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User Manual

Somatic Mutation Viewer

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Somatic Mutation Viewer

Introduction

This document provides the general guidelines on how to use the Somatic Mutation Viewer application.

Somatic Mutation Viewer is a tool to visualize somatic mutation MutScores and MutCalls across a set of supplied OncoScan FFPE Assay OSCHP files.

In addition to visualizing the MutScores, the tool also enables you to edit the thresholds used to make the MutCalls and save its updated calls to OSCHP files.

You can view the data grouped by marker or by sample, and you can select individual data points for more information. Summary statistics are provided by sample or by marker. Sort these tables by the field of interest to quickly find rows of interest.

System Requirements

Operating System
Windows® 7 Professional (64-bit) with Service Pack 1 installed

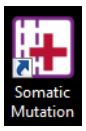
Installing Somatic Mutation Viewer

To install Somatic Mutation Viewer:

1. Go to **www.affymetrix.com** and navigate to the Somatic Mutation Viewer location.
2. Locate and download the zipped Somatic Mutation Viewer software package.
3. Unzip the file, then double-click **SomaticMutationApp.exe** to install it.
4. Follow the directions provided by the installer.

Starting Somatic Mutation Viewer

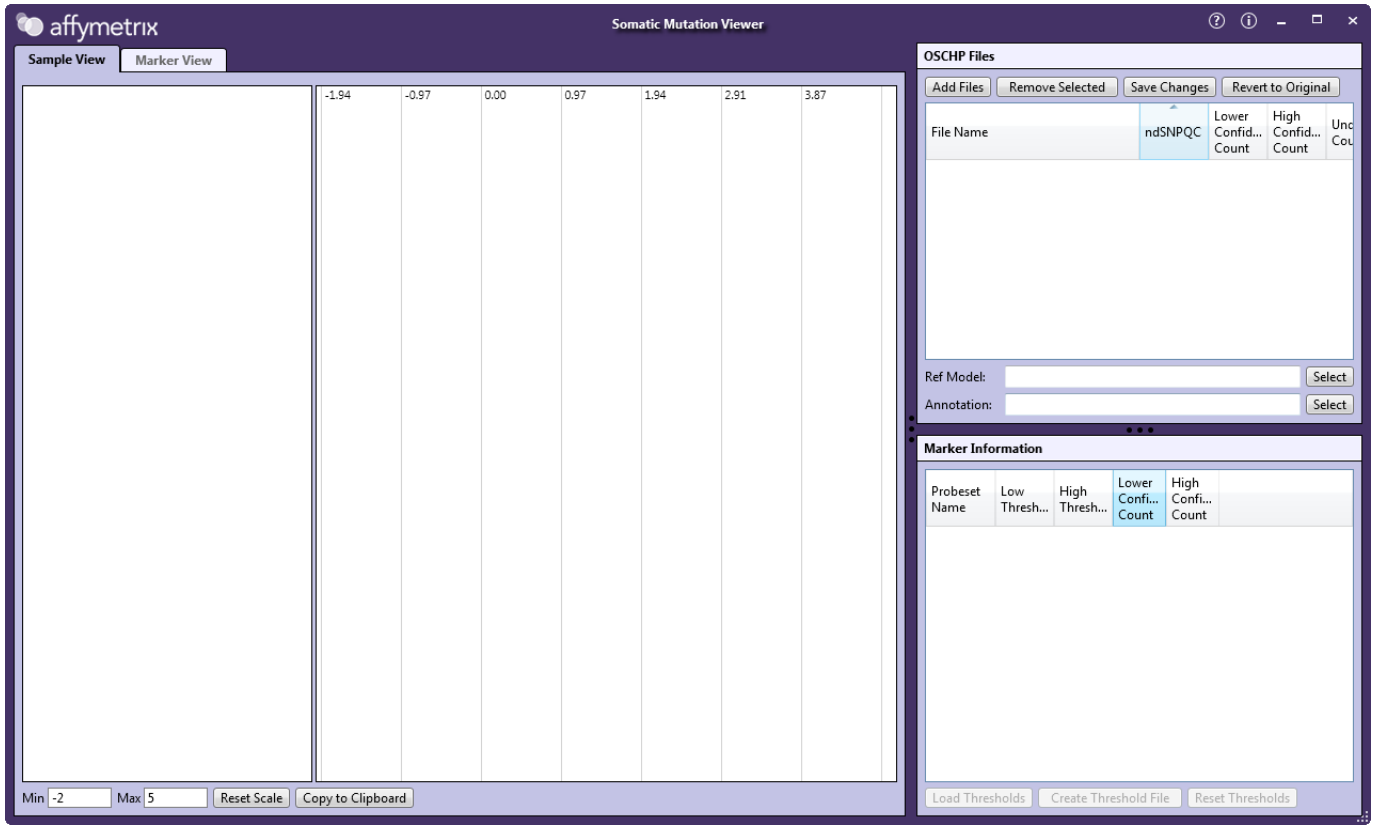
To start Somatic Mutation Viewer:

1. Locate the Somatic Mutation Viewer Shortcut 

on your system's Desktop, then double-click on it.

   The Viewer opens. (Figure 1.1 on page 3)

Figure 1.1 Somatic Mutation Viewer- Main window

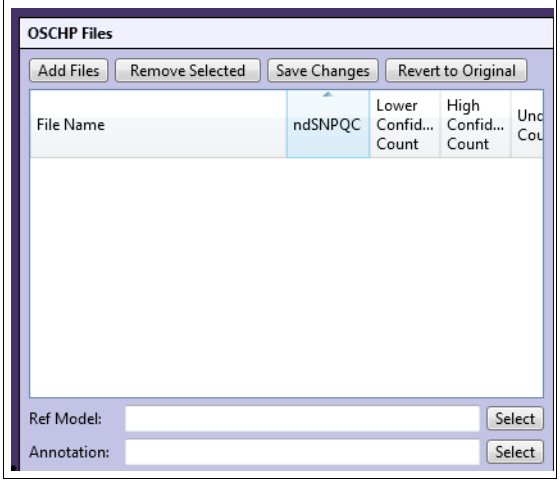


Setting Up the Viewer

IMPORTANT: The Somatic Mutation Viewer requires OncoScan array data (OSCHP files).

