applied biosystems

Diomni™ Design and Analysis (RUO) Software v3.0

USER GUIDE

Desktop configuration

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Revision B

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Revision history: MAN0030162 B (English)

Revision	Date	Description
B	16 June 2025	• The name of the copy number variation analysis module was corrected to Copy Number Variation Analysis Module (project). • More instructions were provided to open a file from the dashboard. • More details were provided in the instructions to send the plate file to the instrument run queue (“Review the plate file and send to the instrument run queue” on page 101). • The information about the algorithm to reduce dye signal cross-talk was updated to indicate that the algorithm does not correct signal saturation (“Overview of the algorithm to reduce dye signal cross-talk” on page 147). • The information about data export in legacy format was corrected (“Export data in legacy format” on page 171). • More information was provided about batch generating plate files (“Batch generate plate files” on page 183). • More information was provided about exporting and importing a list of instruments (“Export the instruments” on page 186 and “Import instruments” on page 187). • The information about analysis plugins was updated to indicate that the software should be run as an administrator to install a plugin. If a plugin is uninstalled, the corresponding analysis tab is not available. • Instructions were added to install Rosetta 2 Software after an upgrade to the macOS™. • Recommended actions were added if files cannot be accessed or saved.
A	13 February 2025	New document for Diomni™ Design and Analysis (RUO) Software v3.0 (desktop configuration).

The information in this guide is subject to change without notice.

Contents

1 About the software

Diomni™ Design and Analysis (RUO) Software 3 is used to set up instrument runs on and analyze data generated with QuantStudio™ real-time PCR systems (see “Instruments compatible with plate files from Diomni™ Design and Analysis (RUO) Software 3” on page 13).

Product description

The Diomni™ Design and Analysis (RUO) Software 3 is used to create plate files to run on a real-time PCR instrument.

The software is used to analyze data files. Primary and secondary analysis can be performed. The software includes the following secondary analysis modules:

- Presence Absence Analysis Module
- Genotyping Analysis Module
- Relative Quantification Analysis Module
- Standard Curve Analysis Module
- Copy Number Variation Analysis Module (project) (available only in a project)
- Genotyping Analysis Module (project) (available only in a project)
- High Resolution Melt Analysis Module (available only with an extra license)

The Copy Number Variation Analysis Module (project) and the Genotyping Analysis Module (project) allow multi-plate analysis.

The software enables setup of project templates. A project template can be used to create a project. A project allows multiple data files to be added and analyzed. Project templates and projects apply to the Copy Number Variation Analysis Module (project) and the Genotyping Analysis Module (project).

Overview of the software configuration

The desktop software is installed on a single computer and can be accessed only from the single computer.

Depending on the configuration when the software is installed, it can be accessed by a single user for a computer or all of the users of the computer.

IMPORTANT! The option to use the Security, Auditing, and E-signature (SAE) Administrator Console is not available for the desktop configuration of Diomni™ Design and Analysis (RUO) Software 3.

There is no file management feature for the desktop configuration. The files are downloaded to the local computer and can be uploaded from the local computer.

Template files and data files are checksum protected. Checksum protection helps to ensure that files produced by the instruments or the software are not edited outside of the system.

A plate file can be sent to the QuantStudio™ 6 Pro Real-Time PCR Instrument or the QuantStudio™ 7 Pro Real-Time PCR Instrument.

The High Resolution Melt Analysis Module is available for the desktop configuration of Diomni™ Design and Analysis (RUO) Software 3. The module requires an extra license.

Other configurations are available.

- Thermo Fisher™ Connect Platform
- On-premises configuration

The desktop configuration of the software can be installed on the same computer as the on-premises configuration. This is not recommended. The data in one configuration are not available in the other configuration if the instances of the software are coinstalled on the same computer.

Instruments compatible with plate files from Diomni™ Design and Analysis (RUO) Software 3

Use the software to create plate files to run on the following instruments:

- QuantStudio™ 7 Pro Real-Time PCR System
- QuantStudio™ 6 Pro Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (all block formats, except the OpenArray™ Plate format)
- QuantStudio™ 7 Flex Real-Time PCR System
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System
- QuantStudio™ 1 Plus Real-Time PCR System (available in China)

Pre-run files for the OpenArray™ Plate cannot be created in the Diomni™ Design and Analysis (RUO) Software 3. For more information, see “Files for the OpenArray™ Plate format” on page 17.

File formats

File extension	File description	File contents	Color
EDMT	Project template	Used as a starting point to create a project. See “Overview of a project template” on page 24. • Instrument • Block type	System template

(continued)

File extension	File description	File contents	Color
EDMT	Project template	• Analysis module • Targets • SNPs • CNVs • Samples • Biogroups	User-created template 
EDT	Plate file (system template or user-created template)	A run setup file that contains information to set up specific experiments. The file can be used as a template to produce multiple experiment runs. • Run method • Samples • Assays • Reagents • Plate layout • Analysis settings	System template  User-created template 
EDM	Project file	Contains all the data files for the runs that are associated with the project. See “Overview of projects” on page 45. • Samples • Assays • Reagents • Common analysis settings • Project-level results (for multiple plates) • Plate layout (single plate) • Results (single plate)	
EDS	Data file	A pre-run or post-run file that contains the data for a single experiment. • Run method • Samples • Assays • Reagents • Plate layout • Analysis settings • Run data • Analysis results	

(continued)

File extension	File description	File contents	Color
JSON	Export settings	Specifies the information that is included when data are exported. See Chapter 15, “Manage export settings”.	

Overview of the file icons

The color of the icon depends on the file format (see “File formats” on page 13).

Icon	Definition
	A system template that cannot be edited.
	An editable file, with security settings enabled.
	An editable file, no security settings enabled.
	A user-created plate file (template).
	A user-created project template.

Data files

If the data file is generated from a plate file that was created with QuantStudio™ Design and Analysis Software 2 or Diomni™ Design and Analysis (RUO) Software 3, it can be opened and analyzed with Diomni™ Design and Analysis (RUO) Software 3. A conversion is not required. For the list of instruments that generate a compatible data file, see “Compatible data files” on page 16 .

If the data file is generated from a plate file that was created with legacy software or legacy instrument software, it must be converted to the updated file format. For more information, see “Compatible data files (legacy file format)” on page 16 and “Convert a legacy file” on page 17.

Compatible data files

The software is compatible with data files from the following real-time PCR instruments, if the plate file for the run was created with QuantStudio™ Design and Analysis Software 2 or Diomni™ Design and Analysis (RUO) Software 3:

- QuantStudio™ 7 Pro Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 6 Pro Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (including TaqMan™ Array Card format, but not including the OpenArray™ Plate format)
- QuantStudio™ 7 Flex Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System
- QuantStudio™ 1 Plus Real-Time PCR System (available in China)

Data files for the OpenArray™ Plate are in the legacy file format. For information about a workflow for the OpenArray™ Plate, see “Files for the OpenArray™ Plate format” on page 17.

Compatible data files (legacy file format)

If a data file is generated from a plate file that was created with legacy software or legacy instrument software, it must be saved as the updated file format.

Data files from the following instruments are compatible after they have been saved as the updated file format:

- QuantStudio™ 7 Flex Real-Time PCR System (including the TaqMan™ Array Card format)
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (including the TaqMan™ Array Card and the OpenArray™ Plate format)
- QuantStudio™ 5 Real-Time PCR System
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System
- QuantStudio™ 1 Plus Real-Time PCR System (available in China)
- StepOnePlus™ Real-Time PCR System
- ViiA™ 7 Real-Time PCR System
- 7500/7500 Fast Real-Time PCR System
- 7900HT Real-Time PCR System

Convert a legacy file

If a legacy data file was converted to the updated file format in QuantStudio™ Design and Analysis Software 2, it does not need to be converted again in Diomni™ Design and Analysis (RUO) Software 3. The file from QuantStudio™ Design and Analysis Software 2 is compatible with Diomni™ Design and Analysis (RUO) Software 3.

1. Open a data file.
   See “Open a data file” on page 132.
2. Click **Actions** ▸ **Save As**.

Files for the OpenArray™ Plate format

Pre-run files for the OpenArray™ Plate format cannot be created with the Diomni™ Design and Analysis (RUO) Software 3.

A data file from the OpenArray™ Plate format can be opened in the Diomni™ Design and Analysis (RUO) Software 3. The plate layout can be saved as a CSV file. This file can be used as an OA.csv file format in the QuantStudio™ 12K Flex Software.

The plate layout can be edited in Diomni™ Design and Analysis (RUO) Software 3.

The sample name or the target name can be edited. A sample layout file can be imported to overwrite the sample layout of the OpenArray™ Plate.

For information about saving the plate layout as a CSV file, see “Export a plate setup file” on page 102.

The Genotyping Analysis Module and the Presence Absence Analysis Module are the only secondary analysis modules that can be applied to a single OpenArray™ Plate.

The analysis modules for a project can be applied to an OpenArray™ Plate. The analysis modules for a project are the Genotyping Analysis Module (project) and the Copy Number Variation Analysis Module (project).

View the software information

1. From any screen, click ⓘ **(Help)** ▸ **About**.
   The **About** dialog box is displayed. The dialog box indicates the version of the software.
2. Click **Close**.

View the end user license agreement

1. From any screen, click ⓘ **(Help)** ▸ **End User License Agreement**.
   The *End User License Agreement* is displayed.
2. Click **Close**.

Navigation in the software

Overview of the pages

The software contains the following tabs on the left side of the page. Each tab corresponds to a page. Click each tab to access the page.

Tab	More information
Dashboard tab	Chapter 3, “View and manage the dashboard”
Run templates tab	• Chapter 6, “Set up a plate file” • Chapter 12, “Manage plate files and data files”
Runs tab	• Chapter 8, “Review and analyze data” • Chapter 12, “Manage plate files and data files”
Project templates tab	• Chapter 4, “View and manage project templates” • Chapter 7, “View the plate setup for a project”
Projects tab	• Chapter 5, “View and manage projects” • Chapter 7, “View the plate setup for a project” • Chapter 9, “Review and analyze a project”
Instruments tab	Chapter 13, “Manage instruments”

Change the view of the files

The following pages display files:

- **Run templates** page
- **Runs** page
- **Project templates** page
- **Projects** page

1. Use the checkboxes in the left pane to filter the files.
   The following filter categories are available:
   - Instrument
   - Block
   - Run mode (available only for run templates and runs)
   - Analysis
2. At the bottom of the left pane, click **Clear all** to clear the filters.
3. Click **(Search)** to access the search field, enter a search term, then press **Enter**.
4. Click **(Close)** to close the search field and remove the search criteria from the displayed files.

5. Click **(List View)** to view the files as a list.
6. In the list view, click a column header to sort by that parameter.
7. Click **(Grid View)** to view the files as a grid.
8. In the grid view, click the dropdown lists in the top-right corner to sort by the parameter and in ascending order or descending order.
9. At the bottom-left of the page, navigate to a different page number.
   - Click the page number.
   - Click **(Back)** or **(Forward)** to navigate through the pages sequentially.
   - Enter a number in the **Go To Page** field, then click **Go**.
10. At the bottom-right of the page, in the **Show** dropdown list, select the number of files that are displayed.

    The total number of files is displayed beside the dropdown list.

Change the view of the tables

Changing the view of the tables applies to the following tables:

- Tables in the **Plate Setup** tab, include samples, targets, SNP assays, and CNV assays
- Tables in the **Quality Check** tab
- Tables in the tabs associated with the analysis modules

1. In any table pane, click a column to sort by the parameter.
2. To sort by a subsequent column, press and hold control on the keyboard, then click the column.

   The order of the sorting is displayed with a number in the column.
3. To sort by a single column after sorting by multiple columns, click a column without pressing control.
4. Click and drag a column to change the order that the columns are displayed in the table.

2 Workflows

Workflow (single plate)

① Set up a plate file
Set up a plate file (page 70)
Select a system template or existing plate file to set up a new plate file (page 70)
Confirm or edit run method (page 71)
Confirm or edit plate setup (page 78)
Review the plate file and send to the instrument run queue (page 101)

② Review and analyze data
Review and analyze data (page 130)
Review results in the Amplification Plot (page 135)
Identify and omit outliers from analysis (page 140)
Export results (page 169)

Workflow (project)

1 Create a project

Create new project (page 49)

Define the project (page 50)

***(Optional)* Import items to the project**

- "Import targets to a project" on page 58
- "Import SNPs to a project" on page 59
- "Import CNVs to a project" on page 60
- "Import samples to a project" on page 61

Add a run to a project (page 62)

Review and analyze a project (page 152)

3 View and manage the dashboard

Overview of the dashboard

The **Dashboard** page is the default page when you launch the software.

The **Dashboard** page contains two panes:

- **Recent Runs** pane
- **Recent Projects** pane

The list of files is displayed for any user that launches the software. The list of files is not specific to the user.

Options for the files on the dashboard

Files can be opened from the dashboard. Files can be removed from the dashboard.

If a file is removed from the dashboard, it is not available from any other page, for example, the **Runs** page or the **Projects** page.

If the file is still available on the computer, it can be opened again in order to view it in the software.

You can navigate to all of the runs or all of the projects from the dashboard (see “Navigate from dashboard” on page 22).

Only the grid view is available on the dashboard. The list view is available for files on the other screens.

Navigate from dashboard

- Open a file from the list of recent runs or recent projects.
  See “Open a file from the dashboard (recent files)” on page 23.
- Open a file from the menu.
  See “Open a file from the dashboard (menu)” on page 23.
- Click **View All**.
  The **View All** button is available in the **Recent Runs** pane and the **Recent Projects** pane.
  The **View All** button in the **Recent Runs** pane opens the **Runs** page.
  The **View All** button in the **Recent Projects** pane opens the **Projects** page.

Open a file from the dashboard (recent files)

In the **Dashboard** page, perform one of the following actions.

- Click the file to open.
  A data file opens in the same window, in the **Quality Check** tab of the file.
  A project file opens in the same window, in the **Run Files** tab of the file.
- Hover over the template, then click ··· **(More Options)** ▸ **Open in new window**.
  A data file opens in a new window, in the **Quality Check** tab of the file.
  A project file opens in a new window, in the **Run Files** tab of the file.

Open a file from the dashboard (menu)

1. In the top-right corner of the **Dashboard** page, click **Open File**.
2. Navigate to the location of the file, then click **Open**.

Rerun a file from the dashboard

Rerunning a file applies only to run files on the **Dashboard** page. The run files are in the **Recent Runs** pane.

Rerunning a file is not available for the OpenArray™ Plate format.

In the **Dashboard** page, perform one of the following actions.

- Hover over the run file, then click ··· **(More Options)** ▸ **Rerun**.
- Hover over the run file, then click ··· **(More Options)** ▸ **Rerun in new window**.

A new template file is created. It is available in the **Recents** tab of the **Run templates** screen.

Remove file from dashboard

If a file is removed from the dashboard, the file can be reopened in order to view it or edit it.

Removing a file from the dashboard also removes it from any other pages that it appeared on. For example, removing a file from the **Recent Projects** pane of the dashboard also removes it from the **Recents** tab of the **Projects** page.

In the **Dashboard** page, hover over the file, then click ··· **(More Options)** ▸ **Remove**.

4 View and manage project templates

Overview of a project template

A project allows multiple run files to be analyzed at the same time.

A project template is a predefined structure that is a starting point to create new projects. It helps to consistently create new projects by providing a standardized starting point.

The software includes system project templates.

Projects are available for copy number variation analysis and genotyping analysis.

A project template is in EDMT file format. It contains the following information:

- Project name (user-created project templates only)
- Analysis modules
- Analysis settings
- Instrument type
- Block type

A project name is not included for system project templates. A system project template must be saved as a user-created project template in order to use it.

You must have the corresponding analysis module installed in order to use a project template. The software includes built-in analysis modules for project analysis. See “About analysis modules” on page 190.

IMPORTANT! We recommend using the system project template that corresponds to your block types when setting up a new project. The analysis settings are optimized for each block type in the system project templates. The block type can be changed when creating a project template but the analysis settings are not updated according to the block type.

The instrument type can be changed without affecting the analysis settings if the block type is not changed.

Overview of primary analysis settings for a project template

Primary analysis settings can be defined for a project template. All of the primary analysis settings are available for a project template.

For example, melt primary analysis settings are available for a project template. When a project is created from the project template, the runs that are added to the project might contain melt curve data.

The run method is not defined in a project template.

In the **General** tab, the **PCR Stage/Step** dropdown list is not available when defining primary analysis settings for a project template. The PCR stage and PCR step depend on the run method and these items is not defined in a project template.

For more information about analysis for a project, see “Overview of data analysis for a project” on page 48.

Overview of the project template summary

The **Project Summary** tab displays the following information in the top pane:

- Number of samples
- Number of targets
- Number of SNPs
- Number of CNVs
- Instrument
- Block types
- Analysis modules

The following information is displayed in the bottom panes:

- Targets, including the following information:
  - Name
  - Color
  - Reporter
  - Quencher
- SNPs, including the following information:
  - Name
  - Color
  - Allele 1 reporter
  - Allele 1 quencher

  - Allele 2 reporter
  - Allele 2 quencher
- CNVs, including the following information:
  - Name
  - Color
  - Test reporter
  - Test quencher
  - Reference reporter
  - Reference quencher
- Samples, including the following information:
  - Name
  - Color
  - Type
  - Quantity
  - Biogroup
- Biogroup, including the following information:
  - Name
  - Color

Sort or filter the project templates

1. Navigate to one of the tabs in the **Project templates** page.
   - **Recents** tab
   - **My Project Files** tab
   - **System Project Templates** tab
2. Use the checkboxes in the left pane to filter the project templates.
   The following filter categories are available for project templates:
   - Instrument
   - Block
   - Analysis
3. At the bottom of the left pane, click **Clear all** to clear the filters.
4. Click **(Search)** to access the search field, enter a search term, then press **Enter**.
5. Click **(Close)** to close the search field and remove the search criteria from the displayed project templates.
6. Click **(List View)** to view the project templates as a list.
7. In the list view, click a column header to sort by that parameter.

8. Click **(Grid View)** to view the project templates as a grid.
9. In the grid view, click the dropdown lists in the top-right corner to sort by the parameter and in ascending order or descending order.
10. At the bottom-left of the page, navigate to a different page number.
    - Click the page number.
    - Click **(Back)** or **(Forward)** to navigate through the pages sequentially.
    - Enter a number in the **Go To Page** field, then click **Go**.
11. At the bottom-right of the page, in the **Show** dropdown list, select the number of project templates that are displayed.

    The total number of project templates is displayed beside the dropdown list.

Create a project template from an existing project template

A project template can be created only from an existing project template or existing project.

To create a project template from a project, see “Create a project template from a project” on page 28.

1. In the **Project templates** screen, select one of the following tabs.
    - **Recent** tab
    - **My Project Files** tab
    - **System Project Templates** tab
2. *(Optional)* Filter and sort the list of templates.

    See “Sort or filter the project templates” on page 26.
3. Select a project template.
4. Set up the project template.
    - Define the project template (see “Define the project template” on page 29).
    - Update the project settings for the project template (see “Update the project settings for a project template” on page 39).
    - Edit the primary analysis settings for the project template (see “Edit the primary analysis settings for a project template” on page 41).

    These items can be edited in the saved project template at a later time.
5. Click **Actions** ▸ **Save As Template**.
6. In the **Save As** dialog box, enter a file name in the **File Name** field.
7. *(Optional)* Click **Browse** to edit the location to save the project template.
8. Click **Save**.

The project template is saved in EDMT file format. It can be used to create another project template or it can be used to create a project.

Create a project template from a project

For information about a project template, see “Overview of a project template” on page 24. A project template is in EDMT file format.

Open or view a project. See “Open a project” on page 50 or “View a project” on page 50.

1. Click **Actions** ▸ **Save As Template**.
2. In the **Save As** dialog box, enter a file name in the **File Name** field.
   The **File Name** field is populated with the original file name appended with ***Copy***.
3. Click **Browse** to change the location to save the file.
4. Click **Save**.

The open project is the template that was saved.

The run files that were included in the project are not included in the project template.

The instruments and block types are included in the project template.

The assay information is included in the project template. The assay information includes the list of the targets, SNPs, and CNVs. For more information about the assays, see “Overview of the project summary” on page 46.

The list of samples and biogroups is included in the project template.

The plate assignment of any items cannot be completed in the project template. The plate assignment must be completed in a project. Only the list of items is included in the project template.

Add the project template to My Project Files list

The **My Project Files** list is on the **Project templates** page.

A system project template cannot be added to the **My Project Files** list. The project template must be saved as a user-created project template before it can be added to the **My Project Files** list.

Open or view a project template. See “Open a project” on page 50 or “View a project” on page 50.
Click **Actions** ▸ **Add To My Project Files**.

The file is displayed in the **My Project Files** tab of the **Project templates** page.

Remove the project template from the My Project Files list

Removing the project template from the **My Project Files** list does not remove it from the software. For example, if the project template is in the **Recents** tab of the **Project templates** page, the project template is not removed from this tab if it is removed from the **My Project Files** list.

A system project template cannot be removed.

Hover over the file, then click ··· **(More Options)** ▸ **Remove**.

Define the project template

The following information can be defined in a project template:

- Targets
- SNPs
- CNVs
- Samples
- Biogroups

The information is included in the project when a project is created from a project template.

Define the targets for a project template

Targets cannot be added to a system project template. Targets can be added only to a user-created project template.

A target that is defined in the project template is available to assign to a well of a run file when a project is created.

The targets define the sequence regions that are detected by the assay.

Each new target is populated with a default name. Each target must have unique name.

Targets can be imported (see “Import targets to a project template” on page 36).

1. In an open project template, select the **Targets** tab.
2. Click + **Add Targets**.
   A new target is added. The new target contains default information.
3. Enter a target name in the **Name** field.
4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
5. Select the reporter dye from the **Reporter** dropdown list.
6. Select the quencher dye from the **Quencher** dropdown list.

7. Click ⊗ **(Remove)** to remove a target from the project template.

8. Click ··· **(More Options)** ▸ **Remove unused targets**.
   If a project template was created from a separate project template or a project, there might be targets from the project template or project that are not used in the new project template. Removing unused targets allows you to have a project summary that matches the targets that are defined within the project template.

Define the SNPs for a project template

Single nucleotide polymorphisms (SNPs) are used in the genotyping application. SNPs define the sequence variants that are detected by the assay.

SNPs cannot be added to a system project template. SNPs can be added only to a user-created project template.

A SNP that is defined in the project template is available to assign to a well of a run file when a project is created.

Each new SNP is populated with a default name. Each SNP must have unique name.

SNPs can be imported (see “Import SNPs to a project template” on page 37).

The allele 1 reporter dye and the allele 2 reporter dye cannot be the same dye.

If there is more than one SNP, the default is the same allele 1 reporter assigned to each SNP and the same allele 2 reporter assigned to each SNP. The reporters for each SNP can be edited.

1. In an open project template, select the **SNPs** tab.

2. Click + **Add SNPs**.
   A new SNP is added. The new SNP contains default information.

3. Enter an SNP name in the **Name** field.

4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the SNPs in the plate layout view.

5. Select the reporter from the **Allele 1 reporter** dropdown list.

6. Select the quencher from the **Allele 1 quencher** dropdown list.

7. Select the reporter from the **Allele 2 reporter** dropdown list.

8. Select the quencher from the **Allele 2 quencher** dropdown list.

9. Click ⊗ **(Remove)** to remove an SNP from the project template.

10. Click ··· **(More Options)** ▸ **Remove unused SNPs**.
    If a project template was created from a separate project template or a project, there might be SNPs from the project template or project that are not used in the new project template. Removing unused SNPs allows you to have a project summary that matches the SNPs that are defined within the project template.

If there is no amplification or low amplification with the SNP assays that were defined, see *Diomni™ Design and Analysis (RUO) Software 3 Genotyping Analysis Module (project) User Guide* (Pub. No. MAN1000138).

Define the CNVs for a project template

Copy number variations (CNVs) cannot be added to a system project template. CNVs can be added only to a user-created project template.

A CNV that is defined in the project template is available to assign to a well of a run file when a project is created.

CNVs define the differences in the number of copies in a particular region of the genome between samples.

Each new CNV is populated with a default name. Each CNV must have unique name.

The test reporter dye and the reference reporter dye must be different.

CNVs can be imported (see “Import CNVs to a project template” on page 38).

CNVs can be converted from SNPs or targets if the run was set up with an instrument software that does not include CNVs. A conversion can be included in the project template. See “Add the CNV assay conversion to the project template” on page 32.

IMPORTANT! If the data are exported from Diomni™ Design and Analysis (RUO) Software 3 for import into AlleleTyper™ Software, the CNV name must end with ***_cn***.

For more information about conversion, see the *Diomni™ Design and Analysis (RUO) Software 3 Copy Number Variation Analysis Module (project) User Guide* (Pub. No. MAN0030169).

1. In an open project template, select the **CNVs** tab.
2. Click **+ Add CNVs**.
   A new CNV is added. The new CNV contains default information.
3. Enter a CNV name in the **Name** field.
4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
5. Select the reference reporter dye from the **Reference Reporter** dropdown list.
6. Select the reference quencher dye from the **Reference Quencher** dropdown list.
7. Select the test reporter dye from the **Test Reporter** dropdown list.
8. Select the test quencher dye from the **Test Quencher** dropdown list.

9. Click ⊗ **(Remove)** to remove a CNV from the project template.

10. Click ··· **(More Options)** ▸ **Remove unused CNVs**.

    If a project template was created from a separate project template or a project, there might be CNVs from the project template or project that are not used in the new project template. Removing unused CNVs allows you to have a project summary that matches the CNVs that are defined within the project template.

Add the CNV assay conversion to the project template

The CNV assay conversion is included in a project when a project is created from the project template. When the CNV assay conversion is included in the project, it is applied to the data files automatically when they are added to the project.

Adding the CNV assay conversion to a project template is optional. The CNV assay conversion can be added to the individual project that is created from the project template (see "Add the CNV assay conversion to the project" on page 54).

The CNV assay conversion can also be added to an individual data file within a project (see "Add the CNV assay conversion to a plate" on page 122). When the CNV assay conversion is added to an individual data file, the conversion rules are applied to all of the data files in the project.

The CNV assay conversion includes pre-defined conversion rules. The rules can be edited or deleted.

1. In the **Project Summary** tab of an open project template, click the **CNV** tab.

2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.

3. In the **CNV Assay Converter** dialog box, click + **Add rule**.
   A new row is displayed in the **CNV Assay Converter** dialog box.

4. Enter the name of the target or SNP assay in the **Target / SNP Assay** field.

5. Enter the name of the CNV reference in the **CNV Reference Name** field.

6. Enter the name of the CNV assay in the **CNV Assay Name** field.
   The content of the fields in each row cannot be duplicated.

7. *(Optional)* Import rules.
   See "Import a CNV assay conversion file for a project template" on page 33.

8. Click ⊗ **(Delete)** in a row to delete a single rule.

9. Click ⊗ **(Delete)** in the table header to delete all of the rules.

10. Click **Apply**.

Import a CNV assay conversion file for a project template

The file must be in CSV format.

The file must contain the following headers:

- **Target / SNP Assay**
- **CNV Reference Name**
- **CNV Assay Name**

The conversion does not overwrite any of the CNVs that were previously defined in the project template. The CNVs that are defined in the CNV assay conversion file are added to the list of CNVs that are defined in the **CNVs** tab.

If a CNV with the same name was previously defined in the project template, the CNV from the CNV assay conversion file is not added to the list of CNVs that are defined in the **CNVs** tab.

1. In the **Project Summary** tab of an open project template, click the **CNV** tab.
2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. In the **CNV Assay Converter** dialog box, click **Import**.
4. In the **Open** dialog box, navigate to the location of the file, then click **Open**.

The **CNV Assay Converter** is populated with the rules for conversion.

Add or remove rules from the rules that were imported, if necessary.

Click **Apply** to apply the imported rules.

Export a CNV assay conversion file from a project template

The file is in CSV format.

The exported file can be imported to other project templates, projects, or plate setup files.

1. In the **Project Summary** tab of an open project template, click the **CNV** tab.
2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. In the **CNV Assay Converter** dialog box, click **Export**.
4. Navigate to the location to save the file, then edit the file name, if necessary.
5. Click **Save**.

Import an Assay Information File (AIF) to a project template

An Assay Information File (AIF) is provided with every TaqMan™ assay order. An AIF does not include sample information.

An AIF can be applied to a project (see “Import an Assay Information File (AIF) to a project” on page 56). An AIF can be applied to an individual plate within a project (see “Import an Assay Information File (AIF)” on page 113).

Note: TaqMan™ Array Card plate setup requires AIF import, as targets cannot be added, assigned, or edited manually for TaqMan™ Array Cards.

Download the AIF for your order at **thermofisher.com/taqmanfiles**.

1. In the **Project Summary** tab of an open project template, click **Import AIF**.
2. Navigate to, then select the previously downloaded AIF file.
3. Click **Open**.

The targets and SNP assays are added to the **Project Summary** tab.

In a project template, the targets and SNP assays are not applied to a plate setup because there are no plates in a project template.

Define the samples for a project template

Samples cannot be added to a system project template. Samples can be added only to a user-created project template.

A sample that is defined in the project template is available to assign to a well of a run file when a project is created.

Each new sample is populated with a default name. Each sample must have unique name.

Samples can be added to a project template if the same samples are included in multiple projects.

Samples can be imported (see “Import samples to a project template” on page 39).

1. In an open project template, select the **Samples** tab.
2. Click + **Add Samples**.
   A new sample is added. The new sample contains default information.
3. Enter a sample name in the **Name** field.
4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the sample in the plate layout view.
5. Select the sample type from the **Type** dropdown list.
   The options for the sample type depend on the experiment type.

6. Select a biogroup from the **Biogroup** dropdown list.

   One or more biogroups must be defined in order for a biogroup to be available in the **Biogroup** dropdown list. For more information, see “Define the biogroups for a project template” on page 35.

7. Click ⊗ **(Remove)** to remove a sample from the project template.

8. Click ··· **(More Options)** ▸ **Remove unused samples**.

   If a project template was created from a separate project template or a project, there might be samples from the project template or project that are not used in the new project template. Removing unused samples allows you to have a project summary that matches the samples that are defined within the project template.

Define the biogroups for a project template

Biogroups cannot be added to a system project template. Biogroups can be added only to a user-created project template.

A biogroup that is defined in the project template is available to assign to a sample of a run file when a project is created.

Each new biogroup is populated with a default name. Each biogroup must have unique name.

Biogroups, or biological replicate groups, are reactions that contain identical components and volumes, but evaluate separate samples of the same biological source. A biogroup is used to categorize samples. A biogroup applies mainly to relative quantification analysis.

1. In an open project template, select the **Biogroup** tab.

2. Click + **Add Biogroup**.

   A new biogroup is added. The new biogroup contains default information.

3. Enter a biogroup name in the **Name** field.

4. Click the color to open the color picker, then select a color.

   The color is not related to the dye. It is to visualize the biogroup in the plate layout view.

5. Click ⊗ **(Remove)** to remove a biogroup from the project template.

After a biogroup has been defined for a project template, a sample can be assigned to the biogroup (see “Define the samples for a project template” on page 34).

Import and export items for a project template

Import targets to a project template

The file must be in CSV format.

If the target is already defined in the project template and is included in the imported file, the target is not duplicated in the project template. A target is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color (in red, green, blue color model format or hexadecimal code)
- Reporter
- Quencher

Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project template, select the **Targets** tab.
2. Click ··· **(More Options)** ▸ **Import Targets Assays**.
3. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The targets from the imported file are added to the project template.

Export targets from a project template

Exported targets can be imported to another project template or another project.

1. In an open project template, select the **Targets** tab.
2. Click ··· **(More Options)** ▸ **Export Targets Assays**.
3. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import SNPs to a project template

The file must be in CSV format.

If the SNP is already defined in the project template and is included in the imported file, the SNP is not duplicated in the project template. An SNP is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color
- Allele 1 Name
- Allele 1 Color
- Allele 1 Reporter
- Allele 1 Quencher
- Allele 2 Name
- Allele 2 Color
- Allele 2 Reporter
- Allele 2 Quencher
- Assay ID
- Gene Symbol
- Gene Name
- NCBI SNP Reference
- Context Sequence
- Comments

The allele 1 name, allele 2 name, assay ID, gene symbol, gene name, NCBI SNP reference, context sequence, and comments fields are optional.

Colors are in red, green, blue color model format or hexadecimal code

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Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project template, select the **SNPs** tab.
2. Click ··· **(More Options)** ▸ **Import SNPs Assays**.
3. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The SNPs from the imported file are added to the project template.

Export SNPs from a project template

Exported SNPs can be imported to another project template or another project.

1. In an open project template, select the **SNPs** tab.
2. Click ··· **(More Options)** ▸ **Export SNPs Assays**.
3. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import CNVs to a project template

The file must be in CSV format.

If the CNV is already defined in the project template and is included in the imported file, the CNV is not duplicated in the project template. A CNV is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color
- Test Name
- Test Color
- Test Reporter
- Test Quencher
- Reference Name
- Reference Color
- Reference Reporter
- Reference Quencher
- Assay ID
- Comments

The following fields can be blank:

- Test Name
- Reference Name
- Assay ID
- Comments

Colors are in red, green, blue color model format or hexadecimal code

---

Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project template, select the **CNVs** tab.
2. Click ··· **(More Options)** ▸ **Import CNVs Assays**.
3. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The CNVs from the imported file are added to the project template.

Export CNVs from a project template

Exported CNVs can be imported to another project template or another project.

1. In an open project template, select the **CNVs** tab.
2. Click ··· **(More Options)** ▸ **Export CNVs Assays**.
3. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import samples to a project template

The file must be in CSV format.

If the sample is already defined in the project template and is included in the imported file, the sample is not duplicated in the project template. A sample is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color (in red, green, blue color model format or hexadecimal code)
- Quantity
- Biogroup

Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

The quantity and biogroup fields are optional.

1. In an open project template, select the **Samples** tab.
2. Click ··· **(More Options)** ▸ **Import Samples Assays**.
3. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The samples from the imported file are added to the project template.

Export samples from a project template

Exported samples can be imported to another project template or another project.

1. In an open project template, select the **Samples** tab.
2. Click ··· **(More Options)** ▸ **Export Samples Assays**.
3. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Update the project settings for a project template

1. In an open project template, click **Actions** ▸ **Project Setting**.
2. In the **Project Setting** dialog box, select one of the following checkboxes in the **Settings** section.
   - **Strict run method compatibility**
   - **Check for conflicting assay**

   For more information about these settings, see “Overview of run method compatibility for a project template” on page 40 and “Overview of conflicting assays for a project template” on page 41.

3. In the **Instrument Type** dropdown list, select an instrument.
4. In the **Block Type** pane, in the **Block Type** dropdown list, select a block type.
5. In the **Block Type** pane, in the **Analysis Module** dropdown list, select an analysis module.

   The analysis module determines how the data are analyzed. The project settings determine how the run files that are added to the project are analyzed.

