to the low inherent Heterozygote call error rate noise, not the true presence of two alleles.	chromosome since it is assumed that there not substantial Y chromosomal 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 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 copy. Will be called LOH where there is 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	 Workflow name: 	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 Please select output file folder				Select a s	Add	Remove analysis results
Output result information Please select output file folder				Select a s	Add	Remove analysis results
Output result information Please select output file folder Optional				Select a s	Add	Remove analysis results
Output result information Please select output file folder	Generate	e Results Summary File	Report format: PDF	Select a s	Add	Remove 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 Select CytoRegions File:	Report format: PDF	Select a s	Add suffix to append to the	Remove 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 Select CytoRegions File: 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 Array type: CytoScanHD_Array Gen	C results 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 Sample_02.CEL Sample_03.CEL Sample_04.CEL 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
 Analyze all CEL files as male Analyze all CEL files as female 	✓ Generate Results Summary File Select CytoRegions File: Select Overlap Map File:	Report format: DOCX	Clear Clear 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 ^{•••} 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 \Documents C:\								
C:\Cytoscan 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 or currently in process. Press OK to name the result file below. Press Cancel to return to the Analysis Setup.	zed and/or were selected for workflows that are pending (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	× esults List
toScanHD_Array Single Sample Analysis: NA33 toScanHD_Array 1. Source File	Current workflow status: Workflow completed successfully	r Logs
orkflow 11/23/2016 04:08:41	. View Re	× esults List
toScanHD_Array Single Sample Analysis: NA33 toScanHD_Array 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 ≤ 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>></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. Loading more files may impact the performance of the software. 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? 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) C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_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) C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_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 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 ⁻ 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
	Ω Φ σ [*] 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 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	33 Sum, mean and median message
	Sum: -381.5232014879366 Mean: -0.1510982976189848 <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 ≤ 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.]			
pplied 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 Please select ou	nformation Itput file folder			"Add CEL	Files" to import	Optiona Select a	files for analyze.	nalysis results	
Output result in Please select ou Manual Recent	nformation Itput file folder ering			"Add CEL	Files" to import	Optiona Select a	files for analyze.	nalysis results	
Output result in Please select ou Manual Recent	nformation Itput file folder ering Recentering TuScar	Log2Ratio Adj.	Adjust this	"Add CEL	Files" to import	Optiona 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 or currently in process. Press OK to name the result file(s) generated by this workflow to the ones suggested 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 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 ∎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	 Genome Version: 	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						Optional Select a s	uffix to a	ppend to the anal	ysis results	
utput resu lease select :\Clinical Sj	It information coutput file folder pecialist Training						Optional 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.

Figure 6 Display (7 EXAMPLE: File 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
	A	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 \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 \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	 Select analysis workflow: 	CytoScanHD_Array Reference Model Creation: NA33	 Set workflow name 	:: 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	 View In 	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 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 \Desktop	OncoSci	anGeneBoundaries.r1.bed	5/5/2014 3:16 PM	27 KB	BED File		
C:\Users\ppavic \Documents							
€ 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 \Desktop C:\Users\ppavic \Documents C:\	Name		Date Modified	Size	Туре		
D:\							1
Create New Folder	File Name: Files of type:	Result *.genereport.txt				•	Save 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)

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

Expression Gene 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 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 Report Column	Description
Segment ID	The unique identifier for the copy number segment.
Segment Start Position	Start position of the overlapping copy number segment in the xxCHP file.
Segment End 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 Overlap	Gene Boundary Size/Segment Size.
% Aberr.Cells (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 (OncoScan only)	TuScan Ploidy is the most likely ploidy state of the tumor before additional aberrations occurred. Algorithmically it is the CN state of the markers identified by the algorithm as normal diploid before %AC and ploidy are determined. When a high ploidy is determined the "normal diploid" is deemed to correspond to a higher CN and the log2 ratio gets adjusted appropriately. If ploidy cannot be determined NA (Not Available) is reported.
Low Diploid Flag (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 (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 (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 importing 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 Attributes button is enabled.
	3.	Click on the Attributes drop down, select Import , then navigate to the corresponding TXT file.
	4.	Click Open .
		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 Attributes drop-down, then select Save .
Exporting to Integrative	The 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 Add Files , then navigate to and highlight the CHP files.
	3.	Click Open .
	4.	Select files by either clicking the Select All , 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 OK .
		Note: To include sample attribute information in the IGV Export, click on Attribute \rightarrow Save 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	 View 	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				•	
201	41015_165517_006_APL01732	2_A0003367_P0	Within Bounds	0.1759291	20.35406	male	33	hg19				. .	
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 Gender Shape By Attribute Threshold Test
Colored by Gender female male Shaped by Threshold Test Within Bounds 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	97 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></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 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)

Figu 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	 Set workflow r 	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	× View Results List
CytoScanHD_Array Mendelian Error Check CytoScanHD_Array 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 Report Column	Description
Analysis Type	Provides the samples being run through the analysis based on the sample key. 0 = Proband, $1 = Mother$, $2 = Father$.
Familial Sample Key	Tells you the parent for which relatedness is being tested. 1= Mother only used in the analysis. 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/ 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 Trio	Number of raw error turned into a percent 119/56482 *100.
Percentage MIE 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 ≤ 0.12	NA Version					
						1				
			Chromosom	e Analysis Suite			×			
			Cog	Collection has comple S 4.0\LogCollection2 s folder: C:\ChAS 4.0	eted successfu 01610150357	lly. The : 54.zip ca	zip file C: n be found 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 Desktop Documents	 ChAS_2.0_files New_CYCHP_files 08-0989_A3_2.cyhd.cychp 09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228(2)_2.cyhd.cychp 09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.cyhd.cychp 11-0810_LC_ONC13B_A6_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC13B_A6_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC41B_A12_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC41B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC41B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC134B_B10_POP#2_CytoScan-PS_20110511(2)_2.cyhd.cychp 11-0816_LC_ONC134B_B10_POP#2_CytoScan-PS_20110511(2)_c.cyhd.cychp 20120730_181709_001_FFPE 3_2.cyhd.cychp 20120730_181709_002_FFPE 6_2.cyhd.cychp
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 determine QC. Are you sure you want to load these files? • C:\Users\ppavic\Desktop\chAS_Data\20120913_17532 (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 Loading more files may impact the performance of the software. 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? 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\11-0816_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) C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_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) C:\Users\ppavic\Desktop\chAS_Data\New_cychp_files\11-0816_LC_ONC41B_A12_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)