Loading OSCHP Files

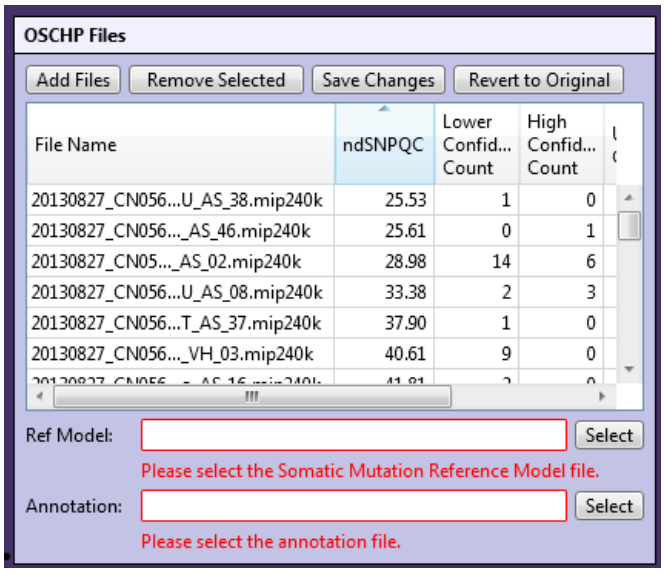
Figure 2.1 OSCHP Files pane



To Load OSCHP Files:

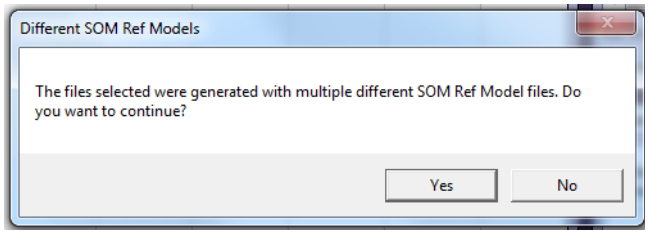
1. Click **Add Files**.
   A File window appears.
2. Navigate to your OSCHP folder, then single click, Ctrl click, Shift click, or Ctrl-A (to select multiple or all OSCHP files).
3. Click **Open**.
   The OSCHP File Name pane is now populated. (Figure 2.2)

Figure 2.2 OSCHP Files pane populated



A warning may appear if the selected OSCHP files were generated with different SOM Ref Model File. (Figure 2.3)

Figure 2.3 OSCHP Files Warning



Do one of the following:

- Click **Yes** to continue.
- Click **No** to remove the current list of OSCHP files.

Assigning a Reference Model File

NOTE: The reference model file supplies the information that generates the Marker View's ox Box Whisker Plot graphic. Make sure to select the same reference model file you used to generate the OSCHP data.

To select a Reference Model file:

1. Click **Select**.

   A File window appears.

   In most cases, the Viewer automatically chooses the appropriate location of the Reference Model file needed, as it is based on the OSCHP files you selected.

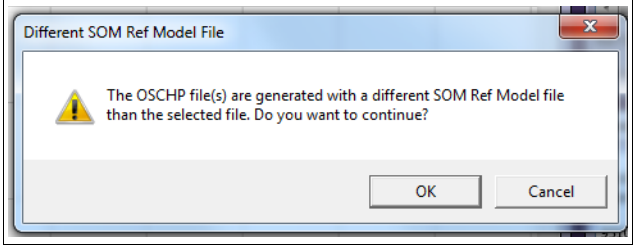
2. Click to select the Reference Model file, then click **Open**.

   If your Reference Model File is not listed in the initial window, use the File window to navigate to the required Reference Model file, then click **Open**.

   The Ref Model field is now populated.

   A warning may appear If the selected SOM Ref Model is not the same as the one used to create the OSCHP files. (Figure 2.4)

Figure 2.4 SOM Ref Model Warning



- Click **Yes** to continue.
- Click **No** to remove the current SOM Ref Model File.

Assigning an Annotation File

IMPORTANT: The annotation file provides information about each marker. Without this file, only the Probeset Name is available.

To assign the Annotation file:

1. Click **Select**.

   A File window appears.

