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 EurekaTM 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	2.83 GHz Intel Pentium Quad Core	16 GB RAM	150 GB HD + data storage	IE 8.0 and above
Service Pack 1	Processor			

*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

	A	В	С	D	E	F	G	Н
1	Sample to we	II mapping information	n file					
2	PlateName	Eureka						
з	PlateBarCode	@011970710123	4567171612309	104631				
4	PlateType		96			For	384 Plat	es, you
5	Items:					mu	st change	this :
6	WellPosition	SampleName	Pagel			Pla	teType 9	6 value
7	A1	SampleA1	PG7_12			to 3	384.	
8	A2	SampleA2	PG7_12					
9	A3	SampleA3	PG7_12					
10	A4	SampleA4	PG7_12					
11	A5	SampleA5	PG7_12					
12	A6	SampleA6	PG7_12					
13	A7	SampleA7	PG7_12					
14	A8	SampleA8	PG7_12					
15	A9	SampleA9	PG7_12					
16	A10	SampleA10	PG7_12					
17	A11	SampleA11	PG7_12					
18	A12	SampleA12	PG7_12					
	4 F	A1toA12-SamePanel1	- 96 🔶				;	•

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

1

8	🛚 affyme	trix Eureka Analysis - Pete	0	()		
	New Analysis	Dashboard Preferences				
	Libron: Faldon	Có Heard Dublich De cursente) Sureka (in sheric Suite) Hitean (
	Library Polder:	Calusers/Public/Documents/Eurekavnarysissuite/Library/				



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*.

 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

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Patrimp Busides			1985		100						100	
Anter Mill Frank Open Sound	Pate Name	where Park Name -						Mapping	Potriar	NR .		
-Deley Family No. or Land Barriers	Sergin Index Panelbecode	2		1								
	Serginized a limit Type:	Name .							Quela	tag parent		
	Sample Index Electe							Tamplete	the lot	100		
								1	Hene			
								1.1	Code			
								10000				
									Co.b.			
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	1 3 3 4 5	4 7 8 8 3		4 15 16 15	18.88	10 11 1	1 11 24		Phone:			
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	100000							40		100		
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	+00000	00000	00000	0000	00	000	000	10		H2H		
	100000	0000	0000	000	00	000	000	40		45		
	00000	00000	00000	2000	00	000	000	42		48		
	100000	00000	20000	2000	000	000	200	44	۰.	NR		
	100000	00000	00000	1000	00	000	000	40	<u>.</u>	A08		
	*00000	00000	00000		200	201	200	10		12		
		00000		2000	200	22	200	44		-		
	.00000	00000	0000	000	i a a	223	200	12		44		
		00000				000	000	40		40		· · · · ·
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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

Mapping Plate Layout	
Quadrant	
Quad staggered	
Quadrant	
Full 384 Plate	

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

	Name:	+
1	Code:	
	Name:	+
2	Code:	
Set	Name:	+
3	Code:	
Set	Name:	+
4	Code:	

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

Set	Name:	Eureka	×	
1	Code:	@0119707101234567171612309104636		
Set	Name:		+	
2	Code:			
Set	Name:		÷	
3	Code:			
Set	Name:		•	
4	Code:			

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

Exan	nple.txt - Notepad		
File E	dit Format View He	lp	
5amp1	e to well mapping	information file	
Plate	vame <plate< td=""><td>Name≻</td><td></td></plate<>	Name≻	
Plate	BarCode <plate< td=""><td>BanCode></td><td></td></plate<>	BanCode>	
Plate	Type 96	our couct	
Items	//		
wellpr	sition Sample	Name Panel	
A01	<samplename></samplename>	<panelname></panelname>	
A02	<samplename></samplename>	<panelname></panelname>	
A03	<samplename></samplename>	<panelname></panelname>	
A04	<samplename></samplename>	<panelname></panelname>	
A05	<samplename></samplename>	<panelname></panelname>	
A06	<samplename></samplename>	<panelname></panelname>	
A07	<samplename></samplename>	<panelname></panelname>	
A08	<samplename></samplename>	<panelname></panelname>	
A09	<samplename></samplename>	<panelname></panelname>	
A10	<samplename></samplename>	<panelname></panelname>	
A11	<samplename></samplename>	<panelname></panelname>	
A1Z	<samplename></samplename>	<panelname></panelname>	
B01	<samplename></samplename>	<panelname></panelname>	
B02	<samplename></samplename>	<panelname></panelname>	
B03	<samblevame></samblevame>	<panelname></panelname>	
B04	<samplename></samplename>	<panelname></panelname>	
B05	<samplename></samplename>	<panelname></panelname>	
B06	<samplename></samplename>	<panelname></panelname>	
B07	<samplename></samplename>	<panelname></panelname>	
B08	<samblevame></samblevame>	<panelname></panelname>	
в09	<samplename></samplename>	<panelname></panelname>	
B10	<samplename></samplename>	<panelname></panelname>	
B11	<samplename></samplename>	<panelname></panelname>	
B12	<samplename></samplename>	<panelname></panelname>	
C01	<samplename></samplename>	<panelname></panelname>	
C02	<samplename></samplename>	<panelname></panelname>	
C03	<samplename></samplename>	<panelname></panelname>	
C04	<samplename></samplename>	<panelname></panelname>	
Ic05	<comp onomos<="" td="" =""><td><danolnamos< td=""><td></td></danolnamos<></td></comp>	<danolnamos< td=""><td></td></danolnamos<>	



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



Settings			×
Plate 1 Color:	•	Circle Samp	le:
Plate 2 Color:	-	Circle Samp	le:
Plate 3 Color:		Circle Samp	le:
Plate 4 Color:	-	Circle Samp	le:
Control Sample Color:	-	Circle Samp	le:
Well Circle Opacity: 50%	5		
Sample Files Default Lo	cation:		
C:\Affymetrix\Eureka			
	_		
		ок	Cancel

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

Settings	×
Plate1 Color:	🗾 💌 Circle Sample: 🛑
Plate 2 Color:	🗾 👻 Circle Sample:
Plate 3 Color:	🗾 👻 Circle Sample: 📃
Plate 4 Color:	🗾 👻 Cite Sample
Control Sample Color:	Circle Sample:
Well Circle Opacity: 509	Available Colors
Sample Files Default Lo Ct\Affymetrik\Eureka	
	Advanced
	Advanced

- 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

Well Circle Opacity: 50%	
	U

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 @0119707101234567171612309104636) 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

Plate Name:	Test_Plate_1
Sample Index Plate Barcode:	1
Sample Index Block Type:	None
Sample Index Block	

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 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

🍋 a	ffyr	net	rıx				Eureka Genotyping Calculator
Plate S	Setup	Run Se	etup				
Add 3 Test_I Test_	84 Plate Plate_1 Plate_2	Open	Saved Save Save	Remove Remove	Plate Name: Sample Index Plate Barcode: Sample Index Block Type: Sample Index Block:	Test_Plate_2	1

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

W	ell Posit	Index	Original	Sample Name	Panel
	A01	1537	A01	SampleA1	PG7 <u>1</u> 2 ▼
	A02	1538	A02	Sample_A2	PG7 <u>1</u> 2 ▼
	A03	1539	A03	Sample_A3	PG7_12 ▼
	A04	1540	A04	Sample_A4	PG7 <u>1</u> 2 •
	A05	1541	A05	Sample_A5	PG7 <u>1</u> 2 ▼
	A06	1542	A06	Sample_A6	PG7 <u>1</u> 2 ▼
	A07	1543	A07	Sample_A7	PG7_12 ▼
	A08	1544	A08	Sample_A8	PG7 <u>1</u> 2 ▼
	A09	1545	A09	Sample_A9	PG7_12 ▼
	A10	1546	A10	Sample_A10	PG7_12 ▼
	A11	1547	A11	Sample_A11	PG7_12 ▼
	A12	1548	A12	Sample_A12	PG7_12 ▼
	B01	1561	B01		-

