applied biosystems

Eureka™ Analysis Suite

User Guide

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Chapter 1 Introduction

Eureka Analysis Suite Overview

Eureka Analysis Suite allows you to perform the following functions:

- Set up your Eureka™ Genotyping Assay plate
- Calculate amounts of each pooled library that will be blended together on a single sequencing lane.
- Run QC and Genotyping Algorithms
- View Cluster Graphs of genotype calls
- Export your Data

The Suite contains three modules:

- **Eureka Genotyping Calculator**—Enables an end user to calculate the amount of each pooled library (one 384 plate pooled = one pooled library) to be mixed together & run on a single NextSeq lane. The calculator takes into account the control library from Illumina (PhiX), number of reads required, DNA concentration, read length, and SNP panel size.
- **Eureka Analysis**—Uses a genotyping algorithm to call genotypes.
- **Eureka Viewer**—Displays analysis summary metrics, Sample summary metrics, and SNP summary metrics, including visualization of cluster plots of the genotypes called.

Minimum Recommended Requirements

64-bit Operating System	Speed	Memory (RAM)	Available Disk Space*	Web browser
Microsoft Windows 7 (64 bit) Professional with Service Pack 1	2.83 GHz Intel Pentium Quad Core Processor	16 GB RAM	150 GB HD + data storage	IE 8.0 and above

*****Minimum storage requirements are for a single run. Total storage space should include additional space for data storage of input and output files from current and previously completed analyses.

Installation Instructions

1. Go to the Eureka Analysis Suite web page on the Thermo Fisher web site.
2. Locate and download the Eureka Analysis Suite software zip package.
3. Unzip the file, then double-click **EurekaAnalysisSuiteSetup.exe**
4. Follow the on-screen instructions to complete the installation.

Creating Sample to Well Mapping Files

IMPORTANT: Before using the Genotyping Calculator, you must first create a file that represents each 96 or 384 plate you want to blend.

1. Open MS Excel or Notepad.
2. Create a new table as you normally would.
   - Your table must contain these five headers:

     Sample to well mapping information file
     PlateName
     PlateBarCode
     PlateType
     Items:

   - Your table must contain these three columns:

     WellPosition
     SampleName
     Panel

NOTE: **If you attempt to import a sample to well mapping file that does not resemble Figure 1.1, an error message (with an option to select a pre-formatted template) appears. (Figure 1.2) To access this template, see *Plate File Template*.**

Figure 1.1 MS Excel table example

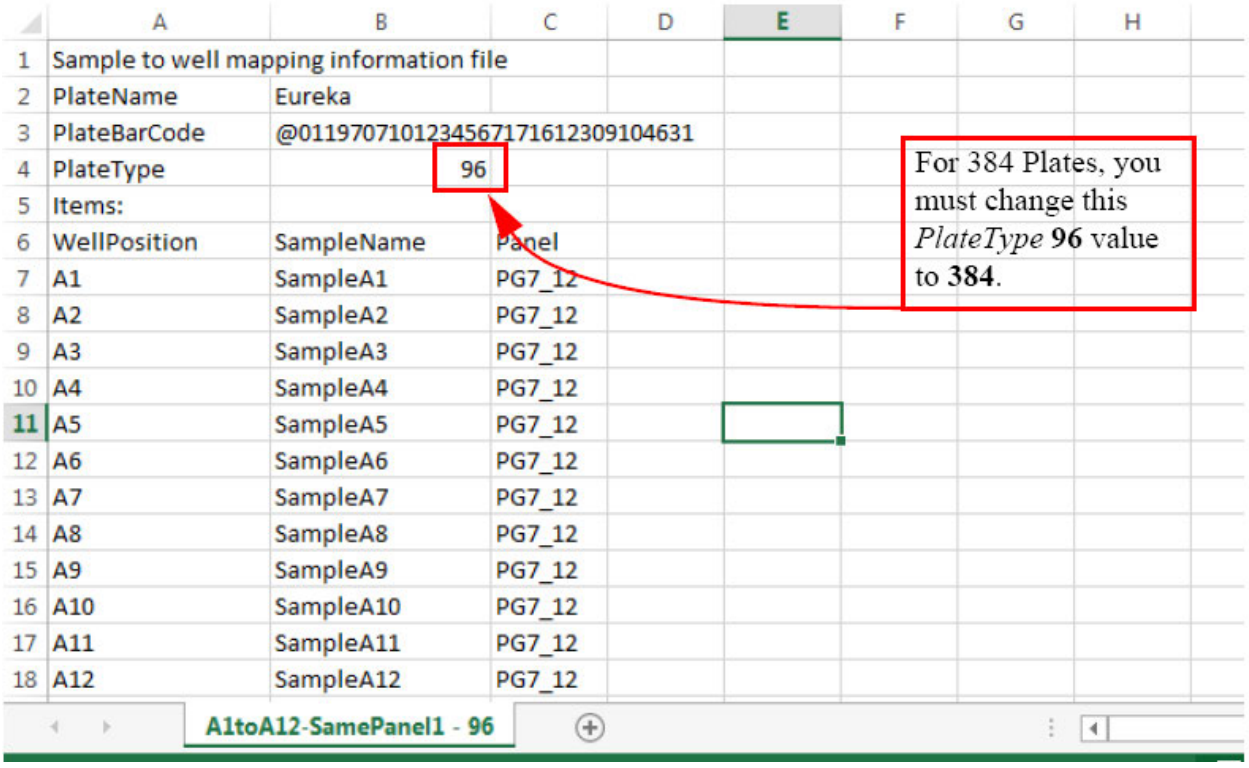
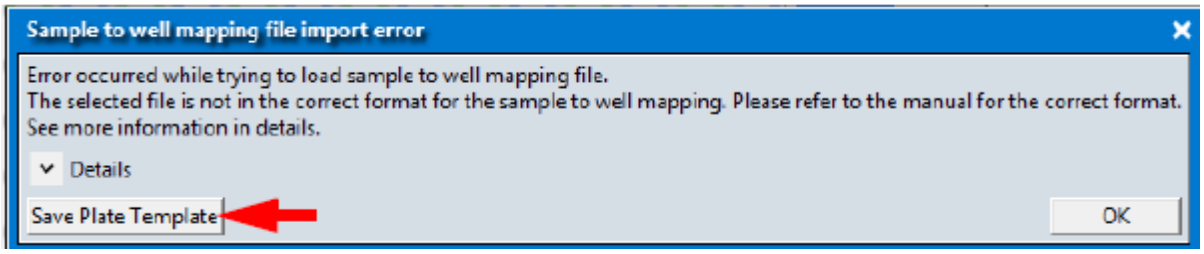


Figure 1.2 Sample to well mapping file import error message



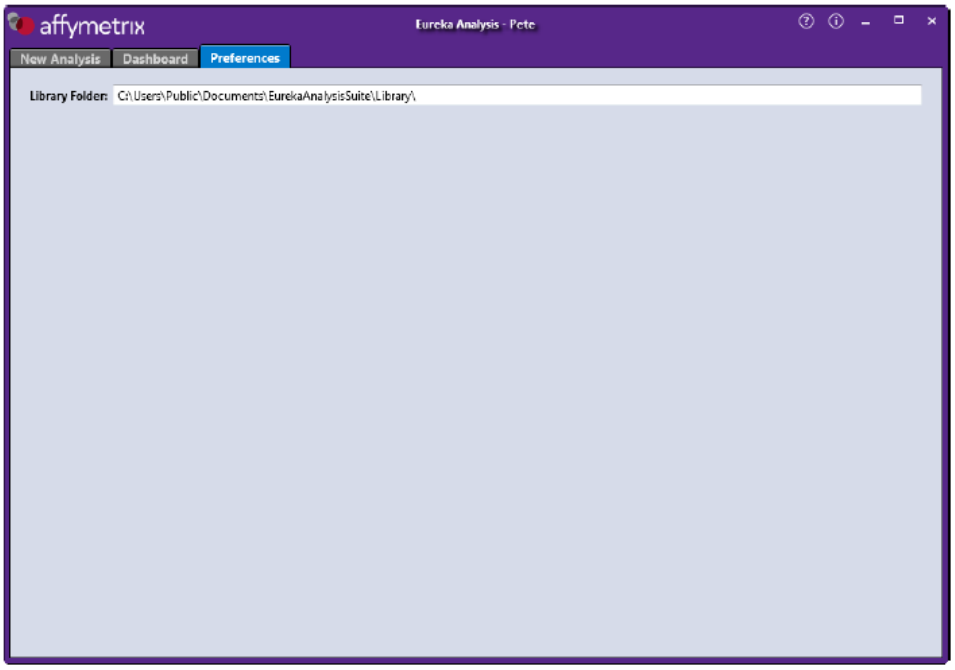
3. After completing your table, you must save it as a tab-delimited.txt file.
4. Open your file and make sure it is formatted correctly, as shown in Figure 1.1.
5. Repeat steps 2–3 to create additional plate text files.

IMPORTANT: Sample Name and Panel fields cannot have blank values. If there is no sample in a particular well, use the word *EMPTY* in the Sample Name field(s). Sample names must be unique. For example, add *Rep1* and *Rep2* to a replicated Sample name.

Viewing your Library Folder Path

In the Eureka Analysis module, click the **Preferences** window tab to view and make note of the assigned library folder path, as shown in Figure 1.3.

Figure 1.3 Main Preferences window



NOTE: The library folder path is auto-assigned during installation and cannot be changed.

Chapter 2 Eureka Genotyping Calculator

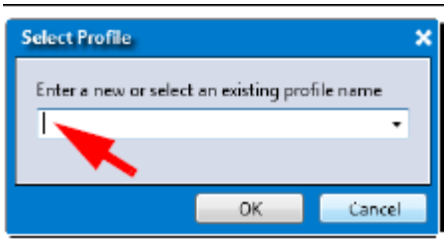
Starting the Genotyping Calculator

IMPORTANT: Before using the Genotyping Calculator, you must first create a file that represents each 96 or 384 plate you want to blend. See *Creating Sample to Well Mapping Files*.

1. Double-click on the Eureka Genotyping Calculator Desktop shortcut icon or click **Start -> All Programs -> Eureka Genotyping Calculator**.

   A **Select Profile** window appears. (Figure 2.1)

Figure 2.1 Select Profile window



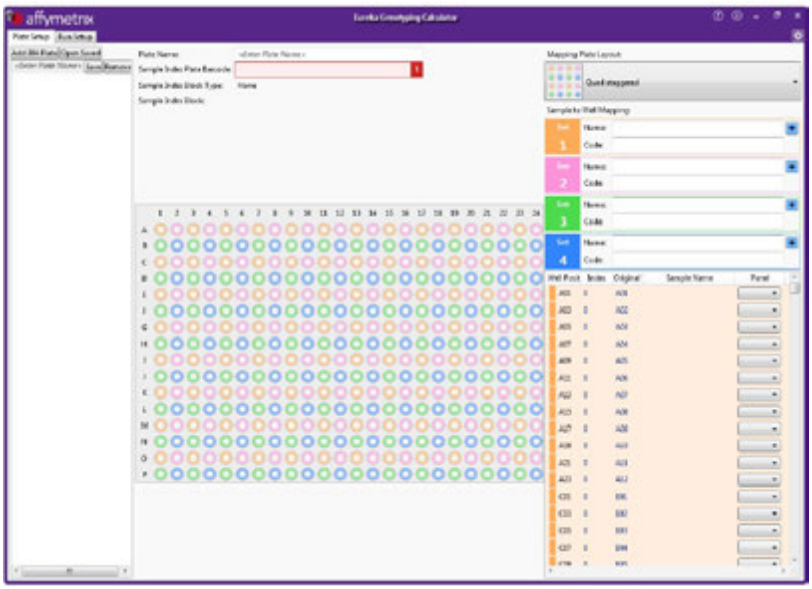
2. Enter a profile name, then click **OK**.

   An Explorer window appears.

3. Navigate to (or create) a folder to save your analyzed files, then click **Select Folder**. This is required after a new installation. If needed, you can change this save location. See *Changing your Default Sample File Folder Location*.

   The Eureka Genotyping Calculator window appears. (Figure 2.2)

Figure 2.2 Main window

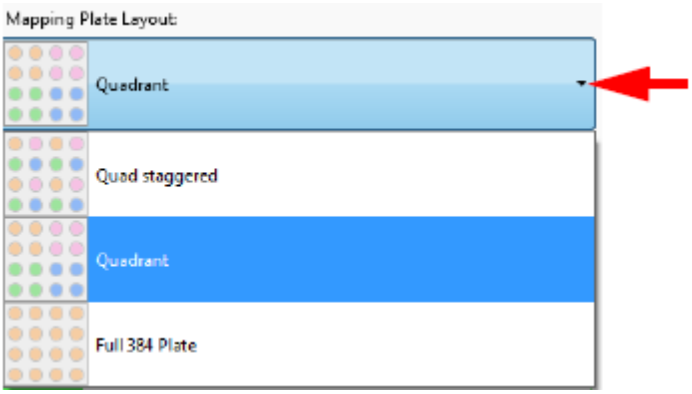


Configuring your 384 Plate(s)

Selecting your 384 Plate Mapping Configuration

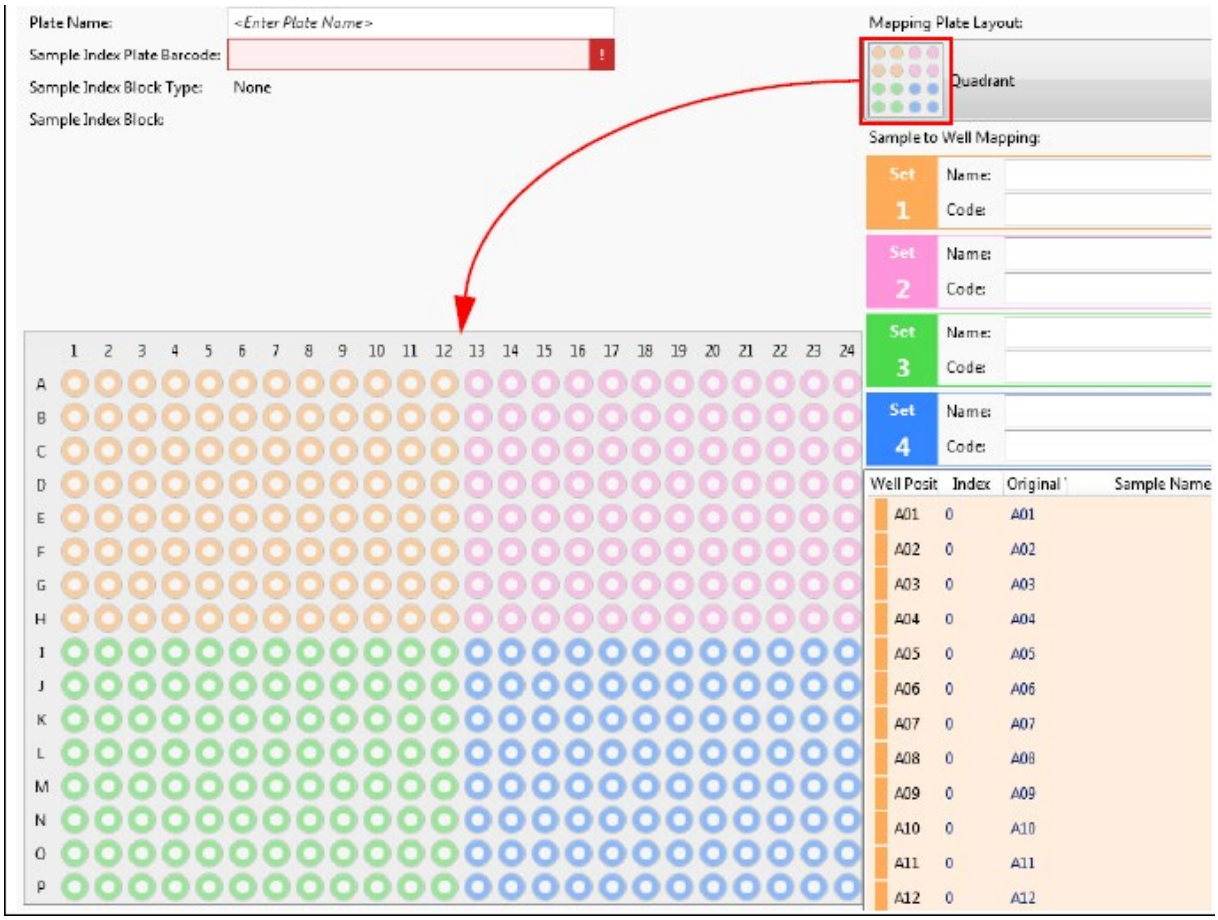
1. Click the **Plate mapping** drop-down menu (Figure 2.3), then click to select the plate mapping configuration you want.

Figure 2.3 Plate mapping - Available plate configurations



Your selection is reflected in the master 384 plate view graphic, as shown in Figure 2.4.

Figure 2.4 Plate mapping - 384 Plate view



Importing your 96 Plate File(s)

1. Click the **Set 1**  button. (Figure 2.5)

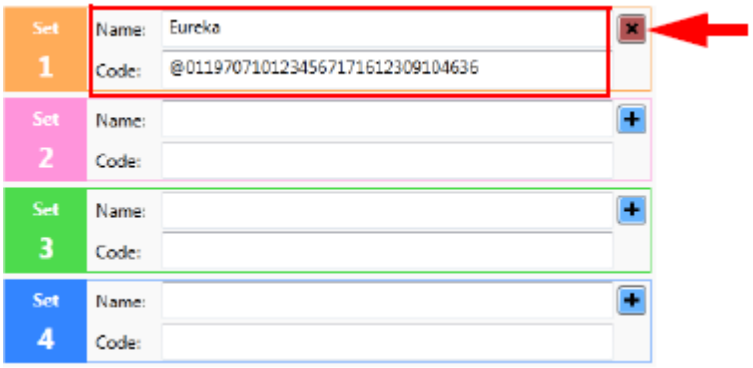
Figure 2.5 Sets 1-4

An Explorer window appears.

2. Navigate to the save location of the 96 plate text file(s) you created earlier. See *Configuring your 384 Plate(s)*.
3. Click on the text file you want to import, then click **Open**.

   The **Name** and **Code** fields auto-populate. (Figure 2.6)

Figure 2.6 Example: Populated Set 1 fields



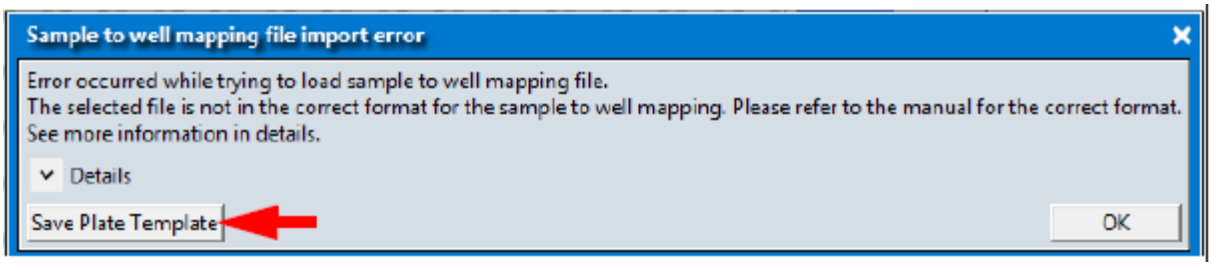
4. Repeat steps 1–3 to populate each additional 96 plate set.

   If needed, click  to remove an imported plate set.

Plate File Template

If you attempt to import a plate file that was not formatted correctly, the following error message appears: (Figure 2.7)

Figure 2.7 Sample to well mapping file import error message



Acknowledge the message, click **OK**, then re-format your text file, as described in *Creating Sample to Well Mapping Files.*

OR

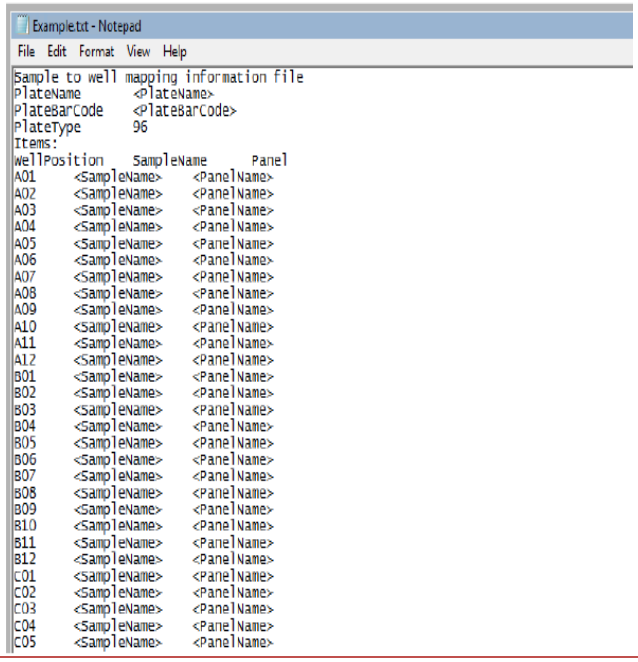
1. Click the Save Plate Template button.

   A **Save template file** window appears.

2. Enter a file name, then click **Save.**
3. Click **OK** to close the error message window.
4. Locate your saved template file, then double-click on it.

   A pre-formatted text file appears (Figure 2.8) and is ready to be opened in MS Excel. If you make edits to the file in MS Excel, make sure you save it as a .TXT file.

Figure 2.8 Plate template of sample to well mapping file import error example



IMPORTANT: Sample Name and Panel fields cannot have blank values. If there is no sample in a particular well, use the word *EMPTY* in the Sample Name field(s).

Custom Settings (Optional)

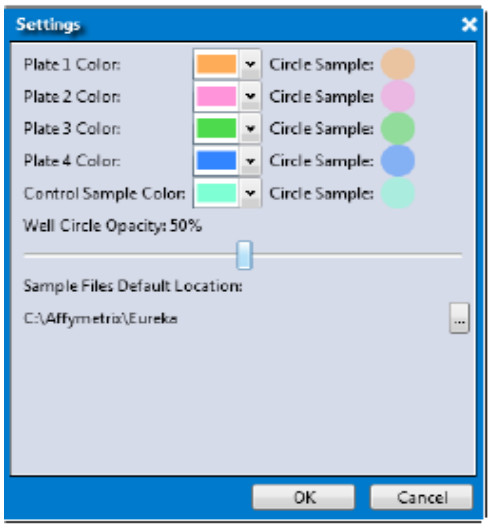
- Changing Plate View Colors.
- Adjusting Well Circle Opacity.
- Changing your Default Sample File Folder Location.

Changing Plate View Colors

1. Click on the  button (upper right corner).

   The Settings window appears. (Figure 2.9)

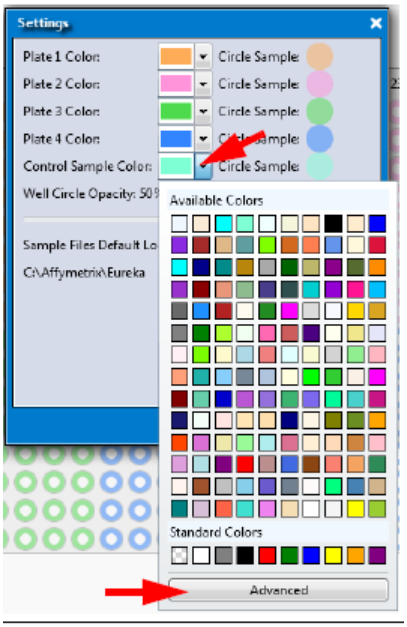
Figure 2.9 Settings Window



2. Click the plate number's drop-down arrow.

   A pallet of available colors appears. (Figure 2.10)

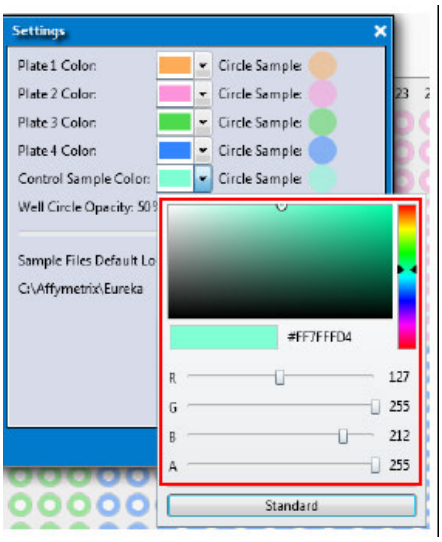
Figure 2.10 Settings Window - Available Colors



3. Click on the color you want, then click **OK** to close the Settings window.
4. Optional: For more color options, click on the **Advanced** button.

   A custom coloring tool appears. (Figure 2.11)

Figure 2.11 Settings Window - Available Colors



5. Use the tool's color mixing features to create the plate number color you want.
6. (Optional) Repeat steps **2-5** to change additional plate number colors.
7. Click anywhere on the Settings window to close the coloring tool and save your custom color.
8. Click **OK** to close the Settings window.

Your newly assigned color is saved and reflected on the application's plate view.

Adjusting Well Circle Opacity

Click, hold, then drag the opacity slider left or right to change the opacity level of your plate well circles. (Figure 2.12)

Figure 2.12 Opacity slider

Changing your Default Sample File Folder Location

1. Click on the  button.

   The Settings window appears.

2. Click the **Browse** button. (Figure 2.13)

Figure 2.13 Sample File Location Browse button

An Explorer window appears.

3. Navigate to (or create) a folder to save your analyzed files, then click **Select Folder**.

   Your default path is now changed.

4. Click **OK** to exit the Settings window.

Entering your Plate Barcode

After importing your 96/384 Plate text files and selecting your mapping configuration, you must scan or manually enter each plate's barcode.

Plate Barcode Breakdown

A Plate Barcode (for example **@01197071012345671716123091046 36**) is defined as follows:

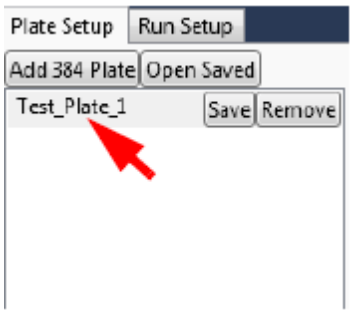
- **@01 =** Start of Barcode code
- **19707 =** Part Number
- **10 =** Separator code
- **1234567 =** Lot number
- **17 =** Separator code
- **161230 =** Expiration Date (YYMMDD)
- **91 =** Separator code
- **04636 =** A five digit incremental serial number based on a 32 digit serial number. A three digit incremental serial number denotes a 30 digit serial number.

1. Enter a **Plate Name**. (Figure 2.14)

Figure 2.14 Plate information

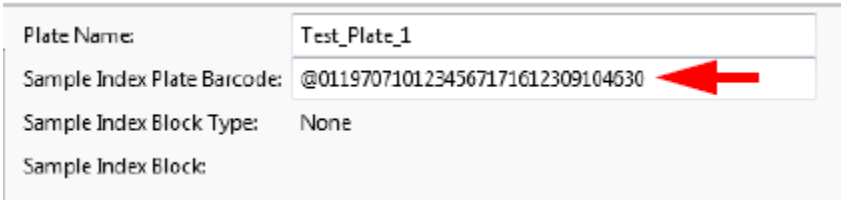
Your assigned plate name is displayed in the Main window's left column. (Figure 2.15)

Figure 2.15 Plate information



2. Use a hand-held scanner to scan the plate's barcode or click inside the **Plate Bar Code** field to enter it manually. (Figure 2.16)

Figure 2.16 Plate information



3. Click the Save button.

   A Save Library Plate window appears.

4. Enter a file name, then click Save.

Adding, Removing, and Opening Saved Plates

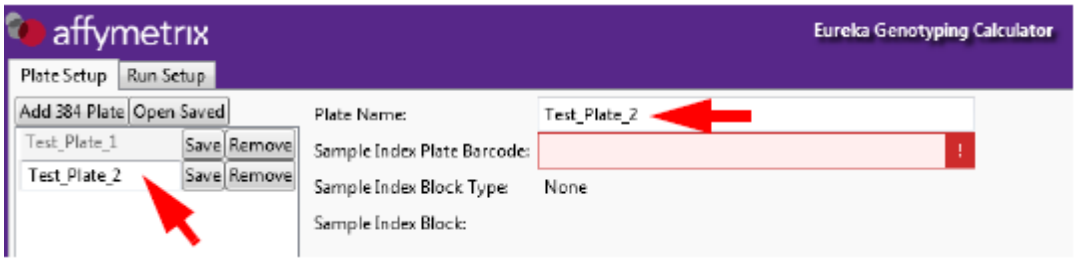
Adding 384 Plates

1. Click the Add 384 Plate button.

   A new ***<Enter Plate Name>*** field appears.

2. Click inside the ***<Enter Plate Name>*** field, then enter a plate name. Your entered plate name appears, as shown in Figure 2.17.

Figure 2.17 Entering a plate name



Removing Plates

1. Click the Remove button.

   The plate is removed from the plate name list.

Opening Saved Plates

1. Click the Open Saved button.

   A Open Saved Library window appears.

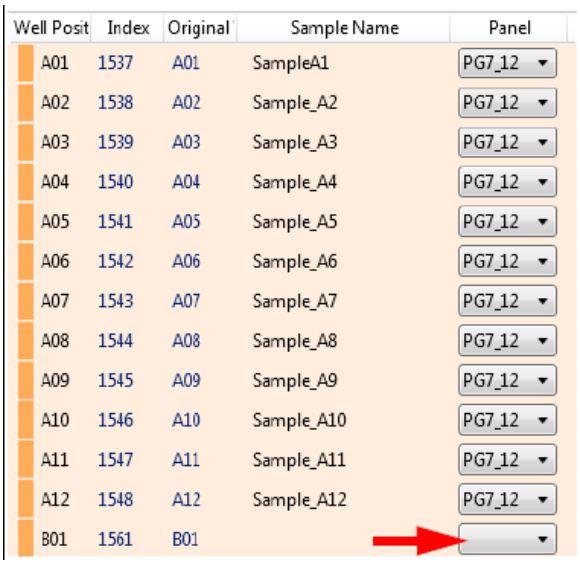
2. Locate, then click on your previously saved plate name, then click **Open**.

   The previously saved plate name appears on the plate name list.

Using the Well Position Table

After importing your 96/384 plate text files, the Well Position table auto-populates. (Figure 2.18)

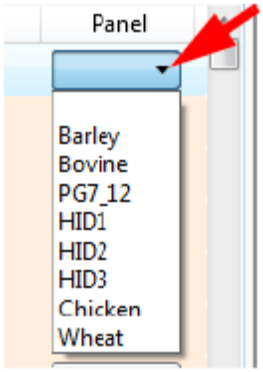
Figure 2.18 Well position table



Changing your Panel Selection

Each Panel is auto-detected and displayed in the Panel drop-down button. The Panel ID is based on your library file. If the Panel drop-down is blank (Figure 2.18), you must assign a panel manually. To do this: Click on the blank drop-down button, then click to select the appropriate Panel selection. (Figure 2.19)

Figure 2.19 Selecting a Panel

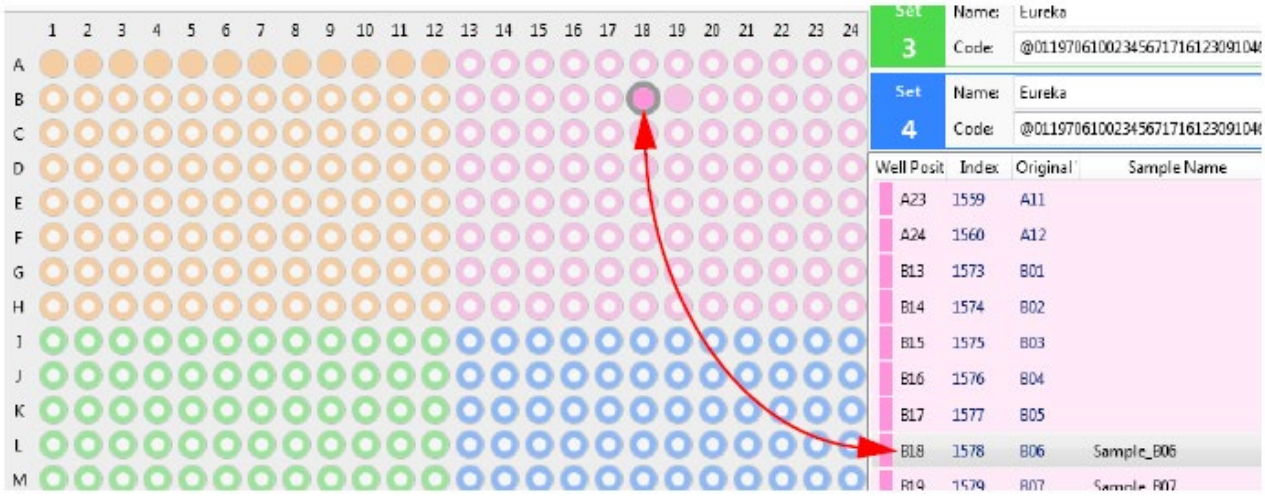


NOTE: If a *Panel* is from an imported file that does not exist in the software's Library file, the Panel field and its drop-down menu will appear blank.

Viewing Specific Well Position Properties

1. Click on any 384 plate well position to view its properties, as shown in Figure 2.20.

Figure 2.20 Plate mapping selection

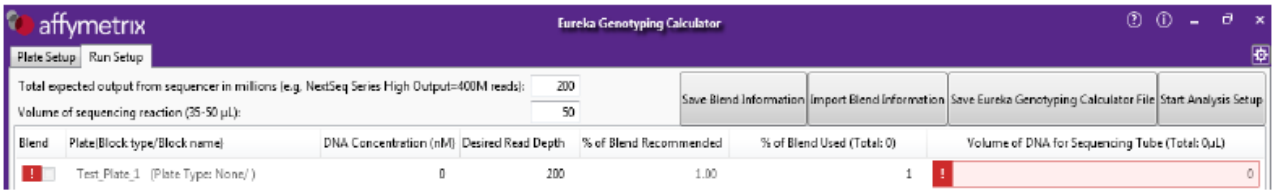


Setting Up your Sequencing Run Calculations

1. After selecting your 384 mapping configuration, importing your 96 Plate text files or 384 Plate text file, and scanning your plate barcode, click the **Run Setup** tab.