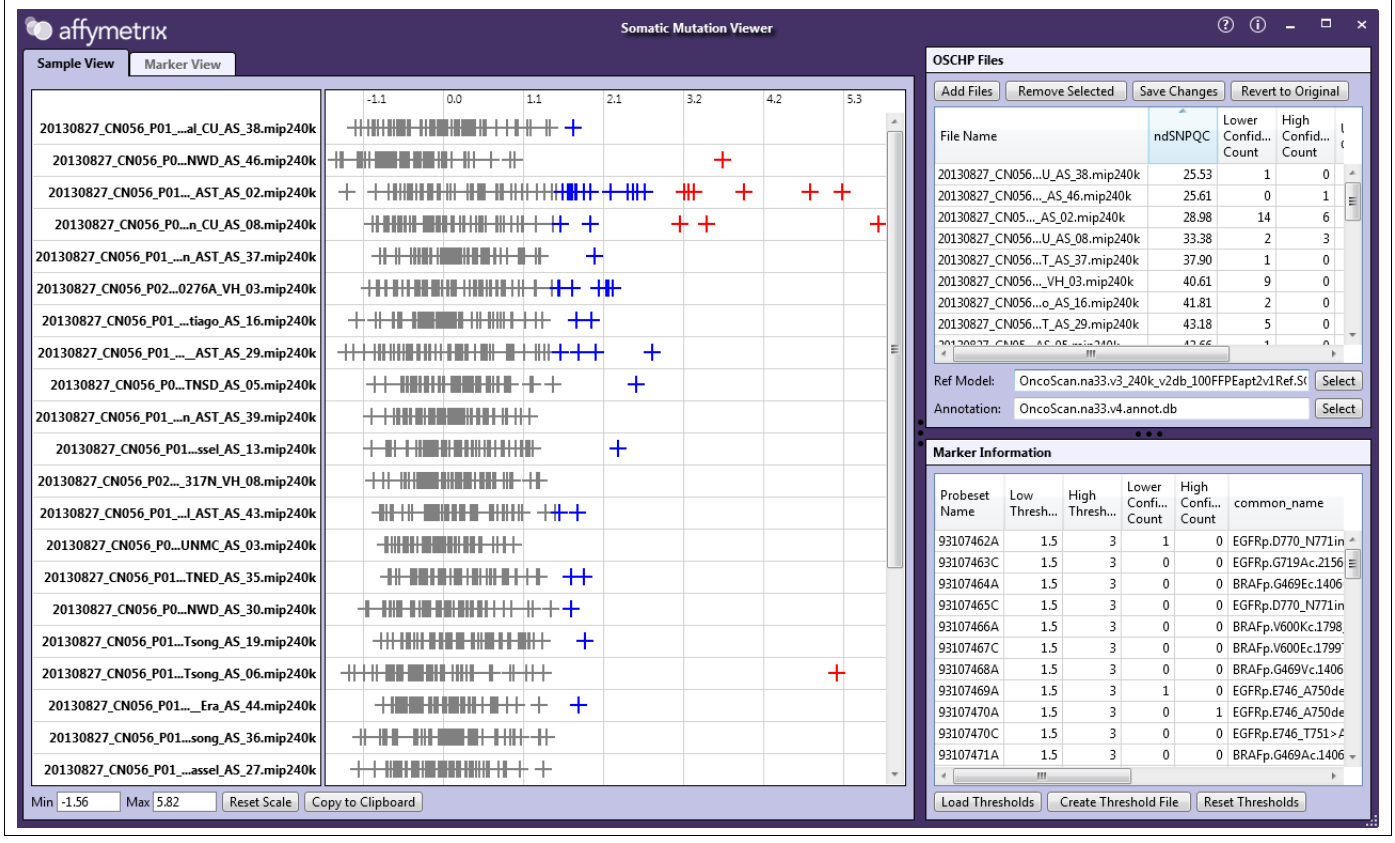
   In most cases, the Viewer automatically chooses the appropriate location of the Annotation file needed, as it is based on the OSCHP files you selected.

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

   If your Annotation File is not listed in the initial window, use the File window to navigate to the required Annotation file, then click **Open**.

   The Somatic Mutation Viewer's 3 window panes are now fully populated. (Figure 2.5)

Figure 2.5 3 Populated Viewer example



Using the Viewer

Different OSCHP files may have used different thresholds and/or somatic mutation reference model files. For display purposes, Somatic Mutation Viewer loads the thresholds from the first OSCHP file loaded.

It is important that all OSCHPs must originate from the same SOM RefModel. If the thresholds are different, a message appears stating the first OSCHP thresholds will be used.

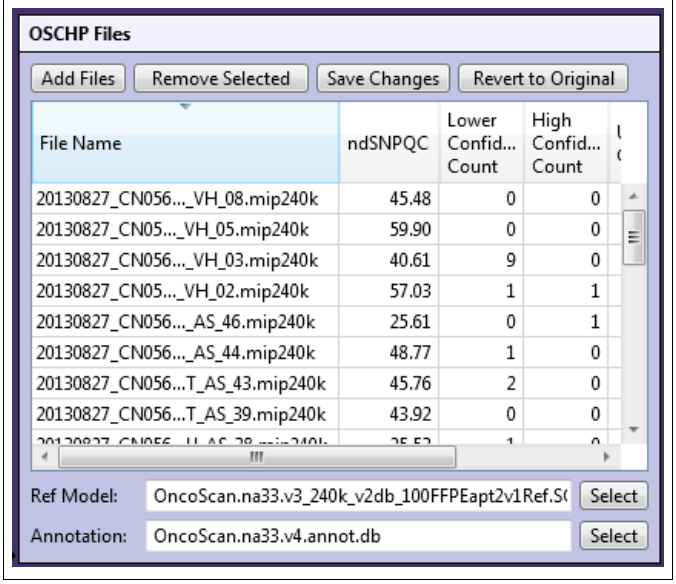
Poorer quality samples will have more false positive calls. Affymetrix recommends that you exclude samples that do not meet recommended QC thresholds, and any additional under-performing samples, from the visualizations and further analysis.

In the OSCHP Files table, sort on ndSNPQC to sort the samples according to their quality. Examine these samples in the Sample View. Consider removing the samples from the study that appear to have a substantial number of false positive calls.

OSCHP Files Window

The OSCHP Files window displays your OncoScan Console generated OSCHP files, the Reference Model File, and Annotation file. (Figure 3.1)

Figure 3.1 OSCHP Files window



OSCHP File Window Columns

The Marker Information window (Figure 3.1) displays the following columns (from left to right):

- **File Name:** Displays the OSCHP file name.

