Aria Real-Time PCR Software

Always make sure you are using the most recent version of the Aria software. Check the Aria software download website at www.agilent.com/en/ariamx-software-download.

User Manual

Revision J1, September 2023

AriaMx: For Research Use Only. Not for use in diagnostic procedures.

AriaDx: For In Vitro Diagnostic Use

Agilent Technologies

1
Getting Started with the Aria Software

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The Aria software

The Aria software features a variety of experiment type options with customized plate and thermal profile setup, and experiment analysis screens that streamline the process of collecting and analyzing data for specific applications. The software capabilities allow you to:

- Quickly set up an experiment using a template or the Import Plate Setup function
- View raw fluorescence data without mathematical correction or calibration factors
- Quantitate the initial template quantity of a DNA target based on a standard curve
- Generate and display normalized relative quantity values on a log(2) fold change chart to assess the effects of an experimental treatment on gene expression levels
- Export any data set directly to Microsoft Excel or to a text file
- View and analyze the data from several experiments together in a single project using the multiple experiment analysis functions
- Use high resolution melt analysis for SNP genotyping in an Allele Discrimination experiment

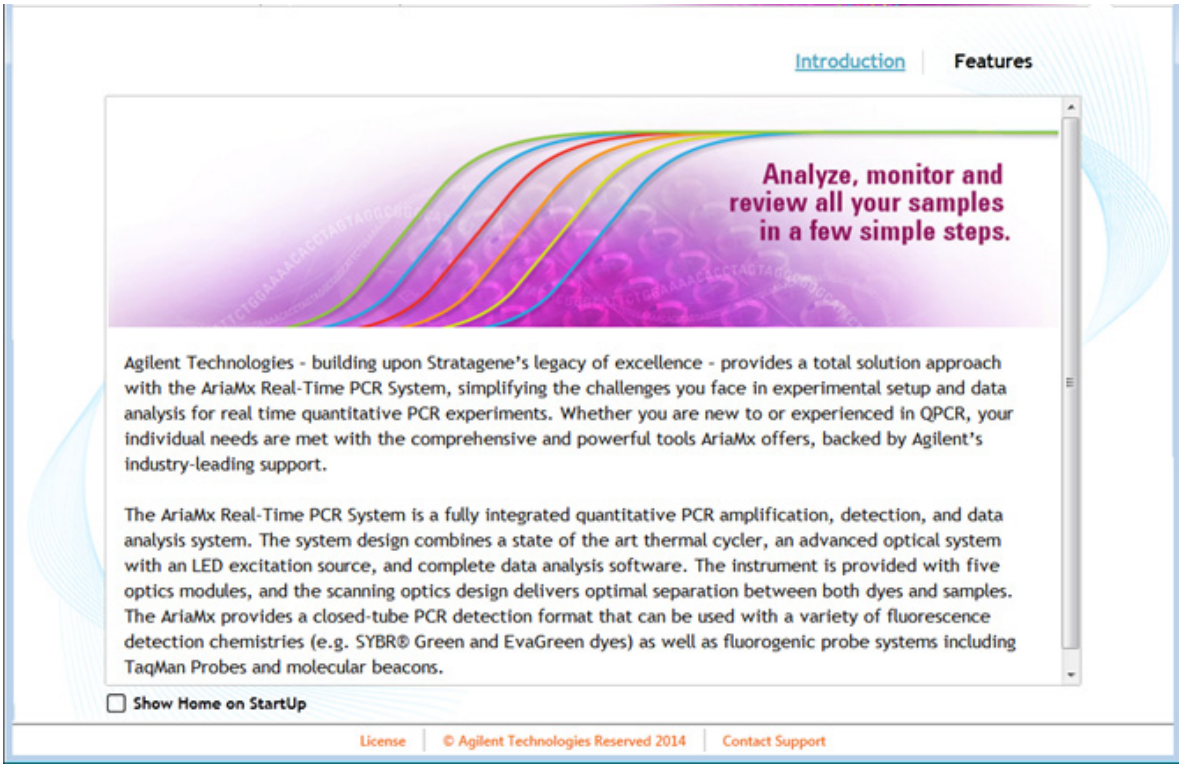
Getting Started with the Aria Software

Introduction to the Aria software

The software's Home screen provides an introduction to the program and a list of program features.

To open the Home screen: Click the Home icon near the upper right corner of the program window.

NOTE If you want the program to open to the Home screen, mark the check box near the bottom of the screen labeled Show Home on StartUp.



Introduction

To view the Introduction, click Introduction (near the top right corner of the Home screen). The text on this screen provides an overview of the AriaMx/AriaDx Real-Time PCR System.

Features

To view the Features, click Features (near the top right corner of the Home screen). The text on this screen lists the features of the Aria software.

Minimum PC requirements

Table 1 lists the minimum PC requirements needed to run the Aria software.

Table 1 Minimum requirements for running the Aria software

Operating system	Windows 10 – with regional format set to English (United States)
Supported architectures	×64 (64 bit)
Programs*	Microsoft .NET Framework 4.0 Microsoft SQL Server 2019 (required for ET software only) Runtime components of Microsoft Visual C++ 2010 Libraries
Processor	2 GHz Dual Core Processor
Working memory (RAM)	2 GB (more is recommended)
Hard disk space	40 GB
Display resolution	1024 × 768 (1280 × 1024 is recommended)

* Installers for Microsoft .NET Framework 4.0 and Microsoft SQL Server 2019 are provided on the Aria Software Download page of the Agilent website. If you do not have the needed Microsoft Visual C++ 2010 components, then the Aria installer will automatically install them to your PC when you initiate installation of the Aria software.

Overview of the user interface

Home screen

The Home screen provides an introduction to the AriaMx/AriaDx system and a list of program features. See "Introduction to the Aria software" on page 16 for detailed information on the Home screen.

Menu toolbar

At the top of the program window are the File and Instrument menus.

- The File menu contains commands for opening, closing, saving, and printing experiments and projects.
- The Instrument menu contains commands for connecting to, configuring, exporting data from, and testing the AriaMx/AriaDx instrument.

Tabs

The user interface of the Aria software allows you to open up to 5 experiments at a time (when experiment files are < 1.5 MB), or one project at a time. The program displays each open experiment or project on its own tab, enabling you to quickly switch between them. Click the icon to the right of the tabs to open a new tab. New tabs open to the Getting Started screen.

Left and right panels

When you are working in an experiment or project, the left side of the screen displays the Experiment Area panel, and the right side of the screen displays a panel with tools for the currently open screen (e.g., the Report Configuration panel is displayed on the Generate Report screen). You can hide these panels by clicking the arrow icon next to the panel name. Click the arrow icon again to expand the panel.

Home/Notifications/Help icons

The top right corner of the program window has 3 icons for quickly accessing the Home screen, viewing any notifications from the program, and opening the Aria help system.

- Click this icon to open the Home screen. Click the icon again to close the Home screen and return to previous screen.

- Click this icon to open a text box displaying any notifications from the program. When you have unread notifications waiting, the icon is dark blue.

- Click this icon to open the program's help system to the topic that pertains to the currently displayed screen.

Getting Started screen

When you open a new tab in the program (from the File menu or the icon), it opens to the Getting Started screen. From this screen you can create a new experiment (from scratch or from a template), create a new multiple experiment analysis project, or open an existing experiment or project. See "Overview of the Getting Started screen" on page 43 for more information.

Experiment Notes / Project Notes

The Experiment Notes icon or Project Notes icons appears in the lower left corner of the screen whenever an experiment or project is open. Clicking the icon opens the **Experiment Notes** or **Project Notes** text box. Use this text box to type your own notes pertaining to the particular experiment or project. Click **Save** to save your notes for later reference.

Help access for the Aria software

The Aria software contains a help system that provides instructions on setting up, running, and analyzing experiments and creating multiple experiment projects. You can also view video tutorials on the Agilent website that describe how to perform some of the most common tasks in the program.

Help system

From any screen or dialog box, click the help icon (?) in the upper right corner of the screen to open the help system. The help system automatically opens to the most relevant topic based on where you are in the program.

You can navigate the help system window from the Contents tab or the Search tab.

- On the Contents tab, use the table of contents on the left side of the window to browse the chapters and topics within the help system.
- On the Search tab, type a search word into the field and click GO to find the help topics that contain the word. If you type multiple words into the field, the program will list help topics that contain all of the words. Use quotes to search for a complete phrase (e.g., "comparative *quantitation*"). You can also use the Boolean operators AND and OR to find topics that contain more than one search word (e.g., *comparative AND quantitation*), or topics that contain any one of multiple search words (e.g., comparative OR quantitation).

The help system contains the following chapters:

- Getting Started with the Aria Software- Contains help topics to familiarize new users with the program and provide instructions for getting started
- How-To Help - Provides step-by-step instructions on how to use the program
- Reference Help - Contains trademark designations and a glossary of QPCR terms
- Troubleshooting and Support - Contains troubleshooting suggestions and a directory for contacting a technical support person in your region

Sample experiments

The Aria software comes with several sample experiment files that include post-run data. The sample experiments are saved to the folder **C:\Users\Public\Public Documents\Agilent Aria\Sample Experiments** during installation of the software. The folder includes a sample experiment for each experiment type and subtype for both the AriaMx (*.*amxd*) and AriaDx (*.*adxd*) software modes. You open the sample experiments in the Aria software to help familiarize yourself with the experimental setup and graphical displays available for each experiment type.

2
Specifying Hardware and Software Settings

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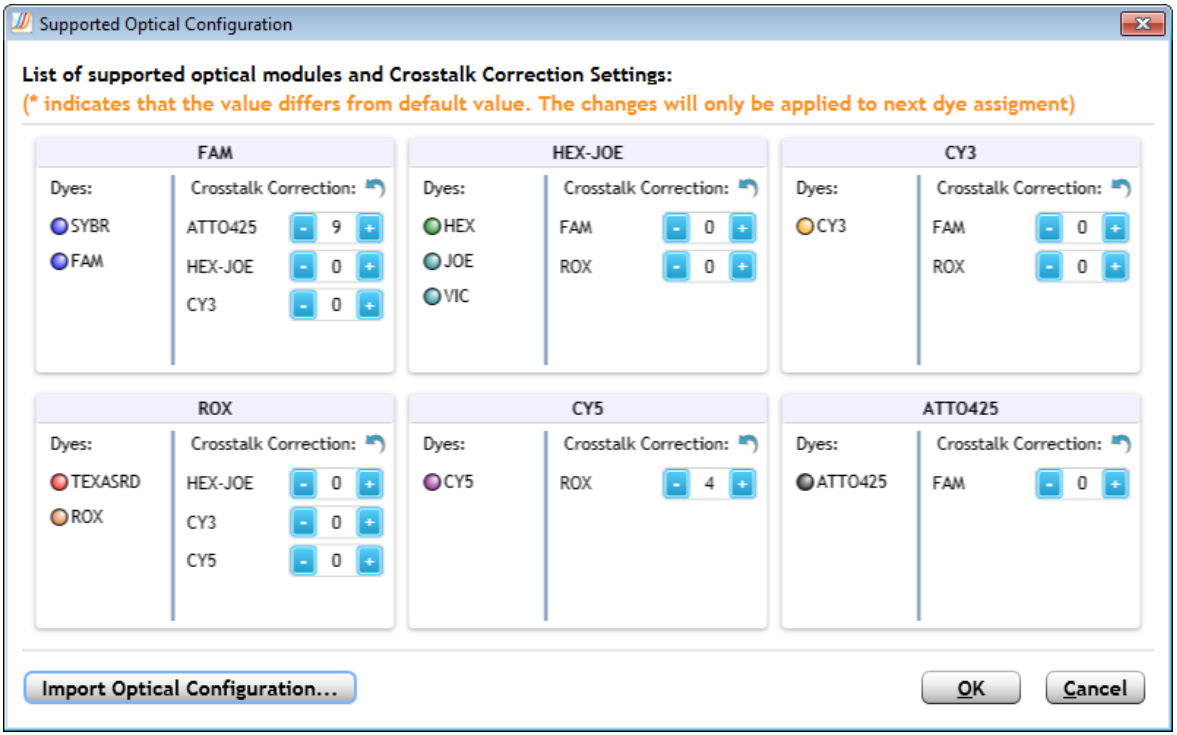
Update instrument optics

Multiple optical modules are available for use with the AriaMx/AriaDx instrument for detection of different dye spectra. In the Supported Optical Configuration dialog box, you can view the optical modules, their currently supported dyes, and the crosstalk correction settings for non-target dyes that could be detected by each module.

Periodically, Agilent may add new supported dyes and optical modules to the AriaMx/AriaDx Real-Time PCR System, and make a new optics configuration file available to you. You can use the Supported Optical Configuration dialog box to load that new file.

To open the Supported Optical Configuration dialog box: At the top of the program window, click **Instrument > Optical Configuration**.



To update the optical configuration file:

1 Open the Supported Optical Configuration dialog box.

2 Click **Import Optical Configuration**.

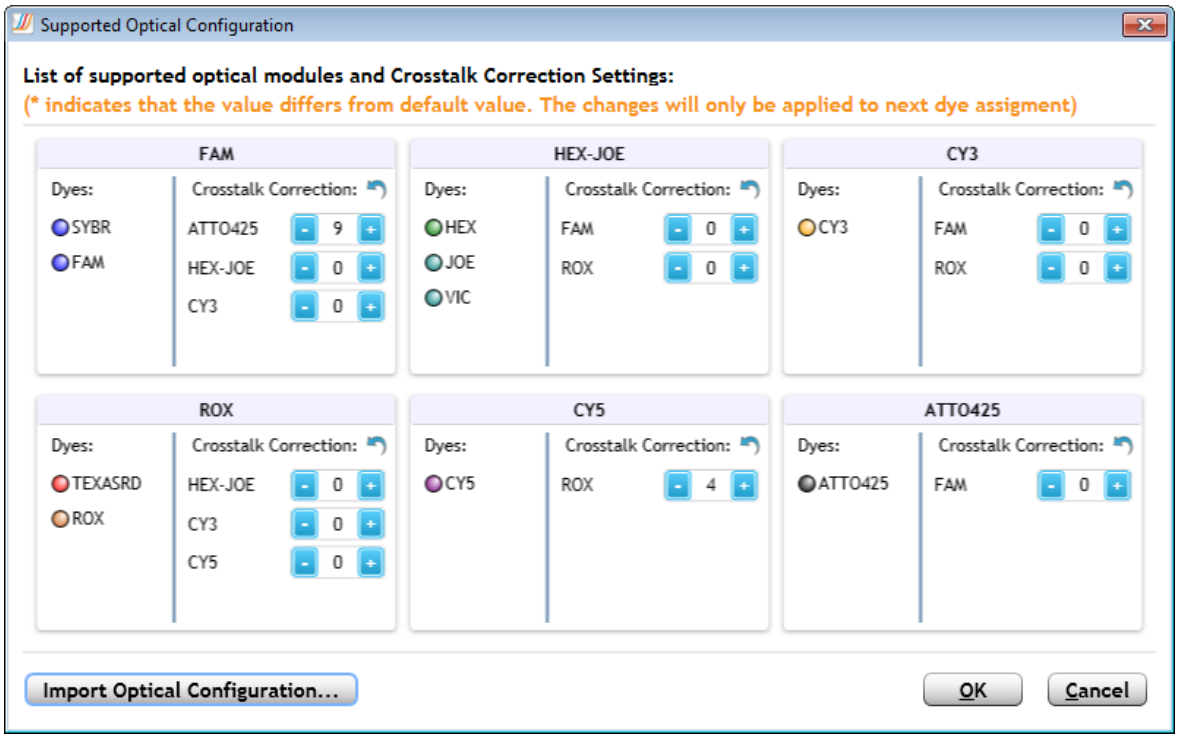
The Open dialog box opens.

3 Browse to the folder of the configuration file that you received from Agilent. Select the file and click **Open**. The Supported Optical Configuration dialog box is updated with the new configuration.

Change the default crosstalk correction settings

Multiple optical modules are available for use with the AriaMx/AriaDx instrument for detection of different dye spectra. In the Supported Optical Configuration dialog box, you can view the optical modules and their currently supported dyes, and change the crosstalk correction settings for non-target dyes that could be detected by each module.

To open the Supported Optical Configuration dialog box: At the top of the program window, click **Instrument > Optical Configuration**.



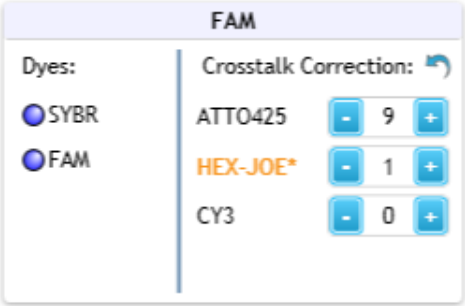
Crosstalk occurs when emission from one dye is detected by two different optical modules (the target optical module and the spillover optical module). A dye is at risk for crosstalk when its emission spectra overlaps that of another dye that is assigned to a different optical module. The Aria software includes crosstalk correction settings, which can help compensate for crosstalk.

The factory settings for crosstalk correction are the default settings, unless the default settings are changed in the Supported Optical Configuration dialog box. Once changed, the new default crosstalk correction settings are applied to all experiments going forward. Alternatively, you can adjust the crosstalk correction settings for an individual post-run experiment using the tools in the Amplification Plots graph. See "Adjust the crosstalk correction settings" on page 201 for instructions.

NOTE The factory settings for crosstalk correction have been optimized to eliminate potential crosstalk for dyes that are part of the default optical configuration. Agilent does not recommend changing the crosstalk correction settings unless you are using a new custom dye and the emission wavelength of that dye could be detected by more than one optical module in the instrument.

To change the default crosstalk correction settings:

1 In the Supported Optical Configuration dialog box, locate the box for the optical module that is or could be reporting crosstalk fluorescence (i.e., the spillover optical module). The dyes that have the potential to crosstalk with that optical module are listed in that box (e.g., the HEX-JOE dye in the FAM optical module).



2 Change the crosstalk correction setting for the dye by adjusting the value in the field.

The values in these fields are percentages of the total raw fluorescence. They will be subtracted from the raw fluorescence signal for the optical module when that dye is used as a target.

Crosstalk correction values that differ from the factory settings are noted with an asterisk (*).

3 Click **OK** to apply your changes and close the dialog box.

To reset the crosstalk correction settings back to the factory settings:

1 In the Supported Optical Configuration dialog box, locate the box for the optical module that you want to reset.

   Crosstalk correction values that differ from the factory settings are noted with an asterisk (*).

2 Within that box, click the reset icon next to **Crosstalk Correction**.

   The crosstalk correction values for all dyes listed in the box are reset to the factory values.

3 Click **OK** to apply your changes and close the dialog box.

NOTE The Supported Optical Configuration dialog box does not include crosstalk correction settings between the HEX-JOE and Cy3 optical modules because the degree of crosstalk is too significant to be adequately corrected. Agilent does not recommend using HEX-JOE and Cy3 together in a multiplex reaction.

Set software preferences

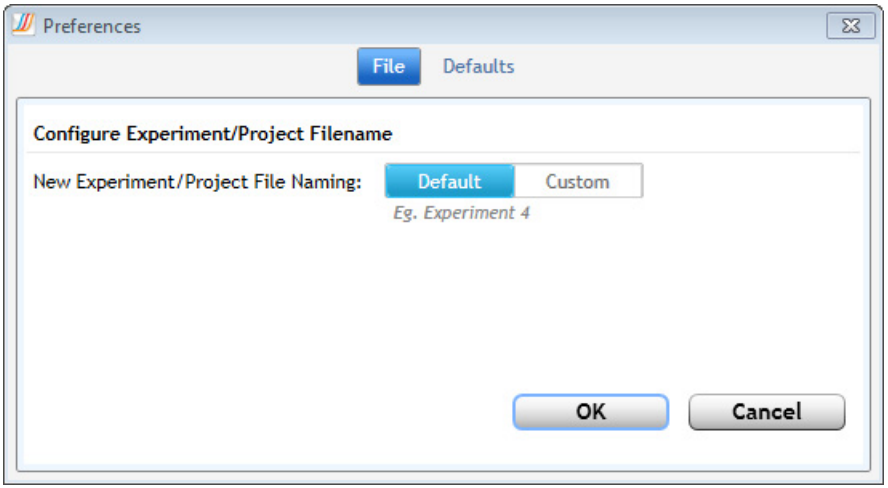
The Preferences dialog box is used for setting your preference on the default file name configuration and for creating and selecting analysis templates.

To open the Preferences dialog box: At the top of the program window, click **File > Preferences**.

Set default file name configuration

To configure the default file name for experiments and projects:

1 In the Preferences dialog box, make sure **File** is selected at the top.



2 Next to **New Experiment/Project File Naming**, click one of the following options:

- **Default** - Select this option to use the program's default file naming system. For experiments, the default file name is “Experiment” followed by the next available number (e.g., “Experiment 3"). For projects, the default file name is “Project” followed by the next available number (e.g., “Project 3").

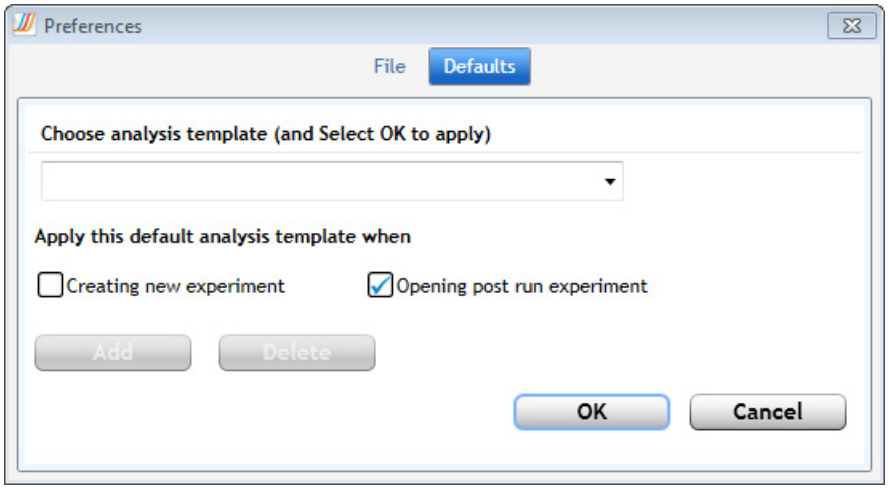
- **Custom** - Select this option to configure your own file naming system by selecting which pieces of information to include in the default file name. Using the check boxes, you can select to include the experiment type, the creation date for the experiment or project (in format MM-DD-YYYY, DD-MM-YYYY, or YYYY-MM-DD), and the time that the experiment or project was created.

3 Click **OK** to close the dialog box and save your changes.

Create and apply analysis templates

To create an analysis template that sets the default analysis settings for experiments:

1 In the Preferences dialog box, select **Defaults** at the top.



2 Type a name for the new template into the field below **Choose analysis template (and Select OK to apply)**.

3 Select when to apply the analysis template using the check boxes. You can mark one check box, both check boxes, or neither check box.

- Mark **Creating new experiment** if you want to apply the analysis template to all newly created experiments going forward.
- Mark **Opening post run experiment** if you want to apply the analysis template anytime a post run experiment is opened in the program.
- Clear both check boxes if you do not want the analysis template to be automatically applied to experiments.

4 Click **Add**.

A message box opens asking you to confirm that you want to create the new template. Click **OK** to continue.

5 Click **OK** to close the Preferences dialog box.

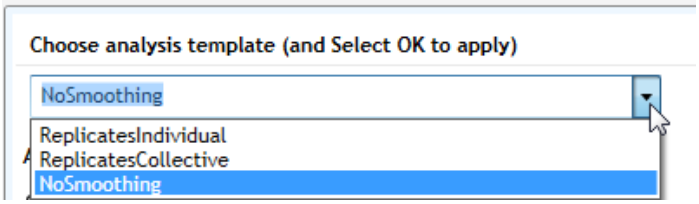
The program will apply the template to your experiments according to the check box selections made in step 3.

Newly created analysis templates have only default analysis settings. See "Configure and apply analysis templates" on page 192 for instructions on configuring the analysis settings in the analysis template.

To select an existing analysis template to be applied to experiments:

1 In the Preferences dialog box, select **Defaults** at the top.

2 In the field below **Choose analysis template (and Select OK to apply)**, click the arrowhead to expand the drop-down list. The list contains all of the existing analysis templates.



3 Select a template from the list.

4 Select when to apply the analysis template using the check boxes. You can mark one check box, both check boxes, or neither check box.

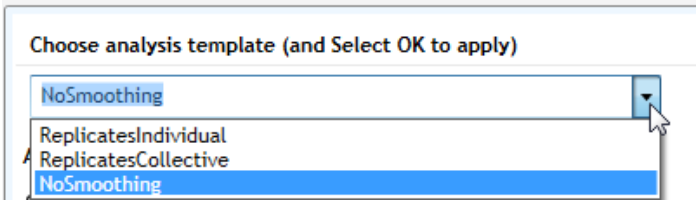
- Mark **Creating new experiment** if you want to apply the analysis template to all newly created experiments going forward.
- Mark **Opening post run experiment** if you want to apply the analysis template anytime a post run experiment is opened in the program.
- Clear both check boxes if you do not want the analysis template to be automatically applied to experiments.

5 Click **OK** to close the Preferences dialog box. The program will apply the template to your experiments according to the check box selections made in step 3. See "Configure and apply analysis templates" on page 192 for instructions on configuring the analysis settings in the analysis template.

To delete an existing analysis template:

1 In the Preferences dialog box, select **Defaults** at the top.

2 In the field below **Choose analysis template (and Select OK to apply)**, click the arrowhead to expand the drop-down list. The list contains all of the existing analysis templates.



3 Select a template from the list.

4 Click **Delete**.

5 In the message box that opens, click **OK** to confirm that you want to delete the selected template.

6 If desired, select a different template from the list, or create a new template.

7 When finished making changes, click **OK** to close the Preferences dialog box.

3

Performing Hardware and Software Tests and HRM Calibrations

Agilent Technologies

Run an instrument qualification test

Running a qualification test is one way to test for instrument errors. You perform the test using a qualification test plate that must be purchased separately from Agilent (part number 5190-7708). The wells of the test plate are pre-filled with the QPCR reagent mixture needed to run the test.

Run the test

Running a qualification test requires preparing the plate, running the experiment on the AriaMx/AriaDx instrument, and checking the results.

To run a qualification test:

1. Prepare the qualification test plate according to the instructions that came with the plate.
2. At the top of the program window, click **Instrument > Qualification Test**.

   A new tab opens for the Qualification Test experiment. The experiment opens to the Thermal Profile screen.
3. Click **Run**.

   The Instrument Explorer dialog box opens.
4. Locate the instrument that you will be using for the run and click **Send Config**.
   - If the instrument is not listed, see "Add instruments to your network" on page 162 for instructions on searching for and adding instruments.
   - If this is the first time you have connected to an instrument since last launching the Aria software, the Login dialog box opens. Select your Username from the drop-down list, type your login password into the Password field, and click **Login**. To log in with a different user account, right-click on the instrument name and click **Log off current user**. You can then log in using the desired user account

     A message box opens notifying you that you must save the experiment before you can connect to the instrument.
5. Click **Save** in the message box.

   The Save As dialog box opens.

6 Select a folder for the experiment and type a name into the File name field (or use the default). Click **Save**.

7 Take the prepared qualification test plate over to the instrument and load it into the thermal block.

8 On the instrument touchscreen, open the primed experiment to the Thermal Profile screen and press **Run Experiment**.

The instrument starts running the qualification test experiment.

9 Return to the PC program. The program directs you to the Raw Data Plots screen, where you can monitor the progress of the run. See "Monitor a run" on page 168.

10 At the end of the run, save the qualification test experiment to your PC (see "Retrieve run data from the instrument" on page 173 for instructions). Open the experiment in the Aria software on your PC.

11 Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. (See "About the Qualification Test graphical data" on page 34 for more information about the graphs on this screen.)

12 Check the panel on the right side of the screen to determine if the qualification test passed.

- If it passed, the panel displays **Results Pass**.
- If it failed, the panel displays **Results Check**.

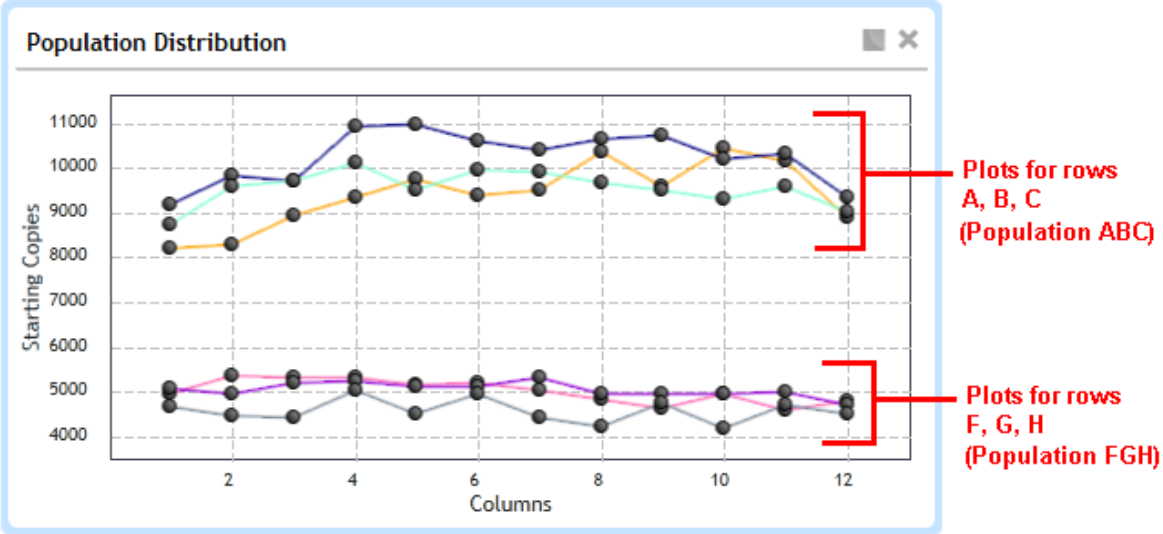
If your qualification test failed, contact Agilent Technical Support for help with troubleshooting. See "Contact Agilent Technical Support".

About the Qualification Test graphical data

For a qualification test, the Graphical Displays screen includes graphs for the Amplification Plots, the Standard Curve, and the Population Distribution.

The Population Distribution graph is unique to Qualification Test experiments. For each row of Unknown wells on the plate, this graph plots the number of initial template copies calculated for that row against the plate's column number. Because all wells in rows A, B, and C are replicates and all wells in rows F, G, and H are replicates, the distribution of the plot lines indicate the variability in initial template calculations across the thermal block within the two populations (the ABC population and the FGH population). In order to pass the qualification test, the

program requires that both populations be at least 98.5% distinct after outlier wells are culled. The percent distinct for the Population Distribution graph is displayed in the panel on the right side of the Graphical Displays screen (next to **% Distinct**). Note that outlier wells are plotted on the Population Distribution graph with a red x.



If your qualification test failed, Agilent's Technical Support staff may use the graphical data to help troubleshoot the cause of the failure.

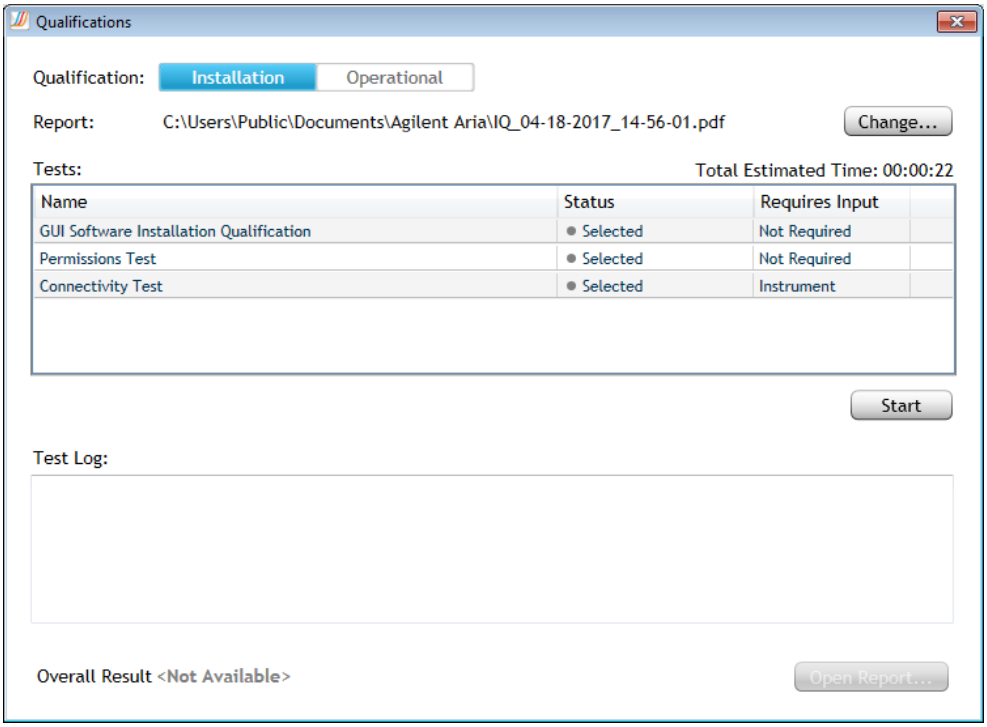
Perform an Installation Qualification test

You can run an Installation Qualification (IQ) test to determine if the Aria software is properly installed on your system. The IQ test is comprised of three separate tests:

- **GUI Software Installation Qualification**: Verifies the integrity of the files and folders that are created as part of Aria software installation
- **Permissions test**: Verifies that the current user has full access to the software's default file path for experiment files
- **Connectivity test**: Tests the communication between software and instrument via FTP or TCP protocol service (if the firmware version is determined to be outdated, a message box opens prompting you to upgrade the firmware)

To perform the IQ test:

1 Click **File > Qualifications**. The Qualifications dialog box opens.



2 Next to **Report** is the file path and file name of the report that the program generates at the end of the test. To select a different folder or file name for the report, click **Change**. Then, in the Save As dialog box, select a different folder and/or file name.

3 Click **Start** to start the test.

The program displays the status of the test in the Test Log area of the dialog box. When finished, the program updates the Status column in the dialog box and displays the overall result (Passed or Failed). If the test failed, contact Agilent Technical Support for assistance.

4 Click **Open Report** to open the PDF report generated by the program.

5 To run the test again, click **Reset** to clear the results of the previous test.

NOTE The Operational option on the Qualifications dialog box has tools to run an Operational Qualification (OQ) test. However, the Operational option is only available to Agilent Service Engineers with an appropriate password.

Run an HRM calibration plate (HCP)

For experiments that include a high resolution melt (HRM) segment, before you can analyze the HRM data on a Difference Plots graph, you must associate the experiment with an HRM calibration plate (HCP) that was run on the same instrument. The purpose of the HCP is to calculate an offset temperature for each well in order to help normalize well-to-well temperature variations. See "About HRM calibration plates" on page 67 for more information.

NOTE A separate HRM software license is required in order to associate an experiment with an HCP. To purchase a software license, contact your Agilent Sales representative. The HRM license option is only available for the AriaMx system. The license is not supported for the AriaDx system.

Prepare the plate

Use the Agilent HRM Calibration Plate

Agilent offers a HRM Calibration Plate that you can use for HRM calibration (Agilent part number 5190-7701). The plate comes pre-aliquoted with a master mix containing EvaGreen dye. See the plate's user manual for more information.

Visit www.agilent.com/genomics for ordering information on the HRM Calibration Plate.

Prepare the plate with your own reagents

Alternatively, you can prepare your own reagent mixture to be used in the HCP run.

The 1x mixture needs to contain a purified amplicon at a concentration of 0.1-0.3 mM. Ideally, the amplicon has a melting temperature (Tm) close to that of the amplicon that you will be analyzing in your Allele Discrimination experiment. The 1x mixture also needs to contain the same DNA binding dye, at the same concentration, that you use in the QPCR reactions for the Allele Discrimination experiment.

Instead of using a purified amplicon in your reagent mixture, you can use a template, primers, and polymerase to produce the amplicon during the HCP run. If you choose this approach, you need to add an amplification segment to the default HCP thermal profile.

Note that the Aria calibration algorithms were tested and optimized using the Agilent HRM Calibration Plate. Using your own HCP reagent mixture may impact results.

Run an HCP

All HCP experiments must be set up and run directly from the instrument (you cannot set up an HCP experiment from the Aria program on your PC).

To run an HCP experiment:

1. On the instrument Home screen, press the HRM Calibration icon.
2. On the subsequent screen, press **Open Default Experiment**.

   The default HCP experiment opens to the Plate Setup screen. All wells are set to the Unknown well type and the SYBR dye is selected for target detection in all wells

   The EvaGreen dye used in the Agilent HCP kit is detectable with the FAM/SYBR optic module.
3. Navigate to the Thermal Profile screen and press **Run Experiment**.

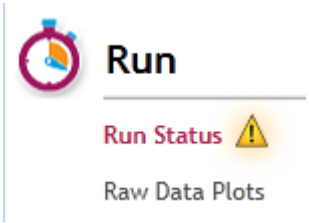
   A message box opens on the touchscreen prompting you to save the experiment. Press **OK** in the message box to save the experiment to the HCP folder.
4. Select a file name for the experiment and press **Save**.

   The instrument starts running the experiment.
5. After the run, a message box opens on the screen notifying you if the HCP passed or failed.
   - If it passed, copy the post-run HCP experiment file to your PC (see "Retrieve run data from the instrument" on page 173 for instructions). You can then use the HCP to calibrate HRM data from an experiment. See "Assign an HRM calibration plate" on page 183 for instructions.

- If it failed, you cannot use the HCP to calibrate HRM data from an experiment. See "If the HCP fails", below, for information on failed HCPs.

If the HCP fails

If the HCP fails the system's quality check, the touchscreen displays a message box at the end of the HCP run notifying you of the failure. You will also see a warning icon (as shown below) if you open the experiment in the Aria program on your PC.



Possible causes of a failed plate include pipetting errors during set up of the plate and amplicon degradation. If you find your HCP runs repeatedly fail, try setting up the plate using the Agilent HRM Calibration Plate. If problems persist, contact Agilent Technical Support (see "Contact Agilent Technical Support" on page 370). Note that you cannot associate a failed HCP with an experiment.

4
Creating/Opening an Experiment

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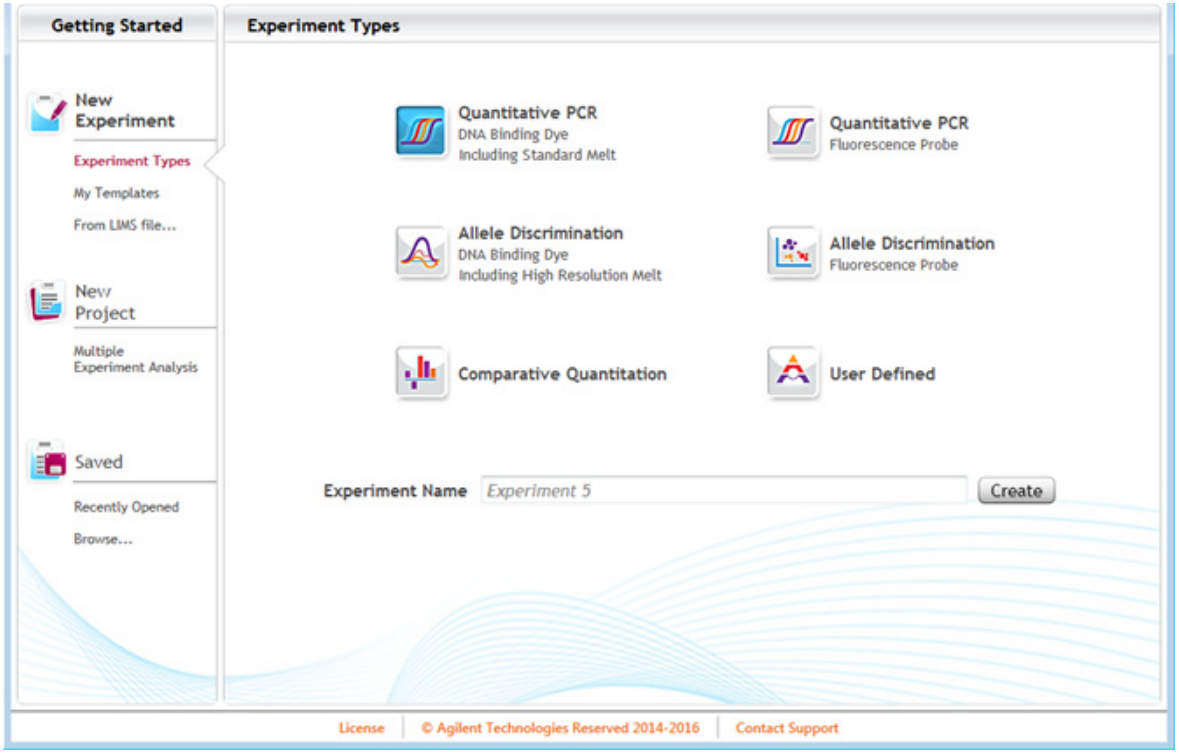
Overview of the Getting Started screen

The tools on the Getting Started screen allow you to create a new experiment (from scratch or from a template), create a new multiple experiment analysis project, or open an existing experiment or project.

To open the Getting Started screen: At the top of the program window, click **File > New**. Alternatively, click the icon to open a new tab in the program. The new tab opens to the Getting Started screen.



Tools available on the Getting Started screen

The content in the center of the screen changes depending on which option is selected in the panel on the left. These options are described in the table below

Option	Description
New Experiment	
Experiment Types	Click this option to create a new experiment based on the desired experiment type. The center of the screen displays the experiment types for selection.
My Templates	Click this option to create a new experiment from a template. The center of the screen displays the template files in the Experiments Templates folder that is created during program installation.
From LIMS file...	Click this option to create a new experiment from a LIMS data file that specifies the plate setup and, optionally, the thermal profile. The center of the screen displays the Import From LIMS Data File wizard.
New Project	
Multiple Experiment Analysis	Click this option to create a new project for analyzing and comparing multiple post-run experiments. The center of the screen displays tools for adding experiments to the new project.
Saved	
Recently Opened	Click this option to open an existing experiment or project that you recently accessed. The center of the screen displays a list of experiments that you have recently opened.
Browse	Click this option to open an existing experiment or project by browsing to a desired folder.
Links at the bottom of the screen	
License	Click this link to open a message box about licensing. In the message box, click **License Agreement** to view the full text of the Aria software license agreement.
© Agilent Technologies	Click this link to open the About message box. This box displays the full version number of the software.
Contact Support	Click this link to create a new email message directed to the Agilent Technical Support group.

About the Aria file types

Three different files types can be created in the Aria program: an experiment file, a protocol template file, and a project file. These file types are summarized below.

Experiment Files (*.*amxd* or *.*adxd*)

In the Aria software, experiments of all types (Quantitative PCR, Comparative Quantitation, Allele Discrimination, and User Defined) are saved as experiment files. When an experiment file is open, the program includes screens for defining the wells of the experimental plate, setting up the thermal profile, running the experiment, and analyzing the results of that run. AriaMx experiment files are given the extension *amxd*, and AriaDx experiment files are given the extension *adxd*.

Template Files (*.*amxt* or *.*adxt*)

In addition to saving an experiment as an experiment file, the plate setup and thermal profile of an experiment can be saved as a template file. Creating a new experiment from a template file allows you to quickly set up new experiments that require a similar plate setup or thermal profile. AriaMx template files are given the extension *amxt*, and AriaDx template files are given the extension *adxt*.

Project Files (*.*amxp* or *.*adxp*)

Multiple experiment analysis projects are saved as project files. Up to 8 post-run experiment files (of the same experiment type) can be added to a project, enabling you to analyze the results side by side or combine results across experiments. AriaMx project files are given the extension *amxp*, and AriaDx project files are given the extension *adxp*.

Quick Start Protocol

How to create, set up, run, analyze, and generate reports for an experiment

1. Create the experiment

- Open a new tab. On the Getting Started screen, under **New Experiment**, click **Experiment Types** (to create a new experiment by the experiment type) or **My Templates** (to create a new experiment from a template).

2. Set up the plate

- After creating the experiment, the experiment opens to the Plate Setup screen.
- Assign the plate properties based on experiment type, including assigning well types, replicate numbers, and dyes/targets. See the topics below for instructions specific to each experiment type.

  "Assign plate properties for a Quantitative PCR DNA Binding Dye experiment" on page 86

  "Assign plate properties for a Quantitative PCR Fluorescence Probe experiment" on page 94

  "Assign plate properties for a Comparative Quantitation experiment" on page 102

  "Assign plate properties for an Allele Discrimination DNA Binding Dye experiment" on page 113

  "Assign plate properties for an Allele Discrimination Fluorescence Probe experiment" on page 122

  "Assign plate properties for a User Defined experiment" on page 131

3. Set up the thermal profile

- Navigate to the Thermal Profile screen. (Click **Thermal Profile** in the Experiment Area panel on the left side of the screen.)
- Edit the thermal profile as desired, or use the default/template thermal profile.

4. Run the experiment

- From the Thermal Profile screen, click **Run**. In the Instrument Explorer dialog box that opens, locate the instrument and click **Send Config**.
- Load your reaction plate into the instrument's thermal block. On the instrument touchscreen, open the primed experiment to the Thermal Profile screen and press **Run**.
- If desired, monitor the progress of the run from your PC.

5. Set the analysis criteria

- When the run is finished, navigate to the Analysis Criteria screen. (Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.)
- Select the wells, well types, and targets to include in the analysis, specify the treatment of replicate wells, and select the data collection marker to use for analysis. If your experiment included a high resolution melt segment (HRM), assign an HRM calibration plate to the experiment.

6. Analyze the data

- Navigate to the Graphical Displays screen. (Click **Graphical Displays** in the Experiment Area panel on the left side of the screen.)
- View the results of the analysis and customize analysis settings for individual graphs. See the topics below for instructions specific to each graph.

"View the Amplification Plots" on page 197

"View the Melt Curve - Raw/Derivative Curve" on page 212

"View the Melt Curve - Difference Plots" on page 218

"View the Standard Curve" on page 225

"View the Relative Quantity" on page 230

"View the Allele Determination graph" on page 235

7. Export the results

- To generate a report of the results, navigate to the Generate Report screen (click **Generate Report** in the Experiment Area panel on the left side of the screen). Configure and create the report according to your selections.
- To export numerical data from the experiment, navigate to the Export Data screen (click **Export Data** in the Experiment Area panel on the left side of the screen). Select the file type and information you want to export.

Create a new experiment

The Getting Started screen allows you to create a new experiment. To create the new experiment from scratch, you start by selecting the experiment type. To create the new experiment from a template or LIMS data file, you start by selecting the template or LIMS data file that you want to use. Once created, you can edit the plate setup and thermal profile for the new experiment.

To open the Getting Started screen: At the top of the program window, click **File > New**, or click the icon, to open a new tab in the program. The new tab opens to the Getting Started screen.

Create an experiment based on experiment type

When you create a new experiment based on experiment type, your selection of the experiment type determines some of the setup options and analysis outputs. The thermal profile of the new experiment is set to the default for the chosen experiment type. The plate setup of the experiment is blank, but the available well types and other well configuration tools on the Plate Setup screen are specific to the experiment type.

To create an experiment based on experiment type:

1. Open the Getting Started screen.
2. Under **New Experiment**, click **Experiment Types**.

   The center of the screen displays all the available experiment types. See "Overview of Experiment Types" on page 59.
3. Click the desired experiment type to select it.
4. In the Experiment Name field, type a name for the new experiment, then click **Create**.

   The program creates the new experiment and opens the experiment to the Plate Setup screen.

NOTE At step 3, you can double-click the desired experiment type to select it with the default experiment name. The program creates the new experiment and opens the experiment to the Plate Setup screen (or to the Thermal Profile screen for User Defined experiments).

Create an experiment from a template

When you create a new experiment from a template, the experiment has the plate setup and thermal profile of the selected template. After you create the experiment, you can edit the plate setup and thermal profile as desired.

The Aria software comes with three sample template files. The templates are available in the folder **C:\Users\Public\Public Documents\Agilent Aria\Experiment Templates**.

To create an experiment from a template:

1 Open the Getting Started screen.

2 Under **New Experiment**, click **My Templates**.

The center of the screen displays the template files in the default template folder.

NOTE You can toggle between displaying the templates in list format and tile format by clicking the icons in top right corner.

 = List view icon

 = Tile view icon

3 In the Experiment Name field, type a name for the new experiment.

4 Select the desired template and create the experiment.

- If the template is in the default folder, click directly on the template to select it and then click **Create** (or double-click directly on the template). The program creates the new experiment and opens the experiment to the Plate Setup screen.
- If the template is not in the currently selected folder, click the Browse to Template icon (shown below) to open the browser window. Browse to the folder of the desired template file. Select the file and click **Open**. The program creates the new experiment and opens the experiment to the Plate Setup screen.

Create an experiment from a LIMS data file

To create a new experiment from a LIMS data file, you must provide a valid file. The file may be generated by exporting a post-run Aria experiment to a LIMS data file (see "Export to a LIMS data file" on page 262), by setting up a text file in the necessary Aria-supported LIMS data file format, or by a LIMS software program. See "LIMS File Format for Aria Software" on page 361 for information on format requirements for LIMS data files used to create new experiments in the Aria program. After you import the file and create the experiment, you can edit the plate setup and thermal profile as desired from the Plate Setup and Thermal Profile screens.

The Aria software comes with sample LIMS data files (text files and CSV files). The files are available in the folder **C:\Users\Public\Public Documents\Agilent Aria\Sample LIMS Import Files.**

To create an experiment from a LIMS data file:

1. Open the Getting Started screen.
2. Under **New Experiment**, click **From LIMS file...**.

   The center of the screen displays the Import From LIMS Data File wizard.
3. In the Filename field under **LIMS data file**, type the file path for the LIMS data file, or click **Browse** to browse to and select the LIMS data file. The file type can be a text file (**.txt*) or a CSV file (**.csv*).

