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OpenArray® Real-Time PCR System

User Guide

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Part Number 4458837 Rev. A
09/2010

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About This Guide

Purpose

The OpenArray® Real-Time PCR System uses fluorescence-based polymerase chain reaction (PCR) reagents to detect targets of interest. You can use the OpenArray system to perform:

- Gene expression experiments, with the TaqMan® OpenArray® Real-Time PCR Plates or SYBR® OpenArray® Real-Time PCR Plates.
- Genotyping experiments, with the TaqMan® OpenArray® Genotyping Plates.

The *OpenArray® Real-Time PCR System User Guide* provides information about the OpenArray system, including step-by-step procedures to:

- Run an OpenArray plate on the OpenArray® Real-Time PCR Instrument.
- Analyze the data with the OpenArray® Real-Time qPCR Analysis Software or the OpenArray® SNP Genotyping Analysis Software.
- Maintain the OpenArray® platform.

Prerequisites

This guide assumes that your OpenArray® platform has been installed by an Applied Biosystems service representative.

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 Appendix C, “Safety” on page 145. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or 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* (see "Safety symbols" on page 146).

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, see "SDSs" on page 153.

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

PART I

Gene Expression Experiments

Performing real-time imaging and analysis

1 Before You Begin

This chapter covers:

- Required system components 15
- Required plates 17
- Workflow 19

Required system components

To perform gene expression experiments with the OpenArray® Real-Time PCR System, you need the following system components:

- OpenArray® platform (this page)
- Software (this page)

Note: If you have the TaqMan® OpenArray® Genotyping System (rather than the OpenArray® Real-Time PCR System), you need the OpenArray® Upgrade to perform gene expression experiments. For more information, see page 16.

OpenArray® platform

For gene expression experiments, the OpenArray® platform includes the:

- **OpenArray® AutoLoader** – Loads cDNA or gDNA sample onto a TaqMan® OpenArray® Real-Time PCR Plate or a SYBR® OpenArray® Real-Time PCR Plate.
- **OpenArray® Case Sealing Station** – Seals the OpenArray® Cases.
- **OpenArray® instrument** (with heat block) – Performs thermal cycling and real-time imaging of the OpenArray plates.
- **Computer** – Connects to the OpenArray® instrument; includes the OpenArray® Real-Time qPCR Analysis Software (see below).

Software

OpenArray® Real-Time qPCR Analysis Software – Controls the OpenArray instrument and analyzes the real-time imaging data.

(If needed) OpenArray® Upgrade

If you have the TaqMan® OpenArray® Genotyping System (rather than the OpenArray® Real-Time PCR System), you need the OpenArray® Upgrade to perform gene expression experiments.

The OpenArray Upgrade transforms a TaqMan® OpenArray® Genotyping System into a dual-use instrument that allows you to perform:

- Imaging of the TaqMan® OpenArray® Genotyping Plates
- Thermal cycling and real-time imaging of the TaqMan® OpenArray® Real-Time PCR Plates or the SYBR® OpenArray® Real-Time PCR Plates

Note: Contact your Applied Biosystems service representative for more information on the OpenArray Upgrade.

Required plates

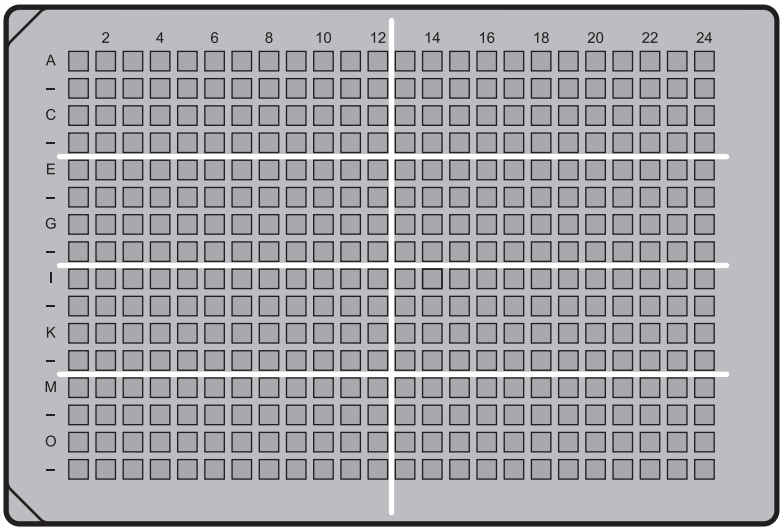
To perform gene expression experiments, the OpenArray system requires two plate types:

- OpenArray® 384-Well Sample Plate (*sample plate*) (this page)
- One of the following OpenArray plates (page 18):
  - TaqMan® OpenArray® Real-Time PCR Plate
  - SYBR® OpenArray® Real-Time PCR Plate

Sample plate

The OpenArray 384-Well Sample Plate is a 384-well reaction plate. You combine the PCR mix (user-prepared) with cDNA or gDNA sample in the sample plate, then use the OpenArray AutoLoader to transfer the mixture from the sample plate to one or more OpenArray plates.

IMPORTANT! The well dimensions of the OpenArray 384-Well Sample Plate are specifically suited for use with the OpenArray AutoLoader. Applied Biosystems does not recommend the use of other microtiter plates with the AutoLoader.



SYBR® and TaqMan® OpenArray® Real-Time PCR Plates

The TaqMan® OpenArray® Real-Time PCR Plate and the SYBR® OpenArray® Real-Time PCR Plate are 63-mm × 19-mm mid-density reaction plates. There are 3072 reaction through-holes in each plate. Individual through-holes are preloaded with a SYBR or TaqMan gene expression assay; each through-hole can accommodate a 33-nL reaction volume.

As shown in the figure below, the OpenArray plates are divided into 48 subarrays; each subarray consists of 64 through-holes. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes.

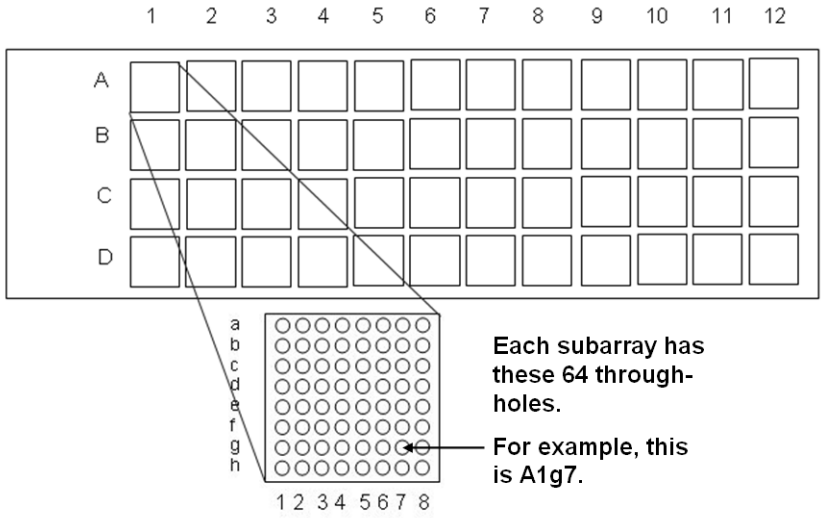


Plate preparation procedures

Refer to the *TaqMan® OpenArray® Real-Time PCR Plate Protocol* or the *SYBR® OpenArray® Real-Time PCR Plate Protocol* for procedures on:

- Preparing the OpenArray 384-Well Sample Plate
- Using the OpenArray AutoLoader to transfer sample from the sample plate to an OpenArray plate.

To obtain the appropriate OpenArray plate protocol, see "Product documentation" on page 159.

IMPORTANT! This User Guide assumes that the OpenArray plate is loaded with sample and ready for real-time imaging.

Workflow

Prepare the plates

1. Prepare an OpenArray® 384-Well Sample Plate.
2. Use the OpenArray® AutoLoader to transfer sample from the sample plate to a TaqMan® OpenArray® Real-Time PCR Plate or to a SYBR® OpenArray® Real-Time PCR Plate.

Note: For procedures, refer to the appropriate OpenArray plate protocol. See "Product documentation" on page 159.

Perform real-time imaging (Chapter 2)

1. Set up the OpenArray® Real-Time qPCR Analysis Software.
2. Place the prepared OpenArray® plate in the OpenArray® instrument
3. Enter sample information in the software.
4. Perform thermal cycling and real-time imaging.

Analyze the run data (Chapter 3)

1. View the results.
2. (Optional) Modify the data.
3. (Optional) Modify project files (*.ncx).
4. (Optional) Publish data.

2 Perform Real-Time Imaging

In this chapter, you set up the OpenArray® Real-Time qPCR Analysis Software, then perform thermal cycling and real-time imaging on the OpenArray® instrument.

This chapter covers:

- About the data files 22
- Set up the software 24
- Place the prepared OpenArray® plate into the instrument 25
- Enter sample information 26
- Perform thermal cycling and real-time imaging 31

About the data files

The OpenArray® Real-Time qPCR Analysis Software uses four types of data files:

- Plate setup file (*.tpf) (this page)
- Project files (*.ncx) (this page)
- Plate data files (*.tpd) (page 23)
- Sample information files (*.csv) (page 23)

Plate setup file (*.tpf)

A plate setup file (*.tpf) contains specific information for individual TaqMan® OpenArray® Real-Time PCR Plates or SYBR® OpenArray® Real-Time PCR Plates, such as:

- Assay IDs
- Gene symbol and name
- Location of each assay in the OpenArray plate

Each plate setup file is named with the serial number of its corresponding OpenArray plate. For example, the plate setup file for an OpenArray plate with the serial number **ABC01** is named **ABC01.tpf**.

Accessing *.tpf files

When you order the OpenArray plates, you must download a plate setup file (*.tpf) for each OpenArray plate in your order, then copy the *.tpf files to the OpenArray system computer (as described on page 24). The OpenArray software uses the *.tpf files to populate the columns in the Assays pane; the software must access the *.tpf file for each OpenArray plate before the OpenArray instrument can perform real-time imaging.

Project files (*.ncx)

Project files (*.ncx) are the files that you view and modify in the OpenArray software. A project file allows you to combine, edit, and save changes to run data from up to 13 plate data files (*.tpd).

Project files contain:

- **Run data** – When you image OpenArray plates, the run data is automatically saved to a plate data file (*.tpd), then copied to the currently open project file (*.ncx).
- **Modifications made to the data** – Within a single project file, you can overlay, view, and edit curves from multiple plate data files (as described in Chapter 3).

To save modifications made to the data, you must save the project file (use the **File ▸ Save** or **File ▸ Save As** function). Otherwise, all your changes are lost. Project file names and save locations are user-defined.

IMPORTANT! The software *copies* the run data from the plate data file to the project file. The files are not linked; that is, modifications that you save to the project file (*.ncx) are not saved to the corresponding plate data file (*.tpd).

Plate data files (*.tpd)

A plate data file (*.tpd) contains run data for a single OpenArray plate. Plate data files are generated by the OpenArray software during imaging.

The software automatically names plate data files with the OpenArray plate serial number. For example, the plate data file for an OpenArray plate with the serial number **ABC01** is named **ABC01.tpd**.

By default, the software saves the *.tpd files to the following location:

<*drive*>:\images\<*run date*>\<*run number*>

where:

<*drive*> is the computer drive on which the OpenArray software is installed. The default installation drive is the C: drive.

<*run date*> is the date the run was performed.

<*run number*> is the chronological run number.

For example, data for the third run on June 15, 2008, is saved to:

C:\images\06-15-08\3

After a run is completed, you can change the *.tpd file name and/or save the *.tpd file to a different location.

Sample information files (*.csv)

The OpenArray software uses comma-delimited files (*.csv) to import and export sample information:

- **Import** – Applied Biosystems recommends that you create sample information files (*.csv) to track your cDNA or gDNA samples. Before performing real-time imaging on the OpenArray plates, you can import the sample information into the OpenArray software. See "Import sample information from a *.csv file" on page 27.
- **Export** – After an imaging run, you can export data from your project. See "Export cycling data" on page 62.

Set up the software

Set up the OpenArray® Real-Time qPCR Analysis Software for each OpenArray plate to be included in the real-time imaging run:

- Start the instrument and software (this page)
- Copy the plate setup file (*.tpf) to your computer (this page)

Start the instrument and software

1. Power on the OpenArray® instrument.
2. Power on the computer, then start the OpenArray® Real-Time qPCR Analysis Software. The software displays a new (empty) project file (*.ncx).
3. Wait for the system to fully start: When the system is ready, "Idle" appears in the software status bar at the bottom of the window. Startup may take a few minutes.

Copy the plate setup file (*.tpf) to your computer

1. For each OpenArray plate in your order, download the plate setup file (*.tpf) from the OpenArray plate product page or from:

   www.appliedbiosystems.com/tpfdownload

   Note: For detailed ordering information, refer to the *TaqMan® OpenArray® Real-Time PCR Plate Protocol* or the *SYBR® OpenArray® Real-Time PCR Plate Protocol*.
2. Open the download location and confirm that there is a *.tpf file for each OpenArray plate in your order.
3. Copy the plate setup files to the **PLATEFILES** folder:

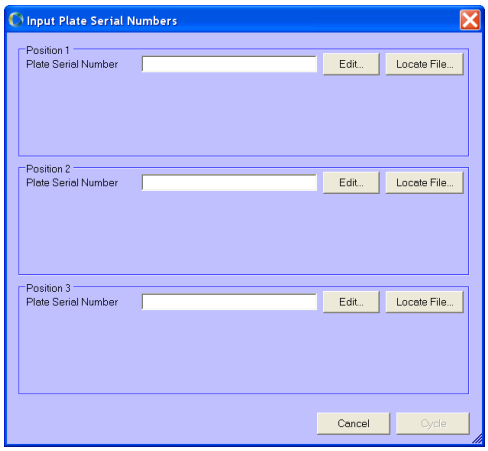
   <*drive*>:\Program Files\BioTrove\PLATEFILES

   where <*drive*> is the computer drive on which the OpenArray software is installed. The default installation drive is the C: drive.

Place the prepared OpenArray® plate into the instrument

1. In the OpenArray software, open a project file (*.ncx). You can open:
   - A new project file – Use the project file automatically opened at startup, or select **File** ▸ **New**.
   - An existing project file (containing data from previous runs) – Select **File** ▸ **Open**, then browse to and open a project file.

2. Click **Cycle** to open the Input Plate Serial Numbers dialog box:

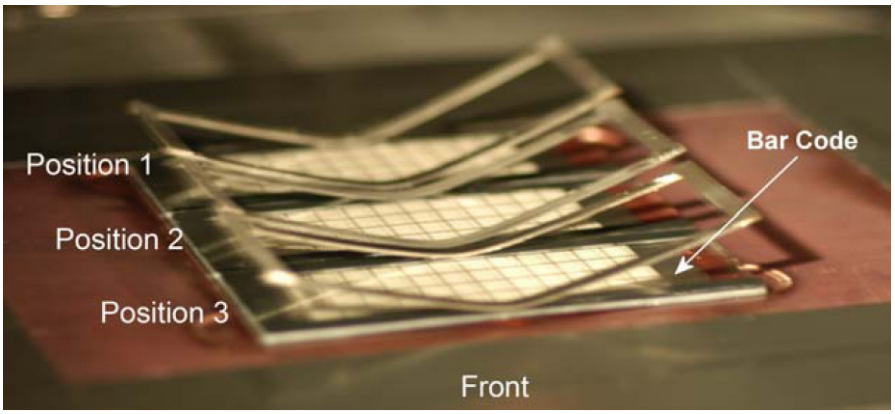


3. At Position 1, enter the serial number for the first OpenArray plate. You can:
   - Click **Locate File**, then browse to and open the plate setup file (*.tpf) that corresponds to the OpenArray plate. The software automatically displays the serial number in the Plate Serial Number field.
   - Type the serial number.
   - Scan the barcode located on the OpenArray plate package.

IMPORTANT! If you enter the serial number by typing or scanning, the *.tpf file *must* be located in the PLATEFILES directory (see "Copy the plate setup file (*.tpf) to your computer" on page 24). Otherwise, the software will not be able to locate the *.tpf file.

Note: The OpenArray software uses the serial numbers to access the appropriate plate setup files (*.tpf). During imaging, the software uses information in the plate setup files to populate the Assays pane in the project file (*.ncx). For information on the Assays pane, see "View data in the Assays pane" on page 34.

4. Open the OpenArray instrument door and lid, then place the OpenArray plate into Position 1. Be sure that:
   - The plate position in the instrument matches the plate position in the software.
   - The barcode is facing up and to the right, and the plate is flush with the right and back edges.



IMPORTANT! If the plates are not positioned correctly, your data results will be adversely affected.

5. Repeat this procedure to enter the serial numbers and place OpenArray plates in Positions 2 and 3.

   Note: If you are running fewer than three OpenArray plates, Applied Biosystems recommends the following: For one plate, use Position 1; for two plates, use Positions 1 and 2.

IMPORTANT! Leave the Input Plate Serial Numbers dialog box open, then proceed to "Enter sample information" below. If you close the dialog box, the information you have entered will be lost.

Enter sample information

Entering sample information allows you to:

- Track the OpenArray® 384-Well Sample Plates, and map the sample plate areas to each TaqMan® or SYBR® OpenArray Real-Time PCR Plate.
- Associate information about the samples with the data results in order to normalize data or compute standard curves and calculate concentrations.

To enter sample information, you can:

- (Recommended) Import sample information from a *.csv file (page 27)
- Manually enter sample information (page 29)

If needed, you can add or delete sample information columns in the Sample Data pane. See Appendix A on page 133.

Import sample information from a *.csv file

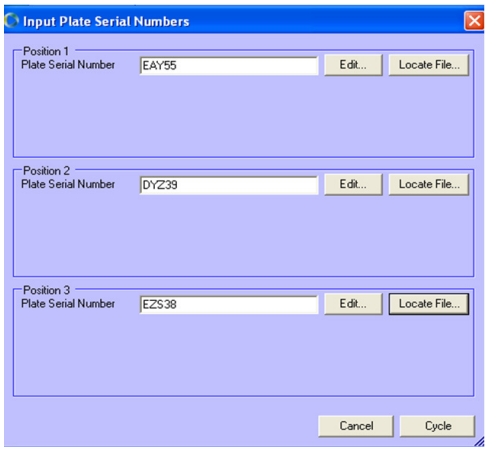
Note: You can import sample information after a run has completed; however, Applied Biosystems recommends that you import the sample information *before* starting the run.

1. If you have not done so already, create a *.csv file.

   Note: For procedures on creating a *.csv file, refer to the *TaqMan® OpenArray® Real-Time PCR Plate Protocol* or the *SYBR® OpenArray® Real-Time PCR Plate Protocol*.

2. At Position 1 of the Input Plate Serial Numbers dialog box, click **Edit**.

   Note: If you are importing sample information after a run: In the Settings pane, click **Edit Sample Info**, then continue with step 3 below.

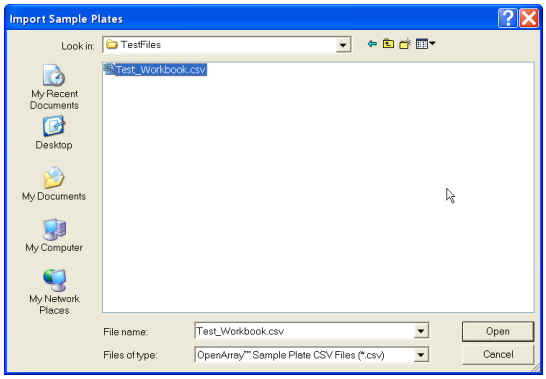


3. In the Sample Information dialog box, select the OpenArray plate of interest from the OpenArray drop-down menu.

4. Import the sample information:

   a. Click **Import**.

   b. In the Import Sample Plates dialog box, browse to and open the *.csv file to import.

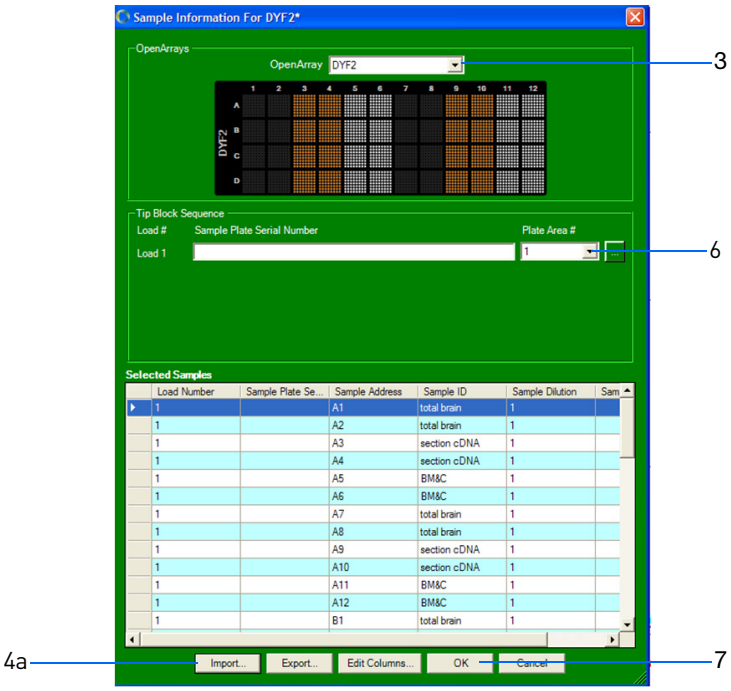


   The sample information appears in the Selected Samples pane of the Sample Information dialog box.

5. (Optional) Edit the sample information in each row.
6. From the Plate Area # drop-down menu, select the 12-well × 4-well area of the sample plate that the samples were transferred from.
7. Click **OK** to close the Sample Information dialog box.
8. Repeat this procedure to import sample information for the remaining OpenArray plates.

IMPORTANT! If you are importing sample information before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform thermal cycling and real-time imaging" on page 31. If you close the dialog box, the information you have entered will be lost.

Figure 1 Sample Information dialog box (the numbers called out in the screen capture refer to the step numbers in the above procedure)

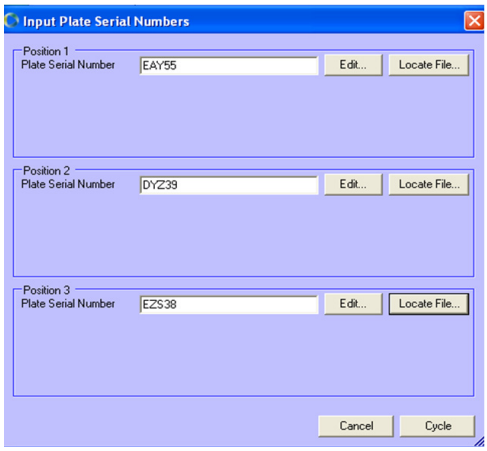


Manually enter sample information

Note: You can manually enter sample information after a run has completed; however, Applied Biosystems recommends that you enter the sample information *before* starting the run.

1. At Position 1 of the Input Plate Serial Numbers dialog box, click **Edit**.

   Note: If you are entering sample information after a run: In the Settings pane, click **Edit Sample Info**, then continue with step 2 below.



2. In the Sample Information dialog box, select the OpenArray plate of interest from the OpenArray drop-down menu.

3. In the Selected Samples pane, enter sample information for each sample:
   a. Double-click inside a field to activate it.
   b. Enter the appropriate information.

   Note: You cannot enter or edit information in the following columns: *Load Number*, *Sample Plate Serial Number*, and *Sample Address*. If you want to add or delete columns, see Appendix A on page 133.

4. Enter the Tip Block Sequence information:
   a. In the Sample Plate Serial Number field, enter the unique identifier for the OpenArray® 384-Well Sample Plate.

      Note: The unique identifier is the one you created when you prepared the sample plates. Refer to the *TaqMan® OpenArray® Real-Time PCR Plate Protocol* or the *SYBR® OpenArray® Real-Time PCR Plate Protocol*.

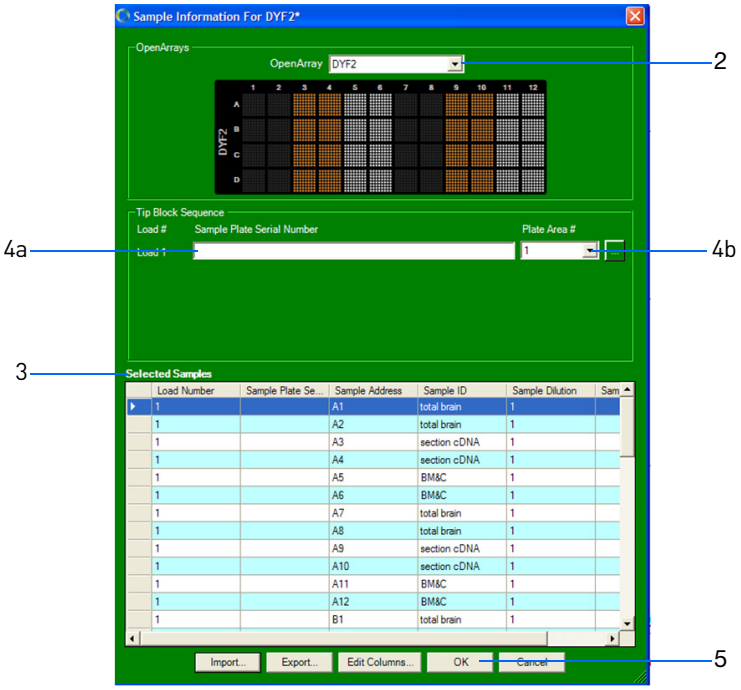
   b. From the Plate Area # drop-down menu, select the 12-well × 4-well area of the sample plate that the samples were transferred from.

5. Click **OK** to close the Sample Information dialog box.

6. Repeat this procedure to manually enter sample information for the remaining OpenArray plates.

IMPORTANT! If you are entering sample information before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform thermal cycling and real-time imaging" on page 31. If you close the dialog box, the information you have entered will be lost.

Figure 2 Sample Information dialog box (the numbers called out in the screen capture refer to the step numbers in the above procedure)



Perform thermal cycling and real-time imaging

During thermal cycling and real-time imaging, the OpenArray® instrument records the amount of fluorescence in each through-hole of the OpenArray plates. The run data are automatically saved to plate data files (*.tpd).

OpenArray® instrument commands

The table below lists the OpenArray software commands that you can use to control the OpenArray instrument.

Command	Description
Stop Cycling	To stop cycling at any time: In the OpenArray software, select **Actions** ▸ **Stop Cycling**. A message appears asking if you want to save the collected data. • Click **Yes** to save the incomplete plate data file (*.tpd). • Click **No** to continue imaging.
Interior Light On/Off (toggle switch)	To turn the light inside the instrument on or off, select **Actions** ▸ **Interior Light On/Off**.