- **ndSNPQC (SNP Quality Control of Normal Diploid Markers):** The metric SNPQC is a measure of how well genotype alleles are resolved in the microarray data. ndSNPQC is the same metric but only applied to normal diploid markers (that is those that have been determined to have Copy Number=2 in the sample). Larger ndSNPQC values are better.
- **Lower Confidence Count:** In the OSCHP Files table, this is the count of ProbeSets for the OSCHP that have a MutCall reporting "Lower confidence," describing the likelihood that the mutation is present.
- **High Confidence Count:** In the OSCHP Files table, this is the count of ProbeSets for the OSCHP that have a MutCall reporting "High confidence," describing the likelihood that the mutation is present.
- **Undetected Count:** This is the count of ProbeSets for the OSCHP that have a MutCall reporting "Undetected," describing the likelihood that the mutation is not present.
- **Error Message:** Reports Somatic Mutation Viewer errors associated with the OSCHP file.

Adding and Removing OSCHP Files

To add OSCHP files to this window:

1. Click **Add Files**.
   A File window appears.
2. Search within the File window that appears by default, or navigate to another folder location. Single click, Ctrl click, or Shift click (to select multiple OSCHP files).
3. Click **Open**.
   The additional OSCHP files are now added.

To remove OSCHP files from this window:

1. Single click, Ctrl click, or Shift click (to select multiple OSCHP files).
2. Click **Remove Selected**.
   The file(s) are removed.

Sorting OSCHP File Window Columns

To sort a column:

1. Click on a header.
   The column is now sorted in an ascending order.
2. Click on the header again to reverse the sorting order.

To move a column:

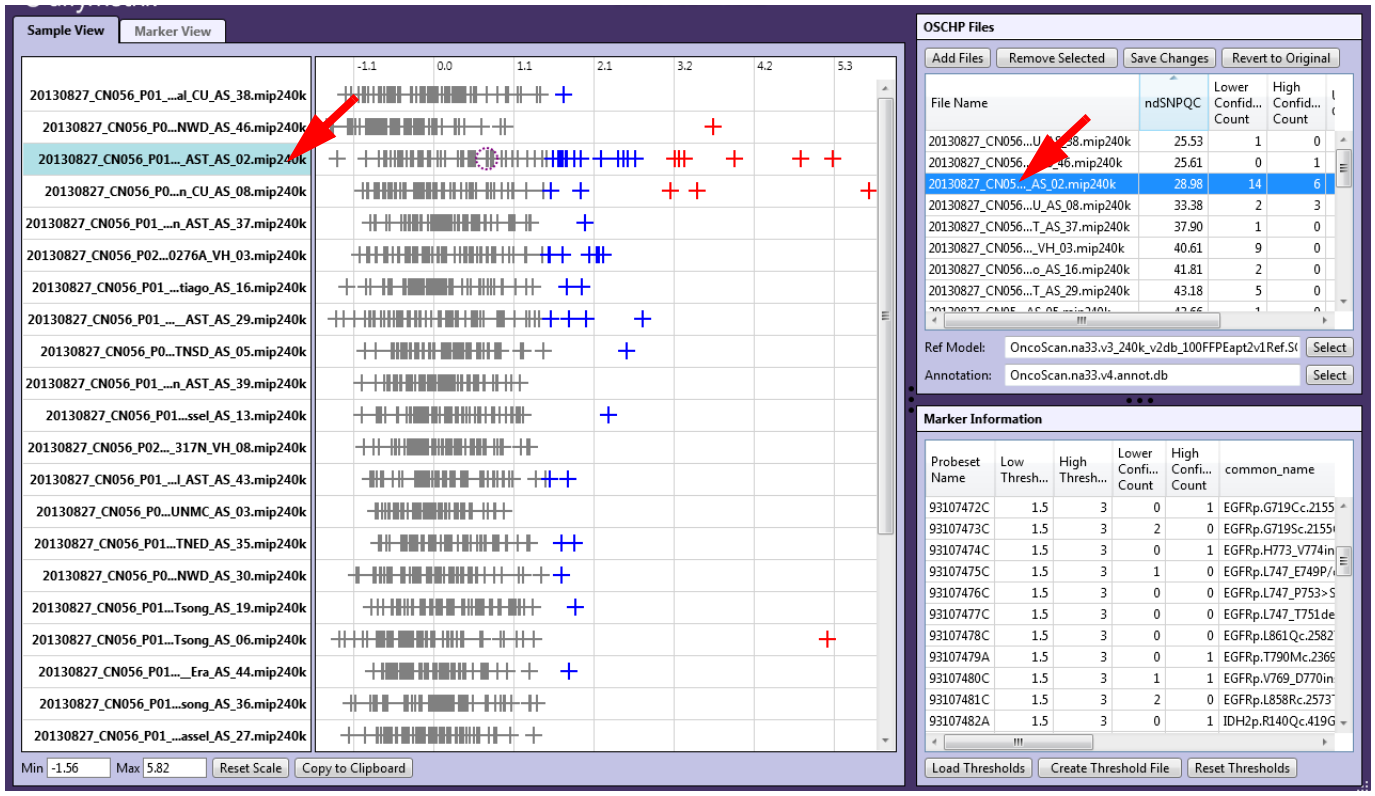
1. Click on a header, then drag it to a desired position within the OSCHP Files window.
2. Release the mouse button.
   The header now resides at its new location within the OSCHP Files window.

Sample View Tab Window

TIP: The OSCHP Files window works in sync with the Sample View pane. Clicking on a sample file name also highlights that file within the Sample View pane, as shown in Figure 3.2.

Your data is also in sync with the Marker Information window. If you click on a marker in the Sample View, that marker is highlighted in the Marker Information window, as shown in Figure 3.2.

Figure 3.2 Sample View window



Using the Sample View Tab Window

The Sample View is ***read-only***.