   If a secondary analysis module is selected, primary analysis is performed in addition to the secondary analysis. If primary analysis is selected, only primary analysis is performed.

   Select **No analysis module** for only primary analysis.
6. Click + **(Add)** to add a block type.

   A maximum of two block types can be included in each project.

   The combination of the block type and the analysis module can be added only one time. The combination of the block type and analysis module cannot be duplicated.

   An analysis module can be applied to only one block type. Two different block types cannot have the same analysis module.
7. Repeat step 4 and step 5 for the block that was added.
8. Click ⊗ **(Remove)** to remove a block type.
9. *(Optional)* Click **Reset**.

   The settings are reset to the original settings for the project template.
10. Click **Save**.

Overview of run method compatibility for a project template

The **Strict run method compatibility** checkbox is selected by default.

The settings are applied to project that are created from the project template. For information about these settings in a project, see “Overview of run method compatibility for a project” on page 65.

Parameter	Required for strict run method compatibility	Required if strict run method compatibility is not set
Sample volume must be the same	Yes	Yes
Run mode must be the same (fast or standard)	Yes	No
The total number of steps must be the same	Yes	Yes
Each step must be within the same type of stage (PCR, hold)	Yes	Yes
Each step must have the same ramp rate, temperature, time	Yes	No
Each step must have the same status for data collection	Yes	Yes
No VeriFlex™ Zones are Auto Delta settings are applied	Yes	No

(continued)

Parameter	Required for strict run method compatibility	Required if strict run method compatibility is not set
The same number of cycles in each PCR stage	Yes	Yes
The filter settings must be the same[1]	Yes	No

[1] See "Confirm or edit filter settings" on page 77.

Overview of conflicting assays for a project template

The **Check for conflicting assay** checkbox is deselected by default.

When enabled, this setting checks that the targets, SNPs, and CNVs are assigned the same dyes.

For example, if FAM™ dye is the reporter dye for one target in one run file and VIC™ dye is the reporter dye for the same target in a second run file, the second run file cannot be added to the project.

When a project is created from the project template, the run file that is used to check for conflicting assays is dependent on the information in the project. For more information, see "Overview of conflicting assays for a project" on page 65.

Selecting the **Check for conflicting assay** checkbox increases the time that is required to add run files to a project.

Edit the primary analysis settings for a project template

View or edit C_q settings for a project template

- The default C_q settings are appropriate for most applications. Edit the threshold and baseline settings for analysis of atypical or unexpected run data.
- For information about C_q analysis, see "About the quantification cycle (Cq)" on page 201.
- For information about C_q settings, see "Cq settings overview" on page 202.
- The **PCR Stage/Step** dropdown list is not available when defining primary analysis settings for a project template. For more information, see "Overview of primary analysis settings for a project template" on page 25.

The following instructions apply to the **General** tab and the **Well Cq** tab of the **Primary Analysis Setting** dialog box.

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Primary Analysis Setting** dialog box, in the **Block Type** dropdown list, select a block type.
3. In the **General** tab, select an option from the **Algorithm Settings** dropdown list.
   - **Relative Threshold**
   - **Baseline Threshold**

4. *(For relative threshold algorithm settings)* Enter a start cycle in the **Default C_{RT} Start Cycle** field.

5. *(For baseline threshold algorithm settings)* To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.

6. *(For baseline threshold algorithm settings)* To edit the settings, make the edits in the appropriate table row.

Option	Action
Use Auto Threshold	Select the checkbox in the **Auto Threshold** column.
Manually set the Threshold	Deselect the checkbox in the **Auto Threshold** column, then edit the value in the **Threshold** column.
Use Auto Baseline	Select the checkbox in the **Auto Baseline** column. • To specify the Baseline Start cycle, Click **AUTO** in the **Baseline Start** column, then enter the cycle number. The software will automatically determine the Baseline end cycle. • To remove the specified Baseline Start cycle, click the cycle number, then delete it. The **Baseline Start** will revert back to **AUTO**.
Manually set the Baseline	Deselect the checkbox in the **Auto Baseline** column, then edit the values in the **Baseline Start** field and the **Baseline End** field.

7. (*Optional*) In the **Well Cq** tab, make the edits in the appropriate table row to apply custom C_q settings to a specific well.

8. Click **Save**.

9. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit melt analysis settings for a project template

For descriptions of the melt analysis settings, see “Melt analysis settings overview” on page 203.

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.

2. In the **Melt** tab, select an option from the **Melt Stage/Step** dropdown list.

3. In the **Multi-Peak Calling** column, select the checkbox.

   The threshold type, peak level, and peak height settings are available only when multi-peak calling is enabled.

4. In the **Threshold Type** column, select one of the following options.

   - **Percentage**
   - **Height**

5. In the **Peak Level (%)** column, enter a value.

   A value can be entered only if **Percentage** was selected in step 4.

6. In the **Peak Height** column, enter a value.

   A value can be entered only if **Height** was selected in step 4.

7. *(Optional)* In the **Melt Peak Parity Factor** field, enter a factor for the applicable targets.

   Note: If the melt peak parity factor is applied to wells without a reaction mix, this can lead to unexpected T_m results.

   A factor of ***0*** turns off the feature. Negative values are not permitted.

   The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.

8. Click **Save**.

9. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit QC alerts setting for a project template

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.

2. In the **QC Alerts** tab, review selections:

   - Curve Quality—(*default*) inactive
   - Results Quality—(*default*) active

   For more information about curve quality and results quality, see “Quality checks” on page 204.

3. (*Optional*) Select the **Curve Quality** checkbox.

4. (*Optional*) Set up the acceptance criteria for the result quality checks.

   a. Ensure that the **Results Quality** checkbox is selected.

   b. Select **Results Quality**.

   c. Click **+ (Add)**.

   d. Select the **Sample Type** from the dropdown list, then select the **Target** from the dropdown list.

      If a rule is set up for a specific sample type, it takes precedence over a rule that is set for all samples.

   e. In the right panel, select the acceptance criteria for each sample type and target combination (see “Acceptance criteria for result quality checks” on page 205).

   f. (*Optional*) Click **✕ (Remove)** to remove a sample type and target combination from the table.

5. Click **Save**.

6. (*Optional*) To reset to the default settings, click **Reset to Default**.

In the **Quality Check** tab, click **Analyze**, then review the QC alerts in the **Well Table**.

View or edit advanced settings for a project template

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Advanced** tab, select and/or enter the following, then click **Save**
   - **Use a variant of primary analysis algorithm via plugin**
   - **Set the Delta-Rn below which curves will be considered Non-Amplified**

   Any curves with the ΔR_n below the threshold are set to non-amplified. This is regardless of whether there is a C_q value.
3. Click **Save**.
4. (*Optional*) To reset to the default settings, click **Reset to Default**.

Reset primary analysis settings for a project template to the default settings

The settings are reverted to the system default settings.

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.
2. Click **Reset to Default**.

Overview of the use of different analysis settings for a project template

The software allows you to apply analysis settings from a different project template or project to a specific project template.

This feature allows you to try different analysis settings quickly.

This feature means that you do not need to manually update analysis settings in order to optimize the project template.

The instrument, block type, samples, and targets or SNP assays must all be the same in order to apply analysis settings from a different project template or project to a current project template.

Use the settings from a project template or a project

The primary analysis settings from the selected project template or project are applied to the open project template.

1. In an open project template, click **Actions** ▸ **Use settings from another EDM/EDMT**.
2. Navigate to the file location, then click **Open File**.

5 View and manage projects

Overview of projects

A project is a location within the software to group the data files and the analysis settings. The data files and analysis settings are grouped together for a specific analysis task.

A project is in EDM file format.

A project allows the analysis of data from multiple plates at the same time.

When a data file is added to the project, the original data file is not edited. The software extracts the information that is required for the project from the data file without editing the original data file. The primary analysis settings that were defined in the original data file are not used.

You can enable or disable multiplate analysis for the Copy Number Variation Analysis Module (project) within a project. Multiplate analysis is always enabled for the Genotyping Analysis Module (project) within a project.

Overview of the Projects page

The **Projects** page contains the **Recents** tab and the **Examples** tab.

The **Recents** tab displays the most recent files when the software is launched.

The **Examples** tab displays files that are included with the software. These example files can be used as a reference.

The following tabs are displayed with you open a project.

- **Run Files** tab
- **Project Summary** tab
- **Plate Setup** tab
- **Quality Check** tab
- A tab associated with the secondary analysis

The **Run Files** tab displays a list of all of the data files that are included in the project. A run can be added or deleted from the project. For more information, see “Overview of the run files in a project” on page 46, “Add a run to a project” on page 62, and “Delete a run from a project” on page 62.

The **Project Summary** tab displays the key information about the project. For more information about the **Project Summary** tab, see “Overview of the project summary” on page 46.

The **Plate Setup** tab displays the information about the physical setup of each plate. You can view the different plates associated with the run files within a project. The **Plate Setup** tab provides a visual representation of the targets, SNPs, CNVs, and samples on each plate. For more information, see “View or edit the plate setup for a project” on page 104.

The **Quality Check** tab displays the different plots associated with the run. You can review the plots, view alerts, and omit wells from the analysis. For more information, see Chapter 9, "Review and analyze a project".

The **Projects** page allows you to perform the following functions:

- Save a copy of the project (see "Save a copy of a project" on page 63)
- Save the project as a template (see "Create a project template from a project" on page 28)

A project template is in EDMT file format. For more information about the project templates, see "Overview of a project template" on page 24.

Overview of the run files in a project

The **Run Files** tab displays a list of the runs that are included in a project.

The following information is displayed for each run file:

- Run name
- Instrument
- Instrument serial number
- Block type
- Plate ID (barcode)
- Date that the run was modified (date that the run was completed, or the date that the run file was edited after the run)

You can add or delete run files from a project from the **Run Files** tab. See "Add a run to a project" on page 62, and "Delete a run from a project" on page 62.

Overview of the project summary

The **Project Summary** tab displays the following information in the top pane:

- Number of runs
- Number of samples
- Number of targets
- Number of SNPs
- Number of CNVs
- Instrument
- Block types
- Analysis modules

The following information is displayed in the bottom panes:

- Targets, including the following information:
  - Name
  - Color
  - Reporter
  - Quencher
- SNPs, including the following information:
  - Name
  - Color
  - Allele 1 reporter
  - Allele 1 quencher
  - Allele 2 reporter
  - Allele 2 quencher
- CNVs, including the following information:
  - Name
  - Color
  - Test reporter
  - Test quencher
  - Reference reporter
  - Reference quencher
- Samples, including the following information:
  - Name
  - Color
  - Type
  - Quantity
  - Biogroup
- Biogroup, including the following information:
  - Name
  - Color

You can add items to the project. See the following sections for more information:

- “Define the project” on page 50
- “Import and export items for a project” on page 58

You can analyze the data from the **Project Summary** tab.

Overview of data analysis for a project

Primary analysis is performed individually for each data file in a project.

The primary analysis settings in a project are applied to a block type. Two block types in a project can have different primary analysis settings.

Secondary analysis is then performed together for the all of the data files in the project.

Replicates across different plates are not consolidated during secondary analysis. The replicates are analyzed separately.

Replicates within the same plate are consolidated during secondary analysis.

The secondary analysis modules that are applied to the project are defined in the project settings. The secondary analysis modules are defined by the block type (see “Update the project settings” on page 63).

Overview of the primary analysis settings for a project

When runs are added to the project that is based on the new project template and the run method is compatible, the primary analysis settings related to the run method are used. When runs are added to the project that is based on the new project template and the run method is not compatible, the stages and steps are automatically adjusted to the first stage and step that matches the run file.

An example is that the melt primary analysis settings are available for a project template because the run files that are added to a project might contain melt curve data.

When the run files are imported into the project, the applicable settings are used based on the run method. The settings that were defined in the project template that match the run method in the run files are used.

For example, if melt curve primary analysis settings were defined and the run file contains a melt curve in the run method, these settings are applied. If the run file does not contain a melt curve in the run method, these settings are not applied.

Sort or filter the projects

1. On the **Projects** page, use the checkboxes in the left pane to filter the projects.

   The following filter categories are available for projects:

   - Instrument
   - Block
   - Analysis

2. At the bottom of the left pane, click **Clear all** to clear the filters.

3. Click **(Search)** to access the search field, enter a search term, then press **Enter**.

4. Click **(Close)** to close the search field and remove the search criteria from the displayed projects.

5. Click **(List View)** to view the projects as a list.
6. In the list view, click a column header to sort by that parameter.
7. Click **(Grid View)** to view the projects as a grid.
8. In the grid view, click the dropdown lists in the top-right corner to sort by the parameter and in ascending order or descending order.
9. At the bottom-left of the page, navigate to a different page number.
   - Click the page number.
   - Click **(Back)** or **(Forward)** to navigate through the pages sequentially.
   - Enter a number in the **Go To Page** field, then click **Go**.
10. At the bottom-right of the page, in the **Show** dropdown list, select the number of projects that are displayed.

    The total number of projects is displayed beside the dropdown list.

Create new project

A new project must be created from a project template. For more information about project templates, see Chapter 4, "View and manage project templates".

1. On the **Project Templates** page, navigate to a project template.
   - **Recents** tab
   - **My Project Files** tab
   - **System Project Templates** tab
2. In the left pane, select the appropriate options to filter the project templates.
   - **Instrument**
   - **Block**
   - **Analysis**
3. Hover over the template, then click one of the following options.
   - **(More Options) ▸ Create New Project**
   - **(More Options) ▸ Create New Project in a New Window**

The new project is opened.

Define the project, if necessary ("Define the project" on page 50). The project might need to be defined if the items were not defined in the project template. The project might need to be defined in order to edit any items that were defined in the project template.

Add the run files to the project (see "Add a run to a project" on page 62).

Save the project (see "Save a project" on page 62).

View a project

A project must be opened in the software before it can be viewed from the **Projects** page.

1. In the **Projects** page, select the **Recents** tab.
2. Click the EDM file.

Open a project

Open a project if the project is not available in the **Recents** tab of the **Projects** page.

A project is in EDM file format.

If you open a file that was previously opened in the software, the original file is opened. A copy is not created in the software. This is based on the file name. The original file is retained even if it is saved in a different location on the computer. In order to create a copy, use the save as feature.

1. On the **Dashboard** page, click **Open File**.
2. Navigate to the location of the EDM file, select the EDM file, then click **Open**.

   All file formats can be opened from the **Dashboard** page. To open a project, ensure that you select an EDM file.

The project is opened with the list of run files displayed. The project can be viewed and edited as soon as it is opened.

The project is displayed in the **Recents** tab of the **Projects** page for future reference. However, it is not necessary close the project, then navigate to the **Projects** page in order to edit the newly opened project.

Define the project

The following information is defined in a project:

- Targets
- SNPs
- CNVs
- Samples
- Biogroups

The information can be added to a project template. The information is included in the project when a project is created from project template.

If a project is created from another project, the information is included in the new project.

The information is added when one or more runs are added to the project.

Unused items can be removed from the project. For example, if a target was included in the project based on the original project template but it is not used in any of the run files, it can be removed. This option is provided so that the user does not need to determine which items are unused. The project summary accurately reflects the items in the run files that are added to the project.

Define the targets for a project

The targets define the sequence regions that are detected by the assay.

When a run file is added to a project, the targets in the run file are added to the project summary.

A target that is defined in the project summary is available in the **Plate Setup** tab of the project to assign to a well.

Each new target is populated with a default name. Each target must have unique name.

Targets can be imported (see “Import targets to a project” on page 58).

Targets that were defined in the project template are included in the project that was created from the project template.

1. In an open project, select the **Project Summary** tab.
2. Select the **Targets** tab.
3. Click **+ Add Targets**.
   A new target is added. The new target contains default information.
4. Enter a target name in the **Name** field.
5. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
6. Select the reporter dye from the **Reporter** dropdown list.
7. Select the quencher dye from the **Quencher** dropdown list.
8. Click ⊗ **(Remove)** to remove a target from the project.

   IMPORTANT! Removing a target from the project summary removes it from the well assignments of the run files.
9. Click ··· **(More Options) ▸ Remove unused targets**.
   Removing unused targets allows you to have a project summary that matches the targets that are included in the run files within the project.

Define the SNPs for a project

Single nucleotide polymorphisms (SNPs) are used in the genotyping application. SNPs define the sequence variants that are detected by the assay.

When a run file is added to a project, SNPs in the run file are added to the project summary.

A SNP that is defined in the project summary is available in the **Plate Setup** tab of the project to assign to a well.

Each new SNP is populated with a default name. Each SNP must have unique name.

The allele 1 reporter dye and the allele 2 reporter dye cannot be the same dye.

If there is more than one SNP, the default is the same allele 1 reporter assigned to each SNP and the same allele 2 reporter assigned to each SNP. The reporters for each SNP can be edited.

SNPs can be imported (see “Import SNPs to a project” on page 59).

1. In an open project, select the **Project Summary** tab.
2. Select the **SNPs** tab.
3. Click + **Add SNPs**.
   A new SNP is added. The new SNP contains default information.
4. Enter an SNP name in the **Name** field.
5. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the SNPs in the plate layout view.
6. Select the reporter from the **Allele 1 reporter** dropdown list.
7. Select the quencher from the **Allele 1 quencher** dropdown list.
8. Select the reporter from the **Allele 2 reporter** dropdown list.
9. Select the quencher from the **Allele 2 quencher** dropdown list.
10. Click ⊗ **(Remove)** to remove an SNP from the project.

    IMPORTANT! Removing an SNP from the project summary removes it from the well assignments of the run files.

11. Click ··· **(More Options)** ▸ **Remove unused SNPs**.
    Removing unused SNPs allows you to have a project summary that matches the targets that are included in the run files within the project.

If there is no amplification or low amplification with the SNP assays that were defined, see *Diomni™ Design and Analysis (RUO) Software 3 Genotyping Analysis Module (project) User Guide* (Pub. No. MAN1000138).

Define the CNVs for a project

Copy number variations (CNVs) define the differences in the number of copies in a particular region of the genome between samples.

When a run file is added to a project, the CNVs in the run file are added to the project summary.

A CNV that is defined in the project summary is available in the **Plate Setup** tab of the project to assign to a well.

Each new CNV is populated with a default name. Each CNV must have unique name.

The test reporter dye and the reference reporter dye must be different.

CNVs can be imported (see “Import CNVs to a project” on page 60).

CNVs can be converted from SNPs or targets if the run was set up with an instrument software that does not include CNVs. A conversion can be included in the project template. See “Add the CNV assay conversion to the project” on page 54.

IMPORTANT! If the data are exported from Diomni™ Design and Analysis (RUO) Software 3 for import into AlleleTyper™ Software, the CNV name must end with ***_cn***.

For more information about conversion, see the *Diomni™ Design and Analysis (RUO) Software 3 Copy Number Variation Analysis Module (project) User Guide* (Pub. No. MAN0030169).

1. In an open project, select the **Project Summary** tab.
2. Select the **CNVs** tab.
3. Click **+ Add CNVs**.
   A new CNV is added. The new CNV contains default information.
4. Enter a CNV name in the **Name** field.
5. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
6. Select the reference reporter dye from the **Reference Reporter** dropdown list.
7. Select the reference quencher dye from the **Reference Quencher** dropdown list.
8. Select the test reporter dye from the **Test Reporter** dropdown list.
9. Select the test quencher dye from the **Test Quencher** dropdown list.

10. Click ⊗ **(Remove)** to remove a CNV from the project.

    IMPORTANT! Removing a CNV from the project summary removes it from the well assignments of the run files.

11. Click ··· **(More Options)** ▸ **Remove unused CNVs**.

    Removing unused CNVs allows you to have a project summary that matches the targets that are included in the run files within the project.

Add the CNV assay conversion to the project

If the project template contained a CNV assay conversion, the project that is created from the template contains the CNV assay conversion.

If the project template contains a CNV assay conversion, the CNV assay conversion can be updated for the project.

If the project template did not contain a CNV assay conversion, a CNV assay conversion can be added to the project. When the CNV assay conversion is included in the project, it is applied to the data files automatically when they are added to the project.

It is not necessary to add the CNV assay conversion to the project. The CNV assay conversion can also be added to an individual data file within a project. When the CNV assay conversion is added to an individual data file, the conversion rules are applied to all of the data files in the project.

The CNV assay conversion includes pre-defined conversion rules. The rules can be edited or deleted.

1. In the **Project Summary** tab of an open project an open project, select the **CNV** tab.
2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. In the **CNV Assay Converter** dialog box, click + **Add rule**.
   A new row is displayed in the **CNV Assay Converter** dialog box.
4. Enter the name of the target or SNP assay in the **Target / SNP Assay** field.
5. Enter the name of the CNV reference in the **CNV Reference Name** field.
6. Enter the name of the CNV assay in the **CNV Assay Name** field.
   The content of the fields in each row cannot be duplicated.
7. *(Optional)* Import rules.
   See “Import a CNV assay conversion file for a project template” on page 33.
8. Click ⊗ **(Delete)** in a row to delete a single rule.
9. Click ⊗ **(Delete)** in the table header to delete all of the rules.
10. Click **Apply**.

Import a CNV assay conversion file for a project

The file must be in CSV format.

The file must contain the following headers:

- **Target / SNP Assay**
- **CNV Reference Name**
- **CNV Assay Name**

The conversion does not overwrite any of the CNVs that were previously defined in the project. The CNVs that are defined in the CNV assay conversion file are added to the list of CNVs that are defined in the **CNVs** tab.

If a CNV with the same name was previously defined in the project, the CNV from the CNV assay conversion file is not added to the list of CNVs that are defined in the **CNVs** tab.

1. In the **Project Summary** tab of an open project template or an open project, click the **CNV** tab.
2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. In the **CNV Assay Converter** dialog box, click **Import**.
4. In the **Open** dialog box, navigate to the location of the file, then click **Open**.

The **CNV Assay Converter** is populated with the rules for conversion.

Add or remove rules from the rules that were imported, if necessary.

Click **Apply** to apply the imported rules.

Export a CNV assay conversion file from a project

The file is in CSV format.

The exported file can be imported to other project templates, projects, or plate setup files.

1. In the **Project Summary** tab of an open project, click the **CNV** tab.
2. Click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. In the **CNV Assay Converter** dialog box, click **Export**.
4. Navigate to the location to save the file, then edit the file name, if necessary.
5. Click **Save**.

Import an Assay Information File (AIF) to a project

An Assay Information File (AIF) is provided with every TaqMan™ assay order. An AIF does not include sample information.

An AIF can be applied to a project template (see “Import an Assay Information File (AIF) to a project template” on page 34). An AIF can be applied to an individual plate within a project (see “Import an Assay Information File (AIF)” on page 113).

Note: TaqMan™ Array Card plate setup requires AIF import, as targets cannot be added, assigned, or edited manually for TaqMan™ Array Cards.

Download the AIF for your order at **thermofisher.com/taqmanfiles**.

1. In the **Project Summary** tab of an open project, click **Import AIF**.
2. Navigate to, then select the previously downloaded AIF file.
3. Click **Open**.

The targets and SNP assays are added to the **Project Summary** tab.

In a project, the targets and SNP assays are not applied to a plate setup.

Define the samples for a project

Each new sample is populated with a default name. Each sample must have unique name.

When a run file is added to a project, the samples in the run file are added to the project summary.

A sample that is defined in the project summary is available in the **Plate Setup** tab of the project to assign to a well.

Samples can be added to a project if the same samples are included in multiple run files.

Samples can be imported (see “Import samples to a project” on page 61).

1. In an open project, select the **Project Summary** tab.
2. Select the **Samples** tab.
3. Click + **Add Samples**.
   A new sample is added. The new sample contains default information.
4. Enter a sample name in the **Name** field.
5. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the sample in the plate layout view.
6. Select the sample type from the **Type** dropdown list.
   The options for the sample type depend on the experiment type.

7. Select a biogroup from the **Biogroup** dropdown list.

   One or more biogroups must be defined in order for a biogroup to be available in the **Biogroup** dropdown list. For more information, see “Define the biogroups for a project” on page 57.

8. Click ⊗ **(Remove)** to remove a sample from the project.

   IMPORTANT! Removing a sample from the project summary removes it from the well assignments of the run files.

9. Click ··· **(More Options)** ▸ **Remove unused samples**.

   Removing unused samples allows you to have a project summary that matches the targets that are included in the run files within the project.

Define the biogroups for a project

Biogroups, or biological replicate groups, are reactions that contain identical components and volumes, but evaluate separate samples of the same biological source. A biogroup is used to categorize samples. A biogroup applies mainly to relative quantification analysis.

Each new biogroup is populated with a default name. Each biogroup must have unique name.

When a run file is added to a project, the biogroups in the run file are added to the project summary.

A biogroup that is defined in the project summary is available in the **Plate Setup** tab of the project to assign to a sample.

1. In an open project, select the **Project Summary** tab.
2. Select the **Biogroup** tab.
3. Click + **Add Biogroup**.

   A new biogroup is added. The new biogroup contains default information.

4. Enter a biogroup name in the **Name** field.
5. Click the color to open the color picker, then select a color.

   The color is not related to the dye. It is to visualize the biogroup in the plate layout view.

6. Click ⊗ **(Remove)** to remove a biogroup from the project.

   IMPORTANT! Removing a biogroup from the project summary removes it from the sample assignments of the run files.

After a biogroup has been defined for a project, a sample can be assigned to the biogroup (see “Define the samples for a project” on page 56).

Import and export items for a project

Import targets to a project

The file must be in CSV format.

If the target is already defined in the project and is included in the imported file, the target is not duplicated in the project. A target is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color (in red, green, blue color model format or hexadecimal code)
- Reporter
- Quencher

Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project, select the **Project Summary** tab.
2. Select the **Targets** tab.
3. Click ··· **(More Options)** ▸ **Import Targets Assays**.
4. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The targets from the imported file are added to the project.

Export targets from a project

Exported targets can be imported to another project template or another project.

1. In an open project, select the **Project Summary** tab.
2. Select the **Targets** tab.
3. Click ··· **(More Options)** ▸ **Export Targets Assays**.
4. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import SNPs to a project

The file must be in CSV format.

If the SNP is already defined in the project and is included in the imported file, the SNP is not duplicated in the project template. An SNP is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color
- Allele 1 Name
- Allele 1 Color
- Allele 1 Reporter
- Allele 1 Quencher
- Allele 2 Name
- Allele 2 Color
- Allele 2 Reporter
- Allele 2 Quencher
- Assay ID
- Gene Symbol
- Gene Name
- NCBI SNP Reference
- Context Sequence
- Comments

The allele 1 name, allele 2 name, assay ID, gene symbol, gene name, NCBI SNP reference, context sequence, and comments fields are optional.

Colors are in red, green, blue color model format or hexadecimal code

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Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project, select the **Project Summary** tab.
2. Select the **SNPs** tab.
3. Click ··· **(More Options)** ▸ **Import SNPs Assays**.
4. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The SNPs from the imported file are added to the project.

Export SNPs from a project

Exported SNPs can be imported to another project template or another project.

1. In an open project, select the **Project Summary** tab.
2. Select the **SNPs** tab.
3. Click ··· **(More Options)** ▸ **Export SNPs Assays**.
4. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import CNVs to a project

The file must be in CSV format.

If the CNV is already defined in the project and is included in the imported file, the CNV is not duplicated in the project template. A CNV is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color
- Test Name
- Test Color
- Test Reporter
- Test Quencher
- Reference Name
- Reference Color
- Reference Reporter
- Reference Quencher
- Assay ID
- Comments

The assay ID and comments fields are optional.

Colors are in red, green, blue color model format or hexadecimal code

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Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

---

1. In an open project, select the **Project Summary** tab.
2. Select the **CNVs** tab.
3. Click ··· **(More Options)** ▸ **Import CNVs Assays**.
4. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The CNVs from the imported file are added to the project.

Export CNVs from a project

Exported CNVs can be imported to another project template or another project.

1. In an open project, select the **Project Summary** tab.
2. Select the **CNVs** tab.
3. Click ··· **(More Options)** ▸ **Export CNVs Assays**.
4. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Import samples to a project

The file must be in CSV format.

If the sample is already defined in the project and is included in the imported file, the sample is not duplicated in the project template. A sample is identified as a duplicate based on the name.

The file must contain the following columns:

- Name
- Color (in red, green, blue color model format or hexadecimal code)
- Quantity
- Biogroup

Note: A color does not need to be defined. If the field in blank in the file, the software applies a default color.

The quantity and biogroup fields are optional.

1. In an open project, select the **Project Summary** tab.
2. Select the **Samples** tab.
3. Click ··· **(More Options)** ▸ **Import Samples Assays**.
4. In the **Open** dialog box, navigate to the file location, select the file, then click **Open**.

The samples from the imported file are added to the project.

Export samples from a project

Exported samples can be imported to another project template or another project.

1. In an open project, select the **Project Summary** tab.
2. Select the **Samples** tab.
3. Click ··· **(More Options)** ▸ **Export Samples Assays**.
4. In the **Save As** dialog box, navigate to the location to save the file, edit the default file name, then click **Save**.

The file is saved in CSV format.

Add a run to a project

A run can be added to a project only one time.

A run that is added to a project does not become available as a recent single plate file.

The instrument and block type are defined in the project settings. The run file must be from the instrument and the block type that are defined in the project settings.

The secondary analysis settings that are applied to the run file are defined in the project settings (see “Update the project settings” on page 63).

The rules for run files that can be added to the project are defined in the project settings. For more information, see “Update the project settings” on page 63.

1. Click + **Add run**.
2. Navigate to the location of the data file, select the data file, then click **Open**.
   Use control-click or shift-click to select multiple data files.

The run file or the run files are listed in the **Run Files** tab of the project.

Delete a run from a project

When the run is deleted from the project, the run file is also deleted from the software.

Open or view a project. See “Open a project” on page 50 or “View a project” on page 50.

1. Select the **Run Files** tab for the project.
2. Delete the run or the runs from the project.
   - Click a row, then click **Delete**.
   - Control-click to select multiple rows, then click **Delete**.

   The **Delete** button is above the table.
3. In the **Confirmation** dialog box, click **OK**.

Reanalyze the data.

Save a project

Open or view a project. See “Open a project” on page 50 or “View a project” on page 50.
Click **Actions** ▸ **Save**.

Save a copy of a project

Open or view a project. See “Open a project” on page 50 or “View a project” on page 50.

1. Click **Actions** ▸ **Save As**.
2. In the **Save As** dialog box, enter a file name in the **File Name** field.
   The **File Name** field is populated with the original file name appended with ***Copy***.
3. Click **Browse** to change the location to save the file.
4. Click **Save**.

The open project is the copy that was saved.

View the project information

The comments are included in the metadata fields when the project data are exported (see “Export data (project)” on page 171).

Open or view a project. See “Open a project” on page 50 or “View a project” on page 50.

1. Click **Actions** ▸ **Project information**.
2. In the **Project information** dialog box, enter information in the **Comment** field.
3. Click **Save**.

View a project summary

For information about the project summary, see “Overview of the project summary” on page 46.

In an open project, click the **Project Summary** tab.

Update the project settings (see “Update the project settings” on page 63) or define the project (see “Define the project” on page 50).

Update the project settings

1. In an open project, click **Actions** ▸ **Project Setting**.
2. In the **Project Setting** dialog box, select one of the following checkboxes in the **Settings** section.
   - **Strict run method compatibility**
   - **Check for conflicting assay**

   The **Strict run method compatibility** setting can be edited only if run files have not been added to the project or if only one run file has been added to the project.

For more information, see “Overview of run method compatibility for a project” on page 65 and “Overview of conflicting assays for a project” on page 65.

3. In the **Instrument Type** dropdown list, select an instrument.
   The instrument type cannot be changed if run files have been added to the project.

4. In the **Block Type** pane, in the **Block Type** dropdown list, select a block type.
   Only the block types that are compatible with the instrument can be selected.

5. In the **Block Type** pane, in the **Analysis Module** dropdown list, select an analysis module.
   The analysis module determines how the data are analyzed. The project settings determine how the run files that are added to the project are analyzed.
   If a secondary analysis module is selected, primary analysis is performed in addition to the secondary analysis. If primary analysis is selected, only primary analysis is performed.
   Select **No analysis module** for only primary analysis.

6. Click ＋ **(Add)** to add a block type.
   A maximum of two block types can be included in each project.
   An analysis module can be applied to only one block type. Two different block types cannot have the same analysis module.
   The block types that are defined in the project settings must match the block types in the run files that are included in the project.

7. Repeat step 4 and step 5 for the block that was added.

8. Click ⊗ **(Remove)** to remove a block type.

9. *(Optional)* Click **Reset** to cancel the changes that were made.
   The values are reset to the default settings for the project.

10. Click **Save**.

Overview of run method compatibility for a project

The **Strict run method compatibility** checkbox is selected by default.

The **Strict run method compatibility** checkbox can be edited only if run files have not been added to the project or if only one run file has been added to the project.

In order to change this setting if there is more than one run file in the project, you can delete the run files. Update the setting, then add the run files back to the project.

Parameter	Required for strict run method compatibility	Required if strict run method compatibility is not set
Sample volume must be the same	Yes	Yes
Run mode must be the same (fast or standard)	Yes	No
The total number of steps must be the same	Yes	Yes
Each step must be within the same type of stage (PCR, hold)	Yes	Yes
Each step must have the same ramp rate, temperature, time	Yes	No
Each step must have the same status for data collection	Yes	Yes
No VeriFlex™ Zones are Auto Delta settings are applied	Yes	No
The same number of cycles in each PCR stage	Yes	Yes
The filter settings must be the same[1]	Yes	No

[1] See "Confirm or edit filter settings" on page 77.

Overview of conflicting assays for a project

The **Check for conflicting assay** checkbox is deselected by default.

Selecting the **Check for conflicting assay** checkbox increases the time that is required to add run files to the project.

This setting checks that the targets, SNPs, and CNVs are assigned the same dyes.

For example, if FAM™ dye is the reporter dye for one target in one run file and VIC™ dye is the reporter dye for the same target in a second run file, the second run file cannot be added to the project.

The **Check for conflicting assay** checkbox can be updated regardless of the number of run files that have been added to the project. This setting does not affect the primary analysis settings.

If the setting is updated after run files have been added, it does not retroactively apply this setting to the existing run files. The updated setting is applied only to any run files that are added after the update is made.

Project setup	Validation
Includes one or more run files **Note:** The plate setup information is populated from the run files.	The software uses the existing run file to check for conflicting assays. The setting is not applied retroactively if it is enabled after run files have been added.
• Includes plate setup information • No run files	The software uses the plate setup to check for conflicting assays.
• No plate setup information • No run files	Multiple run files can be imported at the same time. One run file is selected by the software are used as the baseline to check for conflicting assays.