Perform thermal cycling and real-time imaging

1. Close the OpenArray® instrument lid and door.
2. In the Input Plate Serial Numbers dialog box, click **Cycle**.

   IMPORTANT! Do not open the instrument door during the run. The run is complete when: (1) The blue LED light on the instrument door is off; and (2) In the software, data appears and the status bar displays a green circle. If you need to stop the run, see "OpenArray® instrument commands" above.

3. When the run is complete, save the project file (*.ncx):
   a. Select **File** ▸ **Save** or **File** ▸ **Save As** to open a save dialog box.
   b. Browse to a save location, enter a file name, then click **Save**.
4. Open the instrument door, then remove the OpenArray plates.

   Note: Applied Biosystems recommends that you discard the OpenArray plates after the run.

3 Analyze the Run Data

In this chapter, you view the data from the real-time imaging run (performed in Chapter 2) in a project file (*.ncx). If the default analysis settings are not suitable for your experiment, you can modify the data. This chapter also explains how to modify the project files and publish data.

This chapter covers:

- View the results. 34
- (Optional) Modify the data 42
- (Optional) Modify project files 57
- (Optional) Publish data 61

View the results

After a real-time imaging run, the OpenArray® Real-Time qPCR Analysis Software automatically analyzes the data for each TaqMan® OpenArray® Real-Time PCR Plate or SYBR® OpenArray® Real-Time PCR Plate in the run. To view the results of the automatic analysis:

- Open a project file (*.ncx) (this page)
- View data in the Assays pane (page 34)
- View data in the Sample Data pane (page 35)
- View data in the Curve pane (page 38)

If the default analysis parameters are not suitable for your experiment, you can modify the data. See "(Optional) Modify the data" on page 42.

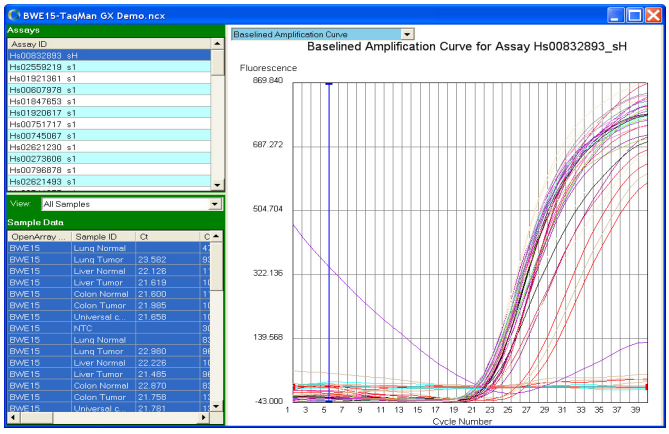
Open a project file (*.ncx)

In the OpenArray software, select **File ▸ Open**, then browse to and open the project file (*.ncx) of interest.

Note: After a run, the OpenArray software automatically opens the project file for that run.

View data in the Assays pane

Each row in the Assays pane represents a specific assay in the project. You can navigate within the Assays pane as described in the table below.

To...	Action	Result/Example
Select individual assays	Click the assay to view.	The data for all samples associated with the selected assays appear in the Sample Data pane; the reactions† appear as curves in the Curve pane. 
Select multiple assays, nonadjacent	Press and hold the **CTRL** key, then click the assays to view.	
Select multiple assays, adjacent	Press and hold the **SHIFT** key, then click the first and last rows of the block of assays to view.	
Select all assays	Press **CTRL + A**.	
Rearrange columns	Click and drag a column heading. For a description of each column in the Assays pane, see page 35.	
Sort rows	Click a column heading.	

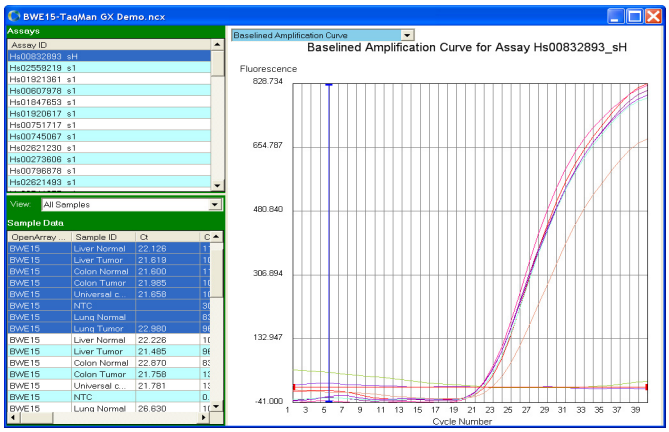
† A reaction is a sample-assay combination; each through-hole in an OpenArray plate contains a single reaction.

Assays pane column descriptions

Column name	Column description
Assay ID	Unique identifier for the assay
Assay RefSeq	The NCBI transcript identification number that corresponds to the gene
Assay Description	A description of the assay
Pathway Panels	For the SYBR® OpenArray® Pathway Panels, the name of the panel

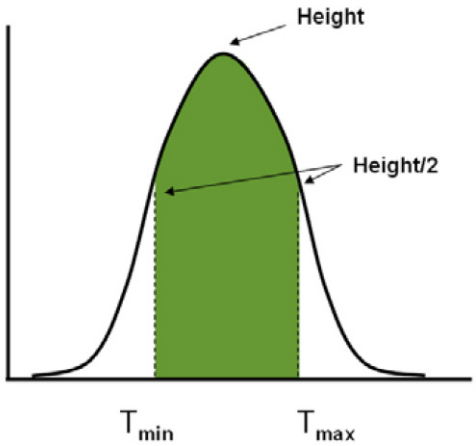
View data in the Sample Data pane

Each row in the Sample Data pane represents a specific sample in the project. You can navigate within the Sample Data pane as described in the table below.

To...	Action	Result/Example
Select an individual sample	Select the assay of interest in the Assays pane, then click the sample to view in the Sample Data pane.	The selected reactions† appear as curves in the Curve pane. The assay remains selected in the Assays pane as you select different samples. You can use this feature to see how different genes behaved for specific experimental conditions. 
Select multiple samples, nonadjacent	Select the assay of interest in the Assays pane, press and hold the **CTRL** key, then click the samples to view in the Sample Data pane.	
Select multiple samples, adjacent	Select the assay of interest in the Assays pane, press and hold the **SHIFT** key, then click the first and last rows of the block of samples to view in the Sample Data pane.	
Select all samples	Press **CTRL + A**.	
Restrict the samples displayed in the Sample Data pane	Select an option from the View drop-down menu: • **All Samples** • **Non Standard Curve Samples** • **Standard Curve Samples**	
Rearrange columns	Click and drag a column heading. For a description of each column in the Samples Data pane, see page 36.	
Add or delete columns	For add and delete procedures, see Appendix A on page 133.	
Sort rows	Click a column heading.	

† A reaction is a sample-assay combination; each through-hole in an OpenArray plate contains a single reaction.

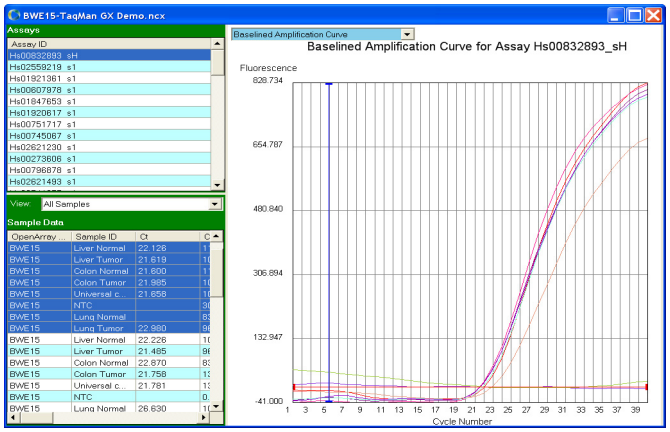
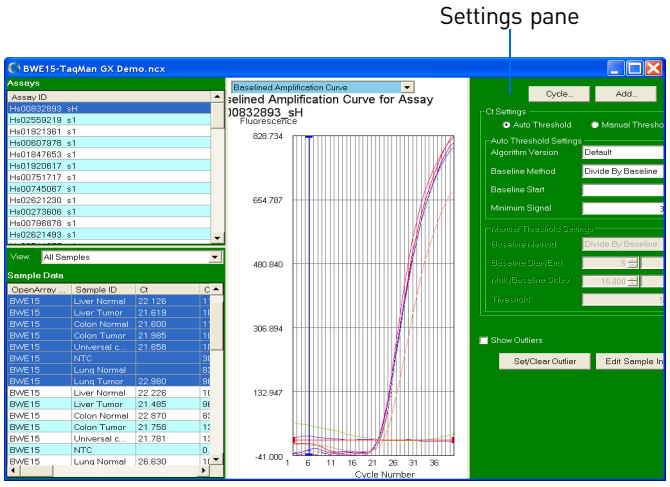
Sample Data pane column descriptions

Column name	Column description
OpenArray Serial Number	An alphanumeric code (for example, **ABC01**) for the TaqMan® or SYBR® OpenArray® Real-Time PCR Plate.
Sample ID	The sample identification (user-defined). Note: To perform normalization or generate a standard curve, Sample IDs must be entered.
C_T	Threshold cycle. The cycle number at which fluorescence is detectable above background fluorescence. The software calculates the initial concentration of target DNA in your DNA sample from the C_T value.
C_T Confidence	The confidence value applied to the threshold cycle. Larger values indicate increased confidence in the results. Values below the minimum signal setting indicate a failed reaction.
Concentration	The known concentration of the sample (entered in the Sample Information and Setup Standard Curves window) or the concentrations calculated from the C_T value and standard curve.
Concentration Unit	The unit of concentration defined in the Setup Standards Curves window.
T_m	Melting temperature. The temperature at which 50% of a DNA fragment denatures (melts). Because DNA fragments have different melting temperatures, you can verify the presence of the specific DNA amplicon and determine if any primer dimers or other non-specific amplification products are present by analyzing a melting curve. The OpenArray instrument generates a melting curve by slowly increasing temperature above the melting point while measuring fluorescence.
T_m Area	The area under the curve (see the green portion in the diagram below) from the temperatures immediately less than and greater than the T_m at which half the height of the T_m is achieved. 
Address	The location of the assay on the TaqMan® or SYBR® OpenArray® Real-Time PCR Plate (for example, **A1a1**).
Sample Plate Serial Number	A unique identifier for the OpenArray® 384-Well Sample Plate (user-defined).

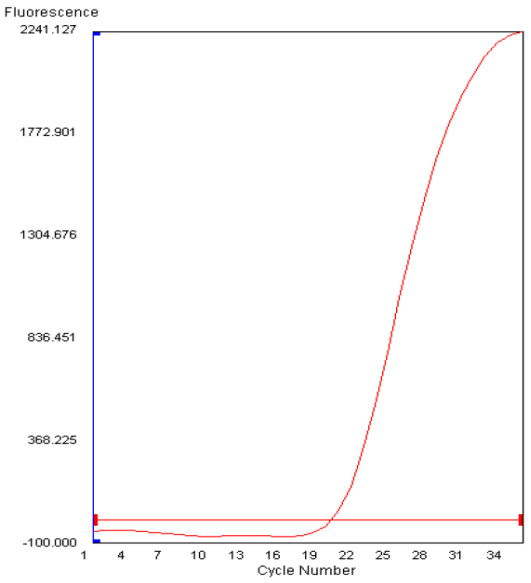
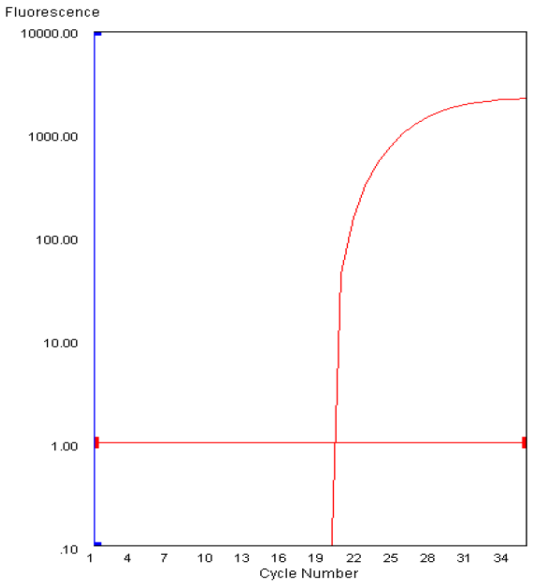
Column name	Column description
Sample Address	The well in the OpenArray® 384-Well Sample Plate from which the sample was transferred (for example, **A1**).
Sample Dilution	The fold dilution of the sample (user-defined). For example, a value of 100 indicates that the sample was diluted 100-fold.
Sample Description	A description of the sample (user-defined).
Is C_T Calculated	Indicates whether or not the software calculated the C_T value. The C_T value is not calculated when: • The Confidence is below the minimum signal value. • The sample was selected as an outlier. • The curve does not cross the threshold.
Is Valid Concentration	A checkmark appears if a concentration value was either calculated by the software or manually entered as part of a standard curve.
Outlier	A checkmark appears if the sample has been marked as an outlier and eliminated from the analysis. For more information, see "Set outliers" on page 55.
Through-Hole Index	A sequential number assigned to each through-hole in the OpenArray plate.
SampleInfo.Properties *<heading>* where: *<heading>* is user-defined	Indicates a new column added by a user. All user-defined columns are prefixed with *SampleInfo.Properties.*

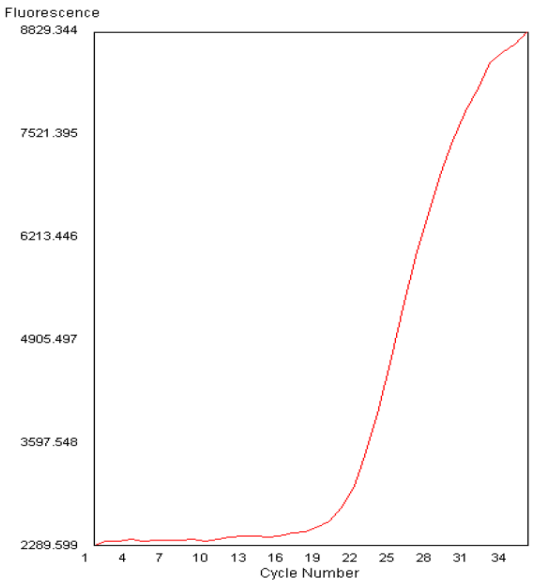
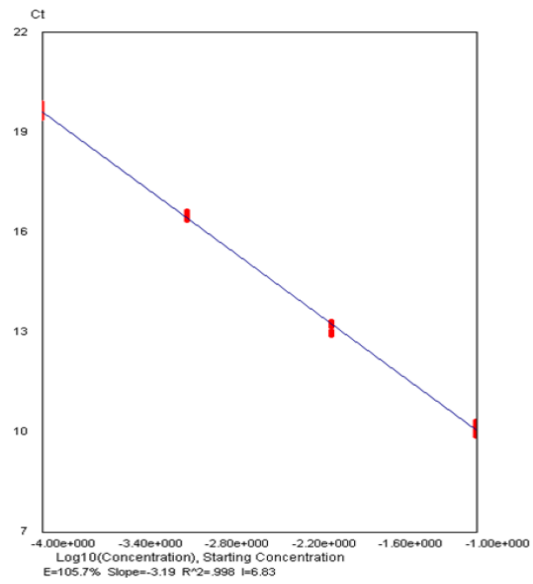
View data in the Curve pane

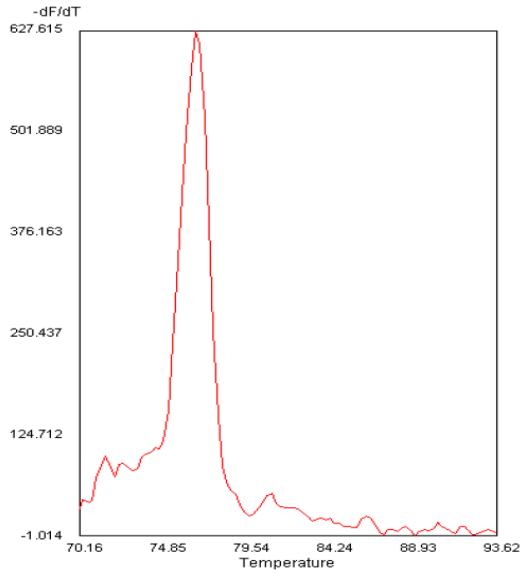
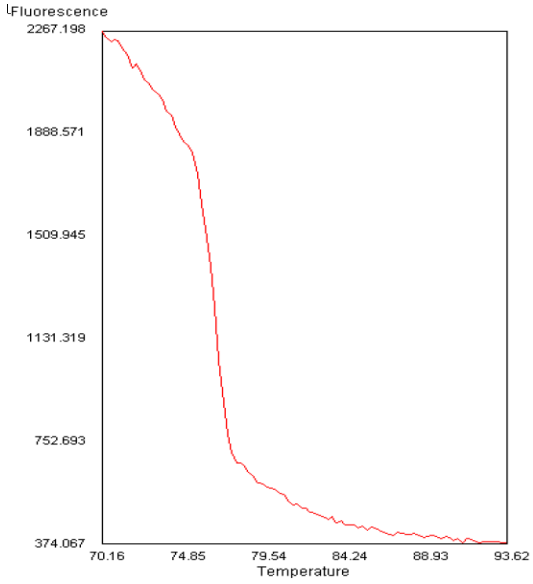
Each curve in the Curve pane represents a specific reaction in the project (a reaction is a sample-assay combination; each through-hole in the OpenArray plate contains a single reaction). You can navigate within the Curve pane as described in the table below.

To...	Action	Result/Example
Select individual reactions	Click the curve to view.	The assay and sample data associated with the selected curves are highlighted in the Assays pane and the Sample Data pane.
Select multiple reactions, nonadjacent	Press the **CTRL** key while clicking the curves to view. Note: You may find it easier to select curves if you first magnify the area of interest. See "Magnify an area of interest" below.	
Magnify an area of interest	Right-click, drag the mouse over the area to view, then release the mouse. To reset the curve to the default size, right-click the area without dragging the mouse.	
View different curve types	Select a curve type from the drop-down menu. For a description of each curve type, see page 39.	
Show or hide the Settings pane	The Settings pane is the right-most pane; it displays the C_T and threshold settings. To show or hide the Settings pane, do one of the following: • Select **View ▸ Show/ Hide Settings** (toggle switch). • Double-click or drag the Settings pane boundary.	
Show or hide gridlines	Select **View ▸ Show/Hide Grid** (toggle switch).	

Curve types

Curve type	Description	Example
Baselined Amplification Curve	Baselined relative fluorescence plotted on a linear scale.	
Baselined Log-Linear Amplification Curve	Baselined relative fluorescence plotted on a log-linear scale.	

Curve type	Description	Example
Amplification Curve	Sigmoidal curve reflecting accumulation of raw fluorescence at each PCR cycle that is proportional to the amount of product generated.	
Standard Curve	Known starting concentrations of DNA plotted against the C_T. This curve is used to determine PCR efficiency and to calculate absolute concentrations of sample from C_T values.	

Curve type	Description	Example
Melting Curve (For SYBR® OpenArray® Real-Time PCR Plates only)	The melting of amplification product at the end of a PCR run. It is plotted as the negative derivative of fluorescence against temperature.	
Raw Melting Curve (For SYBR® OpenArray® Real-Time PCR Plates only)	The raw fluorescence intensity decrease that results from slowly increasing temperature from below the melting point of the products to a temperature above the melting point.	

(Optional) Modify the data

If the default analysis settings are not suitable for your experiment, you can modify the data as follows:

- Normalize the data (this page)
- Modify standard curves (page 49)
- Set outliers (page 55)
- Adjust the CT settings (page 56)

IMPORTANT! The software applies data modifications only to the assay you are viewing, not to the entire project. To modify data for the entire project, see "(Optional) Modify project files" on page 57.

Save your changes to the project file (*.ncx)

After modifying the data, be sure to save the project file (*.ncx) if you want to save your changes. If you do not save the project file, all your changes are lost when you close the project file.

IMPORTANT! The software *copies* the run data from the plate data file to the project file. The files are not linked; that is, modifications you save to the project file (*.ncx) are not saved to the corresponding plate data file (*.tpd).

1. Select a save option:
   - **File ▸ Save** to save the changes to the current project file.
   - **File ▸ Save As** to save the changes to a new project file. The File ▸ Save As option allows you to perform multiple analyses of the same plate data file (*.tpd).
2. For the **File ▸ Save As** option, browse to a save location, enter a file name, then click **Save**.

Normalize the data

Normalizing the data allows you to compute relative expression values. To normalize the data:

- Enter sample IDs (page 43)
- Calculate the DCT data and view the DCT graph (page 43)

  and/or

  Calculate the DDCT data and view the DDCT graph (page 46)

  Note: The ΔC_T and $\Delta\Delta C_T$ are calculated independently, so you can calculate either or both values.
- (Optional) Save the normalization data to *.csv files (page 48)

Enter sample IDs

You must enter sample IDs in the software before you can normalize the data. If the project file (*.ncx) does not contain sample IDs, enter the IDs before continuing with this procedure. See "Enter sample information" on page 26.

Calculate the ΔC_T data and view the ΔC_T graph

1. Select **Actions ▸ View Normalization** to open the Normalization dialog box.

   Note: If prompted to assign sample IDs, see "Enter sample information" on page 26.

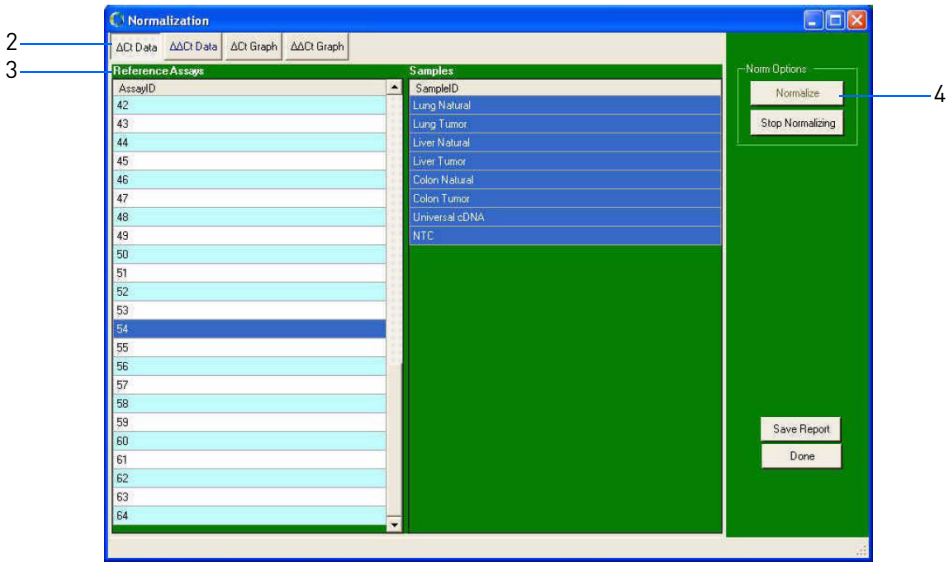
2. Click the **ΔC_T Data** tab.

3. In the Reference Assays table, select the reference assay to normalize against. The Samples table lists all the samples for which the reference assay was measured.

4. In the Samples table, select the samples to normalize against the reference assay, then click **Normalize**.

5. To see existing normalization relationships, move the pointer over the normalized samples. The related assays and samples are highlighted.

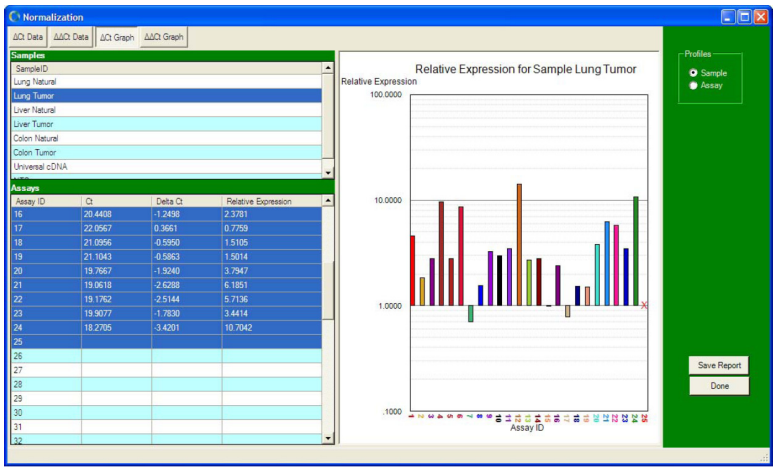
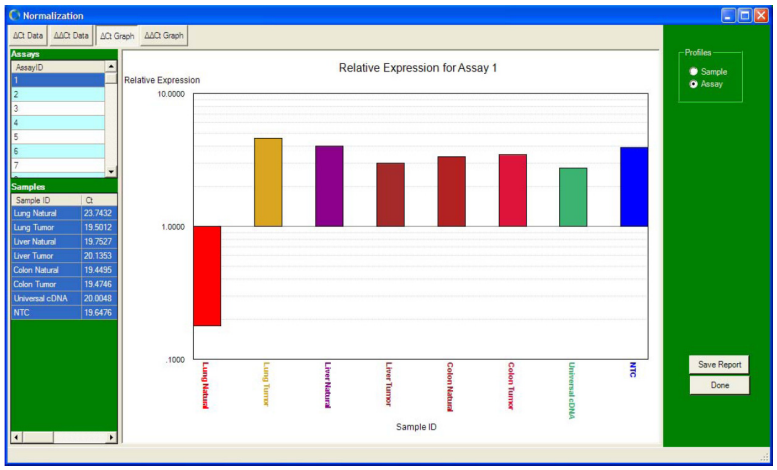
Figure 3 Normalization dialog box, ΔC_T Data tab (the numbers called out in the screen capture refer to the step numbers in the above procedure)



6. View the results:
   a. Click the **ΔC_T Graph** tab.
   b. In the Profiles box, select **Sample**. From the Assays table, select the assays to view.
   c. In the Profiles box, select **Assay**. From the Samples table, select the samples to view.

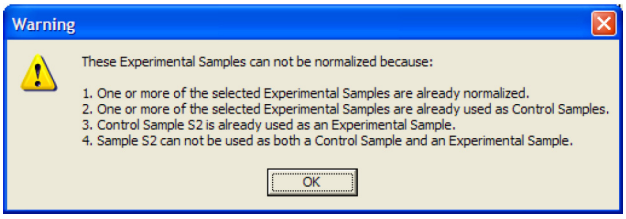
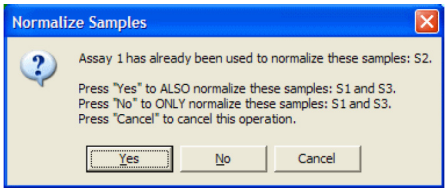
   The Sample and Assay Profiles are described in the table below.

   Note: If an assay or sample is unrelated, the Relative Expression graph is blank and the status bar indicates that the assay or sample is not normalized. See "Normalization rules for DCT data" on page 45.

