OpenArray™ Plate experiments

USER GUIDE

TrueMark™ OpenArray™ Plate and custom miRNA OpenArray™ panels

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: 4470935 E (English)

Revision	Date	Description
E	1 October 2024	• Sealing instructions were updated throughout document. • Minor verbiage updates throughout document.
D	26 March 2024	• The plates were rebranded to TrueMark™ OpenArray™ Plate. • The OpenArray™ Sample Tracker Software was removed. • Information was added for OpenArray™ AccuFill™ Software v2.0. • The instructions to download the setup files were updated. • The locations of the template files and example files were updated. • The SuperScript™ VILO™ cDNA Synthesis Kit was removed. • The storage conditions for the OpenArray™ AccuFill™ System Tips was updated to one week after the box is opened. • The real-time PCR system user guide was updated to *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832). • The instructions to seal the OpenArray™ Plate were updated. The protective film on the outside of the lid is removed after sealing the plates, but before performing real-time PCR. • QuantStudio™ Design and Analysis Software v2.7 or later was added as an option to view the OpenArray™ Plate images. • The location of the software was updated to `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12KFlex Software`, where <...> is the installation drive. • The information about the default installation drive was updated. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer. • The PCR instrument in the genotyping section was updated to ProFlex™ 2 × Flat PCR System. • The stability of a loaded and sealed TrueMark™ OpenArray™ Plate in the genotyping section was updated to 24 hours (“Seal the TrueMark™ OpenArray™ Plate” on page 114) to be consistent with the information provided in the list of storage conditions (“Storage conditions” on page 106). • The QuantStudio™ 12K Flex OpenArray™ microRNA Starter Kit was removed. The information was updated to provide a procedure for custom miRNA OpenArray™ panels. • Instructions were added to review the QC images before analyzing the experiment results.. • The digital PCR kit was removed. • The safety appendix was updated. The information specific to the QuantStudio™ 12K Flex Real-Time PCR Instrument was removed because this document covers experiment setup. For information about symbols and standards, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).
C	22 April 2014	Baseline for this revision history.

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

Contents

About this guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Each part in this guide provides instructions for performing experiments on the QuantStudio™ 12K Flex Real–Time PCR System using OpenArray™ Plate formats. The following parts are provided in this guide.

- "QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit" on page 12—Prepare samples, prepare the sample plate, run and analyze a gene expression experiment.
- "QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit" on page 88—Prepare samples, prepare the sample plate, run and analyze a genotyping experiment.
- "Custom miRNA OpenArray™ panels" on page 165—Prepare samples, prepare the sample plate, run and analyze a microRNA experiment.
- Appendix A, "Ordering information"
- Appendix B, "Plate information"
- Appendix C, "PCR good laboratory practices"
- Appendix D, "Safety"
- Appendix E, "Documentation and support"

The instructions for gene expression experiments and genotyping experiments are specific to the starter kits. We recommend that you use the starter kits to familiarize yourself with the QuantStudio™ 12K Flex Real–Time PCR System. After performing the starter kit experiments, you can follow the instructions in this guide to perform your own experiments. Tips for running your own experiments are provided at various points in this guide.

Part I

QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit

Introduction

QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit

The QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit contains materials that are required to perform two experiments on the QuantStudio™ 12K Flex Real–Time PCR System, from sample preparation to data analysis, unless otherwise shown in Table 1 on page 14. The materials include TrueMark™ OpenArray™ Plate formats, reagents, and accessories. The components allow for a typical setup for two gene expression experiments.

Table 1 Components of the QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit.

Component	Cat. No.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit	4469604	• TaqMan™ OpenArray™ Real-Time PCR Master Mix, 2X, 1.5 mL • TaqMan™ OpenArray™ Human Endogenous Control Panels (2 plates) • Human cDNA controls (brain, liver, lung, and placenta) • QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit (Cat. No. 4469586)	Contains reagents to conduct two gene expression experiments on the QuantStudio™ 12K Flex Real–Time PCR System, using the TaqMan™ OpenArray™ Human Endogenous Control Panels as an example. This kit contains human cDNA control samples.
QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit	4469586	• OpenArray™ Case Lid (6 lids) • OpenArray™ Plugs (6 plugs) • OpenArray™ Carriers (1 or 2 carriers) • QuantStudio™ Immersion Fluid(6 syringes) • OpenArray™ Immersion Fluid Tip • OpenArray™ AccuFill™ System Tips (1 box of 384 tips) • OpenArray™ 384-well Sample Plate (10 plates) • OpenArray™ 384-Well Plate Seals (10 seals)	Contains accessories to assemble TrueMark™ OpenArray™ Plate formats for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

Plates

The instructions in this document use three types of plates. The plates are listed described in detail in Appendix B, “Plate information”.

- MicroAmp™ Optical 96-Well Reaction Plate (96-well plate)
  A non-optical 96-well reaction plate can also be used.
- OpenArray™ 384-well Sample Plate (384-well plate, for QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)
- TrueMark™ OpenArray™ Plate (OpenArray™ Plate)

File formats

The files are used to track your assays and samples. The QuantStudio™ 12K Flex Software (included with the QuantStudio™ 12K Flex Real–Time PCR System) contains example files for each starter kit experiment type.

The instructions in this guide use four types of files.

- Sample information file (CSV)—Contains the sample layout information for the plate. Allows input of Sample IDs. See page 15.
- Transcript plate file (TPF)—Contains the information for gene expression runs. Allows input of Assay IDs and thermal cycling protocol. See page 16.
- Template file (EDT)—Includes complete setup information (samples, assays, and cycling protocol) saved as a template. See page 17.
- Experiment file or data file (EDS)—A complete data file. See page 18.

Additional files (AIF, TXT) are available for selection if you use the batch experiment setup in the software to create and run your own experiments.

Sample information file (CSV)

We recommend that you create or use a comma-delimited file (CSV) to track your cDNA or gDNA samples. Use a sample information file to perform these tasks.

- Track where samples and controls are located in the 96-well plate. See Chapter 2, “Prepare the nucleic acid samples”.
- Map the sample locations, depending on the TrueMark™ OpenArray™ Plate format being used.
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate. See Chapter 3, “Prepare the OpenArray™ 384-well Sample Plate”.
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each TrueMark™ OpenArray™ Plate. See Chapter 4, “Prepare the TrueMark™ OpenArray™ Plate”.
- Associate information about the samples with the data results to normalize data or compute standard curves and calculate concentrations.

IMPORTANT! To enable accurate results, you must correctly track sample information from plate to plate.

For OpenArray™ AccuFill™ Software v2.0, all sample tracking and mapping features are available in this software. The OpenArray™ Sample Tracker Software is not used for OpenArray™ AccuFill™ Software v2.0.

- OpenArray™ 384-well Sample Plate—Integrate this file with a plate setup file in the OpenArray™ AccuFill™ Software. See “Prepare the plate setup files” on page 36.
- TrueMark™ OpenArray™ Plate—Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see “Start a run from the software” on page 51), or after the run is complete.

Note: To track sample information for the starter kit experiments, use the example CSV files supplied with the QuantStudio™ 12K Flex Software.

Plate setup file (TPF)

Use an OpenArray™ Plate setup file

Plate setup files (TPF) contain the assay information for individual OpenArray™ Plate formats, including the gene symbol, gene name, assay ID, and location of each assay on the plate.

- Use the OpenArray™ AccuFill™ Software to integrate the sample information from a 384-well plate file (CSV) with the assay information in the plate setup file. See “Prepare the plate setup files” on page 36).
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (EDS file). See “Start a run from the software” on page 51).

Access the starter kit plate setup files

1. Go to **thermofisher.com/OA-platefiles**.
2. In the **Select your product** dropdown list, select **TaqMan OpenArray Inventoried**.
3. Enter the serial numbers, then click **Download**.

Download your own plate setup files

To process a TrueMark™ OpenArray™ Plate on the QuantStudio™ 12K Flex Real–Time PCR System, you must download the specific plate files that correspond to your plate and experiment type. Gene expression experiments use transcript plate files (TPF).

Note:

- The OpenArray™ AccuFill™ Software v2.0 includes a feature to download the TPF files. The computer running the software must be connected to the internet.
- The OpenArray™ AccuFill™ Software v2.0 includes a feature to create a TPF file from a template.
- For more information about these features, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

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1. Go to **thermofisher.com/OA-platefiles**.
2. Select one of the following options.
   - **Custom**
   - **Inventoried**

3. Enter the following information.

Product	Information
Custom	a. Enter the ***Lot number*** or ***Batch number***. b. Enter one ***Serial number*** from the lot. Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

4. *(Custom gene expression plates only)* Select a target name option.
   - **With microbial target names**
   - **Without microbial target names**

   The microbial target name selection is not displayed if inventoried products are selected.

5. Click **Download**.

The downloaded files are in a compressed ZIP folder.

Template file (EDT)

An experiment document template file (EDT) contains predefined experiment setup information, such as experiment type, assay names, and run method.

You can access a template to create a new experiment from the two locations.

- QuantStudio™ 12K Flex Instrument touchscreen. See “Start a run from the instrument touchscreen” on page 55.
- QuantStudio™ 12K Flex Software. See “Start a run from the software” on page 51.

  To create and run the starter kit experiments, use the example template files supplied with the QuantStudio™ 12K Flex Software.

  The example template files are at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray`, where <...> is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Experiment file (EDS)

An experiment document single file (EDS) is an electronic record used by the QuantStudio™ 12K Flex Software that contains information about a particular TrueMark™ OpenArray™ Plate that was run on the QuantStudio™ 12K Flex Instrument. The EDS file includes the following information.

- Metadata
  - Name
  - Barcode
  - Comments
- Experiment setup
  - Well contents
  - Assay definitions
- Run method (thermal cycling protocol)
- Run results
- Analysis protocol
- Analysis results
- Audit records
- Other plate-specific data

Use an EDS file to perform these tasks.

- Create and run an experiment using the QuantStudio™ 12K Flex Software (see “Start a run from the software” on page 51).

  To create and run the starter kit experiments, use the example template files (EDT, see “Template file (EDT)” on page 17) supplied with the software.
- Create and run an experiment using the QuantStudio™ 12K Flex Instrument touchscreen (see “Start a run from the instrument touchscreen” on page 55).
- Analyze the experiment results in the QuantStudio™ 12K Flex Software (see Chapter 6, “Analyze the experiment results”).

  To view and analyze results for the starter kit experiments, use the example experiment files supplied with the QuantStudio™ 12K Flex Software.

Starter kit data files

Use the example files supplied with the QuantStudio™ 12K Flex Software to perform the tasks that are described in this guide.

Table 2 QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit data files referenced in this guide

File type	Description	File name	Location[1]	Used in
TPF	Transcript plate file	—	Download (see "Access the starter kit plate setup files" on page 16)	Chapter 3, "Prepare the OpenArray™ 384-well Sample Plate"
CSV	96-well sample information file	—	These files can be generated in the OpenArray™ AccuFill™ Software v2.0.	Chapter 3, "Prepare the OpenArray™ 384-well Sample Plate"
EDT	Experiment template	`TaqMan Gene Expression.edt`	`<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray`	Chapter 5, "Perform the instrument run"
EDS	Experiment data file	`Gene Expression Starter Kit Example.eds`	`<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\examples\Gene Expression`	Chapter 6, "Analyze the experiment results"

[1] <...> is the drive on which the software is installed. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Workflow

QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit

Prepare the nucleic acid samples (page 21)

The workflow for sample preparation varies, depending on starting from the MicroAmp™ Optical 96-Well Reaction Plate using cDNA or your own DNA.

Prepare the OpenArray™ 384-well Sample Plate (page 28)

Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray™ 384-well Sample Plate.

Prepare the TrueMark™ OpenArray™ Plate (page 33)

- Prepare for sample transfer using 384-well plates.
- Transfer the samples from 384-well plates to OpenArray™ plates using OpenArray™ AccuFill™ Software.
- Seal the OpenArray™ plates.
- Complete sample transfer for the remaining OpenArray™ plates.

Perform the instrument run (page 47)

- Prepare the QuantStudio™ 12K Flex Software.
- Load the OpenArray™ plates into the instrument.
- Perform the instrument run.
- *(Optional)* Monitor the experiment run.
- Unload the OpenArray™ plates from the instrument.
- *(Optional)* Transfer the experiment results to the computer.

Analyze the experiment results (page 63)

2 Prepare the nucleic acid samples

In this chapter, you prepare the nucleic acid samples for your QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit experiment.

Perform the starter kit experiment

When you perform the gene expression starter kit experiment, use the human cDNA control samples (brain, liver, lung, and placenta) provided in the QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit (see Table 1 on page 14).

Workflow

Prepare nucleic acid samples for starter kit experiment

Obtain human cDNA control samples from the starter kit (see Required materials (page 21))

(Optional) Transfer the cDNA samples to a 96-well reaction plate (page 22)

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Cat. No.
Starting material: Human cDNA control samples (brain, liver, lung, and placenta) from the starter kit	Included in the kit
MicroAmp™ Optical 96-Well Reaction Plate	4316813

(Optional) Transfer the cDNA samples to a 96-well reaction plate

To load cDNA samples and controls into a TrueMark™ OpenArray™ Plate, pipet the samples into a 96-well reaction plate.

Enter sample information for the 96-well reaction plate directly into the OpenArray™ AccuFill™ Software v2.0. See “Track the samples” on page 29.

Use the following cDNA samples and controls.

1	2	3	4	5	6
NTC	HB	HLiv	HLun	HP	NTC

- HB = Human Brain
- HLiv = Human Liver
- HLun = Human Lung
- HP = Human Placenta
- NTC = No Template Control

1. Prepare the PCR reaction mix with each of the four cDNA samples in strip cap tubes or in one row of a MicroAmp™ Optical 96-Well Reaction Plate.

   There is 10 µL per sample of excess volume.

Component	Volume per subarray per sample	Number of subarrays per sample	Total volume
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X)	2.5 µL	8	25.0 µL
Water	1.3 µL	8	13.0 µL
cDNA	1.2 µL	8	12.0 µL
Total volume	**5.0 µL**	**—**	**50.0 µL**

2. Prepare the PCR reaction mix with two no template controls (NTCs) in strip cap tubes or in one row of a MicroAmp™ Optical 96-Well Reaction Plate.

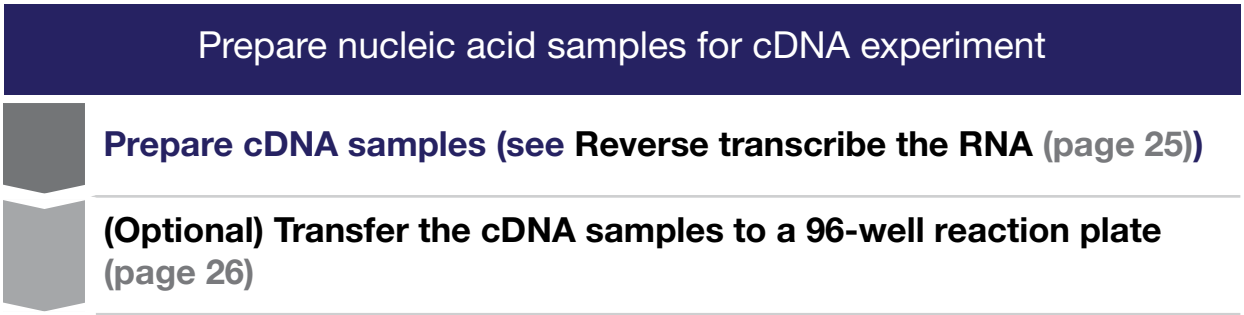
Component	Volume per subarray per sample	Number of subarrays per sample	Total volume
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X)	2.5 µL	8	25.0 µL
Water	2.5 µL	8	25.0 µL
Total volume	**5.0 µL**	**—**	**50.0 µL**

Proceed to Chapter 3, “Prepare the OpenArray™ 384-well Sample Plate”.

Perform your own experiments with cDNA

When you prepare cDNA samples for your own gene expression experiments, note the items described in the following sections.

Workflow



Required materials

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Item	Source
Starting material: Total RNA	User-supplied
One of the following kits: • *(Recommended)* High-Capacity cDNA Reverse Transcription Kit • High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	• 4368813 • 4374967
RNase-free water	**MLS**
Incubator or thermal cycler	**MLS**
MicroAmp™ Optical 96-Well Reaction Plate	4316813
MicroAmp™ Clear Adhesive Film	4306311

RNA quantity

The recommended quantity of starting material (total RNA, for preparing cDNA) is 10 µL at a concentration of 200 ng/µL (2 µg total). To prepare cDNA from RNA, see “Reverse transcribe the RNA” on page 25.

Guidelines for RNA quality

RNA that is used for gene expression experiments should have these characteristics.

- Is extracted from the raw material of interest using an optimized protocol.
- Does not contain PCR inhibitors.
- Has an $A_{260/230}$ ratio between 2.0 and 2.4.
- Has an $A_{260/280}$ ratio between 1.8 and 2.1.
- Has an RNA Integrity Number (RIN) that is between 6.5 and 10.

Reverse transcribe the RNA

For other reverse transcription kits, see the documentation for the kit.

1. Thaw the reverse transcription kit components and the total RNA on ice.
2. Combine the following components to prepare a 2X reverse transcription mix.

Component		Volume for 1 reaction	Stock concentration	Final concentration (2X mix)
High-Capacity cDNA Reverse Transcription Kit	10X RT Buffer	2.0 µL	10X	2X
	10X RT Random Primers	2.0 µL	10X	2X
	25X dNTP Mix	1.0 µL	25X	2X
	MultiScribe™ Reverse Transcriptase, 50 U/µL	1.0 µL	50 U/µL	5 U/µL
RNase-free water		4.0 µL	—	—
Total volume of 2X reverse transcription mix		**10.0 µL**	—	—

3. Transfer 10 µL of reverse transcription mix to each well of the MicroAmp™ Optical 96-Well Reaction Plate.
4. Add 10 µL of total RNA to each well of the MicroAmp™ Optical 96-Well Reaction Plate.
   The total volume per reaction is 20 µL.
5. Seal the plate with MicroAmp™ Clear Adhesive Film, then incubate at room temperature for 10 minutes.
6. Incubate at 37°C for 2 hours.
7. Place on ice for 5 minutes, then centrifuge briefly.
8. Incubate at 75°C for 10 minutes.
9. Place on ice for 5 minutes, then centrifuge briefly.

cDNA can be stored at –20°C for up to 2 months.

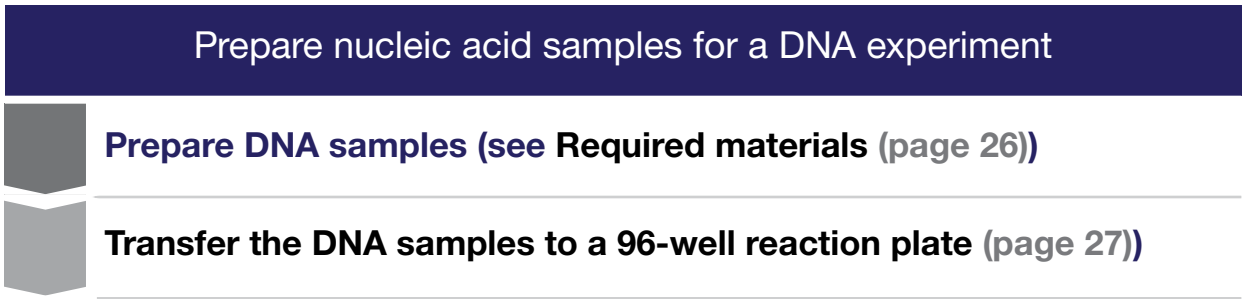
(Optional) Transfer the cDNA samples to a 96-well reaction plate

- Transfer 2.0 µL of the prepared cDNA into the appropriate number of wells of a MicroAmp™ Optical 96-Well Reaction Plate, depending on the TrueMark™ OpenArray™ Plate format being used.
- *(Recommended)* Create a sample information file (CSV) to track where the samples are in the 96-well sample plate. For OpenArray™ AccuFill™ Software v2.0, samples are mapped in the OpenArray™ AccuFill™ Software. See *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).
- Proceed to Chapter 3, “Prepare the OpenArray™ 384-well Sample Plate”.

Perform your own experiments with DNA

When you prepare DNA samples (genomic or plasmid) for your own real-time PCR experiments, note items described in the following sections.

Workflow



Required materials

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Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Starting material: gDNA or plasmid DNA	User-supplied
MicroAmp™ Optical 96-Well Reaction Plate	4316813

DNA quality

DNA that is used for real-time PCR experiments should have the following characteristics.

- Is extracted from the raw material that you are testing with an optimized protocol; salting out procedures and crude lysates are not recommended.
- Does not contain PCR inhibitors.
- Has an $A_{260/230}$ ratio between 1.7 and 1.9.
- Has an $A_{260/280}$ ratio between 1.7 and 1.9.
- Is intact as visualized by gel electrophoresis.
- Has no nucleases present; is stabilized for storage.
- Has good gel image.

DNA quantity

We recommend that you quantify the amount of gDNA in the samples.

- The recommended quantity of starting material (cDNA, gDNA or plasmid DNA) is 10 µL at a concentration of approximately 100 ng/µL.
- For accurate results, it is important to normalize all DNA samples in an experiment so that each through-hole receives the same input quantity of sample.

Transfer the DNA samples to a 96-well reaction plate

- Transfer 5.0 µL of the DNA samples into the appropriate number of wells of a MicroAmp™ Optical 96-Well Reaction Plate, depending on the TrueMark™ OpenArray™ Plate format being used.

  See Appendix B, “Plate information”.

- *(Recommended)* Create a sample information file (CSV) to track where the samples are in the 96-well sample plate. For OpenArray™ AccuFill™ Software v2.0, samples are mapped in the OpenArray™ AccuFill™ Software. See *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

- Proceed to Chapter 3, “Prepare the OpenArray™ 384-well Sample Plate”.

3 Prepare the OpenArray™ 384-well Sample Plate

In this chapter, you use an 8– or 12–channel pipette to transfer the nucleic acid samples from the 96-well reaction plates to the OpenArray™ 384-well Sample Plate. The plates are described in detail in Appendix B, “Plate information”.

You track the sample locations from the 96-well reaction plates to the appropriate locations in the OpenArray™ 384-well Sample Plate. The workflow for preparing the OpenArray™ 384-well Sample Plate varies, depending on the starter kit or the experiment type in use.

Workflow

Prepare the OpenArray™ 384-well Sample Plate

1. **Track the samples** (page 29)
2. **Prepare the PCR Reaction Mix** (page 31)
3. **Transfer the samples to the OpenArray™ 384-well Sample Plate and add the PCR mix** (page 31)

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
96-well reaction plates, containing prepared cDNA samples	User-supplied
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X), 1.5 mL	4462159[1]
OpenArray™ 384-well Sample Plates	4406947[1]
OpenArray™ 384-Well Plate Seals	4469876[1]
RT-PCR Grade Water	AM9935[2]
Fine-tip marker	**MLS**

[1] Provided in the starter kit.
[2] Not provided in the starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For OpenArray™ AccuFill™ Software v2.0, samples are tracked in the OpenArray™ AccuFill™ Software. The samples are tracked in the **Map Plates** tab.

For more information about OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Navigate to the **Full Run** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Gene expression**.
2. In the **Plate format** section, select a format for your experiment.

Experiment type	Format
Starter kit experiments	**Gene Expression—56**
Your own experiments	• **Gene Expression—18** • **Gene Expression—56** • **Gene Expression—112** • **Gene Expression—168** • **Gene Expression—224**

3. In the **Pipettor** section, select a type of pipette.
   - **Fixed**
   - **Adjustable**

   The number of OpenArray™ Plate formats displayed in the **Add your OpenArray plate serial numbers** section depends on the selections made in the previous steps.

4. In the **Add your OpenArray Plate serial numbers** section, click **Choose File**, navigate to the location of the TPF file, then select the file.

   Repeat for each TPF file.

5. In the **Add your sample plates - optional** section, click **Choose File**, navigate to the location of the CSV file, then select the file.

   If the sample plate file is not imported, the samples must be added manually.

   The file must be a CSV file for a 96-well format.

   The format of the sample plate file is validated. For information about the required format, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

   The name of the file is displayed in the **Select file** field.

6. Repeat step 5 for each CSV file.

7. Click **Next**.

   The **Map plates** pane is displayed.

8. *(Optional)* Add or edit the sample name.

9. Label the OpenArray™ 384-well Sample Plate with a fine-tip marker.

   Based on the tracking information on the **Map plates** pane, mark the sections of the OpenArray™ 384-well Sample Plate to transfer samples from the 96-well reaction plates.

10. Click **Next**.

The **Start run** pane is displayed.

Set up the samples in the OpenArray™ 384-well Sample Plate, then prepare to load the OpenArray™ Plate formats in the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.

Prepare the PCR Reaction Mix

1. Gently invert the tube of TaqMan™ OpenArray™ Real-Time PCR Master Mix several times.
2. Combine the following components to prepare the PCR Reaction Mix.

Component	Volume for 1 area of the 384-well sample plate[1]
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X)	132.0 µL
RT-PCR Grade Water	68.6 µL
Total volume of PCR Reaction Mix	**200.6 µL**

[1] One area of a 384-well sample plate corresponds to a single OpenArray™ Plate. See Appendix B, "Plate information".

3. Mix well by pipetting up and down.

Transfer the samples to the OpenArray™ 384-well Sample Plate and add the PCR mix

Thaw the cDNA samples at room temperature.

1. Vortex the cDNA samples to mix, then centrifuge for 1 minute at 1,000 *x* g to collect the contents at the bottom of the tube.
2. Review the concentration of the normalized samples. The recommended starting concentration for human cDNA, gDNA, and plasmid DNA samples is approximately 100 ng/µL.

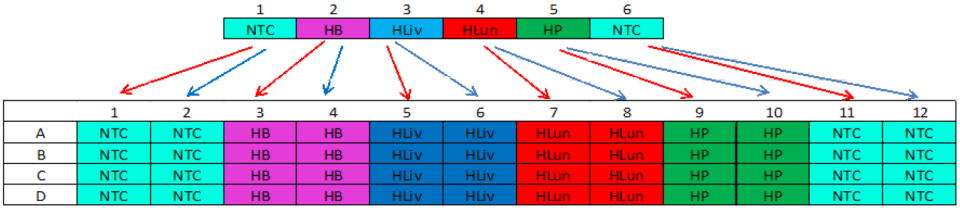
   Note: For optimal results, normalize all cDNA, gDNA, and plasmid DNA samples in an experiment. For example, if you use 200 ng/µL total RNA starting material and assume 100% efficiency in the reverse transcription reaction, you should obtain a human cDNA concentration of approximately 100 ng/µL equivalent to the total RNA.

3. Load the OpenArray™ 384-well Sample Plate based on the plate layout.

   See "Track the samples" on page 29.

   a. Add 5 µL of each PCR sample to the 384-well sample plate (see "Prepare the PCR Reaction Mix" on page 31).

b. Using 6 tips from an 8- or 12-channel pipette, transfer the normalized cDNA, gDNA, or plasmid DNA samples from the 96-well reaction plate to the OpenArray™ 384-well Sample Plate.



Component	Volume per 384-well sample plate well[1]	
	Format 56 (starter kit experiment)	Format 18 (in triplicate) and remaining formats
Prepared PCR mix	3.8 µL	3.8 µL
Normalized human cDNA, gDNA, or plasmid DNA samples	1.2 µL	1.2 µL
Total volume	**5.0 µL**	**5.0 µL**

[1] One well of a 384-well sample plate corresponds to one subarray of an TrueMark™ OpenArray™ Plate. The number of subarrays required depends on the format of the TrueMark™ OpenArray™ Plate. For detailed information about the TrueMark™ OpenArray™ Plate, see Appendix B, “Plate information”.

4. Seal the sample plate, vortex gently to mix, then centrifuge for 1 minute at 2,000 *x* g to eliminate bubbles and to collect the contents at the bottom of the wells.

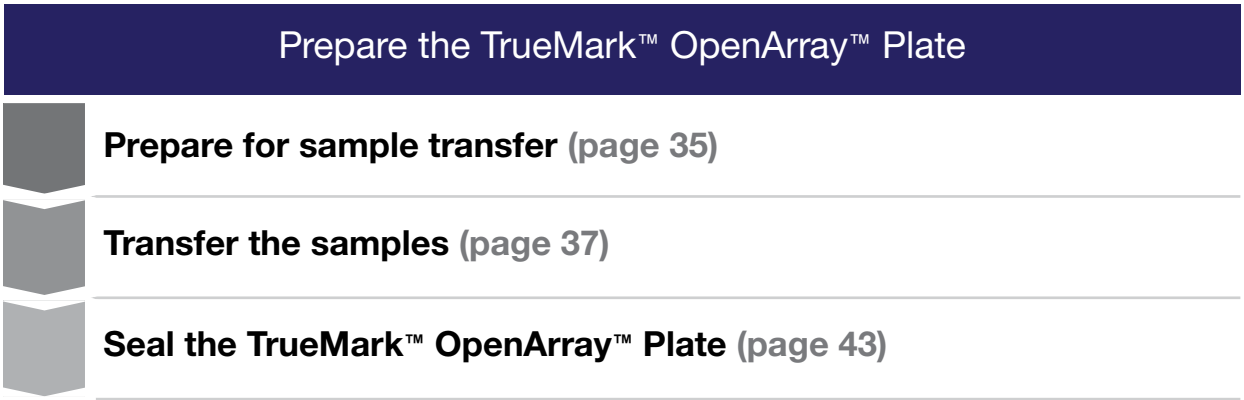
5. Place the sample plate on ice for up to 1 hour.

Proceed to Chapter 4, “Prepare the TrueMark™ OpenArray™ Plate”.

4 Prepare the TrueMark™ OpenArray™ Plate

In this chapter, you use the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System to transfer the nucleic acid samples from the OpenArray™ 384-well Sample Plate to the OpenArray™ Plate formats. The workflow is the same for all OpenArray™ Plate formats.

Workflow



Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
TrueMark™ OpenArray™ Plate	See Appendix A, “Ordering information”.
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System	4471021
QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit The accessories kit contains the following components: • OpenArray™ Case Lid (6 lids) • OpenArray™ Plugs (6 plugs) • OpenArray™ Carriers (2 carriers) • OpenArray™ Immersion Fluid (6 syringes) • OpenArray™ Immersion Fluid Tip • OpenArray™ AccuFill™ System Tips (1 box of 384 tips) • OpenArray™ 384-well Sample Plate (10 plates) • OpenArray™ 384-Well Plate Seals (10 seals)	4469586
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
Foil seals	**MLS**
Bleach (10%)	**MLS**
Ethanol	**MLS**
Fine-tip marker	**MLS**
Razor blade	**MLS**
Powder-free gloves	**MLS**
Laboratory-grade wipes	**MLS**
Safety glasses	**MLS**
Tweezers or forceps (for removing foil sections from the 384-well sample plate)	**MLS**

Storage conditions

Item	Condition	Storage
OpenArray™ Plate	Frozen, unopened	Store at –20°C until the expiration date provided on the product label.
	Thawed, unopened	Store at room temperature for up to 24 hours.
	Thawed, opened	Store at room temperature for up to 1 hour.
	Loaded and sealed, pre-run	Store at room temperature, protected from light, for up to 1 hour.
OpenArray™ Immersion Fluid	Unopened	Store at room temperature until the expiration date provided on the product label.
	Opened	Store at room temperature. Do not store any remaining immersion fluid. Use the amount required, then discard the remainder.
OpenArray™ AccuFill™ System Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Use tips within one week.

Prepare for sample transfer

Guidelines for handling the OpenArray™ Plate

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Plate.

- Hold the OpenArray™ Case by the edges.
- Do not touch the through-holes of the OpenArray™ Plate.
- Load and seal an OpenArray™ Plate within *one hour* after opening the package.
- If you drop a loaded OpenArray™ Plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray™ Plate if it becomes dislodged from the case.

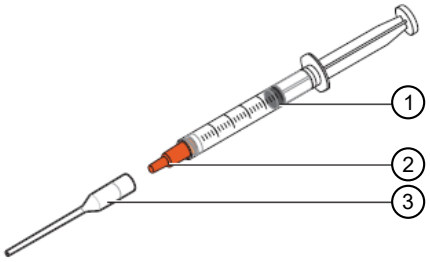
Prepare the equipment and plates

Ensure that the OpenArray™ 384-well Sample Plate, the OpenArray™ AccuFill™ System Tips, and OpenArray™ Plate holder are completely clean and dry.

1. Remove an OpenArray™ Plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).

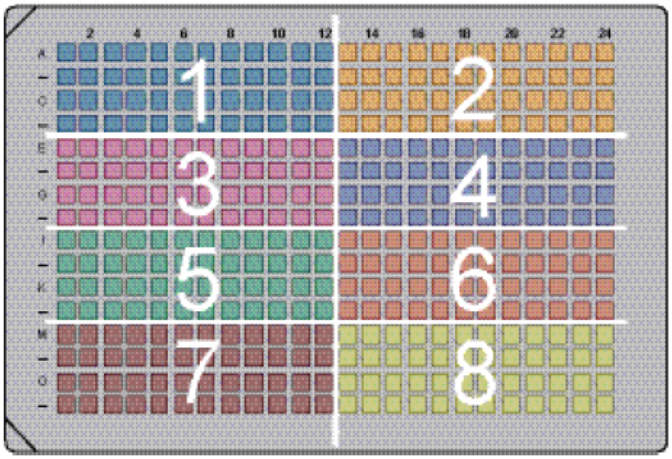
2. Prepare a syringe containing OpenArray™ Immersion Fluid.
   a. Remove the cap from the syringe containing OpenArray™ Immersion Fluid.

b. Remove the cap and attach the tip to the syringe. Place the assembly on a clean surface.



① OpenArray™ Immersion Fluid
② Cap (remove)
③ Syringe tip (attach)

3. Score or cut the foil seal of the OpenArray™ 384-well Sample Plate into the 8 sections shown below, then place the plate on ice to keep the samples cold.



Prepare the plate setup files

- OpenArray™ AccuFill™ Software v2.0 allows the transfer of samples without a sample plate file. The QuantStudio™ 12K Flex Instrument requires a sample plate file if the real-time PCR run is started with a TPF file or an EDT file.
- For your own gene expression experiments, the following plate setup files can be used to transfer samples with the OpenArray™ AccuFill™ Software
  - OpenArray™ 384-well sample information file (CSV, see “Track the samples” on page 29)
  - OpenArray™ plate setup file (TPF, see “Use an OpenArray™ Plate setup file” on page 16)
- *(Optional)* If you created a CSV file in the OpenArray™ AccuFill™ Software (see “Track the samples” on page 29), you can import the sample information in this file directly into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

Transfer the samples

Start the system

Note: If the samples were tracked immediately before this section, the system might be on (see "Track the samples" on page 29). The sample tracking and sample transfer functions are both done with OpenArray™ AccuFill™ Software if OpenArray™ AccuFill™ Software v2.0 is used.

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips. See "Set up the system" on page 37.

The instrument does not initiate a self-test immediately after starting the software. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

The other features in the software can be accessed after starting the software without a self-test.

1. Ensure that the instrument door is closed.
2. Power on the instrument, if it is off.
3. Start the OpenArray™ AccuFill™ Software ( ).
   The software checks the computer and connections as the system starts.

Proceed to set up the system (see "Set up the system" on page 37).

Set up the system

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

1. Open the instrument door, empty the waste bin, then place the waste bin back on the instrument deck.

CAUTION! Wear appropriate personal protective equipment while handling the waste bin.

2. Ensure that the sample plate holder and the OpenArray™ Plate holders are empty.
3. Place the sample plate in the sample plate holder on the instrument deck, with the notch to the left. Do not stack sample plates.
4. Place each OpenArray™ Plate in an OpenArray™ Plate holder.

5. Replace the tip boxes, if necessary.

   Each tip box contains 384 tips, divided into 8 sections.

   When setting up a run, the status of the tip boxes is confirmed in the software. A full tip box is recommended when starting a run.

6. Remove the cover from each tip box.

   Ensure that the tip box covers are removed from the instrument deck.

   IMPORTANT!
   - Do not reuse tips.
   - Use tips within one week of opening the box.
   - Discard any unused tips within one week.

7. Close the instrument door.

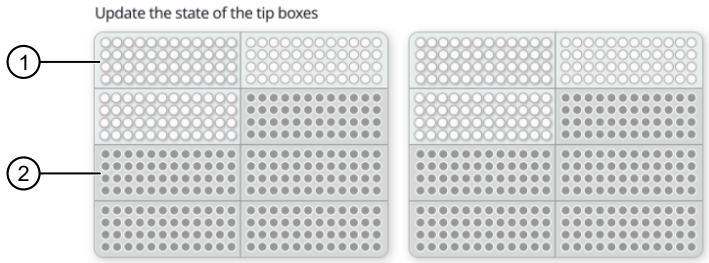
The system is ready to start a run. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes. The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box that is full.
② Section of the tip box that is empty.

2. *(Optional)* Click **Auto-fill tip boxes**.

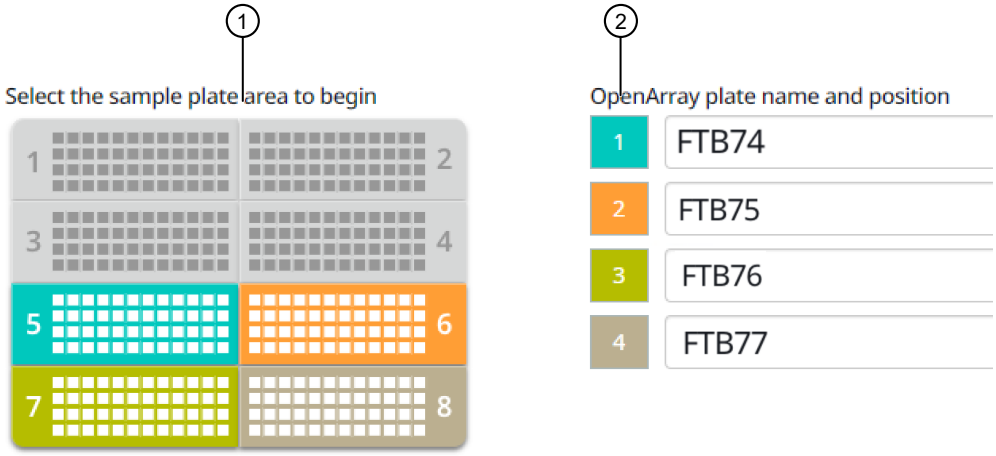
   The status of all sections of the tips boxes is set to full.

3. Select the first section of the sample plate to be used to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple plates are filled during a run. The software selects the total number of sections that correspond with the total number of plates.

   In the following example, section 5 was selected. The group of sections 5, 6, 7, and 8 is highlighted by the software because four plates are being filled.

The position box displays the color that corresponds to the section of the sample plate.



① Sample plate section (section 5, 6, 7, and 8 are highlighted).
② Corresponding plates.

4. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   Note: Do not remove the foil from all the sections of the sample plate at once.

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of these conditions.

   - The waste bin is not in position.
   - The sample plate is not in position.
   - The plates are not in position.
   - There are more plates on the instrument deck than are defined in the experiment setup.

The **Deck** screen is displayed.

For a description of the run progress, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see “Seal the TrueMark™ OpenArray™ Plate” on page 43).

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument

After an OpenArray™ Plate is filled, the **Remove plate and foil** dialog box is displayed (see Figure 1).

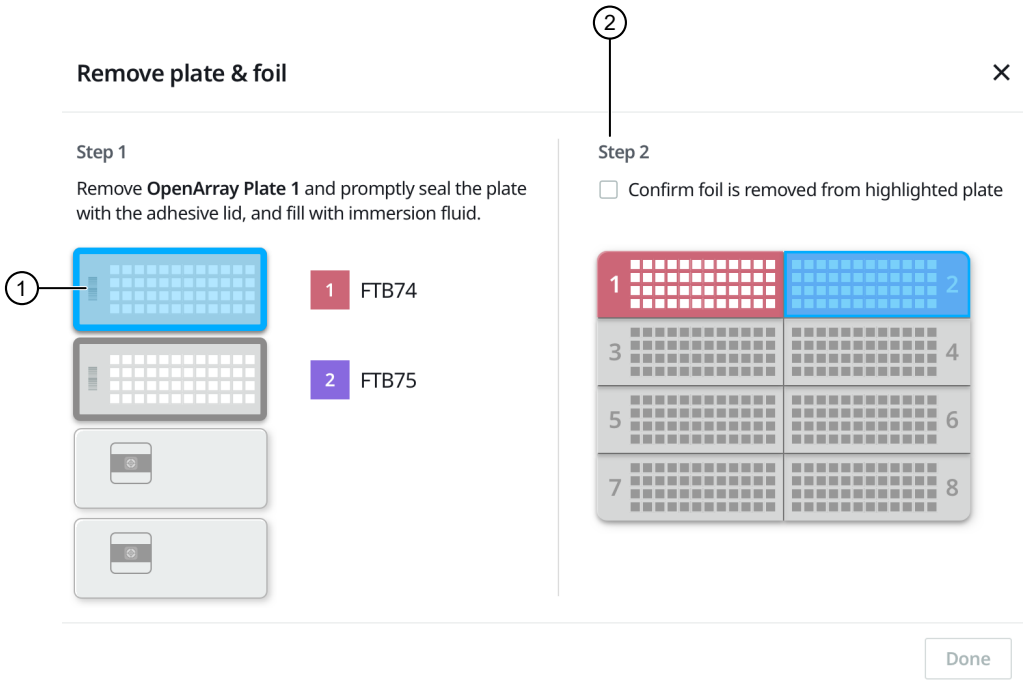


Figure 1 Remove plate and foil dialog box.

① OpenArray™ Plate to remove from the instrument.
② **Confirm foil is removed from highlighted plate section** checkbox.

Remove each OpenArray™ Plate *immediately* after it has been filled, even if the run was set up to fill multiple plates.

After the last OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed (see Figure 2).

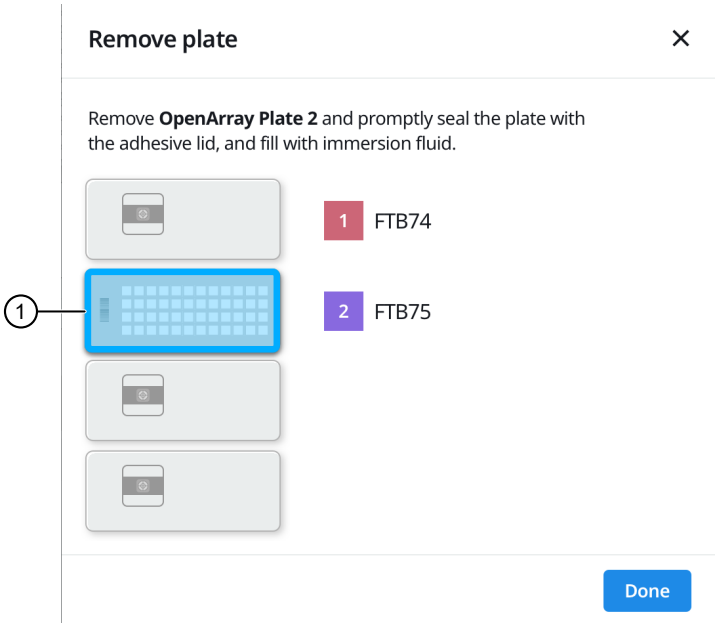


Figure 2 Remove plate dialog box

① OpenArray™ Plate to remove from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate immediately, to avoid evaporation within the plate.

