



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:

 Locate the Somatic Mutation Viewer Shortcut double-click on it.

The Viewer opens. (Figure 1.1 on page 3)



on your system's Desktop, then

Figure 1.1 Somatic Mutation Vie	wer- Main windo	W		
🕲 affymetrix		Somatic Mutation Vi	iewer	0 ① – □ ×
Sample View Marker View			OS	CHP Files
	.94 -0.97 0.0	0 0.97 1.94 2.	.91 3.87 Fi	dd Files Remove Selected Save Changes Revert to Original Ile Name ndSNPQC Confid High Count Count Count Count
			Ret	f Model: Select
			An	notation: Select
			Ma	arker Information
			P	robeset Low High Thresh High Confi Count Count
Min -2 Max 5 Reset Scale Copy t	to Clipboard		L	oad Thresholds Create Threshold File Reset Thresholds

Setting Up the Viewer

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

Loading OSCHP Files

Figure 2.1 OSCHP Files pane							
OSCHP Files							
Add Files Remove Selected	Save Changes	Revert	to Origina	1			
File Name	ndSNPQC	Lower Confid Count	High Confid Count	Unc Cou			
Pef Model			S.	lect			
Annotation:			Se	lect			

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)

igure 2.2 OSCHP Files pane populated								
OSCHP Files								
Add Files Remove Selected Save Changes Revert to Original								
File Name		ndSNPQC	Lower Confid Count	High Confid Count	l C			
20130827_CN	056U_AS_38.mip240k	25.53	1	0				
20130827_CN	056AS_46.mip240k	25.61	0	1				
20130827_CN	05AS_02.mip240k	28.98	14	6				
20130827_CN	056U_AS_08.mip240k	33.38	2	3				
20130827_CN	056T_AS_37.mip240k	37.90	1	0				
20130827_CN	056VH_03.mip240k	40.61	9	0	_			
20120027_CN	INEE - AC 16	/1 01	ì					
Ref Model:	Ref Model: Select							
Please select the Somatic Mutation Reference Model								
Annotation:				Se	lect			
	Please select the annota	tion file.						

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						
Different SOM Ref Models		x				
The files selected were generated with multiple diff you want to continue?	ferent SOM Ref Mod	el files. Do				
	Yes	No				

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						
Different SOM Ref Model File	×					
The OSCHP file(s) are generated with than the selected file. Do you want to	:h a different SOM Ref Model file to continue?					
_	OK Cancel					