   The Run Setup window tab appears. (Figure 2.21)

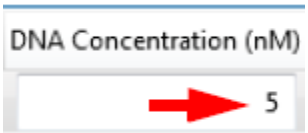
Figure 2.21 Run Setup window tab



NOTE: You can edit most of the text fields on the Run Setup window tab. The non-editable fields are used for auto-generated calculations.

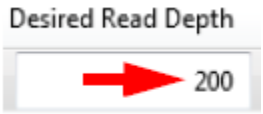
2. Click inside the **DNA concentration** field, then enter the DNA concentration for the pool. (Figure 2.22)

Figure 2.22 DNA concentration



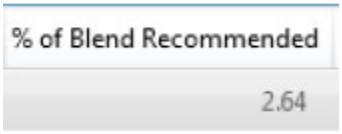
3. Click inside the **Desired read depth** field, then enter a value. (Figure 2.23)

Figure 2.23 Desired read depth



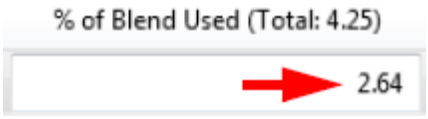
The software auto-calculates the % **of blend Recommended** field based on the number of samples, loci, and read depth, as shown in Figure 2.24.

Figure 2.24 % of blend Recommended



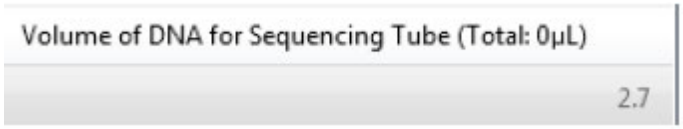
4. If needed, click inside the % **of blend Used** field (Figure 2.25), then enter a value. The software updates the percentage total automatically when a % of blend Used plate field is modified.

Figure 2.25 % of blend Used



The software calculates and displays the volume of pooled libraries to add (based on percentage and concentration values entered), as shown in Figure 2.26.

Figure 2.26 Volume of DNA for Sequencing



NOTE: The Volume of DNA used for the Sequencing Tube cannot be greater than the volume of the sequencing reaction.

5. All plates recorded in the Plate Setup window tab are auto-populated in the Plate column. Make sure the plates you want to include in your sequencing run calculations are checked, as shown in Figure 2.27.

Figure 2.27 Plate check boxes

Saving your Calculations for Analysis

After setting up your plate and its sequencing run calculations, do one of the following:

- Save your blending information for importing into the Eureka Analysis and Viewer application.
- Save your current table information for later use/editing.

Saving your Blending Information

1. Click the **Save Blend Information** button. (Figure 2.28)

Figure 2.28 Save Blend Information

A Save As window appears.

2. Navigate to a network drive or a location that is easily accessible.
3. Enter a filename, then click **Save**.

Saving your Calculator File

1. Click the Save Eureka Genotyping Calculator File button. (Figure 2.29)

Figure 2.29 Save in Table

A Save As window appears.

2. Navigate to a network drive or a location that is easily accessible.
3. Enter a filename, then click **Save**.

A Save As window appears.

4. Navigate to a network drive or a location that is easily accessible.
5. Enter a filename, then click **Save**.

NOTE: Genotyping Calculator Files use the file extension *.brfc*.

If you click the *Start Analysis Setup* button without saving your Eureka Genotyping Calculator file (as described above), the .brfc file is auto-saved in your default folder as:

Run-date-time.brfc

Loading Previously Saved Blend File

1. Click the **Import Blend Information** button. (Figure 2.30)

Figure 2.30 Import Blend Information

An Open window appears.

2. Locate the blend information file you want to import into the table, then click to select it.
3. Click **Open**.

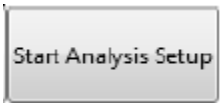
   The table populates.

Running your Calculations

- Click the **Start Analysis Setup** button (Figure 2.31)

  Alternatively, click **Start ->All Programs -> Affymetrix -> Eureka Analysis Suite -> Eureka Analysis,** then continue to *Importing your Eureka Genotyping Calculator File*.

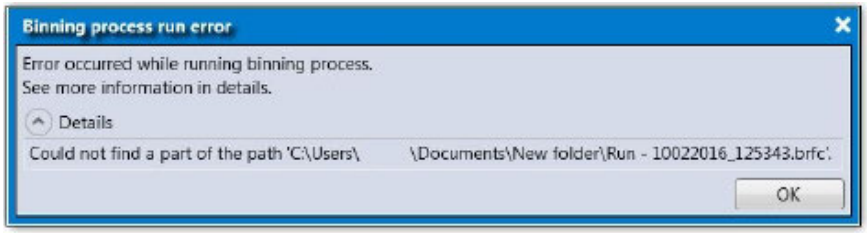
Figure 2.31 Start Analysis Setup



NOTE: After you click the *Start Analysis Setup* button, a brfc file with the name format of *Run-date-time.brfc* is saved in the default folder you set earlier.

After clicking on the Start Analysis Setup button, the Eureka Analysis module opens automatically. For instructions on how to setup your analysis, continue to *Setting Up an Analysis*.

NOTE: If you click on the *Start Analysis Setup* button and a *Binning process run error* message appears, click OK to acknowledge it, then change your Default Sample File Folder location.



To do this, go to *Changing your Default Sample File Folder Location*. After changing the location, click on the *Start Analysis Setup* button again.

Chapter 3 Eureka Analysis

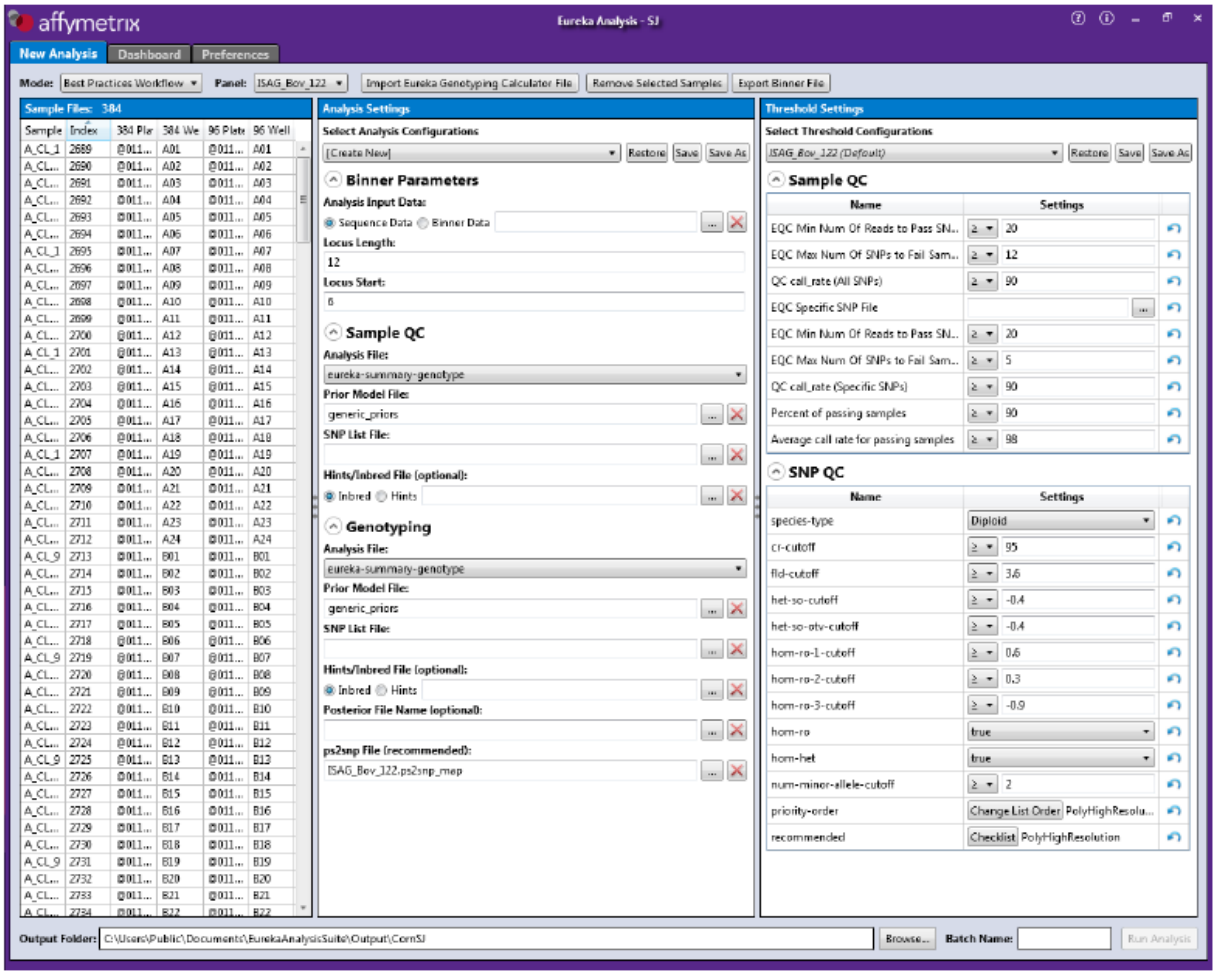
Setting up an Analysis

After you click the Genotyping Calculator's **Start Analysis Setup** button, the Eureka Analysis window auto-populates with your blend information, as shown in Figure 3.1.

Continue to *Selecting a Mode (Workflow)* for instructions on how to set up your analysis.

If the Analysis module was not launched (opened) from the Genotyping Calculator, go to *Importing your Eureka Genotyping Calculator File*.

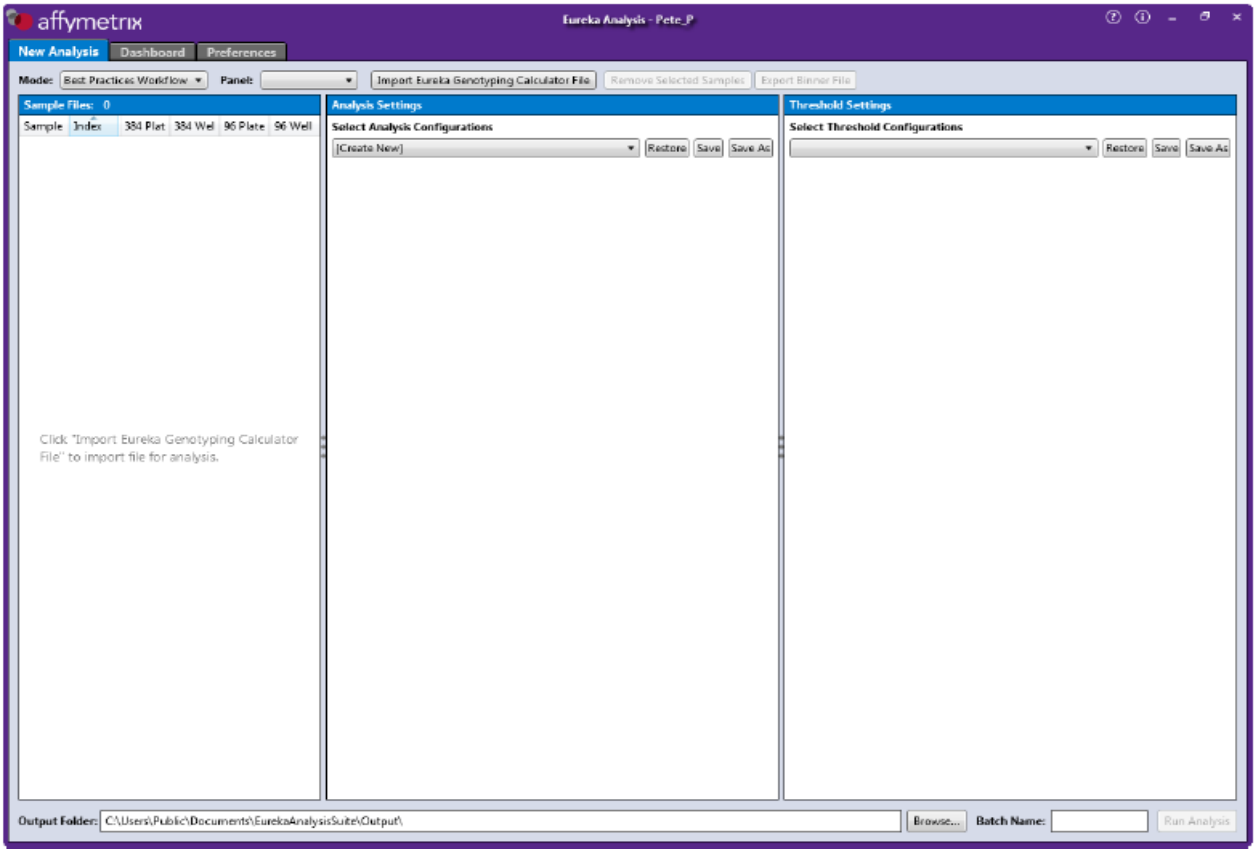
Figure 3.1 New Analysis window tab



Importing your Eureka Genotyping Calculator File

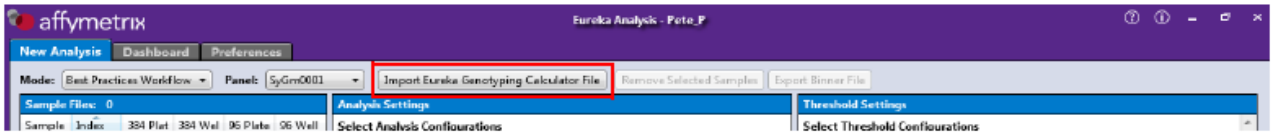
IMPORTANT: If the Analysis module was not launched (opened) from the Genotyping Calculator, the New Analysis window appears as shown in Figure 3.2. At this point, you must import a .brfc file that was generated using the Eureka Genotyping Calculator module.

Figure 3.2 New Analysis window tab – unpopulated



1. Click Import Eureka Genotyping Calculator File. (Figure 3.3)

Figure 3.3 New Analysis window tab - Import Eureka Genotyping Calculator File button



An Add Eureka Genotyping Files window appears.

2. Locate the **.brfc** file you want to import, then click on it.

   The .brfc name appears in the **File name** field.

3. Click **Open**.

   The New Analysis window tab populates, as shown in Figure 3.1.

Selecting a Mode (Workflow)

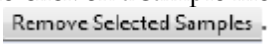
- Click the 

drop-down menu, to select the workflow you want to use.
  - **Best Practices Workflow (Default):** This workflow performs quality control analysis for samples and plates, genotypes those samples which pass the defined QC thresholds, and then categorizes the probe sets to identify those whose genotypes are recommended for statistical tests in downstream study.
  - **Eureka Sample QC:** This workflow performs the quality control analysis for samples and plates. Note this workflow does not produce genotype calls for the passing samples.
  - **Genotyping:** This performs genotyping on the imported sample files, regardless of the sample and plate QC metrics. Note: Including samples that do not pass defined QC thresholds may reduce the quality of the results for passing samples.

NOTE: In subsequent runs, the application defaults to the last Mode (Workflow) used.

Removing Selected Sample Files

Use this option to remove unwanted sample files.

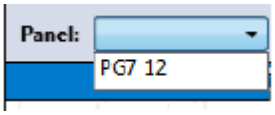
- Single-click on a sample file or **Ctrl+click, Shift+click,** or press **Ctrl+a** (to select multiple files), then click 

.

Selecting a Panel

- Click the **Panel** drop-down (Figure 3.4) to select the Panel you want to use.

Figure 3.4 Panel drop-down menu example

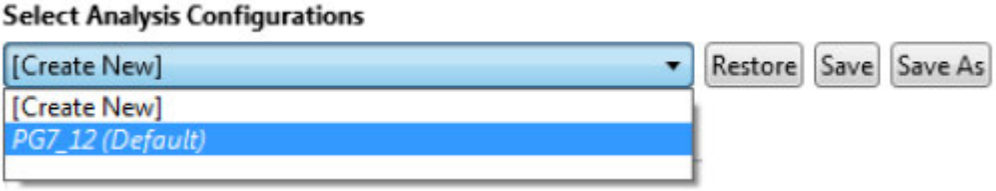


Setting Up an Analysis Configuration

- From the Select Analysis Configuration drop-down menu (Figure 3.5), click to select the option that best matches your sample files.

NOTE: All default fields displayed in the Analysis Settings window pane are based on your imported sample files and panel selection.

Figure 3.5 Select an analysis configuration drop-down menu

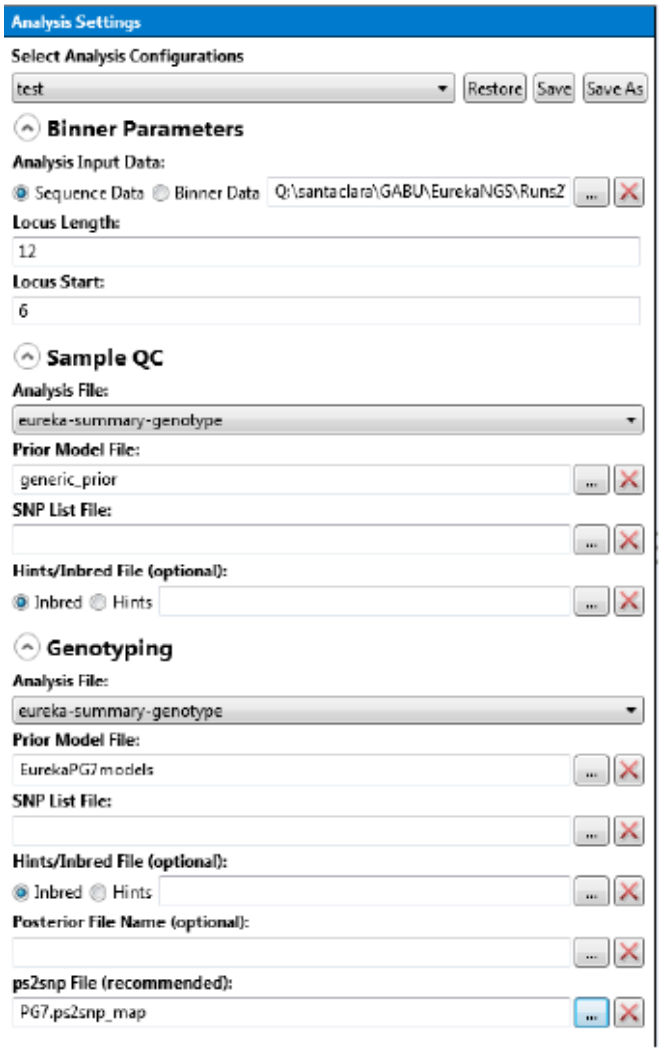


(Optional) If you want to create a new analysis configuration, click **[Create New]**.

NOTE: New analysis configurations require manual entry of all Analysis Setting fields. For more information, see *Using the Analysis Settings Fields*.

After selecting the appropriate configuration default, the Analysis Setting pane auto-populates, as shown in Figure 3.6.

Figure 3.6 Auto-populated Analysis Setting pane example



Using the Analysis Settings Fields

Follow the instructions below to create a new analysis configuration or edit a pre-populated field(s).

Binner Parameters

1. From the **Analysis Input Data,** click to select the radio button that coincides with your input type.
   - Select **Sequence Data** when starting an analysis from Sequencing **Read Data**.

     If this is your very first attempt analyzing a set of samples, you must select this **Sequence Data** option.
   - Select **Binner Data** when starting from a binning file.
2. Click the **Analysis Input Data**  button.

   An Explorer window appears.
3. Navigate and select your Sequence Data Path folder or Binning.txt, then click **Select Folder/File.**

   Your assigned path is displayed.
4. (Optional) Click inside the **Locus Start** field to change the assigned default value.

   Locus Start represents the nucleotide where the allele-locus barcode starts. The default is 6.
5. (Optional) Click inside the **Locus Length** field to change the assigned default value.

   Locus Length represents the nucleotide length of the allele-locus barcode. The default is 12.

Exporting a Command Line Binner File

At this point, you can export your completed binner parameters as a binary JSON file and imported sample files for use in a command line application.

To do this:

1. Enter a 

.
2. Click 

.

   An Explorer window appears.
3. Navigate to an easily accessible location.
4. Click **Save**.

   Your file is now ready for use in a command line application.

Use Nibls.exe to run the binner from a command line. The Nibls.exe is part of the **Applied Biosystems Array Power Tools (APT) Software Package** available at our web site.

For more information on the **JSON** file, see the **Eureka Analysis Suite Command Line Script Help Guide** (included in the Eureka Analysis Suite software zip package).

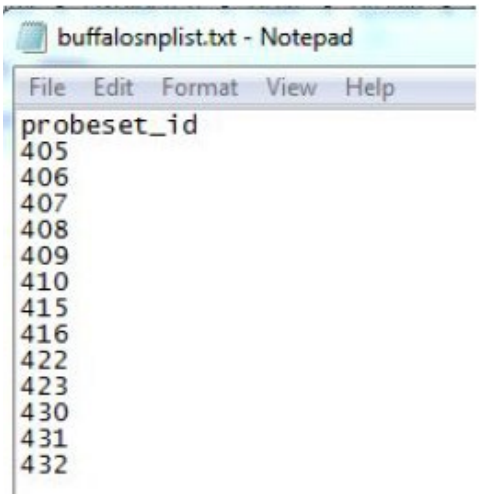
Sample QC Fields

1. Click the **Analysis File** drop-down button to select the appropriate XML file.
2. Click the **Prior Model File**  button.
   The Prior Model File window appears.
3. Navigate and select the appropriate file, then click **Open**.
   Your newly assigned filename is displayed.
4. (Optional) Click the **SNP List File**  button.
   The SNP List File window appears.
5. Navigate and select the appropriate file, then click **Open**.
   Your newly assigned filename is displayed.
6. (Optional) Click the **Hints/Inbred File**  button.
   The Hints/Inbred File window appears.
   To create a customized Inbred file, see Appendix A, *Inbred File Generation Tool*.
7. Navigate and select the appropriate file, then click **Open**.
   Your newly assigned path is displayed.

Genotyping Fields

1. Click the **Analysis File** drop-down button to select the appropriate XML file.
2. Click the **Prior Model File**  button.
   The Prior Model File window appears.
3. Navigate and select the appropriate file, then click **Open**.
   Your newly assigned filename is displayed.
4. (Optional) Click the **SNP List File**  button.
   The SNP List File window appears.
5. Navigate and select the appropriate SNP List file.
   Your SNP List file must be a one column text file with a **probeset_id** header, as shown in Figure 3.7.

Figure 3.7 One column SNP List file example



For more information on how to create a SNP List, see *Managing your SNP List*.

6. Click **Open**.

   Your newly assigned filename is displayed.

7. (Optional) Click the **Hints/Inbred File** button.

   The Hints/Inbred File window appears.

   To create a customized Inbred file, see Appendix A, *Inbred File Generation Tool*.

8. Navigate and select the appropriate file, then click **Open**.

   Your assigned filename is displayed.

9. (Optional) Click the **Posterior File Name** button.

   The Posterior File Name window appears.

10. Navigate to a location for your posterior file, enter a name, then click **Open**.

    Your assigned filename is displayed.

11. (Recommended) Click the **ps2snp File** button.

    The ps2snp File window appears.

12. Navigate to your ps2snp-file location, then click **Open**.

    Your newly assigned filename is displayed.

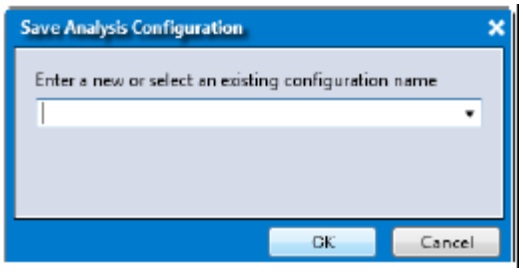
Saving your Analysis Configuration

1. After editing your Analysis Configuration settings, click (top of Analysis Setting pane). (Figure 3.8)

Figure 3.8 Analysis Configuration Save window

The following window appears: (Figure 3.9)

Figure 3.9 Analysis Configuration Save window



2. Enter a new configuration name or use the drop-down to select an existing name, then click **OK**. Your saved analysis configuration name is now stored (an can be accessed) in the **[Create New]** drop-down menu.

Modifying an Existing Analysis Configuration

- Click the **Select Analysis Configuration** drop-down, then click to select the saved analysis configuration you want to modify.

Do one or more of the following to modify an existing analysis configuration:

- Click the applicable File field's **browse** button to navigate to a different location, then click **Open** to reassign its path.
- If needed, click a File field's  button to delete a displayed path setting.
- Click the **Restore** button to return to the last saved values of the analysis configuration file.
- Click the **Save** button to overwrite your previously saved configuration.
- Click the **Save As** button to save your modified configuration with a different name. [Recommended]

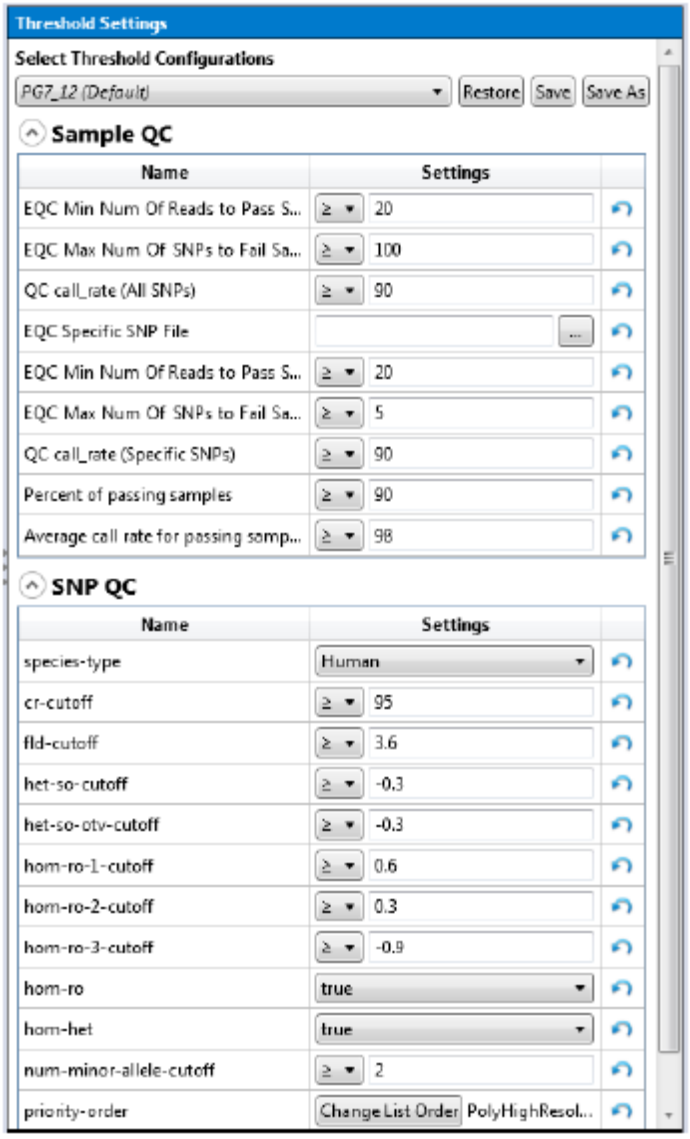
Selecting your Threshold

- Click the **Select Threshold Configuration** drop-down (Figure 3.10), then click to select the threshold that coincides with your selected panel.

Customizing Threshold Settings

The **Threshold Setting** pane (Figure 3.10) populates (with default settings based on your Panel).

Figure 3.10 Automated QC Mode Threshold Settings pane example



Sample QC

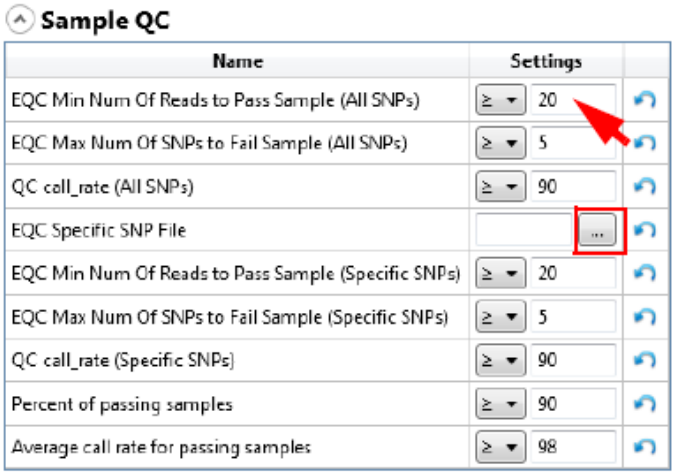
All the Sample QC Threshold Settings are populated with default values based on your Panel. See Appendix B, *Definitions* for Sample QC Name definitions.

NOTE: The ≥ *greater than or equal* to value is preset and cannot be changed.

1. Click inside each text field to enter a different value, as shown in Figure 3.11.

   Click the text field's button to return its value back to its last saved value within the threshold configuration file.

Figure 3.11 Threshold Name text field example



2. (Optional) Click the **EQC Specific SNP File** ... button to perform **EQC** on a specific subset of SNPs.

   The **EQC** Specific **SNP** File window appears.

3. Navigate and select the appropriate file, then click **Open**.

   Your newly assigned filename is displayed.

SNP QC

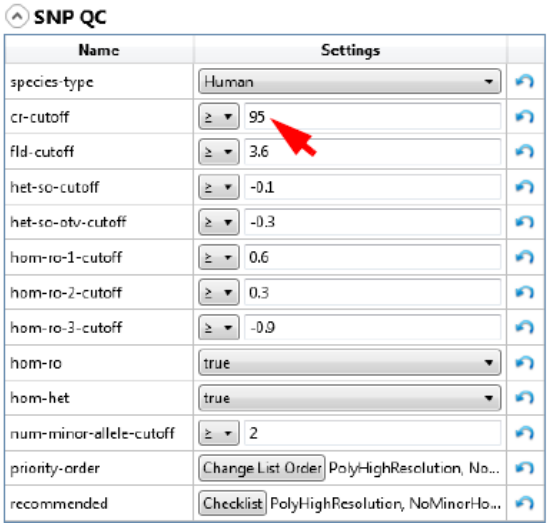
All the SNP QC Threshold Settings are auto-populated with default values. See Appendix B, *Definitions* for SNP QC Name definitions.

NOTE: The ≥ *greater than or equal to* value is preset and cannot be changed.

1. Click inside each text field to enter a different value, as shown in Figure 3.12.

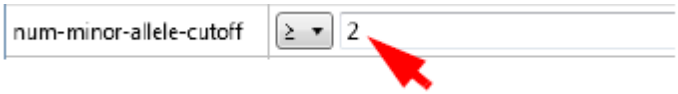
   Click the text field's button to return its value back to its last saved value within the threshold configuration file.

Figure 3.12 SNP QC text fields



2. Use the **hom-ro and hom-het** drop-down menus to change their **True** or **False** values.
3. Click inside the **num-minor-allele-cutoff** text field to enter a different value, as shown in Figure 3.13.

Figure 3.13 SNP QC text fields



4. The **priority-order** option allows you to change the order of categories when determining which probesets are selected as the best probeset for a **SNP**. To change the priority-order of your **SNP QC** Metric, click Change List Order.

   The following window appears: (Figure 3.14)

Figure 3.14 Change the Priority Order window

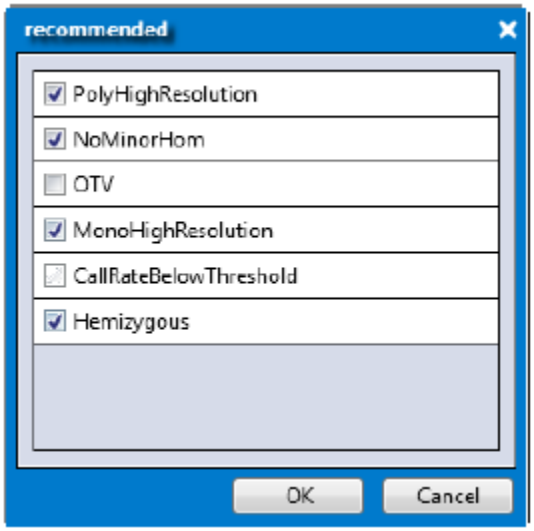
5. Click and hold onto the selection you want to move, then drag and drop it into its new position. After you get the order of priority you want, click **OK**.

   Click the priority-order field's button to return the list back to its default priority.

6. Use the **recommended** checklist to choose the Classification conversion types for your analysis. To change the recommended options, click Checklist.

   The following window appears: (Figure 3.15)

Figure 3.15 Recommended window



7. Click to check/uncheck the available recommended options, then click **OK**.

NOTE: If all *recommended* options are *unchecked*, the software uses the following default values:

For Human: *PolyHighResolution*, *NoMinorHom*, *MonoHighResolution*, *and Hemizygous*.

For Diploid: *PolyHighResolution*

For Polyploid: *PolyHighResolution*

Assigning an Output Folder Path

1. Click the **Output Folder** path's **Browse** button. (Figure 3.16)

Figure 3.16 Output Folder field

An Explorer window appears.

2. Navigate to the recommended path C:\Users\Public\Documents\EurekaAnalysisSuite\Output, then click **Select Folder**.

Your selected output folder path is now displayed.

Adding Sub-Folders

TIP: To better organize your output results, you can add sub-folders to your newly assigned output result path's folder.

To add sub-folders to your newly assigned result path's folder:

1. Click the Output Folder's **browse** button to return to your assigned output path and/or folder.
2. In the Explorer window, click **New Folder**.
3. Enter a sub-folder name.
4. Click **Select Folder**.

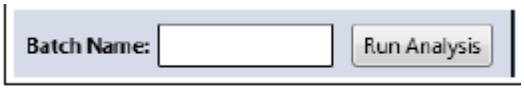
   The newly created sub-folder now appears in the output result information window.
5. Repeat the above steps 1–4 to add more sub-folders, then click **Select Folder**.

Assigning a Batch Name

The batch file is produced while your analysis is running and includes all the necessary files needed to view your analysis in the Eureka Analysis Suite Viewer.

