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# TaqMan® OpenArray® Genotyping

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## SUBJECT: Troubleshooting Guide



**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

## Revision history

| Revision | Date | Description |
|---|---|---|
| A.0 | February 2015 | New publication |

## Tools for troubleshooting

### Troubleshoot with cycling and imaging run images

Many problems with OpenArray® results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run.

The QC images are fluorescent or reflected light images taken before, during and after cycling, and may require adjustment to make image features visible. To view the images, we recommend that you install the free program ImageJ, which allows you to easily manipulate the images in ways that other image viewers cannot.

1. In the QuantStudio® Software, with the experiment in question open, click **Export QC Images** in the Export tab, then select a new folder for the exported images.

   IMPORTANT! Select a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. Use ImageJ to view the images of interest.

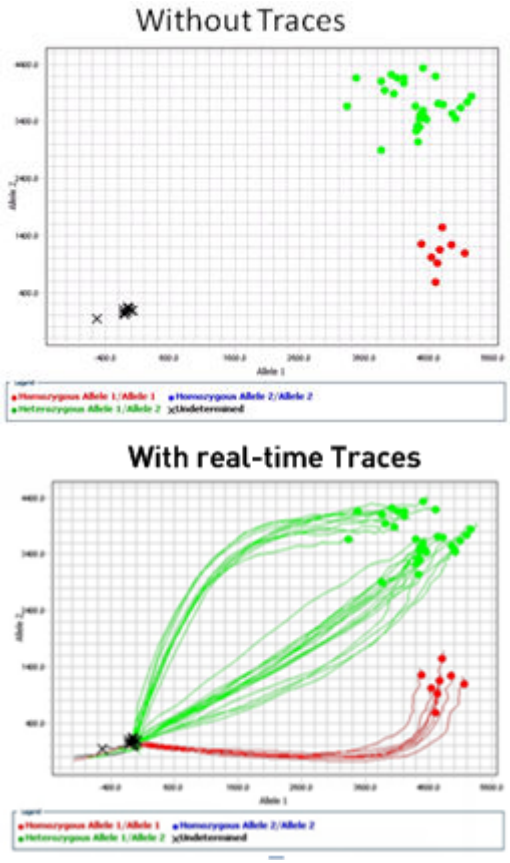
| To ... | View image ... | Image description |
|---|---|---|
| Confirm the identity of images within a folder | BARCODE IMAGE.tiff | Reflected light image of the entire OpenArray® plate |
| Assess the loading quality | PRE-READ_CHANNEL_4.tiff<br>POST-READ_CHANNEL_4.tiff | Pre and post ROX™ images |
| Check for existing contamination on the case and/or heated cover | s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff[1] | Pre-run reflected light spotfinding image (used by the software for determining the location of the holes) |
| Identify potential leaks or other contamination | s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff[1] | Mid-run reflected light spotfinding image |
| | s04_c001_t02_p0001_m2_x3_e1_cp#_spotfind.tiff[1] | Post-run reflected light spotfinding image |
| Look at patterns in the florescent data (e.g. gradients) | STAGEx_CYCLEy_CHANNEL_z.tiff | FAM™ (z=1) or VIC® (z=2) images at a particular cycle (y) of a particular stage (x) of the run |

[1] "cp#" in the image file name refers to the position (1–4) within the QuantStudio® 12K Flex instrument.

3. If needed, adjust the images for brightness and/or contrast to make image features visible. Open the image in ImageJ, select **Image ▸ Adjust Brightness/ Contrast** (or press **Ctrl+Shift+C**), then click **Auto** or adjust the sliders until the features of interest in the image are visible.

## Using real-time traces for troubleshooting

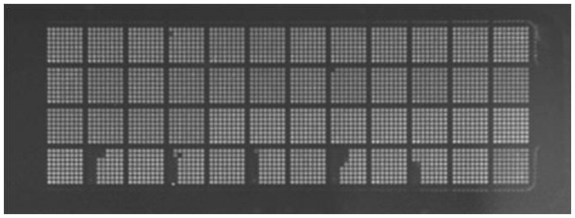
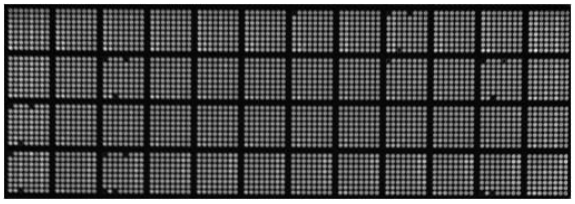
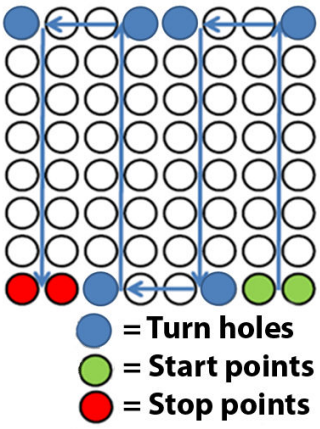
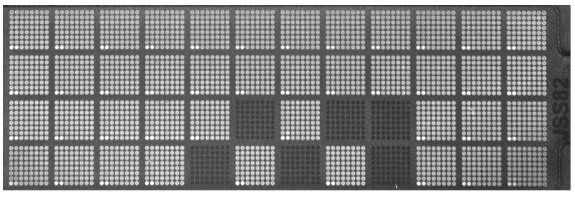
Real-time traces can be used to identify outlier data points and make manual calls. In the example below, a review of the data from an earlier cycle number helped to determine the correct genotypes for several samples. The QuantStudio® 12K Flex Software algorithm determined that many samples were heterozygous (top). However, the real-time traces (bottom) show signal saturation of the homozygous FAM™ cluster towards the heterozygous cluster, providing good evidence to change some of the calls from heterozygous to homozygous for the FAM™ allele.