   The program populates the fields in the **Experiment setup** and **Thermal profile setup** areas of the LIMS Data File wizard using the available information from the selected file. Note that the file may not include all experiment details.
4. (Optional) Edit the information in the Experiment setup fields as desired.
   - In the Name field, type a name for the experiment. If the imported LIMS data file specified the experiment name, then the field is populated with that name.
   - In the Type drop-down list, select an experiment type. If the imported LIMS data file specified the experiment type, then the drop-down list is set to that selection. See "Overview of Experiment Types" on page 59 for descriptions of the Aria experiment type options.

- In the Notes field, type any experiment notes that you want associated with the new experiment. If the imported LIMS data file included experiment notes, then the field is populated with those notes.

5 Click **Next**.

The screen displays the plate setup information for the experiment.

6 (Optional) Edit the Reference Dye selection and other target information as permitted for the experiment type.

7 Click **Finish**.

The program creates the new experiment and opens the experiment to the Plate Setup screen. A message box opens confirming that the experiment has been successfully imported. Click **OK** to close the message box.

Open an existing experiment

The program allows you to open up to 5 experiments at a time, or one project at a time. The program displays each open experiment or project on its own tab.

To open an experiment in a new tab:

1 Click the icon to the right of the tabs to open a new tab.

The new tab opens to the Getting Started screen.

2 Click one of the options under **Saved:**

- To open an existing experiment that you recently accessed, click **Recently Opened**. The center of the screen displays a list of experiments and projects that you have recently opened. Double-click the experiment you want to open. The program opens the experiment to the Plate Setup screen.
- To browse to the folder of the experiment, click **Browse**. The Open dialog box opens. Browse to the folder location of the experiment. Select the experiment and click **Open**. The dialog box closes and the program opens the experiment to the Plate Setup screen.

To open an experiment in an already open tab:

1 From the toolbar, click **File > Open**.

The Open dialog box opens. If an experiment or project is currently open in the tab, the program closes that experiment or project, and prompts you to save any changes.

2 Browse to the folder location of the experiment. Select the experiment and click **Open**.

The dialog box closes and the program opens the experiment to the Plate Setup screen.

Save a copy of an existing experiment

You can use the Save As command to copy the open experiment and save it with a new experiment name.

To copy an existing experiment using the Save As command:

1. Open the existing experiment that you want to copy.
2. Click **File > Save As**.

   The Save As dialog box opens.
3. Select a folder for the new experiment and type a name into the file name field.
4. Click **Save**.

   The program saves the open experiment under the new name.

Create a template from an existing experiment

You can use the Save As Template command to create a template file based on the plate setup and thermal profile of an existing experiment. You can later use the template to quickly create new experiments. See "Create an experiment from a template" on page 50.

To create a template from an existing experiment using the Save As Template command:

1. Open the existing experiment that you want to create a template from.
2. Click **File > Save As Template**.

   The Save As dialog box opens. If you are running the AriaMx mode of the software, the file type set to **AriaMx Template Files (*.amxt)**. If you are running the AriaDx mode of the software, the file type set to **AriaDx Template Files (*.adxt)**.
3. Select a folder for the new template and type a name into the file name field.
4. Click **Save**.

   The dialog box closes and the program saves the new template file (*.*amxt* or *.*adxt*) to the designated folder.

Convert an experiment to a new experiment type

The Convert Experiment Type command can convert a post-run experiment into another experiment type. This command is useful when the experiment was set up as one type and the data needs to be re-analyzed as a different experiment type. The program applies the analysis algorithms and display options based on the new experiment type.

To convert an experiment to a new experiment type:

1. Open the existing post-run experiment that you want to convert.
2. Click **File > Convert Experiment Type**, and in the sub-menu, select the new experiment type.

   A message box opens notifying you that the conversion was successful and that some well types may have changed.
3. Click **OK** to close the message box.

   The program creates a new experiment file for the converted experiment and opens the experiment to the Plate Setup screen. By default, the new experiment has the same name as the parent experiment with the word “Converted” added at the beginning.
4. Click **File > Save As**.

   The Save As dialog box opens.
5. Select a folder for the new experiment. Type a name into the file name field or use the default file name.
6. Click **Save**.

   The program saves the new experiment to the designated folder.

5
Selecting an Experiment Type

Overview of Experiment Types

The Aria program offers a variety of experiment types. Each experiment type was designed for a specific application and has its own unique options for experimental setup and analysis that are specialized for that application. The Aria experiment types are summarized below.

Quantitative PCR

The Quantitative PCR experiment type is the best choice when you need to determine the exact quantity of a particular DNA target in the experimental template samples. This experiment type uses a standard curve produced with samples of known template quantity to derive the initial quantity of the target in the experimental sample.For more information, see "The Quantitative PCR Experiment Type" on page 61.

The program offers two sub-types for the Quantitative PCR experiment type: *DNA Binding Dye Including Standard Melt* and *Fluorescence Probe*. These sub-types differ by the type of chemistry used to detect the PCR products. In the *DNA Binding Dye Including Standard Melt* sub-type, detection is based on signal from a double-stranded DNA binding dye, e.g., SYBR Green, and the default thermal profile includes a melt curve. In the *Fluorescence Probe* sub-type, a target-specific probe, e.g., a TaqMan probe, is used for target detection.

Comparative Quantitation

The Comparative Quantitation experiment type is best used for comparing levels of RNA or DNA across samples when you do not require information about the absolute amount of target. Most common is the comparison of amounts of mRNA in treated versus untreated, or normal versus diseased cells or tissues. The program will ask you to identify which wells contain the control sample (called the "calibrator") and which wells contain the associated experimental sample (called the "unknown"). For accurate results, you need to normalize the data to the quantity of a target gene (called a "normalizer") that is known to be unaffected by the experimental conditions, such as a housekeeping gene. For more information, see "The Comparative Quantitation Experiment Type" on page 63.

Allele Discrimination - Fluorescence Probes

The Allele Discrimination experiment type is used to discriminate between two alleles in a DNA sample. For more information, see "The Allele Discrimination - Fluorescence Probe Experiment Type" on page 69.

The program offers two sub-types for the Allele Discrimination experiment type. In the sub-type *Fluorescence Probe*, you use two fluorogenic probes labeled with different dyes to discriminate between two alleles in a DNA sample. For example, if amplification in an unknown DNA sample is detected for the dye identifying the wild-type allele but not for the dye identifying a mutant allele, the sample can be designated as wild-type homozygous.

Allele Discrimination - DNA Binding Dye with High-Resolution Melt

The Allele Discrimination experiment type is used to discriminate between two alleles in a DNA sample. For more information, see "The Allele Discrimination - DNA Binding Dye Experiment Type" on page 67.

The program offers two sub-types for the Allele Discrimination experiment type. In the sub-type *Fluorescence Probe*, you use two fluorogenic probes labeled with different dyes to discriminate between two alleles in a DNA sample. For example, if amplification in an unknown DNA sample is detected for the dye identifying the wild-type allele but not for the dye identifying a mutant allele, the sample can be designated as wild-type homozygous

User Defined

The User Defined experiment type provides the greatest flexibility in setup and analysis of an experiment. All the well types and other plate setup options that are available across the other experiment types are available on the Plate Setup screen in a User Defined experiment. Similarly, on the Thermal Profile screen, you can add any type of segment to the thermal profile, and on the Graphical Displays screen, you can view the results for any of the experiment type-specific graphs.

The Quantitative PCR Experiment Type

In Quantitative PCR experiments, the instrument detects the fluorescence of one or more dyes or fluorophores during each cycle of the thermal cycling process and a fluorescence value is reported for each dye/fluorophore at each cycle. Generally, you want the instrument to acquire fluorescence readings during the annealing stage of thermal cycling.

You can quantify the initial copy numbers of RNA or DNA targets based on quantification cycle (Cq) determinations. The Cq is defined as the cycle at which a statistically-significant increase in fluorescence is first detected. The threshold cycle is inversely proportional to the log of the initial copy number. In other words, the more template that is present initially, the fewer the number of cycles required for the fluorescence signal to be detectable above background. The Aria program offers both automatic and manual methods for determination of the threshold fluorescence level that is used to determine Cq values.

Typical Quantitative PCR experiments use a standard curve to quantitate the amount of target present in an experimental sample (called the "unknown" sample since the quantity of the target is unknown). In this method, you set up the plate to amplify a series of standards to generate a standard curve that relates initial template quantity to Cq. You generate the standards by serial dilution of a template sample that contains a known quantity of the target under investigation. The program then uses the standard curve to derive the initial template quantity of this target in the unknown samples based on their Cq values.

Multiplexing quantitative PCR experiments

The instrument records fluorescence readings for each sample on all five optical modules. This allows you to use multiplex PCR for quantitation of multiple targets in the same well by using spectrally-distinct dyes to detect each target. The Aria program reports each target in each well separately on amplification plots and other graphical results displays.

Well types for Quantitative PCR experiments

Well Type	Description
Unknown	Contains a complete reaction mixture including a test template that contains an unknown amount of the target-of-interest.
Buffer	Contains only buffer; used to monitor the background fluorescence attributable to the buffer.
NAC	No amplification control; contains all reaction components except DNA polymerase.
NTC	No template control; contains all reaction components except the template nucleic acid. This well type is useful for detecting amplicon contamination.
Standard	Contains a complete reaction mixture including a known concentration of the target-of-interest. This well type is used to generate a standard curve, which is then used to relate the quantification cycle (Cq) to initial template quantity in Unknown wells and calculate the amplification efficiency.
No RT	No reverse transcriptase control; contains all QRT-PCR reaction components except reverse transcriptase. In one-step RT-PCR assays, this control is useful for assessing levels of genomic DNA carryover that may contribute to fluorescence increase in the sample.

The Comparative Quantitation Experiment Type

The Comparative Quantitation experiment type provides an efficient method for comparing levels of RNA or DNA across samples when you do not require information about the absolute amount of target in any sample. The most common application is the comparison of amounts of mRNA in treated versus untreated, or normal versus diseased cells or tissues.

For many gene expression studies, your experiments do not require you to determine the absolute amount of a target in a particular sample; evidence of a relative increase or decrease in expression, compared to a sample of reference, is sufficient. The sample of reference is referred to as the calibrator. For example, in a study in which a large number of compounds are screened for the ability to induce the expression of a certain set of genes in HeLa cells, the calibrator might be a nucleic acid sample isolated from an untreated HeLa cell culture. In a study involving the expression of a cancer marker gene, the calibrator might be a nucleic acid sample isolated from the normal, non-diseased part of the organ, whereas the test samples (referred to in the program as Unknowns) are nucleic acids isolated from the diseased tissue of the same patient. The expression level of the target-of-interest (i.e., the gene you are studying) in the calibrator is defined as 1× (or 1.0). Expression levels in all unknown samples are reported as a fold difference relative to this calibrator benchmark.

Normalizing chance variations in target levels

The quantity of a target-of-interest present across a set of independently-isolated samples is subject to many variables such as sample-to-sample differences in total amount of input nucleic acid and differences in the efficiency of RNA extraction or reverse transcription. You can include a normalizer target in the assay to reduce the effect of these spurious variations that do not reflect true differences in target abundance as a result of experimental treatment. Any gene with little to no variance in expression due to the experimental treatment can serve as a normalizer. (Commonly-used examples include housekeeping genes such as GAPDH, cyclophilin, GUS, TFIID, or 18S, or 28S ribosomal RNA.) The abundance of the normalizer and the target-of-interest should be similar.

In a typical Comparative Quantitation experiment, you would set up the wells containing the calibrator sample to run alongside a variety of

unknown samples to test the effect of some variable on the expression level of one or more genes of interest. You can set up the reactions amplify the normalizer target in the same well as the target-of-interest (using multiplexing) or set up the reactions to amplify the normalizer and the target-of-interest in different wells.

During analysis, the program automatically adjusts the levels of the target-of-interest in both Unknown and Calibrator wells to compensate for differences in the levels of the normalizer. The program then compares the normalized value for each unknown sample to the normalized calibrator value, and reports the relative quantity for each unknown. (The expression level of the target-of-interest in the Calibrator wells is set to 1.0.)

Establish the amplification efficiencies of the normalizer target and the target-of-interest before you use a particular normalizer in a Comparative Quantitation experiment.

See the following section ("Determining amplification efficiencies for the targets of interest and normalizer targets") for more information.

Determining amplification efficiencies for the targets of interest and normalizer targets

In developing a Comparative Quantitation experiment, it is important that the amplification efficiencies of the target-of-interest and the normalizer target are reproducible and, ideally, very similar. To measure the amplification efficiency for each target, generate a standard curve in a Quantitative PCR experiment. When running a standard curve for the sole purpose of determining the amplification efficiency for a particular target, it is not necessary to know the exact quantity of your targets. Instead, you can use serially diluted template as the standard samples and assign the standard quantities in Plate Setup using the "relative" unit designation. The program then analyzes the standard curve data and calculates the amplification efficiency from the slope of the curve. (See "View the R-squared values, slopes, and amplification efficiencies" on page 227 for more information on deriving amplification efficiencies from standard curves.)

If differences in amplification efficiency exist

In an idealized Comparative Quantitation experiment, the amplification efficiencies for the target-of-interest and normalizer target must be

identical in order to allow a direct correction of target levels across samples. However, if you find from your standard curves that the target-of-interest and normalizer have different amplification efficiencies, the program allows you to compensate for this difference using the settings under Amplification Efficiencies in the Graphical Displays screen. To access these settings, expand the menu in the panel on the right side of the Graphic Displays screen. (See "Enter the amplification efficiencies for the targets " on page 233 for detailed instructions.)

Including biological replicates in comparative quantitation

In QPCR, biological replicates are template samples that were isolated independently but from biologically-identical sources (sources that are genetically identical are of the same cell type and were treated identically during experimentation). For example, two samples of cDNA that were isolated from the same tissue source in two different mice that were exposed to identical conditions and have the same genotype would be biological replicates. Biological replicates help you determine the level of variability in gene expression for your specific experiment that is due to uncontrolled biological variation from sample to sample.

When setting up the plate for a Comparative Quantitation experiment, you may designate two or more samples as biological replicates while assigning the sample names. Samples that are biological replicates are assigned the same sample name but have different biological replicate ID numbers. During analysis, the program treats biological replicates independently as different samples.

If the experiment includes multiple biological replicates of the calibrator sample, you can designate only one of the samples within the set of replicates as the calibrator. You can change the assignment of the calibrator after the run if you want to re-analyze the results using a different calibrator designation.

Well types for Comparative Quantitation experiments

Well Type	Description
Unknown	Contains a complete reaction mixture including a test template that contains an unknown amount of the target-of-interest.
Calibrator	Contains a complete reaction mixture including an unknown amount of the target-of-interest. The level of a target-of-interest in the calibrator wells is set to 1.0 for comparison to the relative quantities in unknown samples.
NTC	No template control; contains all reaction components except the template nucleic acid.
Standard	Contains a complete reaction mixture including a known concentration of the target-of-interest. This well type is used to generate a standard curve, which is then used to relate the quantification cycle (Cq) to initial template quantity in Unknown wells and calculate the amplification efficiency.
No RT	No reverse transcriptase control; contains all QRT-PCR reaction components except reverse transcriptase. In one-step RT-PCR assays, this control is useful for assessing levels of genomic DNA carryover that may contribute to fluorescence increase in the sample.
NAC	No amplification control; contains all reaction components except DNA polymerase.
Buffer	Contains only buffer; used to monitor the background fluorescence attributable to the buffer.

The Allele Discrimination - DNA Binding Dye Experiment Type

The Allele Discrimination - DNA Binding Dye experiment type is used to discriminate between two alleles of a gene in a genomic DNA or cDNA sample using a double-stranded DNA binding dye, such as SYBR Green or EvaGreen dye, and a high-resolution melt (HRM).

HRM analysis is a technique used for genotyping samples that include a single nucleotide polymorphism (SNP) in the DNA sequence. Applications that may use HRM analysis include species identification, mutation screening, and haplotype characterization. When using the allele discrimination experiment type for HRM analysis, you set up the experiment to amplify all alleles in the same well using the same set of primers, and the program detects all alleles using the same double-stranded DNA-binding dye (such as SYBR Green or EvaGreen dye). The thermal profile includes a melt segment so that melt curves of the targets can be generated. Even DNA amplicons that differ in sequence by only a single nucleotide will yield slightly different melt curves. An Allele Discrimination experiment that uses HRM analysis needs to include positive control samples for each base pair possibility at the SNP location (homozygous as well as heterozygous positive control samples).

On the Graphical Displays screen, Difference Plots show the difference in fluorescence between two plots during a melt ramp (Y axis) as a function of temperature (X axis). By graphing the difference in fluorescence, the program can detect even slight differences between two melt curves. You can use the difference plots to compare samples of unknown genotype to positive control samples, and visually determine which genotype group the target in the unknown sample falls into.

NOTE A separate HRM software license is required in order to view the Difference Plots. To purchase a software license, contact your Agilent Sales representative.

About HRM calibration plates

During the HRM segment of the thermal profile, the instrument ramps the temperature of the thermal block in 0.2°C increments. At any given target temperature, very slight differences in the exact temperature may exist from well to well across the thermal block. The purpose of an HRM calibration plate (HCP) is to calculate an offset temperature for each well

in order to help normalize these temperature variations. The offset temperature is the difference between the Tm (melting temperature) in any given well and the average Tm for the plate. When you associate your Allele Discrimination experiment with an HCP experiment, the program subtracts the offset temperature from the calculated Tm in each well. This normalization improves the accuracy and clarity of the difference plots and the raw/derivative melt curves.

In order to calculate the offset temperatures, an HCP must contain the identical amplicon in each well. During the HCP run, that amplicon is melted in an HRM segment. The program then calculates a Tm for each well and an average Tm for the plate.

All HCP experiments must be set up and run directly from the instrument (you cannot set up an HCP experiment from your PC). See "Run an HRM calibration plate (HCP)" on page 38 for instructions.

You can use the same HCP experiment for multiple Allele Discrimination experiments. For each instrument, Agilent recommends running a new HCP experiment at least once per year.

Well types for Allele Discrimination - DNA Binding Dye experiments

Well Type	Description
Unknown	Contains a complete reaction mixture including a test template that contains an unknown amount of the specific target.
NTC	No template control; contains all reaction components except the template nucleic acid. This control is useful for detecting amplicon contamination.
Homo Allele A	Contains a complete reaction mixture with a positive control template that is known to be homozygous for allele A.
Home Allele B	Contains a complete reaction mixture with a positive control template that is known to be homozygous for allele B.
Hetero	Contains a complete reaction mixture with a heterozygous positive control template that is known to include both allele A and allele B.

The Allele Discrimination - Fluorescence Probe Experiment Type

The Allele Discrimination experiment type is used to discriminate between two alleles of a gene in a genomic DNA or cDNA sample. An Allele Discrimination experiment can be performed two different ways: using fluorescent probes (as described below) or using a double-stranded DNA binding dye.

Using the probe method for allele discrimination, two fluorescent probes labeled with two spectrally distinct dyes are used to discriminate between the two alleles and, subsequently, determine the genotype of a sample. For example, if the program detects amplification in an unknown DNA sample for the dye identifying the wild-type allele but not for the dye identifying a mutant allele, the program designates the sample as wild-type homozygous. If the program detects amplification in an unknown DNA sample for the dye identifying the mutant allele but not for the dye identifying the wild-type allele, the program designates the sample as mutant homozygous. If the program detects amplification for both dyes, it designates the unknown sample as heterozygous for the two alleles. With properly designed probes, this assay is sensitive enough to detect a single-base difference (single nucleotide polymorphism or SNP) between two alleles.

The program uses the quantification cycle (Cq) value for each target in each sample to determine the genotypes of the samples. A Cq value of 24 to 32 is expected for a sample that contains the specific allele recognized by the probe. A Cq value equal to the final cycle of the PCR reaction (typically 40) or equal to the Cq of the negative control samples indicates the absence of a specific allele. The program displays the results in the Allele Determination graph, which is a scatter plot that shows the Cq for the dye specific to one allele plotted against the Cq for the dye specific to the other allele. The program groups plotted points according to their positions on the graph, providing a convenient visualization of samples which share the same genotype (allelic composition).

In the analysis of real-time Allele Discrimination experiments that use fluorescence probes (e.g., TaqMan probes), you would typically monitor and report fluorescence at the end of the annealing/extension step. At that point, the polymerase has already extended across the template and hydrolyzed any probe that had annealed.

Well types for Allele Discrimination - Fluorescence Probe experiments

Well Type	Description
Unknown	Contains a complete reaction mixture including a test template that contains an unknown amount of the specific target.
NTC	No template control; contains all reaction components except the template nucleic acid. This control is useful for detecting amplicon contamination.
Homo Allele A	Contains a complete reaction mixture with a positive control template that is known to be homozygous for allele A.
Home Allele B	Contains a complete reaction mixture with a positive control template that is known to be homozygous for allele B.
Hetero	Contains a complete reaction mixture with a heterozygous positive control template that is known to include both allele A and allele B.

The User Defined Experiment Type

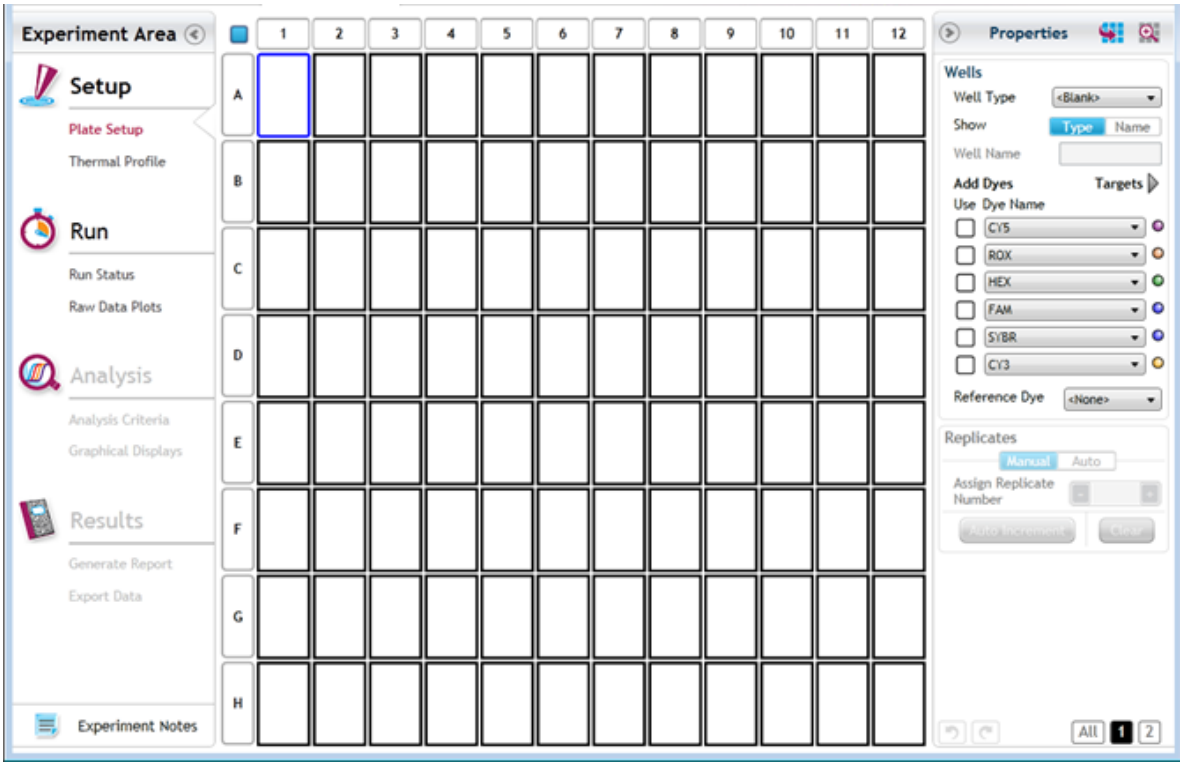
The User Defined experiment type provides the greatest flexibility in setup and analysis of an experiment. All the well types and other plate setup options that are available across the other three experiment types are available on the Plate Setup screen in a User Defined experiment. Similarly, on the Thermal Profile screen, you can add any type of segment to the thermal profile, and on the Graphical Displays screen, you can view the results for any of the experiment type-specific graphs.

6
Setting Up the Plate

Overview of the Plate Setup screen

The Plate Setup screen has tools for assigning properties to the wells of the plate so that the program can properly analyze your data.

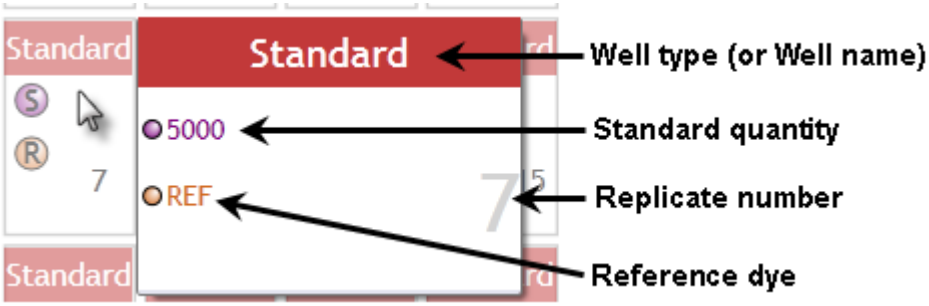
To open the Plate Setup screen: With an experiment or project file open, click **Plate Setup** in the Experiment Area panel on the left side of the screen.



The plate map

In the center of the Plate Setup screen is a representation of the wells of a 96-well plate. This diagram, called a plate map, is used for showing which wells in the plate are in use in the current experiment, what kind of sample is in each well, which dyes are being used for detection in the wells, and what targets those dyes are detecting.

The properties assigned to the wells are indicated in the plate map. To see more detailed information on the properties of a particular well, such as target names and standard quantities, hover your cursor over the well.



You can also open a mini plate map to view the details in a selected set of wells.

Well type

All wells that are in-use in the experiment need to be assigned a well type. This assignment indicates to the program the type of reaction in the well. For example, the Unknown well type is used for experimental templates in which the quantity of the target(s) is unknown. When the **Show** setting on the Plate Setup Properties panel is set to **Type**, the well type appears at the top of the well.

The available well types vary depending on the experiment type.

Well name / Sample name

If desired, you can assign names to the wells of the plate. When the **Show** setting on the Plate Setup Properties panel is set to **Name**, the well name appears at the top of the well. The Comparative Quantitation, Allele

Discrimination, and User Defined experiment types also allow you to assign sample names to designate the template sample used in each well. The program displays the sample name at the top of each well when the **Show** setting is set to **Sample**.

Target information

All experiment types require, at a minimum, that you designate the dyes being used for detection in the wells. You may also assign a name to the target being detected by each dye. If the same dye will be used to amplify multiple targets in the experiment, assigning a unique name to each target allows the program to distinguish between these targets during analysis.

Replicate number

If some of the wells on the plate are technical replicates of each other (i.e., they have the exact same reaction components), you can designate the replicate wells during plate setup by assigning them the same replicate number. During analysis, you can choose to have the results from replicate wells averaged together at each cycle, or you can treat replicates separately to monitor for well-to-well variation among identical reactions.

Invalid Sets: When assigning replicates, if you see a flashing red warning icon in the Properties panel next to **Replicates**, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

Note that technical replicates are different from biological replicates. The assignment of biological replicate IDs is unique to Comparative Quantitation (and User Defined) experiments.

Reference dye

Passive reference dyes are used for normalization of the fluorescence signal in order to compensate for non-PCR related variations in fluorescence, such as pipetting variation from well to well. Typically, most experiments use ROX as the reference dye.

If you will be adding a dye to the wells of your plate as a passive reference dye, you need to assign a reference dye in the plate setup. The assignment of that dye as a reference dye is indicated in the wells by the target name REF. Note that if you assign a reference dye, you must assign it to all wells in use on the plate.

The Properties panel

The panel on the right side of the Plate Setup screen has the tools for assigning properties to the wells in the plate map. The content of this panel depends on the experiment type. See the following help topics for information on your experiment type:

"Assign plate properties for a Quantitative PCR DNA Binding Dye experiment" on page 86

"Assign plate properties for a Quantitative PCR Fluorescence Probe experiment" on page 94

"Assign plate properties for a Comparative Quantitation experiment" on page 102

"Assign plate properties for an Allele Discrimination DNA Binding Dye experiment" on page 113

"Assign plate properties for an Allele Discrimination Fluorescence Probe experiment" on page 122

"Assign plate properties for a User Defined experiment" on page 131

To hide the Properties panel, click the arrow icon in the upper left corner of the panel. Click the arrow again to display the Properties panel.

Additional tools for setting up a plate

Copy/Paste

To copy well information, first select the wells that contain the information to be copied. Then, press **Ctrl+c**, or right-click on the plate map and click **Copy**, to copy the properties of the selected wells to the clipboard. You can later paste the properties into other wells within the same experiment or in another open experiment.

To paste well information from the clipboard to a set of wells, first select the wells that you want to paste into. Then, press **Ctrl+v**, or right-click on the plate map and click **Paste**, to paste the well information into a selected set of wells. You can paste into wells within the same experiment, or into wells of an experiment that is open in another tab of the program (provided that the properties of the wells are compatible with the experiment type).

See "Select and view wells in the plate map" on page 79 for instructions on selecting sub-sets of wells on the plate map.

Undo/Redo

Click the Undo icon in the lower right corner of the screen to undo your most recent action. You can click the icon multiple times to undo multiple, consecutive actions.

Click the Redo icon in the lower right corner of the screen to redo the most recent action that you reversed using the Undo tool. You can click the icon multiple times to redo multiple, consecutive actions.

You can also access the Undo and Redo commands by right-clicking anywhere on the plate map.

Clear Selected Well or Clear Plate

To clear all the properties assigned to a well or set of wells, select the well(s) on the plate map, right-click, and then click **Clear Selected Well**. Alternatively, select the well(s) on the plate map and press **Delete** to clear the properties.

To clear all properties assigned to all wells in the plate, right-click anywhere on the plate map and click **Clear Plate**.

Import a plate setup

The Aria program allows you to set up the plate on the Plate Setup screen by importing the plate setup of any existing experiment of the same experiment type. After you import the plate setup, you can edit the well properties as desired.

To open the Plate Setup screen: With an experiment or project file open, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

To import a plate setup:

1. In the Properties panel of the Plate Setup screen, click the Import Plate Setup icon.

   The Open dialog box opens.

2. Browse to the folder of the existing experiment with the plate setup that you want to import. Select the file and click **Open**.

   The dialog box closes and the program imports the plate setup into the currently open experiment.

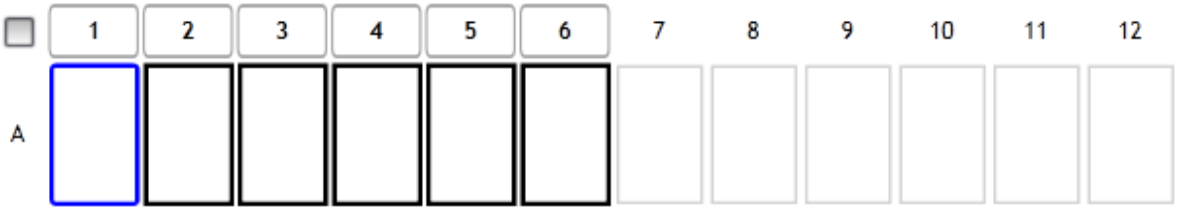
Select and view wells in the plate map

When setting up your plate on the Plate Setup screen, you may need to select a sub-set of wells in the 96-well plate map in order to assign properties to specific wells. You can select and unselect wells by clicking directly on the plate map. You may also need to view certain wells of the plate map in more detail, which can also be done from the Plate Setup screen.

To open the Plate Setup screen: With an experiment or project file open, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Select wells in the plate map

When a new experiment is first opened, all wells on the plate are already selected. Selected wells appear highlighted on the plate map (see wells A1 through A6 in the image below) while unselected wells appear grayed out (see wells A7 through A12 in the image below).



To select all wells when some wells are not already selected:

1 Click the small square in the upper left-hand corner of the plate. (If all wells on the plate are already selected, clicking this button will unselect all wells.)

To select an entire row or column of wells:

1 Click the corresponding row header (A–H) or column header (1–12).

To select a range of adjacent wells:

1 Click and hold the left mouse button as you drag the cursor across the wells to be selected.

   A visible marking rectangle appears.

2 When all of the required wells are included in the rectangle, release the left mouse button.

The range of wells is selected.

To select a group of non-contiguous wells:

1 Press **Ctrl** as you click individually on each of the wells to be selected.

Unselect wells in the plate map

Use one of the following approaches to unselect wells:

- To unselect individual wells, press **Ctrl** as you click on the wells to be unselected.
- To unselect a selected row or column, click on the header for that row or column.
- To rapidly unselect all wells, click twice on the button in the upper left-hand corner of the plate. This will select and then unselect all wells.

View details of a well or wells

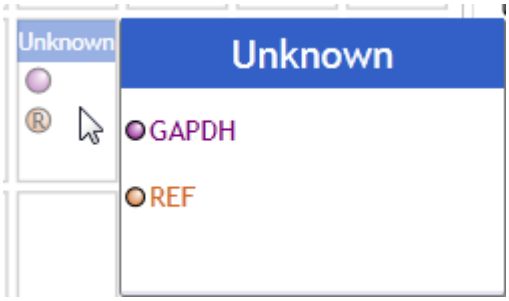
When the plate map is displaying all 96 wells, details such as target names and standard quantities are not displayed. The Plate Setup screen offers multiple ways to view the detailed properties of a single well or multiple wells.

View details of an individual well

To view the detailed properties of an individual well:

- Hover the cursor over the well.

  The program opens a larger schematic of the well that displays the properties assigned to the well.



Zoom in/out on the plate map

You can zoom in and zoom out on the wells of the plate map using the commands on the plate map short-cut menu.

To zoom in on the wells of the plate map:

1 Right-click anywhere on the plate map.

   The short-cut menu opens.

2 Click **Zoom In**.

   The program zooms in on the plate map, displaying fewer wells in more detail.

3 If desired, repeat steps 1-2 to zoom in further.

To zoom out on the wells of the plate map:

1 Right-click anywhere on the plate map.

   The short-cut menu opens.

2 Click **Zoom Out**.

   The program zooms out on the plate map.

3 If desired, repeat steps 1-2 to zoom out until the plate map displays all 96 wells.

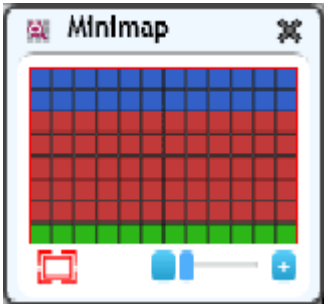
View a mini plate map

The minimap tool allows you to limit the plate map to specific wells, which enables you to view more detailed information on the properties of those wells.

To view a mini plate map:

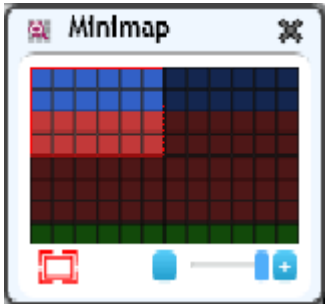
1 In the Properties panel, click the Minimap icon.

The Minimap box opens. This box displays a small schematic of the plate map.



2 In the Minimap box, click the zoom scroll bar to zoom in on the wells in the minimap.

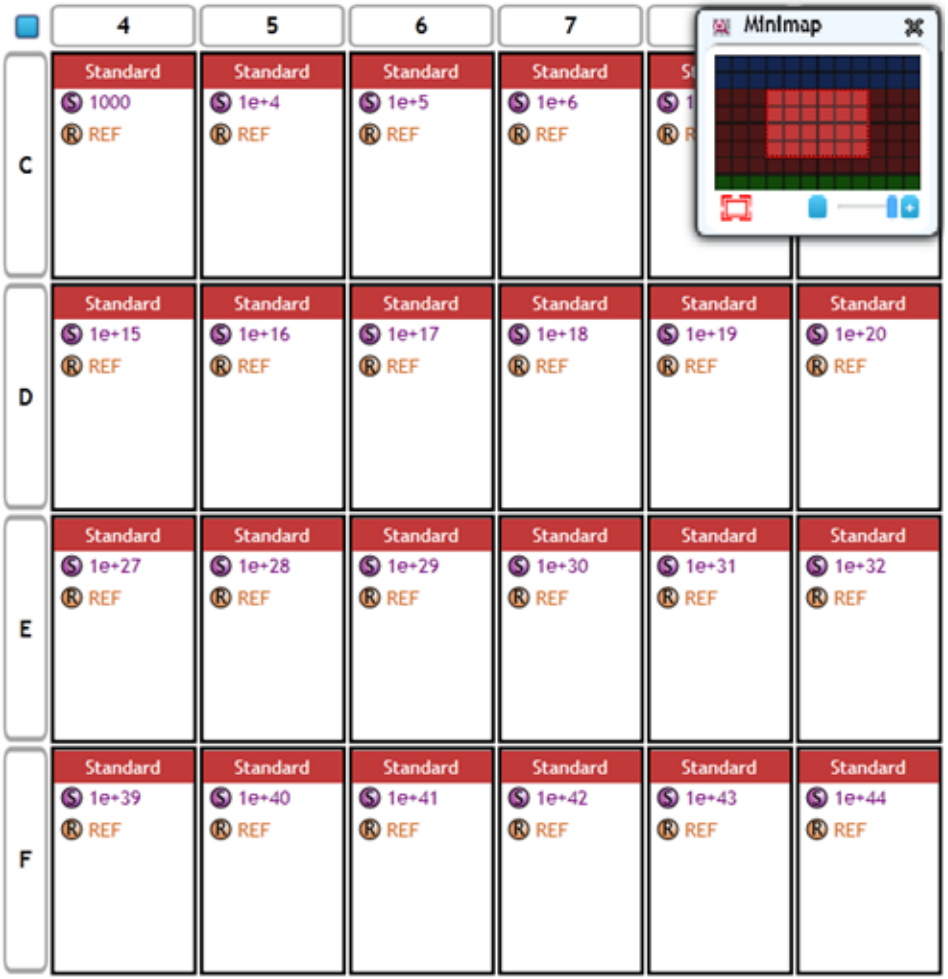
The red box outlines the wells to be included in the minimap.



3 Click and drag the red box to capture the wells that you want to view in more detail.

4 Click the X in the upper right corner of the Minimap box to close the box.

The plate map on the Plate Setup screen displays only the wells selected in the Minimap box.



To restore the mini plate map to the full 96 wells:

1 In the Properties panel, click the Minimap icon.

The Minimap box opens.

2 Click the Maximize icon in the lower left corner of the Minimap box.

3 Click the X in the upper right corner of the Minimap box to close the box.

The plate map displays all 96 wells.

Export the plate map image

The image of the Plate Setup screen's plate map can be exported to a Microsoft PowerPoint presentation.

To open the Plate Setup screen: With an experiment or project file open, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

To export an image of the plate map to PowerPoint:

- From the Plate Setup screen, right-click anywhere on the plate map. In the short-cut menu, click **Send Image to PowerPoint**.

  PowerPoint opens to a new presentation file with the plate map image on the slide.

Assign plate properties for a Quantitative PCR DNA Binding Dye experiment

The controls on Plate Setup screen's Properties panel allow you to create a customized plate setup for experiments of the type *Quantitative PCR - DNA Binding Dye Including Standard Melt*.

To open the Plate Setup screen: When you create a new Quantitative PCR experiment, you will automatically be directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment. See "Well types for Quantitative PCR experiments" on page 62 for a description of the available well types.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1-2 for all well types to be included in the experiment.

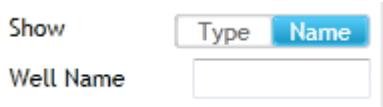
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

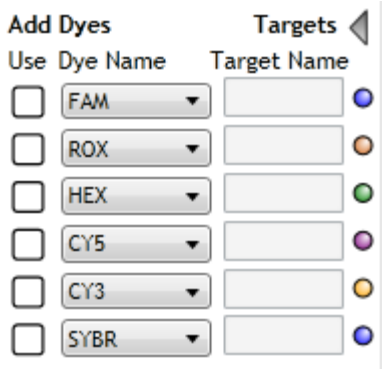
The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

5 Click **OK** in the message box to close it.

The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1 On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel, under **Add Dyes**, mark the Use check box for the dye used for target detection in the selected wells.

3 If the fields for entering target names are not displayed, click the arrow next to **Targets**.

The fields appear to the right of the dye names.

4 For the marked dye, type a name into the adjacent Target Name field. The program assigns the target name to the selected wells.

   If you do not assign a target name, the program uses the dye name as the target name.

5 Repeat steps 1-4 for all wells included in use in the experiment.

6 (Optional) Select a new color to associate with a target:

   a Click the colored dot to right of the Target Name field.

   b In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Assign replicates

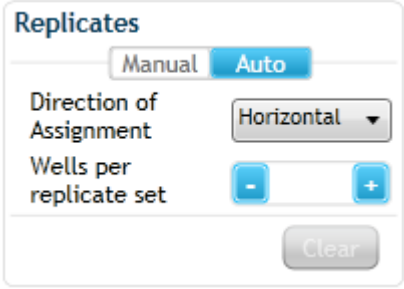
The Aria program uses replicate ID numbers to denote technical replicates. Technical replicates are QPCR reaction tubes containing identical reaction components and set up using a template from the exact same biological sample source. While biological replicates measure the variability in the experimental results due to uncontrolled biological variation from sample to sample, technical replicates are used to measure the variability in results that is introduced during the process of experimental setup.

You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1. On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.
2. In the Properties panel under **Replicates**, select **Auto**.



3. In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.

- Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
- Select **Vertical** if the replicate reactions will be arranged vertically in columns.

4 In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

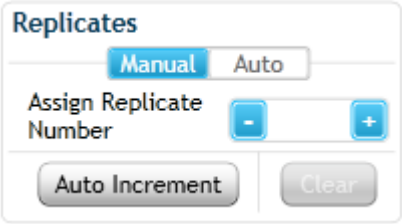
The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

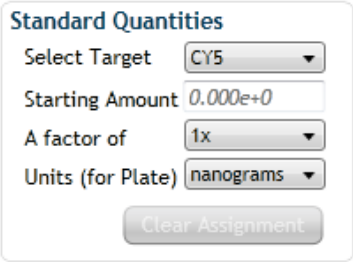
5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign quantities to Standard wells

In order to generate a standard curve from your data, you need to assign the initial template quantity to each Standard well.



To assign the standard quantities for a target in the Standard wells:

1 On the Plate Setup screen, select the Standard wells. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Standard Quantities**, select which target in the selected wells is the standard target (i.e., the target of known quantity in the template).

3 In the Starting Amount field, type in the quantity of the standard target present in the first replicate set of Standard wells. You will be asked to specify the units for this amount in step 5.

NOTE This quantity must be either the highest quantity or the lowest quantity in the dilution series of the standard template sample.

4 In the drop-down list labeled **A factor of**, select the dilution factor used to generate the dilution series of the standard template. For example, if each standard quantity is separated by a factor of 10, select **10x**. Negative dilution factors are used to specify a decrease in quantity from the starting amount while positive dilution factors specify an increase from the starting amount.

5 In the drop-down list labeled **Units (for Plate)**, select the units of the quantity entered in the Starting Amount field. Note that all Standard wells on the plate must use the same units.

To clear the assigned standard quantity from one or more wells:

- Select the well(s) and click **Clear**.

Assign plate properties for a Quantitative PCR Fluorescence Probe experiment

The controls on Plate Setup screen's Properties panel allow you to create a customized plate setup for experiments of the type *Quantitative PCR - Fluorescence Probe*.

To open the Plate Setup screen: When you create a new Quantitative PCR experiment, you will automatically be directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment. See "Well types for Quantitative PCR experiments" on page 62 for a description of the available well types.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1 and 2 for all well types to be included in the experiment.

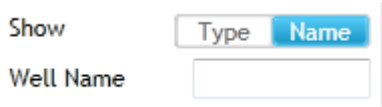
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

You must assign a well to a well type before you can assign it a well name.

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

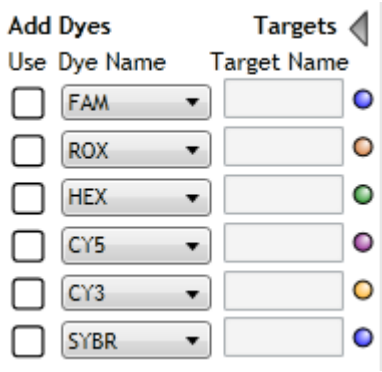
The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

5 Click **OK** in the message box to close it.

The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1 On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 Under **Add Dyes**, mark the Use check boxes for the dyes used for target detection in the selected wells.

3 If the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.

4 For the marked dyes, type a name into the adjacent Target Name field. The program assigns the target names to the selected wells.

   If you do not assign a target name to a marked dye, the program uses the dye name as the target name.

5 Repeat steps 1-4 for all wells included in use in the experiment.

6 (Optional) Select a new color to associate with a target:

   a Click the colored dot to right of the Target Name field.

   b In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Assign replicates

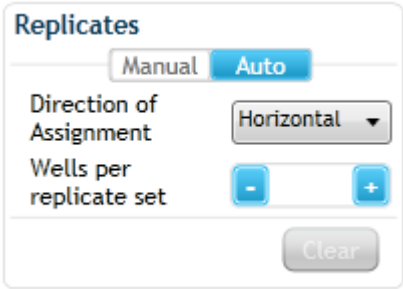
Replicates are wells that contain identical reaction components (repeats). You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1 On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.

2 In the Properties panel under **Replicates**, select **Auto**.



3 In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.

- Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
- Select **Vertical** if the replicate reactions will be arranged vertically in columns.

4 In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

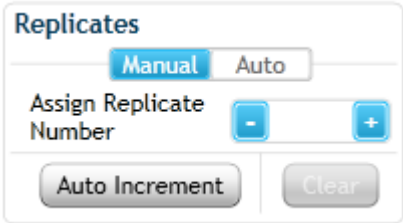
The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

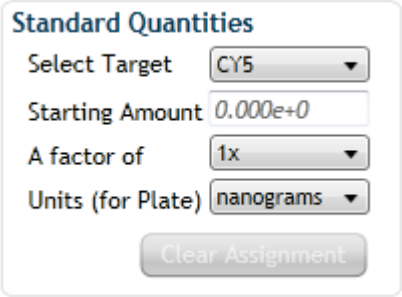
5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign quantities to Standard wells

In order to generate a standard curve from your data, you need to assign the initial template quantity to each Standard well.



To assign the standard quantities for a target in the Standard wells:

1 On the Plate Setup screen, select the Standard wells. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Standard Quantities**, select which target in the selected wells is the standard target (i.e., the target of known quantity in the template).

3 In the Starting Amount field, type in the quantity of the standard target present in the first replicate set of Standard wells. You will be asked to specify the units for this amount in step 5.

NOTE This quantity must be either the highest quantity or the lowest quantity in the dilution series of the standard template sample.

4 In the drop-down list labeled **A factor of**, select the dilution factor used to generate the dilution series of the standard template. For example, if each standard quantity is separated by a factor of 10, select **10x**. Negative dilution factors are used to specify a decrease in quantity from the starting amount while positive dilution factors specify an increase from the starting amount.

5 In the drop-down list labeled **Units (for Plate)**, select the units of the quantity entered in the Starting Amount field. Note that all Standard wells on the plate must use the same units.

To clear the assigned standard quantity from one or more wells:

- Select the well(s) and click **Clear**.

Assign plate properties for a Comparative Quantitation experiment

The program analyzes comparative quantitation data based on how the targets and well types are assigned in the Plate Setup screen. For a particular target-of-interest, the program measures the quantity of the target in an Unknown well relative to the level of that target in the Calibrator well.

To open the Plate Setup screen: When you create a new Comparative Quantitation experiment, you will automatically be directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

NOTE

In comparative quantitation, it is important for the amplification efficiencies of the target-of-interest and the normalizer target to be reproducible and, ideally, very similar. If you do not know the amplification efficiency for all of your targets, run a standard curve. See"Determining amplification efficiencies for the targets of interest and normalizer targets" on page 64 for more information.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment. See "Well types for Comparative Quantitation experiments" on page 66 for a description of the available well types.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1 and 2 for all well types to be included in the experiment.

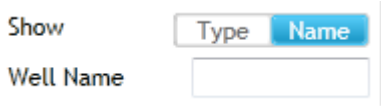
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

You must assign a well to a well type before you can assign it a well name.

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

   The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

   The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

   The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

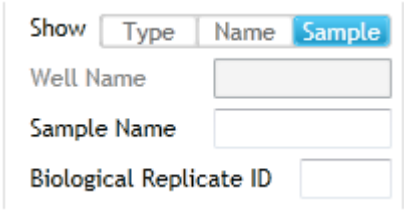
5 Click **OK** in the message box to close it.

   The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

Assign sample names and biological replicates

Once well types have been assigned, you can specify the sample in each well by assigning sample names. Each unique template sample included in the experiment can be assigned its own sample name. *If the normalizer target and the target-of-interest are being amplified in different wells, be sure to assign the same sample name to these wells so the data are normalized properly during analysis.*

For samples that are biological replicates, assign the same sample name to the wells but give the wells different biological replicate ID numbers to keep them differentiated. Biological replicates are template samples that were isolated independently but from biologically-identical sources (see "Including biological replicates in comparative quantitation" on page 65 for more information on biological replicates).



To assign sample names manually:

1. On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

   The Sample Name field becomes available for typing.

2. Select a group of wells that contain the same template sample or a group of wells containing samples that are biological replicates. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

3. In the Sample Name field, type in a name for the sample. Press **Enter**.

   The sample name appears at the top the selected wells.

   Sample names must not start with the number zero (0).

4. Repeat steps 2-3 for all samples to be included in the experiment.

To assign sample names in batch mode (using barcode reader or manual input):

1. If inputting sample names with a barcode reader, set up the barcode reader and barcodes.

   The barcodes correspond to the sample names to be inputted into the plate setup. Each time you read a barcode, it is inputted into the Aria software as the sample name for the selected well.

   a. Connect the barcode reader directly to the PC on which the Aria software is installed.

   b. Make sure the barcodes are within reach of the barcode scanner.

2 On the Plate Setup screen, assign wells to a well type before assigning sample names in batch mode.

3 Select all the wells to which you want to assign sample names. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

4 Right-click on the plate map. In the menu that opens, click **Input Sample Name in Batch Mode**.

   Next to **Show**, the Sample option is automatically selected and the cursor is in the Sample Name field.