- Enter a name in the **Batch Name** field. (Figure 3.17)

Figure 3.17 Enter a Batch Name

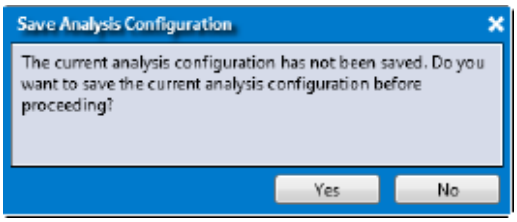


NOTE: A folder (with the same name as your entered batch name) is auto-generated during the analysis process. This folder includes all the necessary files needed to view your analysis results in the Viewer.

Running your Analysis

- Click Run Analysis.
  - If you have not saved any changes to your configured Analysis Settings, a Save Analysis Configuration window appears. (Figure 3.18)

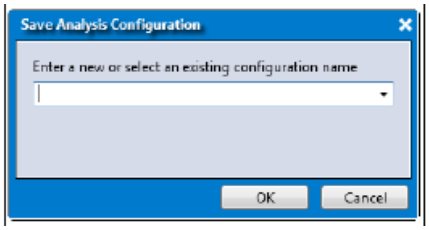
Figure 3.18 Save Analysis Configuration prompt window



  - Click **No** to continue the analysis without saving your configuration.
  - Click **Yes**. (Recommended) Figure 3.19 appears.

    Enter a new analysis name or use the drop-down to select a previously saved name, then click **OK**.

Figure 3.19 Save Analysis Configuration window

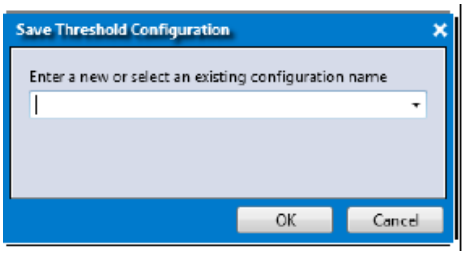


  - If you have not saved any changes to your configured Threshold Settings, a Save Threshold Configuration window appears. (Figure 3.20) Click **Yes**. Click **No** to continue the analysis without saving your configuration.

Figure 3.20 Save Threshold Settings prompt window

- Enter a new threshold name or use the drop-down to select a previously saved name, then click **OK**.

Figure 3.21 Save Threshold Settings window



The Dashboard window/tab appears and shows the status of your running analysis. (Figure 3.22)

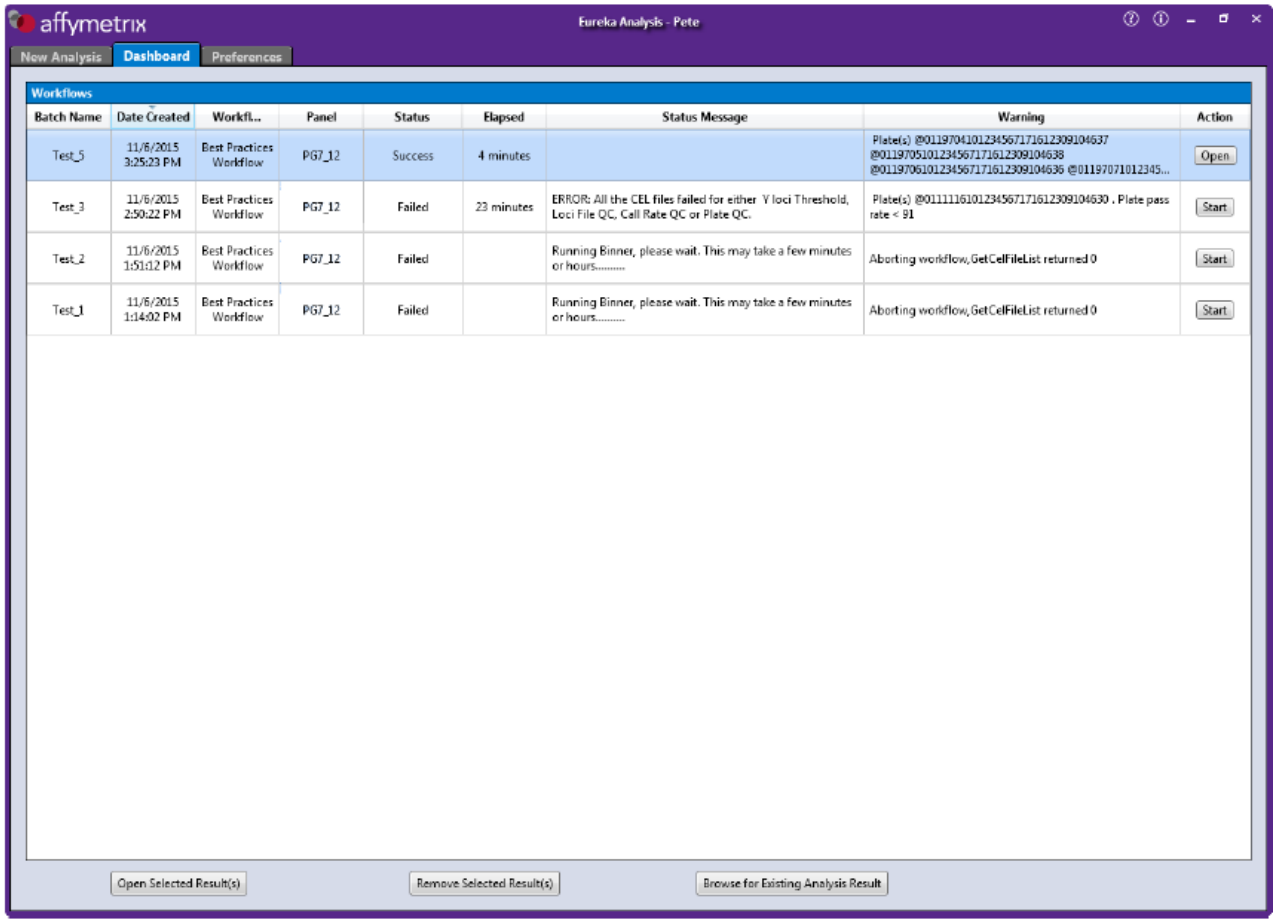
To cancel a running analysis, click Stop. ***It may take several minutes for the Stop command to complete.***

Figure 3.22 Dashboard window/tab - Status bar and Stop button example

Using the Dashboard Window Tab

The Dashboard tab window displays existing results. (Figure 3.23)

Figure 3.23 Dashboard window



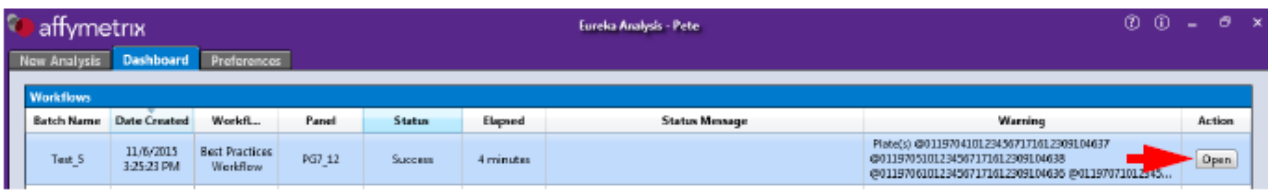
Open Selected Result(s)

Do one of the following to open a selected Successful result:

- Single-click on a study, then click **Open Selected Result(s)**
- Double-click on a study to open it.
- Right-click on a study, then click . Open . (Figure 3.24)

TIP: Click on any of the Workflow header columns to sort your listed results.

Figure 3.24 Dashboard window/tab



After a few moments, the Viewer opens and displays your study (as you last left it).

To open a previously failed result, use the *Browsing For Existing Analysis Results* feature.

Browsing For Existing Analysis Results

Do the following if a result is not listed on the Dashboard:

1. Click browse for Existing Analysis Result.

TIP: Navigate to the Workflows sub-folder to locate other User Profile folders (that may contain more recent results).

The Select Analysis Result File window appears.

2. Click on a recently successful or failed analysis, then click **Select Folder**.

   After a few moments, your analysis opens as you last left it.

3. After your analysis has successfully completed. click 

. Alternatively, click to highlight the completed analysis, then click **Open Selected Result(s)**.

   The Eureka Analysis Suite Viewer appears.

   For instructions on how to use the Viewer, continue to Chapter 4, *Eureka Viewer*.

Remove Selected Result(s)

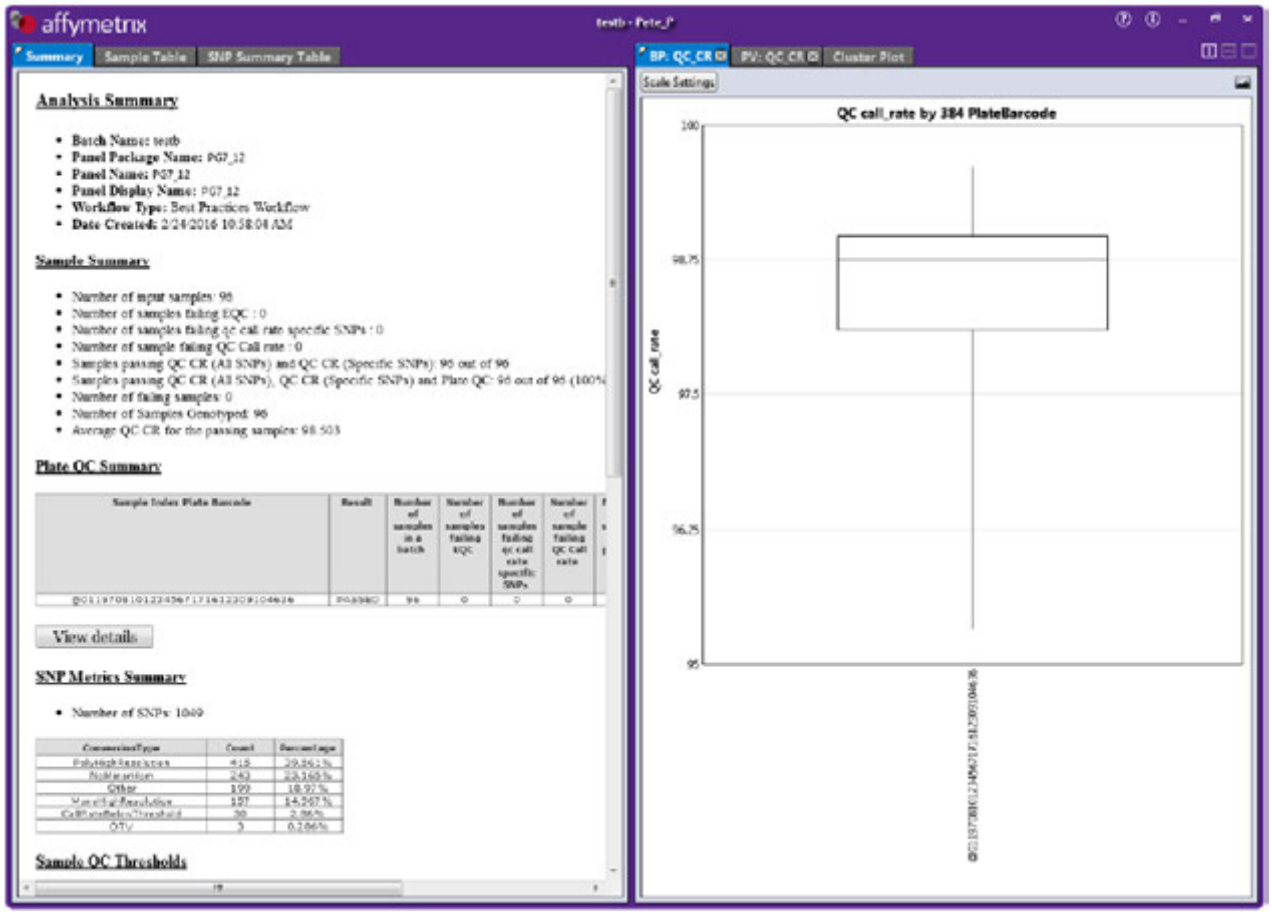
Do one of the following to remove a selected result:

- Single-click to highlight the analysis you want to remove, then click **Remove Selected Result(s)**.
- Right-click on the highlighted analysis, then click **Remove from List**.

Chapter 4 Eureka Viewer

After setting up and successfully running an analysis, as described in Chapter 3, the Eureka Analysis Suite Viewer opens. (Figure 4.1)

Figure 4.1 Main Viewer window



Viewing Options

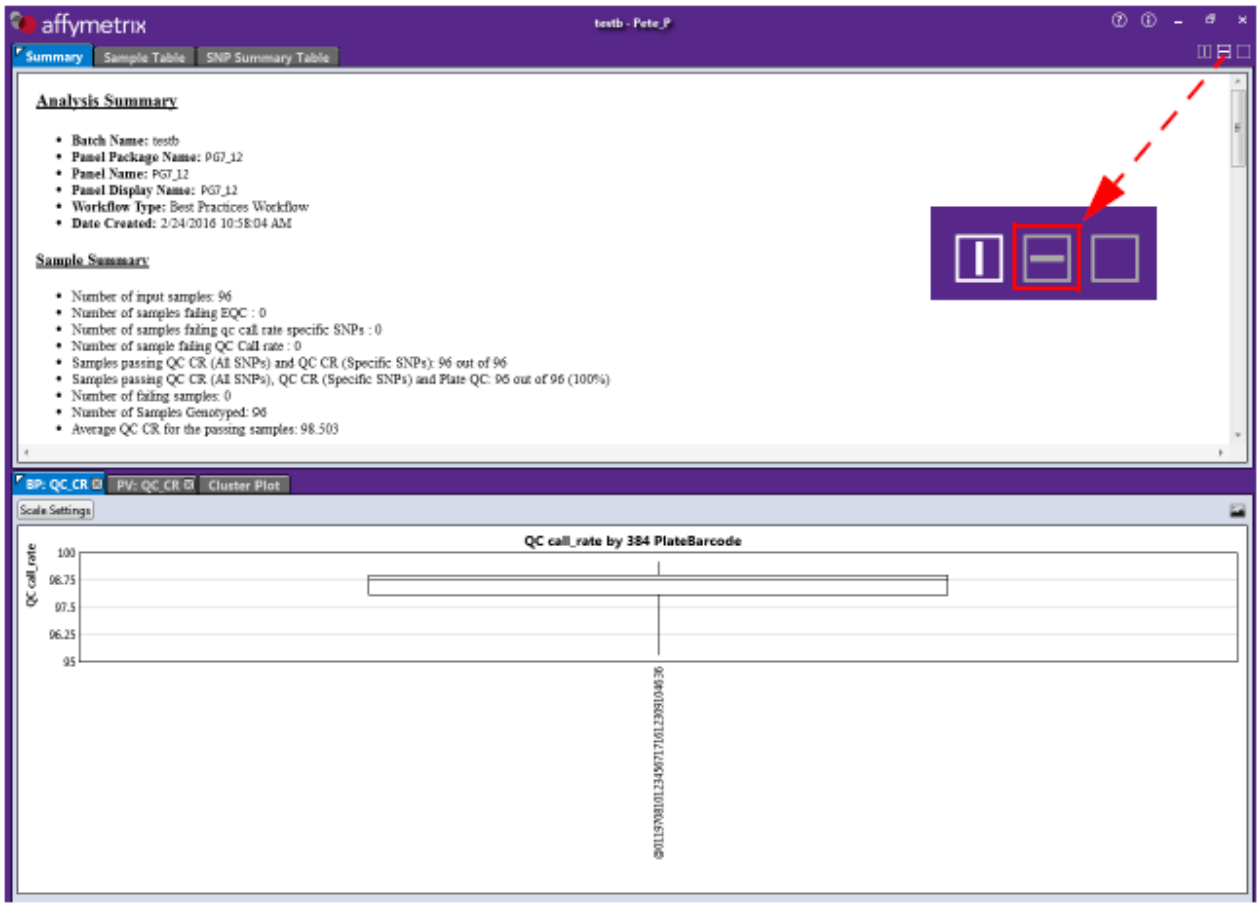
As shown in Figure 4.1, the Viewer (by default) displays a **side-by-side** split-screen configuration.

Split-Screen Options

To change side by side split-screen to a top and bottom configuration:

1. Click the **Horizontal Split** icon. (Figure 4.2)

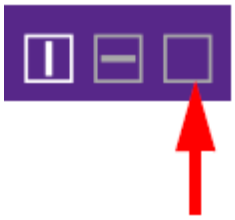
Figure 4.2 Split Horizontal View icon and window layout example



To disable the split-screen:

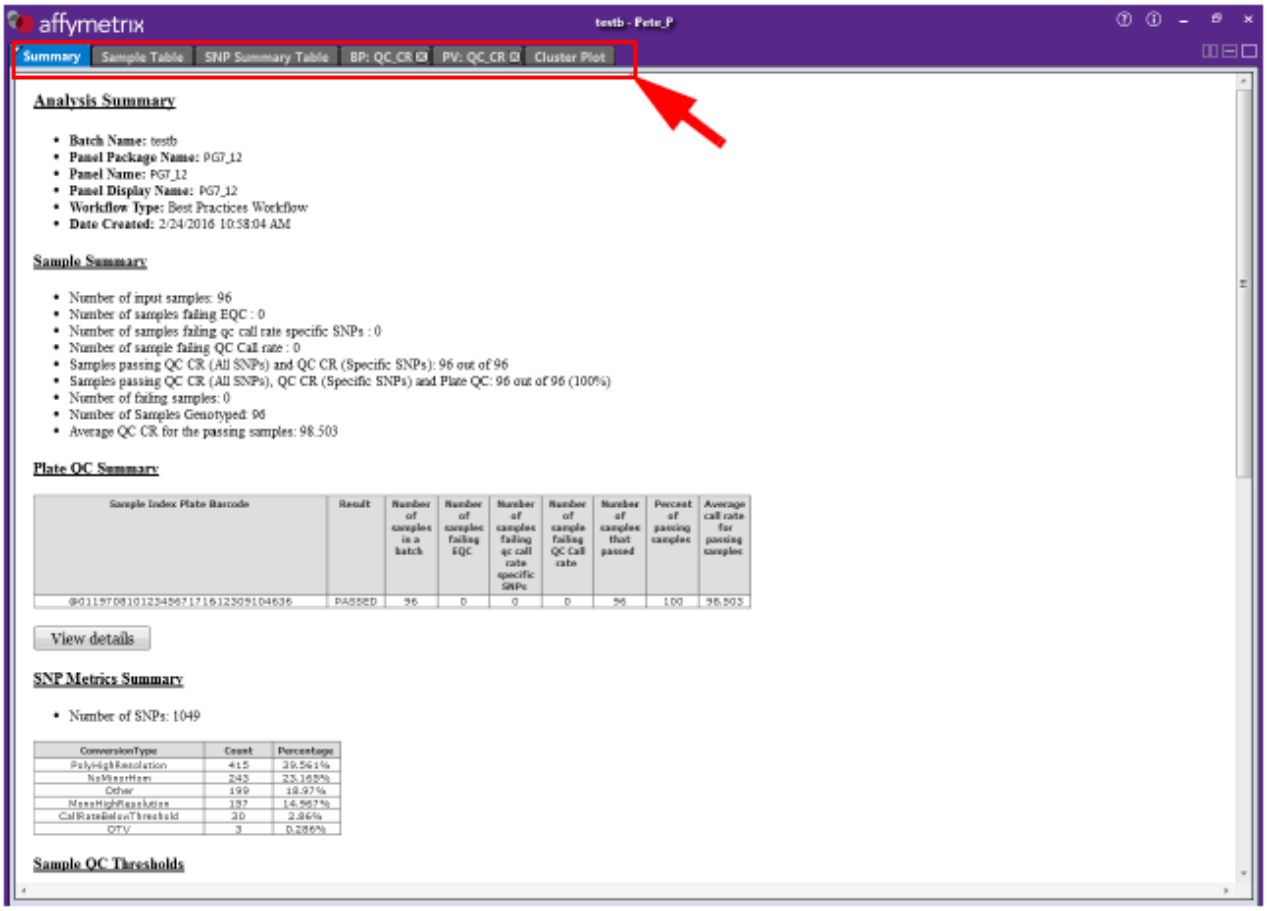
1. Click the **Disable Split-Screen** icon. (Figure 4.3)

Figure 4.3 Disable Split-Screen icon



The split-screen becomes 1 window. (Figure 4.4)

Figure 4.4 Full window view example



2. Click on any window tab (Figure 4.4) to view it in full window mode.

   To return to the default side by side split-screen configuration:

   Click the **Vertical Split** icon. (Figure 4.5)

Figure 4.5 Vertical Split icon

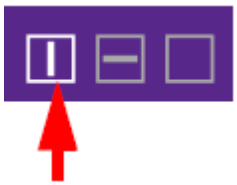
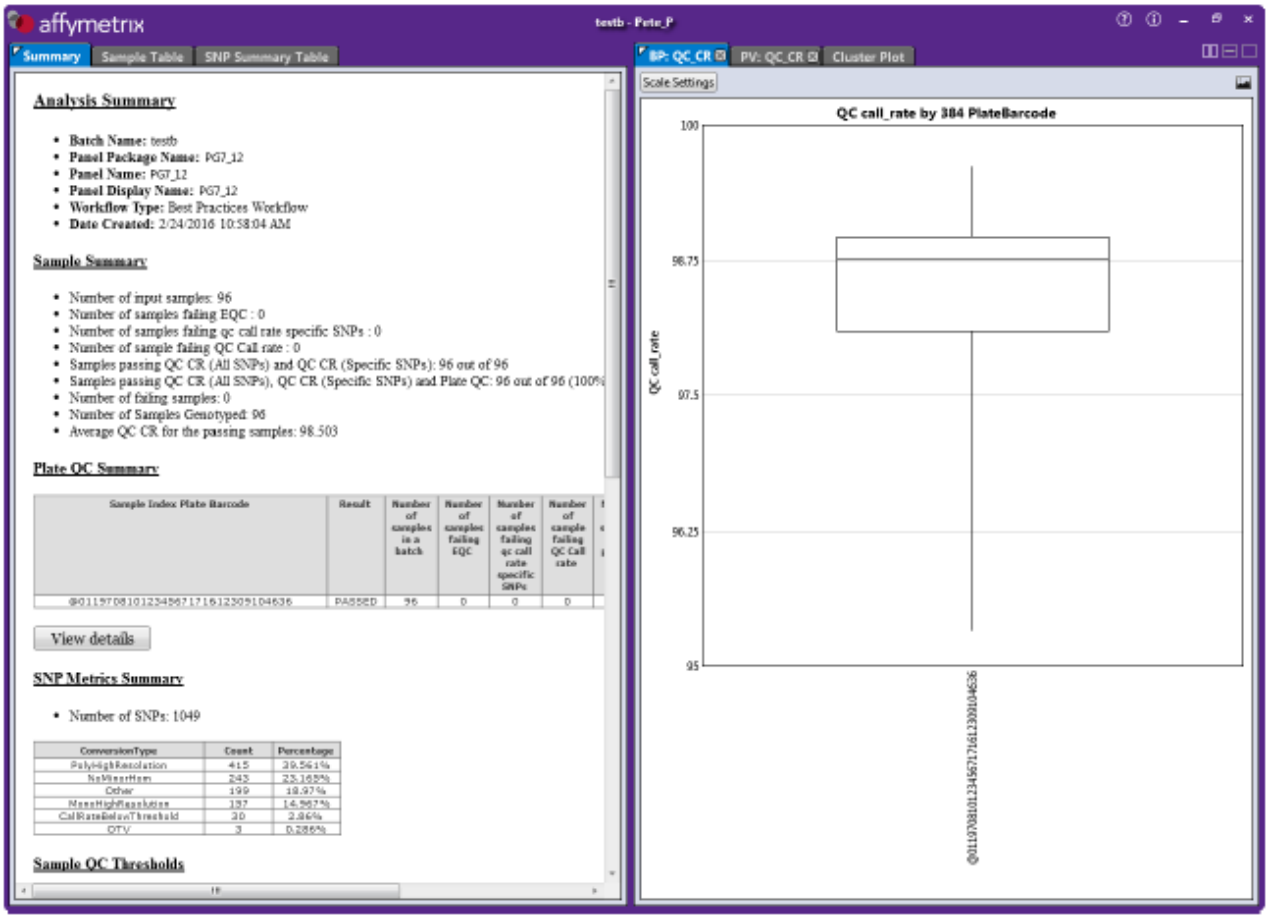


Figure 4.6 Split Vertical View example



Changing a Tab Window to a Full Screen Windows

To toggle a tab window to full screen:

1. Locate the tab you want to make full screen.
2. Click on a tab's white triangle. (Figure 4.7)

Figure 4.7 Tab to window toggle icon

   The window tab is now a window.

3. Double-click anywhere along the top of the window to change it to full screen.

To toggle a full screen window to its default tab window:

1. Double-click anywhere along the top of the window.
2. Click the **X** button (top right) to close the window.

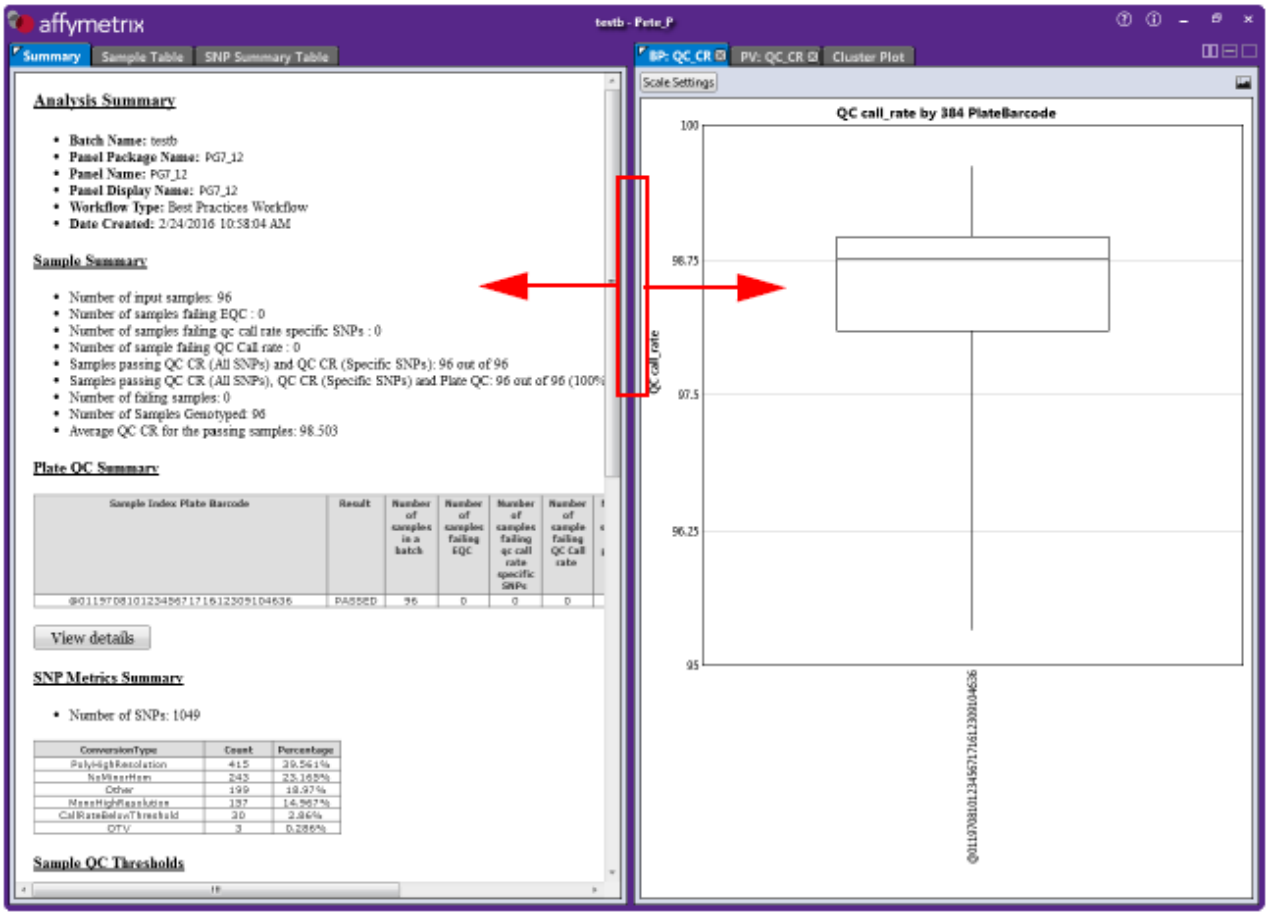
   The window returns to its default tab window and position.

Adjusting the Window Size

To change the size of a window pane:

- Click, hold, then drag the edge of the window pane (Figure 4.8) to resize it.

Figure 4.8 Split Vertical View example



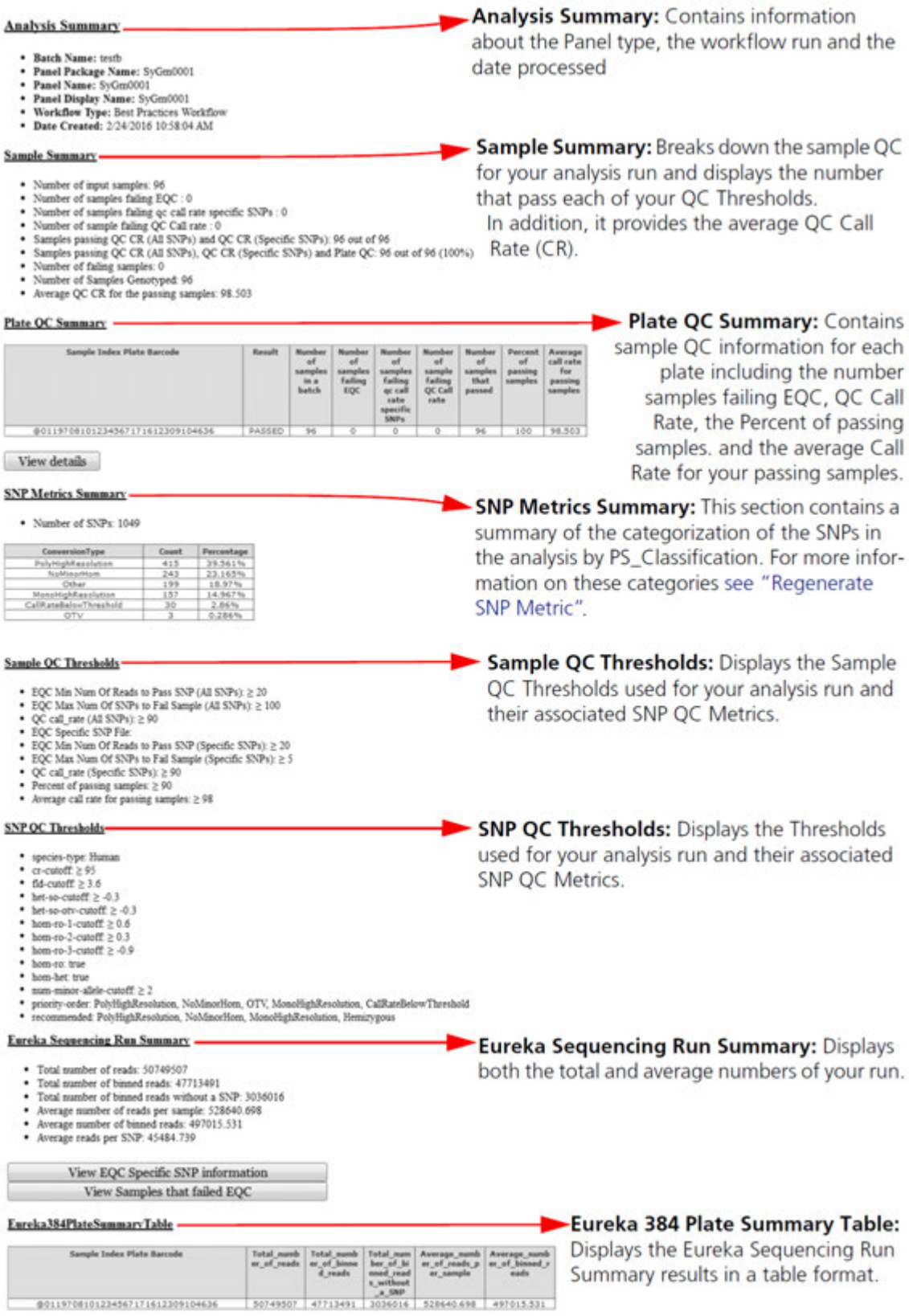
Using the Summary Window

The Summary window tab (Figure 4.9) displays a summary snapshot of your analysis, including detailed threshold values, and tables based on your analysis.

Data Analysis Summary

NOTE: Each workflow type reports different information within the *Analysis Summary* window tab. Figure 4.9 is an example of a *Best Practices* workflow.

Figure 4.9 Summary window tab overview

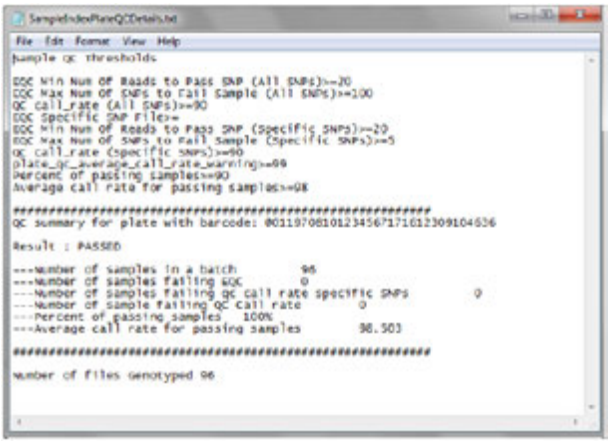


Viewing the Plate Barcode Table Details

- In the Summary window tab, click the View Details button.

  A MS Notepad window opens. (Figure 4.10)

Figure 4.10 MS Notepad window



Viewing Eureka QC Specific SNP Information

1. In the Summary window tab, click the View EQC Specific SNP information button.

   A MS Notepad window opens. (Figure 4.11)

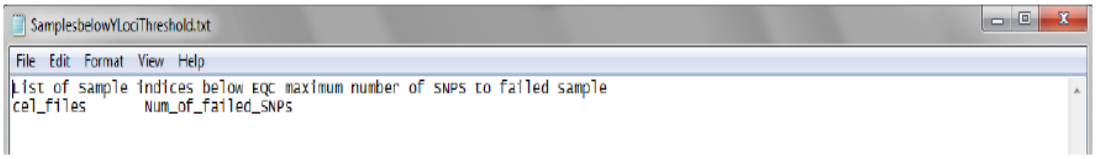
Figure 4.11 MS Notepad window

Viewing Samples that Failed Eureka QC

- In the Summary window tab, click the View Samples that failed EQC button.