Edit the primary analysis settings for a project

View or edit C_q settings for a project

- The default C_q settings are appropriate for most applications. Edit the threshold and baseline settings for analysis of atypical or unexpected run data.
- For information about C_q analysis, see “About the quantification cycle (Cq)” on page 201.
- For information about C_q settings, see “Cq settings overview” on page 202.
- The **PCR Stage/Step** dropdown list is available when defining primary analysis settings for a project. It is not available when defining primary analysis settings for a project template. For more information, see “Overview of primary analysis settings for a project template” on page 25.

The following instructions apply to the **General** tab and the **Well Cq** tab of the **Primary Analysis Setting** dialog box.

1. Open a project, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **General** tab, select an option from the **PCR Stage/Step** dropdown list.
3. In the **Primary Analysis Setting** dialog box, in the **Block Type** dropdown list, select a block type.
4. In the **General** tab, select an option from the **Algorithm Settings** dropdown list.
   - **Relative Threshold**
   - **Baseline Threshold**
5. *(For relative threshold algorithm settings)* Select the PCR stage and step from the **PCR Stage/Step** dropdown list.
6. *(For relative threshold algorithm settings)* Enter a start cycle in the **Default C_{RT} Start Cycle** field.

7. *(For baseline threshold algorithm settings)* To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.

8. *(For baseline threshold algorithm settings)* To edit the settings, make the edits in the appropriate table row.

Option	Action
Use Auto Threshold	Select the checkbox in the **Auto Threshold** column.
Manually set the Threshold	Deselect the checkbox in the **Auto Threshold** column, then edit the value in the **Threshold** column.
Use Auto Baseline	Select the checkbox in the **Auto Baseline** column. • To specify the Baseline Start cycle, Click **AUTO** in the **Baseline Start** column, then enter the cycle number. The software will automatically determine the Baseline end cycle. • To remove the specified Baseline Start cycle, click the cycle number, then delete it. The **Baseline Start** will revert back to **AUTO**.
Manually set the Baseline	Deselect the checkbox in the **Auto Baseline** column, then edit the values in the **Baseline Start** field and the **Baseline End** field.

9. (*Optional*) In the **Well Cq** tab, make the edits in the appropriate table row to apply custom C_q settings to a specific well.

10. Click **Save**.

11. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit melt analysis settings for a project

For descriptions of the melt analysis settings, see “Melt analysis settings overview” on page 203.

1. Open a project, then click **Actions** ▸ **Primary Analysis Setting**.

2. In the **Melt** tab, select an option from the **Melt Stage/Step** dropdown list.

3. In the **Multi-Peak Calling** column, select the checkbox.

   The threshold type, peak level, and peak height settings are available only when multi-peak calling is enabled.

4. In the **Threshold Type** column, select one of the following options.

   - **Percentage**
   - **Height**

5. In the **Peak Level (%)** column, enter a value.

   A value can be entered only if **Percentage** was selected in step 4.

6. In the **Peak Height** column, enter a value.

   A value can be entered only if **Height** was selected in step 4.

7. *(Optional)* In the **Melt Peak Parity Factor** field, enter a factor for the applicable targets.

   Note: If the melt peak parity factor is applied to wells without a reaction mix, this can lead to unexpected T_m results.

   A factor of ***0*** turns off the feature. Negative values are not permitted.

   The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.

8. Click **Save**.

9. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit QC alerts setting for a project

1. Open a project, then click **Actions** ▸ **Primary Analysis Setting**.

2. In the **QC Alerts** tab, review selections:

   - Curve Quality—(*default*) inactive
   - Results Quality—(*default*) active

   For more information about curve quality and results quality, see “Quality checks” on page 204.

3. (*Optional*) Select the **Curve Quality** checkbox.

4. (*Optional*) Set up the acceptance criteria for the result quality checks.

   a. Ensure that the **Results Quality** checkbox is selected.

   b. Select **Results Quality**.

   c. Click **+ (Add)**.

   d. Select the **Sample Type** from the dropdown list, then select the **Target** from the dropdown list.

      If a rule is set up for a specific sample type, it takes precedence over a rule that is set for all samples.

   e. In the right panel, select the acceptance criteria for each sample type and target combination (see “Acceptance criteria for result quality checks” on page 205).

   f. (*Optional*) Click **✕ (Remove)** to remove a sample type and target combination from the table.

5. Click **Save**.

6. (*Optional*) To reset to the default settings, click **Reset to Default**.

In the **Quality Check** tab, click **Analyze**, then review the QC alerts in the **Well Table**.

View or edit advanced settings for a project

1. Open a project template, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Advanced** tab, select and/or enter the following, then click **Save**
   - **Use a variant of primary analysis algorithm via plugin**
   - **Set the Delta-Rn below which curves will be considered Non-Amplified**

   Any curves with the ΔR_n below the threshold are set to non-amplified. This is regardless of whether there is a C_q value.
3. Click **Save**.
4. (*Optional*) To reset to the default settings, click **Reset to Default**.

Reset primary analysis settings for a project to the default settings

The settings are reverted to the system default settings.

1. Open a project, then click **Actions** ▸ **Primary Analysis Setting**.
2. Click **Reset to Default**.

Overview of the use of different analysis settings for a project

The software allows you to apply analysis settings from a different project template or project to a specific project.

This feature allows you to try different analysis settings quickly.

This feature means that you do not need to manually update analysis settings in order to optimize the project.

The instrument, block type, samples, and targets or SNP assays must all be the same in order to apply analysis settings from a different project template or project to a current project.

Use the settings from a project template or a project

The primary analysis settings from the selected project template or project are applied to the open project.

1. In an open project, click **Actions** ▸ **Use settings from another EDM/EDMT**.
2. Navigate to the file location, then click **Open File**.

6 Set up a plate file

Select a system template or existing plate file to set up a new plate file

Plate files cannot be created for the OpenArray™ Plate format with the Diomni™ Design and Analysis (RUO) Software 3. For information about a workflow for the OpenArray™ Plate format, see “Files for the OpenArray™ Plate format” on page 17.

A limited number of items in the data files for the OpenArray™ Plate format can be edited. It is noted in each section if this format can be edited.

A new plate file must be created from a system template or a previously created plate file. For more information about system templates and plate files, see “Overview of system templates and plate files” on page 180.

IMPORTANT! You must select a system template or a plate file that corresponds to your instrument, block, and run mode. These properties are not editable once the plate file has been created.

Not all of the variations of the EDT files and EDS files are provided in the galleries with the software installer.

1. Click **Run templates**.
   The **Plate Gallery** is displayed.

2. In the left pane, select the appropriate options to filter the system template and plate file lists.
   - **Instrument**
   - **Block**
   - **Run Mode**
   - **Analysis**

   Note: Thermal protocol, plate setup, and post-run analysis options are independent of analysis module selection. Analysis module selection can be changed at any point during plate file set or post-run analysis (see “Select an analysis module (single plate)” on page 191).

3. Navigate to appropriate **Plate Gallery** tab.

Tab	Description
Recents	Contains plate files that were recently opened. Recently opened plate files from **System Templates** and **My Plate Files** do not populate this tab. Click a plate file to open it. The plate file can be edited, then saved, or saved as a new plate file.
My Plate Files	Contains plate files that were previously saved to the **My Plate Files** tab. Click a plate file to open it. The plate file can be edited, then saved, or saved as a new plate file.
System Templates	Contains system templates, non-editable plate files that are included with the software. Click a system template to automatically generate a new plate file that can be edited, then saved.

Note: Click 🔍 to search for a plate file based on a tag (see "Edit plate file or data file information" on page 100).

4. Select a system template or plate file to open. To view all options for opening the plate file, hover over the plate file, then click ··· **(More Options)**.

The plate file opens in the **Run Method** tab.

Confirm or edit run method

Overview of the run method

The run method cannot be edited in a data file (post-run file).

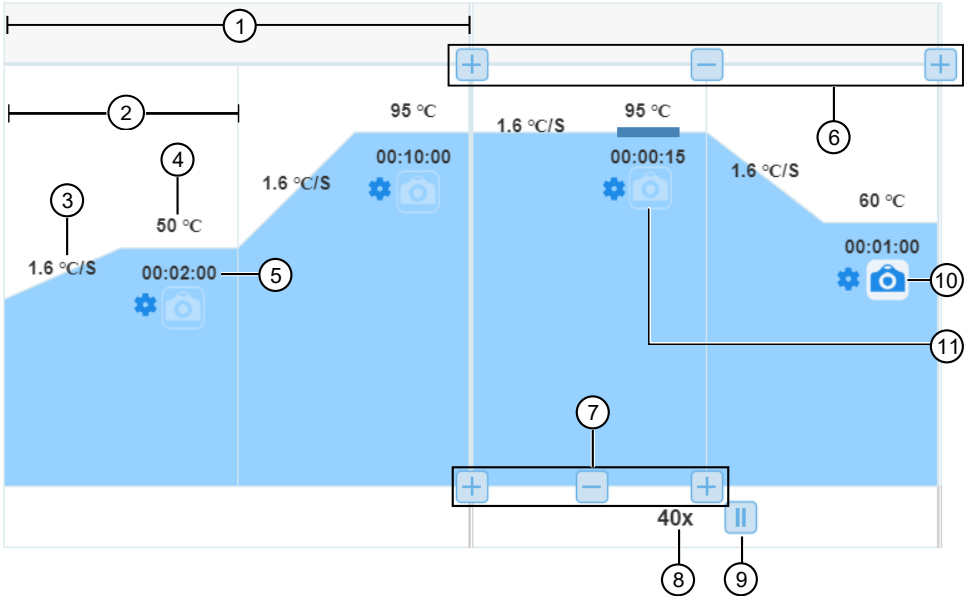
A run method has the following requirements:

- The run method requires at least one step.
- An infinite hold can be added but it must be at the end of the run.
- A run method can have only one pre-read stage and one post-read stage. If the run method contains both a pre-read stage and post-read stage, the pre-read stage must be before the post-read stage.
- Only one of an Auto Delta or a VeriFlex™ Zone can be added. These items cannot both be present in the run method.

A minimum hold time is calculated based on the minimum exposure time for each filter that is used.

Run method elements

Edit run method elements in the **Run Method** tab.

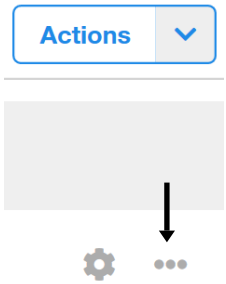


① Stage
② Step within a stage
③ Temperature ramp rate of a step
④ Temperature of a step
⑤ Time length of a step
⑥ Add/remove stage
⑦ Add/remove step
⑧ Number of PCR cycles
⑨ PCR stage pause cycle
⑩ Data collection on
⑪ Data collection off

Apply the recommended run method for your master mix

Override the current run method with the run method that we recommend for use with your master mix, instrument, block, and run mode. The recommended run method can differ depending on the application being used. We recommend that you confirm the run method in the master mix user guide.

1. On the right side of the **Run Method** tab, click ··· **(More Options)** ▸ **Select Master Mix**.



2. Search for your master mix by name or catalog number, or select a master mix from the list.
3. (*Optional*) To add the master mix to the reagents table in the **Plate Setup** tab, select **Append selected master mix to reagent table**.
   The master mix is added to the Reagent Table in the **Plate Setup** tab (see “Edit reagent information” on page 97).
4. Click **Apply**.

The run method is updated to the recommended run method for the selected master mix.

Edit temperature ramp rate, temperature, and time length for a step

1. In the **Run Method** tab, in the step of interest, click the temperature ramp rate, temperature, or time length element.
   For more information about elements in the run method, see “Run method elements” on page 72.
2. Enter the value, then click outside the element to stop editing.
   Each filter set has a minimum exposure time. A warning is displayed if the time is shorter than the minimum exposure time.

Add or remove a step

1. In the **Run Method** tab, hover over the stage for which you want to edit steps to view the + and − buttons.
   - The buttons at the top of the stage control adding and removing stages.
   - The buttons at the bottom of the stage control adding and removing steps.
   - For more information about elements in the run method, see “Run method elements” on page 72.
2. At the insert location, click + at the bottom of the stage.

3. (*Optional*) Edit the temperature ramp rate, temperature, or time length of the new step (see "Edit temperature ramp rate, temperature, and time length for a step" on page 73).

4. To remove a step, click − at the bottom of the step.

Add or remove a stage

1. In the **Run Method** tab, hover over where you want to insert the stage to view the + and − buttons.
   - The buttons at the top of the stage control adding and removing stages.
   - The buttons at the bottom of the stage control adding and removing steps.
   - For more information about elements in the run method, see "Run method elements" on page 72.

2. At the insert location, click + at the top of the stages.

3. Select the type of stage from the list.

Option	Description
Hold	• Multiple hold stages can be added.
Pre-Read	• Only one pre-read stage can be added. • A pre-read stage can only be added to the beginning of the run method.
Post-Read	• Only one post-read stage can be added. • A post-read stage can only be added after the final PCR stage of the run method.
Infinite	• Only one infinite hold stage can be added. • An infinite hold stage can only be added to the end of the run method.
PCR	• Multiple PCR stages can be added.
Melt Curve	• Multiple melt curve stages can be added.

4. (*Optional*) Edit the temperature ramp rate, temperature, or time length for steps of the new stage (see "Edit temperature ramp rate, temperature, and time length for a step" on page 73).

5. To remove a stage, hover of the stage of interest, then click − at the top of the stage.

Turn data collection on or off

The data collection status can affect whether a data file can be added to a project. See "Overview of run method compatibility for a project template" on page 40 and "Overview of run method compatibility for a project" on page 65.

In the **Run Method** tab, click the camera icon for the stages and steps where data is collected.

See "Run method elements" on page 72.

If more than one data collection point is selected, ensure that the primary analysis settings are set up to use the correct step and stage for the baseline analysis. See “View or edit Cq settings” on page 144 and “View or edit Cq settings” on page 164.

Add, edit, or remove a pause cycle in a PCR stage

1. In a legacy template, in the **Run Method** tab, click ⏸ at the bottom of the PCR stage.
   For more information about elements in the run method, see “Run method elements” on page 72.
2. To add a pause to the PCR stage, select **Pause Cycle**.
3. To edit a pause, enter a pause temperature between 4°C and 99.9°C.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the plate temperature can reach 100°C. To access the plate during a run pause, enter room temperature as the pause temperature and allow the plate to cool to room temperature before handling.

4. Enter the appropriate cycle after which the pause will occur.
5. Click **Save**.
6. (*Optional*) To remove a pause, click ⏸, then deselect **Pause Cycle**.

Add, edit, or remove a pause cycle in a PCR step

1. In a template, in the **Run Method** tab, click ⚙ **(Advanced Setting)** in a step.

   Note: Any changes apply only to the step in which you clicked.

2. In the **Pause Setting** tab, select **Pause Cycle**.

   Note: **Pause Setting** is not available for the melt curve dissociation step.

3. In the **Pause Temperature** field, enter a pause temperature between 4°C and 99.9°C.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the plate temperature can reach 100°C. To access the plate during a run pause, enter room temperature as the pause temperature and allow the plate to cool to room temperature before handling.

4. In the **Pause After Cycle** field, enter the appropriate cycle after which the pause will occur.
5. Click **Save**.
   A PCR step with a pause cycle applied to it is denoted with ⏸ in the top-left corner of the step.
6. (*Optional*) To remove a pause cycle, deselect **Pause Cycle**.

Edit the ramp increment for the melt curve dissociation step

1. In the **Run Method** tab, in the **Melt** stage, click the name of the ramp increment method, then select one of the following options:

Option	Description
Continuous (default)	Continuously increases the temperature by the ramp increment (°C/sec).
Step and Hold	Increases the temperature by the ramp increment (°C), then holds at that temperature for the specified time.
No. of Data Points per Degree	Increases the temperature by the ramp increment (°C) and collects the specified number of data points per degree increased.

2. Edit the temperature ramp increment.
   a. Click the ramp increment element in the **Dissociation** step.
   b. Enter a value or use the up/down arrows (default is 0.15°C/s).
3. (*Step and Hold only*) Edit the hold time after each temperature increase.
   a. Click the time field next to **Step and Hold**.
   b. Enter a value or use the up/down arrows (default is 5 seconds).
4. (*No. of Data Points per Degree only*) Edit the number of data points to be collected with each degree increase.
   a. Click the number of data points element in the **Dissociation** step.
   b. Enter a value or use the up/down arrows (default is 10 data points).

Enable, edit, or disable Auto Delta

Auto Delta enables the incremental increase or decrease of the temperature or hold time for a step in a PCR stage.

Not all instruments support Auto Delta. For specific information about Auto Delta, see the instrument documentation.

1. In the **Run Method** tab, click **(Advanced Setting)** in a step.

   Note: Any changes apply only to the step in which you clicked.

2. In the **Auto Delta Settings** tab, select **Enable Auto delta**.
3. In the **Temperature Delta** field, enter the numerical difference in the temperature. The software indicates the appropriate range.
4. In the **Time Delta** field, enter the numerical difference in the time. The software indicates the appropriate range.
5. In the **Starting Cycle** field, enter the first cycle to which you want Auto Delta settings to apply.

6. Click **Save**.
   A PCR step with an Auto Delta setting applied to it is denoted with A.

7. (*Optional*) To remove Auto Delta, deselect **Enable Auto delta**.

Enable, edit, or disable VeriFlex™ zones

VeriFlex™ Zones enable independent temperature zones ≤5°C of adjacent zones.

- The number of VeriFlex™ zones depends on the instrument. For specific information about VeriFlex™ zones, see the instrument documentation.
- VeriFlex™ zones are only available for 96-well blocks.

1. In the **Run Method** tab, click **(Advanced Setting)** in a step.

   Note: Any changes apply only to the step in which you clicked.

2. In the **VeriFlex™ Zones** tab, select **Enable VeriFlex™**.

3. In the **Adjusted Temperature (°C)** fields, enter the adjusted temperature.

4. Click **Save**.
   A step with VeriFlex™ Zones applied to it is denoted with V.

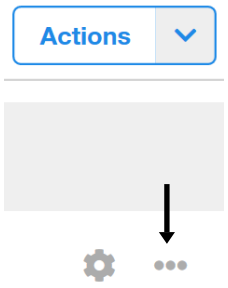
5. (*Optional*) To remove VeriFlex™ Zones, deselect **Enable VeriFlex™**.

Confirm or edit filter settings

The need to edit optical filter settings is rare, and it is for advanced or custom uses only. For more information about instrument supported dyes and their calibration and optical filter selection, see the instrument documentation.

Use the optical filters settings to select a filter set to match the profile of a custom dye.

1. On the right side of the **Run Method** tab, click **(More Options)** ▸ **Filter Settings**.



   The excitation (x) and emission (m) wavelengths that correspond to each filter are shown on the screen.

2. Select the checkboxes to enable or disable filters.

   IMPORTANT! If you select the wrong filters, you cannot correct the selection and retrieve data after a run has been completed.

3. Click **Save**.

Confirm or edit plate setup

Note: The table view is not available for the OpenArray™ Plate format. The table view is noted by the icon.

Edit the view

Edit the grid view

The grid view is displayed in the **Plate Setup** tab. It matches the plate layout of a physical plate.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click **(Grid View)**.

1. In the top-right corner of the plate layout pane, click **(Zoom In)** and **(Zoom Out)**.
2. Click the percentage value, then use the slider to select a scaling based on a numerical value.
3. Click **(Reset)** to reset the zoom to fit the screen width.

4. Click ⚙ **(Settings)**, the select the following options from the dialog box.

Section	Option
Show	• **Sample Color** • **Target Color** or **SNP Assay Color** Select one, none, or all of the options.
Tab/Auto Fill Direction	• **Horizontal** • **Vertical** Select one option. This option selects the direction of the next well that is selected when **Tab** or **Enter** is pressed.
Dock Table	• **To Right** • **To Bottom** Select one option. This option selects whether the tables are displayed on the right of the screen or on the bottom of the screen. The tables include the **Samples** table, the **Targets** table, and the **SNP Assays** table.

5. If any of the wells display an invalid setup, hover over the warning icon to view the reason.

Edit the table view

The table view is displayed in the **Plate Setup** tab.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click ☰ **(Table View)**.

1. In the top-right corner, click **View**.
2. Select or deselect the checkbox associated with each item to display in the table view.

Table	Options
Target	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Target Name** checkbox • **Target Reporter** checkbox • **Target Quencher** checkbox • **Task** checkbox • **Quantity** checkbox
SNP	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Assay Name** checkbox • **Allele 1 Name** • **Allele 1 Reporter** checkbox • **Allele 1 Quencher** checkbox • **Allele 2 Name** • **Allele 2 Reporter** checkbox • **Allele 2 Quencher** checkbox • **Task** checkbox

(continued)

Table	Options
CNV	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Assay Name** checkbox • **Reference Name** • **Reference Reporter** checkbox • **Reference Quencher** checkbox • **Test Name** • **Test Reporter** checkbox • **Test Quencher** checkbox • **Task** checkbox

3. Click ⚙ **(Settings)**, the select the following options from the **Dock Table** dialog box.

   - **To Right** radio button
   - **To Bottom** radio button

   This setting selects whether the tables are displayed on the right of the screen or on the bottom of the screen.

   The tables include the **Samples** table, the **Targets** table, the **SNP Assays** table, and the **CNV Assays** table.

Select plate wells or ports

- Select plate wells or ports in the ⠿ **(Grid View)**.

Note: Selecting ports is only available for TaqMan™ Array Card plate setup.

To	Action
Select a single well	Click a well in the plate
Select multiple wells	Click-drag in the plate
Select contiguous wells	Shift-click wells in the plate
Select non-contiguous wells	PC: Ctrl-click wells in the plate Mac: Cmd-click wells in the plate
Select a column of wells	Click a column header
Select all wells	Click the top-left corner of the plate grid
Select a block of wells	Click a well to define a corner, then shift-click another well on the opposite corner
Select a single port[1]	Click a cell in the **Port** column

(continued)

To	Action
Select multiple ports[1]	Click-drag in the **Port** column
Select all ports[1]	Click the **Port** column header

[1] For TaqMan™ Array Cards only.

- Select plate wells in the ☰ **(Table View)**.

  This view is not available for the OpenArray™ Plate format.

To	Action
Select a single well	Click a row in the table
Select contiguous wells	Shift-click rows in the table
Select non-contiguous wells	PC: Ctrl-click rows in the table Mac: Cmd-click rows in the table
Deselect a single well	PC: Ctrl-click the selected row Mac: Cmd-click the selected row

Add samples and assign to plate wells

Each well can contain only one sample.

Import a plate setup file (samples)

Import a plate setup file that was previously exported from the software (see “Export a plate setup file” on page 102), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

A sample layout can be imported for the OpenArray™ Plate format. Targets or SNP assays cannot be imported for an OpenArray™ Plate format.

For all of the other formats, the plate setup file can include both samples and targets or SNP assays.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Import Plate Setup**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Import Sample Layout**.

2. Navigate to, then select the file.
3. Click **OK** to confirm that the plate setup is overwritten.

Copy samples from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **Samples** table, click ··· **(More Options)** ▸ **Copy all samples**.

The information is available in the clipboard. It can be pasted into another program for reference.

Paste samples into the plate layout

You can copy samples from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **Samples** table, click ··· **(More Options)** ▸ **Paste samples**.

The samples are copied to the **Samples** table. They must be assigned to the plate layout (see “Manually add or assign a sample to a well or port” on page 84.

Remove unused samples

The samples that were not assigned to a well can be removed from the **Samples** table.

In the **Samples** table, click ··· **(More Options)** ▸ **Remove unused samples**.

Manually add samples to the Samples table

Each sample name in the **Samples** table must be unique. Do not add multiple entries for technical replicates.

Adding a single sample is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, select an option in the upper right corner of the **Samples** table.

Option	Description
Add a single sample	Click + **(Add)**.
Copy/paste multiple samples	a. Copy the sample information from one of the following sources: • Previously created plate file or data file—Click ··· **(More Options)** ▸ **Copy all Samples** in the upper-right corner of **Samples** table. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] b. Click ··· **(More Options)** ▸ **Paste Samples**.

[1] Column headers must match the column headers in the **Samples** table.

Note:
- Click ··· **(More Options)** ▸ **Export Samples** to export samples.
- Click ··· **(More Options)** ▸ **Import Samples** to import samples.

2. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 85).

Note: The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

3. To remove a sample from the table, click ✕ **(Remove)** in the last column.

Manually add or assign a sample to a well or port

Sample assignment by port is available only for TaqMan™ Array Card plate setup.

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, perform one of the following actions.
   - Select one or more wells in the **(Grid View)** or in the **(Table View)**.
   - Select one or more ports in the **(Grid View)**.

2. Assign a sample to the selected well or port.

Note: The user can only assign up to eight samples by port in the **(Grid View)** for TaqMan™ Array Card plate setup.

Option	Description
(Grid View)	
Sample not defined	Enter the sample name in the text field.
Sample previously defined	• Start typing the sample name, then select the sample name from the autocomplete list. • Select the checkbox of the sample in the **Samples** table.
(Table View)	
Sample previously defined	Select the checkbox of the sample in the **Samples** table.

3. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 85).

Note: The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

Edit sample name, color, and type

The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

The sample name, color, and type can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click the sample name in the **Name** column.
2. Enter a new name, then press **Enter**.
3. Click the sample color in the **Color** column, then select a color from the color picker.
4. Select a sample type from the **Type** column dropdown list.
   - **Unknown** (default)
   - **Standard**

     Note: A standard sample requires that you enter a value in the **Quantity** column of the **Samples** table. The software uses this value to populate the **Quantity** field for standard target tasks in the **Targets** table.
   - **Negative Control**
   - **Positive Control**
   - **Positive 1/1**—A sample that is homozygous for allele 1.
   - **Positive 2/2**—A sample that is homozygous for allele 2.
   - **Positive 1/2**—A sample that is heterozygous for allele 1 and 2.

IMPORTANT! Editing the sample type after a run can affect the validity of the plate setup.

Add a custom attribute to samples

Custom attributes can be added for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click ··· **(More Options)** ▸ **Add Custom Attribute**.
2. In the **Add Custom Attribute** window, enter the custom attribute name, then click **Done**.
   A column for the custom attribute is added to **Samples** table, and a new tab for the custom attribute is created.
3. Select an option to define the choices for the custom attribute:

Option	Description
Define in the **Samples** table	In the **Samples** table, define the custom attribute in the appropriate field in the custom attribute column.
Define in the custom attribute tab	a. In the custom attribute tab, click + **(Add)**. b. In the table, click in the field to edit the custom attribute name.

   The custom attribute options are added to the following locations:
   - **Samples** table, in the dropdown list in the custom attribute column
   - Table in the custom attribute tab
4. In the **Samples** table, for each sample, select the custom attribute from the dropdown list.
5. (*Optional*) Edit the custom attribute name or color.
   a. Select the custom attribute tab.
   b. In the table, click a field to edit.

Remove a custom attribute

The custom attribute is removed. All values associated with the custom attribute are also removed.
In the **Plate Setup** tab, in the **Samples** table, click ··· **(More Options)** ▸ **Remove Custom Attribute**.

Add targets or SNP assays and assign to plate wells

Overview default dye assignment

A default reporter dye and a default quencher dye are assigned if there are no targets defined in the **Targets** table.

If a melt curve stage is present in the run method, the reporter dye is set to **SYBR** and the quencher is set to **None**.

If a melt curve stage is not present in the run method, the reporter dye is set to **FAM** and the quencher is set to **NFQ-MGB**.

If targets are defined in the **Targets** table, a new target is assigned the same the reporter dye and quencher as the previous target that was defined. This applies regardless of whether a melt curve stage is present in the run method.

Note: Targets cannot be defined for the TaqMan™ Array Card format and the OpenArray™ Plate format.

Import plate setup from TaqMan™ files

Import assay information using your TaqMan™ assay order details. The plate setup information extracted from TaqMan™ files is the same as the information in the Assay Information File (AIF), and does not include sample information.

Importing the TaqMan™ files is not available for the OpenArray™ Plate format.

Note: Using this feature requires an internet connection.

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Import TaqMan™ assay/plates & card files**.

2. Select a product from the dropdown list, then enter the required information.

Product	Required Information
TaqMan™ Assays	• Sales Order Number • Rack/Plate ID
Fixed TaqMan™ Array Cards	• Part Number • Lot Number
Custom Gene Expression TaqMan™ Array Cards	• Sales Order Number • Lot Number
Custom Advanced miRNA TaqMan™ Array Cards	• Sales Order Number • Lot Number
Fixed TaqMan™ Array Plates	• Part Number • Batch Number

(continued)

Product	Required Information
Custom TaqMan™ Array Plates	• Sales Order Number • Batch Number

3. Click **Import Plate Setup**.

Import an Assay Information File (AIF)

An Assay Information File (AIF) is provided with every TaqMan™ assay order. An AIF does not include sample information.

Importing an AIF overwrites targets or SNP assays. Importing an AIF does not change the samples.

Note: TaqMan™ Array Card plate setup requires AIF import, as targets cannot be added, assigned, or edited manually for TaqMan™ Array Cards.

Prior to plate setup, download the AIF for your order at **thermofisher.com/taqmanfiles**.

Note: To directly import AIF information into the **Plate Setup** without having to first download the file, see “Import plate setup from TaqMan™ files” on page 87.

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Import AIF**.
2. Navigate to, then select the previously downloaded AIF file.
3. Click **Open**.
   If the plate setup contained targets or assays, the **Confirmation** dialog box is displayed.
4. In the **Confirmation** dialog box, click **OK**.

Import a plate setup file

Import a plate setup file that was previously exported from the software (see “Export a plate setup file” on page 102), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Import Plate Setup**.
2. Navigate to, then select the file.
3. Click **OK** to confirm that the plate setup is overwritten.

Copy targets or SNP assays from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ··· **(More Options)** ▸ **Copy all targets**
   - ··· **(More Options)** ▸ **Copy all SNP assays**

The information is available in the clipboard. It can be pasted into another program for reference.

Paste targets or SNP assays in the plate layout

You can copy targets or SNP assays from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

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1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ··· **(More Options)** ▸ **Paste targets**
   - ··· **(More Options)** ▸ **Paste SNP assays**

The targets or SNP assays are copied to the respective table. They must be added to the plate layout (see “Manually add or assign a target or SNP assay to a well” on page 90).

Manually add targets or SNP assays to the Targets or SNP Assays table

Targets cannot be added manually for TaqMan™ Array Card plate setup. To add targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

Targets cannot be added manually for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, select **Target** or **SNP** to display the appropriate table in the right pane.
2. Select an option in the upper right corner of the **Targets** or **SNP Assays** table.

Option	Description
Add a single target or SNP assay	Click + **(Add)**.
Copy/paste multiple targets or SNP assays	a. Copy the information from one of the following sources: • **Targets** or **SNP Assays** table from plate file or data file—Click ··· **(More Options)** ▸ **Copy all Targets/SNP Assays** in the upper-right corner of **Targets** or **SNP Assay** table. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] b. Click ··· **(More Options)** ▸ **Paste Targets/SNP Assays**.

[1] Column headers must match the column headers in the **Targets** or **SNP Assays** table.

Note:

- Click ··· **(More Options)** ▸ **Export Targets** to export targets.
- Click ··· **(More Options)** ▸ **Export SNP Assays** to export SNP assays.
- Click ··· **(More Options)** ▸ **Import Targets** to import targets.
- Click ··· **(More Options)** ▸ **Import SNP Assays** to import SNP assays.

3. Click in a cell in the table to edit the attributes for the target or SNP assay.
4. To remove a target or SNP assay, click ✕ **(Remove)**.

Manually add or assign a target or SNP assay to a well

Targets cannot be added or assigned manually for TaqMan™ Array Card plate setup. To add or assign targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

This is not available for the OpenArray™ Plate format.

If a passive reference is assigned to the plate, each well can have the number of targets or SNP assays that correspond to the number of optical filters minus one. The passive reference dye requires one optical filter.

If a passive reference is not assigned to the plate, each well can have the number of targets or SNP assays that correspond to the number of optical filters.

Two targets or SNP assays with the same reporter dye cannot be assigned to the same well.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or the **(Table View)**.

2. Assign the target or SNP assay to the selected well.

Option	Description
(Grid View)	
Target or SNP assay not defined	Enter the target or SNP assay in the text field.
Target or SNP assay previously defined	• Select the target or SNP assay from the dropdown list. • Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table.
(Table View)	
Target or SNP assay previously defined	Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table.

Note: In **Targets** table or **SNP Assays** table, change the default selections for the reporter and quencher dyes and for tasks (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

(*Optional*) Edit the task assigned to a target or SNP assay in one or more wells

The software automatically assigns a task to the target or SNP assay in a well based on the sample type in that well. The automatic task assignment can be edited, if needed (except for TaqMan™ Array Card plate setup and the OpenArray™ Plate format).

A target or SNP assay can have only one task.

1. In the **Plate Setup** tab, in the plate layout pane, select plate wells in the **(Grid View)** or the **(Table View)** (see “Select plate wells or ports” on page 81).

   If selecting multiple wells, only select well that have the same target or SNP assay, and the same sample type.

2. In the **Targets** or **SNP Assays** table, confirm that the checkbox of the target or SNP assay is selected.

3. Select a detection task from the **Task** column dropdown list.

   The available task options depend on the sample type in the selected well (see “Edit sample name, color, and type” on page 85).

Task	Description
Unknown (default)	The well contains an unknown sample.
Standard[1]	The well contains a sample with known standard quantities. **Note:** The quantity for the standard sample should be entered in the **Samples** table. For each target, the value entered in **Quantity** column in the **Targets** table must be the same for every well.
Negative Control	The well contains water or buffer instead of sample.
Positive Control	The well contains a positive control.
Internal positive control (IPC) [2]	The well contains a short synthetic DNA template. The IPC is used to distinguish between true negative results and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.
Blocked IPC	The well contains an IPC blocking agent, which blocks amplification of the IPC.
Positive 1/1	The well contains a sample homozygous for allele 1.
Positive 2/2	The well contains a sample homozygous for allele 2.
Positive 1/2	The well contains a sample heterozygous for allele 1 and 2.

[1] For standard curve and relative standard curve analysis only.
[2] For presence/absence analysis only.

Edit the SNP assay

Some edits to the SNP assay can be made without opening the **Edit SNP Assay** dialog box (see “Edit the target or SNP assay name and color” on page 93).

1. In the **Plate Setup** tab, in the **SNP Assays** table, click **(Edit)** for the row associated with the SNP assay to edit.
2. In the **Edit SNP Assay** dialog box, enter or edit the following information.
   - The SNP assay name in the **SNP assay name** field
   - A color from the color picker
   - The assay ID in the **Assay ID** field
3. Enter or edit the following information.
   - Gene symbol in the **Gene Symbol** field
   - Gene name in the **Gene Name** field
   - NCBI SNP reference in the **NCBI SNP Reference** field
   - Context sequence in the **Context Sequence** field
4. Enter or edit the following information for allele 1.
   - Allele 1 name or base in the **Allele 1 name or base** field
   - A color from the color picker
   - The reporter dye from the **Reporter** dropdown list
   - The quencher dye from the **Quencher** dropdown list
5. Enter or edit the following information for allele 2.
   - Allele 1 name or base in the **Allele 2 name or base** field
   - A color from the color picker
   - The reporter dye from the **Reporter** dropdown list
   - The quencher dye from the **Quencher** dropdown list
6. *(Optional)* Enter comments in the **Comments** field.
7. Click **Save**.