Profile	Description	Example
Sample	The Sample Profile displays: • A graph of the relative expression of the selected assays for a particular sample. A red X on the x-axis indicates that the relative expression for an assay cannot be calculated. • The C_T, ΔC_T, and relative expression data in the Assays table.	
Assay	The Assay Profile displays: • A graph of the relative expression of the selected samples for a particular assay. A red X on the x-axis indicates that the relative expression for the assay of the corresponding sample cannot be calculated. • The C_T, ΔC_T, and relative expression data in the Samples table.	

Normalization rules for ΔC_T data

When calculating the ΔC_T data, the following normalization rules apply:

Rule	Description	Action
A sample can be normalized to only one assay at a time.	If you try to normalize a sample to more than one assay, the following error message appears: 	Break the existing relationship before creating the new one: 1. Select the normalized assay. 2. Select the samples to break from the assay. 3. Click **Stop Normalizing**. The selected samples become unrelated to the assay. For example, if there is a relationship between assay A and samples a, b, and c and you want to create a relationship between assay B and sample a, you first need to break the relationship between assay A and sample a.
Assays can be related to multiple samples.	If you select an assay that is already related to a sample or samples, the following message appears: 	• To add the samples to the assay normalization, click **Yes**. • To break the previous relationship and establish one with only the currently selected samples, click **No**.

Calculate the $\Delta\Delta C_T$ data and view the $\Delta\Delta C_T$ graph

1. Select **Actions ▸ View Normalization** to open the Normalization dialog box.

   Note: If prompted to assign sample IDs, see "Enter sample information" on page 26.

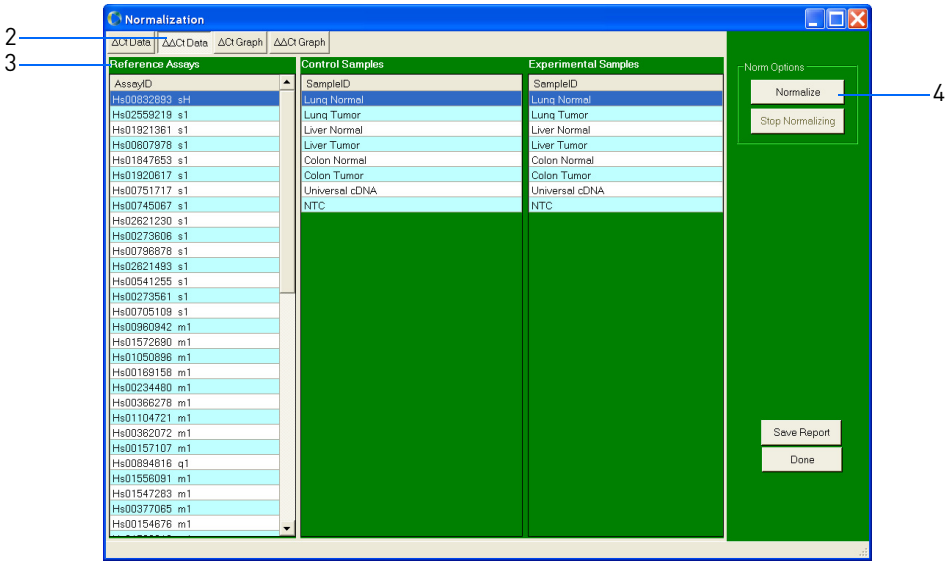
2. Click the **$\Delta\Delta C_T$ Data** tab.

3. In the Reference Assays table, select the reference assay to normalize against.

4. The Control and Experimental Samples tables list all the samples for which the reference assay was applied. Select the control and experimental samples to compare (for example, healthy tissue vs. diseased tissue), then click **Normalize**.

5. To see existing normalization relationships, move the pointer over the Experimental Samples table. The related control samples and reference assays are highlighted.

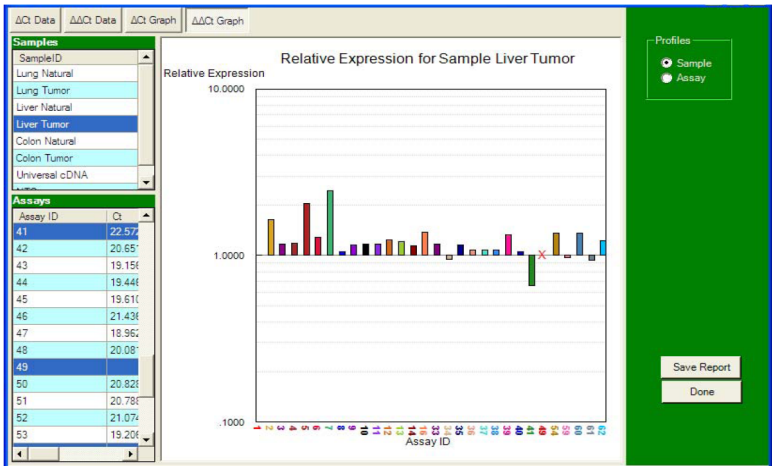
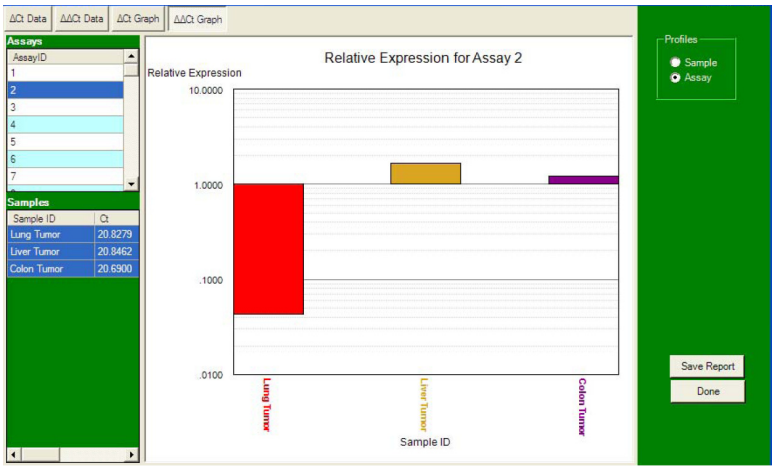
Figure 4 Normalization dialog box, $\Delta\Delta C_T$ Graph tab (the numbers called out in the screen capture refer to the step numbers in the above procedure)



6. View the results:
   a. Click the **$\Delta\Delta C_T$ Graph** tab.
   b. In the Profiles box, select **Sample**. From the Assays table, select the assays to view.
   c. In the Profiles box, select **Assay**. From the Samples table, select the samples to view.

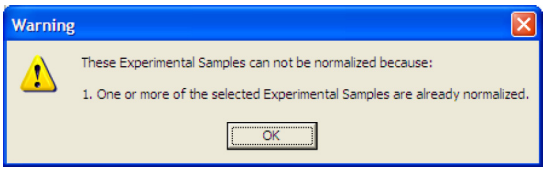
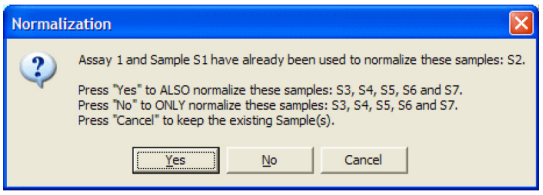
   The Sample and Assay Profiles are described in the table below.

   Note: If an assay or sample is unrelated, the Relative Expression graph is blank and the status bar indicates that the assay or sample is not normalized. See "Normalization rules for DDCT data" on page 48.

Profile	Description	Example
Sample	The Sample Profile displays: • A graph of the relative expression of the selected assays for a particular sample. A red X on the x-axis indicates that the relative expression for an assay cannot be calculated. • The C_T, $\Delta\Delta C_T$, and relative expression data in the Assays table.	
Assay	The Assay Profile displays: • A graph of the relative expression of the selected samples for a particular assay. A red X on the x-axis indicates that the relative expression for the assay of the corresponding sample cannot be calculated. • The C_T, $\Delta\Delta C_T$, and relative expression data in the Samples table.	

Normalization rules for $\Delta\Delta C_T$ data

When calculating the $\Delta\Delta C_T$ data, the following normalization rules apply:

Rule	Description	Action
A sample can be normalized to only one control-experimental pair at a time.	In either of these situations, the following error message appears: 	Break the existing relationship before creating the new one: 1. Select the samples to break from the assay. 2. Click **Stop Normalizing**. The selected samples become unrelated to the assay. For example, if there is a relationship between assay A and samples a1/a2, b1/b2, and c1/c2 and you want to create a relationship between assay B and sample a1/a2, you first need to break the relationship between assay A and sample a1/a2.
A sample cannot be a control and an experimental sample in the same or different relationships.		
Control-experiment pairs can be related to multiple samples.	If you select an assay control-experiment pair that is already related to a sample or samples, the following message appears: 	• To add the assay pairs to the assay normalization, click **Yes**. • To break the previous relationship and establish one with only the currently selected assay pair, click **No**.

(Optional) Save the normalization data to *.csv files

Data for ΔC_T and $\Delta\Delta C_T$ are saved in separate *.csv files. If you calculate only ΔC_T data or only $\Delta\Delta C_T$ data, only one *.csv file is generated.

1. Click **Save Report**.
2. Browse to a save location, then click **Save**.

   IMPORTANT! If you have previously exported reports for ΔC_T or $\Delta\Delta C_T$ data in the same folder, be sure to rename the existing reports. If you do not rename the existing reports, they will be overwritten by the current reports.

3. Click **Done** to close the Normalization dialog box.

Modify standard curves

The OpenArray software plots known starting concentrations of DNA against the C_T to generate a standard curve. The standard curve is used to determine PCR efficiency and to calculate absolute concentrations of sample from C_T values.

About standard curves

The OpenArray software displays the Efficiency, Slope, R^2, and Intercept values for the selected reactions below the standard curve. The software uses these values to determine the starting sample concentrations, as follows:

C_T = slope × log10 (conc.) + intercept

conc. =10((C_T –intercept)/slope)

Note: For information on viewing the standard curve, see "View data in the Curve pane" on page 38.

The Efficiency, Slope, R^2, and Intercept are defined as follows:

Item	Definition
Efficiency	The percent of the theoretical maximum that the reaction produces copies of the sample. The efficiency is calculated by the equation: $E = 10^{(-1/slope)} - 1$ The efficiency should be between 90 and 110%, meaning the sample was doubled each cycle. An efficiency value between 90 and 110% corresponds to a slope of –3.1 to –3.6 in the C_T vs. log starting DNA amount standard curve. Efficiency should be similar for all assays that you are comparing. Factors that affect efficiency include: • Length of the amplicon • Presence of inhibitors • Secondary structure • Primer design
Slope	Slope of the line C_T vs. Log10 (concentration), used for efficiency calculation. 100% efficiency is defined at a slope of –3.32.
R^2	The quality of the fit of a line to the standard curve data. Values approaching 1 are better fits.
Intercept	The C_T at which the line intersects with a 0 Log10 (concentration).

Standard curve design overview

When you design a standard curve for absolute quantification, Applied Biosystems recommends that you use known concentrations of DNA standard molecules (for example, recombinant plasmid DNA, genomic DNA, RT-PCR product, and synthetic-oligonucleotides).

To design a standard curve:

1. Prepare a series of 4- to 6-point serial 2-, 5-, or 10-fold dilutions of a control template at a known concentration. The concentration range should cover the expected concentration range of the sample.

2. Run the serial dilutions with the unknown samples, then record the resulting C_T values.

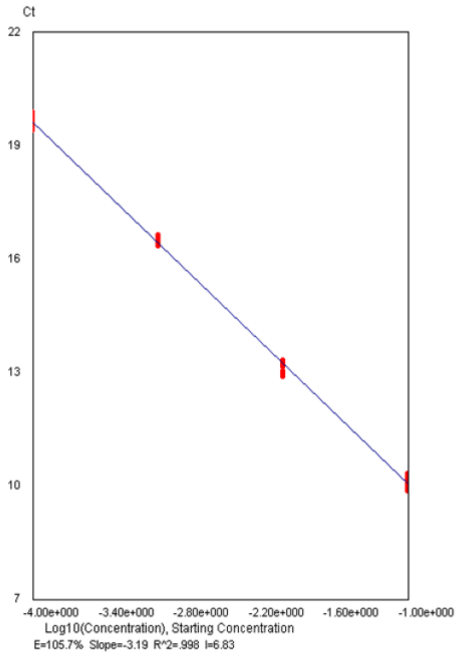
3. Create a plot of C_T vs. the logarithm of the starting template concentration according to "Set up a standard curve" on page 51. This plot results in a straight line, which is a linear regression line through the data points.

4. Extrapolate the number of target gene copies from the standard curve equation.

The figure below shows a standard curve generated from an amplicon pool with the presented assay demonstrated near 100% efficiency. The interpretation of the C_T values generated from a standard template of known concentration on the OpenArray system using SYBR® Green I chemistry is:

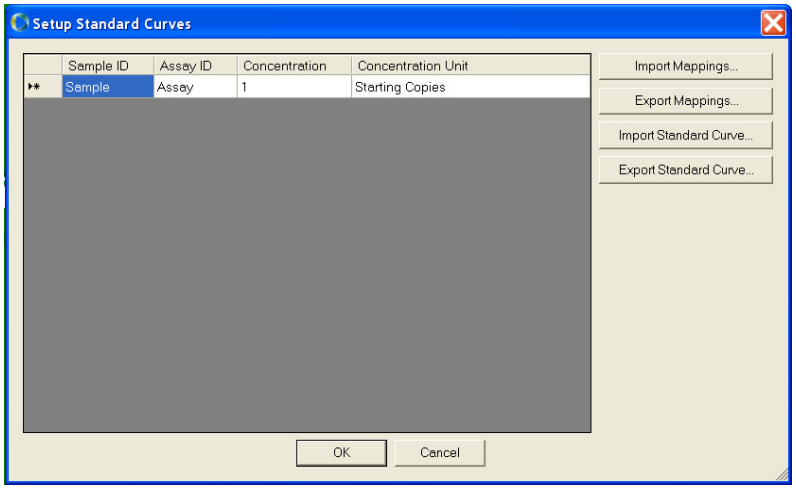
- 10^5 = C_T of 9.4
- 10^4 = C_T of 12.5
- 10^3 = C_T of 15.9
- 10^2 = C_T of 19.3

Note: For TaqMan® chemistries, the C_T values are two cycles later.



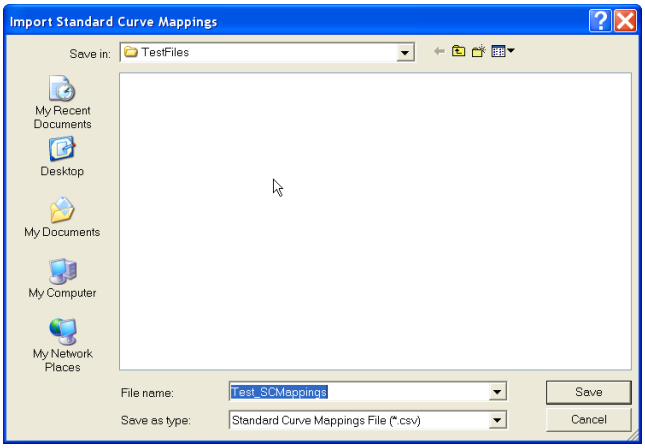
Set up a standard curve

1. Open the project file (*.ncx) of interest.
2. Edit the sample ID and dilution information for each OpenArray plate in the project:
   a. In the Settings pane, click **Edit Sample Info** to open the Sample Information dialog box.
   b. From the OpenArray drop-down menu, select the OpenArray plate of interest.
   c. Edit the Sample ID column so that all samples in the standard curve have the same Sample ID.
   d. In the Sample Dilution column, enter a value for all standard curve samples. A dilution value of 1 indicates no dilution, 10 indicates a 10-fold dilution, and so on.
   e. Click **OK** to accept the changes.
3. Select **Actions** ▸ **Setup Standard Curves** to open the Setup Standard Curves dialog box.
4. Complete the Sample ID, Assay ID, Concentration, and Concentration Unit columns. You can complete the columns by doing one of the following:
   - Import a mapping file (*.csv) (page 52)
   - Manually enter the information (page 54)



Import a mapping file (*.csv)

1. Generate a *.csv file to use as a template:
   a. In the Setup Standard Curves dialog box, click **Export Mappings**.
   b. In the Import Standard Curve Mappings dialog box, browse to a save location, enter a file name, then click **Save**.



   The software populates the Setup Standard Curves dialog box with information from the currently open project file (*.ncx).
   c. Click **OK**. The software exports the mapping information to a *.csv file.

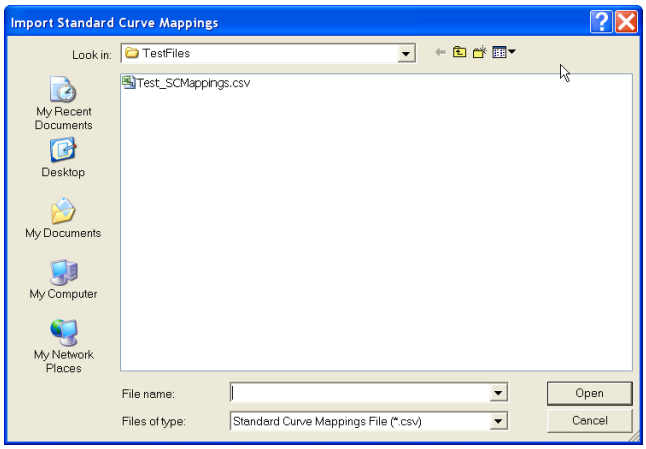
2. Edit the generated *.csv file:
   a. Browse to and open the generated *.csv file in a spreadsheet application (such as Microsoft® Excel® Software).
   b. Edit the columns as needed. Optionally, you can cut and paste information from other documents.

   IMPORTANT! The sample IDs must match exactly the sample IDs in the sample data.

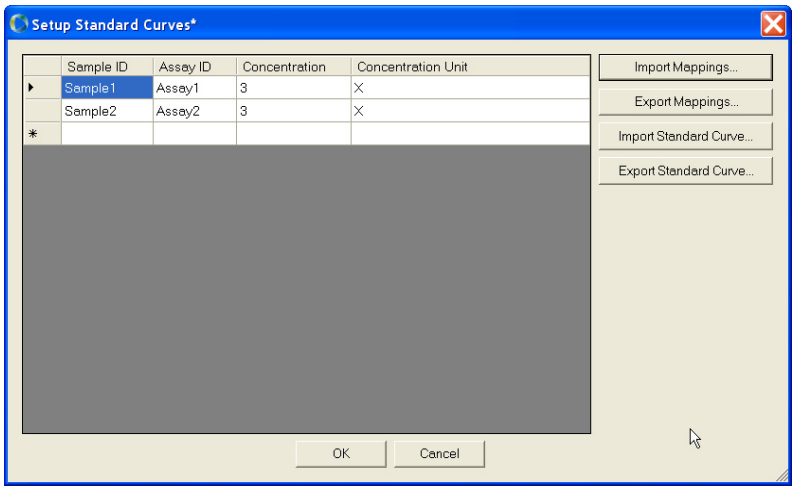
   c. Save the data as a *.csv file.

3. Import the edited *.csv file into the OpenArray software:
   a. Open the project file (*.ncx) of interest.
   b. Select **Actions ▸ Setup Standard Curves** to open the Setup Standard Curves dialog box.
   c. In the Setup Standard Curves dialog box, click **Import Mappings**.

d. In the Import Standard Curve Mappings dialog box, browse to and open the *.csv file.



The software populates the Setup Standard Curves dialog box with information from the imported *.csv file.



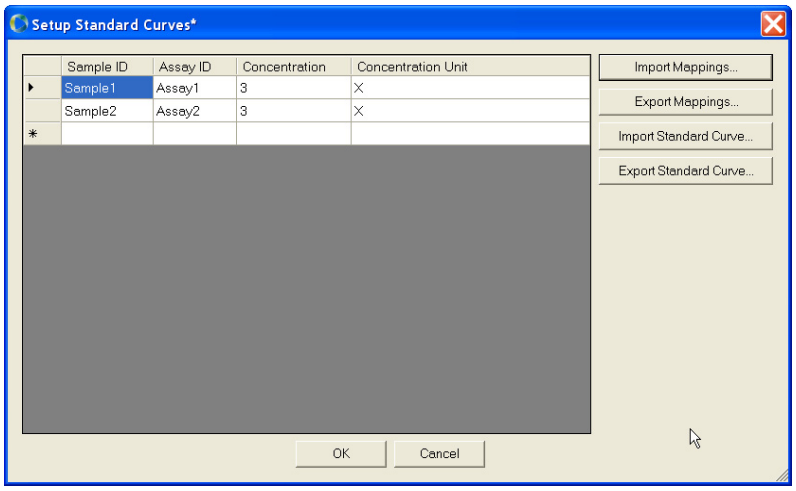
e. Click **OK** to close the Setup Standard Curve dialog box.

Manually enter the information

In the Setup Standard Curves dialog box:

1. Enter the sample IDs and assay IDs.
2. Enter the highest starting concentration of all samples applied to an assay. This is the concentration of the samples before dilution.

   Note: The dilution series for all samples applied to an assay must have the same starting concentration. You can analyze assays with sample dilution series that have different starting concentrations by separating them into different projects.
3. Enter the concentration unit (for example, 1× or ng/µL).
4. Click **OK** to close the Setup Standard Curves dialog box.



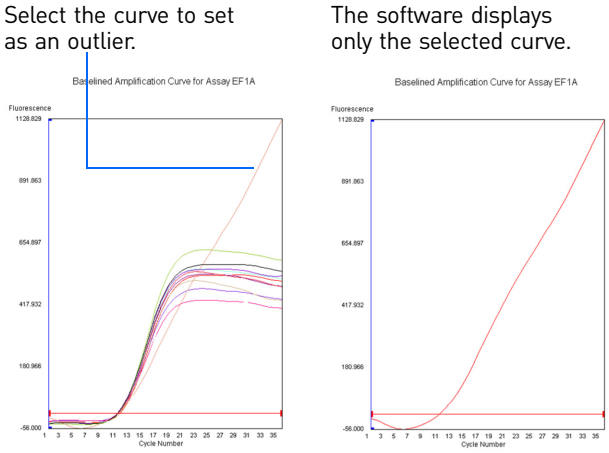
Set outliers

You can set the following reactions as outliers:

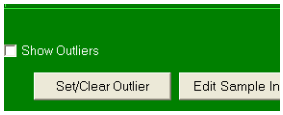
- Reactions with data that is not consistent with other reactions
- Reactions that have very high C_T values

The software does not include outliers in the data results.

1. In the Sample Data pane or Curve pane, select the reactions to set as outliers.



2. At the bottom of the Settings pane, click **Set/Clear Outlier**.



   The software:

   - Checks the Outlier column in the Sample Data pane for each selected sample.
   - Displays the Curve pane graphs without the outliers.

3. To view the outliers, select the **Show Outliers** checkbox. The outliers appear in the Curve pane graphs.

   Note: Outliers do not appear in the Standard Curve graph even when **Show Outliers** is checked.

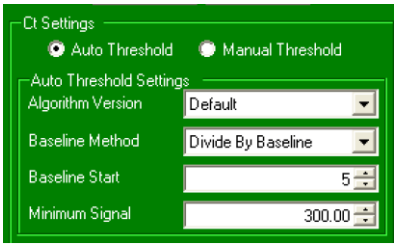
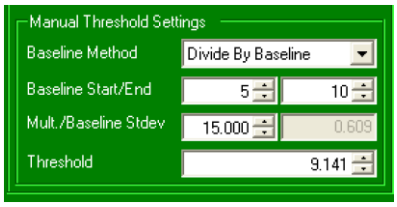
4. To include an outlier back in the analysis:

   a. In the Sample Data pane, select the sample.

   b. In the Settings pane, click **Set/Clear Outlier**. The software recalculates the standard curve.

Adjust the C_T settings

1. In the Sample Data pane or Curve pane, select the samples with the C_T values to change.
2. In the Settings pane, select **Auto Threshold** or **Manual Threshold**, then change the parameters as needed. The software reanalyzes the data with the new parameters.

Setting	Parameter	Definition
Auto Threshold 	Algorithm Version	**Default** – Automatically determines the amplification curve cycle where the values exceed baseline fluorescence (the C_T). **Custom** – An alternative algorithm that uses more amplification curve data to characterize the C_T.
	Baseline Method	Determines how the selected baseline will normalize the amplification curve. The portion of the amplification curve specified by the **Baseline Start** parameter (described below) determines a line that is subtracted from the whole amplification curve. The **Subtract Baseline** method determines a flat line. The line determined by the **Divide by Baseline** method is sloped according to the values in the baseline.
	Baseline Start	The earliest cycle used for baselining and the standard deviation calculation. The Baseline Start is represented by a blue, vertical line on the baselined curves.
	Minimum Signal	If a C_T confidence is below this value, no valid C_T is computed.
Manual Threshold 	Baseline Method	Determines how the selected baseline will normalize the amplification curve. The portion of the amplification curve specified by the **Baseline Start/End** parameter (described below) determines a line that is subtracted from the whole amplification curve. The **Subtract Baseline** method determines a flat line. The line determined by the **Divide by Baseline** method is sloped according to the values in the baseline.
	Baseline Start/ End	Earliest/Latest cycle used for baselining and the standard deviation calculation. Represented by blue vertical lines on the baselined curves.
	Mult./ Baseline Stdev	Value used to determine the threshold (Mult. = Multiplier). Average of the standard deviations of normalized baselines for each curve.
	Threshold	Fluorescence value that defines the C_T found at the cycle at which the amplification curve crosses this fluorescence value. Threshold = Multiplier * Baseline Stdev.

(Optional) Modify project files

Project files (*.ncx) are the files that you view and modify in the OpenArray software. (For a detailed description of project files, see page 22.) You can modify project files as follows:

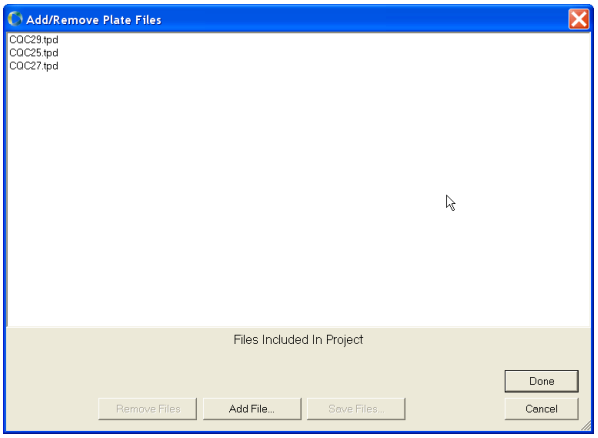
- Add plate data files (*.tpd) (this page)
- Remove plate data files (*.tpd) (page 58)
- Modify project settings (page 59)

Add plate data files (*.tpd)

1. In the OpenArray software, click **Add** to open the Add/Remove Plate Files dialog box. The software displays the plate data files (*.tpd) currently in the project.
2. Click **Add File**.
3. Browse to and select the plate data files to add. A project can contain up to 13 plate data files.