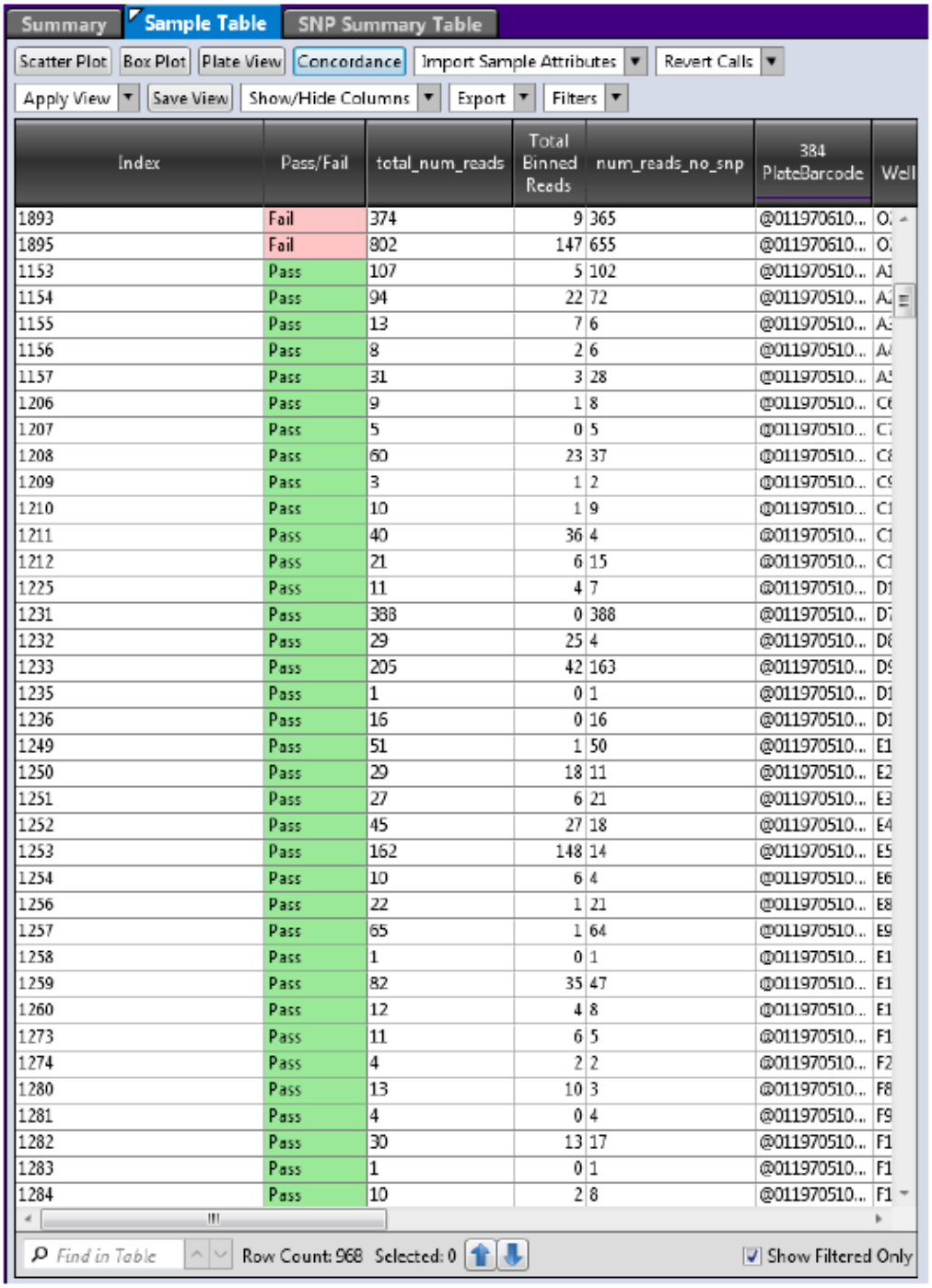
  A MS Notepad window opens. (Figure 4.12)

Figure 4.12 MS Notepad window



Using the Sample Table

Figure 4.13 Sample Table window tab



Index	Pass/Fail	total_num_reads	Total Binned Reads	num_reads_no_snp	384 PlateBarcode	Well
1893	Fail	374	9	365	@011970610...	O.
1895	Fail	802	147	655	@011970610...	O.
1153	Pass	107	5	102	@011970510...	A1
1154	Pass	94	22	72	@011970510...	A2
1155	Pass	13	7	6	@011970510...	A3
1156	Pass	8	2	6	@011970510...	A4
1157	Pass	31	3	28	@011970510...	A5
1206	Pass	9	1	8	@011970510...	C6
1207	Pass	5	0	5	@011970510...	C7
1208	Pass	60	23	37	@011970510...	C8
1209	Pass	3	1	2	@011970510...	C9
1210	Pass	10	1	9	@011970510...	C1
1211	Pass	40	36	4	@011970510...	C1
1212	Pass	21	6	15	@011970510...	C1
1225	Pass	11	4	7	@011970510...	D1
1231	Pass	388	0	388	@011970510...	D7
1232	Pass	29	25	4	@011970510...	D8
1233	Pass	205	42	163	@011970510...	D9
1235	Pass	1	0	1	@011970510...	D1
1236	Pass	16	0	16	@011970510...	D1
1249	Pass	51	1	50	@011970510...	E1
1250	Pass	29	18	11	@011970510...	E2
1251	Pass	27	6	21	@011970510...	E3
1252	Pass	45	27	18	@011970510...	E4
1253	Pass	162	148	14	@011970510...	E5
1254	Pass	10	6	4	@011970510...	E6
1256	Pass	22	1	21	@011970510...	E8
1257	Pass	65	1	64	@011970510...	E9
1258	Pass	1	0	1	@011970510...	E1
1259	Pass	82	35	47	@011970510...	E1
1260	Pass	12	4	8	@011970510...	E1
1273	Pass	11	6	5	@011970510...	F1
1274	Pass	4	2	2	@011970510...	F2
1280	Pass	13	10	3	@011970510...	F8
1281	Pass	4	0	4	@011970510...	F9
1282	Pass	30	13	17	@011970510...	F1
1283	Pass	1	0	1	@011970510...	F1
1284	Pass	10	2	8	@011970510...	F1

NOTE: Depending on the Threshold values you set (prior to running your analysis), color-coded Pass or Fail cells may appear in the table, as shown in Figure 4.13.

Importing Sample Attributes

To import sample attributes into your Sample Table:

1. Click the Import Sample Attributes drop-down.
2. Click to select Import from CSV/Tab-Delimited Text File.

   An Explorer window appears.
3. Navigate to the applicable file location, then click **Open**.

   Your Sample Attributes are now in inserted into the Sample Table.

IMPORTANT: If you are exporting genotypes into a PLINK format, make sure your Sample Attributes include an Index ID and Pedigree Information (Family ID, Individual ID, Father ID, Mother ID, Sex, and Affection Status).

Column Headers

The Sample Table includes default columns, as shown in Figure 4.14.

Figure 4.14 Sample Table columns

Index	Pass/Fail	total_num_reads	Total Binned Reads	num_reads_no_snp	384 PlateBarcode	384 WellPosition	QC call_rate	call_rate	QC het_rate	het_rate
2996	Pass	17062	13643	3419	@011970910...	M20	90.984	90.984	24.59	24.59
3019	Pass	15454	12021	3433	@011970910...	N19	94.262	94.262	27.049	27.049
2995	Pass	20465	17098	3367	@011970910...	M19	93.443	93.443	27.869	27.869
3012	Pass	15386	12322	3064	@011970910...	N12	91.803	91.803	28.689	28.689
2877	Pass	26203	22800	3403	@011970910...	H21	98.361	98.361	31.148	31.148
2878	Pass	22247	19499	2748	@011970910...	H22	99.18	99.18	31.967	31.967
2988	Pass	19698	15656	4042	@011970910...	M12	93.443	93.443	32.787	32.787
2971	Pass	27811	24145	3666	@011970910...	L19	97.541	97.541	32.787	32.787
2854	Pass	24876	21807	3069	@011970910...	G22	100	100	32.787	32.787
2972	Pass	24498	21709	2789	@011970910...	L20	99.18	99.18	33.607	33.607
2853	Pass	26527	23731	2796	@011970910...	G21	100	100	33.607	33.607
3011	Pass	17642	14058	3584	@011970910...	N11	94.262	94.262	34.426	34.426
2948	Pass	27759	24520	3239	@011970910...	K20	99.18	99.18	34.426	34.426
2965	Pass	16630	12799	3831	@011970910...	L13	94.262	94.262	35.246	35.246
2918	Pass	23046	20320	2726	@011970910...	J14	95.902	95.902	35.246	35.246
2768	Pass	24245	21487	2758	@011970910...	D08	97.541	97.541	35.246	35.246
2939	Pass	29992	26924	3068	@011970910...	K11	97.541	97.541	35.246	35.246
2800	Pass	21687	19108	2579	@011970910...	E16	98.361	98.361	35.246	35.246
2805	Pass	25200	22519	2681	@011970910...	E21	98.361	98.361	35.246	35.246

Showing/Hiding Sample Table Columns

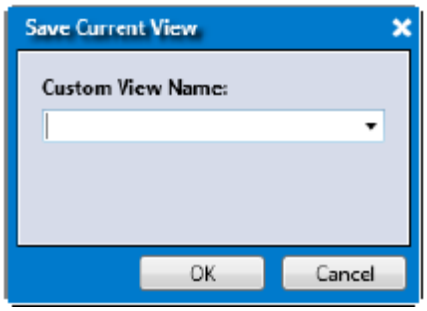
1. Click the **Show/Hide Columns** drop-down menu.
2. Click each available column name's check box to show it or remove it from the table. For definitions of the available columns, see Appendix B, *Definitions*.
3. Click outside the **Show/Hide Columns** drop-down menu to close it.

Saving your Customized Sample Table Column View

1. Click Save View.

   The following window appears: (Figure 4.15)

Figure 4.15 Save New Custom View



2. Enter a name for your custom table view, then click **OK**.

   Your newly saved name is now added to the **Apply View** drop-down menu.

Showing All Available Columns within the Sample Table

- Click the **Apply View** drop-down menu, then select **All Columns View**.

Rearranging Columns

1. Click on a column you want to move.
2. Drag it (left or right) to its new location.
3. Release the mouse button.

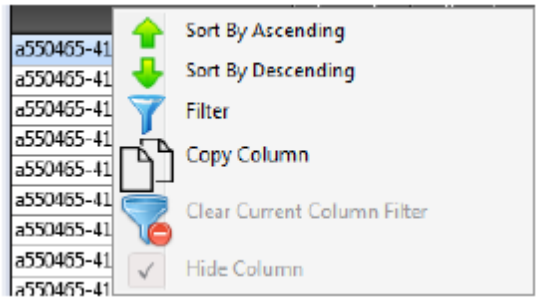
   The column is now in its new position.

Sorting Columns

1. Select a column, then right-click on it.

   The following window appears: (Figure 4.16)

Figure 4.16 Right-click Column Menu



2. Click to select either **Sort By Ascending** (A-Z) or **Sort By Descending** (Z-A).

Single-Click Sorting Method

- Single-click on a column header to sort its data in an ascending order. Single-click on the same column header to sort its data in a descending order.

Hiding a Column

1. Select the column you want to hide from the table, then right-click on it.

   The right-click menu appears. (Figure 4.16)

2. Click the **Hide Column** check box to remove it from the table.

Filtering Column Data

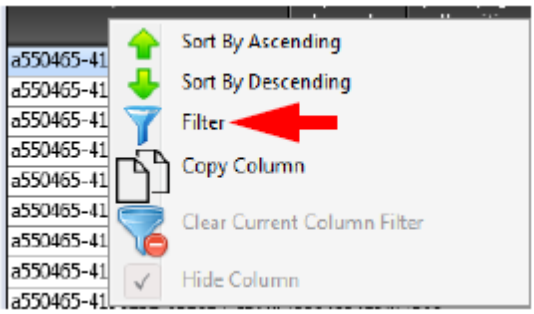
NOTE: All Sample Table columns are filterable.

Adding Filters (Method 1)

1. Select a column, then right-click on it.

   The following window appears: (Figure 4.17)

Figure 4.17 Right-click Column Menu

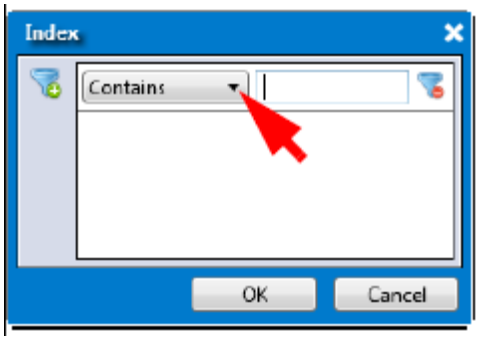


2. Click Filter.

Text-Based Columns

If the column you want to filter contains text-based data, the Contains drop-down menu appears. (Figure 4.18)

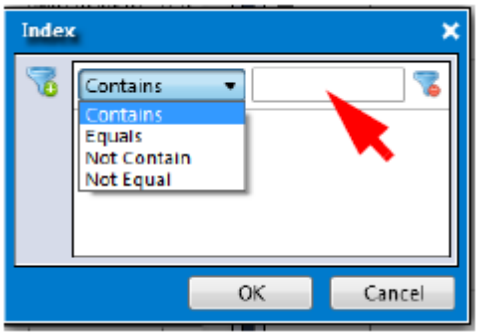
Figure 4.18 Filter Properties



To apply a filter to a text-based column:

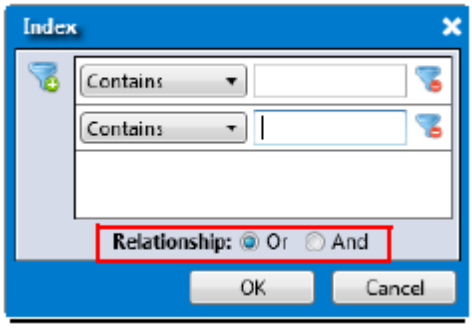
1. Click the **Contains** drop-down menu to select a filtering property. (Figure 4.19)

Figure 4.19 Drop-down Menu



2. Click inside the text entry box  to enter a value. (Figure 4.19)
3. (Optional) Click  to add additional filters.

Figure 4.20 Or or And Relationship Logic

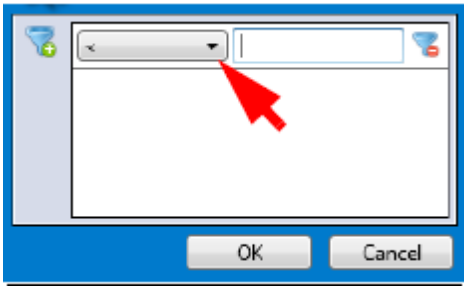


4. Click the **Or** or **And** radio button to choose **Or** or **AND** relationship logic. (Figure 4.20)
5. Repeat steps 1–4 as needed.
6. To remove a filter(s), click  .

Numeric Data Columns

If the column you want to filter contains numeric data, a symbol drop-down menu appears. (Figure 4.21)

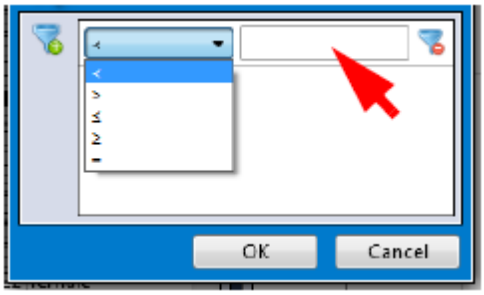
Figure 4.21 Filter Properties



To apply a filter to a value-based column:

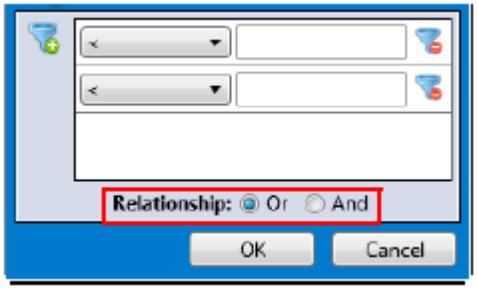
1. Click the **Symbol Value** drop-down menu to select the filtering symbol you want. (Figure 4.22)

Figure 4.22 Drop-down Menu



2. Click inside the text entry box  to enter the value(s). (Figure 4.22)
3. (Optional) Click  to add filter(s).

Figure 4.23 Or or And Relationship Logic



4. Click the **Or** or **And** radio button to choose **Or** or **AND** relationship logic. (Figure 4.23)
5. If needed, repeat steps 1-4.
6. Click **OK**.

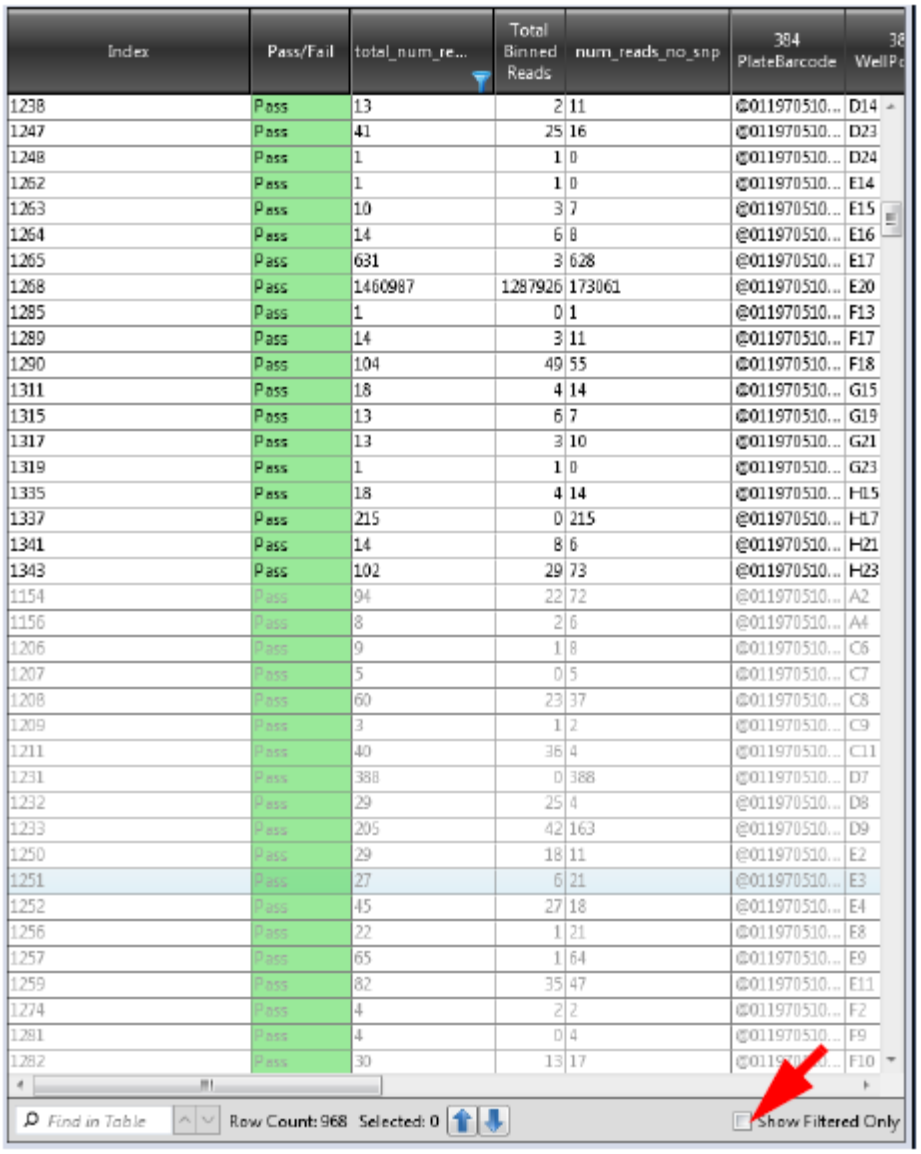
To remove a filter(s), click  .

Showing Filtered Data Only

- Click the **Show Filtered Only** check box 

to show only the data that passes the filters.
- Uncheck this box to show all data, including data that did not pass your filter criteria setting(s). In this mode, data that passes the filter appears in light gray, as shown in Figure 4.24.

Figure 4.24 Sample Table window tab - Show Filter Only unchecked example



Index	Pass/Fail	total_num_re...	Total Binned Reads	num_reads_no_snp	384 PlateBarcode	38 WellPo
1238	Pass	13	2	11	@011970510...	D14
1247	Pass	41	25	16	@011970510...	D23
1248	Pass	1	1	0	@011970510...	D24
1262	Pass	1	1	0	@011970510...	E14
1263	Pass	10	3	7	@011970510...	E15
1264	Pass	14	6	8	@011970510...	E16
1265	Pass	631	3	628	@011970510...	E17
1268	Pass	1460987	1287926	173061	@011970510...	E20
1285	Pass	1	0	1	@011970510...	F13
1289	Pass	14	3	11	@011970510...	F17
1290	Pass	104	49	55	@011970510...	F18
1311	Pass	18	4	14	@011970510...	G15
1315	Pass	13	6	7	@011970510...	G19
1317	Pass	13	3	10	@011970510...	G21
1319	Pass	1	1	0	@011970510...	G23
1335	Pass	18	4	14	@011970510...	H15
1337	Pass	215	0	215	@011970510...	H17
1341	Pass	14	8	6	@011970510...	H21
1343	Pass	102	29	73	@011970510...	H23
1154	Pass	94	22	72	@011970510...	A2
1156	Pass	8	2	6	@011970510...	A4
1206	Pass	9	1	8	@011970510...	C6
1207	Pass	5	0	5	@011970510...	C7
1208	Pass	60	23	37	@011970510...	C8
1209	Pass	3	1	2	@011970510...	C9
1211	Pass	40	36	4	@011970510...	C11
1231	Pass	388	0	388	@011970510...	D7
1232	Pass	29	25	4	@011970510...	D8
1233	Pass	205	42	163	@011970510...	D9
1250	Pass	29	18	11	@011970510...	E2
1251	Pass	27	6	21	@011970510...	E3
1252	Pass	45	27	18	@011970510...	E4
1256	Pass	22	1	21	@011970510...	E8
1257	Pass	65	1	64	@011970510...	E9
1259	Pass	82	35	47	@011970510...	E11
1274	Pass	4	2	2	@011970510...	F2
1281	Pass	4	0	4	@011970510...	F9
1282	Pass	30	13	17	@011970510...	F10

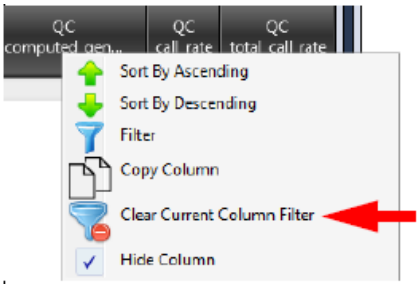
Find in Table Row Count: 968 Selected: 0 Show Filtered Only

Clearing an Individual Filter

1. Right-click on the filtered column you want to clear.

   The following window appears: (Figure 4.25)

Figure 4.25 Right-click Column Menu



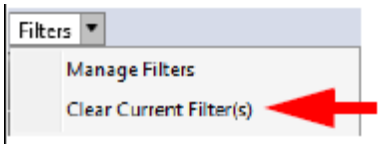
2. Click Clear Current Column Filter.

   The filter is removed.

Clearing All Current Filters

- Click the Filters drop-down, then select **Clear Current Filters**. (Figure 4.26)

Figure 4.26 Filters Menu



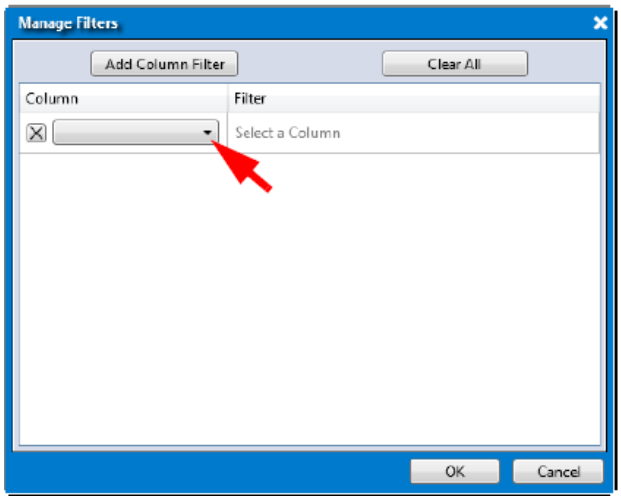
Adding Filters (Method 2)

TIP: Use this method if you want to change more than one of your Sample Table column filters at the same time.

1. Click the **Filters** drop-down menu, then click **Manage Filters**.

   The Manage Filters window appears. (Figure 4.27)

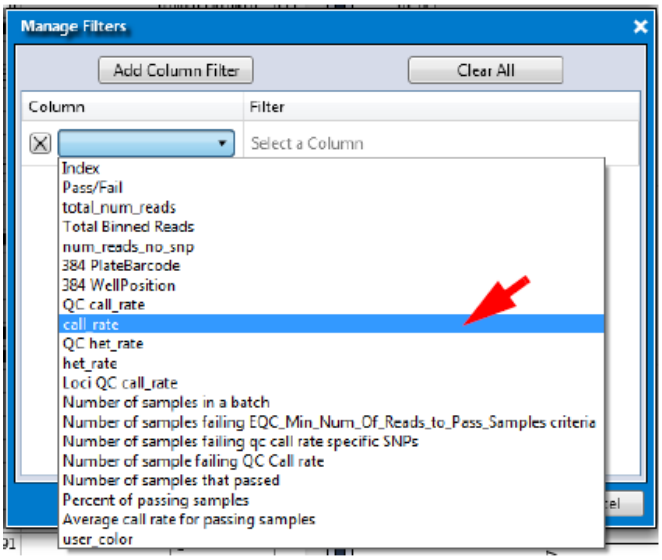
Figure 4.27 Manage Filters window



NOTE: If the column you want to filter contains text-based data, the Contains drop-down menu appears. If the column you want to filter contains numeric data, a symbol drop-down menu appears.

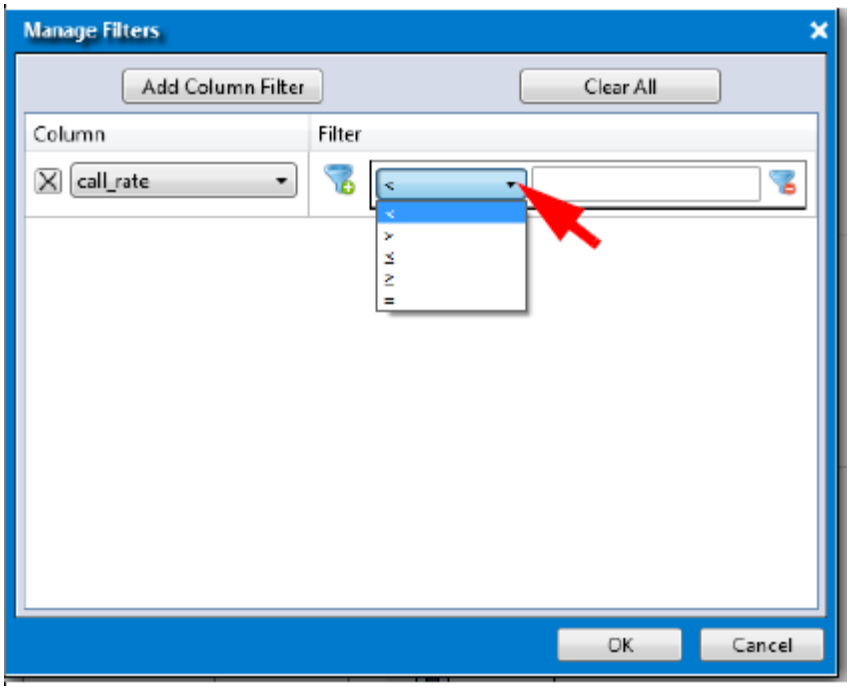
2. Click the **Column** drop-down, then click to select the Column name you want to create a filter for. (Figure 4.28)

Figure 4.28 Manage Filters window - Select a Column



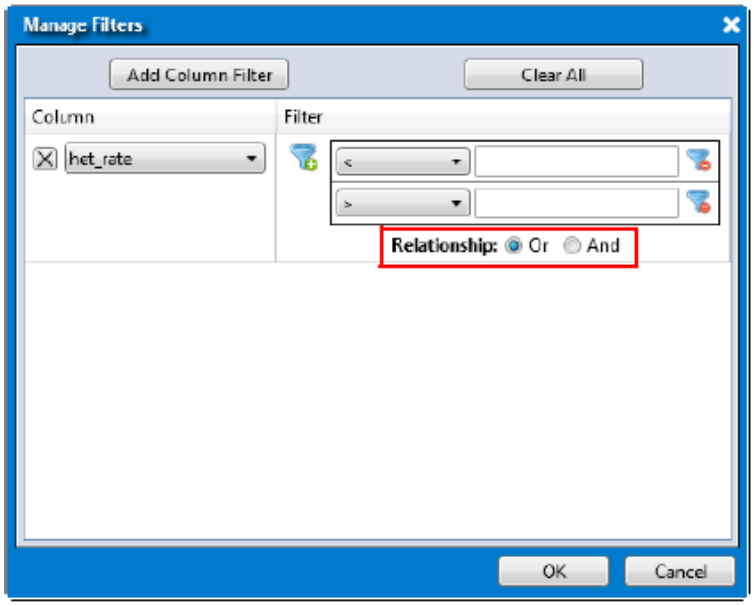
3. Click the **Symbol Value** drop-down menu to select the filtering symbol you want. (Figure 4.29)

Figure 4.29 Manage Filters window - Select a Symbol



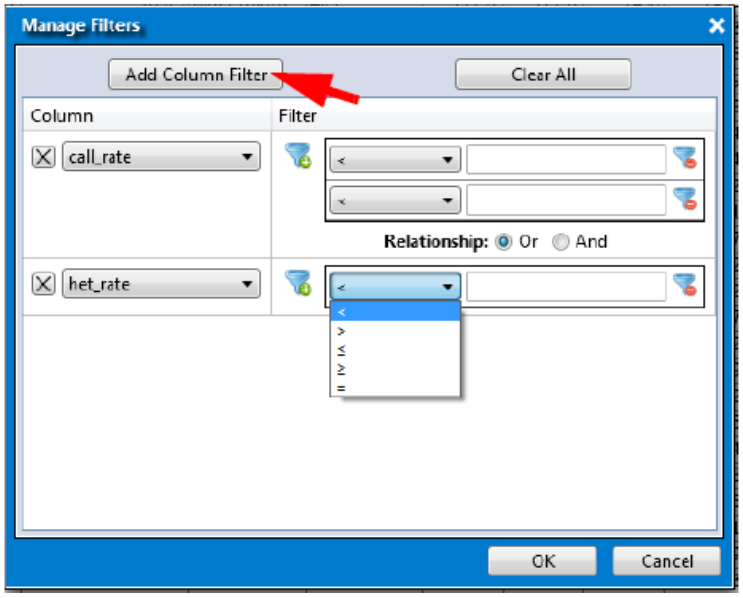
4. Click inside the text entry box  to enter new value(s). (Figure 4.29)
5. (Optional) If you want to add an additional filter to a column, click  .
6. Click the **Or** or **And** radio button to choose **Or** or **AND** relationship logic. (Figure 4.30)

Figure 4.30 Manage Filters window - OR or AND Relationship



7. If needed, click **Add Column Filter**, then repeat the above steps. (Figure 4.31)

Figure 4.31 Manage Filters window - Adding another Column Filter



8. Click **OK**.

   To remove a filter(s), click  .

   Click **Clear All** to remove ALL filters in the Manage Filters window.

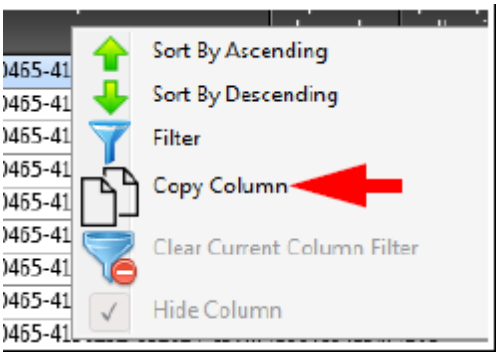
Copying Column Data

To copy column data to your clipboard:

1. Click to select a column you want to copy to a clipboard, then right-click on it.

   The following window appears: (Figure 4.32)

Figure 4.32 Right-click Column Menu



2. Click Copy Column.

   The column data is now ready for pasting (Ctrl v).

Setting User Colors

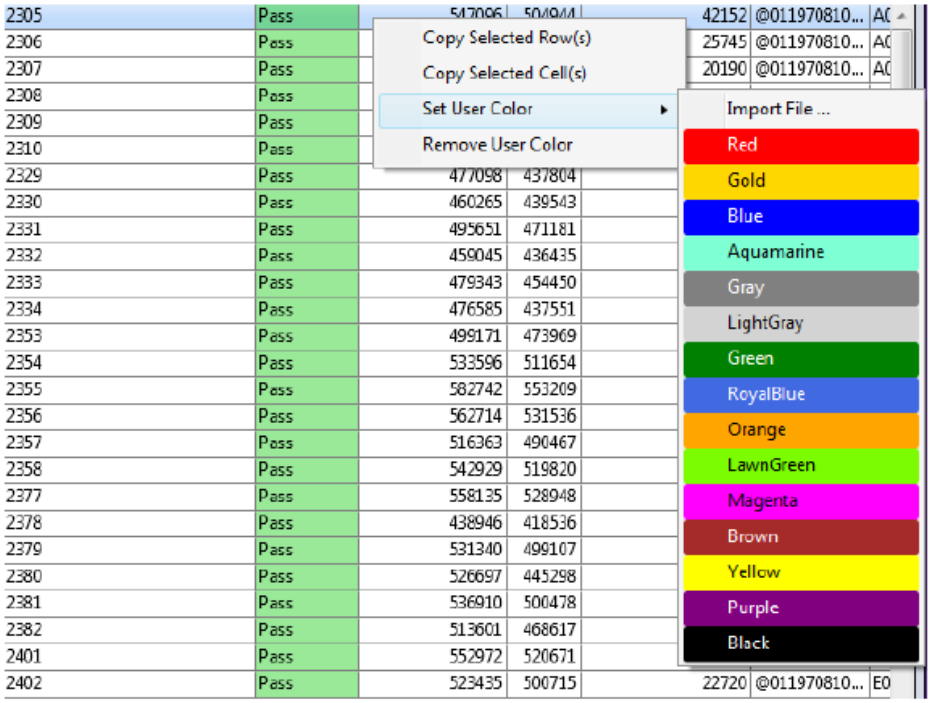
Use this feature to more easily identify different sets between the Sample Table and Cluster Plot.