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

Panel	
Barley Bovine PG7_12 HID1 HID2	
HID3 Chicken	
Wheat	



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

🐌 af	fymetrix		Euro	eka Genotyping Calculator			2	(i)	- 0	×
Plate Set	tup Run Setup									¢
Total eq	pected output from sequencer in millions (e.g. Ne	Course Dia		Internet Direct Information		land		C		
Volume	of sequencing reaction (35-50 µL):		50	Save Big	na intormation	import blend information	Save Eureka Genotyping Calculator Fi	e Start A	Analysis	Secup
Blend	Plate(Block type/Block name)	DNA Concentration (nM) De	esired Read Depth	% of Blend Recommended	% of Ble	nd Used (Total: 0)	Volume of DNA for Sequencing To	be (Tota	d: OµL)	
	Test_Plate_1 (Plate Type: None/)	0	200	1.00		1 !				0



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

% of Blend Recomm	nended
	2.64

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

Blend	Plate(Block type/Block name)	
	Test_Plate_1 (Plate Type: EG Set 1/B)	
	Test_Plate_2 (Plate Type: EG Set 1/A)	

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

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

Save Eureka Genotyping Calculator File

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

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.

Binning process run error	د د
Error occurred while running binning process. See more information in details.	
Could not find a part of the path 'C:\Users\	\Documents\New folder\Run - 10022016_125343.brfc'.
	ОК

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

🍋 aff	, yme	trix					Eurek	a Analysis - SJ				e i -	er ×
New An	alysis	Dashbo	ard	Preferer	ices								
Mode:	Best Pra	ctices Work	flow •	Panel:	ISAG_Bov	122 •	Import Eureka Genetyping Calculator File	Remove Selected Samples Exp	oort Binner File				
Sample	Files: 3	184				Ana	alysis Settings		Threshold Settings				
Sample	Index	384 Pla	384 We	96 Plate	96 Well	Sel	lect Analysis Configurations		Select Threshold Con	figurations			
A_CL1	2689	0011	A01	@011	A01 *	[C	reate New!	 Restore Save Save As 	ISAG Boy 122 (Defou	10		 Restore Save 	Save Ac
A_CL	2690	@011	A02	@011	A02	6	Binner Parameters		Sample OC	-			
A CL.	2091 2692	0011	405	0011	A03 E	An I	alwir Input Data		- Sample de			6	
A_CL	2693	©011	A05	©011	A05		Someone Data Caler		Nam	e		Settings	
A_CL	2694	©011	A05	©011	A06		such and the		EQC Min Num Of Re	ads to Pass SN	2 🔻	20	ົ
A.CLI	2695	©011	A07	©011	A07	12	, , , , , , , , , , , , , , , , , , ,		EQC Max Num Of Si	VPs to Fail Sam	2 🔻	12	5
A_CL	2696	Ø011	A.08	©011	A08	1	- 		CC call rate (All SNR	r)	2.	90	
A_CL	2097	0011	A09	©011	A09		cus starts		ge cargate (All arth	•)			- 1
A CL	2000	0011.	A11	0011	411				EQC Specific SNP File				5
A CL.	2700	8011	A12	8011	A12	6	Sample QC		EQC Min Num Of Re	ads to Pass SN	2 🔻	20	5
A CL 1	2701	8011	A13	8011	A13	Ani	alvsis File:			0.000		r	
A.CL	2702	8011	A14	8011	A14	eu	reka-summany-genotype	•	EQC Max Num Of SI	IPs to Fail Sam	2 ¥	2	• 7
A_CL	2703	@011	A15	@011	A15	Priv	or Model File:		QC call_rate (Specific	SNPs}	2 🔻	90	•
A_CL	2704	Q011	A16	0011	A16		energy of the		Percent of passion sa	moles	2 4	90	0
A_CL	2705	@011	A17	0011	A17	90	D Line Cher	··· ^	r creatin or pairing a	inpres.	-		
A_CL.	2706	0011	A18	2011	A18	SN	P LBL FIR:		Average call rate for	passing samples	2 *	98	ົ
ACL	2707	0011	A19 A20	0011	A19 A20				S END OC				
A CL.	2709	0011	420	0011	420	Hin	nts/Inbred File (optional):		Sine QC				
A.CL	2710	0011	A22	0011	A22		Inbred 🔘 Hints	X	- Nam	e		Settings	
A_CL	2711	Ø011	A23	©011	A23	16	Genotyping		species-type		Diploi	id 🔹	1
A_CL	2712	Ø011	A24	©011	A24		white Gar		cr-cutoff		5 .	05	
A.CL.9	2713	Ø011	B01	©011	B01.	Ana	arysis rite:	-	Cr-Cuton			55	
A_CL	2714	Ø011	B02	©011	802	eu	ireka-summary-genotype	•	fld-cutoff		2 -	3.5	2
A_CL	2715	©011	803	©011	803	PO	or Model File:		het-so-cutoff		2 -	-0.4	2
A CL	2716	0011	805	0011	805	96	enenc_priors		had an also and H			8.4	
A CL	2718	6011.	B06	6011.	806	SN	P List File:		net-so-otv-cutoff			-0.4	- 1
A_CL.9	2719	0011	B07	8011	807			×	hom-ro-1-cutoff		2 -	0.5	2
A_CL	2720	8011	B08	8011	808	Hin	nts/Inbred File [optional]:		hom-ra-2-cutoff		2 +	0,3	2
A_CL	2721	8011	B09	8011	809	۲	Inbred 🛞 Hints	🗙					
A_CL	2722	0011	B10	0011	B10	Pos	sterior File Name (optional):		hom-ro-3-cutoff		5 +	-0.9	•
A_CL	2723	0011	B11	0011	B11			🗙	hom-ro		true		2
A_CL.	2724	2011	B12	2011	812	ps2	2snp File (recommended):		hom-bet		true		
A CL	2726	0011	813 R14	0011	B13	IS.	AG_Bov_122.ps2snp_map	×	nom-net		oue		
A CL.	2727	0011	B15	0011	B15				num-minor-allele-cu	toff	2 🔻	2	•
A_CL	2728	0011	B16	0011	816				priority-order		Chang	ge List Order PolyHighResolu	•
A_CL	2729	Ø011	B17	Ø011	817						-	En Del LEel Developer	
A_CL	2730	©011	818	0011	818				recommended		Check	aise PolymighResolution	• 7
A.CL.9	2731	©011	B19	Ø011	819								
A_CL	2732	©011	B20	Ø011	820								
A_CL	2733	0011	B21	0011	8/1								
LACL	12/54		D//	5011	844						_		
Output	Folder:	C:\Users\Pi	ablic\Doc	ouments\	EurekaAnaly	sisSuit	te\Output\CornSJ			Browse Bat	ch Nan	Run	Analysis

Importing your Eureka Genotyping Calculator File

I

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

٩	e affymetrix	Eureka /	Analysis - Pete_P	@ @ = @ ×
	New Analysis Dashboard Preferences			
I	Mode: Best Practices Workflow - Panel: SyGm0001	Import Euroka Genotyping Calculator File	Remove Selected Samples Export Binner File	
I	Sample Files: 0 A	nalysis Settings	Threshold Settings	
L	Sample Index 384 Plat 384 Wel 95 Plate 96 Well Se	elect Analysis Configurations	Select Threshold Configurations	

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)

Mode: Best Practices 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
 Remove Selected Samples .