   The first well among the group of selected wells is actively selected for input, as indicating by the flashing outline around the well.

5 Input the sample name for the actively selected well.

   - If inputting sample names using a barcode reader, scan the barcode corresponding to the sample name.
   - If inputting sample names manually, type the sample name then press **Enter**.

   The inputted sample name is assigned. The next well among the group of selected wells is now actively selected for input.

6 Repeat step 5 for all selected wells to be assigned a sample name.

7 After inputting the sample name for the last selected well, right-click on the plate map. In the menu that opens, clear the check box next to **Input Sample Name in Batch Mode**.

To assign biological replicate ID numbers:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

   The Biological Replicate ID field becomes available for typing.

2 Among a group of biological replicate wells, select the first sub-set of wells that require the same biological replicate ID. (A separate ID is to be assigned to each biological replicate sample within the group.)

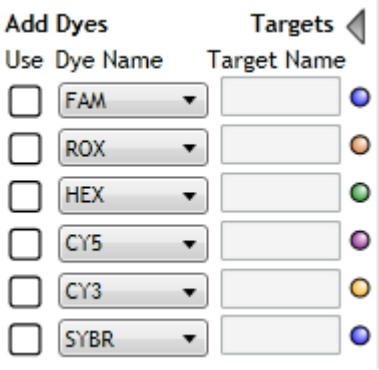
3 In the **Biological Replicate ID** field, type in a number to be assigned to the selected wells. Press **Enter**.

   The program adds the biological replicate ID number to the end of the sample name at the top of the selected well(s).

4 Repeat steps 2-3 for the remaining wells that require a biological replicate ID assignment.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1. On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Under **Add Dyes**, mark the Use check boxes for the dyes used for target detection in the selected wells.
3. If the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.
4. For the marked dyes, type a name into the adjacent Target Name field. The program assigns the target names to the selected wells.

   If you do not assign a target name to a marked dye, the program uses the dye name as the target name.
5. Repeat steps 1-4 for all wells included in use in the experiment.
6. (Optional) Select a new color to associate with a target:
   - **a** Click the colored dot to right of the Target Name field.
   - **b** In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Designate the normalizer

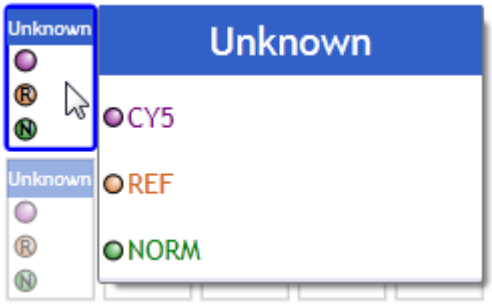
In order to normalize the quantity level of your target-of-interest to a normalizer target, you need to amplify the normalizer in both the Unknown wells and the Calibrator wells. You can amplify the normalizer in the same well as the target-of-interest (if the two targets are detected with spectrally distinct dyes) or you can amplify them in separate wells that contain the same template sample.

When you designate multiple normalizers in the same well, the program first calculates the normalized target-of-interest levels for each normalizer separately, and then calculates the geometric mean of all normalized values. The program then uses the geometric mean for the Relative Quantity calculations.

To assign a normalizer target:

1. On the Plate Setup screen, select the wells that will be used for amplification of the normalizer target.
2. In the **Normalizer Dye** drop-down list on the Properties panel, select the dye (or dyes) that is to be used for detection of the normalizer target.

   The program assigns the target name NORM to the selected wells and displays an N in the wells on the plate map.



Assign replicates

Replicates are wells that contain identical reaction components (repeats). You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1. On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.
2. In the Properties panel under **Replicates**, select **Auto**.
3. In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.
   - Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
   - Select **Vertical** if the replicate reactions will be arranged vertically in columns.
4. In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

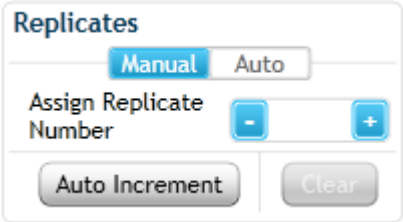
The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

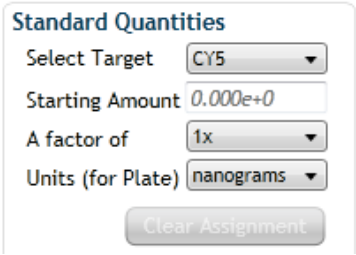
5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign quantities to Standard wells

If your Comparative Quantitation experiment includes running a set of standards in order to calculate amplification efficiencies of your targets, you need to assign the initial template quantity to each Standard well.



To assign the standard quantities for a target in the Standard wells:

1 On the Plate Setup screen, select the Standard wells. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Standard Quantities**, select which target in the selected wells is the standard target (i.e., the target of known quantity in the template).

3 In the Starting Amount field, type in the quantity of the standard target present in the first replicate set of Standard wells. You will be asked to specify the units for this amount in step 5.

NOTE This quantity must be either the highest quantity or the lowest quantity in the dilution series of the standard template sample.

4 In the drop-down list labeled **A factor of**, select the dilution factor used to generate the dilution series of the standard template. For example, if each standard quantity is separated by a factor of 10, select **10x**. Negative dilution factors are used to specify a decrease in quantity from the starting amount while positive dilution factors specify an increase from the starting amount.

5 In the drop-down list labeled **Units (for Plate)**, select the units of the quantity entered in the Starting Amount field. Note that all Standard wells on the plate must use the same units.

To clear the assigned standard quantity from one or more wells:

- Select the well(s) and click **Clear**.

Assign plate properties for an Allele Discrimination DNA Binding Dye experiment

The controls on Plate Setup screen's Properties panel allow you to create a customized plate setup for experiments of the type *Allele Discrimination - DNA Binding Dye Including High Resolution Melt*.

To open the Plate Setup screen: When you create a new Allele Discrimination experiment, you will automatically be directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment. See "Well types for Allele Discrimination - DNA Binding Dye experiments" on page 68 for a description of the available well types.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1 and 2 for all well types to be included in the experiment.

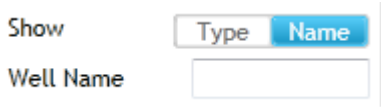
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

You must assign a well to a well type before you can assign it a well name.

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

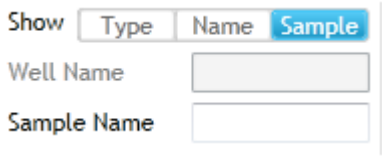
The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

5 Click **OK** in the message box to close it.

The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

Assign sample names

After you assign well types, you can specify the sample in each well by assigning sample names. Each unique template sample included in the experiment can be assigned its own sample name.



To assign sample names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

The Sample Name field becomes available for typing.

2 Select all the wells in the plate map that contain the same template sample. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

3 In the Sample Name field, type in a name for the sample. Press **Enter**.

The sample name appears at the top of the selected wells.

Sample names must not start with the number zero (0).

4 Repeat steps 2-3 for all samples to be included in the experiment.

To assign sample names in batch mode (using barcode reader or manual input):

1. If inputting sample names with a barcode reader, set up the barcode reader and barcodes.

   The barcodes correspond to the sample names to be inputted into the plate setup. Each time you read a barcode, it is inputted into the Aria software as the sample name for the selected well.

   a. Connect the barcode reader directly to the PC on which the Aria software is installed.

   b. Make sure the barcodes are within reach of the barcode scanner.

2. On the Plate Setup screen, assign wells to a well type before assigning sample names in batch mode.

3. Select all the wells to which you want to assign sample names. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

4. Right-click on the plate map. In the menu that opens, click **Input Sample Name in Batch Mode**.

   Next to **Show**, the Sample option is automatically selected and the cursor is in the Sample Name field.

   The first well among the group of selected wells is actively selected for input, as indicating by the flashing outline around the well.

5. Input the sample name for the actively selected well.

   - If inputting sample names using a barcode reader, scan the barcode corresponding to the sample name.
   - If inputting sample names manually, type the sample name then press **Enter**.

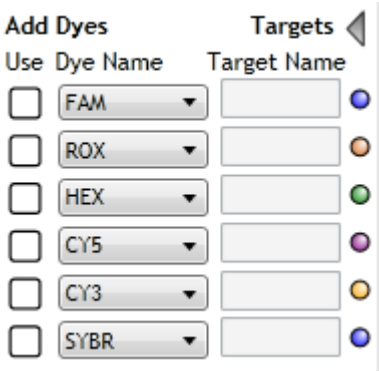
   The inputted sample name is assigned. The next well among the group of selected wells is now actively selected for input.

6. Repeat step 5 for all selected wells to be assigned a sample name.

7. After inputting the sample name for the last selected well, right-click on the plate map. In the menu that opens, clear the check box next to **Input Sample Name in Batch Mode**.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1. On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Under **Add Dyes**, mark the Use check box for the dye used for target detection in the selected wells.
3. If the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.
4. For the marked dye, type a name into the adjacent Target Name field. The program assigns the target name to the selected wells.

   If you do not assign a target name, the program uses the dye name as the target name.
5. Repeat steps 1-4 for all wells included in use in the experiment.

6 (Optional) Select a new color to associate with a target:

a Click the colored dot to right of the Target Name field.

b In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Assign replicates

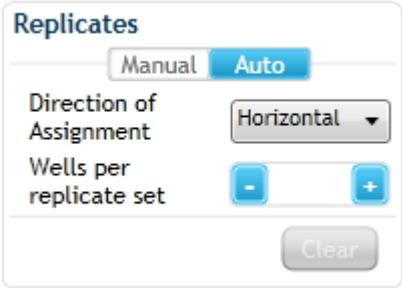
Replicates are wells that contain identical reaction components (repeats). You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1 On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.

2 In the Properties panel under **Replicates**, select **Auto**.



3 In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.

- Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
- Select **Vertical** if the replicate reactions will be arranged vertically in columns.

4 In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

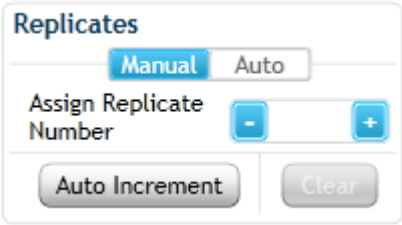
The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign plate properties for an Allele Discrimination Fluorescence Probe experiment

The controls on Plate Setup screen's Properties panel allow you to create a customized plate setup for experiments of the type *Allele Discrimination - Fluorescence Probe*.

To open the Plate Setup screen: When you create a new Allele Discrimination experiment, you will automatically be directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment. See "Well types for Allele Discrimination - Fluorescence Probe experiments" on page 70 for a description of the available well types.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1 through 2 for all well types to be included in the experiment.

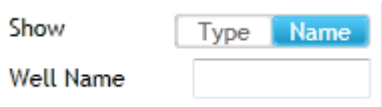
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

You must assign a well to a well type before you can assign it a well name.

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

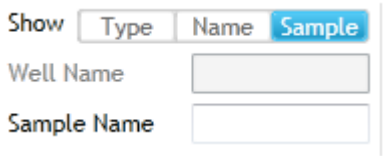
The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

5 Click **OK** in the message box to close it.

The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

Assign sample names

After you assign well types, you can specify the sample in each well by assigning sample names. Each unique template sample included in the experiment can be assigned its own sample name. If the two alleles are being amplified in separate wells, the sample name is used to associate the wells amplifying Allele A with the wells amplifying Allele B from the same template.



To assign sample names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

The Sample Name field becomes available for typing.

2 Select all the wells in the plate map that contain the same template sample. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

3 In the Sample Name field, type in a name for the sample. Press **Enter**.

The sample name appears at the top of the selected wells.

Sample names must not start with the number zero (0).

4 Repeat steps 2-3 for all samples to be included in the experiment.

To assign sample names in batch mode (using barcode reader or manual input):

1. If inputting sample names with a barcode reader, set up the barcode reader and barcodes.

   The barcodes correspond to the sample names to be inputted into the plate setup. Each time you read a barcode, it is inputted into the Aria software as the sample name for the selected well.

   a. Connect the barcode reader directly to the PC on which the Aria software is installed.

   b. Make sure the barcodes are within reach of the barcode scanner.

2. On the Plate Setup screen, assign wells to a well type before assigning sample names in batch mode.

3. Select all the wells to which you want to assign sample names. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

4. Right-click on the plate map. In the menu that opens, click **Input Sample Name in Batch Mode**.

   Next to **Show**, the Sample option is automatically selected and the cursor is in the Sample Name field.

   The first well among the group of selected wells is actively selected for input, as indicating by the flashing outline around the well.

5. Input the sample name for the actively selected well.

   - If inputting sample names using a barcode reader, scan the barcode corresponding to the sample name.
   - If inputting sample names manually, type the sample name then press **Enter**.

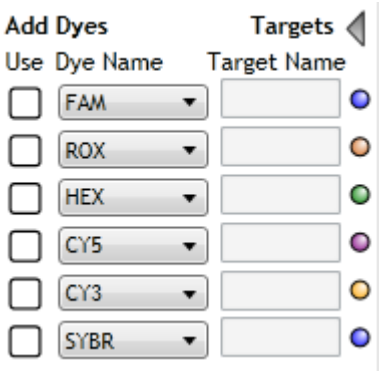
   The inputted sample name is assigned. The next well among the group of selected wells is now actively selected for input.

6. Repeat step 5 for all selected wells to be assigned a sample name.

7. After inputting the sample name for the last selected well, right-click on the plate map. In the menu that opens, clear the check box next to **Input Sample Name in Batch Mode**.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1. On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Under **Add Dyes**, mark the Use check box for the dye used for target detection in the selected wells.
3. If the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.
4. For the marked dye, type a name into the adjacent Target Name field. The program assigns the target name to the selected wells.

   If you do not assign a target name, the program uses the dye name as the target name.
5. Repeat steps 1-4 for all wells included in use in the experiment.

6 (Optional) Select a new color to associate with a target:

a Click the colored dot to right of the Target Name field.

b In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Assign Alleles

Use the drop-down lists under **Allele** to indicate which target is to be designated as Allele A and which target is Allele B.

To assign alleles:

1 In the **Dye/Target Name** drop-down list for Allele A, select the target that represents allele A.

2 In the **Dye/Target Name** drop-down list for Allele B, select the target that represents allele B.

Assign replicates

Replicates are wells that contain identical reaction components (repeats). You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1 On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.

2 In the Properties panel under **Replicates**, select **Auto**.

3 In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.

- Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
- Select **Vertical** if the replicate reactions will be arranged vertically in columns.

4 In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

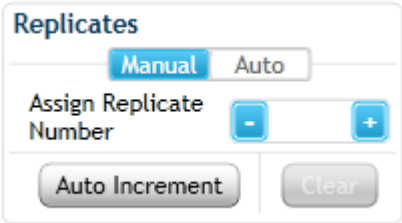
The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign plate properties for a User Defined experiment

The controls on Plate Setup screen's Properties panel allow you to create a customized plate setup for experiments of the type *User Defined*.

To open the Plate Setup screen: Click **Plate Setup** in the Experiment Area panel on the left side of the screen.

Assign well types

Use the **Well Types** drop-down list to assign well types to all the wells used in the experiment.

To assign well types:

1. On the Plate Setup screen, select all the wells in the plate map that are of the same type. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. Select a well type from the **Well Types** drop-down list in the Properties panel.

   When the **Show** setting on the Properties panel is set to **Type**, the well type appears at the top of the selected wells.
3. Repeat steps 1 and 2 for all well types to be included in the experiment.

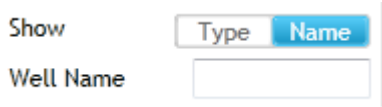
Assign well names

After you assign wells to a well type you can, if desired, assign custom well names. Well names can be assigned manually or they can be imported from an Excel spreadsheet or comma-delimited text file.

Assign wells to a well type before assigning well names.

To assign well names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Name**.



The Well Name field becomes available for typing.

2 Select all the wells in the plate map that you want to assign to the same well name. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

You must assign a well to a well type before you can assign it a well name.

3 In the Well Name field, type the well name for the selected wells. Press **Enter**.

The Well Name appears at the top of the selected wells.

Well names must not start with the number zero (0).

4 Repeat steps 2-3 for all well names that you want to assign.

To assign well names by importing an Excel spreadsheet or comma-delimited text file:

1 Create the Excel spreadsheet or comma-delimited text file and save it to a location that is accessible while working in the Aria software.

The file must be formatted as shown below with well IDs on the left and well names on the right. The well IDs may appear in any order but must use the syntax A1–H12.

Excel spreadsheet

	A	B
1	Well ID	Well Name
2	A1	Reference RNA (1)
3	B1	Reference RNA (10)
4	C1	Reference RNA (100)

Text file

```
A1,Reference RNA (1)
B1,Reference RNA (10)
C1,Reference RNA (100)
```

2 On the Plate Setup screen, right-click on the plate map. In the menu that opens, click **Import Well Name**.

The Open dialog box opens.

3 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Text, Excel Workbook, or Excel 97-2003 Workbook).

4 Browse to the file created in step 1. Select the file and click **Open**.

The software imports the well names from the file into the experiment. A message box opens notifying you that the import was successful.

5 Click **OK** in the message box to close it.

The plate map displays the imported well names. For any wells that have not yet been assigned a well type, the well name remains blanks until a well type is assigned.

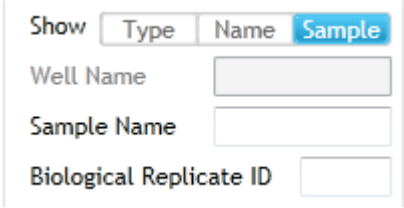
Assign sample names and biological replicates

Once well types have been assigned, you can specify the sample in each well by assigning sample names. Each unique template sample included in the experiment can be assigned its own sample name.

If your experiment is designed for comparative quantitation, and the normalizer target and the target-of-interest are being amplified in different wells, be sure to assign the same sample name to these wells so the data are normalized properly during analysis.

If your experiment is designed for allele determination, and the two alleles are being amplified in separate wells, the sample name is used to associate the wells amplifying Allele A with the wells amplifying Allele B from the same template.

For samples that are biological replicates, assign the same sample name to the wells but give the wells different biological replicate ID numbers to keep them differentiated. Biological replicates are template samples that were isolated independently but from biologically-identical sources (see "Including biological replicates in comparative quantitation" on page 65 for more information on biological replicates).



To assign sample names manually:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

   The Sample Name field becomes available for typing.

2 Select a group of wells that contain the same template sample or a group of wells containing samples that are biological replicates. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

3 In the Sample Name field, type in a name for the sample. Press **Enter**.

   The sample name appears at the top the selected wells.

   Sample names must not start with the number zero (0).

4 Repeat steps 2-3 for all samples to be included in the experiment.

To assign sample names in batch mode (using barcode reader or manual input):

1 If inputting sample names with a barcode reader, set up the barcode reader and barcodes.

   The barcodes correspond to the sample names to be inputted into the plate setup. Each time you read a barcode, it is inputted into the Aria software as the sample name for the selected well.

   a Connect the barcode reader directly to the PC on which the Aria software is installed.

   b Make sure the barcodes are within reach of the barcode scanner.

2 On the Plate Setup screen, assign wells to a well type before assigning sample names in batch mode.

3 Select all the wells to which you want to assign sample names. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

4 Right-click on the plate map. In the menu that opens, click **Input Sample Name in Batch Mode**.

   Next to **Show**, the Sample option is automatically selected and the cursor is in the Sample Name field.

   The first well among the group of selected wells is actively selected for input, as indicating by the flashing outline around the well.

5 Input the sample name for the actively selected well.

   - If inputting sample names using a barcode reader, scan the barcode corresponding to the sample name.
   - If inputting sample names manually, type the sample name then press **Enter**.

   The inputted sample name is assigned. The next well among the group of selected wells is now actively selected for input.

6 Repeat step 5 for all selected wells to be assigned a sample name.

7 After inputting the sample name for the last selected well, right-click on the plate map. In the menu that opens, clear the check box next to **Input Sample Name in Batch Mode**.

To assign biological replicate ID numbers:

1 On the Properties panel of the Plate Setup screen, next to **Show**, select **Sample**.

   The Biological Replicate ID field becomes available for typing.

2 Among a group of biological replicate wells, select the first sub-set of wells that require the same biological replicate ID. (A separate ID is to be assigned to each biological replicate sample within the group.)

3 In the **Biological Replicate ID** field, type in a number to be assigned to the selected wells. Press **Enter**.

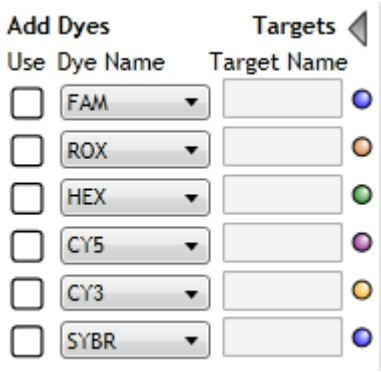
   The program adds the biological replicate ID number to the end of the sample name at the top of the selected well(s).

4 Repeat steps 2-3 for the remaining wells that require a biological replicate ID assignment.

Assign dyes/targets

Use the check boxes, drop-down lists, and fields under **Assign Dyes/Targets** to indicate which dyes are being used in each well and what

target each dye is detecting. Dye assignments are required, but target name assignments are optional. *If different wells will be using the same dye to detect different targets, assigning a unique name to each target enables the program to treat each target separately during analysis.*



To assign dyes and target names:

1 On the Plate Setup screen, select all the wells in the plate map that contain the same target. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 Under **Add Dyes**, mark the Use check box for the dye used for target detection in the selected wells.

3 If the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.

4 For the marked dye, type a name into the adjacent Target Name field. The program assigns the target name to the selected wells.

   If you do not assign a target name, the program uses the dye name as the target name.

5 Repeat steps 1-4 for all wells included in use in the experiment.

6 (Optional) Select a new color to associate with a target:

   a Click the colored dot to right of the Target Name field.

   b In the selection box that opens, click the desired color, or click **Advanced** for more color options.

Select a reference dye

You can include a reference dye (e.g., ROX dye) to normalize the fluorescence signal of the reporter dye.

To assign a reference dye:

- On the Plate Setup screen, select the reference dye from the **Reference Dye** drop-down list in the Properties panel.

  The program assigns the target name REF to all wells in use in the experiment and displays an R (Ⓡ) in the wells of the plate map to indicate that the well contains a reference dye.

Designate the normalizer

In order to normalize the quantity level of your target-of-interest to a normalizer target, you need to amplify the normalizer in both the Unknown wells and the Calibrator wells. You can amplify the normalizer in the same well as the target-of-interest (if the two targets are detected with spectrally distinct dyes) or you can amplify them in separate wells that contain the same template sample.

To assign a normalizer target:

1. On the Plate Setup screen, select the wells that will be used for amplification of the normalizer target.
2. In the **Normalizer Dye** drop-down list on the Properties panel, select the dye that is to be used for detection of the normalizer target.

   The program assigns the target name NORM to the selected wells and displays an N in the plate map.

Assign Alleles

Use the drop-down lists under **Allele** to indicate which target is to be designated as Allele A and which target is Allele B.

To assign alleles:

1 In the **Dye/Target Name** drop-down list for Allele A, select the target that represents allele A.

2 In the **Dye/Target Name** drop-down list for Allele B, select the target that represents allele B.

Assign replicates

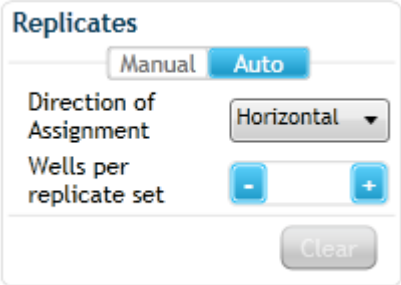
Replicates are wells that contain identical reaction components (repeats). You can assign replicates using the *Manual* option or the *Auto* option. When you designate replicate wells on the Plate Setup screen, you can set the analysis criteria to average results from those wells or treat the wells separately.

NOTE When assigning replicates, if you see a flashing red warning icon in the Properties panel next to Replicates, you have an invalid replicate set on the plate. To be valid, all the wells of a set must be of the same well type and have the same target assignments. Hover your cursor over the warning icon to view specific information on why the program has called a replicate set invalid.

To assign replicates with the Auto option:

1 On the Plate Setup screen, select all the wells on the plate map that have the same number of wells per replicate set.

2 In the Properties panel under **Replicates**, select **Auto**.



3 In the **Direction of Assignment** drop-down list, specify how the replicate wells are arranged on the plate.

- Select **Horizontal** if the replicate reactions will be arranged horizontally in rows.
- Select **Vertical** if the replicate reactions will be arranged vertically in columns.

4 In the **Wells per replicate set** field, type the number of replicate wells per reaction, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

5 If desired, make adjustment to the auto-assignments in any of the wells of the plate by switching to the **Manual** option and manually assigning replicate numbers to those wells.

To manually assign replicates:

1 On the Plate Setup screen, select a set of wells that are part of the same replicate set. Make sure that the selected wells are of the same well type and contain identical targets. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).

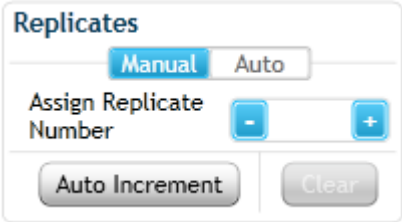
3 In the **Assign Replicate Number** field, type in the desired replicate number for the selected wells, or click the +/- buttons to enter the desired number.

The assigned replicate number appears in each selected well.

To assign replicates using Auto Increment:

1 On the Plate Setup screen, assign well types as needed for your experiment.

2 In the Properties panel under **Replicates**, select **Manual** (if not already selected).



3 Click **Auto Increment**.

When you hover your cursor anywhere on the plate map, an icon of the number 1 appears next to the cursor.

4 With the cursor, click and drag across the group of wells that you want to assign as replicate number 1.

The program assigns the wells to replicate number 1, and the icon next to the cursor changes to a number 2.

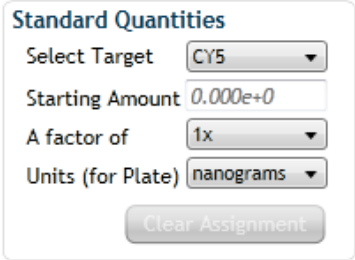
5 Click and drag across the group of wells that you want to assign as replicate number 2.

The program assigns the wells to replicate number 2, and the icon next to the cursor changes to a number 3.

6 Continue assigning replicate numbers for the remainder of the plate. When finished, click **Auto Increment** to turn off the Auto Increment function.

Assign quantities to Standard wells

In order to generate a standard curve from your data, you need to assign the initial template quantity to each Standard well.



To assign the standard quantities for a target in the Standard wells:

1. On the Plate Setup screen, select the Standard wells. (For instructions on well selection, see "Select and view wells in the plate map" on page 79.)
2. In the Properties panel under **Standard Quantities**, select which target in the selected wells is the standard target (i.e., the target of known quantity in the template).
3. In the Starting Amount field, type in the quantity of the standard target present in the first replicate set of Standard wells. You will be asked to specify the units for this amount step 5.

NOTE This quantity must be either the highest quantity or the lowest quantity in the dilution series of the standard template sample.

4. In the drop-down list labeled **A factor of**, select the dilution factor used to generate the dilution series of the standard template. For example, if each standard quantity is separated by a factor of 10, select **10x**. Negative dilution factors are used to specify a decrease in quantity from the starting amount while positive dilution factors specify an increase from the starting amount.
5. In the drop-down list labeled **Units (for Plate)**, select the units of the quantity entered in the Starting Amount field. Note that all Standard wells on the plate must use the same units.

To clear the assigned standard quantity from one or more wells:

- Select the well(s) and click **Clear**.

7
Setting Up the Thermal Profile

Agilent Technologies

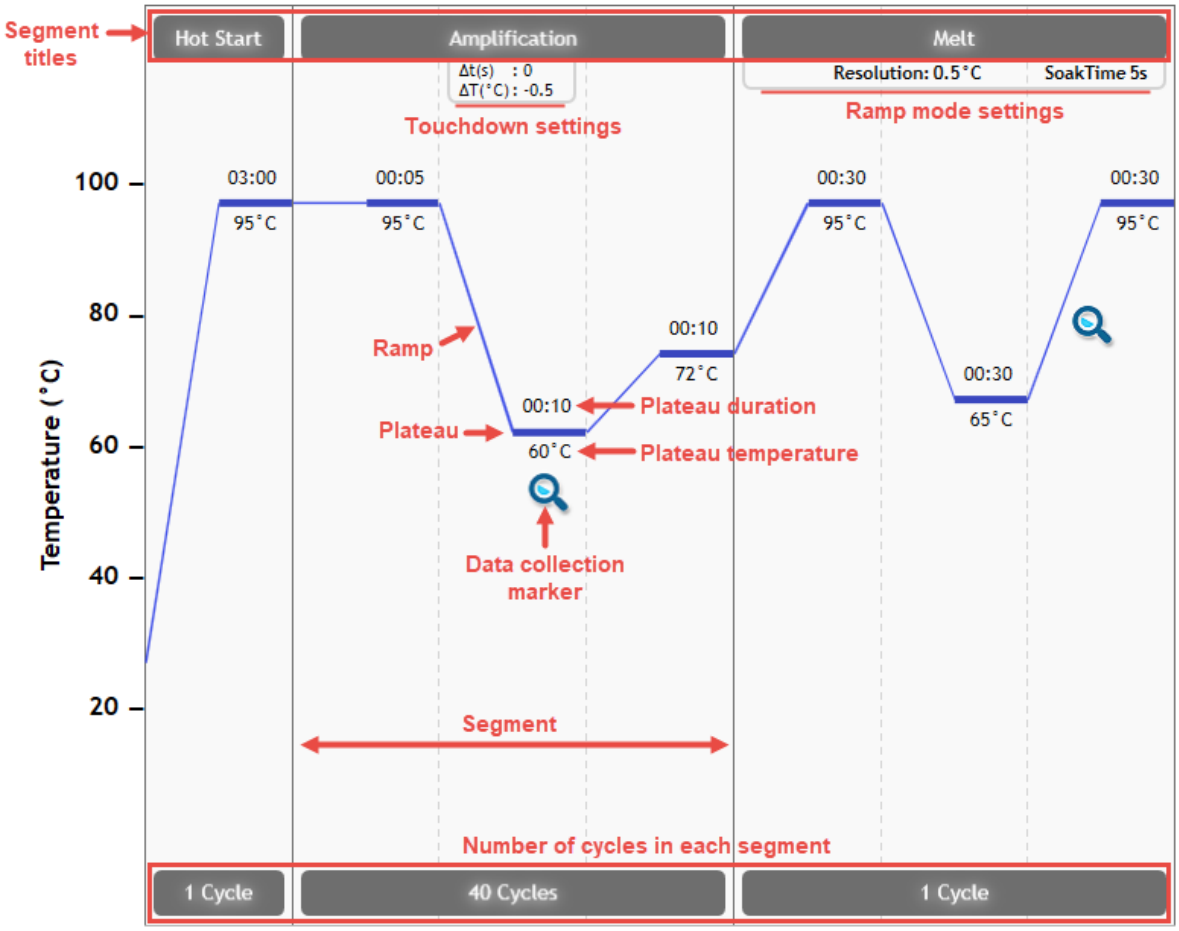
Set up the thermal profile

In the center of the Thermal Profile screen is a visual representation of the temperature cycling program that directs the instrument to incubate samples at specific temperatures for specific times.

To open the Thermal Profile screen: With an experiment or project file open, click **Thermal Profile** in the Experiment Area panel on the left side of the screen.

When you create a new experiment based on experiment type, a default thermal profile is automatically assigned by the program. The default thermal profile is one that is typical for the needs of that experiment type. (The exception is User Defined experiments. Instead of assigning a default thermal profile, the program prompts you to select a thermal profile from a set of defaults). If you created the experiment from a template, the default thermal profile is that of the template. You can also import a thermal profile from an existing experiment (see "Import a thermal profile" on page 146). In any pre-run experiment, you can edit the thermal profile to fit your needs (see "Edit the thermal profile" on page 147).

Elements of a Thermal Profile



Plateau

A plateau is a temperature held by the instrument for a specified duration. It is represented with a solid horizontal line in the thermal profile with the duration of the plateau displayed directly above the line and the temperature of the plateau displayed directly below the line. The valid range for a plateau duration is 1 second to greater than 18 hours. The valid temperature range is 25.0° (ambient) to 99.9°C.

NOTE During plateaus with a temperature lower than 40°C, the heated lid of the thermal block remains off, even when the instrument Hot Top Setting is enabled.

Ramp

A ramp is the transition between two plateaus. When you add a new plateau to the thermal profile, the program automatically adds ramps leading to and from the new plateau temperature. Likewise, when you delete a segment or plateau, the program removes the associated ramps to connect adjacent plateaus.

Segment and Number of Cycles

A segment is a group of plateaus and the intervening ramps that has been set to cycle at least one time. Segments are delineated in the thermal profile by solid vertical lines. A cycle is one pass through a segment. The cycle number for the segment is displayed at the bottom of the thermal profile.

The table below lists the types of segments and the maximum number of cycles allowed for each segment type.

Table: Available segment types

Segment Type	Description	Maximum # of cycles
Amp. 2-Step	Amplification using 2 plateaus: one for denaturation and one for annealing/extension	255*
Amp. 3-Step	Amplification using 3 plateaus: one for denaturation, one for annealing, and one for extension	255*
Melt	Melt/dissociation of PCR products	1
Hot Start	Hot start activation of PCR enzyme	1
UDG (DNA)	Uracil-DNA glycosylation reaction; in a reaction in which UNG (uracil-N-glycosylase) was used to digest amplicon carryover from a previous PCR amplification, you can add this segment to the beginning of the thermal profile to heat inactivate the UNG enzyme	1
RT	Reverse transcription of RNA into DNA	1

* The thermal profile cannot include more than 5 segments that have >50 cycles.

Data Collection Marker

A data collection marker is a magnifying glass icon that indicates the points designated for fluorescence data collection by the instrument. For data collection markers on a plateau, the instrument collects fluorescence data at the end of the plateau duration. The minimum duration for a plateau that includes data collection is 3 seconds. For data collection markers on a melt ramp (only melt segment ramps can accept collection markers), the instrument collects fluorescence data throughout the ramp period.

Touchdown Settings

Touchdown settings allow you to incrementally change the duration or temperature of a plateau with each cycle within the segment. Touchdown settings can be applied to a plateau in an amplification segment (see "Edit touchdown settings (plateaus in amplification segments only)" on page 152). When an amplification segment includes a plateau with touchdown properties, the settings are displayed at the top of the segment in the thermal profile.

Ramp Mode Settings

Ramp mode settings are applicable to melt segments that include data collection (see "Edit ramp properties (melt segments only)" on page 154). These settings include the resolution and soak time, and they are displayed at the top of the melt segment in the thermal profile.

Import a thermal profile

From the Thermal Profile screen, you can import a thermal profile setup from an existing pre-run or post-run experiment file or template file. Once imported, you can edit the thermal profile as desired (see "Edit the thermal profile" on page 147).

The experiment or template used for import must be the same experiment type as the current experiment and have a valid thermal profile (review "Elements of a Thermal Profile" on page 144 for information on restrictions).

To import a thermal profile setup:

1 Right-click on the thermal profile display. In the menu that opens, click **Import Thermal Profile Setup**.

   The Open dialog box opens.

2 At the bottom of the dialog box, use the drop-down list to select the appropriate file type (Experiment Files or Template Files).

3 Browse to the file from which you want to import the thermal profile setup. Select the file and click **Open**. The software imports the thermal profile into the experiment.

   A message box opens notifying you that the import was successful.

4 Click **OK** in the message box to close it.

   The thermal profile display is updated to the imported setup.

Edit the thermal profile

From the Thermal Profile screen, you can edit any element of the thermal profile, including adding and deleting segments and plateaus and changing plateau temperatures and durations.

Edit plateaus

To edit a plateau temperature:

1 In the display, click directly on the plateau temperature that you want to edit.

   The temperature becomes an editable field.

2 Type the desired temperature into the field, or click the +/- buttons until you reach the desired temperature.

3 Press **Enter**, or click anywhere outside of the field.

   You can also adjust plateau temperature by clicking on the plateau and dragging it up or down to a new temperature.

To edit a plateau duration:

1 In the display, click directly on the plateau duration that you want to edit (durations are displayed as *minutes:seconds*).

   The duration becomes an editable field.

2 Type the desired duration for the plateau into the field, or click the +/- buttons until you reach the desired duration.

When typing in the duration, you can either type the number of seconds and let the program convert it to the *minutes:seconds* format (e.g., an entry of “120” would be converted to “2:00”), or you can type in the duration using the *minutes:seconds* format.

3 Press **Enter**, or click anywhere outside of the field.

To add a plateau:

1 On the display, locate the segment to which you want to add a plateau.

2 Within that segment, select the existing plateau that needs to precede the new plateau. (To select a plateau, click directly on it. The plateau becomes red in the display.)

If you need the new plateau to be the first plateau in the segment, skip step 2 and go directly to step 3.

3 Right-click within the same segment.

A short-cut menu opens.

4 Click **Add Plateau**.

The program adds a new plateau immediately after the selected plateau (or to the start of the segment if no plateau is selected). The new plateau has a 25°C temperature and a 30-second duration. The program allows up to 20 plateaus per segment and up to 150 total plateaus in a thermal profile.

5 Edit the temperature and duration of the new plateau as needed. See the instructions above (*To edit a plateau temperature* and *To edit a plateau duration*).

To remove a plateau:

1 On the display, select the plateau that you want to remove. (To select a plateau, click directly on it. The plateau becomes red in the display.)

2 Right-click on the segment containing the selected plateau.

A short-cut menu opens.

3 Click **Remove Plateau**.

The program deletes the selected plateau from the thermal profile.

NOTE For instructions on editing the touchdown properties of a plateau in an amplification segment, see "Edit touchdown settings (plateaus in amplification segments only)" on page 152.

Edit segments

To add a segment:

1. On the display, hover your cursor over an existing segment that will be adjacent to the new segment.
2. Click the + icon (shown below) on either the left side or right side of the existing segment.

   Clicking the icon on the left adds the new segment just before the existing segment. Clicking the icon on the right adds the new segment just after the existing segment.

The program opens a placeholder for the new segment (shown below) listing the available segment types.

3 In the placeholder, click the type of segment that you want to add.

The program adds the new segment to the thermal profile. The program allows up to 20 segments in a thermal profile.

Refer to the table in "Segment and Number of Cycles" for a description of each available segment type.

4 Edit the plateaus or number of cycles for the segment as desired.

To remove a segment:

1 On the display, hover your cursor over the segment that you want to remove.

   An X appears at the top of the segment to the right of the segment name.

2 Click the X.

   The program deletes the segment from the thermal profile.

To move a segment:

1 Click and drag the segment that you want to move to the left or right.

   The purple line indicates where in the thermal profile you have dragged the segment.

2 When the purple line is in the desired location, release the left mouse button to drop the segment into that position.

To change the number of steps in an amplification segment:

1 On the display, right-click on the amplification segment.

   A short-cut menu opens.

2 If you want to change from a 3-step amplification to a 2-step amplification, click **Change to Amp. 2-Step**. If you want to change from a 2-step amplification to a 3-step amplification, click **Change to Amp. 3-Step**.

   The program adjusts the number of plateaus in the thermal profile according to your selection.

   A 3-step amplification segment has 3 plateaus: one for denaturation, one for annealing, and one for extension. A 2-step amplification segment has only 2 plateaus: one for denaturation and a combined annealing/extension plateau.

To change the number of cycles for an Amplification segment:

1 On the display, click directly on the number of cycles shown at the bottom of the segment.

   The cycle number becomes an editable field.

2 Type the desired number of cycles into the field, or click the +/- buttons until you reach the desired number.

3 Press **Enter**, or click anywhere outside of the field.

Edit data collection markers

To add a data collection marker:

1. On the display, hover the cursor over the plateau or ramp where you want to add a new data collection marker.

   A grayed out image of a data collection marker appears on the display.

2. Click the image of the data collection marker.

   The program adds the data collection marker to the thermal profile.

   Only melt segments can have a data collection marker assigned to a ramp.

   You can add up to 20 data collection markers to an amplification segment.

To remove a data collection marker:

- On the display, click directly on the data collection marker that you want to remove.

  The program removes the data collection marker from the thermal profile.

Edit touchdown settings (plateaus in amplification segments only)

1. On the display, locate the amplification segment that contains the plateau to be edited. Make sure that the segment has already been assigned the desired number of cycles.

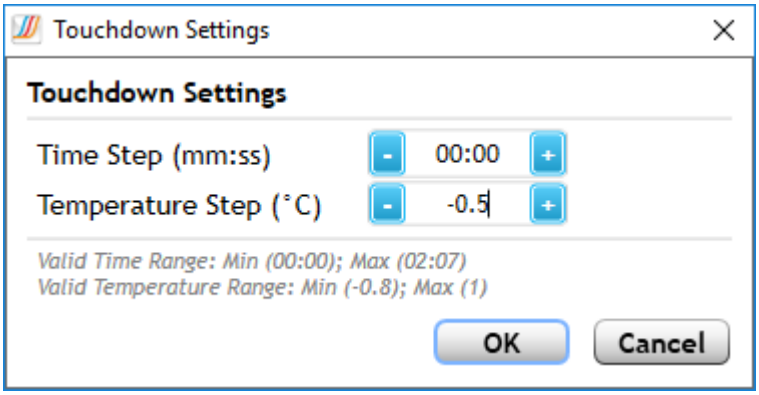
   The number of cycles assigned to the segment impacts the touchdown settings. See the note, Best practices for touchdown PCR.

2. Select the desired plateau within the amplification segment. (To select a plateau, click directly on it. The plateau becomes red in the display.)
3. Right-click on the segment containing the selected plateau.

   A short-cut menu opens.

4. Click **Touchdown Settings**.

The Touchdown Settings dialog box opens.



5 In the **Time Step (mm:ss)** field, add an increment to the duration of the plateau. The permitted range for the selected plateau (called the *Valid Time Range*) is displayed near the bottom of the Touchdown Settings dialog box. This range is based on the initial duration of the plateau and the number of cycles assigned to the segment. The maximum value for the range is designed to ensure that by the last cycle, the duration of the plateau does not exceed the maximum allowed duration.

   During the run, the duration of the plateau increases with each cycle by the specified number of minutes and seconds (mm:ss). For example, enter a Time Step of 00:05 to increase the duration of the plateau by an additional 5 seconds with each cycle.

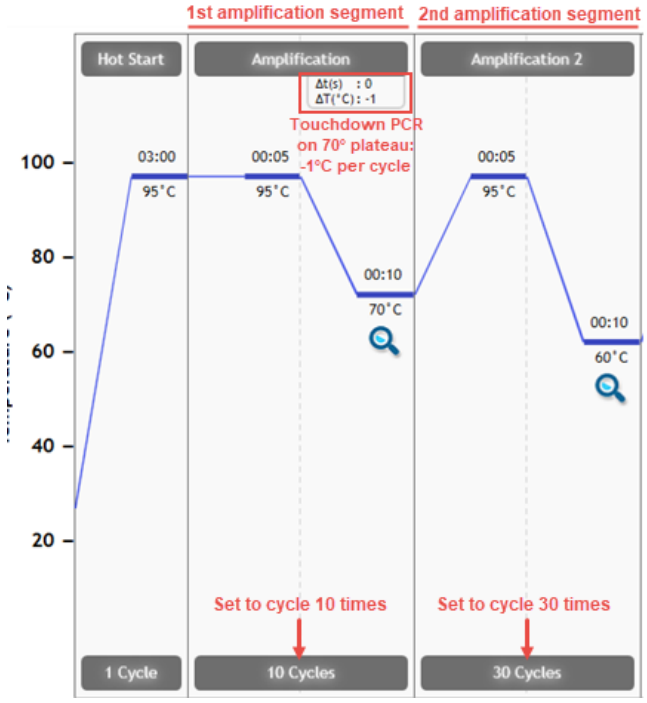
6 In the **Temperature Step (°C)** field, add an increment to the temperature of the plateau. The permitted range for the selected plateau (called the *Valid Temperature Range*) is displayed near the bottom of the Touchdown Settings dialog box. This range is based on the initial temperature of the plateau and the number of cycles assigned to the segment. The range values are designed to ensure that by the last cycle, the temperature of the plateau does not exceed the maximum allowed temperature of 99.9°C, or fall below the minimum allowed temperature of 25°C.

   During the run, the temperature of the plateau increases (for positive numbers) or decreases (for negative numbers) with each cycle by the specified number of degrees. For example, enter a Temperature Step of –0.5 to decrease the temperature of the plateau by 0.5°C with each cycle.

7 Click **OK** to close the dialog box and save your changes.

Best practices for touchdown PCR

If touchdown PCR is only required until a particular plateau duration or plateau temperature is reached, set up the thermal profile to include two amplification segments, and only program the first amplification segment to include touchdown settings. For example, if the desired thermal profile has 40 amplification cycles, with an initial annealing temperature of 70°C and a final annealing temperature of 60°C, include two amplification segments in the thermal profile. In the first amplification segment, set the segment to cycle 10 times. Include an annealing plateau of 70°C, and to this plateau, add touchdown PCR settings with a temperature step of -1°C so that the plateau temperature is 61°C during the 10th cycle. In the second amplification segment, set the segment to cycle 30 times. Include an annealing plateau of 60°C and do not add any touchdown PCR settings.



Edit ramp properties (melt segments only)

If you are collecting data during a ramp within a melt or high resolution melt segment, you can edit the resolution and the soak time. The

resolution sets the temperature increments of the ramp. The soak time is the length of time that the instrument holds each incremental temperature during the ramp.

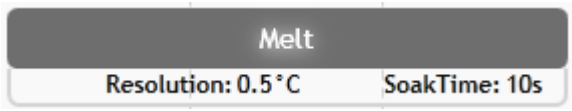
NOTE

Melt segments can have only one data collection marker.

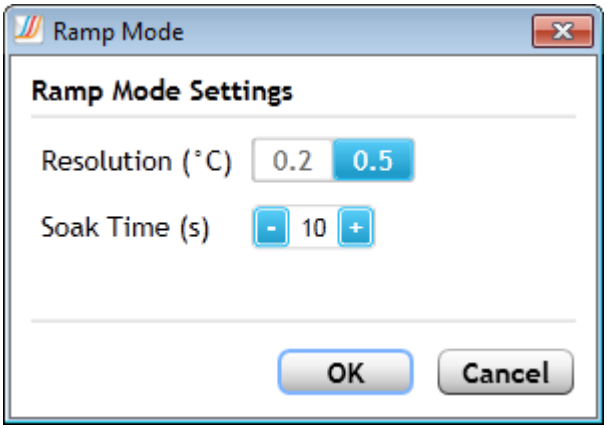
You can only change the ramp resolution if the experiment is an Allele Discrimination - DNA Binding Dye experiment or a User Defined experiment.

To change the resolution of a ramp:

1 On the melt segment, click the area below the segment name that displays the resolution and soak time.



The Ramp Mode dialog box opens.



2 Next to **Resolution (°C)**, select **0.2** to set the ramp to 0.2°C increments, or select **0.5** to set the ramp to 0.5°C increments.

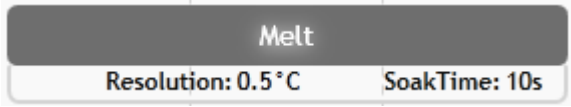
A resolution of 0.2 yields a high resolution melt curve.

3 Click **OK** to close the dialog box and save your changes.

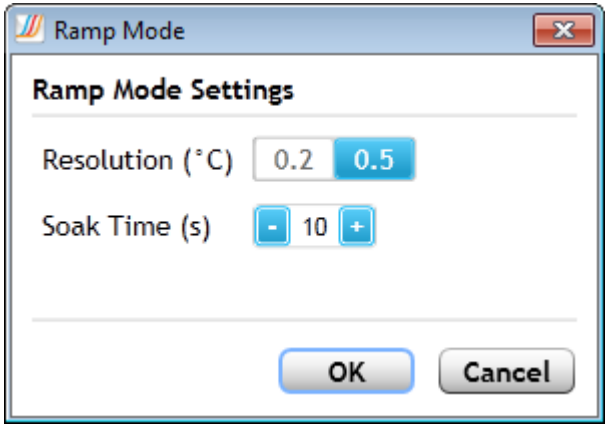
Melt segments with a resolution of 0.2 are named *High Resolution Melt*. Melt segments with a resolution of 0.5 are named *Melt*.

To change the soak time for a ramp:

1 On the melt segment, click the area below the segment name that displays the resolution and soak time.



The Ramp Mode dialog box opens.



2 Next to **Soak Time**, type in the desired number of seconds for the soak time, or click the +/- buttons to adjust the value in the field.

The soak time is the amount of time that the instrument spends at each temperature increment during the ramp.

3 Click **OK** to close the dialog box and save your changes.

Restore the default thermal profile

To restore the thermal profile to the default for the experiment type:

1 Right-click anywhere on the thermal profile display.

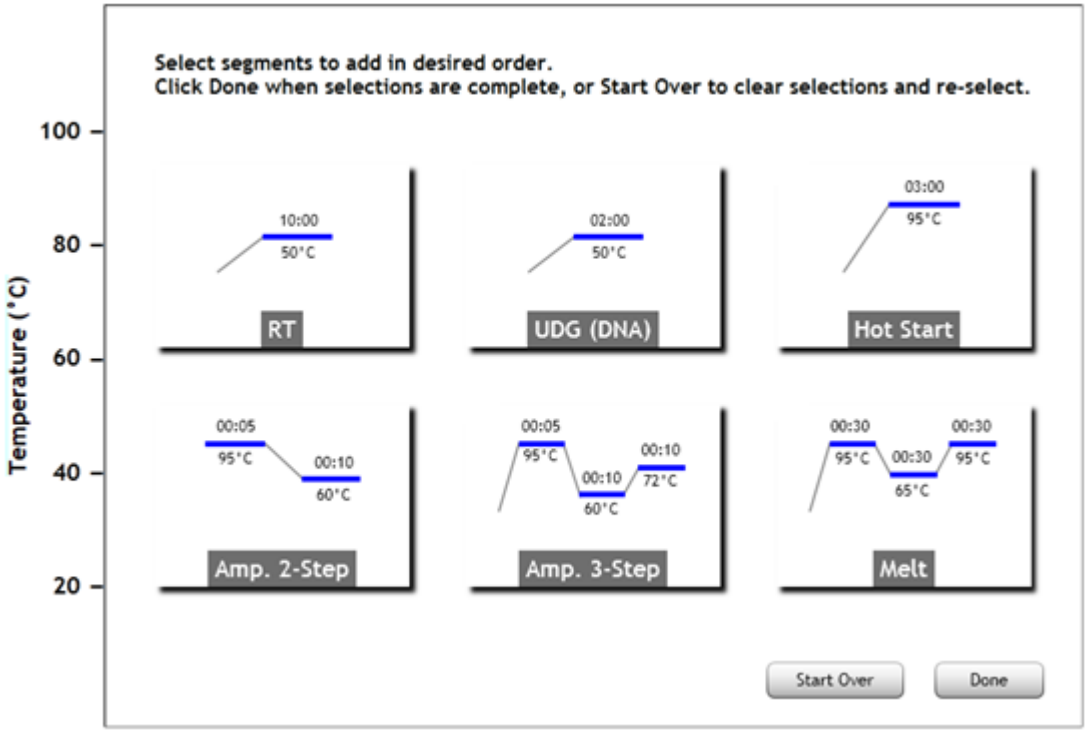
A short-cut menu opens.