   One of the following dialog boxes is displayed:

   - The **Remove plate and foil** dialog box.
   - The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.

   The run does not proceed under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The plates are not in position
   - There are more plates on the instrument deck than are defined in the experiment setup

   The instrument proceeds to load the next OpenArray™ Plate.

6. Repeat step 1 to step 5 for each OpenArray™ Plate to be loaded.

After all of the plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

A loaded TPF is generated for each OpenArray™ Plate. The loaded TPF file corresponds to the original TPF file that was imported for the run. The files are exported to the folder that was designed in the **Preferences**.

Note: Some workflows might not generate a loaded TPF file. For more information about the workflows available for the OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Seal the TrueMark™ OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

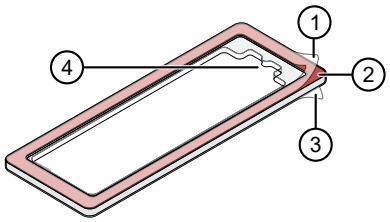
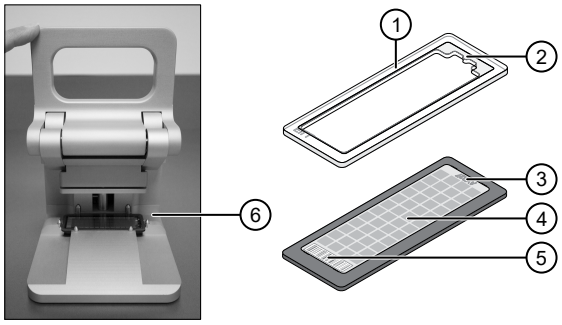


Figure 3 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

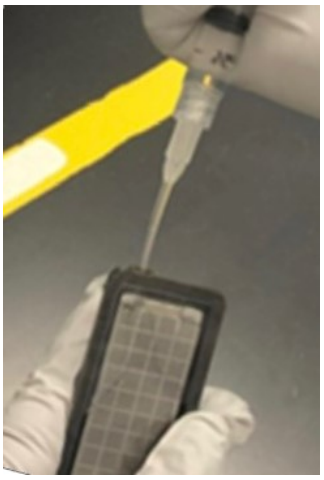
   Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

   IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.

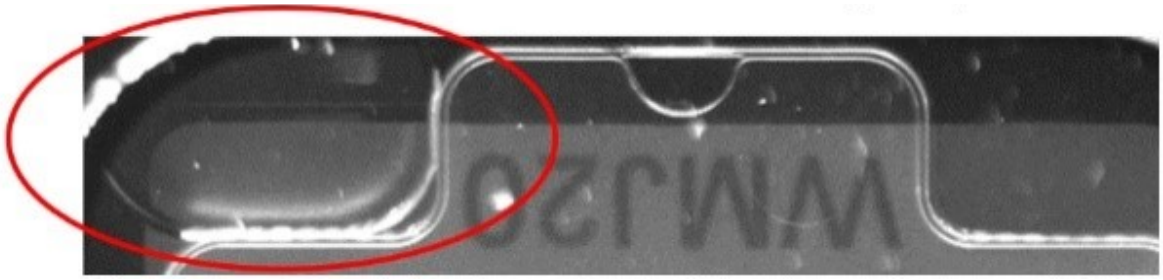


   The syringe tip must be in front of the array when filling the case with immersion fluid.

9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

   IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

   Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

The plate is ready for PCR.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (more than 6) of OpenArray™ Plates, follow these guidelines.

- To help avoid mistakes when entering sample information in the OpenArray™ AccuFill™ Software, load the OpenArray™ Plates in alphanumeric order (according to the OpenArray™ Plate serial number).
- Seal each OpenArray™ Plate immediately after loading is completed, while the other OpenArray™ Plates are loaded.

  IMPORTANT! To avoid evaporation, seal the OpenArray™ Plate with the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0, add the OpenArray™ Immersion Fluid, plug the case, then place the case in a vertical position.

- Use the OpenArray™ Carriers to transport up to four loaded OpenArray™ Plates to the QuantStudio™ 12K Flex Instrument.
- After loading is complete, you can use a large bin to properly discard any used OpenArray™ AccuFill™ System Tips.

  For cleaning procedures, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

5 Perform the instrument run

In this chapter, you run the OpenArray™ Plate formats on the QuantStudio™ 12K Flex Instrument. During the run, the instrument performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all OpenArray™ Plate formats.

Workflow

Perform the instrument run

1. **Prepare the QuantStudio™ 12K Flex Software** (page 48)
2. **Load the TrueMark™ OpenArray™ Plate into the instrument** (page 50)
3. **Run OpenArray™ Plate formats** (page 50)
4. **(Optional) Monitor experiments** (page 56)
5. **Unload the TrueMark™ OpenArray™ Plate from the instrument** (page 60)
6. **(Optional) Transfer experiment results** (page 60)

Prepare the QuantStudio™ 12K Flex Software

(Optional) Select OpenArray™ block run preferences

Preferences provide user-access to the settings that govern how the QuantStudio™ 12K Flex Software functions. This section summarizes only those preferences that apply to experiments with OpenArray™ Plate formats.

For detailed information about the preferences, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

1. Double-click **(QuantStudio™ 12K Flex Software shortcut)** to start the software.
2. In the toolbar, click **Tools** ▸ **Preferences**, then select the **OpenArray** tab.
3. Complete the tab, as needed.

Settings	Description
Setup Folder	Define the absolute path to the default folder from which the software imports experiment setup files. The **Import** dialog box opens to the import folder when invoked from the software.
Experiment Folder	Define the absolute path to the default folder to which the software reads or writes experiment files. The **Open** and **Save** dialog boxes open to the data folder when invoked from the software.
Passive Reference	Define the dye to use as the passive reference. The default is set to **None**. While the software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray™ experiments.
Default Browse File Type list	Define the file type that the **Import**, **Open**, and **Save** dialog boxes select by default when invoked from the software.
Apply experiment template (EDT) to all OpenArray experiment checkbox	If selected, the software applies the Run Method defined in the selected experiment template (EDT) to all OpenArray™ experiments. For more information about OpenArray™ experiment templates, see the software help.

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

IMPORTANT! You must restart the software for preference changes to take effect.

Access the Instrument Console

The **Instrument Console** displays every QuantStudio™ 12K Flex Real-Time PCR Instrument discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups.

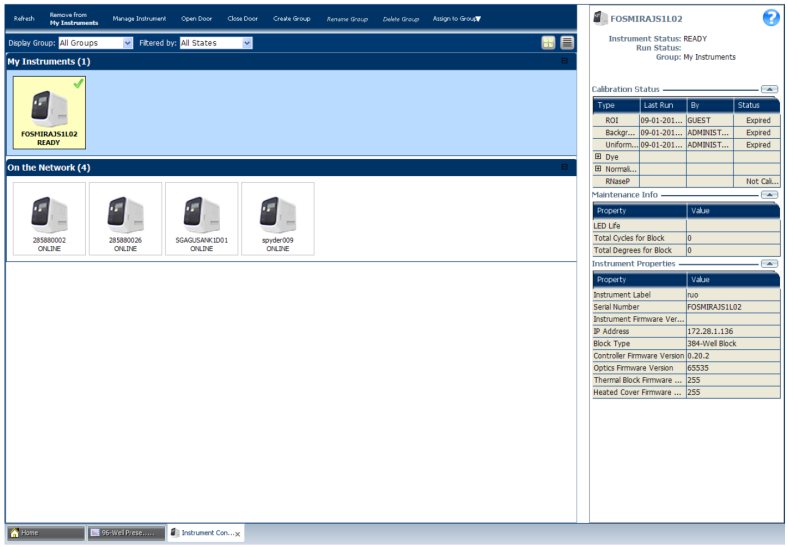
- **On the Network**—All instruments available on the network.
- **My Instruments**—Instruments you have selected to monitor.

To start and monitor a run on an instrument, you must move the instrument from the **On the Network** group to the **My Instruments** group or a custom group that you create.

To access the **Instrument Console** and enable monitoring of a networked instrument:

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. On the **Home** tab, from the **Tools** menu, select **Instrument Console**.
   If you do not see an instrument, click **Refresh** in the **Instrument Console** toolbar.



3. If needed, move an instrument from the **On the Network** group to a group that can be monitored:
   a. Click the instrument of interest, then click **Assign to Group** in the **Instrument Console** toolbar.

   b. Select the **My Instruments** group or a personal group in the drop-down list.

Note: Alternatively, you can select the icon of the instrument that you want to add to the **My Instruments** list, then click **Add to My Instruments**. Similarly, click **Remove from My Instruments** to remove an instrument from the **My Instruments** list. You can also drag and drop the instrument icon into **My Instruments** or into the group created by you.

The instrument is now monitored. The status is indicated by an icon in the upper right corner. For detailed information about the **Instrument Console**, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

Enable or change the notification settings

You can configure the QuantStudio™ 12K Flex Software to alert you by email when the QuantStudio™ 12K Flex Real-Time PCR Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the notification settings feature, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Load the TrueMark™ OpenArray™ Plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

IMPORTANT! Wear powder-free gloves when you handle the OpenArray™ Plate.

IMPORTANT! The instrument should be loaded and unloaded only by operators who have been warned of the moving parts hazard and who have been adequately trained.

1. Open the plate adapter on the instrument. Touch on the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software, to allow the plate adapter to come out from the instrument side.
2. Remove the clear protective film from the outside of the OpenArray™ Case (sealed plate + lid).
3. Place the OpenArray™ Plate or plates on the plate adapter.
   - Ensure that each plate is properly aligned in the plate adapter.
   - Ensure that the plate barcode is facing up and toward the front of the instrument.
4. Close the plate adapter on the instrument. Touch on the instrument touchscreen, or click **Close Door** in the **Instrument Console** of the software, to retract the plate adapter back into the instrument.

Run OpenArray™ Plate formats

You can run OpenArray™ Plate formats in one of these ways.

- From the QuantStudio™ 12K Flex Software. See “Start a run from the software” on page 51.
- From the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen. See “Start a run from the instrument touchscreen” on page 55.

Note: The starter kit experiments in this guide run OpenArray™ Plate formats from the QuantStudio™ 12K Flex Software.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Real-Time PCR Instrument is in operation.

Start a run from the software

There are two ways to create and run an OpenArray™ experiment (EDS) from the QuantStudio™ 12K Flex Software.

- For the starter kit experiments, use a template file (EDT). See “Use a template file” on page 51.
- For your own experiments:
  - (Recommended) Use an OpenArray™ plate setup file (TPF). See “Use an OpenArray™ plate setup file” on page 54.
  - *(Optional)* Use a template file (EDT). See “Use a template file” on page 51.
  - Use the **Batch Experiment Setup Utility**. See the *QuantStudio™ 12K Flex Software Help* (click or press **F1)**.

Use a template file

You can use a template file (EDT) to create a new OpenArray™ experiment, then import the sample and assay information for the OpenArray™ Plate or plates before starting the run, or after the run is complete.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. On the **Home** tab, on the **Experiment** menu, select **Create From Template**.
3. Navigate to and select the template file (EDT) that you want to use, then click **Open**.
   A new experiment is created using the setup information from the template.

   Note: To access the starter kit templates, navigate to the templates folder located at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates`
   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

   ---

4. In the **Experiment Properties** screen, scan the OpenArray™ Plate barcode or type the OpenArray™ Plate serial number.
5. In the **Samples** screen, do either of the following:
   - *(Recommended)* Click **Import** above the sample table, navigate to and select the OpenArray™ sample information file (CSV) that you want to use, then click **Select File**.
     For the gene expression starter kit experiments, use the OpenArray™ CSV files that were created in OpenArray™ AccuFill™ Software v2.0. See “Track the samples” on page 29.
   - In the sample table, click in a cell in the **Sample Name** column, then enter a new name.

6. From the open experiment, select **File ▸ Import Plate Setup**.
   a. Click **Browse**, navigate to and select the gene expression starter kit plate setup file that you want to use.

      Gene Expression Source File (TPF)—Corresponds to the plate setup file associated with gene expression OpenArray™ Plate formats.

      For the gene expression starter kit experiments and for your own experiments, download the appropriate plate setup files from the Thermo Fisher Scientific website. See “Use an OpenArray™ Plate setup file” on page 16.

   b. Click **Select**, then click **Start Import**.

   c. If your experiment already contains plate setup information, the software asks whether to replace the plate setup information with the data from the file. Click **Yes** to replace the plate setup information.

7. Select **File ▸ Save As**, enter a file name, select a location for the experiment file (EDS), then click **Save**.

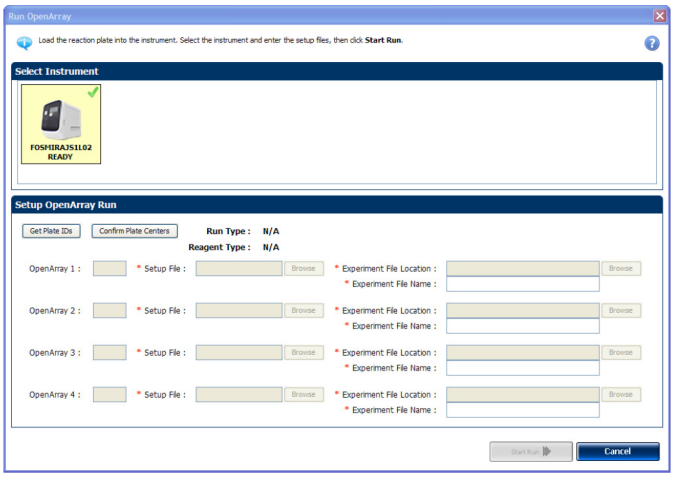
Proceed to “Start the OpenArray™ run” on page 52.

Start the OpenArray™ run

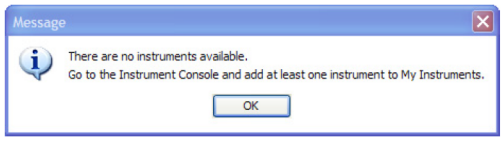
Ensure that the default browse file type is set to EDS. See “(Optional) Select OpenArray™ block run preferences” on page 48.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. On the **Home** tab, from the **Run** menu, select **OpenArray**.



Note: Be sure to add an instrument to **My Instruments** in the **Instrument Console** before you run an experiment. See “Access the Instrument Console” on page 48. If no instrument is selected, a warning appears.



3. In the **Select Instrument** pane, select the instrument that you want to use to run the experiment.

4. Complete the **Setup OpenArray Run** pane.
   - Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate formats that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray™ Plate formats that you want to run. For each plate image in the **Confirm OA Plate Centers** dialog box, click **Continue** if the red box is aligned to the center of the plate. If the box is not in the center of the plate, click **OK**, eject the carrier, rearrange the plates, then click **Get Plate IDs**.
   - *(Optional)* Click **Browse**, then navigate on your computer to select the appropriate setup files. The setup file was created in “Use a template file” on page 51.

   Depending on the number of OpenArray™ Plate formats loaded in the instrument, the barcodes of those plates are populated.

   IMPORTANT! If the QuantStudio™ 12K Flex Real-Time PCR Instrument does not detect a barcode, repeat the barcode read.

5. Click **Start Run**.

Use an OpenArray™ plate setup file

The OpenArray™ AccuFill™ Software integrates the sample information into an OpenArray™ Plate setup file (TPF). You can save the newly created Loaded_tpf files to the OpenArray™ Plate File Input Folder that you selected in the **Preferences** dialog box of the OpenArray™ AccuFill™ Software. Configure this location in the QuantStudio™ 12K Flex Software preferences to upload the integrated plate setup file into the QuantStudio™ 12K Flex Software, then run the file.

Note: You can import a CSV file into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

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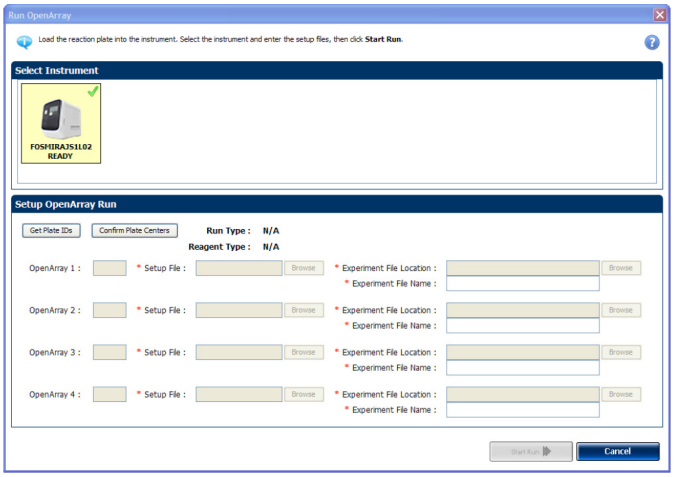
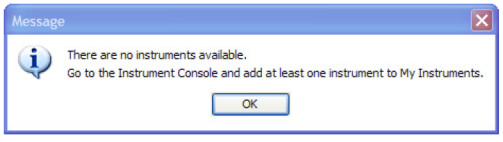
1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. On the **Home** tab, from the **Run** menu, select **OpenArray**.



Note: Be sure to add an instrument to **My Instruments** in the **Instrument Console** before you run an experiment. See “Access the Instrument Console” on page 48. If no instrument is selected, a warning appears.



3. In the **Select Instrument** pane, select the instrument that you want to use to run the experiment.

4. Complete the **Setup OpenArray Run** pane.
   - Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate formats that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray™ Plate formats that you want to run. For each plate image in the **Confirm OA Plate Centers** dialog box, click **Continue** if the red box is aligned to the center of the plate. If the box is not in the center of the plate, click **OK**, eject the carrier, rearrange the plates, then click **Get Plate IDs**.
   - *(Optional)* Click **Browse**, then navigate on your computer to select the appropriate OpenArray™ Plate setup files (TPF).

     Note: When the setup file is selected, the **Experiment File Location** and **Experiment File Name** are populated. To set the default **Experiment File Location**, go to **Tools** ▸ **Preferences** ▸ **OpenArray** ▸ **Experiment Folder**. In the **Setup OpenArray Run** pane, to select another location for the experiment file, click **Browse**. You can also enter an experiment file name of your choice.

   Depending on the number of OpenArray™ Plate formats loaded in the instrument, the barcodes of those plates are populated.

   IMPORTANT! If the QuantStudio™ 12K Flex Real-Time PCR Instrument does not detect a barcode, repeat the barcode read.

5. Click **Start Run**.

Start a run from the instrument touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen.

- “Start a run on the instrument touchscreen from an existing experiment” on page 55
- “Start a run on the instrument touchscreen from a template” on page 56
- “Start a run on the instrument touchscreen from a shortcut” on page 56

The starter kit experiments in this guide start a run from the QuantStudio™ 12K Flex Software.

Start a run on the instrument touchscreen from an existing experiment

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.

   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.

2. In the **Home** screen, touch **Run OpenArray Plates**.

   The instrument retrieves the barcodes and scans for existing experiments with the same barcodes.

3. If experiments with the same barcode cannot be found, touch **Source Input** to select a template to use.

4. Touch Start Run **(Start Run Now)** to start the run.

IMPORTANT! If the instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the instrument touchscreen. Do not proceed if a barcode is not detected by the instrument.

Start a run on the instrument touchscreen from a template

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.

Note: If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.

2. In the **Home** screen, touch **(View Templates).**
3. In the **View Templates** screen, touch **(Folders)** to display the folders containing the template files.
4. Touch any of the folders to display the templates in that folder.
5. In the **View Templates** screen, select the desired template, then touch Start Run.
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
6. Touch Start Run to start the run.

Start a run on the instrument touchscreen from a shortcut

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.
   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.
2. In the **Home** screen, touch any of the shortcuts that have been set to an OpenArray™ template.
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
3. Touch Start Run **(Start Run Now)** to start the run.

(Optional) Monitor experiments

You can monitor an OpenArray™ experiment run in three ways.

- From the **Run** screen of the QuantStudio™ 12K Flex Software, while the experiment is in progress. See “Monitor an experiment from the software Run screen” on page 57.
- From the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, in the same way that you run the experiment. See “Monitor an experiment from the instrument touchscreen” on page 58.
- From the **Instrument Console** of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen). See “Monitor an experiment from the Instrument Console” on page 57.

Note: If connection between the instrument and the QuantStudio™ 12K Flex Software is lost during an experiment, remove and then add the instrument to the **My Instruments** list, or restart the software. You can then resume monitoring the experiment.

Monitor an experiment from the software Run screen

To monitor the **Amplification Plot** of the experiment that you are running, in the QuantStudio™ 12K Flex Software, from the **Run Experiment Menu**, click **Amplification Plot**.

Monitor an experiment from the Instrument Console

1. In the QuantStudio™ 12K Flex Software **Home** tab, from the **Tools** menu, click **Instrument Console**.
2. In the **Instrument Console**, select the icon of the instrument that you are using to run the experiment, then click **Manage Instrument**, or double-click the instrument icon.

   You must add the instrument to a group that can be monitored before you can manage it (see “Access the Instrument Console” on page 48).
3. In the **Instrument Manager** screen, click **Monitor Run** to access the **Run** screen.

You can view the progress of the run in real time from the **Run** screen. During the run, periodically view the **Amplification Plot** (see “Monitor the Amplification Plot” on page 57) available from the software for potential problems.

Task	Action
To stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN**. • In the **Stop Run** dialog box, click one of the following: – **Stop Immediately** to stop the run immediately. – **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold. – **Cancel** to continue the run.
To view amplification data in real time	Select **Amplification Plot**. See “Monitor the Amplification Plot” on page 57 below.

Monitor the Amplification Plot

To view data in the **Amplification Plot**, click **Amplification Plot** from the **Run Experiment** menu, select the **Plate Layout** tab, then select the wells to view. You can view up to four OpenArray™ experiments per run. Click the different tabs to view the **Amplification Plot** for each experiment.

Use the **Amplification Plot** to view sample amplification as the instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the **Amplification Plot** shows the data for the wells selected in the **Plate Layout** tab. The plot contrasts normalized dye fluorescence (ΔRn) and cycle number.

The **Amplification Plot** is useful for identifying and examining abnormal amplification, including:

- Increased fluorescence in negative control wells.
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

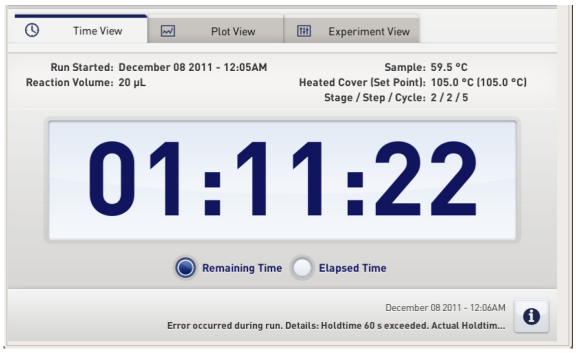
Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

Monitor an experiment from the instrument touchscreen

The QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the barcodes (or Plate IDs) of the TrueMark™ OpenArray™ Plate formats for the run, the date and time at which the run started, the time remaining in the run, and other information.

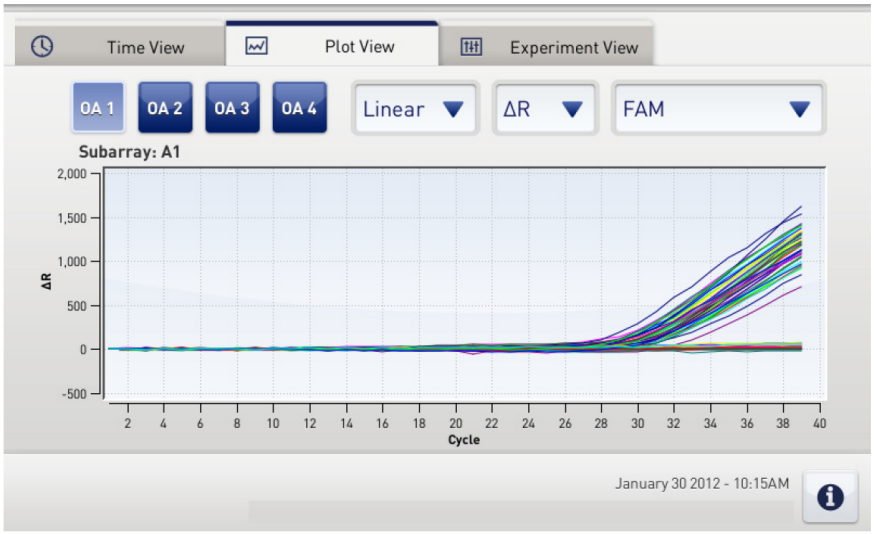
Task	Action
To display the experiment names in the run	Touch **Experiment View**.
To show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
To display the time elapsed and the time remaining in the run	Touch the **Time View** tab, then touch **Experiment View** to return to the previous screen.
To stop the run	Touch **STOP** to stop the run immediately.
View the Events Log	Touch the status bar to display the events log.

Time view



Plot view

The **Plot View** displays the **Amplification Plot** in real time.



You can change the plot using the drop-down menus that are available on the **Plot View** tab.

Touch…	To…
	Change the data displayed on the y-axis. Select either **R** (reporter) or **ΔR** (baseline-corrected reporter). For OpenArray™ experiments, the data is not normalized.
	Change the reporter dye displayed in the plot. Only the dyes that are used in your experiment are shown.
	View the run events that occurred during the run. Touch  again to close the event list.

Unload the TrueMark™ OpenArray™ Plate from the instrument

About completed runs

After the run is complete, if you started the run from the QuantStudio™ 12K Flex Software, close the run and reopen the EDS file to display the **Amplification Plot**. See Chapter 6, "Analyze the experiment results".

If you started the run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, see "(Optional) Transfer experiment results" on page 60.

Unload the instrument

When the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the **Home** screen, unload the TrueMark™ OpenArray™ Plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

1. Touch on the instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software.
2. Remove the OpenArray™ Plate from the plate adapter.
3. Touch or click **Close Door** to retract the plate adapter back into the instrument.

   If the instrument does not eject the plate, remove the plate.

   a. Power off the instrument.

   b. Wait for 15 minutes, then power on the instrument and eject the plate.

   c. If the instrument does not eject the plate, power off and unplug the instrument, then open the access door.

   d. Wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.

(Optional) Transfer experiment results

If you started a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, transfer the experiment data to the computer for analysis after the run is complete. You can transfer the experiment results in either of the following two ways.

- "Download the experiment from the instrument over the network" on page 61
- "Transfer the experiment from the instrument to the computer with a USB drive" on page 61

Download the experiment from the instrument over the network

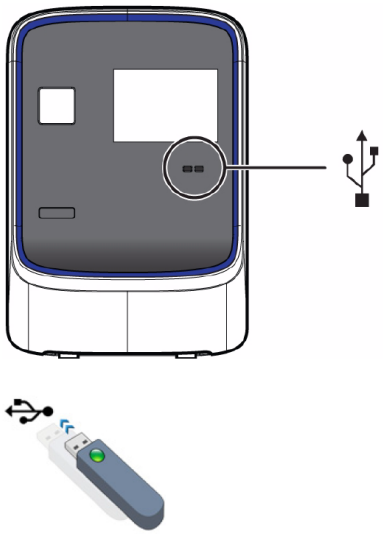
1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. From **My Instruments** list, select the instrument icon of the QuantStudio™ 12K Flex Real-Time PCR Instrument that you just used to run the experiment.
3. Click **Manage Instrument**.
4. In the **Instrument Manager**, click **Manage Files**.
5. In the **Experiments** panel, select the experiment to download, then click **Download**.
6. In the **Save** dialog box, select the folder to hold the experiment results, then click **Save**, then navigate to the experiments folder location.

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Transfer the experiment from the instrument to the computer with a USB drive

1. If not already connected to the QuantStudio™ 12K Flex Real-Time PCR Instrument, connect a USB drive to the USB port.



2. Touch the instrument touchscreen to activate it.
3. If the touchscreen is not at the **Main Menu**, touch **(Home)**.
4. In the **Main Menu**, touch **(Collect Results)** to save the data to the USB drive.

5. Select one or multiple experiments (by touching them). Then touch **(Save to USB)** to copy selected experiments to the USB drive.

   Note: If your instrument cannot find the USB drive, remove the USB drive, then try again. If the instrument still does not recognize the USB drive, try another USB drive.

6. Touch **(Home)** to return to the **Main Menu**.

7. Remove the USB drive from the instrument, then connect it to one of the USB ports on your computer.

8. In the computer desktop, use the Windows™ Explorer to open the USB drive.

9. Copy the example experiment file to:

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

6 Analyze the experiment results

Analyze the run data

This section includes general information and instructions about how to analyze the gene expression example experiment provided with the QuantStudio™ 12K Flex Software. For specific instructions see “Analyze gene expression experiment results” on page 72.

View the data from the EDS file. If the default analysis settings are not suitable for your experiment, you can modify the data. You can also modify the project files, publish data, and export data for downstream analysis using the ExpressionSuite™ Software.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**.

QC images can be viewed in QuantStudio™ Design and Analysis Software v2.7 and later.

1. In the QuantStudio™ 12K Flex Software  **Export** screen, click **Browse**, then create a uniquely–named folder for the QC images export.

   IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.

2. Click **Export QC Images** at the bottom of the screen.

3. View the following ROX™ image to check for loading quality issues:
   - `POST-READ_CHANNEL_4.tiff`

4. Check the following spotfind images for leaks or other displaced sample issues.
   - `s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff`
   - `s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff`

   Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfind image to determine whether the issue existed before cycling:
   - `s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff`

6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfind images:
   - `STAGE2_CYCLE1_CHANNEL_1.tiff`
   - `STAGE2_CYCLE40_CHANNEL_1.tiff`
7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

View the results

After an experiment run, close the run and reopen the EDS file to display the **Amplification Plot**.

Note: For auto-analysis of data, after a run, go to **Tools** ▸ **Preferences** ▸ **Experiment**, then select the **Auto Analysis** checkbox. By default, **Auto Analysis** is always enabled. To reanalyze the data, select all wells in the plate layout, then click **Analyze**.

Set up the EDS file

If you run a gene expression experiment using an EDT file, you must integrate the sample names and Assay IDs into the resulting EDS file.

For Assay IDs, you can import the TPF file of that OpenArray™ Plate into the EDS file before or after the run.

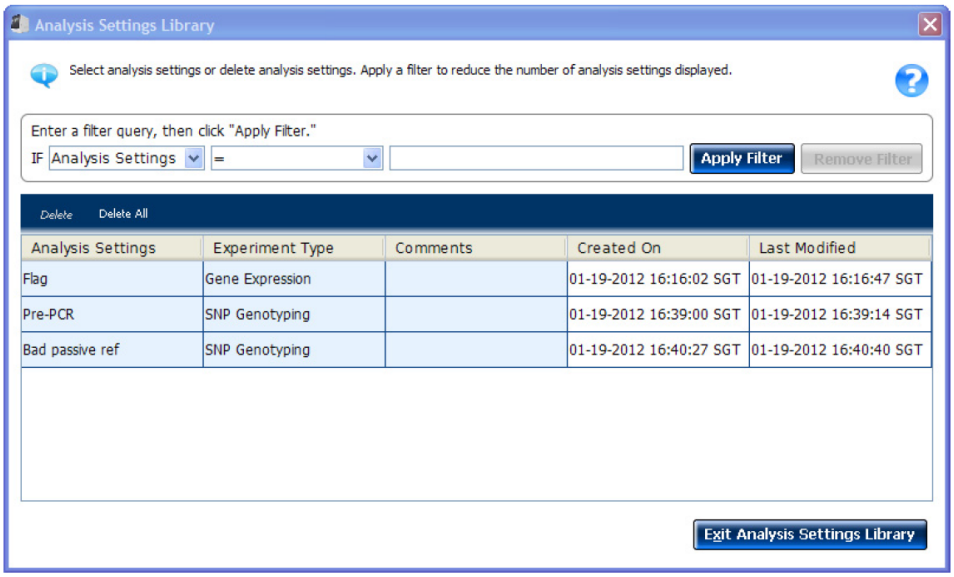
For sample names:

- You can import the OpenArray™ Plate format from the CSV file for the corresponding plate.
- If you use the OpenArray™ AccuFill™ Software for sample integration, navigate to the appropriate folder containing the Loaded TPF file. A Loaded TPF file is one that has sample names integrated into the file using the OpenArray™ AccuFill™ Software.

Change analysis settings

Analysis settings are different for each experiment type. If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment. You can save the changed analysis settings to the **Analysis Settings Library** to use them in other experiments.

Use the **Analysis Settings Library** dialog box to apply a filter to reduce the number of setting protocols that are displayed. Access the **Analysis Settings Library** from the **Tools** menu.



1. From the **Experiment Menu**, select **Analysis**.
2. On the **Analysis** screen, click **Analysis Settings**.
3. In the **Analysis Settings** dialog box, change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes to the **Analysis Settings Library**.

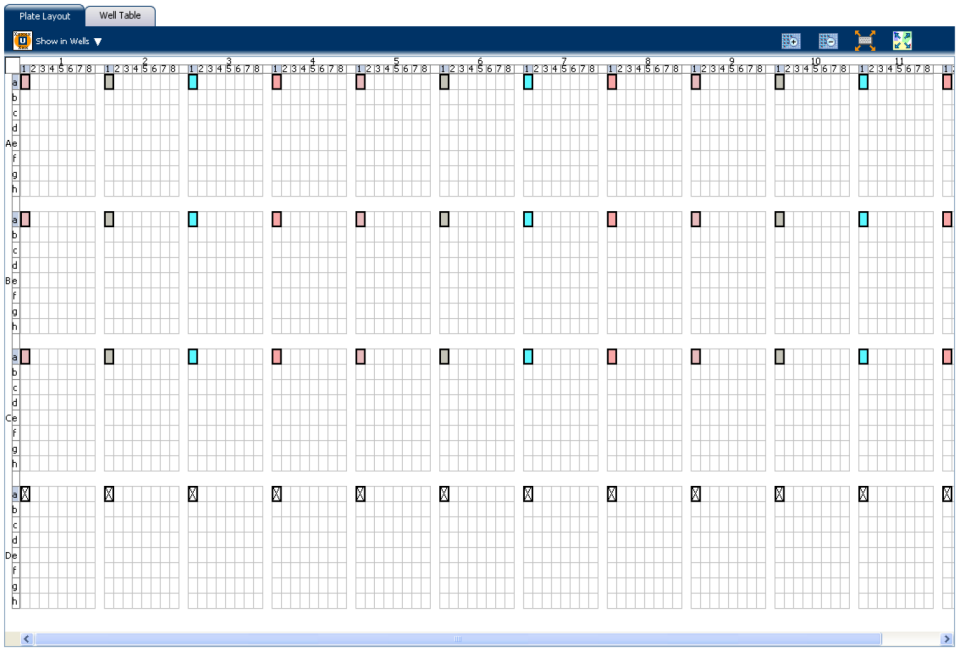
To import the analysis settings that you have previously saved to the **Analysis Settings Library**, in the **Analysis Settings** dialog box, click **Load from Library**.

Display wells

To display specific wells in the analysis plots, select the wells in the **Plate Layout** tab.

- To select specific well type, use the **Show in Wells** drop-down menu.
  - For gene expression and miRNA experiments, select **Sample Color** or **Target Color**.
  - For genotyping experiments, select **Sample Color** or **Assay Color**.
- To select a single well, click the well in the **Plate Layout** tab.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the **Plate Layout** tab.
- To select all wells, click the upper left corner of the **Plate Layout** tab.

This example shows the **Plate Layout** tab for a gene expression experiment.



Expand view of a plot or wells

- Click to expand the plot view, on the left side of the screen.
- Click to expand the **Targets**, **Samples**, and **Subarrays** view on the right side of the screen.
- Click to expand the **Plate Layout** or **Well Table** view on the lower half of the screen.
- Click to expand the Plots and **Targets**, **Samples**, and **Subarrays** view on the upper half of the screen.
- Click to expand and collapse the **Plot** or **Plate Layout** view.

Edit plot properties

Use the **Plot Properties** dialog box on the **Analysis** screen to edit plot settings, such as the font and color of the plot text, and the labels on the x-axis and y-axis.

1. Click on the **Analyze** screen (the icon appears above the plot).
2. In the **Plot Properties** dialog box, edit the settings under the **General**, **X Axis**, and **Y Axis** tab.
   - Click the **X Axis** tab to edit the x-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
   - Click the **Y Axis** tab to edit the y-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
3. Click **OK**.

Publish the analyzed data

Task	Click
Save a plot as an image file.	
Print a plot.	
Copy a plot to the clipboard.	
Print a report.	
Export data.	

Task	Go to	Then
Print the plate layout.	**File** ▸ **Print**	Select the background color, then click **Print**.
Create slides.	**File** ▸ **Send to PowerPoint**	Select the slides for your presentation, then click **Create Slides**.
Print a report.	**File** ▸ **Print Report**	Select data for the report, then click **Print Report**.

(Optional) Export an experiment

Use the **Export** feature to export experiment data from the QuantStudio™ 12K Flex Software. Select to export in the QuantStudio™ 12K Flex (TXT or XLSX) or RDML (no file selection) format.

You can export the following experiment data in a comma-separated file format (CSV).

- Sample setup data
- Raw data
- Amplification data
- Multicomponent data
- Results

You can also export plate images collected during the run as TIF files and use them for troubleshooting purposes. To export plate images, first create an export folder on your hard drive. In the **Export** screen, click **Browse**, navigate to the folder that you created, then click **Export QC Images**.

You can view the images using a public domain software program such as ImageJ (**http://rsb.info.nih.gov/ij/**). For more information about QC images, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

You can view images in QuantStudio™ Design and Analysis Software v2.7 and later. For more information, see *QuantStudio™ Design and Analysis Software 2 User Guide* (Pub. No. MAN0018200).

If you select the **Auto Export** option before running an experiment, the data are exported to the location that you specified. If you do not select the **Auto Export** option, the analyzed data are not exported automatically.

1. Open the experiment file that contains the data to export, then from the **Experiment Menu**, click **Export.**

2. Select the format for exported data.
   - **QuantStudio 12k Flex format**—Supports TXT and XLSX data.
   - **RDML format** (Real Time Data Markup Language)—Supports only XML type of data.

3. Select to export all data in one file or in separate files for each data type.
   - All data types are exported in **one file.**
     - If you select the XLS format, a worksheet is created for each data type.
     - If you select the TXT format, the data are grouped by data type.
   - Each data type is exported in a **separate file.** If you select to export three different data types (for example, Results, Amplification, and Multicomponent), three separate files are created. You can select the export file type (XLS, XLSX, or TXT) to export from the **File Type** menu.

     Note: You cannot use an exported XLS or an XLSX file when importing plate setup information.

4. Select **Yes** to include or **No** to exclude bookmarked data from analysis in the export set.

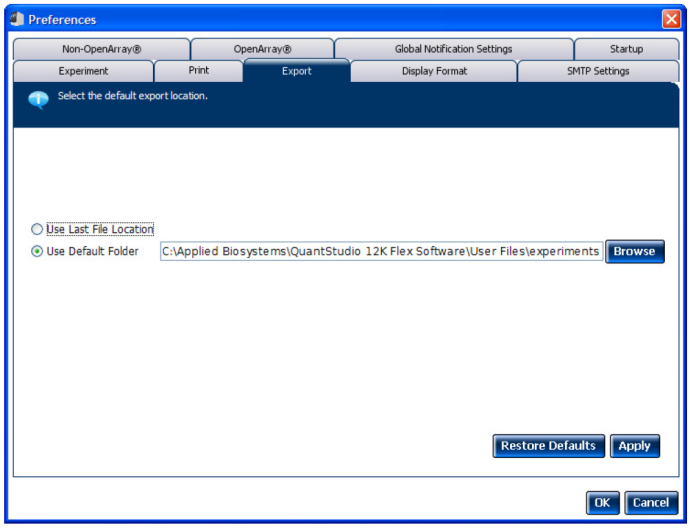
   The **Filter Bookmark Data** feature lets you include only the data bookmarked during analysis in the export set.

5. *(Optional)* Select the **Open file(s) when export is complete** checkbox to open the file when export is complete.

6. Enter a file name and location.
   a. In **Export File Name**, enter a name for the export file.

b. In **Export File Location** accept the default, or click **Browse** if you do not want to save the export file in the default export folder.

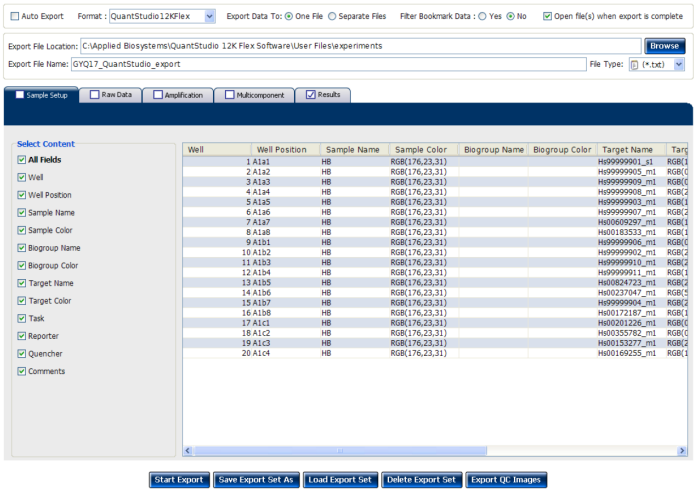
Note: To set up the export file location, go to **Tools** ▸ **Preferences**, then select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** checkbox.



7. Select the type of data to export.

Selection	Data type to export
Sample setup	Well, sample name, sample color, and target name of samples in the plate.
Raw data	Raw fluorescence data for each filter, for each cycle.
Amplification data	Amplification results, such as dC_T values, R, or ΔR.
Multicomponent data	Fluorescence data for each dye, for each cycle.
Results	Results information, such as C_T values, Rn, or calls. Results data are not available for export until the run status is complete and the data are analyzed.

The completed **Export** screen for a gene expression experiment should look like this.



8. *(Optional)* After you have defined the export properties or after you have moved the table headings order, you can save those export settings as an export set. Click **Save Export Set As**.