- 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							
🐚 affymetrix		Somatic M	utation View	er			× □ - 〔) ③
Sample View Marker View							OSCHP Files
	-1.1 0.0 1.1	2.1	3.2	4.2	5.3		Add Files Remove Selected Save Changes Revert to Original
20130827_CN056_P01al_CU_AS_38.mip240k					4		File Name ndSNPQC Confid {
20130827_CN056_P0NWD_AS_46.mip240k			+				20130827 CN056 LLAS 38 min240k 25 53 1 0
20130827_CN056_P01AST_AS_02.mip240k	-+	+ + + + + + +	₩ +	+ -	+		20130827_CN056AS_46.mip240k 25.61 0 1 =
20130827_CN056_P0n_CU_AS_08.mip240k		+	• +		+		20130827_CN05AS_02.mip240k 28.98 14 6
20130827 CN056 P01n AST AS 37.mip240k							20130827_CN056U_AS_08.mip240k 33.38 2 3
20120827 CN056 B02 02764 VII 02							20130827_CN056VH_03.mip240k 40.61 9 0
20150627_CN050_F020276A_VH_05.imip240k							20130827_CN056o_AS_16.mip240k 41.81 2 0
20130827_CN056_P01tiago_AS_16.mip240k							20130827_CN056T_AS_29.mip240k 43.18 5 0
20130827_CN056_P01AST_AS_29.mip240k	╽╶┼┼┼┼╢╫╫╫╫╫╢┼╫╫┼╫╫╴╫┼╶╢┼╊╌╉╼╋	- +					• H
20130827_CN056_P0TNSD_AS_05.mip240k		+					Ref Model: OncoScan.na33.v3_240k_v2db_100FFPEapt2v1Ref.S(Select
20130827_CN056_P01n_AST_AS_39.mip240k							Annotation: OncoScan.na33.v4.annot.db Select
20130827_CN056_P01ssel_AS_13.mip240k		+					Marker Information
20130827_CN056_P02317N_VH_08.mip240k							Lower High
20130827_CN056_P01I_AST_AS_43.mip240k	╶╫┦┼╢╴══╬╢╢╢╢╴╢╢╢╢╸╶┼╫╸╇╸						Probeset Low High Name Thresh Thresh Confi Confi common_name Count Count
20130827_CN056_P0UNMC_AS_03.mip240k							93107462A 1.5 3 1 0 EGFRp.D770_N771in *
20130827_CN056_P01TNED_AS_35.mip240k						1	93107463C 1.5 3 0 0 EGFRp.G719Ac.2156 = 93107464A 1.5 3 0 0 BRAEp G469Ec 1406
20130827 CN056 P0NWD AS 30.mip240k							93107465C 1.5 3 0 0 EGFRp.D770_N771in
20130827 CN056 P01 Tsong AS 19 min240k							93107466A 1.5 3 0 0 BRAFp.V600Kc.1798
20120827 (NOE6 DO1 T AS O5							93107467C 1.5 3 0 0 BRAFp.V600Ec.1799 93107468A 1.5 3 0 0 BRAFp.G469Vc.1406
20130627_CN050_P011solig_A5_00.inip240k				<u>т</u>			93107469A 1.5 3 1 0 EGFRp.E746_A750de
20130827_CN056_P01Era_AS_44.mip240k							93107470A 1.5 3 0 1 EGFRp.E746_A750de
20130827_CN056_P01song_AS_36.mip240k							93107470C 1.5 3 0 0 EGFRp.E746_T751>A 93107471A 1.5 3 0 0 BRAEp.G469Ac.1406 -
20130827_CN056_P01assel_AS_27.mip240k							
Min -1.56 Max 5.82 Reset Scale C	opy to Clipboard						Load Thresholds Create Threshold File Reset Thresholds

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 underperforming 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									
٢	OSCHP Files									
	Add Files Remove Selected	Save Changes	Revert	t to Origina						
	File Name	ndSNPQC	Lower Confid Count	High Confid Count	l c					
I	20130827_CN056VH_08.mip240k	45.48	0	0	*					
I	20130827_CN05VH_05.mip240k	59.90	0	0	=					
I	20130827_CN056VH_03.mip240k	40.61	9	0						
I	20130827_CN05VH_02.mip240k	57.03	1	1						
I	20130827_CN056AS_46.mip240k	25.61	0	1						
I	20130827_CN056AS_44.mip240k	48.77	1	0						
I	20130827_CN056T_AS_43.mip240k	45.76	2	0						
I	20130827_CN056T_AS_39.mip240k	43.92	0	0						
I	20120027 CNOES II AS 20									
	Ref Model: OncoScan.na33.v3_24	0k_v2db_100F	FPEapt2v1	Ref.S(Se	lect					
	Annotation: OncoScan.na33.v4.annot.db Select									