Edit the target or SNP assay name and color

The target or SNP name and color can be edited for the OpenArray™ Plate format.

Additional edits can be made to the SNP assays (see “Edit the SNP assay” on page 93).

1. In the **Plate Setup** tab, in the **Targets** table or the **SNP Assays** table, click the name in the **Target** column or the **SNP Assays** column.
2. Enter a new name, then press **Enter**.
3. Click the sample color in the **Color** column, then select a color from the color picker.

4. For SNP assays, click the following fields to edit the values.
   - **Allele 1 Reporter** field
   - **Allele 1 Quencher** field
   - **Allele 2 Reporter** field
   - **Allele 2 Quencher** field
5. Click **Save**.

Manage target dyes

Managing the target dyes is not available for the OpenArray™ Plate format.

To add a custom dye from the library, ensure that the custom dye has been added (see "Manage preferences for custom dyes" on page 198).

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Manage Dyes**.
   The **Manage Dyes** dialog box is displayed.
2. View system dyes in the **System Dyes** tab.
   All of the system dyes are available to set up the plate file.
3. Add a custom dye.

Option	Instructions
Add a custom dye from the library.	a. Click the **Custom Dyes** tab. b. Select the **Show custom dyes from the library, and click to import** checkbox. c. In the **Library Dye Name** column click the dye to add. The dye and the details of the dye are displayed in the table on the right. d. Click **Close**.
Add a new custom dye.	a. Click the **Custom Dyes** tab. b. Select or deselect the **Show custom dyes from the library, and click to import** checkbox. If the checkbox is selected, it allows you to see the dyes that are available in the library. c. Click ＋ **(Add)**. d. Edit the following fields: • Enter a name in the **Dye Name** field. • Select a color from the color picker. • Select a type from the **Type** dropdown list. • Enter a wavelength in the **Wavelength** field. e. Click **Close**.

If a new custom dye is added when setting up a plate file, it is not applied to the system. A custom dye must be added in the **Preferences** page in order to apply to the system.

4. (*Optional*) Click ✕ **(Remove)** ▸ **OK** to remove a dye from the table.

5. Click **Close**.

Add CNVs and assign to plate wells

Copy number variation analysis can be performed only within a project. The CNVs can be defined on an individual run file before adding the run file to a project.

The following options are available to add CNVs to an individual run file:

- Copy the CNVs, then paste the CNVs
- Manually add CNVs, then assign the CNVs to the plate wells
- Import CNVs, then assign the CNVs to the plate wells

If the plate setup contains targets or SNPs, a CNV with the same dye as the target or SNP cannot be added. CNVs can be converted from SNPs or targets if the run was set up with an instrument software that does not include CNVs.

A CNV assay converter is available for run files within a project. For more information, see “Add the CNV assay conversion to a plate” on page 122.

IMPORTANT! If the data are exported for import into AlleleTyper™ Software, the CNV name must end with _***cn***.

Copy CNVs from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.

2. In the **CNVs** table , click ··· **(More Options)** ▸ **Copy CNV assays**.

The information is available in the clipboard. It can be pasted into another program for reference. The information can be pasted into a different run file.

Paste CNVs in the plate layout

You can copy CNVs from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

If the plate was set up with SNP assays, the CNVs cannot be pasted if they have the same dyes assigned. It is recommended to use the CNV assay conversion tool. See “Add the CNV assay conversion to a plate” on page 122.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **CNVs** table, click ··· **(More Options)** ▸ **Paste CNV assays**

The CNVs are copied to the table. They must be added to the plate layout (see “Manually assign a CNV to a well” on page 97).

Manually add CNVs to the table

CNVs cannot be added manually for TaqMan™ Array Card plate setup. To add targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

CNVs cannot be added manually for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, **CNV** to display the appropriate CNV table in the right pane.
2. Click + **(Add)**.
   A new row is displayed in the table with default information.
3. Enter a CNV name in the **Name** field.
4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
5. Select the reference reporter dye from the **Reference Reporter** dropdown list.
6. Select the reference quencher dye from the **Reference Quencher** dropdown list.
7. Select the test reporter dye from the **Test Reporter** dropdown list.
8. Select the test quencher dye from the **Test Quencher** dropdown list.
9. Click ✎ **(Edit)** to open the **Edit CNV Assay** dialog box.
   The **Edit CNV Assay** dialog box contains the information that was available in step 3 to step 6.

10. Edit the following items in the **Edit CNV Assay** dialog box.
    - Enter an assay ID in the **Assay ID** field
    - Enter a name or base in the **Reference** field
    - Enter a name or base in the **Test** field
    - Select a reference color
    - Select a test color
    - Add comments to the **Comments** field
11. Click ⊗ **(Remove)** to remove a CNV from the table.

Manually assign a CNV to a well

CNVs cannot be added or assigned manually for TaqMan™ Array Card plate setup. To add or assign targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

This is not available for the OpenArray™ Plate format.

If the plate was set up with SNP assays, the CNVs cannot be pasted if they have the same dyes assigned. It is recommended to use the CNV assay conversion tool. See “Add the CNV assay conversion to a plate” on page 122.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or the **(Table View)**.
2. Assign the CNV to the selected well.

Option	Description
(Grid View)	
Target or SNP assay not defined	Enter the CNV in the text field.
Target or SNP assay previously defined	• Select the target or SNP assay from the dropdown list. • Select the checkbox of the CNV in the **CNV** table.
(Table View)	
Target or SNP assay previously defined	Select the checkbox of the CNV in the **CNV** table.

Edit reagent information

Reagents can only be edited for the TaqMan™ Array Card format and the OpenArray™ Plate format. Reagents cannot be added.

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.
2. In the **Reagents** table, perform one of the following actions.
    - Click + **(Add)**.
    - Click ··· **(More Options)** ▸ **Export Reagents** to export reagents.
    - Click ··· **(More Options)** ▸ **Import Reagents** to import reagents.
    - Click ··· **(More Options)** ▸ **Scan Reagents** to scan reagents.

3. If you are manually adding reagents or editing reagents, enter the following information in the table.
   - Name
   - Type
   - Barcode
   - Part Number
   - Lot Number
   - Expiration Date

   Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

   For more information about setting up the recommended run method for your master mix, see "Apply the recommended run method for your master mix" on page 73.

4. If you are scanning the reagent barcode, in the **Scan Reagent** dialog box, select or deselect the **Enable automatic parsing** checkbox.

5. If you are scanning the reagent barcode, when the **Scan Reagent** dialog box is displayed, use a barcode scanner to scan the reagent label.

   Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

   For more information about setting up the recommended run method for your master mix, see "Apply the recommended run method for your master mix" on page 73.

   The fields in the **Scan Reagent** dialog box are populated.

6. In the **Scan Reagent** dialog box, click **Add**.

7. (*Optional*) Click ✕ **(Remove)** in the row of a reagent to delete it from the table.

Assign a reagent to a well

Assigning a reagent not available for the TaqMan™ Array Card format or the OpenArray™ Plate format.

Reagents that are assigned to wells are not included in an exported plate layout or a printed plate layout.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or in the **(Table View)**.

2. Select the checkbox of the reagent in the **Reagents** table.

Select a passive reference

The passive reference is set for the plate. The default passive reference is ROX™ dye.

1. In the upper-left corner of the **Plate Setup** tab, select a passive reference from the dropdown list.

2. (*Optional*) Save the plate file or data file.

Set up the standard curve

A standard curve is only required for standard curve or relative standard curve analysis. See the appropriate analysis module for more information.

Note:

- Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve.
- You can also set up the standard curve during sample setup (see “Add samples and assign to plate wells” on page 82).

1. In the **Plate Setup** tab, in the plate setup pane, click ··· **(More Options)** ▸ **Standard Curve Setup**. The **Standard Curve Wizard** opens.
2. In the **Standard Curve Wizard** pane, enter the sample name prefix.
3. Select the target for the standard curve.

Option	Instructions
Target previously defined	Select the target from the dropdown list.
Target not previously defined	1. Type the target name, then press **Enter**. 2. Select a reporter from the dropdown list. 3. Select a quencher from the dropdown list.

4. Adjust the parameters for the dilution series if needed.
   - **Number of points**—5 recommended
   - **Number of replicates**—3 recommended
   - **Starting Quantity**—The highest or lowest standard quantity, without units.

     Note: The quantity must be greater than 0.

   - **Serial Factor**

     Note: The serial factor calculates quantities for all standard curve points.

     – Starting quantity is the highest value—Select 1:10 to 1:2.
     – Starting quantity is the lowest value—Select 2× to 10×.
5. Select an option to select the wells for the standard.
   - Select **Automatically**.
   - Select **Manually**, then select wells using the displayed plate layout.
6. Select to arrange the standards in **Rows** or **Columns**.
7. Click **Apply Standard Curve**, then click **Close** to return to the **Plate Setup** tab.

Add biogroups and assign samples

Biogroups, or Biological Replicate Groups, are reactions that contain identical components and volumes, but evaluate separate samples of the same biological source. Biogroups can be used in relative quantification analysis.

1. In the **Plate Setup** tab, in the upper right pane, select an option to add biogroups:

Option	Description
Add biogroups in the **Samples** table	In the **Samples** table, enter the new biogroup name in the **Biogroup** field. The biogroup is added to the following locations: • **Samples** table **Biogroup** dropdown list • **Biogroup** table
Add biogroups in the **Biogroup** table	a. Click **Biogroup**. b. In the **Biogroup** table, click + **(Add)**.

2. In the **Samples** table, for each sample in a biogroup, select a biogroup from the dropdown list.
3. (*Optional*) Edit the biogroup name or color.
   a. Click **Biogroup**.
   b. In the **Biogroup** table, click a field to edit.

Edit plate file or data file information

The plate file can be edited for the OpenArray™ Plate format.

1. Click **Actions** ▸ **Plate Information**.
2. In the **Plate Information** dialog box, edit the **Experiment Name** field.
3. (*Optional*) Click in the **Barcode** field, then perform one of the following steps.
   - Scan the plate barcode with a barcode scanner.
   - Manually enter the plate barcode.
4. (*Optional*) Add a tag.
5. (*Optional*) Enter a comment in the **Comment** field.
6. Click **Save**.

Save the updated plate file or data file.

Review the plate file and send to the instrument run queue

The instrument run queue is available only for the QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument.

1. In the **Run Summary** tab, review the run method selections, then edit if needed (see “Confirm or edit run method” on page 71).
2. Review the plate setup, then edit if needed (see “Confirm or edit plate setup” on page 78).
3. (*Optional*) Click the barcode field, then scan the plate barcode or manually enter the barcode.
4. (*Optional*) Select **Add to My Plates Gallery**.
5. Select an instrument or more than one instrument from the list.

   Only the instruments that are compatible with the plate file are displayed.

   If the instrument does not appear on the list, click **Refresh**.

   If the instrument does not appear on the list after refreshing the list, click **System** ▸ **Instruments** to add a new instrument (see “Add an instrument” on page 185).
6. Click **Send to Run Queue**.
7. *(If the plate file was not saved)* In the **Save As** dialog box, edit the file name and destination, then click **Save**.
8. Click **Done** to close **Run Sent** dialog box.

Start the run on an instrument. For specifics on starting an instrument run, see the instrument documentation.

Flip the plate setup

The feature to flip the plate setup rotates the assignments 180° so that A1 is moved to H12 in a 96-well plate. A1 is moved to P24 in a 384-well plate.

The feature to flip the plate setup does not edit any assignments in the well. All of the items assigned to a well are moved.

1. In an open run, select the **Plate Setup** tab.
2. At the top-right corner of the grid view or the table view of the plate layout, click ··· **(More Options)** ▸ **Flip Plate Setup**.

Export a plate setup file

Export a plate setup file to use during future plate setups (see “Import a plate setup file (samples)” on page 82).

The following files types can be exported:

- TXT—text format
- CSV—comma-separated values format

Samples are exported for the OpenArray™ Plate format. The targets are not exported.

Export the sample layout to use for sample integration in the QuantStudio™ 12K Flex Software.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Export Plate Setup**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Export Sample Layout**.

2. Name the file, navigate to the desired folder location, select the file format, then click **Save**.

Print the layout

Printing the layout saves the information in one of the following file formats:

- XLSX
- PDF

For the OpenArray™ Plate format, each cell in an XLSX file format represents a subarray.

Samples are represented for the OpenArray™ Plate format. The targets are not represented.

These files are for reference. To export in a format that can be imported, see “Export a plate setup file” on page 102.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Print Layout**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Print Sample Layout**.

2. Name the file, then navigate to the desired folder location.

3. Select the file format.
    - Select the **XLSX** radio button
    - Select the **PDF** radio button
4. If PDF file format was selected, select the paper size.
    - Select the **A4** radio button
    - Select the **Letter** radio button
5. Click **Save**.

7 View the plate setup for a project

View or edit the plate setup for a project

Note: The table view is not available for the OpenArray™ Plate format. The table view is indicated by the ☰ icon.

Select the run file

In the **Plate Setup** tab of an open project, perform one of the following actions.

- Select the file from the **Run file** dropdown list.
- Use the arrows on the right side of the **Run file** dropdown list to navigate through the run files. The arrows are disabled when there is only one run file in the project.

Edit the view

Edit the grid view

The grid view is displayed in the **Plate Setup** tab. It matches the plate layout of a physical plate.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click **(Grid View)**.

1. In the top-right corner of the plate layout pane, click **(Zoom In)** and **(Zoom Out)**.
2. Click the percentage value, then use the slider to select a scaling based on a numerical value.
3. Click **(Reset)** to reset the zoom to fit the screen width.

4. Click ⚙ **(Settings)**, the select the following options from the dialog box.

Section	Option
Show	• **Sample Color** • **Target Color** or **SNP Assay Color** Select one, none, or all of the options.
Tab/Auto Fill Direction	• **Horizontal** • **Vertical** Select one option. This option selects the direction of the next well that is selected when **Tab** or **Enter** is pressed.
Dock Table	• **To Right** • **To Bottom** Select one option. This option selects whether the tables are displayed on the right of the screen or on the bottom of the screen. The tables include the **Samples** table, the **Targets** table, and the **SNP Assays** table.

5. If any of the wells display an invalid setup, hover over the warning icon to view the reason.

Edit the table view

The table view is displayed in the **Plate Setup** tab.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click ☰ **(Table View)**.

1. In the top-right corner, click **View**.
2. Select or deselect the checkbox associated with each item to display in the table view.

Table	Options
Target	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Target Name** checkbox • **Target Reporter** checkbox • **Target Quencher** checkbox • **Task** checkbox • **Quantity** checkbox
SNP	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Assay Name** checkbox • **Allele 1 Name** • **Allele 1 Reporter** checkbox • **Allele 1 Quencher** checkbox • **Allele 2 Name** • **Allele 2 Reporter** checkbox • **Allele 2 Quencher** checkbox • **Task** checkbox

(continued)

Table	Options
CNV	• **Well** checkbox • **Sample Name** checkbox • **Sample Type** checkbox • **Assay Name** checkbox • **Reference Name** • **Reference Reporter** checkbox • **Reference Quencher** checkbox • **Test Name** • **Test Reporter** checkbox • **Test Quencher** checkbox • **Task** checkbox

3. Click **(Settings)**, the select the following options from the **Dock Table** dialog box.

   - **To Right** radio button
   - **To Bottom** radio button

   This setting selects whether the tables are displayed on the right of the screen or on the bottom of the screen.

   The tables include the **Samples** table, the **Targets** table, the **SNP Assays** table, and the **CNV Assays** table.

Select plate wells or ports

- Select plate wells or ports in the **(Grid View)**.

Note: Selecting ports is only available for TaqMan™ Array Card plate setup.

To	Action
Select a single well	Click a well in the plate
Select multiple wells	Click-drag in the plate
Select contiguous wells	Shift-click wells in the plate
Select non-contiguous wells	PC: Ctrl-click wells in the plate Mac: Cmd-click wells in the plate
Select a column of wells	Click a column header
Select all wells	Click the top-left corner of the plate grid
Select a block of wells	Click a well to define a corner, then shift-click another well on the opposite corner
Select a single port[1]	Click a cell in the **Port** column

(continued)

To	Action
Select multiple ports[1]	Click-drag in the **Port** column
Select all ports[1]	Click the **Port** column header

[1] For TaqMan™ Array Cards only.

- Select plate wells in the ☰ **(Table View)**.

  This view is not available for the OpenArray™ Plate format.

To	Action
Select a single well	Click a row in the table
Select contiguous wells	Shift-click rows in the table
Select non-contiguous wells	PC: Ctrl-click rows in the table Mac: Cmd-click rows in the table
Deselect a single well	PC: Ctrl-click the selected row Mac: Cmd-click the selected row

Add samples and assign to plate wells

Each well can contain only one sample.

Import a plate setup file (samples)

Import a plate setup file that was previously exported from the software (see “Export a plate setup file” on page 102), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

A sample layout can be imported for the OpenArray™ Plate format. Targets or SNP assays cannot be imported for an OpenArray™ Plate format.

For all of the other formats, the plate setup file can include both samples and targets or SNP assays.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Import Plate Setup**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Import Sample Layout**.

2. Navigate to, then select the file.
3. Click **OK** to confirm that the plate setup is overwritten.

Copy samples from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **Samples** table, click ··· **(More Options)** ▸ **Copy all samples**.

The information is available in the clipboard. It can be pasted into another program for reference.

Paste samples into the plate layout

You can copy samples from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **Samples** table, click ··· **(More Options)** ▸ **Paste samples**.

The samples are copied to the **Samples** table. They must be assigned to the plate layout (see “Manually add or assign a sample to a well or port” on page 84.

Remove unused samples

The samples that were not assigned to a well can be removed from the **Samples** table.

In the **Samples** table, click ··· **(More Options)** ▸ **Remove unused samples**.

Manually add samples to the Samples table

Each sample name in the **Samples** table must be unique. Do not add multiple entries for technical replicates.

Adding a single sample is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, select an option in the upper right corner of the **Samples** table.

Option	Description
Add a single sample	Click + **(Add)**.
Copy/paste multiple samples	a. Copy the sample information from one of the following sources: • Previously created plate file or data file—Click ··· **(More Options)** ▸ **Copy all Samples** in the upper-right corner of **Samples** table. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] b. Click ··· **(More Options)** ▸ **Paste Samples**.

[1] Column headers must match the column headers in the **Samples** table.

Note:
- Click ··· **(More Options)** ▸ **Export Samples** to export samples.
- Click ··· **(More Options)** ▸ **Import Samples** to import samples.

2. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 85).

Note: The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

3. To remove a sample from the table, click ✕ **(Remove)** in the last column.

Manually add or assign a sample to a well or port

Sample assignment by port is available only for TaqMan™ Array Card plate setup.

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, perform one of the following actions.
   - Select one or more wells in the **(Grid View)** or in the **(Table View)**.
   - Select one or more ports in the **(Grid View)**.

2. Assign a sample to the selected well or port.

   Note: The user can only assign up to eight samples by port in the **(Grid View)** for TaqMan™ Array Card plate setup.

Option	Description
(Grid View)	
Sample not defined	Enter the sample name in the text field.
Sample previously defined	• Start typing the sample name, then select the sample name from the autocomplete list. • Select the checkbox of the sample in the **Samples** table.
(Table View)	
Sample previously defined	Select the checkbox of the sample in the **Samples** table.

3. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 85).

   Note: The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

Edit sample name, color, and type

The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 92).

The sample name, color, and type can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click the sample name in the **Name** column.
2. Enter a new name, then press **Enter**.
3. Click the sample color in the **Color** column, then select a color from the color picker.
4. Select a sample type from the **Type** column dropdown list.
   - **Unknown** (default)
   - **Standard**

     Note: A standard sample requires that you enter a value in the **Quantity** column of the **Samples** table. The software uses this value to populate the **Quantity** field for standard target tasks in the **Targets** table.
   - **Negative Control**
   - **Positive Control**
   - **Positive 1/1**—A sample that is homozygous for allele 1.
   - **Positive 2/2**—A sample that is homozygous for allele 2.
   - **Positive 1/2**—A sample that is heterozygous for allele 1 and 2.

IMPORTANT! Editing the sample type after a run can affect the validity of the plate setup.

Add a custom attribute to samples

Custom attributes can be added for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click ··· **(More Options)** ▸ **Add Custom Attribute**.
2. In the **Add Custom Attribute** window, enter the custom attribute name, then click **Done**.
   A column for the custom attribute is added to **Samples** table, and a new tab for the custom attribute is created.
3. Select an option to define the choices for the custom attribute:

Option	Description
Define in the **Samples** table	In the **Samples** table, define the custom attribute in the appropriate field in the custom attribute column.
Define in the custom attribute tab	a. In the custom attribute tab, click + **(Add)**. b. In the table, click in the field to edit the custom attribute name.

   The custom attribute options are added to the following locations:
   - **Samples** table, in the dropdown list in the custom attribute column
   - Table in the custom attribute tab
4. In the **Samples** table, for each sample, select the custom attribute from the dropdown list.
5. (*Optional*) Edit the custom attribute name or color.
   a. Select the custom attribute tab.
   b. In the table, click a field to edit.

Remove a custom attribute

The custom attribute is removed. All values associated with the custom attribute are also removed.
In the **Plate Setup** tab, in the **Samples** table, click ··· **(More Options)** ▸ **Remove Custom Attribute**.

Add targets or SNP assays and assign to plate wells

Import plate setup from TaqMan™ files

Import assay information using your TaqMan™ assay order details. The plate setup information extracted from TaqMan™ files is the same as the information in the Assay Information File (AIF), and does not include sample information.

Importing the TaqMan™ files is not available for the OpenArray™ Plate format.

Note: Using this feature requires an internet connection.

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Import TaqMan™ assay/plates & card files**.
2. Select a product from the dropdown list, then enter the required information.

Product	Required Information
TaqMan™ Assays	• Sales Order Number • Rack/Plate ID
Fixed TaqMan™ Array Cards	• Part Number • Lot Number
Custom Gene Expression TaqMan™ Array Cards	• Sales Order Number • Lot Number
Custom Advanced miRNA TaqMan™ Array Cards	• Sales Order Number • Lot Number
Fixed TaqMan™ Array Plates	• Part Number • Batch Number
Custom TaqMan™ Array Plates	• Sales Order Number • Batch Number

3. Click **Import Plate Setup**.

Import an Assay Information File (AIF)

An Assay Information File (AIF) is provided with every TaqMan™ assay order. An AIF does not include sample information.

The software checks the AIF for the assay ID and conflicts in dye assignment.

- If the assay ID and dye information in the plate setup matches the AIF, the AIF is imported.
- If the assay ID in the AIF is new, the AIF is imported and the software uses the new assay ID.
- If the assay ID matches the AIF but the dye information does not match the AIF, the AIF is not imported.

Note: TaqMan™ Array Card plate setup requires AIF import, as targets cannot be added, assigned, or edited manually for TaqMan™ Array Cards.

Prior to plate setup, download the AIF for your order at **thermofisher.com/taqmanfiles**.

Note: To directly import AIF information into the **Plate Setup** without having to first download the file, see "Import plate setup from TaqMan™ files" on page 87.

1. In the **Plate Setup** tab, select the data file from the dropdown list.
2. In the plate layout pane, click ··· **(More Options)** ▸ **Import AIF**.
3. Navigate to, then select the previously downloaded AIF file.
4. Click **Open**.
   If the plate setup contained targets or assays, the **Confirmation** dialog box is displayed.
5. In the **Confirmation** dialog box, click **OK**.

Import a plate setup file

Import a plate setup file that was previously exported from the software (see "Export a plate setup file" on page 102), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, select a data file from the dropdown list.
2. In the plate layout pane, click ··· **(More Options)** ▸ **Import Plate Setup**.
3. Navigate to, then select the file.
4. Click **OK** to confirm that the plate setup is overwritten.

Copy targets or SNP assays from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ··· **(More Options)** ▸ **Copy all targets**
   - ··· **(More Options)** ▸ **Copy all SNP assays**

The information is available in the clipboard. It can be pasted into another program for reference.

Paste targets or SNP assays in the plate layout

You can copy targets or SNP assays from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ··· **(More Options)** ▸ **Paste targets**
   - ··· **(More Options)** ▸ **Paste SNP assays**

The targets or SNP assays are copied to the respective table. They must be added to the plate layout (see “Manually add or assign a target or SNP assay to a well” on page 90).

Manually add targets or SNP assays to the Targets or SNP Assays table

Targets cannot be added manually for TaqMan™ Array Card plate setup. To add targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

Targets cannot be added manually for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, select **Target** or **SNP** to display the appropriate table in the right pane.
2. Select an option in the upper right corner of the **Targets** or **SNP Assays** table.

Option	Description
Add a single target or SNP assay	Click + **(Add)**.
Copy/paste multiple targets or SNP assays	a. Copy the information from one of the following sources: • **Targets** or **SNP Assays** table from plate file or data file—Click ··· **(More Options)** ▸ **Copy all Targets/SNP Assays** in the upper-right corner of **Targets** or **SNP Assay** table. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] b. Click ··· **(More Options)** ▸ **Paste Targets/SNP Assays**.

[1] Column headers must match the column headers in the **Targets** or **SNP Assays** table.

Note:

- Click ··· **(More Options)** ▸ **Export Targets** to export targets.
- Click ··· **(More Options)** ▸ **Export SNP Assays** to export SNP assays.
- Click ··· **(More Options)** ▸ **Import Targets** to import targets.
- Click ··· **(More Options)** ▸ **Import SNP Assays** to import SNP assays.

3. Click in a cell in the table to edit the attributes for the target or SNP assay.
4. To remove a target or SNP assay, click ✕ **(Remove)**.

Manually add or assign a target or SNP assay to a well

Targets cannot be added or assigned manually for TaqMan™ Array Card plate setup. To add or assign targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

This is not available for the OpenArray™ Plate format.

If a passive reference is assigned to the plate, each well can have the number of targets or SNP assays that correspond to the number of optical filters minus one. The passive reference dye requires one optical filter.

If a passive reference is not assigned to the plate, each well can have the number of targets or SNP assays that correspond to the number of optical filters.

Two targets or SNP assays with the same reporter dye cannot be assigned to the same well.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or the **(Table View)**.
2. Assign the target or SNP assay to the selected well.

Option	Description
(Grid View)	
Target or SNP assay not defined	Enter the target or SNP assay in the text field.
Target or SNP assay previously defined	• Select the target or SNP assay from the dropdown list. • Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table.
(Table View)	
Target or SNP assay previously defined	Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table.

Note: In **Targets** table or **SNP Assays** table, change the default selections for the reporter and quencher dyes and for tasks (see “(Optional) Edit the task assigned to a target or SNP assay in one or more wells” on page 92).

(*Optional*) Edit the task assigned to a target or SNP assay in one or more wells

The software automatically assigns a task to the target or SNP assay in a well based on the sample type in that well. The automatic task assignment can be edited, if needed (except for TaqMan™ Array Card plate setup and the OpenArray™ Plate format).

A target or SNP assay can have only one task.

1. In the **Plate Setup** tab, in the plate layout pane, select plate wells in the **(Grid View)** or the **(Table View)** (see "Select plate wells or ports" on page 81).

   If selecting multiple wells, only select well that have the same target or SNP assay, and the same sample type.

2. In the **Targets** or **SNP Assays** table, confirm that the checkbox of the target or SNP assay is selected.

3. Select a detection task from the **Task** column dropdown list.

   The available task options depend on the sample type in the selected well (see "Edit sample name, color, and type" on page 85).

Task	Description
Unknown (default)	The well contains an unknown sample.
Standard[1]	The well contains a sample with known standard quantities. **Note:** The quantity for the standard sample should be entered in the **Samples** table. For each target, the value entered in **Quantity** column in the **Targets** table must be the same for every well.
Negative Control	The well contains water or buffer instead of sample.
Positive Control	The well contains a positive control.
Internal positive control (IPC) [2]	The well contains a short synthetic DNA template. The IPC is used to distinguish between true negative results and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.
Blocked IPC	The well contains an IPC blocking agent, which blocks amplification of the IPC.
Positive 1/1	The well contains a sample homozygous for allele 1.
Positive 2/2	The well contains a sample homozygous for allele 2.
Positive 1/2	The well contains a sample heterozygous for allele 1 and 2.

[1] For standard curve and relative standard curve analysis only.

[2] For presence/absence analysis only.

Edit the SNP assay

Some edits to the SNP assay can be made without opening the **Edit SNP Assay** dialog box (see “Edit the target or SNP assay name and color” on page 93).

1. In the **Plate Setup** tab, in the **SNP Assays** table, click **(Edit)** for the row associated with the SNP assay to edit.
2. In the **Edit SNP Assay** dialog box, enter or edit the following information.
   - The SNP assay name in the **SNP assay name** field
   - A color from the color picker
   - The assay ID in the **Assay ID** field
3. Enter or edit the following information.
   - Gene symbol in the **Gene Symbol** field
   - Gene name in the **Gene Name** field
   - NCBI SNP reference in the **NCBI SNP Reference** field
   - Context sequence in the **Context Sequence** field
4. Enter or edit the following information for allele 1.
   - Allele 1 name or base in the **Allele 1 name or base** field
   - A color from the color picker
   - The reporter dye from the **Reporter** dropdown list
   - The quencher dye from the **Quencher** dropdown list
5. Enter or edit the following information for allele 2.
   - Allele 1 name or base in the **Allele 2 name or base** field
   - A color from the color picker
   - The reporter dye from the **Reporter** dropdown list
   - The quencher dye from the **Quencher** dropdown list
6. *(Optional)* Enter comments in the **Comments** field.
7. Click **Save**.

Edit the target or SNP assay name and color

The target or SNP name and color can be edited for the OpenArray™ Plate format.

Additional edits can be made to the SNP assays (see “Edit the SNP assay” on page 93).

1. In the **Plate Setup** tab, in the **Targets** table or the **SNP Assays** table, click the name in the **Target** column or the **SNP Assays** column.
2. Enter a new name, then press **Enter**.
3. Click the sample color in the **Color** column, then select a color from the color picker.

4. For SNP assays, click the following fields to edit the values.
   - **Allele 1 Reporter** field
   - **Allele 1 Quencher** field
   - **Allele 2 Reporter** field
   - **Allele 2 Quencher** field
5. Click **Save**.

Manage target dyes

Managing the target dyes is not available for the OpenArray™ Plate format.

To add a custom dye from the library, ensure that the custom dye has been added (see "Manage preferences for custom dyes" on page 198).

1. In the **Plate Setup** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Manage Dyes**.
   The **Manage Dyes** dialog box is displayed.
2. View system dyes in the **System Dyes** tab.
   All of the system dyes are available to set up the plate file.
3. Add a custom dye.

Option	Instructions
Add a custom dye from the library.	a. Click the **Custom Dyes** tab. b. Select the **Show custom dyes from the library, and click to import** checkbox. c. In the **Library Dye Name** column click the dye to add. The dye and the details of the dye are displayed in the table on the right. d. Click **Close**.
Add a new custom dye.	a. Click the **Custom Dyes** tab. b. Select or deselect the **Show custom dyes from the library, and click to import** checkbox. If the checkbox is selected, it allows you to see the dyes that are available in the library. c. Click ＋ **(Add)**. d. Edit the following fields: • Enter a name in the **Dye Name** field. • Select a color from the color picker. • Select a type from the **Type** dropdown list. • Enter a wavelength in the **Wavelength** field. e. Click **Close**.

If a new custom dye is added when setting up a plate file, it is not applied to the system. A custom dye must be added in the **Preferences** page in order to apply to the system.

4. (*Optional*) Click ✕ **(Remove)** ▸ **OK** to remove a dye from the table.
5. Click **Close**.

Add CNVs and assign to plate wells

The following options are available to add CNVs:

- Copy the CNVs, then paste the CNVs
- Manually add CNVs, then assign the CNVs to the plate wells
- Import CNVs, then assign the CNVs to the plate wells
- Use the CNV assay converter tool

IMPORTANT! If the data are exported for import into AlleleTyper™ Software, the CNV name must end with _***cn***.

Copy CNVs from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **CNVs** table , click ··· **(More Options)** ▸ **Copy CNV assays**.

The information is available in the clipboard. It can be pasted into another program for reference. The information can be pasted into a different run file.

Paste CNVs in the plate layout

You can copy CNVs from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

If the plate was set up with SNP assays, the CNVs cannot be pasted if they have the same dyes assigned. It is recommended to use the CNV assay conversion tool. See “Add the CNV assay conversion to a plate” on page 122.

Note: Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

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1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **CNVs** table, click ··· **(More Options)** ▸ **Paste CNV assays**

The CNVs are copied to the table. They must be added to the plate layout (see “Manually assign a CNV to a well” on page 97).

Manually add CNVs to the table

CNVs cannot be added manually for TaqMan™ Array Card plate setup. To add targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

CNVs cannot be added manually for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, **CNV** to display the appropriate CNV table in the right pane.
2. Click + **(Add)**.
   A new row is displayed in the table with default information.
3. Enter a CNV name in the **Name** field.
4. Click the color to open the color picker, then select a color.
   The color is not related to the dye. It is to visualize the targets in the plate layout view.
5. Select the reference reporter dye from the **Reference Reporter** dropdown list.
6. Select the reference quencher dye from the **Reference Quencher** dropdown list.
7. Select the test reporter dye from the **Test Reporter** dropdown list.
8. Select the test quencher dye from the **Test Quencher** dropdown list.
9. Click ✎ **(Edit)** to open the **Edit CNV Assay** dialog box.
   The **Edit CNV Assay** dialog box contains the information that was available in step 3 to step 6.
10. Edit the following items in the **Edit CNV Assay** dialog box.
    - Enter an assay ID in the **Assay ID** field
    - Enter a name or base in the **Reference** field
    - Enter a name or base in the **Test** field
    - Select a reference color
    - Select a test color
    - Add comments to the **Comments** field
11. Click ⊗ **(Remove)** to remove a CNV from the table.

Manually assign a CNV to a well

CNVs cannot be added or assigned manually for TaqMan™ Array Card plate setup. To add or assign targets for TaqMan™ Array Cards, see “Import an Assay Information File (AIF)” on page 88.

This is not available for the OpenArray™ Plate format.

If the plate was set up with SNP assays, the CNVs cannot be pasted if they have the same dyes assigned. It is recommended to use the CNV assay conversion tool. See “Add the CNV assay conversion to a plate” on page 122.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or the **(Table View)**.
2. Assign the CNV to the selected well.

Option	Description
(Grid View)	
Target or SNP assay not defined	Enter the CNV in the text field.
Target or SNP assay previously defined	• Select the target or SNP assay from the dropdown list. • Select the checkbox of the CNV in the **CNV** table.
(Table View)	
Target or SNP assay previously defined	Select the checkbox of the CNV in the **CNV** table.