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. 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	 For the CytoScan 750K and HD Arrays, the default smoothing and joining rules are: Smooth Gain or Loss CNState runs to the most common marker value, then generate segments. Join any "split" CNState runs separated by no more than 50 normal-state markers. Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp Skip segments from the smoothing operation for all arrays that have a CN < 1. For SNP 6 arrays, the default smoothing rule: Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.
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 aberrant segments more distant than this number 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 more than this number of markers of normal state data	If this option is chosen, only Gain or Loss CNState Runs which are separated by less than a threshold number of markers of normal state data will be joined. For example, if the marker threshold is set at 50, then CNState runs separated by more than 50 markers of normal state data will not be joined.
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	 For the CytoScan 750K and HD Arrays, the default smoothing and joining rules are: Smooth Gain or Loss CNState runs to the most common marker value, then generate segments. Join any "split" CNState runs separated by no more than 50 normal-state markers. Join Gain or Loss CNState runs interrupted by normal state data which are separated from each other by no more than 200 kbp For SNP 6 arrays, the default smoothing rule: Smooth Gain or Loss CNState runs to the most common marker value, then generate segments.
Limit the joining of CNState data (which flanks normal state data) to not join aberrant segments 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. 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
	MAPD is a QC metric for all probes used to determine copy number that is derived from both polymorphic (SNP) and non-polymorphic (CN) probes
	Waviness SD 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.
	Cel Pair Check 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)
	DQC (DishQC) 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.
	QC Call Rate is the percentage of autosomal SNPs with a call other than NoCall (measured at the Sample QC step).
	SMN MAPD 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.
	SMN WavinessSD 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.
	Note: 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><</u> 0.25	<u>≥</u> 15.0	<u><</u> 0.12	-	-
CytoScan XON Arrays	<u><</u> 0.20	<u>≥</u> 10.0	<u><</u> 0.08	-	-
CytoScan (Normal Diploid Analysis)	<u><</u> 0.25	<u>></u> 15.0	<u><</u> 0.12	<u>></u> 15.0	<u><</u> 0.12
CytoScan HTCMA	=< 0.28	>= 10	= 0.07	= -	= -
CytoScan Optima Array	<u><</u> 0.29	<u>></u> 8.5	-	-	-
Genome-Wide Human Array SNP 6.0	<u><</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><</u> 0.3	<u>></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: 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 Not Filtered No Value
(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 Parameters: 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.	
 Disorder-Causing OMIM ® Genes OMIM ® Phenotype Loci OMIM ® Region Phenotype Loci Protein Coding Ensembl Genes Protein Coding Genes Recurrent/Curated Regions Segmental Duplications Triplosensitivity III sno/miRNA Markers Cytobands 	



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 [*] 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 Named Settin	
Named Setur	ly -
酱 Standard	v
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 NetAffx Genomic Annotation database and its hg version currently loaded in the ChAS Browser (the database is not array-specific)	SmoothSignal: 11-0810_LC_ONC13B_A Restricted Mode Indicator	6_PoP#2_CytoScan-PS_20110511.cyhd.cychp, Nearest chromosome positi Cursor Position information	user Pete User Profile 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
	p11:1	9 10
	q11.1	11 12
		△ 13
	q12	15 16
CancerSample_01_CytoScanHD cythd ND.cychp: Copy Number State (regments)	q21.31	▲ 18 19
CancerSample_01_Oyb/ScanHD_syndh D.syndh. LOH (segments)	q21.32 q21.33	20
Details View	g22	△ 22 X
HD Default LOH	633 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 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 file selected as the CytoRegion file. Includes information on detected segments 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.
	 "Graphs table" on page 341: Displays marker data for the loaded and selected xxCHP files.
	 "Variants table" on page 346: Displays somatic mutation data from OncoScan CNV Plus and Carrier Variant data from CytoScan HTCMA arrays.
	 Query Samples Table: Displays sample level results from searches performed on the ChAS DB.
	 Query Segments Table: Displays segment level results from searches performed on the ChAS DB.
Selected chromosome 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 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 data files (for example, proportion of each chromosome found to be in the state 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 Some
Sample_07.cyhd.cychp: Copy Number State	
RenalCell_04.OSCHP: Log2 Ratio 1.5 Sample_07.cyhd.cychp: Log2 Ratio	q12
Sample_07.cyhd.cychp: Mosaic Copy Number State (segments)	_ \$
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 Save WGV state	noose Data		×	1			S
1 – Load a WGV state 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 star
	Alternating Colors						
-1- To	ose Data	Top Row Line		·			
-1.5	Weighted Log2 Ratio	SmoothSignal	v	. .			
1.5- 1-	in: -1.5 Max: 1.5	Min: 0 Max: 4 Show gridlines Show zero lin	ne Will			New Yo	
0.5 - M	liddle Row Dots	Middle Row Line	_				
	Allele Difference	<no line=""></no>	~	in the second			
	in: -1.8 Max: 1.8 ✔ Show gridlines ✔ Show zero line	Min: 0 Max: 4 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>	v			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 Background Dots Single Color 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				
" _{GV} /	Whole Genome View - Ca	ancerSample_01_CytoScanHD.cyhd.N			
File	View				
₿₽ ₽ <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 WGV state(s)	1. Select View \rightarrow Load a WGV State.
	2. Choose a saved WGV state from the drop-down list.
	3. Click OK to apply the WGV State (or click Cancel to keep the current display).
Deleting a saved	1. Click on View \rightarrow Delete a WGV State .
WGV state	 Select the saved WGV state you want to delete from the drop-down list, then click OK.
	A message appears asking if you are sure you want to delete it.
	3. Click OK or click Cancel 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 1.5 Adds a reference line 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 -			
0.5	Choose a comparison file	×	<u>ى بەر مەرىكە مەرىمە مەرىمە -</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^{p1} 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[™] 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 View and Edit annotations in this file CytoRegions for Targeted XON analysis Include in CytoRegions Set File as Overlap Map View/Edit Properties Set Custom Color 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) Note: 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		