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

Select Analysis Configurations



(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

Analysis Settings	
Select Analysis Configurations	
test	▼ Restore Save Save As
Binner Parameters	
Analysis Input Data:	
Sequence Data Binner Data	Q:\santaclara\GABU\EurekaNGS\Runs2 🗙
Locus Length:	
12	
Locus Start:	
6	
Sample OC	
Analysis Filer	
eureka-summany-gengbyse	
Prior Model File:	
generic prior	
SNP List File:	
Hints/Inbred File (optional):	
Inbred I Hints	🗙
o	
Genotyping	
Analysis File:	
eureka-summary-genotype	•
Prior Model File:	
EurekaPG7 models	
SNP List File:	
III A- Land Black	🗙
Hints/Inbred File (optional):	
Inbred Hints	🗙
rostenor file Name (optional):	
na Dana Ella (annana and a A)	
pszsnp rile (recommended):	
Por.pszsnp_map	×

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 Batch Name:
- 2. Click Export Binner File

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.
- Navigate and select the appropriate file, then click Open. Your newly assigned filename is displayed.
- 4. (Optional) Click the SNP List File ^t und button.
 The SNP List File window appears.
- Navigate and select the appropriate file, then click **Open**. Your newly assigned filename is displayed.
- 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.

- 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.
- 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

в	ffalos	nplist.txt -	Notep	ad
File	Edit	Format	View	Help
orob	eset	_id		
105				
406				
407				
408				
409				
410				
415				
416				
422				
423				
430				
431				
432				

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.

- Navigate and select the appropriate file, then click Open.
 Your assigned filename is displayed.
- (Optional) Click the Posterior File Name under 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 witton.The ps2snp File window appears.
- 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

Analysis Settings	
Select Analysis Configurations	
[Create New]	 Restore Save Save As

The following window appears: (Figure 3.9)

Figure 3.9 Analysis Configuration Save window

Save Analysis Configuration	×
Enter a new or select an existing configuration name	
	•
ск Са	ncel

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

Threshold Settings		
Select Threshold Configurations		
PG7_12 (Default)	 Restore Save Save 	ive As
 Sample QC 		
Name	Settings	
EQC Min Num Of Reads to Pass S	≥ ▼ 20	ຄ
EQC Max Num Of SNPs to Fail Sa	≥ ▼ 100	ก
QC call_rate (All SNPs)	≥ ▼ 90	ຄ
EQC Specific SNP File		ຄ
EQC Min Num Of Reads to Pass S	2 • 20	ຄ
EQC Max Num Of SNPs to Fail Sa	٤ 🔹 5	ຄ
QC call_rate (Specific SNPs)	2 • 90	ຄ
Percent of passing samples	≥ ▼ 90	ຄ
Average call rate for passing samp	≥ ▼ 98	ก
SNP QC		
Name	Settings	
species-type	Human 🔹	5
cr-cutoff	2 • 95	ຄ
fld-cutoff	≥ ▼ 3.6	5
het-so-cutoff	≥ ▼ -0.3	ຄ
het-so-otv-cutoff	≥ ▼ -0.3	ຄ
hom-ro-1-cutoff	≥ ▼ 0.6	ก
hom-ro-2-cutoff	≥ ▼ 0.3	ຄ
hom-ro-3-cutoff	2 -0.9	5
hom-ro	true •	ຄ
hom-het	true 🔹	ຄ
num-minor-allele-cutoff	2 • 2	ຄ
priority-order	Change List Order PolyHighResol	5

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 erater 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

🔿 Sample QC		
Name	Settings	
EQC Min Num Of Reads to Pass Sample (All SNPs)	≥ ▼ 20	•
EQC Max Num Of SNPs to Fail Sample (All SNPs)	≥ - 5	5
QC call_rate (All SNPs)	≥ ▼ 90	\$
EQC Specific SNP File		5
EQC Min Num Of Reads to Pass Sample (Specific SNPs)	≥ ▼ 20	\$
EQC Max Num Of SNPs to Fail Sample (Specific SNPs)	≥ ▼ 5	5
QC call_rate (Specific SNPs)	≥ ▼ 90	\$
Percent of passing samples	≥ ▼ 90	\$
Average call rate for passing samples	≥ ▼ 98	•

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.

 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.

D	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

🔿 SNP QC		
Name	Settings	
species-type	(Human 👻	າ
cr-cutoff	2 95	5
fld-cutoff	2 • 3.6	n
het-so-cutoff	2 .0.1	•
het-so-otv-cutoff	≥ ▼ -0.3	5
hom-ro-1-cutoff	≥ - 0.6	\$
hom-ro-2-cutoff	≥ ▼ 0.3	5
hom-ro-3-cutoff	€.0- ▼ ≤	\$
hom-ro	true 🔹	5
hom-het	(true 🔹	5
num-minor-allele-cutoff	≥ ▼ 2	5
priority-order	Change List Order PolyHighResolution, No	ค
recommended	Checklist PolyHighResolution, NoMinorHo	າ

- 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

num-minor-allele-cutoff	≥ ▼ 2

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

Click on a name, then drag and drop it to its new position. After your list is set, click OK.
Name
PolyHighResolution
NoMinorHom
OTV
MonoHighResolution
CallRateBelowThreshold
DK Cancel

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

The following window appears: (Figure 3.15)

Figure 3.15 Recommended window

recommended	×
RolyHighResolution	
OTV	
MonoHighResolution	
CallRateBelowThreshold	
Hemizygous	
OK	Cancel

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

Output Folder: C:\Users\Public\Documents\EurekaAnalysisSuite\Output\

Browse...

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

Batch Name:	Bun Analysis
baten name.	Ren Analysis



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

Save Analysis Configuration	>			
Enter a new or select an existing configuration name				
1	•			
OK Cane	el			

- 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

Save Threshold Configuration		×
Enter a new or select an existin	g configuration	name
1		•
	ОК	Cancel

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

To cancel a running analysis, click 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

🐌 affymetrıx	affymetrix Eureka Analysis - Pete 🕐 🛈) - 		
New Analysis Dash	oard Preferenc	:es					
Workflows							
Batch Name Date C	eated Workfi	Panel	Status	Elapsed	Status Message	Warning	Action
Test_5 11/6/. 3:25:23	015 Best Practice PM Workflow	^s PG7_12	Success	4 minutes		Plate(s) @0119704101234567171612309104637 @0119705101234567171612309104638 @0119706101234567171612309104636 @01197071012345	Open
Test_3 11/6/. 2:50:22	015 Best Practice PM Workflow	⁵ PG7_12	Failed	23 minutes	ERROR: All the CEL files failed for either Y loci Threshold, Loci File QC, Call Rate QC or Plate QC.	Plate(s) @0111116101234567171612309104630 . Plate pas rate < 91	is Start
Test_2 11/6/. 1:51:12	015 Best Practice PM Workflow	⁵ PG7_12	Failed		Running Binner, please wait. This may take a few minutes or hours	Aborting workflow, GetCelFileList returned 0	Start
Test_1 11/6/. 1:14:02	015 Best Practice PM Workflow	^s PG7_12	Failed		Running Binner, please wait. This may take a few minutes or hours	Aborting workflow, GetCelFileList returned 0	Start
Open S	iected Result(s)		Remove	Selected Result(s) Browse for Existing Analysis Re	suit	

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

🍋 affyrne	etrix		_			Eureka Analysis - Pete	00	- 0	×
New Analysis Workflows	Dashboard	Preferences							
Batch Name	Date Created	WorkfL	Panel	Status	Elapsed	Status Message	Warning	Action	1
Test_5	11/6/2015 3-25-23 PM	Best Practices Workflow	PG7_12	Success	4 minutes		Piste(s) @0119704101234307171012309104637 @0119705101234507171612309104638 @0119705101234507171612309104636 @01197071012345	Open	

۸

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.