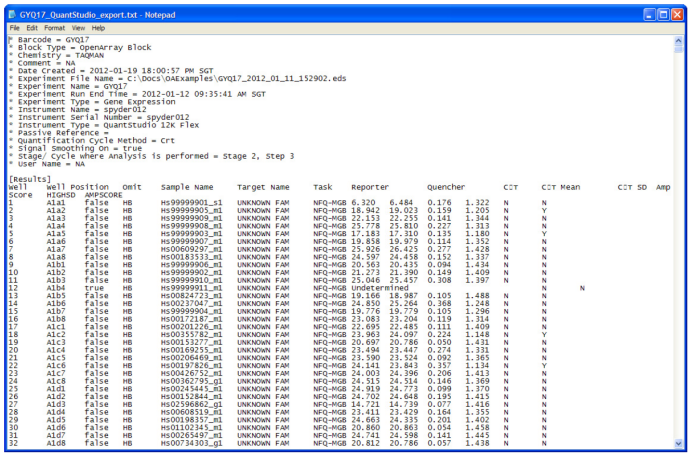
9. Later you can import the heading order into another file by clicking **Load Export Set**. You can also delete export settings by clicking **Delete Export Set.**

10. *(Optional)* Click **Export QC Images** to export quality control (QC) images in experiment files (EDS).

    QC images include calibration images, a barcode image, and images taken during PCR. You can view the images to check sample loading and assay spotting. View PCR images to validate your data.

11. Click **Start Export.**

    The exported file, when opened in Notepad, should look like this.



Perform downstream analysis (secondary analysis)

You can perform downstream analysis of experiments that have been run on any real-time PCR system by using the ExpressionSuite™ Software.

Use the ExpressionSuite™ Software to efficiently analyze, edit, and conduct a study of a large number of gene expression.

- Import data from the QuantStudio™ 12K Flex Software project files, then manage the data in a database.
- Search the database for assays using specific search criteria.
- Easily view data in a variety of ways (plots, statistics, status codes, and so on).
- Edit data. Your edits are saved to the database.
- Overlay data from multiple plates.
- Export data.

ExpressionSuite™ Software is available for download from the Thermo Fisher Scientific website.

Analyze gene expression experiment results

In this section you use the gene expression example experiment files provided with the QuantStudio™ 12K Flex Software to analyze the experiment results.

Workflow

Analyze gene expression experiment results

- **Access the example experiment file** (page 72)
- **Assess amplification results on the Amplification Plot** (page 73)
- **Identify well problems using the Well Table** (page 75)
- **Confirm accurate dye signal using the Multicomponent Plot** (page 76)
- **Determine signal accuracy using the Raw Data Plot** (page 78)
- **Review the flags in the QC Summary** (page 79)
- **(Optional) Adjust parameters to reanalyze your own experiments** (page 82)

Access the example experiment file

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. From the **Home** screen, click **Open,**, then browse to the **Gene Expression** examples folder.

   `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12KFlex Software\examples\Gene Expression`
3. Open the `Gene Expression Starter Kit Example.eds` file.

Set up the EDS file

If you run a gene expression experiment using an EDT file, you must integrate the sample names and Assay IDs into the resulting EDS file.

For Assay IDs, you can import the TPF file of that OpenArray™ Plate into the EDS file before or after the run.

For sample names:

- You can import the OpenArray™ Plate format from the CSV file for the corresponding plate.
- If you use the OpenArray™ AccuFill™ Software for sample integration, navigate to the appropriate folder containing the Loaded TPF file. A Loaded TPF file is one that has sample names integrated into the file using the OpenArray™ AccuFill™ Software.

Assess amplification results on the Amplification Plot

The **Amplification Plot** displays amplification of all samples in the selected wells. View the **Amplification Plot** for the example experiment to evaluate the quality of the amplification curve and to check for outliers.

Three plots are available.

- **ΔR vs Cycle**—ΔR is the magnitude of fluorescence signal generated by the reporter at each cycle during the PCR amplification. This plot displays ΔR as a function of cycle number. Use this plot to identify and examine irregular amplification and to view C_{RT} values for the run.
- **R vs Cycle**—R is the fluorescence signal from the reporter dye. This plot displays R as a function of cycle number. Use this plot to identify and examine irregular amplification.
- **C_{RT} vs Well**—C_{RT} is the PCR cycle number at which the fluorescence meets the threshold in the amplification plot. This plot displays C_{RT} as a function of well position. Use this plot to locate outlying amplification (outliers).

Each plot can be viewed as a linear or log10 graph type.

View the Amplification Plot

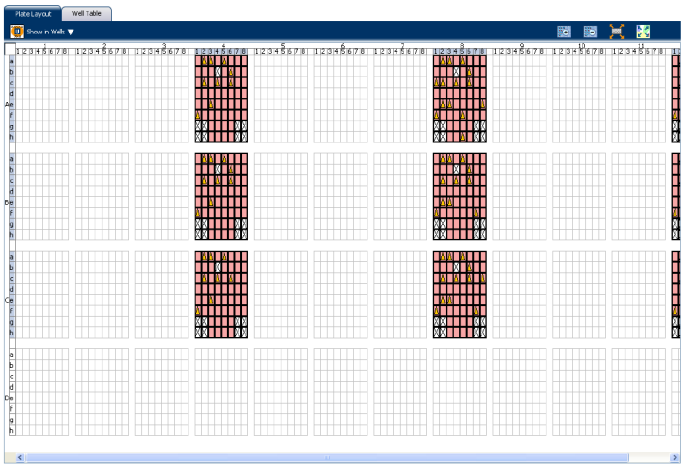
1. From the **Experiment Menu**, select **Analysis** ▸ **Amplification Plot**.

   If no data are displayed, click **Analyze**.

2. Display the HPp wells in the **Amplification Plot**.
   a. Click the **Plate Layout** tab.

b. Select **Show in Wells ▸ Sample Color**.

The **Plate Layout** screen should look like this.



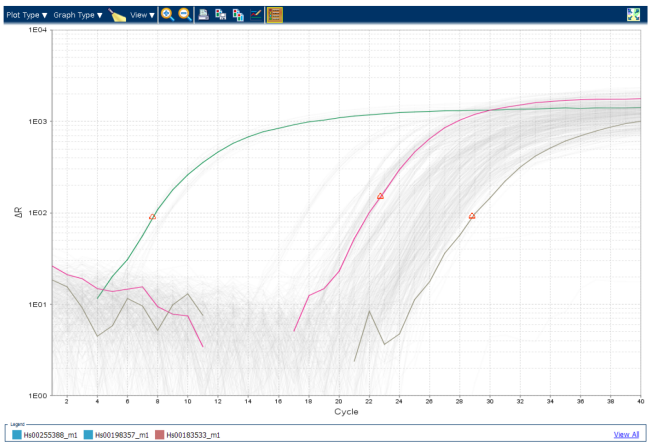
3. In the **Amplification Plot**, make the following selections.

Menu	Selection
Plot Type	**ΔR vs Cycle** (default)
Graph Type	**Log** (default)
View	**Target Color** (default) **Show Unselection** (default)

4. View the C_{RT} values.

a. Click **View ▸ Show C_{RT}**.

b. Verify that the C_{RT} value that is reported matches its occurrence (the triangle icon) on the plot.



5. Repeat steps 2 through 4 on page 73 for the HLiv, HLun, HB, and NTC wells.

Identify well problems using the Well Table

The **Well Table** displays the following information for each well in the reaction plate.

- The sample name, target name, task, and dyes.
- The calculated threshold cycle (C_{RT}) and quantity values.
- Flags.

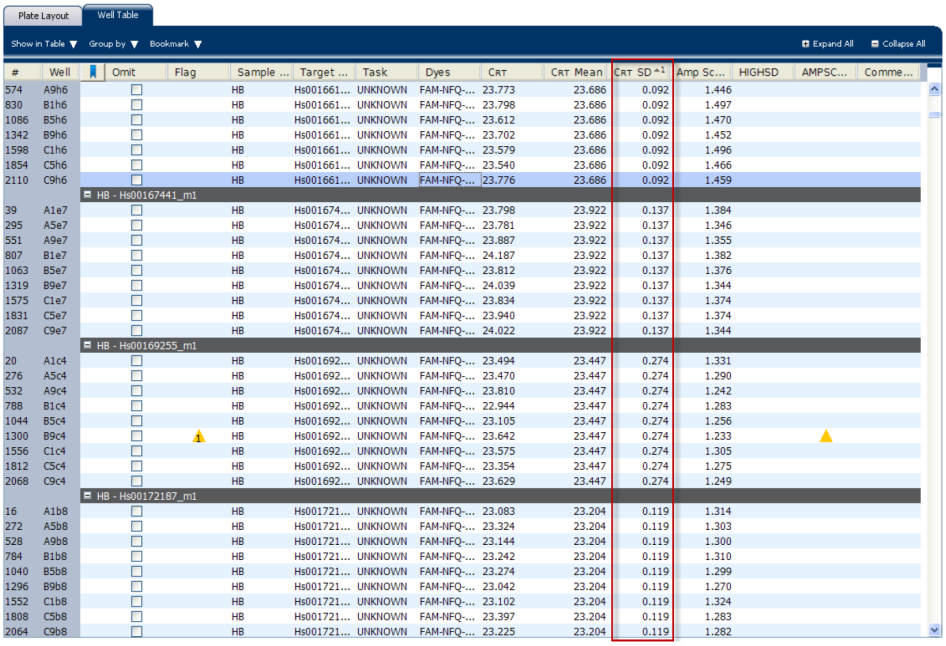
Example experiment values and flags

Review the **Well Table** to evaluate the C_{RT} precision of the replicate groups and AMPSCORE values.

The software produces the AMPSCORE flag if the amplification in the linear region is below a certain threshold, corresponding to the score set in the analysis settings. For robust amplification, AMPSCORE values should be ≥1.24.

View the Well Table

1. From the **Experiment Menu**, select **Analysis** ▸ **Amplification Plot**, then click the **Well Table** tab.
2. From the **Group By** menu, select **Replicate**.
3. Look at the **C_{RT} SD** column to evaluate the C_{RT} precision of the replicate groups.
   In the example experiment, the C_{RT} SD have the expected value of <0.5.



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	CRT	CRT Mean	CRT SD	Amp Sc...	HIGHSD	AMPSC...	Comme...
574	A9h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.773	23.686	0.092	1.446			
830	B1h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.798	23.686	0.092	1.497			
1086	B5h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.612	23.686	0.092	1.470			
1342	B9h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.702	23.686	0.092	1.452			
1598	C1h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.579	23.686	0.092	1.496			
1854	C5h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.540	23.686	0.092	1.466			
2110	C9h6			HB	Hs001661...	UNKNOWN	FAM-NFQ-...	23.776	23.686	0.092	1.459			
		HB - Hs00167441_m1												
39	A1e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.798	23.922	0.137	1.384			
295	A5e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.781	23.922	0.137	1.346			
551	A9e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.887	23.922	0.137	1.355			
807	B1e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	24.187	23.922	0.137	1.382			
1063	B5e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.812	23.922	0.137	1.376			
1319	B9e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	24.039	23.922	0.137	1.344			
1575	C1e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.834	23.922	0.137	1.374			
1831	C5e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	23.940	23.922	0.137	1.374			
2087	C9e7			HB	Hs001674...	UNKNOWN	FAM-NFQ-...	24.022	23.922	0.137	1.344			
		HB - Hs00169255_m1												
20	A1c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.494	23.447	0.274	1.331			
276	A5c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.470	23.447	0.274	1.290			
532	A9c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.810	23.447	0.274	1.242			
788	B1c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	22.944	23.447	0.274	1.283			
1044	B5c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.105	23.447	0.274	1.256			
1300	B9c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.642	23.447	0.274	1.233			
1556	C1c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.575	23.447	0.274	1.305			
1812	C5c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.354	23.447	0.274	1.275			
2068	C9c4			HB	Hs001692...	UNKNOWN	FAM-NFQ-...	23.629	23.447	0.274	1.249			
		HB - Hs00172187_m1												
16	A1b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.083	23.204	0.119	1.314			
272	A5b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.324	23.204	0.119	1.303			
528	A9b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.144	23.204	0.119	1.300			
784	B1b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.242	23.204	0.119	1.310			
1040	B5b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.274	23.204	0.119	1.299			
1296	B9b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.042	23.204	0.119	1.270			
1552	C1b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.102	23.204	0.119	1.324			
1808	C5b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.397	23.204	0.119	1.283			
2064	C9b8			HB	Hs001721...	UNKNOWN	FAM-NFQ-...	23.225	23.204	0.119	1.282			

Assess the Well Table in your own experiments

When you analyze your own OpenArray™ gene expression experiment, look for standard deviation in the replicate groups (C_{RT} SD values). If needed, omit outliers.

Confirm accurate dye signal using the Multicomponent Plot

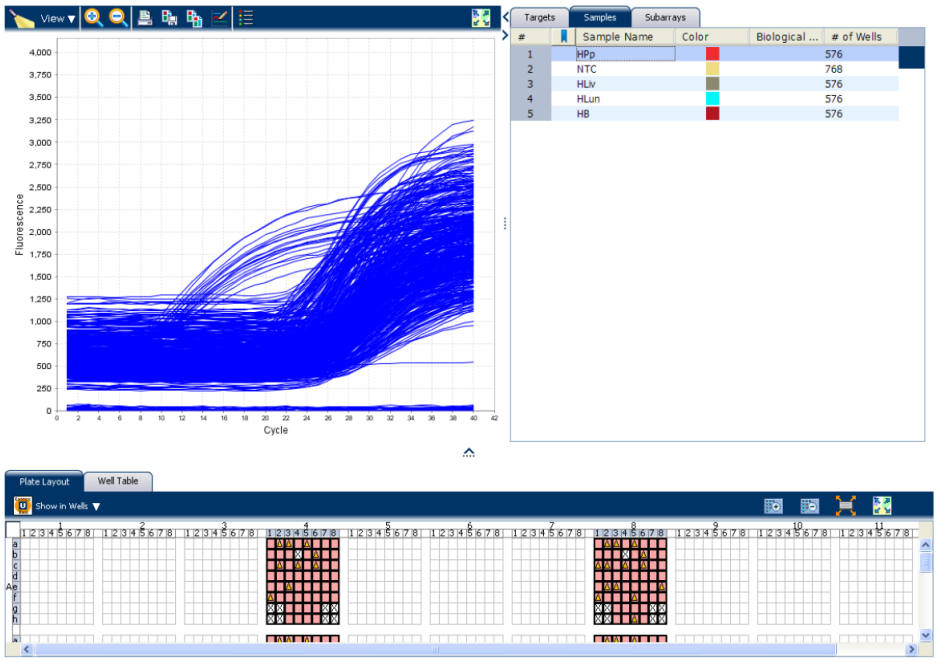
The **Multicomponent Plot** displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

In the OpenArray™ gene expression example experiment, review the **Multicomponent Plot** for:

- FAM™ dye (reporter)
- Spikes, dips, and/or sudden changes
- Amplification in the negative control wells

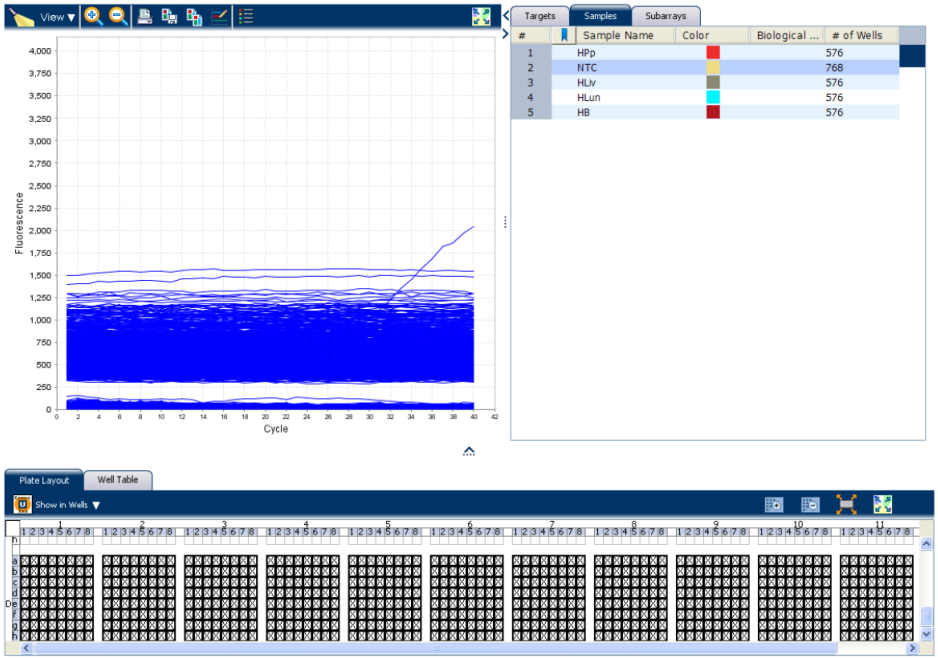
View the Multicomponent Plot

1. From the **Experiment Menu**, select **Analysis** ▸ **Multicomponent Plot**.
   If no data are displayed, click **Analyze**.
2. Display the unknown and standard wells, one at a time, in the **Multicomponent Plot**.
   a. Click the **Plate Layout** tab.
   b. Select one well in the plate layout. The well is shown in the **Multicomponent Plot**.
      If you select multiple wells, the **Multicomponent Plot** displays the data for all selected wells simultaneously.
3. Click **View** ▸ **Dye Color**.
4. Click **Show a legend for the plot** (default).
   This is a toggle button. When the legend is displayed, the button changes to **Hide the plot legend**.
5. Check the FAM™ dye signals.
   In the gene expression example experiment, the FAM™ dye signal increases throughout the PCR process, indicating normal amplification.



6. Select the negative control wells one at time and check for amplification.

   In the gene expression example experiment, there is no amplification in the negative control wells.



Tips for confirming dye accuracy in your own experiment

When you analyze your own experiment, look for these results.

- Reporter dye—The reporter dye fluorescence level should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- Irregularities in the signal—There should not be any spikes, dips, and/or sudden changes in the fluorescent signal.
- Negative Control wells—There should not be any amplification in the negative control wells.

Determine signal accuracy using the Raw Data Plot

The **Raw Data Plot** displays the raw fluorescence signal (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

In the OpenArray™ gene expression example experiment, review the **Raw Data Plot** for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

View the Raw Data Plot

1. From the **Experiment Menu**, select **Analysis** ▸ **Raw Data Plot**.
   If no data are displayed, click **Analyze**.
2. To display all wells in the **Raw Data Plot**, click the upper left corner of the plate layout in the **Plate Layout** tab.
3. Click **Show a legend for the plot** (default).
   The legend displays the color code for each row of the reaction plate (see the legend in the **Raw Data Plot**).
   This is a toggle button. When the legend is displayed, the button changes to **Hide the plot legend**.
4. Click and drag the **Show Cycle** pointer from cycle 1 to cycle 40.
   In the example experiment, the stable increase in signal from filter 1 corresponds to the FAM™ dye filter.

Tips for determining signal accuracy in your own experiment

When you analyze your own OpenArray™ gene expression experiment, look for the following conditions in each filter.

- Characteristic signal growth.
- No abrupt changes or dips.

Review the flags in the QC Summary

The **QC Summary** displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment. Review the **QC Summary** in the OpenArray™ gene expression example experiment for any flags triggered by the experiment data.

The following wells have data that triggered the HIGHSD flag.

A2b3, A3a7, A3b3, A3d5, A3f2, A3h3, A4a3, A4c4, A4e3, A6b3, A7a7, A7b3, A7d5, A7f2, A7h3, A8a3, A8c4, A8e3, A10b3, A11a7, A11b3, A11d5, A11f2, A11h3, A12a3, A12c4, A12e3, B2b3, B3a7, B3b3, B3d5, B3f2, B3h3, B4a3, B4c4, B4e3, B6b3, B7a7, B7b3, B7d5, B7f2, B7h3, B8a3, B8c4, B8e3, B10b3, B11a7, B11b3, B11d5, B11f2, B11h3, B12a3, B12c4, B12e3, C2b3, C3b3, C3d5, C3f2, C3h3, C4a3, C4c4, C4e3, C6b3, C7a7, C7b3, C7d5, C7f2, C7h3, C8a3, C8c4, C8e3, C10b3, C11a7, C11b3, C11d5, C11f2, C11h3, C12a3, C12c4, and C12e3

The following wells have data that triggered the AMPSCORE flag.

A1a2, A1a5, A1c2, A1c6, A1f1, A2a2, A2a5, A2b6, A2c2, A2c6, A2f1, A2f5, A3a2, A3a5, A3c2, A3c6, A3f1, A4a2, A4a5, A4b6, A4c2, A4c6, A4f1, A5a2, A5a5, A5b6, A5c2, A5c6, A5f1, A6a2, A6a5, A6b6, A6c2, A6c6, A6e2, A6f1, A7a2, A7a5, A7c2, A7c6, A7e2, A7f1, A7f5, A8a2, A8a5, A8b6, A8c1, A8c2, A8c6, A8e2, A8e8, A8f1, A8f5, A8h5, A9a2, A9a5, A9b6, A9c2, A9c6, A9e2, A9f1, A10a2, A10a5, A10b6, A10c2, A10c4, A10c6, A10e2, A10f1, A10f5, A10f7, A11a2, A11a5, A11b6, A11c2, A11c4, A11c6, A11e2, A11f1, A11f5, A11f7, A12a2, A12a5, A12c2, A12c6, A12f1, B1a2, B1a5, B1b6, B1c2, B1c6, B1f1, B2a2, B2a5, B2c2, B2c6, B2f1, B3a2, B3a5, B3c2, B3c6, B3f1, B4a2, B4a5, B4b6, B4c2, B4c6, B4f1, B5a2, B5a5, B5b6, B5c2, B5c6, B5f1, B6a2, B6a5, B6b6, B6c2, B6c6, B6e2, B6f1, B6f7, B7a2, B7a5, B7b6, B7c2, B7c6, B7e2, B7f1, B7f5, B7f7, B8a2, B8a5, B8b6, B8c2, B8c4, B8c6, B8e2,

B8f1, B8f7, B9a2, B9a5, B9b6, B9c2, B9c4, B9c6, B9e2, B9f1, B9f5, B10a2, B10a5, B10b6, B10b7, B10c2, B10c4, B10c6, B10e2, B10e8, B10f1, B10f5, B10f7, B11a2, B11a5, B11b6, B11c2, B11c4, B11c6, B11e2, B11e8, B11f1, B11f5, B11f7, B12a2, B12a5, B12b6, B12c2, B12c6, B12f1, B12f7, C1a2, C1a5, C1b6, C1c2, C1c6, C1e2, C1f1, C1f5, C2a2, C2a5, C2c2, C2c6, C2f1, C3a2, C3a5, C3a7, C3c2, C3c6, C3f1, C4a2, C4a5, C4c2, C4c6, C4f1, C5a2, C5a5, C5b6, C5c2, C5c6, C5e2, C5f1, C6a2, C6a5, C6b6, C6c2, C6c6, C6e2, C6f1, C7a2, C7a5, C7b6, C7b7, C7c2, C7c6, C7d3, C7e2, C7f1, C8a2, C8a5, C8b6, C8c2, C8c6, C8c8, C8e2, C8f1, C8f7, C9a2, C9a5, C9b6, C9c2, C9c6, C9e2, C9f1, C9f5, C9f7, C10a2, C10a5, C10b6, C10b7, C10c2, C10c4, C10c6, C10c8, C10e2, C10e8, C10f1, C10f5, C10f7, C11a2, C11a5, C11b6, C11c2, C11c4, C11c6, C11c8, C11e2, C11e8, C11f1, C11f5, C11f7, C12a2, C12a5, C12c2, C12c6, and C12f1

View the QC Summary

1. From the **Experiment Menu**, select **Analysis ▸ QC Summary**.

   If no data are displayed, click **Analyze.**

2. Review the **Flags Summary**.

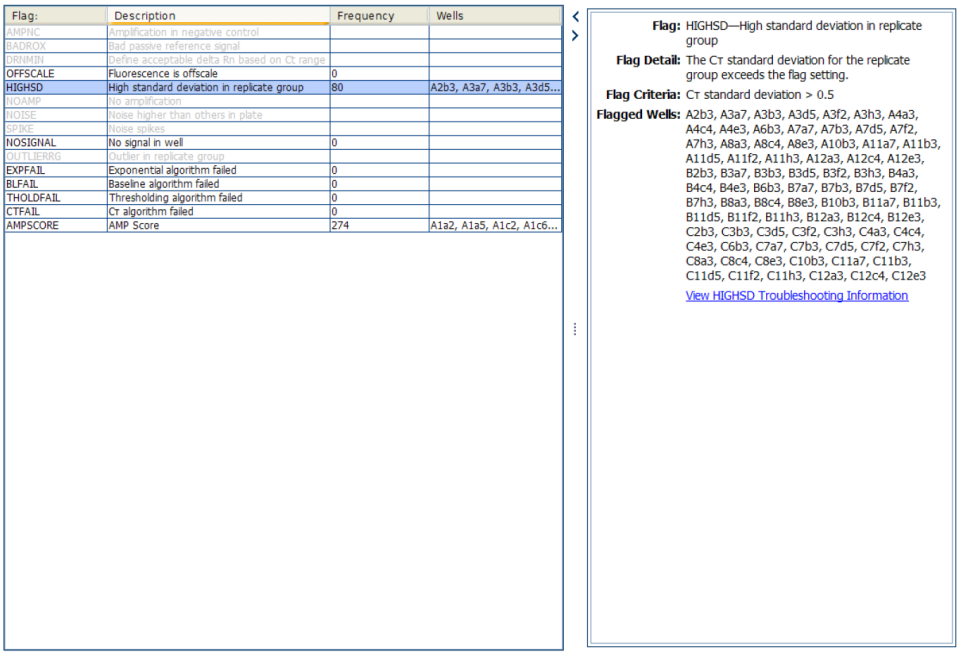
   Note: A 0 displayed in the **Frequency** column indicates that the flag does not appear in the experiment. If the frequency is >0, the flag appears somewhere in the experiment; the well position is listed in the **Wells** column.

   In the example experiment, there are 354 flagged wells.

3. In the **Flag Details** table, click each flag with a frequency >0 to see detailed information about the flag. In the example experiment, the HIGHSD flag appears 80 times and the AMPSCORE flag appears 274 times.

4. *(Optional)* For those flags with a frequency >0, click the troubleshooting link to view information about correcting the flag.

   The **QC Summary** for the example experiment looks like this.



Possible flags

The flags listed below may be triggered by the gene expression experiment data.

Flag	Description
Preprocessing flag	
OFFSCALE	Fluorescence is off scale.
Primary analysis flags	
BADROX	Bad passive reference signal.
NOAMP	No amplification.
NOISE	Noise is higher than others in plate.
SPIKE	Noise spikes.
NOSIGNAL	No signal in well.
EXPFAIL	Exponential algorithm failed.
BLFAIL	Baseline algorithm failed.
THOLDFAIL	Thresholding algorithm failed.
CTFAIL	C_T algorithm failed.
AMPSCORE	Amplification in the linear region is below a specific threshold, corresponding to the score set in the analysis settings.

(continued)

Flag	Description
Secondary analysis flags	
OUTLIERRG	Outlier in replicate group.
AMPNC	Amplification in the negative control.
HIGHSD	High standard deviation in replicate group.

The AMPNC, BADROX, DRNMIN, NOAMP, NOISE, SPIKE, and OUTLIERRG flags, by default, are not in use for the gene expression experiment.

For the **Relative Threshold** algorithm, the EXPFAIL, BLFAIL, THOLDFAIL, and CTFAIL flags are not reported, but they appear in the **QC Summary** (by default, a 0 is displayed in the **Frequency** column for each flag).

(Optional) Adjust parameters to reanalyze your own experiments

The **Analysis Settings** dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

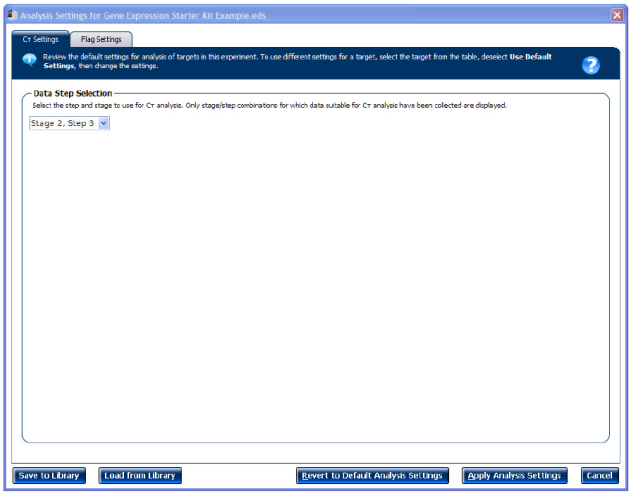
If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment.

View the analysis settings

1. From the **Experiment Menu**, select **Analysis**.
2. Click **Analysis** ▸ **Analysis Settings** to open the **Analysis Settings** dialog box.

   In the example experiment, the default analysis settings are used for each tab.

   - **C_T Settings**
   - **Flag Settings**



3. View and, if needed, change the analysis settings.

   See “Adjust analysis settings” on page 83.

   You can save changes to the analysis settings to the **Analysis Settings Library** for later use. See “Change analysis settings” on page 64.

4. Click **Apply Analysis Settings** to apply the current analysis settings.

   You can go back to the default analysis settings, by clicking **Revert to Default Analysis Settings**.

Adjust analysis settings

Adjust C_T settings

Use the **Data Step Selection** feature to select one stage/step combination for C_T analysis when there is more than one data collection point in the run method.

Adjust flag settings

Use the **Flag Settings** tab to adjust flag settings.

- Adjust the sensitivity so that more wells or fewer wells are flagged.
- Change the flags that are applied by the QuantStudio™ 12K Flex Software.

1. In the **Flag Settings** tab, in the **Use** column, select the checkboxes for flags to apply during analysis.

2. *(Optional)* If an attribute, condition, and value are listed for a flag, specify the setting for applying the flag.

   If you choose to adjust the setting for applying a flag, make minor adjustments as you evaluate the appropriate setting.

3. In the **Reject Well** column, select the checkboxes if you want the software to reject wells with the flag.

   After you have rejected the flagged wells, analysis results depend on factors such as the experiment type and flag type. For example, rejecting wells flagged by HIGHSD in experiments using the Standard Deviation calculations may change the result of C_{RT} SD. For some flags, analysis results calculated before the well is rejected are maintained.

4. In the **Analysis Settings** dialog box, click **Apply Analysis Settings**.

   If the run status is complete, the data are reanalyzed.

   The **Flag Settings** tab looks like this:

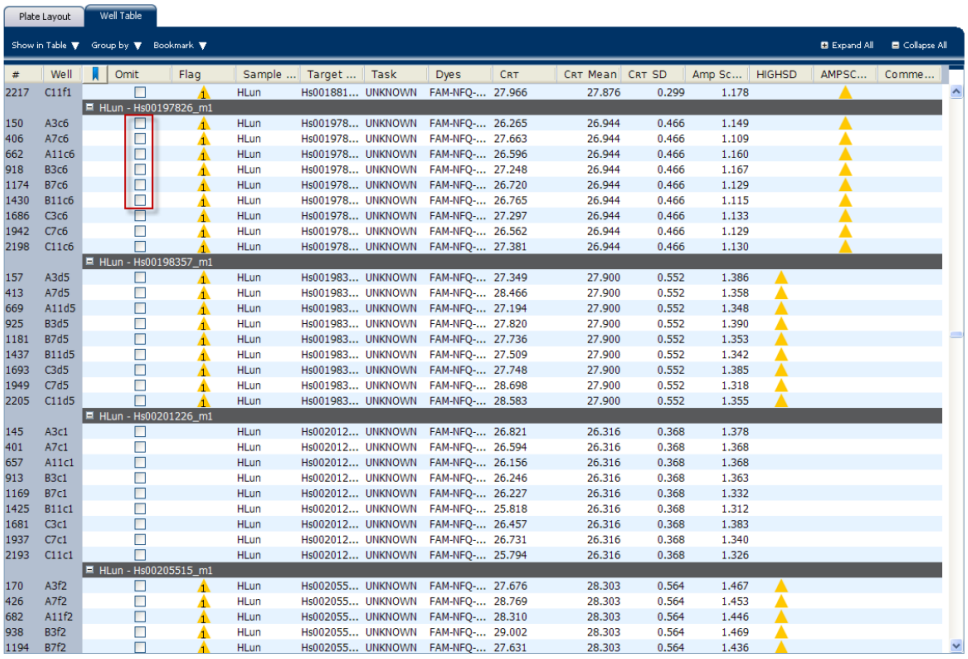
Improve C_{RT} precision by omitting wells

Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_{RT} values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outliers can result in erroneous measurements. To enable C_{RT} precision, omit the outliers from the analysis.

In the OpenArray™ gene expression example experiment, there are 354 outliers. You can remove these wells from analysis.

1. From the **Experiment Menu**, select **Analysis ▸ Amplification Plot**.
   If no data are displayed, click **Analyze.**
2. In the **Amplification Plot**, select **Plot Type ▸ C_{RT} vs Well**.
3. Select the **Well Table** tab.
4. In the **Well Table**, identify outliers.
   a. Select **Group By ▸ Replicate**.
   b. Look for outliers in the replicate group (ensure that they are flagged).

c. Select the **Omit** checkbox next to outlying well or wells.



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	CRT	CRT Mean	CRT SD	Amp Sc...	HIGHSD	AMPSC...	Comme...
2217	C11f1	☐	⚠	HLun	Hs001881...	UNKNOWN	FAM-NFQ-...	27.966	27.876	0.299	1.178		▲	
		HLun - Hs00197826_m1												
150	A3c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	26.265	26.944	0.466	1.149		▲	
406	A7c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	27.663	26.944	0.466	1.109		▲	
662	A11c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	26.596	26.944	0.466	1.160		▲	
918	B3c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	27.248	26.944	0.466	1.167		▲	
1174	B7c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	26.720	26.944	0.466	1.129		▲	
1430	B11c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	26.765	26.944	0.466	1.115		▲	
1686	C3c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	27.297	26.944	0.466	1.133		▲	
1942	C7c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	26.562	26.944	0.466	1.129		▲	
2198	C11c6	☐	⚠	HLun	Hs001978...	UNKNOWN	FAM-NFQ-...	27.381	26.944	0.466	1.130		▲	
		HLun - Hs00198357_m1												
157	A3d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.349	27.900	0.552	1.386	▲		
413	A7d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	28.466	27.900	0.552	1.358	▲		
669	A11d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.194	27.900	0.552	1.348	▲		
925	B3d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.820	27.900	0.552	1.390	▲		
1181	B7d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.736	27.900	0.552	1.353	▲		
1437	B11d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.509	27.900	0.552	1.342	▲		
1693	C3d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	27.748	27.900	0.552	1.385	▲		
1949	C7d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	28.698	27.900	0.552	1.318	▲		
2205	C11d5	☐	⚠	HLun	Hs001983...	UNKNOWN	FAM-NFQ-...	28.583	27.900	0.552	1.355	▲		
		HLun - Hs00201226_m1												
145	A3c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.821	26.316	0.368	1.378			
401	A7c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.594	26.316	0.368	1.368			
657	A11c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.156	26.316	0.368	1.368			
913	B3c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.246	26.316	0.368	1.363			
1169	B7c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.227	26.316	0.368	1.332			
1425	B11c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	25.818	26.316	0.368	1.312			
1681	C3c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.457	26.316	0.368	1.383			
1937	C7c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	26.731	26.316	0.368	1.340			
2193	C11c1	☐	⚠	HLun	Hs002012...	UNKNOWN	FAM-NFQ-...	25.794	26.316	0.368	1.326			
		HLun - Hs00205515_m1												
170	A3f2	☐	⚠	HLun	Hs002055...	UNKNOWN	FAM-NFQ-...	27.676	28.303	0.564	1.467	▲		
426	A7f2	☐	⚠	HLun	Hs002055...	UNKNOWN	FAM-NFQ-...	28.769	28.303	0.564	1.453	▲		
682	A11f2	☐	⚠	HLun	Hs002055...	UNKNOWN	FAM-NFQ-...	28.310	28.303	0.564	1.446	▲		
938	B3f2	☐	⚠	HLun	Hs002055...	UNKNOWN	FAM-NFQ-...	29.002	28.303	0.564	1.469	▲		
1194	B7f2	☐	⚠	HLun	Hs002055...	UNKNOWN	FAM-NFQ-...	27.631	28.303	0.564	1.436	▲		

5. With the outlying well or wells removed from the analysis, click **Analyze** to reanalyze the experiment data.

Note: You can also omit undesirable wells in an experiment from the **Plate Layout**. To omit a well from the **Plate Layout**, right-click the well and select **Omit**

Export the analyzed data

1. Open the OpenArray™ gene expression example experiment file that you analyzed in Chapter 5, “Perform the instrument run”.

2. In the **Experiment Menu**, click **Export**.

Note: To export data automatically after analysis, during experiment setup or before running the experiment, select the **Auto Export** checkbox. **Auto Export** is unchecked for the example experiment.

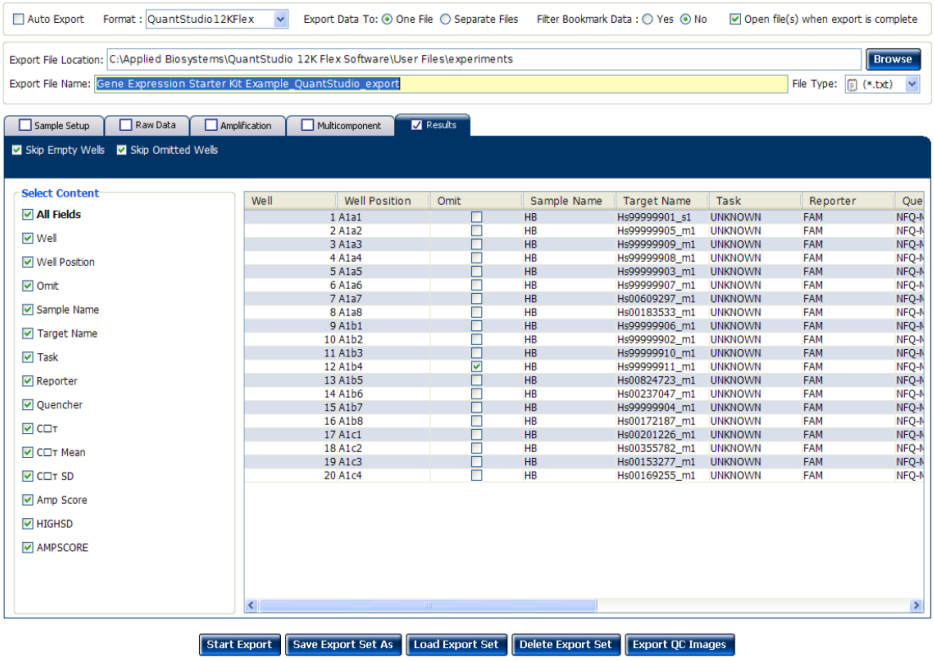
---

3. In the top of the screen, select **Format** ▸ **QuantStudio 12K Flex format**.

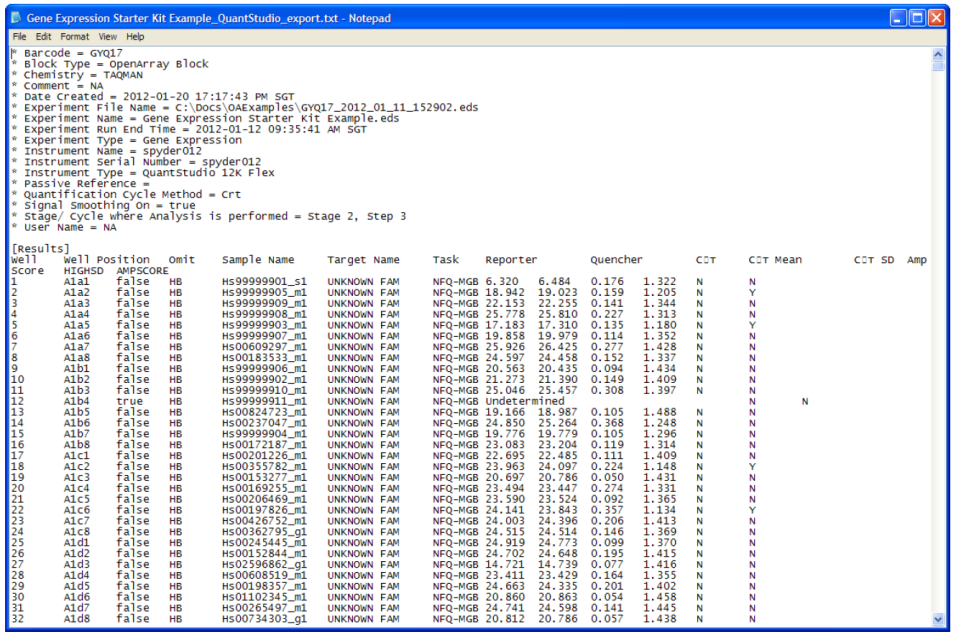
4. Complete the **Export** screen.

Item	Entry
Export Data To	**One File**
Filter Bookmark Data	**No**
Export File Location	`<...>:\Applied Biosystems\QuantStudio 12K Flex Software\experiments`
Export File Name	`Gene Expression Starter Kit Example_QuantStudio_export`
File Type	**(*.txt)**
Select Data to export/ Select Content	Select the **All Fields** checkbox or select a specific set of checkboxes.

The completed **Export** screen should look like this.



When opened in Notepad, the exported file should look like this.