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.
- 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											
Sample View Marker View							OSCHP Files	;			
	-1.1 0.0 1.1	2.1	3.2	4.2	5.3		Add Files	Remove Selected	Save Change	Revert	to Original
20130827_CN056_P01al_CU_AS_38.mip240k	-14-11-11-11-11-11-11-11-11-11-11-11-11-					*	File Name		ndSNPQC	Lower Confid	High Confid
20130827_CN056_P0NWD_AS_46.mip240k			+				20130827 0	N056U \$ -8.min2	10k 25.53	1	Count 0 A
20130827_CN056_P01AST_AS_02.mip240k	┽╶┼╫╫╫╫╫╫╫╫╢╢	+ 1111	+++ +	· + ·	+		20130827_0	N05646.mip240	k 25.61	. 0	1 =
20130827_CN056_P0n_CU_AS_08.mip240k			++		+		20130827_0	N05AS_02.mip2401	: 28.98	14	6
20130827 CN056 P01n AST AS 37.mip240k							20130827_0	N056U_AS_08.mip2	10k 33.38	2	3
							20130827_C	N056VH_03.mip24	0k 40.61	9	0
20130827_CN056_P020276A_VH_03.mip240k							20130827_0	N056o_AS_16.mip24	l0k 41.81	2	0
20130827_CN056_P01tiago_AS_16.mip240k							20130827_C	N056T_AS_29.mip24	l0k 43.18	5	0 +
20130827_CN056_P01AST_AS_29.mip240k		+				E	4	III III	1126		•
20130827_CN056_P0TNSD_AS_05.mip240k		+					Ref Model:	OncoScan.na33.v3	_240k_v2db_100	FFPEapt2v1R	ef.S(Select
20130827_CN056_P01n_AST_AS_39.mip240k							Annotation:	OncoScan.na33.v4	.annot.db		Select
20130827_CN056_P01ssel_AS_13.mip240k		+					Marker Info	ormation			
20130827_CN056_P02317N_VH_08.mip240k						н	Probeset	Low High	Lower High		
20130827_CN056_P01I_AST_AS_43.mip240k	╶╫╂┼╫╌╫╫╫╂╂╋╶╫┼╟┼╢╴╶┼┽╋╌╋╴						Name	Thresh Thresh	Count Count	commoi t	n_name
20130827_CN056_P0UNMC_AS_03.mip240k							93107472C	1.5 3	0	1 EGFRp.G	719Cc.2155 🔺
20130827 CN056 P01TNED AS 35.mip240k						-	93107473C	1.5 3	2	0 EGFRp.G	719Sc.2155
20120927 (N056 P0 NWD AS 20 min240k							93107474C 93107475C	1.5 5	1	0 EGFRp.L	747 E749P/
20130827_CN030_F0NWD_N3_30.IIIIp240k							93107476C	1.5 3	0	0 EGFRp.L	747_P753>S
20130827_CN056_P01Tsong_AS_19.mip240k							93107477C	1.5 3	0	0 EGFRp.L	747_T751de
20130827_CN056_P01Tsong_AS_06.mip240k				-	F		93107478C	1.5 3	0	0 EGFRp.L8	361Qc.2582
20130827_CN056_P01Era_AS_44.mip240k							93107479A 93107480C	1.5 3	1	1 EGFRp.V	7901VIC.2305 769 D770in
20130827_CN056_P01song_AS_36.mip240k							93107481C	1.5 3	2	0 EGFRp.L8	358Rc.2573
20130827_CN056_P01assel_AS_27.mip240k						-	93107482A	1.5 3	0	1 IDH2p.R1	140Qc.419G →
Min -1.56 Max 5.82 Reset Scale Copy to Clipboard Load Thresholds Create Threshold File Reset Thresholds											

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:

 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.

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Figure 3.3 Sample View window