After your analysis has successfully completed. click Open.
 After your 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)



🐌 affy	etrix	textb - Pete_P	00- ª ×
Summary	Sample Table SNP Summary Table		
Analy • B • P • P • V • E • Sample • N • N • N • N • N • N • N • N	Summary Name: For J2 Package Name: P67_J2 Package Name: P67_J2 Display Name: P67_J2 Display Name: P67_J2 Display Name: P67_J3 Control (1999) Created: 2/24/2016 10:38:04 AM Create: 2/24/2016 AM Create: 2/24/2	2 1 96 (100%)	
	PV-OC CE D Cluster Plot		
Scale Settin	The second statement of the		E
S 100	QC	C call_rate by 384 PlateBarcode	
10 98.75 0 97.5 96.25			
95		9E9R016KET10/LT//STEETID18K/STT0@	

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

affymetrix texts-PeterP	
Summary Sample Table SNP Summary Table BP: QC_CR Ø PV: QC_CR Ø Cluster Plot	
Analysis Summary	
 Batch Name: testb Panel Package Name: P67_12 Panel Name: P67_12 Panel Display Smare: P67_12 Ponel Display Smare: P67_12 Workflow Type: Best Practices Workflow Date Created: 2/24/2016 10:38:04 Ab4 	
Sample Summary	
Number of input samples: 96 Number of samples faling EQC : 0 Number of samples faling qC call rate specific SNPs : 0 Number of sample faling qC call rate : 0 Samples passing QC CR (All SNPs) and QC CR (Specific SNPs): 96 out of 96 Samples passing QC CR (All SNPs), QC CR (Specific SNPs): 96 out of 96 Samples passing QC CR (All SNPs), QC CR (Specific SNPs) and Plate QC: 96 out of 96 (100%) Number of Samples Genotyped: 96 Average QC CR for the passing samples: 98.503 Plate OC Summary	
Sample Index Plate Barcode Result Number Number Number Mumber Mumber Mumber Standber Percent of of af af af af af is a falling falling that camples hatch EQC after a state camples camples camples camples provide the state camples sate camples camples camples acting falling falling that camples sate camples sate camples camples provide camples sate cample	Average all rate for posting caregion
@0119708101234567171612309104636 PA65ED 96 0 0 0 96 100	26.203
View details	
SNP Metrics Summary	
 Number of SNPs: 1049 	
ConversionType Count Percentage Pab/eightEndation 44.5 20.564.06 Nablestrian 242.23.1029. Other 199.9 103.0756. MentifyEnsition 127.14.5979. CalRateBalestriantics 127.14.5979. CalRateBalestriantics 127.0 Sample OC Thresholds 3	

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

🔏 affymetrix	textb	Pote P	() () _ # ×
Summary Sample Table SNP Summary Table		EP: QC CR II PV: QC CR II Cluster Plot	0080
Analysis Summary	*	Scale Settings	III RA Dista Barrada
 Batch Name: testb Panel Package Name: PG7_12 Panel Name: PG7_12 Panel Display Name: PG7_12 Panel Display Name: PG7_12 Workflow Type: Best Practices Workflow Date Created: 2/24/2016 10:38/04 AM 		100	
Sample Summary		98.75	
 Number of input samples: 96 Number of samples failing EQC:0 Number of samples failing QC Call rate specific SNPs:0 Number of sample failing QC Call rate:0 Samples passing QC CR (All SNPs) and QC CR (Specific SNPs):96 out of 96 Samples passing QC CR (All SNPs), QC CR (Specific SNPs) and Plate QC: 96 out of 96 (10 Number of failing samples:0 Number of samples Genotyped: 96 Average QC CR for the passing samples: 98.503 	E 10%	हैं। 19 8 97.5	
Sarapio Index Plate Barcode Result Vamber Number Number Number Second of		96.25	
00119708101234567171612309104636 PA6582D 96 D 0 D	1		
View details			
SNP Metrics Summary		95	899
 Number of SNPs: 1049 			016062
ConversionType Count Parcentage Pab/sightenolation 415 20,551% Northightenolation 242 23,162% Other 199 14,07% Calification 32 2,452% Calification 32 2,45% Other 3 0,226% Other 3 0,226%			
4 19	и 3		

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

_			
Q	-	Σ	

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

SamplesbelowYLociThreshold.txt	- C X
File Edit: Format View Help	
List of sample indices below EQC maximum number of SNPS to failed sample cel_files Num_of_failed_SNPs	Å

Using the Sample Table

Figure 4.13 Sample Table window tab

Summary Sample Table	SNP Su	mmary Table				
Scatter Plot Box Plot Plate Vie	ew Concord	ance Import Sam	ple Attrib	utes 🔻 Revert Calls	•	
Apply View Save View SI	how/Hide Co	lumns 🔻 Export	• Filte	rs 🔻		
			Tatal			
Index	Pass/Fail	total num reads	Binned	num reads no snp	384 DL L D	
			Reads	,	PlateBarcode	wai
1893	Fail	374	9	365	@011970610	0
1895	Fail	902	147	655	@011970610	0.
1153	Pass	107	5	102	@011970510	A1
1154	Pass	94	22	72	@011970510	Ai E
1155	Pass	13	/	6	@011970510	A:
1150	Pass	8	2	0	@011970510	A.
1206	Pass	0	1	8	@011970510	â
1200	Pacc	5	0	5	@011970510	õ
1208	Pass	60	23	37	@011970510	a
1209	Pass	3	1	2	@011970510	C
1210	Pass	10	1	9	@011970510	a
1211	Pass	40	36	4	@011970510	a
1212	Pass	21	6	15	@011970510	CI
1225	Pass	11	4	7	@011970510	D1
1231	Pass	388	0	388	@011970510	Di
1232	Pass	29	25	4	@011970510	DE
1233	Pass	205	42	163	@011970510	D:
1235	Pass	15	0	16	@011970510	D1
1249	Pass	51	1	50	@011970510	F1
1250	Pass	29	18	11	@011970510	E
1251	Pass	27	6	21	@011970510	E
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	ES
1258	Pass	1	0	1	@011970510	E1
1259	Pass	82	35	47	@011970510	E1
1260	Pass	12	4	8	@011970510	E
1273	Pass	4	0	2	@011970510	F1 E2
1280	Pass	7	10	3	@011970510	F8
1281	Pass	4	10	4	@011970510	F9
1282	Pass	30	13	17	@011970510	F1
1283	Pass	1	0	1	@011970510	F1
1284	Pass	10	2	8	@011970510	F1 -
× [Þ.
	w Count: 968	Selected: 0 👔 🚶	L)		Show Filtered	Only



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	К11	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

G

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

Index	4	×
3	Contains Contains Equals Not Contain Not Equal	
		OK Cancel

- 2. Click inside the text entry box to enter a value. (Figure 4.19)
- 3. (Optional) Click $\overline{}$ to add additional filters.

