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TaqMan® OpenArray® Genotyping

Troubleshooting Guide

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About This Guide

Purpose

The *TaqMan® OpenArray® Genotyping Troubleshooting Guide* provides information for troubleshooting the OpenArray® system, including:

- Troubleshooting information for loading TaqMan® OpenArray® Genotyping Plates.
- Troubleshooting information for data analysis in the OpenArray® SNP Genotyping Analysis Software.

Prerequisites

This guide assumes that you have access to and are familiar with the procedures provided in the *TaqMan® OpenArray® Genotyping Getting Started Guide* (see "System documentation" on page 67).

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

Safety information

Note: For general safety information, see this section and the "Safety" Appendix in the *TaqMan® OpenArray® Genotyping Getting Started Guide*. When a hazard symbol and hazard type appear by an instrument hazard, see the "Safety" Appendix in the Getting Started Guide for the complete alert on the instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments*.

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, refer to the "Safety" Appendix in the *TaqMan® OpenArray® Genotyping Getting Started Guide*.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**CAUTION!** Hot surface.	**ATTENTION!** Surface brûlante.
	**CAUTION!** Class 2(II) visible and/or invisible laser radiation present when using the instrument and barcode scanner. Do not stare directly into the beam or view directly with optical instruments.	**ATTENTION!** Rayonnement visible ou invisible d'un faisceau laser de Classe 2(II) en cas d'ouverture et de neutralisation des dispositifs de sécurité. Ne pas regarder le faisceau directement ou au travers d'un instrument optique.
	**CAUTION!** UV LIGHT HAZARD. UV light may harm your skin and eyes. Keep at least 25 cm distance.	**ATTENTION!** Dangers liés aux rayons UV. Les rayons UV peuvent endommager votre peau et vos yeux. Gardez une distance de plus de 25 cm.

Hazard symbol	English	Français
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

1 Guidelines for the TaqMan® OpenArray® Genotyping Plates

This chapter provides:

- Guidelines for handling and loading the TaqMan® OpenArray® Genotyping Plates (page 10)
- Detailed procedures for viewing the ROX™ dye image file, using the ImageJ software (page 13)

  The image file can help you find problems caused by incorrectly loading and/or handling the genotyping plates.
- A troubleshooting table that illustrates common problems that can be seen in the ROX dye image files (page 18)

Workflow

This chapter is intended to be used as supplemental information for Chapter 3 of the *TaqMan® OpenArray® Genotyping Getting Started Guide* (PN 4377476), "Prepare the TaqMan® OpenArray® Genotyping Plates." The workflow is:

1. Prepare for loading.
2. Place a TaqMan® OpenArray® Genotyping Plate in an OpenArray® AutoLoader Plate Holder.
3. Load the OpenArray® AutoLoader Tip Blocks.
4. Run the OpenArray® AutoLoader.
5. Seal the TaqMan® OpenArray® Genotyping Plates.

Guidelines for handling the plates

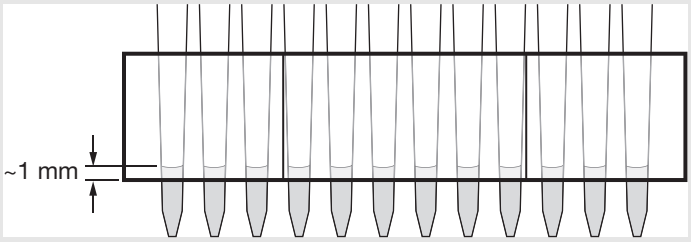
To ensure that you obtain successful results with the genotyping plates, follow the handling procedures provided in Chapter 3 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*. In particular, be sure to follow the guidelines listed below.

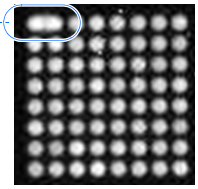
- Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the genotyping plate during loading.
- Hold the genotyping plate by the edges, at the end opposite from the barcode. Do not touch the through-holes
- *Within 1 hour after opening the plate packaging*, load the genotyping plate with sample, place the loaded plate in a TaqMan® OpenArray® Genotyping Case, then seal the case.
- If you drop a loaded genotyping plate, discard it in the appropriate waste container.

Guidelines for loading the plates

To ensure the genotyping plates are successfully loaded with sample, follow the loading procedures provided in Chapter 3 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*. In particular, be sure to follow the guidelines listed below.

Procedure in Chapter 3 of the Getting Started Guide	Guideline
"Prepare for loading"	Before you begin the sample loading procedure, be sure the OpenArray® Plate Guide Set, OpenArray® AutoLoader Tip Blocks, and OpenArray® AutoLoader Plate Holder are completely clean and dry. Residual water prevents the samples from loading correctly into the genotyping plates. Detailed cleaning procedures are in Appendix A of the *TaqMan® OpenArray® Genotyping Getting Started Guide*.
"Place a TaqMan® OpenArray® Genotyping Plate in a plate holder"	Properly align the genotyping plate in the plate holder. The genotyping plate should reach all the way to the right of the plate holder.

Procedure in Chapter 3 of the Getting Started Guide	Guideline
"Load the OpenArray® AutoLoader Tip Blocks"	After placing the plate guide on the OpenArray® 384-Well Sample Plate, be sure that the plate guide sits flat on the benchtop. The plate guide should not be tilted by the sample plate beneath it. To check the plate guide position, gently slide the plate guide across the benchtop. If the plate guide is not aligned correctly, it slips toward the base of the sample plate.
	Do not press firmly when inserting the OpenArray® Loader Tips into the tip block. Let the tips drop into the tip block slots.
	After the tips are placed into the tip block, slide the tip block up and down (25 to 50 times), until the tips are filled to 1 mm above the bottom edge of the tip block. Visually inspect the tip block to ensure that: • The tips are filled to 1 mm above the bottom edge of the tip block. • There are no air bubbles. You can remove the tip block to look at the tips. When you replace the tip block to load more sample, be sure to: • Level the tip heights. • Keep the tip block in the same orientation. If you turn the tip block around, the samples will mix together and become contaminated. 
"Run the OpenArray® AutoLoader – Set up the AutoLoader"	When placing the tip block into the AutoLoader: **1.** Align the tip block with the metal guide pins on the AutoLoader. **2.** Bring the tip block straight down, without tilting it. **IMPORTANT!** Perform this step slowly and evenly to prevent improper sample loading (for example, too much sample or not enough sample).
	Be sure the tip heights are level: • Gently slide your finger across the tops of the tips so that the tip heights are level. *Or* • Gently rest another tip block on top of the tips until the tip heights are level, then remove the tip block.

Procedure in Chapter 3 of the Getting Started Guide	Guideline
"Run the OpenArray® AutoLoader – Load the sample"	When removing the tip block, slowly pull the tip block straight up, without any rocking motion. To prevent rocking, it may be helpful to hold the tip block with your index finger and thumb, and press your remaining fingers against the AutoLoader surface. Removing the tip block in this manner may prevent liquid bridges in the stop position. Liquid bridge 
	When removing the genotyping plate from the plate holder: 1. Place the plate holder on a flat surface. 2. Push the latch down, then carefully lift the genotyping plate from the plate holder with one hand. 3. With the other hand, grasp the edge of the genotyping plate and lift it out.
"Seal the TaqMan® OpenArray® Genotyping Case"	When inserting the genotyping plate into the genotyping case, align the genotyping plate with the grooves in the case. Misalignment may cause surface rubbing, loss of samples, and/or contamination.

View the image file to find potential problems

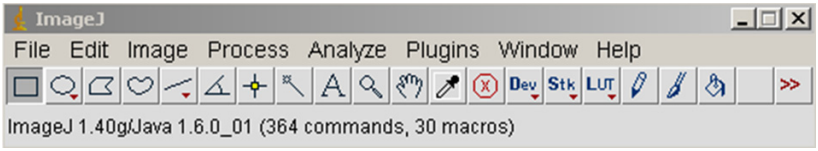
The ROX™ dye image files generated by the OpenArray® system can reveal potential problems with an experiment. Use the ImageJ software to open the ROX dye image files.

Note: The ImageJ software is installed on the computer with the OpenArray® SNP Genotyping Analysis Software. You can also download a free copy of the ImageJ software from the Internet (from your Internet browser, search for **ImageJ**).

View the ROX™ dye image file

1. On the computer desktop, double-click 

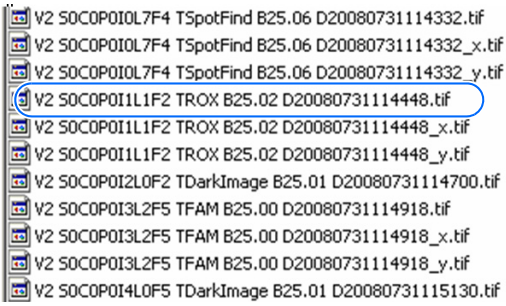
to start the ImageJ software. The ImageJ toolbar is displayed.



2. Navigate to and open the Images folder: **C:\Images**.

3. Locate the ROX dye image file of interest.

   The ROX dye image file is a *.tif file. The file name includes "TROX" and the date the file was created.

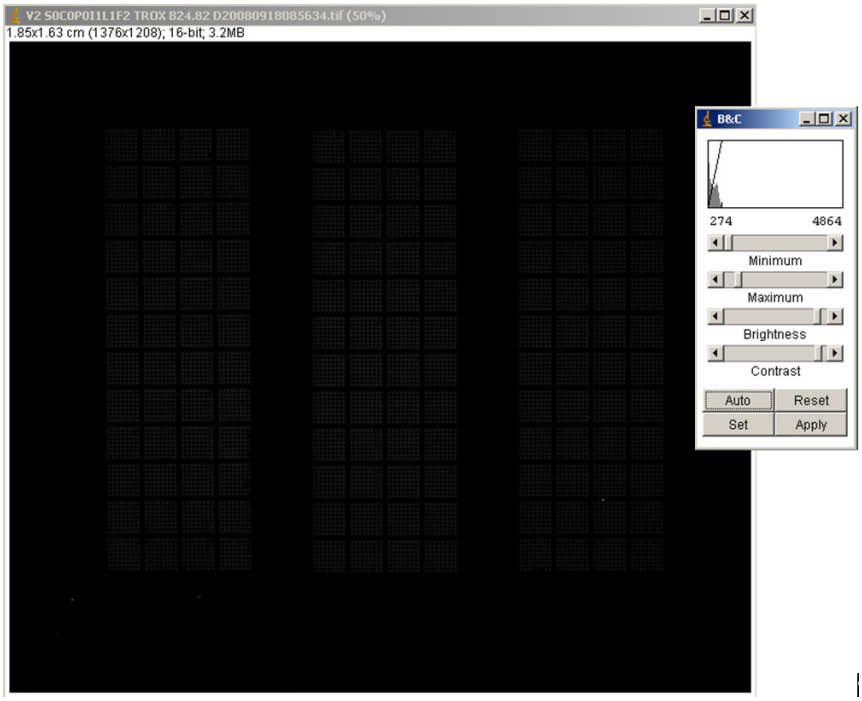


4. To view the image file:
   - In the Images folder, select the image file, then drag and drop the file on top of the ImageJ toolbar.

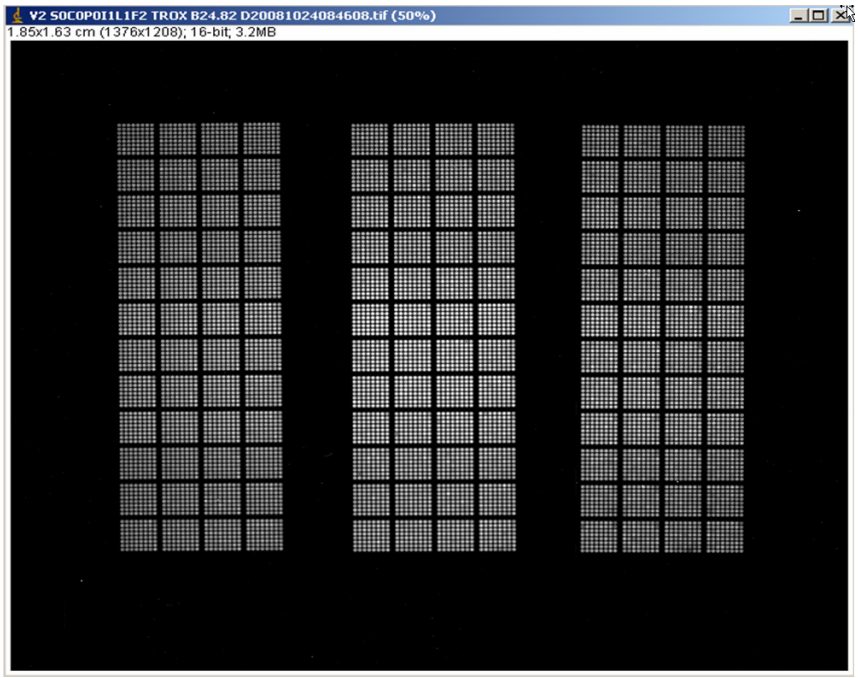
     Or
   - In the ImageJ toolbar, select **File** ▸ **Open**, then navigate to and open the image file.

The image opens in a dark window along with the B&C (Brightness and Contrast) toolbar.

Note: If the B&C toolbar is not automatically displayed: from the ImageJ toolbar, select **Image ▸ Adjust ▸ Brightness and Contrast**.



5. In the B&C toolbar, click **Auto** to brighten the image.



(Optional) View the SpotFind image file

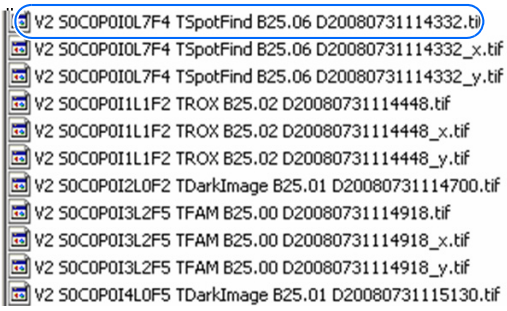
If you are not able to view the barcodes using the B&C toolbar, use the ImageJ software to open the SpotFind image file. Viewing the barcodes allows you to verify the orientation of the genotyping plates (see "About the image file" on page 16).

1. Navigate to and open the Images folder: **C:\Images**.

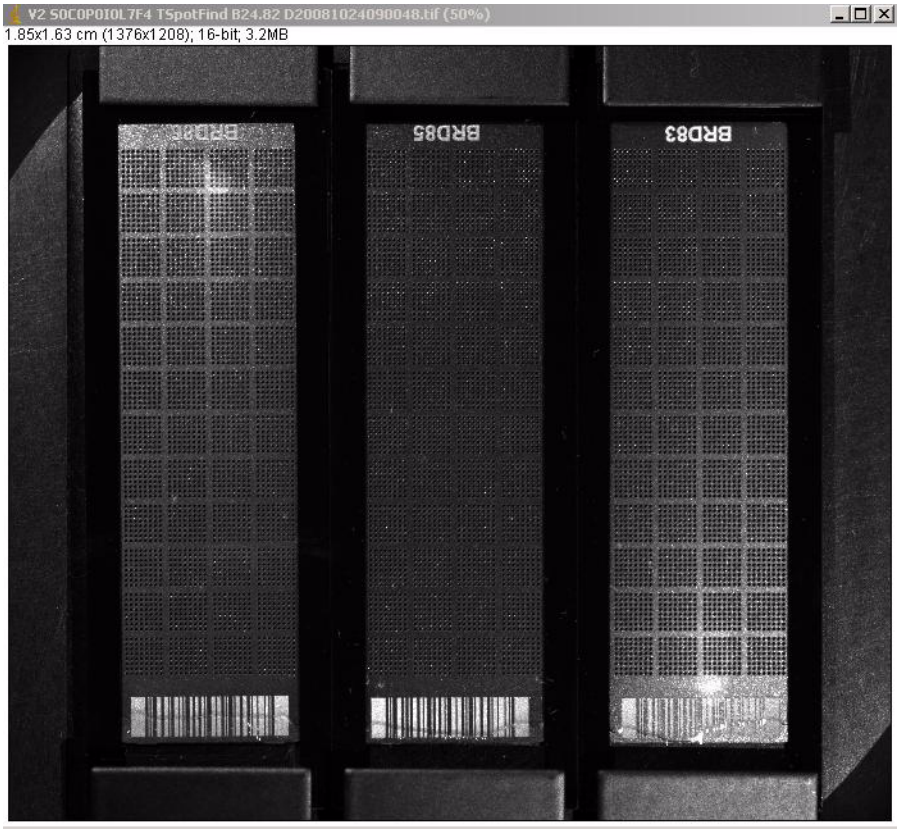
2. Locate the SpotFind image file of interest.