🖲 affymetrix	Somatic Mutation Viewer	() () _ □ ×
Sample View Marker View		OSCHP Files
	-11 0.0 1.1 2.1 3.2 4.2 5.3	Add Files Remove Selected Save Changes Revert to Original
20130827_CN056_P01al_CU_AS_38.mip240k		File Name ndSNPQC Lower High L Confid Confid t
20130827_CN056_P0NWD_AS_46.mip240k		20130827 CN056 LLAS 38 min240k 25 53 1 0
20130827_CN056_P01AST_AS_02.mip240k	╡┼╫╫╫╫╫╫╫╠╠╟╢┼╫ ╢┼┼┼╢┼ ╺╫╴┽╴┽ _╴ ╈╸╵	20130827_CN056AS_46.mip240k 25.61 0 1 =
20130827 CN056 P0n CU AS 08.mip240k		20130827_CN05AS_02.mip240k 28.98 14 6
20120827 (NOSE POL - AST AS 27		20130827_CN056U_AS_08.mip240k 33.38 2 3
20150627_CN050_F0111_A51_A5_57.111p240k	IDH2p.R140Qc.419G>A	20130827_CN056I_AS_37.mip240k 37.90 1 0
20130827_CN056_P020276A_VH_03.mip240k	Score: 3.458112 Call: HighConfidence	20130827 CN056o AS 16.mip240k 41.81 2 0
20130827_CN056_P01tiago_AS_16.mip240k		20130827_CN056T_AS_29.mip240k 43.18 5 0
20130827_CN056_P01AST_AS_29.mip240k	<u>+++++101010000000000000000000000000000</u>	
20130827_CN056_P0TNSD_AS_05.mip240k	++- 	Ref Model: OncoScan.na33.v3_240k_v2db_100FFPEapt2v1Ref.St Select
20130827_CN056_P01n_AST_AS_39.mip240k		Annotation: OncoScan.na33.v4.annot.db Select
20130827 CN056 P01ssel AS 13.mip240k		Marker Information
20130827 CN056 P02 317N VH 08.min240k		
20130827_CN056_P01I_AST_AS_43.mip240k		Probeset Low High Confi Confi common_name Name Thresh Thresh Count Count
20130827_CN056_P0UNMC_AS_03.mip240k		93107472C 1.5 3 0 1 EGFRp.G719Cc.2155 ^
20130827 CN056 P01 TNED AS 35 min240k		93107473C 1.5 3 2 0 EGFRp.G719Sc.2155
		93107474C 1.5 3 0 1 EGFRp.H773_V774in 92107475C 1.5 2 1 0 EGFRp.1747_E740P.(
20130827_CN056_P0NWD_AS_30.mip240k		93107476C 1.5 3 0 0 EGFRp.L747_C745F7
20130827_CN056_P01Tsong_AS_19.mip240k		93107477C 1.5 3 0 0 EGFRp.L747_T751de
20130827_CN056_P01Tsong_AS_06.mip240k		93107478C 1.5 3 0 0 EGFRp.L861Qc.2582
20130827_CN056_P01Era_AS_44.mip240k		9310/4/9A 1.5 3 0 1 EGFRp.T790Mc.2369 93107480C 1.5 3 1 1 EGFRp.V769.D770in
20130827 CN056 P01song AS 36.min240k		93107481C 1.5 3 2 0 EGFRp.L858Rc.2573
		93107482A 1.5 3 0 1 IDH2p.R140Qc.419G -
20130027_CN030_P01assei_A5_27.mip240k		Land Thresholds Create Threshold File Peret Thresholds
Min -1.30 Max 2.82 Keset Scale	opy to Ciipboard	

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								
\oplus	\oplus		÷					
93107495C KRASp.Q61R/Pc.182A > Score: 1.314487 Call: Undetected	G/C 93107 Scores Call: L	7497A p.G12S/Cc.34G> 2.734969 .owerConfidence	A/T 9310748 IDH2p.R Score: 3.4 Call: High	2A 140Qc.419G>A 58112 hConfidence				

Marker Information Window

Figure 3.5 Marker Information window.										
Marker Information										
Probeset Name	Low Thresh	High Thresh	Lower Confi Count	High Confi Count	common_name					
93107462A	1.5	3	1	0	EGFRp.D770_N771in 🔺					
93107463C	1.5	3	0	0	EGFRp.G719Ac.2156 ≡					
93107464A	1.5	3	0	0	BRAFp.G469Ec.1406					
93107465C	1.5	3	0	0	EGFRp.D770_N771in					
93107466A	1.5	3	0	0	BRAFp.V600Kc.1798					
93107467C	1.5	3	0	0	BRAFp.V600Ec.1799					
93107468A	1.5	3	0	0	BRAFp.G469Vc.1406					
93107469A	1.5	3	1	0	EGFRp.E746_A750de					
93107470A	1.5	3	0	1	EGFRp.E746_A750de					
93107470C	1.5	3	0	0	EGFRp.E746_T751>A					
93107471A	1.5	3	0	0	BRAFp.G469Ac.1406 +					
•					•					
Load Thres	holds	Create Thre	eshold Fil	e Rese	t Thresholds					