Add the CNV assay conversion to a plate

If the project template or the project did not contain a CNV assay conversion, a CNV assay conversion can be added to the plate.

When the CNV assay conversion is added to an individual data file within a project, the conversion rules are applied to all of the data files in the project.

The CNV assay conversion includes pre-defined conversion rules. The rules can be edited or deleted.

1. In an open project, select the **Plate Setup** tab.
2. Select the run file.
   - Select the file from the **Run file** dropdown list.
   - Use the arrows on the right side of the **Run file** dropdown list to navigate through the run files. The arrows are disabled when there is only one run file in the project.
3. At the top-right corner of the grid view or the table view of the plate layout, click **(More Options) ▸ CNV Assay Converter**.
4. In the **CNV Assay Converter** dialog box, click **Add rule**.
   A new row is displayed in the **CNV Assay Converter** dialog box.
5. Enter the name of the target or SNP assay in the **Target / SNP Assay** field.

6. Enter the name of the CNV reference in the **CNV Reference Name** field.
7. Enter the name of the CNV assay in the **CNV Assay Name** field.
   The content of the fields in each row cannot be duplicated.
8. *(Optional)* Import rules.
   See “Import a CNV assay conversion file for a plate” on page 123.
9. Click ⊗ **(Delete)** in a row to delete a single rule.
10. Click ⊗ **(Delete)** in the table header to delete all of the rules.
11. Click **Apply**.

Import a CNV assay conversion file for a plate

The file must be in CSV format.

The file must contain the following headers:

- **Target / SNP Assay**
- **CNV Reference Name**
- **CNV Assay Name**

The conversion does not overwrite any of the CNVs that were previously defined for the plate. The CNVs that are defined in the CNV assay conversion file are added to the list of CNVs that are defined in the **CNVs** tab.

If a CNV with the same name was previously defined in the project, the CNV from the CNV assay conversion file is not added to the list of CNVs that are defined in the **CNVs** tab.

1. In the **Plate Setup** tab of an open project, select the file from the **Run file** dropdown list.
2. In the plate layout pane, click ··· **(More Options)** ▸ **CNV Assay Converter**.
3. At the top-right corner of the grid view or the table view of the plate layout, click ··· **(More Options)** ▸ **CNV Assay Converter**.
4. In the **CNV Assay Converter** dialog box, click **Import**.
5. In the **Open** dialog box, navigate to the location of the file, then click **Open**.

The **CNV Assay Converter** is populated with the rules for conversion.

Add or remove rules from the rules that were imported, if necessary.

Click **Apply** to apply the imported rules.

Export a CNV assay conversion file from a plate

The file is in CSV format.

The exported file can be imported to other project templates, projects, or plate setup files.

1. In the **Plate Setup** tab of an open project, at the top-right corner of the grid view or the table view of the plate layout, click ··· **(More Options)** ▸ **CNV Assay Converter**.
2. In the **CNV Assay Converter** dialog box, click **Export**.
3. Navigate to the location to save the file, then edit the file name, if necessary.
4. Click **Save**.

Edit reagent information

Reagents can only be edited for the TaqMan™ Array Card format and the OpenArray™ Plate format. Reagents cannot be added.

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.
2. In the **Reagents** table, perform one of the following actions.
   - Click + **(Add)**.
   - Click ··· **(More Options)** ▸ **Export Reagents** to export reagents.
   - Click ··· **(More Options)** ▸ **Import Reagents** to import reagents.
   - Click ··· **(More Options)** ▸ **Scan Reagents** to scan reagents.
3. If you are manually adding reagents or editing reagents, enter the following information in the table.
   - Name
   - Type
   - Barcode
   - Part Number
   - Lot Number
   - Expiration Date

   Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.
   For more information about setting up the recommended run method for your master mix, see “Apply the recommended run method for your master mix” on page 73.
4. If you are scanning the reagent barcode, in the **Scan Reagent** dialog box, select or deselect the **Enable automatic parsing** checkbox.
5. If you are scanning the reagent barcode, when the **Scan Reagent** dialog box is displayed, use a barcode scanner to scan the reagent label.

   Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

For more information about setting up the recommended run method for your master mix, see “Apply the recommended run method for your master mix” on page 73.

The fields in the **Scan Reagent** dialog box are populated.

6. In the **Scan Reagent** dialog box, click **Add**.
7. (*Optional*) Click ✕ **(Remove)** in the row of a reagent to delete it from the table.

Assign a reagent to a well

Assigning a reagent not available for the OpenArray™ Plate format.

Reagents that are assigned to wells are not included in an exported plate layout or a printed plate layout.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or in the **(Table View)**.
2. Select the checkbox of the reagent in the **Reagents** table.

Select a passive reference

The passive reference is set for the plate. The default passive reference is ROX™ dye.

1. In the upper-left corner of the **Plate Setup** tab, select a passive reference from the dropdown list.
2. (*Optional*) Save the plate file or data file.

Set up the standard curve

A standard curve is only required for standard curve or relative standard curve analysis. See the appropriate analysis module for more information.

Note:

- Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve.
- You can also set up the standard curve during sample setup (see “Add samples and assign to plate wells” on page 82).

1. In the **Plate Setup** tab, in the plate setup pane, click ··· **(More Options)** ▸ **Standard Curve Setup**. The **Standard Curve Wizard** opens.
2. In the **Standard Curve Wizard** pane, enter the sample name prefix.

3. Select the target for the standard curve.

Option	Instructions
Target previously defined	Select the target from the dropdown list.
Target not previously defined	1. Type the target name, then press **Enter**. 2. Select a reporter from the dropdown list. 3. Select a quencher from the dropdown list.

4. Adjust the parameters for the dilution series if needed.
   - **Number of points**—5 recommended
   - **Number of replicates**—3 recommended
   - **Starting Quantity**—The highest or lowest standard quantity, without units.

     Note: The quantity must be greater than 0.

   - **Serial Factor**

     Note: The serial factor calculates quantities for all standard curve points.

     – Starting quantity is the highest value—Select 1:10 to 1:2.
     – Starting quantity is the lowest value—Select 2× to 10×.

5. Select an option to select the wells for the standard.
   - Select **Automatically**.
   - Select **Manually**, then select wells using the displayed plate layout.

6. Select to arrange the standards in **Rows** or **Columns**.

7. Click **Apply Standard Curve**, then click **Close** to return to the **Plate Setup** tab.

Add biogroups and assign samples

Biogroups, or Biological Replicate Groups, are reactions that contain identical components and volumes, but evaluate separate samples of the same biological source. Biogroups can be used in relative quantification analysis.

Biogroups and assigned samples can be defined in a project template or a project. They can be edited for the run file. They can be defined for the run file if they were not defined in the project template or the project.

1. In the **Plate Setup** tab, in the upper right pane, select an option to add biogroups:

Option	Description
Add biogroups in the **Samples** table	In the **Samples** table, enter the new biogroup name in the **Biogroup** field. The biogroup is added to the following locations: • **Samples** table **Biogroup** dropdown list • **Biogroup** table
Add biogroups in the **Biogroup** table	a. Click **Biogroup**. b. In the **Biogroup** table, click + **(Add)**.

2. In the **Samples** table, for each sample in a biogroup, select a biogroup from the dropdown list.

3. (*Optional*) Edit the biogroup name or color.
   a. Click **Biogroup**.
   b. In the **Biogroup** table, click a field to edit.

Flip the plate setup in a project

The feature to flip the plate setup rotates the assignments 180° so that A1 is moved to H12 in a 96-well plate. A1 is moved to P24 in a 384-well plate.

The feature to flip the plate setup does not edit any assignments in the well. All of the items assigned to a well are moved.

Flipping a plate setup applies only to the plate in the project that was selected. It does not apply to all of the plates. If more than one plate should be flipped, each plate must be flipped individually.

1. In an open project, select the **Plate Setup** tab.

2. Select the run file.
   - Select the file from the **Run file** dropdown list.
   - Use the arrows on the right side of the **Run file** dropdown list to navigate through the run files. The arrows are disabled when there is only one run file in the project.

3. At the top-right corner of the grid view or the table view of the plate layout, click ··· **(More Options)** ▸ **Flip Plate Setup**.

Export a plate setup file for a project

Export a plate setup file to use during future plate setups (see “Import a plate setup file (samples)” on page 82).

The following files types can be exported:

- TXT—text format
- CSV—comma-separated values format

A plate setup file can be exported only one at a time. The export feature does not export all the plate setup files from the project.

Samples are exported for the OpenArray™ Plate format. The targets are not exported.

Export the sample layout to use for sample integration in the QuantStudio™ 12K Flex Software.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Export Plate Setup**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Export Sample Layout**.

2. Name the file, navigate to the desired folder location, select the file format, then click **Save**.

Print the layout

Printing the layout saves the information in one of the following file formats:

- XLSX
- PDF

For the OpenArray™ Plate format, each cell in an XLSX file format represents a subarray.

Samples are represented for the OpenArray™ Plate format. The targets are not represented.

These files are for reference. To export in a format that can be imported, see “Export a plate setup file” on page 102.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

Format	Procedure
96-well, 0.2-mL plate 96-well, 0.1-mL plate 384-well plate TaqMan™ Array Card	Click ··· **(More Options)** ▸ **Print Layout**.
OpenArray™ Plate	Click ··· **(More Options)** ▸ **Print Sample Layout**.

2. Name the file, then navigate to the desired folder location.
3. Select the file format.
    - Select the **XLSX** radio button
    - Select the **PDF** radio button
4. If PDF file format was selected, select the paper size.
    - Select the **A4** radio button
    - Select the **Letter** radio button
5. Click **Save**.

Review and analyze data

We recommend that you analyze data using the default analysis settings. If the default analysis settings are not suitable for the data, modify the analysis settings, then reanalyze the data.

For information about general procedures to analyze data in the **Quality Check** tab, see Chapter 11, “General procedures to analyze data in the Quality Check tab”.

Workflow: General procedures to review analysis results

The software automatically analyzes run data using the analysis settings that are specified during plate file set up. The software then displays analysis results in the **Quality Check** tab.

IMPORTANT! If you omit wells, click **Analyze** to reanalyze the data.

Review results

Review results in the Amplification Plot (page 135)
Review results in the Amplification Plot to confirm or correct threshold and baseline settings.

Identify and omit outliers from analysis (page 140)
Review data for outliers and (*optional*) omit wells.

Review results in the multicomponent plot (page 140)
(*Optional*) View the Multicomponent Plot to review the dye signal profile.

Review results in the raw data plot (page 141)
(*Optional*) View the Raw Data Plot to review the signal profile.

Review QC alerts in the well table (page 144)
(*Optional*) Review flags and QC alerts.

Edit primary analysis settings (page 144)
(*Optional*) Edit analysis settings.

Perform additional analysis (page 151)
(*Optional*) Perform additional analysis.

Open a data file

After a data file is opened, it appears on the **Runs** page.

You can open a data file directly from the **Runs** page if the file appears on the **Runs** page.

If you open a data file that was previously opened in the software, the original data file is opened. A copy is not created in the software. This is based on the file name. The original file is retained even if it is saved in a different location on the computer. In order to create a copy, use the save as feature.

1. Select the **Dashboard** tab, then click **Open File**.
2. Navigate to, then open a data file.

Option	Description
Open data files that were automatically transferred to the software from the instrument.	The data file is saved to the same location as the plate file that was used for the instrument run.
Open data files that were manually transferred from the instrument.	Navigate to the location that was selected when the data files were transferred from the instrument.

The data file opens and the analysis results are displayed in the **Quality Check** tab.

The data file is added to the **Runs** page, and appears in the **Recents** tab.

Options during analysis of results

Update the page layout

The **Customize Page Layout** dialog box has two sides. The left side displays the options that are available. The right side displays the layout.

1. In the **Quality Check** tab or analysis module tab of an open run file, click **Actions** ▸ **Page Layout Setting**.
2. In the **Customize Page Layout** dialog box, drag an item from the left side to the right side in order to display the item.
3. Click and drag an item on the right side to arrange the display.
4. *(Optional)* Click **Reset to Default** to display the items according to the default.
5. Click **Save**.

Change the view of the results tables

1. In any table pane, click a column to sort by the parameter.
2. To sort by a subsequent column, press and hold control on the keyboard, then click the column. The order of the sorting is displayed with a number in the column.

3. To sort by a single column after sorting by multiple columns, click a column without pressing control.

4. Click and drag a column to change the order that the columns are displayed in the table.

Edit the view of the plot

The view for all of the plots can be adjusted.

1. Use the zoom buttons to zoom in or out.
   - Click **(Zoom In)**.
   - Click **(Zoom Out)**.

2. Click **(More Options) ▸ Reset Zoom**.

3. Click **(Drag)** to move the plot if you are zoomed in.

4. Click **(Select)** to select a data point on the plot.
   The single data plot is displayed on the plot. The corresponding item is highlighted in the plate layout pane and the table pane.

5. Click and drag a section of the data plot.
   The data within that section are displayed. The corresponding wells in the plate layout pane and the table pane are highlighted.

6. To revert the plot to display all data points, click a point in the plot that does not contain a curve.

7. Click **(Settings)** to update the display of the plot.
   The available settings vary, depending on the type of plot.
   - Plot title
   - Color by
   - Y value
     - ΔRn
     - Rn
   - Y scale
     - Log
     - Linear
   - Thickness of the lines
   - Maximum number of curves
     Setting a maximum number of curves allows the data to be displayed more quickly when there is a large number of samples and a large number of targets.

- Show
  - Legend
  - Cq mark

    If enabled, the location where the amplification curve crosses the threshold is marked. If the amplification curve does not cross the threshold mark, the Cq mark is not displayed.
  - Unselected

    If a single data point is selected to display in the plot, the remaining data plots are displayed in gray.
  - Tooltip

    The information about the data is displayed when you hover over the data in the plot.
  - Replicates of selected

    The plot from the data that is selected is displayed in the plot. The plots from any replicates associated with the selected data are also displayed.
  - Threshold

    The threshold is displayed on the plot.
  - Baseline

    The baseline is displayed on the plot.
- Edit the labels for the x-axis and the y-axis
- Automatically adjust the range that is displayed for the x-axis and the y-axis

  If the range is not set to be automatically adjusted, the minimum and maximum values are specified.

ΔRn is the magnitude of normalized fluorescence signal, relative to the baseline fluorescence, generated by the reporter at each cycle during the PCR amplification. It can be used to identify and examine irregular amplification. It can also be used to view the threshold values for the run.

Rn is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference, if a passive reference is used. It can be used to identify and examine irregular amplification. It can also be used to view the baseline values for the run.

8. *(Optional)* Click **(Settings)** ▸ **Reset Settings**.

Select by subarray

When viewing the **Quality Check** tab for data from the OpenArray™ Plate format, the option to view by subarray is provided.

1. In the **Quality Check** tab, in the plate layout pane, toggle **Select by Subarray** on.
2. Click the subarray of interest.
3. Select multiple subarrays, if required.
   - Use the control + click function to select multiple subarrays.
   - Use the click + drag function to select multiple subarrays.
4. To select subarrays and single cells, select the subarrays, toggle **Select by Subarray** off, then select single cells.

The items within the selected subarray or subarrays are highlighted in blue in the well table view. The plots display the results from the selected subarray or subarrays.

Review results in the Amplification Plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

For more information about the **Amplification Plot**, see “Amplification Plot overview” on page 207.

Evaluate the overall shape of the curves in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Click ⚙ **(Settings)**, then make the following selections:
   - **Color By**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**
3. *(Optional)* Click ⚙ **(Settings)**, then select a value from the **Max Curves** dropdown list.
   The default value for the **Max Curves** dropdown list is **384 x 2**. If **All** is selected in the **Max Curves** dropdown list, it can take time to load all of the curves, especially for the OpenArray™ Plate format.
4. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
5. Review the overall shape of the curves in the amplification plot.
   For more information about the amplification plot, see “Amplification Plot overview” on page 207.

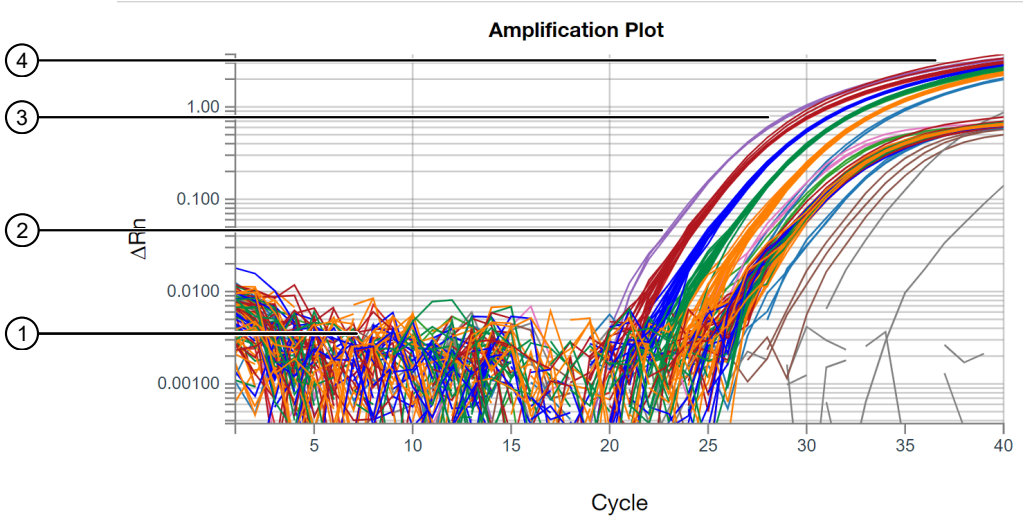


Figure 1 Typical amplification plot A typical amplification curve has four distinct sections:

① Baseline
② Exponential (geometric) phase
③ Linear phase
④ Plateau phase

Review the amplification status for each well

Note: Amp Status is only applicable for analysis that includes a PCR stage.

In the **Quality Check** tab, in the **Well Table**, review the amplification status of each well.

The **Amp Status** column displays one of four values:

Amplification status value	Description
Amp	Target amplified.
No Amp	Target did not amplify.
Inconclusive	Unable to determine if amplification occurred. Review run data.
N/A	One of the following occurred. • The well was omitted from analysis. • Insufficient cycle number to determine if amplification occurred.

Review or edit threshold settings in the amplification plot

The default analysis setting is for automatic threshold. To set the threshold manually, see "View or edit Cq settings" on page 144.

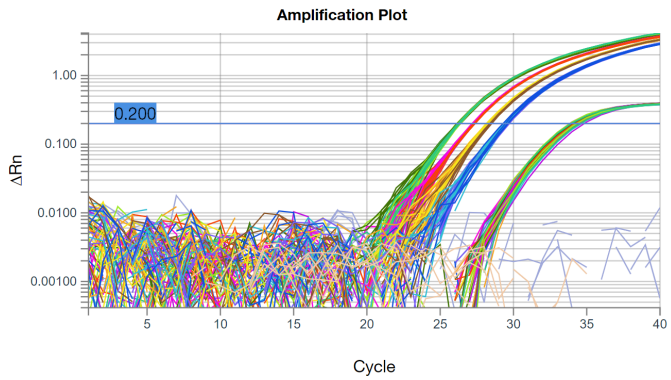
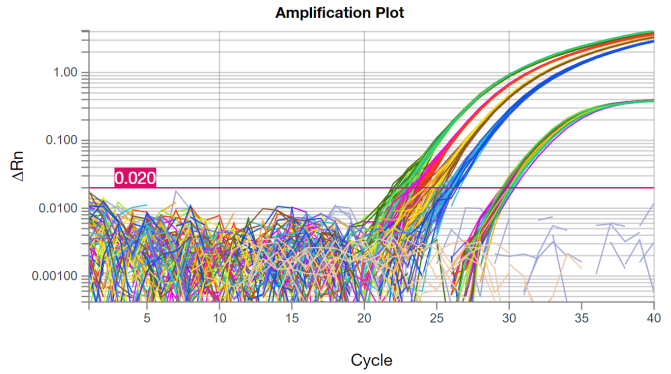
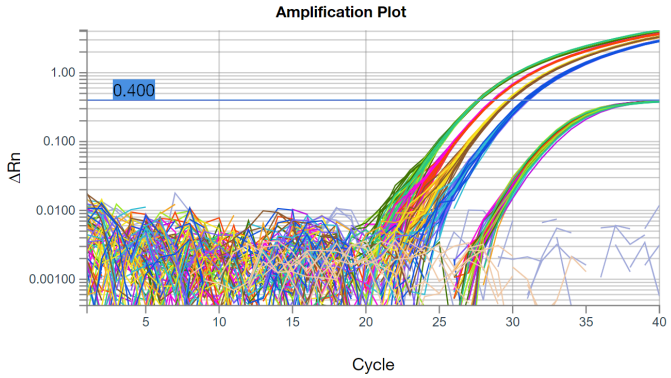
The threshold values can be edited only if the baseline threshold is selected as the algorithm (see "View or edit Cq settings" on page 144).

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Click **(Settings)**, then make the following selections:
   - **Plot Color**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**

   The amplification plot is displayed for all wells.
3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

4. Review the threshold values to determine if editing is necessary. A threshold set above or below the optimum can increase the standard deviation of the replicate groups.

Table 1 Examples of threshold settings

Threshold setting evaluation	Example
Threshold set correctly.	
Threshold set too low.	
Threshold set too high.	

5. (*Optional*) Adjust the threshold in the exponential phase of the amplification curve.

Note: For easier viewing, ensure that the **Y Scale** is set to log (default), not linear.

- Click-drag the threshold bar into the exponential phase of the curve.
- Edit the C_q analysis settings (see “View or edit Cq settings” on page 144).

Review or edit baseline settings in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections:
   - **Y Value**: **Rn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
   - **Baseline**

   The baseline can be selected only if the baseline threshold is selected as the algorithm (see "View or edit Cq settings" on page 144).

   Note: The start and end cycles are used to calculate the baseline.

   The amplification plot is displayed for the selected wells in the **Plate Layout** pane.
   The start and end cycles display for each well.
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. (*Optional*) Adjust the start and end cycle values for the baseline (see "View or edit Cq settings" on page 144).

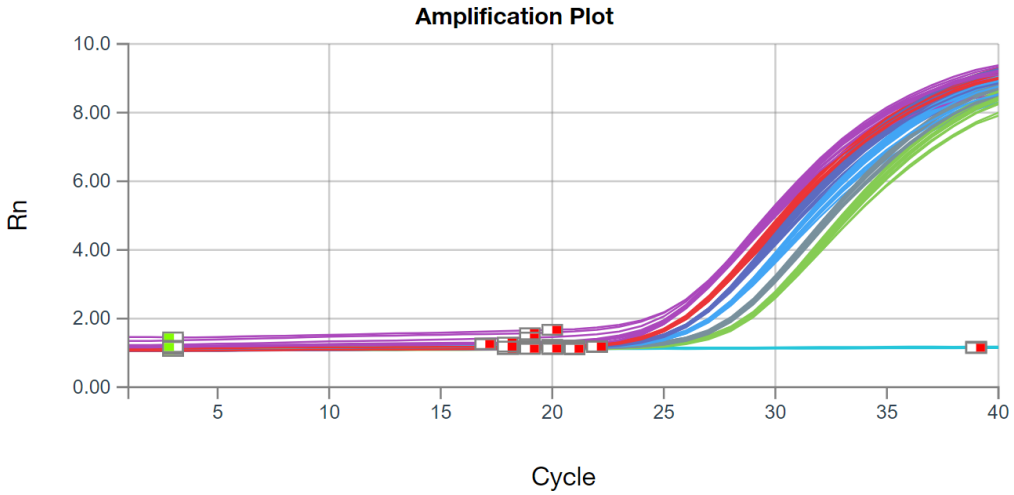


Figure 2 Example of correct baseline Set the end cycle a few cycles before the cycle number where significant fluorescence signal is detected.

Optimize display of negative controls in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Target**
   - Deselect **Show: Threshold**
   - Deselect **Show: Baseline**
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. In either the **Plate Layout** or **Well Table**, select the negative control wells (wells that should not have amplification for a particular target).
5. In the plot pane, click ⚙ **(Settings)**, then make the following selections in the **Y Axis** tab.
   a. Deselect **Auto-adjust range**.
   b. Enter **Minimum value** of –1.
   c. Enter **Maximum value** of 2.

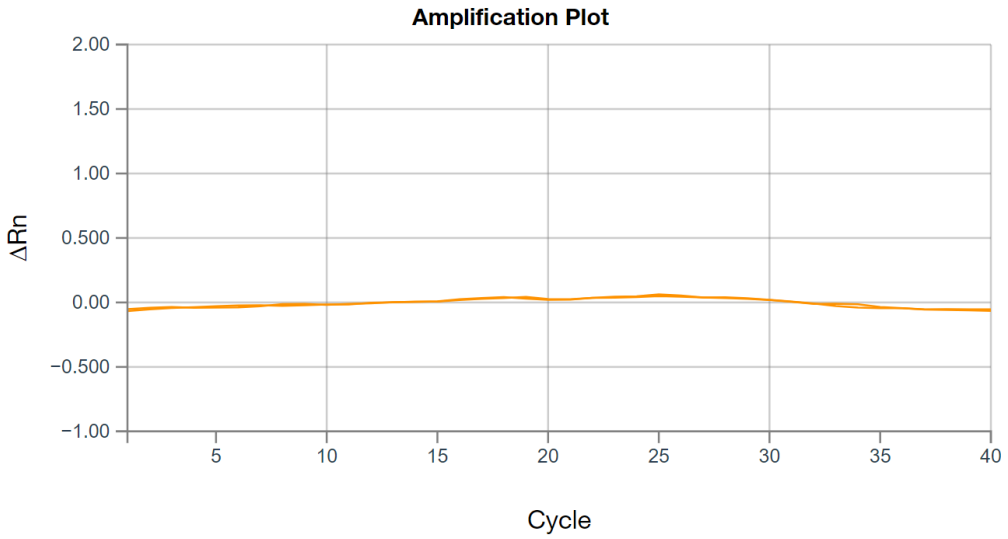


Figure 3 Example amplification plot of negative controls The linear plot displays the amplification plot for negative controls as smooth lines. The expanded y-axis displays low levels of amplification.

Identify and omit outliers from analysis

Outlier wells have C_q values that differ significantly from the average for the associated replicate wells. To support C_q precision, consider omitting the outliers from analysis.

1. In the **Quality Check** tab, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click **(Settings)**, then make the following selections to configure the plot:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
4. To identify outliers in the **Plate Layout**, select **Cq** from the dropdown list.
   The C_q values for each well are color-coded according to the value.
5. Omit outliers.
   - In the **Well Table**, select **Omit** in the row of the outlier well.
   - In the **Plate Layout**, select a well or multiple wells, then select ··· **(More Options)** ▸ **Omit Wells**.
   - In the amplification plot, click and drag around the data to omit. The selected data are displayed in the **Well Table** and the **Plate Layout**. Omit the wells in the **Well Table** or the **Plate Layout**.
6. Click **Analyze** to reanalyze the run data with any outliers removed.

Review results in the multicomponent plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Click **(Settings)**, then select **Dye** from the **Color By** dropdown list.
   The **Multicomponent Plot** is displayed for all wells.
3. (*Optional*) To edit the dyes that are displayed in the plot, click **Dyes**, then select dyes from the dropdown list.
4. *(Optional)* Click **(Settings)**, then select a value from the **Max Curves** dropdown list.
   The default value for the **Max Curves** dropdown list is **384 x 2**. If **All** is selected in the **Max Curves** dropdown list, it can take time to load all of the curves, especially for the OpenArray™ Plate format.
5. *(Optional)* To show or hide the background grid in the multicomponent plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

6. In the **Plate Layout**, select wells one at a time, then examine the **Multicomponent Plot** for the following plot characteristics.

Plot characteristic	Description
Passive reference dye	The passive reference dye fluorescence signal should remain relatively constant throughout the PCR process.
Reporter dye	The reporter dye fluorescence signal should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
Irregularities in the signal	Spikes, dips, and/or sudden changes in the fluorescence signal may have an impact on the data.
Negative control wells	The negative control wells should show no significant increase in fluorescence signal.

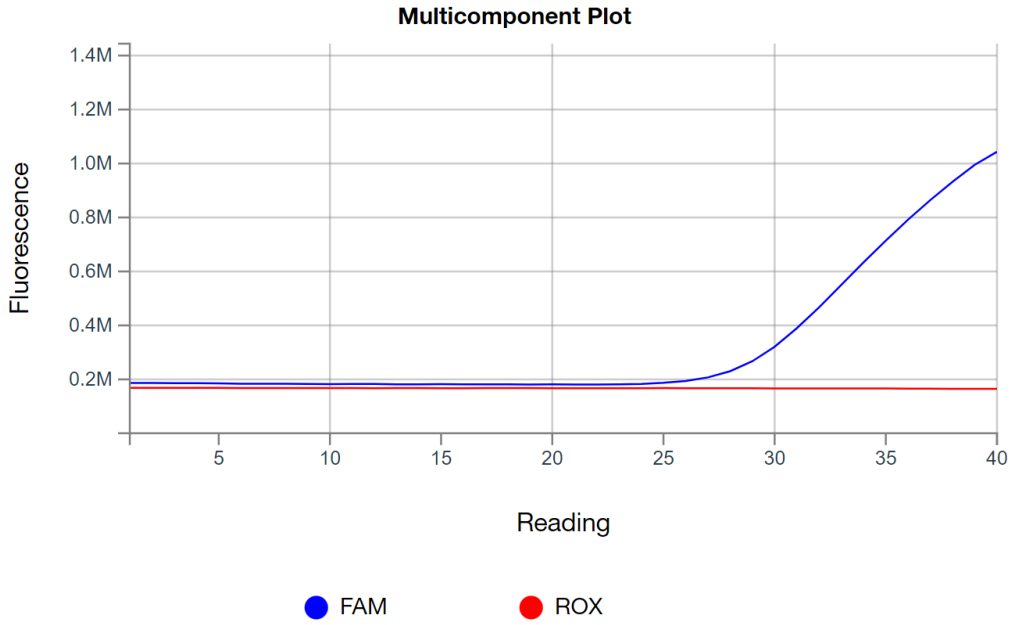


Figure 4 Example multicomponent plot (single well)

Review results in the raw data plot

For more information about the raw data plot, see “Raw Data Plot overview” on page 208.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.
2. *(Optional)* To show or hide the background grid in the raw data plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
3. Click-drag the **Cycle Number** slider from cycle 1 to cycle 40, then confirm that each filter displays the characteristic signal increase.

   For more information on each filter set, see the instrument documentation.

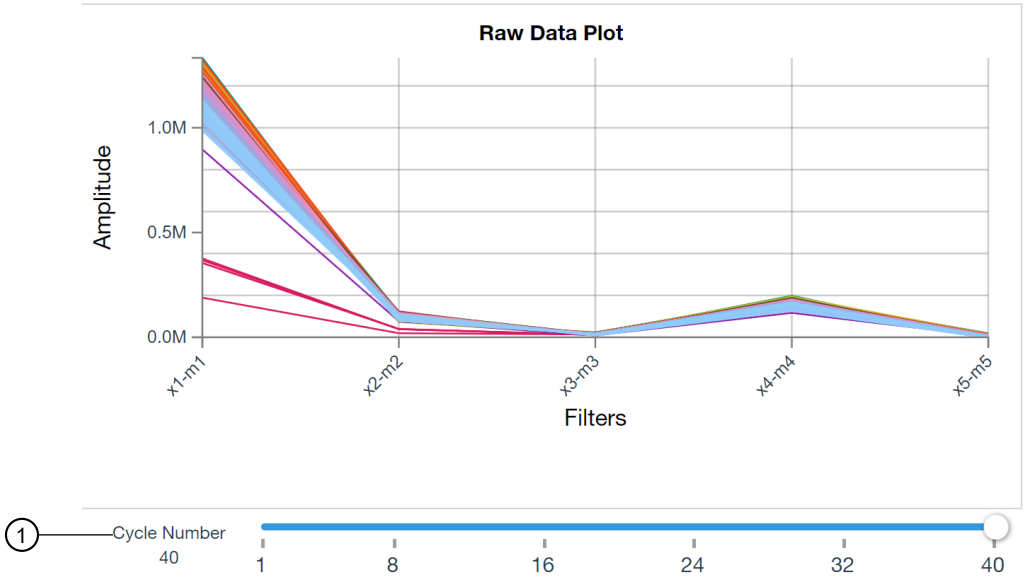


Figure 5 Example Raw Data Plot

① Slider to select the cycle

Review results in the melt curve plot

For custom experiments with more than one melt curve stage. For more information about the melt curve plot and the melt peak parity factor, see “Melt Curve Plot overview” on page 209.

For analysis with more than one melt curve stage, select the melt curve stage to analyze in the analysis settings (see “View or edit melt analysis settings” on page 148).

1. In the **Quality Check** tab, in the plot pane, select **Melt Curve Plot** from the dropdown list.
2. In the plot pane, click **(Settings)**, then make the following selections:
   - **Color By**: **Sample**, **Target**, or **Well**
   - **Plot Type**: **Derivative**
3. *(Optional)* To show or hide the background grid in the melt curve plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
4. Review the plot for evidence of unexpected multiple peaks, which can indicate non-specific amplification or formation of primer-dimers.
5. Review the **Well Table** for the calculated T_m in each well.

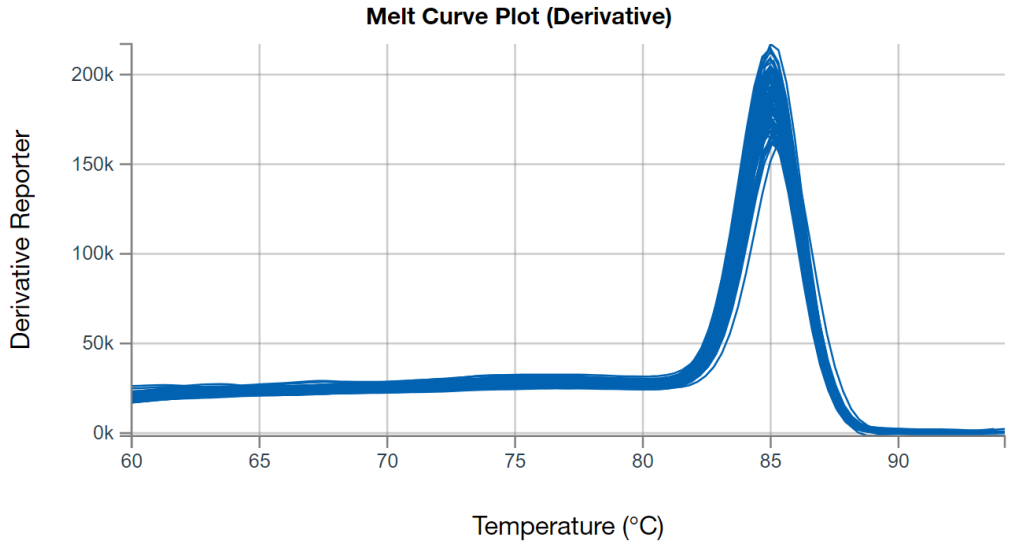


Figure 6 Example Melt Curve Plot

Mark wells

Wells can be marked in an individual run file. Wells can also be marked in individual run files within a project.