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 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) Note: 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 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.	
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) Note: 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 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) Note: 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 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 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 The position that is highlighted in the graphs table. 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
With no added/removed markers:
Diagonal Pattern
With added/removed markers:
Diamond Pattern
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
Coordinates: 1000000 1,000,000
Genome Names: GRCh37/GRCh38 hg19/hg38
Bance Separator: 100,200 100-200 |
| No buffer Number of bases: 10000 Percent of segment length ¹ ¹ | 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 When zooming to a segment, optionally add a buffer on both the left and right sides. O No buffer 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 ♥ Î₽♥♂ Sample01.cyhd.cychp ♥ Î₽♥♂ Sample02.cyhd.cychp ♥ Ĩ₽♥♂ GeneList bed ♥ Ĩ₽↓ Regions.bed ♥ Ŷ DGV	Sample Data 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
 □ ii sno/miRNA ✓ Markers ✓ ∞∞ Cytobands 	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	Export File as AED	- 1		aptStateReferenceTextOut	Text	false
	HED	Export file as ALD	- 1		Autonomo LOH	Decimal Number	0.021010429
	A 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 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 _c	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 γ appears next to the segment type name, as shown in Figure 185.

Figure 185 The funnel graphic denotes filter have been applied.	′S
Data Types 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 Types Allele Difference Allele Peaks Allele Peaks Allele Peaks Allele Peaks Copy Number State Copy Number State	Reset to Default
Style Bar Line Points Min/Max/Avg Big Dots Stairstep Auto-Size Dots Threshold (bases) 10,000,000 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 Types Allele Difference Allele Peaks E BAF Copy Number State E Log2 Ratio E LOH E LOH E SmoothSignal E Weighted Log2 Ratio	
Style Bar Line Points Min/Max/Avg Big Dots Stairstep Auto-Size Dots Threshold (bases) 10,000,000 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.					

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Column	Description				
	Segments				
File	File the segment was detected in.				
CN State	Copy Number State (not displayed for LOH segment types). 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.				
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 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. Segments completely overlapped by an Overlap Map item are 100% overlapped. This number is used for Filtering Segments out by "Overlap".				
Overlap Map Items (% of 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 Genes	List of OMIM Genes designated with a Phenotype Map Key of 3				

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Column	Description
Disorder Causing OMIM 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 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 Location	The coordinate location of 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.
Following Marker	The array marker just below the segment in the data track used as input for the segment. Note: This column is only applicable to CNState Gain and Loss segments.
Following Marker Location	The coordinate location of the array marker just below the segment in the data track used as input for the segment. 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 Ratio	The mean of all the Weighted Log2 Ratio values contained in the segment.
Microarray 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 Items	Number of Overlap Map items which share genomic coordinates with the segment.
% of Overlaps Map Item 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 (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. 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 Key	Indicates how this phenotype was placed on the map.				
OMIM Phenotype Locus 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 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 1 TaqMan [®] 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 [Hs] Human Gene TCEB2 Assay Type ?	SNP ID rs8017	Gene Loca TCEB2 Chr. on C	tion 16: 2771572 SRCh38	SNP Type Transition Substitution, Intron, Mis-sense Mutation, UTR 3, Intragenic	Assay Type Functionally Tested	Availability Catalog # Made to Order 4351379 S: 300 rxns ▼ Price (USD): 259.00 Check your price > 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 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 Option	Function
Hide All Segments 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. Note: This option is only available with a CytoRegions file is assigned.
Hide LOH where 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 Level	 Based on the Level Assignment to the region in the genome. Level 1: Medical Research exome and cancer Level 2: ClinVar genes not covered in Level 1 Level 3: Other OMIM genes Level 4: Opportunistic regions from Refseq/UCSC/Enseble/LOVD. 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.
9

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 Marker Count 50 5000 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
~	✓	
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 08-0989_A3na32.3.cyhd.cychp: Copy Number State 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 An en stransport programme fragment in ser en se 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 Anticipa de la companya de la company Anticipa de la companya de la company
 -1.5 Cancer_ONC134B_CytoScan_20110511.cyhd.cychp: Allele Differ -1.5 	ence	and the state of the state of the second
1.5 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 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). This information will not be applied to the merged segment 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_	€	Zoom to selection Selection Details	Ctrl+Space Ctrl+D	eig	phte	d Log:	2 R	atio												
	4	<mark>☆</mark>	Add to a File Un-do Merge									•						÷			ł
		*	Delete Segment View/Edit Annotation Properties																		
		⇔ ₽	Edit start/end 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 ⁰¹ 797 - 5988 38 - 499 ⁸⁴	Sample_07.cyhd Array Type Cytos Loss 6: 257,064 Type Location Size (kbp) CN State 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 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_ 1.5 0.5	20110511.cyhd.c 11-0816_LC_O Array Type Cyto Loss 8: 35,021	ychp: Weighted Log2 Ratic NC134B_B10_PoP#2_Cyl ScanHD_Array 410 - 36,455,932	toScan-PS_20110511.cyhd.cychp
1.5 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-PS_	Type Location	Loss 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 - 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 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 - Change Copy Number - Pick a state 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 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F	PS_20110511.cyhd.cychp: Copy Number State (segments PS_20110511.cyhd.cychp: Mosaic Copy Number State (se	>> >> >> >> >> >> >> >> >> >>
11-0816_LC_ONC134B_B10_PoP#2_CytoScan- - 1.5 - 0.5 - 0.5 - 1.5 - 1.5 - 1.5 11-0816_LC_ONC134B_B10_PoP#2_CytoScan- - 1.5 - 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 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
1.5 11-0816_LC_ONC134B_B10_PoP#2_CytoScan-F 	Promot 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	61 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	p15.31	8,304	10,0
×		3.00	▲ Cain	5	p15.1	1,949	- 1,1
×		3.00	▲ Cain	6	p14.3	15,225	10,7
		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 Som Mut ♥ III Query Samples ♥ III Query Segments ♥				
		. T		F -4		DD Court Dath	Cine (In
	CN State	 Type 	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	Σ 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		
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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	