Gene Expression Starter Kit Example_QuantStudio_export.txt - Notepad

File Edit Format View Help

```
* Barcode = GYQ17
* Block Type = OpenArray Block
* Chemistry = TAQMAN
* Comment = NA
* Date Created = 2012-01-20 17:17:43 PM SGT
* Experiment File Name = C:\Docs\OAExamples\GYQ17_2012_01_11_152902.eds
* Experiment Name = Gene Expression Starter Kit Example.eds
* Experiment Run End Time = 2012-01-12 09:35:41 AM SGT
* Experiment Type = Gene Expression
* Instrument Name = spyder012
* Instrument Serial Number = spyder012
* Instrument Type = QuantStudio 12K Flex
* Passive Reference =
* Quantification Cycle Method = Crt
* Signal Smoothing On = true
* Stage/ Cycle where Analysis is performed = Stage 2, Step 3
* User Name = NA

[Results]
Well    Well Position   Omit    Sample Name     Target Name     Task    Reporter        Quencher        CÐT     CÐT Mean        CÐT SD  Amp
Score   HIGHSD  AMPSCORE
1       A1a1    false   HB      Hs99999901_s1   UNKNOWN FAM     NFQ-MGB 6.320   6.484   0.176   1.322   N       N
2       A1a2    false   HB      Hs99999905_m1   UNKNOWN FAM     NFQ-MGB 18.942  19.023  0.159   1.205   N       Y
3       A1a3    false   HB      Hs99999909_m1   UNKNOWN FAM     NFQ-MGB 22.153  22.255  0.141   1.344   N       N
4       A1a4    false   HB      Hs99999908_m1   UNKNOWN FAM     NFQ-MGB 25.778  25.810  0.227   1.313   N       N
5       A1a5    false   HB      Hs99999903_m1   UNKNOWN FAM     NFQ-MGB 17.183  17.310  0.135   1.180   N       Y
6       A1a6    false   HB      Hs99999907_m1   UNKNOWN FAM     NFQ-MGB 19.858  19.979  0.114   1.352   N       N
7       A1a7    false   HB      Hs00609297_m1   UNKNOWN FAM     NFQ-MGB 25.926  26.425  0.277   1.428   N       N
8       A1a8    false   HB      Hs00183533_m1   UNKNOWN FAM     NFQ-MGB 24.597  24.458  0.152   1.337   N       N
9       A1b1    false   HB      Hs99999906_m1   UNKNOWN FAM     NFQ-MGB 20.563  20.435  0.094   1.434   N       N
10      A1b2    false   HB      Hs99999902_m1   UNKNOWN FAM     NFQ-MGB 21.273  21.390  0.149   1.409   N       N
11      A1b3    false   HB      Hs99999910_m1   UNKNOWN FAM     NFQ-MGB 25.046  25.457  0.308   1.397   N       N
12      A1b4    true    HB      Hs99999911_m1   UNKNOWN FAM     NFQ-MGB Undetermined                    N       N
13      A1b5    false   HB      Hs00824723_m1   UNKNOWN FAM     NFQ-MGB 19.166  18.987  0.105   1.488   N       N
14      A1b6    false   HB      Hs00237047_m1   UNKNOWN FAM     NFQ-MGB 24.850  25.264  0.368   1.248   N       N
15      A1b7    false   HB      Hs99999904_m1   UNKNOWN FAM     NFQ-MGB 19.776  19.779  0.105   1.296   N       N
16      A1b8    false   HB      Hs00172187_m1   UNKNOWN FAM     NFQ-MGB 23.083  23.204  0.119   1.314   N       N
17      A1c1    false   HB      Hs00201226_m1   UNKNOWN FAM     NFQ-MGB 22.695  22.485  0.111   1.409   N       N
18      A1c2    false   HB      Hs00355782_m1   UNKNOWN FAM     NFQ-MGB 23.963  24.097  0.224   1.148   N       Y
19      A1c3    false   HB      Hs00153277_m1   UNKNOWN FAM     NFQ-MGB 20.697  20.786  0.050   1.431   N       N
20      A1c4    false   HB      Hs00169255_m1   UNKNOWN FAM     NFQ-MGB 23.494  23.447  0.274   1.331   N       N
21      A1c5    false   HB      Hs00206469_m1   UNKNOWN FAM     NFQ-MGB 23.590  23.524  0.092   1.365   N       N
22      A1c6    false   HB      Hs00197826_m1   UNKNOWN FAM     NFQ-MGB 24.141  23.843  0.357   1.134   N       Y
23      A1c7    false   HB      Hs00426752_m1   UNKNOWN FAM     NFQ-MGB 24.003  24.396  0.206   1.413   N       N
24      A1c8    false   HB      Hs00362795_g1   UNKNOWN FAM     NFQ-MGB 24.515  24.514  0.146   1.369   N       N
25      A1d1    false   HB      Hs00245445_m1   UNKNOWN FAM     NFQ-MGB 24.919  24.773  0.099   1.370   N       N
26      A1d2    false   HB      Hs00152844_m1   UNKNOWN FAM     NFQ-MGB 24.702  24.648  0.195   1.415   N       N
27      A1d3    false   HB      Hs02596862_g1   UNKNOWN FAM     NFQ-MGB 14.721  14.739  0.077   1.416   N       N
28      A1d4    false   HB      Hs00608519_m1   UNKNOWN FAM     NFQ-MGB 23.411  23.429  0.164   1.355   N       N
29      A1d5    false   HB      Hs00198357_m1   UNKNOWN FAM     NFQ-MGB 24.663  24.335  0.201   1.402   N       N
30      A1d6    false   HB      Hs01102345_m1   UNKNOWN FAM     NFQ-MGB 20.860  20.863  0.054   1.458   N       N
31      A1d7    false   HB      Hs00265497_m1   UNKNOWN FAM     NFQ-MGB 24.741  24.598  0.141   1.445   N       N
32      A1d8    false   HB      Hs00734303_g1   UNKNOWN FAM     NFQ-MGB 20.812  20.786  0.057   1.438   N       N
```

Part II

QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit

Introduction

QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit

Each QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit contains materials that are required to perform two experiments on the QuantStudio™ 12K Flex Real–Time PCR System, from sample preparation to data analysis, unless otherwise shown in Table 3 on page 90. The components allow for a typical setup for two genotyping experiments.

Table 3 Components of the QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit

Starter kit (2–20 components)	Source	Kit contents	Description
QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit	4469605	• TaqMan™ OpenArray™ Genotyping Master Mix, 2X • TaqMan™ OpenArray™ Genotyping Training Plate, QuantStudio™ 12K Flex (2 plates) • QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit (Cat. No. 4469586)	Contains reagents to conduct two real-time genotyping experiments on the QuantStudio™ 12K Flex Real–Time PCR System, using the TaqMan™ OpenArray™ Genotyping Training Plate, QuantStudio™ 12K Flex as an example. This kit does not contain samples.
QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit	4469586	• OpenArray™ Case Lid (6 lids) • OpenArray™ Plugs (6 plugs) • OpenArray™ Carriers (1 or 2 carriers) • QuantStudio™ Immersion Fluid(6 syringes) • OpenArray™ Immersion Fluid Tip • OpenArray™ AccuFill™ System Tips (1 box of 384 tips) • OpenArray™ 384-well Sample Plate (10 plates) • OpenArray™ 384-Well Plate Seals (10 seals)	Contains accessories to assemble TrueMark™ OpenArray™ Plate formats for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

Plates

The instructions in this document use three types of plates. The plates are described in detail in Appendix B, “Plate information”.

- MicroAmp™ Optical 96-Well Reaction Plate (96-well plate)
  A non-optical 96-well reaction plate can also be used.
- OpenArray™ 384-well Sample Plate (384-well plate, for QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)
- TrueMark™ OpenArray™ Plate (OpenArray™ Plate)

File formats

The files are used to track your assays and samples. The QuantStudio™ 12K Flex Software (included with the QuantStudio™ 12K Flex Real–Time PCR System) contains example data files for each starter kit experiment type.

The instructions in this guide use four types of files.

- Sample information file (CSV)—Contains the sample layout information for the plate. Allows input of Sample IDs. See page 91).
- SNP plate file (SPF)—Contains the information for genotyping runs. Allows input of Assay IDs and thermal cycling protocol. See page 92.
- Template file (EDT)—Includes complete setup information (samples, assays and cycling protocol) saved as a template. See page 93).
- Experiment file or data file (EDS)—A complete data file. See page 93.

Additional files (AIF, TXT) are available for selection if you use the batch experiment setup in the software to create and run your own experiments.

Note: Use the AIF file to assign VIC™ and FAM™ dyes to the correct SNP alleles.

Sample information file (CSV)

We recommend that you create or use a comma-delimited file (CSV) to track your cDNA or gDNA samples. Use a sample information file to perform these tasks.

- Track where samples and controls are located in the 96-well plate. See Chapter 8, “Prepare the nucleic acid samples”.
- Map the sample locations, depending on the TrueMark™ OpenArray™ Plate format being used:
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate. See Chapter 9, “Prepare the OpenArray™ 384-well Sample Plate”.
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each OpenArray™ Plate. See Chapter 10, “Prepare the TrueMark™ OpenArray™ Plate”.
- Associate information about the samples with the data results, to normalize data or compute standard curves and calculate concentrations.

IMPORTANT! To enable accurate results, you must correctly track sample information from plate to plate.

For OpenArray™ AccuFill™ Software v2.0, all sample tracking and mapping features are available in this software. The OpenArray™ Sample Tracker Software is not used for OpenArray™ AccuFill™ Software v2.0.

- OpenArray™ 384-well Sample Plate—Integrate this file with a plate setup file in the OpenArray™ AccuFill™ Software (see “Prepare the plate setup files” on page 107).
- TrueMark™ OpenArray™ Plate—Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see “Start a run from the software” on page 123, or after the run is complete.

Note: To track sample information for the starter kit experiments, use the example CSV files supplied with the QuantStudio™ 12K Flex Software.

Plate setup file (SPF)

Use an OpenArray™ Plate setup file

Plate setup files (SPF) contain the assay information for individual OpenArray™ Plate formats, including the gene symbol, gene name, assay ID, and location of each assay on the plate.

- Use the OpenArray™ AccuFill™ Software to integrate the sample information from a 384-well plate file (CSV) with the assay information in the plate setup file. See “Prepare the plate setup files” on page 107.
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (EDS). See “Start a run from the software” on page 123.

Access the starter kit plate setup files

1. Go to **thermofisher.com/OA-platefiles**.
2. In the **Select your product** dropdown list, select **TaqMan OpenArray Inventoried**.
3. Enter the serial numbers, then click **Download**.

Download your own plate setup files

To process a TrueMark™ OpenArray™ Plate on the QuantStudio™ 12K Flex Real–Time PCR System, you must download the specific plate files that correspond to your plate and experiment type. Genotyping experiments use SNP plate files (SPF).

Note:

- The OpenArray™ AccuFill™ Software v2.0 includes a feature to download the SPF files. The computer running the software must be connected to the internet.
- The OpenArray™ AccuFill™ Software v2.0 includes a feature to create a SPF file from a template.
- For more information about these features, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

1. Go to **thermofisher.com/OA-platefiles**.
2. Select one of the following options.
   - **Custom**
   - **Inventoried**

3. Enter the following information.

Product	Information
Custom	a. Enter the ***Lot number*** or ***Batch number***. b. Enter one ***Serial number*** from the lot. Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

4. Click **Download**.

The downloaded files are in a compressed ZIP folder.

Template file (EDT)

An experiment document template file (EDT) contains predefined experiment setup information, such as experiment type, assay names, and run method.

You can access a template to create a new experiment from the two locations.

- QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen. See “Start a run from the instrument touchscreen” on page 127.
- QuantStudio™ 12K Flex Software. See “Experiment file (EDS)” on page 93.

  To create and run the starter kit experiments, use the example template files supplied with the QuantStudio™ 12K Flex Software.

  The example template files are located at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray`, where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Experiment file (EDS)

An experiment document single file (EDS) is an electronic record used by the QuantStudio™ 12K Flex Software that contains all information about a particular TrueMark™ OpenArray™ Plate run on the QuantStudio™ 12K Flex Real-Time PCR Instrument. The EDS file includes the following information.

- Metadata
  - Name
  - Barcode
  - Comments
- Experiment setup
  - Well contents
  - Assay definitions
- Run method (thermal cycling protocol)

- Run results
- Analysis protocol
- Analysis results
- Audit records
- Other plate-specific data

Use an EDS file to perform these tasks.

- Create and run an experiment using the QuantStudio™ 12K Flex Software (see “Start a run from the software” on page 123).

  To create and run the starter kit experiments, use the example template files (EDT, see “Template file (EDT)” on page 93) supplied with the software.
- Create and run an experiment using the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen (see “Start a run from the instrument touchscreen” on page 127).
- Analyze the experiment results in the QuantStudio™ 12K Flex Software (see Chapter 12, “Analyze the experiment results”).

  To view and analyze results for the starter kit experiments, use the example experiment files supplied with the QuantStudio™ 12K Flex Software.

Starter kit data files

Use the example files supplied with the QuantStudio™ 12K Flex Software to perform the tasks that are described in this guide.

Table 4 QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit data files referenced in this guide.

File type	Description	File name	Location[1]	Used in
SPF	SNP plate file	—	Download (see "Access the starter kit plate setup files" on page 92)	Chapter 9, "Prepare the OpenArray™ 384-well Sample Plate"
CSV	96-well sample information file	—	These files can be generated in the OpenArray™ AccuFill™ Software v2.0.	Chapter 9, "Prepare the OpenArray™ 384-well Sample Plate"
EDT	Experiment template	`Genotyping.edt`	`<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K FlexSoftware\templates\OpenArray`	Chapter 10, "Prepare the TrueMark™ OpenArray™ Plate"
EDS	Experiment data file	`Genotyping Starter Kit Example.eds`	`<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\examples\Genotyping`	Chapter 12, "Analyze the experiment results"

[1] `<...>` is the drive on which the software is installed. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Workflow

QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit

Prepare the nucleic acid samples (page 97)

The workflow for sample preparation varies, depending on starting from the MicroAmp™ Optical 96-Well Reaction Plate using cDNA or your own DNA.

Prepare the OpenArray™ 384-well Sample Plate (page 100)

Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray™ 384-well Sample Plate.

Prepare the TrueMark™ OpenArray™ Plate (page 104)

- Prepare for sample transfer using 384-well plates.
- Transfer the samples from 384-well plates to TrueMark™ OpenArray™ Plate formats using the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.
- Seal the TrueMark™ OpenArray™ Plate formats
- Complete sample transfer for the remaining TrueMark™ OpenArray™ Plate formats.

Perform the instrument run (page 118)

- Prepare the QuantStudio™ 12K Flex Software.
- Load the TrueMark™ OpenArray™ Plate formats into the instrument.
- Run the TrueMark™ OpenArray™ Plate formats.
- (Optional) Monitor the run.
- Unload the TrueMark™ OpenArray™ Plate formats from the instrument.
- Transfer the experiments results to the computer.

Analyze the experiment results (page 136)

8 Prepare the nucleic acid samples

In this chapter, you prepare the nucleic acid samples for your experiment using the QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit.

Workflow

Prepare nucleic acid samples for a genotyping experiment

Measure the gDNA quality and quantity

See “DNA quality” on page 98 and “DNA quantity” on page 98.

Transfer the gDNA samples to a 96-well reaction plate (page 98)

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Starting material: human gDNA	User-supplied
MicroAmp™ Optical 96-Well Reaction Plate	4316813
DNase-free, sterile-filtered water	**MLS**
ProFlex™ 2 × Flat PCR System	4484078

DNA quality

DNA that is used with genotyping experiments should have the following characteristics:

- The DNA is extracted from the raw material that you are testing with an optimized protocol. Salting out procedures and crude lysates are not recommended.
- The DNA does not contain PCR inhibitors.
- The DNA has an $A_{260/230}$ ratio between 1.7 and 1.9.
- The DNA has an $A_{260/280}$ ratio between 1.7 and 1.9.
- The DNA is intact as visualized by gel electrophoresis.
- The DNA has not been heated above 60°C. Temperatures above 60°C can cause degradation.

DNA quantity

We recommend that you quantify and normalize the amount of gDNA in your human DNA samples, for use with genotyping experiments.

- The recommended amount of template for each single (through-hole) reaction in a TrueMark™ OpenArray™ Plate is 250 copies of the haploid genome, equivalent to 0.84 ng for human DNA samples.

  The recommended starting concentration for human DNA samples is 50 ng/µL to obtain 250 copies of the haploid genome per through-hole.
- For optimal cluster plot results, it is important to normalize all gDNA samples in an experiment so that each through-hole receives the same input quantity of sample.

To quantify the amount of gDNA in your human DNA samples, perform one of the following items:

- Generate an A_{260} reading using a UV spectrophotometer.
- Generate a Qubit™ dsDNA HS assay reading using a Qubit™ Fluorometer.
- Quantification by RNase real-time PCR experiment

See the appropriate instrument or chemistry kit user guide for detailed instructions about performing the DNA quantitation.

Transfer the gDNA samples to a 96-well reaction plate

To load gDNA samples and controls into a TrueMark™ OpenArray™ Plate, pipet the samples into a 96-well reaction plate.

1. Transfer 5.0 µL of the gDNA samples and controls into the appropriate number of wells of a MicroAmp™ Optical 96-Well Reaction Plate, based on the OpenArray™ Plate format being used.

   See Appendix B, “Plate information”.

Note: For the starter kit experiment, the Format 64 OpenArray™ genotyping plate format is used to test a maximum of 48 samples against the TaqMan™ OpenArray™ Genotyping Training Plate, QuantStudio™ 12K Flex, a fixed content panel containing 64 TaqMan™ SNP Genotyping Assays.

2. *(Recommended)* Create a sample information file (CSV) to track where the samples are in the 96-well sample plate.

   A CSV file can be created in OpenArray™ AccuFill™ Software v2.0.

   For more information, see "Sample information file (CSV)" on page 91.

Proceed to Chapter 9, "Prepare the OpenArray™ 384-well Sample Plate".

Perform your own experiments

When you prepare samples for your own genotyping experiments, note the following modifications.

- For gDNA samples of other organisms, proportionally adjust the starting concentration based on the organism genome size relative to the human genome size (approximately 3×10^9 base pairs in the haploid genome)

  Note: To obtain the genome size for other species, go to **genomesize.com** or **cvalues.science.kew.org/**.

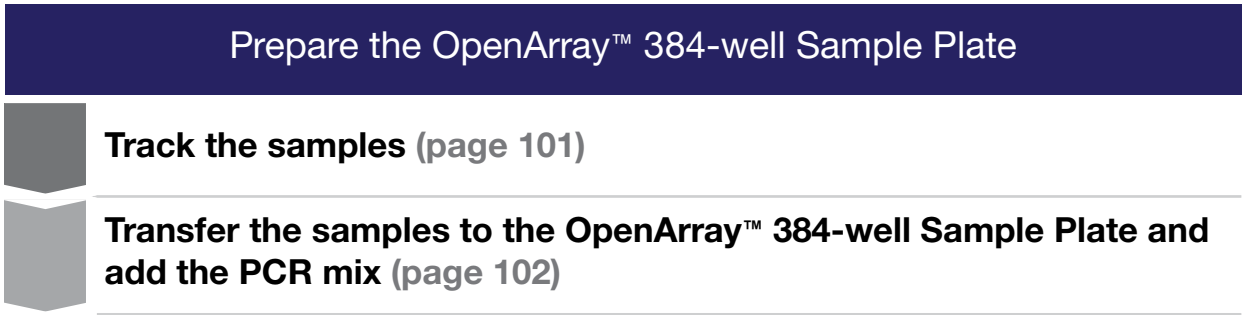
- Transfer 5.0 µL of the gDNA samples and controls into the appropriate number of wells of a MicroAmp™ Optical 96-Well Reaction Plate, based on the OpenArray™ genotyping plate format being used. See Appendix B, "Plate information".

9 Prepare the OpenArray™ 384-well Sample Plate

In this chapter, you use an 8- or 12-channel pipette to transfer the nucleic acid samples from the 96-well reaction plates to OpenArray™ 384-well Sample Plate formats. The plates are described in detail in Appendix B, “Plate information”.

You also track the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates. The workflow for preparing the 384-well sample plate varies, depending on the starter kit (or experiment type) in use.

Workflow



Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source[1]
96-well reaction plates, containing prepared gDNA samples	User-supplied (see “Required materials” on page 97)
2× TaqMan™ OpenArray™ Genotyping Master Mix	4404846
OpenArray™ 384-well Sample Plate	4406947
OpenArray™ 384-Well Plate Seals	4469876
Fine-tip marker	**MLS**

[1] Provided in starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For OpenArray™ AccuFill™ Software v2.0, samples are tracked in the OpenArray™ AccuFill™ Software. The samples are tracked in the **Map Plates** tab.

For more information about OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Navigate to the **Full Run** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Genotyping**.
2. In the **Plate format** section, select a format for your experiment.

Experiment type	Format
Starter kit experiments	**Genotyping—64**
Your own experiments	• **Genotyping—16** • **Genotyping—32** • **Genotyping—64** • **Genotyping—128** • **Genotyping—192** • **Genotyping—256**

3. In the **Pipettor** section, select a type of pipette.
   - **Fixed**
   - **Adjustable**

   The number of OpenArray™ Plate formats displayed in the **Add your OpenArray plate serial numbers** section depends on the selections made in the previous steps.

4. In the **Add your OpenArray Plate serial numbers** section, click **Choose File**, navigate to the location of the SPF file, then select the file.

   Repeat for each SPF file.

5. In the **Add your sample plates - optional** section, click **Choose File**, navigate to the location of the CSV file, then select the file.

   If the sample plate file is not imported, the samples must be added manually.

   The file must be a CSV file for a 96-well format.

   The format of the sample plate file is validated. For information about the required format, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

   The file name is displayed in the **Select file** field.

6. Repeat step 5 for each CSV file.

7. Click **Next**.
   The **Map plates** pane is displayed.

8. *(Optional)* Add or edit the sample name.

9. Label the OpenArray™ 384-well Sample Plate with a fine-tip marker.

   Based on the tracking information on the **Map plates** pane, mark the sections of the OpenArray™ 384-well Sample Plate to transfer samples from the 96-well reaction plates.

10. Click **Next**.

The **Start run** pane is displayed.

Set up the samples in the OpenArray™ 384-well Sample Plate, then prepare to load the OpenArray™ Plate formats in the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.

Transfer the samples to the OpenArray™ 384-well Sample Plate and add the PCR mix

Thaw the 96-well reaction plate containing prepared gDNA samples at room temperature.

1. Vortex the gDNA samples to mix, then centrifuge for 1 minute at 1,000 *x* g to collect the contents at the bottom of the tube.

2. Review the concentration of the normalized gDNA samples. The recommended starting concentration for human gDNA samples is 50 ng/µL.

   Note: For optimal results, normalize all gDNA samples in an experiment. For human gDNA, ensure that the samples are close to the recommended starting concentration of 50 ng/µL. For gDNA samples of other organisms, proportionally adjust the starting concentration based on the organism's genome size relative to the human genome size (*approximately 3 × 10^9 base pairs in the haploid genome*).

3. Mix the 2× TaqMan™ OpenArray™ Genotyping Master Mix by gently inverting the bottle 10 times.

4. Load the OpenArray™ 384-well Sample Plate based on the plate layout.

   See “Track the samples” on page 101.

   a. Add the master mix to the 384-well sample plate.

   b. Using a 12-channel pipette, transfer the normalized gDNA samples from the 96-well reaction plate to the 384-well sample plate.

Component	Volume per 384-well sample plate well[1]		
	Format 64 *(starter kit experiment)* and larger formats	Format 16	Format 32
2✕ TaqMan™ OpenArray™ Genotyping Master Mix	2.5 µL	1.5 µL	2.0 µL
Normalized gDNA sample*(human gDNA starting concentration = 50 ng/µL)*	2.5 µL	1.5	2.0 µL
Total volume	**5.0 µL**	**3.0 µL**	**4.0 µL**

[1] One well of a 384-well sample plate corresponds to one subarray of a TrueMark™ OpenArray™ Plate . The number of subarrays required depends on the format of the TrueMark™ OpenArray™ Plate. For more information, see Appendix B, “Plate information”.

5. Seal the sample plate, vortex gently to mix, then centrifuge for 1 minute at 1,000 *x* g to eliminate bubbles and to collect the contents at the bottom of the wells.

6. Place the sample plate on ice for up to 1 hour.

Proceed to Chapter 10, “Prepare the TrueMark™ OpenArray™ Plate”.

10 Prepare the TrueMark™ OpenArray™ Plate

In this chapter, you use the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System to transfer the nucleic acid samples from the OpenArray™ 384-well Sample Plate to the TrueMark™ OpenArray™ Plate. The workflow is the same for all OpenArray™ Plate formats.

Workflow

Prepare the TrueMark™ OpenArray™ Plate

Prepare for sample transfer (page 106)

Transfer the samples (page 108)

Seal the TrueMark™ OpenArray™ Plate (page 114)

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
TrueMark™ OpenArray™ Plate[1]	See Appendix A, “Ordering information”.
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System	4471021
QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit The accessories kit contains: • OpenArray™ Case Lid (6 lids) • OpenArray™ Plugs (6 plugs) • OpenArray™ Carriers (2 carriers) • QuantStudio™ Immersion Fluid (6 syringes) • OpenArray™ Immersion Fluid Tip • OpenArray™ AccuFill™ System Tips (1 box of 384 tips) • OpenArray™ 384-well Sample Plate (10 plates) • OpenArray™ 384-Well Plate Seals (10 seals)	4469586
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
Foil seals	**MLS**
Bleach (10%)	**MLS**
Ethanol	**MLS**
Fine-tip marker	**MLS**
Razor blade	**MLS**
Powder-free gloves	**MLS**
Laboratory-grade wipes	**MLS**
Safety glasses	**MLS**
Tweezers or forceps (for removing foil sections from the 384-well sample plate)	**MLS**

[1] For more information, see Appendix B, “Plate information”.

Storage conditions

The following materials require special storage conditions.

Item		Storage
TrueMark™ OpenArray™ Plate	Frozen, unopened	Store at –20°C until the expiration date provided on the product label.
	Thawed, unopened	Store at room temperature for up to 24 hours.
	Thawed, opened	Store at room temperature for up to 1 hour.
	Loaded and sealed, pre-run	Store at room temperature, protected from light, for up to 24 hours.
OpenArray™ Immersion Fluid	Unopened	Store at room temperature until the expiration date provided on the product label.
	Opened	Store at room temperature. Do not store any remaining immersion fluid. Use the amount required, then discard the remainder.
OpenArray™ AccuFill™ System Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Use tips within one week.

Prepare for sample transfer

Guidelines for handling the OpenArray™ Plate

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Plate.

- Hold the OpenArray™ Case by the edges.
- Do not touch the through-holes of the OpenArray™ Plate.
- Load and seal an OpenArray™ Plate within *one hour* after opening the package.
- If you drop a loaded OpenArray™ Plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray™ Plate if it becomes dislodged from the case.

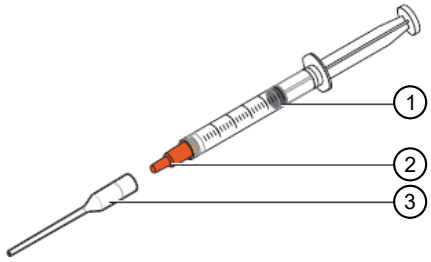
Prepare the equipment and plates

Ensure that the OpenArray™ 384-well Sample Plate, the OpenArray™ AccuFill™ System Tips, and OpenArray™ Plate holder are completely clean and dry.

1. Remove an OpenArray™ Plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).

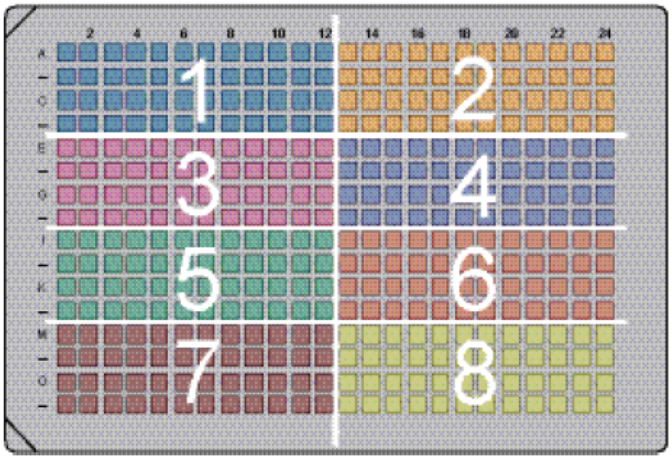
2. Prepare a syringe containing OpenArray™ Immersion Fluid.
   a. Remove the cap from the syringe containing OpenArray™ Immersion Fluid.

b. Remove the cap and attach the tip to the syringe. Place the assembly on a clean surface.



① OpenArray™ Immersion Fluid
② Cap (remove)
③ Syringe tip (attach)

3. Score or cut the foil seal of the OpenArray™ 384-well Sample Plate into the 8 sections shown below, then place the plate on ice to keep the samples cold.



Prepare the plate setup files

- OpenArray™ AccuFill™ Software v2.0 allows the transfer of samples without a sample plate file. The QuantStudio™ 12K Flex Instrument requires a sample plate file if the real-time PCR run is started with an SPF file or an EDT file.
- For your own genotyping experiments, the following plate setup files can be used to transfer samples with the OpenArray™ AccuFill™ Software
  - OpenArray™ 384-well sample information file (CSV, see “Track the samples” on page 101)
  - OpenArray™ plate setup file (SPF, see “Use an OpenArray™ Plate setup file” on page 92)
- *(Optional)* If you created a CSV file in the OpenArray™ AccuFill™ Software (see “Track the samples” on page 101), you can import the sample information in this file directly into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

Transfer the samples

Start the system

Note: If the samples were tracked immediately before this section, the system might be on (see "Track the samples" on page 101). The sample tracking and sample transfer functions are both done with OpenArray™ AccuFill™ Software if OpenArray™ AccuFill™ Software v2.0 is used.

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips. See "Set up the system" on page 37.

The instrument does not initiate a self-test immediately after starting the software. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

The other features in the software can be accessed after starting the software without a self-test.

1. Ensure that the instrument door is closed.
2. Power on the instrument, if it is off.
3. Start the OpenArray™ AccuFill™ Software ( ).
   The software checks the computer and connections as the system starts.

Proceed to set up the system.

Set up the system

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

1. Open the instrument door, empty the waste bin, then place the waste bin back on the instrument deck.

CAUTION! Wear appropriate personal protective equipment while handling the waste bin.

2. Ensure that the sample plate holder and the OpenArray™ Plate holders are empty.
3. Place the sample plate in the sample plate holder on the instrument deck, with the notch to the left. Do not stack sample plates.
4. Place each OpenArray™ Plate in an OpenArray™ Plate holder.

5. Replace the tip boxes, if necessary.

   Each tip box contains 384 tips, divided into 8 sections.

   When setting up a run, the status of the tip boxes is confirmed in the software. A full tip box is recommended when starting a run.

6. Remove the cover from each tip box.

   Ensure that the tip box covers are removed from the instrument deck.

   IMPORTANT!
   - Do not reuse tips.
   - Use tips within one week of opening the box.
   - Discard any unused tips within one week.

7. Close the instrument door.

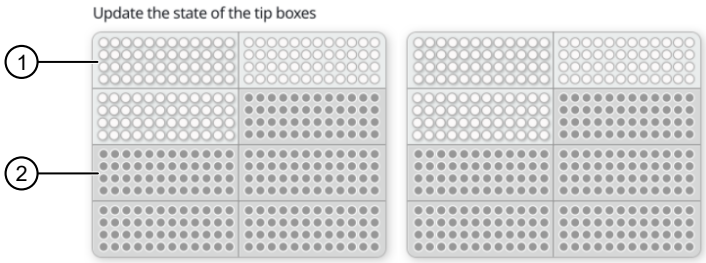
The system is ready to start a run. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes. The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box that is full.
② Section of the tip box that is empty.

2. *(Optional)* Click **Auto-fill tip boxes**.

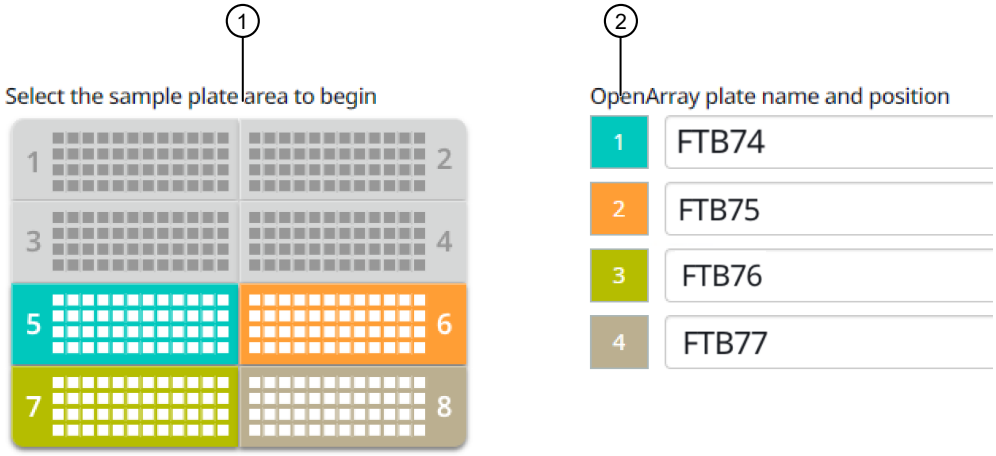
   The status of all sections of the tips boxes is set to full.

3. Select the first section of the sample plate to be used to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple plates are filled during a run. The software selects the total number of sections that correspond with the total number of plates.

   In the following example, section 5 was selected. The group of sections 5, 6, 7, and 8 is highlighted by the software because four plates are being filled.

The position box displays the color that corresponds to the section of the sample plate.



① Sample plate section (section 5, 6, 7, and 8 are highlighted).
② Corresponding plates.

4. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   Note: Do not remove the foil from all the sections of the sample plate at once.

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of these conditions.

   - The waste bin is not in position.
   - The sample plate is not in position.
   - The plates are not in position.
   - There are more plates on the instrument deck than are defined in the experiment setup.

The **Deck** screen is displayed.

For a description of the run progress, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see “Seal the TrueMark™ OpenArray™ Plate” on page 43).

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument

After an OpenArray™ Plate is filled, the **Remove plate and foil** dialog box is displayed (see Figure 4).

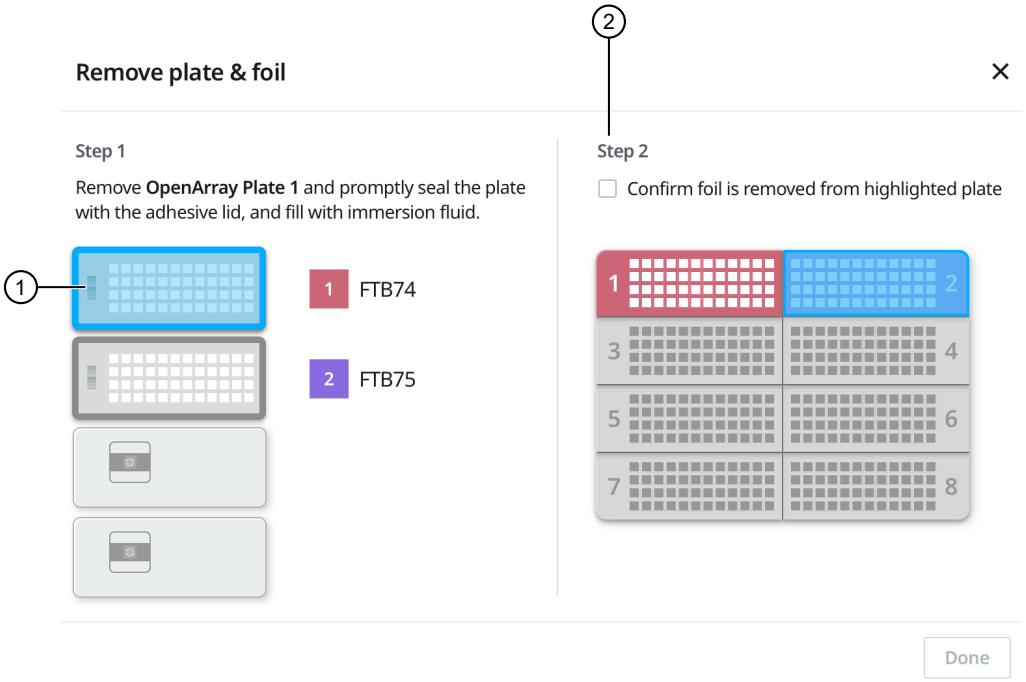


Figure 4 Remove plate and foil dialog box.

① OpenArray™ Plate to remove from the instrument.
② **Confirm foil is removed from highlighted plate section** checkbox.

Remove each OpenArray™ Plate *immediately* after it has been filled, even if the run was set up to fill multiple plates.

After the last OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed (see Figure 5).

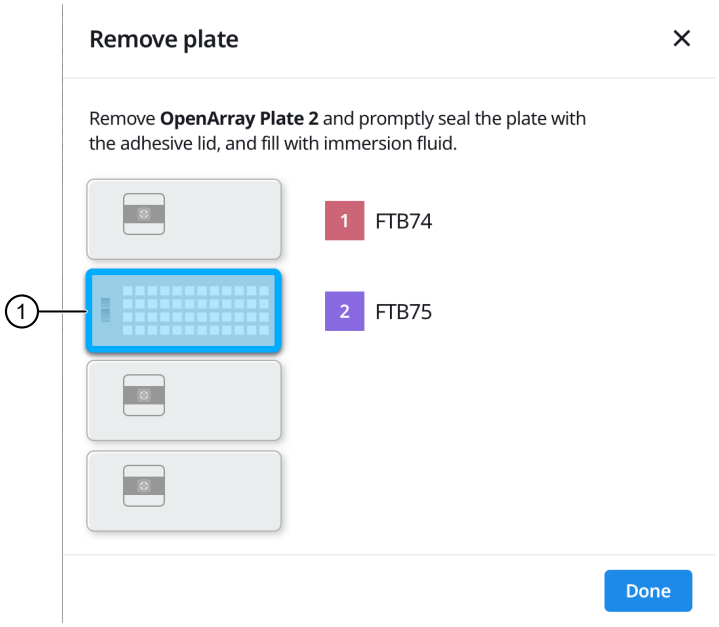


Figure 5 Remove plate dialog box

① OpenArray™ Plate to remove from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate immediately, to avoid evaporation within the plate.

   One of the following dialog boxes is displayed:

   - The **Remove plate and foil** dialog box.
   - The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.

   The run does not proceed under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The plates are not in position
   - There are more plates on the instrument deck than are defined in the experiment setup

   The instrument proceeds to load the next OpenArray™ Plate.

6. Repeat step 1 to step 5 for each OpenArray™ Plate to be loaded.

After all of the plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

A loaded SPF is generated for each OpenArray™ Plate. The loaded SPF file corresponds to the original SPF file that was imported for the run. The files are exported to the folder that was designed in the **Preferences**.

Note: Some workflows might not generate a loaded SPF file. For more information about the workflows available for the OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Seal the TrueMark™ OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

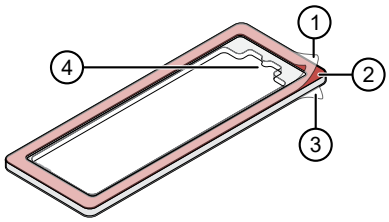
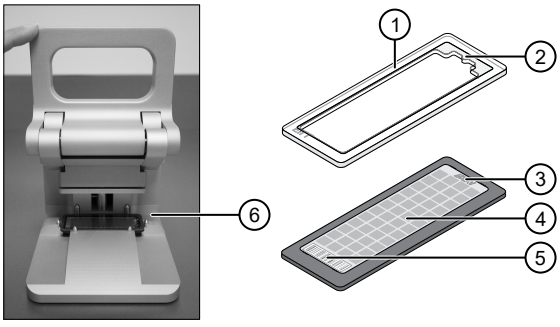


Figure 6 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

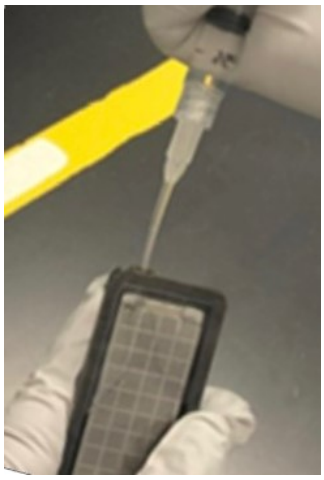
   Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

   IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.

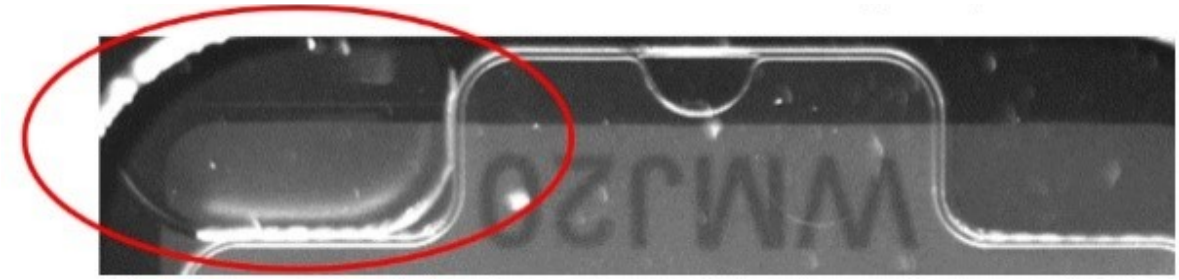


   The syringe tip must be in front of the array when filling the case with immersion fluid.

9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

   IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

   Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

The plate is ready for PCR.

Note: For genotyping experiments, you can store loaded and sealed plates at room temperature, protected from light, for up to 24 hours.

Proceed to Chapter 11, “Perform the instrument run”.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (more than 6) of OpenArray™ Plates, follow these guidelines.

- To help avoid mistakes when entering sample information in the OpenArray™ AccuFill™ Software, load the OpenArray™ Plates in alphanumeric order (according to the OpenArray™ Plate serial number).
- Seal each OpenArray™ Plate immediately after loading is completed, while the other OpenArray™ Plates are loaded.

  IMPORTANT! To avoid evaporation, seal the OpenArray™ Plate with the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0, add the OpenArray™ Immersion Fluid, plug the case, then place the case in a vertical position.

- Use the OpenArray™ Carriers to transport up to four loaded OpenArray™ Plates to the QuantStudio™ 12K Flex Instrument.
- After loading is complete, you can use a large bin to properly discard any used OpenArray™ AccuFill™ System Tips.

  For cleaning procedures, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Perform the instrument run

In this chapter, you run the QuantStudio™ 12K Flex TrueMark™ OpenArray™ Plate formats on the QuantStudio™ 12K Flex Real–Time PCR System. During the run, the system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all OpenArray™ Plate formats.

Workflow

Perform the instrument run

- **Prepare the QuantStudio™ 12K flex software** (page 119)
- **Load the TrueMark™ OpenArray™ Plate into the instrument** (page 199)
- **Run the OpenArray™ Plate formats** (page 122)
- **(Optional) Cycle offline genotyping experiments** (page 129)
- **(Optional) Monitor experiments** (page 130)
- **Unload the TrueMark™ OpenArray™ Plate from the instrument** (page 133)
- **(Optional) Transfer experiment results** (page 133)

Prepare the QuantStudio™ 12K flex software

(Optional) Select OpenArray™ block run preferences

Preferences provide user-access to the settings that govern how the QuantStudio™ 12K Flex Software functions. This section summarizes only those preferences that apply to experiments with OpenArray™ Plate formats.

For detailed information about the preferences, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1**).

1. Double-click **(QuantStudio™ 12K Flex Software shortcut)** to start the software.
2. In the toolbar, click **Tools** ▸ **Preferences**, then select the **OpenArray** tab.