   The SpotFind image file is a *.tif file. The name of the SpotFind image file:

   - Is similar to the name of the ROX dye image file, but includes "SpotFind" instead of "TROX."
   - Includes the date the file was created.



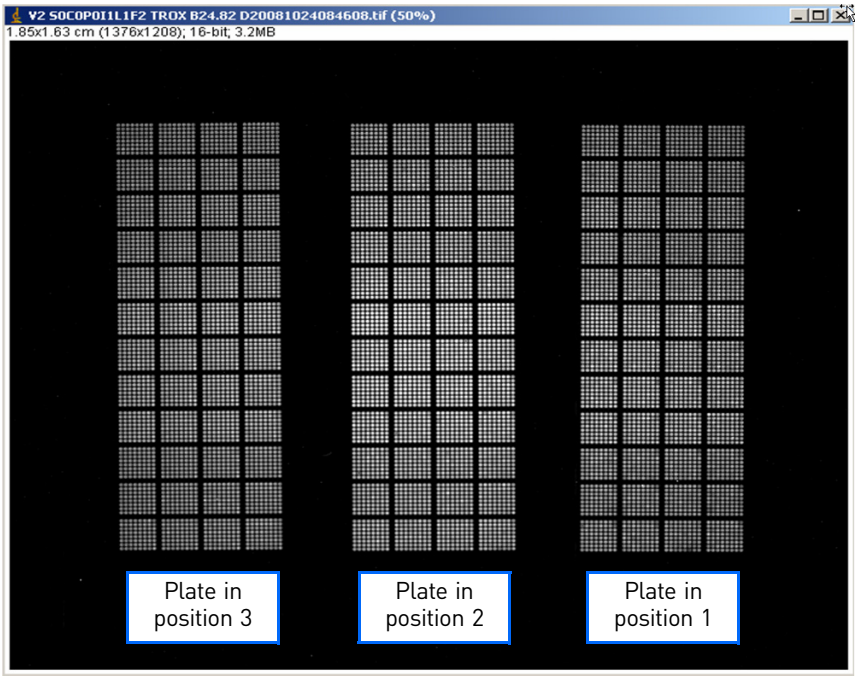
3. Double-click the file name to view the SpotFind image file:



About the image file

Each ROX dye image file contains up to three genotyping plates. In the image file, the genotyping plates should be oriented as follows:

- The barcode end of the plate is at the bottom of the image; the serial number end of the plate is at the top of the image.
- The right image corresponds to the genotyping plate in position 1 of the OpenArray® instrument. Position 1 is at the back of the instrument.
- The middle image corresponds to the genotyping plate in position 2 of the OpenArray® instrument. Position 2 is in the middle of the instrument.
- The left image corresponds to the genotyping plate in position 3 of the OpenArray® instrument. Position 3 is at the front of the instrument (closest to the door).



In the default view, you can see the overall appearance and loading patterns of the genotyping plates. To:

Zoom in – Press the **Ctrl** and **+** keys.

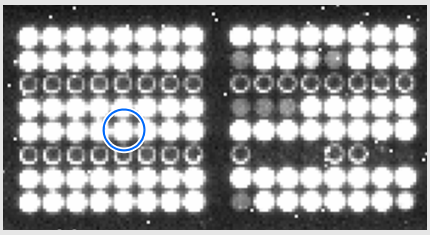
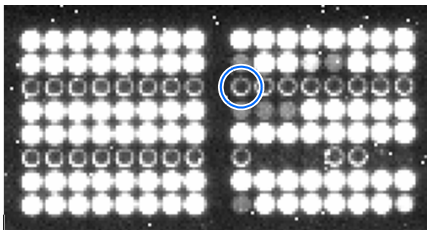
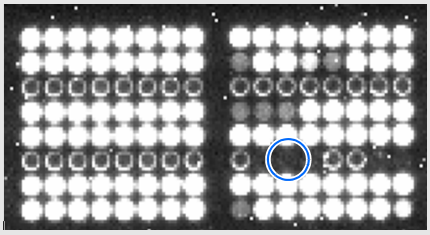
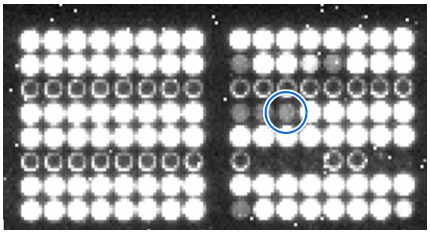
Zoom out – Press the **Ctrl** and **-** keys.

Ensure that the assays have been spotted

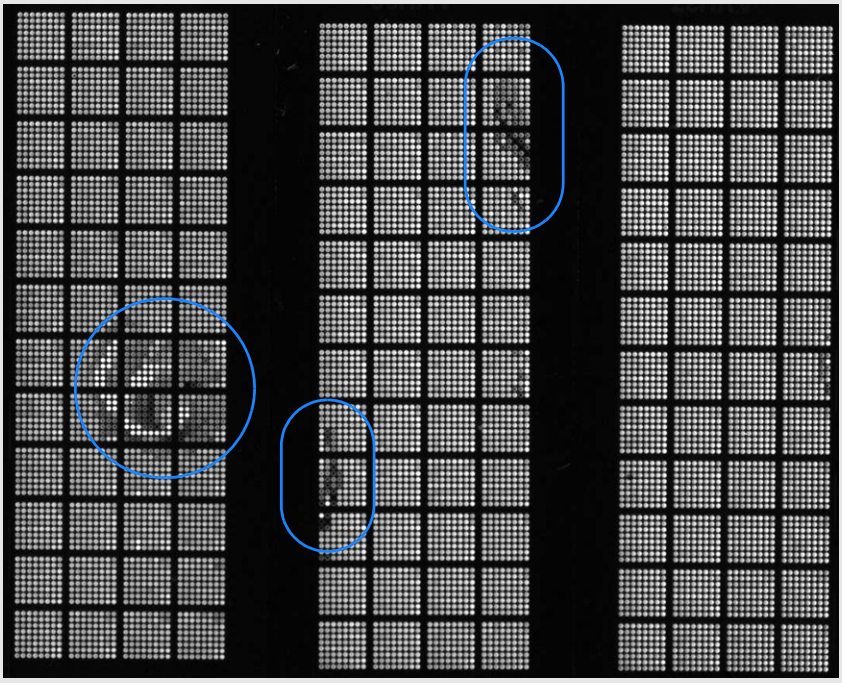
To ensure that the TaqMan® SNP Genotyping Assays have been spotted on the genotyping plate, use the ImageJ software to examine each through-hole for evidence of the ROX dye.

1. Open the image file of interest, as described on page 13.
2. (Optional) Press the **Ctrl** and **+** keys to zoom in on the image.

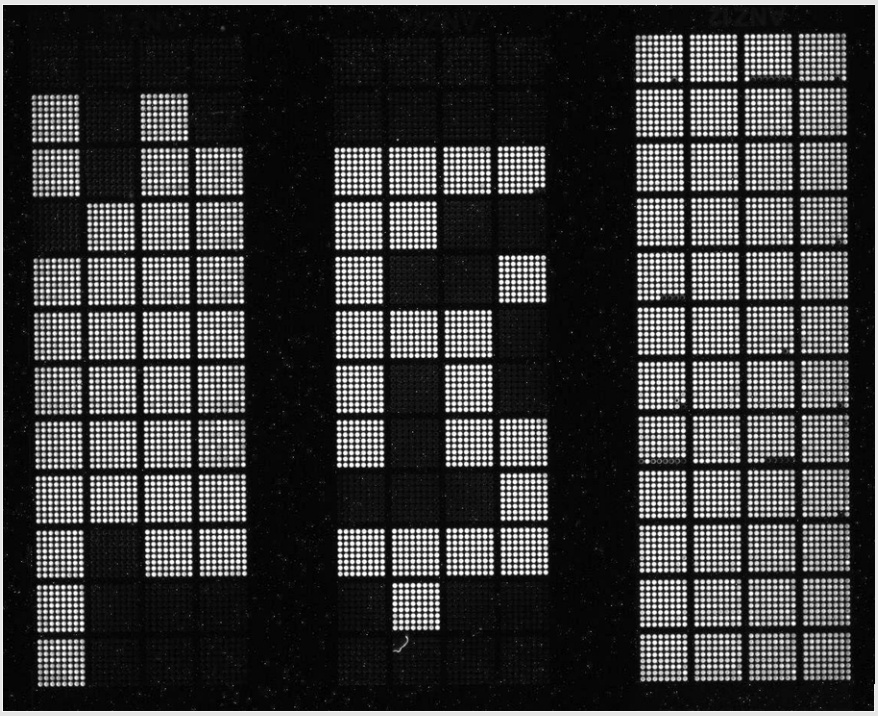
3. View each through-hole to determine if an assay has been spotted:

If the through-hole is...	Then the through-hole is...	Example
Extremely bright	Fully loaded. The through-hole contains an assay, sample, and master mix.	
Dark, but there is a light-colored ring around it	Partially loaded. The through-hole contains an assay, but it does not contain sample or master mix.	
Completely dark, and there is no light-colored ring around it	Empty. The through-hole does not contain an assay, sample, or master mix.	
Slightly brighter than an empty through-hole, but less bright than a fully loaded through-hole	Partially loaded. The through-hole contains sample and master mix, but it does not contain an assay.	

Troubleshoot using the image files

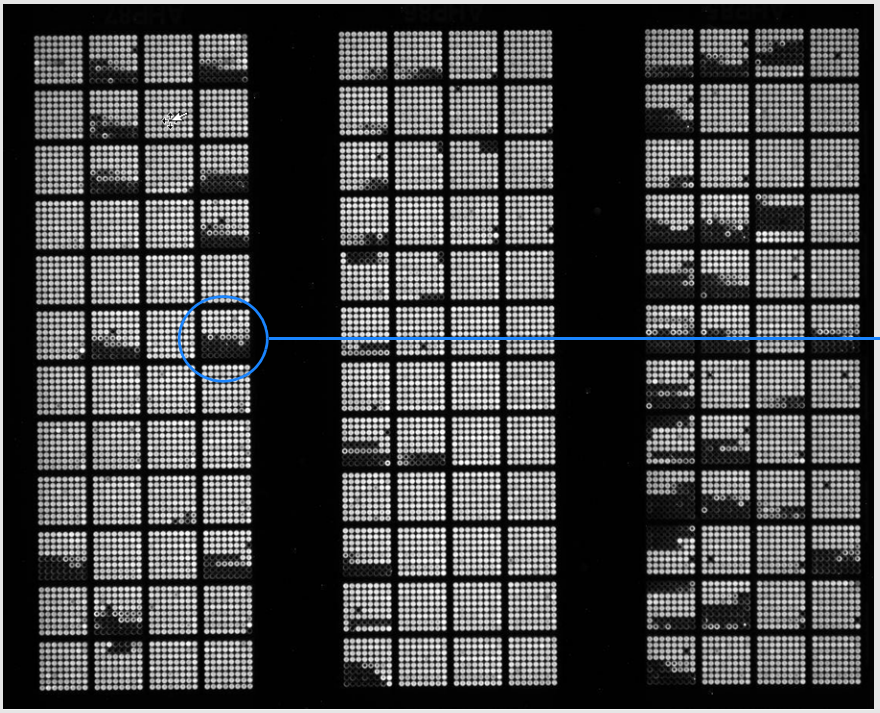
Observation	Possible cause	Recommended solution
Volume in the through-holes is drawn out	You touched the through-holes on the loaded genotyping plate.	• Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the genotyping plate. • Hold the genotyping plate by the edges, at the end opposite from the barcode. Do not touch the through-holes
	When inserting the genotyping plate into the genotyping case, you squeezed the case too tightly.	Hold the case by its sides only and do not squeeze the case.
 Circled areas show where the volume in the through-holes is drawn out.		

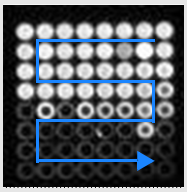
Observation	Possible cause	Recommended solution
Missing subarrays	• The OpenArray® Loader Tips were not leveled while in the OpenArray® AutoLoader Tip Block. • Excessive pressure was used when leveling the tips.	After the tip block is placed on the AutoLoader, level the tips in one of two ways: • *Gently* glide your index finger over the top of the tips. • *Gently* rest an unused tip block on top of the tips and remove.
	No sample was loaded into the tips.	After you load and level the tips, confirm that the tips are loaded to 1 mm. If there are empty tips or tips not filled to 1 mm, return the tip block to the plate guide and tap again to load and level the tips. If any tips remain empty, note the empty tips for future sample loading.
	The tip block may be dirty or may not be completely dry after cleaning. A dirty or wet tip block can cause "sticky" tips that do not sit correctly in the tip block.	Ensure that the tip blocks are completely dry. Use 100% ethanol as the final rinse and allow the tip blocks to completely dry before using them. To expedite drying, use nitrogen gas from a tank and air gun.



Dark areas show where the subarrays are missing

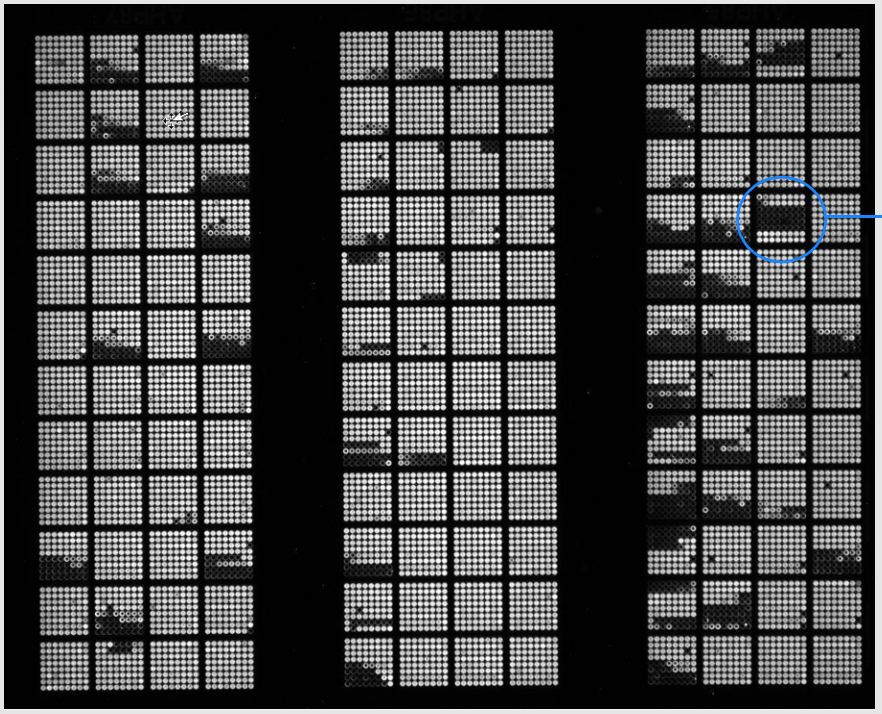
Observation	Possible cause	Recommended solution
Progressively empty through-holes. In the figure below, note the direction of loading (indicated by the blue arrow); as sample is loaded to the left, volume has run out and the through-holes are empty.	Not enough volume was loaded into the OpenArray® Loader Tips.	• Be sure to use the correct volume: 5 µL per through-hole. Volumes less than 5 µL result in poorly loaded subarrays. • The volume level must be at least 1 mm above the bottom of the tip block. Be sure to tap 25 to 50 times and examine the level prior to loading onto the AutoLoader.