   Note: To select multiple plate data files, press and hold the **CTRL** or **SHIFT** key.
4. Click **Open**. The software copies the run data from the selected plate data files to the project file.

   Note: When you add a plate data file, the software *copies* the run data from the plate data file to the project file. The files are not linked; that is, any changes you make in the project file (*.ncx) are not made in the corresponding plate data file (*.tpd).
5. Click **Done**. The software reanalyzes the revised group of plate data files.



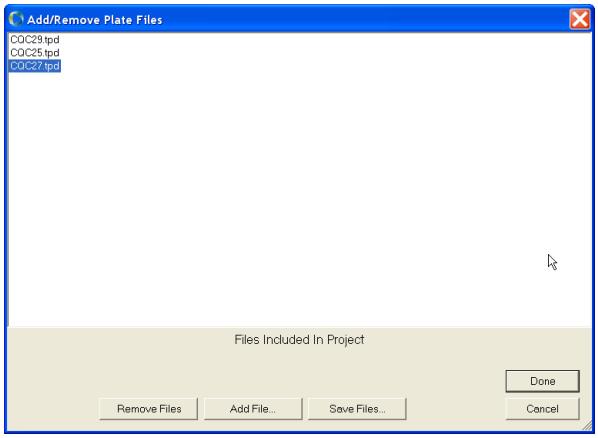
Remove plate data files (*.tpd)

1. In the OpenArray software, click **Add** to open the Add/Remove Plate Files dialog box. The software displays the plate data files (*.tpd) currently in the project.
2. Select the plate data files to remove.

   Note: To select multiple plate data files, press and hold the **CTRL** or **SHIFT** key.
3. Select a remove option:
   - Click **Remove Files** to remove the run data for the selected plate data files from the project file.

     IMPORTANT! When you remove a plate data file from a project, the results for the samples in that plate data file are lost.

   - Click **Save Files** to remove the run data for the selected plate data files from the project file, but save the plate data file to another location.
4. Click **Done**. The software reanalyzes the revised group of plate data files.



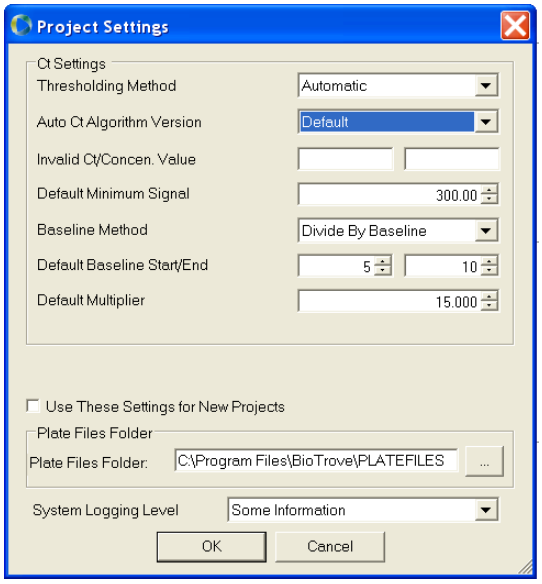
Modify project settings

IMPORTANT! When you modify the project settings, the software applies the settings to all reactions in the current project file. You can optionally select to apply the settings to all new project files.

Edit project settings

1. Select **Edit ▸ Project Settings**.
2. In the Project Settings dialog box, enter your changes. For a description of each setting, see "Project Settings dialog box" on page 60.
3. Click **OK**, then confirm your changes at the prompt.

The software reanalyzes the data with the new project settings.



Project Settings dialog box

Setting	Description
Thresholding Method	Determines whether the Automatic or Manual Method will be used to determine C_T. In Automatic Mode, you specify the Baseline Start and a Minimum Signal that determines whether a reaction successfully completed.
Auto C_T Algorithm Version	• **Default** – Automatically determines the amplification curve cycle where the values exceed baseline fluorescence (the C_T). • **Custom** – An alternative algorithm that uses more amplification curve data to characterize the C_T.
Invalid Ct/Concen. Value	The value displayed when a C_T cannot be calculated.
Default Minimum Signal	If a C_T Confidence is below this value, no valid C_T is computed.
Baseline Method	Determines how the selected baseline will normalize the amplification curve. The portion of the amplification curve specified by the baseline start/end analysis parameter determines a line that is subtracted from the whole amplification curve. • **Subtract Baseline** – Determines a flat line. • **Divide by Baseline** – The line is sloped according to the values in the baseline
Default Baseline Start/ End	Earliest/Latest cycle used for baselining and the standard deviation calculation. Represented by blue vertical lines on the baselined plots
Default Multiplier	Value used to determine the threshold.
Use These Settings for New Projects	When this box is selected, the settings become the default settings for new projects.
Plate Files Folder	The location of the plate files folder.
System Logging Level	The amount of technical support information to collect. The system default is Some Information. Only adjust this value when asked by an Applied Biosystems representative.

(Optional) Publish data

Publish data for use in reports, spreadsheets, and so on. You can:

- Copy and paste data (this page)
- Export *.csv files (this page)
- Export and import standard curve data files (page 63)

Copy and paste data

Copy/paste curve pane graphs

You can copy and paste the Curve pane graphs into other software applications, such as Microsoft® PowerPoint® Software.

1. In the Curve pane, select a curve type from the drop-down menu.
2. Click the Curve pane graph, then press **CTRL+C** or select **Edit ▸ Copy**.
3. Paste the graph into an appropriate software application.

Copy/paste table rows

You can copy and paste table rows from the Assays pane or Sample Data pane into other software applications, such as Microsoft® Excel® Software.

1. In the Assays or Sample Data pane, select one or more rows, then press **CTRL+C** or select **Edit ▸ Copy**.
2. Paste the information into an appropriate software application.

Export *.csv files

You can export data from your project as a comma-delimited file (*.csv). You can export:

- All data (this page)
- Only cycling data (this page)

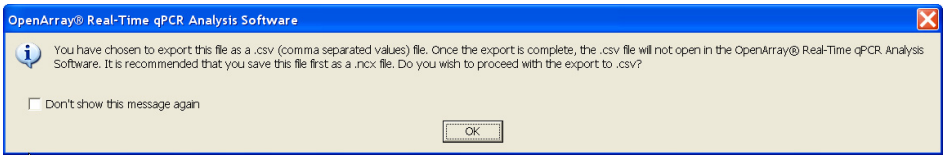
Note: The 2003 version of the Microsoft® Excel® Software cannot import all of the columns. If you are exporting to the 2003 version, you can export only the cycling data.

Export all data

When you export all data, the *.csv file includes (but is not limited to) the following:

- Assay information from the plate setup file (*.tpf)
- Sample information
- Fluorescence intensity data

1. Select **File** ▸ **Export CSV**. The following message appears:



   Note: Exported *.csv files cannot be reopened in the OpenArray software. Applied Biosystems recommends that you save the project file (*.ncx) before exporting the *.csv file.

2. Click **OK** to close the message.

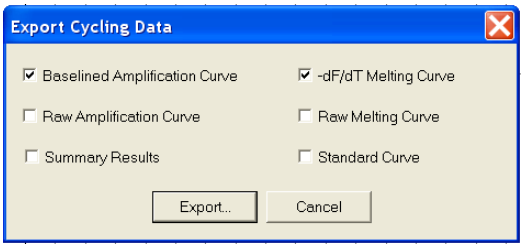
3. In the Export CSV dialog box, browse to a save location, enter a file name, then click **Save**.

Export cycling data

To export only cycling data:

1. Select **File** ▸ **Export Cycling Data**.

2. In the Export Cycling Data dialog box, select the data to export, then click **Export**.



3. Browse to a save location, then click **OK**. The software exports a separate *.csv file for each selection.

   Note: If you are exporting data from TaqMan OpenArray Real-Time PCR Plates, no data are exported for the -dF/dT Melting Curve or the Raw Melting Curve.

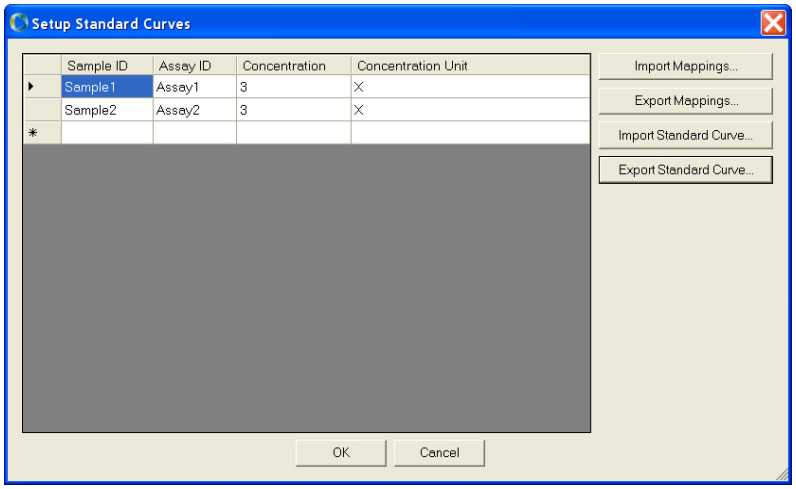
Export and import standard curve data files

You can export the standard curve and related plate file information from one project, then import the information into another project.

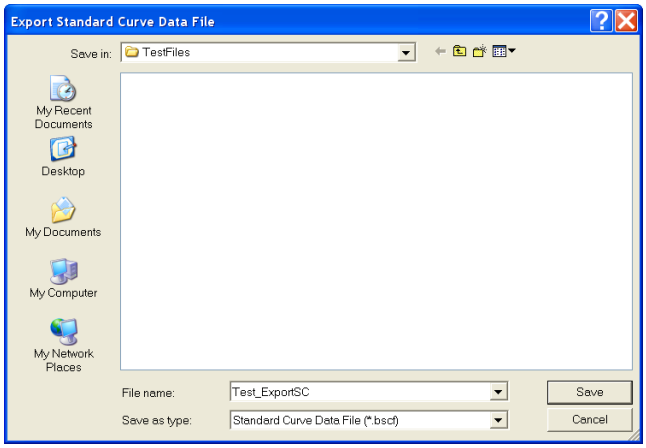
Export a standard curve data file

To export a standard curve data file (*.bscf):

1. Open the project file (*.ncx) of interest.
2. Select **Actions** ▸ **Setup Standard Curves**.
3. In the Setup Standard Curves dialog box, click **Export Standard Curve**.



4. In the Export Standard Curve Data File dialog box, browse to a save location, enter a file name, then click **Save**.

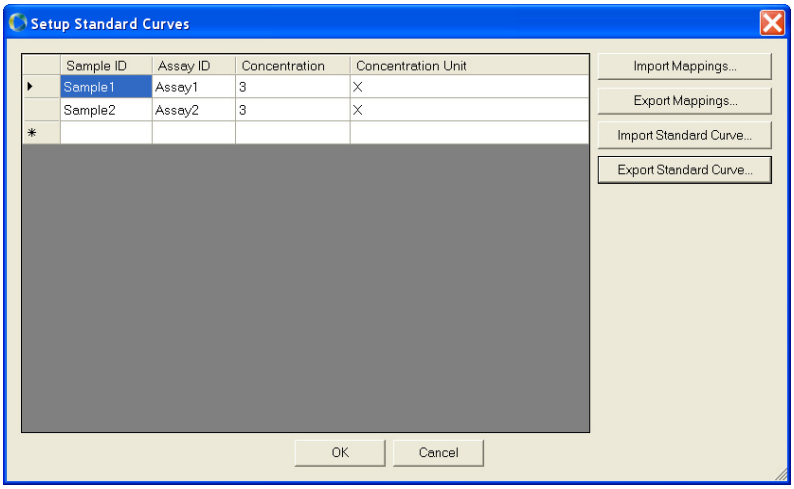


5. Click **OK** to close the Setup Standard Curves dialog box.

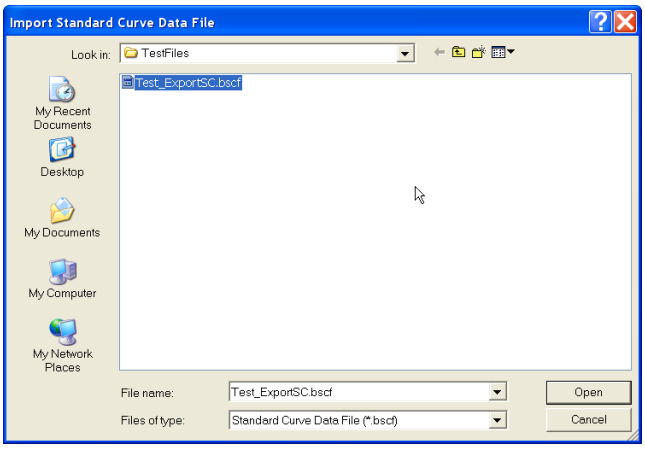
Import a standard curve data file

To import a standard curve data file (*.bscf):

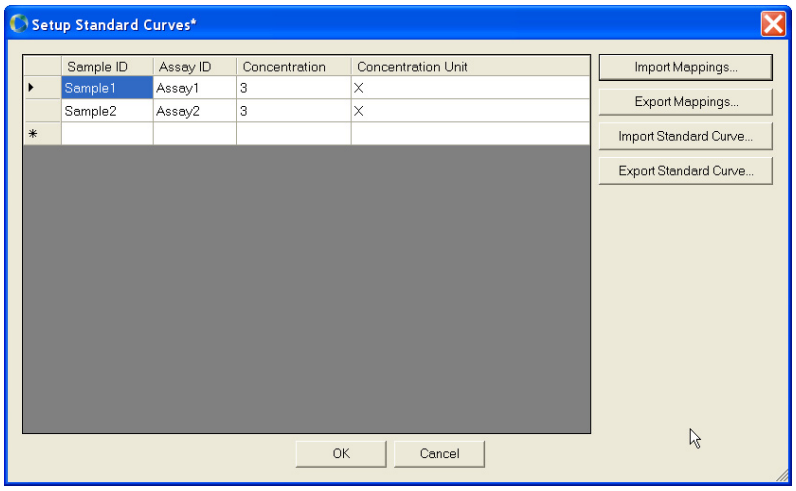
1. Open the project file (*.ncx) of interest.
2. Select **Actions ▸ Setup Standard Curves**.
3. In the Setup Standard Curves dialog box, click **Import Standard Curve**.



4. In the Import Standard Curve Data File dialog box, browse to and open the *.bscf file of interest.



The software populates the Setup Standard Curves dialog box with information from the imported *.bscf file, and calculates concentrations for the mapped samples.



5. Click **OK** to close the Setup Standard Curves dialog box.

Part Number 4458837 Rev. A 08/2010

PART II

Genotyping Experiments

Performing imaging and analysis

4 Before You Begin

This chapter covers:

- Required system components 71
- Required plates 72
- Workflow 74

Required system components

To perform genotyping experiments with the OpenArray® Real-Time PCR System, you need the following system components:

- OpenArray® platform (this page)
- Software (this page)
- Thermal cycler (page 72)

OpenArray® platform

For genotyping experiments, the OpenArray® platform includes the:

- **OpenArray® AutoLoader** – Loads DNA sample onto a TaqMan® OpenArray® Genotyping Plate.
- **OpenArray® Case Sealing Station** – Seals the OpenArray® Cases.
- **OpenArray® instrument** – Performs imaging of the OpenArray plates.
- **Computer** – Connects to the OpenArray® instrument; includes the OpenArray® SNP Genotyping Analysis Software (see below).

Software

- **OpenArray® SNP Genotyping Analysis Software** – Controls the OpenArray instrument and analyzes the imaging data.
- (Optional) **Applied Biosystems TaqMan® Genotyper Software** – Performs downstream analysis; for more information, see page 114.

Thermal cycler (purchased separately)

Genotyping experiments require two steps: thermal cycling (PCR amplification), followed by endpoint detection of the resulting fluorescence signals.

While the OpenArray® instrument performs the endpoint detection, you need a standalone thermal cycler to perform PCR amplification. Purchase a thermal cycler that has been qualified for use with the TaqMan OpenArray Genotyping Plates. The following thermal cyclers are qualified for use with the OpenArray plates:

- Dual Flat Block GeneAmp® PCR System 9700
- Thermo Electron PX2 thermal cycler

Note: Contact your Applied Biosystems service representative for more information on the thermal cyclers.

Required plates

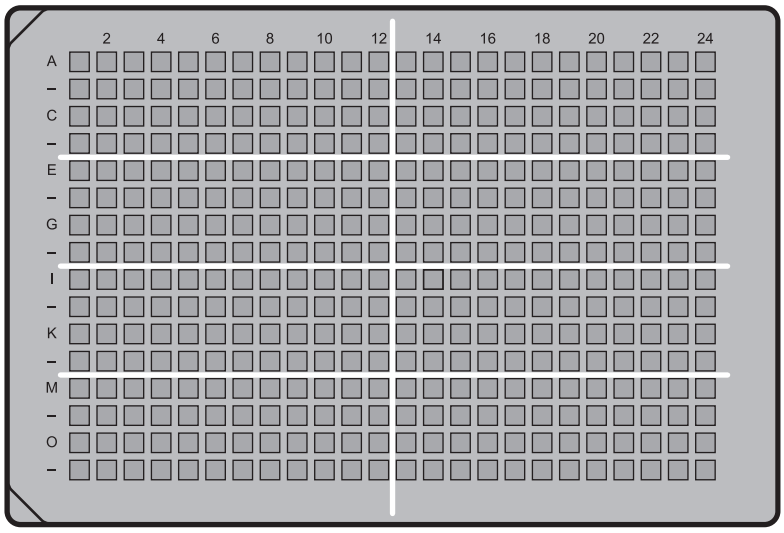
To perform genotyping experiments, the OpenArray system requires two plate types:

- OpenArray® 384-Well Sample Plate (*sample plate*) (this page)
- TaqMan® OpenArray® Genotyping Plate (*OpenArray plate*) (page 73)

Sample plate

The OpenArray 384-Well Sample Plate is a 384-well reaction plate. You combine the TaqMan® OpenArray® Master Mix with DNA sample in the sample plate, then use the OpenArray AutoLoader to transfer the mixture from the sample plate to one or more OpenArray plates.

IMPORTANT! The well dimensions of the OpenArray 384-Well Sample Plates are specifically suited for use with the OpenArray AutoLoader. Applied Biosystems does not recommend the use of other microtiter plates with the AutoLoader.



TaqMan® OpenArray® Genotyping Plate

The TaqMan® OpenArray® Genotyping Plate is a 63-mm × 19-mm mid-density reaction plate. There are 3072 reaction through-holes in the plate; individual through-holes are preloaded with a TaqMan genotyping assay, and each can accommodate a 33-nL reaction volume.

As shown in the figure below, the OpenArray plate is divided into 48 subarrays; each subarray consists of 64 through-holes. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes.

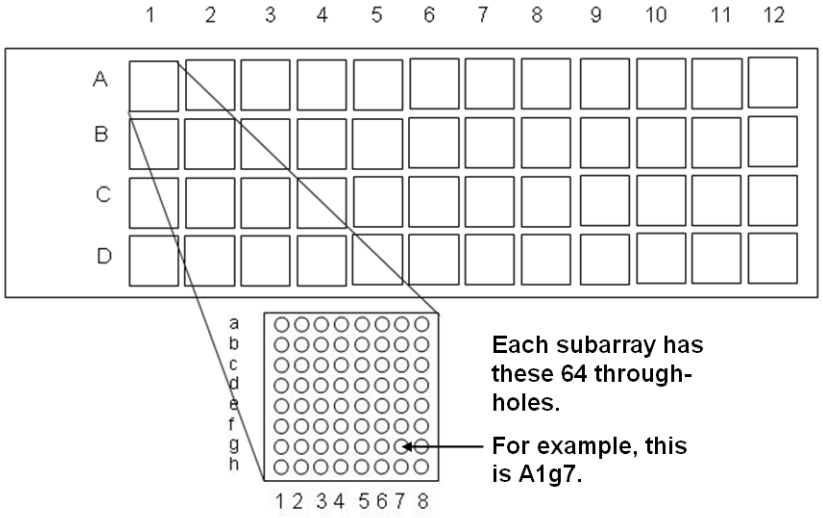


Plate preparation procedures

Refer to the *TaqMan® OpenArray® Genotyping Getting Started Guide* for procedures on:

- Preparing the OpenArray 384-Well Sample Plate
- Using the OpenArray AutoLoader to transfer sample from the sample plate to an OpenArray plate

To obtain the Getting Started Guide, see "Product documentation" on page 159.

IMPORTANT! This User Guide assumes that the OpenArray plate is loaded with sample and ready for imaging.

Workflow

Prepare the plates

1. Prepare an OpenArray® 384-Well Sample Plate.
2. Use the OpenArray® AutoLoader to transfer sample from the sample plate to a TaqMan® OpenArray® Genotyping Plate.

Note: For procedures, refer to the *TaqMan® OpenArray® Genotyping Getting Started Guide*. See "Product documentation" on page 159.

Perform imaging (Chapter 5)

1. Set up the OpenArray® SNP Genotyping Analysis Software.
2. Place the prepared OpenArray® plates in the OpenArray® instrument
3. Enter sample information in the software.
4. Perform imaging.

Analyze the run data (Chapter 6)

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 TaqMan® Genotyper Software.

5 Perform Imaging

In this chapter, you set up the OpenArray® SNP Genotyping Analysis Software, then perform imaging on the OpenArray® instrument.

This chapter covers:

- About the data files 76
- Set up the software 78
- Place the prepared OpenArray® plates into the instrument 79
- Enter sample information 81
- Perform imaging 87

About the data files

The OpenArray® SNP Genotyping Analysis Software uses four types of data files:

- Plate setup files (*.spf) (this page)
- Project files (*.nix) (this page)
- Plate data files (*.spd) (page 77)
- Sample information files (*.csv) (page 77)

Plate setup files (*.spf)

A plate setup file (*.spf) contains specific information for individual TaqMan® OpenArray® Genotyping Plates, such as:

- Assay IDs
- Reporter 1 and 2 sequences
- Gene symbol and name
- Location of each assay in the OpenArray plate

Each plate setup file is named with the serial number of its corresponding OpenArray plate. For example, the plate setup file for an OpenArray plate with the serial number **ABC01** is named **ABC01.spf**.

Accessing *.spf files

When you order the OpenArray plates, you must download a plate setup file (*.spf) for each OpenArray plate in your order, then copy the *.spf files to the OpenArray system computer (as described on page 78). The OpenArray software uses the *.spf files to populate the columns in the Assays pane; the software must access the *.spf file for each OpenArray plate before the OpenArray instrument can perform imaging.

Project files (*.nix)

Project files (*.nix) are the files that you view and modify in the OpenArray software. A project file allows you to combine, edit, and save changes to run data from up to 50 plate data files (*.spd).

Project files contain:

- **Run data** – When you image OpenArray plates, the run data is automatically saved to a plate data file (*.spd), then copied to the currently open project file (*.nix).
- **Modifications made to the data** – Within a single project file, you can overlay, view, and edit cluster plots from multiple plate data files (as described in Chapter 6).

To save modifications made to the data, you must save the project file (use the **File ▸ Save** or **File ▸ Save As** function). Otherwise, all your changes are lost. Project file names and save locations are user-defined.

IMPORTANT! The software *copies* the run data from the plate data file to the project file. The files are not linked; that is, modifications that you save to the project file (*.nix) are not saved to the corresponding plate data file (*.spd).

Plate data files (*.spd)

A plate data file (*.spd) contains run data for a single OpenArray plate. Plate data files are generated by the OpenArray software during imaging.

The software automatically names plate data files with the OpenArray plate serial number. For example, the plate data file for an OpenArray plate with the serial number **ABC01** is named **ABC01.spd**.

By default, the software saves the *.spd files to the following location:

<*drive*>:\images\<*run date*>\<*run number*>

where:

<*drive*> is the computer drive on which the OpenArray software is installed. The default installation drive is the C: drive.

<*run date*> is the date the run was performed.

<*run number*> is the chronological run number.

For example, data for the third run on June 15, 2008, is saved to:

C:\images\06-15-08\3

After a run is completed, you can change the *.spd file name and/or save the *.spd file to a different location.

Sample information files (*.csv)

The OpenArray software uses comma-delimited files (*.csv) to import and export sample information:

- **Import** – Applied Biosystems recommends that you create sample information files (*.csv) to track your DNA samples. Before imaging the OpenArray plates, you can import the sample information into the OpenArray software. See "Import sample information from a *.csv file" on page 81.
- **Export** – After an imaging run, you can export data from your project. See "Export *.csv files" on page 113.

Set up the software

Set up the OpenArray® SNP Genotyping Analysis Software for each OpenArray plate to be included in the imaging run:

- Start the instrument and software (this page)
- Copy the plate setup file (*.spf) to your computer (this page)

Start the instrument and software

1. Power on the OpenArray® instrument.
2. Power on the computer, then start the OpenArray® SNP Genotyping Analysis Software. The software displays a new (empty) project file (*.nix).
3. Wait for the system to fully start: When the system is ready, "Idle" appears in the software status bar at the bottom of the window. Startup may take a few minutes.

Copy the plate setup file (*.spf) to your computer

1. For each OpenArray plate in your order, download the plate setup file (*.spf) from the OpenArray plate product page or from:

   www.appliedbiosystems.com/spfdownload

   Note: For detailed ordering information, refer to the *TaqMan® OpenArray® Genotyping Plates Ordering Guide*.
2. Open the download location and confirm that there is an *.spf file for each OpenArray plate in your order.
3. Copy the plate setup files to the **PLATEFILES** folder:

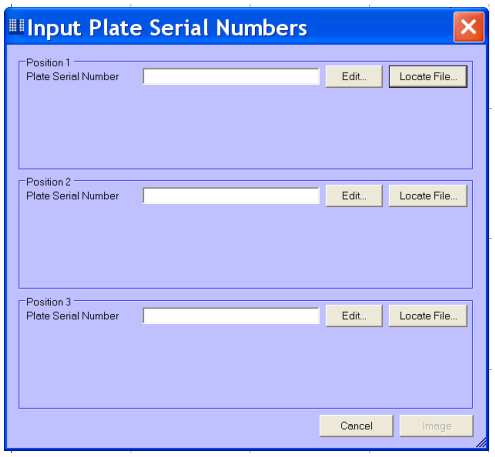
   <*drive*>:\Program Files\BioTrove\PLATEFILES

   where <*drive*> is the computer drive on which the OpenArray software is installed. The default installation drive is the C: drive.