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 Likely Pathogenic Unknown Significance Likely Benign 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			
	🗟 🗎 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 💷 🎚 🛊 🚮	V				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	, 1	
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 🔢 🕴 🛊	M			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 🎙 🏾	▼ ♥ ↓ 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 34C2_0N_100T_W	3.00	▲ Gain ▲ Gain	chr12:189400-1104608 chr12:1216632-50589836	
34C2_0N_100T_W 34C2_0N_100T_W	3.00	▲ Gain ▼ Loss	chr12:50895901-133818115 chr13:19084823-83475931	
34C2_0N_100T_W	3.00	▲ Gain	chr15:94993371-97760294	
34C2_0N_1001_W 34C2_0N_100T_W	1.00	▼ Loss ▼ Loss	chr17:400959-27580756	Deletion 13 Deletion 13q
34C2_0N_100T_W 34C2_0N_100T_W	3.00	▲ Gain ▲ Gain	chr17:27600648-35975176 chr17:36165624-37832222	Chromosomal abnormality
34C2_0N_100T_W	4.00	▲ Gain ▲ Gain	chr17:37898475-38563079 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 dh
	11 interpret Loss1.0_Constitutional_Blood_01_CytoScanHD.cy	h 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 # for dbSNP 129 database on hg18 on chr6 # near the gene SLC22A1 (chosen at random) # The columns ave:	
# Sequence Min Max Name # The "browser" line is ignored by ChAS, used by UCSC Browser. # The "track" line is used by both ChAS and UCSC Browser.	
# ChAS ignores all parameters except "db". # UCSC Browser also uses "name", "description" and others. # # Specify genome version on track line as: db="hg18" #	
browser position chr6:160460899-160501368 track_db="hg18" pame="SNPs near SLC22A1" description="test" chr6 150457853 160462854 re41262793	
chr6 160462997 160462998 rs34447885 chr6 160463023 160463024 rs34570655 # This comment is ignored	[•] hg version
chr6 160463069 160463070 rs35888596 chr6 160463079 160463080 rs2297373 # 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 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: – CN loss or gain – XON loss or gain – Mosaic loss or gain – LOH
Segment Min	Starting position of segment.



CytoRegion Table Column	Description
Segment Max	Ending position of segment. 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).
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 touching 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. Successfully created "C:\CHAS 3.3\Charcot_Marie_Panel_File.aed".
	1 gene symbol(s) cannot be found: 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.
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
<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.
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"
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 EXP Files ✓ Start of Cytosc ✓ Bull DECIPH ✓ B* UNY La	Analysis ChAS DB Preferences Help ✓ S ↓ ▼ ✓ ✓ ▲ anHD_Group2_2 ↓ ★ ★ ★ ★ ★ anHD_Group2_2 ↓ ★ ★ ★ ★ ★ ★ ★ b Cases.aed ↓ ★ ↓ ★ ★ ↓ ★ ↓			
XON Reg	Close Save ≟ Expand Annotation Track 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	×
 ✓ DGV DGV-GS Gain DGV-GS Loss ☑ Ensembl Genes ☑ Genes I Banloinsufficiency 	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							
🗄 🐻 🗎 📗		~						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 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 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: CN loss or gain Mosaicism LOH
Segment Min	Starting position of segment.
Segment Max	Ending position of segment. 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).
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%. 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 Look in: My Docum My Compu My Netwo	Desktop ients der rk Places	
File <u>N</u> ame: Files of <u>T</u> ype:	MyAnnotations 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 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 seg85	Category 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 –		
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		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 –		
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)

Figure 328 Right-click menu for View/Edit Annotation Properties in the Detail View							
noScan-PS 20110511.cyhd.cychp.aed ≷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. Note: Always use alphanumeric characters and underscores. Avoid the use of odd characters. (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	v	v	
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 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 CytoScanDDG2PGeneList.r1.aed	
Genome	NetAffx Build
Array	Modified 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 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 eneSymbolList ((IM) 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) Text Text	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) Text Text			-			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) nut_sontax_cds (COSMIC) ame (General) ote (General) isorder (OMIM) eneSymbol (OMIM) eneSymbol List (OMIM)	name (General)	Text					
nut_syntax_aa (COSMIC) ame (General) ote (General) isorder (OMIM) eneSymbol (OMIM) eneSymbolList (OMIM)	nutScore (Biology)						
ame (General) ote (General) isorder (OMIM) eneSymbol (OMIM) eneSymbolList (OMIM)	nut_syntax_aa (COSMIC)						
ote (General) isorder (OMIM) eneSymbol (OMIM) eneSymbolList (OMIM)	name (General)						
iisorder (OMIM) eneSymbol (OMIM) 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 AED/BED Annotation Property Name 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 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
 ✓ □ ● Bet ✓ □ ● ▲ ✓ □ ● ▲ ✓ □ ● ▲ ✓ □ ● ▲ 	a13_F_01_Cyt Beta15_M A s from car	o_VH.cychp Close 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 - 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 Genes	mor vs Lung_9yr_	Normal_20140930.a	aed		TC06000935.hg.	1		
Lung_9yr_Tur Genes	mor vs Lung_9yr_	Normal_20140930.a	aed	CEES	ТС06000935.hg . Туре	1 DownRegulated		
Lung_9yr_Tur	nor vs Lung_9yr_	Normal_20140930.a	aed	CEES	TC06000935.hg. Type Location	1 DownRegulated 6: 118,869,442 - 118,881,893		 ₩1Δ1←
Lung_9yr_Tur Genes	mor vs Lung_9yr_	SLC35F1→	aed		TC06000935.hg. Type Location Size (kbp)	1 DownRegulated 6: 118,869,442 - 118,881,893 12	- + 	N1A1← +
Genes	nor vs Lung_9yr_ 118200kb	SLC35F1-+ 118400kb	aed	CEES HHIIIII CE HHIIIIIIIIIIIIIIIIIIIIIIIIII	TC06000935.hg. Type Location Size (kbp) TAC Fold Change	1 DownRegulated 6: 118,869,442 - 118,881,893 12 -4.390		NIAI← ↓↓↓↓↓↓↓ 119600kb
Lung_9yr_Tur Genes +# 118000kb	118200kb	SLC35F1+ 118400kb	aed		TC06000935.hg. Type Location Size (kbp) TAC Fold Change TAC P-Value	1 DownRegulated 6: 118,869,442 - 118,881,893 12 -4.390 0.0		NIAI← ↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓↓
Lung_9yr_Tur Genes 	nor vs Lung_9yr_ 118200kb	SLC35F1-+ 118400kb 922.2	aed		TC06000935.hg. Type Location Size (kbp) TAC Fold Change TAC P-Value TAC Gene Symbol	1 DownRegulated 6: 118,869,442 - 118,881,893 12 -4.390 0.0 PLN		NIA1← +