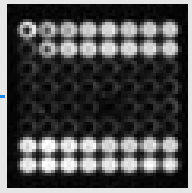




Enlarged view of the circled subarray. The arrow indicates the direction that the AutoLoader loaded the samples in.

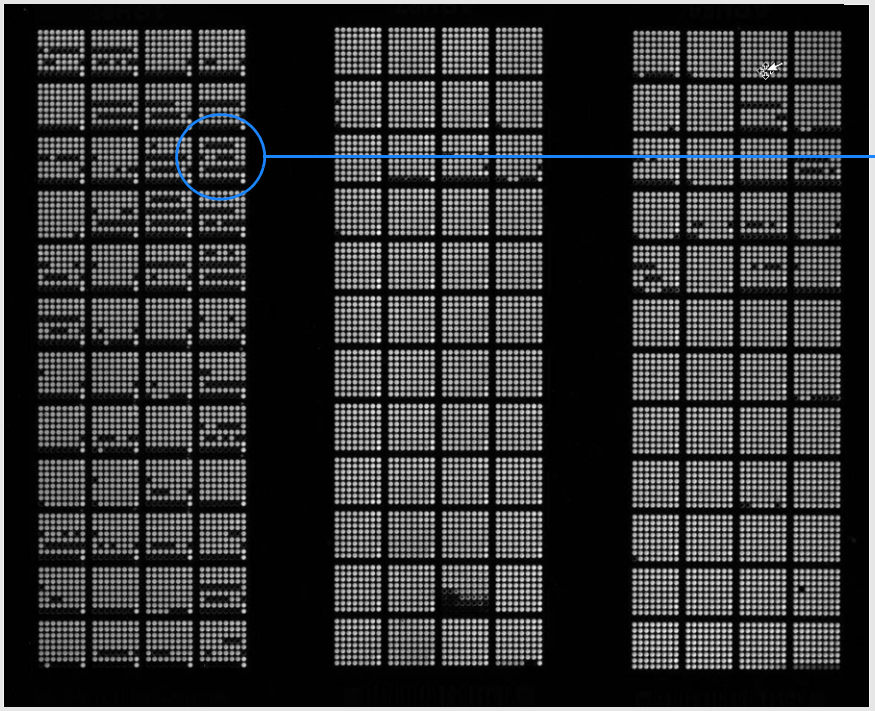
Observation	Possible cause	Recommended solution
Empty through-holes in the middle of a subarray	Air bubbles were in the OpenArray® Loader Tips.	• Use proper pipetting techniques to avoid introducing bubbles when loading volume into the 384-well sample plates. • Be sure to spin the 384-well sample plates before you load the genotyping plates. Spin at 1000 rpm for 1 minute.

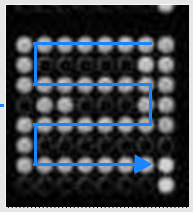




Enlarged view of the circled subarray.

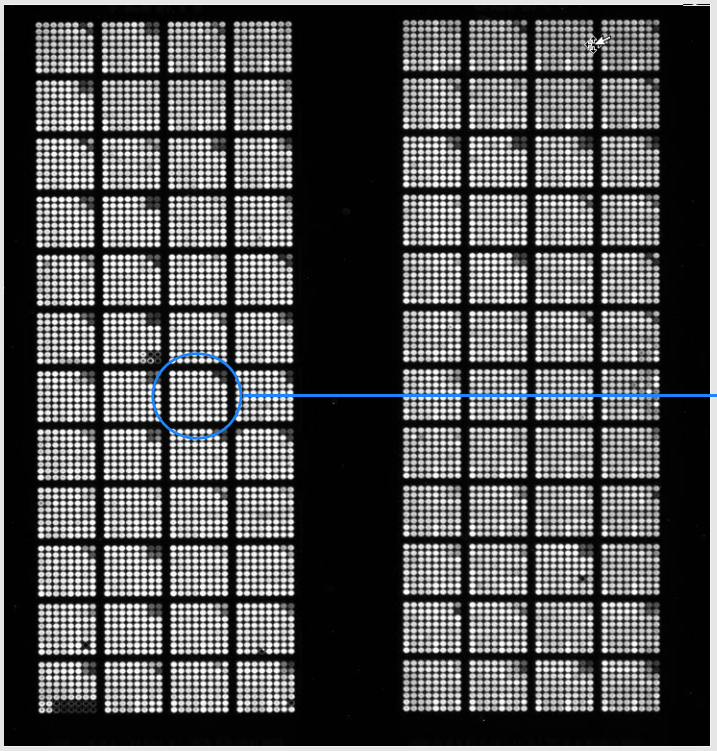
Observation	Possible cause	Recommended solution
A serpentine pattern appears in one or more subarrays.	The genotyping plate is not sitting tightly against the stop block on the right side of the plate holder.	Use tweezers to push the genotyping plate as far to the right as possible.
	Too many bubbles and/or foam in the tip.	There is not enough volume in the sample plate. Correct the volumes as needed on the rest of the sample plate. To ensure that all the volume is at the bottom of the wells, centrifuge the sample plate before loading the tips.
	The AutoLoader is misaligned.	Contact Applied Biosystems Technical Support for servicing.

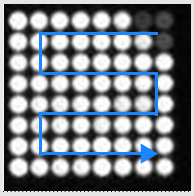




Enlarged view of the circled subarray. The arrow indicates the direction that the AutoLoader loaded the samples in.

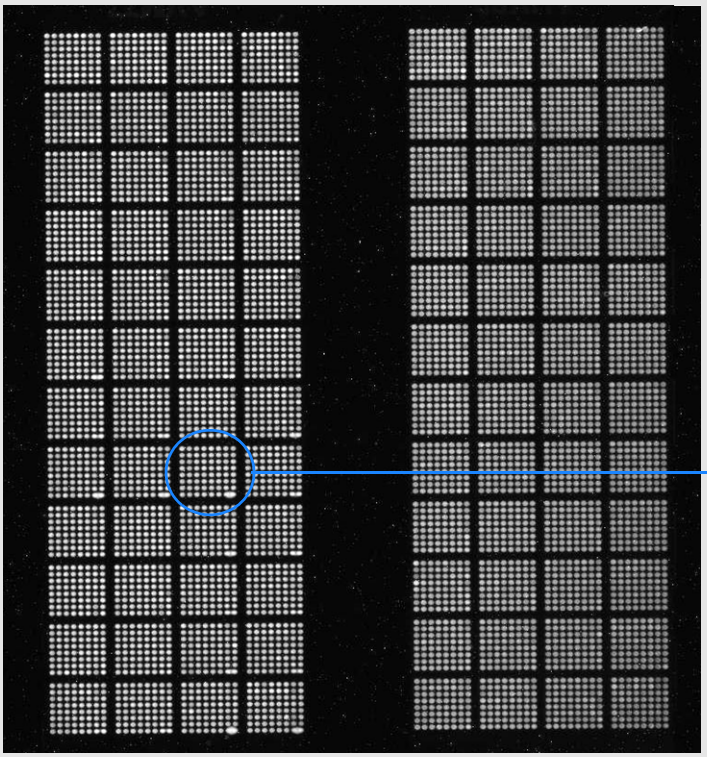
Observation	Possible cause	Recommended solution
Most of the start positions show low or no sample loaded.	• The tips were not leveled. • The tips were pressed too hard in the tip block and/or during tip leveling. • There are air gaps and/or bubbles in the ends of the tips.	• When loading the tip block, allow the tips to fall into the tip block after ejecting them from the pipette. • When leveling the tips, gently move your index finger over the top or use an unused tip block placed on the top of the tips for leveling. Do not apply pressure when performing these actions. • Before you place the tip block in the AutoLoader, confirm that the ends of the tips are loaded (no air gaps).

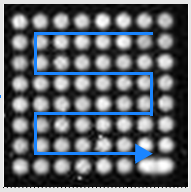




Enlarged view of the circled subarray. The arrow indicates the direction that the AutoLoader loaded the samples in.

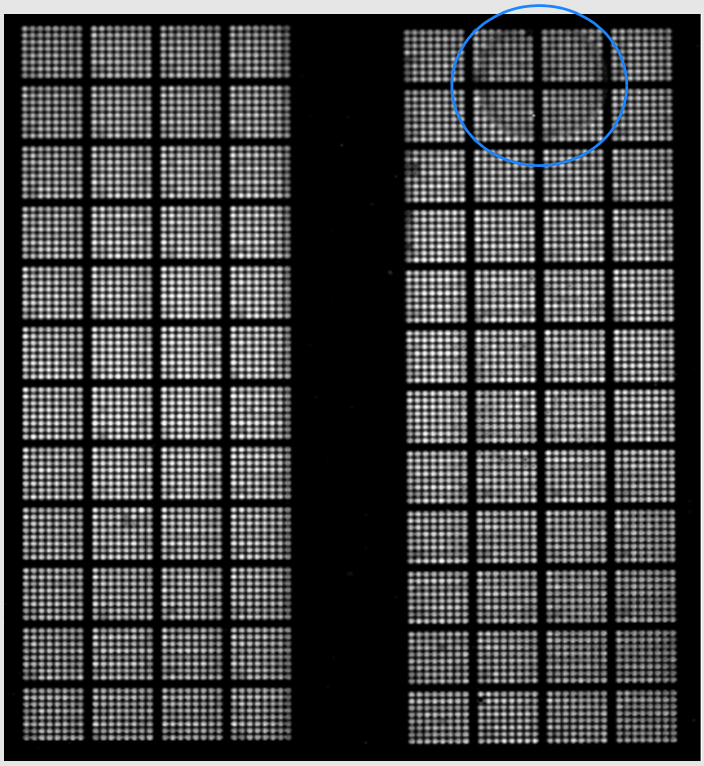
Observation	Possible cause	Recommended solution
Liquid bridges appear at the stop location on the sample plate surface.	The tip block was removed too quickly, leaving excess volume.	Remove the tip block slowly. See the guidelines on page 12.

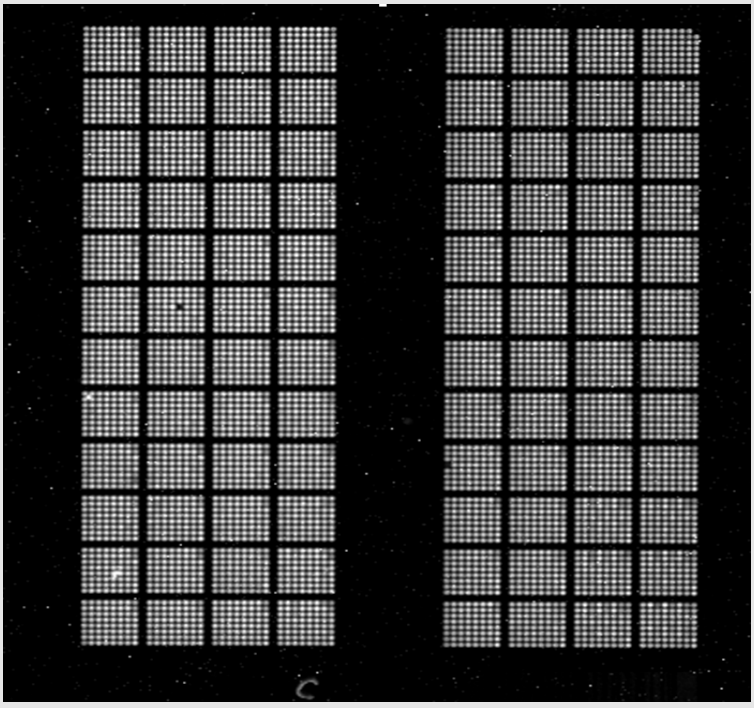




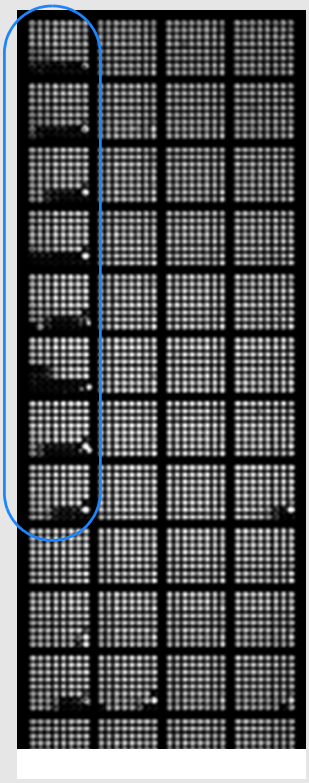
Enlarged view of the circled subarray. The arrow indicates the direction that the AutoLoader loaded the samples in.

Observation	Possible cause	Recommended solution
Bubbles appear to float around on the sample plate surface.	Bubbles formed when the genotyping case was sealed with the OpenArray® Sealing Glue.	• When filling a case with glue, be sure to follow the illustrated procedures in Chapter 3 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*. • If you notice a bubble after filling the case with glue, use a clean pipette to remove the bubble before curing the glue.

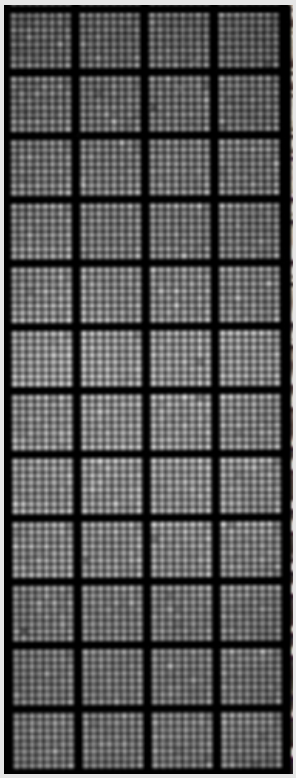


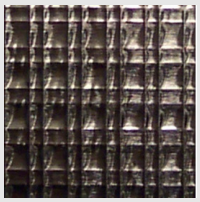
Observation	Possible cause	Recommended solution
Numerous white specks appear throughout the image.	The lens is dirty/dusty.	Over time, dust and other debris can accumulate on the lens inside the OpenArray® instrument. Although this debris is not detrimental to the overall data, it can affect various datapoints. Regularly clean both sides of the lens using a lint-free cloth.
		

Observation	Possible cause	Recommended solution
There are empty through-holes along the outer edges of the genotyping plate	Evaporation occurred because the sample plates were not properly sealed. **Note:** Evaporation usually occurs along the borders of the sample plates.	Be sure to properly seal the sample plates. If you need to store the sample plates, store them as follows: • Store sample plates containing sample only (2.5 µL) at –20 °C. • Store sample plates containing sample and master mix (5.0 µL) at 4 °C.



The sample plate is not properly sealed.





The sample plate is properly sealed.

2 Guidelines for Data Analysis

This chapter provides:

- An brief overview of genotyping experiments and scatter plots in the OpenArray® SNP Genotyping Analysis Software (page 30)
- Tips for performing data analysis in the OpenArray software (page 34)
- A troubleshooting table that illustrates common problems that can be seen in the scatter plots in the OpenArray software (page 42)
- Information about degraded DNA (page 55)
- Procedures for determining sample population size (page 57)

Workflow

This chapter is intended to be used as supplemental information for Chapter 5 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*, "Analyze the Run Data." The workflow is:

1. View the results.
2. (Optional) Modify clustering parameters.
3. (Optional) Modify project files (*.nix).
4. (Optional) Publish data.
5. (Optional) Perform downstream analysis using the AutoCaller™ Software.