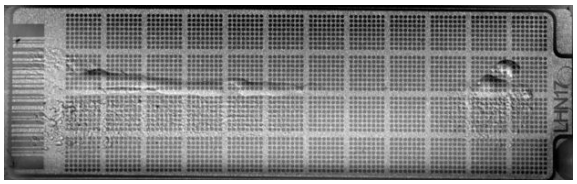
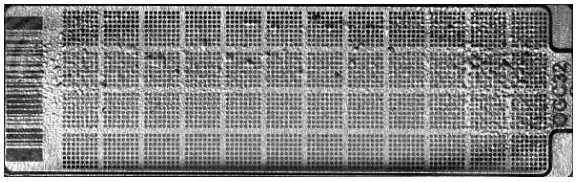
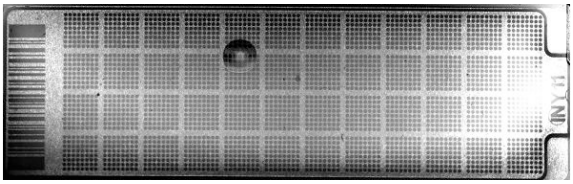

SNP assay real-time traces can also be viewed using the genotyping data analysis tools in the Applied Biosystems® qPCR Analysis Module, which is accessed from the **www.lifetechnologies.com** web site.

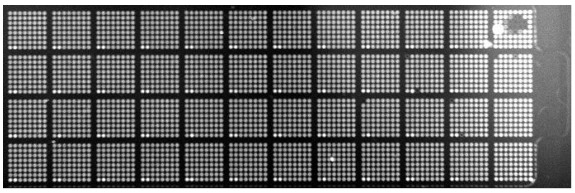
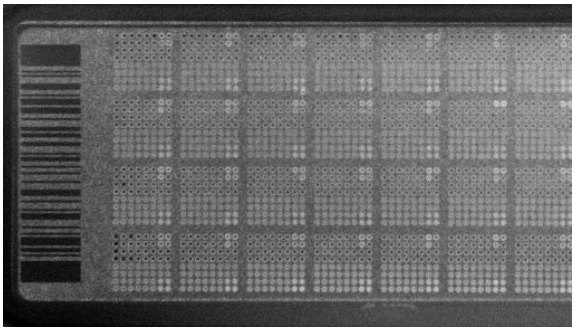
As a rule of thumb, a $C_T$ value of ~25 indicates that the sample input is approximately 250 haploid copies, which is the recommended input amount per 33-nL reaction for OpenArray® genotyping plates (that is, 825 pg of DNA is loaded per PCR reaction when a stock solution of 50 ng/µL is used).

# AccuFill™ instrument plate loading errors

| Observation | Possible cause | Recommended action |
|---|---|---|
| Empty through-holes<br>  | Not enough volume of sample was added to the 384-well sample plate. | Ensure that proper pipetting techniques are performed. Ensure that there are no air bubbles in the pipette tips after sample aspiration. |
| | Reaction mix (sample + master mix) is not at the bottom of the 384-well sample plate. | Ensure that the sample plate is centrifuged at 1000 rpm for 60 seconds. |
| Turn-holes are repeatedly missed<br>  | AccuFill™ instrument is aligned too far to the left or right.<br><br>Systematic loading problems can occur with the AccuFill™ instrument, indicating a need for service. For example, when turn-holes (where the AccuFill™ instrument changes direction during sample loading; see Load Path image below) are repeatedly missed across multiple subarrays, service is required.<br>  | Contact your local field service engineer to resolve the issue. |
| Entire subarrays are missing<br>  | Sample/Master Mix not added to particular wells in the 384-well sample plate. | Visually inspect the sample plate to confirm that the wells have sample/ master mix. |
| | Stuck tip mandrel on AccuFill™ instrument may need cleaning. | Contact your local field service engineer. |
| | Pipette tip not loaded on mandrel. | Contact your local field service engineer if this happens regularly (infrequent occurrences can be due to a poorly molded tip). |