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		
P 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 Gain. Marker Count ≥ 50, Size (kbp) ≥ 50. Loss. Marker Count ≥ 25, Size (kbp) ≥ 25. 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 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 Chosen Use In Export File Name/ID CN State Type Full Location DB Count Both Score (Exon Region) Size (kbp) Marker Count CytoRegions Call Table Sorting Optionally select one or more columns for sorting data i Sort by: CN State Then by: Chromosome	Available % of Overlap Map Item covered by Segment BACs Call Approval Chromosome Curation By Curation Time Cytoband End Cytoband Start DB Coverage Count DB Overlap Count DGV End Marker New York Start DESCENDING V 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						
 Image: Segments Segments CytoRegions Cy						
File [■♥ ♂ 11-0810_L ■♥ ♂ 11-0810_L ■♥ ♂ 11-0810_L ■♥ ♂ 11-0810_L	▼ CN State 3.00 3.00 3.00 3.00	Type ▲ Gain ▲ Gain ▲ Gain ▲ Gain	Chromosome	000	Hide "Chromosome" 6, Show all columns 5, Expand "Chromosome" 8, Auto-size all columns 4, Restore defaults Select columns Save table state Apply table state > 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
■0 11-0810_LC_ONC13B_A6	3.00	🛦 Gain	2	p15	159	212	USP34, XPO1
∎ ♥♂ 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	
ີ 0 d [*] 11-0810_LC_ONC13B_A6	3.00	🛦 Gain	10	q11.22	1,332	276	SYT15, GPRIN
■0 [*] 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
■0 [*] 11-0810_LC_ONC13B_A6	3.00	🔺 Gain	17	q21.31	572	72	KIAA1267, LO
○ 0 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 (Mean: 412.829 kbp Median: 259.952 kbp						∢ > ₀	
11-0810_LC_ONC13B_A6_PoP#	5_20 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 - 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 Chosen Use In Export File Name/ID CN State Type Full Location DB Count Both Score (Exon Region) Size (kbp) Marker Count CytoRegions Call	able' and 'Chosen'. Re-order entries in 'Chosen' by dragging. Available % of Overlap Map Item covered by Segment BACs Call Approval Chromosome Curation By Curation Time Cytoband End Cytoband Start DB Coverlap Count DB Overlap Count DGV End Marker
Table Sorting Optionally select one or more columns for sorting data 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	 CN State 	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										
	p	Σ	1 🛊 👪	V							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				v ,	~
<												>	

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 10th item to indicate that some data are not displayed in order to save calculation time. For example, "..." will follow the 10th 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 Item covered by 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 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). 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 (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 Coverage	The number of segments in the database meeting the Minimum Percent Coverage and the selected Filter Criteria.
Filtered DB 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. Note: This column is only applicable to CNState Gain and Loss segments.
Following Marker Location	The coordinate location of the array marker just below the segment in the data track used as input for the segment. 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 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 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 Time	The time and date when the Segment was last materially modified.
Мах	End position of segment. 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).
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 Distance	Length of the segment in base pairs divided by the number of markers in the segment.
Mean Weighted 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 Nomenclature	An ISCN-based description of the segment.
Microarray Nomenclature ISCN 2013	ISCN 2013 based description of the segment.
Min	Start position of segment.
Number of Overlap 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 Loci	List of OMIM Phenotype Loci that share coordinates with the segment.
OMIM Region 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 (% of 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.
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 Location	The coordinate location of 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.
Precision (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 Genes	List of protein coding RefSeq genes from the Genes track that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Protein Coding Genes Count	Number of Protein Coding RefSeq genes that shapre coordinates with the segment.
Protein Coding Ensembl Genes	List of protein coding Ensembl Genes annotations that share coordinates with the segment. Identically named gene isoforms are NOT repeated.
Protein Coding Ensembl Genes Count	Number of Protein Coding Ensembl Genes that share coordinates with the segment.
Sample UUID	Unique identifier for the CHP file.
Segment 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 Duplications	List of Segmental Duplications that share coordinates with the segment.
Size (kbp)	Size of the segment.
Smoothed/Joined/ 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 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	 Whether marker is located in a CytoRegion or not: ✓ In CytoRegion ➤ Not in CytoRegion. See "Using CytoRegions" on page 263 for more information.
Markers	Marker ID. Right-click to link to NetAffx information about the marker. 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 ▼ № ♀ 09-1420_B2_Phase4CustomerPanel_CytoScan_PS_20110228.hg38. □ № ♂ 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. There will be separate data columns for each CYCHP file in the exported file.
Separate File for each 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 Chromosome and Separate File for 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 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): MutScore.old = (measured quantile normalized signal - median signal for this marker in the reference model file) / (95th percentile signal for this marker in the reference model file - median 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): 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 СҮТО2	1.0.0	
Region Files	e (ChAS) 	c S S	reated (General) modifie at Jul 03 07:56:1 Sat Ju	sd (General) 103 13:56:5 Summary Data		<u>></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 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 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 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 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 Algorithm	Call	Gender Call Confidence
ChAS 1.X, 2.X and 3.X/4.X CytoScan Arrays	Y-gender	male/female	Yes
GTC 3.0 to GTC 4.1/ Genome- Wide SNP Array 6.0	affymetrix- chipsummary-Gender	male/female/unknown	No
GTC 2.1/ Genome-Wide SNP Array 6.0	affymetrix- chipsummary-hasY	male/female	No
GTC 2.0/ Genome-Wide SNP Array 6.0	affymetrix- 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

*	\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	M 🗹 🗆				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 N		