3. Complete the tab, as needed.

Settings	Description
Setup Folder	Define the absolute path to the default folder from which the software imports experiment setup files. The **Import** dialog box opens to the import folder when invoked from the software.
Experiment Folder	Define the absolute path to the default folder to which the software reads or writes experiment files. The **Open** and **Save** dialog boxes open to the data folder when invoked from the software.
Passive Reference	Define the dye to use as the passive reference. The default is set to **None**. While the software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray™ experiments.
Default Browse File Type list	Define the file type that the **Import**, **Open**, and **Save** dialog boxes select by default when invoked from the software.
Apply experiment template (EDT) to all OpenArray experiment checkbox	If selected, the software applies the Run Method defined in the selected experiment template (EDT) to all OpenArray™ experiments. For more information about OpenArray™ experiment templates, see the software help.
Always include Amplification stage for Genotyping experiment checkbox	*(Genotyping experiments only)* If selected, the software adds an Amplification stage to the Run Method for all OpenArray™ genotyping experiments. If deselected, you must perform amplification on another instrument. For more information about Run Method settings, see the software help.
Always include Pre-Read stage for Genotyping experiment checkbox	*(Genotyping experiments only)* If selected, the software adds a Pre-Read stage to the Run Method for all OpenArray™ genotyping experiments. For more information about Run Method settings, see the software help.

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

IMPORTANT! You must restart the software for preference changes to take effect.

Access the Instrument Console

The **Instrument Console** displays every QuantStudio™ 12K Flex Real-Time PCR Instrument discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups.

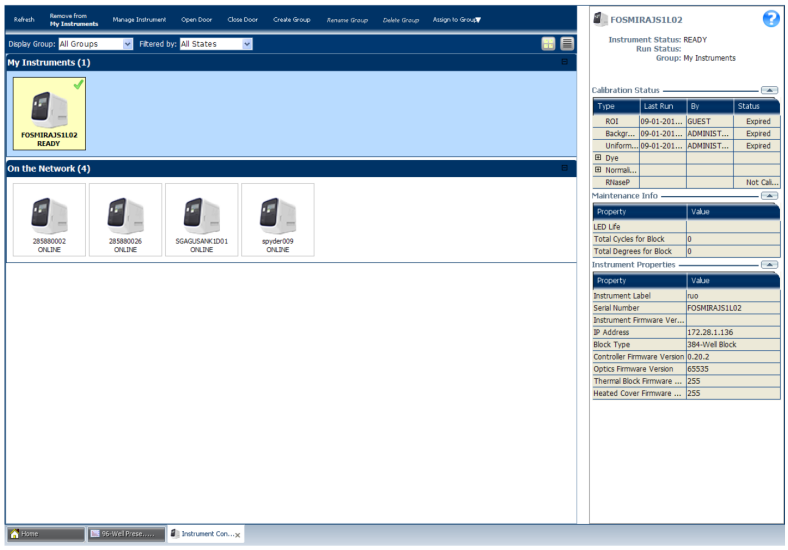
- **On the Network**—All instruments available on the network.
- **My Instruments**—Instruments you have selected to monitor.

To start and monitor a run on an instrument, you must move the instrument from the **On the Network** group to the **My Instruments** group or a custom group that you create.

To access the **Instrument Console** and enable monitoring of a networked instrument:

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. On the **Home** tab, from the **Tools** menu, select **Instrument Console**.
   If you do not see an instrument, click **Refresh** in the **Instrument Console** toolbar.



3. If needed, move an instrument from the **On the Network** group to a group that can be monitored:
   a. Click the instrument of interest, then click **Assign to Group** in the **Instrument Console** toolbar.
   b. Select the **My Instruments** group or a personal group in the drop-down list.

Note: Alternatively, you can select the icon of the instrument that you want to add to the **My Instruments** list, then click **Add to My Instruments**. Similarly, click **Remove from My Instruments** to remove an instrument from the **My Instruments** list. You can also drag and drop the instrument icon into **My Instruments** or into the group created by you.

The instrument is now monitored. The status is indicated by an icon in the upper right corner. For detailed information about the **Instrument Console**, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

Enable or change the notification settings

You can configure the QuantStudio™ 12K Flex Software to alert you by email when the QuantStudio™ 12K Flex Real-Time PCR Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the notification settings feature, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Load the OpenArray™ Plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

IMPORTANT! Wear powder-free gloves when you handle the OpenArray™ Plate.

IMPORTANT! The instrument should be loaded and unloaded only by operators who have been warned of the moving parts hazard and who have been adequately trained.

1. Open the plate adapter on the instrument. Touch on the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software, to allow the plate adapter to come out from the instrument side.
2. Remove the clear protective film from the outside of the OpenArray™ Case (sealed plate + lid).
3. Place the OpenArray™ Plate or plates on the plate adapter.
   - Ensure that each plate is properly aligned in the plate adapter.
   - Ensure that the plate barcode is facing up and toward the front of the instrument.
4. Close the plate adapter on the instrument. Touch on the instrument touchscreen, or click **Close Door** in the **Instrument Console** of the software, to retract the plate adapter back into the instrument.

Run the OpenArray™ Plate formats

You can run TrueMark™ OpenArray™ Plate formats in two ways.

- From the QuantStudio™ 12K Flex Software. See “Start a run from the software” on page 123.
- From the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen. See “Monitor an experiment from the instrument touchscreen” on page 131.

Note: The starter kit experiments in this guide run OpenArray™ Plate formats from the QuantStudio™ 12K Flex Software.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Real-Time PCR Instrument is in operation.

Start a run from the software

There are two ways to create and run an OpenArray™ experiment (EDS) from the QuantStudio™ 12K Flex Software.

- For the starter kit experiments, use a template file (EDT). See “Use a template file” on page 123
- For your own experiments:
  - Use a template file (EDT). See “Use a template file” on page 123.
  - Use an OpenArray™ plate setup file (SPF). See “Use an OpenArray™ Plate setup file” on page 126
  - Use the **Batch Experiment Setup Utility**. See the *QuantStudio™ 12K Flex Software Help* (click or press **F1**)

Use a template file

You can use a template file (EDT) to create a new OpenArray™ experiment, then import the sample and assay information for an OpenArray™ Plate or plates before starting the run, or after the run is complete.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. On the **Home** tab, select **Create From Template**.
3. Navigate to and select the template file (EDT) that you want to use, then click **Open**.
   A new experiment is created using the setup information from the template.

   Note: To access the starter kit templates, navigate to the templates folder located at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates`
   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

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4. In the **Experiment Properties** screen, scan the OpenArray™ Plate barcode or type the OpenArray™ Plate serial number.
5. In the **Samples** screen, do either of the following:
   - *(Recommended)* Click **Import** above the sample table, navigate to and select the OpenArray™ sample information file (CSV) that you want to use, then click **Select File**.
     For the genotyping starter kit experiments, use the OpenArray™ CSV files that were created in OpenArray™ AccuFill™ Software v2.0. (see “Track the samples” on page 101).
   - In the sample table, click in a cell in the **Sample Name** column, then enter a new name.

6. From the open experiment, select **File ▸ Import Plate Setup**.
   a. Click **Browse**, navigate to and select the genotyping starter kit plate setup file that you want to use.

      Genotyping Source File (SPF)—Corresponds to the plate setup file associated with genotyping OpenArray™ Plate formats.

      For the genotyping starter kit experiments and for your own experiments, download the appropriate plate setup files from the Thermo Fisher Scientific website. See “Plate setup file (SPF)” on page 92.

   b. Click **Select**, then click **Start Import**.

   c. If your experiment already contains plate setup information, the software asks you to replace the plate setup with the data from the file. Click **Yes** to replace the plate setup information.

7. Select **File ▸ Save As**, enter a file name, select a location for the experiment file (EDS), then click **Save**.

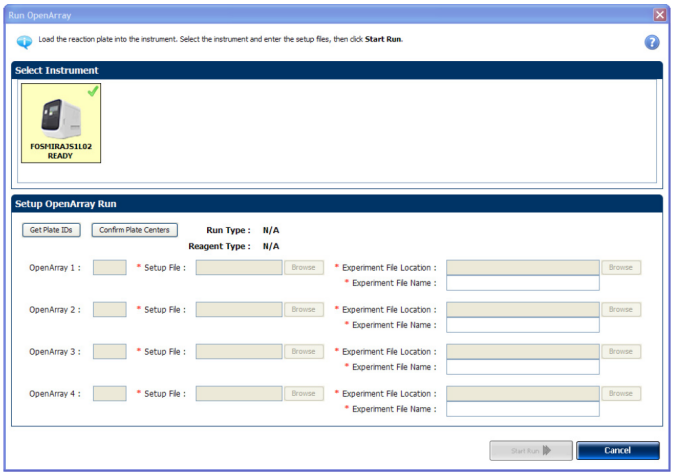
Proceed to “Start the OpenArray™ run” on page 124.

Start the OpenArray™ run

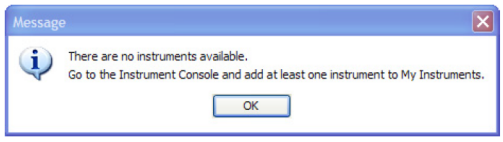
Ensure that the default browse file type is set to EDS. See “(Optional) Select OpenArray™ block run preferences” on page 119.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. On the **Home** tab, from the **Run** menu, select **OpenArray**.



Note: Be sure to add an instrument to **My Instruments** in the **Instrument Console** before you run an experiment. See “Access the Instrument Console” on page 48. If no instrument is selected, a warning appears.



3. In the **Select Instrument** pane, select the instrument that you want to use to run the experiment.

4. Complete the **Setup OpenArray Run** pane.
   - Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate formats that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray™ Plate formats that you want to run. For each plate image in the **Confirm OA Plate Centers** dialog box, click **Continue** if the red box is aligned to the center of the plate. If the box is not in the center of the plate, click **OK**, eject the carrier, rearrange the plates, then click **Get Plate IDs**.
   - *(Optional)* Click **Browse**, then navigate on your computer to select the appropriate setup files. The setup file was created in “Use a template file” on page 51.

   Depending on the number of OpenArray™ Plate formats loaded in the instrument, the barcodes of those plates are populated.

   IMPORTANT! If the QuantStudio™ 12K Flex Real-Time PCR Instrument does not detect a barcode, repeat the barcode read.

5. Click **Start Run**.

Use an OpenArray™ Plate setup file

The OpenArray™ AccuFill™ Software integrates the sample information into an OpenArray™ Plate setup file (SPF). You can save the newly created Loaded_spf files to the OpenArray™ Plate File Input Folder that you selected in the **Preferences** dialog box of the OpenArray™ AccuFill™ Software. Configure this location in the QuantStudio™ 12K Flex Software preferences to upload the integrated plate setup file into the QuantStudio™ 12K Flex Software, then run the file.

Note: You can import a CSV file into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

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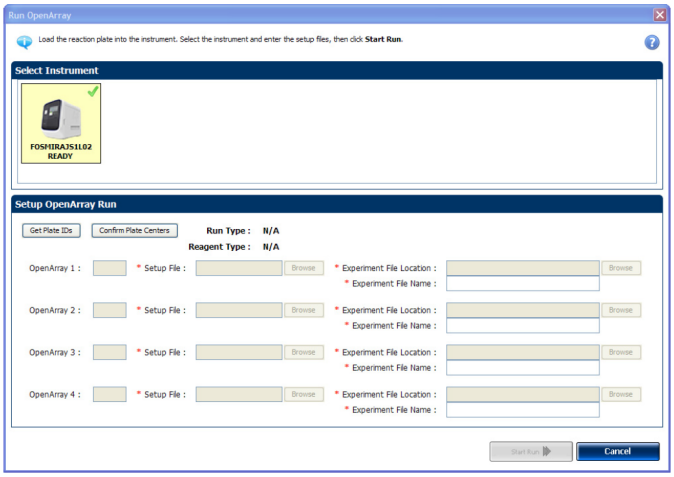
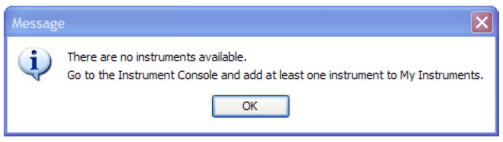
1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. On the **Home** tab, from the **Run** menu, select **OpenArray**.



Note: Be sure to add an instrument to **My Instruments** in the **Instrument Console** before running an experiment. See “Access the Instrument Console” on page 120. If no instrument is selected, a warning appears.



3. In the **Select Instrument** pane, select the instrument that you want to use to run the experiment.

4. Complete the **Setup OpenArray Run** pane.
   - Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate formats that you want to run.
   - *(Optional)* **Click Confirm Plate Centers** to view the center of the OpenArray™ Plate formats that you want to run. For each plate image in the **Confirm OA Plate Centers** dialog box, click **Continue** if the red box is aligned to the center of the plate. If the box is not in the center of the plate, click **OK**, eject the carrier, rearrange the plates, then click **Get Plate IDs**.
   - *(Optional)* Click **Browse**, then navigate on your computer to select the appropriate OpenArray™ Plate setup files (SPF or TPF).

     Note: When the setup file is selected, **Experiment File Location** and **Experiment File Name** are populated. To set the default **Experiment File Location**, go to **Tools** ▸ **Preferences** ▸ **OpenArray** ▸ **Experiment Folder**. In the **Setup OpenArray Run** pane, to select another location for the experiment file, click **Browse**. You can also enter an experiment file name of your choice.

   Depending on the number of OpenArray™ Plate formats loaded in the instrument, the barcode of those plates is populated.

   IMPORTANT! If the QuantStudio™ 12K Flex Real-Time PCR Instrument does not detect a barcode, repeat the barcode read.

5. Click **Start Run**.

Start a run from the instrument touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen:

- “Start a run on the instrument touchscreen from an existing experiment” on page 127
- “Start a run on the instrument touchscreen from a template” on page 128
- “Start a run on the instrument touchscreen from a shortcut” on page 128

Note: The starter kit experiments in this guide start a run from the QuantStudio™ 12K Flex Software.

Start a run on the instrument touchscreen from an existing experiment

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.
   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.

2. In the **Home** screen, touch **Run OpenArray Plates**.
   The instrument retrieves the barcodes and scans for existing experiments with the same barcodes.

3. If experiments with the same barcode cannot be found, touch **Source Input** to select a template to use.

4. Touch Start Run **(Start Run Now)** to start the run.

IMPORTANT! If the instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the instrument touchscreen. Do not proceed if a barcode is not detected by the instrument.

Start a run on the instrument touchscreen from a template

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.

Note: If the touchscreen is not at the **Main Menu** screen, touch **(Home).**

2. In the **Home** screen, touch **(View Templates).**
3. In the **View Templates** screen, touch (**Folders)** to display the folders containing the template files.
4. Touch any of the folders to display the templates in that folder.
5. In the **View Templates** screen, select the desired template, then touch Start Run **(Start Run).**
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
6. Touch Start Run **(Start Run Now)** to start the run.

Start a run on the instrument touchscreen from a shortcut

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.
   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.
2. In the **Home** screen, touch any of the shortcuts that have been set to an OpenArray™ template.
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
3. Touch Start Run **(Start Run Now)** to start the run.

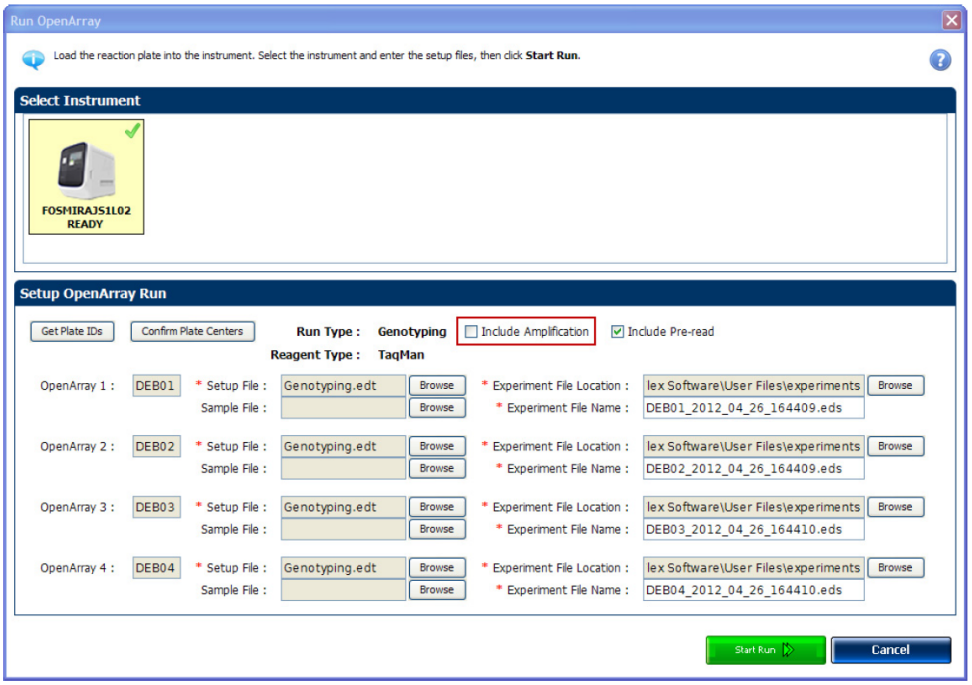
(Optional) Cycle offline genotyping experiments

You can cycle OpenArray™ Plate formats on the ProFlex™ 2 × Flat PCR System.

The thermal protocol to be used for offline cycling is as follows.

Stage	Step	Temp	Time	Ramp rate	Cycles
Pre-PCR hold	1	93.0°C	10 minutes	100%	—
PCR cycles	1	95.0°C	45 seconds	84%	50 cycles
	2	94.0°C	13 seconds	100%	—
	3	53.5°C	2 minutes and 14 seconds	44%	—
Pre-PCR hold	1	25.0°C	2 minutes	100%	—

Note: To image plates on the QuantStudio™ 12K Flex Real-Time PCR Instrument, in the QuantStudio™ 12K Flex Software, uncheck the **Include Amplification** checkbox on the **Run** screen.



(Optional) Monitor experiments

You can monitor an OpenArray™ experiment run in three ways.

- From the **Run** screen of the QuantStudio™ 12K Flex Software, while the experiment is in progress. See “Monitor an experiment from the software Run screen” on page 130.
- From the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, in the same way that you run the experiment. See “Monitor an experiment from the instrument touchscreen” on page 131.
- From the **Instrument Console** of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen). See “Monitor an OpenArray™ experiment run from the Instrument Console” on page 130.

Note: If there is loss of connection during an experiment, remove and then add the instrument to the **My Instruments** list, or restart the QuantStudio™ 12K Flex Software. Then resume monitoring the experiment run.

Monitor an experiment from the software Run screen

To monitor the **Amplification Plot** of the experiment that you are running, in the QuantStudio™ 12K Flex Software, from the **Run Experiment Menu**, click **Amplification Plot**.

Monitor an OpenArray™ experiment run from the Instrument Console

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. In the **Home** tab, in the **Tools** menu, click **Instrument Console**.
3. In the **Instrument Console**, select the icon of the instrument that you are using to run the experiment, then click **Manage Instrument** or double-click the instrument icon.

   Note: You must add the instrument to a group that can be monitored before you can manage it. See “Access the Instrument Console” on page 120.

4. In the **Instrument Manager**, click **Monitor Run** to access the **Run** screen.

   You can view the progress of the run in real time from the **Run** screen. During the run, periodically view the **Amplification Plot** for potential problems. See “Monitor the Amplification Plot” on page 131).

Task	Action
To stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN.** • In the **Stop Run** dialog box, click one of the following: – **Stop Immediately** to stop the run immediately. – **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold.
To view amplification data in real time	Select **Amplification Plot**.

Monitor the Amplification Plot

To view data in the **Amplification Plot**, click **Amplification Plot** from the **Run Experiment** menu, select the **Plate Layout** tab, then select the wells to view. You can view up to four OpenArray™ experiments per run. Click the different tabs to view the **Amplification Plot** for each experiment.

Use the **Amplification Plot** to view sample amplification as the instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the **Amplification Plot** shows the data for the wells selected in the **Plate Layout** tab. The plot contrasts normalized dye fluorescence (ΔRn) and cycle number.

The **Amplification Plot** is useful for identifying and examining abnormal amplification, including:

- Increased fluorescence in negative control wells.
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

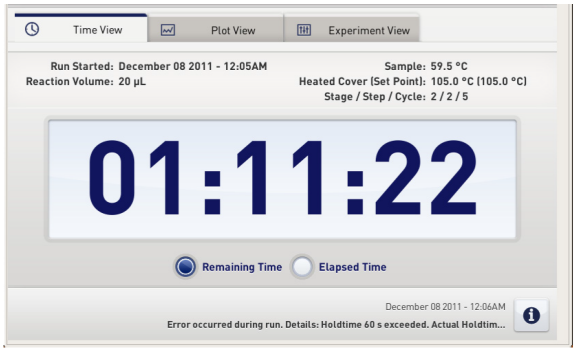
Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the *QuantStudio™ 12K Flex Software Help* (click ? or press **F1).**

Monitor an experiment from the instrument touchscreen

The QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the barcodes (or Plate IDs) of the TrueMark™ OpenArray™ Plate formats for the run, the date and time at which the run started, the time remaining in the run, and other information.

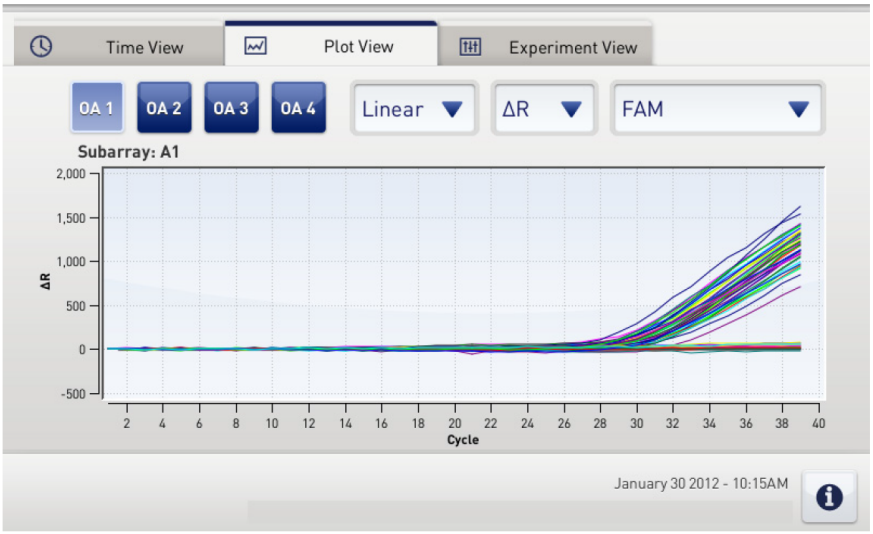
Task	Action
To display the experiment names in the run	Touch **Experiment View**.
To show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
To display the time elapsed and the time remaining in the run	Touch the **Time View** tab, then touch **Experiment View** to return to the previous screen.
To stop the run	Touch **STOP** to stop the run immediately.
View the Events Log	Touch the status bar to display the events log.

Time view



Plot view

The **Plot View** displays the **Amplification Plot** in real time.



You can change the plot using the drop-down menus that are available on the **Plot View** tab.

Touch…	To…
ΔR ▼	Change the data displayed on the y-axis. Select either **R** (reporter) or **ΔR** (baseline-corrected reporter). For OpenArray™ experiments, the data is not normalized.
FAM ▼	Change the reporter dye displayed in the plot. Only the dyes that are used in your experiment are shown.
ℹ	View the run events that occurred during the run. Touch ℹ again to close the event list.

Unload the TrueMark™ OpenArray™ Plate from the instrument

About completed runs

After the run is complete, if you started the run from the:

- QuantStudio™ 12K Flex Software, close the run and reopen the EDS file to display the **Allelic Discrimination Plot** screen (for genotyping experiments). See Chapter 12, "Analyze the experiment results".
- QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, see "(Optional) Transfer experiment results" on page 133.

Unload the instrument

When the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the **Home** screen, unload the TrueMark™ OpenArray™ Plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

1. Touch ⏏ on the instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software.
2. Remove the OpenArray™ Plate from the plate adapter.
3. Touch ⏏ or click **Close Door** to retract the plate adapter back into the instrument.

   If the instrument does not eject the plate, remove the plate.

   a. Power off the instrument.

   b. Wait for 15 minutes, then power on the instrument and eject the plate.

   c. If the instrument does not eject the plate, power off and unplug the instrument, then open the access door.

   d. Wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.

(Optional) Transfer experiment results

If you started a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, transfer the experiment data to the computer for analysis after the run is complete. You can transfer the experiment results in either of the following two ways:

- "Download the experiment from the instrument over the network" on page 134
- "Transfer the experiment from the instrument to the computer with a USB drive" on page 134

Download the experiment from the instrument over the network

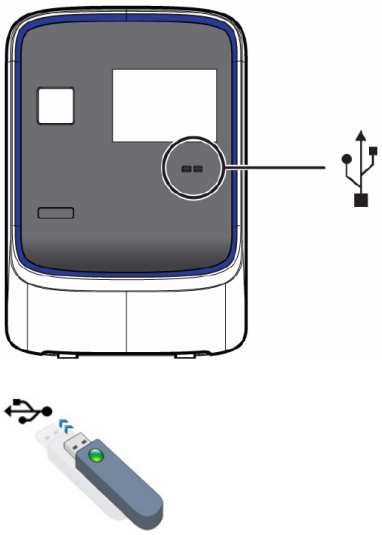
1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. From **My Instruments** list, select the instrument icon of the QuantStudio™ 12K Flex Real-Time PCR Instrument that you just used to run the experiment.
3. Click **Manage Instrument**.
4. In the **Instrument Manager**, click **Manage Files**.
5. In the **Experiments** panel, select the experiment to download, then click **Download**.
6. In the **Save** dialog box, select the folder to hold the experiment results, then click **Save**, then navigate to the experiments folder location.

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Transfer the experiment from the instrument to the computer with a USB drive

1. If not already connected to the QuantStudio™ 12K Flex Real-Time PCR Instrument, connect a USB drive to the USB port.



2. Touch the instrument touchscreen to activate it.
3. If the touchscreen is not at the **Main Menu**, touch **(Home)**.
4. In the **Main Menu**, touch **(Collect Results)** to save the data to the USB drive.

5. Select one or multiple experiments (by touching them). Then touch **(Save to USB)** to copy selected experiments to the USB drive.

   Note: If your instrument cannot find the USB drive, remove the USB drive, then try again. If the instrument still does not recognize the USB drive, try another USB drive.

6. Touch **(Home)** to return to the **Main Menu**.

7. Remove the USB drive from the instrument, then connect it to one of the USB ports on your computer.

8. In the computer desktop, use the Windows™ Explorer to open the USB drive.

9. Copy the example experiment file to:

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

12 Analyze the experiment results

Analyze the run data

This section includes general information and instructions about how to analyze the example experiments provided with the QuantStudio™ 12K Flex Software. For specific instructions, see “Analyze genotyping experiment results” on page 145.

View the data from the EDS file. If the default analysis settings are not suitable for your experiment, you can modify the data. You can also modify the project files, publish data, and export data for downstream analysis using the ExpressionSuite™ Software and TaqMan™ Genotyper Software.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**.

QC images can be viewed in QuantStudio™ Design and Analysis Software v2.7 and later.

1. In the QuantStudio™ 12K Flex Software  **Export** screen, click **Browse**, then create a uniquely–named folder for the QC images export.

   IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.

2. Click **Export QC Images** at the bottom of the screen.

3. View the following ROX™ image to check for loading quality issues:
   - `POST-READ_CHANNEL_4.tiff`

4. Check the following spotfind images for leaks or other displaced sample issues.
   - `s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff`
   - `s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff`

   Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfind image to determine whether the issue existed before cycling:
   - `s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff`

6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfind images:
   - `STAGE2_CYCLE1_CHANNEL_1.tiff`
   - `STAGE2_CYCLE40_CHANNEL_1.tiff`
7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

View the results

After an experiment run, close the run and reopen the EDS file to display the **Allelic Discrimination Plot** (for genotyping experiments).

Note: For auto-analysis of data, after a run, go to **Tools** ▸ **Preferences** ▸ **Experiment**, then select the **Auto Analysis** checkbox. By default, **Auto Analysis** is always enabled. To reanalyze the data, select all the wells in the plate layout, then click **Analyze**.

Set up the EDS file

If you run a genotyping experiment using an EDT file, you must integrate the sample names and Assay IDs into the resulting EDS file.

For Assay IDs, you can import the SPF file of that OpenArray™ Plate into the EDS file before or after the run.

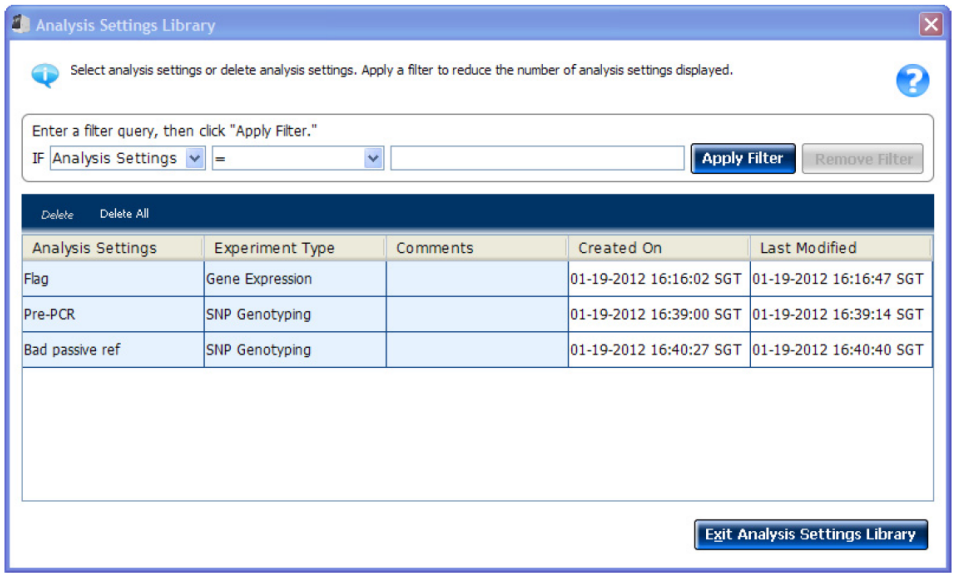
For sample names:

- You can import the OpenArray™ Plate format from the CSV file for the corresponding plate.
- If you use the OpenArray™ AccuFill™ Software for sample integration, navigate to the appropriate folder containing the Loaded SPF file. A Loaded SPF file is one that has sample names integrated into the file using the OpenArray™ AccuFill™ Software.

Change analysis settings

Analysis settings are different for each experiment type. If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment. You can save the changed analysis settings to the **Analysis Settings Library** to use them in other experiments.

Use the **Analysis Settings Library** dialog box to apply a filter to reduce the number of setting protocols that are displayed. Access the **Analysis Settings Library** from the **Tools** menu.



1. From the **Experiment Menu**, select **Analysis**.
2. On the **Analysis** screen, click **Analysis Settings**.
3. In the **Analysis Settings** dialog box, change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes to the **Analysis Settings Library**.

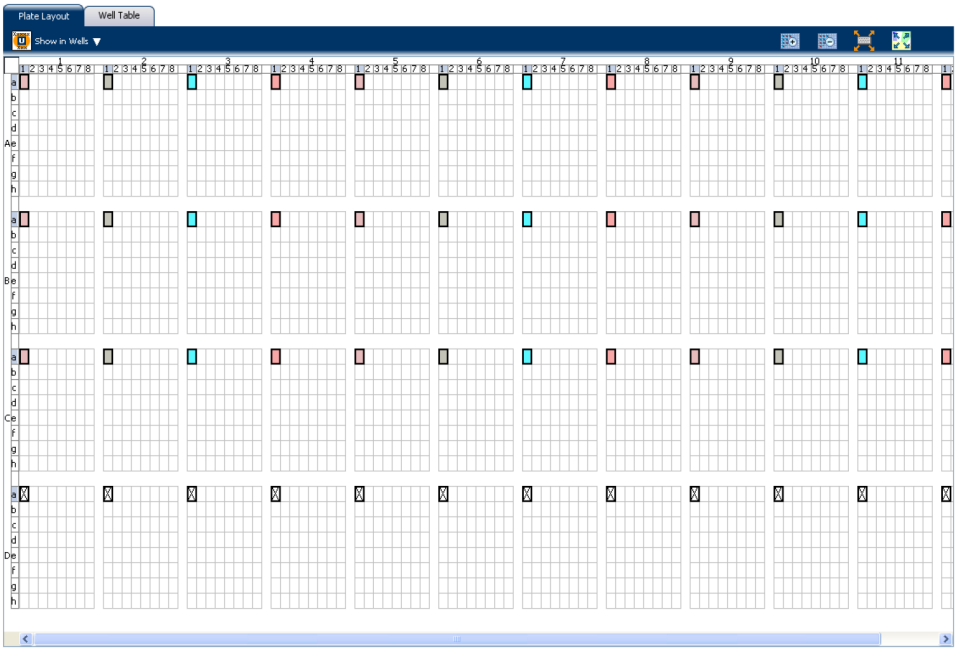
To import the analysis settings that you have previously saved to the **Analysis Settings Library**, in the **Analysis Settings** dialog box, click **Load from Library**.

Display wells

To display specific wells in the analysis plots, select the wells in the **Plate Layout** tab.

- To select specific well type, use the **Show in Wells** drop-down menu.
  - For gene expression and miRNA experiments, select **Sample Color** or **Target Color**.
  - For genotyping experiments, select **Sample Color** or **Assay Color**.
- To select a single well, click the well in the **Plate Layout** tab.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the **Plate Layout** tab.
- To select all wells, click the upper left corner of the **Plate Layout** tab.

This example shows the **Plate Layout** tab for a genotyping experiment.



Expand view of a plot or wells

- Click to expand the plot view, on the left side of the screen.
- Click to expand the **Targets**, **Samples**, and **Subarrays** view on the right side of the screen.
- Click to expand the **Plate Layout** or **Well Table** view on the lower half of the screen.
- Click to expand the Plots and **Targets**, **Samples**, and **Subarrays** view on the upper half of the screen.
- Click to expand and collapse the **Plot** or **Plate Layout** view.

Edit plot properties

Use the **Plot Properties** dialog box on the **Analysis** screen to edit plot settings, such as the font and color of the plot text, and the labels on the x-axis and y-axis.

1. Click on the **Analyze** screen (the icon appears above the plot).
2. In the **Plot Properties** dialog box, edit the settings under the **General**, **X Axis**, and **Y Axis** tab.
   - Click the **X Axis** tab to edit the x-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
   - Click the **Y Axis** tab to edit the y-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
3. Click **OK**.

Publish the analyzed data

Task	Click
Save a plot as an image file.	
Print a plot.	
Copy a plot to the clipboard.	
Print a report.	
Export data.	

Task	Go to	Then
Print the plate layout.	**File** ▸ **Print**	Select the background color, then click **Print**.
Create slides.	**File** ▸ **Send to PowerPoint**	Select the slides for your presentation, then click **Create Slides**.
Print a report.	**File** ▸ **Print Report**	Select data for the report, then click **Print Report**.

(Optional) Export an experiment

Use the **Export** feature to export experiment data from the QuantStudio™ 12K Flex Software. You can export experiment data in the QuantStudio™ 12K Flex format (TXT or XLSX) or RDML format (no file selection).

You can export the following experiment data in a comma-separated file format (CSV).

- Sample Setup data
- Raw data
- Amplification data
- Multicomponent data
- Results

You can also export plate images collected during the run as TIF files to use them for troubleshooting. To export plate images, first create an export folder on your hard drive. In the **Export** screen, click **Browse**, navigate to the folder that you created, then click **Export QC Images**.

You can view the images using a public domain software program such as ImageJ (**imagej.nih.gov/ig**). See the *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832) for more information on QC Images.

If you selected the **Auto Export** option before running an experiment, the data is automatically exported to the location that you specified. If you did not select the **Auto Export** option, the analyzed data is not exported automatically.

1. Open the experiment file that contains the data to export, then from the **Experiment Menu**, click **Export.**

2. Select the format for exported data.
   - **QuantStudio 12k Flex format**—Supports TXT and XLSX data.
   - **RDML format** (Real Time Data Markup Language)—Supports only XML type of data.

3. Select to export all data in one file or in separate files for each data type.
   - All data types are exported in **one file.**
     - If you select the XLS format, a worksheet is created for each data type.
     - If you select the TXT format, the data are grouped by data type.
   - Each data type is exported in a **separate file**. If you select three different data types (for example, Results, Amplification, and Multicomponent) to export, three separate files are created. From **File Type**, select the export file type (XLS, XLSX, or TXT) to export.

     Note: You cannot use an exported XLS or XLSX file when importing plate setup information.

4. Select **Yes** to include or **No** to exclude bookmarked data from analysis in the export set.

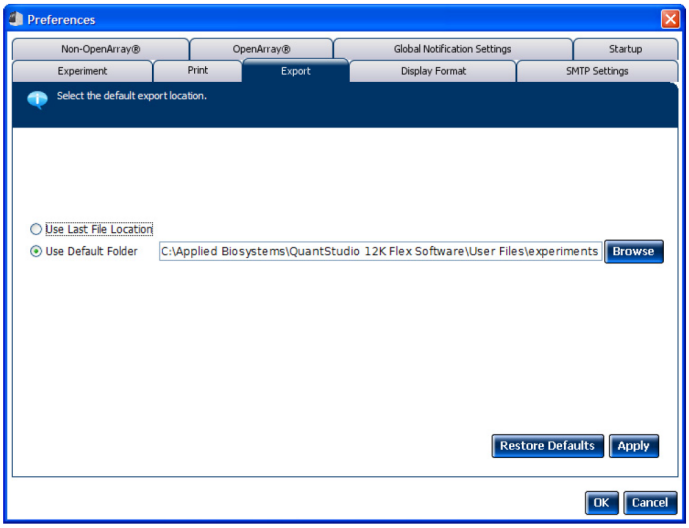
   The **Filter Bookmark Data** feature lets you include only the data bookmarked during analysis in the export set.

5. *(Optional)* Select the **Open file(s) when export is complete** checkbox to open the file when export is complete.

6. Enter a file name and location.
   a. In **Export File Name**, enter a name for the export file.

b. In **Export File Location** accept the default, or click **Browse** if you do not want to save the export file in the default export folder.

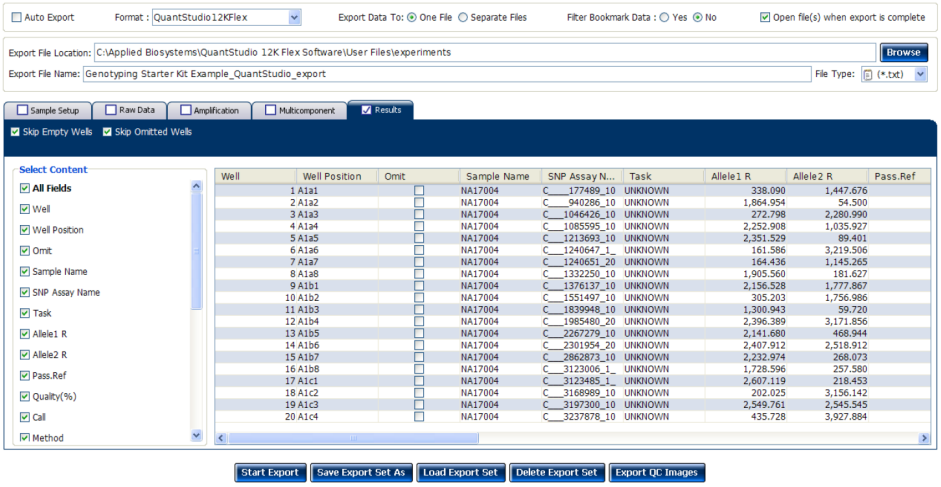
Note: To set up the **Export File Location**, go to **Tools ▸ Preferences**, then select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** checkbox.



7. Select the type of data to export.

Type of data	Data to export
Sample setup	Well, sample name, sample color, and target name of samples in the plate.
Raw data	Raw fluorescence data for each filter, for each cycle.
Amplification data	Amplification results, such as dC_T values, R, or ΔR.
Multicomponent data	Fluorescence data for each dye, for each cycle.
Results	Results information, such as C_T values, Rn, or calls. Results data are not available for export until the run status is complete and the data are analyzed.

The completed **Export** screen for a genotyping experiment should look like this.