Figure 4.20 Or or And Relationship Logic

Index	د ک
7	Contains 🔹
	Contains 🔹 📔
	Kelationship: Or O And
	OK Cancel

- 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 $\overline{\mathbf{s}}$.

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 ⁵⁶ 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

✓ Show Filtered Only

to show only the data that passes the

- Click the **Show Filtered Only** check box 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 T	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	D	©011970510	D24
1262	Pass	1	1	D	©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	D	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	D	@011970510	G23
1335	Pass	18	4	14	@011970510	H15
1337	Pass	215	D	215	@011970510	H117
1341	Pass	14	8	6	@011970510	H21
1343	Pass	102	29	73	@011970510	H23
1154	Plass	94	22	72	@011970510	A2
1156	Plass	8	2	6	@011970510	A4
1206	Pass	9	1	8	©011970510	C6
1207	Pass	5	D	5	@011970510	C7
1208	Pass	60	23	37	@011970510	C8
1209	Pass	3	1	2	@011970510	0
1211	Pass	40	36	4	@011970510	C11
1231	Pass	388	D	388	@011970510	D7
1232	Plass	29	25	4	@011970510	D8
1233	Pass	205	42	163	@011970510	D9
1250	P ass	29	18	11	@011970510	E2
1251	Plass	27	6	21	@011970510	E3
1252	Plass	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	Ø011970510	F2
1281	Pass	4	D	4	@011970510	F9
1282	Pass	30	13	17	@011970F.d	F10 -
* III						F.
₽ Find in Table ∧ ∨ Ro	w Count: 968	Selected: 0 👔	•		Show Filter	ed 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)





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**

Manage Filters		×
Add Column Filter	Cl	ear All
Column Filter		
		OK Cancel

- 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 \overline{s} .
- 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

Manage Filters		×
Add Column Filter	Cle	ar All
Column	filter	
🗙 het_rate 🔹 🔹	₹	3
	> •	7
	Relationship: 🔘 Or	🔊 🔘 And
		OK Cancel

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

Manage Filters			>
Add Column Filter		Clear All	
Column	Filter		
🗙 calLrate 🔹 🔻	< ▼		3
	× •	•	8
	Relation	nship: 🖲 Or 🔘 And	
🗶 het_rate 🔹 🔻	70 <	•	3
	>		
	2		
	-	_	
		ОК	Cancel

8. Click **OK**.

To remove a filter(s), click $\overline{}$.

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

2305	Pass	5/170.06 50	молл	42152 @011970810 A(-
2306	Pass	Copy Selected	Row(s)	25745 @011970810 A(
2307	Pass	Copy Selected	Cell(s)	20190 @011970810 AC
2308	Pass	Set User Color		Insport Eile
2309	Pass	Set User Color	•	Import File
2310	Pass	Remove User C	Color	Red
2329	Pass	477098 43	37804	Gold
2330	Pass	460265 43	39543	Plue
2331	Pass	495651 47	/1181	Blue
2332	Pass	459045 43	36435	Aquamarine
2333	Pass	479343 45	54450	Gray
2334	Pass	476585 43	37551	LightGrav
2353	Pass	499171 47	73969	Lightolay
2354	Pass	533596 51	1654	Green
2355	Pass	582742 55	53209	RoyalBlue
2356	Pass	562714 53	31536	Orange
2357	Pass	516363 49	90467	Olange
2358	Pass	542929 51	19820	LawnGreen
2377	Pass	558135 52	28948	Magenta
2378	Pass	438946 41	18536	Pressee
2379	Pass	531340 49	99107	Drown
2380	Pass	526697 44	15298	Yellow
2381	Pass	536910 50	00478	Purple
2382	Pass	513601 46	58617	Diank
2401	Pass	552972 52	20671	BINCK
2402	Pass	523435 50	0715	22720 @011970810 E0

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

1	affymetrix Summary Sample Table SNP Summary Table									
ľ	Summary Sample Table Style Summary Table Scatter Plot Box Plot Plate View Concordance Import Sample Attributes Revert Calls Apply View Save View Show/Hide Columns Export Filters									
	Loci QC call_rate	Number of samples	Number of samples failing EQC_Min_Num_Of_Reads criteria	Number of samples	Number of sample failing	Number of samples	Percent of passing	Aver call 🔶 rate f	user_color	
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
	0	384	0	380	1	3	0.781	42.623		
				202		-	A 704	10.000	1	

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

File	Edit Format	View Help	
cel 230 230 230 230	_files 5 red 6 black 7 red 8 black	user_color	^
230 231 232	9 red 0 black 9 red		

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**.

1

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)

2305	Pass	547096 504944	42152 @011970810 A(~
2306	Pass	Copy Selected Row(s)	25745 @011970810 A(
2307	Pass	Copy Selected Cell(s)	20190 @011970810
2308	Pass	Set User Color	1 Import File
2309	Pass	Set Oser Color	F Import File
2310	Pass	Remove User Color	Red
2329	Pass	477098 437804	Gold
2330	Pass	460265 439543	Plue
2331	Pass	495651 471181	Ditte
2332	Pass	459045 436435	Aquamarine
2333	Pass	479343 454450	Gray
2334	Pass	476585 437551	LinktCons
2353	Pass	499171 473969	Lightoray
2354	Pass	533596 511654	Green
2355	Pass	582742 553209	RoyalBlue
2356	Pass	562714 531536	- Course
2357	Pass	516363 490467	Orange
2358	Pass	542929 519820	LawnGreen
2377	Pass	558135 528948	Magenta
2378	Pass	438946 418536	Press
2379	Pass	531340 499107	brown
2380	Pass	526697 445298	Yellow
2381	Pass	536910 500478	Purple
2382	Pass	513601 468617	Disal
2401	Pass	552972 520671	BIBICIX
2402	Pass	523435 500715	22720 @011970810 E0