Assigning a Color to a Sample

1. Right-click on the sample you want to assign a color to.

   A menu appears. (Figure 4.33)

Figure 4.33 Right-click menu - Set User Color



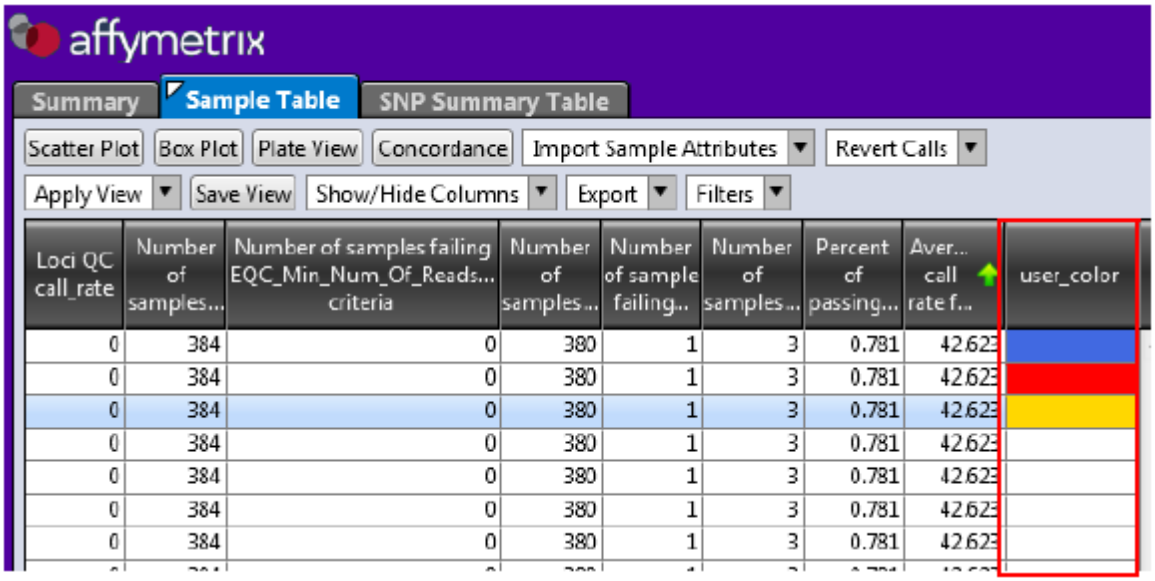
2. Mouse over Set User Color.

   A color pallet appears.

3. Click on the color you want.

   A user_color column is automatically added to your Sample Table and contains your sample's newly assigned color, as shown in Figure 4.34.

Figure 4.34 Right-click menu - Set User Color

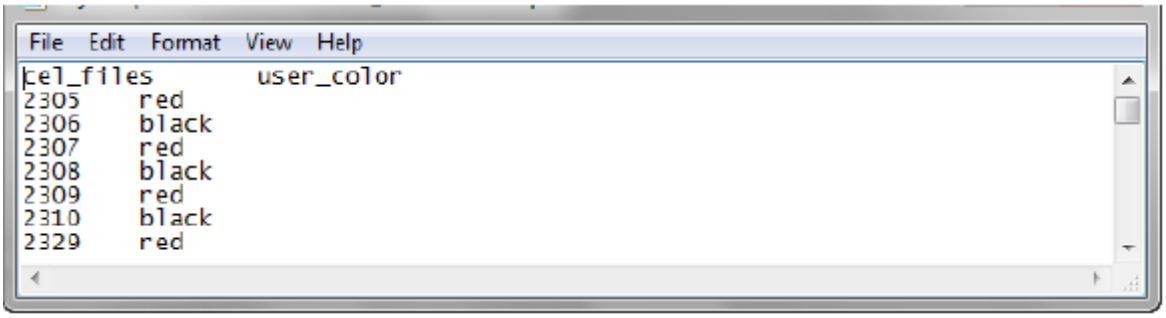


Importing Assigned Colors

Use this feature if you want to assign colors to a large number of samples or if your Sample Table contains a vast amount of samples and you want to assign a color to only a few samples.

1. Use MS Excel or Notepad (as you normally would) to create a 2-column table. (Figure 4.35).

Figure 4.35 2-column example in Notepad



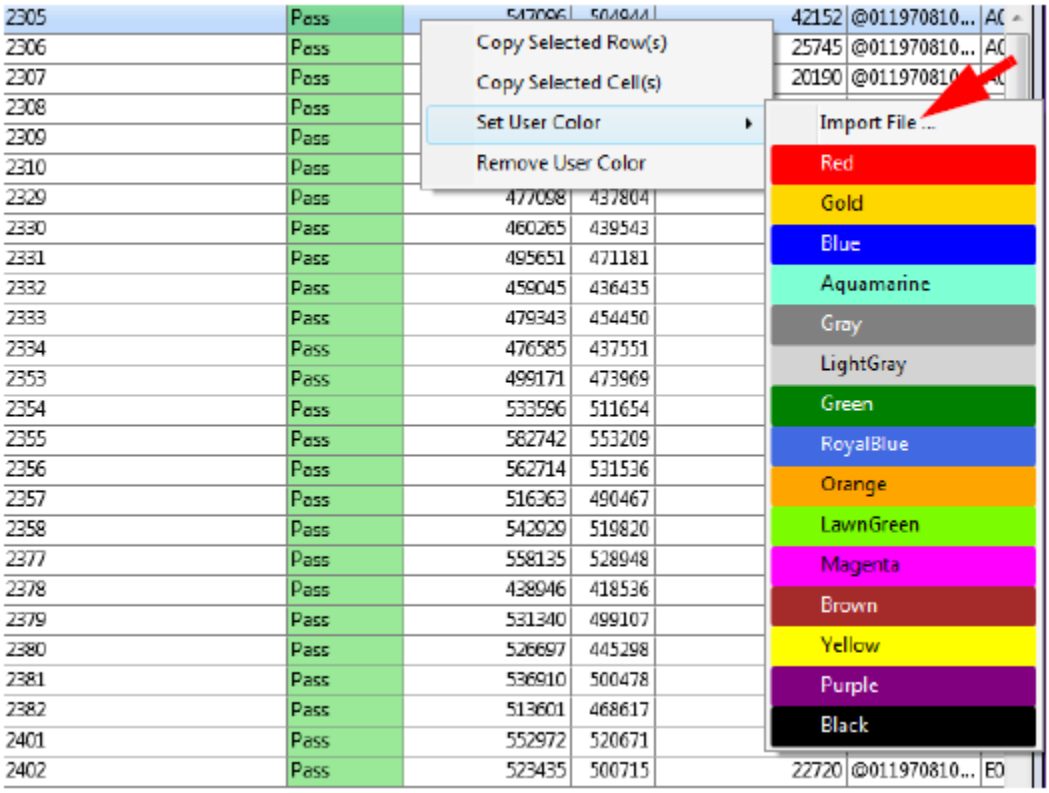
2. Save your two column table as a tab-delimited .txt file to an easily accessible location. Again, your table must be only two columns with the headers **cel_files** and **user-color**.

IMPORTANT: Your *user_color* entries must match the color pallet naming conventions shown in Figure 4.36. Example: *RoyalBlue* not *Royal Blue*.

3. At the Sample Table, right-click on any sample.

   A menu appears. (Figure 4.36)

Figure 4.36 Right-click menu - Set User Color - Import File



4. Mouse over Set User Color.
5. Click on Import File...

   An **Import User Colors** Explorer window appears.
6. Click to highlight your .txt file, then click **Open**.

   The 2-column table entries are now incorporated into the Sample Table.
7. Scroll the Sample Table right to see the added **user_color** column and assigned sample colors.

Removing an Assigned User Color

1. From the Sample Table, right-click on the sample containing the color you want to remove.

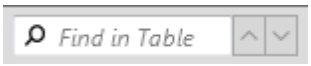
   A menu appears.
2. Click Remove User Color.

   Your previously assigned sample color is now removed.

Searching Keywords

NOTE: The Find in Table tool can locate exact (case insensitive) matches. It also accepts wild- card (*) characters to aid in your search. Example: ABC*

To search for a keyword within your table:

1. Click inside the 

text field (bottom left corner of table).
2. Enter a keyword or number.
3. Click the **Up** or **Down** button.

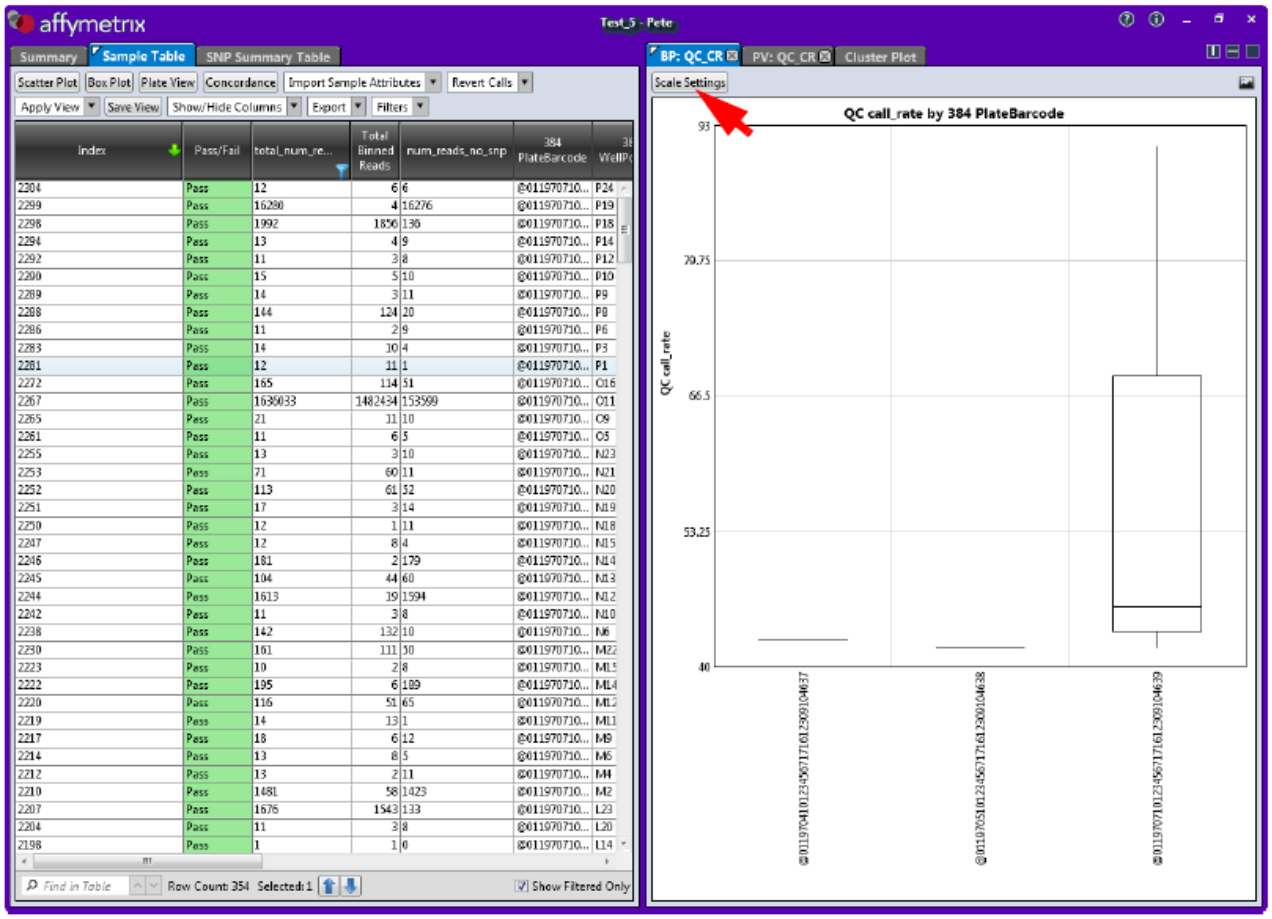
   When a match is found, the appropriate table entry is highlighted. If a graph is displayed, the appropriate graph point is also highlighted.

Using the Box Plots

Viewing the Default Box Plots

By default, the Viewer generates 1 Box Plot.

Figure 4.37 Table and Box Plot 1



NOTE: You you cannot change a plot's axis values after it has been created. However, you can change its scale and coloring properties. See Changing the Box Plot's Scale Setting Ranges.

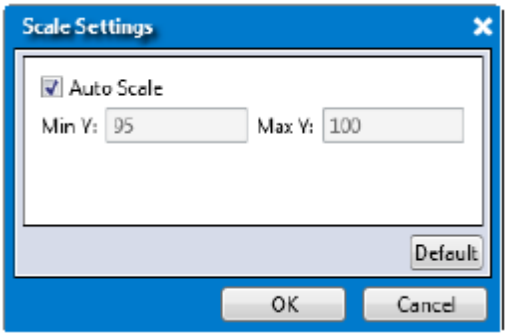
To change a Box Plot's axis properties, you must create a new Box Plot. See *Adding a New Box Plot*.

Changing the Box Plot's Scale Setting Ranges

1. Click the **Scale Settings** button.

   The following window appears. (Figure 4.38)

Figure 4.38 Scale Settings window



   By default, the window displays your current range values.

2. Enter your new ranges in the appropriate text fields.
3. Click **OK**.

   Your new settings are now reflected within the Box Plot. Modified Set Scale values are auto-saved.

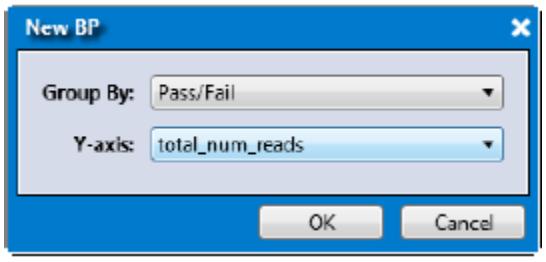
   If needed, click the Default button to return all values to their default ranges.

Adding a New Box Plot

1. Click the **Box Plot** button.

   The following window appears: (Figure 4.39)

Figure 4.39 Box Plot Versus menus



2. Click the **Group By** drop-down menu to select the X-axis for your new Box Plot.

   Your X-axis selection determines your new Box Plot's boxes and whiskers, based on the data group of values that are compiled.

3. Click the **Y-axis** drop-down menu to select the Y-axis you want.

   For Group By and Y-axis definitions, see *Use the following table of definitions to help select your Sample table columns* in Appendix B.

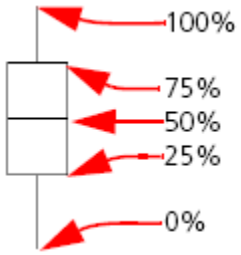
4. Click **OK**.

   A new Box Plot window tab is created.

Reading Box Plot Percentiles

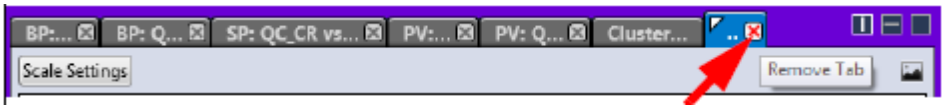
(Figure 4.40)

Figure 4.40 Box Plot percentiles



At any time, click X to remove a window/tab. (Figure 4.41)

Figure 4.41 New Window/Tab



Saving the current Box Plot view as a .PNG file

1. Click the **Save Image** button.

   An Explorer window appears.

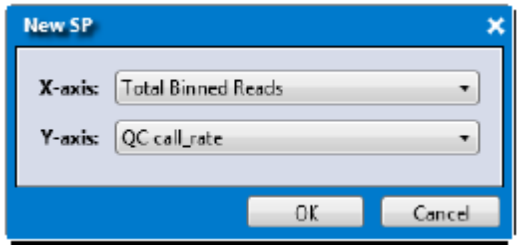
2. Navigate to where you want to save the **.PNG** file, enter a filename, then click **Save**.

Using the Scatter Plot (Optional)

1. From the Sample Table window tab, click the button.

   The following window appears: (Figure 4.42)

Figure 4.42 Scatter Plot Versus menus



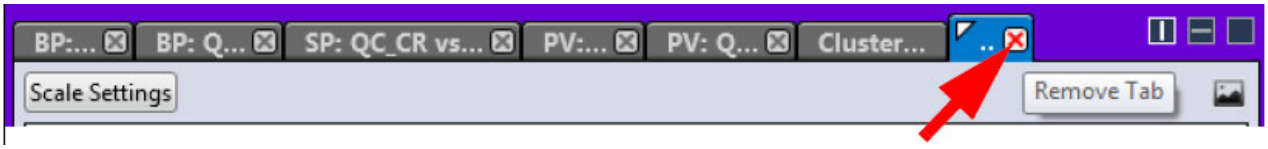
2. Use the drop-down menus to select your Plot's versus scenario (X and Y axis). See Appendix B, *Definitions* for definitions.

3. Click **OK**.

   A new Scatter Plot window tab is created.

   At any time, click X to remove a window/tab. (Figure 4.43)

Figure 4.43 New Window/Tab

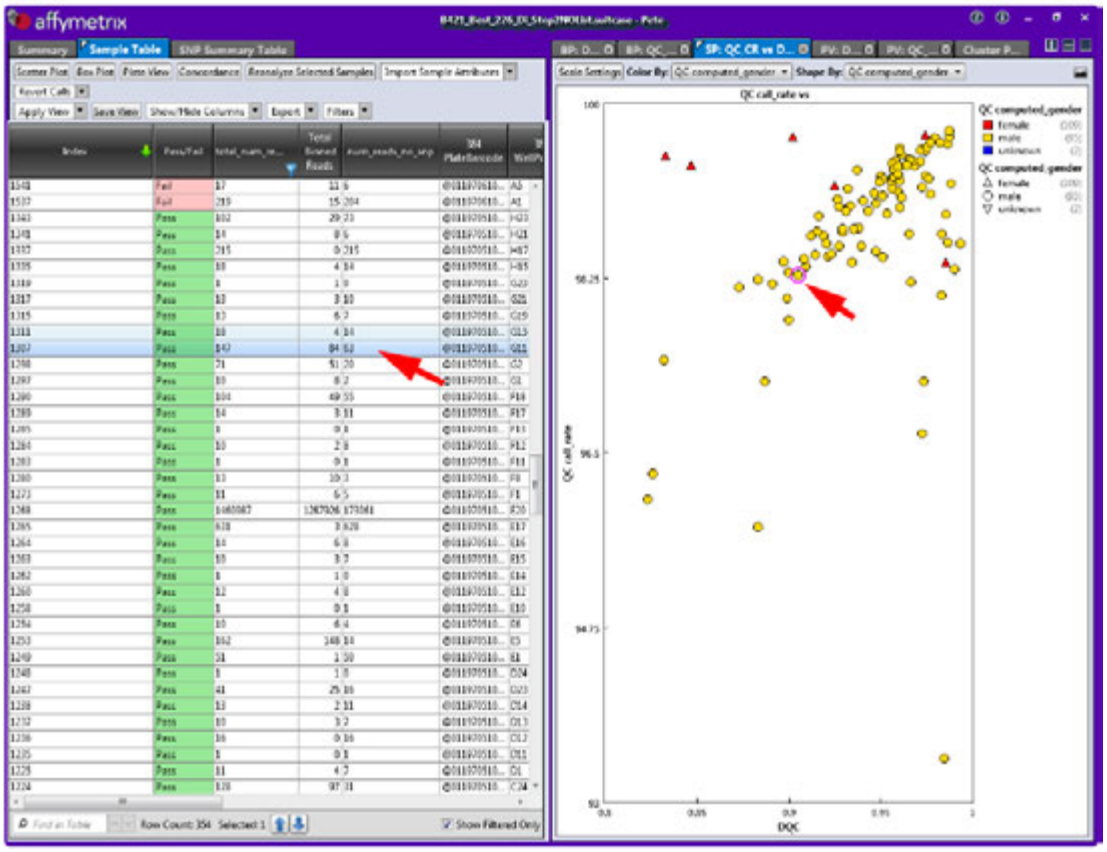


The Scatter Plot window tab appears. (Figure 4.44)

Viewing the Scatter Plot

- Click to highlight a table entry to view its location within the Scatter Plot or click on a data point to highlight its corresponding entry in the Sample Table.

Figure 4.44 Table and Scatter Plot



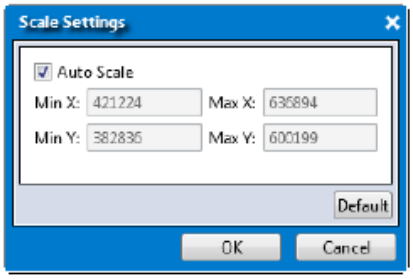
NOTE: You cannot change the default Scatter Plot's pre-defined X and Y definitions, however you can change its *Scale Settings* and *Color By and Shape By* configuration. To change a Scatter Plot's axis properties, you must create a new Scatter Plot.

Changing the Scatter Plot's Setting Ranges and Views

1. Click the **Scale Settings** button.

   The following window appears. (Figure 4.45)

Figure 4.45 Scale Settings window



   By default, the window displays your current range values.

2. Enter your new ranges in the appropriate text fields.
3. Click **OK**.

   Your new settings are now reflected within the Scatter Plot. Modified Set scale values are auto-saved.

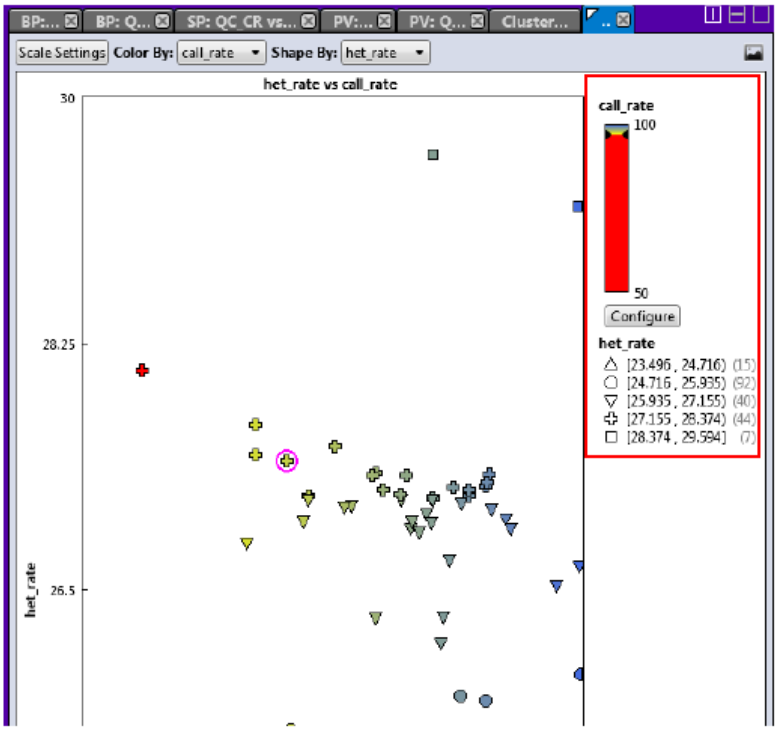
   If needed, click the **Default** button to return all values to their default ranges.

4. Click the **Color By** and **Shape By** drop-down menus to select the combination view you want.

> **NOTE:** Your imported sample attributes are also available for use within the Color By and Shape By drop-down menus. For information on importing sample attributes, go to *Importing Sample Attributes*.

   A legend appears within the plot. (Figure 4.46)

Figure 4.46 Color By and Shape By Legend example



The graph can display up to 10 different colors and up to 10 different shapes. If the attributes selected for display have more than 10 categories, categories 1 through 9 are displayed normally, but categories 10 and higher get grouped together.

If your study has more than 10 values:

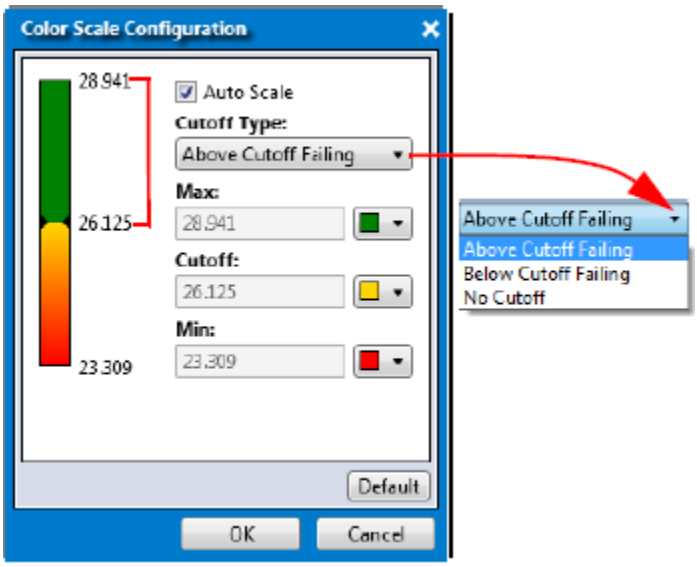
- If the value is text, the software takes the first nine values and assigns each a color or shape. The remaining values are put into a bin called "Other¨. All values in the Other bin have the same color or shape.
- If the value is a date or number, the software divides the range of data into 10 equal bins and assigns a color or shape to each bin. If the data includes one or more outliers, it is possible to have one value in a particular bin and all other values in another bin.

Customizing Color By Settings

1. Click Configure.

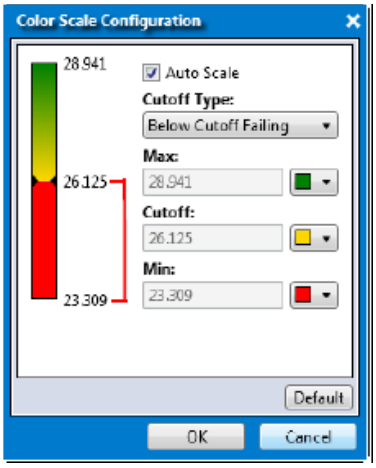
   The following window appears: (Figure 4.47)

Figure 4.47 Color By options



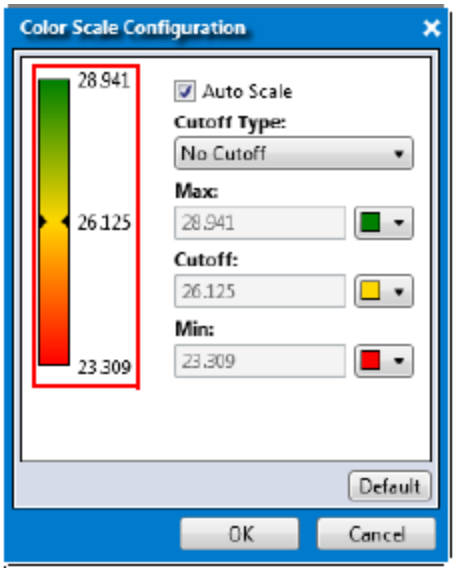
2. Use the provided text fields and color drop-down menus to customize your Color By selection.
   - **Auto Scale** checkbox (when checked) uses the actual minimum (lower bound) and maximum (upper bound) as your min/max scale. Uncheck the Auto Scale checkbox to enter your min and max number scales in the provided fields.
   - Click the **Cutoff Type** drop-down menu to select your cutoff preference.
     - **Above Cutoff Failing** - This presents a hard visual cutoff graph of all values that fail ABOVE the Cutoff value entered. The Above Cutoff data is represented by the color defined for Max. (Green in Figure 4.47)
     - **Below Cutoff Failing** - This presents a hard visual cutoff graph of all values that fail BELOW the Cutoff value entered. The Below Cutoff data is represented by the color defined for Min. (Red in Figure 4.48)

Figure 4.48 Below Cutoff



- **No Cutoff**—This presents a smooth 3-point gradient of your defined Max, Min, and colors. (Figure 4.49)

Figure 4.49 No Cutoff



3. Click **OK**.

   Your Cutoff preference, entered values, and color selections are now displayed on the graph and saved for future use. If needed, click the **Default** button to revert ALL values back to their factory setting.

Saving the current Scatter Plot View as a .PNG file

1. Click the **Save Image**  button.

   An Explorer window appears.

2. Navigate to where you want to save the **.PNG** file, enter a filename, then click **OK**.

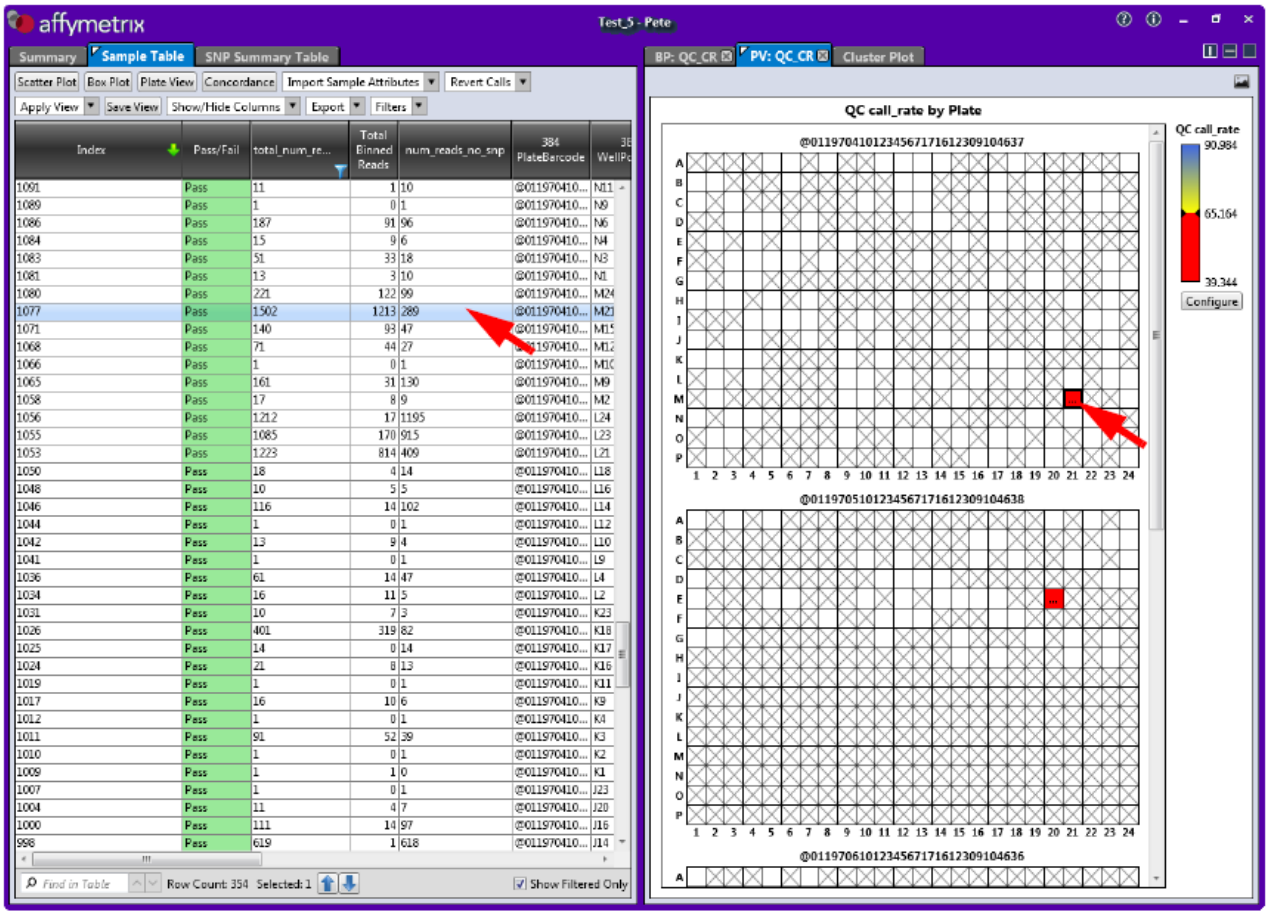
Using the Plate View

By default, the Viewer generates one Plate View. To display a different metric you must create a new Plate View. For more details, see *Adding a New Plate View Metric*.

Viewing the Plate View

- Click to highlight a table entry to view its location within the Plate View or click on a plate position to highlight its corresponding table entry. (Figure 4.50)

Figure 4.50 Table and Plate View 1



NOTE: You cannot change a default Plate View, however you can change its Scale Settings, as well as gradient and coloring. See *To customize your Plate View settings.*

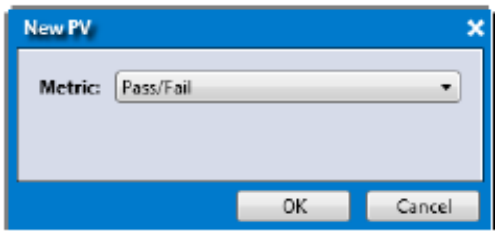
Adding a New Plate View Metric

The default Plate Views cannot be altered, therefore you must click the **Plate View** button to create a new Plate View to reflect your Metric change.

1. Click the **Plate View** button.

   The following window appears: (Figure 4.51)

Figure 4.51 Plate View Metric setting

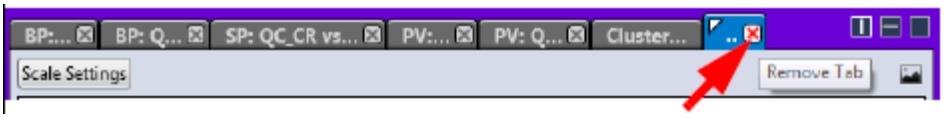


2. Use the drop-down menus to select your Plate View's Metric setting. See the tables in Appendix B, *Definitions* for Metric definition.
3. Click **OK**.