Place the prepared OpenArray® plates into the instrument

1. In the OpenArray software, open a project file (*.nix). You can open:
   - A new project file – Use the project file automatically opened at startup, or select **File ▸ New**.
   - An existing project file (containing data from previous runs) – Select **File ▸ Open**, then browse to and open a project file.

2. Click **Image** to open the Input Plate Serial Numbers dialog box.

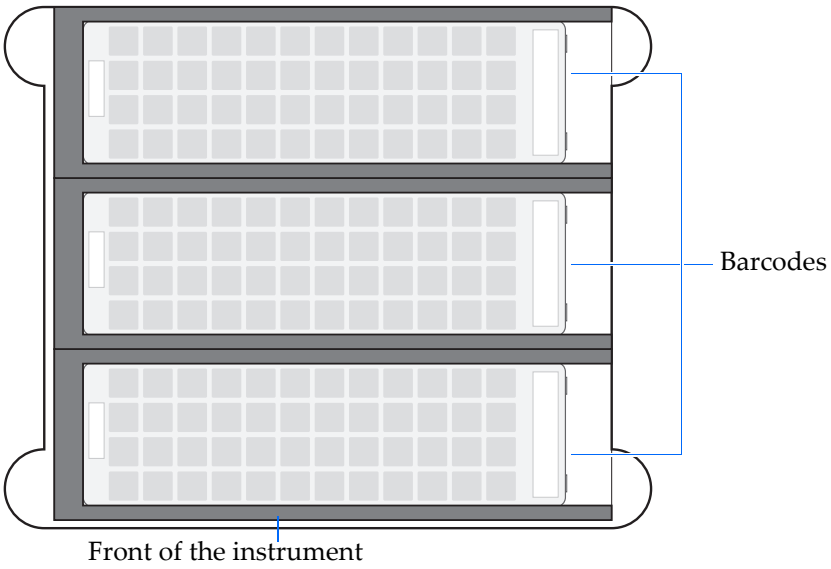


3. At Position 1, enter the serial number for the first OpenArray plate. You can:
   - Click **Locate File**, then browse to and open the plate setup file (*.spf) that corresponds to the OpenArray plate. The software automatically displays the serial number in the Plate Serial Number field.
   - Type the serial number.
   - Scan the barcode located on the OpenArray plate package.

IMPORTANT! If you enter the serial number by typing or scanning, the *.spf file *must* be located in the PLATEFILES directory (see "Copy the plate setup file (*.spf) to your computer" on page 78). Otherwise, the software will not be able to locate the *.spf file.

Note: The OpenArray software uses the serial numbers to access the appropriate plate setup files (*.spf). During imaging, the software uses information in the plate setup files to populate the Assays pane in the project file (*.nix). For information on the Assays pane, see "View data in the Assays pane" on page 91.

4. Open the OpenArray instrument door and lid, then place the OpenArray plate into Position 1. Be sure that:
   - The plate position in the instrument matches the plate position in the software.
   - The barcode is facing up and to the right, and the plate is flush with the right and back edges.



IMPORTANT! If the plates are not positioned correctly, your data results will be adversely affected.

5. Repeat this procedure to enter the serial numbers and place OpenArray plates in Positions 2 and 3.

   Note: If you are running fewer than three OpenArray plates, Applied Biosystems recommends the following: For one plate, use Position 1; for two plates, use Positions 1 and 2.

IMPORTANT! Leave the Input Plate Serial Numbers dialog box open, then proceed to "Enter sample information" on page 81. If you close the dialog box, the information you have entered will be lost.

Enter sample information

Entering sample information allows you to:

- Track the OpenArray® 384-Well Sample Plates, and map the sample plate areas to each OpenArray plate.
- Associate information about the samples with the data results.

To enter sample information, you can:

- (Recommended) Import sample information from a *.csv file (this page)
- Manually enter sample information (page 84)

If needed, you can add or delete sample information columns in the Samples pane. See Appendix A on page 133.

Import sample information from a *.csv file

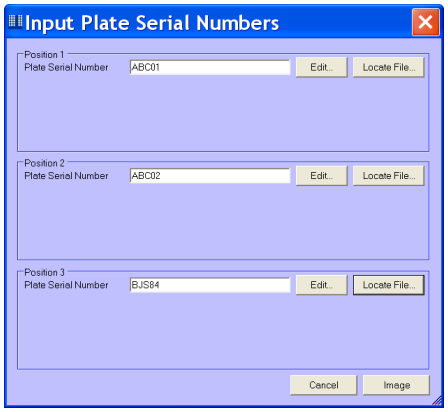
Note: You can import sample information after a run has completed; however, Applied Biosystems recommends that you import the sample information *before* starting the run.

1. If you have not done so already, create a *.csv file.

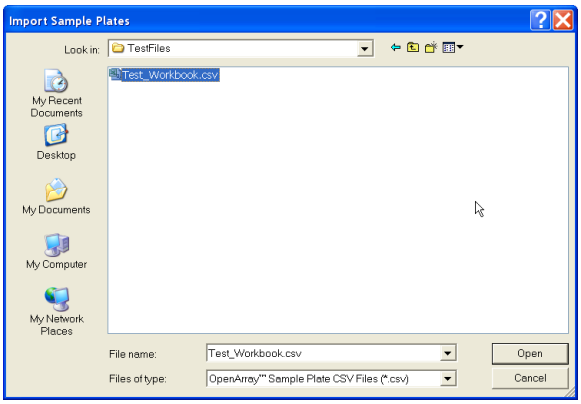
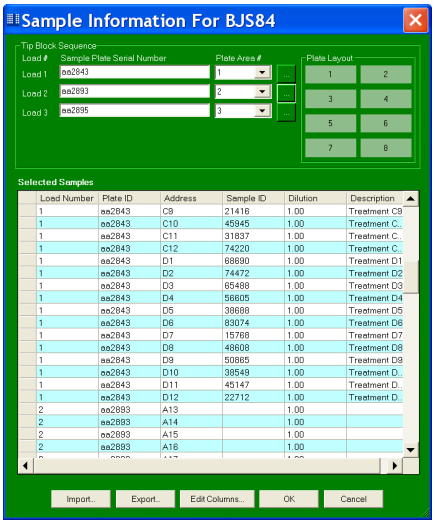
   Note: For procedures on creating a *.csv file, refer to the *TaqMan® OpenArray® Genotyping Getting Started Guide*.

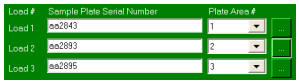
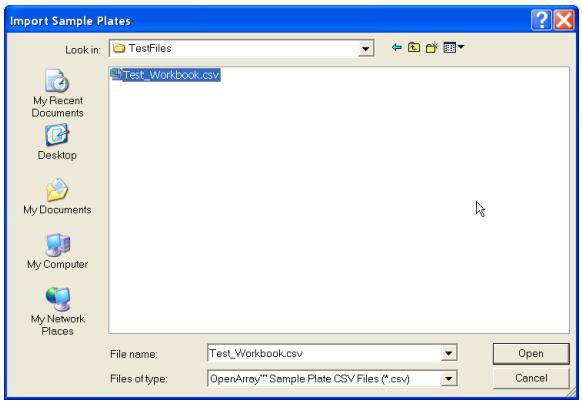
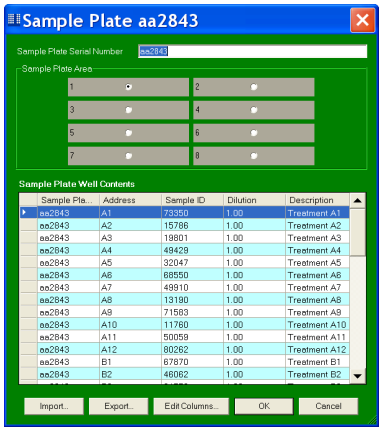
2. At Position 1 of the Input Plate Serial Numbers dialog box, click **Edit**.

   Note: If you are importing sample information after a run: Below the Samples pane, click **Edit**, then continue with step 3 on page 82.



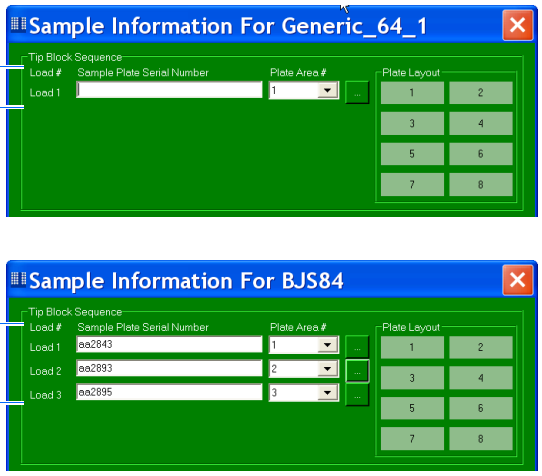
3. Import the sample information according to the appropriate procedure below.

If you want to...	Then...
Import the sample information for all loads at one time (1 to 3 loads)	1. In the Sample Information dialog box, click **Import**. 2. In the Import Sample Plates dialog box, browse to and open the *.csv file to import. IMPORTANT! Be sure to select a *.csv file that contains sample information for all of the required loads.  The sample information appears in the Selected Samples pane of the Sample Information dialog box.  3. (Optional) Edit the sample information in each row.

If you want to...	Then...
Import the sample information for each load separately	For *each* load: 1. In the Sample Information dialog box, click the … icon next to the load number of interest.  2. In the Sample Plate dialog box, click **Import**. 3. In the Import Sample Plates dialog box, browse to and open the *.csv file to import.  The sample information appears in the Sample Plate Well Contents pane of the Sample Plate dialog box.  4. (Optional) Edit the sample information in each row. 5. Click **OK** to close the Sample Plate dialog box.

4. For *each* load: From the Plate Area # drop-down menu, select the 12-well × 4-well area of the sample plate that the samples were transferred from.

The software displays 1 to 3 load numbers (Load 1, Load 2, and Load 3), depending on the format of the OpenArray plate.



5. Click **OK** to close the Sample Information dialog box.

6. Repeat this procedure to import sample information for the remaining OpenArray plates.

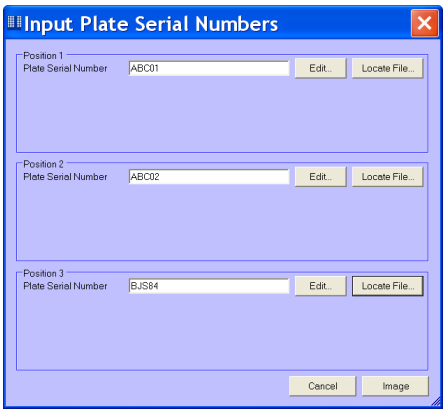
IMPORTANT! If you are importing sample information before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform imaging" on page 87. If you close the dialog box, the information you have entered will be lost.

Manually enter sample information

Note: You can manually enter sample information after a run has completed; however, Applied Biosystems recommends that you enter the sample information *before* starting the run.

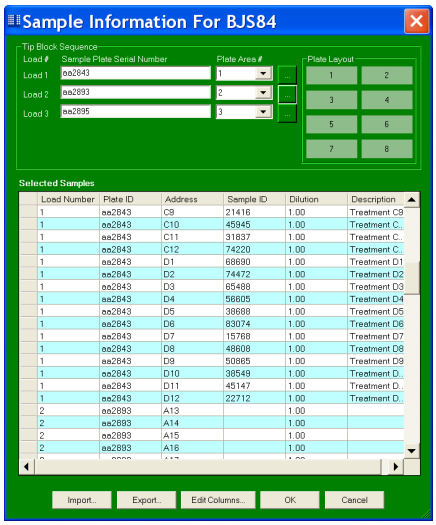
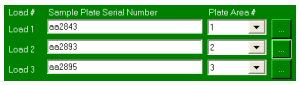
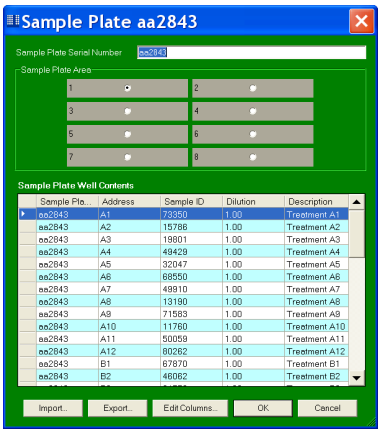
1. At Position 1 of the Input Plate Serial Numbers dialog box, click **Edit**.

   Note: If you are entering sample information after a run: Under the Samples pane, click **Edit**, then continue with step 2 on page 85.



2. Enter the sample information according to the appropriate procedure below.

   Note: You cannot enter or edit information in the following columns: *Load Number*, *Plate ID*, and *Address*. If you want to add or delete columns, see Appendix A on page 133.

If you want to...	Then...
Enter sample information for all loads at one time (1 to 3 loads)	In the Sample Information dialog box, edit the desired fields in the Selected Samples pane: 1. Double-click inside the field to activate it. 2. Enter the appropriate information. 
Enter sample information for each load separately	For *each* load: 1. In the Sample Information dialog box, click the … icon next to the load number of interest.  2. In the Sample Plate dialog box, edit the desired fields in the Sample Plate Well Contents pane: a. Double-click inside the field to activate it. b. Enter the appropriate information.  3. Click **OK** to close the Sample Plate dialog box.

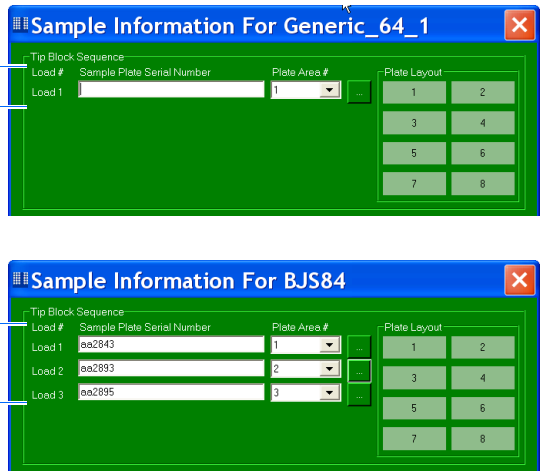
3. In the Sample Information dialog box, enter identifying information for *each* load number:

   a. 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. Refer to the *TaqMan® OpenArray® Genotyping Getting Started Guide*.

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

The software displays 1 to 3 load numbers (Load 1, Load 2, and Load 3), depending on the format of the OpenArray plate.



4. Click **OK** to close the Sample Information dialog box.

5. Repeat this procedure to manually enter sample information for the remaining OpenArray plates.

IMPORTANT! If you are entering sample information before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform imaging" on page 87. If you close the dialog box, the information you have entered will be lost.

Perform imaging

During imaging, the OpenArray® instrument records the amount of fluorescence in each through-hole of the OpenArray plates. The run data are automatically saved to the plate data file (*.spd).

OpenArray® instrument commands

The table below is a summary of OpenArray software commands that you can use to control the OpenArray® instrument.

Command	Description
Stop Imaging	To stop imaging at any time: In the OpenArray software, select **Actions ▸ Stop Imaging**. A message appears asking if you want to save the collected data. • Click **Yes** to save the incomplete plate data file (*.spd). • Click **No** to continue imaging.
Interior Light On/Off (toggle switch)	To turn the light inside the instrument on or off, select **Actions ▸ Interior Light On/Off.**

Perform imaging

1. Close the OpenArray® instrument lid and door.
2. In the Input Plate Serial Numbers dialog box, click **Image**.

   IMPORTANT! Do not open the instrument door during the run. The run is complete when: (1) The blue LED light on the instrument door is off; and (2) In the software, data appears and the status bar displays a green circle. If you need to stop the run, see "OpenArray® instrument commands" above.

3. When the run is complete, save the project file (*.nix):
   a. Select **File ▸ Save** or **File ▸ Save As** to open a save dialog box.
   b. Browse to a save location, enter a file name, then click **Save**.
4. Open the instrument door, then remove the OpenArray plates.

   Note: Applied Biosystems recommends that you temporarily save the OpenArray plates until you have reviewed the data. If you store the plates in the dark at 4 °C, you can re-image the plates for up to 5 days.

6 Analyze the Run Data

In this chapter, you view the data from the imaging run (performed in Chapter 5) in a project file (*.nix). If the automatic calls are not suitable for your experiment, you can modify the clustering parameters. This chapter also explains how to modify the project files, publish data, and export data for downstream analysis using the Applied Biosystems TaqMan® Genotyper Software.

This chapter covers:

- View the results. 90
- (Optional) Modify clustering parameters 97
- (Optional) Modify project files 108
- (Optional) Publish data 111
- (Optional) Perform downstream analysis. 114

View the results

After an imaging run, the OpenArray® SNP Genotyping Analysis Software automatically calls the genotypes for each TaqMan® OpenArray® Genotyping Plate in the run. To view the results of the automatic analysis:

- Open a project file (*.nix) (this page)
- View data in the Assays pane (this page)
- View data in the Samples pane (page 93)
- View data in the Scatter Plot pane (page 95)

If the automatic calls are not suitable for your experiment, see "(Optional) Modify clustering parameters" on page 97.

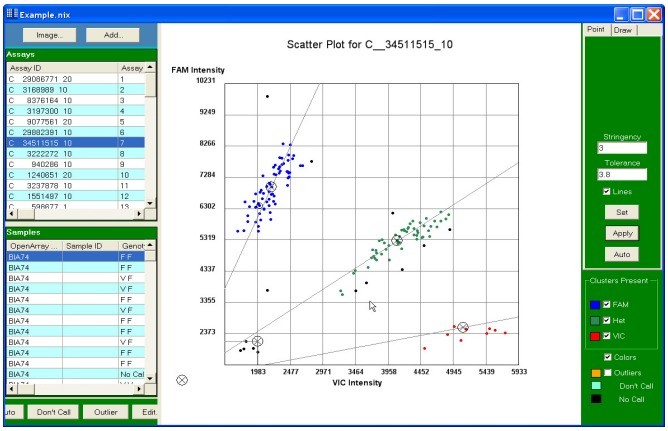
Open a project file (*.nix)

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

Note: After a run, the OpenArray software automatically opens the project file for that run.

View data in the Assays pane

Each row in the Assays pane represents a specific assay in the project. You can navigate within the Assays pane as described in the table below.

To...	Action	Result/Example
Select individual assays	Click the assay to view.	The data for all samples associated with the selected assays appear in the Samples pane; the reactions† appear as data points in the Scatter Plot pane. 
Enter the allele nucleotide sequences detected by each assay	1. Click in the appropriate sequence column: **Reporter 1 Sequence** or **Reporter 2 Sequence**. 2. Enter the appropriate letter for the reporter dye: **F** (FAM™ dye), **V** (VIC® dye), or **N** (non-specific).	
Rearrange columns	Click and drag a column heading. For a description of each column in the Assays pane, see page 92.	
Sort rows	Click a column heading.	

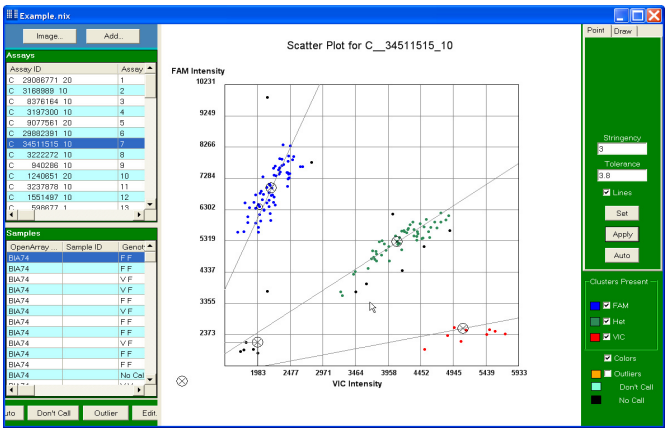
† A reaction is a sample-assay combination; each through-hole in an OpenArray plate contains a single reaction.

Assays pane column descriptions

Column name	Column description
Assay ID	Unique identifier for the assay (may be user-defined for custom assays).
Study Name	The name of your sales order (user-defined).
Assay Number	A sequential number assigned to each assay in the OpenArray plate.
Reporter 1 Sequence	The nucleotide sequence of reporter 1.
Reporter 2 Sequence	The nucleotide sequence of reporter 2.
Gene Symbol	LocusLink symbol for the associated gene.
Gene Name	LocusLink gene name.
Chromosome	Chromosome on which the gene or SNP is found.
NCBI SNP Reference	Reference ID from the NCBI-dbSNP database.
Cytogenetic Band	Chromosomal band location of the gene. If the cytogenetic band is not available, the chromosome number is listed instead.
SNP Type	Type of SNP, based on Celera Assembly: Acceptor Splice Site, Donor Splice Site, Intergenic/Unknown, Intron, Mis-sense Mutation, Nonsense Mutation, Putative UTR 5ESilent Mutation, UTR 3′ , UTR 5′ .
Order Number	Customer sales order number.
VIC SEQUENCE	The nucleotide polymorphism assayed by the probe labeled with the VIC® dye, if provided in the *.spf file. If not provided, this field displays the letter V.
FAM SEQUENCE	The nucleotide polymorphism assayed by the probe labeled with the FAM™ dye, if provided in the *.spf file. If not provided, this field displays the letter F.
Minor Allele Freq - Caucasian	The frequency with which the minor allele occurs in the Caucasian population.
Minor Allele Freq - African American	The frequency with which the minor allele occurs in the African American population.
Minor Allele Freq - Japanese	The frequency with which the minor allele occurs in the Japanese population.
Minor Allele Freq - Chinese	The frequency with which the minor allele occurs in the Chinese population.

View data in the Samples pane

Each row in the Samples pane represents a specific sample in the project. You can navigate within the Samples pane as described in the table below.

To...	Action	Result/Example
Select an individual sample	Select the assay of interest in the Assays pane, then click the sample to view in the Sample Data pane.	In the Scatter Plot, the software displays a black circle around the data points for the selected reactions†. The assay remains selected in the Assays pane as you select different samples in the Samples pane. You can use this feature to see how different genes behaved for specific experimental conditions. 
Select multiple samples, nonadjacent	Select the assay of interest in the Assays pane, press and hold the **CTRL** key, then click the samples to view in the Sample Data pane.	
Select multiple samples, adjacent	Select the assay of interest in the Assays pane, press the **SHIFT** key, then click the first and last rows of the block of samples to view in the Sample Data pane.	
Rearrange columns	Click and drag a column heading. For a description of each column in the Samples pane, see page 94.	
Sort rows	Click a column heading.	
Add or delete columns	For add and delete procedures, see Appendix A on page 133.	

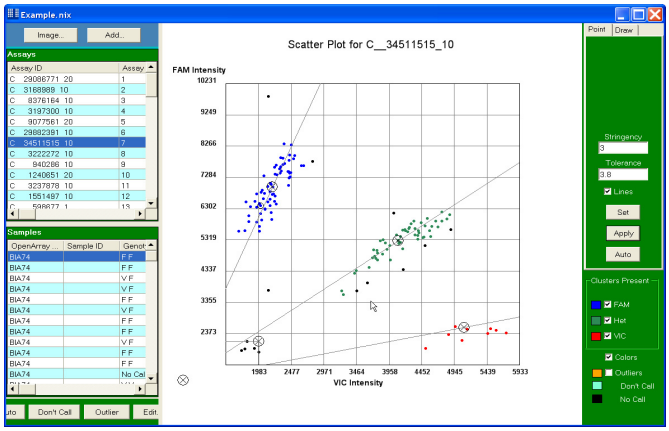
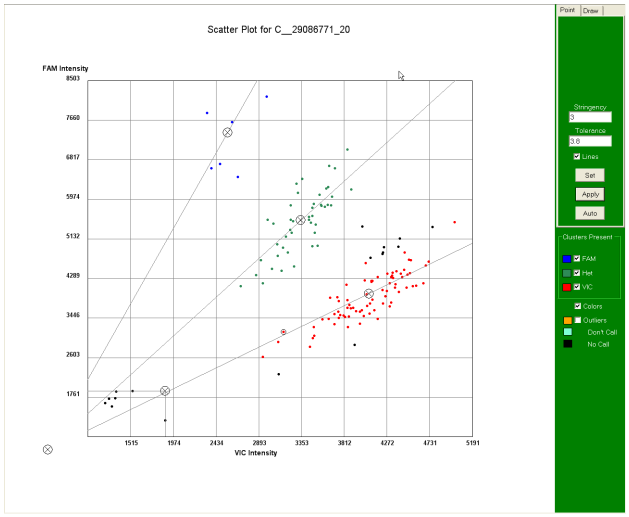
† A reaction is a sample-assay combination; each through-hole in an OpenArray plate contains a single reaction.

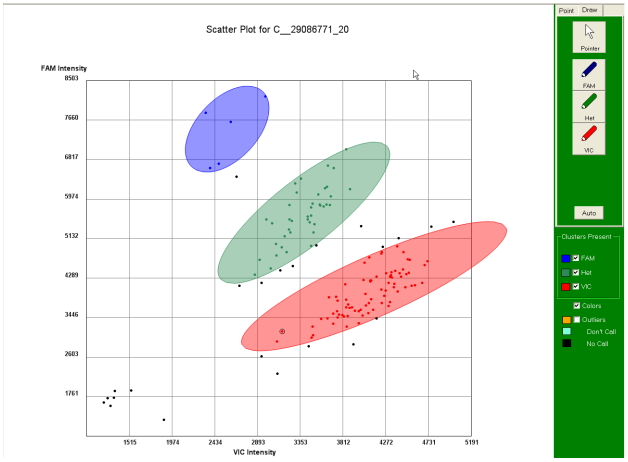
Samples pane column descriptions

Column name	Column description
OpenArray Serial Number	An alphanumeric code (for example, **ABC01**) for the TaqMan® OpenArray® Genotyping Plate.
Sample ID	The sample identification (user-defined).
Genotype String	The genotype call made for the sample by the software or by a user: **VV** = VIC® dye homozygote **VF** = Heterozygote **FF** = FAM™ dye homozygote **No Call** or **Don't Call** = No genotype is called for the sample, and the samples are excluded from the analysis **Outlier** = The sample is set as an outlier **A**, **C**, **G**, **N**, or **T** = Allele information
Consensus Genotype String	The calculated genotype result for all assay replicates.
Replicate ID	Reserved for future use.
Address	The location of the assay on the TaqMan® OpenArray® Genotyping Plate (for example, **A1a1**).
Distance To Cluster Center	The distance between the data point and the appropriate genotyping cluster line.
Confidence	A measurement between 0 and 1. Larger values indicate close proximity to the cluster line compared with other data points in the cluster.
Distance To Nearest Cluster In STDs	The distance between a data point and the **nearest** cluster line, expressed as standard deviation units. The standard deviation is calculated using all data points in the relevant cluster.
Distance To Next Nearest Cluster In STDs	The distance between a data point and the *next* **nearest** cluster line, expressed as standard deviation units. The standard deviation is calculated using all data points in the relevant cluster.
Through-Hole Index	The identification number of the through-hole in which the assay was cycled and imaged.
VIC, FAM	The measurement of indicated dye fluorescence detected by the OpenArray® instrument.
Sample Plate Serial Number	An alphanumeric code for the OpenArray® 384-Well Sample Plate (user-defined).
Sample Address	The well in the OpenArray® 384-Well Sample Plate from which the sample was transferred.
Sample Dilution	The sample concentration (user-defined).
Sample Description	A description of the sample (user-defined).
SampleInfo.Properties *<heading>* where: *<heading>* is user-defined	Indicates a new column added by a user. All user-defined columns are prefixed with *SampleInfo.Properties.*

View data in the Scatter Plot pane

Each data point in the Scatter Plot pane represents a specific reaction in the project (a reaction is a sample-assay combination; each through-hole in the OpenArray plate contains a single reaction). You can navigate within the Scatter Plot pane as follows:

To...	Action	Result/Example
Select individual reactions	Click the data point to view.	The assay and sample data associated with the selected data points are highlighted in the Assays pane and the Samples pane.
Select multiple reactions	Press the **CTRL** key while clicking the data points to view.	
Group data points by their angle about a clustering axis	Select the **Point** tab.	Clusters are described by lines between their clustering axis and automatically determined cluster centers. 