2 Click **Restore Default Thermal Profile**.

The program sets the thermal profile to the default for the experiment type.

Select segments for a blank thermal profile

If you delete all segments in a thermal profile, or if you create a new User Defined experiment, the thermal profile screen appears as shown below.

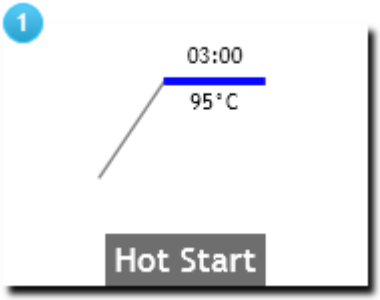


On this screen, you select the segments you want to use to create a new thermal profile.

To select segments to add to a blank thermal profile:

1 From the screen shown above, click on the segment type that you want to be the first segment in the thermal profile.

   The program marks the segment with a number “1” in the upper left corner, as in the example image shown below.



2 Click on the segment type that you want to be the second segment in the thermal profile.

The program marks the segment with a number “2” in the upper left corner.

3 Continue clicking on segments to build the thermal profile. If you need to redo an assignment, click **Start Over**. When finished building the thermal profile click **Done**.

The program generates the thermal profile based on your selections and displays it on the Thermal Profile screen.

4 Edit the thermal profile as needed.

Export the thermal profile image

The image of the thermal profile can be exported to a Microsoft PowerPoint presentation.

To export an image of the thermal profile to PowerPoint:

- From the Thermal Profile screen, right click anywhere on the display. In the short-cut menu, click **Send Image to PowerPoint**.

  PowerPoint opens to a new presentation file with the thermal profile image on the slide.

8
Running and Monitoring Experiments

Overview of the Instrument Explorer dialog box

The Instrument Explorer dialog box has tools for performing a variety of functions that require connecting to an instrument through a local network.

To open the Instrument Explorer dialog box:

- For starting and monitoring a run, open the Thermal Profile, Run Status, or Raw Data Plots screen and click Run.
- For other Instrument Explorer functions, at the top of the program window, click **Instrument > Instrument Explorer**. (Starting a run is not enabled when you open the dialog box from the Instrument menu. However, opening the dialog box from the Instrument menu does allow you to monitor a run that has already been started.)

See the following help topics for instructions on specific tasks that require the use of the Instrument Explorer dialog box.

"Add instruments to your network" on page 162

"Start, stop, or pause a run" on page 165

"Monitor a run" on page 168

"Retrieve run data from the instrument" on page 173

"Export instrument data to a CSV file" on page 175

Add instruments to your network

The Instrument Explorer dialog box lists the instruments connected to your subnetwork. If the instrument you need is not displayed in the list, the dialog box has tools to search for and add a new instrument.

To open the Instrument Explorer dialog box: At the top of the program window, click **Instrument > Instrument Explorer**. (Note that in order to start a run, you must open the dialog box by clicking **Run** on the Thermal Profile, Run Status, or Raw Data Plots screen. Starting a run is not enabled when you open the dialog box from the Instrument menu. However, opening the dialog box from the Instrument menu does allow you to monitor a run that has already been started.)

Add a new instrument based on its IP address

If you do not see the instrument you are looking for listed on the Instrument Explorer dialog box, you can search for and add an instrument by its IP address.

NOTE You can look up the IP address of an instrument through the touchscreen. Near the bottom right corner of the Home screen, press the Connections button (shown below). The pop-up box that opens includes the instrument's IP address.

To search for and connect to an instrument based on its IP address:

1 In the Instrument Explorer dialog box, click the Add Instrument icon.

The Add Instrument dialog box opens.

2 In the Instrument field, type the IP address of the instrument you want to connect to, then click **Add**.

The Add Instrument dialog box closes and the instrument is listed in the Instrument Explorer dialog box.

Add a new instrument based on its port number

If you do not see the instrument you are looking for listed on the Instrument Explorer dialog box, you can search for and add instruments that are connected to the network on a different port number.

To select port numbers on which the application searches for available instruments:

1 In the Instrument Explorer dialog box, click the Discovery Port icon, 

   The Discovery Port dialog box opens. The marked port numbers are the ones on which the program currently searches for instruments.

2 Mark the port number you want to search.
   - If the port number is already listed in the Discovery Port dialog box, mark the check box in the Use column.
   - If the port number is not listed, click the Add icon to add additional ports.
   - For any listed ports that you do not want included in the search, clear the check box next to the port number or select the port number and click the Delete icon .

3 Click **OK** to save your changes and close the Discovery Port dialog box. The instruments from the selected port number are listed in the Instrument Explorer dialog box.

View information about an instrument

For the available instruments listed in the Instrument Explorer dialog box, you can view information about the instrument including:

- The optical modules that are installed on the instrument
- The instrument serial number
- The IP address for the instrument
- The firmware version installed on the instrument

To view instrument information:

1 In the Instrument Explorer dialog box, click the Instrument Information icon next to the desired instrument.

The Instrument Information dialog box opens.

Start, stop, or pause a run

Starting a run causes the instrument to initiate the thermal profile protocol.

Start a run

To start running an experiment:

1. Set up the experiment in the program (either using the PC version of the Aria program or using the touchscreen program) and set up the PCR reactions in the 96-well reaction plate.

   Setting up the experiment in the software requires creating the experiment file, setting up the plate and, setting up the thermal profile.

2. Start the run:

   - If you are working from the PC version of the program:

     a. Open the Thermal Profile screen, Run Status screen, or Raw Data Plots screen.

     b. Click **Run**.

     The Instrument Explorer dialog box opens.

     If the open experiment is a post-run experiment, you will first be prompted to save the experiment with a new name.

     c. Locate the instrument that you will be using for the run and click **Send Config**. (See "Add instruments to your network" on page 162 for instructions on searching for and adding instruments.)

     - If this is the first time you have connected to an instrument since last launching the Aria program, the Login dialog box opens. Select your Username from the drop-down list, type your login password into the Password field, and click **Login**. To log in with a different user account, right-click on the instrument name and click **Log off current user**. You can then log in using the desired user account.
     - If the experiment is new, you will be prompted to save the experiment before proceeding.

     d. Take your reaction plate over to the instrument and load it into the thermal block.

e On the instrument touchscreen, open the primed experiment to the Thermal Profile screen and press **Run Experiment**.The instrument starts running the experiment.

f Return to the PC program. The program directs you to the Run Status screen, where you can monitor the progress of the run. See "Monitor a run" on page 168.

- If you are working from the touchscreen version of the program:

  a Load your reaction plate into the instrument's thermal block.

  b On the Thermal Profile screen, press **Run Experiment.**
  The instrument starts running the experiment. You can monitor the progress of the run from your PC. See "Monitor a run" on page 168 for instructions.

Cancel a run

To stop an in-progress run:

1 Make sure you are monitoring the run. See "Monitor a run" on page 168.

2 On the Run Status or Raw Data Plots screen, click **Stop Run.**

A message box opens asking you to confirm that you want to stop the run.

3 In the message box, if you do not want to save the partial data collected during the run, mark the check box labeled Discard Instrument Data. If you do want to save the partial data, leave the check box unmarked.

4 Click **Abort Experiment** in the message box to stop the run.

If you chose to save the partial data, you can view it on the Graphical Displays screen.

Pause a run

To pause an in-progress run:

1. Make sure you are monitoring the run. See "Monitor a run" on page 168.
2. On the Run Status or Raw Data Plots screen, click **Pause.**

   A message box opens asking you to confirm that you want to pause the run.
3. In the message box, click **Pause Run**.

   The program pauses the run. When you are ready to resume running the experiment, click **Resume**.

Monitor a run

The Run Status and Raw Data Plots screens allow you to start a run and to monitor the progress of a run. The Run Status screen shows the progression of the run through the thermal profile. The Raw Data Plots screen displays the amplification or melt data in each well as it is collected by the instrument in real-time.

To open the Run Status or Raw Data Plots screen: Click **Run Status** or **Raw Data Plots** in the Experiment Area panel on the left side of the screen.

NOTE

Only one computer can monitor the run on a particular instrument at any one time.

If you are monitoring a run when the run completes, the instrument will automatically transfer the run data to the PC, unless you logged into the instrument using the *guest* account. If you logged in as *guest*, you must retrieve the data in order to view the results on your PC. See "Retrieve run data from the instrument" on page 173 for more information.

If you are monitoring a run, and someone stops the run from the instrument touchscreen, the program opens a message notifying you that the run has been stopped. The message will indicate whether or not the user who stopped the run selected to save the partial data from the run. If partial data was saved, you will need to retrieve it. See "Retrieve run data from the instrument" on page 173.

Connect to the running instrument

To connect to the instrument that is running the experiment that you want to monitor:

1. At the top of the program window, click **Instrument > Instrument Explorer**.
2. Locate the instrument and click **Monitor Run**. (If the instrument is not listed, see "Add instruments to your network" on page 162 for instructions on searching for and adding instruments.)

   If this is the first time you have connected to an instrument since last launching the Aria program, the Login dialog box opens. Select your Username from the drop-down list, type your login password into the Password field, and click **Login**.

The program directs you to the Run Status screen.

Monitor a run by viewing its progress through the thermal profile

The Run Status screen allows you to monitor a run by viewing the instrument's progress through the thermal profile of the experiment.

To monitor the run status:

1 Connect to the running instrument.

2 Open the Run Status screen.

In the center of the screen is a representation of the thermal profile for the experiment. As an experiment is running, the progression of the experiment through the thermal profile protocol is indicated on the display.

The top of the screen lists the time remaining in the run and the current temperature. The bottom of each segment shows how many cycles of the segment have been completed.

Monitor a run by viewing the raw data plots

The Raw Data Plots screen allows you to monitor a run by watching the real-time changes in fluorescence levels in the individual wells. The program displays these changes as raw data plots, which are graphs that plot the fluorescence values on the Y-axis in relative fluorescence units (RFU).

To monitor the raw data plots:

1 Connect to the running instrument.

2 Open the Raw Data Plots screen.

On the left side of the screen is a representation of the experiment plate. During the run, the raw data are plotted and displayed in each well. The Y-axis of the plots shows the level of raw fluorescence. If monitoring data collection for an Amplification segment, the X-axis plots the cycle number. If monitoring data collection for a melt segment, the X-axis plots the temperature.

On the right side of the screen is an expanded view of the raw data plots within a selected well or a set of selected wells. Hover your cursor over any individual plot line in this view to see which well and target that line refers to. Hover your cursor over a well on the plate to highlight the plot line for that well in the raw data plots.

Change the display options for the raw data plots

Change which data collection marker is used for the plots

By default, the raw data plots show the data collected during the amplification segment of the thermal profile.

To select a different data collection marker to display in the raw data plots:

1 On the Raw Data Plots screen, hover your cursor over the Data Collection Marker icon at the bottom of the screen.

A window opens displaying the thermal profile with data collection markers.

2 Click the data collection marker that you want to use for the raw data plots.

The window closes and the program uses the selected data collection marker in the raw data plots.

Select which targets to include in the plots

By default, the raw data for all targets in use on the plate are included in the plots.

To select specific targets to display in the raw data plots:

1 On the Raw Data Plots screen, hover your cursor over the Display Targets icon at the bottom of the screen.

A window opens showing all targets in use on the plate.

2 For any targets that you do not want displayed, clear the check box next to the target name. You can remark the check box at any time to add the target back to the raw data plots.

Select which well types to include

By default, the raw data plots are displayed for all well types in use on the plate.

To show the raw data for only specific well types:

1 On the Raw Data Plots screen, hover your cursor over the Display Well Types icon at the bottom of the screen.

A window opens showing all well types in use on the plate.

2 For any well types that you do not want to include, clear the check box next to the well type name. You can remark the check box at any time to add the well type back to the raw data plots.

Change the scale or orientation of the axes

By default, the X and Y axes of the graph on the right side of the Raw Data Plots screen are oriented in ascending order and the ranges of the axes adjust automatically based on the plots being displayed.

To change the orientation of the X or Y axis:

1 Right-click anywhere on the large graph of the Raw Data Plots screen.

A short-cut menu opens.

2 Click **Axis Options > Reverse Orientation in X-Axis** or **Axis Options > Reverse Orientation of Y-Axis**.

The program reverse the orientation of the selected axis.

To change the scale of the X or Y axis:

1 Right-click anywhere on the large graph of the Raw Data Plots screen.

A short-cut menu opens.

2 Click **Axis Options > Customize Scale**.

The Graph Properties dialog box opens to the Axis Options tab.

3 Under the heading for the desired axis, change the Autoscale setting to **Manual**.

4 In the Min and Max fields, type the desired minimum and maximum values for the scale of the axis.

5 Click **Close** to save your changes and close the Graph Properties dialog box.

The program sets the scale of the axis to the new values.

Stop monitoring a run

When you stop monitoring a run, the instrument continues running the experiment and the run becomes available for monitoring from another PC.

To stop monitoring a run, do one of the following:

- Close the experiment.
- From the Run Status or Raw Data Plots screen, click **Stop Monitor**. In the message box that opens, click **Yes** to confirm that you want to stop monitoring the run.

Retrieve run data from the instrument

If you are monitoring a run from your PC when the run completes, the instrument will automatically transfer the run data to the PC program (unless you logged into the instrument using the *guest* account). If you are not monitoring the run, the data from the run is saved to the instrument and you must retrieve it to your PC in order to analyze the experiment results. You can retrieve the data through the network, or by copying it to a USB drive.

Retrieve data through the network

If the instrument is connected to the same network as your PC, you can retrieve data by connecting to the instrument remotely.

To retrieve the data from a run by remotely connecting to the instrument from your PC:

1. Make sure the experiment file is not open on the instrument.
2. From your PC, click **Instrument > Instrument Explorer**.

   The Instrument Explorer dialog box opens.
3. Locate the instrument on which you ran the experiment, and in that row, click the File Explorer icon.

   The File Explorer dialog box opens.

   If this is the first time you have connected to an instrument since last launching the application, the Login dialog box opens. Select your Username from the drop-down list, type your login password into the Password field, and click **Login**. After logging in, the File Explorer dialog box opens.
4. In the list of folders on the left side of the File Explorer dialog box, browse to the folder of the experiment on the instrument.

   The root folder for the experiment is the folder for the user who ran the experiment. The subfolder is named for the experiment type.
5. Click directly on the experiment for which you want to transfer the run data.
6. Click **Copy & Delete**.

The program transfers the post-run experiment file from the instrument to the default experiment storage folder on your PC (the program deletes the file from the instrument in the process). If the pre-run experiment is already saved to your PC, the program saves the post-run experiment file with a bracketed number appended to the end of the file name (e.g., *Experiment1[1]*).

7 Open the experiment file in the Aria program to view the results of the run.

Retrieve data using a USB drive

You can connect a USB drive to the instrument and save the data to the drive, and then transfer it to your PC. This approach is the only way to retrieve data from an instrument that is not network-connected.

1 Insert an external USB drive (FAT format) into the USB port on the front of the instrument.

2 On the instrument touchscreen, open the folder with the post-run experiment file.

3 Copy the experiment to the USB drive.

After successful transfer of the file, delete the file from the instrument to avoid filling the instrument's hard drive.

4 Remove the USB drive from the instrument and insert it into your PC.

5 Move the experiment file to a folder of your choice.

6 Open the experiment file in the Aria program to view the results of the run.

Export instrument data to a CSV file

For each post-run experiment, the program stores the raw data for all dyes in all wells at each data collection point. To view this data, export it to a CSV file and then open it in Excel.

Export instrument data by column

To export instrument data by column:

1 Click **Instrument > Export Instrument Data > By Columns**.

The Export Instrument Data By Columns dialog box opens.

2 Select a folder and file name for the CSV file and click **Save**.

The program generates the file and opens it in Microsoft Excel.

Export instrument data by target

To export instrument data by target:

1 Click **Instrument > Export Instrument Data > By Target**.

The Export Instrument Data By Target dialog box opens.

2 Select a folder and file name for the CSV file and click **Save**.

The program generates the file and opens it in Microsoft Excel.

Export instrument data by wells

To export instrument data by wells:

1 Click **Instrument > Export Instrument Data > By Wells**.

The Export Instrument Data By Wells dialog box opens.

2 Select a folder and file name for the CSV file and click **Save**.

The program generates the file and opens it in Microsoft Excel.

9
Setting Analysis Criteria

Overview of the Analysis Criteria screen

Your selections on the Analysis Criteria screen determine the settings that the program uses for data analysis.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

NOTE The Analysis Criteria screen is only available in post-run experiments and in-progress experiments that have completed at least one point of data collection.

The Analysis Criteria screen has an image of the plate map that is based on the settings on the Plate Setup screen. At the bottom of the screen are icons that provide access to menus for making selections on which data you want included in the results. Results are displayed on the Graphical Displays screen.

See the following help topics for instructions on specific tasks for setting the analysis criteria:

"Select the wells and well types to include in analysis" on page 179

"Select the targets to include in analysis" on page 180

"Select which data collection points to analyze" on page 181

"Choose a treatment for replicate wells" on page 182

"Assign an HRM calibration plate" on page 183 (only for experiments with HRM segment)

Note that if you wish to use the default analysis settings, you need only select the wells to analyze.

Toggle display of plate map wells

The plate map on the Analysis Criteria screen can display either the dye/target information and replicate number in each well, or it can display the Cq value(s) calculated in each well. Your selection does not impact the results shown on the Graphical Displays screen.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

To toggle between the two display options for the plate map on the Analysis Criteria screen:

1 On the Analysis Criteria screen, click the Display toggle button at the bottom of the screen. When the button looks like the image on the left, the program is displaying the dye/target information and replicate number in each well. When the button looks like the image on the right, the program is displaying the Cq value(s) calculated in each well.

Select the wells and well types to include in analysis

From the Analysis Criteria screen, you can select which wells and well types to include in the results displayed on the Graphical Displays screen.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

Select wells for analysis using the plate map

The program's analysis algorithms only use the wells that are selected in the plate map on the Analysis Criteria screen.

Well selection on the Analysis Criteria screen works that same way as it does on the Plate Setup screen. See "Select wells in the plate map" on page 79 for instructions on well selection.

Select wells for analysis based on well type

You can select specific wells to include in the analysis based on well type.

To select specific well types:

1. On the Analysis Criteria screen, hover your cursor over the Display Well Types icon at the bottom of the screen.

   A window opens showing all well types in use on the plate.
2. For any well types that you do not want to include, clear the check box next to the well type name. You can remark the check box at any time to reselect those wells.

Select the targets to include in analysis

From the Analysis Criteria screen, or from the Graphical Displays screen, you can select which targets to include in the results displayed on the Graphical Displays screen.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

To select specific targets:

1 On the Analysis Criteria screen, hover your cursor over the Display Targets icon at the bottom of the screen.

A window opens showing all targets in use on the plate.

2 For any targets that you do not want included in the analysis, clear the check box next to the target name. You can remark the check box at any time to reselect those targets.

Select which data collection points to analyze

If your thermal profile includes multiple amplification collection points or multiple melt collection points, you can specify on the Analysis Criteria screen, or on the Graphical Displays screen, which data collection point to use for analysis.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

To select which data collection point to use for analysis of amplification data:

1 On the Analysis Criteria screen, hover your cursor over the Data Collection Marker icon at the bottom of the screen.

A window opens displaying the thermal profile with data collection markers.

2 Click the data collection marker within an amplification segment that you want to use for analysis of amplification data.

The window closes and the program uses the selected data collection marker to analyze amplification data.

To select which data collection point to use for analysis of melt data:

1 On the Analysis Criteria screen, hover your cursor over the Data Collection Marker icon at the bottom of the screen.

A window opens displaying the thermal profile with data collection markers.

2 Click the data collection marker within a melt segment that you want to use for analysis of melt data.

The window closes and the program uses the selected data collection marker to analyze melt data.

Choose a treatment for replicate wells

Data from wells identified as replicates in the plate setup can be treated either individually (each well separate) or collectively (replicate wells averaged at each cycle) during analysis. You can choose a treatment for replicate wells from the Analysis Criteria screen, or from the Graphical Displays screen.

To open the Analysis Criteria screen: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

To specify that replicates be treated individually or collectively:

- On the Analysis Criteria screen, click the Replicates toggle button at the bottom of the screen.

  When the button looks like the image on the left, the program treats them individually. When the button looks like the image on the right, the program treats them collectively.

Assign an HRM calibration plate

For an experiment that includes a high resolution melt (HRM) segment, in order to view the Melt Curve - Difference Plots graph, the program requires you to assign an HRM calibration plate (HCP) to the experiment.

In order to assign an HCP to an experiment, the HCP must:

- pass the system's quality check
- be run on the same instrument as your experiment with the HRM segment
- have an identical soak time and plateau time in the HRM segment as the experiment

NOTE A separate HRM software license is required in order to assign an HCP. To purchase a software license, contact your Agilent Sales representative. The HRM license option is only available for the AriaMx system. The license is not supported for the AriaDx system.

Assign an HCP for new experiments

The first time you attempt to open the Analysis Criteria or Graphical Displays screen for a new post-run experiment that includes an HRM segment, the following message box opens on your screen.

To assign an HCP from the message box:

1. In the message box, click **Import**.

   The Open dialog box opens.

2. Browse to the HCP file that you want to assign. Select the file and click **Open**.

The program assigns the HCP to the experiment and calibrates the melt data accordingly.

If you click **Cancel** in the message box shown above, you can still assign an HCP using the HCP icon on the Analysis Criteria or Graphical Displays screen. See instructions below.

Assign an HCP using the HCP icon

The HCP icon is available on the Analysis Criteria and Graphical Displays screens for all experiments that include an HRM segment.

To assign an HCP:

1 On the Analysis Criteria or Graphical Displays screen, click the HCP icon at the bottom of the screen.

The Open dialog box opens.

2 Browse to the HCP file that you want to assign. Select the file and click **Open**.

The program assigns the HCP to the experiment and calibrates the melt data accordingly.

To assign a recently used HCP:

1 On the Analysis Criteria or Graphical Displays screen, click the arrowhead next to the HCP icon at the bottom of the screen.

2 In the menu that opens, hover your cursor over **Recently Used Data**.

A list of recently used HCPs opens.

3 Click the HCP that you want to assign to the current experiment.

The program assigns the HCP to the experiment and calibrates the melt data accordingly.

To clear an HCP assignment:

1 On the Analysis Criteria or Graphical Displays screen, click the arrowhead next to the HCP icon at the bottom of the screen.

2 In the menu that opens, click **Clear Calibration Data**.

The experiment no longer has an HCP assigned. If desired, you can now assign a different HCP to the experiment.

10
Viewing Graphical Displays of the Results

Agilent Technologies

Overview of the Graphical Displays screen

The Graphical Displays screen shows the results of the experiment displayed in a series of graphs. For each graph, the screen includes tools for setting certain analysis parameters. The screen also includes a result table, with configurable columns of data, that you can export to an Excel spreadsheet.

To open the Graphical Displays screen: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen.

NOTE The Graphical Displays screen is only available in post-run experiments and in-progress experiments that have completed at least one point of data collection.

Graphs

The exact set of graphs available on the Graphical Displays screen varies depending on the experiment type, but all experiments have a graph of the amplification plots, and all experiments with a melt segment have a graph of the melt curves.

See the topics below for detailed information on specific graphs:

"View the Amplification Plots" on page 197

"View the Melt Curve - Raw/Derivative Curve" on page 212

"View the Melt Curve - Difference Plots" on page 218

"View the Standard Curve" on page 225

"View the Relative Quantity" on page 230

"View the Allele Determination graph" on page 235

By default, data from all of the wells that you selected on the Analysis Criteria screen are displayed in the graphs. You can limit the graphs to only display data from particular wells or replicate sets by selecting specific rows in the result table (press **Ctrl** to select multiple rows). The result table is described below.

Result table

The result table on the right side of the Graphical Displays screen shows results for each well (if replicates are treated individually) or replicate set (if replicates are treated collectively) that you selected on the Analysis Criteria screen (see "Select the wells and well types to include in analysis" on page 179).

Row selection

You can select individual rows within the results table to limit the graphs to displaying data only from particular wells/replicate sets. Click directly on a row to select it. Press **Ctrl** to select multiple rows. By default, all rows are initially selected. Click the Select All icon at the top of the result table to reselect all rows.

Data columns

You can configure which columns of data are included in the table. Click the Column Options icon at the top of the result table to open the Column Options dialog box. In the dialog box, mark the columns that you want to include in the result table.

You can freeze one or more columns on the left side of the result table so that as you scroll through the table horizontally, the frozen columns are always visible. Right-click on the header of the right-most column that you want to freeze and click **Freeze Column**. To unfreeze, right-click again and click **Unfreeze Column**.

Sorting

You can sort the data in the result table. Click directly on the header of the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The column headers selected for sorting are highlighted in blue.

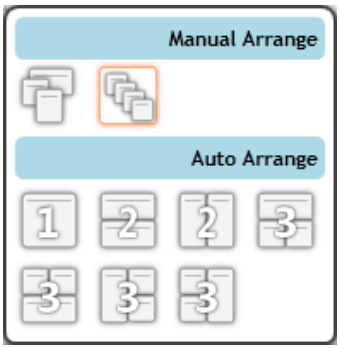
Display options

When you have multiple graphs selected for viewing on the Graphical Displays screen, you can manually drag and drop the graphs to new positions on the screen using your cursor (the Manual Arrange feature). Alternatively, you can select for the program to automatically arrange the

graphs based on the desired number of graphs per screen (the Auto Arrange feature).

To access the options for manually and automatically arranging the graphs, click the icon (shown below) at the bottom of the Graphical Display screen.

The following menu opens. The options under **Manual Arrange** and **Auto Arrange** are described below.



Manual Arrange

Under **Manual Arrange**, you have two arrangement options:

	Floating arrangement - This option allows you to move the graphs to any location on the screen by dragging and dropping them with your cursor.
	Cascade arrangement - This option sets the graphs in a cascading arrangement. You can move the graphs by dragging and dropping with your cursor.

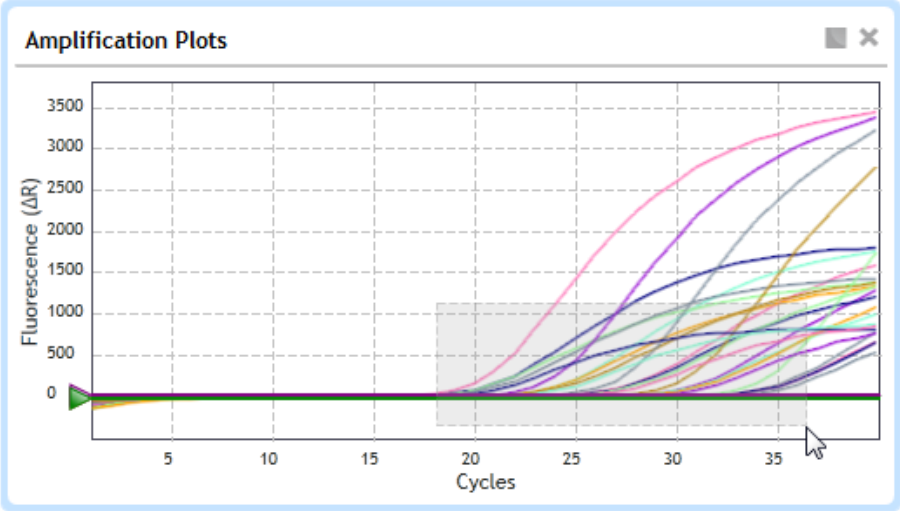
Auto Arrange

Under **Auto Arrange**, the options determine the number of graphs displayed on the screen (1, 2, 3, or 4). The image in each icon shows the arrangement of the graphs associated with that option. When you select to display more than one graph at a time, you can reorder the positions of the graphs by dragging and dropping one graph on top of another with your cursor.

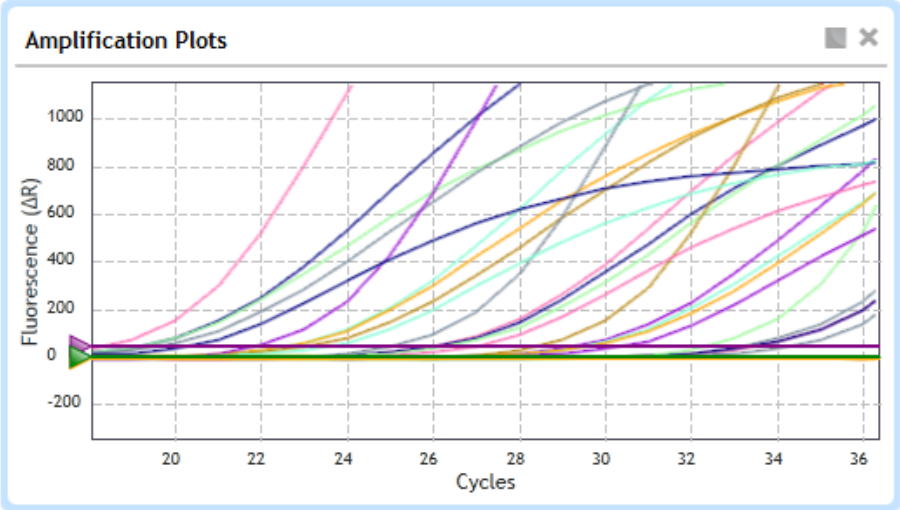
Zooming

Within a graph you can zoom in on a particular region of interest.

Drag your cursor across the region of interest, as shown below.



The program then zooms in on the selected region.



To reset the zoom level, right-click anywhere on the graph and click **Reset Zoom**.

For more information on the display options available for the graphs on the Graphical Displays screen, see "Customize graph properties" on page 240.

Configure and apply analysis templates

An analysis template is a pre-configured set of analysis settings that can be automatically applied to experiments. The analysis settings that can be defined in an analysis template are:

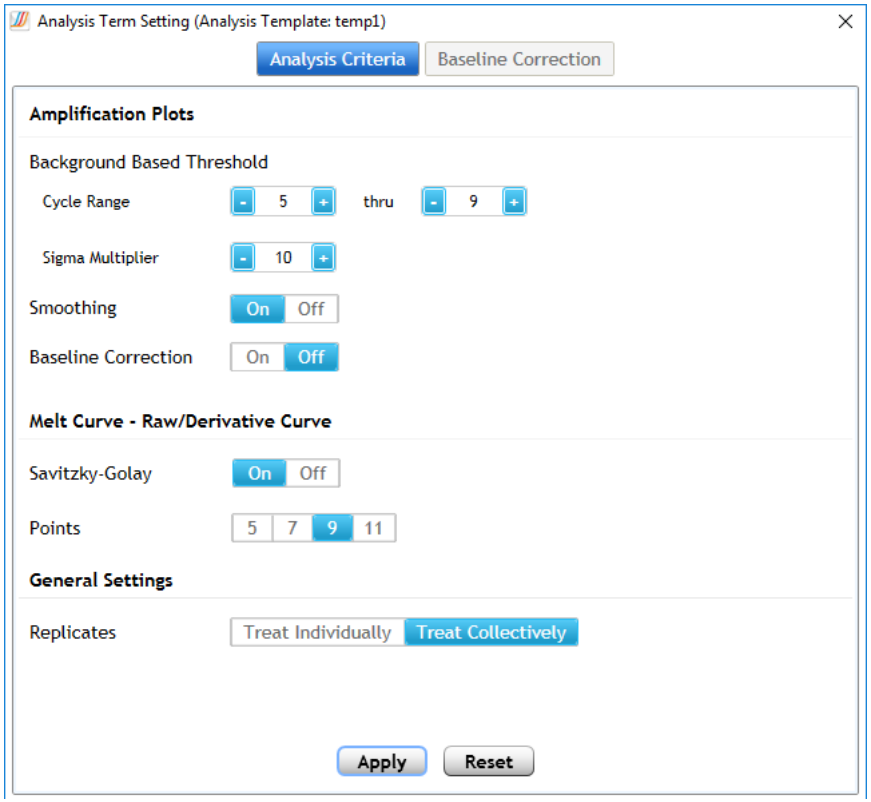
- The cycle range and sigma multiplier used for background based thresholds
- Option and settings for the use of Savitzky-Golay smoothing of melt curves
- Treatment of replicate wells
- The baseline cycle range used for baseline correction.

NOTE The settings in a newly created analysis template (i.e., settings for background based threshold, Savitzky-Golay smoothing, and treatment of replicates) use default analysis criteria.

You can create analysis templates from the Preferences dialog box. The Preferences dialog box also has tools for selecting which analysis template to apply to experiments and how and if the template is applied to experiments automatically. See "Set software preferences" on page 28 for instructions.

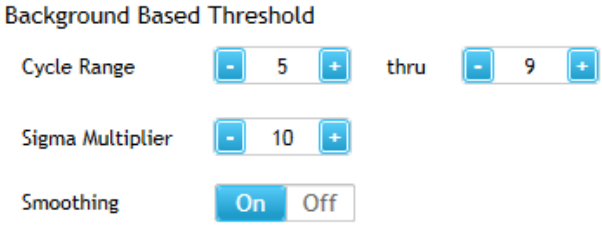
Once an analysis template has been created and selected in the Preferences dialog box, you can customize its analysis settings from the Analysis Term Setting dialog box.

To open the Analysis Term Setting dialog box: At the top of the program window, click **File > Analysis Term Settings**. The title bar indicates which analysis template is the one that is selected.



Configure an analysis template in the Analysis Term Settings dialog box

1 (Optional) Adjust the background based threshold for the amplification plots as desired. Note that the cycle range needs to be in the flat baseline range in order for the resulting thresholds to be accurate.



- At the top of the Analysis Term Settings dialog box, select **Analysis Criteria**.
- Under the **Background Based Threshold** heading, in the first field next to **Cycle Range**, type the cycle number that will be the first cycle in the range, or click the +/- buttons to adjust the value in the field to the desired cycle number.
- In the second field, type the cycle number that will be the last cycle in the range, or click the +/- buttons to adjust the value in the field to the desired cycle number.
- In the Sigma Multiplier field, type the desired value for the sigma multiplier, or click the +/- buttons to adjust the value in the field to the desired cycle number.
- Next to **Smoothing**, select **On** to turn on curve smoothing for the amplification plots, or select **Off** to turn off curve smoothing.
- Click **Apply** to save changes.

2 (Optional) Set the Savitzky-Golay smoothing options for melt curves as desired.

- At the top of the Analysis Term Settings dialog box, select **Analysis Criteria**.
- Under **Melt Curve - Raw/Derivative Curve**, next to **Savitzky-Golay**, select **On** to turn on smoothing for the melt curves, or select **Off** to turn off smoothing.
- If you selected **On**, next to **Points**, select the number of data points on each melt curve that you want the algorithm to use in the smoothing calculations. A higher number of points leads to a smoother (i.e., more manipulated) curve.
- Click **Apply** to save changes.

3 (Optional) Designate how data from replicate wells are treated during analysis.

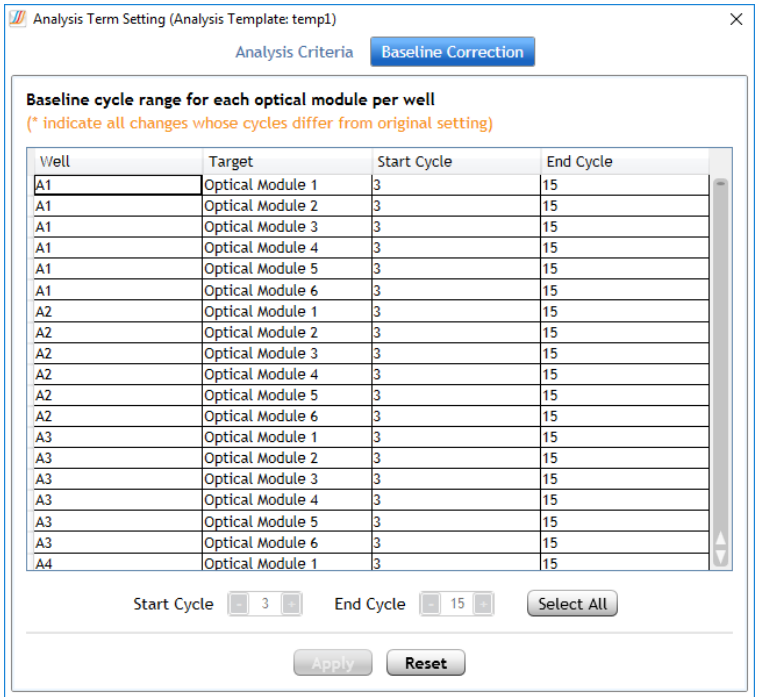
- At the top of the Analysis Term Settings dialog box, select **Analysis Criteria**.
- Under **General Settings**, select **Treat Individually** to analyze each well separately, or select **Treat Collectively** to average the data at each cycle.
- Click **Apply** to save changes.

4 (Optional) Set the cycle range used for baseline correction.

Newly created analysis templates have the baseline cycle range set to cycles 3–15. To obtain the optimal baseline value, you may need to adjust the baseline cycle range in individual experiments. See "Adjust the baseline correction settings" on page 199.

- Under **Amplification Plots**, set **Baseline Correction** to **On**. This enables viewing and editing of the baseline correction cycle range for the template.
- At the top of the Analysis Term Settings dialog box, select **Baseline Correction**.

  The dialog box displays the settings for the baseline cycle range.



- Select the plots (a plot is one target in one well) that you want to adjust by clicking the appropriate row in the table. Press **Ctrl** while clicking to select more than one plot. Click **Select All** to select all plots in the table.
- In the Start Cycle field at the bottom of the dialog box, type the cycle number that will be the first cycle in the baseline cycle range, or click the +/- buttons to adjust the value in the field to the desired cycle number.
- In the End Cycle field, type the cycle number that will be the last cycle in the baseline cycle range, or click the +/- buttons to adjust the value in the field to the desired cycle number.
- Click **Apply** to save changes.

5 When finished configuring the analysis template, click the red X in the top right corner to close the Analysis Term Settings dialog box.

View the Amplification Plots

The Amplification Plots graph on the Graphical Displays screen shows a plot of fluorescence (Y-axis) versus cycles (X-axis) for each target in each well (or in each set of replicate wells) at a single data collection point. The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the amplification plots.

To view the Amplification Plots: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Amplification Plots graph is not already displayed, click the Amplification Plots icon at the bottom of the screen.

View data for a single data point

Each curve on the amplification plots is the plot for a single target in a single well or replicate set. Each data point that makes up the curve is a fluorescence value (plotted on the Y-axis) measured at a particular cycle number (plotted on the X-axis). The program connects these data points to draw the curves displayed on the graph.

To view a summary of the data for a single data point on a plot:

1. Select the Amplification Plots graph on the Graphical Displays screen.
2. Hover your cursor over an individual plot on the graph.

   A tooltip opens displaying the following information:

   - Well ID and/or replicate number of the plot
   - Target/dye
   - Well type
   - Fluorescence data type for the plot followed by the X and Y coordinates for the data point where your cursor is located
   - (If replicates are being treated individually) Baseline range (starting cycle number, ending cycle number) used to calculate baseline-corrected fluorescence values for that plot

Select a fluorescence data type

For the Y-axis of the amplification plots, you can select to use fluorescence values that have been baseline-corrected and/or normalized to a reference dye (if included).

To select the type of fluorescence data displayed in the amplification plots:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, select one of the options next to **Fluorescence Term**. The possible options are:

R - raw fluorescence

ΔR - baseline-corrected raw fluorescence

Rn - normalized fluorescence (normalized to the reference dye)

ΔRn - baseline-corrected and normalized fluorescence

If the thermal profile for your experiment included multiple data collection points during amplification, see "Select which data collection points to analyze" on page 181 for instructions on specifying which data collection point to use for generating the amplification plots.

Specify the use of smoothing

You can select to apply a curve-smoothing algorithm to the amplification plots. Smoothing alters the shapes of the amplification plots by decreasing the effects of signal noise. The algorithm is based on a moving average calculation with 5 averaging points.

The smoothing option is turned on by default.

To turn smoothing on or off:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, next to **Smoothing**, select **On** to turn on smoothing, or select **Off** to turn off smoothing.

Adjust the graph properties

You can adjust certain properties of the Amplification Plots graph (e.g., the scales of the axes, the graph title, and the background color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

Adjust the baseline correction settings

By default, the program automatically determines which cycles to use to calculate the baseline in order to produce baseline-corrected fluorescence (ΔR) data for each well/target combination. For each target in each well, the raw fluorescence data over a specific range of cycles are fit to a line using a linear least mean squares algorithm to produce a baseline. The value of the baseline function is calculated for every cycle and subtracted from the raw fluorescence to produce baseline-corrected fluorescence (ΔR).

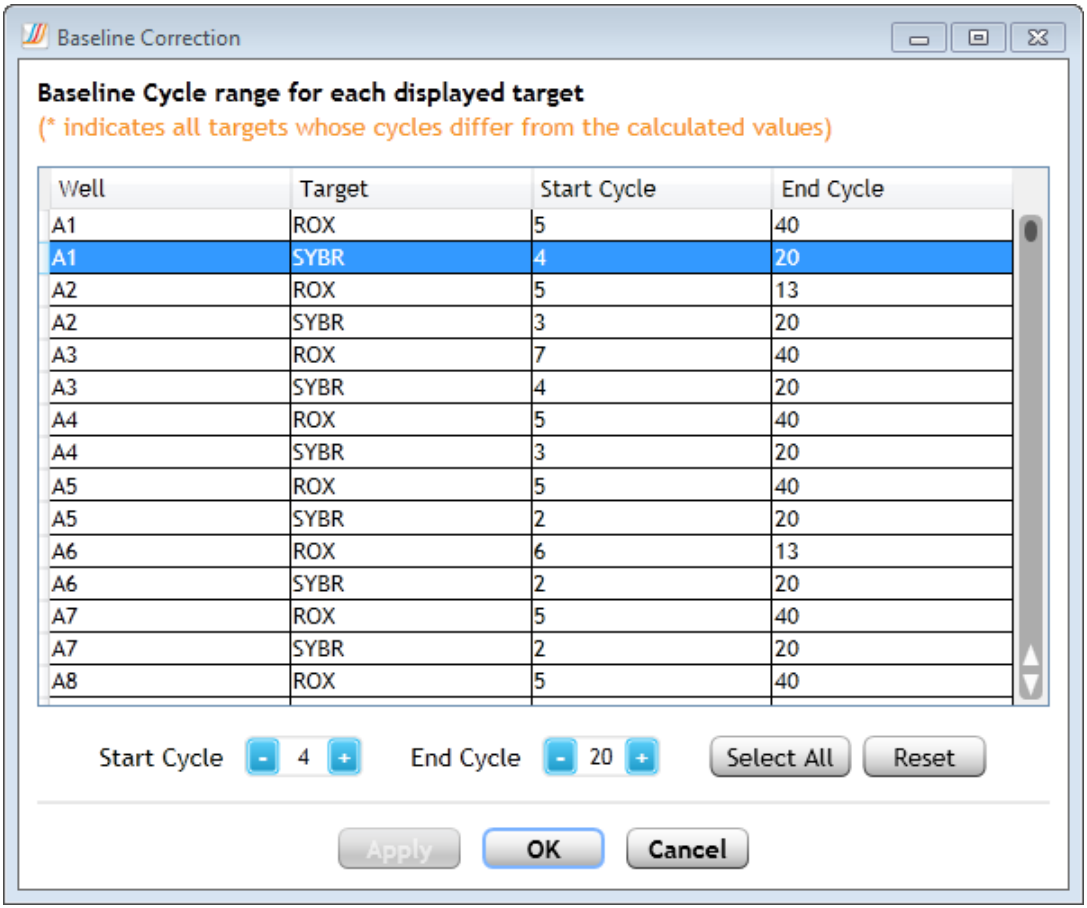
You can view and adjust these software-determined cycle ranges (known as adaptive values) from the Baseline Correction dialog box. You can adjust the cycle range for individual plots, or set all plots to the same cycle range.

NOTE If the Cq value for a target is within the baseline cycle range, the Aria software calls the target as "no Cq." In such cases, adjust the baseline cycle range for that target to make sure that the Cq is not within the baseline cycle range.

To open the Baseline Correction dialog box:

1. Select the Amplification Plots graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Next to **Baseline Correction**, click **Adjust**. (An asterisk on the Adjust button indicates that the baseline settings have already been manually adjusted.)

   The Baseline Correction dialog box opens.



To adjust the baseline cycle range:

1 Open the Baseline Correction dialog box.

2 Select the plots (a plot is one target in one well) that you want to adjust by clicking the appropriate row in the table. Press **Ctrl** while clicking to select more than one plot. Click **Select All** to select all plots in the table.

3 In the Start Cycle field at the bottom of the dialog box, type the cycle number that will be the first cycle in the baseline cycle range, or click the +/- buttons to adjust the value in the field to the desired cycle number.

4 In the End Cycle field, type the cycle number that will be the last cycle in the baseline cycle range, or click the +/- buttons to adjust the value in the field to the desired cycle number.

5 Click **Apply** to apply this baseline cycle range to the selected plots.

The program updates the table to show the new cycle range and adds an asterisk (*) to the Well column to indicate that the cycle range for that plot differs from the adaptive values.

To set the baseline cycle range back to adaptive values:

1 Open the Baseline Correction dialog box.

2 Select the plots (a plot is one target in one well) that you want to reset to the adaptive values by clicking the appropriate line in the table. Press **Ctrl** while clicking to select more than one plot. Click **Select All** to select all plots in the table.

3 Click **Reset**.

The program updates the cycle ranges listed in the table back to the adaptive values.

Adjust the crosstalk correction settings

Crosstalk occurs when emission from one dye is detected by two different optical modules (the target optical module and the spillover optical module). A dye is at risk for crosstalk when its emission wavelength overlaps that of another dye that is assigned to a different optical module. The Aria program includes crosstalk correction settings, which can help compensate for crosstalk.

NOTE The factory settings for crosstalk correction have been optimized to eliminate potential crosstalk for dyes that are part of the default optical configuration. Agilent does not recommend changing the crosstalk correction settings unless you are using a new custom dye and the emission wavelength of that dye could be detected by more than one optical module in the instrument.

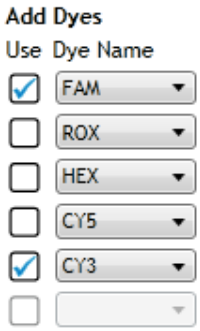
You can adjust the crosstalk correction settings for an individual experiment from the Amplification Plots using the instructions provided here. Alternatively, you can change the default crosstalk correction settings for all new experiments going forward from the Supported Optical

Configuration dialog box. The factory settings for crosstalk correction are the default settings, unless the default settings are changed in the Supported Optical Configuration dialog box. See "Change the default crosstalk correction settings" on page 25 for instructions.

If you suspect that your custom dye may be detected by more than one optical module, perform the following set of steps to eliminate crosstalk:

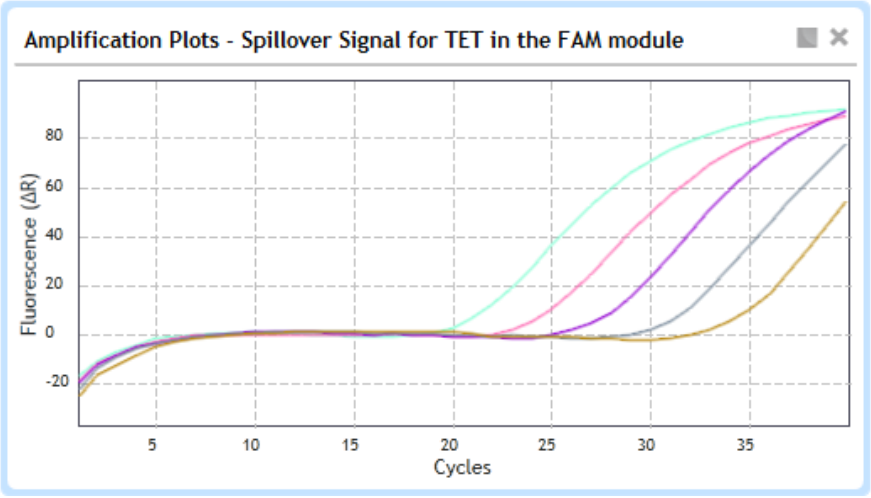
1 Run an experiment in which the custom dye is the only dye used in the reactions. In the Plate Setup of the experiment, assign a target to the spillover optical module as well as the target optical module for the dye.

   For example, if you are using the custom dye TET, then CY3 is the target optical module and FAM is the spillover optical module. In the Plate Setup, mark CY3 and FAM, as shown in the image below.