   The new Plate View window tab appears.

   At any time, click X to remove a window/tab. (Figure 4.52)

Figure 4.52 New Window/Tab

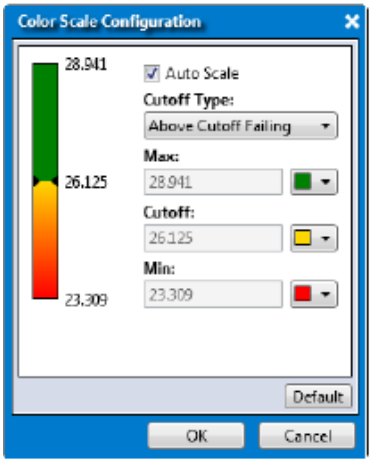


To customize your Plate View settings:

1. Click Configure.

   The following window appears: (Figure 4.53)

Figure 4.53 Color Scale options



2. Use the provided text fields and color drop-down menus to customize your Color By selection.
   - **Auto Scale** checkbox (when checked) uses the actual minimum (lower bound) and maximum (upper bound) as your min/max scale. Uncheck the Auto Scale checkbox to enter your min and max number scales in the provided fields. Note: If the Auto Scale checkbox remains unchecked, you must enter new scale limits for each subsequent analysis.

- Click the **Cutoff Type** drop-down menu to select the appropriate cutoff (based on the custom Cutoff value you entered).

3. Click **OK**.

   Your new preferences are now displayed and saved for future use.

   At any time, click the **Default** button to revert all the Color Scale Configuration window values back to their factory setting.

Saving the current Plate View as a .PNG file

1. Click the **Save Image** button.

   An Explorer window appears.

2. Navigate to where you want to save the **.PNG** file, enter a filename, then click **OK**.

Performing Concordance Checks

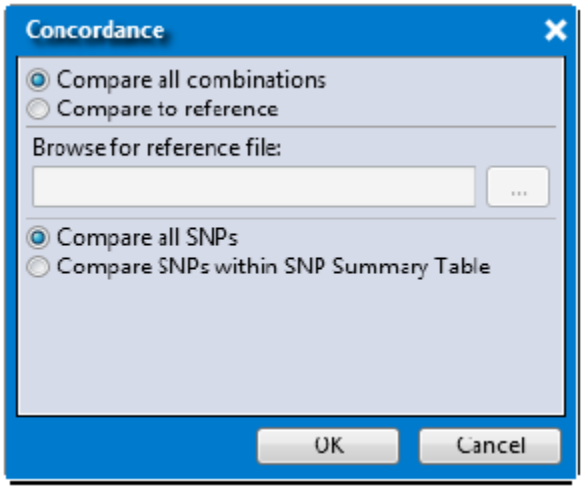
- **Compare all combinations** allows you to compare the SNP calls for all samples. The concordance between all pairwise comparisons for the samples in the dataset/suitcase are reported.
- **Compare to reference** allows you to compare every sample to a single reference file.

Running a Concordance Check

- Click the **Concordance** button.

  The following window appears: (Figure 4.54)

Figure 4.54 Concordance window



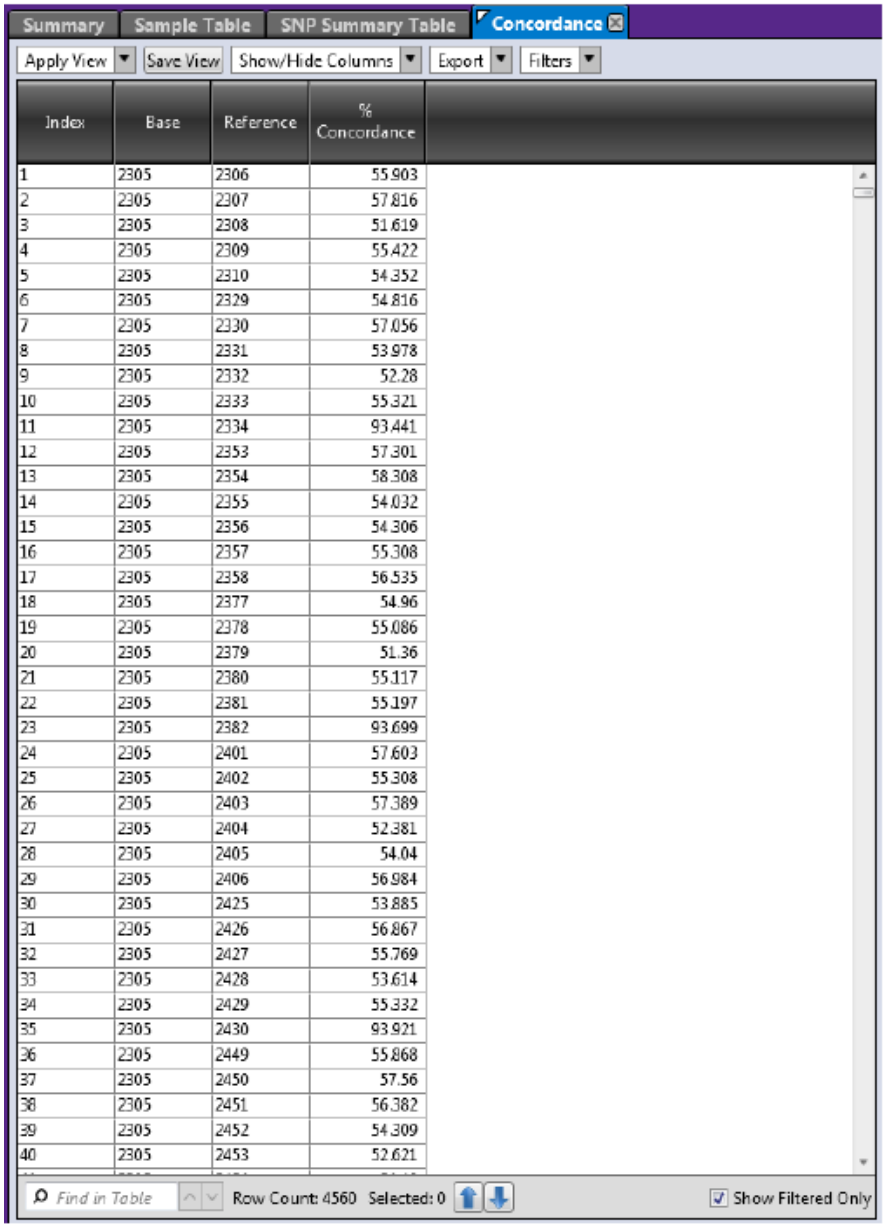
To compare all combinations:

1. Make sure the **Compare all combinations** radio button is selected.
2. By default, the **Compare all SNPs** button is selected. If needed, click the **Compare signature SNPs within the SNP Summary Table** radio button.
3. Click **OK**.

   After a few moments, the following Concordance window tab appears: (Figure 4.55)

NOTE: The amount of time to calculate concordance is proportional to the number of samples and SNPs. It is highly recommended you use <1000 SNPs for an All versus All concordance check.

Figure 4.55 Default Concordance Table example



Index	Base	Reference	% Concordance
1	2305	2306	55.903
2	2305	2307	57.816
3	2305	2308	51.619
4	2305	2309	55.422
5	2305	2310	54.352
6	2305	2329	54.816
7	2305	2330	57.056
8	2305	2331	53.978
9	2305	2332	52.28
10	2305	2333	55.321
11	2305	2334	93.441
12	2305	2353	57.301
13	2305	2354	58.308
14	2305	2355	54.032
15	2305	2356	54.306
16	2305	2357	55.308
17	2305	2358	56.535
18	2305	2377	54.96
19	2305	2378	55.086
20	2305	2379	51.36
21	2305	2380	55.117
22	2305	2381	55.197
23	2305	2382	93.699
24	2305	2401	57.603
25	2305	2402	55.308
26	2305	2403	57.389
27	2305	2404	52.381
28	2305	2405	54.04
29	2305	2406	56.984
30	2305	2425	53.885
31	2305	2426	56.867
32	2305	2427	55.769
33	2305	2428	53.614
34	2305	2429	55.332
35	2305	2430	93.921
36	2305	2449	55.868
37	2305	2450	57.56
38	2305	2451	56.382
39	2305	2452	54.309
40	2305	2453	52.621

For definitions of the Concordance columns, see *Concordance*.

To compare to reference:

1. Click the **Compare to reference** button.

   The browse for the reference file field is now activated.

2. Click the **Browse** button.

   A Windows Explorer window appears.

3. Navigate to the appropriate reference file location, then click **Open**.

   Your Reference file is displayed.

NOTE: The reference file you select can have more than two columns, however only the first two columns are used during the compare to reference concordance check.

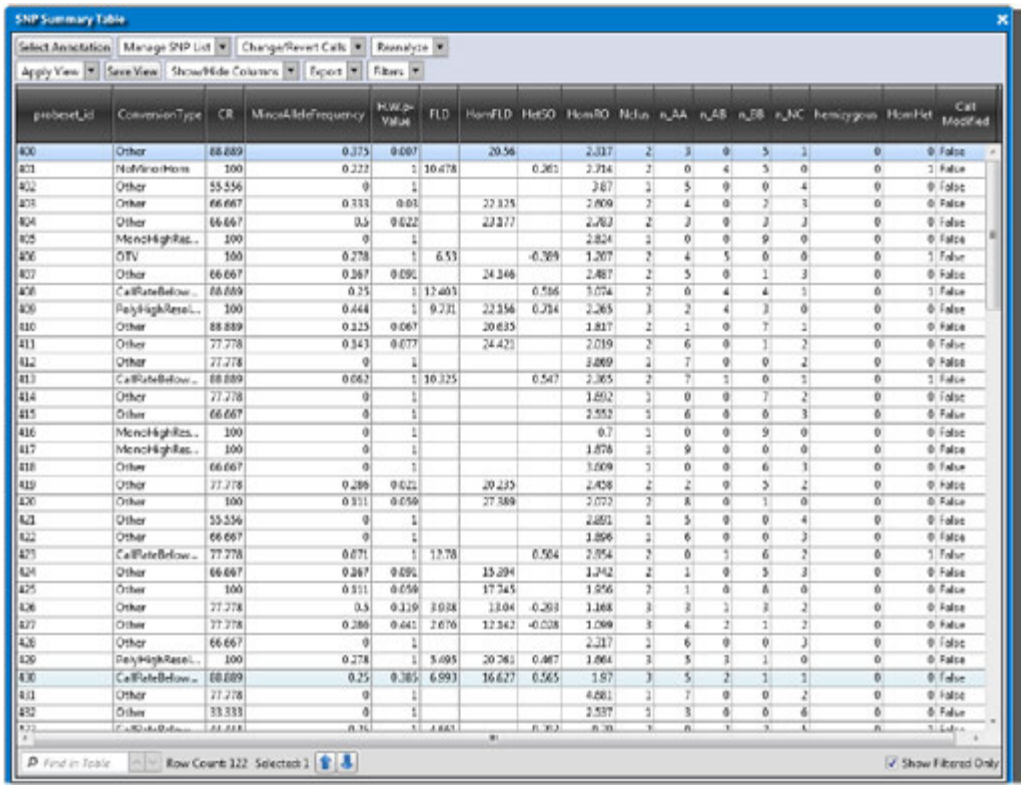
The first column header must be "Probe Set ID" or 'SNP' or 'probeset_it' and the second column header must contain 'Call' or Consensus". The calls must be one of AA, AB, BB or NN in order to run.

4. By default, the **Compare all SNPs** button is selected. If needed, click the **Compare signature SNPs within the SNP Summary Table** radio button.
5. Click **OK**.

   After a few moments, a Concordance window tab updates.

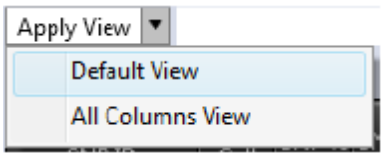
Using the SNP Summary Table

Figure 4.56 SNP Summary Table window



Setting Up your SNP Summary Table

Figure 4.57 Apply View drop-down

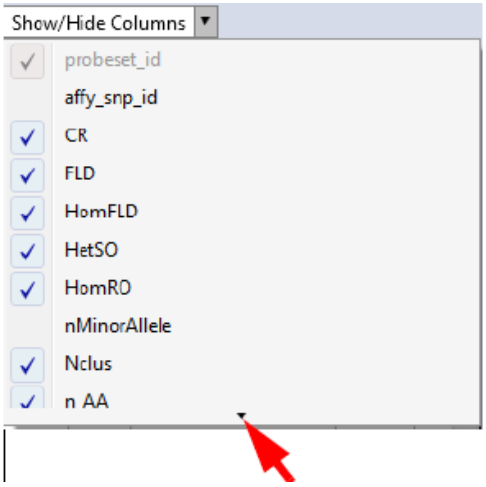


1. Click the **Apply View** drop-down (Figure 4.57).

   The Default View is the initial table view and includes a preset number of columns.
2. Click **All Columns View** to display the maximum available columns.

Adding and Removing Table Columns

Figure 4.58 Show/Hide Columns drop-down



1. Click the **Show/Hide Columns** drop-down (Figure 4.58)

   A list of available columns appear.

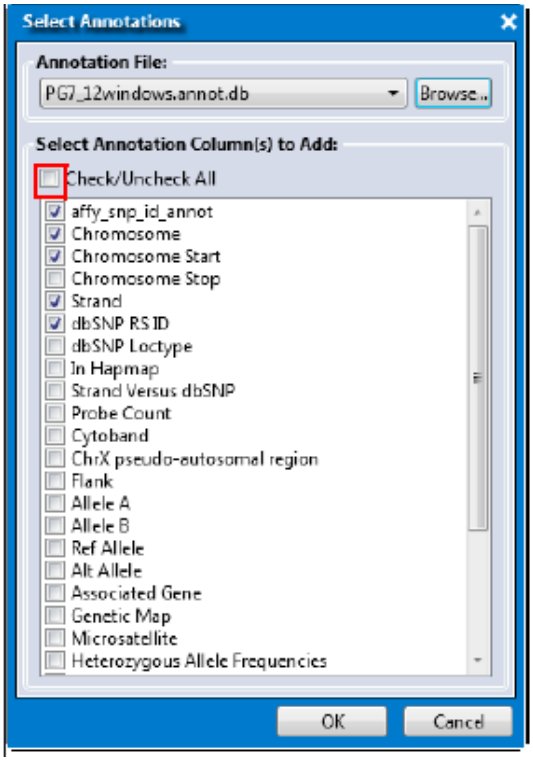
2. Click the check box next to the column(s) you want to add (Show) on the table. Click to uncheck a column you want to remove (Hide) from the table. Mouse over the menu's down arrow to reveal more available column choices. For column definitions, see *SNP Summary Table.*

Selecting Annotations

1. Click the **Select Annotation** button.

   The following window appears. (Figure 4.59)

Figure 4.59 Available Annotations



2. Select the appropriate Annotation File from the drop-down menu list.
3. Click the check box next to the Annotation Column(s) you want to add to the table or click the **Check/Uncheck All** check box (Figure 4.59) to add or remove ALL available annotations. For annotation definitions, see page 102.
4. Click **OK**.

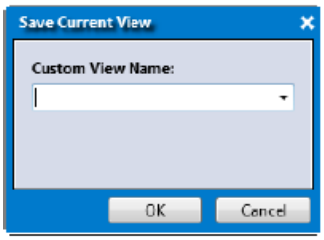
   Your selected annotation columns are now added to the right side of the SNP Summary Table.

Saving your Table Column View

1. After you have your preferred SNP Summary Table columns set, click **Save View**.

   The following window appears: (Figure 4.60)

Figure 4.60 Save New Custom View



2. Enter a name, then click **OK**.

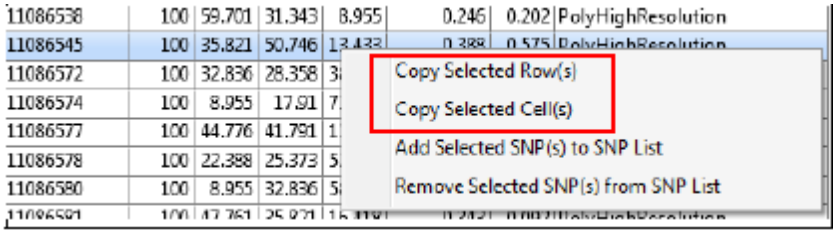
   Your custom table view is now saved and stored inside the Apply View menu for future use.

Copying Selected Row(s)

1. Right-click on a row you want to copy. Optional: Single-click on a row or **Ctrl+click, Shift+click,** or press **Ctrl+a** (to select multiple rows).

   A menu appears. (Figure 4.61)

Figure 4.61 Right-click menu



2. Click Copy Selected Row(s).

   The row data is now ready for pasting (Ctrl v).

Copying Selected Cell(s)

1. Right-click on a cell you want to copy. Optional: Single-click on a row or **Ctrl+click, Shift+click**, or press **Ctrl+a** (to select multiple cells).

   A menu appears. (Figure 4.61)

2. Click Copy Selected Cell(s).

   The cell data is now ready for pasting (Ctrl v).

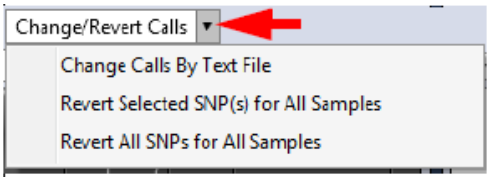
Changing or Reverting Genotype Calls

Reverting Calls changes them back to what they were originally called by the algorithm. No other history is saved, only its current and original values.

- Click the **Change/Revert Calls** drop-down.

  The following menu appears: (Figure 4.62)

Figure 4.62 Revert Calls drop-down menu



To change genotype calls by text file:

1. Click Change Calls By Text File.

   An Explorer window appears.

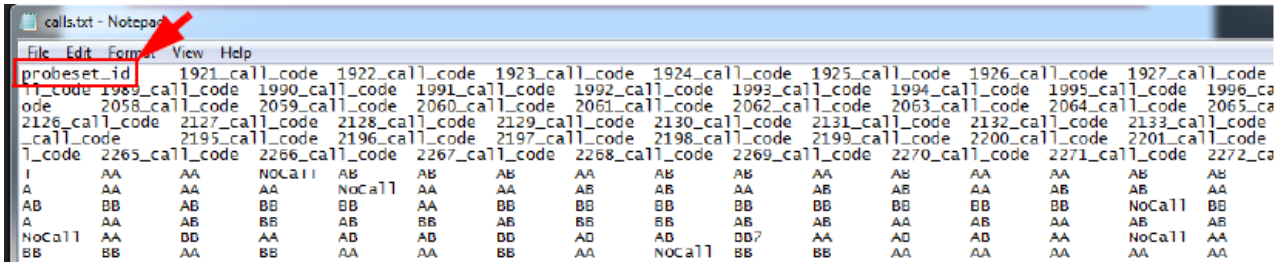
2. Navigate to the text file's location.

IMPORTANT: Your Change Calls by Text File must start with the header probeset_id and only numeric or alphabetic codes for the genotype.

Samples must be listed by index (not by sample name).

Your Change Calls by Text File cannot contain any annotation columns.

Figure 4.63 Change Call by Text file example shown in Excel (as a tab-delimited text file)



3. Click **Open**.

   Your Genotype Calls are now changed and reflected in the Cluster Plot.

To revert selected SNPs for all samples:

1. Single-click on a SNP file or **Ctrl+click, Shift+click,** or press **Ctrl+a** (to select multiple files) within the SNP Sample Table.
2. Click **Revert Selected SNP(s) for All Samples**.

To revert all SNPs for all samples:

Use this feature to perform a master SNPs reset.

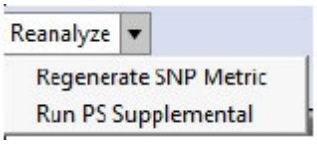
- Click Revert All SNPs for All Samples.

IMPORTANT: Once *Revert Calls* is performed, the selected calls will be reverted to original calls. This cannot be undone.

Regenerating your SNP Summary Table Data

Click the **Reanalyze** drop-down menu (Figure 4.64) post-processing options.

Figure 4.64 Reanalyze drop-down menu



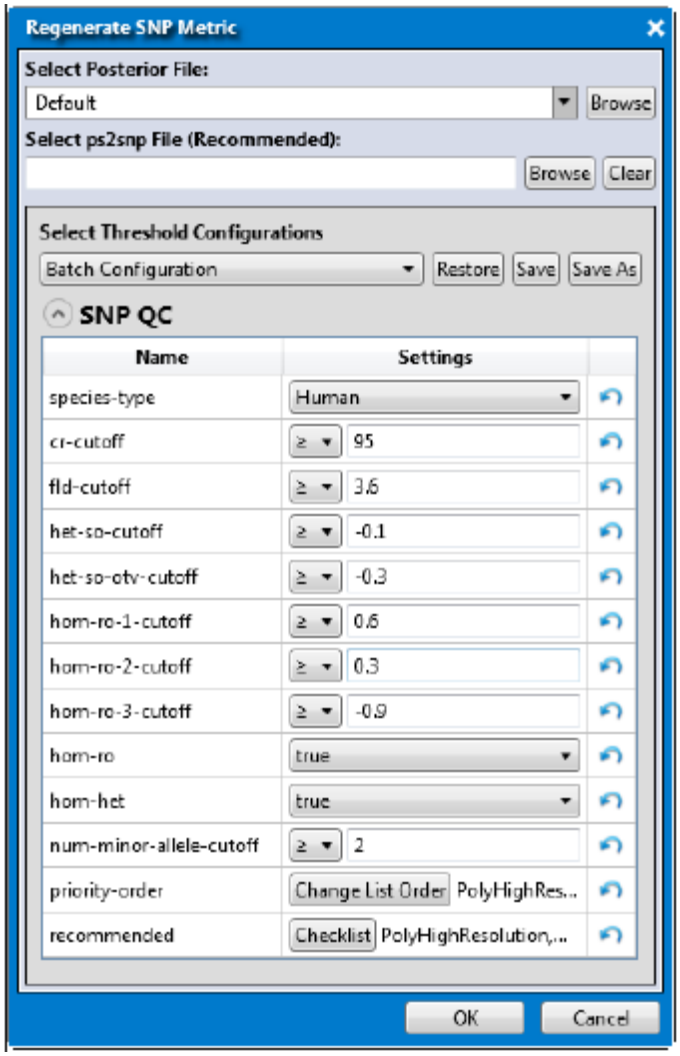
Regenerate SNP Metric

The Regenerate SNP Metric allows for modification of SNP Metric thresholds to be applied, better classifying SNPs into the six main categories.

- Click Regenerate SNP Metric.

  The Regenerate SNP Metric appears. (Figure 4.65)

Figure 4.65 Regenerate SNP Metric window



1. Use the drop-down to select the appropriate Posterior File or click its **browse** button.

   An Explorer window appears.

2. Navigate to Posterior File you want to use, then click **Open**.
3. Choose an appropriate ps2snp File (recommended), as described in *Saving your Analysis Configuration*.
4. Select the Threshold Configuration you want to use, as described in *Customizing Threshold Settings*.
5. Use the **SNP QC** drop-down menus, and text fields to setup the regeneration of your SNP Metric.
6. To change the priority-order of your **SNP QC** Metric, click Change List Order.

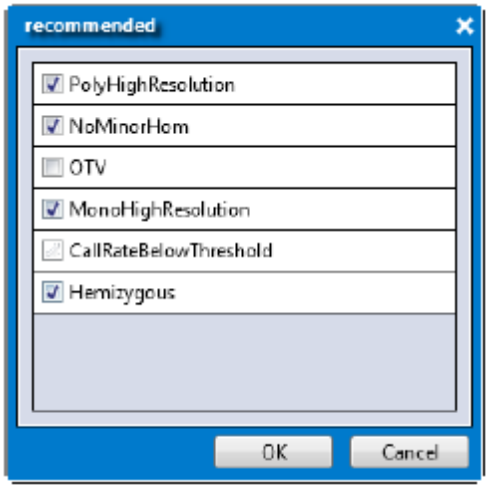
   The following window appears: (Figure 4.66)

Figure 4.66 Change the Priority Order window

7. Click and hold onto the selection you want to move, then drag and drop it into its new position. After you get the order of priority you want, click **OK**.
8. To change the recommended options, click Checklist.

   The following window appears: (Figure 4.66)

Figure 4.67 Recommended window



9. Click to check/uncheck the available recommended options. At anytime, click a field's button to return its value back to its default setting. See *SNP QC* for their definitions.
10. Click **OK**.

NOTE: After clicking OK, *n_AA*, *n_AB*, *n_BB*, *n_NC* in the SNP Summary Table are updated, however the *SNP Call Rate/MAF/nMinorAllele* are not updated.

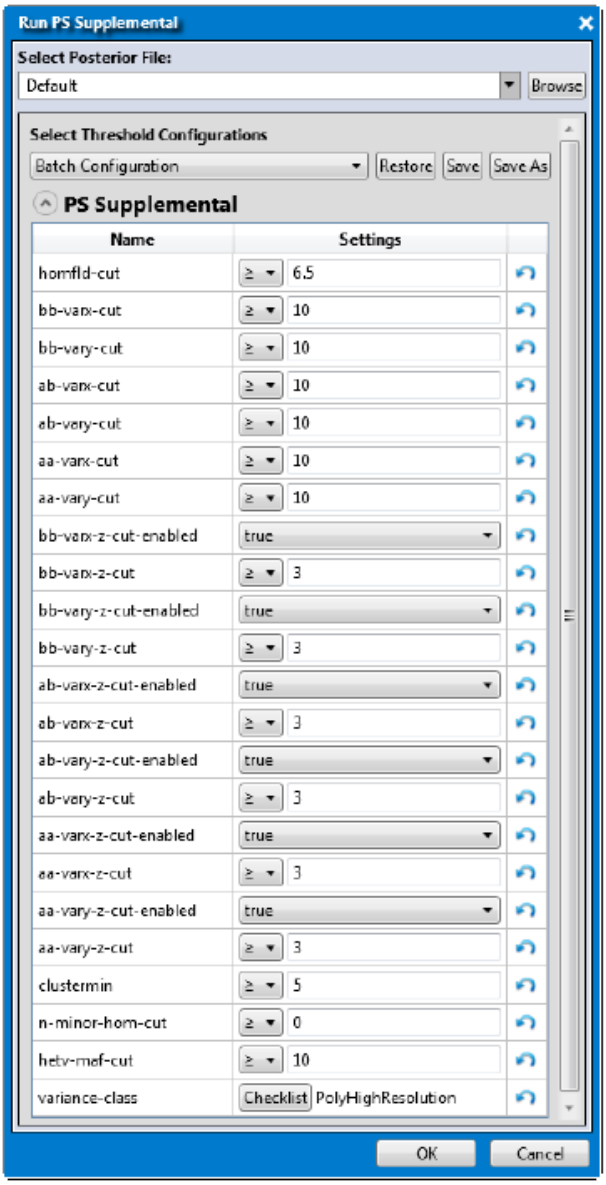
Running PS Supplemental

The PS_Supplemental performs further classification that may be needed for polyploid organisms, complex genomes, or inbred populations.

- Click Run PS Supplemental.

  The following window appears: (Figure 4.68)

Figure 4.68 Run PS Supplemental window



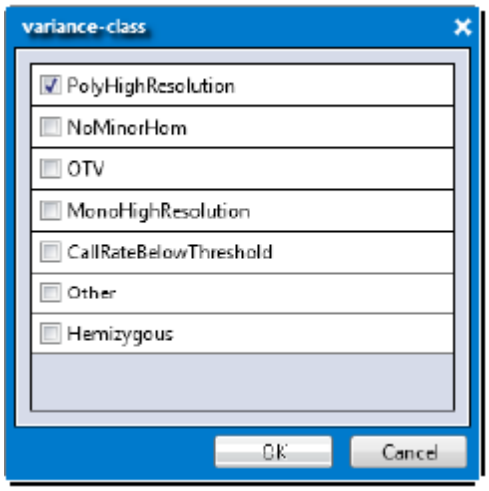
1. Use the drop-down to select the appropriate Posterior File or click its **browse** button.

   An Explorer window appears.

2. Navigate to Posterior File you want to use, then click **Open**.
3. Select the Threshold Configuration you want to use, as described in *Customizing Threshold Settings*.
4. Use the **PS Supplemental** drop-down menus, and text fields to setup and run your **PS** Supplemental.
5. To change the variance-class options, click Checklist .

   The following window appears: (Figure 4.66)

Figure 4.69 Variance-class window



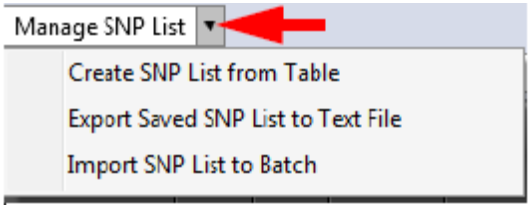
6. Click to check/uncheck the available variance-class options.
7. Click **OK**.

Managing your SNP List

The Eureka Analysis Suite enables lists of SNPs to be saved with the application.

Use the Manage SNP List drop-down menu (Figure 4.70) to perform one of the following:

Figure 4.70 Manage SNP List drop- down menu

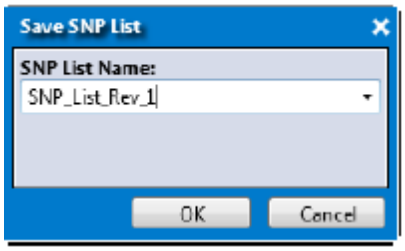


Saving your current SNP List

1. To save all SNPs currently displayed in the SNP Summary Table, click **Create SNP List from Table**.

   The following window appears: (Figure 4.71)

Figure 4.71 Save SNP List



2. Enter a name, then click **OK**.

Creating a SNP list from Annotation File

1. After importing your annotation file into your SNP Summary table, filter your results to display only your SNPs of interest.
2. Click the **Manage SNP List** drop-down menu, then select **Create SNP List**.

   A Save SNP List window appears.
3. Enter a SNP List Name, then click **OK**.
4. Proceed to *Exporting your SNP List*.

Exporting your SNP List

Before exporting a SNP List you must first create one. If no SNP Lists are detected, a message box appears. Click **OK** to acknowledge the message, then go to *Saving your current SNP List* to create a SNP List.

1. Click Export Saved SNP List to Text File.

   An Explorer window appears.
2. Navigate to an export location, enter a name, then click **Save**.

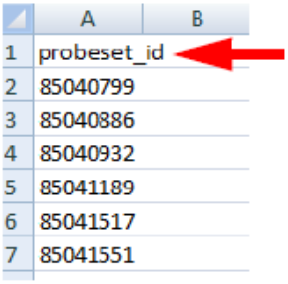
Importing a SNP List

1. Click Import SNP List to Batch.

   An Explorer window appears.
2. Navigate to your SNP List location containing your tab-delimited text file.

   Your first row/column header must be labeled **probeset_id**, as shown in Figure 4.72, otherwise an error message appears.

Figure 4.72 Required probeset_id column header



3. Click **Open**.

   Your imported SNP List now appears in the SNP Summary Table.

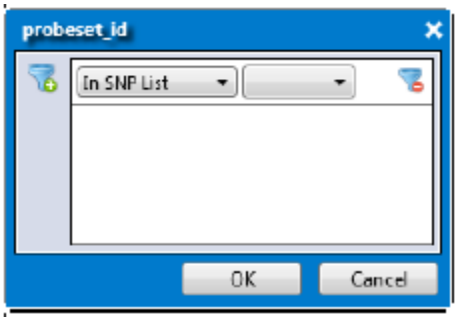
Using your Saved SNP List

Displaying SNPs in a SNP list

1. Click to select the **probeset_id** column, then right-click on it.
2. Click **Filter**.

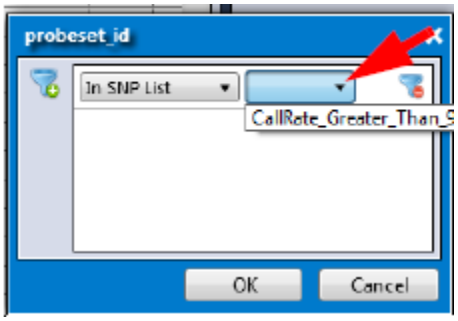
   The Filter window appears. (Figure 4.73)

Figure 4.73 Filter window



3. Add your previously saved SNP List by selecting it from the drop-down list. (Figure 4.74)

Figure 4.74 Saved SNP List drop-down



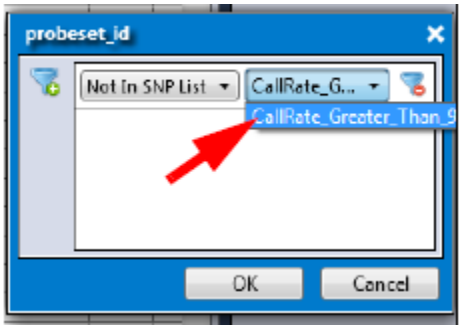
4. Click **OK**.

   Only the SNPs in your SNP List are displayed in the SNP Summary Table.

Displaying SNPs that are not in your SNP List

1. Click to select the **probeset_id** column, then right-click on it.
2. Click **Filter**.
3. Click the **In SNP List** drop-down, then click to select **Not in SNP List**. (Figure 4.75)
4. Click the Saved SNP List drop-down, select your saved SNP List, then click **OK**.

Figure 4.75 Saved SNP List drop-down

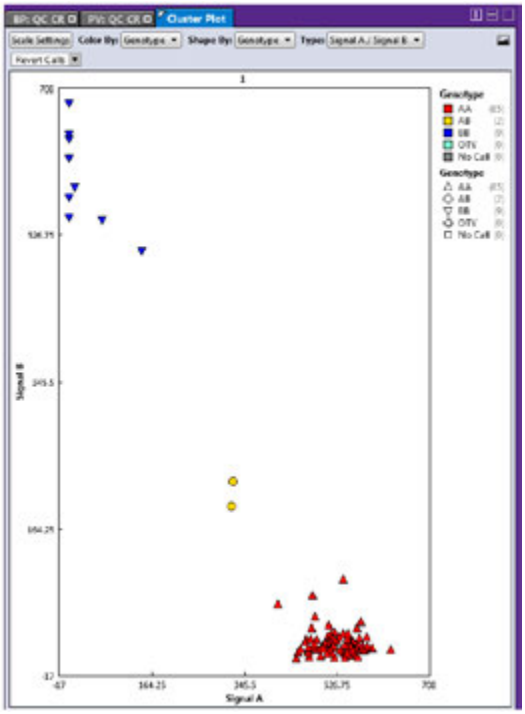


Your SNPs from the SNP List are no longer displayed in the SNP Summary Table.