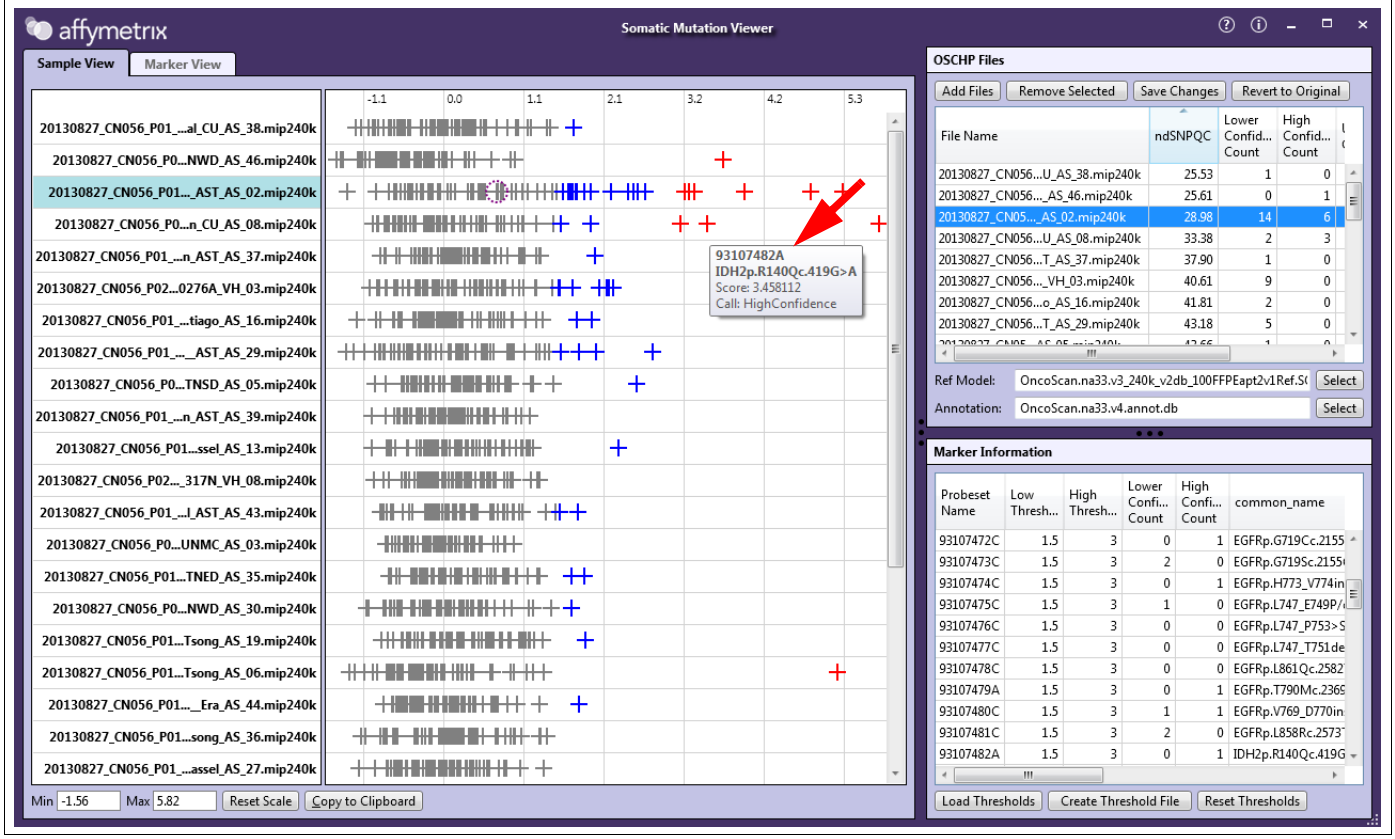
To view the Calls from an OSCHP File:

1. Click on the desired OSCHP file from the OSCHP File window pane or from the left pane of the Sample View window, as shown in Figure 3.2.

To view specific calls:

1. Mouse over a call to reveal its score and Call property. Click on the call to see its Probeset Name and properties within the Marker Information window, as shown in Figure 3.3.

Figure 3.3 Sample View window

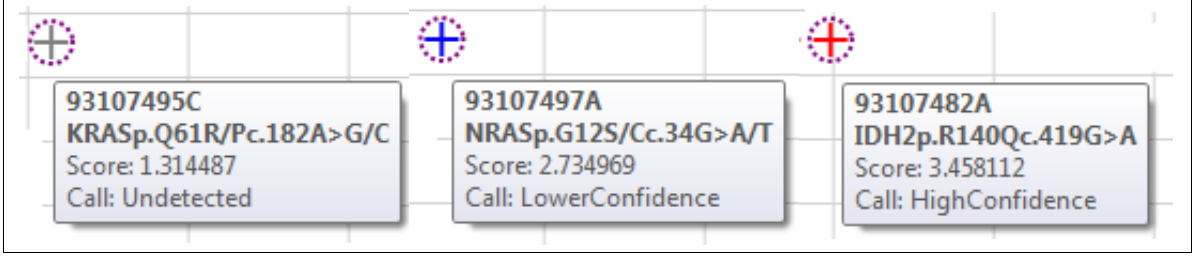


MutScores (Scores) and MutCalls (Calls) - Overview

The x-axis signal is the MutScore from the OSCHP file. A MutScore is a measure of the signal response of the marker relative to the expected signal distribution of this marker in the absence of the mutation. It is calculated as follows:

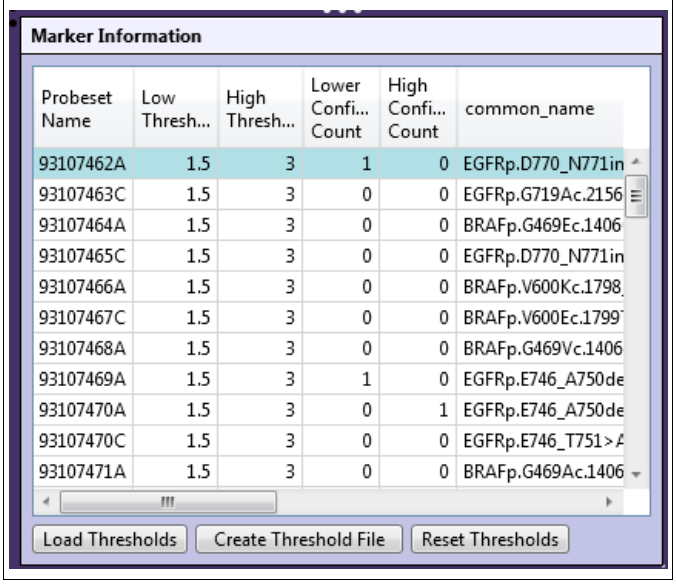
- MutScore = (measured quantile normalized signal - median signal for this marker in the reference model file) **/** (95th percentile signal for this marker in the reference model file - median signal for this marker in the reference model file).
- A higher MutScore for the same marker indicates greater confidence that the mutation is present, and is correlated with higher % mutant allele. However, as each marker's signal is normalized to its own marker's reference signal distribution, it is not appropriate to compare MutScores between different markers to assign relative % mutant loads. Different markers will have different detection sensitivities.
- A MutCall is displayed as ***Undetected*** if the MutScore is below the Low Confidence threshold.
- A MutCall is reported as ***HighConfidence*** if greater than or equal to the High Confidence threshold.
- If the MutCall is equal to or greater than the Low Confidence threshold and is less than the High Confidence threshold, the MutCall is reported as ***LowerConfidence***. (Figure 3.4)

Figure 3.4 MutCall and MutScore examples



Marker Information Window

Figure 3.5 Marker Information window.



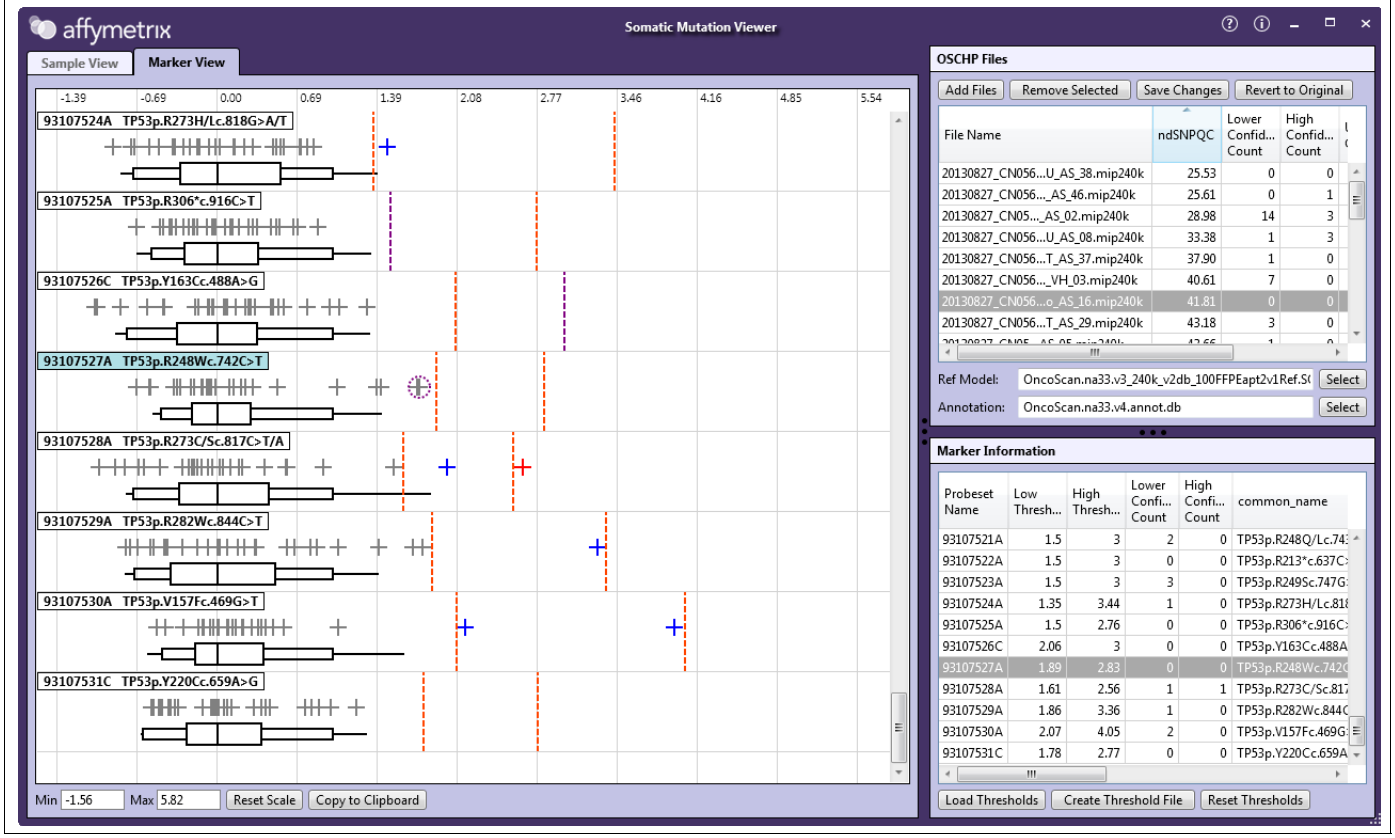
The Marker Information window (Figure 3.5) displays the following columns (from left to right):