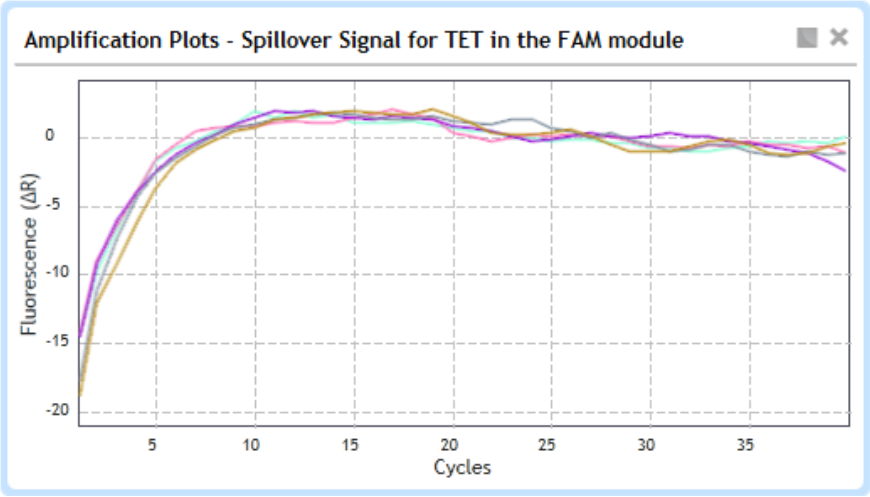
2 After running the experiment, view the results in the Amplification Plots graph to determine if there is an increase in fluorescence detected by the spillover optical module.

   In the example Amplification Plots graph below, only the plots for the spillover optical module (FAM) are displayed. A low level of fluorescence from the TET dye is being detected by the FAM optical module.



3 Adjust the crosstalk correction setting for the spillover optical module until that optical module no longer detects an increase in fluorescence (i.e., the amplification plot for the optical module is as close to flat as possible in the graph). See instructions in "To adjust the crosstalk correction settings:" on page 204 for details on how to perform this step.

In the example Amplification Plots graph below, the crosstalk correction setting for CY3 in the FAM optical module has been adjusted and the plots are now nearly flat.



4 For all experiments that include this dye, make sure that the crosstalk correction setting for the spillover optical module is set to the value determined in Step 3 above. To change the default crosstalk correction settings for future experiments, see "Change the default crosstalk correction settings" on page 25.

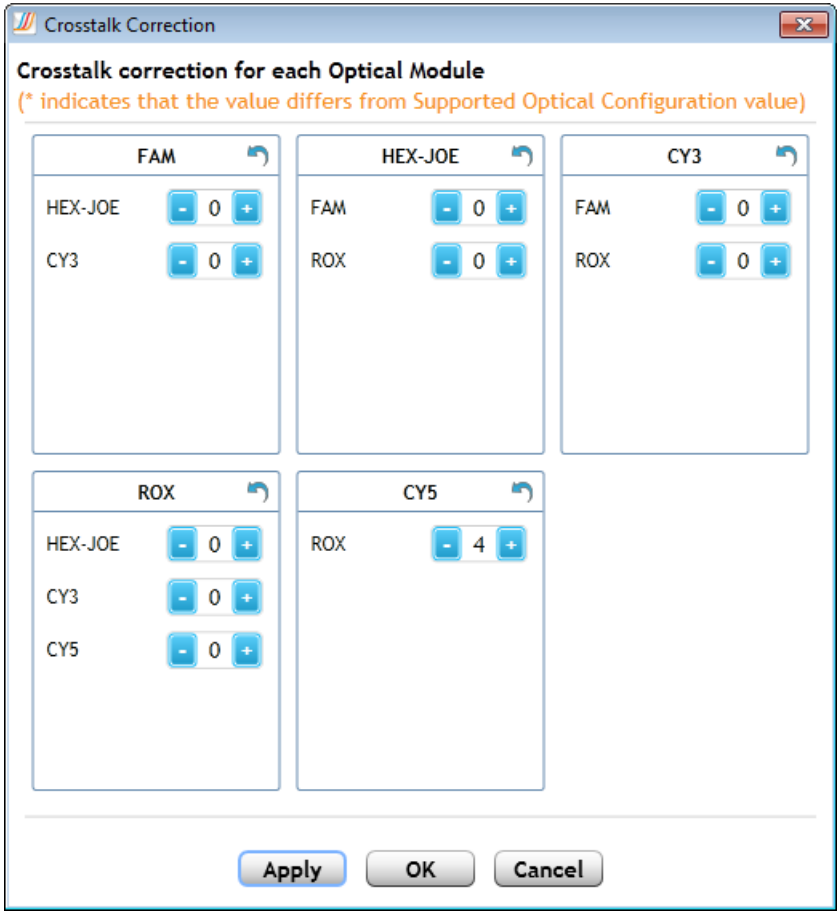
To adjust the crosstalk correction settings:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Crosstalk Correction**, click **Adjust**.

The Crosstalk Correction dialog box opens. The dialog box only displays the settings for the optical modules used in the experiment. For each optical module that is displayed, the current crosstalk correction setting for each dye is listed.



4 In the dialog box, locate the box for the optical module that is reporting crosstalk fluorescence (i.e., the spillover optical module). The dyes that have the potential to crosstalk with that optical module are listed in that box (e.g., the ROX dye in the CY5 optical module).

5 Change the crosstalk correction setting for the dye by adjusting the value in the field.

The values in these fields are percentages of the total raw fluorescence. They will be subtracted from the raw fluorescence signal for the spillover optical module when that dye is used as a target.

Crosstalk correction values that differ from the default values specified in the Supported Optical Configuration dialog box are noted with an asterisk (*). To reset a value back to its default, click the reset icon.

6 Click **Apply** to apply or changes, or click **OK** to apply your changes and close the dialog box.

Select the scale (linear or log) of the Y-axis

By default, the fluorescence values on the Y-axis of the amplification plots are in linear scale. You can quickly toggle between linear scale and log scale from the Graphical Displays screen.

To select the scale of the Y-axis:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Graph Type**, select **Linear** to plot the fluorescence values on a linear scale, or select **Log** to plot the fluorescence values on a log scale. The program adjust the amplification plots according to your selection.

Manually adjust threshold fluorescence values

The threshold fluorescence value for a target determines the target's Cq value. The Cq is the cycle number at which the fluorescence level passes the threshold. You can manually adjust the threshold fluorescence values while viewing the amplification plots.

To adjust the threshold fluorescence value for a target by typing the desired value:

1. Select the Amplification Plots graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Under **Threshold Fluorescence**, type a new value into the field next to the desired target, or click on the +/- buttons to adjust the value. *If the lock icon is in the locked position for the target, click on it to unlock before the adjusting threshold. See* *"Lock or unlock the threshold fluorescence values"* on page 210 *for more information.*

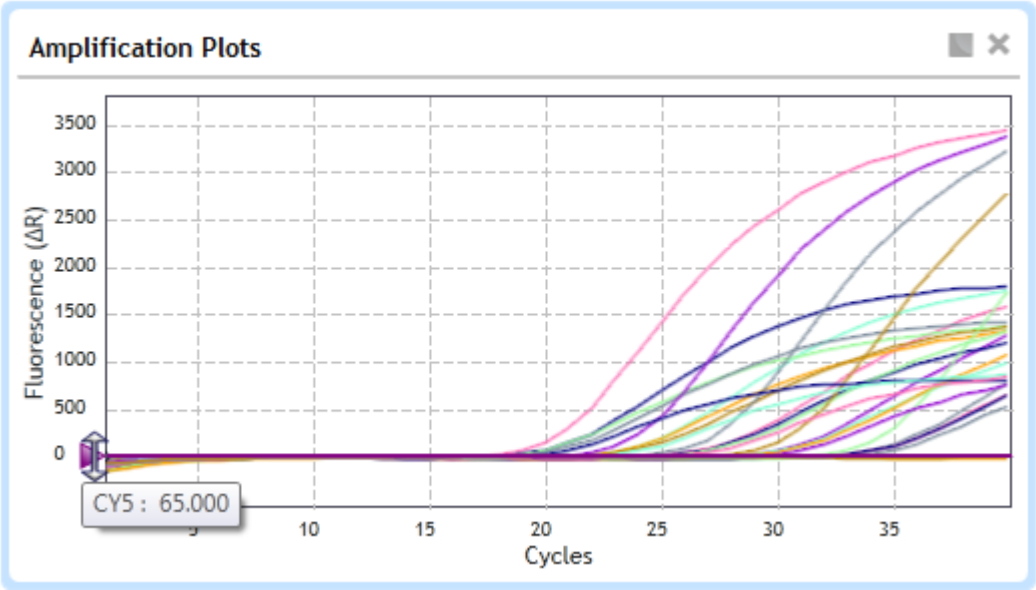
   The position of the corresponding horizontal line on the amplification plots adjusts to the new threshold fluorescence value.

To adjust the threshold fluorescence value for a target by dragging the threshold line on the Amplification Plots graph:

1. Select the Amplification Plots graph on the Graphical Displays screen.

   The threshold fluorescence level for each target is displayed on the graph as a horizontal line with a triangle marker along the Y-axis.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Under **Threshold Fluorescence**, locate the target that you want to adjust. If the lock icon next to the target is in the locked position, click on it to unlock it.
4. On the graph, hover your cursor over the triangle marker for the threshold line that you want to adjust.

   The cursor becomes a vertical, double-side arrow, and a tooltip box opens displaying the name of the target and its current threshold fluorescence value.



5 Click and drag the triangle marker to the desired position on the Y-axis.

The program sets the threshold fluorescence value for that target its new Y-axis value. The program also updates the value listed for that target in the right panel.

To reset the threshold fluorescence value for a target back to the default value calculated by the program:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Under **Threshold Fluorescence**, click the Recalculate icon next to the desired target.

The program resets the threshold fluorescence value in the field back to the default value and adjusts the position of the corresponding horizontal line on the Amplification Plots graph.

Adjust threshold fluorescence values by altering the algorithm settings

The default threshold fluorescence values calculated by the program are background based thresholds. Background based thresholds are based on the level of fluorescence noise in the background. The program calculates the background noise from the levels of fluorescence present in the selected wells (the wells included in the analysis) during the early cycles of PCR before product begins to accumulate and the fluorescence levels rise. By default, the cycles that the application uses for calculating the background noise are cycles 5 through 9.

To calculate the threshold fluorescence values, the program then multiplies the standard deviation (sigma) of the raw fluorescence in the background cycles by a mathematical constant known as a sigma multiplier. The default sigma multiplier is 10.

You can adjust the cycles used for calculating background noise and the sigma multiplier used in the algorithm from the Graphical Displays screen. Adjusting these settings results in a change to the threshold fluorescence values.

To change the range of cycles used for determining background noise:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

The current cycle range is displayed in the Cycle Range fields under **Background Based Threshold.**

3 In the first field next to **Cycle Range**, type the cycle number that will be the first cycle in the range, or click the +/- buttons to adjust the value in the field to the desired cycle number.

The program automatically recalculates the background noise and adjusts the threshold fluorescence values for all targets that have not been locked.

4 In the second field, type the cycle number that will be the last cycle in the range, or click the +/- buttons to adjust the value in the field to the desired cycle number.

   The program automatically recalculates the background noise and adjusts the threshold fluorescence values for all targets that have not been locked.

NOTE To set an accurate threshold, you need to set the cycle range to be in the flat baseline range for all plots.

To change the value of the sigma multiplier:

1 Select the Amplification Plots graph on the Graphical Displays screen.
2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3 In the Sigma Multiplier field under **Background Based Threshold**, type the desired value for the sigma multiplier, or click the +/- buttons to adjust the value in the field to the desired cycle number.

   The program automatically recalculates the background noise and adjusts the threshold fluorescence values for all targets.

Lock or unlock the threshold fluorescence values

You can lock the threshold fluorescence value for a given target. When locked, the threshold fluorescence value is fixed and the functions related to manually adjusting the threshold for that target are disabled. Additionally, if you make changes to the dataset or adjust any of the algorithm settings, the program does not recalculate the threshold fluorescence for that target.

To lock the threshold fluorescence value for a particular target:

1 Select the Amplification Plots graph on the Graphical Displays screen.
2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3 Under the **Threshold Fluorescence**, click the lock icon next to the target name.

   The image of the lock icon changes from unlocked ( ) to locked ( ).

To unlock the threshold fluorescence value for a particular target:

1. Select the Amplification Plots graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Under the **Threshold Fluorescence**, click the lock icon next to the target name.

   The image of the lock icon changes from locked ( ) to unlocked ( ).

NOTE The settings for the threshold fluorescence values and baseline cycle ranges on the Amplification Plots graph directly impact Cq value calls. Prior to finalizing an analysis of experimental results, verify that these settings align with the analysis requirements for your assay.

View the Melt Curve - Raw/Derivative Curve

For experiments that include a melt segment in the thermal profile, the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen displays the fluorescence data collected during the melt segment (Y-axis) as a function of temperature (X-axis). The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the melt curves.

To view the Melt Curve - Raw/Derivative Curve: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Melt Curve - Raw/Derivative Curve graph is not already displayed, click the icon for this graph at the bottom of the screen.

About raw/derivative curves

When using SYBR Green or EvaGreen dye for detection, the raw/derivative curves are used to verify that the predominant PCR products are amplicons of the intended target. The graph plots fluorescence or the first derivative of the fluorescence versus temperature. The temperatures plotted on the graph are typically from a melt segment ramp that included a data collection point.

Plotting results based on the first derivative multiplied by -1 [fluorescence term **-R´(T)** or **-Rn´(T)**] allows you to view the amplification products as peaks on the graph, with each peak centered on the melting temperature (Tm) for that product. Products with a Tm of 80°C or higher correspond to the larger PCR products, and can usually be assigned as specific DNA product. Products displaying melting temperatures of <75°C correspond to non-specific DNA products, such as primer dimers. It is important to note, however, that these populations are not necessarily homogeneous, and may contain multiple PCR product species.

View data for a single data point

Each curve on the graph is the plot for a single target in a single well or replicate set. Each data point that makes up the curve is a fluorescence value (plotted on the Y-axis) measured at a particular temperature (plotted on the X-axis). The program connects these data points to draw the raw/derivative curves displayed on the graph.

To view a summary of the data for a single data point on a plot:

1. Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.
2. Hover your cursor over an individual plot on the graph.

   A tooltip opens displaying the following information:

   - The well ID or replicate number for the plot and the target name
   - The fluorescence data type for the plot followed by the X and Y coordinates for the data point where your cursor is located
   - The Tm of each product identified by the algorithm

Select a fluorescence data type

For the Y-axis of the raw/derivative curves, you can select to use raw or normalized fluorescence values or the first derivative of those values multiplied by -1. Typically, the graphs are plotted using first derivative values multiplied by -1.

To select the type of fluorescence data displayed in the Raw/Derivative Curve:

1. Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.
2. In the right panel, select one of the options next to **Fluorescence Term**. The possible options are:

   R- Raw fluorescence

   Rn- Normalized fluorescence (normalized to reference dye)

   -R´(T)- First derivative of the raw fluorescence multiplied by -1

   -Rn´(T)- First derivative of the normalized fluorescence multiplied by -1

See "Select which data collection points to analyze" on page 181 for information on specifying which data collection point to use for generating the raw/derivative curves.

Adjust the graph properties

You can adjust certain properties of the Melt Curve - Raw/Derivative Curve graph (e.g., the scales of the axes, the graph title, and the back ground color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

Specify the use of smoothing

You can select to apply a Savitzky-Golay curve-smoothing algorithm to the raw/derivative curves [for a reference, see Savitzky and Golay, *Analytical Chemistry*, 1964; 36(8):1627-1639]. Smoothing alters the shapes of the curves by decreasing the effects of signal noise. The smoothing option is turned on by default.

To turn smoothing on or off:

1. Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Next to **Savitzky-Golay**, select **On** to turn on smoothing, or select **Off** to turn off smoothing.
4. If you selected **On**, next to **Number of Points**, select the number of data points on each melt curve that you want the algorithm to use in the smoothing calculations.

   The options are 5, 7, 9, and 11. A higher number of points leads to a smoother (i.e., more manipulated) curve.

Normalize the fluorescence values

To facilitate comparison among the curves on the graph, you can select to normalize the fluorescence values on the Y-axis. Normalization rescales the Y-axis value of each data point in each curve such that the minimum

fluorescence value for each plot is set to 0 and the maximum fluorescence value is set to 1. The normalization option is turned off by default.

To turn normalization on or off:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Normalization**, select **On** to turn on normalization, or select **Off** to turn off normalization.

Adjust the range of the X-axis

You can quickly modify the range of temperatures displayed on the X-axis.

To adjust the range of the X-axis:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 In the fields below **Temperature Range**, type in the desired lower temperature and upper temperature for the X-axis range, or click the +/- buttons to adjust the temperatures in the fields to the desired values.

The program adjusts the range of the X-axis accordingly.

Adjust product melting temperature settings

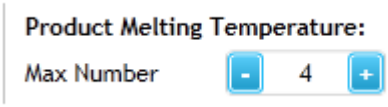
Some of the settings that the program uses to identify amplification products and calculate melting temperatures are adjustable. You can change the maximum number of amplification products for which the program reports a melting temperature. When basing plots on the negative derivative of fluorescence, you can set a minimum height that a product peak must reach in order for the program to consider it an amplification product.

To change the maximum number of amplification products for a which a melting temperature is reported:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 In the Max Number field, below the Product Melting Temperature heading, type in the desired maximum number of products, or click the +/- buttons to adjust the number of products in the field to the desired value. The default value is 4. The highest value allowed is 6.



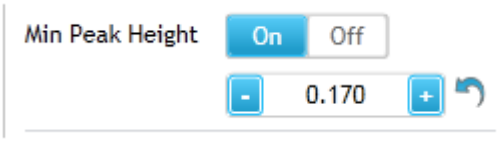
To set a minimum peak height for an amplification product:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Min Peak Height**, select **On** to turn on the minimum peak height option.

A new field appears for entering the minimum peak height, and a horizontal line appears on the graph marking the minimum height.



4 In the field, type the desired minimum peak height, or click the +/- buttons to adjust the value in the field. (Clicking the Reset icon sets the Minimum Peak Height value to the lowest peak among the set of highest peaks across all selected wells.)

The height of the horizontal line on the graph adjusts accordingly, and the program only counts a peak as an amplification product if the peak crosses that horizontal line.

View the Melt Curve - Difference Plots

The Melt Curve - Difference Plots graph on the Graphical Displays screen is typically used for analysis of Allele Discrimination experiments that use a DNA binding dye and a high resolution melt (HRM) segment to distinguish between alleles. The graph displays the difference in fluorescence between two plots during a melt ramp (Y-axis) as a function of temperature (X-axis). The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the melt curves.

The Melt Curve - Difference Plots graph is only available for experiments with:

- A high resolution melt (HRM) segment in the thermal profile
- An associated HRM calibration plate (HCP); see "Assign an HRM calibration plate" on page 183.

To view the Melt Curve - Difference Plots: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Melt Curve - Difference Plots graph is not already displayed, click the icon for this graph at the bottom of the screen.

If the Difference Plots icon has a warning symbol ⚠ next to it, then you cannot open the Difference Plots until you assign an HRM calibration plate (HCP) to the experiment. See "Assign an HRM calibration plate" on page 183 for instructions.

About difference plots

Difference plots are a useful way of viewing the results of an Allele Discrimination experiment that uses high resolution melt (HRM) analysis for SNP (single nucleotide polymorphism) genotyping. The values plotted on the Y-axis are the difference in fluorescence between a target in one well (or replicate set) and a control target from a designated well/replicate set. Because the plots display the difference in fluorescence, you can detect even slight differences between two plots. Consequently, this graph allows you to use HRM analysis to distinguish between product populations that differ in sequence by as little as a single nucleotide.

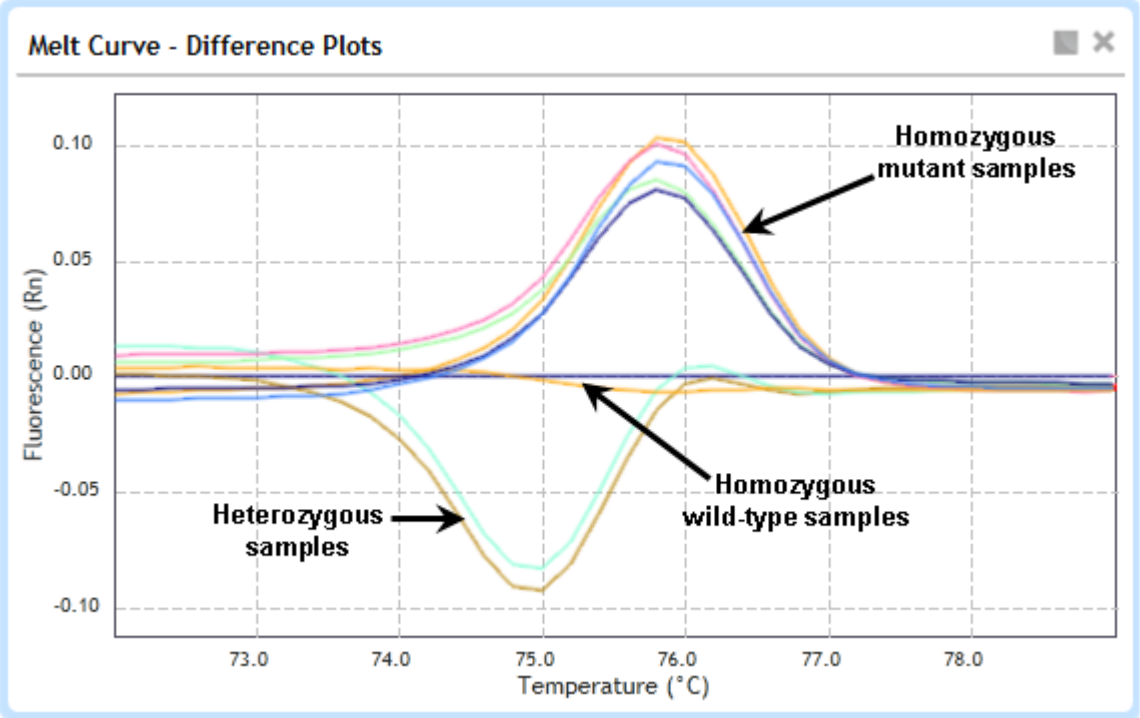
The equation for determining the Y-axis value at each temperature is:

Diff(T) = R(T) – compR(T)

where R(T) is the fluorescence value for the target at temperature T, and compR(T) is the value for the control target at temperature T.

Note that the fluorescence value can be any fluorescence term [R, Rn, –R´(T) or –Rn´(T)].

The figure below displays a difference plot for a class 4 SNP (A>T). Note the distinctly different plot shape for each genotype group.



To use HRM analysis for SNP genotyping, your experiment needs to include positive control samples for each base pair possibility (homozygous as well as heterozygous positive control samples). Then, when viewing the difference plots, designate the target in one of the homozygous positive control samples as the control target. You can then compare samples of unknown genotype to the samples of known genotype to visually determine the correct genotype group for the target in the unknown sample.

Select a fluorescence data type

For the Y-axis of the difference plots, you can select to use raw or normalized fluorescence values.

To select the type of fluorescence data displayed in the Difference Plots:

1. Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.
2. In the right panel, select one of the options next to **Fluorescence Term**. The possible options are:

   R-Raw fluorescence

   Rn-Normalized fluorescence (normalized to reference dye)

Assign the control target

The values plotted on the Y-axis are the difference in fluorescence values between a target in a particular well (or replicate set) and a specified control target. You can designate the control sample and target using the using the tools in the panel on the right side of the screen. Typically, a target from a homozygous positive control sample is selected as the control.

To assign the control target:

1. Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the Control Target settings.
3. Under **Control Target**, use the drop-down lists to specify the well or replicate set that contains the control sample and the target in that well/replicate set that is detecting the control target.
   a. In the **Well Type** drop-down list, select the well type in which the control target was amplified. Typically, one of the homozygous control wells is selected (Homo Allele A or Homo Allele B).
   b. In the **Replicate** or **Well Id** drop-down list, select the specific well or replicate set in which the control target was amplified.
   c. In the **Target** drop-down list, select the dye or target name for the control target.

View data for a single data point

Each curve on the graph is the plot for a single target in a single well or replicate set. Each data point that makes up the curve is a fluorescence value for that target/well measured at a particular temperature (plotted on the X-axis). The program connects these data points to draw the raw/derivative curves displayed on the graph.

To view a summary of the data for a single data point on a plot:

1. Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.
2. Hover your cursor over an individual plot on the graph.

   A tooltip opens displaying the following information:

   - The well ID or replicate number for the plot and the dye/target name
   - The fluorescence data type for the plot followed by the X and Y coordinates for the data point where your cursor is located
   - The Tm of each product identified by the algorithm

Manually assign Unknowns to a genotype call

Typically, you can visually examine the difference plots to determine the genotype of the samples in the Unknown wells. Once you have made your determinations, you can assign a genotype call to the Unknown wells/replicate sets in the program.

Add the Call column to the Results table

Before assigning genotype calls, you may want to add the Call column to the Results Table so you can easily see the call assignments. The calls you apply appear in this column.

To add the Call column:

1. Click the Column Option icon above the table to open the Column Options dialog box.
2. In the dialog box, mark **Call.**
3. Click **OK.**

   The dialog box closes and the Call column appears in the Results Table.

Apply calls

To apply a genotype call to a single Unknown well or replicate set:

1 Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.

2 In the Results Table, locate the row for the well or replicate set of interest, and right-click directly on that row.

3 In the pop-up menu that opens, click **Apply Call to Current Item**, then click the genotype that you want to assign (Homozygous A, Homozygous B, or Heterozygous).

   If the Results Table is displaying the Call column, the call you applied appears in that column.

To apply a genotype call to multiple Unknown wells or replicates sets:

1 Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.

2 In the Results Table, add the Check column (if not already added).

   a Click the Column Option icon above the table to open the Column Options dialog box.

   b Mark **Check** and click **OK**.

3 In the Results Table, locate the wells or replicate sets that you want to assign to the same genotype call. For those wells or replicate sets, mark the check box in the Check column.

4 Right-click on the Results Table or on the Difference Plots graph.

5 In the pop-up menu that opens, click **Apply Call to All Checked Items**, then click the genotype that you want to assign (Homozygous A, Homozygous B, or Heterozygous).

   If the Results Table is displaying the Call column, then the call you applied appears in that column.

Clear calls

You can remove calls that were manually applied to Unknown wells or replicate sets.

To clear a call for a single Unknown well or replicate set:

1 Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.

2 In the Results Table, locate the row for the well or replicate set of interest, and right-click directly on that row.

3 In the pop-up menu that opens, click **Apply Call to Current Item > Clear Call**.

   If the Results Table is displaying the Call column, the call no longer appears in that column.

To clear a call from multiple Unknown wells or replicate sets:

1 Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.

2 In the Results Table, add the Check column (if not already added).

   a Click the Column Option icon above the table to open the Column Options dialog box.

   b Mark **Check** and click **OK**.

   c In the Results Table, locate the wells or replicate sets for which you want to clear the manually applied genotype call. For those wells or replicate sets, mark the check box in the Check column.

   d Right-click on the Results Table or on the Difference Plots graph.

   e In the pop-up menu that opens, click **Clear All Checked Items**.

   If the Results Table is displaying the Call column, the calls no longer appears in that column.

Edit manual call settings

From the HRM Manual Calling dialog box, you can edit the name and color that is used to for each genotype in the Difference Plots graph and the result table.

1 Select the Melt Curve - Difference Plots graph on the Graphical Displays screen.

2 Right-click on the Results Table or on the Difference Plots graph.

3 In the pop-up menu that opens, click **Edit Manual Call Settings**.

   The HRM Manual Calling dialog box opens.

4 For the genotype that you want to assign to a different name, type the desired name into the field.

5 For the genotype that you want to assign to a different color, expand the drop-down list to view the color options.

6 Click directly on the color that you want to assign.

7 Click **OK** in the dialog box.

The dialog box closes and color coding in the difference plots is updated.

Adjust the graph properties

You can adjust certain properties of the Melt Curve - Difference Plots graph (e.g., the scales of the axes, the graph title, and the background color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

View the Standard Curve

For Quantitative PCR experiments, Comparative Quantitation experiments, and User Defined experiments that include a set of Standard wells, the Graphical Displays screen includes a Standard Curve graph. This graph is a plot of the Cq (Y-axis) versus the log of the initial template quantity in the Standard wells (X-axis). Each plot is a target in a Standard well or replicate set. The graph also plots the Cq values from the Unknown wells. The program uses a least mean squares curve fitting algorithm to generate the standard curves. The panel on the right side of the screen has tools for adjusting some of the analysis parameters.

To view the Standard Curve: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Standard Curve graph is not already displayed, click the Standard Curve icon at the bottom of the screen.

NOTE Only the Standard wells that you selected in the Analysis Criteria screen are included in the Standard Curve graph.

View data for a single data point

Each curve on the graph is the plot for a single target in a single Standard well or replicate set. The square-shaped markers denote the data points from the Standard wells that make up the curve. Each of these data points is a fluorescence value (typically log scale) plotted against the initial template quantity as entered on the Plate Setup screen. The program connects these data points to draw the curves displayed on the graph. The triangle-shaped markers on the graph denote Cq values from Unknown wells.

To view a summary of the data for a single data point from a Standard well:

1. Select the Standard Curve graph on the Graphical Displays screen.
2. Hover your cursor over the square marker for the data point of interest.

   A tooltip opens displaying the following information:

- The well ID, replicate number, target name, and well type (or sample name) for the plot
- The initial template quantity as entered on the Plate Setup screen (i.e., the X-axis coordinate)
- The quantification cycle for the plot (i.e., the Y-axis coordinate)

To view a summary of the data for a single data point from an Unknown well:

1 Select the Standard Curve graph on the Graphical Displays screen.

2 Hover your cursor over the triangle marker for the data point of interest.

A tooltip opens displaying the following information:

- The well ID, replicate number, target name, and well type (or sample name) for the plot
- The initial template quantity as calculated by the program (i.e., the X-axis coordinate)
- The quantification cycle for the plot (i.e., the Y-axis coordinate)

Select a fluorescence data type

For the Y-axis of the amplification plots, you can use baseline-corrected fluorescence values with or without normalization to a reference dye.

To select the type of fluorescence data displayed in the Standard Curve graph:

1 Select the Standard Curve graph on the Graphical Displays screen.

2 In the right panel, select one of the options next to **Fluorescence Term**. The possible options are:

ΔR - baseline-corrected raw fluorescence

ΔRn - baseline-corrected, normalized fluorescence

See "Select which data collection points to analyze" on page 181 for information on specifying which data collection point to use for generating the standard curve.

View the R-squared values, slopes, and amplification efficiencies

The Target Information Table displays information about each target on the Standard Curve graph. This information includes the amplification efficiency (which is calculated from the slope), the R-squared (R^2) value, the slope of the standard curve plot, and the point where it intercepts the Y-axis.

Target Information Table

Target	Efficiency	R^2	Slope	Intercept
FAM	94.41	0.999	-3.464	20.54
ROX	92.1	0.999	-3.527	20.48
HEX	99.39	0.999	-3.337	20.77
CY5	95.53	0.999	-3.434	21.26
CY3	93.92	0.998	-3.477	20.9

The R^2 value is an indicator of the quality of the fit of the standard curve to the standard data points plotted. The value is always between 0 and 1, and the closer the value is to 1, the better the fit of the line.

The slope of the curve is directly related to the average amplification efficiency throughout the cycling reaction. The program uses the following equation to calculate slope:

$y = m*\log(x) + b$, where m is the slope of the line

PCR efficiency is the percentage of template molecules that are doubled every cycle. The equation that relates the slope to amplification efficiency is:

$\text{PCR efficiency} = 10(-1/\text{slope}) - 1$

Based on this equation, a PCR reaction with 100% efficiency results in a standard curve with a slope of -3.322.

To view the Target Information Table:

1 Select the Standard Curve graph on the Graphical Displays screen.

The table is displayed in the panel on the right side of the screen.

Adjust the graph properties

You can adjust certain properties of the Standard Curve graph (e.g., the scales of the axes, the graph title, and the background color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

Manually adjust threshold fluorescence values

The threshold fluorescence value for a target determines the target's Cq value. The Cq is the cycle number at which the fluorescence level passes the threshold. You can manually adjust the threshold fluorescence values while viewing the standard curves.

To adjust the threshold fluorescence value for a target by typing the desired value:

1. Select the Amplification Plots graph on the Graphical Displays screen, and unlock the threshold fluorescence values that you want to manually adjust.
2. Select the Standard Curve graph.
3. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
4. Under **Threshold Fluorescence**, type a new value into the field next to the desired target, or click on the +/- buttons to adjust the value.

   The program adjusts the standard curves according to the new values.

To reset the threshold fluorescence value for a target back to the default value calculated by the program:

1. Select the Standard Curve graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Under **Threshold Fluorescence**, click the Recalculate icon next to the desired target.

   The program resets the threshold fluorescence value in the field back to the default value and adjusts the standard curves accordingly.

Display and adjust confidence intervals

You can select to display the confidence intervals for each plot on the Standard Curve graph as hashed lines. These lines show the range of initial quantity values at a particular Cq that cannot be statistically distinguished from the fit line with more certainty than the specified confidence level. The width of the confidence interval is an indicator of the quality of the fit of the data to the standard curve.

The default confidence level is 99%. When you select to display the confidence intervals, you can adjust the confidence level.

To display confidence intervals, the program must treat replicates individually.

To display confidence intervals:

1. Select the Standard Curve graph on the Graphical Displays screen.
2. In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.
3. Under **Threshold Fluorescence**, mark **Show confidence interval**.

   The program displays the hashed lines on the Standard Curve graph indicating the confidence intervals.

   If you had selected to treat replicates collectively, the program will prompt you to treat them individually in order to display the intervals.

To adjust the confidence level:

1. Display the confidence intervals on the Standard Curve graph (see instructions above).
2. Next to **Level %**, type the desired confidence level into the field, or click on the +/- buttons to adjust the value in the field.

   The program updates the positions of the confidence interval lines on the graph to reflect the new confidence level.

View the Relative Quantity

For Comparative Quantitation experiments and User Defined experiments that include a set of Calibrator wells, the Graphical Displays screen includes a Relative Quantity chart. This chart is a bar graph that shows the amount of target present in the experimental samples (the Unknown wells) relative to the associated reference sample (the Calibrator wells) after the program has normalized the quantities using data from the normalizer target.

To view the Relative Quantity: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Relative Quantity chart is not already displayed, click the Relative Quantity icon at the bottom of the screen.

About relative quantities

Each bar on the graph is a target within a well or replicate set. The expression level of the target-of-interest in the Calibrator wells is defined 1.0. In the Unknown wells, the expression levels of the target-of-interest are reported on the Y-axis as a fold difference relative to the calibrator benchmark.

How you set up the wells on the Plate Setup screen impacts how the program compares samples in the Relative Quantity chart.

- If you designate a separate calibrator sample for each unique target, the program only compares Unknown wells with that same target to those Calibrator wells. This allows you to run multiple targets on the same plate and analyze them independently.
- If you set up multiple Calibrator wells/replicate sets with the same target name, the program will average the Cq values of those Calibrator wells and calculate the relative quantities of that target in the Unknown wells relative to the average.

Select a fluorescence data type

For the Y-axis of the Relative Quantity chart, you can use baseline-corrected fluorescence values with or without normalization to a reference dye.

To select the type of fluorescence data displayed in the Relative Quantity chart:

1 Select the Relative Quantities chart on the Graphical Displays screen.

2 In the right panel, select one of the options next to **Fluorescence Term**. The possible options are:

   ΔR - baseline-corrected raw fluorescence

   ΔRn - baseline-corrected, normalized fluorescence

Set the Y-axis scale for the Relative Quantity chart

By default, the program displays the Y-axis in linear scale. In this scale, you can view the quantity of an target as a fold change in expression level relative to the calibrator. This view is convenient for assessing increases in expression levels of a target relative to the calibrator.

Alternatively, you can set the Y-axis to display the target quantities on a base 2-logarithmic scale. This view is convenient for viewing target expression levels that decrease or increase relative to the calibrator.

To display the relative quantities of the targets in the Unknown wells on a base 2-logarithmic scale:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel next to **Chart Type**, select **Log2**.

   The program updates the Y-axis values on the chart.

To reset the chart to display relative quantities as a fold change relative to the quantities in the Calibrator wells:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel next to **Chart Type**, select **Fold**.

   The program updates the Y-axis values on the chart.

Add error bars to the Relative Quantity chart

When you are treating replicate wells collectively, you can select to display error bars on the relative quantities that reflect the deviation among the replicates. The program calculates the error bars on the relative quantities from the error bars on the Cqs, which it calculates from the deviation on each fluorescence measurement at each cycle and estimates on the imprecision of the normalizers.

To add error bars:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 Make sure that the program is set to treat replicates collectively. (See "Choose a treatment for replicate wells" on page 182.)

3 In the right panel next to **Error Bar**, select **On**.

The program add error bars to the chart.

To remove error bars:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel next to **Error Bar**, select **Off**.

The program removes the error bars from the chart.

Adjust the graph properties

You can adjust certain properties of the Relative Quantities chart (e.g., the scales of the axes, the graph title, and the background color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

Select the algorithm method

The program provides multiple algorithm options for calculating the relative quantities.

To select an algorithm for the relative quantity calculations:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Mode**, select the method you want to use. The options are described briefly below.

ΔΔCq (Livak) - The ΔΔCq method, also called the Livak method, relies on two assumptions. The first assumption is that both the normalizer and the target-of-interest have amplification efficiencies at or near 100% and that the efficiencies of the two targets do not differ by more than 5%. The second assumption is that the amplification efficiency of a target is consistent from one run to the next. Any run-to-run variance is not included in the calculations. The ΔΔCq method is often referred to as an approximation method and requires a validation step to confirm that efficiencies of your normalizer and target-of-interest are similar. This method is only available if the wells selected on the Analysis Criteria screen include a normalizer target.

ΔCq - The ΔCq method is similar to the ΔΔCq method in that it relies on the same mathematical assumptions about efficiencies and consistency. Unlike the ΔΔCq method, however, the relative quantity in the calibrator sample is not set to 1.0. This method is only available if the wells selected on the Analysis Criteria screen *do not* include a normalizer target.

Pfaffl - With the Pfaffl method, the program takes into account the amplification efficiencies of the normalizer and target-of-interest when calculating the relative quantities. This method is a good choice for Comparative Quantitation experiments in which the normalizer and target-of-interest differ in amplification efficiency. The Pfaffl method does not, however, incorporate run-to-run variations in amplification efficiency. For a reference, see: Pfaffl, M. W. (2001) *Nucleic Acids Res.* 29(9):e45. This method is only available if the wells selected on the Analysis Criteria screen include a normalizer target.

Enter the amplification efficiencies for the targets

If you selected the Pfaffl algorithm method to calculate the relative quantities, you need to enter the amplification efficiency of each target. If you determined the efficiencies based on a standard curve that was run in another experiment, you can enter the efficiencies manually. If the current experiment includes a standard curve to determine the efficiencies, you can specify to use the efficiencies derived from that data.

To enter the amplification efficiencies manually:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Mode**, select **Pfaffl** to enable the fields in the Amplification Efficiencies table.

4 In the Amplification Efficiencies table, enter the efficiency of each target using either of the following approaches:

- In the Slope column, type the slope of the target, or click the +/- buttons to adjust the value in the field. The program automatically adjusts the value in the Efficiency (%) column by relating slope to amplification efficiency.
- In the Efficiency (%) column, type the amplification efficiency of the target, or click the +/- buttons to adjust the value in the field. The program automatically adjusts the value in the Slope column by relating amplification efficiency to slope.

To enter the amplification efficiencies from a standard curve included in the experiment:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 Next to **Mode**, select **Pfaffl** to enable the fields in the Amplification Efficiencies table.

4 Below the Amplification Efficiencies table, mark the check box labeled **Apply Std Curve Efficiencies to CQ Results**.

View the Allele Determination graph

For Allele Discrimination experiments and User Defined experiments that include wells with allele designations, the Graphical Displays screen includes an Allele Determination graph. This graph is useful for viewing the genotype results in Allele Discrimination experiments that use two differentially-labeled fluorescent probes to detect the two different alleles. Each plotted point on the graph represents the coordinates of either the fluorescence values or Cq values for the two targets. For example, the X-axis may correspond to the Cq of the Allele A target while the Y-axis corresponds to the Cq of the Allele B target and the plotted point (x,y) corresponds to the coordinates describing the two Cq values determined for a given well. The position of the data point on the graph indicates the presence or absence of each allele.

To view the Allele Determination: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Allele Determination graph is not already displayed, click the icon for this graph at the bottom of the screen.

View data for a single data point

If you set up the experiment to amplify the two alleles within the same well, then each data point on the Allele Determination graph represents a single well or replicate set. If you set up the experiment to amplify the two alleles in separate wells that contain the same sample, then each data point represents a single sample.

To view a summary of the data for a single data point on a plot:

1. Select the Allele Determination graph on the Graphical Displays screen.
2. Hover your cursor over an individual data point on the graph.

   A tooltip opens displaying the following information:

   - The well ID or replicate number for the data point
   - The fluorescence data type for the plot followed by the X and Y coordinates for the data point
   - The well name as specified on the Plate Setup screen

- The genotype assigned by the program to that target in that well/replicate

Select data type and fluorescence type to display

Each plotted point on the graph represents the coordinates of either the fluorescence values or Cq values for the two targets. You can select which type of data (fluorescence or Cq) you want displayed on the graph.

To select a data type and fluorescence type:

1. Select the Allele Determination graph on the Graphical Displays screen.
2. In the right panel, next to **Display by**, select **Fluorescence** to plot the fluorescence values, or select **Cq** to plot the Cq values.

   Based on your selection, the program displays the options for fluorescence data type.
3. Select one of the fluorescence data type options.
   - The possible options if you selected to display by Fluorescence:

     R last - the final raw fluorescence reading as measured in the last cycle

     ΔR last - the final baseline-corrected fluorescence reading as measured in the last cycle

     Rn last - the final normalized fluorescence reading as measured in the last cycle

     ΔRn last - the final baseline-corrected, normalized fluorescence reading as measured in the last cycle

     R last / R first - the final fluorescence reading divided by the initial fluorescence reading
   - The possible options if you selected to display by Cq:

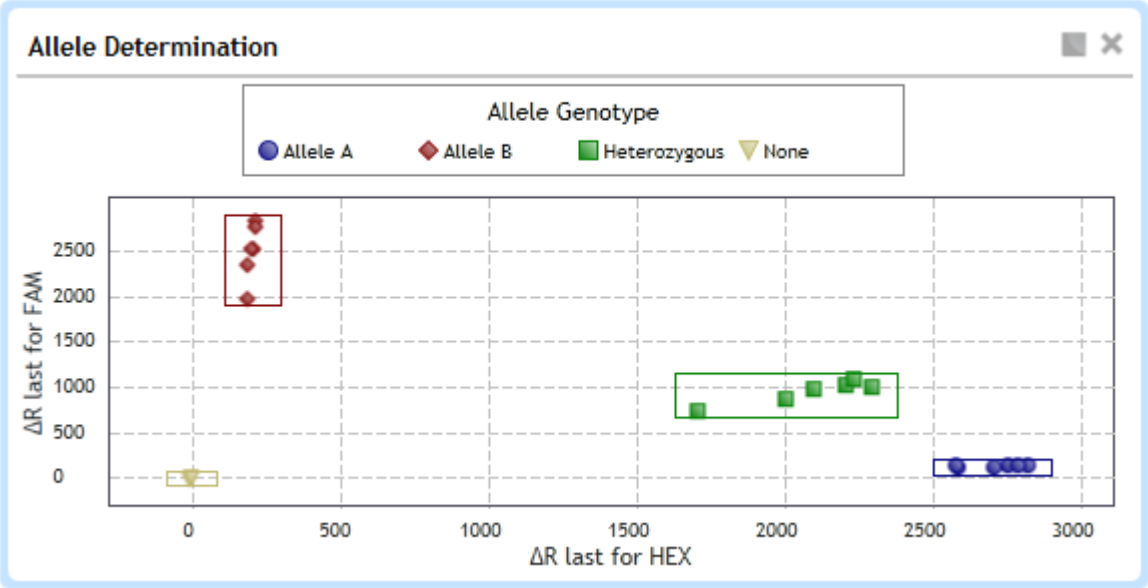
     ΔR - Cq of the baseline-corrected raw fluorescence plot

     ΔRn - Cq of the baseline-corrected and normalized fluorescence plot

See "Select which data collection points to analyze" on page 181 for information on specifying which data collection point to use for generating the amplification plots.

Display genotype groups on the graph

You can include on the graph colored rectangles that group data points with the same assigned genotype (allelic composition). You can allow the program to automatically determine the positions of these rectangles, or you can manually position them yourself.



To include colored rectangles that are automatically determined by the program:

1 Select the Allele Determination graph on the Graphical Displays screen.

2 In the right panel, next to **Genotype Calls**, select **Auto**.

The program adds the rectangles to the graph, grouping data points with the same genotype call.

To include colored rectangles and manually position them:

1 Select the Allele Determination graph on the Graphical Displays screen.

2 In the right panel, next to **Genotype Calls**, select **Manual**.

The program adds the rectangles to the graph, grouping data points with the same genotype call.

3 Move the border of the any of the rectangles to include or exclude data points.

   a Hover your cursor over the border that you want to reposition.

   b Click and drag the border to a new position.

To specify which genotype groups have a rectangle displayed on the graph:

1 Select the Allele Determination graph on the Graphical Displays screen.

2 Right-click on the graph.

   A short-cut menu opens. The menu includes a list of the possible genotypes (Allele A, Allele B, Both, and None). The genotypes that are already represented on the graph with a rectangle have a check mark next to them.

3 Click on the genotypes in the short-cut menu to add or remove rectangles as desired.

   Each time you click a genotype, the short-cut menu closes and the program updates the rectangles on the graph. Re-open the short-cut menu to make further changes.

Adjust the graph properties

You can adjust certain properties of the Allele Determination graph (e.g., the graph title, and the background color) through the short-cut menu and the Graph Properties dialog box. See "Customize graph properties" on page 240 for more information.

Adjust the last cycle

When you select to display the graph by fluorescence (rather than Cq), the cycle number entered in the Last Cycle field specifies which cycle the program uses for the fluorescence values plotted on the graph.

The last cycle selection only affects the Allele Determine graph. It does not affect the amplification plots or the Cq values calculated by the program.

By default, the cycle number in this field is the last cycle of the segment being analyzed, but you can specify a different cycle number.

To adjust the last cycle:

1 Select the Allele Determination graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 In the field next to **Last Cycle**, type in the desired cycle number, or click the +/- buttons to adjust the cycle number in the field.

Rename the genotype groups

By default, the genotypes are given the default names **Allele A** (for the allele A homozygous group), **Allele B** (for the allele B homozygous group) and **Heterozygous** (for the A/B heterozygous group). You can assign new names to the genotype groups.

To assign new names to the genotype groups:

1 Select the Allele Determination graph on the Graphical Displays screen.

2 In the right panel, click the downward arrowhead above the result table to display the advanced analysis parameter settings.

3 In the fields below **Rename Genotypes**, type the desired name for each genotype group.

- Allele A - the group that is homozygous for allele A
- Allele B - the group that is homozygous for allele B
- Hetero - the group that is heterozygous for alleles A and B

Customize graph properties

You can customize many of the properties of the graphs on the Graphical Displays screen. Customization options are available through a short-cut menu and on the Graph Properties dialog box.

Customize graph properties using the short-cut menu

To open the graphs short-cut menu: From the Graphical Displays screen, right-click on the graph whose properties you want to edit.

The commands available through the graphs short-cut menu are described in the table below.

Command or Command set	Description
Reset Zoom	If you zoomed in on a graph, this command resets the zoom level of the graph back to 100%. You can also reset the zoom from the Graph Properties dialog box. (See "Zooming" on page 190 for instructions on how to zoom)
Add Marker at X-Axis Add Marker at Y-Axis	These two commands are only available for Melt Curve graphs (Difference Plots and Raw/Derivative Curve). The commands add a vertical marker (X-axis marker) or a horizontal marker (Y-axis marker) to the graph.
Check Item	This command is used for manual genotype calling in the Melt Curve - Difference Plots graphs. It is only available when you right-click on an Unknown well or replicate set in the Results Table. The command adds a check mark to the selected well or replicate set. Once you check items, use the Apply Call to All Checked Items command to assign a genotype call. Alternatively, you can add a check mark to a well or replicate set using the Check column in the Results Table.
Apply Call to Current Item > Homozygous A Homozygous B Heterozygous Clear Call	These commands are used for manual genotype calling in the Melt Curve - Difference Plots graphs. They are only available when you right-click on an Unknown well or replicate set in the Results Table. Select one of the genotypes (Homozygous A, Homozygous B, or Heterozygous) to assign a call, or select Clear Call to remove an existing call.

Apply Call to All Checked Items> Homozygous A Homozygous B Heterozygous Clear Call	These commands are used for manual genotype calling in the Melt Curve - Difference Plots graphs. If you added a check mark to specific wells or replicate sets (using the Check Item command or the Check column in the Results Table), use these commands to assign the checked items to a genotype (Homozygous A, Homozygous B, or Heterozygous), or to remove genotype calls from the checked items (Clear Call command).
Clear Checked Items	This command is used for manual genotype calling in the Melt Curve - Difference Plots graphs. If you added a check mark to specific wells or replicate sets (using the Check Item command or the Check column in the Results Table), use this command to remove genotype calls from the checked items.
Edit Manual Call Settings	This command is used for manual genotype calling in the Melt Curve - Difference Plots graphs. It opens the HRM Manual Call Settings dialog box, which allows you to assign a color and name to each genotype for use in the difference plots.
Allele A Allele B Both None	This set of four options is only available for Allele Determination graphs. Use the options to specify which genotype groups on the graph are visually grouped together with a rectangle. See "Display genotype groups on the graph" on page 237 for more information.
Axis Options > Enable X-Axis Log Scale Enable Y-Axis Log Scale	These two commands are part of the Axis Options sub-menu. Click directly on one of these commands to toggle between a linear scale and a log scale for the X and Y axes of the graph. When a command has a check mark next to it, that indicates that the axis is set to a log scale. The absence of a check mark indicates that the axis is set to a linear scale.
Axis Options > Reverse Orientation in X-Axis Reverse Orientation in Y-Axis	These two commands are part of the Axis Options sub-menu. Click directly on one of these commands to toggle the orientation of the X and Y axes of the graph. When a command has a check mark next to it, that indicates that the axis is oriented in the reverse direction. The absence of a check mark indicates that the axis is oriented in the forward (ascending) direction.
Axis Options > Enable X-Axis AutoScale Enable Y-Axis AutoScale	These two commands are part of the Axis Options sub-menu. Click directly on one of these commands to turn on or off the AutoScale functionality. When turned on, this functionality causes the program to automatically set the range of the axis based on the data points present on the graph. When a command has a check mark next to it, that indicates that AutoScale is on. The absence of a check mark indicates that AutoScale is off.
Axis Options > Customize Scale	This command, which is part of the Axis Options sub-menu, opens the Graph Properties dialog box to the Axis Options tab. You can customize the scale and range of the axes using the tools on this tab. See "Customize the graph axes" on page 244.