Using the Cluster Plot

The Cluster Plot (Figure 4.76) displays the SNP calls for selected samples as a set of points in the clustering space used for making the calls. A visual inspection of select Cluster Plots, aids in identifying problematic SNPs and allows you to manually change calls.

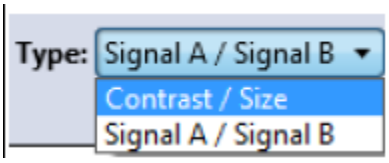
Figure 4.76 Cluster window tab



Choosing your Cluster Plot Type

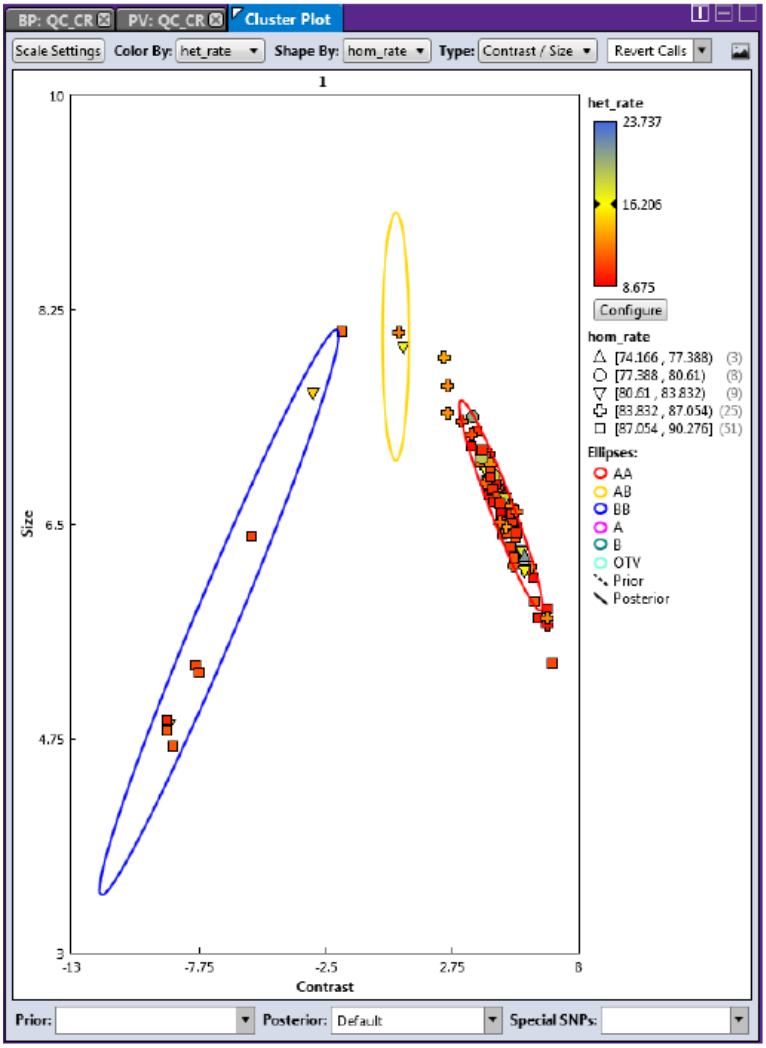
By default, the Cluster Plot type is set to Signal A/Signal B. To change the plot to a different type, click the Type drop-down menu, then select **Contrast/Size**.

Figure 4.77 Cluster Plot Type



The Cluster Plot's X and Y axis are now changed, as shown in Figure 4.78.

Figure 4.78 Cluster window tab

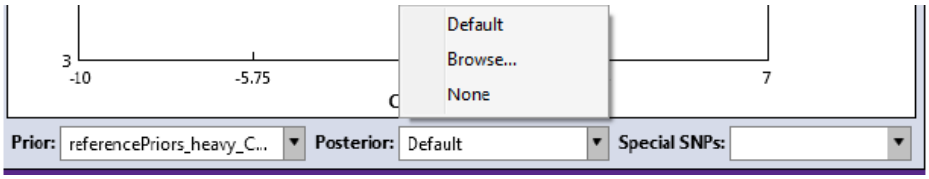


Displaying Cluster Model Data (Contrast/Size Plot Only)

By default, the **Prior, Posterior,** and **Special SNPs** drop-down selections are preset to best suit the currently displayed Cluster Graph.

- Click the appropriate drop-down menu (Figure 4.79), then click to select a new setting.

Figure 4.79 Cluster Model Data drop-down menus



- If you select **Browse**, an Explorer window appears. Navigate to your folder location as you normally would then click **Open** to display your data within the graph.
- Selecting **None**, conceals (hides) the selected graph data.

Displaying a SNP Cluster Plot that Corresponds with a SNP

1. In the SNP Summary Table, click on row (SNP) of interest.

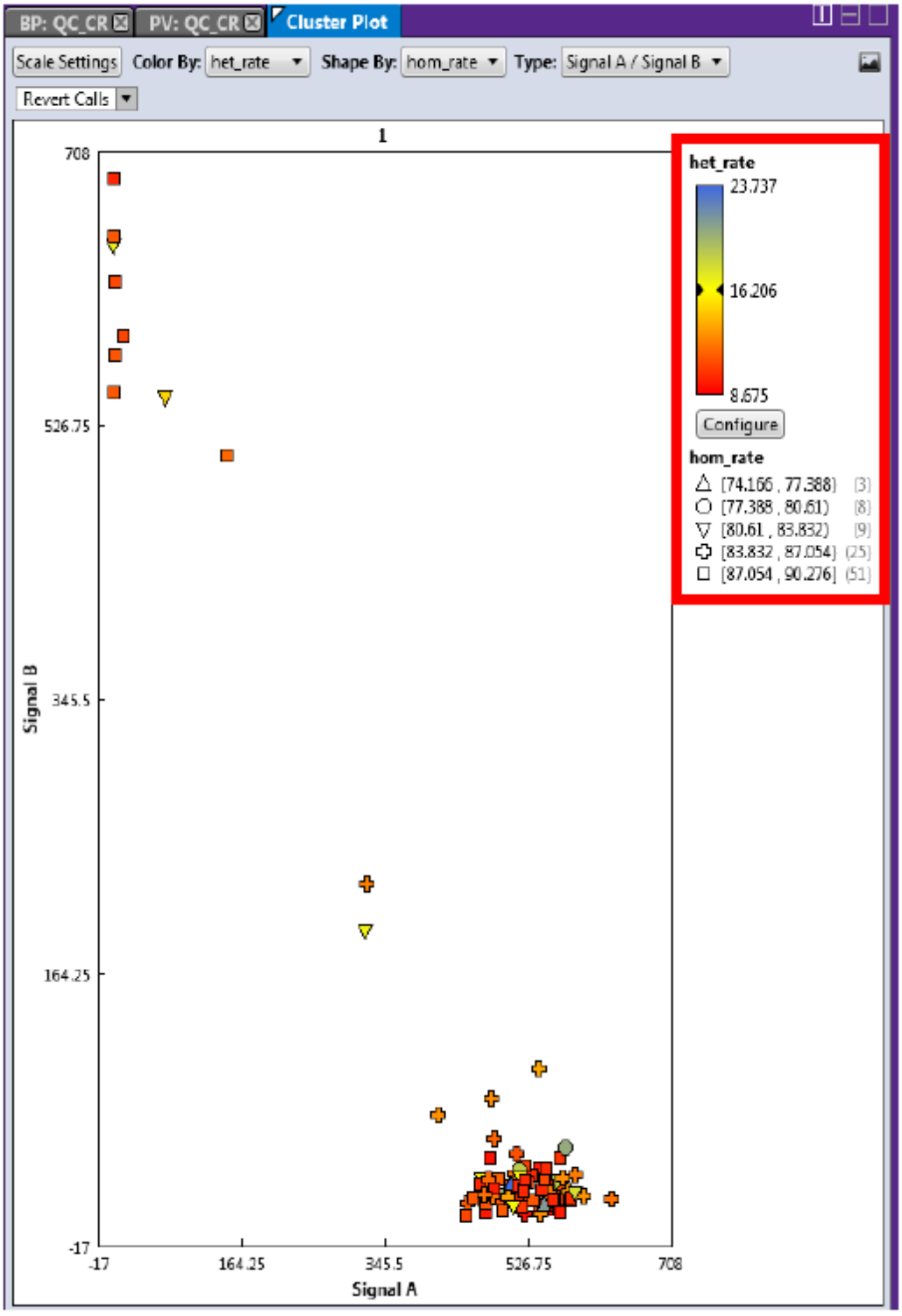
TIP: Use the arrow keys on the keyboard to toggle through the list. As you toggle through the list, the Cluster Plot auto-updates to match your selected SNP**.**

2. Click the **Color By** and **Shape By** drop-down menus to select the combination view you want.

NOTE: Your imported sample attributes are also available for use within the Color By and Shape By drop-down menus. For information on importing sample attributes, go to Importing Sample Attributes.

The appropriate legend appears within the plot. (Figure 4.80)

Figure 4.80 Color By and Shape By Legend example

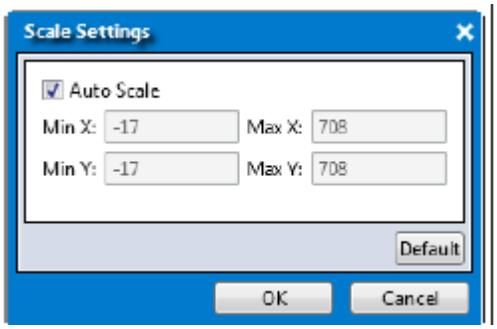


Setting New Scale Setting Ranges

1. Click the **Scale Settings** button.

   The following window appears. (Figure 4.38)

Figure 4.81 Scale Settings window



   By default, the window displays your current range values.

2. Enter your new ranges in the appropriate text fields.
3. Click **OK**.

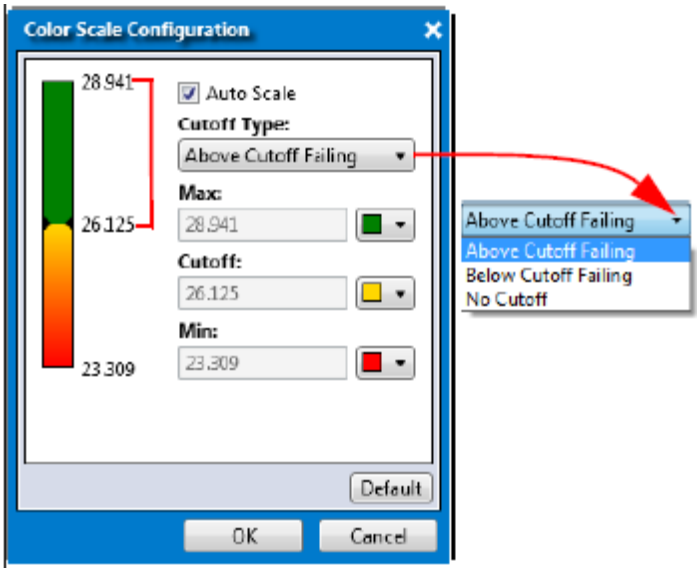
   Click the **Default** button to return all values to their default ranges.

Customizing Color By Settings

1. Click **Configure**.

   The following window appears: (Figure 4.47)

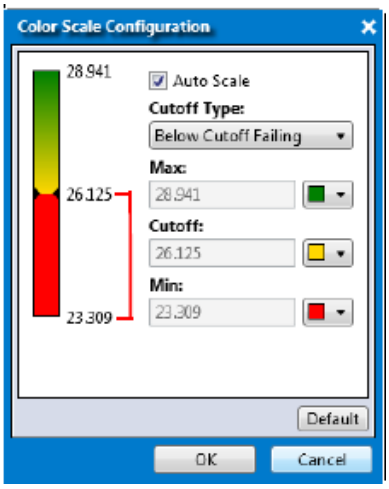
Figure 4.82 Color By options



2. Use the provided text fields and color drop-down menus to customize your Color By selection.
   - **Auto Scale** check box (when checked) uses the actual minimum (lower bound) and maximum (upper bound) as your min/max scale. Uncheck the Auto Scale check box to enter your min and max number scales in the provided fields. Note: If the Auto Scale check box remains unchecked, you must enter new scale limits for each subsequent analysis.
   - Click the **Cutoff Type** drop-down menu to select your cutoff preference.

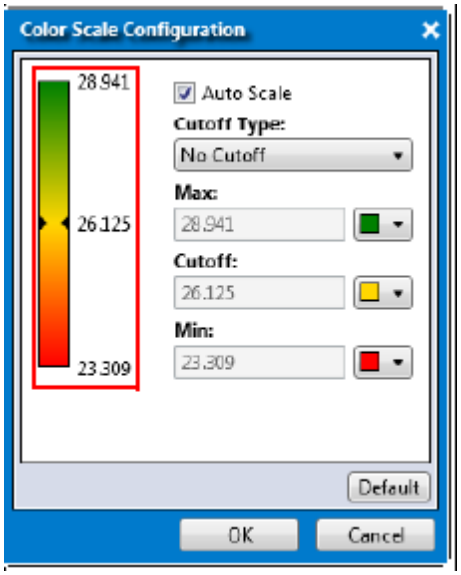
- **Above Cutoff Failing**—This presents a hard visual cutoff graph of all values that fail ABOVE the Cutoff value entered. The Above Cutoff data is represented by the color defined for Max. (Green in Figure 4.47)
- **Below Cutoff Failing**—This presents a hard visual cutoff graph of all values that fail BELOW the Cutoff value entered. The Below Cutoff data is represented by the color defined for Min. (Red in Figure 4.48)

Figure 4.83 Below Cutoff



- **No Cutoff**—This presents a smooth 3-point gradient of your defined Max, Min, and colors. (Figure 4.49).

Figure 4.84 No Cutoff



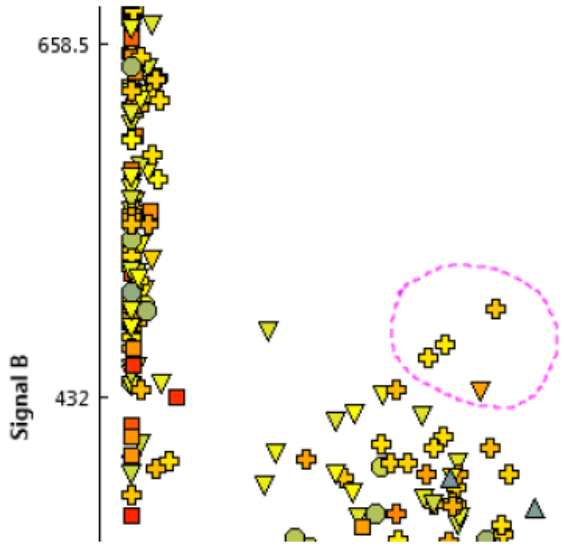
3. Click **OK**.

   Your Cutoff preference, entered values, and color selections are now displayed on the graph and saved for future use. If needed, click the **Default** button to revert ALL values back to their factory setting.

Selecting Multiple Samples in a Cluster Plot

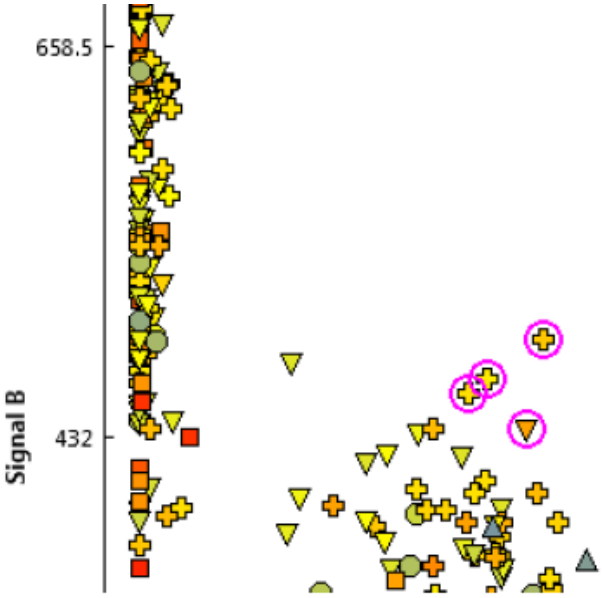
1. Drag the cursor around a group of samples to draw a pink-dotted closed shape around them. (Figure 4.85)

Figure 4.85 Selecting multiple samples



2. Release the mouse button to select the group of lassoed samples. (Figure 4.86)

Figure 4.86 Selecting multiple samples



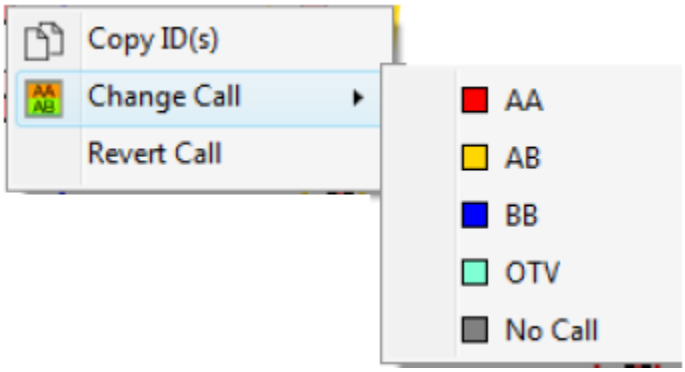
Changing a Sample's Call for a Single SNP

1. Highlight the sample or samples you want to modify, then right-click on them.

   A menu appears.

2. Click **Change Call**, then move your cursor to the right, then click to select a different call, an OTV (Off Target Variant), or No Call. (Figure 4.87)

Figure 4.87 Change Call menu



The Call is now changed, but not the position. The image may or may not change, as it depends on the Color By and Shape By options you selected.

Reverting a Single Call

1. Single-click to highlight the Call you want to revert back, then right-click on it.

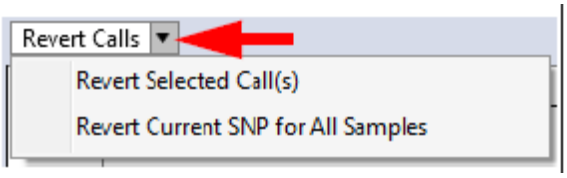
   A menu appears.

2. Click Revert Call.

Reverting Multiple Calls

1. Drag the cursor around a group of samples to draw a pink-dotted closed lasso shape around them.
2. Release the mouse button to highlight your selected samples.
3. Click the **Revert Call** drop-down menu (Figure 4.88), then click to select **Revert Selected Call(s)**.

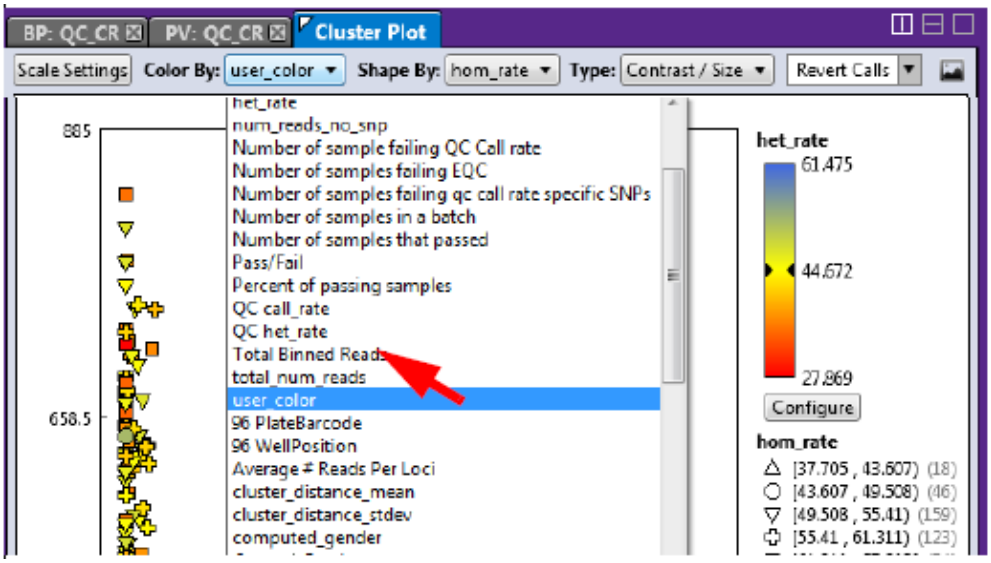
Figure 4.88 Revert Calls drop-down menu



Viewing User Colors in the Cluster Plot

1. From the Cluster Plot, click the **Color By** drop-down menu. (Figure 4.89)

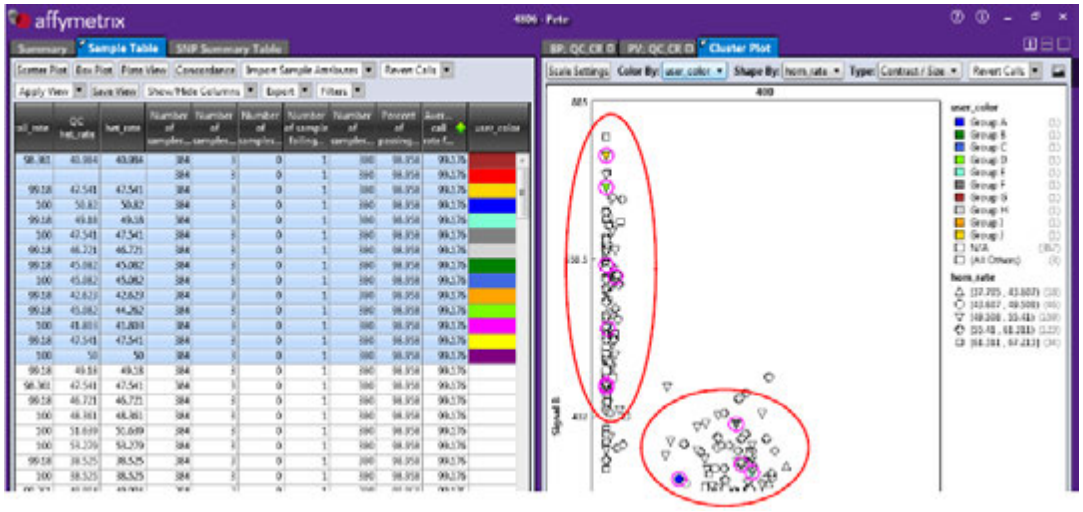
Figure 4.89 Color By menu - user_color



2. From the Sample Table, single-click on a color-coded sample file or **Ctrl+click, Shift+click,** or press **Ctrl+a** (to select multiple color-coded sample files).

   Each highlighted sample and its assigned color are now displayed in the Cluster Plot, as shown in Figure 4.90.

Figure 4.90 user_color shown in Sample Table and Cluster Plot



Saving the Current Cluster Plot View as a .PNG file

1. Click the **Save Image**  button.

   An Explorer window appears.

2. Navigate to where you want to save the **.PNG** file, enter a filename, then click **OK**.

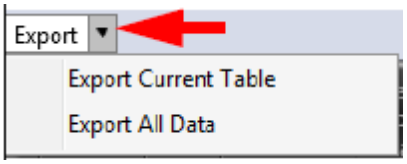
Chapter 5 Exporting

Using the Sample Table Export Options

1. Click the **Export** drop-down.

   Your Export options appear. (Figure 5.1)

Figure 5.1 Sample Table Export Menu



2. Click Export Current Table or Export All Data.

   A **Save As** window appears.

3. Click on an existing folder or click **New Folder** to choose a new save location.
4. Type a filename for the table, then click **Save**.

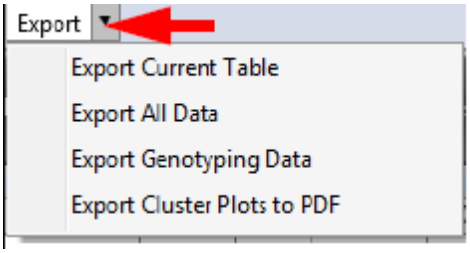
   The table data is now saved as a tab-delimited text file.

Using the SNP Summary Table Export Options

- Click the **Export** drop-down.

  The following window appears: (Figure 5.2)

Figure 5.2 SNP Summary Table Export menu



Exporting the Current Table

To export the columns and rows currently displayed in the table:

1. Click **Export Current Table**.

   An Explorer window appears.

2. Enter a filename, then click **Save**.

   The current table data is now saved and exported as a tab-delimited text file.

Exporting All Data

To export all columns and rows, including hidden and filtered data:

1. Click **Export All Data**.

   An Explorer window appears.

2. Enter a filename, then click **Save**.

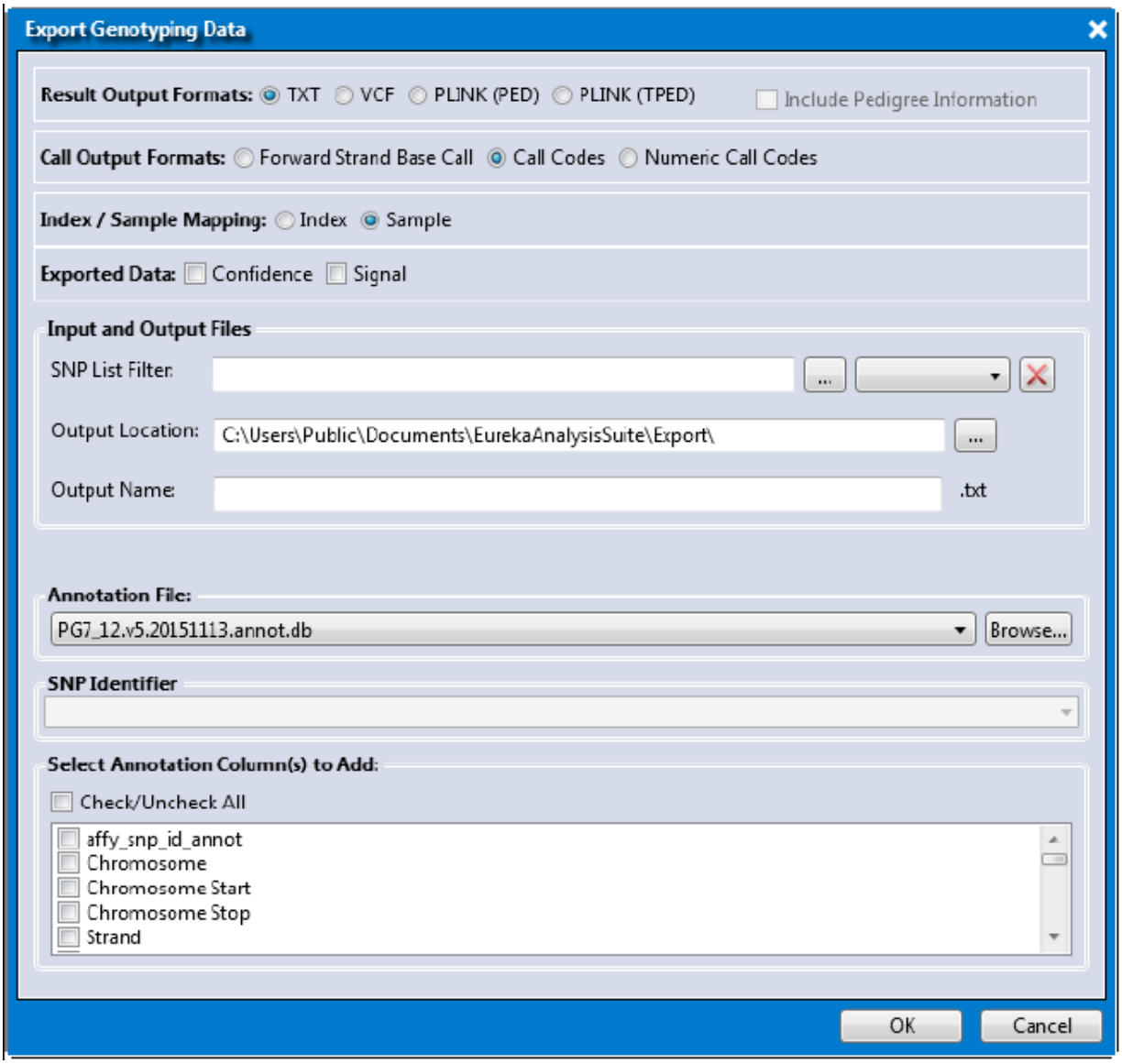
   All data in the table (displayed or not) is saved and exported as a tab-delimited text file.

NOTE: Not all options are available and are dependent on the export format you select and its applicable format restrictions.

- Click Export Genotyping Data.

  The Export Genotyping Data window appears. (Figure 5.3)

Figure 5.3 Export Genotype Data window



Result Output Formats

Figure 5.4 Result Output Format selections

1. Click the radio button to select the Result Output Format you want to use.
2. (Optional) If you selected a PLINK format, make sure you click the **Include Pedigree Information** check box. Not checking this box may require special handling (within PLINK) to make your exported output work properly.

IMPORTANT: If you are exporting genotypes into a PLINK format, make sure your Sample Attributes include an Index ID and Pedigree Information (Family ID, Individual ID, Father ID, Mother ID, Sex, and Affection Status).

Call Output Formats

- Click the radio button to select the appropriate Call Output Format (Figure 5.5) you want to use.

Figure 5.5 Call Output Format selections

NOTE: Numeric Call Codes are exported using the following representation: -1 = NoCall, 0 = AA, 1 = AB, and 2 = BB.

Index/Sample Mapping

- Click the **Index** radio button to export Index information or click the **Sample** radio button to export your sample filenames. (Figure 5.6)

Figure 5.6 Index or Sample mapping selections

Exported Data Selections

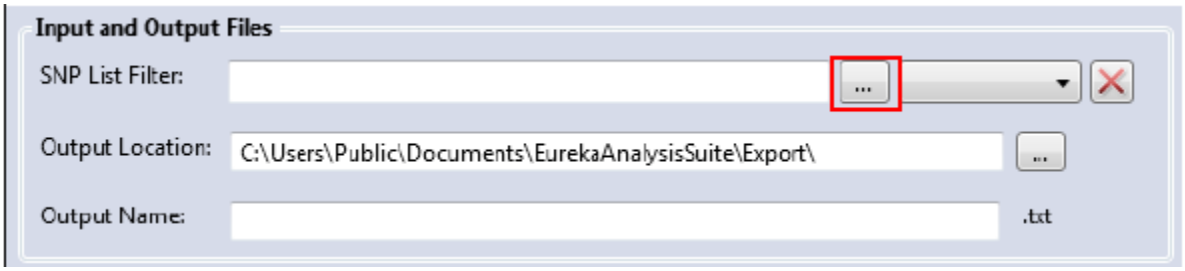
- Click inside the check box(es) to check the additional type(s) of Exported Data (Figure 5.7) you want to include.

Figure 5.7 Exported Data selections

Input and Output Files

(Figure 5.8)

Figure 5.8 Input and Output Files selections



SNP List Filter (Optional)

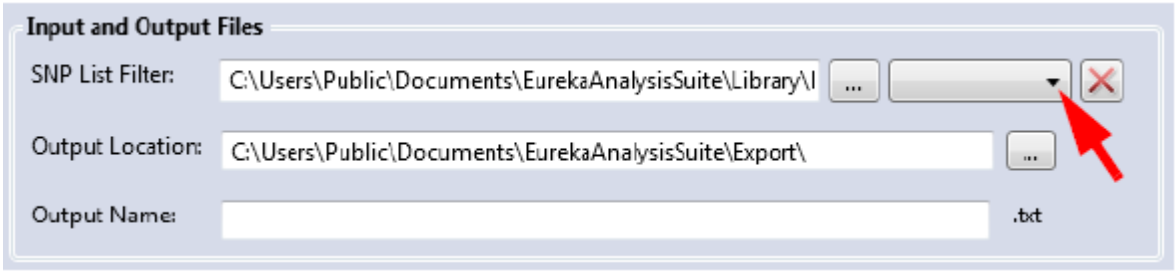
1. To restrict the output to a list of SNPs (probeset_IDs) contained in a file, click the SNP List Filter field's **browse** button (Figure 5.8).

   An Explorer window appears.

2. Navigate to the SNP Filter List location, then click **Open**.

   The SNP Filter List field is now populated. (Figure 5.9)

Figure 5.9 SNP List Filter and Output path



3. Click the SNP List Filter's drop-down menu to view and select a previously saved SNP List. (Figure 5.9)

Output Location (Required)

1. Click the Output Location field's **browse** button.

   An Explorer window appears.

2. Navigate to an output location, create a new folder if needed, then click **Select Folder** button.

   The Output Location path is displayed. (Figure 5.9)

Output Name (Required)

- Use the output name already in the Output Name field, or click inside the field to enter a new name.

! NOTE: Your output name's file extension reflects the Results Output Format you selected in Step 1.

Changing the SNP Identifier

- Click the drop-down arrow, then click to select the SNP Identifier you want to use. (Figure 5.10)

Figure 5.10 SNP Identifier

Changing the Current Annotation File (Optional)

1. To change the currently displayed Annotation File, click the Annotation File field's **browse** button (Figure 5.11).

   An Explorer window appears.