To...	Action	Result/Example
Group data points by their inclusion in a cluster	Select the **Draw** tab.	Inclusion is represented by an ellipse or hand-drawn shape. 
Change the data point color display	In the Point or Draw tab: • Select the **Colors** checkbox to display colors for the data points by genotype. For a description of the colors, see "Data point color descriptions" below. • Deselect the **Colors** checkbox to display all data points in gray.	
Zoom in or out	• **Zoom in** – Right-click in the corner of the area you want to view, drag diagonally across the area, then release the mouse. The selected area will be enlarged. • **Zoom out** – Right-click any where in the Scatter Plot. The entire plot reappears.	

Data point color descriptions

The color of each data point in the Scatter Plot pane indicates the genotype calls made by the software.

Color	Color description
Blue	FAM™ dye homozygous
Green	FAM™ and VIC® dye heterozygous
Red	VIC® dye homozygous
Orange	Outlier
Cyan	Don't Call *Don't Call* samples are set by the user (see page 103). *Don't Call* samples are excluded from the analysis.
Black	No Call *No Call* samples are set by the software if the sample's data point is outside the range of all clusters or within the range of two or more clusters. *No Call* samples are excluded from the analysis.

(Optional) Modify clustering parameters

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 follows:

- Set outliers (page 98)
- Adjust genotyping clusters (page 99)
- Adjust stringency (page 102)
- Adjust tolerance (page 103)
- Set Don't Call samples (page 103)
- Exclude genotyping clusters from analysis (page 105)
- Draw genotyping clusters (page 107)

IMPORTANT! The software applies modifications to the clustering parameters only to the assay you are viewing, not to the entire project. To change the default settings for the entire project, see "Set project parameters" on page 109.

Save your changes to the project file (*.nix)

After modifying the clustering parameters, be sure to save the project file (*.nix) if you want to save your changes. If you do not save the project file, all your changes are lost when you close the project file.

IMPORTANT! The software *copies* the run data from the plate data file to the project file. The files are not linked; that is, modifications you save to the project file (*.nix) are not saved to the corresponding plate data file (*.spd).

1. Select a save option:
   - **File ▸ Save** to save the changes to the current project file.
   - **File ▸ Save As** to save the changes to a new project file. The File ▸ Save As function allows you to perform multiple analyses of the same plate data file (*.spd).
2. For the **File ▸ Save As** option, browse to a save location, enter a file name, then click **Save**.

About the Auto functions

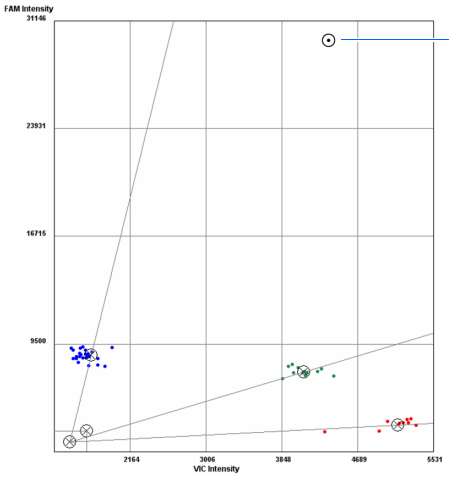
There are three Auto functions in the OpenArray software:

- **Auto button in the Samples pane** – The software re-calls a sample that a user has labeled *Don't Call* or *Outlier*, per the current settings. To use this Auto function, select the sample, then click **Auto** in the Samples pane.
- **Auto button in the Draw or Point tab** – The software determines genotype calling parameters for the current assay and re-calls the genotypes (*FAM, Het, VIC,* or *No Call*) for all samples applied to the current assay. To use this Auto function, select the assay, then click **Auto** in the Draw or Point tab.
- **Auto Reclassify All Assays** – The software determines the genotype calling parameters for all assays and re-calls the genotypes (*FAM, Het, VIC,* or *No Call*) for all samples. To use this Auto function, select **Action** ▸ **Auto Reclassify All Assays**.

Set outliers

If your data includes one or more data points that are very different from that of most of the other data points, you can set them as outliers. The software does not call outliers.

1. In the Samples pane or Scatter Plot pane, select the samples to set as outliers.
2. In the Samples pane, click **Outlier**. The software:
   - Labels each selected sample as *Outlier* in the Samples pane.
   - Removes the outliers from view in the Scatter Plot pane.
   - Recalculates the clusters without the outliers.



The fluorescence of this data point is different from the others. Select it, then click **Outlier**.

3. To view the outliers, select the **Outliers** checkbox in the Point or Draw tab. The outliers appear in the Scatter plot as orange data points.
4. To include an outlier back in the analysis:
   a. Select the sample.
   b. Click **Auto** in the Samples pane.

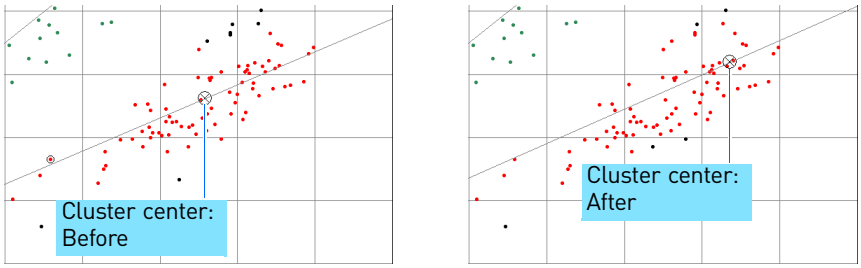
Adjust genotyping clusters

Use the Point and Draw tab tools to adjust genotyping clusters. You can:

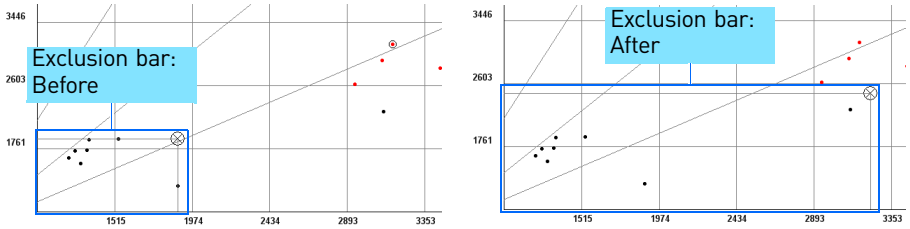
- Drag and drop to move the clusters and exclusion bars (this page)
- Use the Auto-Classification Wizard to move the clusters and exclusion bars (this page)
- Modify the cluster shapes (page 100)

Drag and drop

1. In the OpenArray software, select the **Point** tab.
2. Drag and drop one of the following:
   - Cluster center

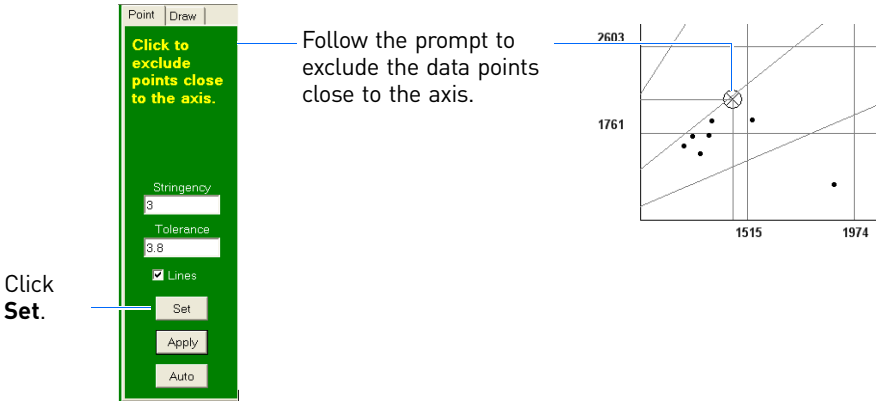


   - Exclusion bar

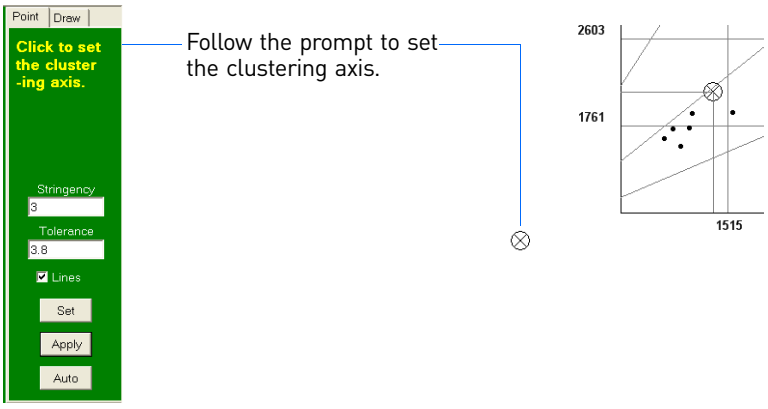


Auto-Classification Wizard

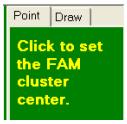
1. In the OpenArray software, select the **Point** tab.
2. Click **Set**.
3. At the prompt, exclude the data points close to the axis: Select a data point where all the data points with less fluorescence will be marked *No Call*. For example, you may want to exclude no template controls (NTCs).



4. At the prompt, set the clustering axis: Click where all the cluster lines appear to intersect. Typically, the cluster lines intersect near the origin.

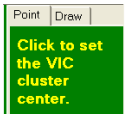


5. At the prompts, click where you want to set the new:
   a. FAM dye cluster center



   b. Heterozygote cluster center

   c. VIC dye cluster center.



6. Repeat the classification procedure to make further changes, then click **Apply** to apply the changes.

Modify the cluster shapes

You can rotate, resize, reshape, and move the ellipses drawn around each genotyping cluster. These adjustments change the genotype call for data points that were outside and are now inside the cluster and for data points that were inside and are now outside the cluster.

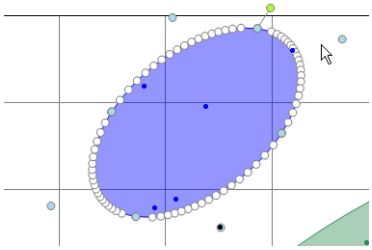
To modify the cluster shapes:

1. In the OpenArray software, select the **Draw** tab. The software automatically draws ellipses for each genotype.

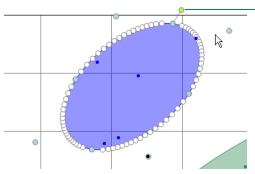
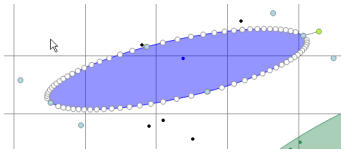
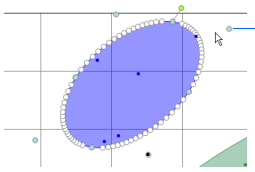
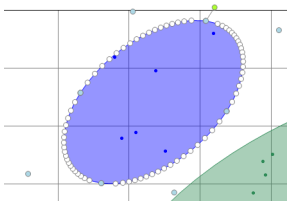
   Note: The software does not call data points that are outside a cluster area or are within more than one cluster area.

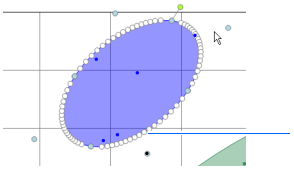
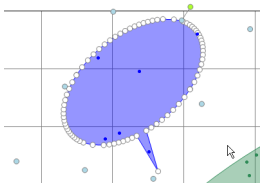
2. Select the cluster to modify. You can:
   - Select  , then click the cluster.
   - Select **Tools** ▸ **Pointer**, then click the cluster.

   The software highlights the selected clusters as shown.



3. Modify the cluster shape as needed:

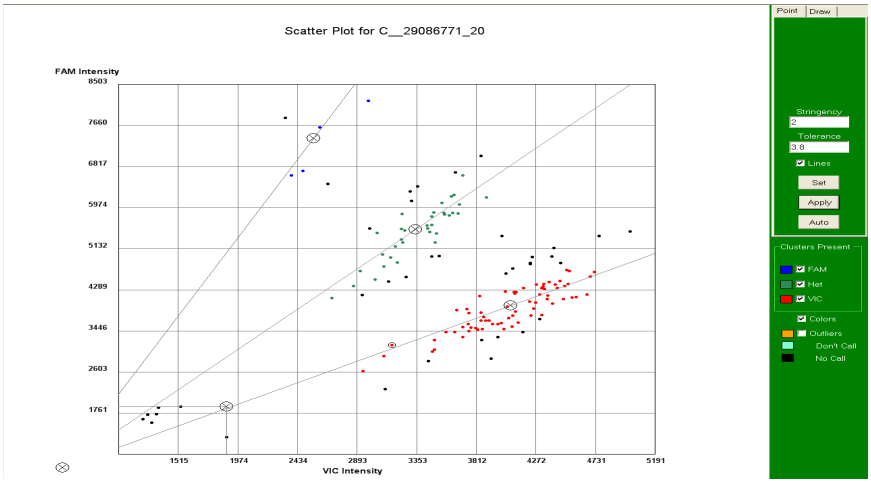
To...	Action	Result/Example
Rotate the cluster	Click and drag the green handle in the direction you want to rotate the cluster.	 Click and drag the green circle. Cluster: Before  Cluster: After
Resize the cluster	Click and drag the appropriate blue circle outward (to enlarge) or inward (to shrink).	 Click and drag a blue circle. Cluster: Before  Cluster: After

To...	Action	Result/Example
Reshape the cluster (for example, to include a nearby point)	Click and drag the appropriate white circle in the direction you want the cluster to be stretched.	 Click and drag a white circle. Cluster: Before  Cluster: After
Move the cluster	Click and drag the cluster to move it to the desired position.	

Adjust stringency

The software assigns *No Call* status to data points that are too far from their cluster line, and excludes the data points from the analysis. You can change the number of standard deviations from cluster lines to the data points that are included in the genotype call.

1. In the OpenArray software, select the **Point** tab.
2. In the Stringency field, enter a positive number (for example, **2**) or enter **Infinity**.
3. Click **Apply**. The software assigns *No Call* status to any data points that are farther from the cluster than the value entered. *No Call* data points are black.

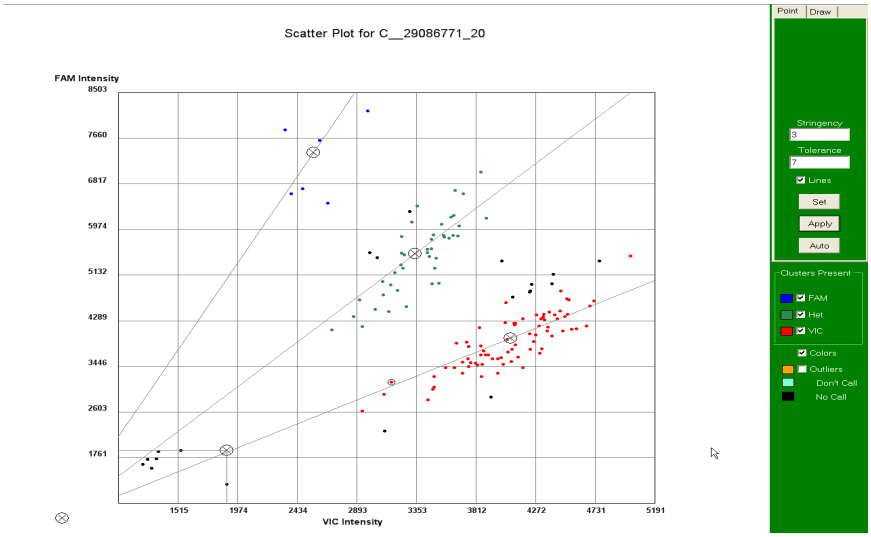


Adjust tolerance

You can adjust how close a data point in one cluster can be to an adjacent cluster line before the software assigns *No Call* status.

1. In the OpenArray software, select the **Point** tab.
2. In the Tolerance field, enter a standard deviation value.

   Note: Larger tolerance values result in more *No Call* data points.
3. Click **Apply**. The software assigns *No Call* status to any points that are within the tolerance value of more than one cluster line. *No Call* data points are black.

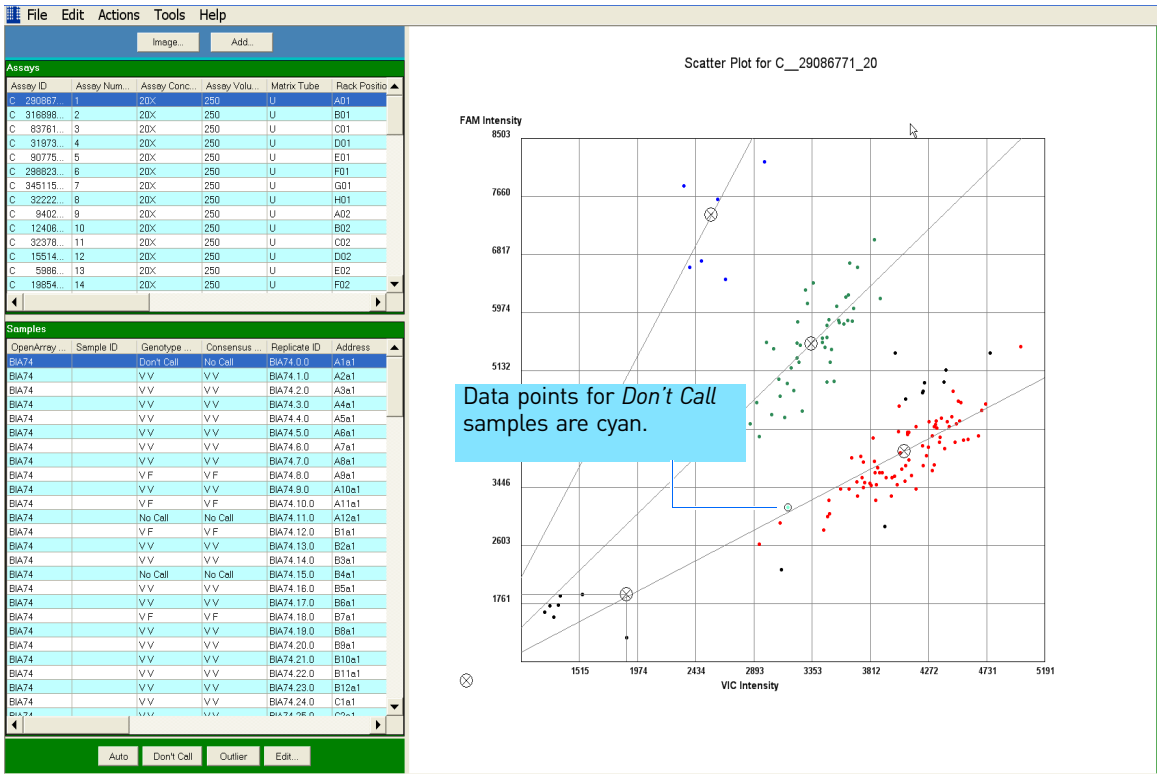


Set *Don't Call* samples

The software does not call samples that you set as *Don't Call* (that is, the software excludes *Don't Call* samples from the analysis.

1. In the Samples pane or Scatter Plot pane, select the sample.
2. Click **Don't Call**. In the Scatter Plot, the data point for the selected sample turns cyan.

3. To include the data point back in the analysis, select it, then click **Auto** in the Samples pane.

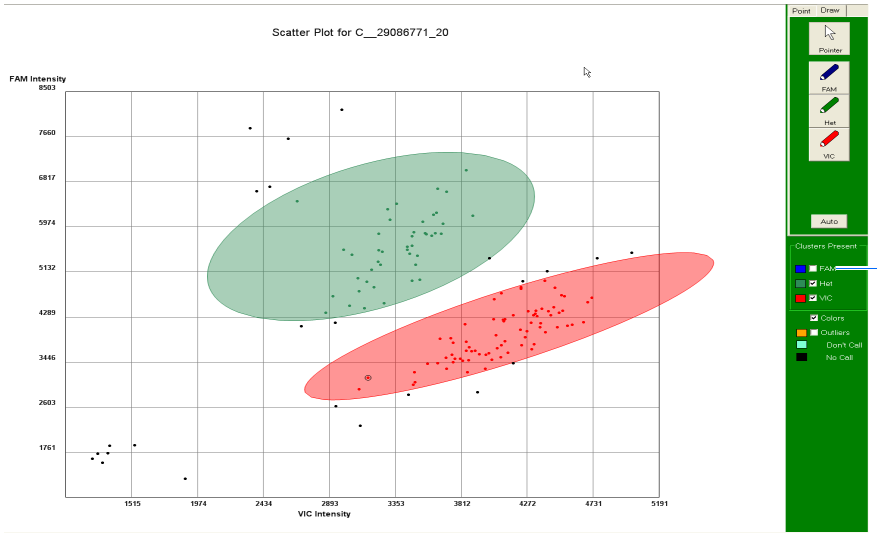


Exclude genotyping clusters from analysis

You can configure the software to identify fewer than three genotypes. For example, if you know your samples do not include any FAM dye homozygotes, you can remove the FAM dye from the analysis.

Exclude a genotyping cluster using the Draw tab

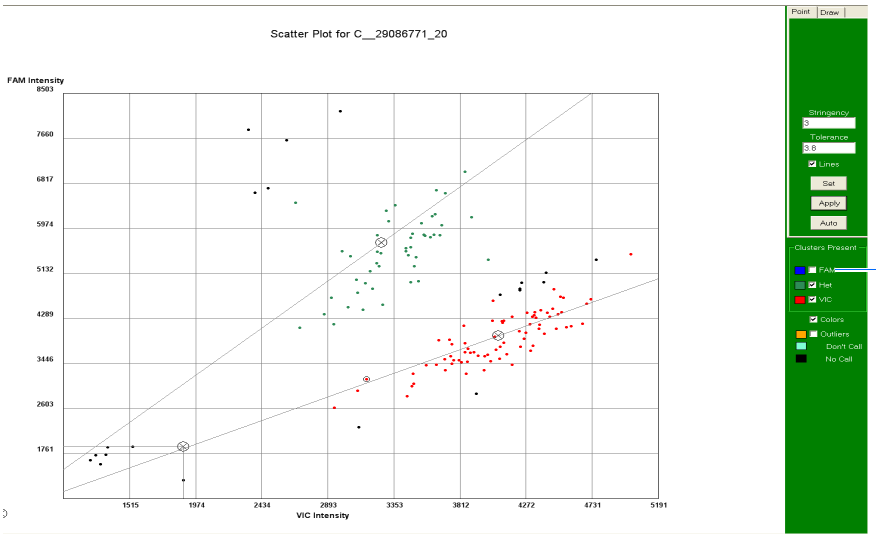
1. Select the **Draw** tab.
2. In the Scatter Plot, select the appropriate genotyping cluster, then press the **DELETE** key. The genotyping cluster disappears from the Scatter Plot; in the Clusters Present area, the corresponding genotype (*FAM*, *Het*, or *VIC*) is automatically deselected. The software analyzes the data without the excluded genotyping cluster.



3. To include the genotyping cluster back in the analysis, do one of the following:
   - In the Point or Draw tab, select the excluded genotype: **FAM**, **Het**, or **VIC**.
   - Redraw the cluster (see "Draw genotyping clusters" on page 107).

Exclude a genotyping cluster using the Clusters Present area

1. Select the **Point** or **Draw** tab.
2. In the Clusters Present area, deselect the genotype (**FAM**, **Het**, or **VIC**) that you do not have. The genotyping cluster disappears from the Scatter Plot. The software analyzes the data without the excluded genotyping cluster.



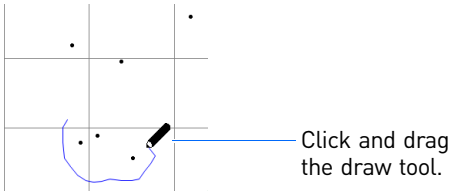
FAM dye homozygote is not present in this assay.

3. To include the genotyping cluster back in the analysis, do one of the following:
   - In the Point or Draw tab, select the excluded genotype: **FAM**, **Het**, or **VIC**.
   - Redraw the cluster (see "Draw genotyping clusters" on page 107).

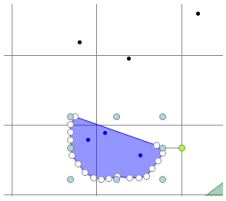
Draw genotyping clusters

Note: When you create a new genotyping cluster, the software automatically deletes the previously configured cluster for that genotype.

1. In the OpenArray software, select the **Draw** tab.
2. Select the appropriate drawing tool for the genotyping cluster you want to recreate (for example,). Or select **Tools** ▸ **Draw** <*dye*> **Tool** (where **<dye>** is **FAM**, **Het**, or **VIC**). The software deletes the previously configured cluster for that genotype.
3. In the Scatter Plot, click and draw a line around all the data points you want to include in the new genotyping cluster.



Cluster: Before



Cluster: After

(Optional) Modify project files

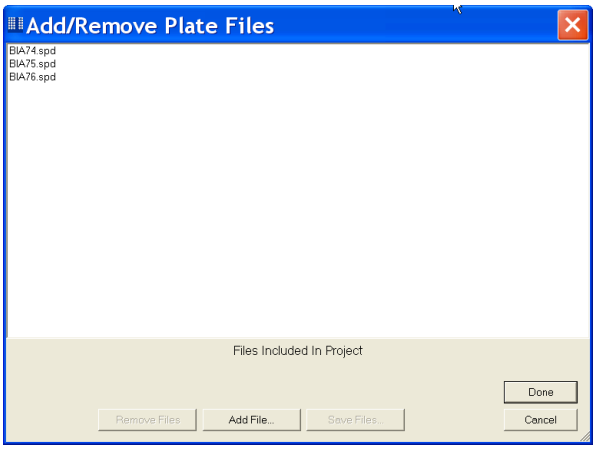
Project files (*.nix) are the files that you view and modify in the OpenArray software. (For a detailed description of project files, see page 76.) You can modify project files as follows:

- Add plate data files (*.spd) (this page)
- Remove plate data files (*.spd) (page 109)
- Set project parameters (page 109)

Add plate data files (*.spd)

1. In the OpenArray software, click **Add** to open the Add/Remove Plate Files dialog box. The software displays the plate data files (*.spd) currently in the project.
2. Click **Add File**, then browse to and select the plate data files to add.