Legend Options > Show Legend	This command is part of the Legend Options sub-menu. Click directly on **Show Legend** to show or hide the graph legend. When the command has a check mark next to it, that indicates that the legend is currently being displayed. The absence of a check mark indicates that the legend is currently hidden.
Legend Options > Top Bottom Left Right	This set of four options is part of the Legend Options sub-menu, and is only available when the Show Legend command is marked (i.e., the legend is shown on the graph). Use these options to set the location of the legend within the graph.
Legend Options > Edit Legend	This command, which is part of the Legend Options sub-menu, opens the Graph Properties dialog box to the Legend Options tab. You can customize the plot colors and legend font size using the tools on this tab. See "Customize the graph legend" on page 247.
Grid Options > Show Both Axis Grid Lines Show X-Axis Grid Lines Show Y-Axis Grid Lines Hide Grid Lines	These four commands are part of the Grid Options sub-menu. Use these commands to set the display of grid lines on the graph. When a command has a check mark next to it, that indicates that it is turned on. The absence of a check mark indicates that it is turned off. When the Show Both Axis Grid Lines command is turned on, the graph displays horizontal and vertical grid lines. When the Hide Grid Lines command is turned on, the graph does not display any grid. When the Show X-Axis Grid Lines or Show Y-Axis Grid Lines command is turned on, only vertical or horizontal grid lines are displayed, respectively.
Grid Options > Solid Grid Lines Dashed Grid Lines	These two options are part of the Grid Options sub-menu. Use these options to set the type of grid lines displayed on the graph. When an option has a check mark next to it, that indicates that it is turned on. The absence of a check mark indicates that it is turned off. When the Solid Grid Lines option is turned on, the grid lines on the graph are solid lines. When the Dashed Grid Lines option is turned on, the grid lines on the graph are dashed lines.
Grid Options > Edit Grid Line Color	This command, which is part of the Grid Options sub-menu, opens the Graph Properties dialog box to the Grid Options tab. You can customize the grid line color using the tools on this tab. See "Customize graph grid lines" on page 246.
Edit Background Color	This command opens the Graph Properties dialog box to the General tab. You can customize the background color on the graph using the tools on this tab. See "Customize general graph properties" on page 243.
Edit Graph Title	This command opens the Graph Properties dialog box to the General tab. You can customize the graph title using the tools on this tab. See "Customize general graph properties" on page 243.

Print Image	This command opens the Print dialog box where you can print a copy of the graph.
Save Image As	This command opens the Save As dialog box, where you can save a jpeg image of the graph.
Send Image To PowerPoint	This command launches Microsoft PowerPoint and creates a new presentation that contains the image of the selected graph.
Send To Excel > Vertical Format Horizontal Format	These two options are part of the Send To Excel sub-menu. Both commands launch Microsoft Excel and create a new spreadsheet that contains the data from the selected graph. Click **Vertical Format** export the graph data in vertical format, or click **Horizontal Format** to export the graph data in horizontal format. Note that some graphs may only have the Vertical Format option available.
Restore Default Settings	This command sets all the graph properties back to their default settings.

Customize graph properties using the Graph Properties dialog box

To open the Graph Properties dialog box: From the Graphical Displays screen, double-click on the graph whose properties you want to edit.

Customize general graph properties

The tools on the General tab of the Graph Properties dialog box allow you to set many of the general properties for the graph, including title and background color.

To edit a graph title:

1 Open the Graph Properties dialog box to the General tab.

2 In the Graph Title field, type the desired title for the graph.

3 Click **Close**.

The dialog box closes and the graph displays the new title.

To customize the background color of a graph:

1 Open the Graph Properties dialog box to the General tab.

2 Expand the Background Color drop-down list to view a menu of standard background colors.

3 Select a background color:

- If the standard menu includes your desired color, click directly on it.

- If you need a color not included on the palette, click **Advanced** to view an advanced menu for color selection. Use the color picker tools to create a custom color.

The color menu closes and the new color is displayed in the Background Color drop-down list.

4 (Optional) Click the Apply To All Plots icon to set the new color as the background for all graphs in the experiment.

5 Click **Close**.

The dialog box closes and the graph displays the new background color.

To reset the zoom level of a graph back to 100%:

1 Open the Graph Properties dialog box to the General tab.

2 Next to **Zoom**, click **Reset**.

3 Click **Close**.

The dialog box closes and the zoom level of the graph is reset to 100%. You can also reset the zoom level from the short-cut menu. See "Zooming" on page 190 for instructions on how to zoom.

Customize the graph axes

The tools on the Axis Options tab of the Graph Properties dialog box allow you to customize the orientations and scales of the X and Y axes.

To set the scale (linear or log) of the X or Y axis:

1 Open the Graph Properties dialog box to the Axis Options tab.

2 Next to **X-Axis Scale** or **Y-Axis Scale**, select **Linear** to plot the values on the selected axis in a linear scale, or select **Log** to plot the values on a log scale.

3 Click **Close**.

The dialog box closes and the program adjusts the graph according to your selection.

To manually set the upper and lower limits on the scale of an axis:

1 Open the Graph Properties dialog box to the Axis Options tab.

2 Under the heading for the desired axis, next to **Autoscale**, select **Manual** (if not already selected) to turn off the Autoscale functionality.

3 In the Min field, type the minimum value for the axis.

4 In the Max field, type the maximum value for the axis.

5 Click **Close**.

The dialog box closes and the program adjusts the scale of the axis according to your entries.

To allow the program to automatically set the scale on an axis:

1 Open the Graph Properties dialog box to the Axis Options tab.

2 Under the heading for the desired axis, next to **Autoscale**, select **Autoscale** (if not already selected). The Autoscale functionality causes the program to automatically set the range of the axis based on the data points present on the graph.

3 Click **Close**.

The dialog box closes and the program automatically adjusts the scale of the axis.

To set the orientation of an axis:

1 Open the Graph Properties dialog box to the Axis Options tab.

2 Under the heading for the desired axis, next to **Reverse Orientation**, select **On** to plot the values on the selected axis in reverse (descending) order, or select **Off** to plot the values or ascending order.

3 Click **Close**.

The dialog box closes and the program adjusts the axis of the graph according to your selection.

To set minimum value of the Y-axis to exclude below-threshold noise from log-scale Amplification Plots graph:

1 For an Amplification Plot graph, open the Graph Properties dialog box to the Axis Options tab.

2 Under the Y-Axis heading, make sure the Y-Axis Scale is set to **Log**.

3 Next to **Noise Cutoff**, select **On**.

4 Click **Close**.

The dialog box closes and the program adjusts the minimum value of the Y-axis to be just below the baseline threshold, which removes the background noise signal plots from the display.

Customize graph grid lines

The tools on the Grid Options tab of the Graph Properties dialog box allow you to customize the appearance of the graph's grid lines.

To select which axes of the graph have grid lines:

1 Open the Graph Properties dialog box to the Grid Options tab.

2 Next to **Grid Lines**, select one of the options described below.

- **Both**: Displays grid lines for the X- and Y-axes.
- **X-Axis**: Displays grid lines for the X-axis only.
- **Y-Axis**: Displays grid lines for the Y-axis only.
- **None**: Does not display grid lines for either axis.

3 Click **Close**.

The dialog box closes and the program adjusts the grid lines according to your selection.

To set the style of the grid lines:

1 Open the Graph Properties dialog box to the Grid Options tab.

2 Next to **Styles**, select one of the options described below.

- **Solid**: Displays the grid as solid lines.
- **Dashed**: Displays the grid as dashed lines.

3 Click **Close**.

The dialog box closes and the program adjusts the grid lines according to your selection.

To customize the color of the grid lines:

1 Open the Graph Properties dialog box to the Grid Options tab.

2 Expand the Color drop-down list to view a menu of standard grid line colors.

3 Select a color:

- If the standard menu includes your desired color, click directly on it.
- If you need a color not included on the standard menu, click **Advanced** to view an advanced menu for color selection. Use the color picker tools to create a custom color.

The color menu closes and the new color is displayed in the Color drop-down list.

These options are not available if you selected to not display grid lines.

4 Click **Close**.

The dialog box closes and the graph displays the grid color.

Customize the graph legend

The tools on the Legend Options tab of the Graph Properties dialog box allow you to customize the graph's legend, including the position of the legend, the font size used in the legend text, and the color assigned to each plot.

To show or hide the legend on a graph:

1 Open the Graph Properties dialog box to the Legend Options tab.

2 Next to **Legend**, select **On** to display a legend on the graph, or select **Off** to hide the legend.

3 Click **Close**.

The dialog box closes and the program shows or hides the legend according to your selection.

To set the position of the legend on a graph:

1 Open the Graph Properties dialog box to the Legend Options tab.

2 Next to **Position**, select where on the graph you want to display the legend. The options are: Top, Bottom, Left, and Right.

These options are not available if you selected to hide the legend.

3 Click **Close**.

The dialog box closes and the program adjusts the position of the legend according to your selection.

To adjust the font size of the legend text:

1 Open the Graph Properties dialog box to the Legend Options tab.

2 Next to **Font Size**, type the desired font size into the field, or click the +/- buttons to adjust the value in the field.

3 Click **Close**.

The dialog box closes and the program adjusts the font size according to your selection.

To change the color assigned to an individual plot:

1 Open the Graph Properties dialog box to the Legend Options tab.
The table under **Plot/Legend Properties** shows each plot color used in the graph (Plot Color column) and the name of the plot as described in the legend (Legend Label column).

2 Locate the plot to which you want to assign a new color, and expand the drop-down list in the Plot Color column.

A menu opens displaying standard plot color options.

3 In the Plot Color column, expand the drop-down list for the desired plot and select a color.

- If the standard menu includes your desired color, click directly on it.
- If you need a color not included on the standard menu, click **Advanced** to view an advanced menu for color selection. Use the color picker tools to create a custom color.

The color menu closes and the new color is displayed in the Plot Color drop-down list.

These options are not available if you selected to hide the legend.

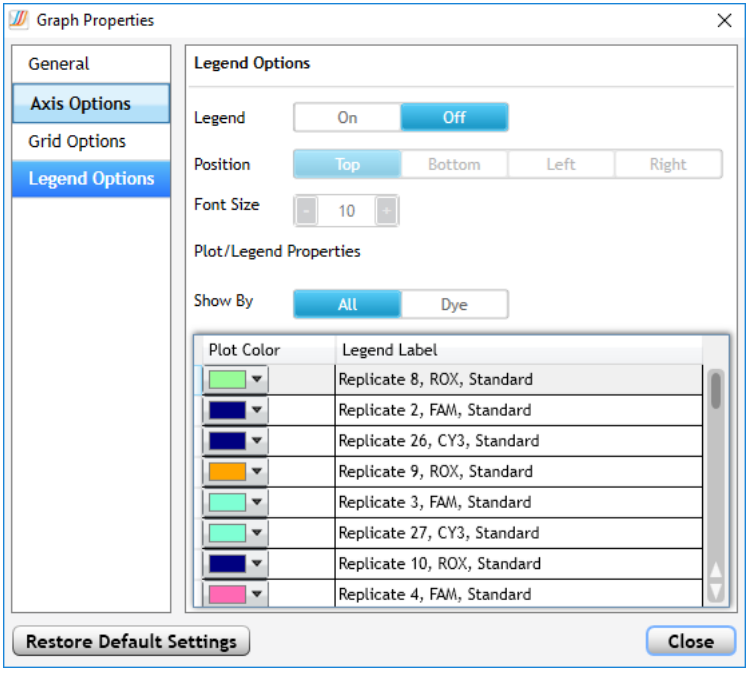
4 Click **Close**.

The dialog box closes and the graph displays the new plot color.

To assign plot color based on dye/target and change the color assigned to all plots of a particular dye/target:

1 For an Amplification Plot or Melt Curve graph, open the Graph Properties dialog box to the Legend Options tab.

The table under **Plot/Legend Properties** shows each plot color used in the graph (Plot Color column) and the name of the plot as described in the legend (Legend Label column).

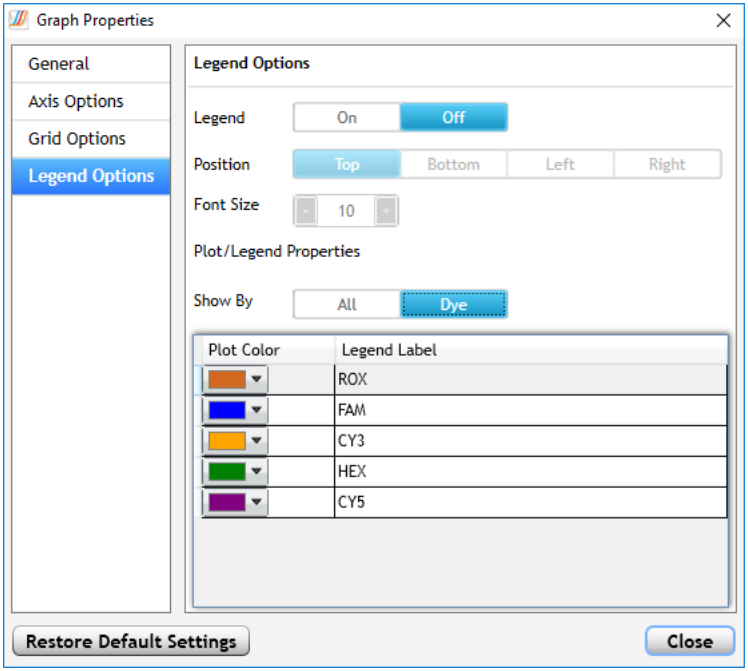


2 Next to **Show By**, click **Dye**.

A message box opens notifying you that the current settings for plot colors, in which plot colors are individually assigned to each well or replicate set, will be removed.

3 Click **Yes** in the message box to proceed.

The table under **Plot/Legend Properties** now shows a separate plot color for each dye or target included in the experiment. This is because, when showing plots by dye, all plots belonging to the same dye or target are displayed in the same color.



4 (Optional) To change the color assigned to a dye/target, in the Plot Color column, expand the drop-down list for the desired dye/target and select a different color.

- If the standard menu includes your desired color, click directly on it.
- If you need a color not included on the standard menu, click **Advanced** to view an advanced menu for color selection.

The color menu closes and the new color is displayed in the Plot Color drop-down list.

5 Click **Close**.

The dialog box closes and the Amplification Plots graph and, if applicable, Melt Curve graph now display all plots of the same dye or target in the same color.

To revert the color scheme back to displaying each plot in a different color, reopen the Graph Properties dialog box to the Legend Options tab. Then, next to **Show By**, click **All**.

11
Generating Reports and Exporting Results

Agilent Technologies

Generate report of results

The Generate Report screen allows you to set up, preview, and create a PDF or PowerPoint report for a post-run experiment. The content and format of the report are highly customizable, and you can save a report configuration for use with additional experiments later on.

To open the Generate Report screen: Click **Generate Report** in the Experiment Area panel on the left side of the screen.

View a preview of the report

The center of the Generate Report screen displays a page-by-page preview of what the report will look like with the current configuration settings. Above each page is the name of the report item displayed on that page.

To view all pages of the report preview, scroll down.

To adjust the display size of the pages, click the +/- buttons at the bottom of the screen.

Select report type

The report can be a PDF or PowerPoint file.

To select the report type:

- In the Report Configuration panel of the Generate Report screen, next to **Report Type**, select **PDF** to select a PDF report, or select **PowerPoint** to select a PowerPoint report.

Generate the report

After you configure the report as desired (see the tasks under "Configure the report" on page 254), you can generate the report file. By default, reports are saved to the folder C:\Users\Public\Public Documents\Agilent Aria\Reports, but you can select a different folder when you generate the report.

To generate the report:

1 At the bottom of the Generate Report screen, click **Generate Report**.

The Save As dialog box opens.

2 Specify a file name and folder for the report file and click **Save**.

The program generates the report and then opens it in the appropriate application (either Microsoft PowerPoint or your default PDF reader).

Configure the report

The program provides numerous ways for you to customize the report configuration. It also allows you to load a report configuration definition to quickly configure the report to a set of previously saved settings.

Load a saved report configuration definition

If you already have a saved report configuration definition on your system that you want to use for the current experiment, you can load that definition from the Generate Report screen. (The saved definition must be for the same experiment type.)

The program comes preloaded with a default report configuration definition that is automatically loaded. You can also configure your own report and save the configuration for later use (see "Create or edit report configuration definitions" on page 257).

To load a report configuration definition:

- In the Report Configuration panel of the Generate Report screen, next to **Definition**, select the saved report configuration definition from the drop-down list.

  The program updates the report preview and the settings under **Items** and **Header & Footer** according to the selected definition.

After you load a definition, you can still make edits to the report configuration.

Select the items to include in the report

The programs offers a variety of items that you can include in the report. Each item takes one or more page in the report. The Cover Page and Tabular Results items are customizable.

To include and exclude item from the report:

- In the Report Configuration Panel of the Generate Report screen, under **Items**, mark the check boxes for the items that you want to include in the report. Clear the check boxes for any items that you do not want to include.

  The preview of the report in the center of the screen includes only the marked items. Above each page is the name of the report item displayed on that page.

To customize the elements of the Cover Page:

1. In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Cover Page check box is marked.
2. Click the icon next to the Cover Page item.

   The Cover Page Options dialog box opens.
3. In the Report Title and Report Description fields, edit the content as desired. The title and description are printed on the cover page.
4. Under **Experiment Information** and **Other Items**, mark the check boxes for the pieces of information that you want to include on the cover page. Clear the check box for any pieces of information that you do not want to include.
5. In the fields labeled Left, Center, and Right, type the text that you want to appear at the bottom of the cover page on the left side, center, and right side.
6. Click **OK** in the Cover Page Options dialog box.

   The dialog box closes and the Cover Page displayed in the report preview includes your changes.

To customize the information included in the Tabular Results:

1. In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Tabular Results check box is marked.
2. Click the icon next to the Tabular Results item.

   The Tabular Results Properties dialog box opens.
3. Next to **Include Target Information**, select **Yes** to include the target information (as shown in the table on the dialog box) with the tabular results, or select **No** to exclude the target information.
4. Under **Tabular Results**, mark the check boxes for the pieces of information that you want to include as columns in the tabular results.

Clear the check box for any pieces of information that you do not want to include.

The table to the left of the check boxes displays a preview of the tabular results based on which columns you select to include.

To quickly mark all check boxes, click **Select All**. To restore the default selections, click **Restore Defaults**.

5 (Optional) To sort the data in the Tabular Results table, click directly on the header of the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The columns selected for sorting are highlighted in blue.

6 Click **OK** in the Tabular Results Properties dialog box.

The dialog box closes and the Tabular Results pages displayed in the report preview include your changes.

To edit the Experiment Notes:

1 In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Experiment Notes check box is marked.

2 Click the icon next to the Experiment Notes item.

The Experiment Notes text box opens.

3 In the text box, type any notes that you want to add to the experiment and include in the report.

4 Click **Save** to save your changes and close the text box.

To show analysis settings:

1 In the Report Configuration Panel of the Generate Report screen, next to **Show Analysis Settings**, select **Yes**.

In the report, the analysis settings for each graph are displayed below the graph.

Select the contents of the header and footer

You can select which pieces of information you want to include in the header and footer on the body pages of report.

To select the contents of the header and footer:

- In the Report Configuration Panel of the Generate Report screen, under **Header & Footer**, mark the check boxes for the pieces of information that you want to include in the header or footer. Clear the check boxes for any pieces of information that you do not want to include.

  If marked, the Experiment Name, Experiment Type, and Run Date appear in the header of the report. The Page Number, if marked, appears in the footer of the report.

Rearrange the pages of the report

You can set the order of the items included in the report by dragging and dropping within the report preview.

To rearrange the pages:

1. At the bottom of the Generate Report screen, click **Rearrange**.

   The program adjusts the display of the report preview to show thumbnails of all items included in the report, with a number next to each item to indicate its order in the report.

   Note that some items take up more than one page. For those items, the number of pages is indicated in parentheses after the item name.
2. For an item that you want moved to a different order, click and drag on the thumbnail. Drop the item into the desired order. Repeat for any other items you want to rearrange.
3. Click **Rearrange** again.

   The program sets the display of the report preview back to the standard mode with the pages displayed in the new arrangement.

Create or edit report configuration definitions

You can save your definitions for the report configuration as a report configuration definition. You can then load the saved definition into other experiments of the same experiment type.

Save changes to the default report configuration definition as a new definition

The program comes preloaded with a default report configuration definition, which is loaded by default for new experiments. When the default definition is loaded, you can still make edits to the report

configuration, but you cannot save the changes to the default definition. Instead, save the report configuration as a new definition.

To save changes to the default definition as a new definition:

1 With the default definition loaded on the Generate Report screen, make your desired changes to the report configuration.

2 In the Report Configuration Panel, next to **Definition**, expand the drop-down list and click **Add New**.

   The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Make sure the Use Current Settings check box is marked.

5 Click **Add**.

   The dialog box closes and the program saves the current report configuration to the new definition.

Save changes to a custom report configuration definition

If you loaded a saved definition (other than the default definition) and then made changes to the report configuration, you can save the changes, either by overriding the existing definition or by creating a new definition.

To save changes to a report configuration by overriding the existing definition:

1 After loading the saved definition on the Generate Report screen and making changes to the report configuration, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save**.

   The program saves the changes that you made to the report configuration to the loaded definition.

To save changes to a report configuration by creating a new definition:

1 After loading the saved definition on the Generate Report screen and making changes to the report configuration, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save As**.

   The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Click **Add**.

The dialog box closes and the program saves the report configuration to the new definition.

Export data/results to an Excel, text, LIMS data, or RDML file

The Export Data screen has tools for exporting numerical data from the experiment to an Excel, text, LIMS data, or RDML file. Data files can include data on the setup of the experiment as well as data from the results of the experiment.

To open the Export Data screen: Click **Export Data** in the Experiment Area panel on the left side of the screen.

Configure the file and export data

Before creating the file, you can configure the file by selecting which data items to include. If desired, you can save the configuration as a data export definition, which you can later load with a future experiment (see Load a saved data export definition).

Select the items to include in the file

To include and exclude item from the file:

- In the Export Configuration Panel of the Export Data screen, under **Items**, mark the check boxes for the items that you want to include in the file. Clear the check boxes for any items that you do not want to include.

  The preview of the file in the center of the screen includes only the marked items. Above each page is the name of the item.

To customize the elements of the Plate Setup, Thermal Profile, Tabular Results, or Experiment Notes:

1. In the Export Configuration Panel of the Export Data screen, under **Items**, make sure that the check box for the item that you want to customize is marked.
2. Click the icon next to the item.

   The Column Options dialog box opens.
3. Mark the check boxes for the elements that you want to include in the exported file. Clear the check boxes for elements that you do not want to include.
4. (Optional) To sort the data for elements that are in table format (e.g., Plate Setup and Tabular Results table), click directly on the header of

the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The columns selected for sorting are highlighted in blue.

Data will be sorted in the exported file in the same manner that they are sorted in the software.

5 (Optional) To only export data from selected rows of a table (e.g., Plate Setup and Tabular Results table), select the individual rows that you want to export. To select a range of adjacent rows, click and hold the left mouse button as you drag the cursor across the rows, or press **Shift** as you select the first and last row in the set. To select multiple rows that are not adjacent to each other, press **Ctrl** as you click individually on each of the rows. To deselect a selected row, press **Ctrl** and click on the row.

6 Click **OK** in the Column Options dialog box.

The dialog box closes and the preview on the Export Data screen includes your changes.

Export to Excel

When you export data/results to Excel, the program automatically launches Microsoft Excel and creates a workbook with each data item displayed on a separate tab within the workbook. You can then save the workbook with the file name and folder location of your choice.

To configure and export data to an Excel file:

1 On the Export Data screen, next to **File Type**, select **Excel**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the file" on page 260.

A preview of the file appears in the center of the screen.

3 Click **Export Data**.

Microsoft Excel opens to the new workbook.

4 In Excel, save the file as desired.

Export to text files

When you export data/results as a text file, the program creates a separate text file for each data item included in the data export configuration. The file names include the experiment name and data item.

The program prompts you to select a folder location for the files. You can then open the files in the text editing program of your choice.

To configure and export data to text files:

1 On the Export Data screen, next to **File Type**, select **Text**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the file" on page 260.

3 Click **Export Data**.

The Browse For Folder dialog box opens.

4 Select the folder where you want to save the text files and click **OK**.

The program creates the files and saves them in the designated folder.

Export to a LIMS data file

A laboratory information management system (LIMS) is a software system for managing and tracking laboratory activities. The Aria program allows you to export data/results from a post-run experiment to a text file or CSV file that can then be loaded into a LIMS program.

To configure and export data to a LIMS data file:

1 On the Export Data screen, next to **File Type**, select **LIMS**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the report", above.

3 In the **File Format** drop-down list, select a file type for the exported LIMS file.

Select **txt** to generate a text file, or select **csv** to generate a CSV file.

4 If you selected **txt** as the File Format, then in the **Data field delimiter** drop-down list, select the character to be used as a delimiter in the text file.

Select **Comma** to use a comma as the delimiting character, or select **Semicolon** to use a semicolon as the delimiting character.

5 Under **Plot data export tabular format**, select the layout of the data in the spreadsheet.

Select **Vertical** to list data in rows, with one data point per row. Select **Horizontal** to list data in columns, with one data point per column.

6 Under **Output**, select between creating a single LIMS data file that contains all the selected items (the **Single file** option) and creating a separate file for each data item (the **Separate files** option).

7 Under **File Naming**, use the drop-down lists to select the structure of the default file name. You can select up to four fields to include in the file name (Field 1 through Field 4). Each field is separated by an underscore character in the file name. Selecting **<None>** for any of the fields excludes that field from the file name.

   If you selected **Separate files** as the output, then the file names also include the data item identifier (e.g., Amplification Plots). If you selected **Single file** as the output, then the file name also includes the term "AllInOne."

8 Click **Export Data**.

   - If you selected **Separate files** as the output, then the Browse For Folder dialog box opens. Select the folder where you want to save the files and click **OK**. The program creates the files and saves them in the designated folder.
   - If you selected **Single file** as the output, then the Save As dialog box opens. Select a folder for the new experiment. Type a name into the file name field or use the default file name, then click **Save**. The program creates the file and saves it in the designated folder.

If desired, you can use the exported LIMS data file to quickly set up future experiments. See "Create an experiment from a LIMS data file" on page 51. To view or edit the LIMS file, open it in Notepad. To open in another application (e.g., Microsoft Excel), set the encoding to Unicode (UTF-8) to ensure that special characters display correctly.

Export data to an RDML file

MIQE guidelines recommend the Real-time PCR Data Markup Language (RDML) file format for publication of QPCR data.

NOTE RDML files can contain a large variety of data items. This help topic does not describe all the available fields that you can include. See www.rdml.org and the publication in Nucleic Acids Research [*Nucleic Acids Res*. 2009 April; 37(7): 2065–2069] for more information on RDML files.

When you export data/results to an RDML file, the program prompts you to select a folder location for the file. You can then open the file in an RDML compliant program.

To configure and export data to an RDML file:

1. On the Export Data screen, next to **File Type**, select **RDML**.

   The Experimenter fields appear in the center of the screen.

2. Type the First Name and Last Name of the experimenter into the fields in the center of the screen.

3. Select and configure the optional fields as desired:

   a. Click the + icon to expand the options for any particular category.

   b. Mark the check boxes for fields that you want to add.

   c. Type the information into the new fields.

4. Click **Export Data**.

   The Save As dialog box opens.

5. Type a file name into the dialog box and select the folder where you want to save the file. Click **Save**. The program creates the file and saves it in the designated folder.

Load a saved data export definition

If you already have a saved data export definition on your system that you want to use for the current experiment, you can load that definition from the Export Results screen. (The saved definition must be for the same experiment type.)

The program comes preloaded with a default data export definition that is automatically loaded. You can also configure a custom definition for later use (see "Create or edit data export definitions", below).

To load a data export definition:

- In the Export Configuration panel of the Export Data screen, next to **Definition**, select the saved data export definition from the drop-down list.

  The program updates the settings in the Export Configuration panel according to the selected definition.

After you load a definition, you can still make edits to the data export configuration.

Create or edit data export definitions

You can save your settings on the Export Data screen as a data export definition. The program saves the definitions to your system, allowing you to use them again with future experiments of the same type.

Save changes to the default data export definition as a new definition

The program comes preloaded with a default data export definition, which is loaded by default for new experiments. When the default definition is loaded, you can still make edits to the data export settings, but you cannot save the changes to the default definition. Instead, save the settings as a new definition.

To save changes to the default data export definition as a new definition:

1. With the default definition loaded on the Export Data screen, make your desired changes to the settings.
2. In the Export Configuration Panel, next to **Definition**, expand the drop-down list and click **Add New**.

   The Add New Definition dialog box opens.
3. In the Definition Name field, type a name for the definition, or use the name provided.
4. Make sure the Use Current Settings check box is marked.
5. Click **Add**.

   The dialog box closes and the program saves the data export configuration to the new definition.

Save changes to a custom data export definition

If you loaded a saved definition (other than the default definition) and then made changes to the data export settings, you can save the changes, either by overriding the existing definition or by creating a new definition.

To save changes to the data export settings by overriding the existing definition:

1 After loading the saved definition on the Export Data screen and making changes to the settings, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save**.

The program saves the changes that you made to the settings to the loaded definition.

To save changes to the data export settings by creating a new definition:

1 After loading the saved definition on the Export Data screen and making changes to the settings, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save As**.

The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Click **Add**.

The dialog box closes and the program saves the data export settings to the new definition.

12
Creating and Setting Up an MEA Project

Agilent Technologies

Quick Start Protocol

How to create, set up, analyze, and generate reports for a multiple experiment analysis project

In the Aria program, a multiple experiment analysis file is referred to as a project. A project consists of one or more post-run experiment files. Project files have the extension *amxp*.

1. Create the project

- Open a new tab, and on the Getting Started screen, click **Multiple Experiment Analysis**.
- Click **Add Experiment** to add the first experiment to the project. Click **Add Experiment** again for each additional experiment that you want to add to the project. (You can also add experiments after you create the project.)
- Enter a name for the project and click **Create**.

2. Set the analysis criteria

- Navigate to the Analysis Criteria screen.
- Select the wells and targets to include in the analysis and specify the treatment of replicate wells.
- If applicable, select the data collection marker to use for analysis (only available when toggle button is set to display only one experiment in the project).

3. Analyze the data

- Navigate to the Graphical Displays screen.
- View the results of the analysis and customize analysis settings for individual graphs.

4. Export the results

- To generate a report of the results, navigate to the Generate Report screen. Configure and create the report according to your selections.
- To export numerical data from the project, navigate to the Export Data screen. Select the file type and information you want to export.

Overview of multiple experiment analysis

Multiple experiment analysis (MEA) is a feature in the Aria software that allows you to display and analyze the results from two or more post-run experiments together in a single file called a project. Project files are given the extension *amxp* (for the AriaMx mode) or *adxp* (for the AriaDx mode.)

The data from the experiments in a project may be grouped together based on target name or maintained as separate data sets. The analysis and display of the data depend on how you set up the experiment plates and how you choose to compare the experiment data.

Applications

MEA in the Aria program was designed for the following purposes:

- In projects comprised of Quantitative PCR experiments, you can use multiple experiment analysis to determine the quantity of a particular target in an unknown sample using a standard curve for the target that was generated in a separate experiment. This capability allows you to compare unknown sample data from multiple experiments to the same standard curve, thereby eliminating the need to run standards with every experiment that includes amplification of that target.
- In projects comprised of Comparative Quantitation experiments, you can normalize the quantity of a target-of-interest to a normalizer target that was run in a separate experiment. The program uses the sample name to associate the target-of-interest wells with the normalizer wells. This capability is particularly useful when you are screening a sample for expression levels of many different target genes and you want to normalize them all to the same normalizer.
- In all project types, you can display results from multiple experiments of the same type side-by-side while still treating the experiments independently. This capability facilitates comparisons between experiments while still keeping the data separate.

Restrictions

You can only use MEA with completed (post-run) experiments that were run on an AriaMx or AriaDx instrument. To be grouped into the same project, experiments must be of the same experiment type and run on the same type of instrument (AriaMx or AriaDx), and their thermal profiles need to be similar (same type of segments in the same order). You can convert experiments to a different type through the Convert Experiment Type command in the File menu.

The program allows you to add up to 8 experiments to a project. Note, however, that only 2 of the 8 experiments can have a file size > 1.5 MB.

The Melt Curve - Difference Plots graph is not available for MEA projects, even if all the experiments in the project include a high resolution melt segment. To view the Difference Plots for an individual experiment in a project, open the experiment.

Guidelines for Comparing Cq Values Across Experiments

In a multiple experiment analysis (MEA) project, the program individually calculates a threshold fluorescence value for each target in each experiment and uses these threshold values to derive the quantification cycle (Cq) values. When the data are compared by target, you may be comparing the Cq values from one experiment to the values from another experiment. Any spurious plate-to-plate variability in fluorescence that is not due to true differences in template concentration between reactions can impact the reliability of such comparisons. Although the program was designed to calculate the threshold values in a way that limits the effects of this variability, to help ensure the validity of your comparisons, you can take steps to reduce variability between experiments (see"Reducing plate-to-plate variability", below). Also consider which method for threshold fluorescence determination is most appropriate for your project (see "Selecting a method for setting threshold fluorescence levels" on page 273).

Reducing plate-to-plate variability

Variability comes in many forms (e.g., signal strength, noise level, background fluorescence, and amplification efficiency) and may result from many sources (e.g., differences in plates, reagents, and assay preparation). The best way to reduce variability is to use good lab technique and high-quality reagents. To further limit variability, use reagents from the same lots when setting up experiments that you plan to directly compare.

To monitor for variability in the amplification efficiency of a target, run several standard curves on different days. The amplification efficiency for a target should be similar from one experiment to the next.

In a Comparative Quantitation project, the Aria program allows you to normalize the quantity of a target-of-interest to a normalizer target that was run on a different experiment. In this kind of plate-to-plate comparison, you are only comparing the ΔCq values between experiments rather than directly comparing Cqs, thus reducing the effects of variability between experiments. For measuring the relative template quantity of a target in an unknown sample, however, the program requires that the calibrator sample be from the same experiment. This requirement avoids

the potential problem of differences in the amplification efficiency between the unknown and calibrator reactions.

Selecting a method for setting threshold fluorescence levels

One of the major concerns when directly comparing the Cq values between experiments is the manner in which the threshold fluorescence levels are set. If you consider a single amplification plot, raising the threshold will give a later Cq, and lowering the threshold will give an earlier Cq. So, reactions with the same starting concentration of template and identical amplification efficiencies will have different Cq values if you set the thresholds differently. Consequently, you do not want to vary the way that you set the thresholds between experiments when you are directly comparing the Cq values. However, variation in the level of background fluorescence between experiments means that it is not always optimal to assign the threshold fluorescence to the same value across all experiments in a project. Review the three methods described below for setting threshold fluorescence levels, than select the approach that best suits your experimental needs.

Method 1) Determine thresholds using a control reaction. The most valid way to ensure equivalent thresholds between all the experiments in a project is to include a control reaction (or reactions) for each target on all the experiments in the project. This reaction must have the same quantity of target in every experiment. You can then manually adjust the thresholds in each experiment so that the well containing the control reaction has the exact same Cq in all experiments. Use of this sort of inter-experiment control is the most accurate way to set thresholds when performing multiple experiment analysis [for a reference see Hellemans et. al., *Genome Biology*, 2007; 8(2):R19]. If it is not possible to include a control reaction with each experiment, follow one of the methods described below.

Method 2) Set the thresholds separately for each experiment. This method is the default for setting the threshold levels in a project. When you create a new project, the program assigns a separate threshold (using a background-based algorithm) to each target in each experiment based on the settings that you provide for the amplification plots. Using this method, the program bases the background-based thresholds on noise levels in the baseline cycle range, so this method is preferable to method

#3 (below) if you observe significant differences in background noise between experiments.

Method 3) Set identical thresholds in all experiments. If you find that the background signal levels are similar between the experiments in your project, you can manually set the threshold level for a target to the same value in all experiments. The tools used for manually adjusting threshold fluorescence levels in a project are the same as those used for an experiment; see "Manually adjust threshold fluorescence values" on page 207.

Create an MEA project

You can create a new MEA project from the Getting Started screen.

To open the Getting Started screen: At the top of the program window click **File > New**, or click the icon, to open a new tab in the program. The new tab opens to the Getting Started screen.

To create a MEA project:

1 Close all open experiments and projects.

2 On the Getting Started screen, under **New Project**, click **Multiple Experiment Analysis**.

   The center of the screen displays the tools for creating a new project.

3 Click **Add Experiment**.

   The Open dialog box opens.

4 Browse to the experiment that you want to add to the project. Select the experiment (press **Ctrl** to select multiple experiments within the same folder) and click **Open**.

   The dialog box closes and the selected experiment appears in the list on the Getting Started screen.

5 Repeat steps 2–3 for all experiments that you want to include in the experiment. You can add up to 8 experiments to a project. Note that only 2 of the 8 experiments can have a file size > 1.5 MB.

   You can also add experiments after you create the project. See "Add or remove experiments from the project" on page 277.

6 In the Project Name field at the bottom of the Getting Started screen, type a name for the new project.

7 Click **Create**.

   The program creates the new project and opens the project to the Plate Setup screen.

Open an existing MEA project

In order to open a project, the program requires you to close all other experiments and tabs.

To open a project from the Getting Started screen:

1 On the Getting Started screen, under **Saved,** click **Browse**.

The Open dialog box opens.

2 Select the project and click **Open**.

The dialog box closes and the program opens the project to the Plate Setup screen. You may be prompted to save any open experiments and/or close any open tabs.

To open a project from the File menu:

1 Click **File > Open**.

The Open dialog box opens. If an experiment or project is currently open in the selected tab, the program closes that experiment or project, and prompts you to save any changes.

2 Select the project and click **Open**.

The dialog box closes and the program opens the project to the Plate Setup screen. You may be prompted to save any open experiments and/or close any open tabs.

Select experiments for a project

You can add or remove experiments from an existing project. You can also include and exclude specific experiments from the project analysis.

Add or remove experiments from the project

When you first create a project, the program prompts you to select the experiments that you want to add to the project (see "Create an MEA project" on page 275). You can also add experiments after the project is created, as well as delete experiments from the project. You can add up to 8 experiments in a single project.

To add experiment to an existing project:

1. Open the project to any screen.
2. In the Experiment Area, next to **Project**, click the icon.

   The Experiments Selection Window opens. This window contains a table listing the experiments that are currently in the project.
3. Click **Add Experiment**.

   The Open dialog box opens.
4. Browse to the folder that contains the experiment you want to add. Select the experiment and click **Open**. To select multiple experiments in a single folder, press **Ctrl** as you select the experiments.

   The Open dialog box closes and the selected experiment appears in the table on the Experiments Selection Window.
5. Repeat steps 3–4 for all experiments that you want to include in the experiment.
6. Click **OK** in the Experiments Selection Window.

   The window closes and the experiments that you added are now listed in the Experiment Area under **Project**.

To remove experiments from an existing project:

1. Open the project to any screen.
2. In the Experiment Area, under **Project**, locate the experiment that you want to remove from the project, and click the adjacent Delete icon.

A message box opens asking you to confirm that you want to remove the selected experiment.

3 Click **Yes** in the message box to continue.

The program removes the experiment from the project.

Include or exclude experiments in the project analysis

The program allows you to quickly select which experiments in the project are included in the program's analysis of the project. This feature allows you to view the effects of excluding one or more experiments without completely removing those experiments from the project.

To include or exclude experiments in the analysis:

1 Open the project to any screen.

2 In the Experiment Area, under **Project**, clear the check box next to any experiments that you want to exclude from the analysis, and mark the check boxes for those experiments that you want included in the analysis.

The program re-analyzes the project data and updates the results accordingly.

Edit the plate setup of experiments in a project

In a multiple experiment analysis project, the setup of each plate affects how the program compares the data across experiments.

To open the Plate Setup screen for a project: When you create a new project, you are automatically directed to the Plate Setup screen. To return to the Plate Setup screen at any time before, during, or after a run, click **Plate Setup** in the Experiment Area panel on the left side of the screen.

The Plate Setup screen for a project is very similar to the Plate Setup screen for an experiment. See "Overview of the Plate Setup screen" on page 73 for descriptions of the elements of the Plate Setup screen.

Select an experiment to edit

On the Plate Setup screen, you can only edit one plate setup for one experiment at a time.

To select an experiment for plate setup editing:

- On the Plate Setup screen, in the Experiment Area under **Project**, click directly on the name of the experiment that you want to edit.

  The program displays the plate map for the selected experiment.

Differentiate between targets across experiments

When you compare experiments by target, the program analyzes the data and displays results by combining data for each target across all experiments included in the project analysis. For this reason, if your experiments include multiple targets that were detected using the same dye, you need to provide unique target names on each plate in order to differentiates between those different targets. (If you do not assign a target name to a marked dye, the program uses the dye name as the target name.)

To assign dyes and target names:

1. On the Plate Setup screen, select the first experiment for editing.
2. Select all the wells in the plate map that contain the same target.

3 Under **Add Dyes**, if the fields for entering target names are not displayed, click the arrow next to **Targets**.

   The fields appear to the right of the dye names.

4 For the dyes that were used to amplify multiple targets across all experiments in the project, type a name into the adjacent Target Name field.

   The program assigns the target names to the selected wells.

5 Repeat steps 1-4 for all wells and experiments included in the project. Make sure to give the same target name to identical targets and different target names to different targets.

Edit plate properties

You can assign plate properties to the experiments of a project in the same way that you assign them for an individual experiment. The tools for assigning these properties are located in the panel on the right side of the Plate Setup screen. The content of this panel depends on the experiment type. See the following help topics for information on your experiment type:

"Assign plate properties for a Quantitative PCR DNA Binding Dye experiment" on page 86

"Assign plate properties for a Quantitative PCR Fluorescence Probe experiment" on page 94

"Assign plate properties for an Allele Discrimination DNA Binding Dye experiment" on page 113

"Assign plate properties for an Allele Discrimination Fluorescence Probe experiment" on page 122

"Assign plate properties for a Comparative Quantitation experiment" on page 102

"Assign plate properties for a User Defined experiment" on page 131

To hide the Properties panel, click the arrow icon in the upper left corner of the panel. Click the arrow again to display the Properties panel.

View the thermal profiles of experiments in a project

Because the experiments in a MEA project are post-run, you cannot edit the thermal profiles. However, you can use the Thermal Profile screen to view the thermal profiles of the experiments.

In the center of the Thermal Profile screen is a visual representation of the temperature cycling program that the instrument used while running the experiment.

To open the Thermal Profile screen for a project: Click **Thermal Profile** in the Experiment Area panel on the left side of the screen.

See "Elements of a Thermal Profile" on page 144 for a description of the elements of a thermal profile image.

To view the thermal profile for an experiment in a project:

1. Open the Thermal Profile screen.
2. In the Experiment Area panel, under **Project**, select the experiment for which you want to view the thermal profile.

   The program displays the thermal profile for the experiment in the center of the screen.

13
Analyzing Multiple Experiment Analysis Project Results

Set analysis criteria for a project

On the Analysis Criteria screen for a project, your selections determine the settings that the program uses for data analysis.

To open the Analysis Criteria screen for a project: Click **Analysis Criteria** in the Experiment Area panel on the left side of the screen.

The Analysis Criteria screen has an image of the plate map for each experiment in the project. The plate maps are based on the settings on the Plate Setup screen. At the bottom of the screen are icons that provide access to menus for making selections on which data you want included in the results displayed on the Graphical Displays screen.

Toggle display between one experiment and all experiments

In a project, the Analysis Criteria screen and Graphical Displays screen includes a toggle button for switching between two distinct modes, as described in the table below.

	When the toggle button looks like this, the program displays the results for all experiments included in the project (unless the check box for the experiment is not marked in the Experiment Area panel. In this mode, the Graphical Displays screen shows only one type of graph at a time (e.g., all the Amplification Plots graphs).
	When the toggle button looks like this, the program displays the results for one experiment at a time. The program displays results for whichever experiment is selected in the Experiment Area panel.

Select the wells and well types to include in analysis

In a project, the method for selecting the wells and well types for analysis is similar to that used for an individual experiment. See “Select wells in the plate map” on page 79 for detailed instructions.

Select the targets to include in analysis

To select specific targets:

1 On the Analysis Criteria screen, hover your cursor over the Display Targets icon at the bottom of the screen.

A window opens showing all targets in use on the plate.

2 For any targets that you do not want included in the analysis, clear the check box next to the target name. You can remark the check box at any time to reselect those targets.

Select which data collection points to analyze

One experiment displayed

If you are displaying only one experiment in the project (see "Toggle display between one experiment and all experiments" on page 283), use the instructions below to select a data collection point for the displayed experiment.

1 Hover your cursor over the Data Collection Marker icon at the bottom of the screen.

A window opens displaying the thermal profile with data collection markers.

2 Click the data collection marker that you want to use for analysis.

All experiments displayed

If you are displaying all experiments in the project, by default, the program uses the data collection point for each experiment that was selected in the experiment at the time the project was created. You cannot change the selected collection point unless you toggle to displaying one experiment, and then change which data collection marker is selected in the displayed experiment.

Choose a treatment for replicate wells

To specify that replicates be treated individually or collectively:

- On the Analysis Criteria screen, click the Replicates toggle button at the bottom of the screen.

  When the button looks like the image on the left, the program treats them individually. When the button looks like the image on the right, the program treats them collectively.

Overview of the Graphical Displays screen for a project

The Graphical Displays screen shows the results of the project displayed in a series of graphs. For each graph, the screen includes tools for setting certain analysis parameters. The screen also includes a result table, with configurable columns of data, that you can export to an Excel spreadsheet.

To open the Graphical Displays screen for a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen.

Graphs

The exact set of graphs available on the Graphical Displays screen varies depending on the type of experiments in the project, but all projects include a graph of the amplification plots, and all project in which the experiments have a melt segment include a graph of the raw/derivative melt curves (note that difference plots are not available in projects).

See the topics below for detailed information on specific graphs:

"Compare amplification plots in a project" on page 291

"Compare raw or derivative melt curves in a project" on page 293

"Compare standard curves in a project" on page 295

"Compare Relative Quantity charts in a project" on page 297

"Compare Allele Determination graphs in a project" on page 299

When all experiments in the project are displayed, the Graphical Displays screen shows one type of graph at a time. However, if you set the toggle button to display only one experiment, the screen can show multiple graphs for that one experiment, similar to when a single experiment file is open.

By default, data from all of the wells that you selected on the Analysis Criteria screen are included in the analysis and displayed in the graphs. You can limit the graphs to only display data from particular wells or replicate sets using the check boxes in the result table. The result table is described below.

Result table

The result table on the right side of the Graphical Displays screen shows results for each well (if replicates are treated individually) or replicate set (if replicates are treated collectively) that you selected on the Analysis Criteria screen (see "Select the wells and well types to include in analysis" on page 283).

Row selection

You can select individual rows within the results table to limit the graphs to displaying data only from particular wells/replicate sets. Click directly on a row to select it. Press **Ctrl** to select multiple rows. By default, all rows are initially selected. Click the Select All icon at the top of the result table to reselect all rows.

Data columns

You can configure which columns of data are included in the table. Click the Column Options icon at the top of the result table to open the Column Options dialog box. In the dialog box, mark the columns that you want to include in the result table.

You can freeze one or more columns on the left side of the result table so that as you scroll through the table horizontally, the frozen columns are always visible. Right-click on the header of the right-most column that you want to freeze and click **Freeze Column**. To unfreeze, right-click again and click **Unfreeze Column**.

Sorting

You can sort the data in the result table. Click directly on the header of the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The column headers selected for sorting are highlighted in blue.

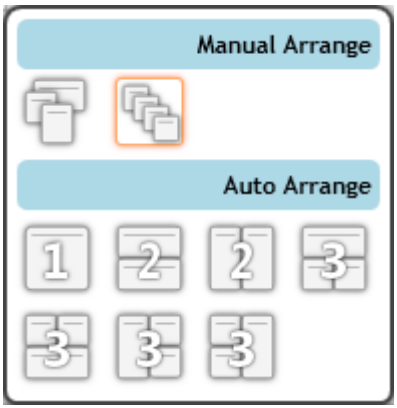
Display options

When you have multiple graphs selected for viewing on the Graphical Displays screen, you can manually drag and drop the graphs to new positions on the screen using your cursor (the Manual Arrange feature). Alternatively, you can select for the program to automatically arrange the

graphs based on the desired number of graphs per screen (the Auto Arrange feature).

To access the options for manually and automatically arranging the graphs, click the icon (shown below) at the bottom of the Graphical Display screen.

The following menu opens. The options under **Manual Arrange** and **Auto Arrange** are described below.



Manual Arrange

Under **Manual Arrange**, you have two arrangement options:

	Floating arrangement - This option allows you to move the graphs to any location on the screen by dragging and dropping them with your cursor.
	Cascade arrangement - This option sets the graphs in a cascading arrangement. You can move the graphs by dragging and dropping with your cursor.