Well	Well Position	Omit	Sample Name	SNP Assay N...	Task	Allele1 R	Allele2 R	Pass.Ref
1	A1a1		NA17004	C___177489_10	UNKNOWN	338.090	1,447.676	
2	A1a2		NA17004	C___940286_10	UNKNOWN	1,864.954	54.500	
3	A1a3		NA17004	C___1046426_10	UNKNOWN	272.798	2,280.990	
4	A1a4		NA17004	C___1085595_10	UNKNOWN	2,252.908	1,035.927	
5	A1a5		NA17004	C___1213693_10	UNKNOWN	2,351.529	89.401	
6	A1a6		NA17004	C___1240647_1_	UNKNOWN	161.586	3,219.506	
7	A1a7		NA17004	C___1240651_20	UNKNOWN	164.436	1,145.265	
8	A1a8		NA17004	C___1332250_10	UNKNOWN	1,905.560	181.627	
9	A1b1		NA17004	C___1376137_10	UNKNOWN	2,156.528	1,777.867	
10	A1b2		NA17004	C___1551497_10	UNKNOWN	305.203	1,756.986	
11	A1b3		NA17004	C___1839948_10	UNKNOWN	1,300.943	59.720	
12	A1b4		NA17004	C___1985480_20	UNKNOWN	2,396.389	3,171.856	
13	A1b5		NA17004	C___2267279_10	UNKNOWN	2,141.680	468.944	
14	A1b6		NA17004	C___2301954_20	UNKNOWN	2,407.912	2,518.912	
15	A1b7		NA17004	C___2862873_10	UNKNOWN	2,232.974	268.073	
16	A1b8		NA17004	C___3123006_1_	UNKNOWN	1,728.596	257.580	
17	A1c1		NA17004	C___3123485_1_	UNKNOWN	2,607.119	218.453	
18	A1c2		NA17004	C___3168989_10	UNKNOWN	202.025	3,156.142	
19	A1c3		NA17004	C___3197300_10	UNKNOWN	2,549.761	2,545.545	
20	A1c4		NA17004	C___3237878_10	UNKNOWN	435.728	3,927.884	

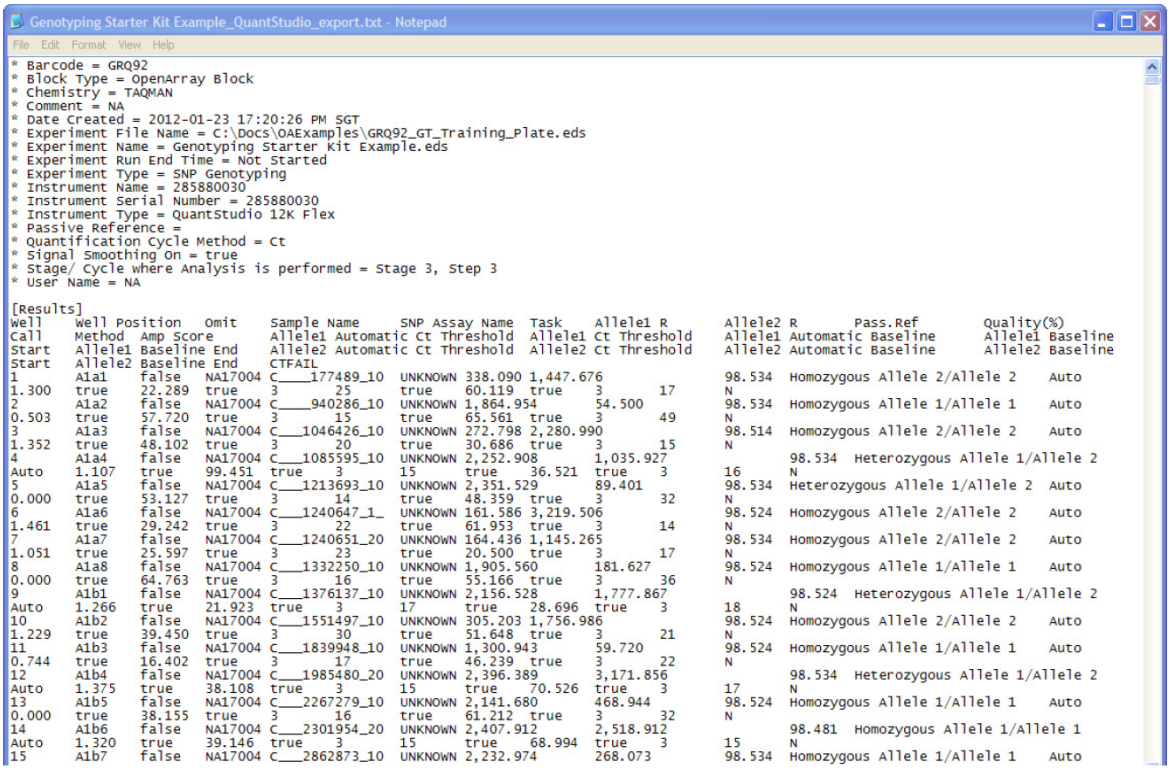
8. *(Optional)* After you have defined the export properties or after moving the table headings order, you can save those export settings as an export set by clicking **Save Export Set As**. Later you can import the heading order into another file by clicking **Load Export Set**. You can also delete export settings by clicking **Delete Export Set**.

9. *(Optional)* Click **Export QC Images** to export quality control (QC) images in experiment files (EDS).

   QC images include calibration images, a barcode image, and images taken during PCR. You can view the images to check sample loading and assay spotting. View PCR images to validate your data.

10. Click **Start Export.**

    The exported file, when opened in Notepad, should look like this.



Genotyping Starter Kit Example_QuantStudio_export.txt - Notepad

File Edit Format View Help

```
* Barcode = GRQ92
* Block Type = OpenArray Block
* Chemistry = TAQMAN
* Comment = NA
* Date Created = 2012-01-23 17:20:26 PM SGT
* Experiment File Name = C:\Docs\OAExamples\GRQ92_GT_Training_Plate.eds
* Experiment Name = Genotyping Starter Kit Example.eds
* Experiment Run End Time = Not Started
* Experiment Type = SNP Genotyping
* Instrument Name = 285880030
* Instrument Serial Number = 285880030
* Instrument Type = QuantStudio 12K Flex
* Passive Reference =
* Quantification Cycle Method = Ct
* Signal Smoothing On = true
* Stage/ Cycle where Analysis is performed = Stage 3, Step 3
* User Name = NA

[Results]
Well    Well Position   Omit    Sample Name     SNP Assay Name  Task    Allele1 R       Allele2 R       Pass.Ref        Quality(%)
Call    Method  Amp Score       Allele1 Automatic Ct Threshold  Allele1 Ct Threshold    Allele1 Automatic Baseline      Allele1 Baseline
Start   Allele1 Baseline End    Allele2 Automatic Ct Threshold  Allele2 Ct Threshold    Allele2 Automatic Baseline      Allele2 Baseline
Start   Allele2 Baseline End    CTFAIL
1       A1a1    false   NA17004 C____177489_10  UNKNOWN 338.090 1,447.676               98.534  Homozygous Allele 2/Allele 2    Auto
1.300   true    22.289  true    3       25      true    60.119  true    3       17      N
2       A1a2    false   NA17004 C____940286_10  UNKNOWN 1,864.954       54.500          98.534  Homozygous Allele 1/Allele 1    Auto
0.503   true    57.720  true    3       15      true    65.561  true    3       49      N
3       A1a3    false   NA17004 C___1046426_10  UNKNOWN 272.798 2,280.990               98.514  Homozygous Allele 2/Allele 2    Auto
1.352   true    48.102  true    3       20      true    30.686  true    3       15      N
4       A1a4    false   NA17004 C___1085595_10  UNKNOWN 2,252.908       1,035.927               98.534  Heterozygous Allele 1/Allele 2
Auto    1.107   true    99.451  true    3       15      true    36.521  true    3       16      N
5       A1a5    false   NA17004 C___1213693_10  UNKNOWN 2,351.529       89.401          98.534  Heterozygous Allele 1/Allele 2  Auto
0.000   true    53.127  true    3       14      true    48.359  true    3       32      N
6       A1a6    false   NA17004 C___1240647_1_  UNKNOWN 161.586 3,219.506               98.524  Homozygous Allele 2/Allele 2    Auto
1.461   true    29.242  true    3       22      true    61.953  true    3       14      N
7       A1a7    false   NA17004 C___1240651_20  UNKNOWN 164.436 1,145.265               98.534  Homozygous Allele 2/Allele 2    Auto
1.051   true    25.597  true    3       23      true    20.500  true    3       17      N
8       A1a8    false   NA17004 C___1332250_10  UNKNOWN 1,905.560       181.627         98.524  Homozygous Allele 1/Allele 1    Auto
0.000   true    64.763  true    3       16      true    55.166  true    3       36      N
9       A1b1    false   NA17004 C___1376137_10  UNKNOWN 2,156.528       1,777.867               98.524  Heterozygous Allele 1/Allele 2
Auto    1.266   true    21.923  true    3       17      true    28.696  true    3       18      N
10      A1b2    false   NA17004 C___1551497_10  UNKNOWN 305.203 1,756.986               98.524  Homozygous Allele 2/Allele 2    Auto
1.229   true    39.450  true    3       30      true    51.648  true    3       21      N
11      A1b3    false   NA17004 C___1839948_10  UNKNOWN 1,300.943       59.720          98.524  Homozygous Allele 1/Allele 1    Auto
0.744   true    16.402  true    3       17      true    46.239  true    3       22      N
12      A1b4    false   NA17004 C___1985480_20  UNKNOWN 2,396.389       3,171.856               98.534  Heterozygous Allele 1/Allele 2
Auto    1.375   true    38.108  true    3       15      true    70.526  true    3       17      N
13      A1b5    false   NA17004 C___2267279_10  UNKNOWN 2,141.680       468.944         98.524  Homozygous Allele 1/Allele 1    Auto
0.000   true    38.155  true    3       16      true    61.212  true    3       32      N
14      A1b6    false   NA17004 C___2301954_20  UNKNOWN 2,407.912       2,518.912               98.481  Homozygous Allele 1/Allele 1
Auto    1.320   true    39.146  true    3       15      true    68.994  true    3       15      N
15      A1b7    false   NA17004 C___2862873_10  UNKNOWN 2,232.974       268.073         98.534  Homozygous Allele 1/Allele 1    Auto
```

Perform downstream analysis (secondary analysis)

You can perform downstream analysis of experiments that have been run on any real-time PCR system with the TaqMan™ Genotyper Software. Use the TaqMan™ Genotyper Software to efficiently analyze, edit, and conduct a study of a large number of genotyping experiments.

- Import data from the QuantStudio™ 12K Flex Software project files, then manage the data in a database.
- Search the database for assays using specific search criteria.
- Easily view data in a variety of ways (plots, statistics, status codes, and so on).
- Edit data. Your edits are saved to the database.
- Overlay data from multiple plates.
- Export data.

TaqMan™ Genotyper Software is available for download from the Thermo Fisher Scientific website. See also Appendix A, “Ordering information”.

For more information about the TaqMan™ Genotyper Software, see the *TaqMan™ Genotyper Software Getting Started Guide* (Pub. No. 4448637).

Analyze genotyping experiment results

In this section, you use the example experiment files provided with the QuantStudio™ 12K Flex Software to analyze the experiment results.

Workflow

Analyze genotyping experiment results

- **Access the example experiment file** (page 146)
- **View and assess clusters in the Allelic Discrimination Plot** (page 146)
- **Confirm setup accuracy using the Plate Layout** (page 149)
- **Assess amplification results on the Amplification Plot** (page 150)
- **Identify well problems using the Well Table** (page 151)
- **Confirm accurate dye signal using the Multicomponent Plot** (page 154)
- **Determine signal accuracy using the Raw Data Plot** (page 155)
- **Review the flags in the QC Summary** (page 156)
- **(Optional) Adjust parameters for reanalysis of your own experiments** (page 159)
- **(Optional) Export an experiment** (page 140)

Access the example experiment file

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. In the **Home** tab, in the **Experiment** menu, click **Open,**, then browse to the experiments folder.
   `<...>:Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\examples\Genotyping`
3. Open the `Genotyping Starter Kit Example.eds` file.

Set up the EDS file

If you run a genotyping experiment using an EDT file, you must integrate the sample names and Assay IDs into the resulting EDS file.

For Assay IDs, you can import the SPF file of that OpenArray™ Plate into the EDS file before or after the run.

For sample names:

- You can import the OpenArray™ Plate format from the CSV file for the corresponding plate.
- If you use the OpenArray™ AccuFill™ Software for sample integration, navigate to the appropriate folder containing the Loaded SPF file. A Loaded SPF file is one that has sample names integrated into the file using the OpenArray™ AccuFill™ Software.

View and assess clusters in the Allelic Discrimination Plot

The **Allelic Discrimination Plot** is a plot of the signal from one allele-specific probe on the x-axis against the signal from the other allele-specific probe on the y-axis.

View the **Allelic Discrimination Plot** to identify clusters.

- Clusters for the three possible genotypes (Allele 1 homozygous, Allele 2 homozygous, and Allele 1/2 heterozygous).
- A cluster for the no template controls.

1. From the **Experiment** menu, select **Analysis ▸ Allelic Discrimination Plot.**
2. Click the **Plate Layout** tab, then click any empty well to select it.
   In the **Allelic Discrimination Plot**, the software highlights all wells that are selected in the **Plate Layout** tab. If the plot displays a single color for all wells, then all wells in the plate layout are selected.
3. In the **Allelic Discrimination Plot**, select **C____177489_10** from the **Assay** menu, on the top-right side of the screen, then enable **Autocaller**.
   If the **Autocaller** is not enabled, the **Allelic Discrimination Plot** displays a crossmark (X – Undetermined) for each sample.
   To enable or disable **Autocaller**, go to **Analysis Settings**. By default, **Autocaller** is enabled but you can edit the default call settings and uncheck the **Autocalling Enabled** checkbox.

The **Allelic Discrimination Plot** displays allele symbols for each sample evaluated for the selected SNP. The samples are grouped on the plot.

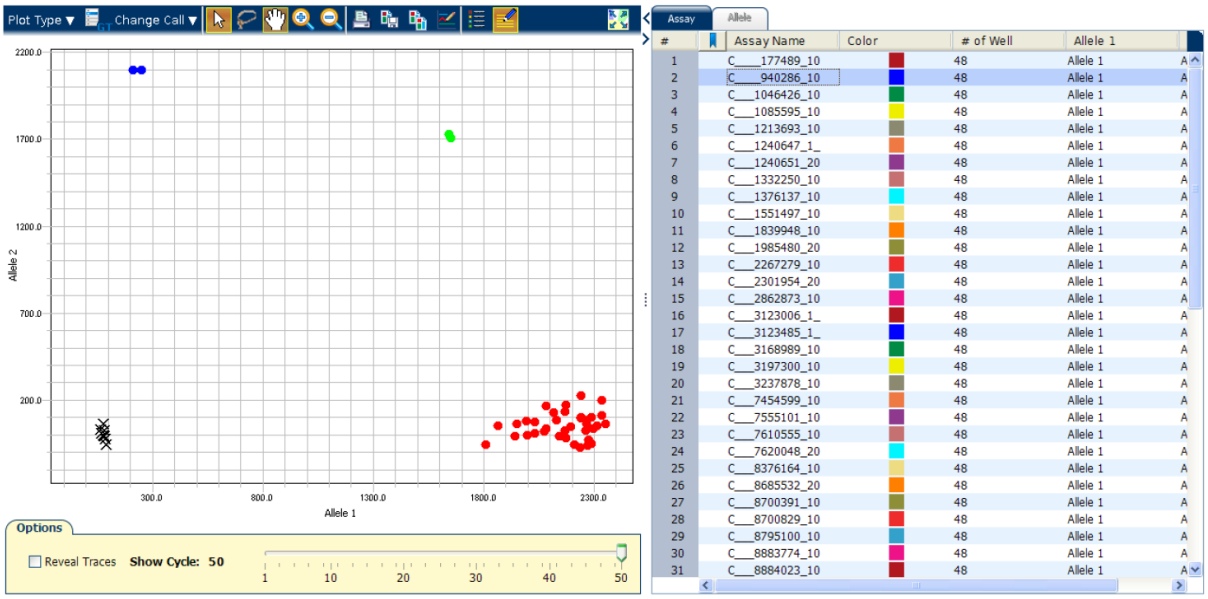
Genotype	Symbol	Location
Homozygous for Allele 1 of the selected SNP assay	(red)	x-axis of the plot
Homozygous for Allele 2 of the selected SNP assay	(blue)	y-axis of the plot
Heterozygous for both alleles of the selected SNP assay (Allele 1 and Allele 2)	(green)	Midway between the homozygote clusters
No Template Control	(black)	Bottom-left corner of the plot
Undetermined	(black)	Anywhere on plot

4. Review each cluster in the plot.
   a. Click and drag a box around the cluster to select the associated wells in the **Plate Layout** and **Well Table**.
   b. Confirm that the expected wells are selected in the **Well Table**.
      For example, if you select the cluster at the bottom-left corner of the plot, only the no-template controls should be selected. The presence of an unknown among the no-template controls may indicate that the sample failed to amplify.
   c. Repeat steps a and b for all other clusters in the plot.

Table 5 Allelic Discrimination Plot elements.

Element	Description
Assay tab	Determines the assay data that the QuantStudio™ 12K Flex Software displays in the plot.
Plot Type dropdown menu	Determines the type of plot (Cartesian or Polar) that the QuantStudio™ 12K Flex Software uses to display the data.
Change Call dropdown menu	When a datapoint is selected, use this menu to assign an allele call to the datapoint within the scatterplot.
Toolbar	Tools for manipulating the scatterplot. • **(Selection tool)**, to select a single data point • **(Selection tool)**, to select multiple data points • **(Reposition)** • **(Zoom in)** • **(Zoom out)**
Legend	Explains the symbols in the scatterplot.
Show Options	Use the **Reveal Traces** option to trace the clusters throughout the PCR process. This option is not active for the example experiment. To activate the option, see “Adjust analysis settings” on page 161.

This is an example of the **Allelic Discrimination Plot** for the OpenArray™ genotyping experiment.



Troubleshoot clustering on the Allelic Discrimination Plot

Do all controls have the correct genotype?

In the example experiment and in your own experiments, confirm that data points cluster as expected.

Clustering in positive controls

1. From the **Well Table**, select the wells containing a positive control to highlight the corresponding data points (symbols) in the **Allelic Discrimination Plot**.
2. Check that the data points for the positive controls cluster along the expected axis of the plot. For example, if you select the **Positive Control Allele 1/Allele 1**, then the controls should cluster along the x-axis.
3. Repeat steps 1 and 2 for the wells containing the other positive controls.

Failed amplification in the unknown samples

1. Select the data points of the cluster in the lower left corner of the Allelic Discrimination Plot to select the corresponding wells in the well table.
2. Check that the selected wells in the well table are the no template controls, and not unknown samples.

Samples clustered with the no template controls

Samples that clustered with the no template controls may:

- Contain no DNA
- Contain PCR inhibitors
- Be homozygous for a sequence deletion
- May not have been set up correctly due to pipetting error

Confirm the results of these samples by retesting them.

Are outliers present?

If the **Allelic Discrimination Plot** contains clusters other than the three representative genotype clusters (heterozygous, homozygous allele 1, and homozygous allele 2), then those can be classified as outliers.

Confirm the results of the associated samples by retesting them.

Note: The results displays are synchronized. For example, selecting a well in the **Plate Layout** selects the corresponding data in the **Well Table** and in the **Allelic Discrimination Plot**.

Confirm setup accuracy using the Plate Layout

Review the experiment results in the **Plate Layout**. The **Plate Layout** displays the assay-specific setup and analysis properties for the experiment in a well format corresponding to the type of reaction plate used for the run.

Click the **Plate Layout** tab in the bottom-half of the screen to display the **Plate Layout**.

Example experiment plate layout values

For the example experiment, confirm that the QuantStudio™ 12K Flex Software called these samples.

- 2 samples as Allele 1 homozygous (🔴).
- 34 samples as Allele 2 homozygous (🔵).
- 4 samples as heterozygous (🟢).
- 8 samples as undetermined (✕).

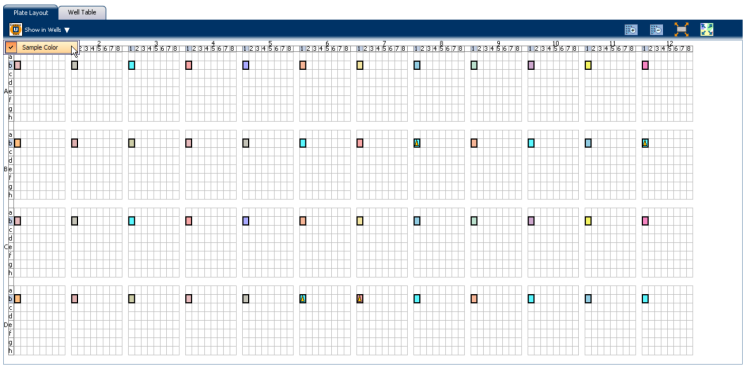
Confirm that no wells of the reaction plate triggered QC flags (▲). The example experiment displays 3 flags.

View the Plate Layout

1. Click the ⟩| and |⟨ icons beside and below the **Allelic Discrimination Plot** to maximize the view of the **Plate Layout**.

2. Click ▸ **Show in Wells** ▸ **Sample Color** to display the sample color in the wells.

   The following figure shows the **Plate Layout** of the OpenArray™ genotyping example experiment.



Tips for troubleshooting plate setup in your own experiment

You can adjust the view of the **Plate Layout**.

- Note the location of any samples that trigger QC flags ⚠. Understanding the position of errors can aid in diagnosing any failures that may occur.
- Select the entire reaction plate, areas of the reaction plate, or specific wells.
  - Click the upper left corner of the reaction plate to select all subarrays.
  - Left-click the mouse and drag across the area to select it.
  - Select **Sample**, **SNP Assay**, or **Task** from the **Show in Wells** menu in the **Plate Layout** to select wells of a specific type using the well-selection criteria.
- Use the (Zoom In), (Zoom Out), (Quick view), and (Fit Plate) buttons to magnify or compress the view of the wells shown.
- Use the arrow tabs to expand the **Plate Layout** to cover the entire screen.

Assess amplification results on the Amplification Plot

The **Amplification Plot** displays amplification of all samples in the selected wells. View the **Amplification Plot** for the example experiment to evaluate the quality of the amplification curve and to check for outliers.

Three plots are available.

- **ΔR vs Cycle**—ΔR is the magnitude of fluorescence signal generated by the reporter at each cycle during the PCR amplification. This plot displays ΔR as a function of cycle number. Use this plot to identify and examine irregular amplification and to view C_{RT} values for the run.
- **R vs Cycle**—R is the fluorescence signal from the reporter dye. This plot displays R as a function of cycle number. Use this plot to identify and examine irregular amplification.
- **C_{RT} vs Well**—C_{RT} is the PCR cycle number at which the fluorescence meets the threshold in the amplification plot. This plot displays C_{RT} as a function of well position. Use this plot to locate outlying amplification (outliers).

Each plot can be viewed as a linear or log10 graph type.

View the Amplification Plot

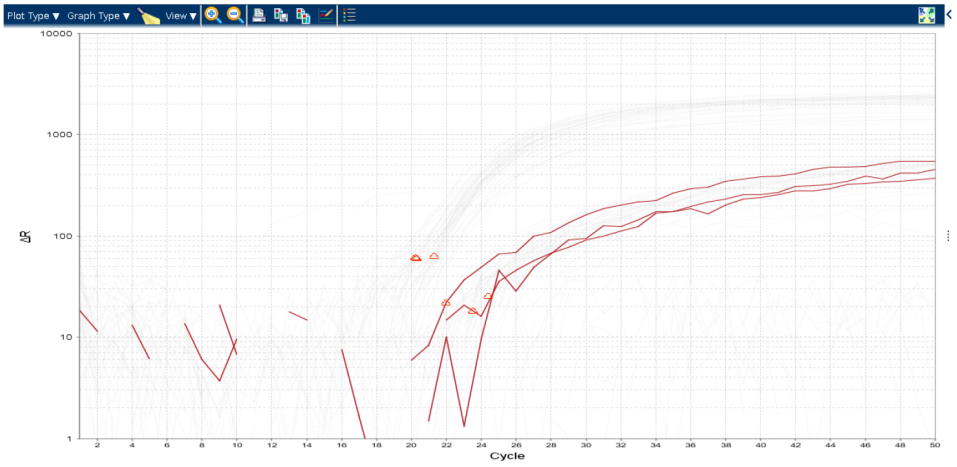
1. From the **Experiment Menu**, select **Analysis** ▸ **Amplification Plot**.

   If no data are displayed, click **Analyze**.

2. In the **Amplification Plot**, select:

Menu	Selection
Plot Type	**ΔR vs Cycle** (default)
Graph Type	**Log** (default)
View	**SNP Allele Color** (default) **Show Unselection** (default)

3. View the C_{RT} values.
   a. From the **View** drop-down, **Show** C_{RT}.
   b. Verify that the C_{RT} value that is reported matches its occurrence (the triangle icon) on the plot.



Identify well problems using the Well Table

Review the details of the experiment results in the **Well Table** and identify any flagged wells. The **Well Table** displays the assay-specific setup and analysis properties for the experiment in a tabular format.

Example experiment values and flags

For the example experiment, look for wells that triggered QC flags (▲). The example experiment has 3 flags.

View the Well Table

1. From the **Experiment Menu**, select **Analysis** ▸ **Amplification Plot**, then click the **Well Table** tab.
2. Click the **Flag** column to sort the data so that the wells that triggered flags appear at the top of the table.
3. Confirm the integrity of the controls.
   a. From the **Group By** menu, select **Task** to organize the table rows by their function on the reaction plate.
   b. Confirm that each of the controls do not display flags ▲.
   c. Click the ▣ icon to collapse the negative and positive controls.
   The **Well Table** for the OpenArray™ genotyping example experiment looks like this.

The **Well Table** shows the following information.

Column	Description
Well	The position of the well on the reaction plate.
Omit	A check mark indicates that the well has been removed from the analysis.
Flag	A ⚠ symbol indicates that the well triggered the number of flags listed inside the symbol.
Sample Name	The name of the sample.
SNP Assay Name	The name of the SNP assay evaluated by the well.
Assay ID	The Assay ID number of the SNP evaluated by the well.
Task	The task assigned to the well (**Unknown**, **No Template Control**, or **Positive Control**).
Allele 1 / 2	The name of the associated allele for the SNP evaluated by the well.
Allele 1 / 2 Dyes	The name of the reporter and quencher dyes of the associated allele for the SNP evaluated by the well.
Allele 1 / 2 R_n	Normalized signal ($_{rn}$) of the reporter dye of the associated allele for the SNP evaluated by the well.
Pass Ref	The signal of the passive reference dye for the well.
Call	The allele call assigned to the sample, where possible calls are: • Homozygous 1/1—Homozygous for allele 1 • Homozygous 2/2—Homozygous for allele 2 • Heterozygous 1/2—Heterozygous • No Template Control • X Undetermined
Quality (%)	The quality value calculated for the genotype call.
Method	The method used to assign the call to the sample (**Auto** if assigned by the QuantStudio™ 12K Flex Software, or **Manual** if applied by a user).

(continued)

Column	Description
Comments	Comments entered for the associated sample well.
Allele 1 / 2 C_T	Threshold cycle (C_T) of the sample for the associated allele for the SNP evaluated by the well.

Identify quality control (QC) problems

The **Well Table** displays columns for QC flags that are triggered by the experimental data. If the experiment data does not trigger a QC flag, then the QuantStudio™ 12K Flex Software does not display a corresponding column for the flag.

A ⚠ in one of the following columns indicates that the associated well triggered the flag.

Flag	Description
BADROX	The well produced a passive reference signal greater than the limit defined in the analysis settings.
OFFSCALE	The well produced a level of fluorescence greater than the QuantStudio™ 12K Flex Real–Time PCR System can measure.
NOSIGNAL	The well did not produce a detectable level of fluorescence.
CLUSTER#	For the SNP evaluated by the well, the number of clusters generated from the experiment data is greater than the limit defined in the analysis settings.
PCFAIL	The positive control did not produce an $_{Rn}$ for the associated allele greater than the limit defined in the analysis settings indicating that the control may have failed to amplify.
SMCLUSTER	The number of data points in the associated cluster is less than the limit defined in the analysis settings.
AMPNC	The negative control has produced an $_{Rn}$ greater than the limit defined in the analysis settings indicating possible amplification.
NOAMP	The well did not produce an $_{Rn}$ for either allele that is greater than the limit defined in the analysis settings indicating that the well may have failed to amplify.
NOISE	The background fluorescence (noise) produced by the well is greater than the other wells on the reaction plate by a factor greater than the limit that is defined in the analysis settings.
SPIKE	The amplification plot for the well contains one or more data points inconsistent with the other points in the plot.
EXPFAIL	The software cannot identify the exponential region of the amplification plot for the well.
BLFAIL	The software cannot calculate the best fit baseline for the data for the well.
THOLDFAIL	The software cannot calculate a threshold for the associated well.
CTFAIL	The software cannot calculate a threshold cycle (C_T) for the associated well.
AMPSCORE	Amplification in the linear region is below a specific threshold, corresponding to the score set in the analysis settings

Tips for analyzing your own experiments

Confirm the integrity of positive controls

When you analyze the example experiment or your own experiment, if you are using positive controls, confirm the integrity of the positive controls.

1. From the **Group By** menu, select **Task** to organize the table rows by their function on the reaction plate.

2. Confirm that the positive controls do not display flags ▲ and that their reporter dye fluorescence (R) is appropriate for the genotype.

   For example, if evaluating the Positive Control Allele 1/Allele 1, you would expect to see significant increase in R_n for the Allele 1 probe and very little for the Allele 2 probe.

Adjust the Well Table

- Review the data for the **Unknown** samples. For each row that displays ▲ in the **Flag** column, note the data and the flag or flags triggered by the associated well.
- Select areas of the table or wells of a specified type. Use one of these methods.
  - Left-click the mouse, then drag across the area of the table that you want to select.
  - In the **Well Table**, from the **Select Wells** menu, select **Sample**, **SNP Assay**, or **Task** to select wells of a specific type using the well-selection tool.
- Select an option from the **Group By** menu to group the rows of the plate layout. To collapse or expand the lists, either click the **+/-** icon next to individual lists, or click **Collapse All** or **Expand All**.
- To omit a well from the analysis, select the **Omit** checkbox for that well. To include the well in the analysis, deselect the **Omit** checkbox.

  You must reanalyze the experiment each time you omit or include a well.

Confirm accurate dye signal using the Multicomponent Plot

The **Multicomponent Plot** displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

In the OpenArray™ genotyping example experiment, review the **Multicomponent Plot** for:

- FAM™ dye (reporter)
- VIC™ dye (reporter)
- Spikes, dips, and/or sudden changes
- Amplification in the no-template control wells

View the Multicomponent Plot

1. From the **Experiment Menu**, select **Analysis** ▸ **Multicomponent Plot**.

   If no data are displayed, click **Analyze**

2. Display the unknown wells one at a time in the **Multicomponent Plot**.
   a. Click the **Plate Layout** tab.

b. Select one well in the plate layout. The well is shown in the **Multicomponent Plot**.

   If you select multiple wells, the **Multicomponent Plot** displays the data for all selected wells simultaneously.

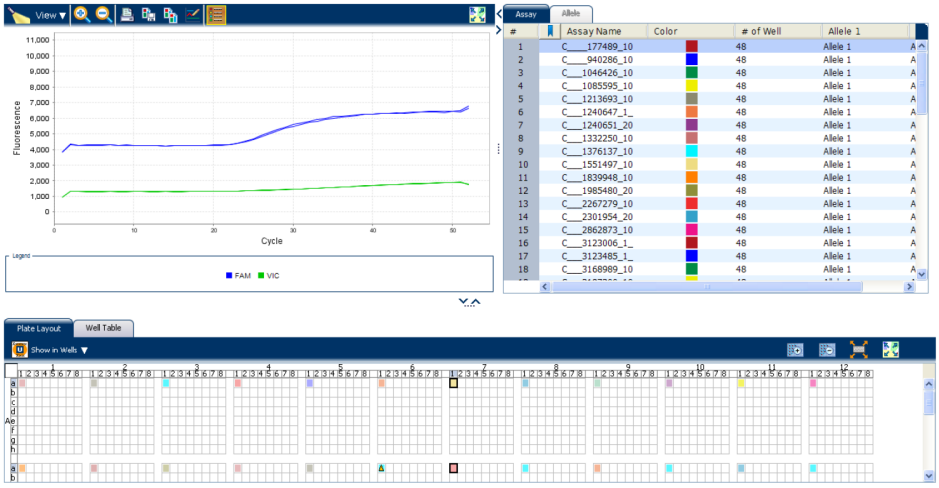
3. Click **View** ▸ **SNP Allele Color**.

4. Click **Show a legend for the plot** (default).

   This is a toggle button. When the legend is displayed, the button changes to **Hide the plot legend**.

5. Check the FAM™ dye signals.

   In the genotyping example experiment, the FAM™ dye signal increases throughout the PCR process, indicating normal amplification.



Tips for confirming dye accuracy in your own experiment

When you analyze your own experiment, look for these results.

- Reporter dye—The reporter dye fluorescence level should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- Irregularities in the signal—There should not be any spikes, dips, and/or sudden changes in the fluorescent signal.
- Negative Control wells—There should not be any amplification in the negative control wells.

Determine signal accuracy using the Raw Data Plot

The **Raw Data Plot** displays the raw fluorescence signal (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

In the OpenArray™ genotyping example experiment, you review the **Raw Data Plot** screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

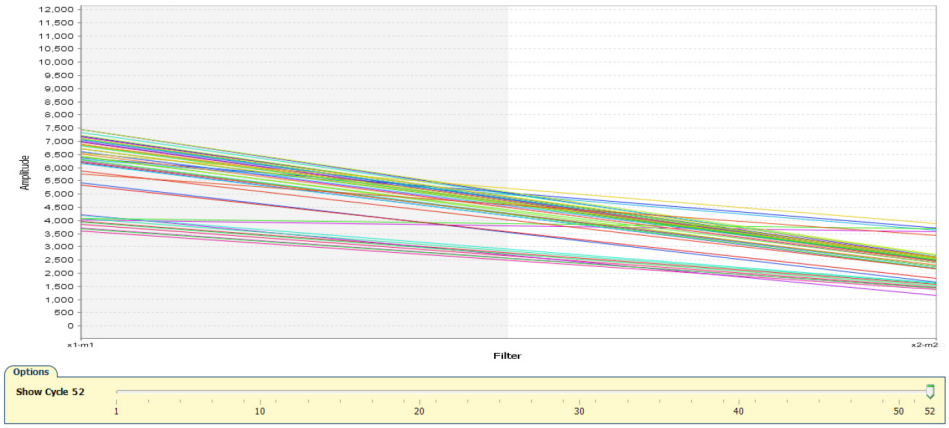
View the Raw Data Plot

1. From the **Experiment Menu**, select **Analysis ▸ Raw Data Plot**.
   If no data are displayed, click **Analyze**

2. To display all wells in the **Raw Data Plot**, click the upper left corner of the plate layout in the **Plate Layout** tab.

3. Click **Show a legend for the plot** (default).
   The legend displays the color code for each row of the reaction plate (see the legend in the **Raw Data Plot**).
   This is a toggle button. When the legend is displayed, the button changes to **Hide the plot legend**.

4. Click and drag the **Show Cycle** pointer from cycle 1 to cycle 52.
   In the example experiment, the stable increase in signal from filter 1 corresponds to the FAM™ dye filter.



Tips for determining signal accuracy in your own experiment

When you analyze your own OpenArray™ genotyping experiment, look for the following conditions in each filter.

- Characteristic signal growth
- No abrupt changes or dips

Review the flags in the QC Summary

The **QC Summary** displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment. Review the **QC Summary** in the OpenArray™ genotyping example experiment for any flags triggered by the experiment data.

The following wells have data that triggered the SMCLUSTER flag.

Ala1, A1a2, A1a4, A1a7, A1c3, A1d5, A1e2, A1f7, A1g4, A1h4, A2a1, A2a2, A2a4, A2a7, A2c3, A2d5, A2e2, A2f7, A2g4, A2h4, A3a1, A3a2, A3a4, A3a7, A3c3, A3d5, A3e2, A3f7, A3g4, A3h4, A4a1, A4a2, A4a4, A4a7, A4c3, A4d5, A4e2, A4f7, A4g4, A4h4, A5a1, A5a2, A5a4, A5a7, A5c3, A5d5, A5e2, A5f7,

A5g4, A5h4, A6a1, A6a2, A6a4, A6a7, A6c3, A6d5, A6e2, A6f7, A6g4, A6h4, A7a1, A7a2, A7a4, A7a7, A7c3, A7d5, A7e2, A7f7, A7g4, A7h4, A8a1, A8a2, A8a4, A8a7, A8c3, A8d5, A8e2, A8f7, A8g4, A8h4, A9a1, A9a2, A9a4, A9a7, A9c3, A9d5, A9e2, A9f7, A9g4, A9h4, A10a1, A10a2, A10a4, A10a7, A10c3, A10d5, A10e2, A10f7, A10g4, A10h4, A11a1, A11a2, A11a4, A11a7, A11c3, A11d5, A11e2, A11f7, A11g4, A11h4, A12a1, A12a2, A12a4, A12a7, A12c3, A12d5, A12e2, A12f7, A12g4, A12h4, B1a1, B1a2, B1a4, B1a7, B1c3, B1d5, B1e2, B1f7, B1g4, B1h4, B2a1, B2a2, B2a4, B2a7, B2c3, B2d5, B2e2, B2f7, B2g4, B2h4, B3a1, B3a2, B3a4, B3a7, B3c3, B3d5, B3e2, B3f7, B3g4, B3h4, B4a1, B4a2, B4a4, B4a7, B4c3, B4d5, B4e2, B4f7, B4g4, B4h4, B5a1, B5a2, B5a4, B5a7, B5c3, B5d5, B5e2, B5f7, B5g4, B5h4, B6a1, B6a2, B6a4, B6a7, B6c3, B6d5, B6e2, B6f7, B6g4, B6h4, B7a1, B7a2, B7a4, B7a7, B7c3, B7d5, B7e2, B7f7, B7g4, B7h4, B8a1, B8a2, B8a4, B8a7, B8c3, B8d5, B8e2, B8f7, B8g4, B8h4, B9a1, B9a2, B9a4, B9a7, B9c3, B9d5, B9e2, B9f7, B9g4, B9h4, B10a1, B10a2, B10a4, B10a7, B10c3, B10d5, B10e2, B10f7, B10g4, B10h4, B11a1, B11a2, B11a4, B11a7, B11c3, B11d5, B11e2, B11f7, B11g4, B11h4, B12a1, B12a2, B12a4, B12a7, B12c3, B12d5, B12e2, B12f7, B12g4, B12h4, C1a1, C1a2, C1a4, C1a7, C1c3, C1d5, C1e2, C1f7, C1g4, C1h4, C2a1, C2a2, C2a4, C2a7, C2c3, C2d5, C2e2, C2f7, C2g4, C2h4, C3a1, C3a2, C3a4, C3a7, C3c3, C3d5, C3e2, C3f7, C3g4, C3h4, C4a1, C4a2, C4a4, C4a7, C4c3, C4d5, C4e2, C4f7, C4g4, C4h4, C5a1, C5a2, C5a4, C5a7, C5c3, C5d5, C5e2, C5f7, C5g4, C5h4, C6a1, C6a2, C6a4, C6a7, C6c3, C6d5, C6e2, C6f7, C6g4, C6h4, C7a1, C7a2, C7a4, C7a7, C7c3, C7d5, C7e2, C7f7, C7g4, C7h4, C8a1, C8a2, C8a4, C8a7, C8c3, C8d5, C8e2, C8f7, C8g4, C8h4, C9a1, C9a2, C9a4, C9a7, C9c3, C9d5, C9e2, C9f7, C9g4, C9h4, C10a1, C10a2, C10a4, C10a7, C10c3, C10d5, C10e2, C10f7, C10g4, C10h4, C11a1, C11a2, C11a4, C11a7, C11c3, C11d5, C11e2, C11f7, C11g4, C11h4, C12a1, C12a2, C12a4, C12a7, C12c3, C12d5, C12e2, C12f7, C12g4, C12h4, D1a1, D1a2, D1a4, D1a7, D1c3, D1d5, D1e2, D1f7, D1g4, D1h4, D2a1, D2a2, D2a4, D2a7, D2c3, D2d5, D2e2, D2f7, D2g4, D2h4, D3a1, D3a2, D3a4, D3a7, D3c3, D3d5, D3e2, D3f7, D3g4, D3h4, D4a1, D4a2, D4a4, D4a7, D4c3, D4d5, D4e2, D4f7, D4g4, D4h4, D5a1, D5a2, D5a4, D5a7, D5c3, D5d5, D5e2, D5f7, D5g4, D5h4, D6a1, D6a2, D6a4, D6a7, D6c3, D6d5, D6e2, D6f7, D6g4, D6h4, D7a1, D7a2, D7a4, D7a7, D7c3, D7d5, D7e2, D7f7, D7g4, D7h4, D8a1, D8a2, D8a4, D8a7, D8c3, D8d5, D8e2, D8f7, D8g4, D8h4, D9a1, D9a2, D9a4, D9a7, D9c3, D9d5, D9e2, D9f7, D9g4, D9h4, D10a1, D10a2, D10a4, D10a7, D10c3, D10d5, D10e2, D10f7, D10g4, D10h4, D11a1, D11a2, D11a4, D11a7, D11c3, D11d5, D11e2, D11f7, D11g4, D11h4, D12a1, D12a2, D12a4, D12a7, D12c3, D12d5, D12e2, D12f7, D12g4, D12h4

The following wells have data that triggered the CTFAIL flag.

A1b8, A1f5, A2b5, A2c6, A2e1, A2f1, A2h7, A3c4, A3c6, A3f1, A3h6, A4a6, A4a8, A4d5, A4d7, A4g2, A4h3, A5a5, A5e8, A5f1, A5f6, A5h6, A6a6, A6d5, A6e7, A6h4, A7b3, A7d7, A7e8, A8a5, A8d3, A8d8, A8e5, A8g2, A8g5, A8h5, A9a7, A9e6, A9f1, A9g2, A9g3, A10a5, A10e6, A10g3, A10g7, A11e8, A11f1, A11g2, A12a7, A12a8, A12c6, A12d3, A12e3, A12e8, A12f2, A12h4, B1a6, B1e6, B1h4, B2c6, B2c7, B3a5, B3e1, B3g2, B4c2, B4d4, B4d5, B4d8, B4f7, B4h4, B6a1, B6b4, B6b8, B6c8, B6d3, B6e1, B6f1, B6f2, B6f8, B6g6, B6g7, B6h2, B6h4, B7b2, B7c6, B7c8, B7g3, B7h2, B8a3, B8b1, B8c1, B8d6, B8d8, B8e1, B8h6, B9a7, B9c2, B9c3, B9f7, B10c6, B10c8, B10e1, B10e5, B10f6, B10f7, B10g2, B10g3, B10h4, B11c4, B11c6, B11c8, B11d4, B11e3, B11e6, B12a5, B12b1, B12c1, B12c5, B12c6, B12d1, B12e1, B12e5, B12g1, B12g2, C1a1, C1b5, C1c1, C1d6, C1g3, C2c6, C2c7, C2f1, C3e3, C3f1, C3g2, C4c6, C4e7, C4h3, C4h6, C5a2, C5a5, C5h6, C6f1, C6g3, C6h4, C7d5, C7f1, C8b2, C8h6, C9b5, C9c3, C9f4, C9g2, C9h7, C10e8, C11a7, C11b2, C11c6, C11c8, C11d3, C11e5, C11e7, C11f4, C11g3, C12a7, C12d8, C12e2, D1e6, D1f5, D2a2, D2e1, D2e4, D2g5, D3c6, D3d8, D3e5, D4d5, D4g3, D5c7, D6a1, D6a7, D6b1, D6c6, D6d4, D6d5, D6f5, D6f6, D6f8, D6h3, D6h4, D7b1, D7g3, D8a7, D8c2, D8d5, D8d7, D8e2, D8g6, D8g7, D8h4, D8h7, D9a5, D10b3, D10c3, D10d5, D10d8, D10e2, D10e5, D10e7, D10g3, D10h3, D11f8, D12c4, D12c6, D12e2, D12g1, D12h3, D12h4, D12h6

View the QC Summary

1. From the **Experiment Menu**, select **Analysis ▸ QC Summary**.

   If no data are displayed, click **Analyze**

2. Review the **Flags Summary**.

   A 0 displayed in the **Frequency** column indicates that the flag does not appear in the experiment. If the frequency is >0, the flag appears somewhere in the experiment; the well position is listed in the **Wells** column.

   In the example experiment, there are 635 flagged wells.