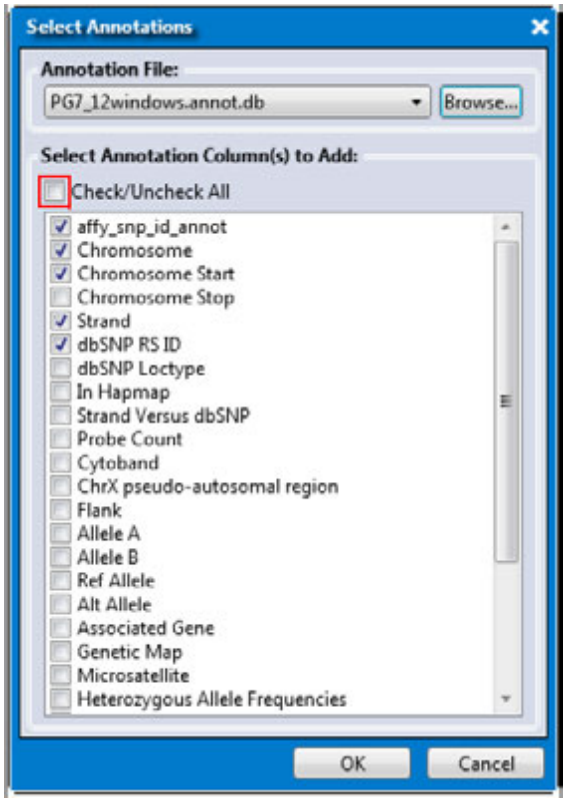
2. Navigate to the appropriate Annotation File location, then click **Open**.

   Your newly selected Annotation file is displayed.

Adding and Removing Annotation Columns

1. Click the check box next to the Annotation Column(s) you want to add to your format results or click to uncheck/remove a column. **Check/Uncheck All** check box to add or remove ALL available annotations. (Figure 5.11)

Figure 5.11 Select Annotation Column(s) pane



2. After the Export Genotype Data form is complete, click **OK**.
3. Your newly exported data now reside in the output location you defined in Step 1.

Exporting Cluster Graphs to PDF

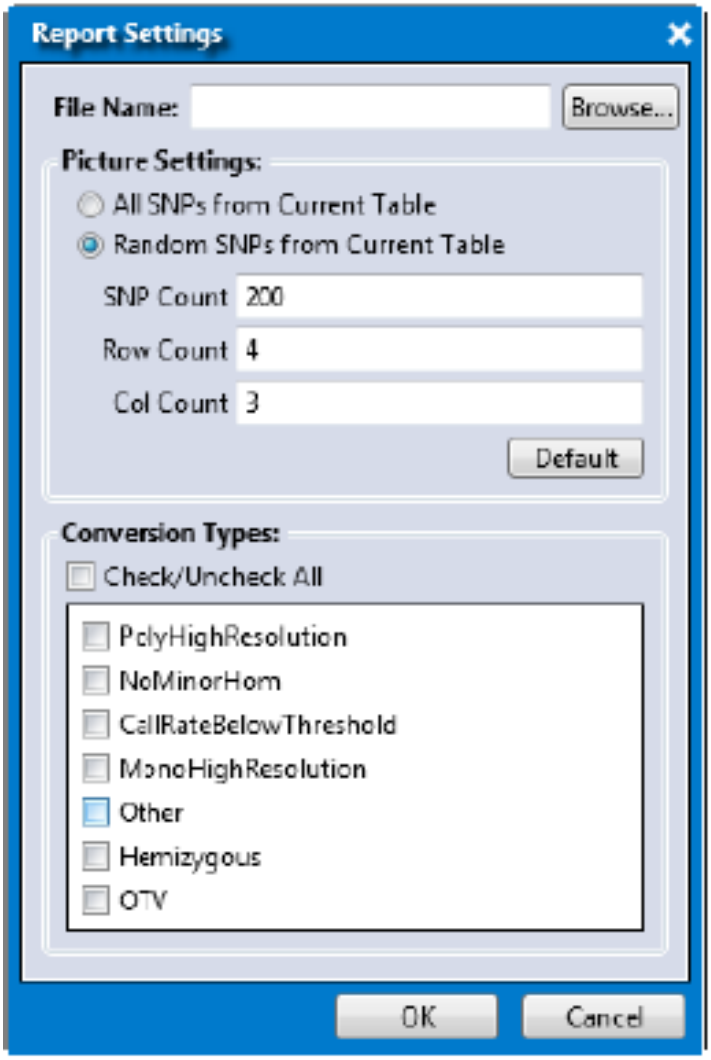
This exporting option generates a specific number of cluster graphs from the selected Conversion Type choices. We recommend examining the cluster graphs of approximately 200 SNPs in each category.

> **NOTE: Export results are based on the type of Cluster Plot graph that you selected, as described in *Choosing your Cluster Plot Type.***

1. Click **Export Cluster Plots to PDF**.

   The following window appears: (Figure 5.12)

Figure 5.12 Report Settings window



2. Click **browse**.

   An Explorer window appears.

3. Navigate to a desired location, then enter a name for your PDF report.

4. Click **Save**.

   You are returned to the **Report Settings** window.

5. In the Picture Settings section, click either:

   - All SNPs from Current Table

- Random SNPs from Current Table

6. Click inside the applicable **Count** field(s) to enter how many cluster plots you want to export.

   Click the **Default** button to revert your modified counts back to their original states.

7. Click to check a Conversion Type(s) you want to add to the report or click the **Check/Uncheck All** check box, then click **OK**.

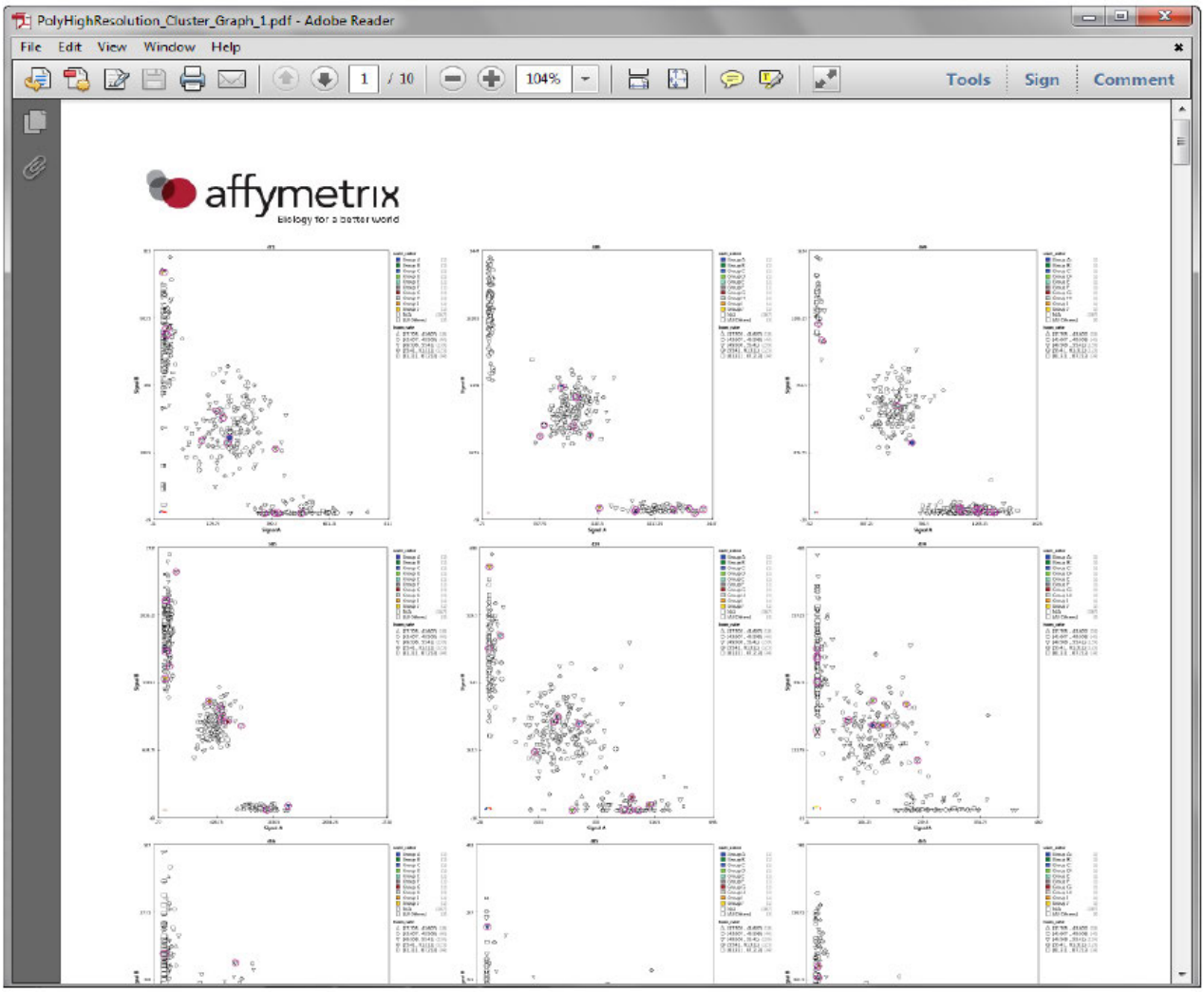
   A Please Wait message and progress bar appear. Allow several minutes if multiple Conversion Type PDF report(s) were selected.

   An Explorer window (where you saved the PDF Report location in Step 3) appears.

8. Double-click on the PDF Report you want to view.

   The PDF Report opens. (Figure 5.13)

Figure 5.13 PDF Report example



Appendix A Inbred File Generation Tool

Introduction

The **Inbred File Generation Tool** is included in the Eureka Analysis Suite software zip package you downloaded earlier. (Step 3)

Use this tool to generate an appropriately formatted inbred file for use in your Eureka Analysis.

How the Tool Works

The Tool uses the information in your BFRC file to replace the Sample names in your Penalty file with corresponding Sample Index IDs. When a generated Inbred file is used, these Sample Index IDs are used in the analysis process.

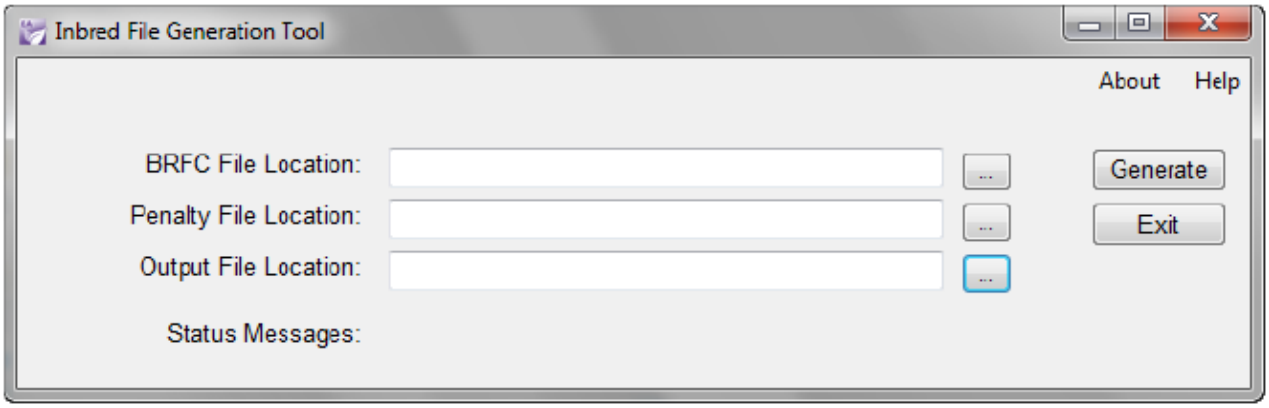
If your Inbred file does not contain Sample Index IDs, your assigned penalties default to 0 and therefore not be used in the analysis process.

Starting the Tool

- Locate the file labeled **InbredFileGenerationTool.exe**, then double-click on it.

  The following window appears: (Figure A.1)

Figure A.1 Inbred File Generation Tool Main window



Assigning File Paths

1. Click the **BRFC File Location** ... button.

   An Explorer window appears.

2. Navigate to the BRFC file save location you setup earlier (Step 3), then click **Open**.

   Your BFRC File Location path is displayed.

3. Click the Penalty File Location ... button.

   An Explorer window appears.

! **IMPORTANT: Your Penalty file must be a tab-delimited 2-column text file.**

Column A must be labeled *Sample* and column B must be labeled *Penalty*, as shown in Figure A.2.

IMPORTANT: Your Inbred file must contain the Sample Index ID and a Penalty value (range from 0-16, 0 for Hybrids - 16 for inbred strains).

Figure A.2 2 column Penalty file example

	A	B
1	Sample	Penalty
2	CG8890_Zea_Strain	16
3	CG8252_Zea_Strain	0
4	CG8287_Zea_Strain	16
5	CG8416_Zea_Strain	16
6	CG8849_Zea_Strain	16
7	CG8808_Zea_Strain	16
8	CG2884_Zea_Strain	16
9	CG2749_Zea_Strain	16
10	CG2626_Zea_Strain	0
11	CG2596_Zea_Strain	16
12	CG2698_Zea_Strain	16

4. Navigate to the location of your penalty file, then click **Open**.

   Your Penalty File Location path is displayed.

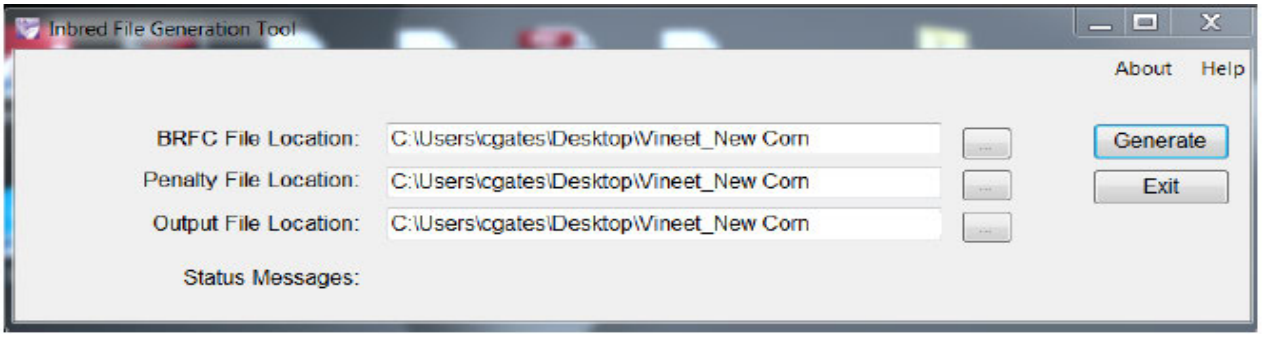
5. Click the **Output File Location**  button.

   An Explorer window appears.

6. Navigate to the output path folder location you want, then click **Save**.

   Your Output File Location path is displayed. (Figure A.3)

Figure A.3 Inbred File Generation Tool Main window

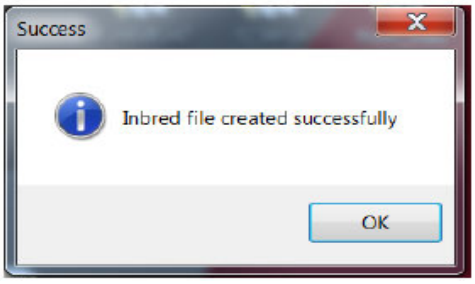


Generating an Inbred File

1. Click Generate.

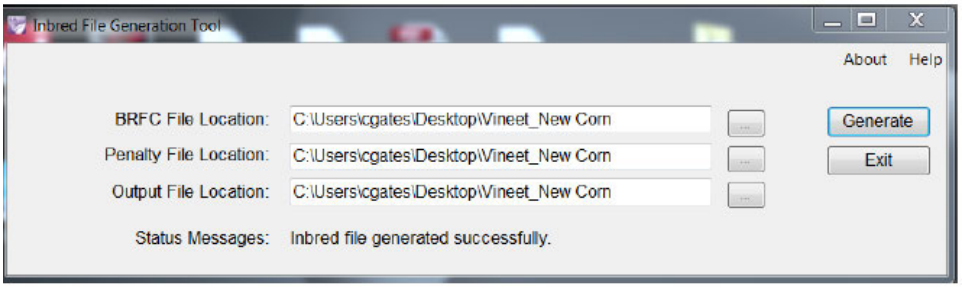
   After a few moments the following message appears: (Figure A.4)

Figure A.4 Inbred File created successfully message



2. Click **OK** to close the message window.

Figure A.5 Inbred File Generation Tool Main window - Inbred file generated successfully



3. Acknowledge the Status Message(s), then click Exit.

Reviewing the Generated Inbred File

- Navigate to your recently assigned Output File Location to open and review your custom Inbred file. (Figure A.6)

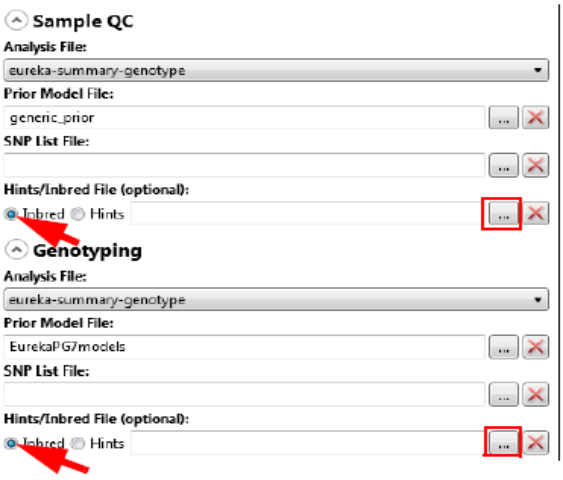
Figure A.6 Inbred File example

	A	B
1	cel_files	inbred_het_penalty
2	2689	16
3	2690	0
4	2691	16
5	2692	16
6	2693	16
7	2694	16
8	2695	16
9	2696	16
10	2697	0
11	2698	16
12	2699	16

Using the Inbred File in your Analysis Setup

There are 2 areas inside the **Analysis Setting** pane to import your newly generated Inbred file, as shown in Figure A.7.

Figure A.7 Analysis Setting pane



1. Click the **Inbred** radio button.
2. Click the **Hints/Inbred File**  button.

   The Hints/Inbred File window appears.
3. Navigate and select your custom Inbred file, then click **Open**.

   The path to your custom Inbred file is displayed.

   For complete instructions on how to setup and run an analysis, refer to Chapter 3, Eureka Analysis.

Appendix B Definitions

Sample Table

Use the following table of definitions to help select your Sample table columns.

Selection	Definition
Index	The unique sequence that is used to identify sample.
Samples	Name of sample.
384 PlateBarCode	Barcode of 384 plate
384 WellPosition	Position of sample in the 384 well plate.
96 PlateBarcode	Barcode of original 96 well sample plate.
96 WellPosition	Position of sample in the original 96 well plate.
Panel	The SNP genotyping panel currently analyzed.
Count A Reads	The number of A alleles read per sample.
Count B reads	The number of B alleles read per sample.
Total Binned Reads	The total of A and B allele reads per sample.
Average # Reads Per Loci	Total binned reads for that locus/total number of samples in the binning file.
Number of Loci with 20 or Fewer Reads	The number of loci with fewer than 20 reads, per sample.
Total_num_reads	The number of reads (including reads with no assigned SNP).
Num_reads_no_snp	The total number of reads without an assigned SNP.
QC_computed_gender	Computed gender for the sample.
QC_call_rate	Call rate at the default or user-specified threshold for autosomal SNPs.
QC_total_call_rate	Call rate at the default or user-specified threshold for all SNPs.
QC_het_rate	Percentage of SNPs called AB (i.e. the heterozygosity) for autosomal SNPs in a Sample QC.
QC_total_het_rate	Percentage of SNPs called AB (i.e., the heterozygosity) for all SNPs.
QC_hom_rate	Percentage of SNPs called AA or BB (i.e. the homozygosity) for autosomal SNPs in a Sample QC.
QC_total_hom_rate	Percentage of SNPs called AA or BB (i.e. the homozygosity) for all SNPs.
QC_cluster_distance_mean	Average distance to the cluster center for the called genotype.
QC_cluster_distance_stdev	Standard deviation of the distance to the cluster center for the called genotype.
Loci Failed QC Num_of_failed_SNPs	The number of SNPs that did not pass QC (within loci).
Pass/Fail	Shows sample that passed or failed your analysis thresholds.
computed_gender	Computed gender for the sample (if available for your selected Panel).

Selection	Definition
call_rate	Call rate (CR) is the ratio of the number of samples assigned a genotype call of either AA, BB or AB for the SNP (i.e. the number of samples that do not have ``No call'') to the number of samples over which a genotype call is attempted for the SNP.
total_call_rate	Call rate at the default or user-specified threshold for all SNPs.
het_rate	Percentage of SNPs called AB (i.e. the heterozygosity) for autosomal SNPs.
total_het_rate	Percentage of SNPs called AB (i.e., the heterozygosity) for all SNPs.
hom_rate	Percentage of SNPs called AA or BB (i.e. the homozygosity) for autosomal SNPs.
total_hom_rate	Percentage of SNPs called AA or BB (i.e. the homozygosity) for all SNPs.
cluster_distance_mean	Average distance to the cluster center for the called genotype.
cluster_distance_stdev	Standard deviation of the distance to the cluster center for the called genotype.
Number of samples in a batch	The number of samples analyzed.
Number of samples failing EQC	Number sample failing EQC within the batch.
Number of samples failing qc call rate specific SNPs	Number sample failing specific SNP qc call rate within the batch.
Number of samples failing QC call rate	The total number of samples in the batch that are failing the QC call rate.
Number of samples that passed	The total number of samples that passed your analysis thresholds settings.
Percent of passing samples	If a plate's percent of passing samples is smaller than this number, all samples on the plate will show a warning in the Sample Table.
Average call rate for passing samples	A plate's average QC call rate of passing samples must be larger than this number to pass plate QC.
User_color	Sample color defined by user.

Annotations

Use the following table of definitions below to help select your annotations.

Column Name	Description
Probe Set ID	The unique identifier for the set of probes used to detect a particular Single Nucleotide Polymorphism (SNP probe sets only).
Affx SNP ID	The unique identifier for the set of probes used to detect a particular Single Nucleotide Polymorphism (SNP). (SNP probe sets only, not available for Axiom™ Genome-Wide Human Array).
dbSNP RS ID	The dbSNP ID that corresponds to this probe set or SNP. The dbSNP at the National Center for Biotechnology Information (NCBI) attempts to maintain a unified and comprehensive view of known single nucleotide polymorphisms (SNPs), small scale insertions/deletions, polymorphic repetitive elements, and microsatellites from TSC and other sources. The dbSNP is updated periodically, and the dbSNP version used for mapping is given in the dbSNP version field. For more information, go to: ***http://www.ncbi.nlm.nih.gov/SNP/*** (SNP probe sets only).
Chromosome	The chromosome on which the SNP is located on the current Genome Version.
Chromosome Start	The nucleotide base start position where the SNP is found. The genomic coordinates given are in relation to the current genome version and may shift as subsequent genome builds are released.
Chromosome Stop	The nucleotide base stop position where the SNP is found. The genomic coordinates given are in relation to the current genome version and may shift as subsequent genome builds are released.
Strand	Genomic strand that the SNP resides on.
Cytoband	Cytoband location of the SNP derived from the SNP physical map and the chromosome band data provided by UCSC.
Strand Vs dbSNP	Indicates whether the SNP is on the same or reverse strand as compared to dbSNP (SNP probe sets only).
ChrX pseudo-autosomal region	SNPs on the X Chromosome which are mapped to the two pseudo-autosomal region have a value of 1 or 2 in this field. All other SNPs are indicated by 0. A value of "1" indicates that the marker maps to the PAR-1 region and a value of "2" indicates that the marker maps to the PAR-2 region. A value of "0" indicates that the marker does not map to either of the two PAR regions.
Flank	The nucleotide sequence surrounding the SNP. This is a 33-mer sequence with 16 nucleotides on either end of the SNP position. The alleles at the SNP position are provided in the brackets (SNP probe sets only).
Allele A	The allele of the SNP that is in lower alphabetical order. When comparing the allele data on NetAffx to the allele data for the corresponding RefSNP record in dbSNP, the alleles reported here could be different from the alleles reported for the corresponding RefSNP on the dbSNP web site. This difference arises mainly from the reference genomic strand that was chosen to define the alleles. To choose the reference genomic strand, we follow a convention based on the alphabetic ordering of the sequence surrounding the SNP. Sometimes the reference strand on the dbSNP is different from NetAffx, and the alleles could represent reverse complement of those provided on dbSNP (SNP probe sets only).

Column Name	Description
Allele B	The allele of the SNP that is in higher alphabetical order. When comparing the allele data on NetAffx to the allele data for the corresponding RefSNP record in dbSNP, the alleles reported here could be different from the alleles reported for the corresponding RefSNP on the dbSNP web site. This difference arises mainly from the reference genomic strand that was chosen to define the alleles. To choose the reference genomic strand, we follow a convention based on the alphabetic ordering of the sequence surrounding the SNP. Sometimes the reference strand on the dbSNP is different from NetAffx, and the alleles could represent reverse complement of those provided on dbSNP (SNP probe sets only).
Associated Gene	SNPs were associated with human genes by comparing the genomic locations of the SNPs to genomic alignments of human mRNA sequences. In cases where the SNP is within a known gene, NetAffx reports the association. Additionally, for genes with exon or CDS annotations, NetAffx reports whether or not the SNP is in an exon, and in the coding region. If the SNP is not within a known gene, NetAffx reports the closest genes in the genomic sequence, and the distance and relationship of the SNP relative to the genes. A SNP is upstream of a gene if it is located closer to the 5' end of the gene and is downstream of a gene if it is located closer to the 3' end of the gene.
Genetic Map	Describes the genetic location of the SNP derived from three separate linkage maps (deCODE, Marshfield, or SLM). The physical distance between the markers is assumed to be linear with their genetic distance. The genetic location is computed using the linkage maps from the latest physical location of the SNP and the neighboring microsatellite markers (SNP probe sets only).
Microsatellite	Describes the nearest microsatellite markers (upstream, downstream and overlapping) for the SNP.
Heterozygous Allele Frequencies	Describes the heterozygous frequency of the allele from Yoruba, Japanese, Han Chinese and CEPH studies using the genotyping arrays. (SNP probe sets only)
Allele Sample Size	Sample size used for Allele Frequency estimates (SNP probe sets only).
Allele Frequencies	Describes the major and minor frequency of the allele from Yoruba, Japanese, Han Chinese and CEPH studies using the genotyping arrays (SNP probe sets only).
Minor Allele	Indicates the Minor Allele of a SNP (SNP probe sets only).
Minor Allele Frequency	The Minor Allele Frequency of a SNP (SNP probe sets only).
OMIM ID	Furnishes OMIM and Morbid Map IDs and their respective gene titles. This database contains information from the Online Mendelian Inheritance in Man® (OMIM®) database, which has been obtained under a license from the Johns Hopkins University. This database/product does not represent the entire, unmodified OMIM® database, which is available in its entirety at www.ncbi.nlm.nih.gov/omim/.

Concordance

Use the table of definitions below to help select your Concordance columns.

Column Name	Description
Base	The first sample in the comparison.
Reference	The second sample in the comparison.
#SNPs Called	Number of SNPs common to both sample and reference files with genotype calls.
#Concordant SNPs	Number of called SNPs that have the same genotype call.
%Concordance	Percentage of called SNPs that have the same genotype call.

Thresholds

Use the table of definitions below to help select Threshold names.

Threshold Name	Description
Sample QC	
EQC Min Num Of Reads to Pass Sample (All SNPs)	Minimum number of reads a SNP must have to be considered a passing SNP.
EQC Max Num Of SNPs to Fail Sample (All SNPs)	For a sample to pass EQC, the maximum number of SNPs (per sample) that are allowed to fail.
QC call_rate (All SNPs)	A samples QC call rate calculated on all autosomal SNPs must be greater than the threshold for the sample to pass genotyping QC.
EQC Specific SNP File	A file containing a list of SNPs to use for EQC and QC Call rate calculations and filtering in addition to the standard autosomal SNPs.
EQC Min Num Of Reads to Pass Sample (Specific SNPs)	Minimum number of reads a SNP from the EQC specific SNP file must have for the SNP to pass EQC.
EQC Max Num Of SNPs to Fail Sample (Specific SNPs)	Maximum number of SNPs from the EQC specific SNP file that can fail and the Sample pass EQC.
QC call_rate (Specific SNPs)	A samples QC call rate calculated on the SNPs in the EQC specific file must be greater than the threshold for the sample to pass genotyping QC.
Percent of passing samples	If a plate's percent of passing samples is smaller than this number, all samples on the plate will show a warning in the Sample Table.
Average call rate for passing samples	A plate's average QC call rate of passing samples must be larger than this number to pass plate QC.
plate_qc_percentsamplespassed	If a plate's percent of passing samples is smaller than this number, all samples on the plate will show a warning in the Sample Table.
plate_qc_averagecallrate	A plate's average QC call rate of passing samples must be larger than this number to pass plate QC.
SNP QC	

Threshold Name	Description
cr-cutoff	Threshold for call rate. If not specified, the default for human is 95 and for diploid and polyploid is 97.
fld-cutoff	Cut-off value for cluster quality (FLD).
het-so-cutoff	Cut-off value for the correctness of the vertical position of the heterozygous cluster (Strength Offset).
het-so-otv-cutoff	Cut-off value for the existence of a fourth cluster below the heterozygous cluster (OTV).
hom-ro-1-cutoff	Cut-off value for the correctness of the horizontal position of the homozygous clusters (Ratio Offset) when a SNP has one genotype.
hom-ro-2-cutoff	Cut-off value for the correctness of the horizontal position of the homozygous clusters when a SNP has two genotypes.
hom-ro-3-cutoff	Cut-off value for the correctness of the horizontal position of the homozygous clusters when a SNP has three genotypes
hom-ro	Flag indicating whether the metric HomRO is used in classification.
hom-het	Flag indicating whether the metric HomHet is used in classification. The HomHet metric identifies two-cluster SNPs/probesets with one homozygote cluster and one heterozygote cluster. This checks if the minor homozygote cluster is missing, which is unreasonable for highly inbred species (e.g. wheat). This metric should be turned on when classifying SNPs/ probesets in highly inbred species.
num-minor-allele-cutoff	The number of minor alleles.
priority-order	When performing probeset selection, the best probeset is selected according to the priority order of probeset conversion types.
recommended	Use this checklist to choose your *ps_classification* conversion types for your analysis.
PolyHighRes	Both homozygous clusters are observed.
MonoHighRes	Passes all thresholds except number of minor alleles.
NoMinorHom	Two-cluster SNP with one heterozygous and one homozygous cluster.
OTV	Heterozygous cluster is populated.
CRBelowThreshold	SNP passes all thresholds except CR; NumMinorAlleles is ignored.
Hemizygous	SNPs from chromosome Y or mitochondrial DNA.

SNP Summary Table

Use the table of definitions below to help select your SNP Summary Table columns.

Column Header	Description
probeset_id	Displays each Probeset's unique identifier.
affy_snp_id	Displays each SNP's unique identifier.
Conversion Type	Provides the category that algorithm has classified a SNP to be in.
CR	Call rate (CR) is the ratio of the number of samples assigned a genotype call of either AA, BB or AB for the SNP (i.e. the number of samples that do not have ``No call'') to the number of samples over which a genotype call is attempted for the SNP.
FLD	Fisher's Linear Discriminant is a measurement of the cluster quality of a SNP. High-quality SNP clusters have well-separated centers, and the clusters are narrow. High-quality clusters can be identified by examining the shape and separation of the SNP posteriors that are produced during genotyping. $Fisher's\ Linear\ Discriminant\ (FLD) = Min(i = aa, bb)\left\{\frac{\left\|M_{ab} - M_i\right\|}{sd}\right\}$
HomFLD	A version of FLD computed for the homozygous genotype clusters. HomFLD is undefined for SNPs without two homozygous clusters.
HomRo	Homozygote Ratio Offset is the distance to zero in the X dimension from the center of the populated homozygous cluster that is closest to zero.
nMinorAllele	The number of minor alleles in a batch.
Nclus	The number of genotype clusters.
n_AA	The number of AA genotypes.
n_AB	The number of AB genotypes.
n_BB	The number of BB genotypes.
n_NC	The number of no calls.
hemizygous	Chromosome Y, W, and mitochondrial genomes produce only two genotype clusters. Example: One represents A and the other represents B.
HomHet	The HomHet metric identifies two-cluster SNPs/probe sets with one homozygote cluster and one heterozygote cluster. This checks if the minor homozygote cluster is missing, which is unreasonable for highly inbred species (example: Wheat). This metric should be turned on when classifying SNPs/probe sets in highly inbred species: default is TRUE.

Column Header	Description
H-W p-value	Hardy Weinberg p-value is a measure of the significance of the discrepancy between the observed ratio or heterozygote calls in a population and the ratio expected if the population was in Hardy Weinberg equilibrium. There are two statistical tests used for HWE. When AA, AB, and BB counts are all >=10, a Chi-squared test is used. When one or more of the AA, AB, and BB counts are <10, an Exact test is used. An Exact test means that the p-value is calculated exactly and not approximated from a population distribution. $$x^2 = \frac{(f^2_{aa} - f_a)^2}{f^2_{aa}} + \frac{(2f_{aa}f_{bb} - f_{ab})^2}{2f_{aa}f_{bb}} + \frac{(f^2_{bb} - f_b)^2}{f^2_{bb}}$$ **Where:** $$f_a = \frac{(\#AA\ Calls)}{Total\ \#\ Calls}$$ $$f_b = \frac{(\#BB\ Calls)}{Total\ \#\ Calls}$$ $$f_{aa} = \frac{(\#AA\ Calls + 0.5 * \#\ AB\ Calls)}{Total\ \#\ Calls}$$ $$f_{bb} = \frac{(\#BB\ Calls + 0.5 * \#\ AB\ Calls)}{Total\ \#\ Calls}$$ $$f_{ab} = \frac{(\#AB\ Calls)}{Total\ \#\ Calls}$$ $$PHW = CDF(x^2)$$ Where CDF is the Cumulative Distributive Function for the chi-squared distribution. The Exact test used is the one implemented in R package "HardyWeinberg" for more information see: [2] Haldane, J., 1954. An exact test for randomness of mating. J. Genet. 52 631-635. [3] Levene, H., 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20 91-94.
H. W. Statistic	If the count of samples for at least one cluster is <10, then H.W. Statistic is 1. The Exact test is used to determine HWE. A 0 value indicates that there are more than 10 samples per cluster and a chi-squared test is used to determine HWE.
MinorAlleleFrequency	The Minor Allele Frequency of a SNP (SNP probe sets only).
Call Modified	Shows whether a call was changed from its original genotype.

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