1. In the **Quality Check** tab, select the wells to mark.
   - In the **Well Table** pane, select one row or multiple rows.
   - In the plate layout pane, select one well or multiple wells.

   Use control-click to select multiple rows or multiple wells.

2. In the **Well Table** pane, perform one of the following actions.

Action	Description
Click ··· **(More Options)** ▸ **Mark Cq as Undetermined**.	The Cq value is changed to undetermined.
Click ··· **(More Options)** ▸ **Mark as Amp**.	The amplification status is changed to amplification. If the Cq value was edited to be undetermined by marking, the Cq value is reverted back to its original value.
Click ··· **(More Options)** ▸ **Mark as No Amp**.	The Cq value is changed to undetermined and the amplification status is change to no amplification.

   A well that has been marked displays a checkmark in the **Annotated** column of the table.

3. To remove marks, select one well or multiple wells, then click ··· **(More Options)** ▸ **Clear Marks**.

Review QC alerts in the well table

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, review the **Well Table** for alerts in the following columns.
   - **Curve Quality** column
   - **Result Quality Issues** column

   For more information about the QC alerts, see "Overview of the result quality checks" on page 204.
2. Adjust the QC alert settings as needed (see "View or edit QC alerts settings" on page 146), then reanalyze.

Review results in the plate layout

1. In the **Quality Check** tab, in the **Plate Layout** pane, select one of the following from the **Color By** dropdown list:
   - **Sample**
   - **Target**
   - **C_q**
   - **C_q Confidence**
   - **Amp Score**
   - **Amp Status**
2. Review the results for each well (see "Acceptance criteria for result quality checks" on page 205).

Edit primary analysis settings

Primary analysis settings include:

- C_q settings
- Melt settings
- QC settings

We recommend that you analyze data with the default analysis settings. If the default analysis settings are not appropriate for the data, modify the analysis settings, then reanalyze the data.

View or edit C_q settings

- The default C_q settings are appropriate for most applications. Edit the threshold and baseline settings for analysis of atypical or unexpected run data.
- For information about C_q analysis, see "About the quantification cycle (Cq)" on page 201.
- For information about C_q settings, see "Cq settings overview" on page 202.

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **General** tab, select an option from the **PCR Stage/Step** dropdown list.
3. Select an option from the **Algorithm Settings** dropdown list.
   - **Relative Threshold**
   - **Baseline Threshold**
4. *(For relative threshold algorithm settings)* Select the PCR stage and step from the **PCR Stage/Step** dropdown list.
5. *(For relative threshold algorithm settings)* Enter a start cycle in the **Default C_{RT} Start Cycle** field.
6. *(For baseline threshold algorithm settings)* To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.
7. *(For baseline threshold algorithm settings)* To edit the settings, make the edits in the appropriate table row.

   One table row corresponds to the default setting. Each additional table row corresponds to a target.

Option	Action
Use Auto Threshold	Select the checkbox in the **Auto Threshold** column.
Manually set the Threshold	Deselect the checkbox in the **Auto Threshold** column, then edit the value in the **Threshold** column.
Use Auto Baseline	Select the checkbox in the **Auto Baseline** column. • To specify the Baseline Start cycle, Click **AUTO** in the **Baseline Start** column, then enter the cycle number. The software will automatically determine the Baseline end cycle. • To remove the specified Baseline Start cycle, click the cycle number, then delete it. The **Baseline Start** will revert back to **AUTO**.
Manually set the Baseline	Deselect the checkbox in the **Auto Baseline** column, then edit the values in the **Baseline Start** field and the **Baseline End** field.

8. (*Optional*) In the **Well Cq** tab, make the edits in the appropriate table row to apply custom C_q settings to a specific well.
9. Click **Save**.
10. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit QC alerts settings

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **QC Alerts** tab, review selections:
   - Curve Quality—(*default*) inactive
   - Results Quality—(*default*) active

   For more information about curve quality and results quality, see “Quality checks” on page 204.
3. (*Optional*) Select the **Curve Quality** checkbox.
4. (*Optional*) Set up the acceptance criteria for the result quality checks.
   a. Ensure that the **Results Quality** checkbox is selected.
   b. Select **Results Quality**.
   c. Click **+ (Add)**.
   d. Select the **Sample Type** from the dropdown list, then select the **Target** from the dropdown list.

      If a rule is set up for a specific sample type, it takes precedence over a rule that is set for all samples.
   e. In the right panel, select the acceptance criteria for each sample type and target combination (see “Acceptance criteria for result quality checks” on page 205).
   f. (*Optional*) Click **✕ (Remove)** to remove a sample type and target combination from the table.
5. Click **Save**.
6. (*Optional*) To reset to the default settings, click **Reset to Default**.

In the **Quality Check** tab, click **Analyze**, then review the QC alerts in the **Well Table**.

View or edit advanced settings

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Advanced** tab, enter a value in the **Use a variant of primary analysis algorithm via plugin** field.
3. Select or deselect the **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox.

   The **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox is deselected by default.

4. If the **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox is selected, enter a value in the field.

   Any curves with the ΔR_n below the threshold are set to non-amplified. This is regardless of whether there is a C_q value.

5. Select the **Reduce dye signal crosstalk by algorithm**.

   Note: Selecting this option can slow down the analysis.

   The algorithm is not available for the OpenArray™ Plate format.

   For a description of this setting, see "Overview of the algorithm to reduce dye signal cross-talk" on page 147.

6. Click **Update** to upload a custom configuration file for the algorithm to reduce dye signal crosstalk.

   Note: A custom configuration file must be obtained from Thermo Fisher Scientific.

   A default configuration file is included in the primary analysis plugin of the software.

   A configuration file is in the CFG file format.

   If a custom configuration file is uploaded, the name of the configuration file is displayed in the dialog box.

7. If a custom configuration file was uploaded, click **Reset** to return to the default configuration file.

   The custom configuration file is removed.

8. Click **Save**.

9. (*Optional*) To reset to the default settings, click **Reset to Default**.

Overview of the algorithm to reduce dye signal cross-talk

Dye signal cross-talk can be observed when the fluorescent signal of an assay does not align with the spectrum of the corresponding dye calibration.

An algorithm is available to reduce the dye signal cross-talk. Using default primary analysis settings, this algorithm is inactive. It can be enabled in the **Advanced** tab.

The algorithm does not correct signal saturation.

The use of the algorithm slows down the analysis.

The impact and effectiveness of this algorithm must be assessed for individual assays.

This algorithm cannot be used for the OpenArray™ Plate format.

The algorithm is not available if any of the following conditions apply:

- The algorithm in the primary analysis plugin is different than what was used for the primary analysis of the data file.
- The configuration file was updated between the time of data analysis and the that the algorithm would be applied.

If the algorithm is applied, the data must be reanalyzed if there are changes to the dyes in the plate setup or if calibrations from another file are used. The reanalysis applies only to the edited wells.

View or edit melt analysis settings

For descriptions of the melt analysis settings, see “Melt analysis settings overview” on page 203.

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Melt** tab, select an option from the **Melt Stage/Step** dropdown list.
3. In the **Multi-Peak Calling** column, select the checkbox.
   The threshold type, peak level, and peak height settings are available only when multi-peak calling is enabled.
4. In the **Threshold Type** column, select one of the following options.
   - **Percentage**
   - **Height**
5. In the **Peak Level (%)** column, enter a value.
   A value can be entered only if **Percentage** was selected in step 4.
6. In the **Peak Height** column, enter a value.
   A value can be entered only if **Height** was selected in step 4.
7. *(Optional)* In the **Melt Peak Parity Factor** field, enter a factor for the applicable targets.

   Note: If the melt peak parity factor is applied to wells without a reaction mix, this can lead to unexpected T_m results.
   A factor of ***0*** turns off the feature. Negative values are not permitted.
   The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.
8. Click **Save**.
9. (*Optional*) To reset to the default settings, click **Reset to Default**.

Use the analysis settings from another file

Apply analysis settings from a plate file or data file to an open data file. If you are analyzing a legacy data file (see “Compatible data files” on page 16), you can use this feature to apply updated analysis settings to the legacy data file before analysis.

The following analysis settings are applied to the data file:

- Primary analysis settings
- Analysis module analysis settings

Applying analysis settings from another file is not available for the OpenArray™ Plate format.

Note: You can only apply analysis settings from another file if the analysis settings are compatible with the new file. Confirm the following before applying analysis settings to the new file:

- The run method consists of the same stages in both files (PCR stage, Melt stage, Pre-Read stage and Post-Read stage).
- The selected analysis module is the same in both files.
- The sample and target/SNP assay information is the same in both files. Because some analysis settings are specific to samples and target/SNP assay setup, remove any sample and target/SNP assay information that is not applicable to the new data file.

1. Open a data file, then click **Actions** ▸ **Use Settings from Another File**.
2. Navigate to the plate file or data file that contains the desired analysis settings.
3. Select the file, then click **Open**.
   The data is reanalyzed using the new analysis settings.
4. Click **Actions** ▸ **Save** to save the new analysis settings to the data file.

View instrument calibration results

Transfer calibration data files from the instrument to a location that is accessible by the software. For more information about instrument calibration, see the instrument documentation.

Calibration results from the OpenArray™ Plate format are not available.

In some cases, you can use calibration data from another instrument for analysis of your data file. For more information, see “Use the calibrations from another file” on page 150.

1. Select the **Dashboard** tab, then click **Open File**.
2. Navigate to the location of the calibration data files were transferred from the instrument, then select the calibration data file.

The calibration data file is opened, and calibration results are displayed.

Review ROI/Uniformity calibration results

1. In the **ROI** tab, select a **Filter Set** from the dropdown list to see the corresponding results.
2. In the **Uniformity** tab, review results in the plot, the **Well Table**, or the **Plate Layout**.

Review Background calibration results

1. Review the calibration properties, including calibration status, in the menu bar.
2. Select the plate wells in the **Plate Layout** or the **Well Table** to view the corresponding curves.
3. Review data in the **Well Table**.
   a. Review the results for each well in tabular format.

b. Sort the wells according to well or normalized fluorescence with each filter.

c. Select wells to review data in the analysis plot.

Review Dye calibration results

1. Review the calibration properties, including calibration status, in the menu bar.
2. Select a Dye row in the **Calibration** table to view the corresponding analysis data plot.
3. Select the plate wells in the **Plate Layout** or the **Well Table** to view the corresponding curves in the plot.
4. Review data in the **Well Table**.
   a. Review the results for each well in tabular format.
   b. Sort the wells according to well or normalized fluorescence with each filter.
   c. Select wells to review data in the analysis plot.

Use the calibrations from another file

The use of calibrations from another file is not available for the OpenArray™ Plate format.

The calibrations must be from a run on the same instrument type and the same block type. The calibrations must contain all of the applicable types of calibration for the instrument and block type.

The original calibrations are retained in the data file. You can revert back to the original calibrations.

Only one additional set of calibrations can be retained in the data file, excluding the original calibrations.

If the calibrations are reverted back to the original ones, the different calibrations are not retained in the data file.

Calibration substitution is used to check for changes in performance after maintenance. Substituting a calibration allows you to determine if a change in performance is due to a new calibration or due to the assay.

1. Open a data file, then click **Actions ▸ Use Calibrations From Another File**.
2. Navigate to the location of the calibration file.
3. Select the file, then click **Open**.
   The data are reanalyzed with the new calibrations.
4. *(Optional)* Click **Actions ▸ Revert to Original Calibrations**.
   The data are reanalyzed with the original calibrations.

Perform additional analysis

Perform additional analysis using the analysis modules.

Select an analysis module (see “Select an analysis module (single plate)” on page 191).

For more information about analysis modules, see “About analysis modules” on page 190.

9 Review and analyze a project

Workflow: Analyze the data for a project

The software automatically analyzes run data using the analysis settings that are specified during the project template setup or the project setup. The software then displays analysis results in the **Quality Check** tab.

Primary analysis is performed individually for each data file in the project. Secondary analysis is then performed for all of the data files in the project.

Replicates across different plates are not consolidated during secondary analysis. The replicates are analyzed separately.

Analyze the data for a project
Open a project (page 50)
Perform analysis for a project (page 155)
Review results in the Amplification Plot (page 156)
Identify and omit outliers from analysis (page 161)
Review results in the multicomponent plot (page 161)
Review results in the raw data plot (page 162)
Review QC alerts in the well table (page 163)
Review results in the plate layout (page 163)
Edit the primary analysis settings for a project (page 164)

Analyze the data for a project

Perform secondary analysis

See “Perform additional analysis” on page 168.

Options during analysis of results

Update the page layout

The **Customize Page Layout** dialog box has two sides. The left side displays the options that are available. The right side displays the layout.

In a project, the top pane of each analysis module tab is fixed. The top pane cannot be removed from the page layout and cannot moved on the page layout.

1. In the **Quality Check** tab or analysis module tab of an open run file or open project file, click **Actions** ▸ **Page Layout Setting**.
2. In the **Customize Page Layout** dialog box, drag an item from the left side to the right side in order to display the item.
3. Click and drag an item on the right side to arrange the display.
4. *(Optional)* Click **Reset to Default** to display the items according to the default.
5. Click **Save**.

Change the view of the results tables

1. In any table pane, click a column to sort by the parameter.
2. To sort by a subsequent column, press and hold control on the keyboard, then click the column. The order of the sorting is displayed with a number in the column.
3. To sort by a single column after sorting by multiple columns, click a column without pressing control.
4. Click and drag a column to change the order that the columns are displayed in the table.

Edit the view of the plot

The view for all of the plots can be adjusted.

1. Use the zoom buttons to zoom in or out.
   - Click **(Zoom In)**.
   - Click **(Zoom Out)**.

2. Click ··· **(More Options)** ▸ **Reset Zoom**.
3. Click **(Drag)** to move the plot if you are zoomed in.
4. Click **(Select)** to select a data point on the plot.
   The single data plot is displayed on the plot. The corresponding item is highlighted in the plate layout pane and the table pane.
5. Click and drag a section of the data plot.
   The data within that section are displayed. The corresponding wells in the plate layout pane and the table pane are highlighted.
6. To revert the plot to display all data points, click a point in the plot that does not contain a curve.
7. Click **(Settings)** to update the display of the plot.
   The available settings vary, depending on the type of plot.
   - Plot title
   - Color by
   - Y value
     - ΔRn
     - Rn
   - Y scale
     - Log
     - Linear
   - Thickness of the lines
   - Maximum number of curves
     Setting a maximum number of curves allows the data to be displayed more quickly when there is a large number of samples and a large number of targets.
   - Show
     - Legend
     - Cq mark
       If enabled, the location where the amplification curve crosses the threshold is marked. If the amplification curve does not cross the threshold mark, the Cq mark is not displayed.
     - Unselected
       If a single data point is selected to display in the plot, the remaining data plots are displayed in gray.
     - Tooltip
       The information about the data is displayed when you hover over the data in the plot.
     - Replicates of selected
       The plot from the data that is selected is displayed in the plot. The plots from any replicates associated with the selected data are also displayed.

  - Threshold
    The threshold is displayed on the plot.
  - Baseline
    The baseline is displayed on the plot.
- Edit the labels for the x-axis and the y-axis
- Automatically adjust the range that is displayed for the x-axis and the y-axis
  If the range is not set to be automatically adjusted, the minimum and maximum values are specified.

ΔRn is the magnitude of normalized fluorescence signal, relative to the baseline fluorescence, generated by the reporter at each cycle during the PCR amplification. It can be used to identify and examine irregular amplification. It can also be used to view the threshold values for the run.

Rn is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference, if a passive reference is used. It can be used to identify and examine irregular amplification. It can also be used to view the baseline values for the run.

8. *(Optional)* Click ⚙ **(Settings)** ▸ **Reset Settings**.

Select by subarray

When viewing the **Quality Check** tab for data from the OpenArray™ Plate format, the option to view by subarray is provided.

1. In the **Quality Check** tab, in the plate layout pane, toggle **Select by Subarray** on.
2. Click the subarray of interest.
3. Select multiple subarrays, if required.
   - Use the control + click function to select multiple subarrays.
   - Use the click + drag function to select multiple subarrays.
4. To select subarrays and single cells, select the subarrays, toggle **Select by Subarray** off, then select single cells.

The items within the selected subarray or subarrays are highlighted in blue in the well table view. The plots display the results from the selected subarray or subarrays.

Perform analysis for a project

If the project has been analyzed, the ✅ is displayed on the **Analyze** button.

If you hover over the **Analyze** button, the analysis modules that were used are displayed, including primary analysis.

If the project has not been analyzed or the analysis settings have been updated, the ⚠ icon is displayed on the **Analyze** button.

In an open project, click **Analyze**.

Review the information on the **Quality Check** page (see “Review QC alerts in the well table” on page 163).

Review results in the Amplification Plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

For more information about the **Amplification Plot**, see “Amplification Plot overview” on page 207.

Evaluate the overall shape of the curves in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Click **(Settings)**, then make the following selections:
   - **Color By**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**
3. *(Optional)* Click **(Settings)**, then select a value from the **Max Curves** dropdown list.
   The default value for the **Max Curves** dropdown list is **384 x 2**. If **All** is selected in the **Max Curves** dropdown list, it can take time to load all of the curves, especially for the OpenArray™ Plate format.
4. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
5. Review the overall shape of the curves in the amplification plot.
   For more information about the amplification plot, see “Amplification Plot overview” on page 207.

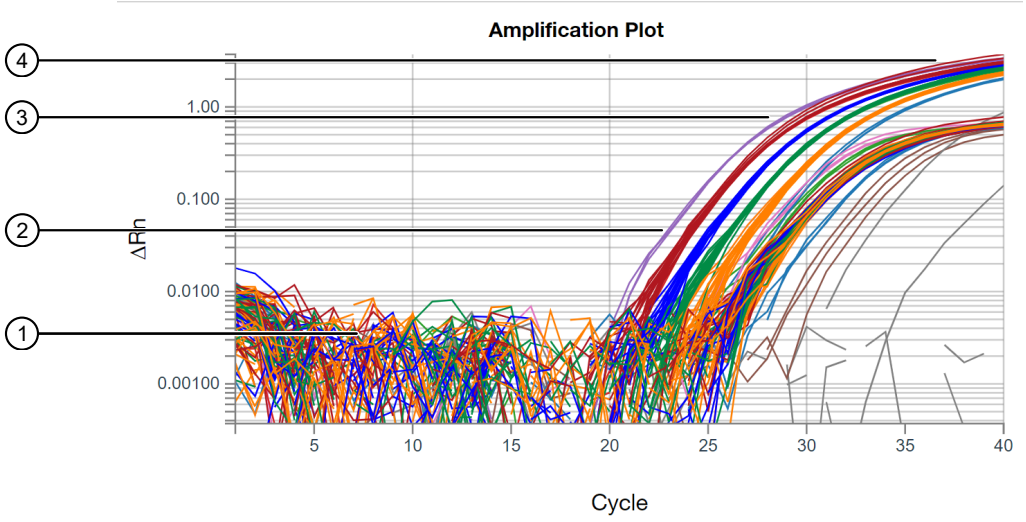


Figure 7 Typical amplification plot A typical amplification curve has four distinct sections:

① Baseline
② Exponential (geometric) phase
③ Linear phase
④ Plateau phase

Review the amplification status for each well

Note: Amp Status is only applicable for analysis that includes a PCR stage.

In the **Quality Check** tab, in the **Well Table**, review the amplification status of each well.

The **Amp Status** column displays one of four values:

Amplification status value	Description
Amp	Target amplified.
No Amp	Target did not amplify.
Inconclusive	Unable to determine if amplification occurred. Review run data.
N/A	One of the following occurred. • The well was omitted from analysis. • Insufficient cycle number to determine if amplification occurred.

Review or edit threshold settings in the amplification plot

The default analysis setting is for automatic threshold. To set the threshold manually, see “View or edit Cq settings” on page 144.

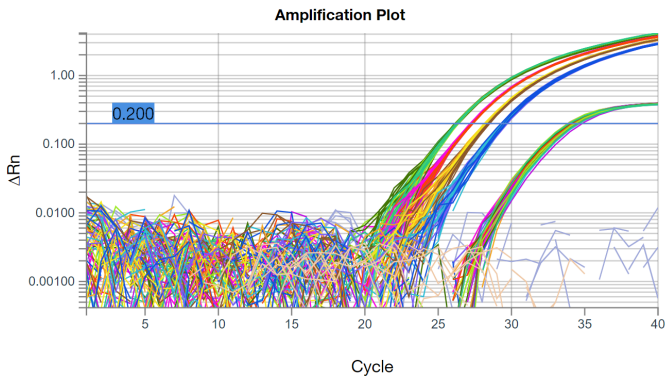
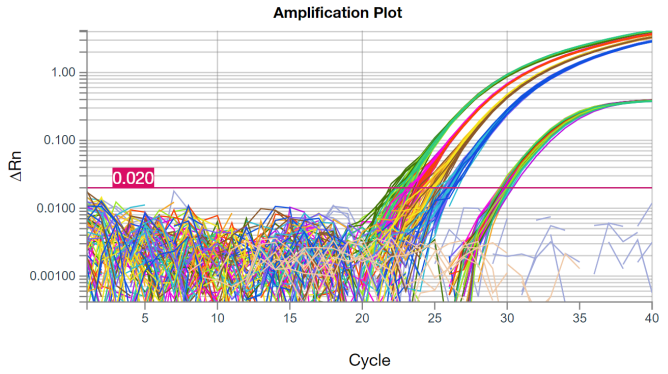
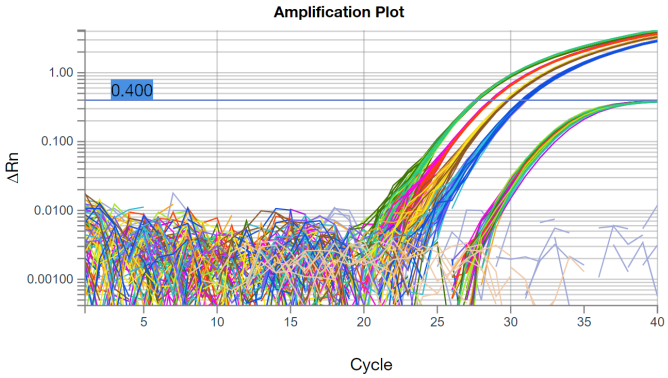
The threshold values can be edited only if the baseline threshold is selected as the algorithm (see “View or edit Cq settings” on page 144).

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Click **(Settings)**, then make the following selections:
   - **Plot Color**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**

   The amplification plot is displayed for all wells.
3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

4. Review the threshold values to determine if editing is necessary. A threshold set above or below the optimum can increase the standard deviation of the replicate groups.

Table 2 Examples of threshold settings

Threshold setting evaluation	Example
Threshold set correctly.	
Threshold set too low.	
Threshold set too high.	

5. (*Optional*) Adjust the threshold in the exponential phase of the amplification curve.

Note: For easier viewing, ensure that the **Y Scale** is set to log (default), not linear.

- Click-drag the threshold bar into the exponential phase of the curve.
- Edit the C_q analysis settings (see “View or edit Cq settings” on page 144).

Review or edit baseline settings in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click **(Settings)**, then make the following selections:
   - **Y Value**: **Rn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
   - **Baseline**

   The baseline can be selected only if the baseline threshold is selected as the algorithm (see "View or edit Cq settings" on page 144).

   Note: The start and end cycles are used to calculate the baseline.

   The amplification plot is displayed for the selected wells in the **Plate Layout** pane.
   The start and end cycles display for each well.
3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
4. (*Optional*) Adjust the start and end cycle values for the baseline (see "View or edit Cq settings" on page 144).

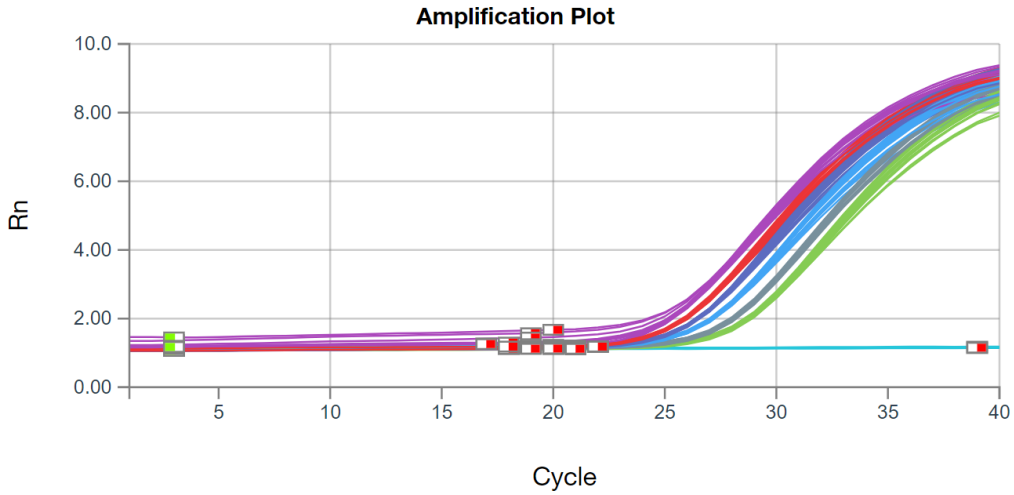


Figure 8 Example of correct baseline Set the end cycle a few cycles before the cycle number where significant fluorescence signal is detected.

Optimize display of negative controls in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Target**
   - Deselect **Show: Threshold**
   - Deselect **Show: Baseline**
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. In either the **Plate Layout** or **Well Table**, select the negative control wells (wells that should not have amplification for a particular target).
5. In the plot pane, click ⚙ **(Settings)**, then make the following selections in the **Y Axis** tab.
   a. Deselect **Auto-adjust range**.
   b. Enter **Minimum value** of –1.
   c. Enter **Maximum value** of 2.

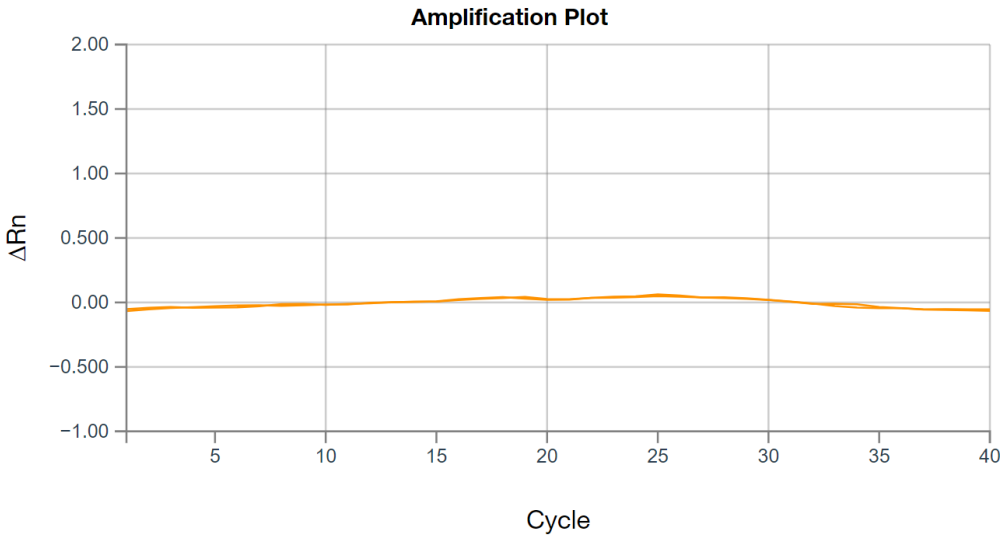


Figure 9 Example amplification plot of negative controls The linear plot displays the amplification plot for negative controls as smooth lines. The expanded y-axis displays low levels of amplification.

Identify and omit outliers from analysis

Outlier wells have C_q values that differ significantly from the average for the associated replicate wells. To support C_q precision, consider omitting the outliers from analysis.

1. In the **Quality Check** tab, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections to configure the plot:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. To identify outliers in the **Plate Layout**, select **Cq** from the dropdown list.
   The C_q values for each well are color-coded according to the value.
5. Omit outliers.
   - In the **Well Table**, select **Omit** in the row of the outlier well.
   - In the **Plate Layout**, select a well or multiple wells, then select ··· **(More Options)** ▸ **Omit Wells**.
   - In the amplification plot, click and drag around the data to omit. The selected data are displayed in the **Well Table** and the **Plate Layout**. Omit the wells in the **Well Table** or the **Plate Layout**.
6. Click **Analyze** to reanalyze the run data with any outliers removed.

Review results in the multicomponent plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Click ⚙ **(Settings)**, then select **Dye** from the **Color By** dropdown list.
   The **Multicomponent Plot** is displayed for all wells.
3. (*Optional*) To edit the dyes that are displayed in the plot, click **Dyes**, then select dyes from the dropdown list.
4. *(Optional)* Click ⚙ **(Settings)**, then select a value from the **Max Curves** dropdown list.
   The default value for the **Max Curves** dropdown list is **384 x 2**. If **All** is selected in the **Max Curves** dropdown list, it can take time to load all of the curves, especially for the OpenArray™ Plate format.
5. *(Optional)* To show or hide the background grid in the multicomponent plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.

6. In the **Plate Layout**, select wells one at a time, then examine the **Multicomponent Plot** for the following plot characteristics.

Plot characteristic	Description
Passive reference dye	The passive reference dye fluorescence signal should remain relatively constant throughout the PCR process.
Reporter dye	The reporter dye fluorescence signal should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
Irregularities in the signal	Spikes, dips, and/or sudden changes in the fluorescence signal may have an impact on the data.
Negative control wells	The negative control wells should show no significant increase in fluorescence signal.

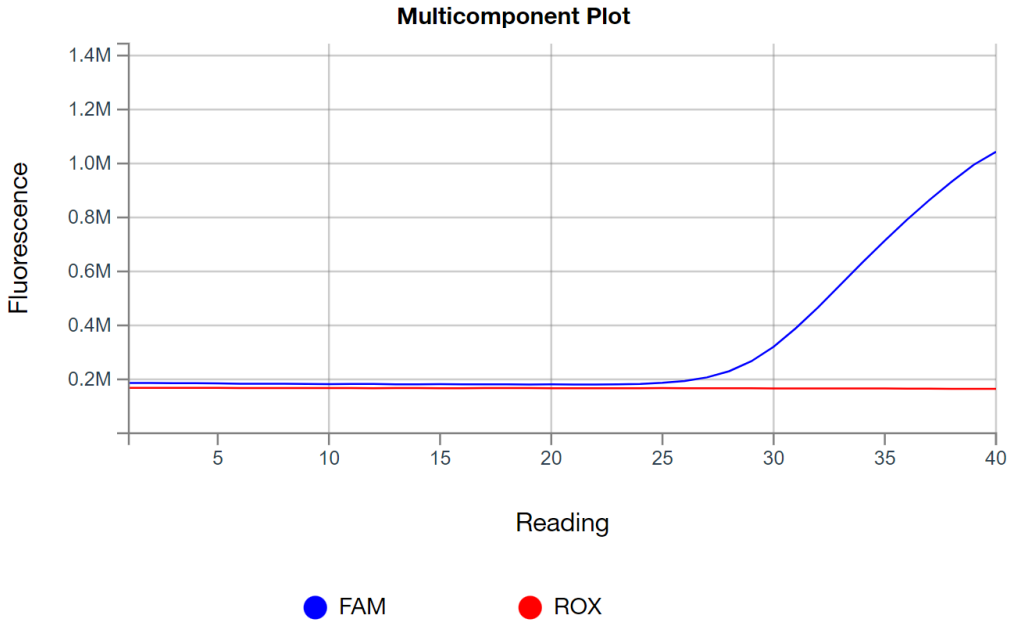


Figure 10 Example multicomponent plot (single well)

Review results in the raw data plot

For more information about the raw data plot, see “Raw Data Plot overview” on page 208.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.
2. *(Optional)* To show or hide the background grid in the raw data plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
3. Click-drag the **Cycle Number** slider from cycle 1 to cycle 40, then confirm that each filter displays the characteristic signal increase.

   For more information on each filter set, see the instrument documentation.

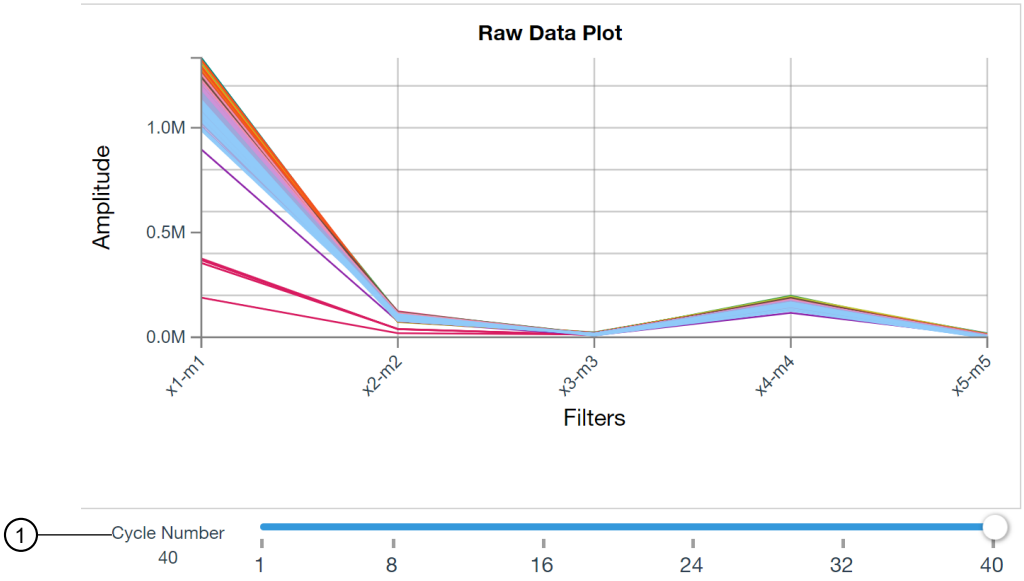


Figure 11 Example Raw Data Plot

① Slider to select the cycle

Review QC alerts in the well table

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, review the **Well Table** for alerts in the following columns.
   - **Curve Quality** column
   - **Result Quality Issues** column

   For more information about the QC alerts, see “Overview of the result quality checks” on page 204.

2. Adjust the QC alert settings as needed (see “View or edit QC alerts settings” on page 146), then reanalyze.

Review results in the plate layout

1. In the **Quality Check** tab, in the **Plate Layout** pane, select one of the following from the **Color By** dropdown list:
   - **Sample**
   - **Target**
   - **C_q**
   - **C_q Confidence**
   - **Amp Score**

- **Amp Status**

2. Review the results for each well (see “Acceptance criteria for result quality checks” on page 205).

Mark wells

Wells can be marked in an individual run file. Wells can also be marked in individual run files within a project.