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. 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. 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. 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 F L	ïle A: ✓ ïle B: ✓ <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: Beta10_F_01_Cyto_VH.cychp Beta15_M_01_Cyto_VH.cychp Beta13_F_01_Cyto_VH.cychp V DGV Genes 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										
		P								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. 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).
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. 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)
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[®] 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 Optional: Associate some or all tiers with calls. Tier 1 v Tier 2 v Tier 3 v Tier 4 v 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 DB-B: Segment has a 'DB Count Both' ≥ 0
✓ DB-F: Segment has a 'Filtered DB Count Both' ≥ 0
OM-3 : 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. 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 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
settingsYou can assign a Call to represent each tier. The contents of the drop-down list was
generated from the Calls Vocabulary list. There are a set of default 'Calls", but this list
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 Level 2	Restore Defaults
Level 3	
Level 4 O OF	Cancel
VOUS	
Probably nothing	
Review Last	
 Review Last 	

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? 6: Review Last 2: Unknown Significance 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 Optional: Associate some or all tiers with calls.	Score to Call 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 2I+: 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 40-DB-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 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

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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	2E+: 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	2H : 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	3B : and overlaps at least this many: 25	
0.9	3C: and overlaps at least this many: 35	
-1	4O-DB-B : Has 'DB Count Both' ≥ 400	
-1	4O-DGV-GS : 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 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 (static value, further assessmen t required)		
2C-1	The Loss copy number segment overlaps the 5'UTR and some CDS of a gene with HI score = 3 in the Haploinsufficiency track. TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates. 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.	0.9		
2C-2	The Loss copy number segment overlaps the 5'UTR, but no CDS of a gene with HI score = 3 in the Haploinsufficiency track. TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates. 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		
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. TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates. 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 for a gene with HI_score = 3 in the Haploinsufficiency track. TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates. 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.	0.3		



Score-based rule/evidence	Description	Default 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. TIP: Right-click on the transcript, choose View/Edit Annotation Properties, then select the Structure tab to view the exons and CDS coordinates. 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 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 Level 4				
	VOUS Probably nothing 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 2: Level 1 6: Review Last 3: Unknown Significance <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: 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 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)	~						
∇							
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 seg132 CyHD_021312T_SS135_401763S	. A Gain 3.00 22 18,648,	21,465,659 2,817 polymorphic (ADGV)	Cogmont Intercept	iono Toblo ^{100%}	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. 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 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 Reporter	Displays the Oncomine Reporter terminology assigned to the segment.
Segment Interpretation	Displays Segment Interpretation assigned to this sample.
% of Overlap Item covered by Segment	The percentage of the Overlap Map Item covered by the segment.
Call from 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 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
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 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 □ Σ □ Σ	24 (Gain); Cove ;	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 100000000000000000000000										
					A								
				Δ									
													^
U				 									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 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 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 👷 🗎 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	 cytoscanhd_array 	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	^

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 X				
0.5 1 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 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 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 <i>No Value</i> blood (constitutional) bone marrow 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 cytoscan_xon_array cytoscanhd_array	Not Filtered
Published		
Segment Interpretation Phenotype Publisher Sample ID File Sexes Female Male Unknown Not Reported	Calls No Value benign likely benign likely pathogenic pathogenic polymorphic (adgv) 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				
✓	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 chrX: 620,972 - 650,195 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 Segment Interpretation					
Phenotype 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 [▼] I Query Samples [▼]	💷 Query Seg	ments N			(
🔠 🔜 😰 🗎 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	E -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 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. This information will be tracked for all highlighted samples. 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 Solution Detected Undetected Gain (XON Region) Loss (XON Region)</table-cell></table-cell></table-cell>	cytoscan_xon_array 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	a		
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.

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- 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 ClinVar Summary Export Signatures Thermo Fisher	r Rules Misc Vocabularies	DB Query	Filtered DB Query	Exports	
New 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 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></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 disting Export					
Existing Exp	ort disting Export					
Existing Exp Add to Exp Table Data Convert t	ort	ments Table				
Existing Exp Add to Ex Table Data	ort disting Export o paragraph style for Seg ow Numbers	ments Table				
Existing Exp Add to Ex Table Data Convert t Include R Hide Y Chror	ort disting Export o paragraph style for Seg ow Numbers nosome	ments Table				
Existing Expr Add to Ex Table Data Convert t Include R Hide Y Chror Hide the	ort disting Export o paragraph style for Seg ow Numbers nosome Y Chromosome in the Ka	ments Table				
Existing Exp Add to Ex- Table Data Convert t Include R Hide Y Chror Hide the	ort disting Export o paragraph style for Seg ow Numbers nosome Y Chromosome in the Ka	ments Table				
Existing Exp. Add to E2 Table Data Convert t Include R Hide Y Chror Hide the Select File	ort o paragraph style for Seg ow Numbers nosome Y Chromosome in the Ka	ryoview				
Existing Exp Add to Ex Table Data Convert t Include R Hide Y Chror Hide the Select File	ort o paragraph style for Seg ow Numbers nosome Y Chromosome in the Ka	ryoview				elect File
Existing Exp Add to Ex Table Data Convert t Include R Hide Y Chror Hide the Select File	ort o paragraph style for Seg .ow Numbers nosome 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.

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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					
clude Sample Properties					
terpretation					
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.

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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 + 🔏 (V							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 1	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	×	q25		~	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	65 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	gure 4	66 1 7	Segme	ent tab	le - Edit	Mo • ov	de OFF rerlap Map 🎙 🔀	Graphs 🎙			
E	🗎 📠 🗎	Σ		• 🔏 🛙	V					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	67 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 	► I