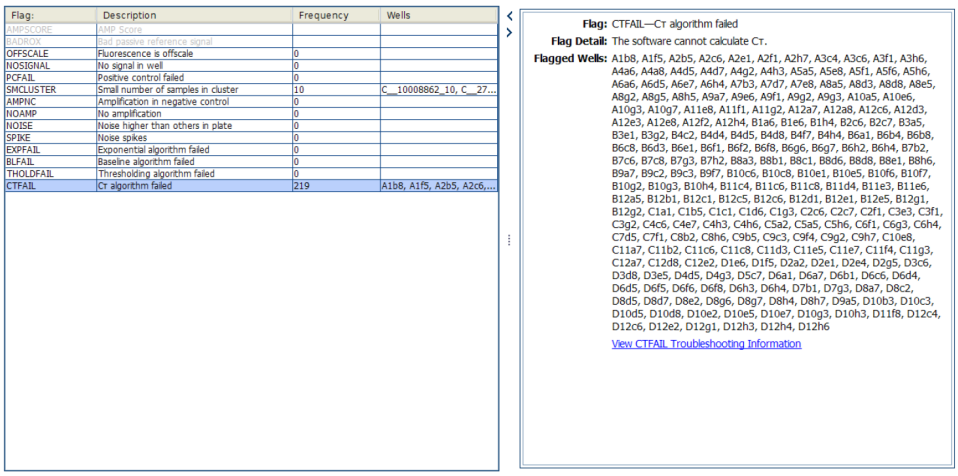
3. In the **Flag Details** table, click each flag with a frequency >0 to display detailed information about the flag.

   In the example experiment, the SMCLUSTER flag appears 416 times and the CTFAIL flag appears 219 times.

   For genotyping experiments, flag appearance is triggered by experiment data or the assay. If a flag has been triggered by the assay, the **Plate Layout** does not display the ⚠ icon. The flag details appear in the **QC Summary**. In the example experiment, some samples have only one allele present and therefore indicate many CTFAIL flags because there is no amplification plot for the second allele.

4. *(Optional)* For those flags with frequency >0, click the troubleshooting link to view information about correcting the flag.

   The **QC Summary** for the OpenArray™ genotyping example experiment looks like this.



Possible flags

The flags listed below may be triggered by the genotyping experiment data.

Flag	Description
Preprocessing flag	
OFFSCALE	Fluorescence is off scale.

(continued)

Flag	Description
Primary analysis flags	
BADROX	Bad passive reference signal.
NOAMP	No amplification.
Flag	
NOISE	Noise higher than others in plate
SPIKE	Noise spikes.
NOSIGNAL	No signal in well.
EXPFAIL	Exponential algorithm failed.
BLFAIL	Baseline algorithm failed.
THOLDFAIL	Thresholding algorithm failed.
CTFAIL	C_T algorithm failed.
AMPSCORE	Amplification in the linear region is below a specific threshold, corresponding to the score set in the analysis settings.
Secondary analysis flags	
AMPNC	Amplification in the negative control.
HIGHSD	High standard deviation in replicate group.

The BADROX and AMPSCORE flags, by default, are not in use for the genotyping experiment.

For the **Relative Threshold** algorithm, the EXPFAIL, BLFAIL, THOLDFAIL, and CTFAIL flags are not reported, but they appear in the **QC Summary** (by default, a 0 is displayed in the **Frequency** column for each flag).

(Optional) Adjust parameters for reanalysis of your own experiments

The **Analysis Settings** dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

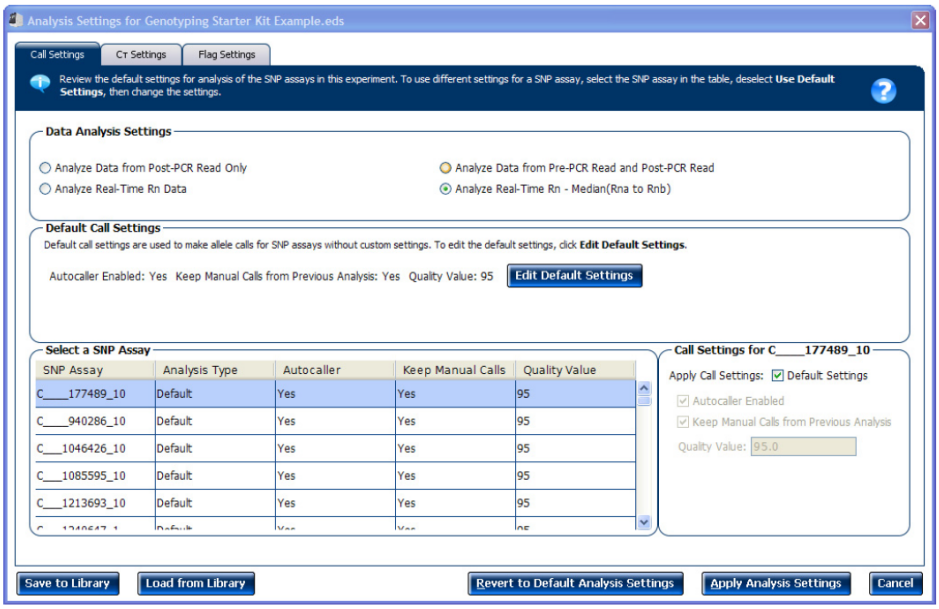
If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment.

View the analysis settings

1. From the **Experiment Menu**, select **Analysis.**

2. Click **Analysis ▸ Analysis Settings** to open the **Analysis Settings** dialog box. In the example experiment, the default analysis settings are used for each tab.
   - **Call Settings**
   - **C_T Settings**
   - **Flag Settings**

   The **Analysis Settings** dialog box for the OpenArray™ genotyping example experiment looks like this.



3. View and, if necessary, change the analysis settings.

   See “Adjust analysis settings” on page 161).

   You can save the changes to the analysis settings to the **Analysis Settings Library** for later use. See “Change analysis settings” on page 137.

4. Click **Apply Analysis Settings** to apply the current analysis settings.

   If necessary, click **Revert to Default Analysis Settings** to go back to the default analysis settings.

Adjust analysis settings

Adjust call settings

Use the **Call Settings** tab to adjust analysis settings.

- Change the default data analysis settings.
  - **Analyze data from Post-PCR Read only**—Select this option if you do not want to use data from the pre-PCR read to determine genotype calls.
  - **Analyze data from Pre-PCR Read and Post-PCR Read**—If you included the pre-PCR read in the run, select this option to use data from the pre-PCR read to determine genotype calls.
  - **Analyze Real-Time Rn Data**—If you included amplification in the run, select this option to use the normalized reporter (Rn) data from the cycling stage to determine genotype calls.
  - **Analyze data from Rn - Avg (Rna - Rnb)**—If you included amplification in the run, select this option to use the subtracted median of the normalized reporter (Rn) data from the cycling stage to determine genotype calls, where Rna to Rnb refers to all the cycles from the Start Cycle Number to the End Cycle Number. The average subtraction provides improved data accuracy.

    Note: To activate the Reveal Traces feature on the **Allelic Discrimination Plot**, select either **Analyze Real-Time Rn Data** or **Analyze data from Rn - Avg (Rna - Rnb)**.
- Edit the default call settings. Click **Edit Default Settings**, then specify the default settings.
  - **Autocaller Enabled**—Select this setting for the software to make genotype calls using the autocaller algorithm.
  - **Keep Manual Calls from Previous Analysis**—If the autocaller is enabled, select to maintain manual calls after reanalysis
  - **Quality Value**—Enter a value to use to make genotype calls. If the confidence value is less than the call setting, the call is undetermined.
- Use custom call settings for an SNP assay.
  - Select one or more SNP assays in the table, then deselect the **Default Settings** checkbox.
  - **Define the custom call settings**.

Adjust C_T settings

Use the **Data Step Selection** feature to select one stage/step combination for C_T analysis when there is more than one data collection point in the run method.

Adjust flag settings

Use the **Flag Settings** tab to:

- Adjust the sensitivity so that more wells or fewer wells are flagged.
- Change the flags that are applied by the QuantStudio™ 12K Flex Software.

1. In the **Flag Settings** tab, in the **Use** column, select the checkboxes for flags to apply during analysis.

2. *(Optional)* If an attribute, condition, and value are listed for a flag, specify the setting for applying the flag.

   If you choose to adjust the setting for applying a flag, make minor adjustments as you evaluate the appropriate setting.

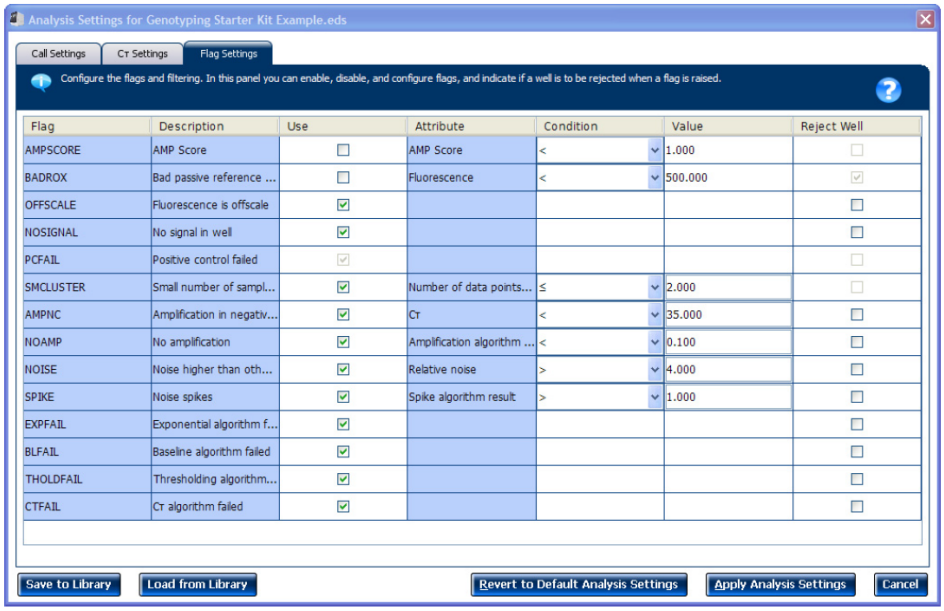
3. In the **Reject Well** column, select the checkboxes if you want the software to reject wells with the flag.

   After you have rejected the flagged wells, analysis results depend on factors such as the experiment type and flag type. For example, rejecting wells flagged by HIGHSD in experiments using the Standard Deviation calculations may change the result of C_{RT} SD. For some flags, analysis results calculated before the well is rejected are maintained.

4. In the **Analysis Settings** dialog box, click **Apply Analysis Settings**.

   If the run status is complete, the data are reanalyzed.

   The **Flag Settings** tab looks like this.



(Optional) Export the analyzed data

1. Open the genotyping example experiment file that you analyzed in Chapter 11, “Perform the instrument run”.

2. In the **Experiment Menu**, click **Export.**

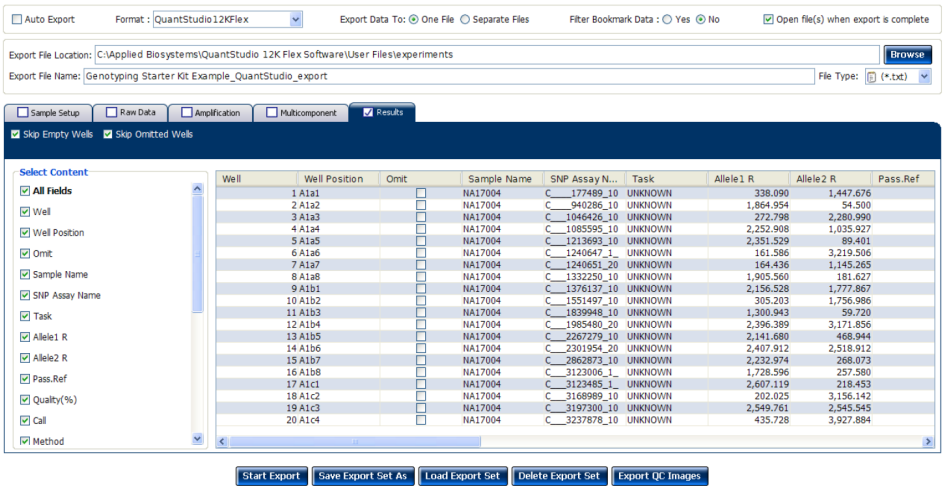
Note: To export data automatically after analysis, select the **Auto Export** checkbox during experiment setup or before running the experiment. Auto export is unchecked for the example experiment.

3. In the top of the screen, select **QuantStudio 12K Flex format**.

4. Complete the **Export** screen.

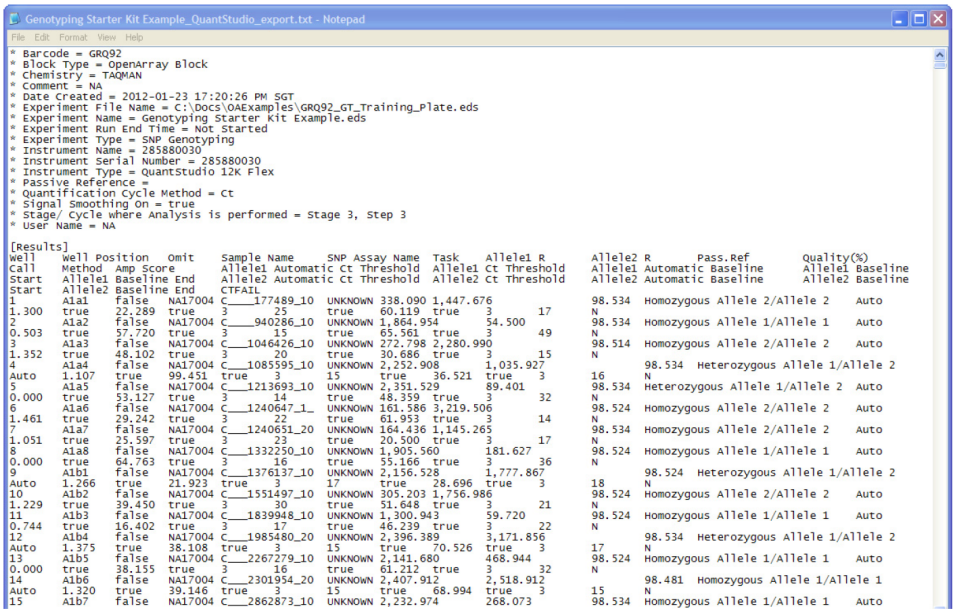
Field or Selection	Entry
Export Data To	**One File**
Filter Bookmark Data	**No**
Export File Location	`<...>\Applied Biosystems\QuantStudio 12K Flex Software\experiments`
Export File Name	`Genotyping Starter Kit Example_QuantStudio_export`
File Type	• **(*.txt)** • **(*.xls)** • **(*.xlsx)**
Select Data to export/ Select Content	Select the **All Fields** checkbox or select a specific set of checkboxes.

The completed **Export** screen should look like this.



5. Click **Start Export**.

The exported TXT file, when opened in Notepad, should look like this.



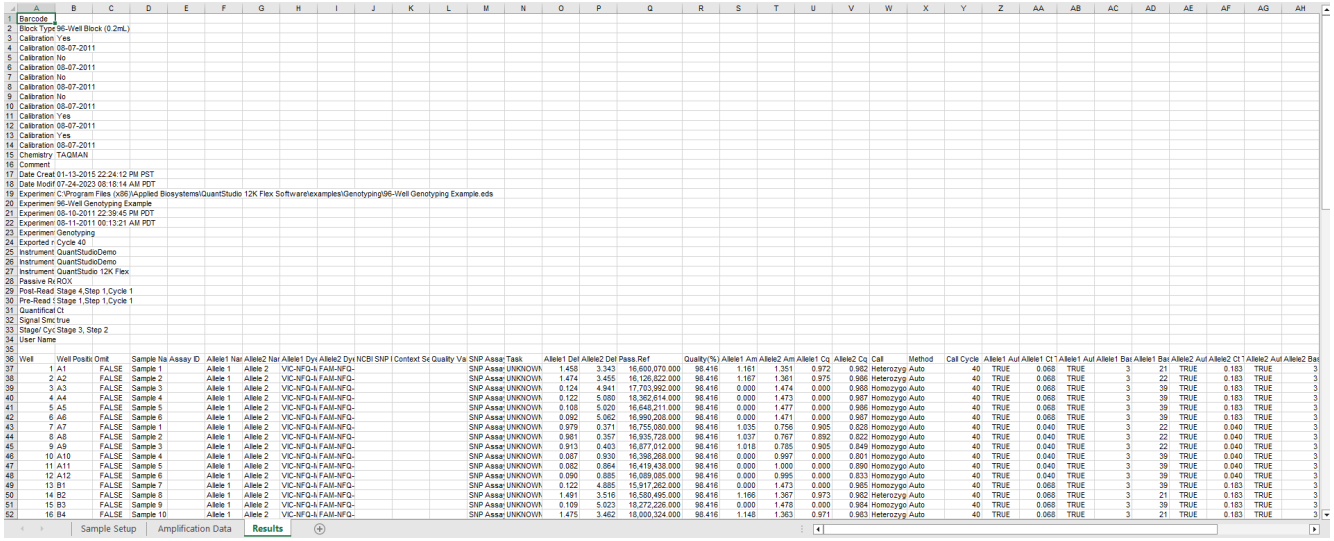
Genotyping Starter Kit Example_QuantStudio_export.txt - Notepad

File Edit Format View Help

```
* Barcode = GRQ92
* Block Type = OpenArray Block
* Chemistry = TAQMAN
* Comment = NA
* Date Created = 2012-01-23 17:20:26 PM SGT
* Experiment File Name = C:\Docs\OAExamples\GRQ92_GT_Training_Plate.eds
* Experiment Name = Genotyping Starter Kit Example.eds
* Experiment Run End Time = Not Started
* Experiment Type = SNP Genotyping
* Instrument Name = 285880030
* Instrument Serial Number = 285880030
* Instrument Type = QuantStudio 12K Flex
* Passive Reference =
* Quantification Cycle Method = Ct
* Signal Smoothing On = true
* Stage/ Cycle Where Analysis is performed = Stage 3, Step 3
* User Name = NA

[Results]
Well    Well Position   Omit    Sample Name     SNP Assay Name  Task    Allele1 R       Allele2 R       Pass.Ref        Quality(%)
Call    Method  Amp Score       Allele1 Automatic Ct Threshold  Allele1 Ct Threshold    Allele1 Automatic Baseline      Allele1 Baseline
Start   Allele1 Baseline End    Allele2 Automatic Ct Threshold  Allele2 Ct Threshold    Allele2 Automatic Baseline      Allele2 Baseline
Start   Allele2 Baseline End    CTFAIL
1       A1a1    false   NA17004 C___177489_10   UNKNOWN 338.090 1,447.676               98.534  Homozygous Allele 2/Allele 2    Auto
1.300   true    22.289  true    3       25      true    60.119  true    3       17      N
2       A1a2    false   NA17004 C___940286_10   UNKNOWN 1,864.954       54.500          98.534  Homozygous Allele 1/Allele 1    Auto
0.503   true    57.720  true    3       15      true    65.561  true    3       49      N
3       A1a3    false   NA17004 C___1046426_10  UNKNOWN 272.798 2,280.990               98.514  Homozygous Allele 2/Allele 2    Auto
1.352   true    48.102  true    3       20      true    30.686  true    3       15      N
4       A1a4    false   NA17004 C___1085595_10  UNKNOWN 2,252.908       1,035.927               98.534  Heterozygous Allele 1/Allele 2
Auto    1.107   true    99.451  true    3       15      true    36.521  true    3       16      N
5       A1a5    false   NA17004 C___1213693_10  UNKNOWN 2,351.529       89.401          98.534  Heterozygous Allele 1/Allele 2  Auto
0.000   true    53.127  true    3       14      true    48.359  true    3       32      N
6       A1a6    false   NA17004 C___1240647_1_  UNKNOWN 161.586 3,219.506               98.524  Homozygous Allele 2/Allele 2    Auto
1.461   true    29.242  true    3       22      true    61.953  true    3       14      N
7       A1a7    false   NA17004 C___1240651_20  UNKNOWN 164.436 1,145.265               98.534  Homozygous Allele 2/Allele 2    Auto
1.051   true    25.597  true    3       23      true    20.500  true    3       17      N
8       A1a8    false   NA17004 C___1332250_10  UNKNOWN 1,905.560       181.627         98.524  Homozygous Allele 1/Allele 1    Auto
0.000   true    64.763  true    3       16      true    55.166  true    3       36      N
9       A1b1    false   NA17004 C___1376137_10  UNKNOWN 2,156.528       1,777.867               98.524  Heterozygous Allele 1/Allele 2
Auto    1.266   true    21.923  true    3       17      true    28.696  true    3       18      N
10      A1b2    false   NA17004 C___1551497_10  UNKNOWN 305.203 1,756.986               98.524  Homozygous Allele 2/Allele 2    Auto
1.229   true    39.450  true    3       30      true    51.648  true    3       21      N
11      A1b3    false   NA17004 C___1839948_10  UNKNOWN 1,300.943       59.720          98.524  Homozygous Allele 1/Allele 1    Auto
0.744   true    16.402  true    3       17      true    46.239  true    3       22      N
12      A1b4    false   NA17004 C___1985480_20  UNKNOWN 2,396.389       3,171.856               98.534  Heterozygous Allele 1/Allele 2
Auto    1.375   true    38.108  true    3       15      true    70.526  true    3       17      N
13      A1b5    false   NA17004 C___2267279_10  UNKNOWN 2,141.680       468.944         98.524  Homozygous Allele 1/Allele 1    Auto
0.000   true    38.155  true    3       16      true    61.212  true    3       32      N
14      A1b6    false   NA17004 C___2301954_20  UNKNOWN 2,407.912       2,518.912               98.481  Homozygous Allele 1/Allele 1
Auto    1.320   true    39.146  true    3       15      true    68.994  true    3       15      N
15      A1b7    false   NA17004 C___2862873_10  UNKNOWN 2,232.974       268.073         98.534  Homozygous Allele 1/Allele 1    Auto
```

The exported XLS file should look like this. There is a separate tab for each data set that was selected for export.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
1	Barcode																																	
2	Block Type	96-Well Block (0.2mL)																																
3	Calibration	Yes																																
4	Calibration	08-07-2011																																
5	Calibration	No																																
6	Calibration	08-07-2011																																
7	Calibration	No																																
8	Calibration	08-07-2011																																
9	Calibration	No																																
10	Calibration	08-07-2011																																
11	Calibration	Yes																																
12	Calibration	08-07-2011																																
13	Calibration	Yes																																
14	Calibration	08-07-2011																																
15	Chemistry	TAQMAN																																
16	Comment																																	
17	Date Creat	01-13-2015 22:24:12 PM PST																																
18	Date Modif	07-24-2023 08:18:14 AM PDT																																
19	Experimen	C:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\examples\Genotyping\96-Well Genotyping Example.eds																																
20	Experimen	96-Well Genotyping Example																																
21	Experimen	08-10-2011 22:39:45 PM PDT																																
22	Experimen	08-11-2011 00:13:21 AM PDT																																
23	Experimen	Genotyping																																
24	Exported r	Cycle 40																																
25	Instrument	QuantStudioDemo																																
26	Instrument	QuantStudioDemo																																
27	Instrument	QuantStudio 12K Flex																																
28	Passive Re	ROX																																
29	Post-Read	Stage 4,Step 1,Cycle 1																																
30	Pre-Read S	Stage 1,Step 1,Cycle 1																																
31	Quantificat	Ct																																
32	Signal Smc	true																																
33	Stage/ Cyc	Stage 3, Step 2																																
34	User Name																																	
35																																		
36	Well	Well Positi	Omit	Sample Na	Assay ID	Allele 1 Nar	Allele 2 Nar	Allele1 Dye	Allele2 Dye	NCBI SNP I	Context Se	Quality Va	SNP Assay	Task	Allele1 Def	Allele2 Def	Pass.Ref	Quality(%)	Allele1 Am	Allele2 Am	Allele1 Cq	Allele2 Cq	Call	Method	Call Cycle	Allele1 Aut	Allele1 Ct T	Allele1 Aut	Allele1 Bas	Allele1 Bas	Allele2 Aut	Allele2 Ct T	Allele2 Aut	Allele2 Bas
37	1	A1	FALSE	Sample 1		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	1.458	3.343	16,600,070.000	98.416	1.161	1.351	0.972	0.982	Heterozyg	Auto	40	TRUE	0.068	TRUE	3	21	TRUE	0.183	TRUE	3
38	2	A2	FALSE	Sample 2		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	1.474	3.455	16,126,822.000	98.416	1.167	1.361	0.975	0.986	Heterozyg	Auto	40	TRUE	0.068	TRUE	3	22	TRUE	0.183	TRUE	3
39	3	A3	FALSE	Sample 3		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.124	4.941	17,703,992.000	98.416	0.000	1.474	0.000	0.988	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
40	4	A4	FALSE	Sample 4		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.122	5.080	18,362,614.000	98.416	0.000	1.473	0.000	0.987	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
41	5	A5	FALSE	Sample 5		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.108	5.020	18,648,211.000	98.416	0.000	1.477	0.000	0.986	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
42	6	A6	FALSE	Sample 6		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.092	5.062	16,990,208.000	98.416	0.000	1.471	0.000	0.987	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
43	7	A7	FALSE	Sample 1		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.979	0.371	16,755,680.000	98.416	1.035	0.756	0.905	0.828	Homozygo	Auto	40	TRUE	0.040	TRUE	3	22	TRUE	0.040	TRUE	3
44	8	A8	FALSE	Sample 2		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.981	0.357	16,935,728.000	98.416	1.037	0.767	0.892	0.822	Homozygo	Auto	40	TRUE	0.040	TRUE	3	22	TRUE	0.040	TRUE	3
45	9	A9	FALSE	Sample 3		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.913	0.403	16,877,012.000	98.416	1.018	0.785	0.905	0.849	Homozygo	Auto	40	TRUE	0.040	TRUE	3	22	TRUE	0.040	TRUE	3
46	10	A10	FALSE	Sample 4		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.087	0.930	16,398,268.000	98.416	0.000	0.997	0.000	0.801	Homozygo	Auto	40	TRUE	0.040	TRUE	3	39	TRUE	0.040	TRUE	3
47	11	A11	FALSE	Sample 5		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.082	0.864	16,419,438.000	98.416	0.000	1.000	0.000	0.890	Homozygo	Auto	40	TRUE	0.040	TRUE	3	39	TRUE	0.040	TRUE	3
48	12	A12	FALSE	Sample 6		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.090	0.885	16,089,085.000	98.416	0.000	0.995	0.000	0.833	Homozygo	Auto	40	TRUE	0.040	TRUE	3	39	TRUE	0.040	TRUE	3
49	13	B1	FALSE	Sample 7		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.122	4.885	15,917,262.000	98.416	0.000	1.473	0.000	0.985	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
50	14	B2	FALSE	Sample 8		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	1.491	3.516	16,580,495.000	98.416	1.166	1.367	0.973	0.982	Heterozyg	Auto	40	TRUE	0.068	TRUE	3	21	TRUE	0.183	TRUE	3
51	15	B3	FALSE	Sample 9		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	0.109	5.023	18,272,226.000	98.416	0.000	1.478	0.000	0.984	Homozygo	Auto	40	TRUE	0.068	TRUE	3	39	TRUE	0.183	TRUE	3
52	16	B4	FALSE	Sample 10		Allele 1	Allele 2	VIC-NFQ-h	FAM-NFQ-				SNP Assay	UNKNOWN	1.475	3.462	18,000,324.000	98.416	1.148	1.363	0.971	0.983	Heterozyg	Auto	40	TRUE	0.068	TRUE	3	21	TRUE	0.183	TRUE	3

Sample Setup | Amplification Data | Results

Part III

Custom miRNA OpenArray™ panels

This section covers miRNA chemistry. For advanced miRNA chemistry, see *TaqMan™ Advanced miRNA Assays User Guide—TrueMark™ OpenArray™ Plates* (Pub. No. MAN0016124).

13

Introduction

Custom miRNA OpenArray™ panels

This part provides information to perform runs with the custom miRNA OpenArray™ panels.

For advanced miRNA chemistry, see *TaqMan™ Advanced miRNA Assays User Guide—TrueMark™ OpenArray™ Plates* (Pub. No. MAN0016124).

Plates

The instructions in this document use three types of plates. The plates are described in detail in Appendix B, “Plate information”.

- MicroAmp™ Optical 96-Well Reaction Plate (96-well plate)

  A non-optical 96-well reaction plate can also be used.
- OpenArray™ 384-well Sample Plate (384-well plate, for QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)
- TrueMark™ OpenArray™ Plate (OpenArray™ Plate)

Data files

Data files are used to track your assays and samples.

The instructions in this guide use four types of data files.

- Sample information file (CSV)—Allows input of Sample IDs. See “Sample information file (CSV)” on page 167.
- Plate setup file (TPF)—Allows input of Assay IDs and cycling protocol. See “Plate setup file (TPF)” on page 167.
- Template file (EDT)—Includes complete setup information (samples, assays and cycling protocol) saved as a template. See “Template file (EDT)” on page 168
- Experiment file (EDS)—A complete data file. See “Experiment file (EDS)” on page 168,.

Additional data files (AIF, TXT) are available for selection if you use the **Batch Experiment Setup Utility** in the software to create and run your own experiments. See the *QuantStudio™ 12K Flex Software Help* (click or press **F1**).

Sample information file (CSV)

We recommend that you create or use a comma-delimited file (CSV) to track your cDNA samples. Use a sample information file to perform these tasks.

- Track where samples and controls are located in the 96-well plate. See Chapter 14, “Prepare the nucleic acid samples”.
- Map the sample locations, depending on the TrueMark™ OpenArray™ Plate format being used.
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate. See Chapter 15, “Prepare the OpenArray™ 384-well Sample Plate”.
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each TrueMark™ OpenArray™ Plate. See Chapter 16, “Prepare the OpenArray™ Plate”.
- Associate information about the samples with the data results to normalize data or to compute standard curves and calculate concentrations.

IMPORTANT! To enable accurate results, you must correctly track sample information from plate to plate.

For OpenArray™ AccuFill™ Software v2.0, all sample tracking and mapping features are available in this software. The OpenArray™ Sample Tracker Software is not used for OpenArray™ AccuFill™ Software v2.0.

- OpenArray™ 384-well Sample Plate—Integrate this file with a plate setup file in the OpenArray™ AccuFill™ Software. See “Prepare the plate setup files” on page 185.
- TrueMark™ OpenArray™ Plate—Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see “Start a run from the software” on page 200), or after the run is complete.

Plate setup file (TPF)

(Optional) Use an OpenArray™ Plate setup file

Plate setup files (TPF) contain the assay information for individual OpenArray™ Plate formats, including the gene symbol, gene name, assay ID, and location of each assay on the plate.

- Use the OpenArray™ AccuFill™ Software to integrate the sample information from a 384-well plate file (CSV) with the assay information in the plate setup file. See “Prepare the plate setup files” on page 185.
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (EDS). See “Start a run from the software” on page 200.

Download your own plate setup files

For miRNA experiments, use template files (EDT) supplied with the QuantStudio™ 12K Flex Software. The files are located at

`<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray`, where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

The miRNA EDT files located at `<...>\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray` include:

- `miRNA_Human.edt`
- `miRNA_Rodent.edt`
- *(Recommended)* Use the EDT file to integrate samples in the OpenArray™ AccuFill™ Software. If you plan to integrate samples, save the file to the OpenArray™ plate file input folder that you selected in the **Preferences** dialog box of the OpenArray™ AccuFill™ Software. The default save location is `<...>:\OpenArray\OpenArray Plates`.
- Use the EDT file directly in the QuantStudio™ 12K Flex Software to start an experiment, then upload samples to the experiment file (EDS) in the software after the experiment is run. See “Use an OpenArray™ Plate setup file” on page 201.

Template file (EDT)

An experiment document template file (EDT) contains predefined experiment setup information, such as experiment type, assay names, and run method.

You can access a template to create a new experiment from the two locations.

- QuantStudio™ 12K Flex Software. See “Start a run from the software” on page 200.
- QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen. See “Start a run from the instrument touchscreen” on page 202.

  The example template files are located at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray`, where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Experiment file (EDS)

An experiment document single file (EDS) is an electronic record used by the QuantStudio™ 12K Flex Software that contains information about a particular OpenArray™ Plate run on the QuantStudio™ 12K Flex Real-Time PCR Instrument.

The EDS file includes the following information:

- Metadata
  - Name
  - Barcode
  - Comments

- Experiment setup
  - Well contents
  - Assay definitions
- Run method (thermal cycling protocol)
- Run results
- Analysis protocol
- Analysis results
- Audit records
- Other plate-specific data

Use an EDS file to perform these tasks.

- Create and run an experiment using the QuantStudio™ 12K Flex Software. See “Start a run from the software” on page 200.

  To create and run the starter kit experiments, use the example template files (EDT) supplied with the QuantStudio™ 12K Flex Software. See “Template file (EDT)” on page 168.
- Create and run an experiment using the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen. See “Start a run from the instrument touchscreen” on page 202.
- Analyze the experiment results in the QuantStudio™ 12K Flex Software. See Chapter 18, “Analyze the experiment results”.

Workflow

Custom miRNA OpenArray™ panels

Prepare the nucleic acid samples (page 171)

The workflow for sample preparation can vary.

Prepare the OpenArray™ 384-well Sample Plate (page 178)

Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray™ 384-well Sample Plate.

Prepare the OpenArray™ Plate (page 181)

- Prepare for sample transfer using 384-well plates.
- Transfer the samples from 384-well plates to the OpenArray™ Plate using the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.
- Seal the OpenArray™ Plate.
- Complete sample transfer for each remaining OpenArray™ Plate.

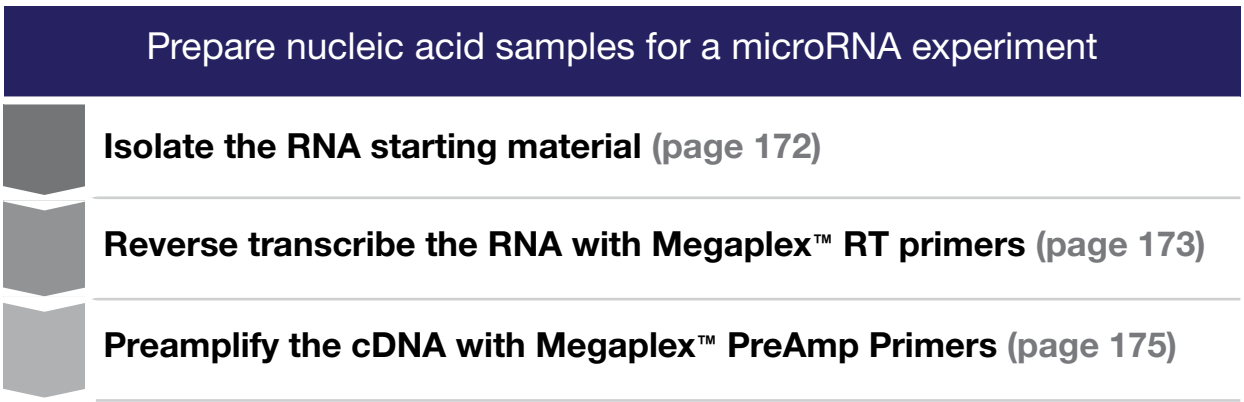
Perform the instrument run (page 195)

- Prepare the QuantStudio™ 12K Flex Software.
- Load the OpenArray™ Plate into the instrument.
- Run the OpenArray™ Plate formats.
- *(Optional)* Monitor the run.
- Unload the OpenArray™ Plate from the instrument.
- Transfer the experiments results to the computer.

Analyze the experiment results (page 210)

14 Prepare the nucleic acid samples

Workflow



Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
One set of Megaplex™ Primer Pools for your selected microRNA panel	See "Megaplex™ Primer Pools" on page 173.
*mir*Vana™ miRNA Isolation Kit, with phenol	AM1560
TaqMan™ MicroRNA Reverse Transcription Kit, 200 reactions	4366596
TaqMan™ PreAmp Master Mix Kit	4384266
MicroAmp™ Optical 96-Well Reaction Plate	4316813
• MicroAmp™ Clear Adhesive Film *or* • MicroAmp™ Optical 8-Cap Strips	• 4306311 *or* • 4323032
Thermal Cycler	**MLS**
0.1× TE pH 8.0	**MLS**

Note: Do not use TaqMan™ Advanced miRNA cDNA Synthesis Kit (Cat. No. A28007). This kit is optimized for advanced microRNA assays.

Isolate the RNA starting material

To prepare RNA samples, use the *mir*Vana™ miRNA Isolation Kit, with phenol for preparing high-quality total RNA containing miRNA species. Follow the total RNA isolation procedure (do *not* follow the option for enrichment for small RNAs).

Before performing a run, import the sample names and save the file as EDS. Use this EDS file to run the miRNA experiment.

Reverse transcribe the RNA with Megaplex™ RT primers

Megaplex™ Primer Pools

The Megaplex™ Primer Pools include content-matched pools of Megaplex™ RT Primers and Megaplex™ PreAmp Primers.

For…	Use…		
	Megaplex™ Primer Pools	**Contents**	**Storage**
Human nucleic acid	Megaplex™ Primer Pools, Human Pools Set v3.0 (Part No. 4444750)	• Megaplex™ RT Primers Pool A • Megaplex™ RT Primers Pool B • Megaplex™ PreAmp Primers Pool A • Megaplex™ PreAmp Primers Pool B	–25°C to –15°C
Rodent nucleic acid	Megaplex™ Primer Pools, Rodent Pools Set v3.0 (Part No. 4444766)		

Set up the RT reactions

In this step, single-stranded cDNA is reverse transcribed from total RNA. Run two reverse transcription (RT) reactions per sample, using Megaplex™ RT Primers Pool A and Pool B.

Each RT reaction has a final volume of 7.5 µL and contains:

- 100 ng (recommended) of total RNA in 3 µL
- 4.5 µL of RT reaction mix, containing reverse transcriptase, Megaplex™ RT Primers Pool A or Pool B, and other reverse transcription reagents

1. Thaw the following on ice.
   - Megaplex™ RT Primers
   - TaqMan™ MicroRNA Reverse Transcription Kit components (do not vortex the MultiScribe™ Reverse Transcriptase)
   - $MgCl_2$ (supplied with the Megaplex™ RT Primers)
2. Combine the following in each of two 1.5-mL microcentrifuge tubes (one for Pool A, the other for Pool B).

RT reaction mix components	Volume per reaction	Volume for 3 reactions[1]
Megaplex™ RT Primers (10×), Pool A	0.75 µL	2.5 µL
dNTPs with dTTP (100 mM)	0.15 µL	0.5 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	1.50 µL	5.1 µL
10× RT Buffer	0.75 µL	2.5 µL
$MgCl_2$ (25 mM)	0.90 µL	3.0 µL
RNase Inhibitor (20 U/µL)	0.09 µL	0.3 µL

(continued)

RT reaction mix components	Volume per reaction	Volume for 3 reactions[1]
Nuclease-free water	0.35 µL	1.2 µL
Total	**4.50 µL**	**15.1 µL**

[1] Includes 12.5% excess for loss from pipetting.

3. Pipet up and down to mix, then briefly centrifuge the tubes.

4. Transfer 4.5 µL of the RT reaction mix into the appropriate number of wells of a MicroAmp™ Optical 96-Well Reaction Plate, as shown in Figure 7 on page 174.

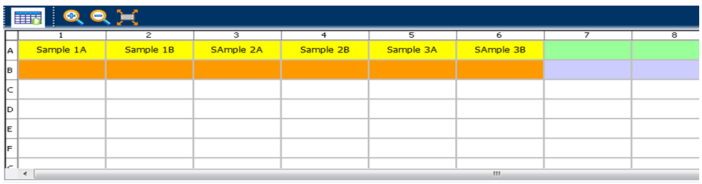


Figure 7 96-well sample plate map (each square represents one sample well).

Note: Each RNA sample is processed in two wells: one for Pool A and one for Pool B. Each 96-well plate can process 48 samples.

5. Add 100 ng of total RNA (recommended amount) in 3 µL of solution to each well containing RT Reaction Mix.

   You can use 3 µL of water for the No Template Control (NTC) reactions.

6. Depending on the number of RT reactions, mix the reactions in one of these ways.
   - Pipet each mixture up and down a few times, then seal the plate using MicroAmp™ Clear Adhesive Film.

     or
   - Seal the plate using MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical 8-Cap Strips, then invert the plate a few times.

Note: Do not use MicroAmp™ Optical Adhesive Film to seal the plate. This film may be difficult to remove after a run in the thermal cycler.

7. Centrifuge the plate briefly to collect the contents at the bottom of the wells, then incubate the plate on ice for 5 minutes.

Run the RT reactions

1. Set up the run method in a thermal cycler using the following conditions.
   - Ramp speed or mode—**9,700** using **Std** or **max** ramp speed.
   - Reaction volume (µL)—**7.5** (enter 8 µL if your instrument accepts only whole number values)

- Thermal cycling conditions:

Stage	Temperature	Time
Cycle (40 cycles)	16°C	2 minutes
	42°C	1 minute
	50°C	1 second
Hold	85°C	5 minutes
Hold	4°C	Hold

2. Load, then run the plate.

If needed, you can store the RT product (cDNA) at –25°C to –15°C for up to 1 month.

Preamplify the cDNA with Megaplex™ PreAmp Primers

Set up the preamplification reactions

In this step, specific cDNA targets are preamplified to increase the quantity of desired cDNA before performing the PCR.

Each preamplification reaction has a final volume of 25 µL and contains:

- 2.5 µL of RT product (cDNA) from "Run the RT reactions" on page 174
- 22.5 µL of preamplification reaction mix, containing Megaplex™ PreAmp Primers Pool A or Pool B and 2× TaqMan™ PreAmp Master Mix

  Use Megaplex™ PreAmp Primers Pools A or Pool B corresponding to the Megaplex™ RT Primers Pool used for reverse transcription.

1. Thaw the Megaplex™ PreAmp Primers on ice and mix by inverting a few times. Centrifuge briefly to collect the contents at the bottom of the tubes.
2. Mix the 2× TaqMan™ PreAmp Master Mix by swirling the bottle.
3. Prepare preamplification reaction mix, one for Pool A and one for Pool B, by combining the following in each of two 1.5-mL microcentrifuge tubes.

Preamplification reaction mix components	Volume for 1 reaction	Volume for 3 reactions[1]
2× TaqMan™ PreAmp Master Mix	12.5 µL	42.4 µL
Megaplex™ PreAmp Primers(10×), Pool A or Pool B[2]	2.5 µL	8.4 µL
Nuclease-free water	7.5 µL	25.3 µL
Total	**22.5 µL**	**76.1 µL**

[1] Includes 12.5% excess for volume loss from pipetting.
[2] Use Pool A in one tube, and Pool B in the other.