About genotyping experiments

A genotyping experiment (also known as an allelic discrimination experiment) is an endpoint experiment used to determine the genotype of unknown samples. With this experiment type, you can differentiate a single nucleotide polymorphism (SNP).

A genotyping experiment determines if unknown samples are:

- Homozygotes (samples having only allele 1)
- Homozygotes (samples having only allele 2)
- Heterozygotes (samples having both allele 1 and allele 2)

About the scatter plots

The OpenArray software genotypes the DNA samples from the genotyping plate simultaneously. The software algorithmically clusters the sample data, then assigns a genotype call to the samples of each cluster according to its position on the plot.

The clustering of datapoints can vary along the X-axis (Allele 1), Y-axis (Allele 2), or diagonal (Allele 1-Allele 2). This variation results from differences in the dye fluorescence intensity after PCR amplification. The table below shows the correlation between fluorescence signals and sequences in a sample.

A substantial increase in...	Indicates...
VIC® dye-labeled probe fluorescence only	Homozygosity for Allele 1
FAM™ dye-labeled probe fluorescence only	Homozygosity for Allele 2
Both VIC® and FAM™ dye-labeled probes fluorescence	Allele 1-Allele 2 heterozygosity

About the dyes

Custom TaqMan® SNP Genotyping Assays

The dye label information for a Custom TaqMan® SNP Genotyping Assay is listed in the Assay Information File (AIF) that ships with the assay:

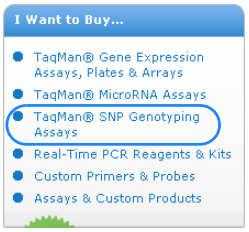
- The VIC® dye-labeled probe is listed under the Reporter 1 sequence
- The FAM™ dye-labeled probe is listed under the Reporter 2 sequence.

Pre-Designed TaqMan® SNP Genotyping Assays

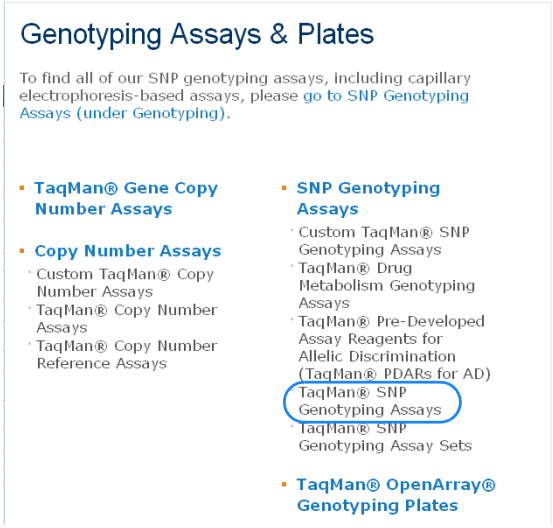
The dye label information for a Pre-Designed TaqMan® SNP Genotyping Assay is listed on the Assay Details page on the Applied Biosystems web site:

1. Go to **www.appliedbiosystems.com**.

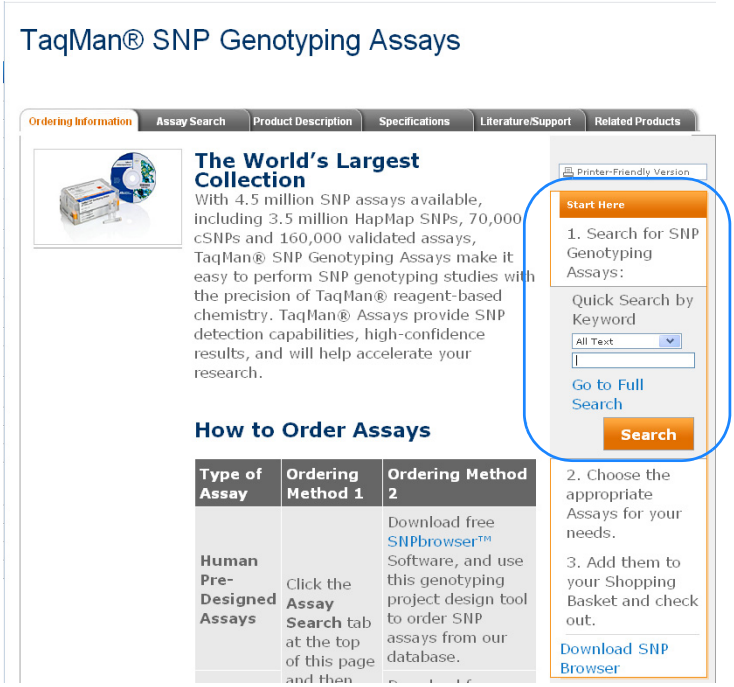
2. In the I Want to Buy box, click **TaqMan® SNP Genotyping Assays**.



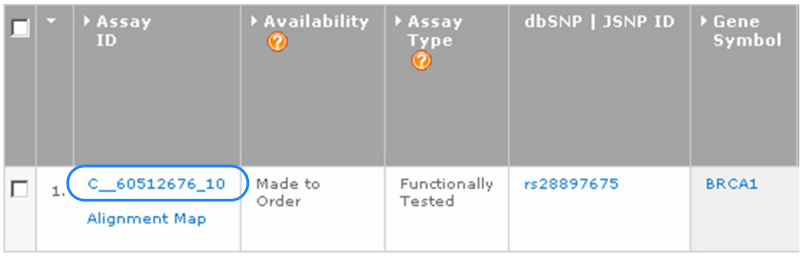
3. Under SNP Genotyping Assays, click **TaqMan® SNP Genotyping Assays**.



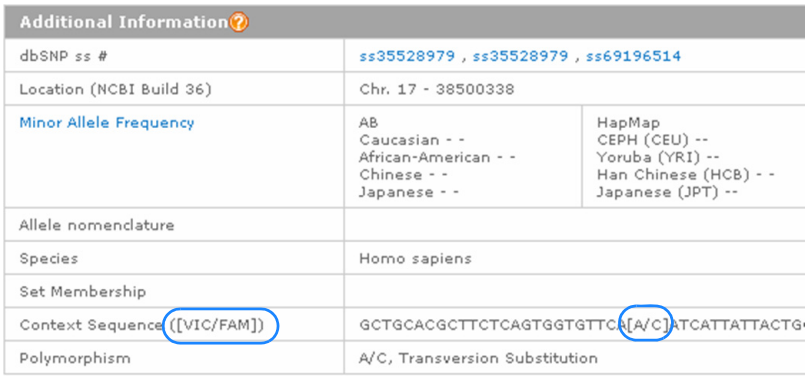
4. On the TaqMan® SNP Genotyping Assays page, follow the prompts to search for the assay of interest.



5. On the search results page, click the assay ID to open the Assay Details page.



On the Assay Details page, the dye label is listed with the context sequence. In the example below, allele 1 (A allele) is detected by the probe labeled with VIC dye, and allele 2 (C allele) is detected by the probe labeled with FAM dye.

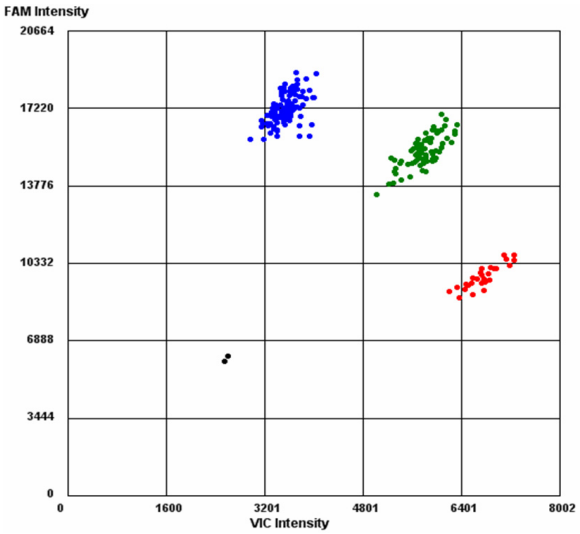


What does a good scatter plot look like?

A good scatter plot shows the following characteristics:

- The no template controls (NTCs) are present and distant from any clusters.
- The clusters are tight (that is, all samples within a cluster are close to the center of the cluster).
- The clusters are clearly separated from one another.
- There are no outliers or failed samples.

The figure below illustrates a good scatter plot.


FAM Intensity
20664
17220
13776
10332
6888
3444
0
0
1600
3201
4801
6401
8002
VIC Intensity

Tips for data analysis

After an imaging run, the OpenArray software automatically calls genotypes. If the automatic calls are not suitable for your experiment, you can modify the clustering parameters as discussed in the *TaqMan® OpenArray® Genotyping Getting Started Guide*. In addition, you may find the following tips helpful when analyzing the data:

Add or edit sample information for individual samples

You can manually add or edit sample information for individual samples, then save the changes to the project file (*.nix) and/or plate data file (*.spd).

Note: You can automatically add sample information for multiple samples by importing a sample information file (*.csv). Refer to Chapter 4 of the *TaqMan® OpenArray® Genotyping Getting Started Guide* or see Appendix B of this guide.

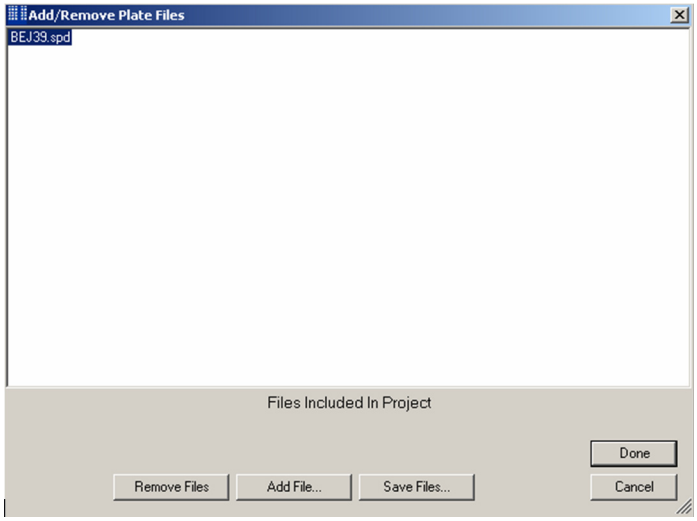
1. Click **Edit** to open the Sample Information dialog box.

2. Double-click the sample ID to edit, then add or edit the information.

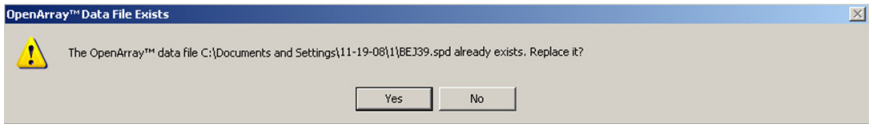
3. Save the *.nix file:
   - Select **File** ▸ **Save** to save the changes to the current *.nix file.

     Or
   - Select **File** ▸ **Save As** to save the changes to a new *.nix file.

4. Save the changes to the plate data file (*.spd):
   a. Click **Add**.
   b. In the Add/Remove Plate Files dialog box, select the appropriate *.spd file, then click **Save Files**.



   c. The Browse For Folder dialog box, select a save location, then click **OK**.
   d. At the prompt, click **Yes** to overwrite the existing *.spd file.

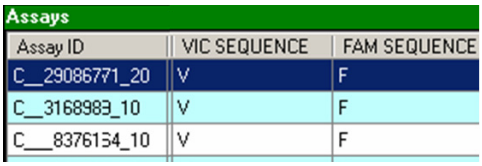


   e. Click **Done**. The *.spd file now contains the new allele information.

Edit the allele information

The OpenArray software automatically populates the VIC SEQUENCE and FAM SEQUENCE columns in the Assays pane as follows:

- **V** for the VIC® dye sequence
- **F** for the FAM™ dye sequence

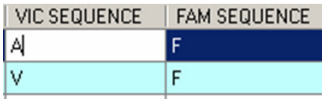


Assays

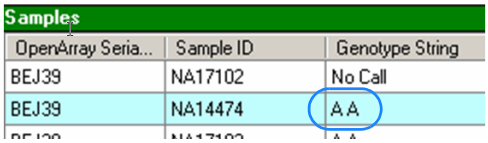
Assay ID	VIC SEQUENCE	FAM SEQUENCE
C__29086771_20	V	F
C__3168983_10	V	F
C___8376134_10	V	F

If needed, you can change the alleles (for downstream analysis, for example):

1. Click in the appropriate cell, then enter the correct allele.



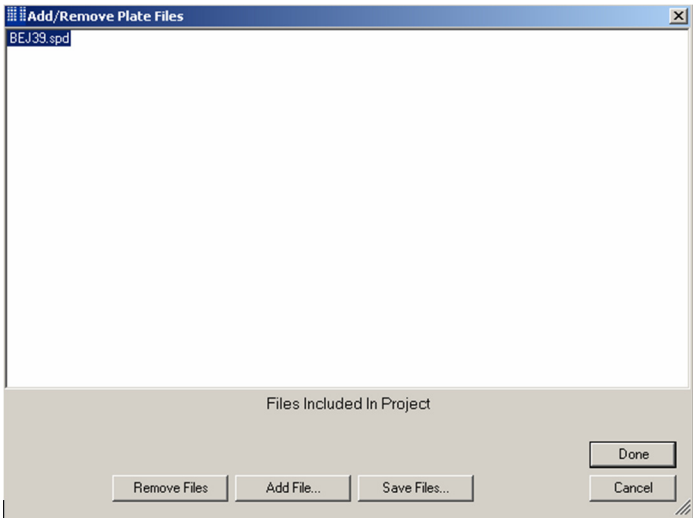
The OpenArray software automatically changes **V** and **F** in the Genotype String column to the appropriate alleles.



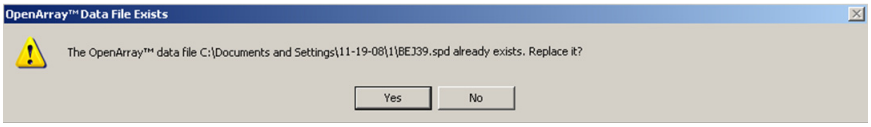
2. Save the *.nix file:
   - Select **File** ▸ **Save** to save the changes to the current *.nix file.

     Or
   - Select **File** ▸ **Save As** to save the changes to a new *.nix file.

3. Save the changes to the plate data file (*.spd):
   a. Click **Add**.
   b. In the Add/Remove Plate Files dialog box, select the appropriate *.spd file, then click **Save Files**.



   c. The Browse For Folder dialog box, select a save location, then click **OK**.
   d. At the prompt, click **Yes** to overwrite the existing *.spd file.

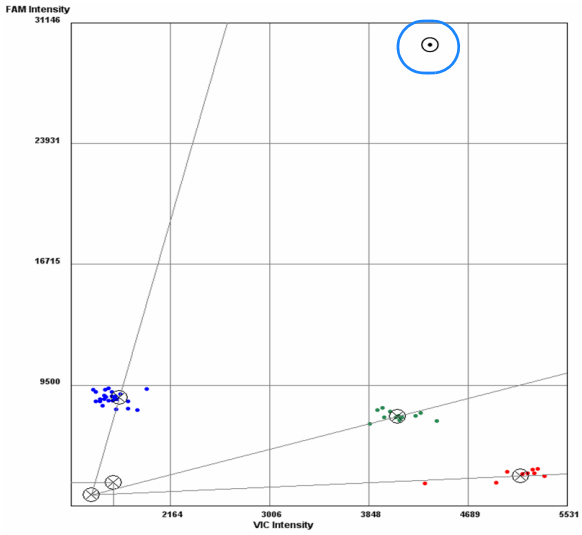


   e. Click **Done**. The *.spd file now contains the new allele information.

Remove outliers to adjust the scale

Marking and removing outliers from the scatter plot enables the OpenArray software to rescale the plot to achieve a greater view of the overall sample set. In the example below, the outlier is circled in blue.