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	 	 	
Existing Export			
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; you will be adding to it or overwriting it. 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	 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 existing ClinVar	 Highlight the profile name in the ClinVar Submission Info list box that is to be modified.
profile	2. In ClinVar Submission Info pane, click the Edit button.
	3. Edit the appropriate fields.
	4. Click OK to save your changes.
Deleting a profile	 Highlight the profile name in the ClinVar Submission Info list box you want to delete.
	2. Click the Delete button.
	3. Click OK to confirm the profile deletion, or click Cancel to keep the profile.
Adding 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 Exports tab, then click the ClinVar 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).
	 From the ClinVar vocabularies drop-down, select the category that you want to add a term(s) to.
	5. Use the text field (at the bottom) to enter the additional term(s).
	6. Click the Add button to add the term to the category's list.
Removing 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 Remove .
Exporting in ClinVar format	There are certain fields that are required before uploading to ClinVar. It is recommended you use the ClinVar Table State 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 Use in Export 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 OK .
	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/ 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/ 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 Filters	CytoRegion Segment Filters	Data Types
Standard	Gain: Marker Count = 50 Size (kbp) = 400; Loss: Marker Count = 50 Size (kbp) = 400	Gain: Marker Count = 50 Size (kbp) = 400 Loss: Marker Count = 50 Size (kbp) = 400	Gain Loss GainMosaic LossMosaic Copy Number State Weighted Log2 Ratio Allele Peaks Allele Difference
High Resolution	Gain: Marker Count = 50 Size (kbp) = 100; Loss: Marker Count = 50 Size (kbp) = 100	Gain: Marker Count = 25 Size (kbp) = 50 Loss: Marker Count = 25 Size (kbp) = 50	Gain Loss GainMosaic LossMosaic Copy Number State Weighted Log2 Ratio
LOH only (3Mb and 50 SNPs)	LOH: Marker Count = 50 Size (kbp) = 3000	LOH: Marker Count = 50 Size (kbp) = 3000	LOH Genotype Calls Allele Peaks Allele Difference
Differential Gains and Losses	Gain: Marker Count = 50 Size (kbp) = 400; Loss: Marker Count = 50 Size (kbp) = 100	Gain: Marker Count = 25 Size (kbp) = 50 Loss: Marker Count = 25 Size (kbp) = 50	Gain Loss GainMosaic LossMosaic Copy Number State Weighted Log2 Ratio



Named Setting	Genome Segment Filters	CytoRegion Segment Filters	Data Types
OncoScan Defaults	0	0	0
XON-Level 1	XON Level: Level 1 = On Level 2-4 = Off XON Gain/Loss Marker: Count = 0 Size (kbp) = 0	0	XON Region Gain, XON Region Loss, LOH Segments, Log2 Ratio, Smooth Signal, 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 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 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 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	186 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	
 Analysis Libraries ChAS Parameters Markers CytoScanHD CELs Music Desktop Pictures Documents Profile_0643 Downloads Recorded TV Favorites Genotypes 	Directory that contains the "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 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 (.chaslink) file, click to highlight it, then click Select File .		
	3. Restart the ChAS Browser to apply the link update(s).		
	Note: For .chaslink file help, contact Technical Support.		
Configuring the HTTP service	The HTTP service may enable external applications to interact with the ChAS Browser. 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.		
	Click the check box to enable the service, then enter the designated Port number.		
	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/. 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 Username:	D Sign out
Sign In to ChAS Username: admin 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 ×	_ D 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:	
 to enable batch uploading of CHP and CHPCAR files. to backup and restore the database. run cleanup scripts. 	
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)			
O Uptime 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 × □ ← → C □ localhost:8099/wicket/bookmarkable/com.affymetris ☆ ≡
CHAS
Status
Maintenance
Administration
Maintenance: Restore Database
Choose File No file chosen
Restores the selected database backup file, overwriting the current database. 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 calhost	×	et/bookmar	kable/com.af	fymetrix 🛣 🔳
CHAS Status					
Maintenance 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 I ▼ L 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 I. Not filtered. All will	e used. I be used.					
LOH. Type	turned off. Will be	e skipped.					
XON Regio Gain (XON	n Levels. These le 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. 0.0.	
Recent Desktop Docu	 HTCMAV2_ 	P40_A0000076_GT6_F01.rhchp i HTCMAv2_P40_A0012100_GT6_ P40_A0000091_GT6_F06.rhchp i HTCMAv2_P40_A0012103_GT6_ P40_A0000623_GT6_F12.rhchp i HTCMAv2_P40_A0012164_GT6_ P40_A0000819_GT6_F03.rhchp i HTCMAv2_P40_A0012165_GT6_ P40_A0000819_GT6_F04.rhchp i HTCMAv2_P40_A0012169_GT6_ P40_A0000943_GT6_F05.rhchp i HTCMAv2_P40_A0012174_GT6_ P40_A0000988_GT6_F02.rhchp i HTCMAv2_P40_A0012175_GT6_ P40_A0001076_GT6_F07.rhchp i HTCMAv2_P40_A0012178_GT6_ P40_A00012064_GT6_A07.rhchp i HTCMAv2_P40_A0012184_GT6_ P40_A0012097_GT6_D07.rhchp i HTCMAv2_P40_A0012185_GT6_ P40_A0012099_GT6_D03.rhchp i HTCMAv2_P40_A0012185_GT6_ 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 CYCHP OSCHP RHCHP XNCHP 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 Tools \rightarrow Test Connection
	A message appears if there is a successful connection to the ChAS database.
Verifying the ChAS database	 From the ChAS Browser, click the Preferences drop-down menu, then select Edit Application Configuration
	The Configuration window appears.
	2. Click the Server 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	
▲ 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	
- → 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. Publish				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	PUBLISHING		
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 				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 Rows	The rows below the Metadata properties are the annotations. Each row is a tab- delimited list of values. Each value must have the correct data type, as described in 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 and other spreadsheet applications	Though not recommended, an AED file may be edited using most spreadsheet programs that support tab-separated value (TSV) files and that recognize a byte order mark (BOM). An AED file can be edited in Microsoft Excel, for example, using the following rules: When loading an AED file into Microsoft Excel as a TSV file, make sure that the Unicode code page for the correct encoding is selected (preferred), or accept the default "Windows (ANSI)" code page (which should still recognize Unicode characters if the correct BOM is present in the file). When saving an AED file from Microsoft Excel, make sure the "Unicode Text" type is selected. This will result in a file encoded in UTF-16LE, which is still a valid AED file as it begins with the appropriate BOM.
Microsoft Notepad and other Text editors	Though not recommended, an AED file may be edited by any text editor that supports Unicode and that uses a byte order mark (BOM) to indicate the charset. The version of Microsoft Notepad in Windows XP, for example, will both correctly read text files marked with a BOM and save text files using the appropriate BOM if the following rules are followed: When saving an AED file from Microsoft Notepad, make sure the encoding is set to "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 Unicode and BOM, and provides a wide range of built-in glyphs for representing Unicode code points that cannot be viewed on most other text editors.
References	
	ISO 8601: 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/ ms776429(VS.85).aspx
	RFC 3986: <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
	RFC 4122: <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
	RFC 4180: <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. Note: For CytoScan HTCMA algorithm and QC, see the RHAS User Guide.
Feature identification and signal extraction	GeneChip Cartridge Microarrays are scanned on the GeneChip Scanner and processed by the GCC scanner software package. GCC aligns a grid on the DAT file (the original scanned image) to identify each microarray feature and calculates the signal from each feature. This process uses the DAT file, containing the raw signal, and creates a CEL file, which contains a single signal intensity for each feature. The CEL file is used for all downstream analyses.
Single sample CytoScan workflow	Beginning with the raw signal data in the CEL file, the Single Sample CytoScan Workflow implements a series of steps that perform probe set summarization, normalization, removal of variation caused by known properties and residual variation, and completing with calling genotypes, copy number segments and LOH segments. A brief overview of each step performed by the CytoScan Workflow is shown in Figure 525 on page 481. In addition, a rough sketch of Analysis Pipeline (for Single Sample Analysis) is demonstrated in Figure 525 on page 481.