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													
🖲 affvmetrix			Somatic N	lutation Viev	ver						?	i) –	
Sample View Marker View							OSCHP Files						
-1.39 -0.69 0.00 0.69	1.39 2.08	2.77	3.46	4.16	4.85	5.54	Add Files	Remove	Selected	Save Cha	nges R	evert to Orig	ginal
93107524A TP53p.R273H/Lc.818G>A/T	+					*	File Name			ndSNP	QC Conf	r High id Confid nt Count	а (i
							20130827_C	N056U_A	S_38.mip24	0k 2	i.53	0	0 -
93107525A TP53p.R306*c.916C>T			1				20130827_C	N056AS	_46.mip2401	k 2	6.61	0	1
-++							20130827_C	N05AS_	02.mip240k	28	1.98	14	3
							20130827_C	N056U_A	S_08.mip24	0k 3	1.38	1	3
		-					20130827_C	N056 VL	(S_37.mip24)	JK 3.	.90	1	0
95107520C TF55p.1105CC.400A>G							20130827_C	N056 o A	S 16 min240	K 40	81	0	0
	- +						20130827 0	N056T A	S 29.mip24	0k 4	.18	3	0
	-						20120027		E:		66	-	0
93107527A TP53p.R248Wc.742C>T													+
+ + +++++++++++++++++++++++++++++++++++	· # 🛞						Ref Model:	OncoSo	an.na33.v3_	240k_v2db_	L00FFPEap	t2v1Ref.S(Sele
							Annotation:	OncoSo	an.na33.v4.a	annot.db			Selec
93107528A TP53p.R273C/Sc.817C>T/A													
	+ +	+					Marker Info	rmation					
93107529A TP53p R282Wc 844C>T			;				Probeset Name	Low Thresh	High Thresh	Lower Hi Confi Co Count Co	gh onfi coi ount	mmon_nam	e
							93107521A	1.5	3	2	0 TP:	53p.R248Q/L	.c.74
		- T					93107522A	1.5	3	0	0 TP:	53p.R213*c.6	537C
				_			93107523A	1.5	3	3	0 TP:	53p.R249Sc.7	747G
93107530A TP53p.V157Fc.469G>T							93107524A	1.35	3.44	1	0 TP:	53p.R273H/L	.c.81
-++++++++++++++++++++++++++++++++++++	- +		+				93107525A	1.5	2.76	0	0 TP:	53p.R306*c.9)16C:
							93107526C	2.06	3	0	0 TP:	53p.Y163Cc.	488A
93107531C_TP53p.Y220Cc.659A>G							93107527A	1.89	2.83	0	0 TP:	53p.R248Wc	.7420
	+						9310/528A	1.61	2.56	1	1 FP:	3p.R2/3C/S	,c.817
	· · · · · · · · · · · · · · · · · · ·					=	93107529A	1.80	5.50	2	0 70	5p.K282Wc	.8440
	_						95107530A	2.07	4.05	2	0 TP:	3p.V220Ccl	650.0
						*	35107531C	1./0	2.17	0	U IP:	op.1220CC) JJA
Min 156 May 5.92 Peret Scale Com	sta Clinhaard			1			Load Three	holds	Croate Three	hold File	Peret Th	resholds	
The set Scale Cop							Load Thres		create miles	noiurile	Cheset In	restions	

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							
	Marker Information							
	Probeset Name	Low Thresh	High Thresh	Lower Confid Count	High Confi Count	chr_id	start	
I	93107469A	1.5	3	1	0	7	55242464	*
I	93107470A	1.5	3	0	1	7	55242465	_
I	93107470C	1.5	3	0	0	7	55242466	≡

3. Enter a new Low Threshold value.

	Figure 3.8 Changing Low Threshold value example 2							
	Marker Info	rmation						
	Probeset Name	Low Thresh	High Thresh	Lower Confid Count	High Confi Count	chr_id	start	
I	93107469A	1.5	3	1	0	7	55242464	*
	93107470A	1.8	3	0	1	7	55242465	_
	93107470C	1.5	3	0	0	7	55242466	Ξ
			-	_	-	_		

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:

 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.



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



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				
Thresholds have been modified.				
The thresholds have been modified. Ad these changes. Do you want to continue	ding or removing files will revert e?			
	OK Cancel			

Do one of the following:

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

F	Figure 3.12 Save Thresholds Warning						
C				~			
S	Save Thresholds						
	Thresholds have b	een modifed. Do you	want to save the	se changes?			
		Yes	No	Cancel			

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



To save your new thresholds:

- 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 Viewir	ng Taskbar	
Min -1.56	Max 5.82	Reset Scale Copy to Clipboard

- 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.



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:

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:

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