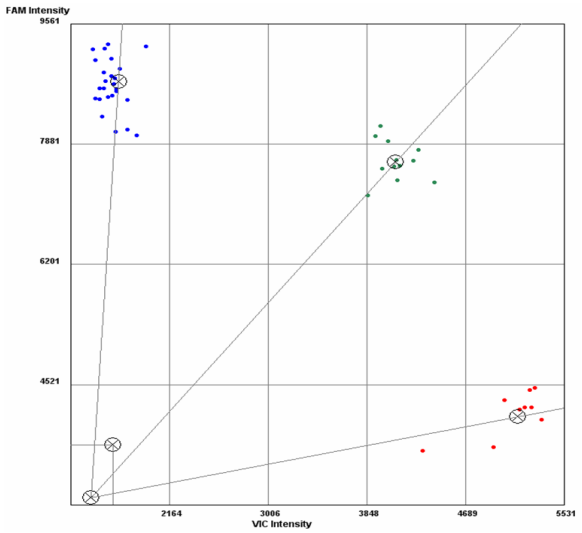
1. In the plot, click the outlier to highlight the sample in the Samples pane.



2. Click **Outlier** (below the Samples pane) to remove the sample from the plot.

The OpenArray software automatically rescales the plot.

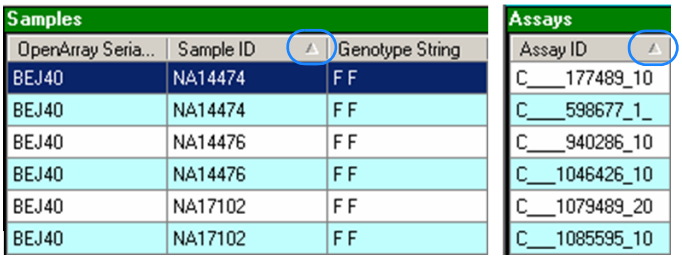
Note: Depending on the location of the outlier, the software may not rescale the plot.



Sort data

Sort the data in the Assays pane or Samples pane:

- To arrange rows in ascending or descending order, click a column heading. A small triangle appears, indicating which column performed the overall sort.

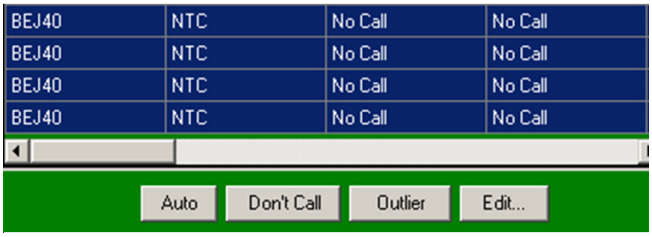


- To rearrange columns, click and drag a column heading to the desired location.

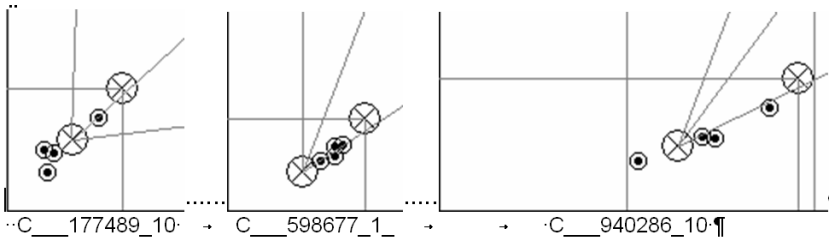
View a sample across assays

You can view the same sample across several assays to evaluate the sample's performance from assay to assay. For example, you may want to track a sample's success rate or ensure that an NTC is always assigned No Call status.

1. In the Samples pane, select the sample(s) to track. The samples are circled in the plot.



2. In the Assays pane, select each assay one at a time. The selected samples remain circled in the plot for each assay that you select:



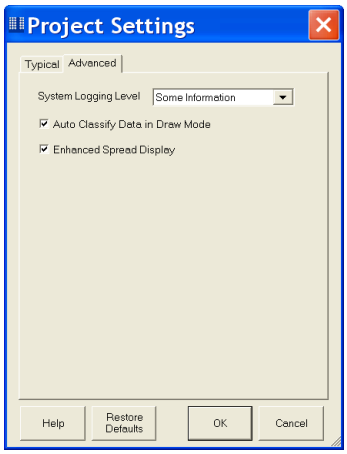
Use the Enhanced Spread Display option

Use the Enhanced Spread Display option to incorporate a spread correction when the clusters appear too close together. The OpenArray software applies the Enhanced Spread Display option to the entire project, not to individual assays.

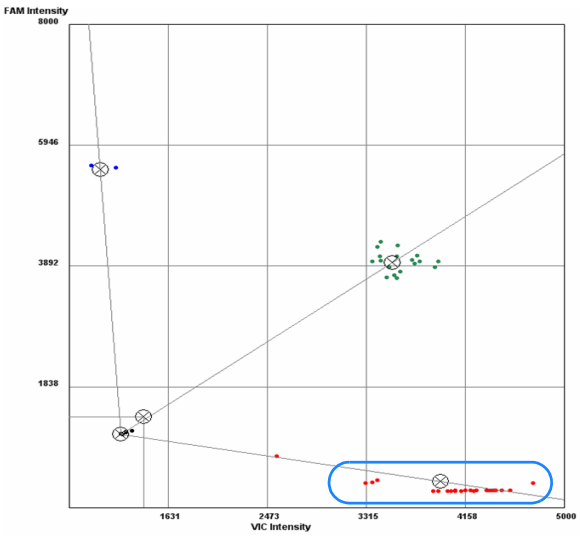
IMPORTANT! Using the Enhanced Spread Display option may adversely affect results when you transfer the data to the Applied Biosystems AutoCaller™ Software.

To turn the Enhanced Spread Display option on:

1. From the menu bar, select **Edit** ▸ **Project Setting**s.
2. In the Project Settings dialog box, select the **Advanced** tab, then select the **Enhanced Spread Display** checkbox.



In the example shown below, the VIC® dye homozygotes are compressed on the X axis.

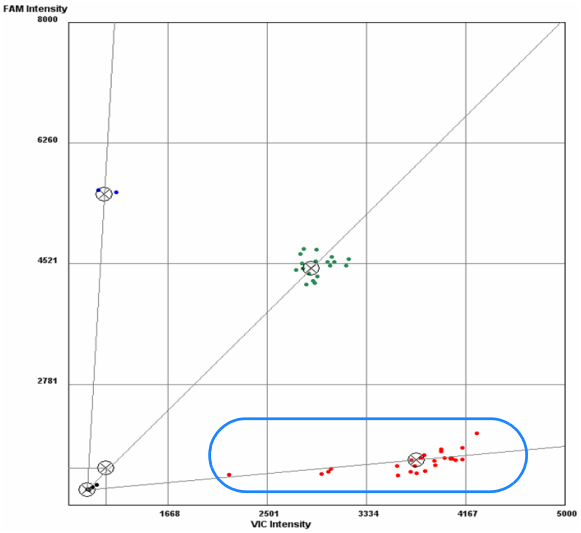


To turn the Enhanced Spread Display option off:

1. From the menu bar, select **Edit** ▸ **Project Setting**s.

2. In the Project Settings dialog box, select the **Advanced** tab, then deselect the **Enhanced Spread Display** checkbox.

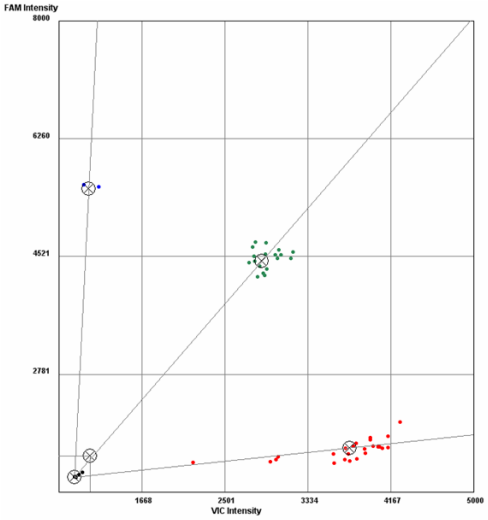
In the example shown below, the VIC dye homozygotes are no longer compressed on the X axis. Therefore, all samples can be correctly genotyped (call vs. no call).



Export genotyping results

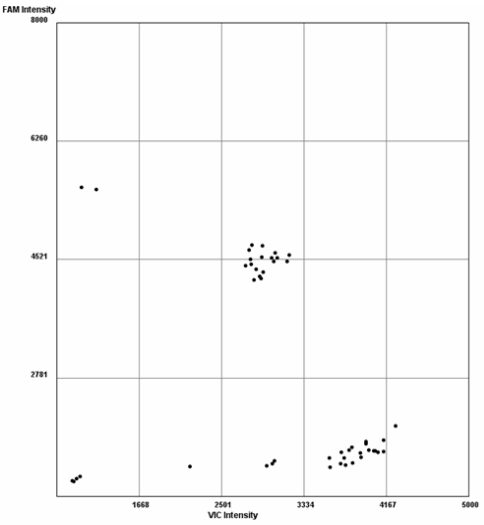
The Point and Draw tabs in the OpenArray software are not connected. For example, when you analyze data in the Point tab, the Draw tab does not reflect that analysis. Before you export genotyping results, be sure that the appropriate tab is active.

- In the example shown below, the data were analyzed in the Point tab, then exported while the Point tab was active. The correct genotypes appear in the exported table.



OpenArray.SerialNumber	Sample.SampleID	C____940286_10.Genotype
BEJ40	NA17102	V V
BEJ40	NA14474	V F
BEJ40	NA17103	F F
BEJ40	NA14476	V V
BEJ40	NA17104	V F
BEJ40	NA17110	V F
BEJ40	NA17105	V F
BEJ40	NA17112	V V
BEJ40	NA17106	V V
BEJ40	NA17113	V V
BEJ40	NA17107	V F
BEJ40	NA17116	V V
BEJ40	NA17114	V V
BEJ40	NA17202	V V

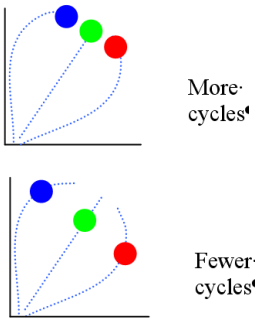
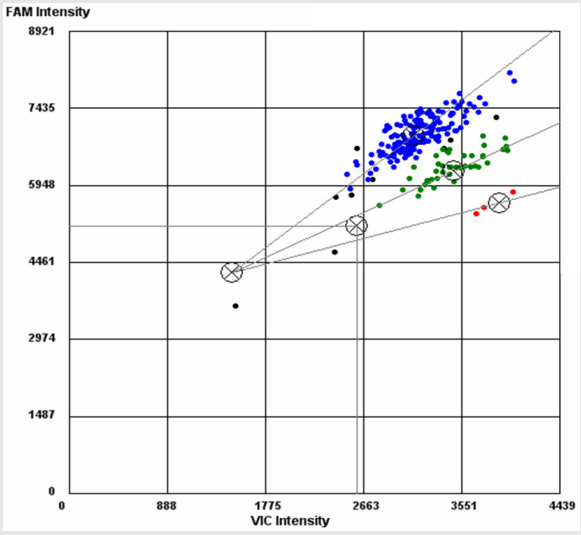
- In the example shown below, the data were analyzed in the Point tab, then exported while the Draw tab was active. Because the data were not analyzed in the Draw tab, the exported data table displays *No Call* for all genotypes.



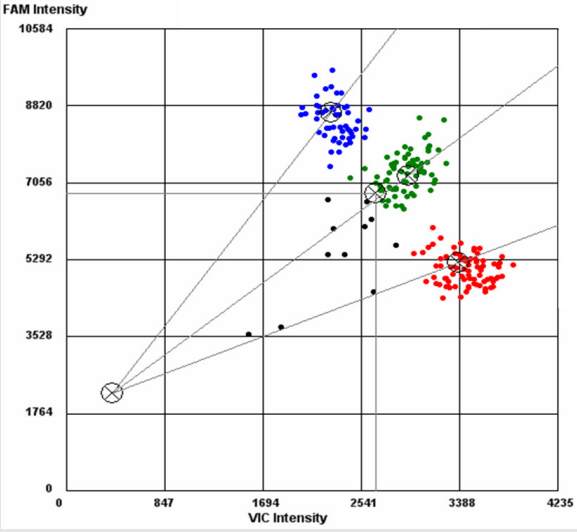
OpenArray.SerialNumber	Sample.SampleID	C____940286_10.Genotype
BEJ40	NA17102	No Call
BEJ40	NA14474	No Call
BEJ40	NA17103	No Call
BEJ40	NA14476	No Call
BEJ40	NA17104	No Call
BEJ40	NA17110	No Call
BEJ40	NA17105	No Call
BEJ40	NA17112	No Call
BEJ40	NA17106	No Call
BEJ40	NA17113	No Call
BEJ40	NA17107	No Call
BEJ40	NA17116	No Call
BEJ40	NA17114	No Call
BEJ40	NA17202	No Call

Troubleshoot using the scatter plots

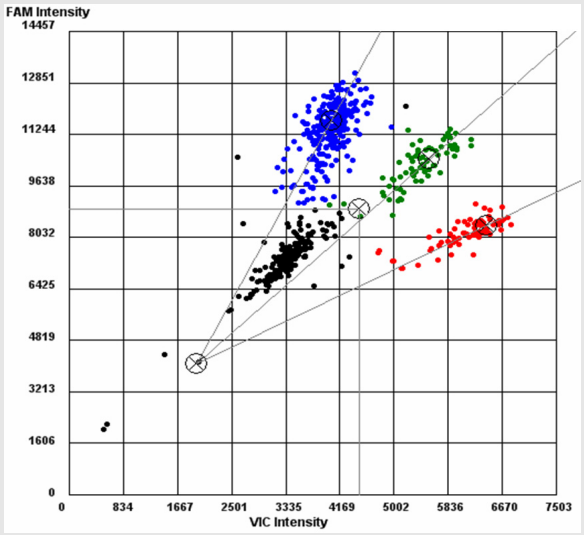
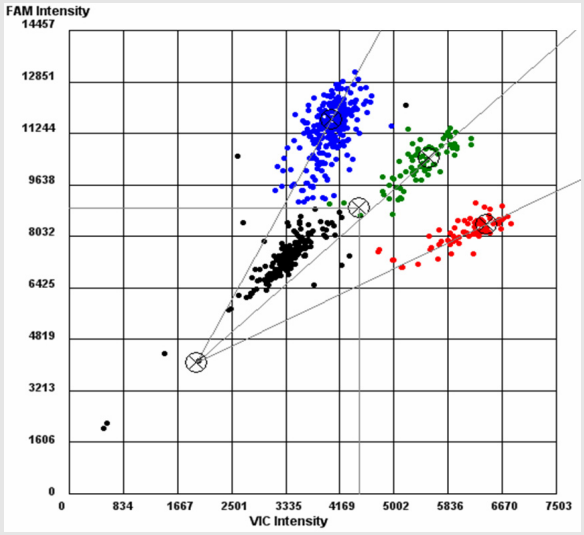
The TaqMan® SNP Genotyping Assays are optimized for use with Applied Biosystems recommended reagents, instruments, and thermal-cycling conditions (refer to the *TaqMan® SNP Genotyping Assays Protocol* for detailed information; see "Documentation and Support" on page 67). If you experience problems with assay performance, be sure that you have followed the Applied Biosystems protocols, then check the troubleshooting table below.