- **Probeset Name:** Affymetrix identifier for the marker.
- **Low Threshold:** Lower confidence MutScore threshold. Measurements with a MutScore below this value are called “Undetected”. Measurements equal to or greater than this threshold but less than the High Threshold are called “Lower confidence,” describing the likelihood that the mutation is present.
- **High Threshold:** High confidence MutScore threshold. Measurements equal to or greater than this threshold are called “High confidence,” describing the likelihood that the mutation is present.
- **Lower Confidence Count:** In the Marker Information table, this is the count of OSCHP files for the ProbeSet that have a MutCall reporting “Lower confidence.”
- **High Confidence Count:** In the Marker Information table, this is the count of OSCHP files for the ProbeSet that have a MutCall reporting “High confidence.”
- **common_name:** Abbreviated description of the mutations to which this ProbeSet is known to respond. The name has the form [Gene]:[amino acid change for mutation]:[cDNA change for mutation]. In the event that the ProbeSet cannot differentiate among multiple mutations to which it can respond, the slash (/) delimits the multiple known mutations.
- **chr_ID:** Chromosome of the mutation.
- **start:** Starting genomic position of the mutation.
- **stop:** Ending genomic position of the mutation.

- **cosmic_id:** The identifier of the mutation as listed in the COSMIC database, which is a catalogue of somatic mutations in cancer. More information on these mutations can be found at http://cancer.sanger.ac.uk
- **channel:** The CEL file from which the signal is measured. **"A"** is the AT CEL, **"C"** is the GC CEL.

TIP: The Marker Information window works in sync with the Marker View pane. Clicking on a sample file name also highlights that file within the Marker View pane, as shown in **Figure 3.6**.

Figure 3.6 File window



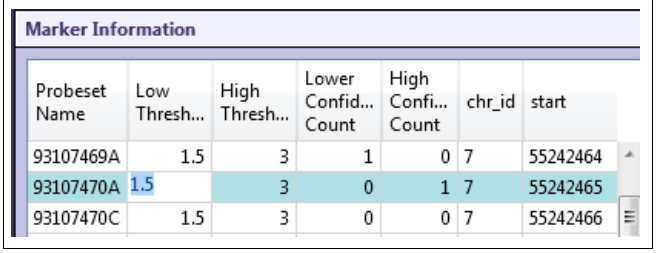
Editing Thresholds in the Marker Information Window

To edit the Low and/or High Thresholds:

1. Click to highlight the Probeset marker you want to edit.

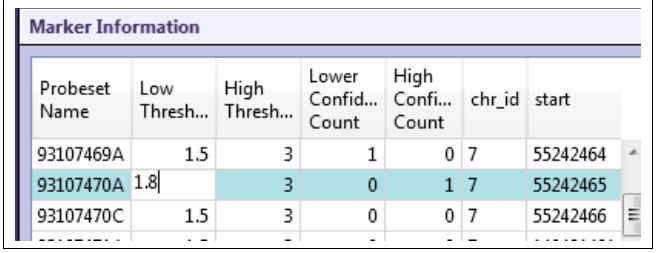
2. Click on the current *Low Threshold* value field.

Figure 3.7 Changing Low Threshold value example 1



3. Enter a new *Low Threshold* value.

Figure 3.8 Changing Low Threshold value example 2



4. Press **Enter**.

   The Low Threshold field value is now changed. This threshold value change (represented by vertical dotted lines) is also reflected in the Marker View window.

5. If you want to change the current *High Threshold* value field, click on the current *High Threshold* value field.
6. Enter a new *High Threshold* value.
7. Press **Enter**.

   The High Threshold field value is now changed. This threshold value change (represented by vertical dotted lines) is also reflected in the Marker View window.

Marker View Tab Window

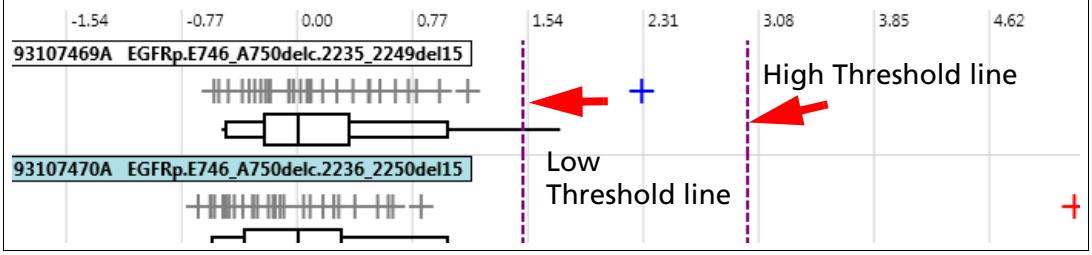
Thresholds for individual markers can be changed either by dragging the vertical dashed threshold lines in the Marker View, or by manually editing the Low and High Threshold values in the Marker Info table.

Editing Thresholds in the Marker View Tab Window

To edit the Low and/or High Thresholds:

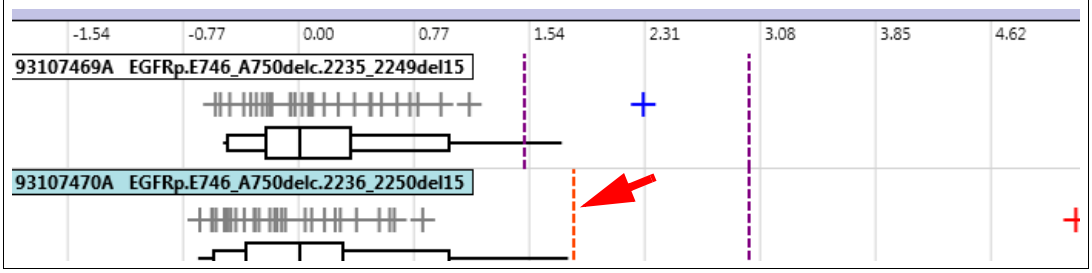
1. Click to highlight the Probeset marker you want to edit.
   Low and High Thresholds are represented by vertical dotted lines, as shown in Figure 3.9.