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

- 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
- 𝒫 Find in Table _____

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

🐌 affymetrix						Test_5	Pete			00-	a ×
Summary Sample Tabl	sNP Su	mmary Table					BP: QC_CR	PV: QC_CR 🖾 Cluster	Plot		
Scatter Plot Box Plot Plate Vi	iew Concord	lance Import Sam	ple Attrib	utes 🔻 Revert Call:			Scale Setting	s			
Apply View 💌 Save View S	how/Hide Co	lumns 💌 Export	▼ Filte	ars 🔻				QC cal	I_rate by 384 PlateBarcode		
			Total		284	26	93				
index 🐣	Pass/Fail	total_num_re	Binned Reads	num_reads_no_snp	PlateBarcode	WellPo					
2304	Pass	12	6	6	@011970710	P24 ~					- 11
2299	Pass	16280	4	16276	©011970710	P19					- 11
2298	Pass	1992	1850	135	@011970710	P18 =					- 11
2294	Pass	13	4	9	@011970710	P14					- 11
2292	Pass	11	3	8	@011970710	P12	79.75				
2290	Pass	15	5	10	©011970710	P10					- 11
2289	Pass	14	3	11	Ø011970710	P9					- 11
2298	Pass	144	124	20	@011970710	P9					- 11
2286	Pass	11	2	9	©011970710	P6					- 11
2283	Pass	14	10	4	@011970710	P3	Ē				- 11
2281	Pass	12	11	1	@011970710	P1	5				- 11
2272	Pass	165	114	51	©011970710	C16	ц.				- 11
2267	Pass.	1636033	1482434	153599	8011970710	011	✓ 66.5				
2265	Pass	21	11	10	2011970710	09					- 11
2261	Pass	11	6	5	@011970710	05					- 11
2255	Pass	13	3	10	©011970710	N23					- 11
2253	Pess	71	60	11	\$011970710	N21					- 11
2252	Pass	113	61	52	@011970710	N20					- 11
2251	Pass	17	3	14	@011970710	N119					- 11
2250	Pass	12	1	11	@011970710	N18	53.25				
2247	Pass	12	8	4	Ø011970710	N15	35.25				- 11
2246	Pass	161	2	179	@011970710	N14					- 11
2245	Pass	104	44	60	©011970710	N113					- 11
2244	Pess	1613	19	1594	@011970710	N12					- 11
2242	Pass	11	3	8	@011970710	NLO					- 11
2238	Pass	142	132	10	©011970710	N6					- 11
2230	Pass	161	111	50	Ø011970710	M22					- 11
2223	Pass	10	2	8	Ø011970710	MLS	40		~		
2222	Pass	195	6	199	@011970710	ML4		63	100	259	
2220	Pass	116	51	65	©011970710	ML2		316	10	310	
2219	Pess	14	13	1	Ø011970710	M11		8	8	<u>10</u>	
2217	Pass	16	6	12	@011970710	M9		10	5	5	
2214	Pass	13	8	5	©011970710	M6		EL	5	EL	
2212	Pass	13	2	11	Ø011970710	644		8	29	<u>8</u>	
2210	Pass	1481	58	1423	2011970710	M2		123	137	123	
2207	Pass	1676	1543	133	@011970710	L23		410.	210	310	
2204	Pass	11	3	8	©011970710	L20		016	102	0/6	
2198	Pess	1	1	0	@011970710	L14 *		10	10	511 11	
×						P.		8	6	8	
P Find in Table ^ V Re	ow Count: 354	Selected: 1 👔	,		🔽 Show Filter	red Only					



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

Scale Settings		×
☑ Auto Scale Min Y: 95	Max Y: 100	
		Default
	ОК	Cancel

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

New BP		×
Group By:	Pass/Fail	•
r-axis:	total_num_reads	
	ОК	Cancel

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

BP: 🖾 BP: Q 🖾 SP: QC_CR vs 🖾 PV: 🖾 PV: Q 🖾 Cluster	٢.	×	
Scale Settings	A		Remove 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

New SP		×
X-axis:	Total Binned Reads	•
Y-axis:	QC call_rate	•
	ОК	Cancel

- 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

BP: 🖾 B	P: Q 🖾	SP: QC_CR vs 🖾	PV: 🖾	PV: Q 🖾	Cluster	۲.,	×		
Scale Settings)							Remove 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



0

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

Scale Set	ttings		×
🔽 Aut	o Scale		
Min X:	421224	Max X:	636894
Min Y:	382836	Max Y:	600199
			Default
		ОК	Cancel

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

Color Scale Confi	guration	×	
28.941	Auto Scale Cutoff Type: Above Cutoff E	ilion	
26 125-	Max: 28.941		Above Cutoff Failing
	Cutoff: 26.125		Above Cutoff Failing Below Cutoff Failing No Cutoff
23 309	Min: 23.309		
	OK	Cancel	

- 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

Color Scale Cont	figuration 🗙
28.941	Auto Scale Cutoff Type: Below Cutoff Failing Max:
26 125	28.941 • • • • • • • • • • • • • • • • • • •
23 309	23.309
	Default
	OK Cancel

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

Figure 4.49 No Cutoff

Color Scale Co	onfiguration	×
28.941	Auto Scale Cutoff Type: No Cutoff	
26 1 25	Max: 28.941 Cutoff:	
23.309	26.125 Min: 23.309	
		Default
	ОК	Cancel

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

New PV		×
Metric: Pass/Fail		•
	ОК	Cancel

- 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

BP: 🖾 BP: Q 🖾 SP: QC_CR vs 🖾 PV: 🖾 PV: Q 🖾 Cluster		3 🗆 🗖 🖉
Scale Settings	1	Remove Tab

To customize your Plate View settings:

1. Click Configure.

The following window appears: (Figure 4.53)

Figure 4.53 Color Scale options

Color Scale Co	nfiguration 🗙
28.941	Auto Scale
	Above Cutoff Failing 🔹
	Max:
26.125	28.941
	Cutoff:
	Min:
23,309	23.309
	Default
	OK Cancel

- 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** witton.

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

Concordance	×
 Compare all combinations Compare to reference 	
Browse for reference file:	
Compare all SNPs Compare SNPs within SNP Summary Table	
UK Cancel	

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

Apply Vie	w 🔻 Save Vie	w/Show/Hie	de Columns 🔻	Export 🔻 Fil	ters 🔻	
			e e			
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			
5	2305	2329	54.816			
7	2305	2330	57.056			
В	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

Select Annotatio	n Manage SNP Lie		Change/Revert Calls *	Reanaly												
Apply View	Save View Show	Hide Co	umos + Boort +	Fibers .	1000											
11.7 80	1 million and the second		AND A DOT			0	Common State		-					0	-	
probeset.id	ConversionType	CR	Minos Allele Trequency	KW.p- Villue	FLD	HamFLD	Het50	Hom20	Nclus	n_AA	n,48	n,58	NC	henizygous	HomHet	Call Modify
00	Other	88.889	0.375	0.007	-	20.56	01-01	2.317	2	- 3	0	5	. 1	0		False
01	NoMine Here	100	0 222	1	10.478		0.261	2.714	. 2	0	- 4	. 5	0	0	1	Falce
62	Other	55.556		1				387	1	- 5	. 0	0	- 4	0		False
103	Other	66.667	0.333	0.03		22.125		2.609	2	4	0	2	3	0		Falce
64	Other	66.667	0.5	0.022		23377		2.783	- 2	3	. 0	1	3	0		False
05	MonoHighRap.	100	9	1				2.824	- 2	0	0	9	0	0		Falce
66	OTV	100	0.278	1	6.53		-0.389	1.207	2	4	- 5	0	ð	0	1	False
07	Other	66.667	0.167	0.091		24,346		2,487	2	5	0	1	. 3	0	0	False
08	CalRateBelow	68.689	0.25	1	12:403	2 2022	0.556	3.074	2	Ó	- 4	4	3	0	3	False
09	PolyHighResoL.	100	0.444	1	9.731	22.156	0.734	2.265)	2	- 4	1	Ô	0	- 0	False
10	Other	88.889	0.125	0.067		20 635		1.817	2	1	0	7	- 1	0	. 0	Falce
13	Other	77.778	0.343	0.077		24.421		2.019	2	6	0	1	2	0		False
12	Other	77.778	9	1				3.069	1	7	9	0	2	0		False
43	CallRateBellow-	88.889	0.062	1	10.325	1	0.547	2.365	2	7	1	0	1	0	3	Falce
14	Other	77.778	0	1				1.892	. 3	0	. 0	7	2	0		False
15	Other	66 667	0	1				2.552	- 1	6	ð	Ó	3	0		False
16	MonoHighRes	100	0	1				0.7	1	0	0	9	0	0	0	False
17	MonoHighRes.	100	0	1				1.878	3	9	ó	0	0	0	. 0	Falce
18	Other	66.667	0	1				3,009	1	0	0	6	3	0		False
19	Other	77.778	0.285	0.021		20 235		2.408	2	2	9	5	2	0		False
20	Other	100	0 311	0.059		27.389		2.072	2	8	0	1	0	0	0	False
21	Other	55-556	0	1				2.891	1	5	0	0	4	0		False
122	Other	66 667	0	1				1.896	- 1	6	0	0	3	0		Falce
23	CallfateBelow-	77 778	0.071	1	12.78		0.504	2.854	2	Ó	3	6	2	0	1	False
24	Other	66.667	0.367	0.091		15.294		1,242	2	1	0	. 5	3	0		False
25	Öther	100	0.511	0.059	-	17.745		1.956	2	- 1	à	6	ð	0		False
126	Other	27.278	0.5	0.339	3.038	12.04	-0.293	1.168	3	3	3	3	2	0		False
127	Other	77.778	0.286	0.441	2076	12 142	-0.028	1.099	3	4	2	1	2	0	0	Falce
26	Other	66.667	0	1				2.317	1	6	0	0	3	0		false
29	RelyHighResel	100	0.278	1	5./95	20 761	0.067	1.664	3	5	3	1	0	0		False
30	CallFateBelow-	683.83	0.25	0.365	6.993	16.627	0.565	1.97)	5	2	1	1	0		false
80	Other	77.778	0	1				4.681	1	7	0	0	2	0		False
32	Other	33.333	0	1				2.537	1	3	- 0	0	6	0		False
02	CARDARA DIRAL	AL ALL	.0.35		1.661		6.302	n 30	- 1		3				1	Lales