   Note: To select multiple plate data files, press and hold the **CTRL** or **SHIFT** key.



3. Click **Open**. The software:
   - Displays the selected plate data files in the Add/Remove Plate Files dialog box.
   - Copies the run data from the plate data file to the project file.

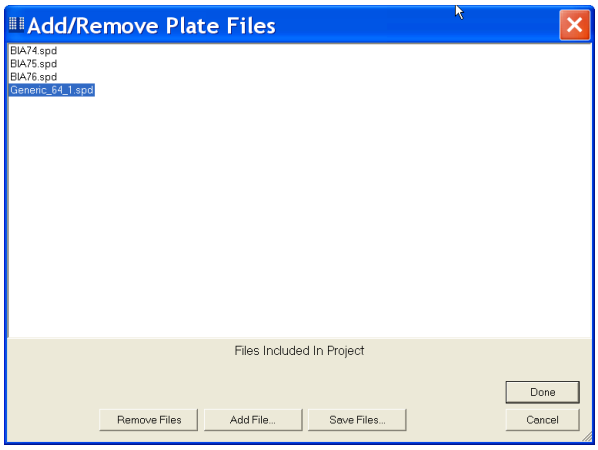
     Note: When you add a plate data file, the software *copies* the run data from the plate data file to the project file. The files are not linked; that is, any changes you make in the project file (*.nix) are not made in the corresponding plate data file (*.spd).
4. Click **Done**. The software automatically calls the genotypes for the revised group of plate data files, using your current settings.

Remove plate data files (*.spd)

1. In the OpenArray software, click **Add** to open the Add/Remove Plate Files dialog box. The software displays the plate data files (*.spd) currently in the project.

2. Select the plate data file to remove, then click **Remove Files**. The software:
   - Removes the selected plate data files from the Add/Remove Plate Files dialog box.
   - Removes the run data for the selected plate data files from the project file.

   Note: When you remove a plate data file from a project, the genotyping calls for the samples in that plate data file are lost. In addition, the genotyping calls for the remaining samples in the project change.



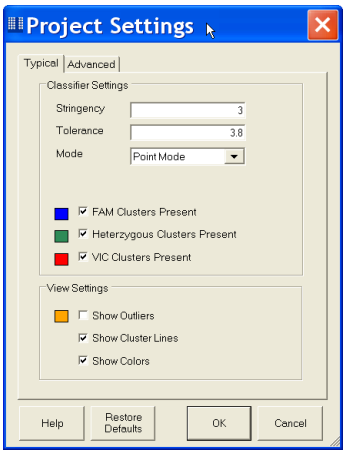
3. Click **Done**. The software automatically calls the genotypes for the revised group of plate data files, using your current settings.

Set project parameters

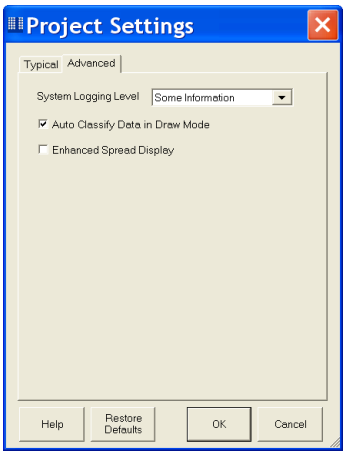
IMPORTANT! When you set project parameters, the settings are applied to the current project file and any *future* project files.

1. Select **Edit ▸ Project Settings** to open the Project Settings dialog box.

2. Select the **Typical** tab, then edit the parameters as needed:

Typical Tab	Parameter	Action
	Stringency	Enter a positive number (for example, **2.0**) or enter **Infinity** to represent a number of standard deviations. After you save the parameters, the software assigns a *No Call* status to any data points that are further from the cluster line than the entered value.
	Tolerance	Enter a positive number (for example, **2.5**) to indicate how close a data point in one cluster may be to an adjacent cluster line. After you save the parameters, the software assigns a *No Call* status to any data points that are within the specified standard deviations of two cluster lines.
	Mode	From the dropdown menu, select the mode (Point or Draw) you most frequently work within as your default. After you save the parameters, the corresponding tab appears in front.
	Clusters Present	Deselect the genotypes you do not have. For example, if you know your samples do not include any FAM dye homozygotes, deselect **FAM**.
	View Settings	Select the items (outliers, cluster lines, colors) to display in the Scatter Plot.

3. Select the **Advanced** tab, then edit the parameters as needed:

Advanced Tab	Parameter	Action
	System Logging Level	The system default is *Some Information*. Only adjust this value when asked by an Applied Biosystems service representative.
	Auto Classify Data in Draw Mode	Deselect this mode if you do not want the software to automatically call genotypes on the Draw tab.
	Enhanced Spread Display	If checked, the software attempts to remove noise from data in the project.

4. Click **OK** to save the parameters. The software applies all parameters to all assays.

(Optional) Publish data

Publish data for use in reports, spreadsheets, and so on. You can:

- Copy and paste Scatter Plots (this page)
- Export genotype tables (this page)
- Export *.csv files (page 113)

Copy and paste Scatter Plots

You can copy and paste the Scatter Plots into other software applications, such as Microsoft® PowerPoint® Software.

1. (Optional) In the OpenArray software, zoom in on an area of the Scatter Plot.
2. Click in the plot area, then select **Edit ▸ Copy**.
3. Paste the Scatter Plot into the appropriate software application.

Export genotype tables

You can export genotype information from your project in a table format. The table includes the following information:

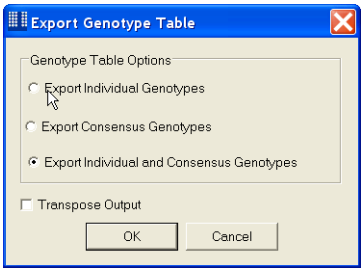
- OpenArray plate serial number
- Sample ID
- Sample description
- Genotype calls

1. Select the appropriate tab to export from (the **Point** or **Draw** tab).

   Note: 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.

2. Select **File ▸ Export Genotype Table** to open the Export Genotype Table dialog box.

3. Select the data to export:
   - **Export Individual Genotypes**
   - **Export Consensus Genotypes**
   - **Export Individual and Consensus Genotypes**.



4. Select the row and column contents:
   - If you want each row to contain sample information and each column to contain assay information, deselect **Transpose Output**.
   - If you want each row to contain assay information and each column to contain sample information, select **Transpose Output**.

5. Click **OK** to open a save dialog box.

6. Browse to a save location, name the file, then click **Save**. A *.csv file is saved to the specified location.

7. To view the exported table, open it in Microsoft® Excel® Software or another spreadsheet application.

	A	B	C	D	E	F
1	OpenArray.SerialNumber	Sample.SampleID	Sample.Description	C__29086771_20.Genotype	C__29086771_20.Consensus Genotype	C__3168989_10.Genotype
2	BIA74			V V	V V	V V
3	BIA74			V V	V V	V V
4	BIA74			V V	V V	V F
5	BIA74			V V	V V	V V
6	BIA74			V V	V V	V F
7	BIA74			V V	V V	V V
8	BIA74			V V	V V	V F
9	BIA74			V V	V V	V F
10	BIA74			V F	V F	V F
11	BIA74			V V	V V	V F
12	BIA74			V F	V F	V V
13	BIA74			No Call	No Call	No Call
14	BIA74			V F	V F	No Call
15	BIA74			V V	V V	No Call
16	BIA74			V V	V V	V V
17	BIA74			No Call	No Call	V F
18	BIA74			V V	V V	V F
19	BIA74			V V	V V	V V

Export *.csv files

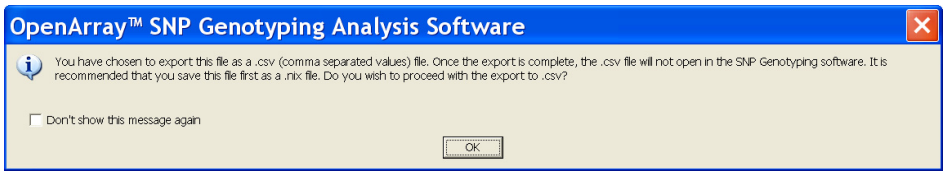
You can export data from your project as a comma-delimited file (*.csv). The *.csv file includes (but is not limited to) the following data:

- Assay information from the plate setup file (*.spf)
- Sample information
- Genotype calls and associated parameters
- Fluorescence intensity data

1. Select the appropriate tab to export from (the **Point** or **Draw** tab).

   Note: 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.

2. Select **File** ▸ **Export CSV**. The following message appears:



   Note: Exported *.csv files cannot be reopened in the OpenArray software. Applied Biosystems recommends that you save the project file (*.nix) before exporting the *.csv file.

3. Click **OK** to close the message.

4. In the Export CSV dialog box, browse to a save location, name the file, then click **Save**. A *.csv file is saved to the specified location.

5. To view the exported *.csv file, open it in Microsoft® Excel® Software or another spreadsheet application.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	VersionInf	VersionInf	VersionInf	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray	OpenArray
2	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
3	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
4	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
5	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
6	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
7	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
8	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
9	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
10	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
11	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
12	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
13	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
14	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
15	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
16	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
17	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
18	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE
19	OpenArray	1.0.181.3	1	BIA74	4	12	8	8	4.5	4.5	0.5	0.5	0.3	TRUE

(Optional) Perform downstream analysis

You can perform downstream analysis with Applied Biosystems TaqMan® Genotyper Software. The TaqMan Genotyper Software is a SNP genotyping analysis tool and client-server program that you can use to efficiently analyze, edit, and compare genotyping assays run on the OpenArray® system.

Features

The TaqMan Genotyper Software allows you to:

- Import data from OpenArray software project files, then manage the data in a database.
- Search the database for assays using specific search criteria.
- Easily view data in a variety ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data.

Export to the TaqMan® Genotyper Software

1. In the OpenArray software, select **File ▸ Export to TaqMan® Genotyper Software** to open a save dialog box.
2. Browse to a save location, name the file, then click **Save**. An *.xml file is saved to the specified location.
3. To import the file into the TaqMan Genotyper Software, refer to the *TaqMan® Genotyper Software Getting Started Guide*.

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PART III
Instrument Maintenance

7 Maintenance

This appendix covers:

- Contact information for preventive maintenance 119
- Required materials 120
- OpenArray® system computer 121
- OpenArray® AutoLoader and accessories 123
- OpenArray® instrument 124

Contact information for preventive maintenance

Contact an Applied Biosystems service representative with questions regarding preventive maintenance of the OpenArray® platform.

You may be asked for your software version, instrument firmware version, and/or instrument serial number. To access this information:

1. Be sure that you are on a computer that is connected to the OpenArray® instrument.
2. In the OpenArray® software, select **Help ▸ About**.

IMPORTANT! Only an Applied Biosystems service representative should clean or service components not covered in this appendix.

Required materials

Product	Source	Part Number
For the OpenArray® system computer		
OpenArray® software installation CD	Applied Biosystems The CD ships with the OpenArray® platform.	20441
Backup storage (for example, an external hard drive)	User-supplied	---
For the OpenArray® AutoLoader and accessories		
Clean, dry cloth	Major laboratory suppliers (MLS)	---
Ethanol†	MLS	---
Bleach, 10%†	MLS	---
(Optional) Filtered 100% compressed nitrogen gas or residue-free compressed air canister, for drying the plate holder, tip blocks, and plate guides	MLS	---
(Optional) Hand-held spray attachment for the compressed gas/air canister	MLS	---
For the OpenArray® instrument		
Powder-free nitrile gloves	MLS	---
M4 hex wrench	MLS	---
12-inch Contec non-laser edge polyknit cloths	VWR	PNHS1212
Ethanol†	MLS	---
Clean, dry cloth	MLS	---

† For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

OpenArray® system computer

Install the software

Follow this procedure for the:

- OpenArray® Real-Time qPCR Analysis Software
- OpenArray® SNP Genotyping Analysis Software

An Applied Biosystems service representative installs the OpenArray software on the system computer. You can also install the OpenArray software on other computers not connected to the instrument (for example, your office computer).

Use the software installation CD that is shipped with the OpenArray® platform. Software installation takes approximately 5 minutes.

1. Insert the software installation CD in your CD drive. A message appears stating that files are being extracted.

   Note: If Microsoft®.Net Runtime v1.1 is not installed, the installation program prompts you to install it. Select **Yes**.

2. Verify that the Installation Wizard appears, but do *not* click **Next** yet.

3. From the **Start** menu, select **My Computer**. If folders are not listed in the left pane, select **Folders** in the My Computer toolbar to make them visible.

4. In the Installation Wizard, click **Next**.

5. Enter your name and organization. If there are multiple user accounts on this computer, select whether to install the software for all users or just yourself, then click **Next**.

6. Select the destination folder for the plate setup files (*.spf or *.tpf), then click **Next**.

7. At the "Do you wish to configure this software to control your <*instrument*>" prompt, select **No**, then click **Next** to install the software.

8. When a message appears stating the software is successfully installed, click **Finish**.

Open the software for the first time

1. Start the OpenArray software:
   - Double-click the software icon on the desktop.

     or
   - Select the software from the Start menu.

2. Click **I Accept** to accept the License Agreement.

3. If you have spyware removal software installed on this computer, you may receive messages regarding changes in the registry. Enable registry updates for the software.

Clean the hard drive

The computer that ships with the OpenArray system contains a 450-GB hard drive. Applied Biosystems recommends that you remove data files from the hard drive as needed. As shown in the table below, the size of the data files varies, depending on the OpenArray software and the file type.

Before removing the data files:

1. Close the OpenArray software.
2. (Recommended) Back up the **images** folder to a secure location (for example, to an external hard drive or to your company network).

Software	Folder	Location	Description	Size
OpenArray® Real-Time qPCR Analysis Software	images	<*drive*>:\images†	Contains run data for TaqMan® OpenArray® Real-Time PCR Plates	1.5 GB
			Contains run data for SYBR® OpenArray Real-Time PCR Plates	2.5 GB
OpenArray® SNP Genotyping Analysis Software	images	<*drive*>:\images†	Contains run data for TaqMan® OpenArray Genotyping Plates	121 MB

† <*drive*> is the computer drive on which the OpenArray software is installed. The default installation drive is the C: drive.

OpenArray® AutoLoader and accessories

Calibrate the AutoLoader

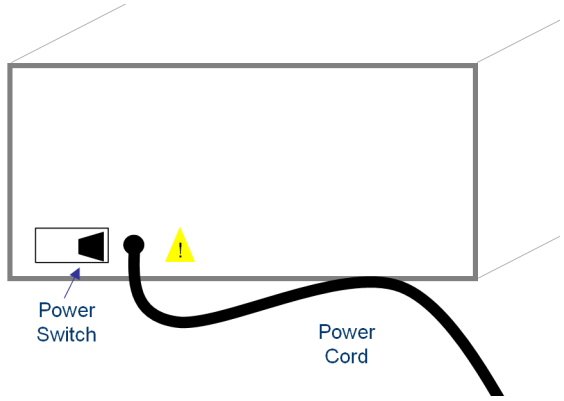
The AutoLoader automatically calibrates each time it is powered on and each time it is stopped. To calibrate the AutoLoader at another time, on the Welcome screen, press the button under *HOME*.

Clean the AutoLoader

Clean the outside of the AutoLoader by wiping with a clean, dry cloth. Do not use solvents.

Do not clean the inside of the AutoLoader. If liquids or other materials spill inside the AutoLoader:

1. Press the power switch on the back of the AutoLoader turn it off, then unplug the power cord from the electrical outlet.



2. Call your Applied Biosystems service representative.

Clean the accessories

After each use, clean the following AutoLoader accessories:

- OpenArray® Plate Guide Set
- OpenArray® AutoLoader Tip Block
- OpenArray® AutoLoader Plate Holder

To clean the AutoLoader accessories:

1. Soak the plate guide, tip block, and/or plate holder in 10% bleach for at least 10 minutes.
2. Rinse with water, then rinse with ethanol.
3. Let the parts completely air dry. If you need them immediately, wipe with paper towels and spray with compressed nitrogen gas.

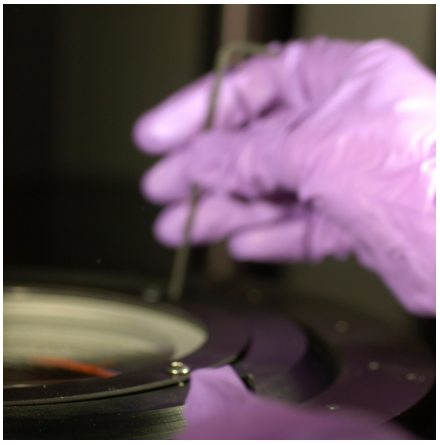
OpenArray® instrument

Clean the lens

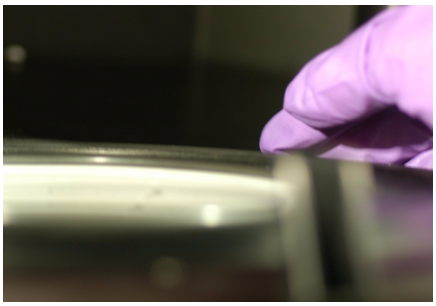
IMPORTANT! The lens is a vital part of the OpenArray instrument and it is easily scratched. Always handle the lens gently and never drop it. If the lens is damaged and needs to be replaced, you will not be able to operate the instrument until Applied Biosystems can ship you a new lens.

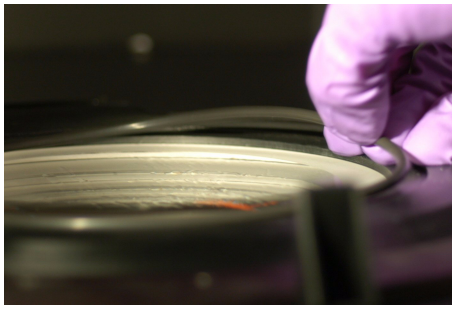
If condensation or dirt builds up on the lens:

1. Put on powder-free nitrile gloves. *Do not use latex gloves*.
2. With an M4 hex wrench, unscrew all six screws on the lid by turning counter-clockwise.

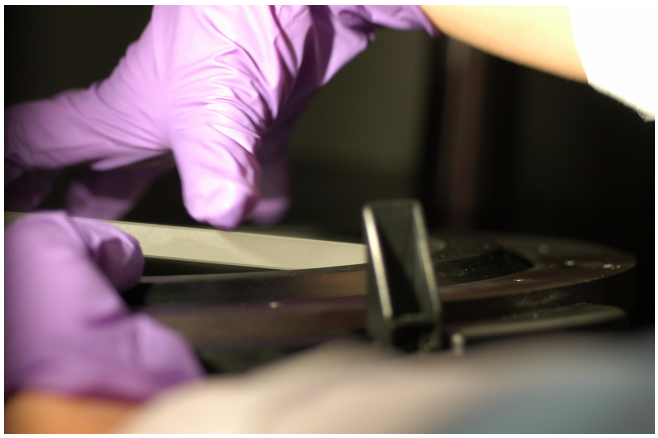


3. Remove the metal ring and the O-ring.

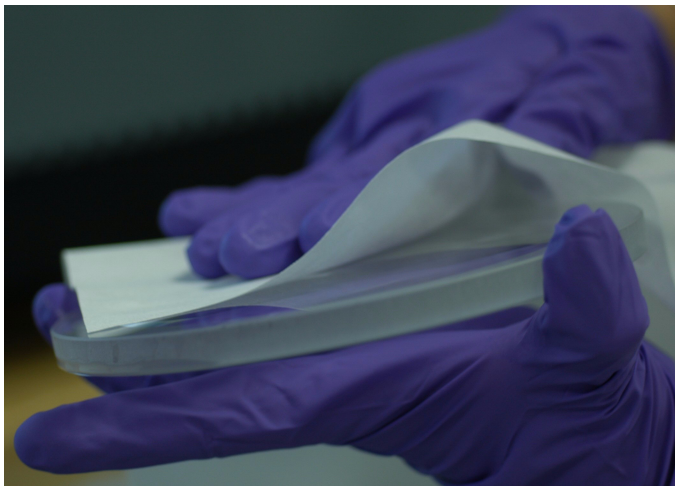




4. Place your hand underneath the lens and carefully pop it out of position. Remove the lens, touching only the outside edge.

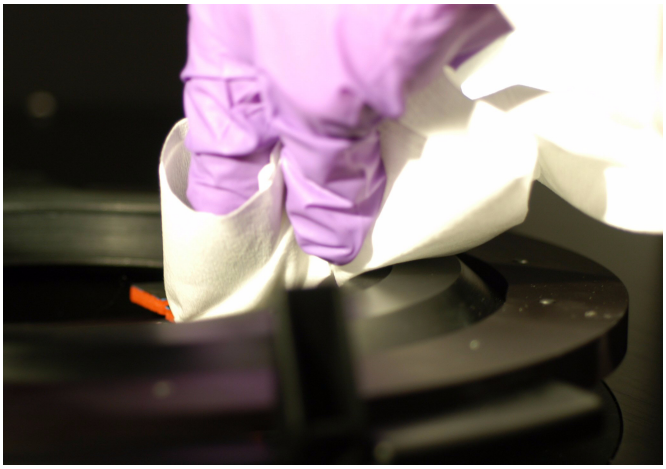


5. Spray a polyknit cloth with ethanol, then wipe the lens until there are no streaks on the lens. Keep the cloth flat to avoid scratching the lens coating; do not fold the cloth.



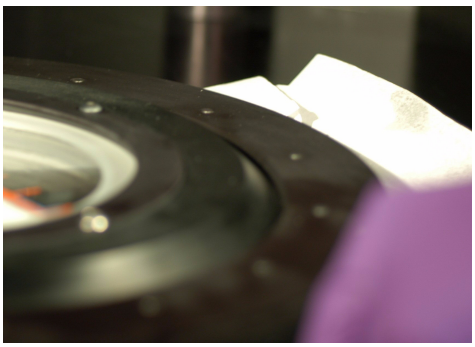
6. Clean the lid:
    a. Spray a polyknit cloth with ethanol.

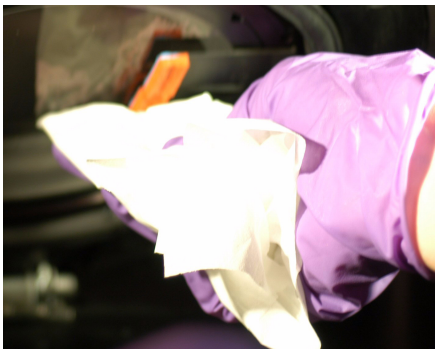
b. Clean the lip of the lid.



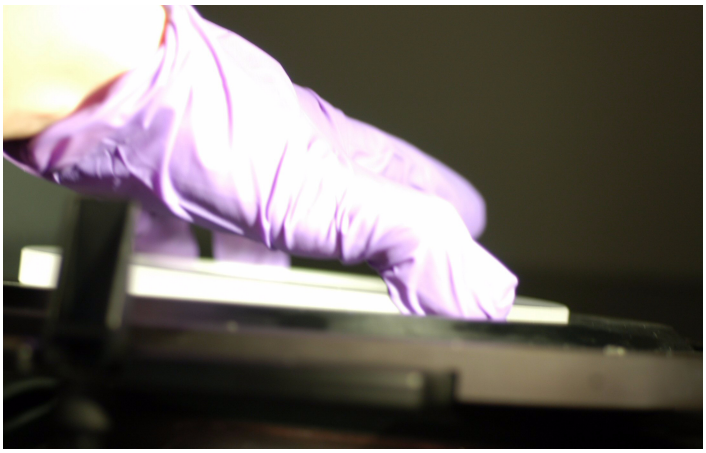
c. Spread the polyknit cloth on the block and close the lid for 10 seconds.

d. Clean the fingers on the top of the lid individually.





7. Be sure that the lens is frosted side up (it should be in a concave position, like a bowl), then place the lens back into the instrument.



8. Place the O-ring on the lip, then place the metal ring on top of the O-ring.

9. With the M4 hex wrench, partially screw in each screw on the lid. After all screws are flush but not tight, screw them in all the way. To reduce pressure on the lens, tighten screws on two opposite sides, then on the other two opposite sides.

Clean the sample block

Clean the instrument sample block by wiping it with a clean, dry cloth. Do not use solvents.

Clean the exterior

Clean the outside of the instrument by wiping it with a clean, dry cloth. Do not use solvents.

Clean the interior after a case failure

This procedure is available only in the OpenArray® Real-Time qPCR Analysis Software.

If fluid leaks from an OpenArray Case, follow this procedure to clean the OpenArray instrument before performing any more runs.

1. Wait for the OpenArray instrument to cool down.
2. Clean the lens according to the procedure on page 124.
3. Remove the OpenArray cases/plates from the instrument, but keep the frames. Discard the cases/plates in an appropriate waste container.
4. In the OpenArray Real-Time qPCR Analysis Software, select **Actions** ▸ **Clean**, then follow the prompts.

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PART IV
Appendices

A Adding or Deleting Sample Information Columns

This appendix covers:

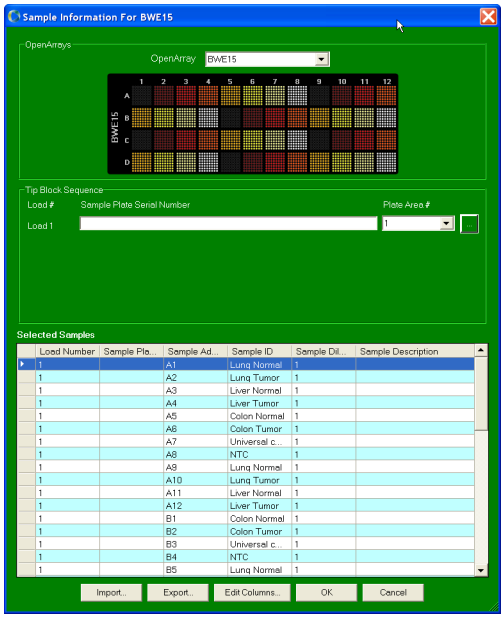
- OpenArray® Real-Time qPCR Analysis Software 133
  - Add columns to the Sample Data pane 133
  - Delete user-created columns from the Sample Data pane 135
- OpenArray® SNP Genotyping Analysis Software 137
  - Add columns to the Samples pane 137
  - Delete user-created columns from the Samples pane 139

OpenArray® Real-Time qPCR Analysis Software

Add columns to the Sample Data pane

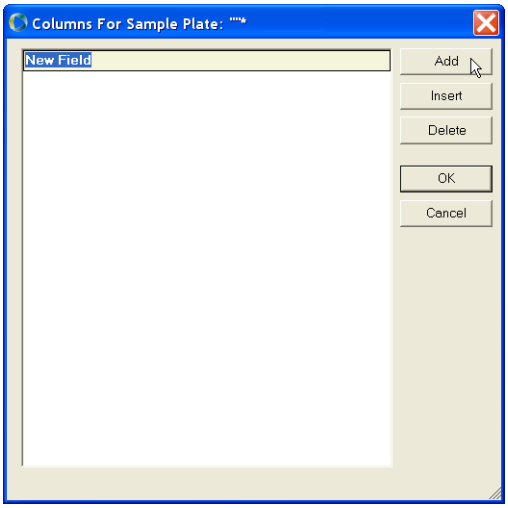
1. Open the Sample Information dialog box:
   - If you are adding columns before a run – Click **Cycle** to open the Input Plate Serial Numbers dialog box, then click **Edit** at the appropriate position.
   - If you are adding columns after a run – In the Settings pane, click **Edit Sample Info**.