Figure 3.9 Marker View - Threshold value lines



2. Click on the current *Low Threshold* line, drag the line to your desired location, then release the mouse button. (Figure 3.10).

Figure 3.10 Marker View - Example: Shifting the Threshold value line

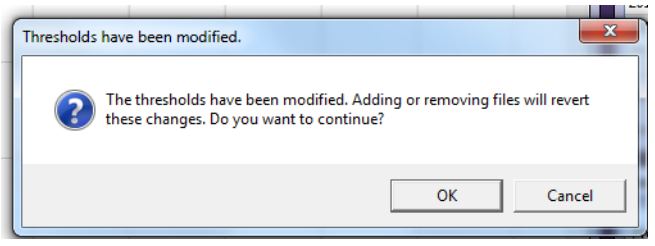


   The Low Threshold field value is now changed. This threshold value change is also reflected in the Marker Information window's corresponding Low Threshold value field.

3. If you want to change the current *High Threshold* value field, click on the current *High Threshold* line, drag the line to your desired location, then release the mouse button.
   The High Threshold field value is now changed. This threshold change is also reflected in the Marker Information window's corresponding High Threshold value field.

A warning may appear when adding or removing an OSCHP file (after its thresholds have been changed). (Figure 3.11)

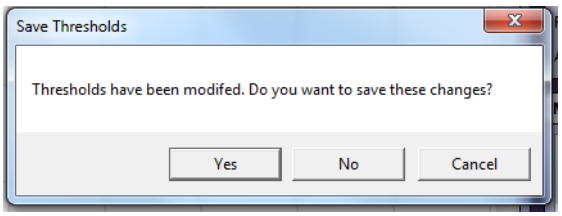
Figure 3.11 Thresholds have been modified Warning



Do one of the following:

- Click **OK** to continue.
- Clicking **Cancel** prompts the following message: (Figure 3.12)

Figure 3.12 Save Thresholds Warning



Do one of the following:

1. Click: **Yes** - Saves the updated calls and thresholds to the OSCHP file and closes the viewer.
2. Click **No** - Does not save the updated thresholds and any changes to the calls and closes the viewer.
3. Click **Cancel** - Does not close the Viewer.

Saving your New Thresholds

TIP: The Viewer always retains your original thresholds. At any time, click *Reset Thresholds* to restore your original threshold values.

To save your new thresholds:

1. At the Marker Information window, click **Create Thresholds**.
   A File window appears.
2. Navigate to a desired save location, enter a filename, then click **Save**.
   Your new Thresholds file is saved as a Tab-delimited text file. You can then load this Thresholds file into Somatic Mutation Viewer at another time, or you can load it into OncoScan Console when setting up additional analyses of CEL files.

To load thresholds:

1. At the Marker Information window, click **Load Thresholds**.

A File window appears.

2. Navigate to the Threshold's location, click on its filename, then click **Open**.
   Your Thresholds file's properties appear in the Marker Information window.

Viewing Tools

- For optimum viewing, each Somatic Mutation Viewer window pane can be easily resized
- The Marker and Sample Views feature a taskbar that can customize your desired view even further.
- The Marker View features a Box Whisker Plot graphic for each Probeset.

Resizing Window Panes

To resize a window pane:

1. Click on the edge of a window you want to resize, then drag it to the size you want.

Taskbar Options

The Marker and Sample Views feature a taskbar (bottom left) that enables you to do the following: (Figure 3.13)

Figure 3.13 Viewing Taskbar

- **Min:** Enter your minimum (starting point) scale size of the currently displayed data.
- **Max:** Enter your maximum (finishing point) scale size of the currently displayed data.
- **Reset Scale:** Returns your view to its original scaled state - as it was - the first time the files were loaded into the Viewer.
- **Copy to Clipboard:** Click to save the current (Marker or Sample) view to your Clipboard for pasting in another application (as a .PNG file).

Box Whisker Plot Graphic

If you have selected a reference model file, the reference signal distribution for each marker in the absence of any mutation is displayed as a box plot in the Marker View.

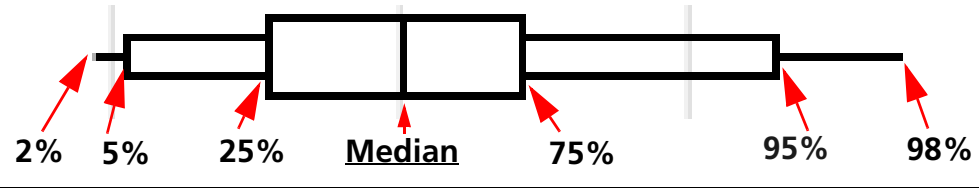
The percentile values marked by the box plot represent the 2%, 5%, 25%, 50%, 75%, 95%, and 98% percentiles of the signal distribution of this marker in the reference. The 50%ile value of the reference will have a MutScore = 0, and the 95%ile value will have a MutScore = 1.

Make sure the reference model file you select is the one used to generate the OSCHP file results you are displaying.

Consider utilizing this plot graphic when determining placement of new threshold values.

Even though each Probeset's plot is uniquely sized, its assigned percentile locations are constant, as shown in Figure 3.14.

Figure 3.14 Box Whisker Plot - Assigned percentiles



Saving your Edited OSCHP Files

TIP: When you use Somatic Mutation Viewer to save changed thresholds and calls to your OSCHP files, the original values are retained in the OSCHP files.

At any time, click *Revert to Original* to restore your original thresholds and calls.

To save your edited OSCHP file set:

1. At the OSCHP Files window, click **Save Changes**.
   A green progress bar appears and your newly edited OSCHP file set is now saved.

To restore your original OSCHP file set:

1. At the OSCHP Files window, click **Revert to Original**.
   A green progress bar appears and your original OSCHP file set is now restored.