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

Show/Hide Columns 🔻	
\checkmark	probeset_id
	affy_snp_id
\checkmark	CR
\checkmark	FLD
\checkmark	HomFLD
\checkmark	HetSO
\checkmark	HomRO
	nMinorAllele
\checkmark	Nclus
1	n AA

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

Save Curren	t View	×
Custom Vi	ew Name:	
		-
	ОК	Cancel

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

11086538	100	59.701	31.343	B.955	0.246 0.202 PolyHighResolution
11086545	100	35.821	50.746	13.123	0.388 0.575 DolyHighResolution
11086572	100	32.836	28.358	3	Copy Selected Row(s)
11086574	100	8.955	17.91	7.	Copy Selected Cell(s)
11086577	100	44.776	41.791	1	Add Colored CND (Sec.
11086578	100	22,388	25,373	5	Add Selected SNP(s) to SNP List
11086580	100	8.955	32.836	5	Remove Selected SNP(s) from SNP List
11/196501	1/20	47 761	25 021	16 775	0 0.2421_0.00201abyEvabPeratution

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)

alls.t	kt - Notepac	(
File Ed	it Format	View Help)												
probes	et_id	1921_ca	ll_code	1922_ca	11_code	1923_ca	ll_code	1924_ca	11_code	1925_ca	ll_code	1926_ca	11_code	1927_ca	ll_code
11_cod	e 1989_Ca	11_code	1990_ca	11_code	1991_ca	11_code	1992_ca	11_code	1993_ca	11_code	1994_ca	11_code	1995_ca	11_code	1996_ca
ode	2058_ca	11_code	2059_ca	11_code	2060_ca	11_code	2061_ca	11_code	2062_ca	11_code	2063_ca	11_code	2064_ca	11_code	2065_ca
2126_C	all_code	2127_ca	11_code	2128_ca	11_code	2129_ca	11_code	2130_ca	11_code	2131_ca	11_code	2132_ca	11_code	2133_ca	11_code
call_	code	2195_ca	11_code	2196_ca	11_code	2197_ca	11_code	2198_ca	11_code	2199_ca	11_code	2200_ca	11_code	2201_ca	11_code
1_code	2265_Ca	11_code	2266_ca	11_code	2267_ca	11_code	2268_ca	11_code	2269_ca	11_code	2270_ca	11_code	2271_ca	11_code	2272_Ca
	AA	AA	NOCALL	AB	AB	AB	AA	AB	AB	AA	AB	AA	AA	AB	AB
A	AA	AA	AA	NoCall	AA	AA	AB	AB	AA	AB	AB	AA	AB	AB	AA
AB	BB	AB	BB	BB	AA	BB	NoCall	BB							
A	AA	AB	BB	AB	BB	AB	BB	BB	AB	AB	AA	AB	AA	AB	AB
NoCal1	AA	BB	AA	AB	AB	BB	AD	AB	BB7	AA	AD	AB	AA	NoCall	AA
BB	BB	AA	BB	AA	AA	BB	AA	NOCall	BB	BB	AA	AA	AA	AA	AA

3. Click Open.

н

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

Regenerate SNP Metric		×
Select Posterior File:		
Default	•	Browse
Select ps2snp File (Recomme	ended):	
	Browse	Clear
Select Threshold Configura	tions	
Batch Configuration	▼ Restore Save Sa	ave As
SNP QC		
Name	Settings	
species-type	Human 🔹	ຄ
cr-cutoff	≥ ▼ 95	ຄ
fld-cutoff	≥ ▼ 3.6	ก
het-so-cutoff	2 🔻 -0.1	ຄ
het-so-otv-cutoff	≥ ▼ -0.3	ຄ
hom-ro-1-cutoff	2 • 0.5	ຄ
hom-ro-2-cutoff	≥ ▼ 0.3	ຄ
hom-ro-3-cutoff	2 • -0.9	ຄ
hom-ro	true 🔻	ຄ
hom-het	true 🔻	ຄ
num-minor-allele-cutoff	2 🔻 2	ຄ
priority-order	Change List Order PolyHighRes	ຄ
recommended	Checklist PolyHighResolution,	5
	ОК С	ancel

- 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

Change the Priority Order					
Click on a name, then drag and drop it to its new position. After your list is set, click OK.	,				
Name					
PolyHighResolution					
NoMinorHom					
OTV					
MonoHighResolution					
CallRateBelowThreshold					
	_				
	-				
0K Cancel					

- 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

recommended	×					
PolyHighResolution						
I NoMinorHom						
III ΟΤV						
MonoHighResolution						
CallRateBelowThreshold						
I Hemizygous						
OK Can	cel					

- 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

Run PS Supplemental			×
Select Posterior File:			
Default		▼ Bro	wse
Select Threshold Configura	tions		*
Batch Configuration	▼ Restore Save S	ove As	
PS Supplementa	1		
Name	Settings		
homfld-cut	≥ ▼ 6.5	ก	
bb-varx-cut	≥ ▼ 10	ຄ	
bb-vary-cut	≥ ▼ 10	ຄ	
ab-varx-cut	2 • 10	ຄ	
ab-vary-cut	≥ ▼ 10	ຄ	
aa-varx-cut	≥ ▼ 10	ຄ	
aa-vary-cut	≥ ▼ 10	ຄ	
bb-varx-z-cut-enabled	true 👻	ก	
bb-varx-z-cut	2 🔻 3	ຄ	
bb-vary-z-cut-enabled	true •	ຄ	Ξ
bb-vary-z-cut	2 • 3	ຄ	
ab-varx-z-cut-enabled	true 🔻	ຄ	
ab-varx-z-cut	2 - 3	ก	
ab-vary-z-cut-enabled	true 🔹	ຄ	
ab-vary-z-cut	2 -	ຄ	
aa-varx-z-cut-enabled	true 🔹	ຄ	
aa-varx-z-cut	≥ ▼ 3	ຄ	
aa-vary-z-cut-enabled	true 🔹	ຄ	
aa-vary-z-cut	2 🔻 3	ຄ	
clustermin	2 - 5	ຄ	
n-minor-hom-cut	2 🔻 0	ຄ	
hetv-maf-cut	≥ ▼ 10	ຄ	
variance-class	Checklist PolyHighResolution	ຄ	*
	OK	Care	