1. In the **Quality Check** tab, select the wells to mark.
   - In the **Well Table** pane, select one row or multiple rows.
   - In the plate layout pane, select one well or multiple wells.

   Use control-click to select multiple rows or multiple wells.

2. In the **Well Table** pane, perform one of the following actions.

Action	Description
Click ··· **(More Options)** ▸ **Mark Cq as Undetermined**.	The Cq value is changed to undetermined.
Click ··· **(More Options)** ▸ **Mark as Amp**.	The amplification status is changed to amplification. If the Cq value was edited to be undetermined by marking, the Cq value is reverted back to its original value.
Click ··· **(More Options)** ▸ **Mark as No Amp**.	The Cq value is changed to undetermined and the amplification status is change to no amplification.

   A well that has been marked displays a checkmark in the **Annotated** column of the table.

3. To remove marks, select one well or multiple wells, then click ··· **(More Options)** ▸ **Clear Marks**.

Edit the primary analysis settings for a project

View or edit C_q settings

- The default C_q settings are appropriate for most applications. Edit the threshold and baseline settings for analysis of atypical or unexpected run data.
- For information about C_q analysis, see “About the quantification cycle (Cq)” on page 201.
- For information about C_q settings, see “Cq settings overview” on page 202.

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Primary Analysis Setting** dialog box, in the **Block Type** dropdown list, select a block type.
3. In the **General** tab, select an option from the **PCR Stage/Step** dropdown list.

4. Select an option from the **Algorithm Settings** dropdown list.
   - **Relative Threshold**
   - **Baseline Threshold**
5. *(For relative threshold algorithm settings)* Select the PCR stage and step from the **PCR Stage/Step** dropdown list.
6. *(For relative threshold algorithm settings)* Enter a start cycle in the **Default C_{RT} Start Cycle** field.
7. *(For baseline threshold algorithm settings)* To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.
8. *(For baseline threshold algorithm settings)* To edit the settings, make the edits in the appropriate table row.

   One table row corresponds to the default setting. Each additional table row corresponds to a target.

Option	Action
Use Auto Threshold	Select the checkbox in the **Auto Threshold** column.
Manually set the Threshold	Deselect the checkbox in the **Auto Threshold** column, then edit the value in the **Threshold** column.
Use Auto Baseline	Select the checkbox in the **Auto Baseline** column. • To specify the Baseline Start cycle, Click **AUTO** in the **Baseline Start** column, then enter the cycle number. The software will automatically determine the Baseline end cycle. • To remove the specified Baseline Start cycle, click the cycle number, then delete it. The **Baseline Start** will revert back to **AUTO**.
Manually set the Baseline	Deselect the checkbox in the **Auto Baseline** column, then edit the values in the **Baseline Start** field and the **Baseline End** field.

9. (*Optional*) In the **Well Cq** tab, make the edits in the appropriate table row to apply custom C_q settings to a specific well.
10. Click **Save**.
11. (*Optional*) To reset to the default settings, click **Reset to Default**.

View or edit QC alerts settings

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **QC Alerts** tab, review selections:
   - Curve Quality—(*default*) inactive
   - Results Quality—(*default*) active

   For more information about curve quality and results quality, see “Quality checks” on page 204.

3. (*Optional*) Select the **Curve Quality** checkbox.
4. (*Optional*) Set up the acceptance criteria for the result quality checks.
   a. Ensure that the **Results Quality** checkbox is selected.
   b. Select **Results Quality**.
   c. Click **+ (Add)**.
   d. Select the **Sample Type** from the dropdown list, then select the **Target** from the dropdown list.
      If a rule is set up for a specific sample type, it takes precedence over a rule that is set for all samples.
   e. In the right panel, select the acceptance criteria for each sample type and target combination (see "Acceptance criteria for result quality checks" on page 205).
   f. (*Optional*) Click **✕ (Remove)** to remove a sample type and target combination from the table.
5. Click **Save**.
6. (*Optional*) To reset to the default settings, click **Reset to Default**.

In the **Quality Check** tab, click **Analyze**, then review the QC alerts in the **Well Table**.

View or edit advanced settings

1. Open a plate file or data file, then click **Actions ▸ Primary Analysis Setting**.
2. In the **Advanced** tab, enter a value in the **Use a variant of primary analysis algorithm via plugin** field.
3. Select or deselect the **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox.
   The **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox is deselected by default.
4. If the **Set the Delta-Rn below which curves will be considered Non-Amplified** checkbox is selected, enter a value in the field.
   Any curves with the ΔR_n below the threshold are set to non-amplified. This is regardless of whether there is a C_q value.
5. Click **Save**.
6. *(Optional)* To reset to the default settings, click **Reset to Default**.

View or edit melt analysis settings

For descriptions of the melt analysis settings, see “Melt analysis settings overview” on page 203.

1. Open a plate file or data file, then click **Actions** ▸ **Primary Analysis Setting**.
2. In the **Melt** tab, select an option from the **Melt Stage/Step** dropdown list.
3. In the **Multi-Peak Calling** column, select the checkbox.
   The threshold type, peak level, and peak height settings are available only when multi-peak calling is enabled.
4. In the **Threshold Type** column, select one of the following options.
   - **Percentage**
   - **Height**
5. In the **Peak Level (%)** column, enter a value.
   A value can be entered only if **Percentage** was selected in step 4.
6. In the **Peak Height** column, enter a value.
   A value can be entered only if **Height** was selected in step 4.
7. *(Optional)* In the **Melt Peak Parity Factor** field, enter a factor for the applicable targets.

   Note: If the melt peak parity factor is applied to wells without a reaction mix, this can lead to unexpected T_m results.
   A factor of ***0*** turns off the feature. Negative values are not permitted.
   The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.
8. Click **Save**.
9. (*Optional*) To reset to the default settings, click **Reset to Default**.

Use the analysis settings from another file

Apply analysis settings from a plate file or data file to an open data file. If you are analyzing a legacy data file (see “Compatible data files” on page 16), you can use this feature to apply updated analysis settings to the legacy data file before analysis.

The following analysis settings are applied to the data file:

- Primary analysis settings
- Analysis module analysis settings

Applying analysis settings from another file is not available for the OpenArray™ Plate format.

Note: You can only apply analysis settings from another file if the analysis settings are compatible with the new file. Confirm the following before applying analysis settings to the new file:

- The run method consists of the same stages in both files (PCR stage, Melt stage, Pre-Read stage and Post-Read stage).
- The selected analysis module is the same in both files.
- The sample and target/SNP assay information is the same in both files. Because some analysis settings are specific to samples and target/SNP assay setup, remove any sample and target/SNP assay information that is not applicable to the new data file.

1. Open a data file, then click **Actions** ▸ **Use Settings from Another File**.
2. Navigate to the plate file or data file that contains the desired analysis settings.
3. Select the file, then click **Open**.
   The data is reanalyzed using the new analysis settings.
4. Click **Actions** ▸ **Save** to save the new analysis settings to the data file.

Perform additional analysis

Perform additional analysis using the analysis modules.

Select an analysis module (see “Select an analysis module (single plate)” on page 191).

For more information about analysis modules, see “About analysis modules” on page 190.

10 Export results

For information about export settings, see Chapter 15, “Manage export settings”.

Export the Well Table

1. In the **Quality Check** tab, in the **Well Table**, click ··· **(More Options)** ▸ **Export**.
2. Name the file, navigate to the desired folder location, then click **Export**.

Export plate layout as an Excel™ spreadsheet

To save an image of the plate layout, see “Export plate layout image” on page 169.

1. In the **Quality Check** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Export Plate View**.
2. In the **Export Plate View** dialog box, enter a file name in the **File Name** field.
3. Click **Browse**, then navigation to a location to save the file.
4. Select the values to include.
5. Click **Export**.

Export plate layout image

To save as an Excel™ spreadsheet, see “Export plate layout as an Excel™ spreadsheet” on page 169.

1. In the **Quality Check** tab, in the plate layout pane, click ··· **(More Options)** ▸ **Save Image**.
2. In the **Save Image** dialog box, enter a file name in the **File Name** field.
3. Select a file format.
   - **PNG** radio button
   - **SVG** radio button
4. Select a size from the **Size** dropdown list.
5. Click **Browse**, then navigation to a location to save the file.
6. Click **Save Image**.

Export images of plots

1. In the **Quality Check** tab, in the plot pane, select a plot from the dropdown list.
2. Click ··· **(More Options)** ▸ **Save Image**.
3. In the **Save Image** dialog box, enter a file name in the **File Name** field.
4. Click **Browse**, then navigation to a location to save the file.
5. Select a file format.
   - **PNG** radio button
   - **SVG** radio button
6. Select a size from the **Size** dropdown list.
7. Click **Save Image**.

Export data (single plate)

Export analyzed data for further analysis. The files that are exported cannot be used for additional analysis within Diomni™ Design and Analysis (RUO) Software 3.

Save the analyzed data file before exporting the results (see “Save a plate file or data file” on page 181).

1. In a data file, click **Actions** ▸ **Export**.
2. In the **Export Plate** dialog box, enter or select the following.
   - **Export Name**
   - **File Format**
   - **Destination**—Click browse to navigate to the location.
   - **Export Setting**—Select an export setting from the dropdown list, or edit the export settings (see “View or edit export settings” on page 193).
3. Click **Export**.

Export data (project)

Export analyzed data for further analysis. The files that are exported cannot be used for additional analysis within Diomni™ Design and Analysis (RUO) Software 3.

The data from multiple plates is combined into a single file when exporting data from a project. The multicomponent data and the raw data from multiple plates are not combined into a single file.

A file is exported for each data type. For example, a file is exported for amplification data, a file is exported for the results.

Save the analyzed project before exporting the results (see "Save a project" on page 62).

1. In a project, click **Actions** ▸ **Export**.
2. In the **Export Project** dialog box, enter or select the following.
   - **Export Name**
   - **File Format**
   - **Destination**—Click **Browse** to navigate to the location.
   - **Export Setting**—Select an export setting from the dropdown list, or edit the export settings (see "View or edit export settings" on page 193).
3. Click **Export**.

Export data in legacy format

The option to export data in legacy format is available only for projects.

The menu option is not available if the project does not have any analysis modules. Analysis modules for a project are set up in the project settings (see "Update the project settings" on page 63).

The data can be used in AlleleTyper™ Software.

The data are in TXT file format.

The data cannot be imported back into Diomni™ Design and Analysis (RUO) Software 3.

IMPORTANT! For import into AlleleTyper™ Software, the CNV name must end with ***_cn***.

Copy number variation data are exported in a single file (TXT format).

Exported files for multiplate analysis contain both single plate results and multiplate results in the same file. If the single plate copy number variation results and the multiplate copy number variation results are not consistent for a specific sample, the result is undetermined in AlleleTyper™ Software. Single plate results can be deleted from the exported file.

Save the analyzed data before exporting the results (see "Save a plate file or data file" on page 181).

1. In a an open project, click **Actions** ▸ **Export in Legacy Format**.
2. In the **Export in Legacy Format** dialog box, enter a file name in the **Export name** field.
   The **Export name** field is populated with a default file name.

3. Select a destination to save the file in the **Folder** field.

4. Select the checkboxes that correspond with the data to export.

   - **Copy Number Variation Results (CopyCaller)** checkbox (selected by default)
   - **Copy Number Variation Results with Cq (CopyCaller)** checkbox (deselected by default)
   - **Genotype Results (TaqMan Genotyper)** checkbox (selected by default)

   The checkboxes that are displayed correspond to the analysis modules in the project. For example, the **Genotype Results (TaqMan Genotyper)** checkbox is not displayed if the project is not set up with genotyping analysis.

   The copy number variation results with Cq data can be imported into AlleleTyper™ Software. The Cq data are not used by AlleleTyper™ Software. The Cq data can be used for other analysis.

5. Click **Export**.

Export data in the RDML format

Export analyzed data in RDML (Real-Time PCR Data Markup Language) format for standard curve, relative standard curve, and comparative C_t analysis.

Exporting data in the RDML format is not available for projects.

Save the analyzed data file before exporting the results (see “Save a plate file or data file” on page 181).

1. In a data file, click **Actions** ▸ **Export to RDML**.

2. Name the file, navigate to the desired folder location, then click **Export**.

Generate a report

Generate a customizable results report.

Generating a report is not available for projects.

1. In a data file, click **Actions** ▸ **Generate report**.

2. (*Optional*) Edit the file name.

3. (*Optional*) To edit the file destination, click **Browse**, then navigate to the desired location.

4. In the **Report Content** pane, select the content to include in the report:

Report Content	Description
Summary	Displays a summary of the experiment, including the following information: • Bar Code • File Name • Run Start Date and Time • Run End Date and Time • Run Duration • Operator • Instrument Name • Instrument Type • Block Type • Block Serial Number • Heated Cover Serial Number • PCR Stage/Step Number • Quantification Cycle Method • Comment • Software Name and Version • Plugin Name and Version • Analysis Date and Time
Well Table	Displays the following information from **Well Table** in the **Quality Check** tab: • Well • Sample • Target • Task • C_q • C_q Confidence • Amplification Score • Amplification Status • C_q Threshold (not applicable if the relative threshold algorithm is selected to calculate the C_q values) • Baseline Start and End (not applicable if the relative threshold algorithm is selected to calculate the C_q values)
Replicate Group Results	Displays the following information from **Replicate Table** in the **Quality Check** tab: • Sample • Target • Number of Replicates • C_q Mean • C_q Standard Deviation

(continued)

Report Content	Description
Plate Layout	Displays the sample name, sample color, target or targets, and target C_q value or values for each well. The subarrays are displayed for the OpenArray™ Plate format. The sample name is displayed on each subarray.
Amplification Plot (dRn)	Displays the Amplification Plot (dRn vs Cycle)[2] If the primary analysis settings are set up for a baseline threshold, the Ct value is displayed on the report. The Ct value is also displayed on the amplification plot in the report. The Ct value is displayed for both automatic threshold values and threshold values that are set manually.[1]
Amplification Plot (Rn)	Displays the Amplification Plot (Rn vs Cycle)[2]
Melt Curve Plot	Displays the Melt Curve Plot (Derivative Reporter vs Temperature)[3] This option only displays if the Run Method includes a Melt Curve stage.
Run Method	Displays the thermal cycling protocol used in the Run Method. (Not applicable for the OpenArray™ Plate format.)
Primary Analysis Settings	Displays the primary analysis information. This includes the PCR state and step, the qualification cycle method, the baseline information, and the threshold information. The QC alerts and advanced settings are displayed. See the following sections: • "View or edit QC alerts settings" on page 146 • "Overview of the result quality checks" on page 204 • "View or edit advanced settings" on page 146
Analysis Module	Displays the plots and analysis results for the selected analysis modules. This option only displays if an analysis module is selected.

[1] For more information, see "View or edit Cq settings" on page 144.
[2] For more information about the Amplification Plot, see "Amplification Plot overview" on page 207.
[3] For more information about the Melt Curve Plot, see "Melt Curve Plot overview" on page 209.

5. Select the wells or the subarrays to include in the report.
   - (*Default*) **All Wells** checkbox.
   - **Customize** checkbox—Select one or more wells or subarrays in the plate layout. The wells or subarrays are colored by sample.

Option	Action
Select one well or subarray	Click the well or subarray
Select multiple contiguous wells or subarrays	Click-drag over the wells or subarrays
Select non-contiguous wells or subarrays	PC: Ctrl-click each well or subarray Mac: Cmd-click each well or subarray

6. Click **Download**.

11 General procedures to analyze data in the **Quality Check** tab

For detailed procedures, see Chapter 8, “Review and analyze data”.

View the post-run summary

A post-run summary is not available for run files that are viewed within a project.

1. Open the data file.
2. In the **Run Summary** tab, view a summary of the run, including the following information:
   - **Run Start** and **Run End**
   - **Operator** and **Block Type**
   - **Heated Cover S/N** and **Block Serial S/N**
   - **Instrument Software** and **Instrument Name**
   - **Run Events** and **Calibration**

Configure the layout of the Quality Check tab

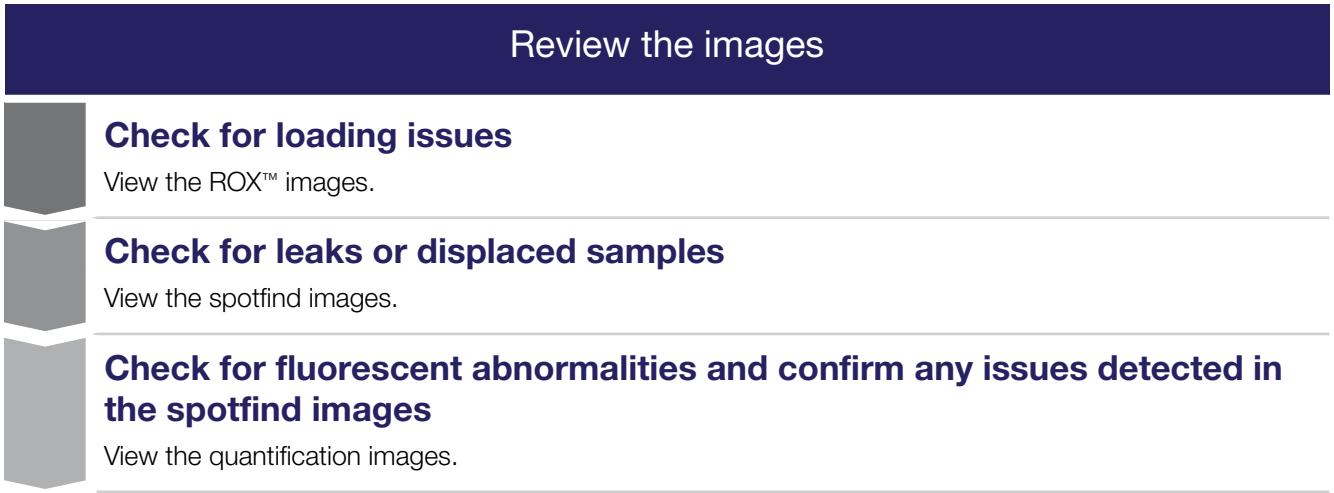
1. In the **Quality Check** tab, click **Actions** ▸ **Page Layout Setting**.
2. Drag up to four options to the display on the right.
   Each option will display in a pane in the **Quality Check** tab.
3. (*Optional*) Drag the panes to rearrange the display in the **Quality Check** tab.
4. Click **Save**.
5. (*Optional*) To reset to the default settings, click **Reset to Default**.

Filter results in the Quality Check tab

1. In the left pane of the **Quality Check** tab, select the following to filter the results.
   - **Sample Type**
   - **QC Alerts**
     Filtering by QC alerts is available only if there is at least one QC alert in the results.
   - **Samples**
   - **Targets**
   - **Biogroups**
   - *(OpenArray™ Plate only)* **View**
2. *(Optional)* To clear the selections, click **Clear all**.

Review the OpenArray™ Plate images

Workflow to review the OpenArray™ Plate images



Overview of OpenArray™ Plate images

OpenArray™ Plate images can be viewed in the software. The images can be used to troubleshoot problems.

Three categories of images are available:

- ROX™ images (contain `_channel_4` in the name)
- Spotfind images (contain `_spotfind` in the name)
- Quantification images (contain `stage`, `cycle`, and `channel` in the name)

The ROX™ images should display uniform fluorescence throughout the OpenArray™ Plate. The following patterns indicate an issue.

Table 3 ROX™ image patterns and possible causes

Pattern	Possible cause
Fluorescence is not displayed in through-holes of the subarray where the AccuFill™ Instrument turns when loading the OpenArray™ Plate.	The AccuFill™ Instrument might be misaligned. Contact Support.
Fluorescence is not displayed in large sections of a subarray near the fill port.	The immersion fluid is injected too quickly or injected without purging the syringe. This causes the sample to be knocked out of the through-holes near the fill port.
Fluorescence is not displayed in large sections of a subarray at the end of the fill path.	There is an insufficient volume of sample and reagents in the sample plate. The tips run out of volume before they reach the end of the fill path.
There are obscured areas at the edge of the OpenArray™ Plate.	The case lid was not aligned correctly in the plate press.
There are very bright spots in a well.	These bright spots are caused by dust or other contaminants.
The wells display a dark center.	There is evaporation. This is caused by low humidity levels and excessive time passing before the immersion fluid is added to the OpenArray™ Plate.

The spotfind images can indicate whether there are leaks. The spotfind images should appear with a uniform pattern. Dark spots indicate leaks. Leaks should be confirmed by reviewing the quantification images.

Quantification images display the fluorescent signals. They can indicate the following issues.

Table 4 Quantification image patterns and possible causes

Pattern	Possible cause
There are very bright spots in a well.	These bright spots are caused by dust or other contaminants.
The wells display a dark center.	There is evaporation. This is caused by low humidity levels and excessive time passing before the immersion fluid is added to the OpenArray™ Plate.
There is variability in the brightness of the signal within the well.	There are leaks. There might be sample and reagent displacement from mishandling.

Review the OpenArray™ Plate images (single plate)

The images are in the **Quality Check** tab.

1. In the left pane, in the **View** dropdown list, select **Plate Image**.
   The image is displayed.
2. In the **Image type** dropdown list, select one of the following options:
   - **ROX Images**
   - **Spotfind Images**
   - **Quant Images**
3. Click the file to view.
   The file is highlighted in blue. The file name is also displayed at the bottom of the image.
4. Use the magnification tools at the top-right corner of the image to zoom in, to zoom out, and to reset the magnification.
5. Click on a subarray to view a magnified image of the subarray.
   The selected subarray is displayed below the image of the full OpenArray™ Plate.
6. Use the sliders to adjust the image brightness and contrast.
   - **Brightness** slider
   - **Contrast** slider

   Edits to the brightness and contrast are retained in the image if the image is exported.
7. Click the **Comments** field to add a comment for an image.

Review the OpenArray™ Plate images (project)

The images are in the **Quality Check** tab.

1. In the plate layout pane, click **View Plate Image**.
2. In the **Image Viewer** dialog box, select the file from the **Select Run File** dropdown list.
3. Select an image in the **Select Image** dropdown list.
4. Use the sliders to adjust the image brightness and contrast.
   - **Brightness** slider
   - **Contrast** slider

   Edits to the brightness and contrast are not retained in the image if the image is exported.

Review individual well results in the Well Table

In the **Quality Check** tab, view results for individual wells in the **Well Table**.

- Click **View** to select the columns that are displayed.
- Click on any column header to sort the table by that value.
- Select a well from the table to highlight the well in the **Plate Layout** or plot.

Note: Well selections are retained when viewing different plots.

Review Replicate Group results

1. In the **Quality Check** tab, click **Replicate Group**.
2. Examine the C_q mean and standard deviation for each replicate group to assess the precision of C_q values.

Configure general plot settings

1. In the **Quality Check** tab, in the plot pane, click **(Settings)**.
2. Edit the following settings in the **General** tab.
   - **Plot Title**
   - **Color By**
   - **Y Value**
   - **Y Scale**
   - **Thickness**
   - **Max Curves**
   - **Show**

   The default value for the **Max Curves** dropdown list is **384 x 2**. If **All** is selected in the **Max Curves** dropdown list, it can take time to load all of the curves, especially for the OpenArray™ Plate format.
3. Edit the following **X Axis** and **Y Axis** settings in their respective tabs.
   - **Label**
   - **Auto-adjust range**—If deselected, select a **Minimum value** and **Maximum value**.
4. Click outside of the dialog box to close.

12 Manage plate files and data files

Overview of system templates and plate files

A plate file contains the information that is necessary to perform an instrument run. A system template is a non-editable plate file that is included with the software. Opening a system template automatically generates a new plate file that can be edited, then saved (see “Select a system template or existing plate file to set up a new plate file” on page 70).

A plate file can contain the following information:

Information type	Properties
Instrument setup	• Instrument type • Block • Run mode
Run Method	• Thermal protocol • Filter settings
Plate Setup	• Sample definitions and well-assignments • Target or SNP assay definitions and well-assignments • Reagent information **Note:** Plate setup information is not included in the system template and must be defined by the user.
Primary Analysis Settings	• C_q settings • Melt settings • QC settings • Advanced settings
Analysis Module	Analysis modules are plugins that enable additional data analysis in the software (see “About analysis modules” on page 190). **Note:** The user can select an analysis module pre- or post-instrument run (“Select an analysis module (single plate)” on page 191).
Additional Information	Plate information — plate barcode and user-defined description

Overview of data files

A data file contains the information from the plate file that was used to perform the instrument run. A data file can also contain the following information:

Information type	Properties
Run summary	• **Run Start** and **Run End** • **Operator** and **Block Type** • **Heated Cover S/N** and **Block Serial S/N** • **Instrument Software** and **Instrument Name** • **Run Events** and **Calibration**
Analysis results	• Data plots • C_q and C_q confidence • Amplification score and status • Melting temperature • Flags
Analysis module results (if applicable)	Data analysis completed using an analysis module.[1]

[1] For more information about analysis modules, see "About analysis modules" on page 190.

Save a plate file or data file

- To save a plate file for the first time, or to save a plate file or data file with a new name, click **Actions ▸ Save As**.
- To save the plate file or data file with the same name, click **Actions ▸ Save**.

Restrict editing of a plate file or data file

IMPORTANT! If you enable restricted editing in a plate file or data file, then you cannot save the file with unrestricted editing. We recommend that you save a backup version of the file before you restrict editing.

If editing is restricted on a plate file, the restriction is carried over to all the data files that are generated from the restricted plate file.

The following items can be edited in a restricted post-run file without a password:

- Barcode
- Create and edit reagent information for the plate
- Create and edit reagent information for an individual well
- Create or delete a sample
- Assign or unassign samples to wells

1. In an open plate file or data file, click **Actions** ▸ **Restrict Editing**.
2. In the **Restrict Editing** window, select the features for which you want to restrict editing.
   - Edit analysis settings
   - Edit target/assay
   - Assign target/assay
   - Edit run method
     The run method is never editable in a data file.

   Note: A blue checkbox (☑) indicates that the function cannot be edited.
3. Enter and confirm a password, then click **Restrict**.

   Note: Record the password because lost passwords cannot be recovered.

   A lock icon (🔒) appears next to the file name in the software menu bar to indicate that restricted editing is enabled.
4. (*Optional*) To update editing restrictions for a file, enter your password, modify the selections, then click **Update**.
   After the file is closed, the password must be entered again in order to edit the file.
5. (*Optional*) To remove editing restrictions from a file, enter your password, then click **Remove**.

Add a plate file to My Plate Files

Save a plate file before adding it to **My Plate Files** (see “Save a plate file or data file” on page 181).
In an open plate file, click **Actions** ▸ **Add to My Plates**.
The plate file appears in the in the **Run Templates** page, in the **My Plate Files** tab.

Search for a plate file or data file

Add a tag to your plate file or data file to enable searching by that tag (see “Edit plate file or data file information” on page 100).

1. Open the **Run templates** page or the **Runs** page.
2. Click 🔍, then enter the tag or tags.
   Plate files or data files with the tag are displayed.

Set up new plate file from a data file

1. Select the **Runs** tab.
2. *(Optional)* Use the filter tools on the left pane to filter the data files.

3. Hover over the data file, then click one of the following options.
   - ··· **(More Options)** ▸ **Rerun**.
   - ··· **(More Options)** ▸ **Rerun in new window**.

   A new plate file is generated.
4. Click **Actions** ▸ **Save As**.
5. In the **Save As** dialog box, enter a file name in the **File Name** field, then select a location to save the file.
6. Click **Save**.

A new plate file is saved.

Remove a data file

1. Select the **Runs** tab.
2. *(Optional)* Use the filter tools on the left pane to filter the data files.
3. Hover over the data file, then click ··· **(More Options)** ▸ **Remove**.

The data file is removed from the software. It is not deleted from the system. The file is still available in the folder where it is saved.

Batch generate plate files

Batch generate multiple EDT files with different barcodes and sample assignments.

If you open a system template, the **Generate Plate Files** button is inactive. A copy of the system template must be saved.

The file must meet the following conditions to batch generate plate files:

- The file must be a template file (see “Set up new plate file from a data file” on page 182)
- The file must be saved (see “Save a plate file or data file” on page 181)
- The file must have a file name
- The file cannot contain any plate setup errors

1. In an open plate file, click **Actions** ▸ **Generate Plate Files**.
   The **Generate Plate Files** dialog box is displayed.
2. In the **Plate file naming** pane, enter or select the following, if needed.
   - **Use Sample Assignment File name as the barcode (only if Sample Assignment File is added)**
   - **Plate File Name prefix**

3. In the **Plate batch generation** pane, in the table:
   - Click **+ (Add)** to add the following.
     - **Add Barcode File**
     - **Add Sample Sheet**
   - Click **✕ (Remove)** to delete a row in the table.
4. To edit the file destination in the **Plate batch generation** pane, click **Browse**, then navigate to the desired location.
5. Click **Generate**.

13 Manage instruments

Overview of instruments

The QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument can be added to the software.

Instrument access must be enabled from the instrument touchscreen before it can be added in the software. For more information, see the following documents or sections:

- *QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide* (Pub. No. MAN0018045)
- "Set up or view the access code for the QuantStudio™ 7 Pro Real-Time PCR Instrument" on page 186
- "Determine the IP address of the QuantStudio™ 7 Pro Real-Time PCR Instrument" on page 186

Obtain the instrument remote access key from the instrument touchscreen or the instrument administrator.

The firewall port `7443` must be open.

Add an instrument

1. Navigate to the **Instruments** page.

   To access instruments from a different screen, click **System** ▸ **Instruments**.

2. Click **Actions** ▸ **Add Instrument**.

3. In the **Add Instrument** dialog box, select an instrument to add using one of the following options:

Option	Description
By discovery	Select an instrument from the list of instruments that are connected to the network.
By IP address	Enter the instrument IP address in the field. See "Determine the IP address of the QuantStudio™ 7 Pro Real-Time PCR Instrument" on page 186.

4. Enter the instrument **Remote Access Key**.

5. Click **Add Instrument**.

Note: If the instrument remote access key is removed, or if instrument access is disabled, the instrument is removed from the software.

Set up or view the access code for the QuantStudio™ 7 Pro Real-Time PCR Instrument

1. In the home screen, tap ⚙ **(Settings)** ▸ **Instrument settings** ▸ **Instrument access**.
2. In the **Instrument Access** screen, set the **Enable access** slider to the **On** position.
   The instrument displays an access code.
3. *(Optional)* Set the **Visible to all users** slider.
   The **Visible to all users** slider is only displayed if the **Enable access** slider is set to the **On** position.
4. Tap **Close**.

Determine the IP address of the QuantStudio™ 7 Pro Real-Time PCR Instrument

In the home screen, tap ⚙ **(Settings)** ▸ **About instrument**.

The **About Instrument** screen is displayed. A wired IP address and a wireless IP address are both displayed on the screen.

Use the wired IP address to add the instrument to the software.

Export the instruments

A list of instruments in exported in CSV file format. The list of instruments can be used as a backup for the instruments connected to the instance of the software. The list can be imported into another instance of the software.

The following information is included in the file:

- Instrument name
- Instrument type
- Block type
- Serial number
- IP address
- Encrypted access code
- Port

1. Navigate to the **Instruments** page.

   To access instruments from a different screen, click **System** ▸ **Instruments**.

2. Click **Actions** ▸ **Export Instruments**.

3. In the **Export Instruments** dialog box, enter a password in the **File password** field, then click **Export**.

   The password is entered when importing the list of instruments. The access codes for the instruments are encrypted. The password allows the access codes for the instruments to be decrypted when importing the list of instruments.

   The password must contain between 6 and 30 characters. It must contain at least one letter and one number. It can contain special characters.

The list of instruments is exported.

Import instruments

1. Navigate to the **Instruments** page.

   To access instruments from a different screen, click **System** ▸ **Instruments**.

2. Click **Actions** ▸ **Import Instruments**.

3. Navigate to the location of the file, then click **Open**.

4. In the **Import Instruments** dialog box, enter the password in the **File password** field.

   The file password is set up when the list of instruments is exported.

5. *(Optional)* Select the **Import from Design and Analysis Software 2.8 and below** checkbox.

   A file password is not required for instruments exported from QuantStudio™ Design and Analysis Software v2.8 and earlier.

6. Click **Import**.

The software attempts to connect to the instrument after the file is successfully imported.

Refresh the list of instruments

Refreshing the list of instruments allows you to view the most recent status for each instrument. It also updates the list of connected instruments, for example, removing an instrument from the list if the instrument was removed.

1. Navigate to the **Instruments** page.

   To access instruments from a different screen, click **System** ▸ **Instruments**.

2. Click **Actions** ▸ **Refresh**.

Review instrument status

For more information about adding an instrument, see “Add an instrument” on page 185.

1. Navigate to the **Instruments** page.
   To access instruments from a different screen, click **System** ▸ **Instruments**.
2. The status for the instrument is displayed:
   - Offline
   - Idle
   - Running
     If running, the remaining time of the run is also displayed.
   - Standby
   - Error
   - Diagnostics

Update the access code

If the access code was updated on the instrument, the new access code must be entered in the software. Entering the new access code enables a continued connection to the instrument.

1. On the **Instruments** page, hover over the top-right corner of an instrument description.
2. Click ··· **(More Options)** ▸ **Update Access Code**.
3. In the **Access Code** dialog box, enter the access code in the **Access Code** field.
4. Click to view or hide the access code as it is being entered.
5. Click **OK**.

View the instrument details

The calibration status and details of compatible instruments can be reviewed in the software. The instrument must be added to the list of instruments.

For more information about adding an instrument, see “Add an instrument” on page 185.

1. Navigate to the **Instruments** page.
   To access instruments from a different screen, click **System** ▸ **Instruments**.
2. Click the instrument.
   The instrument details are displayed.
   - Instrument serial number
   - Instrument software
   - LED life
   - Block serial number
   - Total block degrees
   - Total block cycles
   - Calibration information, including the following items:
     - Calibration type
     - Calibration status
     - Run date
     - Expiration date
3. Click **(Back)** to return to the **Instruments** page.

Remove an instrument

1. Navigate to the **Instruments** page.
   To access instruments from a different screen, click **System** ▸ **Instruments**.
2. Hover over the instrument, then click **(More Options)** ▸ **Remove Instrument**.

14 Manage analysis modules

To see all of the analysis modules that are installed, click ⚙ **(Settings)** ▸ **Plugins**.

About analysis modules

Analysis modules are plugins that enable additional data analysis using Diomni™ Design and Analysis (RUO) Software 3.

- To see all of the analysis modules that are installed, click ⚙ **(System)** ▸ **Plugins**.
- To select an analysis module for a single plate, see “Select an analysis module (single plate)” on page 191.
- Analysis modules for a project are defined in the project settings (see “Update the project settings” on page 63).