Auto Arrange

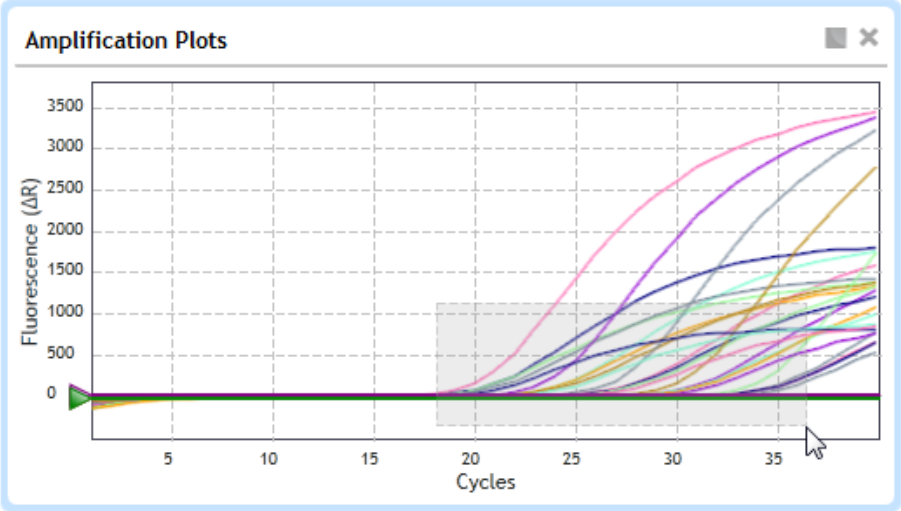
Under **Auto Arrange**, the options determine the number of graphs displayed on the screen (1, 2, 3, or 4). The image in each icon shows the arrangement of the graphs associated with that option. When you select to display more than one graph at a time, you can reorder the positions of

the graphs by dragging and dropping one graph on top of another with your cursor.

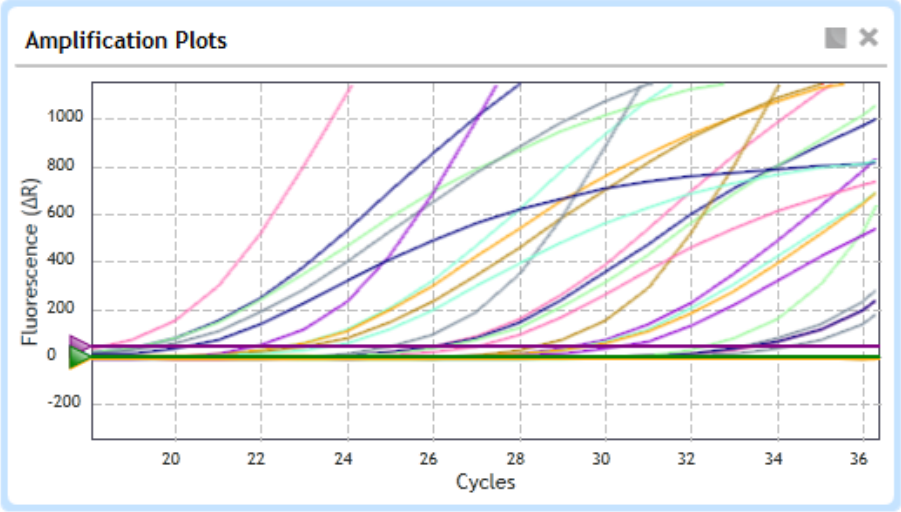
Zooming

Within a graph you can zoom in on a particular region of interest.

Drag your cursor across the region of interest, as shown below.



The program then zooms in on the selected region.



To reset the zoom level, right-click anywhere on the graph and click **Reset Zoom**.

For more information on the display options available for the graphs on the Graphical Displays screen, see "Customize graph properties" on page 240.

Compare amplification plots in a project

The Amplification Plots graphs on the Graphical Displays screen shows a plot of fluorescence (Y axis) versus cycles (X axis). The display of the graphs is dependent on how you select to compare the data.

To view the Amplification Plots for a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Amplification Plots graph is not already displayed, click the Amplification Plots icon at the bottom of the screen.

The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the amplification plots. These tools are the same as those that are available for the amplification plots for a single experiment. See "View the Amplification Plots" on page 197 for instructions.

Compare amplification plots by experiment

When you compare the amplification data by experiment, the program generates a separate Amplification Plots graph for each experiment in the project, and analyzes the data from each experiment separately.

To compare by experiment:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Experiment**.

Compare amplification plots by target

When you compare the amplification data by target, the program generates a separate graph for each target. If multiple experiments in the project amplified the same target, the program displays the amplification plots from these different experiments on the same graph. (Make sure each unique target has a unique name. See "Differentiate between targets across experiments" on page 279. This view provides a good way to compare how the same target performed in different experiments. Note that comparing

amplification plots by target, rather than by experiment, does not impact the Cq values that the program calculates for each plot.

To compare by experiment:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Target**.

Consolidate the amplification plots

When you select to consolidate the amplification data, the program displays all of the amplification plots from all experiments on a single graph. The program calculates the Cq values in the same manner as it does when you compare the data by experiment or by target.

To consolidate the amplification plots:

1 Select the Amplification Plots graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Consolidated**.

Compare raw or derivative melt curves in a project

For projects in which the experiments include a melt segment in the thermal profile, the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen displays the fluorescence data collected during the melt segment (Y-axis) as a function of temperature (X-axis). You can use the raw or derivative melt curves to verify that the predominant PCR products are amplicons of the intended target.

For projects comprised of experiments that include a high-resolution melt (HRM) segment, you cannot associate or disassociate an HRM calibration plate with the experiments once the project is created. Make the HCP associations in the individual experiments before you create the project. Note that the Melt Curve - Difference Plots graph is not available in projects.

To view the Melt Curve - Raw/Derivative Curve in a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Melt Curve - Raw/Derivative Curve graph is not already displayed, click the icon for this graph at the bottom of the screen.

The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the melt curves. These tools are the same as those that are available for the Melt Curve - Raw/Derivative Curve graph for a single experiment. See "View the Melt Curve - Raw/Derivative Curve" on page 212 for instructions.

Compare raw or derivative melt curves by experiment

When you compare the melt data by experiment, the program generates a separate graph for each experiment in the project and analyzes the data from each experiment separately.

To compare by experiment:

1. Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.
2. In the right panel, next to **Compare**, select **Experiment**.

Compare raw or derivative melt curves by target

When you compare the melt data by target, the program generates a separate graph for each target. If multiple experiments in the project amplified the same target, the program displays the melt curves from these different experiments on the same graph. (Make sure each unique target has a unique name. See "Differentiate between targets across experiments" on page 279. This view provides a good way to compare how the same target performed in different experiments. Note that comparing melt curves by target, rather than by experiment, does not impact the Tm values that the program calculates for each curve.

To compare by target:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Target**.

Consolidate the raw or derivative melt curves

When you select to consolidate the melt data, the program displays all of the melt curves from all experiments on a single graph. The program calculates the Tm values in the same manner as it does when you compare the data by experiment or by target.

To consolidate the melt curves:

1 Select the Melt Curve - Raw/Derivative Curve graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Consolidated**.

Compare standard curves in a project

For projects comprised of Quantitative PCR experiments, Comparative Quantitation experiments, or User Defined experiments that include a set of Standard wells, the Graphical Displays screen includes a Standard Curve graph.

The Standard Curve graph shows a plot of the Cq (Y-axis) versus the log of the initial template quantity in the Standard wells. The graph also plots the Cq values from the Unknown wells. The program uses a least mean squares curve fitting algorithm to generate the standard curves. The panel on the right side of the screen has tools for adjusting some of the analysis parameters.

To view the Standard Curve for a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Standard Curve graph is not already displayed, click the Standard Curve icon at the bottom of the screen.

The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the standard curves. These tools are the same as those that are available for the Standard Curve graph for a single experiment. See "View the Standard Curve" on page 225 for instructions.

Compare standard curves by experiment

When you compare the standard curves by experiment, the program generates a separate graph for each experiment in the project, and analyzes the data from each experiment separately. Note that any unknown samples are only compared to Standard wells that were run on the same plate.

To compare by experiment:

1 Select the Standard Curve graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Experiment**.

Compare standard curves by target

When you compare the standard curves by target, the program generates a graph for each target in the project. Thus, all the Cq values for a single target in all Standard and Unknown wells are plotted on the same graph.

The initial template quantities in the Unknown wells are based on the data from the Standard wells for the same target, regardless of whether the Unknown and Standard wells were run on the same plate. If Standard samples for the same target were run on multiple experiments in the project (and they have all been included in analysis), the program plots the Standard wells together on the same curve and calculates initial template quantities of the target in the Unknown wells based on all the collective Standard data from all experiments.

To compare by target:

1 Select the Standard Curve graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Target**.

Consolidate the standard curves

When you select to consolidate the standard curves, the program displays all of the standard curves from all experiments on a single graph. The program calculates the template quantities in the same manner as it does when you compare the data by target.

To consolidate the standard curves:

1 Select the Standard Curve graph on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Consolidated**.

Compare Relative Quantity charts in a project

For projects comprised of Comparative Quantitation experiments or User Defined experiments that include a set of Calibrator wells, the Graphical Displays screen includes a Relative Quantity chart. This chart is a bar graph that shows the amount of target present in the experimental samples (the Unknown wells) relative to the associated reference sample (the Calibrator wells) after the program has normalized the quantities using data from the normalizer target.

To view the Relative Quantity for a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Relative Quantity chart is not already displayed, click the Relative Quantity icon at the bottom of the screen.

The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the relative quantities. These tools are the same as those that are available for the Relative Quantity chart for a single experiment. See "View the Relative Quantity" on page 230 for instructions.

Compare relative quantities by experiment

When you compare the relative quantities by experiment, the program generates a separate chart for each experiment in the project, and analyzes the data from each experiment separately. All targets (except the normalizer) that were run in both an Unknown well and a Calibrator well on the same experiment are included in the chart for that experiment. The program normalizes the data for a target-of-interest to the normalizer target that was run on the same experiment.

To compare by target:

1. Select the Relative Quantity chart on the Graphical Displays screen.
2. In the right panel, next to **Compare**, select **Target**.

Compare relative quantities by target

When you compare the relative quantities by target, the program generates a graph for each target-of-interest that was run in both an Unknown well and a Calibrator well on the same experiment (the program does not compare Unknown wells to Calibrator wells from a different experiment).

In a by-target comparison, the program uses the following guidelines for normalizing the data for a target-of-interest:

- If a normalizer target is designated in the same well as the target-of-interest, the program uses that normalizer for normalization.
- If a normalizer target is not found in the same well as the target-of-interest, the program uses a normalizer from wells of the same sample name within the same experiment.
- If a normalizer target is not found in wells of the same sample name within the same experiment, the program uses a normalizer from wells with the same sample name that were run on a different experiment in the project.

To compare by target:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Target**.

Consolidate the relative quantities

When you select to consolidate the relative quantities, the program displays all of the quantities from all experiments on a single chart. The program calculates the relative quantities in the same manner as it does when you compare the data by target.

To consolidate the relative quantities:

1 Select the Relative Quantity chart on the Graphical Displays screen.

2 In the right panel, next to **Compare**, select **Consolidated**.

Compare Allele Determination graphs in a project

For projects comprised of Allele Discrimination experiments or User Defined experiments that include wells with allele designations, the Graphical Displays screen includes an Allele Determination graph. This graph is useful for viewing the genotype results in Allele Discrimination experiments that use two differentially-labeled fluorescent probes to detect the two different alleles. Each plotted point on the graph represents the coordinates of either the fluorescence values or Cq values for the two targets. The position of the data point on the graph indicates the presence or absence of each allele.

To view the Allele Determinations in a project: Click **Graphical Displays** in the Experiment Area panel on the left side of the screen. If the Allele Determination graph is not already displayed, click the icon for this graph at the bottom of the screen.

The panel on the right side of the screen has tools for adjusting some of the analysis parameters for the allele determinations. These tools are the same as those that are available for the Allele Determination graph for a single experiment. See "View the Allele Determination graph" on page 235 for instructions.

In a project, allele determinations are always compared by experiment. The program generates a separate graph for each experiment in the project, and analyzes the data from each experiment separately.

14
Generating Multiple Experiment Analysis Reports and Exporting Results

Agilent Technologies

Generate report of MEA project results

When working in a project, the Generate Report screen allows you to set up, preview, and create a PDF or PowerPoint report for the project. The content and format of the report are highly customizable, and you can save a report configuration for use with additional projects later on.

To open the Generate Report screen: Click **Generate Report** in the Experiment Area panel on the left side of the screen.

View a preview of the report

The center of the Generate Report screen displays a page-by-page preview of what the report will look like with the current configuration settings. Above each page is the name of the report item displayed on that page.

To view all pages of the report preview, scroll down.

To adjust the display size of the pages, click the +/- buttons at the bottom of the screen.

Select report type

The report can be a PDF or PowerPoint file.

To select the report type:

- In the Report Configuration panel of the Generate Report screen, next to **Report Type**, select **PDF** to select a PDF report, or select **PowerPoint** to select a PowerPoint report.

Generate the report

After you configure the report as desired (see the tasks under "Configure the report"), you can generate the report file. By default, reports are saved to the folder C:\Users\Public\Public Documents\Agilent Aria\Reports, but you can select a different folder when you generate the report.

To generate the report:

1 At the bottom of the Generate Report screen, click **Generate Report**.

The Save As dialog box opens.

2 Specify a file name and folder for the report file and click **Save**.

The program generates the report and then opens it in the appropriate application (either Microsoft PowerPoint or your default PDF reader).

Configure the report

The program provides numerous ways for you to customize the report configuration. It also allows you to load a report configuration definition to quickly configure the report to a set of previously saved settings.

Load a saved report configuration definition

If you already have a saved report configuration definition on your system that you want to use for the current project, you can load that definition from the Generate Report screen. (The saved definition must be for the same experiment type.)

The program comes preloaded with a default report configuration definition that is automatically loaded. You can also configure your own report and save the definition for later use (see "Create or edit report configuration definitions" on page 305).

To load a report configuration definition:

- In the Report Configuration panel of the Generate Report screen, next to **Definition**, select the saved report configuration definition from the drop-down list.

  The program updates the report preview and the settings under **Items** and **Header & Footer** according to the selected definition.

After you load a definition, you can still make edits to the report configuration.

Select the items to include in the report

The programs offers a variety of items that you can include in the report. Each item takes one or more page in the report. The Cover Page and Tabular Results items are customizable.

To include and exclude item from the report:

- In the Report Configuration Panel of the Generate Report screen, under **Items**, mark the check boxes for the items that you want to include in the report. Clear the check boxes for any items that you do not want to include.

  The preview of the report in the center of the screen includes only the marked items. Above each page is the name of the report item displayed on that page.

To customize the elements of the Cover Page:

1. In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Cover Page check box is marked.
2. Click the icon next to the Cover Page item.

   The Cover Page Options dialog box opens.
3. In the Report Title and Report Description fields, edit the content as desired. The title and description are printed on the cover page.
4. Under **Other Items**, mark the check boxes for the pieces of information that you want to include on the cover page. Clear the check box for any pieces of information that you do not want to include.
5. In the fields labeled Left, Center, and Right, type the text that you want to appear at the bottom of the cover page on the left side, center, and right side.
6. Click **OK** in the Cover Page Options dialog box.

   The dialog box closes and the Cover Page displayed in the report preview includes your changes.

To customize the information included in the Tabular Results:

1. In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Tabular Results check box is marked.
2. Click the icon next to the Tabular Results item.

   The Tabular Results Properties dialog box opens.
3. Next to **Include Target Information**, select **Yes** to include the target information (as shown in the table on the dialog box) with the tabular results, or select **No** to exclude the target information.
4. Under **Tabular Results**, mark the check boxes for the pieces of information that you want to include as columns in the tabular results.

Clear the check box for any pieces of information that you do not want to include.

The table to the left of the check boxes displays a preview of the tabular results based on which columns you select to include.

To quickly mark all check boxes, click **Select All**. To restore the default selections, click **Restore Defaults**.

5 (Optional) To sort the data in the Tabular Results table, click directly on the header of the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The columns selected for sorting are highlighted in blue.

6 Click **OK** in the Tabular Results Properties dialog box.

The dialog box closes and the Tabular Results pages displayed in the report preview include your changes.

To edit the Project Notes:

1 In the Report Configuration Panel of the Generate Report screen, under **Items**, make sure that the Project Notes check box is marked.

2 Click the icon next to the Project Notes item.

The Project Notes text box opens.

3 In the text box, type any notes that you want to add to the project and include in the report.

4 Click **Save** to save your changes and close the text box.

To show analysis settings:

1 In the Report Configuration Panel of the Generate Report screen, next to **Show Analysis Settings**, select **Yes**.

In the report, the analysis settings for each graph are displayed below the graph.

Select the contents of the header and footer

You can select which pieces of information you want to include in the report's header and footer.

To select the contents of the header and footer:

- In the Report Configuration Panel of the Generate Report screen, under **Header & Footer**, mark the check boxes for the pieces of information that you want to include in the header or footer. Clear the check boxes for any pieces of information that you do not want to include.

  If marked, the Project Name and Analysis Date appear in the header of the report. The Page Number, if marked, appears in the footer of the report.

Rearrange the pages of the report

You can set the order of the items included in the report by dragging and dropping within the report preview.

To rearrange the pages:

1. At the bottom of the Generate Report screen, click **Rearrange**.

   The program adjusts the display of the report preview to show thumbnails of all items included in the report, with a number next to each item to indicate its order in the report.

   Note that some items take up more than one page. For those items, the number of pages is indicated in parentheses after the item name.
2. For an item that you want moved to a different order, click and drag on the thumbnail. Drop the item into the desired order. Repeat for any other items you want to rearrange.
3. Click **Rearrange** again.

   The program sets the display of the report preview back to the standard mode with the pages displayed in the new arrangement.

Create or edit report configuration definitions

You can save your custom report configuration as a report configuration definition. You can then load the saved definition into other projects of the same experiment type.

Save changes to the default report configuration definition as a new definition

The program comes preloaded with a default report configuration definition, which is loaded by default for new projects. When the default definition is loaded, you can still make edits to the report configuration,

but you cannot save the changes to the default definition. Instead, save the report configuration as a new definition.

To save changes to the default definition as a new definition:

1 With the default definition loaded on the Generate Report screen, make your desired changes to the report configuration.

2 In the Report Configuration Panel, next to **Definition**, expand the drop-down list and click **Add New**.

   The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Make sure the Use Current Settings check box is marked.

5 Click **Add**.

   The dialog box closes and the program saves the current report configuration to the new definition.

Save changes to a custom report configuration definition

If you loaded a saved definition (other than the default definition) and then made changes to the report configuration, you can save the changes, either by overriding the existing definition or by creating a new definition.

To save changes to a report configuration by overriding the existing definition:

1 After loading the saved definition on the Generate Report screen and making changes to the report configuration, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save**.

   The program saves the changes that you made to the report configuration to the loaded definition.

To save changes to a report configuration by creating a new definition:

1 After loading the saved definition on the Generate Report screen and making changes to the report configuration, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save As**.

   The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Click **Add**.

The dialog box closes and the program saves the report configuration to the new definition.

Export MEA data/results to an Excel, text, or LIMS data file

When working in a project, the Export Data screen has tools for exporting numerical data from the experiments to an Excel, text, or LIMS data file. Data files can include data on the setup of the experiments as well as data from the results of the project.

To open the Export Data screen: Click **Export Data** in the Experiment Area panel on the left side of the screen.

Configure the file and export data

Before creating the file, you can configure the file by selecting which data items to include. If desired, you can save the configuration as a data export definition, which you can later load with a future project (see "Load a saved data export definition " on page 311).

Select the items to include in the file

To include and exclude item from the file:

- In the Export Configuration Panel of the Export Data screen, under **Items**, mark the check boxes for the items that you want to include in the file. Clear the check boxes for any items that you do not want to include.

  The preview of the file in the center of the screen includes only the marked items. Above each page is the name of the item.

To customize the elements of the Plate Setup, Thermal Profile, Tabular Results, Project Notes, or Experiment Notes:

1. In the Export Configuration Panel of the Export Data screen, under **Items**, make sure that the check box for the item that you want to customize is marked.
2. Click the icon next to the item.

   The Column Options dialog box opens.
3. Mark the check boxes for the elements that you want to include in the exported file. Clear the check boxes for elements that you do not want to include.
4. (Optional) To sort the data for elements that are in table format (e.g., Plate Setup and Tabular Results table), click directly on the header of

the column on which you want to sort. To designate a second column for secondary sorting, press **Shift** then click the header of the second column. The columns selected for sorting are highlighted in blue.

Data will be sorted in the exported file in the same manner that they are sorted on the software.

5 (Optional) To only export data from selected rows of a table (e.g., Plate Setup and Tabular Results table), select the individual rows that you want to export. To select a range of adjacent rows, click and hold the left mouse button as you drag the cursor across the rows, or press **Shift** as you select the first and last row in the set. To select multiple rows that are not adjacent to each other, press **Ctrl** as you click individually on each of the rows. To deselect a selected row, press **Ctrl** and click on the row.

6 Click **OK** in the Column Options dialog box.

The dialog box closes and the preview on the Export Data screen includes your changes.

Export to Excel

When you export data/results to Excel, the program automatically launches Microsoft Excel and creates a workbook with each data item displayed on a separate tab within the workbook. You can then save the workbook with the file name and folder location of your choice.

To configure and export data to an Excel file:

1 On the Export Data screen, next to **File Type**, select **Excel**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the file" on page 308.

A preview of the file appears in the center of the screen.

3 Click **Export Data**.

Microsoft Excel opens to the new workbook.

4 In Excel, save the file as desired.

Export to text files

When you export data/results as a text file, the program creates a separate text file for each data item included in the data export configuration. The file names include the project name and data item. The

program prompts you to select a folder location for the files. You can then open the files in the text editing program of your choice.

To configure and export data to text files:

1 On the Export Data screen, next to **File Type**, select **Text**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the file" on page 308.

3 Click **Export Data**.

The Browse For Folder dialog box opens.

4 Select the folder where you want to save the text files and click **OK**.

The program creates the files and saves them in the designated folder.

Export to a LIMS data file

A laboratory information management system (LIMS) is a software system for managing and tracking laboratory activities. The Aria program allows you to export data/results from a project to a text file that can then be loaded into a LIMS program.

To configure and export data to a LIMS data file:

1 On the Export Data screen, next to **File Type**, select **LIMS**.

2 Under **Items**, mark the items that you want to include in the file. See "Select the items to include in the file", above.

3 In the **Data field delimiter** drop-down list, select the character to be used as a delimiter in the text file.

Select **Comma** to use a comma as the delimiting character, or select **Semicolon** to use a semicolon as the delimiting character.

4 Under **Plot data export tabular format**, select the layout of the data in the spreadsheet.

Select **Vertical** to list data in rows, with one data point per row. Select **Horizontal** to list data in columns, with one data point per column.

5 Under **Output**, select between creating a single LIMS data file that contains all the selected items (the **Single file** option) and creating a separate file for each data item (the **Separate files** option).

6 Under **File Naming**, use the drop-down lists to select the structure of the default file name. You can select up to four fields to include in the file name (Field 1 through Field 4). Each field is separated by an

underscore character in the file name. Selecting **<None>** for any of the fields excludes that field from the file name.

If you selected **Separate files** as the output, then the file names also include the data item identifier (e.g., Amplification Plots). If you selected **Single file** as the output, then the file name also includes the term "AllInOne."

7 Click **Export Data**.

- If you selected **Separate files** as the output, then the Browse For Folder dialog box opens. Select the folder where you want to save the files and click **OK**. The program creates the files and saves them in the designated folder.
- If you selected **Single file** as the output, then the Save As dialog box opens. Select a folder for the new experiment. Type a name into the file name field or use the default file name, then click **Save**. The program creates the file and saves it in the designated folder.

If desired, you can use the exported LIMS data file to quickly set up future experiments. See "Create an experiment from a LIMS data file" on page 51. To view or edit the LIMS file, open it in Notepad. To open in another application (e.g., Microsoft Excel), set the encoding to Unicode (UTF-8) to ensure that special characters display correctly.

Load a saved data export definition

If you already have a saved data export definition on your system that you want to use for the current project, you can load that definition from the Export Results screen. (The saved definition must be for the same experiment type.)

The program comes preloaded with a default data export definition that is automatically loaded. You can also configure a custom definition for later use (see "Create or edit data export definitions", below).

To load a data export definition:

- In the Export Configuration panel of the Export Data screen, next to **Definition**, select the saved data export definition from the drop-down list.

  The program updates the settings in the Export Configuration panel according to the selected definition.

After you load a definition, you can still make edits to the data export configuration.

Create or edit data export definitions

You can save your settings on the Export Data screen as a data export definition. The program saves the definitions to your system, allowing you to use them again with future projects of the same experiment type.

Save changes to the default data export definition as a new definition

The program comes preloaded with a default data export definition, which is loaded by default for new projects. When the default definition is loaded, you can still make edits to the data export settings, but you cannot save the changes to the default definition. Instead, save the settings as a new definition.

To save changes to the default data export definition as a new definition:

1. With the default definition loaded on the Export Data screen, make your desired changes to the settings.
2. In the Export Configuration Panel, next to **Definition**, expand the drop-down list and click **Add New**.

   The Add New Definition dialog box opens.
3. In the Definition Name field, type a name for the definition, or use the name provided.
4. Make sure the Use Current Settings check box is marked.
5. Click **Add**.

   The dialog box closes and the program saves the data export configuration to the new definition.

Save changes to a custom data export definition

If you loaded a saved definition (other than the default definition) and then made changes to the data export settings, you can save the changes, either by overriding the existing definition or by creating a new definition.

To save changes to the data export settings by overriding the existing definition:

1 After loading the saved definition on the Export Data screen and making changes to the settings, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save**.

The program saves the changes that you made to the settings to the loaded definition.

To save changes to the data export settings by creating a new definition:

1 After loading the saved definition on the Export Data screen and making changes to the settings, click the arrow next to the Save icon to expand the drop-down list.

2 Click **Save As**.

The Add New Definition dialog box opens.

3 In the Definition Name field, type a name for the definition, or use the name provided.

4 Click **Add**.

The dialog box closes and the program saves the data export settings to the new definition.

15
“How-To” Examples for Multiple Experiment Analysis Projects

Agilent Technologies

Example 1

How to find the initial template quantity of a target in an unknown sample using a standard curve from a separate experiment

In a project that includes an experiment with a set of Standard wells, the program can calculate the initial template quantity of a target in an Unknown well from a different experiment in the project. This example walks you through the process of determining those initial template quantities.

Step 1: Create a project containing the experiments

Create the project and make sure to add the two experiments to the project: the experiment containing the unknown samples and the experiment containing the standard samples for the same target. See "Create an MEA project" on page 275 for detailed instructions.

Step 2: Ensure target names are properly assigned

The program can only compare the Unknown wells to the Standard wells if both well types have the same target name assigned to the same dye position. The identical target name indicates that these wells amplified the same target.

View the target name assignments in each experiment and ensure that the target in the Unknown wells and the target in the Standard wells have been assigned the same name. See "Edit the plate setup of experiments in a project" on page 279.

Step 3: Select the analysis criteria

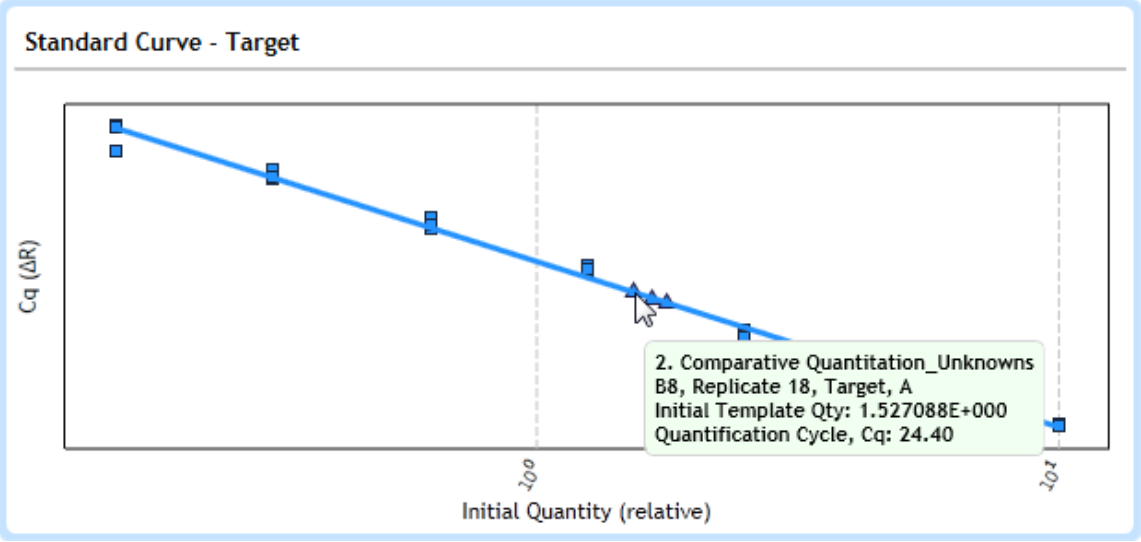
Once you have ensured that the target name assignments are correct, you can set the analysis criteria for the project on the Analysis Criteria screen. Instructions on the controls and settings in this screen can be found in "Set analysis criteria for a project" on page 283.

Step 4: View the initial template quantity calculations in the standard curve

To view the standard curve, open the Graphical Displays screen. If the Standard Curve graph is not already displayed, click the Standard Curve icon at the bottom of the screen.

In the right panel, next to **Fluorescence Term,** select the fluorescence data type to be used for analysis. Then, next to **Compare**, select **Target**.

When compared by target, the program analyzes the data across experiments by target name. The Cq values from the Unknown wells for your target of interest are plotted on the same graph as the standard curve for this target that was generated from the Standard wells run on the other experiment. You can move your cursor over the data point for an Unknown well or replicate set to view the initial template quantity calculated for the target in that well or replicate set (see below).



Example 2

How to normalize target quantities in Unknown and Calibrator wells using a normalizer target from a separate experiment

The Comparative Quantitation experiment type is ideal for comparing amounts of mRNA of a target-of-interest in *treated vs. untreated* or *normal vs. diseased* cells or tissues. In these studies, the control sample is referred to as the calibrator, and the test samples are referred to as the unknown samples. To help correct for spurious differences in the level of a target-of-interest that are not due to the experimental condition being tested, it is important to amplify a normalizer target from each calibrator and unknown sample. An ideal normalizer target is one that you know is not differentially expressed as a result of your experimental conditions (e.g., a housekeeping gene).

In a MEA project composed of Comparative Quantitation experiments (or User Defined experiments designed to determine relative quantities), the data for a target-of-interest can be normalized to data from a normalizer target that was run in a separate experiment. This example walks you through the process for setting up a project in which the target-of-interest and normalizer targets are on different experiments.

Step 1: Create the Project

Create the project and make sure to add the two experiments to the project: the experiment in which the target-of-interest was amplified in the unknown and calibrator samples and the experiment in which the normalizer target was amplified in the same unknown and calibrator samples. See "Create an MEA project" on page 275 for detailed instructions.

Step 2: Ensure the correct target is designated as the normalizer

View the plate setup for the experiment that includes the normalizer target. Make sure that the wells in which the normalizer was amplified have the correct dye assigned in the **Normalizer Dye** drop-down list. Ensure that no other targets are also designated as a normalizer in either experiment in the project.

Step 3: Ensure the sample name assignments are correct

To normalize data for a target-of-interest to a normalizer target that was amplified in a different experiment, be sure to assign the same sample name to the wells on both plates that contain the same template sample.

You can assign sample names from the Plate Setup screen. See "Assign sample names and biological replicates" on page 104.

Step 4: Select the analysis criteria

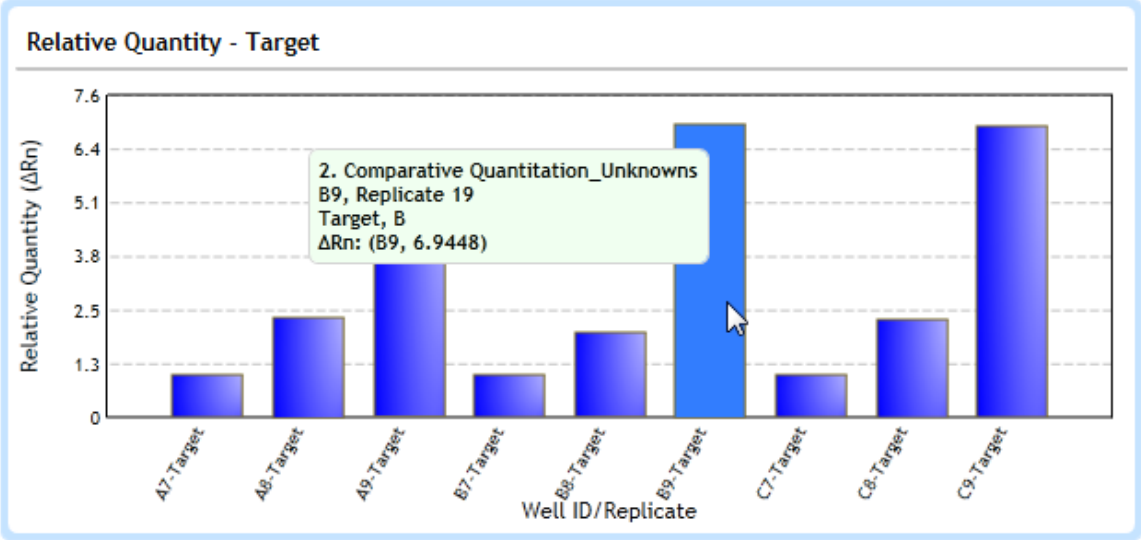
Once you have ensured that the normalizer and sample name assignments are correct, you can set the analysis criteria for the project on the Analysis Criteria screen. Instructions on the controls and settings in this screen an be found in "Set analysis criteria for a project" on page 283.

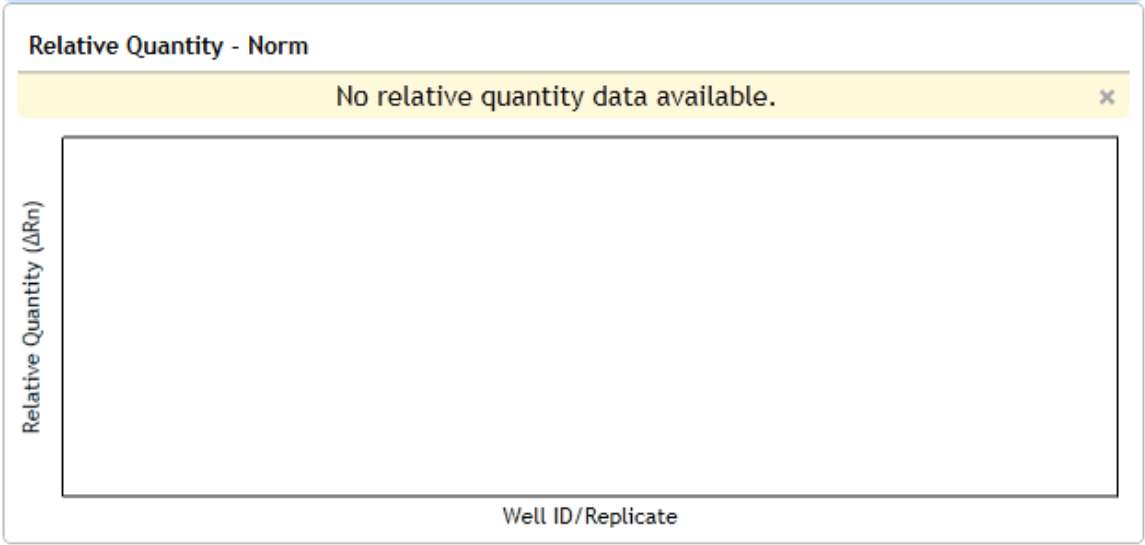
Step 5: View the relative quantity calculations

To view the relative quantities in the Unknown and Calibrator wells, open the Graphical Displays screen. If the Relative Quantity chart is not already displayed, click the Relative Quantity icon at the bottom of the screen.

In the right panel, next to **Fluorescence Term,** select the fluorescence data type to be used for analysis. Then, next to **Compare**, select **Target**.

When compared by target, the program analyzes the data across experiments by target name and generates a separate Relative Quantity chart for each target. The program normalizes the data for the target-of-interest using the normalizer from the same sample. You can hover your cursor over a bar on the chart for the target-of-interest to view the relative quantity calculated for the target in that well or replicate set. The program generates a graph for the normalizer target, but no data are plotted on it.


Relative Quantity - Target
Relative Quantity (ΔRn)
7.6
6.4
5.1
3.8
2.5
1.3
0
2. Comparative Quantitation_Unknowns
B9, Replicate 19
Target, B
ΔRn: (B9, 6.9448)
A7-Target
A8-Target
A9-Target
B7-Target
B8-Target
B9-Target
C7-Target
C8-Target
C9-Target
Well ID/Replicate


Relative Quantity - Norm
No relative quantity data available.
Relative Quantity (ΔRn)
Well ID/Replicate

16
Help for the Aria ET (Electronic Tracking) Software

Agilent Technologies

Overview of the Aria ET software

Agilent offers the Aria ET (Electronic Tracking) software component, an optional upgrade providing security features such as user authentication, database data storage, and audit trail records. For instructions on installing the ET version of the Aria software, see the AriaMx/AriaDx Real-Time PCR System Setup and User's Guide.

If you are running the Aria ET software component, you have access to the following electronic tracking functions:

- Controlled access to the Aria software through identification of all application administrators and users, each with a unique username and encrypted password
- Controlled database access through identification of all databases using a unique user ID and encrypted password
- Experiment storage, retrieval, and deletion to/from defined database(s)
- Chronological and permanent audit trail recording of administrator and user actions that create, modify, or delete experiments, as well as error recording
- Database management, including adding, removing, and switching databases (switching databases is available at login)
- Controlled access to a defined database through administrator and user management
- Report generation for audit trail, user account, and error logs
- Report generation for experiment results with electronic signature (e-signature)

The help topics listed below provide information and instructions on using the program features that are unique to the ET component.

"Open an experiment in the Aria ET software" on page 323

"Import and export experiments in the Aria ET software" on page 326

"Lock or log out of the Aria ET software" on page 328

"Change your password in the Aria ET software" on page 330

"Create a multiple experiment analysis project in the Aria ET software" on page 331

For administrators, the following help topics provide information on administrative functions.

“Manage users in the Aria ET software” on page 332

“Archive and restore experiments in the Aria ET software” on page 339

“View audit trails and system logs in the Aria ET software” on page 343

“Add and remove databases in the Aria ET software” on page 348

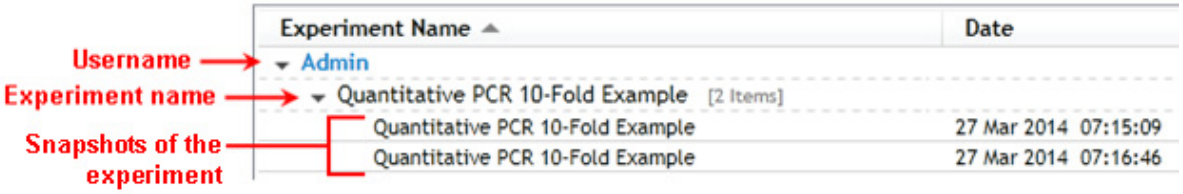
“View transaction logs in the Aria ET software” on page 352

Open an experiment in the Aria ET software

The Aria ET program tracks the actions taken on a post-run experiment using a primary database with controlled access. If the experiment you want to open is stored in the primary database, you can open it directly from the database. If you want to open a post-run experiment that is saved to a file system (i.e., a local or network folder), you first must import the experiment into the database. The instructions in this help topic describe how to open experiments from the primary database. See "Import and export experiments in the Aria ET software" on page 326 for instructions on importing experiments.

Open an experiment

You can open an experiment using multiple approaches. When you open an experiment, you have the option to open the latest snapshot of the experiment (i.e., the most recent version) or an earlier snapshot of the experiment. Refer to the image below for an explanation of how the program organizes experiments and snapshots in its browsers.



Open the latest snapshot of an experiment

You can open the most recent version of an experiment using multiple approaches.

To open the latest snapshot of an experiment from the Getting Started screen:

1. Click the icon to the right of the tabs to open a new tab.

   The new tab opens to the Getting Started screen.

2. Click one of the options under **Saved:**

- To open an existing experiment that you recently accessed, click **Recently Opened**. In the bottom panel of the screen, under **Database**, is a list of post-run experiments that you have recently opened. Double-click the experiment you want to open. The program opens the experiment to the Plate Setup screen.
- To browse to the folder of the experiment, click **Browse Database**. The Browse Database dialog box opens with a list of all experiments in the primary database, organized by user (see image above). Click the experiment name (not a snapshot below an experiment) to select it. Then, click **Open**. The dialog box closes and the program opens the experiment to the Plate Setup screen.

To open the latest snapshot of an experiment from the File menu:

1 From the toolbar, click **File > Open**.

The Open Experiment dialog box opens with a list of all experiments in the primary database, organized by user (see image above).

2 Click the experiment name (not a snapshot below an experiment) to select it. Then, click **Open**. The dialog box closes and the program opens the experiment to the Plate Setup screen.

Open an earlier snapshot of an experiment

You can open a previous snapshot of an experiment using multiple approaches.

To open an earlier snapshot of an experiment from the Getting Started screen:

1 Click the icon to the right of the tabs to open a new tab.

The new tab opens to the Getting Started screen.

2 Under **Saved**, click **Browse Database**. The Browse Database dialog box opens with a list of all experiments in the primary database, organized by user (see image above). Expand the node for the desired experiment to view the snapshots available for the experiment.

The date and time stamp for the snapshot are listed in the Date column.

3 Click a snapshot to select it, then click **Open**. The dialog box closes and the program opens the experiment to the Plate Setup screen.

To open an earlier snapshot of an experiment from the File menu:

1 From the toolbar, click **File > Open**.

The Open Experiment dialog box opens with a list of all experiments in the primary database, organized by user (see image above).

2 Expand the node for the desired experiment to view the snapshots available for the experiment.

The date and time stamp for the snapshot are listed in the Date column.

3 Click a snapshot to select it, then click **Open**. The dialog box closes and the program opens the experiment to the Plate Setup screen.

Import and export experiments in the Aria ET software

The Aria ET software tracks the actions taken on a post-run experiment using a primary database with controlled access. If you want to open a post-run experiment that is saved to a local or network folder, you first must import the experiment into the database. Similarly, if you want to make an experiment available outside of the database, you must first export it to a local or network folder.

Import experiments into the database

Importing a post-run experiment into the database creates a controlled copy of the experiment and makes it available for opening in the Aria ET program.

To import an experiment from a folder into the database:

1. From the toolbar, click **File > Import From File System**.

   The Open dialog box opens.

2. Browse to the folder location of the experiment. Select the experiment and click **Open**.

   The dialog box closes. The program creates a copy of the experiment and saves it to the primary database.

   You can now open the experiment as described in "Open an experiment in the Aria ET software" on page 323.

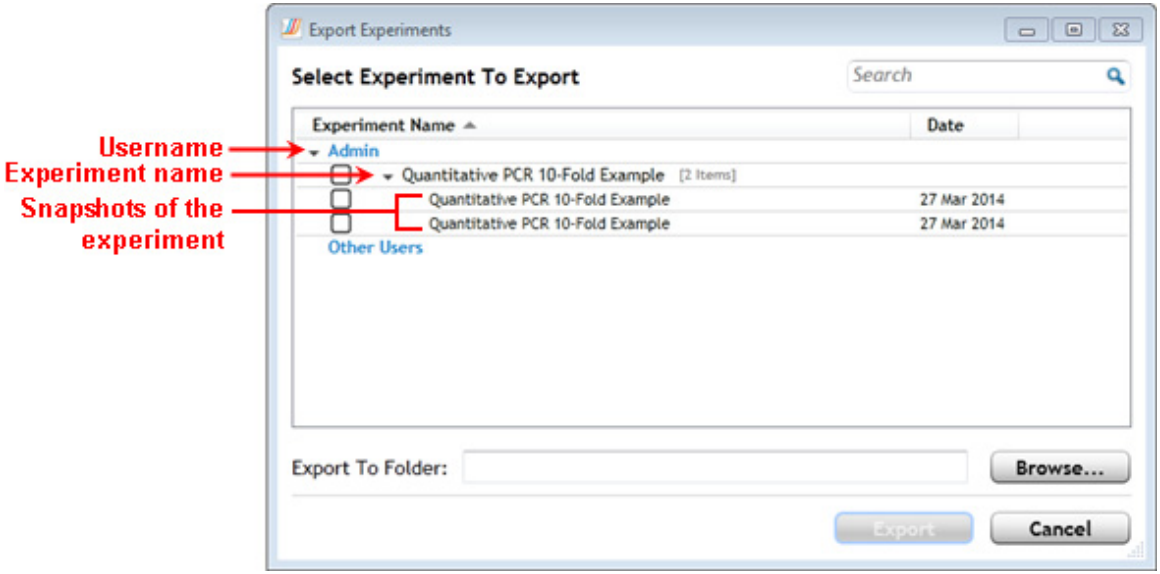
Export experiments from the database

Exporting an experiment creates an uncontrolled copy of the experiment. You may need to export an experiment in order to make it available for viewing to users outside your network.

To export an experiment from the database to a folder:

1 From the toolbar, click **File > Export To File System**.

The Export Experiments dialog box opens, listing all experiments in the primary database, organized by user.



2 Mark the check box for the experiment or snapshot that you want to export. Marking the experiment will export all snapshots of the experiment. Marking a snapshot will export only that snapshot. The date stamp for the snapshots are noted in the Date column.

3 Click **Browse**.

The Browse For Folder dialog box opens.

4 Browse to the folder where you want to save the exported experiments. Select the folder and click **Open**.

The Browse For Folder dialog box closes and the file path for the selected folder is listed in the Export To Folder field on the Export Experiments dialog box.

5 Click **Export**.

The dialog box closes. The program creates a copy of the experiment and saves it to the specified folder. The file name of the exported experiment includes a date and time stamp.

Lock or log out of the Aria ET software

When the Auto lockout feature is enabled, the program automatically locks and requires users to re-enter their password if the program is idle for longer than a set period of time (60 minutes is the default). You can also lock the program, or log out completely. For example, if you need to step away from your computer, or need to secure it for any reason, you may want to lock the program or log out.

Lock the program

Locking the program makes that session of the program inaccessible without logging out the currently logged-in user. To unlock the program, users must re-enter the user password. Only the logged-in user or an administrator can unlock a locked session of the program.

To lock the program:

- From the toolbar, click **File > Lock**.

  The program locks and the Unlock dialog box opens.

To unlock the program:

1. In the Password field of the Unlock dialog box, type the password for the logged-in user. Alternatively, if you are an administrator, type your administrator username and password into the fields.
2. Click **Login**.

Log out of the program

Logging out of the program logs out the currently logged-in user. Any user can log back in after the current user logs out.

To log out of the program:

- From the toolbar, click **File > Logout**.

  The program logs out the current user and the Login dialog box opens.

To log in to the program:

1 In the Login dialog box, type your username and password into the fields.

2 Click **Login**.

Change your password in the Aria ET software

By default, the program automatically prompts users to reset the password for their account every 90 days. However, users can change the password for their Aria ET account at any time using the instructions provided here.

NOTE Administrators can reset the password for any user account at any time. See "Change a user's password" on page 337 for instructions.

To change the password for your Aria ET user account:

1. From the toolbar, click **File > Logout**.

   The program logs you out and the Login dialog box opens.

2. In the Login dialog box, click **Change Password**.

   The Change Password dialog box opens.

3. Type your Username, Old Password, and New Password into the fields. Type the new password again into the Confirm Password field. Passwords must be 6-15 alphanumeric characters in length and include at least one number.

4. Click **OK**.

   The Change Password dialog box closes and you are returned to the Login dialog box.

5. Type your Username and Password into the fields, using your new password.

6. Click **Login**.

Create a multiple experiment analysis project in the Aria ET software

Multiple experiment analysis (MEA) is a feature in the Aria software that allows you to display and analyze the results from two or more post-run experiments together in a single file called a project. See "Overview of multiple experiment analysis" on page 270 for a description of MEA.

Although the ET version of the Aria software allows you to create MEA projects and analyze the data in those projects, the projects are not stored in a database and the program does not electronically track the actions taken on projects.

To create an MEA project in the Aria ET program:

1. Export the post-run experiments to be added to the project from the database to a file system. See "Export experiments from the database" on page 326.
2. Create the project in the same manner as that used for the standard version of the Aria software. See "Create an MEA project" on page 275.

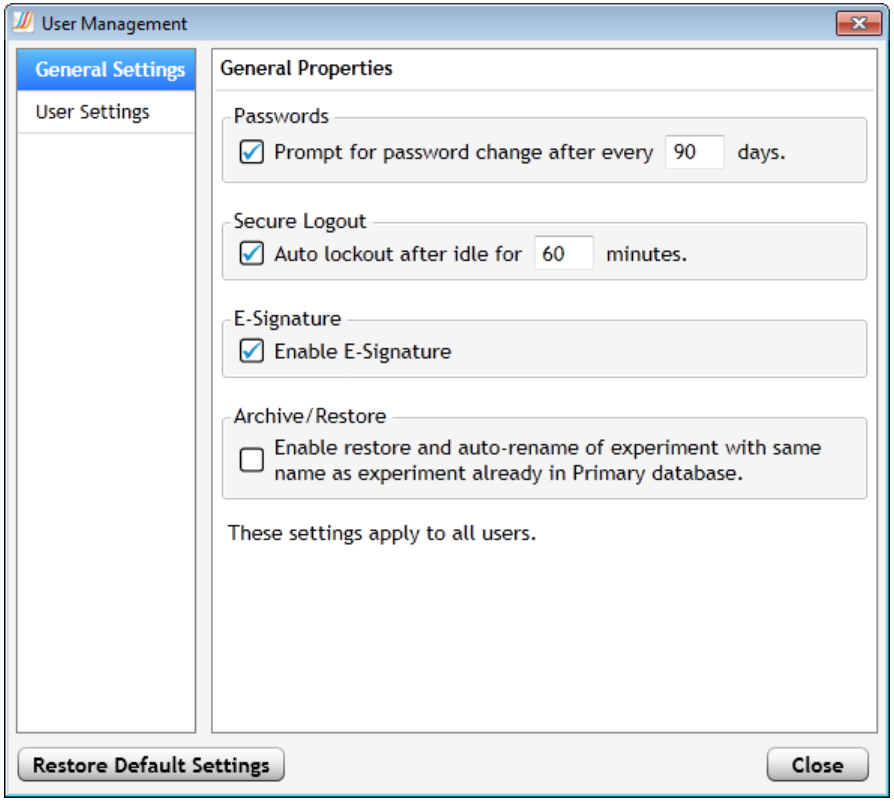
Manage users in the Aria ET software

If you are running the electronic tracking (ET) version of the Aria software, and you logged in using an administrator account, you have access to User Management dialog box for managing users and account settings.