Observation	Possible cause	Recommended solution
Poor cluster separation across most assays. Clusters are too close together to properly determine the genotypes.	The overall fluorescence intensity is strong. For some assays, the trajectories of the clusters merge when too many cycles are run. 	Adjust the thermal-cycling conditions: • Reduce the number of cycles. *And/or* • Increase the annealing Tm. **Note:** You cannot adjust the thermal-cycling conditions for the OpenArray® platform; therefore, you may need to redesign the assay or you may need to perform genotyping on an SDS platform that allows you to adjust the thermal-cycling conditions.
	The genotyping plate was left at room temperature for too long after thermal cycling.	After thermal cycling, store the genotyping plates at 4 °C, in the dark, for up to 72 hours.
		

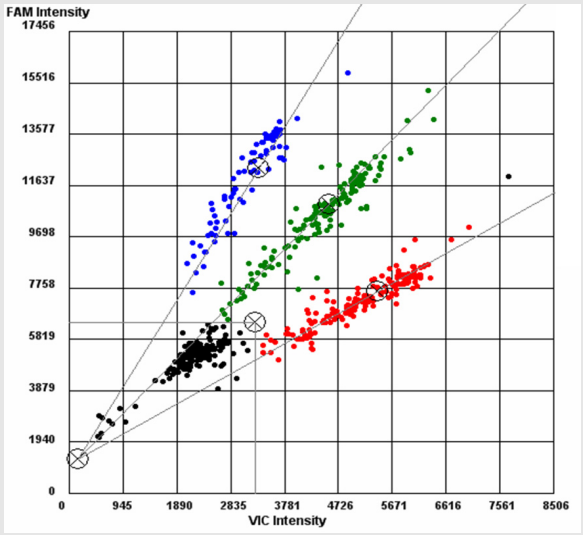
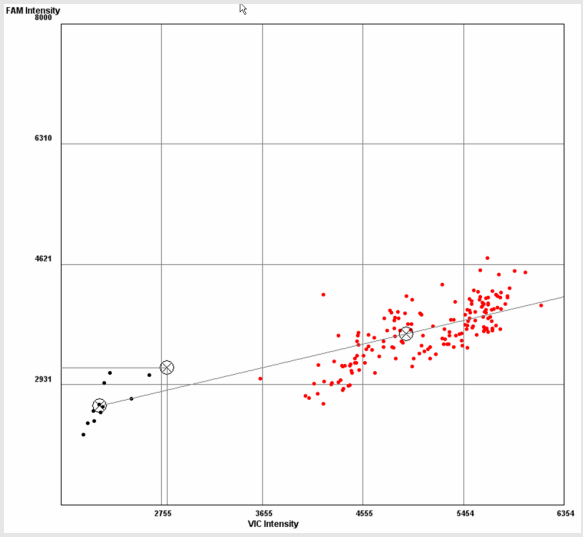
Observation	Possible cause	Recommended solution
Diffuse clusters. Samples within a given cluster are not close to the center.	Degraded DNA. Degraded DNA may not amplify as efficiently as high-quality DNA, so fluorescence intensities vary.	Perform a gel analysis to visualize quality of the DNA. Re-extract those that are degraded or remove from the analysis. See "DNA preparation" on page 55.
	Genomic DNA is not properly quantitated. Samples with differing concentrations result in varied fluorescence intensities. Samples with lower starting quantities exponentially amplify lower yields compared to samples with higher starting quantities.	Use a high-quality spectrophotometer or perform an RNase P quantitation assay to determine the concentration of each sample. Normalize as needed (refer to the *User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit* (see page 67).
	Pipetting errors. Poorly calibrated pipettes, incorrect pipette tips, or inefficient technique result in varied volumes pipetted into the sample plate, and in varied genomic DNA concentrations.	• Ensure that all pipettes are calibrated on a routine basis and use the recommended pipette tips. Consult the pipette manufacturer for proper testing and maintenance. • Check the ROX™ dye levels. Variation in the ROX dye levels may indicate pipetting errors.
	Expired reagents.	Replace with fresh reagents.
	Evaporation has occurred prior to loading the genotyping plate in the case.	Check the ROX™ dye levels after imaging. Variation in the ROX dye levels may indicate evaporation.
	Analyzing too many *.spd files in one view may cause the clusters to become too diffuse for proper genotyping analysis.	Reduce the number of *.spd files. Applied Biosystems recommends analyzing no more than 10 *.spd files in one view.



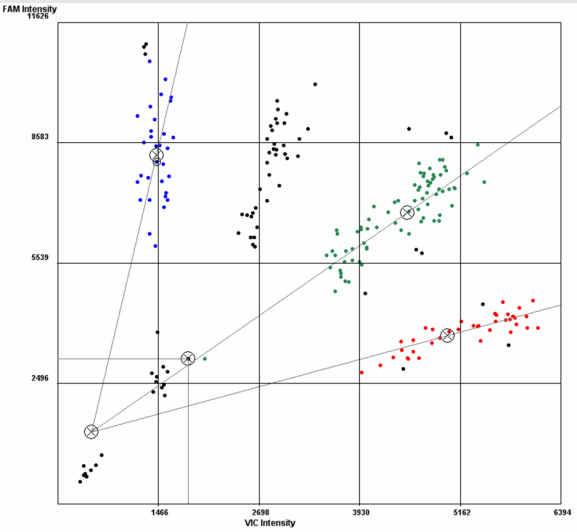
Observation	Possible cause	Recommended solution
Too many failed samples. More than the expected number of samples failed to properly amplify.	Degraded DNA. Degraded DNA may not amplify as efficiently as high-quality DNA, so fluorescence intensities vary.	Perform a gel analysis to visualize quality of the DNA. Re-extract those that are degraded or remove from the analysis. See "DNA preparation" on page 55.
	Genomic DNA is not properly quantitated. Samples with differing concentrations result in varied fluorescence intensities. Samples with lower starting quantities exponentially amplify lower yields compared to samples with higher starting quantities.	Use a high-quality spectrophotometer or perform an RNase P quantitation assay to determine the concentration of each sample. Normalize as needed (refer to the *User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit* (see page 67).
	Pipetting errors. Poorly calibrated pipettes, incorrect pipette tips, or inefficient technique result in varied volumes pipetted into the sample plate, and in varied genomic DNA concentrations.	• Ensure that all pipettes are calibrated on a routine basis and use the recommended pipette tips. Consult the pipette manufacturer for proper testing and maintenance. • Check the ROX™ dye levels. Variation in the ROX dye levels may indicate pipetting errors.
	Expired reagents.	Replace with fresh reagents.
	Evaporation has occurred prior to loading the genotyping plate in the case.	Check the ROX™ dye levels after imaging. Variation in the ROX dye levels may indicate evaporation.
	PCR inhibitors, ranging from organics to non-organics, can cause samples to fail amplification.	Examine the purity of the DNA by checking the: • A_{260}/A_{280} ratio, which should be between 1.7 and 1.9. A ratio <1.7 indicates protein contamination. • A_{260}/A_{230} ratio, which should be similar to the A_{260}/A_{280} ratio. When this ratio is <1.7, salts, solvents, and alcohols may be present. Evaluate the current DNA extraction method and consider an alternative protocol.

Observation	Possible cause	Recommended solution
		
High fluorescence signal in the NTCs	Non-specific probe cleavage.	Perform proper bioinformatics on the sequence, evaluate the SNP design, and consider redesigning the assay. Refer to *Bioinformatic Evaluation of a Sequence for Custom TaqMan® SNP Genotyping Assays*.
	The NTC is contaminated.	Examine other assays for high fluorescence signal in the NTCs. Consider replacing the water used for the NTCs (the water may be a possible source of contamination).
	The probe is degraded.	Store the genotyping plates correctly. Refer to the *TaqMan® OpenArray® Genotyping Getting Started Guide*for storage information.
	The genotyping plate was incorrectly stored.	
		

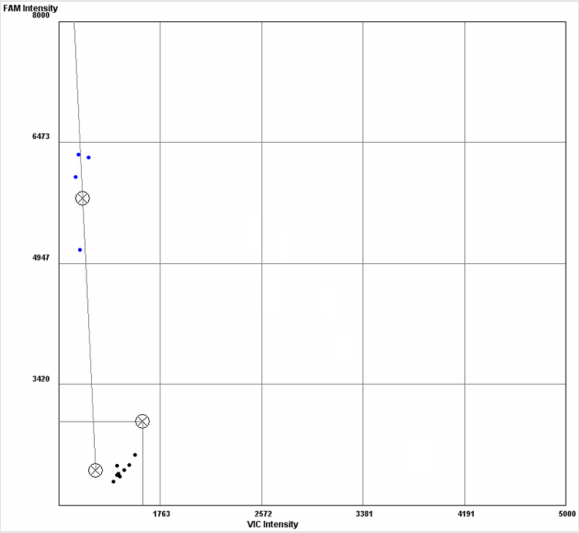
Observation	Possible cause	Recommended solution
Trailing comet/tail. Samples within a given cluster are spread along the center line with varying fluorescence intensities.	(Leading cause) Genomic DNA is not properly quantitated. Samples with differing concentrations result in varied fluorescence intensities. Samples with lower starting quantities exponentially amplify lower yields compared to samples with higher starting quantities.	Use a high-quality spectrophotometer or perform an RNase P quantitation assay to determine the concentration of each sample. Normalize as needed (refer to the *User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit* (see page 67).
	Pipetting errors. Poorly calibrated pipettes, incorrect pipette tips, or inefficient technique result in varied volumes pipetted into the sample plate, and in varied genomic DNA concentrations.	• Ensure that all pipettes are calibrated on a routine basis and use the recommended pipette tips. Consult the pipette manufacturer for proper testing and maintenance. • Check the ROX™ dye levels. Variation in the ROX dye levels may indicate pipetting errors.
	Expired reagents.	Replace with fresh reagents.
	Evaporation has occurred prior to loading the genotyping plate in the case.	Check the ROX™ dye levels after imaging. Variation in the ROX dye levels may indicate evaporation.
	PCR inhibitors, ranging from organics to non-organics, can cause samples to fail amplification.	Examine the purity of the DNA by checking the: • A_{260}/A_{280} ratio, which should be between 1.7 and 1.9. A ratio <1.7 indicates protein contamination. • A_{260}/A_{230} ratio, which should be similar to the A_{260}/A_{280} ratio. When this ratio is <1.7, salts, solvents, and alcohols may be present. Evaluate the current DNA extraction method and consider an alternative protocol.
	Degraded DNA. Degraded DNA may not amplify as efficiently as high-quality DNA, so fluorescence intensities vary.	Perform a gel analysis to visualize quality of the DNA. Re-extract those that are degraded or remove from the analysis. See "DNA preparation" on page 55.

Observation	Possible cause	Recommended solution
		
A single cluster appears in the plot.	The minor allele frequency (MAF) may be low.	Check the MAF for the SNP. A larger sample size may be required to see the minor allele. See "Sample population size" on page 57.
	The SNP may be a pseudo-SNP, non-polymorphic SNP, or non-informative SNP for the target population.	Verify that it is a SNP using dbSNP and examine the SNP for a population-specific MAF. **Note:** dbSNP is an NIH database; go to **www.ncbi.nlm.nih.gov/projects/SNP**.
		

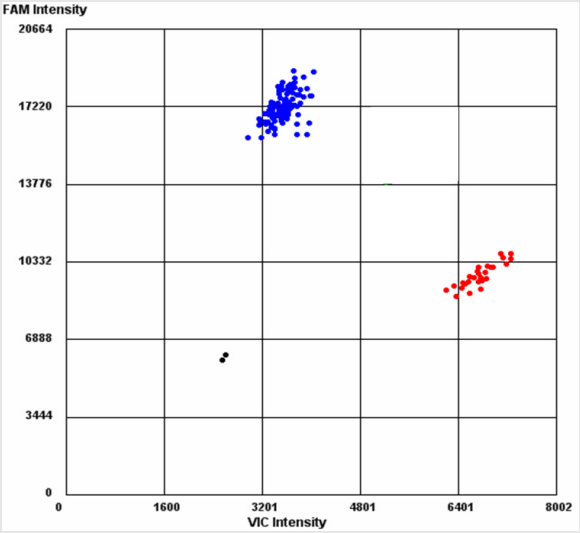
Observation	Possible cause	Recommended solution
More than three clusters appear in the plot.	A non-target SNP under a primer or probe may result in off-cluster data. The location of the non-target SNP under the primer or probe, as well as the MAF, influences the extent to which the cluster pattern is atypical. The number of individuals exhibiting this pattern depends on the allele frequency of the non-target SNP. You may see additional clusters ("angle clusters") or a lack of amplification of the sample when there is an additional polymorphism under the primer.	• To confirm the presence of another SNP under the probe or primer, repeat the experiment and evaluate overall assay performance: – Do the assay results appear in tight clusters? – Do clusters have good separation? • Perform proper bioinformatics on the sequence, evaluate the SNP design, and consider redesigning the assay. Refer to *Bioinformatic Evaluation of a Sequence for Custom TaqMan® SNP Genotyping Assays*. • Verify the presence of the outlier. Examine the sample's performance in other assays to rule out problems caused by this particular sample, such as sample impurity or degradation. Search the public databases (for example dbSNP) to see if the additional SNP has been discovered. Perform comparative sequencing on the subjects to identify any undocumented SNPs present under the primer or probe. The presence of extra SNPs may cause angle clusters or vector clusters. **Note:** dbSNP is an NIH database; go to **www.ncbi.nlm.nih.gov/projects/SNP**.
	A SNP under a probe can result in an outlier that falls between the heterozygote and one of the homozygotes (an angle cluster) or an outlier that has the same angle as a cluster but trails behind the main cluster (a vector cluster).	
	The occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or any chromosome number that is not an exact multiple of the haploid number.	



Observation	Possible cause	Recommended solution
The assay is not in Hardy-Weinberg Equilibrium (HWE).	A SNP is on the X chromosome. An assay detecting a SNP on the X chromosome detects two alleles in female samples, but only one allele in male samples. Males are hemizygous for this SNP (only one copy).	Confirm that you have selected the appropriate TaqMan® SNP Genotyping Assay and confirm its location in the genome.
	A SNP is on the Y chromosome. The assay detects a SNP on the Y chromosome. Only male samples are detected; they are hemizygous (one copy only) for this SNP. There are no heterozygotes for this SNP. The female samples do not amplify at all, but cluster with the NTCs.	