There are two types of analysis modules:

Analysis Module type	Description
Built-in	Analysis modules that pre-installed in the software. These analysis modules cannot be uninstalled. The latest versions of the analysis modules are included with the software installer. When a new version of the software is installed, the latest versions of the analysis modules are installed. Previous versions of the analysis modules are not retained.
User-installed	Analysis modules that are installed by the user. These analysis modules can be uninstalled.

The following analysis modules are available to use with the software:

Analysis Module	Type	Analysis type	Description
Primary analysis	Built-in	Single plate and project	Calculates dye signals, Cq values, and other primary results from the filter signals.
Standard curve	Built-in	Single plate	Use to determine absolute target quantity in test samples.
Genotyping	Built-in	Single plate	Use to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence.
Genotyping project	Built-in	Project	Use to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence.
Presence absence	Built-in	Single plate	Use to determine the presence or absence of a target nucleic acid sequence in a sample.

(continued)

Analysis Module	Type	Analysis type	Description
Relative quantification	Built-in	Single plate	Use to determine the relative quantity of a target of interest in a test sample relative to a reference sample. The analysis module supports relative quantification using either comparative CT (ΔΔCT) analysis or relative standard curve analysis.
Copy number variation project	Built-in	Project	Use to detect and measure copy number variation of specific sequences in genomes.
High resolution melt	User-installed	Single plate	Use to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence. This analysis module must be reinstalled when a new version of the software is installed. **Note:** You must purchase an extra license key to perform analysis using this module.

For detailed information about the analysis modules, see one of the following sources:

- Analysis module user guide
- Analysis module help—With an analysis module selected, click ⓘ **Help**, then select the analysis module help.

Select an analysis module (single plate)

The analysis module selection can be selected in either the plate file or the data file.

Analysis modules for a project are defined in the project settings (see “Update the project settings” on page 63).

The genotyping analysis module and the presence absence analysis module are the only ones that are available for OpenArray™ Plate data files.

The genotyping analysis module is automatically applied to data files from OpenArray™ Plate genotyping runs.

1. In a plate file or data file, click **Actions** ▸ **Analysis Modules**.
2. In the **Analysis Modules** window, select the analysis module, then click **OK**.
3. Save the plate file or data file keep the analysis module choice.

Install a new analysis module plugin

Go to **thermofisher.com/us/en/home/global/forms/life-science/quantstudio-6-7-pro-software** to download the file and purchase a license registration code.

Note: Currently, the High Resolution Melt Analysis Module is the only analysis module available for user-installation.

Start the Diomni™ Design and Analysis (RUO) Software 3 as an administrator to install an analysis module plugin.

1. In any screen, click ⚙ **(System)** ▸ **Plugins**.
2. In the **Plugins** screen, click **Actions** ▸ **Install**.
3. Navigate to, then select the plugin ZIP file.
4. Click **Open**.
5. In the **Install** dialog box, select the **Accept End User License Agreement** checkbox, then click **Next**.
6. In the **Confirmation** dialog box, click **OK**.
   The software restarts.
7. Enter the license registration information, then click **Install** ▸ **OK**.

To select and open an analysis module, see “Select an analysis module (single plate)” on page 191.

Uninstall an analysis module plugin

The analysis tab for the analysis module is not available after the analysis module plugin is uninstalled. This includes existing data files that used the analysis module plugin.

Note: Only user-installed analysis module plugins can be uninstalled. Built-in analysis modules cannot be uninstalled by the user.

1. In any screen, click ⚙ **(System)** ▸ **Plugins**.
2. Hover over the analysis module, then click ··· **(More Options)** ▸ **Uninstall**.
3. In the **Confirmation** dialog box, click **OK**.

15 Manage export settings

About export settings

Export settings designate the data to include in the exported results. The software includes several built-in export settings files in the Export Settings library (click **(System)** ▸ **Export Settings** to view).

The following built-in export settings are available:

- `Default Export Setting` (single plate)
- `CopyCaller Export Setting` (single plate)
- `LIMS Result Only` (single plate)
- `Default Project Export Setting` (project)

To edit the default settings, you must create a new export settings file (see "View or edit export settings" on page 193).

Export settings can be applied to data exported by the software or by an instrument.

View files in the Export Settings library

1. In any screen, click **(System)** ▸ **Export Settings**.

   Note: If you have a data file open, you are prompted to save, then close the file. To view or edit export settings without closing the current data file, see "View or edit export settings" on page 193.

2. Click an export settings file to view.

3. (*Optional*) In the open export settings file, create a new export settings file, or edit an existing custom export settings file.

View or edit export settings

View or edit export settings while a data file or project is open. You can also view or edit export settings from the **Export Settings** library (see "View files in the Export Settings library" on page 193).

1. Open a data file or a project.

2. Click **Actions** ▸ **Export**.

3. In the dialog box, select an option from the **Export Setting** dropdown list, then click **Customize**.

4. In the **Export Setting** dropdown list, select an export setting to edit or to use as a starting point to create a new export setting.

5. In the **View** dropdown list, select **Result**.

6. In the **Analysis Module** dropdown list, select the an analysis module to edit.

   The options that are displayed in the **Analysis Module** dropdown list depend on the analysis modules that are used in the data file or the project.

7. In the **Include** dropdown list, select or deselect the data to include in the export.

   The options that are displayed in the **Include** dropdown list depend on the analysis module that is selected in step 6.

   Each item is exported as a separate file.

   Deselected items do not have an associated tab that can be edited in the dialog box.

8. View the data in each tab.

   See “Data that can be included in an export” on page 195.

9. In the **Select Columns** pane, select or deselect the checkbox associated with the column in the table.

   Items with deselected checkboxes are not included in the exported data.

10. In the **Select Columns** pane, click an item in order to edit the text that is displayed in the exported file.

    The updated name is displayed in the pane that displays the results.

11. Click and drag a column header in order to change the order of the columns in the table.

12. In the **View** dropdown list, select **Metadata**.

13. In the **Select Fields** pane, select the checkbox associated with each item in order to display it in the exported file.

14. In the **Select Fields** pane, click an item in order to edit the text that is displayed in the exported file.

    The updated name is displayed in the **Metadata Fields** pane.

15. Click the **Options** dropdown list to select the options for the values that are displayed in the exported plate.

    a. Select or deselect the **Round values by** checkbox, then enter a number in the **Decimal places** field.

    b. Select or deselect the **Use double quote** checkbox.

    c. Select or deselect the **Use two digits in well position** checkbox.

16. Click the **Options** dropdown list to select the information to include in the exported plate.
    - **Section header** checkbox
    - **Empty wells** checkbox
    - **Omitted wells** checkbox

17. *(Optional)* Click **Save As**, enter a name in the **Export Name** field of the **Save As** dialog box, then click **OK**.
    The saved settings are available as a selection in the **Export Setting** dropdown list.

18. Click **Download** to continue exporting results, or click **Close**.

Data that can be included in an export

Analysis module	Data
Primary	• Results • Amplification data • Multicomponent • Raw data • Replicate group result
Standard curve	Standard curve result
Genotyping (single plate)	Genotyping result
Presence absence	• Target call • Well call • Sample call • Control status
Relative quantification	• RQ replicate group result • Sample group result
Genotyping (project)	• Genotyping result • Genotyping samples • Genotyping SNP assays • Genotyping run files • Genotyping control status • Genotyping reference samples
Copy number variation	• CNV single plate table • CNV single plate sample result • CNV single plate well result • CNV multi plate table • CNV multi plate sample result • CNV multi plate well result

Download an export settings file

An export settings file can be imported into the **Export Settings** library or into the QuantStudio™ 6 Pro Real-Time PCR System or the QuantStudio™ 7 Pro Real-Time PCR System.

1. In any screen, click ⚙ **(System)** ▸ **Export Settings**.
2. Hover over the export settings file, then click ··· **(More Options)** ▸ **Download**.
3. Name the export settings file, navigate to the desired folder location, then click **Save**.

- To import an export settings file into the software, see “Import an export settings file” on page 196.
- To import an export settings file into the QuantStudio™ 6 Pro Real-Time PCR System or the QuantStudio™ 7 Pro Real-Time PCR System, see the instrument user guide.

Import an export settings file

Import a previously downloaded export settings file (see “Download an export settings file” on page 196).

1. In any screen, click ⚙ **(System)** ▸ **Export Settings**.
2. Click **Actions** ▸ **Import Setting**.
3. Navigate to the export settings file, then click **Open**.

Delete export settings

Built-in export settings cannot be deleted.

1. In any screen, click ⚙ **(System)** ▸ **Export Settings**.
2. Hover over the file, then click ··· **(More Options)** ▸ **Delete**.
3. In the **Confirmation** dialog box, click **OK**.

16 Manage the preferences

Overview of the preferences

Preferences for the following items can be updated.

- Importing an AIF
- Custom dyes
- Plate file format
- Result export
- Report generation (not available for projects)
- Destinations to save the files

For the desktop software configuration, the preferences are set up for any user that launches the software. The preferences are not specific to the user.

Manage preferences for AIF import

This setting determines the preferred target name when importing an assay information file (AIF).

The assay ID is used as the target name if the assay name and gene symbol are not available in the AIF.

1. In any screen, click **(System)** ▸ **Preferences**.
2. In the **AIF Import** pane, select one of the following items.
   - **Gene Symbol & Assay ID** radio button
   - **Gene Symbol** radio button
   - **Assay Name** radio button
   - **Assay ID** radio button

   The **Assay ID** radio button is selected by default.
3. Click **Apply**.
4. Click **Reset** to reset to the default settings.

Manage preferences for custom dyes

After a custom dye is added to the system, it is available in the **Manage Dyes** dialog box when a plate file is set up.

1. In any screen, click ⚙ **(System)** ▸ **Preferences**.
2. In the **Custom Dyes** pane, click + **(Add)**.
   A new row is added to the table that contains the list of custom dyes.
3. Edit the following fields in the new row.
   - Enter a name in the **Dye Name** field.
   - Select a color from the color picker.
   - Select a type from the **Type** dropdown list.
     - Reporter
     - Quencher
     - Both
   - Enter a wavelength in the **Wavelength** field.
   - *(For a quencher)* Select or deselect the **Fluorescence** checkbox.
     Fluorescence is required for a reporter or both types of dyes.
4. Click ⊗ **(Remove)** to remove a dye.
5. Click **Reset** to reset the default settings.

Manage preferences for the plate file format

1. In any screen, click ⚙ **(System)** ▸ **Preferences**.
2. In the **Plate File Format** pane, select the **Create plate file (.edt) in legacy format for QuantStudio™ 1/3/5/6 Flex/7 Flex/12K Flex** checkbox.
3. Click **Apply**.
4. Click **Reset** to reset the default settings.

Manage preferences for the results export

1. In any screen, click ⚙ **(System)** ▸ **Preferences**.
2. In the **Result Export** pane, select one of the following options.
   - **File name with date and time stamp** radio button
   - **File name only** radio button

   The default setting is the file name only.

3. Click **Apply**.
4. Click **Reset** to reset the default settings.

Manage preferences for the report generation

A report applies only to a single plate data file. A report cannot be generated for a project.

1. In any screen, click **(System)** ▸ **Preferences**.
2. In the **Report Generation** pane, enter a title in the **Title** field.
3. Select a paper size from the **Paper Size** dropdown list.
   - **A4**
   - **Letter**
4. Select an option for the logo.
   - **Use the default report logo** radio button
   - **Use a customized report logo** radio button
5. *(For customized report logo)* Click **Upload file here**, then navigate to the location of the file.
   The file can be in a JPEG file format or a PNG file format. The file size must be less than 500 KB. The dimension are 1021 px × 218 px.
6. Click **Preview** to preview the report, then click **Close**.
7. Click **Apply**.
8. Click **Reset** to reset the default settings.

Manage preferences for the destination to save the files

If a destination is not defined, the software uses the last location as the destination to save files.

1. In any screen, click **(System)** ▸ **Preferences**.
2. In the **File Save Destinations** pane, select the **Use the following location as default for <…>** checkbox, where <…> is the item that is being selected.
   The following items are available to set a default destination to save files.
   - Export
   - RDML export
   - Report
   - Save as
3. Click **Browse**, navigate to the folder location, then click **Select folder**.

4. Click **Apply**.
5. Click **Reset** to reset the default settings.

Package the log files

The log files can be packaged for troubleshooting purposes.

1. From any screen, click **(Help)** ▸ **Package Log File**.
2. In the **Save As** dialog box, navigate to a location to save the file, then click **Save**.

The log files are saved in a compressed ZIP folder.

17 About data analysis

For information about additional analysis using an analysis module, select the analysis module to view the relevant help information (see “Select an analysis module (single plate)” on page 191).

About the quantification cycle (C_q)

The quantification cycle (C_q) is used for gene expression metrics quantification analysis. Algorithm-specific calculations of C_q values are used as the primary input values for quantification analysis.

Algorithm	Description
Baseline Threshold	C_q (C_t) is calculated using the PCR cycle number at which the fluorescence signal meets the threshold in the amplification plot.
Relative Threshold	C_q (C_{rt}) is calculated using the PCR cycle number for the threshold calculated from the modeled amplification efficiency profile.

For more information about C_q analysis settings, see “Cq settings overview” on page 202.

About melt curve analysis

Use melt curve analysis to determine the melting temperature (T_m) of the amplification products of a PCR that used intercalating dyes.

Melting temperature (T_m) is the temperature at which 50% of the DNA is double-stranded and 50% is dissociated into single-stranded DNA. The melt curve of a single amplification product displays a single peak at the T_m of the product. Multiple peaks in a melt curve experiment indicate additional amplification products, usually from non-specific amplification or formation of primer-dimers.

Multi-peak calling can be used when more than one product is expected in a reaction.

Melt curve analysis is included in the primary software analysis.

1. The software plots a melt curve based on the fluorescence of the dye with respect to change in temperature.
2. Using the melt curve, the software calculates the melting temperature (T_m).

A melt peak parity factor is available. It normalizes the scaling of the melt curve peaks in multiplex assays when the dyes fluoresce at different amplitudes. It helps to make the peak heights more comparable across multiple dyes and targets.

Primary analysis settings overview

C_q settings overview

The default C_q settings are appropriate for most applications. Edit the settings for analysis of not typical or unexpected run data.

Note: The run data must include a PCR stage to perform C_q analysis.

Table 5 C_q settings

Setting	Description
PCR Stage/Step	If there is more than one PCR stage/step with data collection, the user selects PCR stage/step from the dropdown list for C_q or C_q analysis.
Baseline threshold analysis	
Algorithm Settings – **Baseline Threshold**	The **Baseline Threshold** Algorithm is used to calculate the C_q values. This algorithm is an expression estimation algorithm that subtracts a baseline component and sets a fluorescence threshold in the exponential region.
Default C_q Settings	Determines how the **Baseline Threshold** Algorithm is set. The default settings are used for targets and wells unless custom values are selected in the **General** or **Well C_q** tabs. For recommendations on adjusting baseline and threshold settings, see "Guidelines for manual threshold and baseline settings for Cq analysis" on page 203.
C_q Settings for Target	• **Default Settings** selected—The default C_q settings are used to calculate the C_q values for the target. • **Default Settings** deselected—The software allows manual setting of the baseline or the threshold. For recommendations for adjusting baseline and threshold settings, see "Guidelines for manual threshold and baseline settings for Cq analysis" on page 203.
Relative threshold analysis	
Algorithm Settings – **Relative Threshold**	The **Relative Threshold** Algorithm is used to calculate the C_q values. This algorithm is a well-based expression estimation algorithm that sets a threshold for each curve individually. The threshold is based on the shape of the amplification curve, regardless of the height or variability of the curve in its early baseline fluorescence.
Default C_q Settings	Determines the default start cycle. The default start cycle is used for targets unless a custom start cycle is indicated in the **Well C_q** tab.

Guidelines for manual threshold and baseline settings for C_q analysis

Setting	Recommendation
Threshold	Enter a value for the threshold so that the threshold is: • Above the background. • Below the plateau and linear phases of the amplification curve. • Within the exponential phase of the amplification curve.
Baseline	While in the linear plot view, select the **Start Cycle** and **End Cycle** values so that the baseline ends before significant fluorescence signal is detected.

Melt analysis settings overview

- The **Melt Peak Parity Factor** field allows the entry of a factor.

  This feature is for multiplex melt curve experiments. It normalizes the scaling of the melt curve peaks in multiplex assays when the dyes fluoresce at different amplitudes. It helps to make the peak heights more comparable across multiple dyes and targets.

  A factor of ***0*** turns off the feature. Negative values are not permitted.

  If the melt peak parity factor is applied to empty wells of a plate, unexpected T_m values can occur.

  The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.

- Enable or disable **Multi-Peak Calling** the **Melt** tab.

Multi-Peak Calling	Description
Active	– More than one PCR product is expected to amplify. – T_m will be determined for more than one peak.
Inactive	– A single PCR product is expected to amplify. – T_m will be determined for one peak.

- (*For multi-peak calling only, using the percentage as the threshold*) Set the value in the **Threshold Type** column to **Percentage**, then adjust the value in the **Peak Level (%)** column.

  Specify a fractional-level value as the additional peak detection threshold. The detected peaks are measured relative to the height of the tallest peak, which has a perfect fractional level of 100%. The default value is 10%.

  For example, if the peak level value is set to 40%, then peaks above 40% of the tallest peak are reported, and peaks below 40% are regarded as noise.

- (*For multi-peak calling only, using the height as the threshold*) Set the value in the **Threshold Type** column to **Height**, then adjust the value in the **Peak Height** column.

  Specify a value as the peak detection threshold.

  The absolute value of the peak is required to be above the value that is set in the **Peak Height** column

To edit the melt analysis settings, see “View or edit melt analysis settings” on page 148.

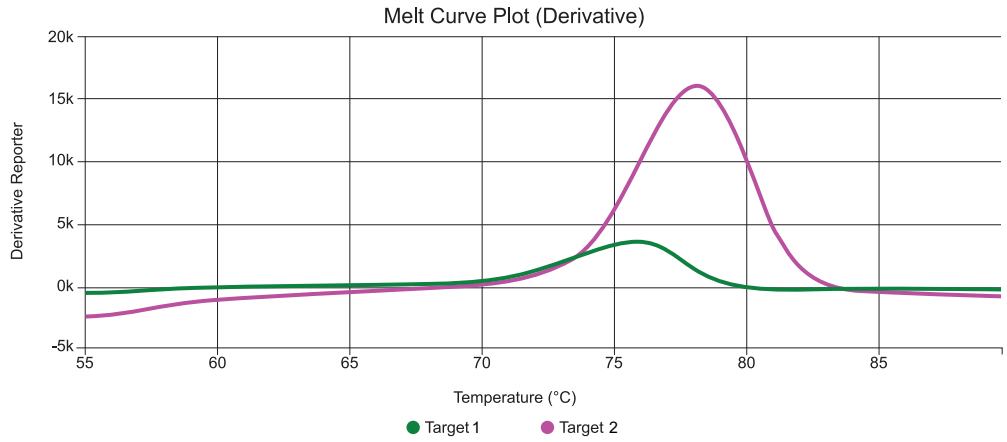


Figure 12 Melt curve before a melt peak parity factor is applied

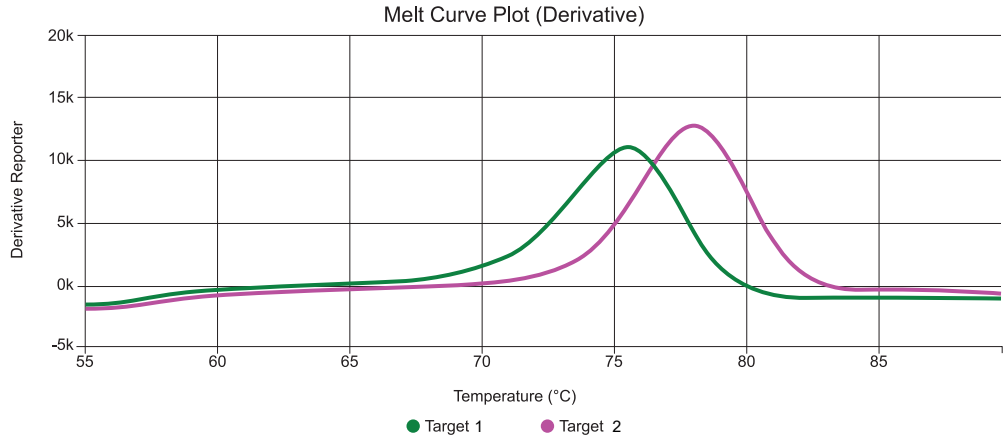


Figure 13 Melt curve after a melt peak parity factor is applied

Quality checks

There are two types of quality checks:

- Result quality check
- Curve quality check

Overview of the result quality checks

The result quality checks are used to describe the quality of the primary analysis results.

The following items can be displayed in the **Result Quality Issues** column of the **Well Table**:

- Amp Score out of range
- Unexpected Amp Status
- Cq Value out of range
- Cq Confidence out of range

- Cq Standard Deviation out of range
- Unexpected Cq Status
- Delta Rn out of range
- Multiple melt peaks
- Replicate group outlier
- Tm out of range
- Pass Ref Change out of range

Note: There can be multiple **Result Quality Issues** for a single well.

Acceptance criteria for result quality checks

Acceptance criteria	Description
C_q should be	• **Expressed** • **Undetermined** Any well outside of the user-selection will be flagged.
C_q Value Range	The C_q Value is the primary input value for quantification analysis. Any well outside of the user-defined minimum and maximum range will be flagged.
C_q Confidence Range	The C_q Confidence is a value that reflects the reliability of the derived C_q. Any well outside of the user-defined minimum and maximum range will be flagged.
C_q Standard Deviation Range	The C_q Standard Deviation is the standard deviation of the C_q of the replicates. Any well outside of the user-defined minimum and maximum range will be flagged.
Amp Status	• **Amp** • **No Amp** • **Inconclusive** Any well outside of the user-selection will be flagged.
Amp Score Range	The Amp Score is a value that indicates the quality of the amplification curve. Any well outside of the user-defined minimum and maximum range will be flagged.
Delta Rn Range	The Delta Rn is the calculated deviation from the baseline. Any well outside of the user-defined minimum and maximum range will be flagged.
Passive Ref Change (%)	Any wells with a change in passive reference greater than the threshold are flagged.
Outlier in Replicate Group	Any wells that have C_q values that differ significantly from the average for the associated replicate wells will be flagged.

(continued)

Acceptance criteria	Description
Tm Range	Tm is the melting temperature calculated in °C. Any well outside of the user-defined minimum and maximum range will be flagged.
Multiple Melt Peak Detection	Any wells that have multiple peaks will be flagged.

Overview of the curve quality checks

The curve quality checks are used to describe the quality of the curve, including passive reference signals and smoothness of the curve.

The following items can be displayed in the **Curve Quality** column of the **Well Table**:

- PRFDROP
- PRFLOW
- NOISE
- NOSIGNAL
- OFFSCALE
- SPIKE

Description of the curve quality flags

Flag	Description
PRFDROP	• Reported for only the PCR data. • The passive reference signal changes near the C_t.
PRFLOW	• Reported for only the PCR data. • The passive reference signal is low.
NOISE	• Reported for only the PCR data. • The noise for a curve is higher than other curves on the plate.
NOSIGNAL	• Reported for the whole run. • There is no signal in the well.
OFFSCALE	• Reported for the whole run. • The fluorescent signal is off the scale.
SPIKE	• Reported for only the PCR data. • There are noise spikes on the curve.

Plots overview

Amplification Plot overview

The **Amplification Plot** displays amplitude of fluorescence by well across a user-defined number of cycles (default 40 cycles). You can use the amplification plot to perform the following tasks:

- Confirm or correct baseline and threshold values.
- Identify outliers.
- Identify and examine abnormal amplification. Abnormal amplification can exhibit one of the following characteristics:
  - Increased fluorescence in negative control wells
  - Absence of detectable fluorescence at an expected cycle

    Note: If you notice abnormal amplification or a complete absence of fluorescence, see the instrument user guide for troubleshooting information.
  - Irregularities due to inefficient reactions or sample contaminants

Three plots are available. Some plots can be viewed as a linear or $\log_{10}$ graph.

Table 6 Amplification Plot types

Plot type	Description	Use to
ΔRn vs Cycle	ΔRn is the magnitude of normalized fluorescence signal, relative to the baseline fluorescence, generated by the reporter at each cycle during the PCR amplification.	• Identify and examine irregular amplification. • View threshold values for the run.
Rn vs Cycle	Rn is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference.	• Identify and examine irregular amplification. • View baseline values for the run.
C_t vs Well	C_t is the PCR cycle number at which the fluorescence meets the threshold in the amplification plot.	• Locate outlying amplification (outliers).

Raw Data Plot overview

The **Raw Data Plot** displays the raw fluorescence signal for each optical filter during each cycle of the real-time PCR read from the real-time PCR instrument.

Raw data are also collected for plate endpoint reads (genotyping and presence absence runs) and for melt curves. Raw data can be collected at any point of the thermal cycling protocol. Raw data are not specific to PCR reads.

Raw data collection is indicated by the camera icon in the run method (see “Run method elements” on page 72).

Raw data have had background and uniformity calibrations applied. They are the data that are used to produce the multicomponent data. Multicomponent data have dye calibrations. If the algorithm to reduce dye signal crosstalk processing was selected, this algorithm is included in multicomponent data.

- The algorithm to reduce dye signal crosstalk is not available for projects.
- The algorithm to reduce dye signal crosstalk off by default.

View the **Raw Data Plot** to perform the following actions:

- Confirm a stable increase in signal (no abrupt changes or dips) from the appropriate filter.
- Confirm that the correct reporter dyes were selected during plate file setup.

Multicomponent Plot overview

The **Multicomponent Plot** displays the complete spectral contribution of each dye over the duration of the thermal cycling protocol.

Multicomponent data are produced whenever raw data are read from the instrument.

Use the **Multicomponent Plot** to obtain the following information.

- Confirm that the signal from the passive reference dye remains unchanged throughout the run.
- Review reporter dye signal for spikes, dips, and/or sudden changes.
- Confirm that no amplification occurs in the negative control wells.

Melt Curve Plot overview

The **Melt Curve Plot** displays the melt curve of the amplification products in the selected wells.

Review the **Melt Curve Plot** to confirm that the amplification products in a well display a single melting temperature (T_m). Multiple peaks in a melt curve indicate non-specific amplification or primer-dimer formation.

Multi-peak calling is available when more than one peak is expected in a reaction. This is when more than one PCR product is expected to amplify.

Table 7 Melt Curve plots

Plot	Description
Derivative Reporter vs. Temperature	Displays the derivative reporter signal in the y-axis as a function of temperature. The peaks in the plot indicate significant decrease in the intercalating dye signal, and therefore the T_m of the amplified products. Use this plot to confirm a single T_m of the amplification products.
Normalized Reporter vs. Temperature	Displays the fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference, as a function of temperature. You can use this plot to check the quality of the fluorescence data.

A Troubleshooting

Observation	Possible cause	Recommended action
High fluorescence signal	The reaction volume is not correct.	Ensure that reaction volumes in the plate are correct and match the volume that is entered in the **Run Method** tab.
	Signals that exceed the limit of normal fluorescence can indicate fluorescent contaminants on the plate or on the sample block.	Examine the bottom of the reaction plate. If there is contamination, prepare and run new plate.
		Identify the location of contamination on the plate or sample block. For detailed instructions, see the instrument documentation.
Inconsistent communication between instrument and computer or instrument and the Thermo Fisher™ Connect Platform	The instrument is configured for *both* wired and wireless network connection.	Ensure only one connectivity option is plugged into the instrument (either an Ethernet cable *or* a wireless adapter, but not both).
		Configure for wired *or* wireless network connection.
	Weak or unstable internet connection, especially if configured for wireless.	Change the configuration to a wired connection.
		Use a wireless network with a stronger or more consistent signal.
The connection between the instrument and the computer is not recognized	The connection is not fully established.	Power the instrument off, then power it on again.
	If using a networked configuration, the instrument and computer are not on the same subnet mask.	Contact your information technologies department to have them ensure that the instrument and computer are on the same subnet mask.
	If using a networked configuration, the instrument or computer has an invalid IP address.	Contact your information technologies department to have them ensure that the IP addresses are valid.
The software does not start after an update to macOS™	The Rosetta 2 Software is absent. This is required for functionality of Diomni™ Design and Analysis (RUO) Software 3 on a macOS™.	Install the Rosetta 2 Software (see “Install the Rosetta 2 Software” on page 212).

Observation	Possible cause	Recommended action
Files cannot be accessed or saved	The user signed in to the computer does not have read and write access to `C:\ProgramData\Design and Analysis`.	Set up read and write access to `C:\ProgramData\Design and Analysis` for each user of the software.
		Create a user group, then set up read and write access to `C:\ProgramData\Design and Analysis` for the user group. Users can be added or removed from the user group as required.

B

Install and manage the software

Overview installation

The desktop software is installed on a single computer and can be accessed only from the single computer.

IMPORTANT! The option to use the Security, Auditing, and E-signature (SAE) Administrator Console is not available for the desktop configuration of Diomni™ Design and Analysis (RUO) Software 3.

Diomni™ Design and Analysis (RUO) Software 3 cannot be downgraded to QuantStudio™ Design and Analysis Software 2. Diomni™ Design and Analysis (RUO) Software 3 must be uninstalled and then QuantStudio™ Design and Analysis Software 2 must be reinstalled.

Recommended computer specifications

The following specifications are recommended:

- Operating system—Windows™ 10 (64-bit)
- Operating system—macOS™ 10.10 or later
- Memory—16 GB RAM
- Hard drive—10 GB storage capacity
- Screen resolution—1920 × 1080 or higher

The on-premises configuration of the software can be installed on the same computer as the desktop configuration. This is not recommended. If both instances are installed on the same computer, the files are not accessible between the configurations. The files must be opened in the desktop configuration. The files must be uploaded to the on-premises configuration.

The desktop configuration does not require any ports to be open.

Install the Rosetta 2 Software

The Rosetta 2 Software might be absent after an upgrade of the macOS™. The Rosetta 2 Software is required for functionality of Diomni™ Design and Analysis (RUO) Software 3 on a macOS™.

1. Sign in to the computer as an administrator.
2. Start the Terminal application.
3. Execute the command ***softwareupdate --install-rosetta***.
4. Follow the instructions to install the Rosetta 2 Software.

5. After the installation is complete, restart the computer.
6. Start Diomni™ Design and Analysis (RUO) Software 3.

Install the software

Download the software installer. The installer is available on the Thermo Fisher™ Connect Platform. The installer is an EXE file.

You need to have administrator privileges for the computer to install the software.

1. Double-click the EXE file.
2. In the **Design and Analysis Setup** dialog box, select one of the following options.
   - **Anyone who uses this computer (all users)** radio button
   - **Only for me <…>** radio button, where <…> is the user name for the computer
3. Click **Install**.
4. Select or deselect the **Run Design and Analysis** checkbox.
   The checkbox is select by default.
5. Click **Finish**.

The license agreement must be accepted the first time that the software is launched.

Software upgrade

The following upgrades are supported:

- QuantStudio™ Design and Analysis Software v2.7 to Diomni™ Design and Analysis (RUO) Software v3.0
- QuantStudio™ Design and Analysis Software v2.8 to Diomni™ Design and Analysis (RUO) Software v3.0

Upgrades from earlier versions of QuantStudio™ Design and Analysis Software 2 to Diomni™ Design and Analysis (RUO) Software v3.0 are not supported.

To upgrade the software, install Diomni™ Design and Analysis (RUO) Software 3 (see “Install the software” on page 213). Uninstalling a previous versions that is supported for an upgrade is not required.

In the desktop configuration, the files are not saved within the software. The files are saved on the local computer drive or on a network drive accessible by the computer.

After an upgrade, recent files might not be available in the **Recents** tabs. Open these files from the local computer drive or network drive to use them.

IMPORTANT! The option to use the security, auditing, and e-signature administrator console is not available for the desktop configuration of Diomni™ Design and Analysis (RUO) Software 3.

The security, auditing, and e-signature administrator console is compatible only with the on-premises configuration of Diomni™ Design and Analysis (RUO) Software 3. For more information, see *Diomni™ Design and Analysis (RUO) Software 3 (On-Premises) User Guide* (Pub. No. MAN1000091).

The files must be imported into the on-premises configuration of Diomni™ Design and Analysis (RUO) Software 3 if you install the on-premises configuration. There is no process to transfer files during the installation of the on-premises configuration. For more information about importing files, see *Diomni™ Design and Analysis (RUO) Software 3 (On-Premises) User Guide* (Pub. No. MAN1000091).

Overview of file backup

In the desktop configuration, the files are not saved within the software. The files are saved on the local computer drive or on a network drive accessible by the computer.

The software program folders do not need to be backed up.

We recommend backing up the drives where the template files and data files are stored.

Follow your institutional policy for backup and retention.

Antivirus software

We recommend the use of antivirus software on the computer that is used for Diomni™ Design and Analysis (RUO) Software 3.

Thermo Fisher Scientific has tested the following antivirus software with Diomni™ Design and Analysis (RUO) Software 3:

- Microsoft™ Defender
- Avast™ Free Antivirus
- McAfee™ Total Protection

Antivirus software other than those listed has not been tested and is not supported. The impact of antivirus software other than those listed has not been established.

Third-party software

We do not recommend installing any third-party software on the computer that is running Diomni™ Design and Analysis (RUO) Software 3. The exception is antivirus software that we recommend (see “Antivirus software” on page 214).

Compatible USB drive formats

The system supports USB drives with formats: FAT, FAT32, and NTFS.

IMPORTANT! Do not use a USB drive with exFAT formatting. It may cause file corruption.

USB drives

USB drives that are used with the instrument or the co-located computer must be kept virus-free and malware-free.

A regular scan of the USB drives with an antivirus software is recommended.

Documentation and support

Related documentation

Document	Publication number
Diomni™ Design and Analysis (RUO) Software 3 (Desktop) User Guide	MAN0030162
Diomni™ Design and Analysis (RUO) Software 3 (On-Premises) User Guide	MAN1000091
Diomni™ Design and Analysis (RUO) 3 User Guide (Thermo Fisher™ Connect Platform)	MAN0030163
Diomni™ Design and Analysis (RUO) Software 3 Copy Number Variation Analysis Module (project) User Guide	MAN0030169
Diomni™ Design and Analysis (RUO) Software 3 Genotyping Analysis Module (project) User Guide	MAN1000138
Diomni™ Design and Analysis (RUO) Software 3 Genotyping Analysis Module User Guide	MAN0030164
Diomni™ Design and Analysis (RUO) Software 3 High Resolution Melt Analysis Module User Guide	MAN0030165
Diomni™ Design and Analysis (RUO) Software 3 Presence Absence Analysis Module User Guide	MAN0030166
Diomni™ Design and Analysis (RUO) Software 3 Relative Quantification Analysis Module User Guide	MAN0030167
Diomni™ Design and Analysis (RUO) Software 3 Standard Curve Analysis Module User Guide	MAN0030168

Customer and technical support

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- Order and web support

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

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.