To open the User Management dialog box: At the top of the program window, click **Admin > User Management**.

Set account properties for all users

The General Settings tab of the User Management dialog box has settings that apply to all users on the database.



Set the password expiration properties

You can change how frequently the program prompts users to reset their passwords. You can also eliminate password expirations so that the program no longer requires users to reset their passwords after a set period of time.

To change the password expiration time:

1 Open the User Management dialog box to the General Settings tab.

2 Under **Passwords**, make sure the check box is marked, and in the field, type the number of days that you want the expiration time to be. The default is 90 days, which means that the program automatically prompts users to reset their password every 90 days.

3 Click **Close** to close the dialog box and apply your changes.

To eliminate password expirations:

1 Open the User Management dialog box to the General Settings tab.

2 Under **Passwords**, clear the check box next to **Prompt for password change every [x] days**.

3 Click **Close** to close the dialog box and apply your changes.

Users will no longer be prompted to change their passwords after a set period of time.

Set the auto lockout properties

You can change the length of idle time required before the program automatically locks and requires users to re-enter their password. You can also disable the automatic lockout feature completely.

To change the length of idle time required before automatic lockout:

1 Open the User Management dialog box to the General Settings tab.

2 Under **Secure Logout**, make sure the check box is marked, and in the field, type the number of minutes for the desired idle time. The default is 60 minutes, which means that the program automatically locks after 60 minutes of inactivity.

3 Click **Close** to close the dialog box and apply your changes.

To disable automatic logout:

1 Open the User Management dialog box to the General Settings tab.
2 Under **Secure Logout**, clear the check box next to **Auto lockout after idle for [x] minute**.
3 Click **Close** to close the dialog box and apply your changes.

   The program no longer locks after a period of inactivity.

Disable or enable the e-signature requirement

By default, the program requires users to re-enter their password before allowing them to print or export an experiment report. This step serves as an electronic signature (e-signature) to verify that the logged-in user approves of the report. The User Management dialog box has tools for disabling and enabling the e-signature requirement.

To disable e-signature:

1 Open the User Management dialog box to the General Settings tab.
2 Under **E-Signature**, clear the check box.
3 Click **Close** to close the dialog box and apply your changes.

   The program no longer requires an e-signature to print or export reports.

To enable e-signature:

1 Open the User Management dialog box to the General Settings tab.
2 Under **E-Signature**, mark the check box.
3 Click **Close** to close the dialog box and apply your changes.

   The program now requires an e-signature to print or export reports.

Enable or disable auto-renaming of restored experiments

The auto-renaming feature is for the automatic renaming of experiments restored to the primary database. If you restore an experiment to the primary database, but the database already contains an experiment of the same name, auto-renaming appends a bracketed number to the name of the restored experiment. When auto-renaming is disabled, you cannot restore an experiment if the primary database already contains an experiment of the same name.

To enable auto-renaming:

1 Open the User Management dialog box to the General Settings tab.
2 Under **Archive/Restore**, mark the check box.
3 Click **Close** to close the dialog box and apply your changes.

Auto-renaming is now enabled.

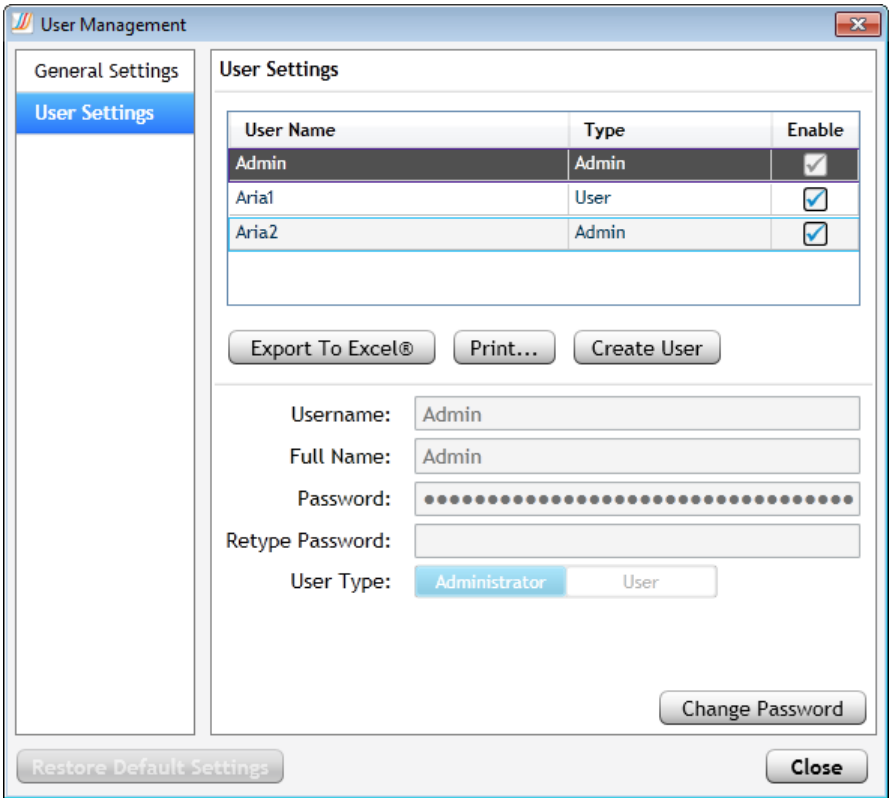
To disable auto-renaming:

1 Open the User Management dialog box to the General Settings tab.
2 Under **Archive/Restore**, clear the check box.
3 Click **Close** to close the dialog box and apply your changes.

Auto-renaming is now disabled.

Manage user accounts

The User Settings tab of the User Management dialog box has tools for creating and editing user accounts.



Create a user account

Create user accounts for each user who needs to log in to the Aria ET program and access the database.

To create a user account:

1 Open the User Management dialog box to the User Settings tab.

2 Click **Create User**.

The fields in the bottom panel of the dialog box (e.g., Username, Full Name, etc.) become editable.

3 Complete all fields.

Username: Type a username for the new account
Full Name: Type the name (first and last) of the person who will be using the account

Password: Type a password for the account (must be 6-15 alphanumeric characters in length and include at least one number)
Retype Password: Type the same password you entered above

4 Next to **User Type**, select the access privileges for the new user
   - Select Administrator to give the user administrator privileges
   - Select User to give the user standard privileges

   Administrator privileges include managing user accounts and databases, archiving and restoring experiments, and viewing audit trails and transaction logs.

5 Click **Close** to close the dialog box and apply your changes.

Disable or enable a user account

Once created, you cannot delete a user account but you can disable the account to prevent the user from being able to log in to the Aria ET program. You can also re-enable a disabled user account.

To disable a user account:

1 Open the User Management dialog box to the User Settings tab.
2 In the table, locate the account that you want to disable and clear the check box in the Enable column.
3 Click **Close** to close the dialog box and apply your changes.

To enable a user account:

1 Open the User Management dialog box to the User Settings tab.
2 In the table, locate the account that you want to enable and mark the check box in the Enable column.
3 Click **Close** to close the dialog box and apply your changes.

Change a user's password

As an administrator, you can change the password for any user account in the database.

To change a password:

1 Open the User Management dialog box to the User Settings tab.
2 In the table, click on the row for the desired user account to select it.

The account information for the selected user is displayed in the bottom panel of the dialog box.

3 Click **Change Password**.

The Password and Retype Password fields become editable.

4 In the Password field, type a new password for the account. Passwords must 6-15 alphanumeric characters in length and contain a number.

5 In the Retype Password field, type the password again.

6 Click **Save** to save the new password.

Print or export the user account table

You can print a copy of the table that appears on the User Settings tab of the User Management dialog box. You can also export a copy of the table to Microsoft Excel.

To print the user account table:

1 Open the User Management dialog box to the User Settings tab.

2 Click **Print**.

The Print dialog box opens.

3 Select a printer and click **Print.**

The program prints a copy of the table.

To export the user account table to Excel:

1 Open the User Management dialog box to the User Settings tab.

2 Click **Export to Excel**.

Excel launches with a copy of the user account table displayed.

3 Save the Excel file, if desired.

Archive and restore experiments in the Aria ET software

If you are running the electronic tracking (ET) version of the Aria software, and you logged in using an administrator account, you can archive experiments to an archive database and restore previously archived experiments.

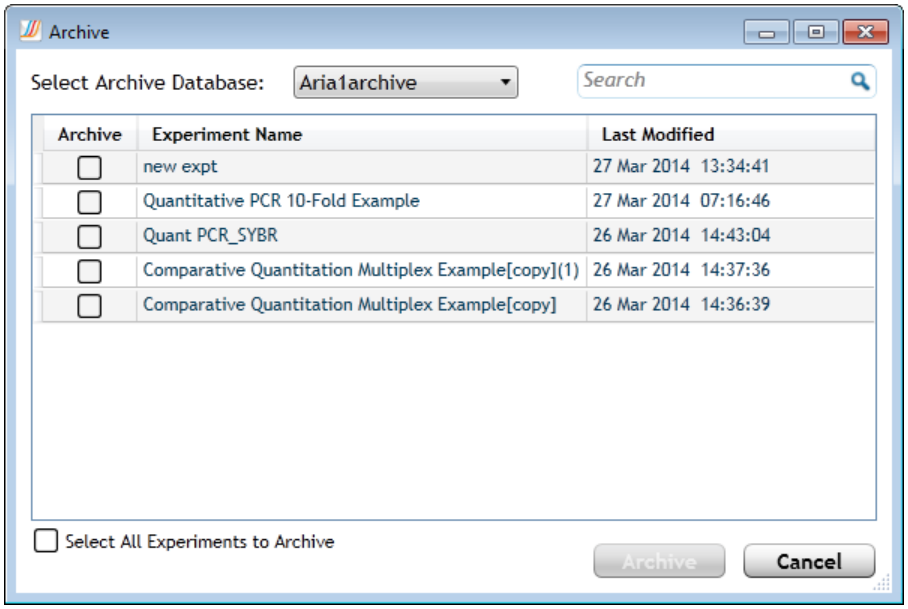
To open the Archive dialog box: At the top of the program window, click **Admin > Archive Experiments**.

To open the Restore dialog box: At the top of the program window, click **Admin > Restore Experiments**.

NOTE In order to archive or restore experiments, your PC must be running MSDTC service. See the AriaMx/AriaDx Setup and User's Guide for instructions on configuring and starting the MSDTC service.

Archive experiments

The Archive dialog box has tools for archiving experiments and creating new archive databases. Archiving an experiment requires moving it from the primary database to an archive database. Once archived, users cannot work in an experiment unless an administrator restores it.



Create an archive database

Before you archive an experiment, you may wish to create a new archive database. Creating an archive database makes it available in the Select Archive Database drop-down list in the Archive dialog box.

To create an archive database:

1. Open the Archive dialog box (Admin > Archive Experiments).
2. In the Select Archive Database drop-down list, click **Create new**. The Create Archive Database dialog box opens.
3. In the Server Name drop-down list, select the appropriate server.
4. In the Password field, type your SQL Server password.
5. In the Database name field, type a name for the new archive database.
6. Click **Create**.

   A message box opens confirming the creation of the new database. Click **OK** to close the message box.

Archive experiments

To archive an experiment:

1. Open the Archive dialog box (Admin > Archive Experiments).
2. In the Select Archive Database drop-down list, select the desired archive database.
3. In the table, mark the check box in the Archive column for the experiments that you want to archive.

   To search for an experiment, type a search term into the Search field at the top of the dialog box.

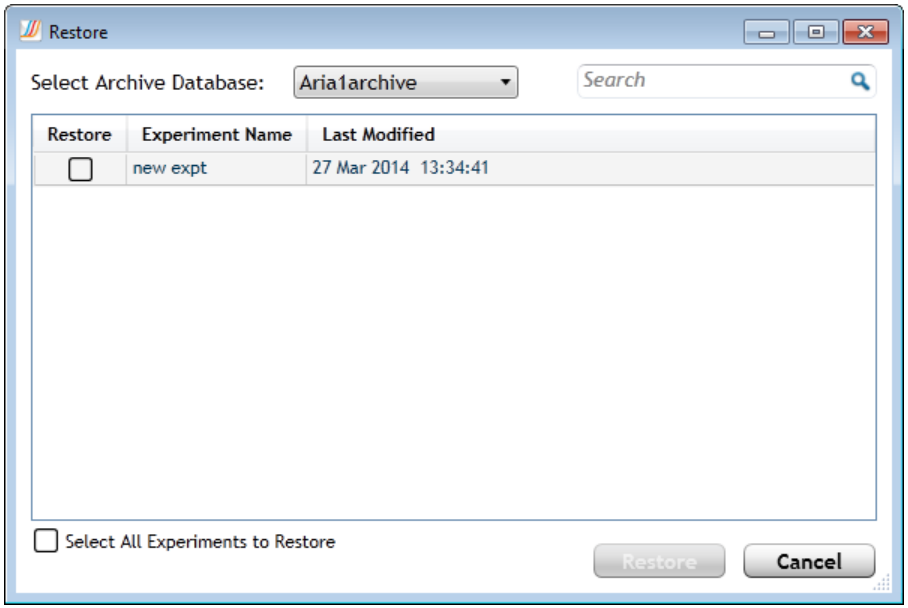
   To archive all experiments, mark **Select All Experiments to Archive** at the bottom of the dialog box.
4. Click **Archive**.

   A message box opens confirming the number of experiments that the program successfully archived.

NOTE In order to archive or restore experiments, your PC must be running MSDTC service. See the AriaMx/AriaDx Setup and User's Guide for instructions on configuring and starting the MSDTC service.

Restore experiments

Using the Restore dialog box, you can take archived experiments out of the archive database and restore them to their original primary database or a different primary database. Restoring experiments makes them available for further editing and analysis.



To restore an archived experiment:

1 Open the Restore dialog box (Admin > Restore Experiments).

2 In the Select Archive Database drop-down list, select the desired archive database.

3 In the table, mark the check box in the Archive column for the experiments that you want to archive.

   To search for an experiment, type a search term into the Search field at the top of the dialog box.

   To restore all experiments, mark **Select All Experiments to Restore** at the bottom of the dialog box.

4 Click **Restore**.

   A message box opens confirming the number of experiments that the program successfully restored.

NOTE In order to archive or restore experiments, your PC must be running MSDTC service. See the AriaMx/AriaDx Setup and User's Guide for instructions on configuring and starting the MSDTC service.

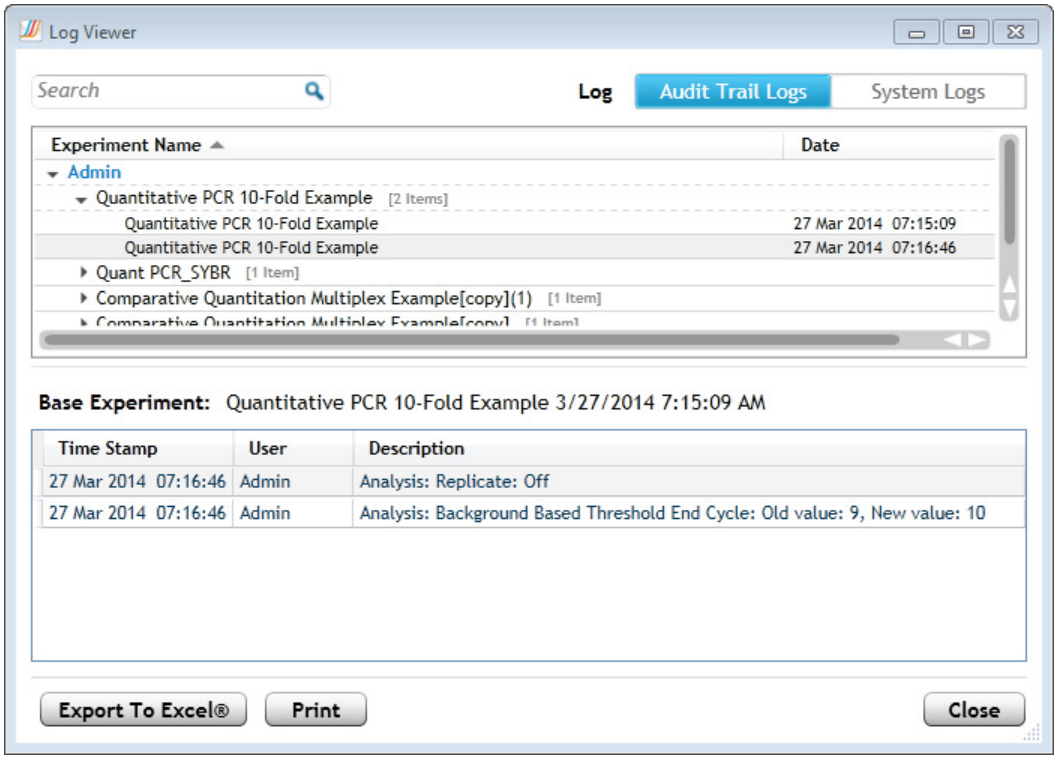
View audit trails and system logs in the Aria ET software

If you are running the electronic tracking (ET) version of the Aria software, and you logged in using an administrator account, you have access to the audit trails and system logs that are available in the Log Viewer dialog box.

To open the Log Viewer dialog box: At the top of the program window, click **Admin > Log Viewer**.

View the audit trail logs

The audit trail logs show all snapshots of an experiment. The program saves a new snapshot of an experiment each time a user performs a tracked action in the experiment (e.g., changing an analysis setting). For each snapshot, the logs indicate the user who performed the action, the date and time of the action, and a description of the action.



Open and navigate the audit trail logs

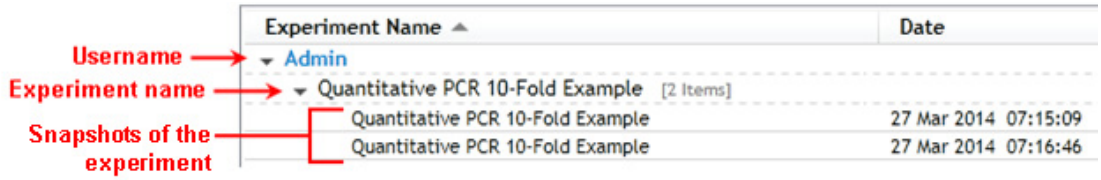
The audit trail logs show all available snapshots for each experiment, organized first by username then by experiment name.

To open the audit trail logs and navigate its contents:

1 Open the Log Viewer dialog box (Admin > Log Viewer).

2 Next to **Log**, select **Audit Trail Logs** (if not already selected).

The table at the top of the dialog box lists the users on the database, with experiments listed under each user, and previous snapshots for the experiment listed below (as described in the image below).



3 Click on an experiment name or snapshot in the table. (To help locate a particular experiment, type a search term into the Search field at the top of the dialog box.)

The table at the bottom of the dialog box shows all actions taken by the user for a selected snapshot (if you selected a snapshot) or for the entire experiment (if you selected an experiment).

Time Stamp	User	Description
27 Mar 2014 07:15:09	Admin	Experiment imported by Admin.
27 Mar 2014 07:16:46	Admin	Analysis: Replicate: Off
27 Mar 2014 07:16:46	Admin	Analysis: Background Based Threshold End Cycle: Old value: 9, New value: 10

Print or export the audit trail logs

You can print a copy of the table that appears at the bottom of the Log View - Audit Trail Logs dialog box. You can also export a copy of the table to Microsoft Excel.

To print the table:

1 Open the Log Viewer dialog box to the Audit Trail Logs.

2 In the table at the top of the dialog box, select an experiment or snapshot (see *Open and navigate the audit trail logs*, above, for detailed instructions).

3 Click **Print**.

The Print dialog box opens.

4 Select a printer and click **Print.**

The program prints a copy of the table.

To export the table to Excel:

1 Open the Log Viewer dialog box to the Audit Trail Logs.

2 In the table at the top of the dialog box, select an experiment or snapshot (see *Open and navigate the audit trail logs*, above, for detailed instructions).

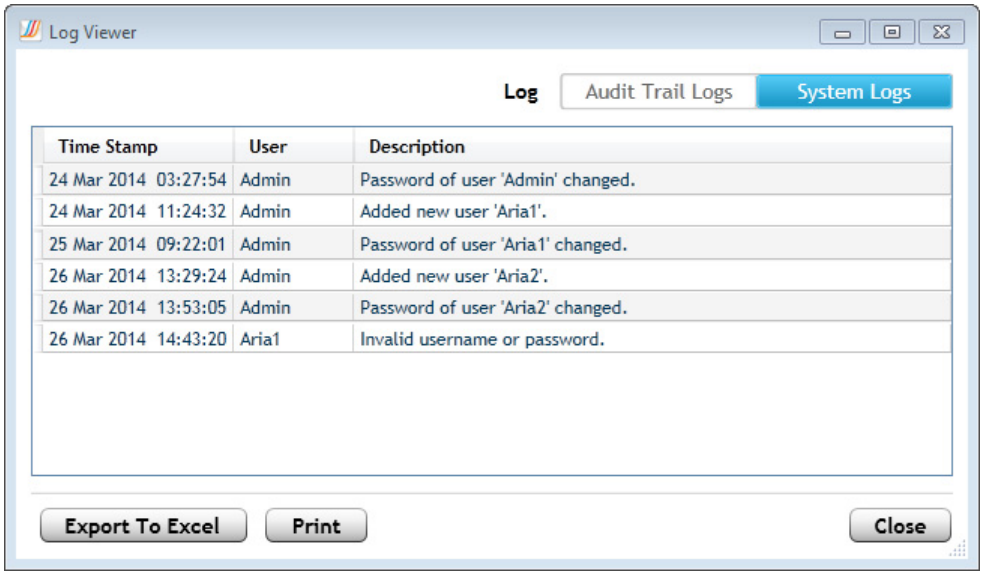
3 Click **Export to Excel**.

Excel launches with a copy of the table displayed.

4 Save the Excel file, if desired.

View the system logs

The system logs show the actions taken to manage user account information as well as any failed login attempts. For each action item, the logs indicate the user who performed the action, the date and time of the action, and a description of the action. The system logs also show the total number of experiments that have been archived or restored and any failed attempts to archive or restore an experiment. For failed archive or restore attempts, the logs list the experiment name and the failure error message.



Open the system logs

To open the system logs:

1 Open the Log Viewer dialog box (Admin > Log Viewer).

2 Next to **Log**, select **System Logs.**

The table displays list system actions in chronological order.

Print or export the system logs

You can print a copy of the system logs table or export a copy to Microsoft Excel.

To print the system logs:

1 Open the Log Viewer dialog box to the System Logs.

2 Click **Print**.

The Print dialog box opens.

3 Select a printer and click **Print.**

The program prints a copy of the table.

To export the system logs to Excel:

1 Open the Log Viewer dialog box to the System Logs.

2 Click **Export to Excel**.

Excel launches with a copy of the table displayed.

3 Save the Excel file, if desired.

Add and remove databases in the Aria ET software

If you are running the electronic tracking (ET) version of the Aria software, and you logged in using an administrator account, you can control which primary and archive databases are available to users on your system.

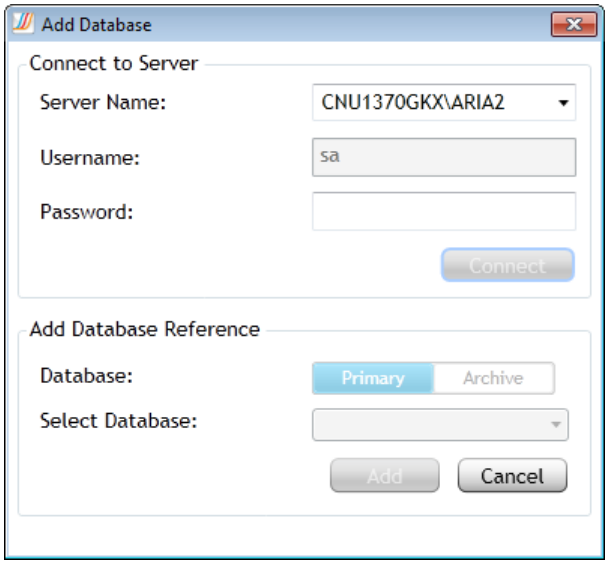
To open the Add Database dialog box: At the top of the program window, click **Admin > Add Database**.

To open the Remove Database dialog box: At the top of the program window, click **Admin > Remove Database**.

Adding a database through the Add Database dialog box does not create a new database; it only makes the database available for use by the Aria ET program on your PC by adding a reference to the database. Similarly, removing a database through the Remove Database dialog box does not delete a database; it only makes the database unavailable for use by the Aria ET program on your PC by removing a reference to the database.

Add an Aria ET database

The Add Database dialog box has tools for adding primary and archive databases.



Add a primary database

Each time a user launches the Aria ET program, the Login dialog box opens, prompting the user to login to an Aria ET database. Using the Add Database dialog box, you can add a database to the list of available databases that appears on the Login dialog box.

To add a primary database:

1. Open the Add Database dialog box (Admin > Add Database).

   The program searches your local machine and network to identify available servers.
2. In the Server Name drop-down list, select the appropriate server.
3. Type your SQL Server password into the Password field.
4. Click **Connect**.
5. Next to **Database**, make sure the Primary option is selected.
6. In the Select Database drop-down, select the desired database. This drop-down lists all primary databases available on the server.

7 Click **Add**.

The dialog box closes and the database is listed in the Login dialog box the next time a user launches the program.

Add an archive database

When you archive an experiment, the Archive dialog box includes a drop-down list of available archive database. You can use the tools on the Add Database dialog box to add an archive database to that drop-down list.

To add an archive database:

1 Open the Add Database dialog box (Admin > Add Database).

The program searches your local machine and network to identify available servers.

2 In the Server Name drop-down list, select the appropriate server.

3 Type your SQL Server password into the Password field.

4 Click **Connect**.

5 Next to **Database**, select **Archive**.

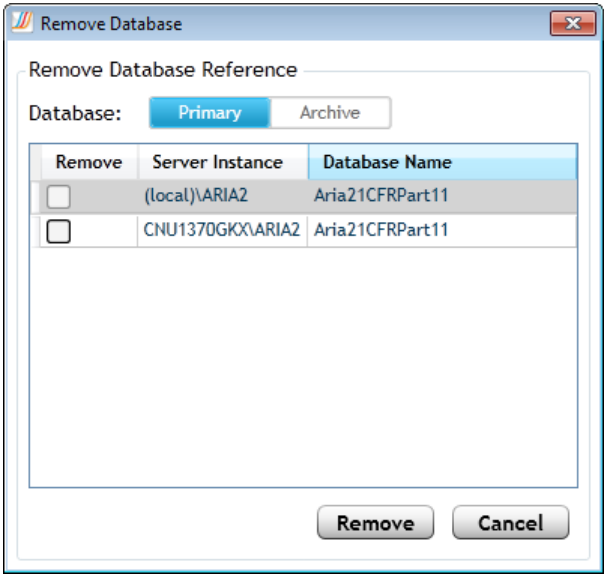
6 In the Select Database drop-down, select the desired database. This drop-down lists all archive databases available on the server.

7 Click **Add**.

The dialog box closes and the database is listed in the Archive dialog box the next time a user archives an experiment.

Remove an Aria ET database

If you have previously added databases, and have multiple databases available on your system, you can remove a database. Removing a primary database makes it no longer available in the Login dialog box that opens when users launch the Aria ET program. Removing an archive database makes it no longer available when users archive or restore an experiment.



To remove a database:

1 Open the Remove Database dialog box (Admin > Remove Database).

2 Next to **Database**, select **Primary** to remove a primary database or **Archive** to remove an archive database.

   The program populates the table with all available primary databases (if **Primary** is selected) or all available archive databases (if **Archive** is selected).

3 In the table, mark the check box in the Remove column for the database that you want to remove. Note that you cannot mark the check box for the currently logged-in database (the row is grayed out in the table).

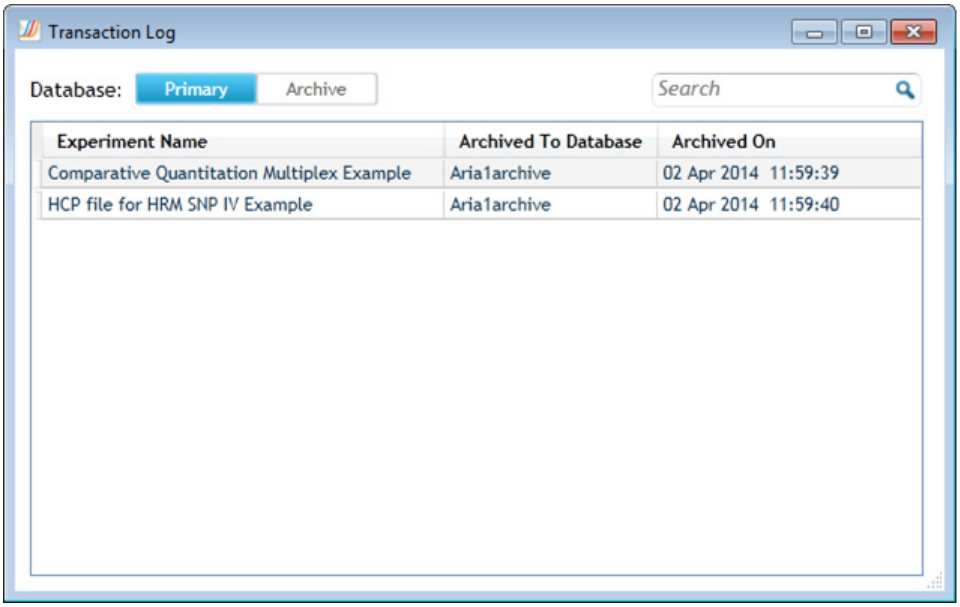
4 Click **Remove**.

   The database is removed from the table.

View transaction logs in the Aria ET software

If you are running the electronic tracking (ET) version of the Aria software, and you logged in using an administrator account, you can view the transaction logs, which track archiving and restoring activities.

To open the Transaction Log dialog box: At the top of the program window, click **Admin > Transaction Log**.



View transaction logs for primary database

The Transaction Log dialog box can display all the experiments that have been archived from the primary database. Note that the logs do not include archived experiments that were later restored to their original primary database.

To view the log of archive transactions:

1 Open the Transaction Logs dialog box (Admin > Transaction Log).

2 Next to **Database**, select **Primary** (if not already selected).

The table lists the experiments that have been archived (Experiment Name column), which database they were archived to (Archived To Database column), and the date and time that they were archived (Archived On column).

To help locate a particular experiment, type a search term into the Search field at the top of the dialog box.

View transaction logs for an archive database

The Transaction Log dialog box can display all the experiments that have been restored from an archive database to the primary database.

To view the log of restoration transactions:

1. Open the Transaction Logs dialog box (Admin > Transaction Log).
2. Next to **Database**, select **Archive.**
3. In the Select Archive Database drop-down list, select the archive database for which you want to view the logs.

   The table lists contains the following columns:

   - Experiment Name: experiments that have been archived/restored
   - Archived From Database: primary database from which the experiment was archived
   - Archived On: date and time that the experiment was archived
   - Restored To Database: primary database to which the experiment was restored
   - Restored On: date and time that the experiment was restored

   To help locate a particular experiment, type a search term into the Search field at the top of the dialog box.

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Reference Help and Troubleshooting & Support

Agilent Technologies

QPCR Glossary

Experiment Type and QPCR Detection Chemistry Terms

Quantitative PCR Experiment Type: Experiments of this type typically use a standard curve to quantitate the amount of target present in an unknown sample with high accuracy using a fluorescently-labeled probe or double-stranded DNA-binding dye for detection. A series of Standard samples, containing dilutions of a known amount of target, are amplified to generate a curve that relates the initial quantity of the specific target to the Cq. The standard curve is then used to derive the initial template quantity in Unknown wells based on their Cq values. This method is sometimes referred to as absolute quantitation or as standard-curve quantitation in the literature. This experiment type is also useful for primer/probe optimization experiments in the absence of a standard curve.

Comparative Quantitation Experiment Type: This experiment type is a form of relative quantitation, comparing the levels of a target gene in test samples (referred to as Unknowns) relative to a sample of reference (referred to as the calibrator). For example, the calibrator sample might contain RNA from untreated cells, while the Unknowns might contain RNA from cells treated with different experimental agents. This experiment type provides an efficient method for comparing levels of RNA or DNA across samples when information about the absolute amounts of target in any sample is not required. This method is used for establishing relative quantitation without the need for repeatedly performing a dilution series standard curve.

Allele Discrimination Experiment Type, Fluorescence Probe: In this experiment type, two fluorescent probes labeled with two spectrally distinct dyes are used to discriminate between the two alleles and, subsequently, determine the genotype of a sample. For example, if the program detects amplification in an unknown DNA sample for the dye identifying the wild-type allele but not for the dye identifying a mutant allele, the program designates the sample as wild-type homozygous.

Allele Discrimination Experiment Type, DNA Binding Dye Including HRM: When using this experiment type, you set up the experiment to amplify all alleles in the same well using the same set of primers, and the program detects all alleles using the same double-stranded DNA-binding dye (such as SYBR Green or EvaGreen dye). The thermal profile includes

a high-resolution melt (HRM) segment so that melt curves of the targets can be generated. Even DNA amplicons that differ in sequence by only a single nucleotide will yield slightly different melt curves. An Allele Discrimination experiment that uses HRM analysis for allele discrimination should include positive control samples for each base pair possibility at the SNP location (homozygous as well as heterozygous positive control samples).

Reference Dye: Passive dye used for normalization of the fluorescence signal of the reporter dye or fluorophore. The reference dye fluoresces at a constant level during the reaction.

Reporter Dye: Fluorescent dye that increases in fluorescence signal as the amount of PCR product increases.

Normalizer: Available in Comparative Quantitation experiments and User Defined experiments, the normalizer is a target that is known to be unaffected by the experimental treatment under investigation, and thus is found in equal quantity across all template samples. Data from the normalizer target is used to normalize the fluorescence signal of the targets of interest.

FRET Chemistry (Fluorescence Resonance Energy Transfer): In FRET chemistry, the excitation of a donor fluorescent dye is transferred to a receptor dye, leading to the fluorescence of the acceptor dye instead of the donor dye. The transfer is possible only if the two dyes are in close proximity.

TaqMan Probes: These are linear fluorescently-labeled hydrolysis probes that can be used to monitor PCR product formation either during or after the amplification process. As the DNA polymerase extends the upstream primer and encounters the downstream probe, the exonuclease activity of the polymerase cleaves the probe. In this event, the reporter fluorophore is released into the reaction solution and is able to fluoresce.

Quencher: A quencher is a moiety that absorbs the energy of the reporter dye in its excited state. The quencher can emit its own fluorescence signal (TAMRA) or emit no fluorescence signal (DABCYL, BHQ).

Well-Types

Unknown: Contains a complete reaction mixture including a test template that contains an unknown amount of the specific target.

Buffer: Contains only buffer, used to monitor the background fluorescence attributable to the buffer.

NAC: No amplification control; contains all reaction components except DNA polymerase.

NPC: No probe control; contains all reaction components except the fluorescence-labeled probe.

NTC: No template control; contains all reaction components except the template nucleic acid.

Standard: Contains a complete reaction mixture including a known concentration of target nucleic acid. Used to generate a standard curve, which is then used to relate the quantification cycle (Cq) to initial template quantity in Unknown wells.

No RT: No reverse transcriptase control; contains all QRT-PCR reaction components except reverse transcriptase.

Allele A+: Available in Allele Discrimination experiments and User Defined experiments. Contains a complete reaction mixtures with a template sample that is a positive control for homozygous allele A.

Allele B+: Available in Allele Discrimination experiments and User Defined experiments. Contains a complete reaction mixtures with a template sample that is a positive control for homozygous allele B.

Mixed+: Available in Allele Discrimination experiments and User Defined experiments. Contains a complete reaction mixtures with a template sample that is a positive control for mixture of allele A and allele B.

Calibrator: Available in Comparative Quantitation experiments and User Defined experiments as the reference sample to which Unknowns are compared. Contains a complete reaction mixture including a characterized target. The level of a target-of-interest in the Calibrator wells is set to 1.0 for comparison to the relative quantities in unknown samples.

Analysis Terms

Standard Curve: The Standard Curve is a plot of the Cq (quantification cycle) on the Y-axis versus the initial template quantity added to standard wells on the X-axis. A best-fit curve is displayed for each dye with data collected in Standard wells.

Amplification Plot: The Amplification Plots view shows a plot of fluorescence versus cycles for each plateau on which data are gathered.

Initial Template Quantity: Provides interpolated quantities of template added to Unknown wells before thermal cycling. The quantities are interpolated from a standard curve based on the calculated Cq values determined for the known quantities of template in the Standard wells.

Baseline Correction: For each well and each path the raw fluorescence data are fit over the specified range of cycles using a linear least mean squares algorithm to produce a baseline. The value of the baseline function is calculated for every cycle and subtracted from the raw fluorescence to produce the baseline corrected fluorescence (dR).

Quantification Cycle (Cq): The Cq is the cycle at which fluorescence is determined to be statistically significant above background signal contributed by the fluorescently labeled oligonucleotides within the PCR reaction. The quantification cycle is inversely proportional to the log of the initial copy number.

Background Cycle Range: The background cycle range specifies the range of cycles of fluorescence data the program uses to calculate the background noise level when using the Background-based threshold algorithm to set the threshold fluorescence. The region specified is typically in the cycle range before exponential amplification occurs. The standard deviation of the raw fluorescence for the specified cycles is calculated and is multiplied by the constant Sigma multiplier for threshold fluorescence.

Replicates: In the Aria program, the term *replicates* refers to technical replicates. Technical replicates are QPCR reaction tubes containing identical reaction components and set up using a template from the exact same biological sample source. While biological replicates measure the variability in the experimental results due to uncontrolled biological variation from sample to sample, technical replicates are used to measure the variability in results that is introduced during the process of experimental setup. Designating replicate wells allows the program to

average results from those wells when the Treat Collectively setting is used.

Collective Replicate Treatment: Collective replicate treatment causes the program to analyze all wells with the same replicate symbol as a group, effectively treating the measurements as all coming from the same well.

Biological Replicates: Biological replicates are template samples that were isolated independently but from biologically-identical sources (that is, sources that are genetically identical, are of the same cell type and were treated identically during experimentation). For example, two samples of cDNA that were isolated from the same tissue source in two different mice that were exposed to identical conditions and have the same genotype would be biological replicates. Biological replicates help you determine the level of variability in gene expression for your specific experiment that is due to uncontrolled biological variation from sample to sample. When setting up the plate for a Comparative Quantitation experiment, you may designate two or more samples as biological replicates while assigning the sample names. Samples that are biological replicates are assigned the same sample name but have different biological replicate ID numbers.

R-squared: The R^2 value is an indication of the fit of the standard curve to the standard data points plotted. The value will always be between 0 and 1. The closer the value is to 1, the better the fit of the line.

Sigma: Sigma is a measurement of the variability (standard deviation) of the fluorescence measured from all wells and more than one cycle. Typically its value is determined from the first few cycles, before the PCR reaction starts to affect the measurement. The Sigma multiplier is a user-defined number that is used to multiply by sigma to create a threshold value for determination of Cq.

p-value: The p-value refers to the probability that the mean of one set of sample data is different than the mean of another set of sample data. The first set of sample data is always the control wells in the analysis selection. When replicates are being treated individually, the second set of sample data consists of a single well (usually an Unknown well). When replicates are being treated collectively, the second set of sample data consists of all of the replicates. If the p-value exceeds the user-specified confidence level, the well/dye is given a (+) call, whereas if the p-value does not exceed the user-specified confidence level, the well/target is given a (-) call.

Confidence Level: The user-defined confidence level for calls is the statistical probability required before the algorithm will call amplification occurrence in a well. The default is 99%.

Multicomponent: Multicomponent is a term used for distinguishing the contribution that each dye and the background makes to the total fluorescence spectra detected.

LIMS File Format for Aria Software

In order for the experiment data in a LIMS data file to be importable into the Aria software, the information in the file must follow a specific format. This topic discusses the format requirements for the LIMS data file sections that are supported by Aria. These formats must be used in a LIMS data file that is imported into the program as part of creating a new experiment.

NOTE When importing a LIMS data file to create a new Aria experiment, the only required data section is Plate Setup. The other sections are optional, and when present they will be imported as part of the new Aria experiment creation, but they are not required in order for the file to be valid and importable.

An Aria-supported LIMS data file can be created by exporting a post-run experiment to a LIMS data file from within the Aria software. This approach ensures that the proper format is used. Once exported, you can edit the file contents as desired. You can also create an Aria-supported LIMS data file by setting up a text file in Microsoft Excel or another spreadsheet or text editing program, or by exporting content from a LIMS software program. If using these approaches, make sure the format of the file is importable into the Aria software before using it to create a new experiment.

Verifying proper integration of LIMS functionality, including import and export of Aria-related data elements, is the responsibility of the end-user.

Plate Setup

To create a new experiment in the Aria software by importing a LIMS data file, the file must include a Plate Setup section.

The content of the first row of the Plate Setup section needs to be "[Plate Setup]". The second row needs to contain the headers, separated by a delimiter. The only headers that are required in order to have a valid LIMS data file are:

- Well – Enter the well ID, e.g., A1
- Dye – Enter the dye used for target detection

If a single well contains multiple dyes/targets, then include a separate row for each, as shown in the example image below.

The following headers are also supported for the Plate Setup section:

- Target
- Replicate
- Sample Name
- Well Name
- Well Type – Enter a predefined well type that is supported for the experiment type (see Chapter 5, “Selecting an Experiment Type” for descriptions of the available well types in each experiment type)
- Starting Amount – Enter a positive floating number; only imported for Standard well types
- Normalizer Dye
- Biological Replicate

[Plate Setup]						
Well	Well Type	Dye	Target	Replicate	Sample Name	Well Name
A2	Standard	FAM	FAM	1		
A2	Standard	ROX	ROX	1		
A2	Standard	CY5	CY5	1		
A3	Standard	FAM	FAM	1		
A3	Standard	ROX	ROX	1		
A3	Standard	CY5	CY5	1		
A4	Standard	FAM	FAM	1		
A4	Standard	ROX	ROX	1		
A4	Standard	CY5	CY5	1		
B2	Standard	FAM	FAM	2		
B2	Standard	ROX	ROX	2		
B2	Standard	CY5	CY5	2		
B3	Standard	FAM	FAM	2		
B3	Standard	ROX	ROX	2		
B3	Standard	CY5	CY5	2		
B4	Standard	FAM	FAM	2		
B4	Standard	ROX	ROX	2		
B4	Standard	CY5	CY5	2		
B6	Calibrator	FAM	FAM	9		
B6	Calibrator	ROX	ROX	9		
B6	Calibrator	CY5	CY5	9		
B7	Unknown	FAM	FAM	10	A	
B7	Unknown	ROX	ROX	10	A	
B7	Unknown	CY5	CY5	10	A	

Experiment Setup

An Aria-supported LIMS data file can also include an Experiment Setup section, which contains information that applies to the entire experiment.

The content of the first row of the Experiment Setup section needs to be "[Experiment Setup]". The second row can contain any of the following headers, separated by a delimiter:

- Type – Enter as: Quantitative PCR (DNA Binding Dye including Melt), Quantitative PCR (Fluorescence Probe), Allele Discrimination (DNA Binding Dye including High Resolution Melt), Allele Discrimination (Fluorescence Probe), Comparative Quantitation, or User-Defined
- Name
- Note
- Reference Dye
- Allele A
- Allele B
- Quantity Unit

Thermal Profile

An Aria-supported LIMS data file can also include a Thermal Profile section, which contains information on the thermal profile.

The content of the first row of the Thermal Profile section needs to be "[Thermal Profile]". The second row can contain any of the following headers, separated by a delimiter:

- Segment – Enter a valid segment type; see “Set up the thermal profile” on page 143
- Plateau – Enter a number 1 through 4 to specify the order of each plateau within the same segment
- Temperature – Enter temperature in °C from 25.0 to 99.0
- Duration – Enter the duration of the plateau numerically as hh:mm:ss, from 00:01 to 18:12:15
- Cycle – Enter a positive integer (maximum of 50 for Amplification segments; maximum of 1 for other segment types)
- DataMarker – Enter No, Plateau, or Ramp

- Resolution – Enter 0.5 for normal melt or 2.0 for HRM (for High Resolution Melt segments, this data field is ignored and 0.2 resolution is automatically used)
- Soak Time – Enter a positive integer from 3 to 30; only imported for Melt segments

The subsequent rows in the file contain the identifiers for each column.

[Thermal Profile]					
Segment	Plateau	Temperature	Duration	Cycle	DataMarker
RT	1	50	0:10:00	1	No
Hot Start	1	95	0:03:00	1	No
Amplification	1	95	0:00:05	40	No
Amplification	2	60	0:00:15	40	Plateau

Default Optical Module

An Aria-supported LIMS data file can also include a Default Optical Module section, which contains information on the optical module setup for the experiment.

The content of the first row of the Default Optical Module section needs to be "[Default Optical Module]". The second row contains the following headers, separated by a delimiter:

- Optical Path – Enter a number 1 through 6 to specify the path of each customized default optical module installed on the instrument
- Optical Dye

The subsequent rows in the file contain the identifiers for each column.

The number of optical modules and the sequence of the optical modules listed in the file need to match those of the instrument on which you will run the experiment. If they do not match, you can manually adjust the modules in the instrument, or, if you transferred the experiment file to the instrument using a USB drive, you can use the **Sync Plate** feature on the Plate Setup screen of the instrument touchscreen.

Threshold Fluorescence

An Aria-supported LIMS data file can also include a Threshold Fluorescence section, which contains information on the target,

fluorescence term, and fluorescence value that will define the threshold fluorescence during analysis of the post-run experiment data.

The content of the first row of the Threshold Fluorescence section needs to be "[Threshold Fluorescence]". The second row contains the following headers, separated by a delimiter:

- TF Target – Enter the target name of the threshold fluorescence target
- TF Term – Enter R, dR, Rn, or dRn
- TF Value – Enter a positive floating number

The subsequent rows in the file contain the identifiers for each column.

Trademarks

SYBR® is a registered trademark of Molecular Probes, Inc.

Troubleshooting and Support

Troubleshooting Guide

Observation	Suggestion
Unable to install the Aria software	You will be unable to install the software if the account that was used to log in to the PC does not have administrative rights. Log in under an account with admin rights. If the computer is on a network, you may have to contact your IT department.
Unable to open post-run Aria files (*.*amxd* or *.*adxd*) on the PC-installed Aria software	Make sure that the Aria software is installed on a PC running the Windows operating system with the regional format set to **English (United States)**.
Very low amplification signal	Low amplification signal could indicate a possible problem with the probe or the stock of dye that was used. A good indication of this type of problem would be if the reference dye is showing good signal but the fluorophore is not. If SYBR Green is being used, verify the concentration. If using a new probe or a new lot of a probe, the problem could be the fluorophore. You can test the probe by performing a nuclease digestion to ensure it is unquenching as expected: incubate 100 nM of probe in 25 µL of 1× buffer with 10 U of DNase or S1 nuclease at room temperature for 30 minutes. This should result in a fluorescence level increase of >5000 RFU. If another probe or a stock of the dye is available, you can also try testing that for comparison. If the probe or dye stock has been stored in a way that might have exposed it to light, low signal could also be due to photobleaching of the fluorophore. If the samples are still available, you can also run these on a gel to make sure you are actually getting amplified product. If not, assay optimization or new reagents may be necessary.
Decreased volume in sample containers at end of run	Sample containers are not vapor tight. Ensure caps/plates are tightly sealed and containers are not malformed. Also ensure that proper plasticware was used.
Unexpected results in one sample	Check the sample container for contaminating material. Check optical clarity of sample container cap. Also check to make sure the liquid inside that sample tube did not evaporate during the run, which would indicate a vapor leak in that particular sample. Vapor leaks can occur if the sample was not sealed properly or incorrect plasticware was used.

No amplification plots are visible in the Graphical Displays screen	This could occur if the wrong data collection point was assigned to the amplification plots. Reset the data collection point for the amplification plots on the Analysis Criteria screen. A lack of amplification plots would also occur if no data collection point was set in the amplification segment on the Thermal Profile Setup screen. Without a data collection point, no data is collected and analysis is impossible. You will also not see any amplification plots if you selected ΔRn data when a reference dye was not defined for the experiment.
Signal fluctuations in amplification plots	Possible sources of signal noise include environmental factors such as vibration of the bench from other instruments, direct sunlight falling on the back of the instrument, or fluctuations in the line power. These problems can normally be resolved by relocating the instrument to a different bench in the lab or, for the case of line power problems, by connecting the instrument to a Power Conditioner or UPS.
In amplification plots, the baseline fluorescence signals gradually decrease across the baseline cycle range	To reduce this effect, enable the instrument's Warm Up feature for the optical modules, which was implemented in version 1.8 of the instrument firmware. From the touchscreen Home screen, go to **Settings > System Settings > Warm Up Settings**. On the Warm Up Settings screen, select **Yes** and press **OK**. The system turns on the optical module LED lights so that the modules are fully warmed up prior to starting an experiment. The warm up is repeated each time the instrument is powered on (after completion of health checks) and after the optical modules door is closed. The process takes approximately 15 minutes. This feature is not recommended as a standard practice for all assays. In some circumstances, however, warming up the optical modules may help signals remain steady throughout the baseline cycle range. Consult with Technical Support if you have questions about this feature.
Low increase in fluorescence with cycling	The probe is not binding the target efficiently. Lower the annealing temperature and verify the melting temperature. Target PCR product is too long; redesign primers to yield a PCR product <150 bp in length. Magnesium concentration is too low; run a titration to optimize concentration. Insufficient or non-specific product is being formed. Verify product formation through gel electrophoresis.

Cq value reported for NTC (no-target control) sample is less than the total number of cycles but the amplification plot curve is horizontal	Review amplification plot and adjust the threshold accordingly.
Increase in fluorescence in control reactions without template	The reaction has been contaminated.

Contact Agilent Technical Support

Agilent Technical Support is available worldwide.

For US and Canada

Call (800)227-9770 (option 3,4,5)

Or send an e-mail to qPCR.support@agilent.com.

For all other regions

Agilent's world-wide Sales and Support Center contact details for your location can be obtained at www.agilent.com/en/contact-us/page.

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