- 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.
- To change the variance-class options, click Checklist
 The following window appears: (Figure 4.66)

The following window appears: (Figur

Figure 4.69 Variance-class window

variance-class					
V PolyHighResolution					
🔲 NoMinerHem					
III ΟΤV					
MonoHighResolution					
CallRateBelowThreshold					
Other					
E Hemizygous					
OK Cancel					

- 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

 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

	Α	В
1	probeset_	id
2	85040799	
3	85040886	
4	85040932	
5	85041189	
6	85041517	
7	85041551	

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

prob	eset_id		
2	In SNP List	• CallRa	te_Greater_Than
		ОК	Cancel

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

3	Default Browse None	7
Prior: referencePriors_heavy_C Posterior:	Default 🔻	Special SNPs:

- 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

Scale Settings		×
👿 Auto Scale		
Min X: -17	Max X:	708
Min Y: -17	Max Y:	708
		Default
	ок	Cancel

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

Color Scale Con	figuration	×	
28.941	Auto Scale Cutoff Type: Above Cutoff I Max: 28.941 Cutoff: 26.125 Min:	Failing V	Above Cutoff Failing Above Cutoff Failing Below Cutoff Failing No Cutoff
23 309	23,309	Default	
	ОК	Cancel	

- 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

Color Scale Cont	iguration	×
28 941 26 125	Auto Scale Cutoff Type: Below Cutoff F Max: 28.941 Cutoff: 26.125 Min: 23.309	ailing •
		Default
	ОК	Cancel

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

Figure 4.84 No Cutoff

Color Scale Configuration			
28.941	Auto Scale Cutoff Type: No Cutoff		
26.125	Max: 28.941		
	Cutoff: 26.125	•	
23 309	Min: 23.309		
		Default	
	ОК	Cancel	

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.

Exporting Genotyping Data

œ

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

Export Genotyping Data 🗙				
Result Output Formats: TXT OVCF OPLINK (PED) OPLINK (TPED)				
Call Output Format	is: 🔘 Forward Strand Base Call 🔘 Call Codes 🔘 Numeric C	Call Codes		
Index / Sample Ma	pping: 🔘 Index 🍥 Sample			
Exported Data: 🔲	Confidence 🔲 Signal			
Input and Output	Files			
SNP List Filter		···· ×		
Output Location:	C:\Users\Public\Documents\EurekaAnalysisSuite\Export\			
Output Name:		.txt		
Annotation File:			_	
PG7_12.v5.201511	13.annot.db	▼ Browse		
SNP Identifier			5	
			<u> </u>	
Select Annotation	Column(s) to Add:			
Check/Unchec	k All			
affy_snp_id_an	not	4		
Chromosome	Start			
Chromosome	Stop	,	,	
		OK Cano	el	

Result Output Formats

Figure 5.4 Result Output Format selections

Result Output Formats:
TXT OVCF OPLINK (PED) PLINK (TPED) Include Pedigree Information

- 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.

1

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

Call Output Formats: O Forward Strand Base Call
Call Codes O Numeric Call Codes



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

```
Index / Sample Mapping: O Index O Sample
```

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

Input and Output	Files	
SNP List Filter:		 - ×
Output Location:	C:\Users\Public\Documents\EurekaAnalysisSuite\Export\	
Output Name:		 .txt

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

Input and Output	Files	
SNP List Filter:	C:\Users\Public\Documents\EurekaAnalysisSuite\Library\I	-
Output Location:	C:\Users\Public\Documents\EurekaAnalysisSuite\Export\	
Output Name:		.txt

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.

 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

SNP Identifier	-
Probe_Set_ID	

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

 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

Report Settings			×	
File Name:			Browse	
Picture Setting	js:			
O All SNPs free	om Curr	ent Table		
Random SI	VPs from	n Current Table	2	
SNP Count	200			
Row Count	4			
Col Count	3			
			Default	
Conversion Typ	pes:			
Check/Uncl	heck All			
PolyHighR	esolutio	n		
📃 NoMinorH	lom			
CallRateBe	lowThre	shold		
📃 MoneHigh	Resolut	ion		
C Other				
📃 Hernizygo	Hernizygous			
ΟΤΟ				
		OK	Cancel	

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

🔭 Inbred File Generation Tool	
	About Help
BRFC File Location: Penalty File Location: Output File Location: Status Messages:	Generate Exit

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.

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

	А	В
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

Inbred File Generation Tool		
		About Help
BRFC File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	 Generate
Penalty File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	 Exit
Output File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	
Status Messages:		

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

Inbred File Generation Tool		_ 🗆 X
		About Help
BRFC File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	 Generate
Penalty File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	 Exit
Output File Location:	C:\Users\cgates\Desktop\Vineet_New Corn	
Status Messages:	Inbred file generated successfully.	

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

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

	А	В	
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

· · · ·	
Analysis File:	
eureka-summary-genotype	•
Prior Model File:	
generic_prior	🗙
SNP List File:	
	··· ×
Hints/Inbred File (optional):	
🔍 Inbred 🔘 Hints	🗙
 Genotyping 	
Analysis File:	
eureka-summary-genotype	•
Prior Model File:	
EurekaPG7models	🗙
SNP List File:	
	X
Hints/Inbred File (optional):	
Quadred ◎ Hints	×
	· · · · · · · · · · · · · · · · · · ·

- 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	o select you	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: <i>http://www.ncbi.nlm.nih.gov/SNP/</i> (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.
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

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.

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

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	Minimum number of reads a SNP must have to be considered a passing SNP.
(All SNPs)	
EQC Max Num Of SNPs to Fail Sample	For a sample to pass EQC, the maximum number of SNPs (per sample) that are allowed to fail.
(All SNPs)	
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	Minimum number of reads a SNP from the EQC specific SNP file must have for the SNP to pass EQC.
(Specific SNPs)	
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 <i>ps_classification</i> 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

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.
	<u>Fisher's Linear Discriminant (FLD)</u> = $Min(i = aa, bb) \left\{ \frac{ M_{ab} - M_i }{sd} \right\}$
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.

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

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.
	$x^{2} = \frac{(f^{2}aa - fa)^{2}}{f^{2}aa} + \frac{(2faafbb - fab)^{2}}{2faafbb} + \frac{(f^{2}bb - fb)^{2}}{f^{2}bb}$
	Where:
	$fa = \frac{(\#AA \ Calls \)}{Total \ \# \ Calls}$
	$fb = \frac{(\#BB \ Calls \)}{Total \ \# \ Calls}$
	$faa = \frac{(\#AA \ Calls + 0.5 * \# AB \ Calls)}{Total \# \ Calls}$
	$jbb = \frac{(\#BB \ Calls + 0.5 * \#AB \ Calls)}{Total \# Calls}$
	$fab = \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.
Obtaining support

Technical support	For the latest services and support information for all locations, visit www.thermofisher.com .
	At the website, you can:
	• Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
	• Search through frequently asked questions (FAQs)
	• Submit a question directly to Technical Support (thermofisher.com/support)
	• Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
	Obtain information about customer training
	Download software updates and patches
Safety Data Sheets (SDS)	Safety Data Sheets (SDSs) are available at thermofisher.com/support .
Limited product warranty	Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms- and-conditions.html . If you have any questions, please contact Life Technologies at www.thermofisher.com/support .



 $thermofisher.com/support \mid thermofisher.com/askaquestion$

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20 December 2018