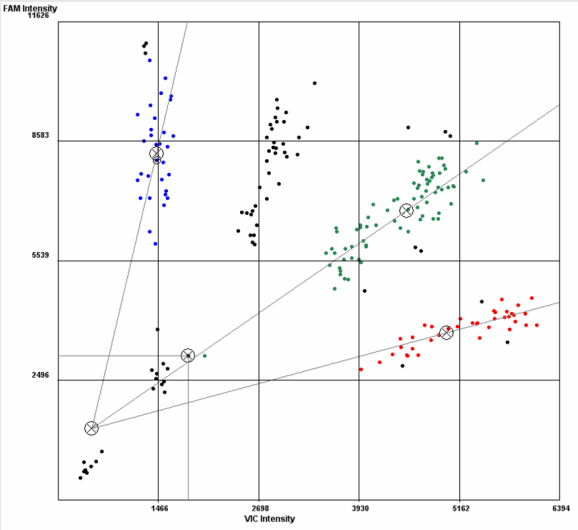


Y chromosome scatter plot

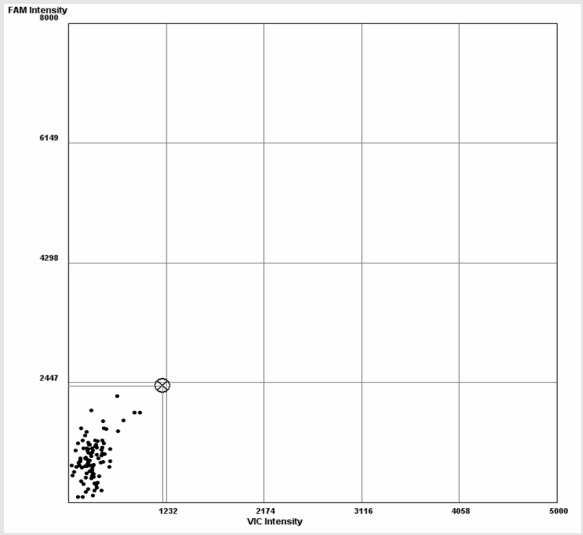


X chromosome scatter plot

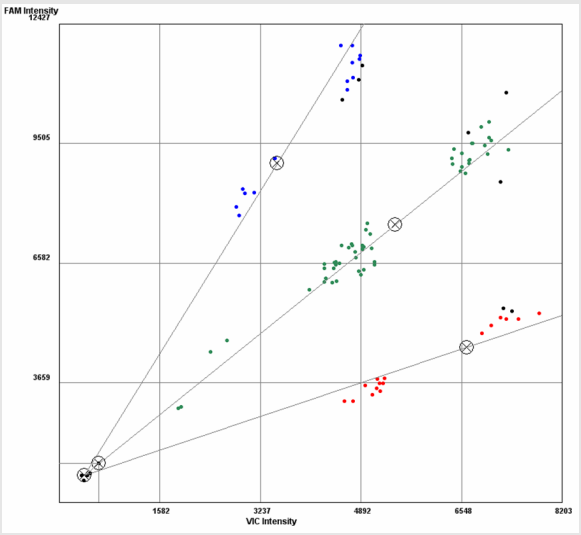
Observation	Possible cause	Recommended solution
A gene has a copy number polymorphism. **Note:** A copy number polymorphism for a gene may or may not appear as an anomaly in the plot.	If an individual is homozygous with more than three copies of the gene and each copy has the same genotype, the data will likely appear in the homozygous cluster. If an individual is heterozygous with an odd number of copies and the copies have different genotypes, the data will likely fall between the clusters for the heterozygote (T:A) and the homozygote (A:A).	1. Evaluate the overall assay performance: • Do the assay results appear in tight clusters? • Do the clusters have good separation? 2. Repeat the experiment to confirm the presence of the off-cluster sample. 3. Examine the sample's performance in other assays to rule out problems caused by this particular sample, such as sample impurity or degradation. 4. Perform a literature search for documentation of copy number polymorphisms for the gene. 5. Perform comparative sequencing on the subjects to identify any undocumented SNPs present under the primer or probe; extra SNPs may cause angle clusters. 6. Perform a TaqMan Gene Copy Number Assay (PN 4331182 – human) on all samples to determine the copy number for the gene in which the polymorphism resides. For species other than human, determine an appropriate Copy Number Assay to use, if possible.

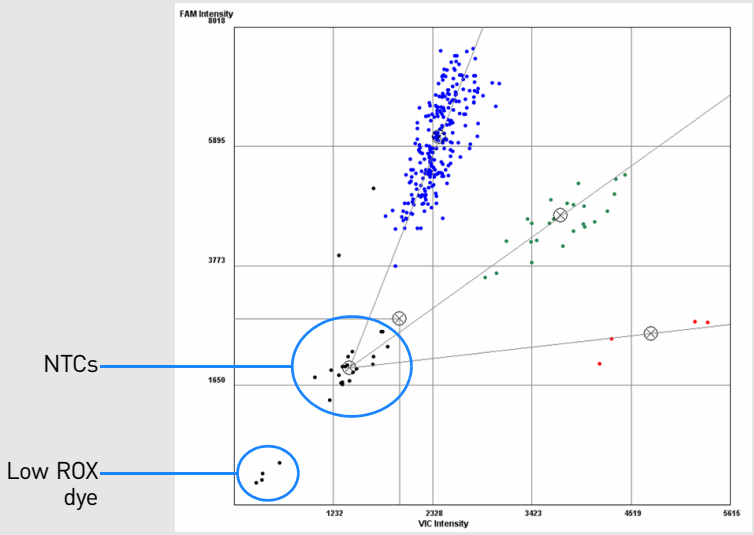


Observation	Possible cause	Recommended solution
All samples failed to amplify.	Numerous problems can cause complete failure of an assay. In addition to the previously mentioned issues in this table, consider the following:	
	A phenol/chloroform DNA extraction method was used.	• Use molecular biology-grade phenol/chloroform, and remove all traces of phenol. • Consider a bead-based or column-based extraction method.
	The DNA sample contains impurities.	Dilute the DNA sample 1:10 to dilute impurities.
	The DNA sample was not properly prepared.	Use an Applied Biosystems control human gDNA (PN 4312660) to determine if the problem arises from the sample preparation.
	Lower-grade reagents were used.	Lower-grade reagents may contain PCR inhibitors. Use molecular biology-grade reagents in all assay-related experiments, including DNA preparation.
	Heparin was used as an anti-coagulant.	If your sample DNA is extracted from blood, do not use Heparin as an anti-coagulant as it can inhibit PCR. Use EDTA as an alternative.
	Samples failed to amplify on the OpenArray® platform, but amplified on a real-time PCR system.	Contact Applied Biosystems Technical Support.



Observation	Possible cause	Recommended solution
Double clustering. Each cluster is comprised of two clusters.	Genomic DNA is not properly quantitated. Samples with differing concentrations result in varied fluorescence intensities. Samples with lower starting quantities exponentially amplify lower yields compared to samples with higher starting quantities.	Use a high-quality spectrophotometer or perform an RNase P quantitation assay to determine the concentration of each sample. Normalize as needed (refer to the *User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit* (see page 67).
	There are differences in fluorescence intensities between two genotyping plates in one project.	• If each genotyping plate was prepared with different master mixes, check the reagent expiration dates. Use only reagents that are not past their expiration date. • Analyze the genotyping plates separately.
	The primer and probe sequences target non-specific sequences.	Targeting non-specific sequences is conventionally not an issue with validated or pre-designed assays. In rare instances, a duplicated region of the genome that is represented in the genomic assembly as a single location produces this result for a pre-designed assay. These duplicated regions may be detected by BLAST analysis of the assay content sequence to the NCBI nr (non-redundant) database sequences.
	A SNP under a probe can result in an outlier that falls between the heterozygote and one of the homozygotes (an angle cluster) or an outlier that has the same angle as a cluster but trails behind the main cluster (a vector cluster).	To confirm the presence of another SNP under the probe or primer, repeat the experiment and evaluate the overall assay performance: • Do the assay results appear in tight clusters? • Do clusters have good separation?

Observation	Possible cause	Recommended solution
		• Perform proper bioinformatics on the sequence, evaluate the SNP design, and consider redesigning the assay. Refer to *Bioinformatic Evaluation of a Sequence for Custom TaqMan® SNP Genotyping Assays*. • Verify the presence of the outlier. Examine the sample's performance in other assays to rule out problems caused by this particular sample, such as sample impurity or degradation. Search the public databases (for example, dbSNP) to see if the additional SNP has been discovered. Perform comparative sequencing on the subjects to identify any undocumented SNPs present under the primer or probe. The presence of extra SNPs may cause angle clusters or vector clusters. **Note:** dbSNP is an NIH database; go to **www.ncbi.nlm.nih.gov/projects/SNP**.

Observation	Possible cause	Recommended solution
Low levels of ROX™ dye	ROX™ dye is included in the TaqMan® OpenArray® Genotyping Master Mix. If assay spotting fails or if sample loading with the OpenArray® AutoLoader fails, the OpenArray software displays low levels of ROX dye in the scatter plot, if the NTCs and all other samples are properly labeled.	To confirm that the plot is reflecting low levels of the ROX dye: 1. Start the ImageJ software, then open the ROX dye image file (*.tif) for the genotyping plate. 2. Compare the through-hole locations (for example, A1c4) of the dark spots in the image file to the sample IDs and locations in the OpenArray software. 3. Note any trends. For more information on using the ImageJ software, see "View the image file to find potential problems" on page 13.
		

DNA preparation

Degraded DNA can affect PCR efficiency due to the presence of fewer template copies; fewer template copies can affect the success of the TaqMan® SNP Genotyping Assays. Degradation can result from:

- Using very old DNA samples
- Using DNA extracted from Formalin Fixed Paraffin Embedded (FFPE) tissue

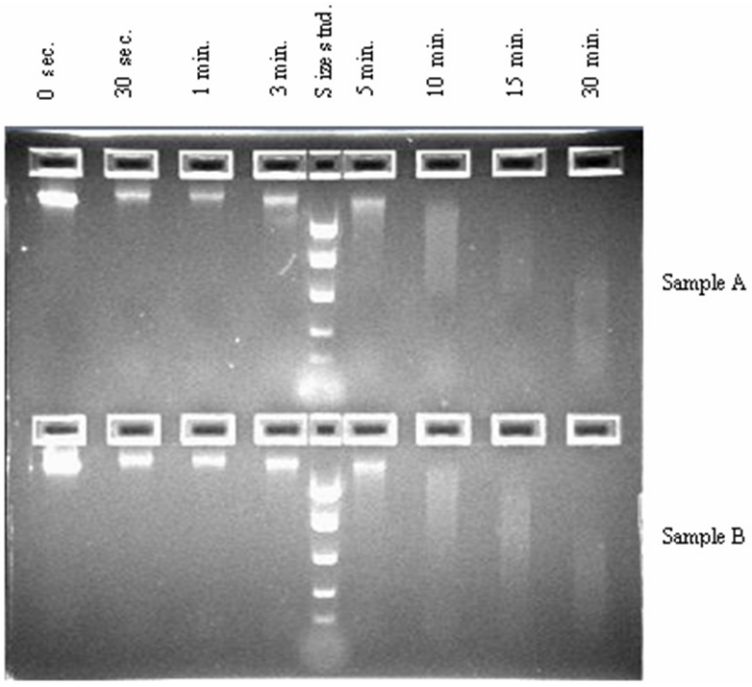
  Note: For information on preparing DNA template from FFPE tissue, refer to the Applied Biosystems publication *DNA Genotyping from Human FFPE Samples – Reliable and Reproducible* (see "Documentation and Support" on page 67).

- Freezing and thawing DNA samples repeatedly
- Leaving DNA samples at room temperature
- Exposing DNA samples to heat, UV irradiation, or physical shearing
- Purifying DNA samples inefficiently so that residual nucleases remain
- Inefficient tissue preservation methods
- The pH level and/or salt concentration in the environment

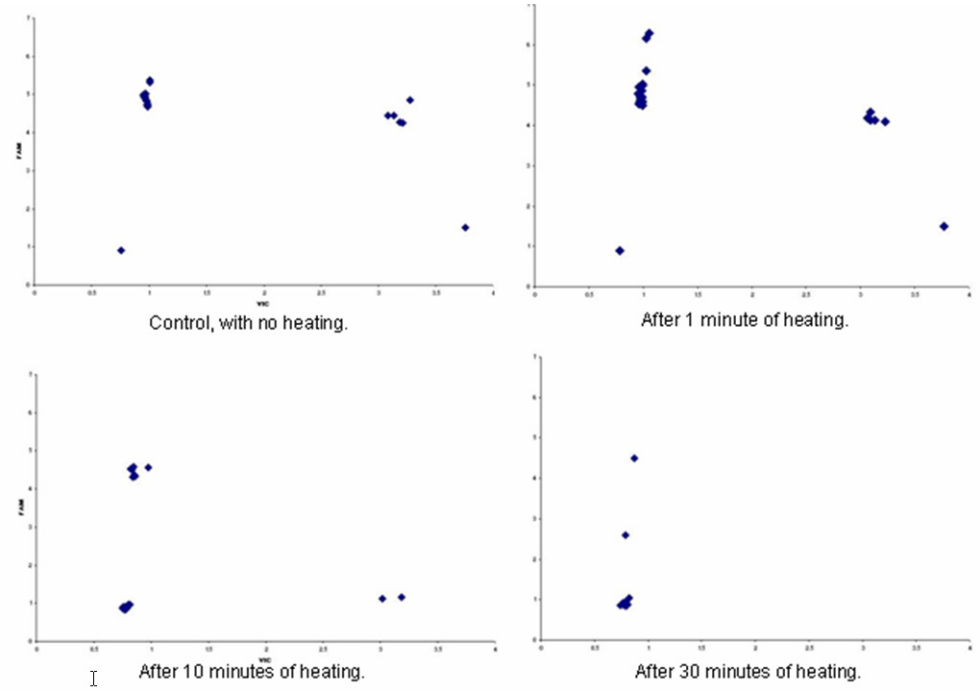
Determine DNA quality

Run an agarose gel to determine if your DNA is degraded. Look for a tight band of high molecular weight; smearing indicates degraded DNA. If the DNA is substantially degraded, use more caution in interpreting your results. If possible, consider repeating the assay using freshly prepared gDNA samples.

Agarose gel stained with ethidium bromide, showing two samples of human gDNA subjected to heating at 99 °C for up to 30 minutes.



The scatter plots shown below illustrate the effects of DNA degradation caused by heating.



Sample population size

Determine the sample population size

To determine if the size of your sample population is large enough to detect the minor allele of interest:

- Go to **www.appliedbiosystems.com**, select the **TaqMan® Genotyping Assays** page, then search for the Minor Allele Frequency (MAF) for your assay.

  Note: Applied Biosystems Custom TaqMan® SNP Genotyping Assays do not contain MAF data.

- Look for the MAF in the Assay Information File distributed with your assays.
- Search for allele frequency data using the public SNP identifier, available on public web sites such as the:
  - dbSNP at **www.ncbi.nlm.nih.gov/SNP/index.html**
  - HapMap project at **www.hapmap.org**

Determine if the minor allele is detectable

Use the Hardy-Weinberg Equilibrium equation to determine if the minor allele is detectable for a sample the size of your test population.

In the Hardy-Weinberg Equilibrium equation, $q2 + 2qp + p2 = 1$, the expected genotype frequencies are $q2$, $2qp$, and $p2$, where q and p represent the allele frequencies.

The values for $q2$, $2qp$, and $p2$ correspond to the fraction of a given population that would be homozygous for the minor allele (qq), heterozygous (qp), and homozygous for the major allele (pp), respectively.

Multiply your sample size by the fraction for each allele to determine the number of individuals with each genotype that you should expect to see. If your sample size is small, you may not be able to detect rare alleles.

Example calculation

For a SNP with a MAF of 5% (0.05), the predicted frequencies are:

- $q2 = 0.0025$
- $2qp = 0.095$
- $p2 = 0.9025$

If you test of 20 gDNA samples from this population, you might expect:

- Homozygotes for the minor allele: 0.0025 x 20 = 0.05, or 0 individuals
- Heterozygotes: 0.095 x 20 = 1.9, or ~2 individuals
- Homozygotes for the major allele: 0.9025 x 20 = 18.05, or ~18 individuals

To detect one homozygote for the minor allele, it would take a sample size of approximately 400 individuals (Sample Size = 1/MAF2).

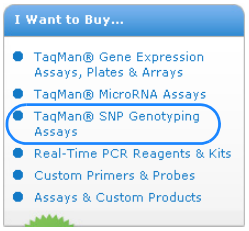
A Access Information about the TaqMan® SNP Genotyping Assays

You can access information about the TaqMan® SNP Genotyping Assays from Applied Biosystems web site.

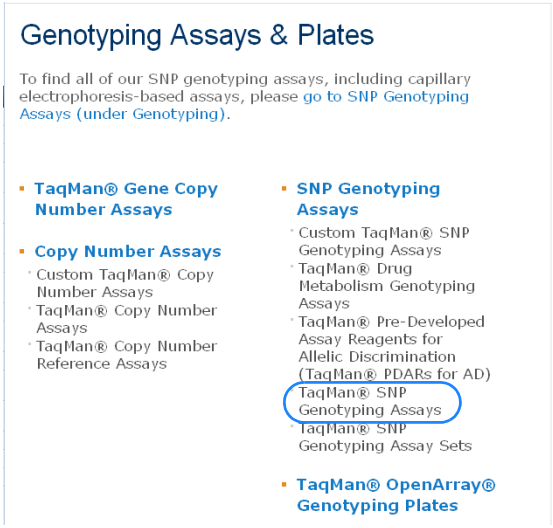
Note: For information on ordering the assays, refer to the *Ordering TaqMan® SNP Genotyping Assays Quick Reference Card* (see "Documentation and Support" on page 67).