2. In the Sample Information dialog box, click **Edit Columns**.



3. Add new columns:
   a. In the Columns For Sample Plate dialog box, click **Add**.
   b. Select **New Field**, then enter the new column name.

      Do not use commas or periods in column names; text is case-sensitive. After real-time imaging, *SampleInfo.Properties.* is prefixed to all new column names in the Sample Data pane to differentiate them from the default columns. If you assign a default column name to a new column, the software automatically renames it. For a description of the default columns in the Sample Data pane, see page 36.
   c. Click **OK**.



4. Click **OK** to close the Sample Information dialog box.
5. Repeat this procedure to add new columns for the remaining OpenArray plates.

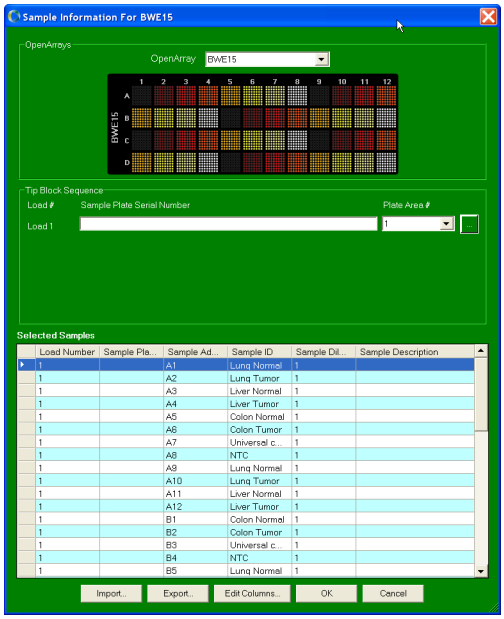
IMPORTANT! If you are adding the columns before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform thermal cycling and real-time imaging" on page 31. If you close the dialog box, the information you have entered will be lost.

Delete user-created columns from the Sample Data pane

Note: You cannot delete any columns created by the software (default columns). For a description of the default columns in the Sample Data pane, see page 36.

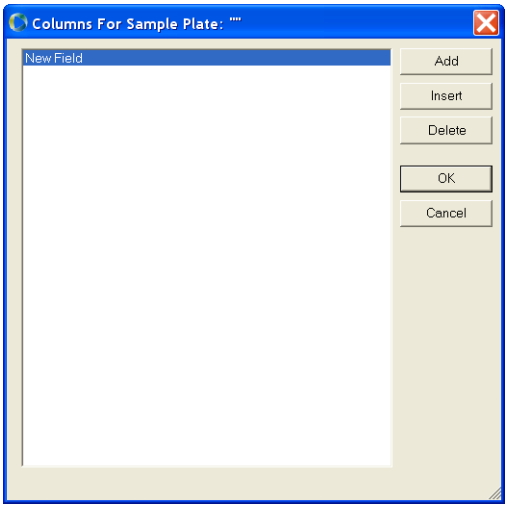
1. Open the Sample Information dialog box:
   - If you are deleting columns before a run – Click **Cycle** to open the Input Plate Serial Numbers dialog box, then click **Edit** at the appropriate position.
   - If you are deleting columns after a run – In the Settings pane, click **Edit Sample Info**.

2. In the Sample Information dialog box, click **Edit Columns**.



3. Delete user-created columns:
   a. In the Columns For Sample Plate dialog box, select the column to delete, then click **Delete**.

b. Click **OK**.



4. Click **OK** to close the Sample Information dialog box.

5. Repeat this procedure to delete user-created columns for the remaining OpenArray plates.

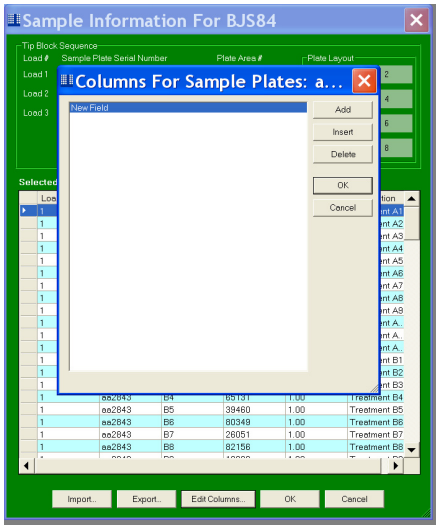
IMPORTANT! If you are deleting the columns before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform thermal cycling and real-time imaging" on page 31. If you close the dialog box, the information you have entered will be lost.

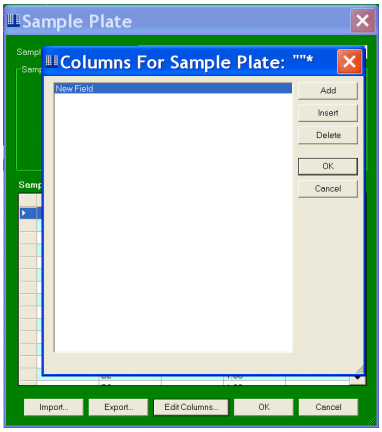
OpenArray® SNP Genotyping Analysis Software

Add columns to the Samples pane

1. Open the Sample Information dialog box:
   - If you are adding columns before a run – Click **Image** to open the Input Plate Serial Numbers dialog box, then click **Edit** at the appropriate position.
   - If you are adding columns after a run – Below the Samples pane, click **Edit**.
2. Add new columns according to the appropriate procedure below.

   Do not use commas or periods in column names; text is case-sensitive. After imaging, *SampleInfo.Properties.* is prefixed to all new column names in the Samples pane to differentiate them from the default columns. If you assign a default column name to a new column, the software automatically renames it. For a description of the default columns in the Samples pane, see page 94.

If you want to...	Then...
Add new columns for all loads at one time (1 to 3 loads)	1. In the Sample Information dialog box, click **Edit Columns**. 2. In the Columns For Sample Plates dialog box, click **Add**, select **New Field**, enter the new column name, then click **OK**. 

If you want to...	Then...
Add new columns for each load separately	For *each* load: 1. In the Sample Information dialog box, click the … icon next to the load number of interest.  2. In the Sample Plate dialog box, click **Edit Columns**. 3. In the Columns For Sample Plate dialog box, click **Add**, select **New Field**, enter the new column name, then click **OK**.  4. Click **OK** to close the Sample Plate dialog box.

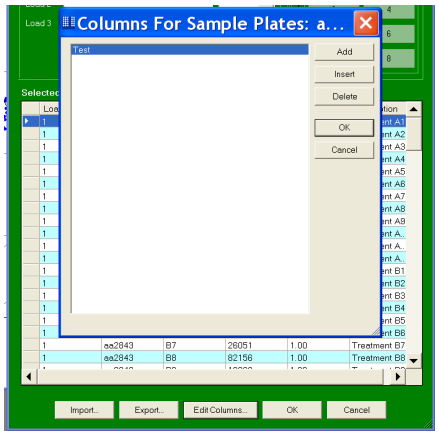
3. Click **OK** to close the Sample Information dialog box.
4. Repeat this procedure to add new columns for the remaining OpenArray plates.

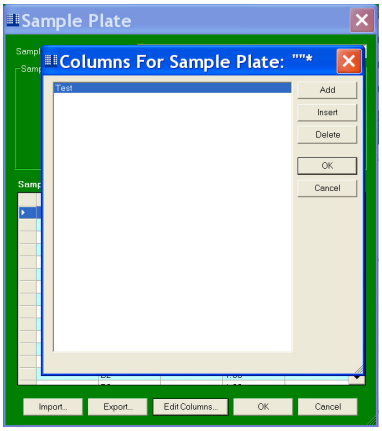
IMPORTANT! If you are adding the columns before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform imaging" on page 87. If you close the dialog box, the information you have entered will be lost.

Delete user-created columns from the Samples pane

Note: You cannot delete any columns created by the software (default columns). For a description of the default columns, see page 94.

1. Open the Sample Information dialog box:
   - If you are deleting columns before a run – Click **Image** to open the Input Plate Serial Numbers dialog box, then click **Edit** at the appropriate position.
   - If you are deleting columns after a run – Below the Samples pane, click **Edit**.
2. Delete user-created columns according to the appropriate procedure below.

If you want to...	Then...
Delete columns for all loads at one time (1 to 3 loads)	1. In the Sample Information dialog box, click **Edit Columns**. 2. In the Columns For Sample Plate dialog box, select the column name, click **Delete**, then click **OK**. 

If you want to...	Then...
Delete columns for each load separately	For *each* load: 1. In the Sample Information dialog box, click the … icon next to the load number of interest.  2. In the Sample Plate dialog box, click **Edit Columns**. 3. In the Columns For Sample Plate dialog box, select the column name, click **Delete**, then click **OK**.  4. Click **OK** to close the Sample Plate dialog box.

3. Click **OK** to close the Sample Information dialog box.
4. Repeat this procedure to delete user-created columns for the remaining OpenArray plates.

IMPORTANT! If you are deleting the columns before a run, leave the Input Plate Serial Numbers dialog box open, then proceed to "Perform imaging" on page 87. If you close the dialog box, the information you have entered will be lost.

B Instrument Warranty Information

Computer configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited product warranty

Limited warranty

Applied Biosystems warrants that all standard components of its OpenArray® system will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its OpenArray® system, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

THE FOREGOING PROVISIONS SET FORTH APPLIED BIOSYSTEMS' SOLE AND EXCLUSIVE REPRESENTATIONS, WARRANTIES, AND OBLIGATIONS WITH RESPECT TO ITS PRODUCTS, AND APPLIED BIOSYSTEMS MAKES NO OTHER WARRANTY OF ANY KIND WHATSOEVER, EXPRESSED OR IMPLIED, INCLUDING WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF DEALING OR USAGE OF TRADE, ALL OF WHICH ARE EXPRESSLY DISCLAIMED.

Warranty limitations

THE REMEDIES PROVIDED HEREIN ARE THE BUYER'S SOLE AND EXCLUSIVE REMEDIES. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE (INCLUDING WITHOUT LIMITATION, ANY TRADE PRACTICE, UNFAIR

COMPETITION, OR OTHER STATUTE OF SIMILAR IMPORT) OR ON ANY OTHER BASIS, FOR DIRECT, INDIRECT, PUNITIVE, INCIDENTAL, MULTIPLE, CONSEQUENTIAL, OR SPECIAL DAMAGES SUSTAINED BY THE BUYER OR ANY OTHER PERSON OR ENTITY, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING WITHOUT LIMITATION, DAMAGES ARISING FROM OR RELATED TO LOSS OF USE, LOSS OF DATA, FAILURE OR INTERRUPTION IN THE OPERATION OF ANY EQUIPMENT OR SOFTWARE, DELAY IN REPAIR OR REPLACEMENT, OR FOR LOSS OF REVENUE OR PROFITS, LOSS OF GOOD WILL, LOSS OF BUSINESS, OR OTHER FINANCIAL LOSS OR PERSONAL INJURY OR PROPERTY DAMAGE.

NO AGENT, EMPLOYEE, OR REPRESENTATIVE OF Applied Biosystems HAS ANY AUTHORITY TO MODIFY THE TERMS OF THIS LIMITED WARRANTY STATEMENT OR TO BIND APPLIED BIOSYSTEMS TO ANY AFFIRMATION, REPRESENTATION, OR WARRANTY CONCERNING THE PRODUCT THAT IS NOT CONTAINED IN THIS LIMITED WARRANTY STATEMENT, AND ANY SUCH MODIFICATION, AFFIRMATION, REPRESENTATION, OR WARRANTY MADE BY ANY AGENT, EMPLOYEE, OR REPRESENTATIVE OF APPLIED BIOSYSTEMS WILL NOT BE BINDING ON APPLIED BIOSYSTEMS, UNLESS IN A WRITING SIGNED BY AN EXECUTIVE OFFICER OF APPLIED BIOSYSTEMS.

THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM APPLIED BIOSYSTEMS AND IS NOT TRANSFERABLE.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, claims, and returns

Damages

If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns

Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

C Safety

This appendix covers:

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  - Symbols on instruments 146
  - Locations of safety labels on instruments 147
  - General instrument safety 148
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Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see "Safety labels on instruments" on page 11). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

Symbol	Description
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of a radiological hazard and to proceed with appropriate caution.
	Indicates the presence of a slipping hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.

Environmental symbols on instruments

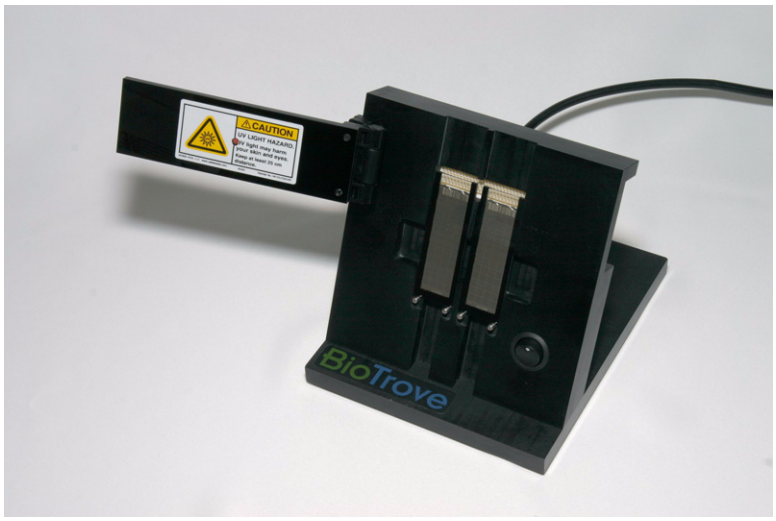
The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Locations of safety labels on instruments

The OpenArray® platform includes the following warning on the OpenArray® Case Sealing Station:

Hazard symbol	English	Français
	**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.



General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

WARNING! PHYSICAL INJURY HAZARD. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

CAUTION! For safety information related to the centrifuge and thermal cycler, refer to the manufacturer's documentation.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

CAUTION! Do not tip the OpenArray® instrument on end. Tipping damages the instrument hardware and electronics and is an unsafe practice.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See "About SDSs" on page 153.

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Compressed gases

WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Solvents and pressurized fluids

WARNING! PHYSICAL INJURY HAZARD. Always wear eye protection when working with solvents or any pressurized fluids.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the OpenArray® instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

WARNING! ELECTRICAL HAZARD. Plug the OpenArray® platform components into properly grounded receptacles with adequate current capacity.

Overvoltage rating

The OpenArray® platform has an installation (overvoltage) category of II, and is classified as portable equipment.

Barcode scanner laser safety

Laser classification

The barcode scanner included with the OpenArray® platform is categorized as a Class 2 (II) laser.

Laser safety requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

WARNING! LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australian EMC Standards

U.S. and Canadian safety standards

The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument have been tested to and comply with the standards:

UL 61010-1:2004, 2nd Edition/CSA-C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use: Part 1: General Requirements."

Canadian EMC standard

The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument have been tested to and comply with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."

European safety and EMC standards

Safety

The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument meet European requirements for safety (Low Voltage Directive 2005/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

The OpenArray® instrument has been tested to and complies with the standard:

EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide.

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class A), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

The OpenArray® AutoLoader, OpenArray® Case Sealing Station, and OpenArray® instrument have been tested to and comply with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 153.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: **www.cdc.gov/biosafety/publications/index.htm**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Part Number 4458837 Rev. A 08/2010

Documentation and Support

Product documentation

Portable document format (PDF) versions of the documents listed in this section are available at **www.appliedbiosystems.com**

Note: To open the PDF versions, use the Adobe Acrobat Reader software available from **www.adobe.com**

OpenArray® Real-Time PCR System

Document	Description	Part number
OpenArray® Real-Time PCR System Troubleshooting Guide	Provides troubleshooting information for the OpenArray® Real-Time PCR System.	4458839
OpenArray® Real-Time PCR System User Guide	Provides procedures for imaging and analyzing OpenArray® plates. Provides maintenance information for the OpenArray® Real-Time PCR System.	4458837
OpenArray® Real-Time PCR System Quick Reference Card: Workflow	Describes the overall workflow and provides brief procedures for performing gene expression experiments with the OpenArray® Real-Time PCR System.	4458838
OpenArray® Real-Time PCR System Quick Reference Card: Using the Sample Tracking Tool	Describes how to use the Sample Tracking Tool to create sample information files (*.csv) and import the files into the OpenArray® Real-Time qPCR Analysis Software.	4460957
SYBR® OpenArray® Real-Time PCR Plates Protocol	Provides procedures for preparing the SYBR® OpenArray® Real-Time PCR Plates.	4458869
TaqMan® OpenArray® Real-Time PCR Plates Protocol	Provides procedures for preparing the TaqMan® OpenArray® Real-Time PCR Plates.	4458840

TaqMan® OpenArray® Genotyping System

Document	Description	Part number
TaqMan® OpenArray® Genotyping Getting Started Guide	Provides procedures for performing genotyping experiments with the TaqMan® OpenArray® Genotyping System.	4377476
TaqMan® OpenArray® Genotyping Plates Ordering Guide	Provides ordering information for the TaqMan® OpenArray® Genotyping Plates.	4400403
TaqMan® OpenArray® Genotyping System Quick Reference Card: Using the Sample Tracking Tool	Describes how to use the Sample Tracking Tool to create sample information files (*.csv) and import the files into the OpenArray® SNP Genotyping Analysis Software.	4460657
TaqMan® OpenArray® Genotyping System Quick Reference Card: Workflow	Describes the overall workflow and provides brief procedures for performing genotyping experiments on the TaqMan® OpenArray® Genotyping System.	4400402
TaqMan® OpenArray® Genotyping System Site Preparation Guide	Provides information on preparing the customer site for the TaqMan® OpenArray® Genotyping System.	4401171
TaqMan® OpenArray® Genotyping Troubleshooting Guide	Provides troubleshooting information for the TaqMan® OpenArray® Genotyping System. To be used in conjunction with the *TaqMan® OpenArray® Genotyping Getting Started Guide*.	4401671

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, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Glossary

C_T	Threshold cycle. In gene expression experiments, the cycle number at which the fluorescence is detectable above background fluorescence. The OpenArray® Real-Time qPCR Analysis Software calculates the initial concentration of target DNA in your sample from the C_T value.
ΔC_T	Delta C_T. The C_T value for any reaction normalized to the reference gene (endogenous control, housekeeping gene, and so on).
$\Delta\Delta C_T$	Delta Delta C_T. The method is used to analyze changes in gene expression. This comparative C_T method involves comparing C_T values of the sample-assay reaction with a control reaction (such as non-treated sample or RNA from normal tissue). C_T values of both reactions are normalized to an appropriate reference gene. For the C_T to be valid, the amplification efficiencies of both reactions must be approximately equal. The equation for C_T calculation is: C_T = Ct, sample-C_T, reference.
R_2	The square of the Pearson product-moment correlation coefficient. R_2 is used as a measure of association between standard curve data and its best fit line.
Tm	Melting temperature. The temperature at which 50% of a DNA fragment denatures (melts). Because DNA fragments have different melting temperatures, you can verify the presence of the specific DNA amplicon and determine if any primer-dimers or other non-specific amplification products are present by analyzing a melt curve. The OpenArray instrument generates a melt curve by slowly increasing temperature above the melting point while measuring fluorescence.
assay	A PCR reaction mix that contains primers to amplify a target and a reagent to detect the amplified target. See also SYBR® Green reagents and TaqMan® reagents.
cluster center	On a Scatter Plot, the user-defined or automatically calculated cluster midpoint of data points for each genotype. Each cluster center appears as a circled X.
cluster lines	On a Scatter Plot, lines that bisect each genotype cluster drawn from the clustering axis to the cluster center.
curve	In the OpenArray® Real-Time qPCR Analysis Software, a line in the Curve pane that represents a reaction. See also reaction.
data point	In the OpenArray® SNP Genotyping Analysis Software, a black dot in the Scatter Plot pane that represents a reaction. See also reaction.
Don't Call	A sample designation in the OpenArray® SNP Genotyping Analysis Software, as set by the user. No genotype is called for the sample, and the sample is excluded from the analysis. *Don't Call* data points are colored cyan in the Scatter Plot pane.

duplicate	An assay is performed "in duplicate" when two through-holes are filled with the same assay/sample combination and a genotype call is made.
efficiency	The percent of the theoretical maximum that the reaction produces copies of the sample. The efficiency is calculated by the equation: $E = 10^{(-1/slope)} - 1$ The efficiency should be between 90 and 110%, meaning the sample was doubled each cycle. An efficiency value between 90 and 110% corresponds to a slope of -3.1 to -3.6 in the C_T vs. log starting DNA amount standard curve. Efficiency should be similar for all assays that you are comparing. Factors that affect efficiency include: • Length of the amplicon • Presence of inhibitors • Secondary structure • Primer design
Entr.	Abbreviation for ENTER used in the OpenArray® AutoLoader display.
frame	Curved metal supports that you affix to the OpenArray cases to ensure that there is good contact between the cases and the thermal block on the OpenArray instrument.
home or homing	An OpenArray® AutoLoader operation that calibrates robotic movement.
intercept	The threshold cycle at which the line intersects with a 0 Log_{10} (concentration). See also CT.
load position	The OpenArray® AutoLoader configuration when you begin loading samples into a TaqMan® OpenArray® Real-Time PCR Plate.
No Call	A sample designation in the OpenArray® SNP Genotyping Analysis Software, as set by the software. No genotype is called for the sample, and the sample is excluded from the analysis. *No Call* data points are colored black in the Scatter Plot pane.
normalization	The use of a reference gene to normalize the results for variable sample amounts and/or variable sample quality. A reference gene is expressed at a constant level across different samples.
OpenArray® 384-Well Sample Plate	A 384-well microtiter plate that you use with the OpenArray® AutoLoader to transfer samples to an OpenArray plate. Also referred to as the *sample plate*.
OpenArray® AutoLoader	Part of the OpenArray® platform. The AutoLoader transfers samples from the OpenArray® 384-Well Sample Plate to an OpenArray plate.
OpenArray® Case Sealing Station	Part of the OpenArray® platform. The sealing station uses UV light to cure the OpenArray® Sealing Glue and seal each OpenArray plate inside an OpenArray case.

OpenArray® instrument	Part of the OpenArray® platform. Refers to one of the following instruments: • **OpenArray® Real-Time PCR System instrument** – Includes a thermal cycling (heat) block. Performs simultaneous thermal cycling and real-time imaging of TaqMan® OpenArray® Real-Time PCR Plates and SYBR® OpenArray® Real-Time PCR Plates. • **TaqMan® OpenArray® Genotyping System instrument** – Does not include a thermal cycling (heat) block. Performs imaging of TaqMan® OpenArray® Genotyping Plates.
OpenArray® plate	Refers to any of the following plates: • SYBR® OpenArray® Real-Time PCR Plate • TaqMan® OpenArray® Real-Time PCR Plate • TaqMan® OpenArray® Genotyping Plate Each OpenArray plate is a 63-mm × 19-mm mid-density reaction plate that consists of individual through-holes that are preloaded with an assay.
OpenArray® software	Refers to either of the OpenArray software applications: • OpenArray® Real-Time qPCR Analysis Software • OpenArray® SNP Genotyping Analysis Software
OpenArray® platform	Refers to all of the instrument components of the system, including: • OpenArray® AutoLoader • OpenArray® Case Sealing Station • OpenArray® instrument • Computer, running the OpenArray® software
Outlier	User designation that a reaction be excluded from the analysis results.
plate guide	When loading sample with the OpenArray® AutoLoader, the part that you place over the sample plates to ensure the correct samples are loaded. Two plate guides are included in the OpenArray® Plate Guide Set.
plate holder	Accurately positions an OpenArray plate for sample loading in the OpenArray® AutoLoader.
project file	A file that you view and modify in the OpenArray software. The file extension is *.ncx or *.nix, depending on the OpenArray software application.
reaction	A specific sample-assay combination. Each through-hole in a prepared OpenArray plate contains a single reaction.
replicate	Experiments performed with the OpenArray® system, in which the same sample/assay combination is performed in multiple through-holes.
sample	The gene/target of interest. The DNA can be from any source (for example, tissue, whole organism, cDNA library).

serial number	The unique alphanumeric identification number that is assigned to each OpenArray plate. This serial number, located opposite the barcode on the OpenArray plate, corresponds to information about the plate in the plate setup file (*.tpf or *.spf).
slope	Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of –3.32 indicates 100% amplification efficiency. See also efficiency.
stringency	In the OpenArray® SNP Genotyping Analysis Software Point tab, the number of standard deviations from cluster lines to the data points that are included in genotype calls. Data points greater than this number of standard deviations are automatically assigned *No Call* status.
subarray	A section in the OpenArray plate that consists of 64 through-holes in an 8 × 8 square configuration. Each OpenArray plate is divided into 48 subarrays.
SYBR® Green reagents	PCR reaction components that consist of two primers designed to amplify the target and SYBR® Green I dye to detect double-stranded DNA.
TaqMan® reagents	PCR reaction components that consist of primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target
target	The nucleic acid sequence to amplify and detect.
threshold	In gene expression experiments, the level of fluorescence above the baseline and within the exponential growth region. See also CT.
through-hole	Bottomless reaction wells in the OpenArray plate. There are 3,072 through-holes in each OpenArray plate; each through-hole contains a single assay. The OpenArray AutoLoader ensures a consistent 33-nL reaction volume in each through-hole. Proprietary plate coatings hold reagents within the through-holes.
tip block	The OpenArray® AutoLoader Tip Block. The tip block holds 48 loader tips for sample loading with the OpenArray® AutoLoader.
tolerance	In the OpenArray® SNP Genotyping Analysis Software Point tab, data points that are too close to more than one cluster line to be accurately genotyped. These data points are automatically assigned *No Call* status. Tolerance is the indicator of excessive closeness, measured in standard deviations.

Part Number 4458837 Rev. A 09/2010

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