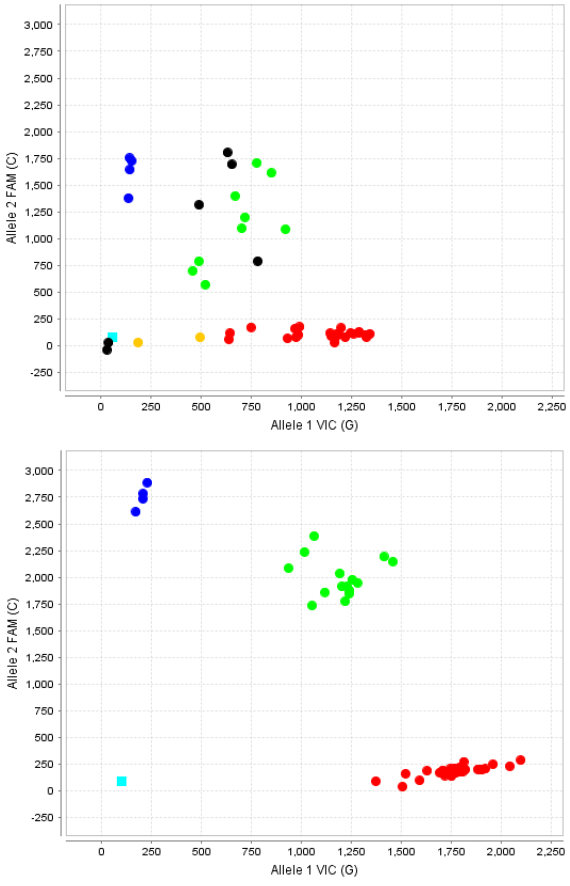
# OpenArray plate assembly and handling errors

| Observation | Possible cause | Recommended action |
|---|---|---|
| Case leaks and bubbles inside the case<br>  <br>  <br>  <br>Improper sealing of theOpenArray® plate in the OpenArray® Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality genotyping data.<br>The images above are examples of OpenArray® plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover windows and obscured the view of the through-holes.<br>The best image in which to detect leaks is the s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff image. This image is taken at the start of cycling, which is where most leaks occur. See "Troubleshoot with cycling and imaging run images" on page 1. | Plate press was not engaged for at least 20 seconds. | Ensure that the plate press is fully engaged for at least 20 seconds on future experiments. |
| | Damaged lid adhesive. | Visually inspect the lid adhesives for defect when the liner has been removed. Ensure that adhesive is not damaged or warped. |
| | Damaged fill port screw gasket. | Visually inspect the screw to ensure that the orange gasket is present and not damaged. |
| | Damaged fill port screw assembly. Breaks off too easily. | The screw may be mis-threaded: Unscrew it and use a new screw assembly. |
| | Oily lid or case from immersion fluid overflow. | Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray® plate is acceptable). |
| | Immersion fluid exposed to air for too long. | Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load. Do not draw air bubbles into the syringe. |
| | Too large of a bubble inside the OpenArray® case after sealing. | Minimize the size of the bubble by tilting the OpenArray® case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case. |
| | Damaged plate press leading to uneven pressure. | Contact your field service engineer if you suspect that your plate press may be damaged. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Sample blow out during the addition of immersion fluid<br>  | The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can actually purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use. | A small amount of immersion fluid should be dispensed onto a paper towel before use to ensure smooth operation of the syringe. |
| Evaporation of reaction mixture in through-holes<br>  | Too much time elapsed before plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentionally left open to the environment to demonstrate the effect of evaporation. "Donuts" are a result of the evaporated fluid in the though-holes. | To minimize the likelihood of evaporation, take the plate off of the AccuFill™ deck, seal the case with the lid, and add immersion fluid as soon as the case is removed from the plate press |

# Troubleshooting unexpected genotyping results

The following table contains troubleshooting tips that pertain to TaqMan® SNP Genotyping Assays run on OpenArray®systems. For a comprehensive guide to troubleshooting SNP assay performance, refer to "Appendix A: Troubleshooting" in the *TaqMan® SNP Genotyping Assays User Guide.*

| Observation | Possible cause | Recommended action |
|---|---|---|
| Diffuse clusters, loss of heterozygosity, and/or non-amplification for many samples in a dataset | Insufficient amount of starting DNA and/or PCR inhibitors present in the sample preparation. | We recommend using 50 ng/µL starting DNA concentration. If it is not possible to increase the concentration of the starting material or remove inhibitors from the sample preparation, preamplify the sample. See the *TaqMan® OpenArray® Genotyping Sample Preamplification User Bulletin* .<br><br>The images show poor quality SNP data (top) that is improved by preamplifying the samples (bottom).<br><br>  |
| Amplification plot is missing expected cluster | Signal saturation caused software to call homozygous alleles as heterozygous (or *vice versa*). | Use real-time traces (see "Using real-time traces for troubleshooting" on page 3) to manually call alleles. |

# Documentation and support

## Related documentation

Portable document format (PDF) versions of this guide and the following publication is also available on **www.lifetechnologies.com**.

| Document | Publication number |
|---|---|
| *Applied Biosystems® QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* | 4470935 |
| *TaqMan® SNP Genotyping Assays User Guide* | MAN0009593 |

**Note:** To open the user documentation, use the Adobe® Reader® software available from **www.adobe.com**

**Note:** For additional documentation, see "Customer and technical support" on page 8.

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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