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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 calculation	Log2 Ratios for each marker are calculated relative to the reference signal profile. The Log2 Ratio is simply Log2(sample _m) – Log2(reference _m), for each marker, "m".
High pass filter image correction	Since most probes map to genomic markers associated with a normal copy number, most Log2 Ratios should be centered at a value of zero. Also, since markers from any genomic region are scattered across the surface of the microarray, regions of altered 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 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 Autosome 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 residual variability 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
segment
algorithmThe algorithm for detection of copy number aberrations in the presence of mosaicism
considers single copy deletions and gains. The algorithm is tuned to be most accurate
when the normal/expected Copy Number State is two. The algorithm targets detection
of changes of approximately 3MB or more in size (for CytoScan HD). Copy number
change events less than this size may be detected; however, sensitivity and specificity
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 GC correction	Systematic changes in Allelic Differences can be related to differences in GC content. For instance, on a given sample Allelic Differences representing AA and BB genotype markers might get progressively closer or further from each other as the GC content changes. It is assumed that such changes represent unwanted variability. The Allelic Difference GC correction determines differences in the structure of the allelic differences associated with GC and then removes these differences. For CytoScan HD the super GC covariate is used. For CytoScan 750K the Local GC covariate is 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 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

2.0.



Array data QC metrics

This section provides a high level overview of the key QC metrics used with the CytoScan arrays.

Median of the
Absolute values
of all Pairwise
Differences
(MAPD)MAPD is a global measure of the variation of all microarray probes across the genome.
It represents the median of the distribution of changes in Log2 Ratio between adjacent
probes. Since it measures differences between adjacent probes, it is a measure of
short-range noise in the microarray data. Based on an empirical testing dataset, we
have determined that array data with MAPD > 0.25 (for CytoScan 750K and HD, MAPD
> 0.29 for CytoScan Optima) has too much noise to provide reliable copy number
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.
Based on an empirical testing dataset, we have determined that array data with
SNPQC < 15 (for CytoScan 750K and HD, SNP QC < 8.5 for CytoScan Optima) is of
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_i as the variance between adjacent probes:

$$Z_i = X_{2i+1} - X_{2i}$$

Waviness-SD is the total variance (X_i) minus the local variance (Z_i):

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.

Chromosome Analysis Suite (ChAS) User Guide

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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	
Chromosome Analysis Suite IFor Research Use Only Not for use in diagnostic procedures 1	- 🗆 X
File View Exports Analysis ChAS DB Preferences Help	
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	281,034 - 56,097,208
, III Karyoview C Segments Cytoregions C POVenap Map Cytoregions C POV	
	3 results 3
File A Common Name A Type Mutation Score Name/ID Chr Min Max	COSMIC ID
■ ♥ ♂ 201401 EGFR:p.G719A:c.2156G>C ● High Confidence 13.593 som-93107463C 7 55,241,707 55,241,708	COSM6239
■ ♥ C 201401 EGFR.p.G719C:c.2155G>T ● High Confidence 11.088 som-93107472C 7 55,241,706 55,241,707	COSM6253 °
Image: Construction of the system	COSM6252 6
¢	< > 7
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20140116_SME161_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_AS_34.RC.OSCHP; Copy Number State	^ 10
-40 Copy Number > 30	11
-20	
201401116 SMF151 240k C10 SMV WD7230 Tum Colorectal CHTNWD AS 34 BC OSCHP1 og2 Batio	13
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F3	15
Electron contraction and the second sec	16
	17
20140116_SME161_240k_C10_SMV_WD7230_Tum_Colorectal_CHTNWD_ASI_34.RC.OSCHP: BAF	18
L Lo.s	10
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20140116_SME161_240K_C10_SMV_WD7230_10m_C000Fectal_CH1NWD_A5_34.KC.OSCHP. Sofialic Mutation (segments)	22
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OMIM® Genes 3 somatic mutations	Y
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ChAS Browser : NetAffx 33.1 (hg19) Restricted Mode: Off Edit Mode: Off	User: CB
ChAS Browser : NetAffx 33.1 (hg19) Restricted Mode: Off Edit Mode: Off	User: CB



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	+ 5 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 	-
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 Use Normal Diploid a	arget Genor malysis for C Upload to Fr	ne Versio ytoScan H ranklin (Ge	n: hg19 D/750K: 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 ^{...} 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 \AutoCelAnalysis\CytoHD\21-3351.CEL to archive folder								
8/11/2023 6:26:48 PM	Copying fil Scientific\E \CHP\2023 archive fold	e: C:\Users\yong: Documents\Proje -08-12T01-25-252 der	xin.yu\OneE cts\ChAS 4.5 Z.cyhd.Copy)rive - Thermo F 5\Autouploader NumberReport.	isher _Test 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 Fisher Scientific\Documents\Projects\ChAS 4.5 \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 Created: Aug 11, 2023 Assay: CytoScan HD HG38	72-7f5b-00187600	1	unassigned		Active)		:
21-3351.cychp (00004f80-29be-47fe 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