Access information on the web site

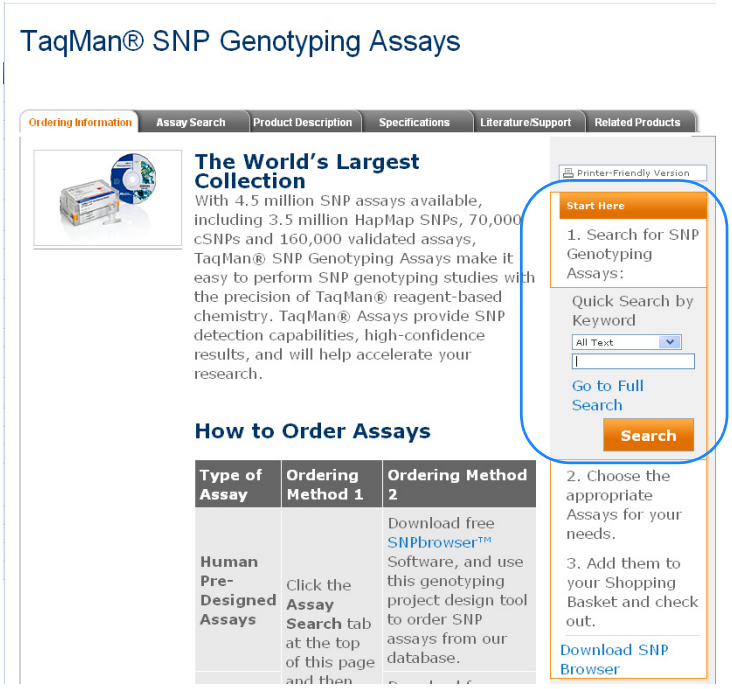
1. Go to **www.appliedbiosystems.com**.
2. In the I Want to Buy box, click **TaqMan® SNP Genotyping Assays**.



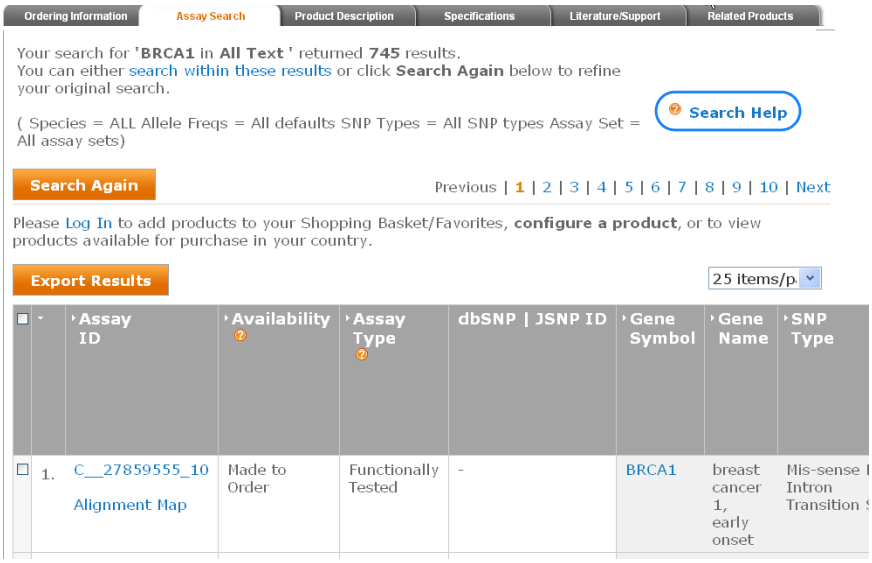
3. Under SNP Genotyping Assays, click **TaqMan® SNP Genotyping Assays**.



4. On the TaqMan® SNP Genotyping Assays page, follow the prompts to search for the assay of interest.



5. On the search results page, view the assay information. For a description of the each column, click . The column descriptions are also provided on page 61.



Column descriptions

Column	Description
Assay ID	The Applied Biosystems Assay ID. This is a link to the SNP Genotyping Assay Details Report.
Mapped ID	Batch ID search results. Indicates which search term corresponds to each search result.
Availability	• Inventoried Assays: These TaqMan® Genomic Assays have been previously manufactured, passed quality control specifications, and are stored in inventory. • Made to Order Assays: These TaqMan® Genomic Assays are manufactured at the time of order. Only assays that pass manufacturing quality control specifications are shipped.
Multiple Scales	Made to Order TaqMan® SNP Genotyping Assays are now offered in multiple scales: Small, Medium, and Large. Once you select your assays and place them in your Shopping Basket, and if multiple scales are available for those assays, you may select their scales in the Shopping Basket.
Assay Type	• Validated Assays: Validated assays are tested against four ethnic populations, consisting of 45 individuals for each population. Minor allele frequencies are determined and published for each individual population tested. Validated assays have at least a minor allele frequency of 5% in one population. • Functionally Tested: Assays for human studies are functionally tested. The function test consists of either 10 or 20 unique DNA samples comprised of a mixed ethnic population and both male and female representation. The functional test data are used to ensure that shipped assays meet minimum functionality criteria only. Minor allele frequency is not calculated due to the small and mixed sample set used in the test. The functional test is similar to the test performed for the Custom TaqMan® SNP Genotyping Assay Service. • TaqMan® Drug Metabolism Assay: DME assays are tested against four ethnic populations, consisting of 45 individuals in each population. Validation data for Caucasian and African-American populations have been performed in replicates.
dbSNP/JSNP ID	The corresponding NCBI dbSNP cluster ID (rs #) and/or JSNP ID. The dbSNP ID is a link to the corresponding record in NCBI dbSNP, and the JSNP ID is a link to the corresponding record in the JSNP® database.
Gene Symbol	The gene symbol (for example, CALCB).
Gene Name	The gene name (for example, calcitonin-related polypeptide).
Minor Allele Frequency	Minor Allele Frequency indicates the number of occurrences of an allele seen in the total number of chromosomes typed at the SNP site. • AB: The Applied Biosystems Minor Allele Frequency data are derived from four ethnic populations up to 45 individuals each: African-American, Caucasian, Chinese, and Japanese. • HapMap: The HapMap Minor Allele Frequency data are derived from specific ethnic populations as part of the International HapMap Project: CEPH (CEU), Yoruba in Ibidan, Nigeria (YRI), Han Chinese in Beijing, China (CHB), and Japanese in Tokyo, Japan (JPT). • AGI: The AGI Minor Allele Frequency data are derived from two ethnic populations of 19-20 individuals each as part of the Applera Genome Resequencing Initiative: Caucasian and African-American. **Note:** Minor Allele Frequency values from different data sets may differ for a particular SNP. This may be because the values were generated by different populations and different sample sets.
Public Location	The SNP location on the NCBI public genome.

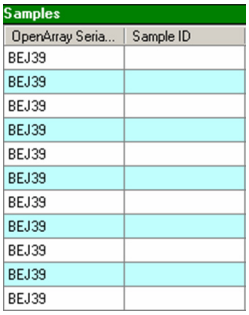
Column	Description
SNP Type	The relationship between the variation and any local gene features. One of the following displays: • acceptor splice site • coding region • donor splice site • intergenic/unknown • intron • mis-sense mutation • nonsense mutation • Pseudoautosomal XY SNP • putative utr 3 • putative utr 5 • repeats • silent mutation • utr 3 • utr 5
Allele Nomenclature	*DME assays only.* The allele nomenclature follows an international nomenclature system created to address the need for a universal system to describe polymorphisms in DNA and protein sequences. The allele nomenclature used indicates the gene name, the allele designation, and the base changes associated with the polymorphism. For example, for *CYP1A1*1C g-3229 G>A*: • CYP1A1 indicates the gene • 1C indicates the allele • g indicates genomic DNA • -3229 indicates the nucleotide position from the start codon • G>A indicates the base change There are three public nomenclature Web sites we referred to when creating the DME product line: • The home page of the Human Cytochrome P450 (CYP) Allele Nomenclature Committee: http://www.imm.ki.se/cypalleles/ • The Arylamine N-Acetyltransferase (NAT) Nomenclature Web site: http://www.louisville.edu/medschool/pharmacology/NAT.html • The home page of the committee mediating the naming of UDP Glucuronosyltransferase: http://som.flinders.edu.au/FUSA/ClinPharm/UGT/ Please note that not all of the polymorphisms included in our DME assay collection have been documented on a nomenclature site; therefore, not all of our assays will be associated with public allele nomenclature.

B Import Sample Information After Imaging

Applied Biosystems strongly recommends that you add sample information *before* you begin imaging. (For procedures, refer to Chapter 4 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*) Adding the sample information before imaging ensures that the information is included in the plate data file (*.spd) that the OpenArray® SNP Genotyping Analysis Software generates during an imaging run. If needed, however, you can import the sample information into a *.nix or *.spd file after imaging is complete.

Determine if the sample information was imported before imaging

To determine if the sample information was imported into a *.nix or *.spd file before imaging, view the Sample ID column in the Samples pane. If the sample information was not imported before imaging, the Sample ID column is empty.



Samples

OpenArray Seria...	Sample ID
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	
BEJ39	

Import the sample information after imaging

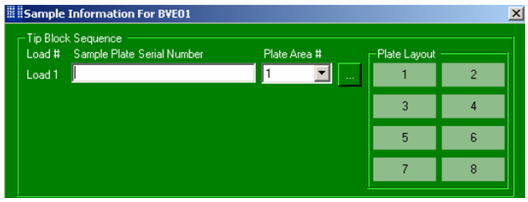
1. If you have not done so already, create a *.csv file according to the procedures in Chapter 2 of the *TaqMan® OpenArray® Genotyping Getting Started Guide*.

2. In the OpenArray software, select **File ▸ Open**, then browse to and open the *.nix file of interest.

3. Click **Edit** (below the Samples pane) to open the Sample Information dialog box.

4. (Optional) In the Sample Plate Serial Number field, enter the unique identifier for each sample plate.

   Note: The unique identifier is the one you created when you prepared the sample plates.

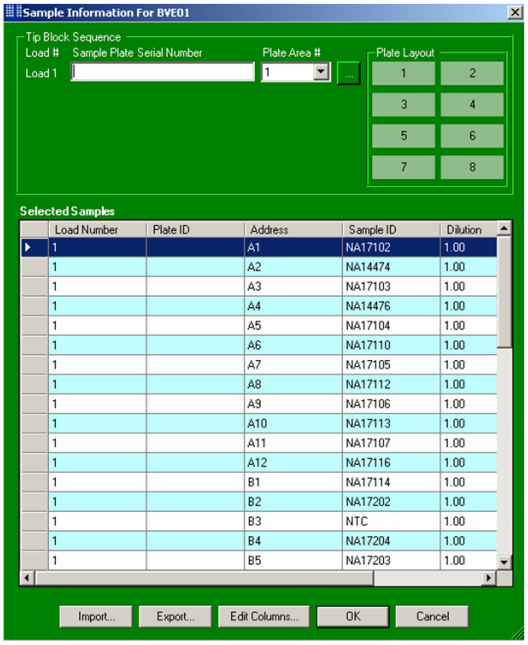


5. Import the sample information:

   a. Click Import to open the Import Sample Plates dialog box.

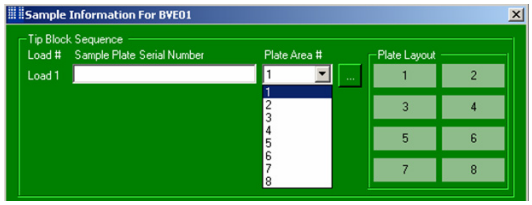
b. Browse to and open the *.csv file to import. The sample information appears in the Selected Samples pane.

IMPORTANT! Be sure to select a *.csv file that contains sample information for all of the required loads.



c. Edit the sample information in each row, as needed.

6. From the Plate Area # dropdown menu, select the 12-well × 4-well area of the sample plate that the samples were transferred from.



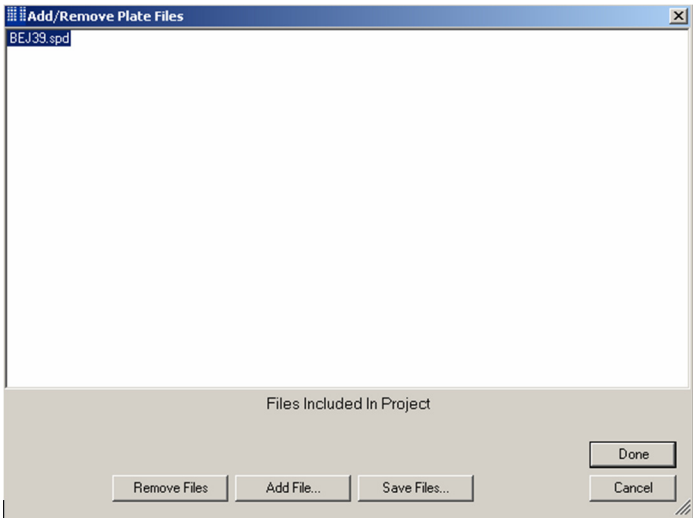
7. Save the *.nix file:
   - Select **File ▸ Save** to save the changes to the current *.nix file.

     Or
   - Select **File ▸ Save As** to save the changes to a new *.nix file.

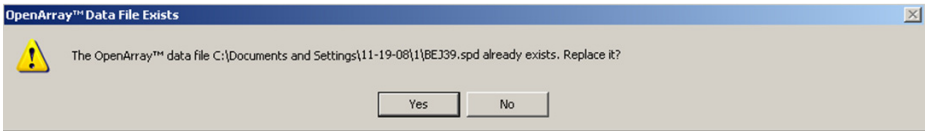
(Recommended) Save the sample information to the *.spd file

After saving the sample information to the *.nix file, Applied Biosystems recommends that you also save the sample information to the *.spd file. Saving the sample information to the *.spd file ensures that the sample information will not be lost, even if the *.nix or *.csv file is deleted.

1. In the OpenArray software, click **Add**.
2. In the Add/Remove Plate Files dialog box, select the appropriate *.spd file, then click **Save Files**.



3. In the Browse For Folder dialog box, select a save location, then click **OK**.
4. At the prompt, click **Yes** to overwrite the existing *.spd file.



5. Click **Done**. The *.spd file now contains the sample information.

Documentation and Support

System documentation

The following documents are available for the OpenArray® system:

Document	Description	Part number
OpenArray® System Site Preparation Guide	Provides information on preparing the customer site for the OpenArray® system.	4401171
TaqMan® OpenArray® Genotyping Troubleshooting Guide	Provides troubleshooting information for TaqMan® OpenArray® Genotyping. To be used in conjunction with the *TaqMan® OpenArray® Genotyping Getting Started Guide*.	4401671
TaqMan® OpenArray® Genotyping Getting Started Guide	Provides procedures for performing TaqMan® OpenArray® Genotyping.	4377476
TaqMan® OpenArray® Genotyping Quick Reference Card	Describes the overall workflow and provides brief procedures for performing TaqMan® OpenArray® Genotyping.	4400402

Related documentation

When using this guide, you may find the documents listed below useful. To obtain this and additional documentation, see "Obtaining support" on page 68.

Document	Part number
Application Note: DNA Genotyping from Human FFPE Samples – Reliable and Reproducible	137AP04-01
Bioinformatic Evaluation of a Sequence for Custom TaqMan® SNP Genotyping Assays	4371003
Ordering TaqMan® SNP Genotyping Assays Quick Reference Card	4374204
TaqMan® SNP Genotyping Assays Protocol	4332856
User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit	4342582

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

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