4. Pipet up and down to mix, then centrifuge the tubes briefly.

5. Pipet 2.5 µL of each RT product into a well of a MicroAmp™ Optical 96-Well Reaction Plate. (Two wells per RNA sample, one for the Pool A RT product and the other for the Pool B product.)

6. Dispense 22.5µL of preamp reaction mix into each well of the 96-well plate containing the corresponding RT product (pool A or pool B).

7. Depending on the number of preamplification reactions, mix the reactions in one of these ways.
   - Pipet each mixture up and down a few times, then seal the plate using MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical 8-Cap Strips.

     or
   - Seal the plate using MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical 8-Cap Strips, then invert the plate a few times.

8. Centrifuge the plate briefly to collect the contents at the bottom of the wells, then incubate the plate on ice for 5 minutes.

Run the preamplification reaction

Set up the run method with the following conditions.

- Ramp speed or mode—**9,700** using **Std** ramp speed
- Reaction volume (µL)—**25**
- Thermal-cycling parameters:

Stage	Temp	Time
Hold	95°C	10 min
Hold	55°C	2 min
Hold	72°C	2 min
Cycle (12 cycles)	95°C	15 sec
	60°C	4 min
Hold[1]	99.9°C	10 min
Hold	4°C	Hold

[1] Required for enzyme inactivation.

Dilute the preamplification products

1. Remove the 96-well plate from the thermal cycler, then briefly centrifuge the plate.

2. For each preamplification reaction, add 156 µL of 0.1× TE pH 8.0 to one well of a new 96-well plate.

3. Transfer 4 µL of each preamplification reaction to a well containing 0.1× TE buffer (final dilution: 1 to 40).

4. Depending on the number of preamplification reactions, mix the diluted products in one of these ways.
   - Pipet up and down a few times, then seal the plate using MicroAmp™ Clear Adhesive Film.

     or
   - Seal the plate using MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical 8-Cap Strips, then invert the plate a few times.

5. Centrifuge the plate briefly to collect the contents at the bottom of the wells, then place the plate on ice.

   If needed, you can store the preamplified product (diluted or undiluted) at 4°C for up to 12 hours, or at –25°C to –15°C for up to 1 week.

Proceed to Chapter 15, “Prepare the OpenArray™ 384-well Sample Plate”.

15 Prepare the OpenArray™ 384-well Sample Plate

In this chapter, you use a 8- or 12-channel pipette to transfer the nucleic acid samples from the 96-well reaction plates to OpenArray™ 384-well Sample Plate formats. The plates are described in detail in Appendix B, “Plate information”.

You also track the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates. The workflow for preparing the 384-well sample plate varies, depending on the experiment type.

Workflow

Prepare the OpenArray™ 384-well Sample Plate

1. **Track the samples** (page 179)
2. **Prepare PCR reaction mix A and B** (page 180)
3. **Transfer the PCR reaction mix** (page 180)

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
96-well reaction plates, containing preamplified cDNA samples	User supplied (see "Required materials" on page 171)
2× TaqMan™ OpenArray™ Real-Time PCR Master Mix, 1.5 mL	4462159
MicroAmp™ Optical 96-Well Reaction Plate	4316813
MicroAmp™ Clear Adhesive Film	4306311
OpenArray™ 384-well Sample Plate	4406947
OpenArray™ 384-Well Plate Seals	4469876
Fine-tip marker	**MLS**

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For OpenArray™ AccuFill™ Software v2.0, samples are tracked in the OpenArray™ AccuFill™ Software. The samples are tracked in the **Map Plates** tab.

For more information about OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

1. Proceed to transfer the samples as shown in Figure 2 on page 180. You do not need to enter sample information from the 96-well sample plates at this time.

   You can edit the sample information directly in the QuantStudio™ 12K Flex Software before starting the run. See Chapter 17, "Perform the instrument run".

2. Use a fine-tip marker to label the 384-well sample plate.
   a. Label the 384-well sample plate with a unique identifier.

   b. Mark the sections of the OpenArray™ 384-well Sample Plate to transfer samples from the 96-well reaction plates with the diluted preamplification product.

Prepare PCR reaction mix A and B

Note: All volumes include 12.5% excess volume to accommodate the loss that occurs during pipetting.

1. If the diluted preamplification products were stored frozen, thaw the 96-well reaction plate completely on ice. Mix by inverting the sealed plate a few times or by gently vortexing, then centrifuge the plate briefly.
2. Mix the TaqMan™ OpenArray™ Real-Time PCR Master Mix by swirling the bottle.
3. For each sample, pipet 22.5 µL of master mix into each of two adjacent wells (one for Pool A and one for Pool B) of a clean 96-well reaction plate.
4. For each sample, pipet:
   - 22.5 µL of diluted Pool A preamplification product into one well of each pair.
   - 22.5 µL of diluted Pool B preamplification product into the other well.
5. Seal the 96-well reaction plate with adhesive film, vortex gently to mix, then centrifuge the plate briefly.

Note: If needed, you can store the sealed 96-well reaction plate at 4°C for up to 12 hours.

Transfer the PCR reaction mix

The PCR reaction mix is transferred as shown in Figure 8 on page 180.

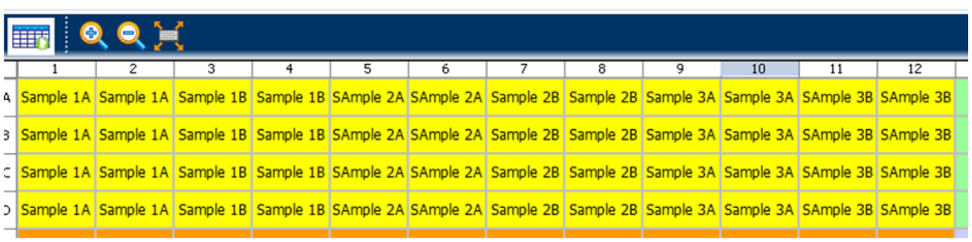


Figure 8 384-well sample plate map (eight wells per sample-pool combination).

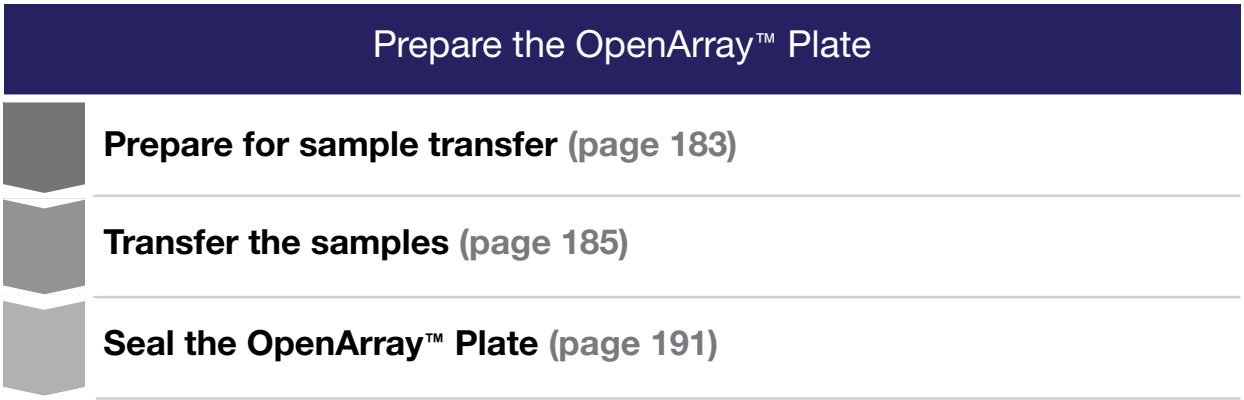
1. Transfer 5 µL of PCR reaction mix A to the following 8 wells of the 384-well sample plate: A1, A2, B1, B2, C1, C2, D1, and D2.
2. Transfer 5 µL of PCR reaction mix B to the following 8 additional wells of the 384-well sample plate: A3, A4, B3, B4, C3, C4, D3, and D4.
3. Cover the sample plate with foil, vortex gently to mix, then centrifuge for 1 minute at 1,000 × *g* to eliminate bubbles.
4. *(Optional)* Place the sample plate on ice, in the dark, for up to 1 hour.

Proceed to Chapter 16, “Prepare the OpenArray™ Plate”.

16 Prepare the OpenArray™ Plate

In this chapter, you use the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System to transfer the nucleic acid samples from the OpenArray™ 384-well Sample Plate to the OpenArray™ Plate. The workflow is the same for all OpenArray™ Plate formats.

Workflow



Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Custom miRNA OpenArray™ panels	See Appendix A, "Ordering information".
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System	4471021
QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit The accessories kit contains: • OpenArray™ Case Lid (6 lids) • OpenArray™ Plugs (6 plugs) • OpenArray™ Carriers (2 carriers) • OpenArray™ Immersion Fluid and OpenArray™ Immersion Fluid Tip (6 syringes) • OpenArray™ AccuFill™ System Tips (1 box of 384 tips) • OpenArray™ 384-well Sample Plate (10 plates) • OpenArray™ 384-Well Plate Seals (10 seals)	4469586
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
Foil seals	**MLS**
Bleach (10%)	**MLS**
Ethanol	**MLS**
Fine-tip marker	**MLS**
Razor blade	**MLS**
Powder-free gloves	**MLS**
Laboratory-grade wipes	**MLS**
Safety glasses	**MLS**
Tweezers or forceps (for removing foil sections from the 384-well sample plate)	**MLS**

Storage conditions

The following materials require special storage conditions.

Item		Storage
Custom miRNA OpenArray™ panels	Frozen, unopened	Store at –20°C until the expiration date provided on the product label.
	Thawed, unopened	Store at room temperature for up to 24 hours.
	Thawed, opened	Store at room temperature for up to 1 hour.
	Loaded and sealed, pre-run	Store at room temperature, protected from light, for up to 1 hour.
QuantStudio™ Immersion Fluid	Unopened	Store at room temperature until the expiration date provided on the product label.
	Opened	Store at room temperature. Do not store any remaining immersion fluid; use the amount required, then discard the remainder.
OpenArray™ AccuFill™ System Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Use tips within one week.

Prepare for sample transfer

Guidelines for handling the OpenArray™ Plate

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Plate.

- Hold the OpenArray™ Case by the edges.
- Do not touch the through-holes of the OpenArray™ Plate.
- Load and seal an OpenArray™ Plate within *one hour* after opening the package.
- If you drop a loaded OpenArray™ Plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray™ Plate if it becomes dislodged from the case.

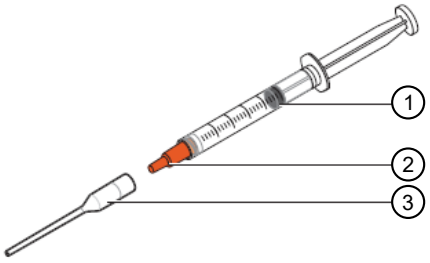
Prepare the equipment and plates

Ensure that the OpenArray™ 384-well Sample Plate, the OpenArray™ AccuFill™ System Tips, and OpenArray™ Plate holder are completely clean and dry.

1. Remove an OpenArray™ Plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).

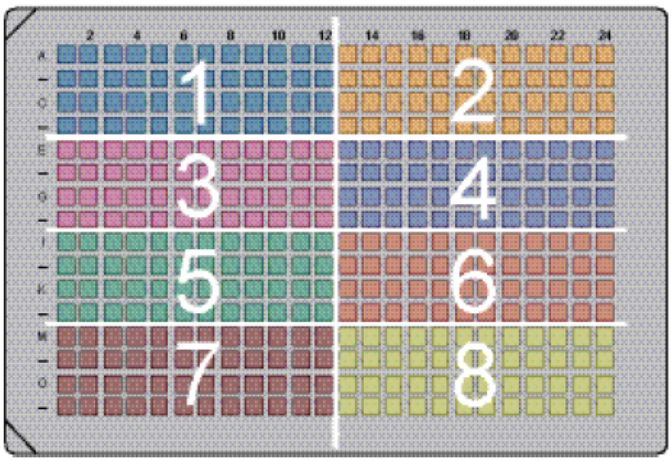
2. Prepare a syringe containing OpenArray™ Immersion Fluid.
   a. Remove the cap from the syringe containing OpenArray™ Immersion Fluid.

   b. Remove the cap and attach the tip to the syringe. Place the assembly on a clean surface.



① OpenArray™ Immersion Fluid
② Cap (remove)
③ Syringe tip (attach)

3. Score or cut the foil seal of the OpenArray™ 384-well Sample Plate into the 8 sections shown below, then place the plate on ice to keep the samples cold.



Prepare the plate setup files

OpenArray™ AccuFill™ Software v2.0 allows the transfer of samples without a sample plate file. The QuantStudio™ 12K Flex Instrument requires a sample plate file if the real-time PCR run is started with a TPF file or an EDT file.

For each OpenArray™ Plate being prepared, note the following.

- *(Recommended)* Use a plate setup file (CSV or TPF) to transfer samples using the OpenArray™ AccuFill™ Software.
  - OpenArray™ 384-well sample information file (CSV). See "Track the samples" on page 179
  - OpenArray™ Plate setup file (TPF). See "(Optional) Use an OpenArray™ Plate setup file" on page 167.
- *(Optional)* If you created a CSV file in the OpenArray™ AccuFill™ Software (see "Track the samples" on page 179), you can import the sample information in this file directly into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

Transfer the samples

Start the system

Note: If the samples were tracked immediately before this section, the system might be on (see "Track the samples" on page 179). The sample tracking and sample transfer functions are both done with OpenArray™ AccuFill™ Software if OpenArray™ AccuFill™ Software v2.0 is used.

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips. See "Set up the system" on page 186.

The instrument does not initiate a self-test immediately after starting the software. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

The other features in the software can be accessed after starting the software without a self-test.

1. Ensure that the instrument door is closed.
2. Power on the instrument, if it is off.
3. Start the OpenArray™ AccuFill™ Software ().
   The software checks the computer and connections as the system starts.

Proceed to set up the system (see "Set up the system" on page 186).

Set up the system

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

1. Open the instrument door, empty the waste bin, then place the waste bin back on the instrument deck.

CAUTION! Wear appropriate personal protective equipment while handling the waste bin.

2. Ensure that the sample plate holder and the OpenArray™ Plate holders are empty.
3. Place the sample plate in the sample plate holder on the instrument deck, with the notch to the left. Do not stack sample plates.
4. Place each OpenArray™ Plate in an OpenArray™ Plate holder.
5. Replace the tip boxes, if necessary.

   Each tip box contains 384 tips, divided into 8 sections.

   When setting up a run, the status of the tip boxes is confirmed in the software. A full tip box is recommended when starting a run.
6. Remove the cover from each tip box.

   Ensure that the tip box covers are removed from the instrument deck.

   IMPORTANT!
   - Do not reuse tips.
   - Use tips within one week of opening the box.
   - Discard any unused tips within one week.
7. Close the instrument door.

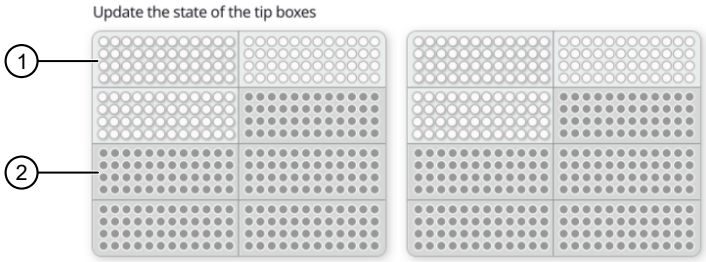
The system is ready to start a run. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes. The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box that is full.
② Section of the tip box that is empty.

2. *(Optional)* Click **Auto-fill tip boxes**.

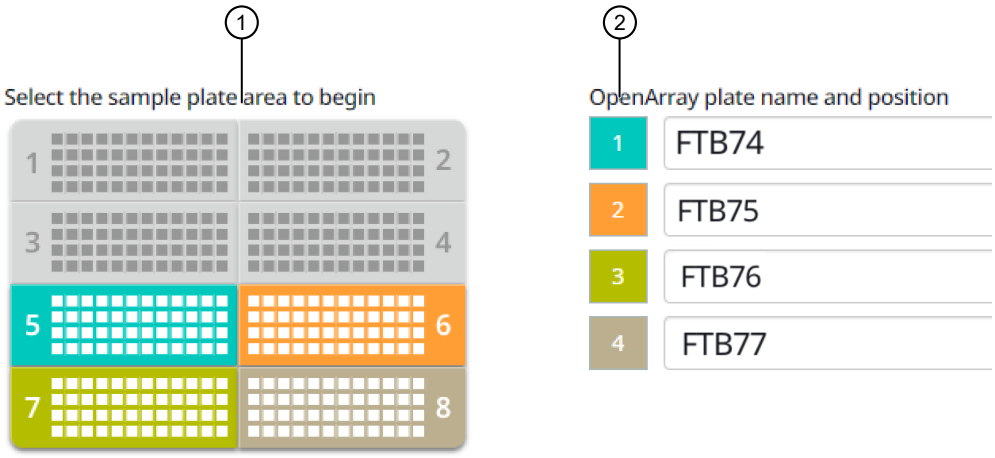
   The status of all sections of the tips boxes is set to full.

3. Select the first section of the sample plate to be used to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple plates are filled during a run. The software selects the total number of sections that correspond with the total number of plates.

   In the following example, section 5 was selected. The group of sections 5, 6, 7, and 8 is highlighted by the software because four plates are being filled.

   The position box displays the color that corresponds to the section of the sample plate.



① Sample plate section (section 5, 6, 7, and 8 are highlighted).
② Corresponding plates.

4. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   Note: Do not remove the foil from all the sections of the sample plate at once.

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of these conditions.

   - The waste bin is not in position.
   - The sample plate is not in position.
   - The plates are not in position.
   - There are more plates on the instrument deck than are defined in the experiment setup.

The **Deck** screen is displayed.

For a description of the run progress, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see "Seal the TrueMark™ OpenArray™ Plate" on page 43).

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument

After an OpenArray™ Plate is filled, the **Remove plate and foil** dialog box is displayed (see Figure 9).

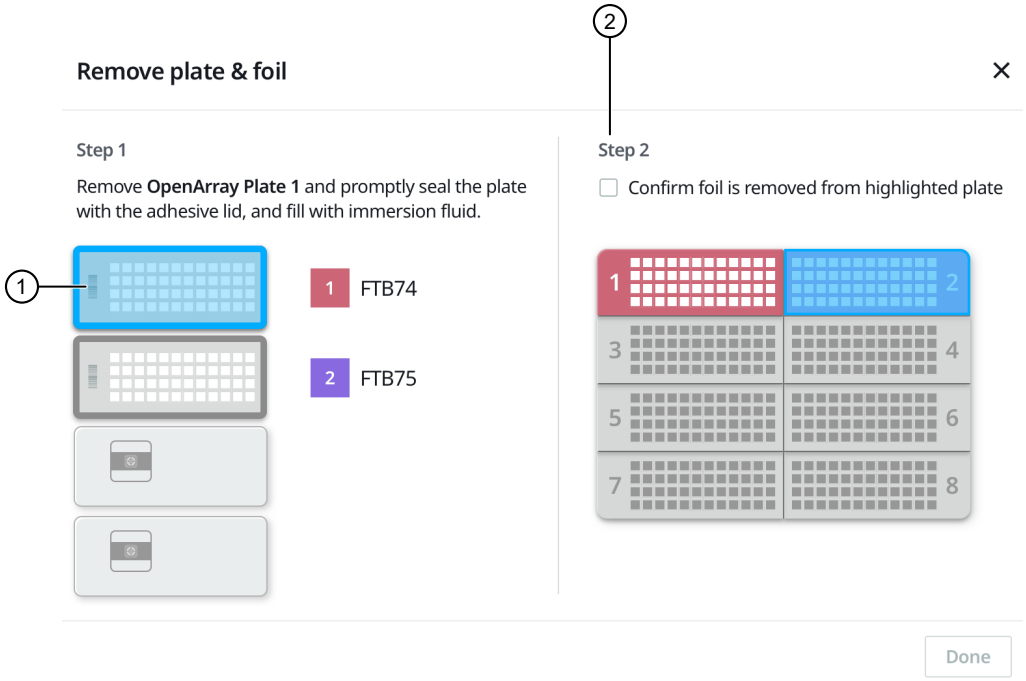


Figure 9 Remove plate and foil dialog box.

① OpenArray™ Plate to remove from the instrument.
② **Confirm foil is removed from highlighted plate section** checkbox.

Remove each OpenArray™ Plate *immediately* after it has been filled, even if the run was set up to fill multiple plates.

After the last OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed (see Figure 10).

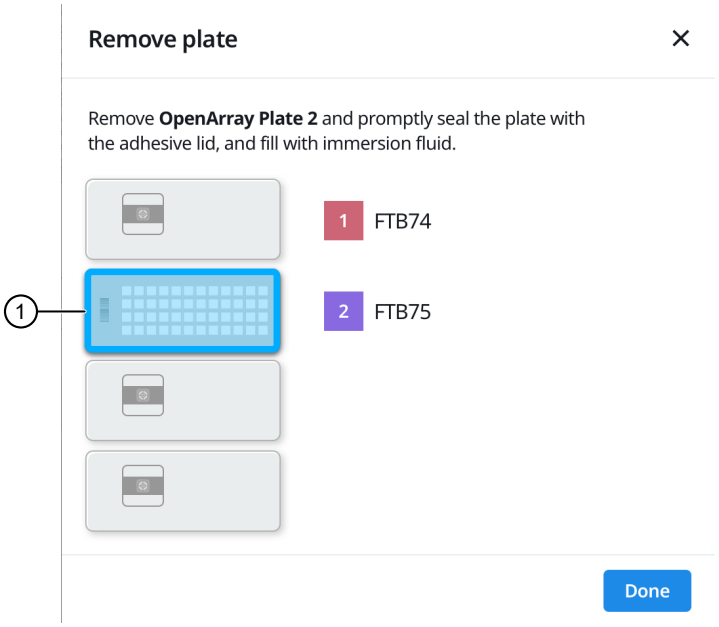


Figure 10 Remove plate dialog box

① OpenArray™ Plate to remove from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate immediately, to avoid evaporation within the plate.

   One of the following dialog boxes is displayed:

   - The **Remove plate and foil** dialog box.
   - The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.

   The run does not proceed under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The plates are not in position
   - There are more plates on the instrument deck than are defined in the experiment setup

   The instrument proceeds to load the next OpenArray™ Plate.

6. Repeat step 1 to step 5 for each OpenArray™ Plate to be loaded.

After all of the plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

A loaded TPF is generated for each OpenArray™ Plate. The loaded TPF file corresponds to the original TPF file that was imported for the run. The files are exported to the folder that was designed in the **Preferences**.

Note: Some workflows might not generate a loaded TPF file. For more information about the workflows available for the OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Seal the OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

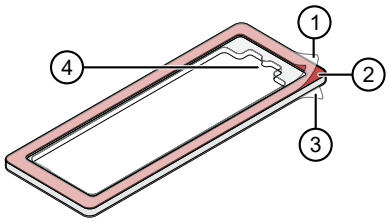
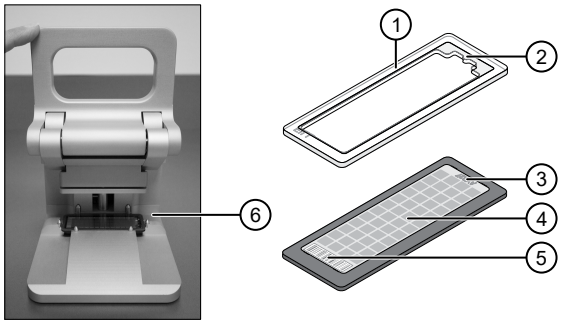


Figure 11 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

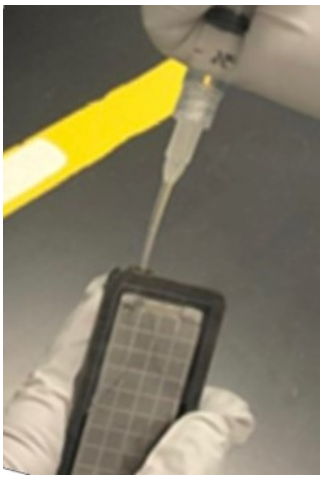
   Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

   IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.

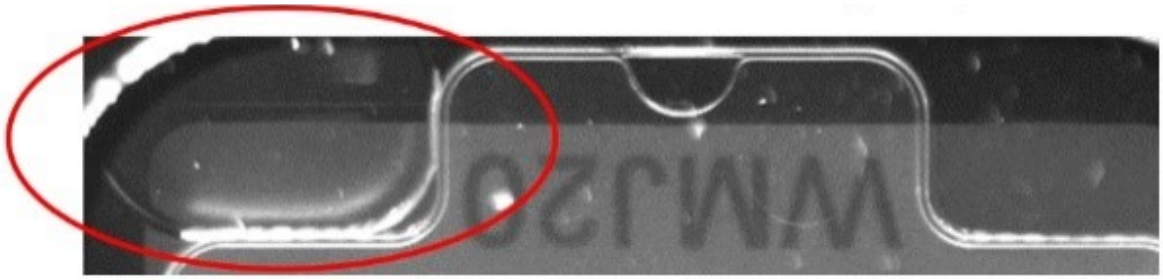


   The syringe tip must be in front of the array when filling the case with immersion fluid.

9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

   IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

   Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

The plate is ready for PCR.

Note: For microRNA experiments, you can store loaded and sealed OpenArray™ Plate formats at room temperature, protected from light, for up to 1 hour.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (more than 6) of OpenArray™ Plates, follow these guidelines.

- To help avoid mistakes when entering sample information in the OpenArray™ AccuFill™ Software, load the OpenArray™ Plates in alphanumeric order (according to the OpenArray™ Plate serial number).
- Seal each OpenArray™ Plate immediately after loading is completed, while the other OpenArray™ Plates are loaded.

  IMPORTANT! To avoid evaporation, seal the OpenArray™ Plate with the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0, add the OpenArray™ Immersion Fluid, plug the case, then place the case in a vertical position.

- Use the OpenArray™ Carriers to transport up to four loaded OpenArray™ Plates to the QuantStudio™ 12K Flex Instrument.
- After loading is complete, you can use a large bin to properly discard any used OpenArray™ AccuFill™ System Tips.

  For cleaning procedures, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Perform the instrument run

In this chapter, you run the QuantStudio™ 12K Flex TrueMark™ OpenArray™ Plate formats on the QuantStudio™ 12K Flex Real–Time PCR System. During the run, the system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all OpenArray™ Plate formats.

Workflow

Perform the instrument run

- **Prepare the QuantStudio™ 12K Flex Software** (page 196)
- **Load the TrueMark™ OpenArray™ Plate into the instrument** (page 199)
- **Run the OpenArray™ Plate formats** (page 199)
- **(Optional) Monitor experiments** (page 203)
- **Unload the OpenArray™ Plate from the instrument** (page 207)
- **(Optional) Transfer experiment results** (page 207)

Prepare the QuantStudio™ 12K Flex Software

(Optional) Select OpenArray™ block run preferences

Preferences provide user-access to the settings that govern how the QuantStudio™ 12K Flex Software functions. This section summarizes only those preferences that apply to experiments with OpenArray™ Plate formats.

For detailed information about the preferences, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

1. Double-click **(QuantStudio™ 12K Flex Software shortcut)** to start the software.
2. In the toolbar, click **Tools** ▸ **Preferences**, then select the **OpenArray** tab.

3. Complete the tab, as needed.

Settings	Description
Setup Folder	Define the absolute path to the default folder from which the software imports experiment setup files. The **Import** dialog box opens to the import folder when invoked from the software.
Experiment Folder	Define the absolute path to the default folder to which the software reads or writes experiment files. The **Open** and **Save** dialog boxes open to the data folder when invoked from the software.
Passive Reference	Define the dye to use as the passive reference. The default is set to **None**. While the software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray™ experiments.
Default Browse File Type list	Define the file type that the **Import**, **Open**, and **Save** dialog boxes select by default when invoked from the software.
Apply experiment template (EDT) to all OpenArray experiment checkbox	If selected, the software applies the Run Method defined in the selected experiment template (EDT) to all OpenArray™ experiments. For more information about OpenArray™ experiment templates, see the software help.
Always include Amplification stage for Genotyping experiment checkbox	*(Genotyping experiments only)* If selected, the software adds an Amplification stage to the Run Method for all OpenArray™ genotyping experiments. If deselected, you must perform amplification on another instrument. For more information about Run Method settings, see the software help.
Always include Pre-Read stage for Genotyping experiment checkbox	*(Genotyping experiments only)* If selected, the software adds a Pre-Read stage to the Run Method for all OpenArray™ genotyping experiments. For more information about Run Method settings, see the software help.

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

IMPORTANT! You must restart the software for preference changes to take effect.

Access the Instrument Console

The **Instrument Console** displays every QuantStudio™ 12K Flex Real-Time PCR Instrument discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups.

- **On the Network**—All instruments available on the network.
- **My Instruments**—Instruments you have selected to monitor.

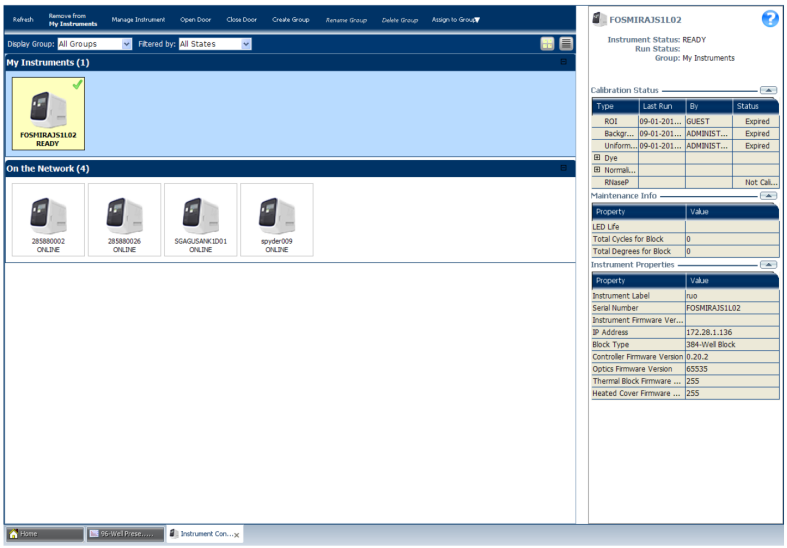
To start and monitor a run on an instrument, you must move the instrument from the **On the Network** group to the **My Instruments** group or a custom group that you create.

To access the **Instrument Console** and enable monitoring of a networked instrument:

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. On the **Home** tab, from the **Tools** menu, select **Instrument Console**.

   If you do not see an instrument, click **Refresh** in the **Instrument Console** toolbar.



3. If needed, move an instrument from the **On the Network** group to a group that can be monitored:

   a. Click the instrument of interest, then click **Assign to Group** in the **Instrument Console** toolbar.

   b. Select the **My Instruments** group or a personal group in the drop-down list.

Note: Alternatively, you can select the icon of the instrument that you want to add to the **My Instruments** list, then click **Add to My Instruments**. Similarly, click **Remove from My Instruments** to remove an instrument from the **My Instruments** list. You can also drag and drop the instrument icon into **My Instruments** or into the group created by you.

The instrument is now monitored. The status is indicated by an icon in the upper right corner. For detailed information about the **Instrument Console**, see the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

Enable or change the notification settings

You can configure the QuantStudio™ 12K Flex Software to alert you by email when the QuantStudio™ 12K Flex Real-Time PCR Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the notification settings feature, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Load the TrueMark™ OpenArray™ Plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

IMPORTANT! Wear powder-free gloves when you handle the OpenArray™ Plate.

IMPORTANT! The instrument should be loaded and unloaded only by operators who have been warned of the moving parts hazard and who have been adequately trained.

1. Open the plate adapter on the instrument. Touch on the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software, to allow the plate adapter to come out from the instrument side.
2. Remove the clear protective film from the outside of the OpenArray™ Case (sealed plate + lid).
3. Place the OpenArray™ Plate or plates on the plate adapter.
   - Ensure that each plate is properly aligned in the plate adapter.
   - Ensure that the plate barcode is facing up and toward the front of the instrument.
4. Close the plate adapter on the instrument. Touch on the instrument touchscreen, or click **Close Door** in the **Instrument Console** of the software, to retract the plate adapter back into the instrument.

Run the OpenArray™ Plate formats

You can run TrueMark™ OpenArray™ Plate formats in two ways.

- “Start a run from the software” on page 200
- “Start a run from the instrument touchscreen” on page 202

Note: The starter kit experiments in this guide run OpenArray™ Plate formats from the QuantStudio™ 12K Flex Software.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Real-Time PCR Instrument is in operation.

Start a run from the software

There are different ways to create and run an OpenArray™ experiment (EDS) from the QuantStudio™ 12K Flex Software.

- *(Recommended)* Use a template file (EDT). See “Use a template file” on page 200.
- Use an OpenArray™ setup file (TPF). See “Use an OpenArray™ Plate setup file” on page 201.
- Use the **Batch Experiment Setup Utility**. See the *QuantStudio™ 12K Flex Software Help* (click or press **F1**).

Use a template file

You can use a template file (EDT) to create a new OpenArray™ experiment, then import the sample and assay information for the OpenArray™ Plate or plates before starting the run, or after the run is complete.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. On the **Home** tab, in the **Experiment** menu, select **Create From Template.**
3. Navigate to and select the template file (EDT) that you want to use, then click **Open.**

   A new experiment is created using the setup information from the template.

   Note: To access the templates, navigate to the templates folder located at `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\templates`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.
4. In the **Experiment Properties** screen, scan the OpenArray™ Plate barcode or type the OpenArray™ Plate serial number.
5. In the **Samples** screen, do either of the following.
   - *(Recommended)* Click **Import** above the sample table, navigate to and select the OpenArray™ sample information file (CSV) that you want to use, then click **Select File**
   - In the sample table, click in a cell in the **Sample Name** column, then enter a new name.
6. From the open experiment, select **File ▸ Import Plate Setup**.
   a. Click **Browse**, navigate to and select the plate setup file that you want to use.

      MicroRNA Source File (TPF)—Corresponds to the plate setup file associated with miRNA OpenArray™ Plate formats.

      Note: Use the EDT template file supplied with the QuantStudio™ 12K Flex Software. See “Plate setup file (TPF)” on page 167.

   b. Click **Select**, then click **Start Import**.

c. If your experiment already contains plate setup information, the software asks whether to replace the plate setup with the data from the file. Click **Yes** to replace the plate setup information.

7. Select **File** ▸ **Save As**, enter a file name, select a location for the experiment file (EDS), then click **Save.**

8. Click **Start Run.**

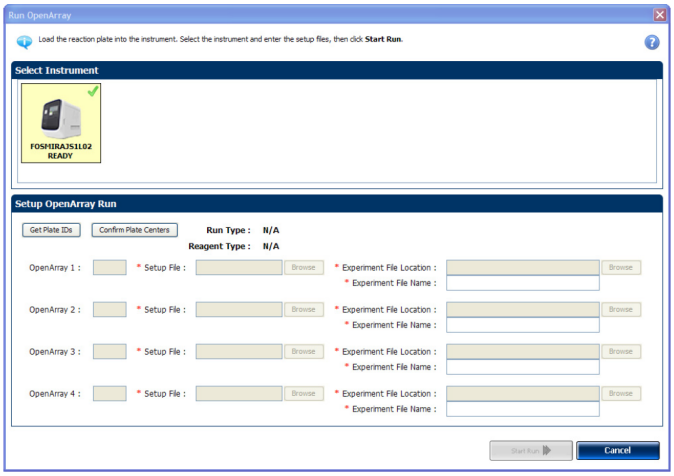
Use an OpenArray™ Plate setup file

The OpenArray™ AccuFill™ Software integrates the sample information into an OpenArray™ Plate setup file (TPF). You can save the newly created Loaded_tpf files to the OpenArray™ Plate File Input Folder that you selected in the **Preferences** dialog box of the OpenArray™ AccuFill™ Software. Configure this location in the QuantStudio™ 12K Flex Software preferences to upload the integrated plate setup file into the QuantStudio™ 12K Flex Software, then run the file.

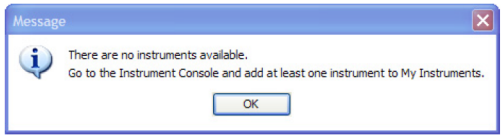
Note: You can import a CSV file into the QuantStudio™ 12K Flex Software before starting the run, or after the run is complete.

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.

2. In the **Home** tab, in the **Run** menu, screen, click **OpenArray**.



Note: Be sure to add an instrument to **My Instruments** in the **Instrument Console** before you run experiment. See “Access the Instrument Console” on page 197. If no instrument is selected, a warning appears.



3. In the **Select Instrument** pane, select the instrument that you want to use to run the experiment.

4. Complete the **Setup OpenArray Run** pane.
   - Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate formats that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray™ Plate formats that you want to run. For each plate image in the **Confirm OA Plate Centers** dialog box, click **Continue** if the red box is aligned to the center of the plate. If the box is not in the center of the plate, click **OK**, eject the carrier, rearrange the plates, then click **Get Plate IDs**
   - *(Optional)* Click **Browse**, then navigate on your computer to and select the appropriate OpenArray™ Plate setup files (TPF).

     Note: When the setup file is selected, **Experiment File Location** and **Experiment File Name** are populated. To set the default **Experiment File Location**, go to **Tools** ▸ **Preferences** ▸ **OpenArray** ▸ **Experiment Folder**. In the **Setup OpenArray Run** pane, to select another location for the experiment file, then click **Browse**. You can also enter an experiment file name of your choice.

   Depending on the number of OpenArray™ Plate formats loaded in the instrument, the barcode of those plates is populated.

   IMPORTANT! If the QuantStudio™ 12K Flex Real-Time PCR Instrument does not detect a barcode, repeat the barcode read.

5. Click **Start Run.**

Start a run from the instrument touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument.

- From existing experiments. See “Start a run on the instrument touchscreen from an existing experiment” on page 202.
- From templates. See “Start a run on the instrument touchscreen from a template” on page 203.
- From shortcuts. See “Start a run on the instrument touchscreen from a shortcut” on page 203.

Start a run on the instrument touchscreen from an existing experiment

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.

   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.

2. In the **Home** screen, touch **Run OpenArray Plates**.

   The instrument retrieves the barcodes and scans for existing experiments with the same barcodes.

3. If experiments with the same barcode cannot be found, touch **Source Input** to select a template to use.

4. Touch **(Start Run Now)** to start the run.

   IMPORTANT! If the instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the instrument touchscreen. Do not proceed if a barcode is not detected by the instrument.

Start a run on the instrument touchscreen from a template

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.

   Note: If the touchscreen is not at the **Main Menu** screen, touch **(Home).**

2. In the **Home** screen, touch **(View Templates).**
3. In the **View Templates** screen, touch **(Folders)** to display the folders containing the template files.
4. Touch any of the folders to display the templates in that folder.
5. In the **View Templates** screen, select the desired template, then touch **(Start Run).**
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
6. Touch **(Start Run Now)** to start the run.

Start a run on the instrument touchscreen from a shortcut

1. Touch the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen to activate it.
   If the touchscreen is not at the **Main Menu** screen, touch **(Home)**.
2. In the **Home** screen, touch any of the shortcuts that have been set to an OpenArray™ template.
   The instrument retrieves the barcodes and creates new experiments based on the template for each plate found.
3. Touch **(Start Run Now)** to start the run.

(Optional) Monitor experiments

You can monitor an OpenArray™ experiment run in three ways.

- From the **Run** screen of the QuantStudio™ 12K Flex Software, while the experiment is in progress. See “Monitor an experiment from the software Run screen” on page 204.
- From the **Instrument Console** of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen). See “Monitor an experiment from the Instrument Console” on page 204.
- From the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, in the same way that you run the experiment. See “Start a run from the instrument touchscreen” on page 202.

Note: If there is loss of connection during an experiment, remove and then add the instrument to the **My Instruments** list, or restart the QuantStudio™ 12K Flex Software. You may then resume monitoring the experiment.

Monitor an experiment from the software Run screen

To monitor the **Amplification Plot** of the experiment that you are running, in the QuantStudio™ 12K Flex Software, from the **Run Experiment Menu**, click **Amplification Plot**.

Monitor an experiment from the Instrument Console

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. In the **Home** tab, in the **Tools** menu, click **Instrument Console**.
3. In the **Instrument Console**, select the icon of the instrument that you are using to run the experiment, then click **Manage Instrument** or double-click the instrument icon.

   You must add the instrument to a group that can be monitored before you can manage it. See “Access the Instrument Console” on page 197.
4. In the **Instrument Manager**, click **Monitor Run** to access the **Run** screen.

You can view the progress of the run in real time from the **Run** screen. During the run, periodically view the **Amplification Plot** (see “Monitor the Amplification Plot” on page 204) available from the software for potential problems.

Task	Action
To stop the run	• In the software, click **STOP RUN**. • In the **Stop Run** dialog box, click one of the following: – **Stop Immediately** to stop the run immediately. – **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold. – **Cancel** to continue the run.
To view amplification data in real time	Select **Amplification Plot**. See “Monitor the Amplification Plot” on page 204.

Monitor the Amplification Plot

To view data in the **Amplification Plot**, click **Amplification Plot** from the **Run Experiment** menu, select the **Plate Layout** tab, then select the wells to view. You can view up to four OpenArray™ experiments per run. Click the different tabs to view the **Amplification Plot** for each experiment.

Use the **Amplification Plot** to view sample amplification as the instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the **Amplification Plot** shows the data for the wells selected in the **Plate Layout** tab. The plot contrasts normalized dye fluorescence (ΔRn) and cycle number.

The **Amplification Plot** is useful for identifying and examining abnormal amplification, including:

- Increased fluorescence in negative control wells.
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

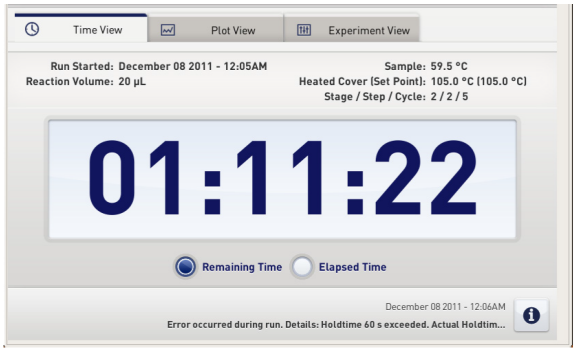
Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the *QuantStudio™ 12K Flex Software Help* (click or press **F1).**

Monitor an experiment from the instrument touchscreen

The QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the barcodes (or Plate IDs) of the TrueMark™ OpenArray™ Plate formats for the run, the date and time at which the run started, the time remaining in the run, and other information.

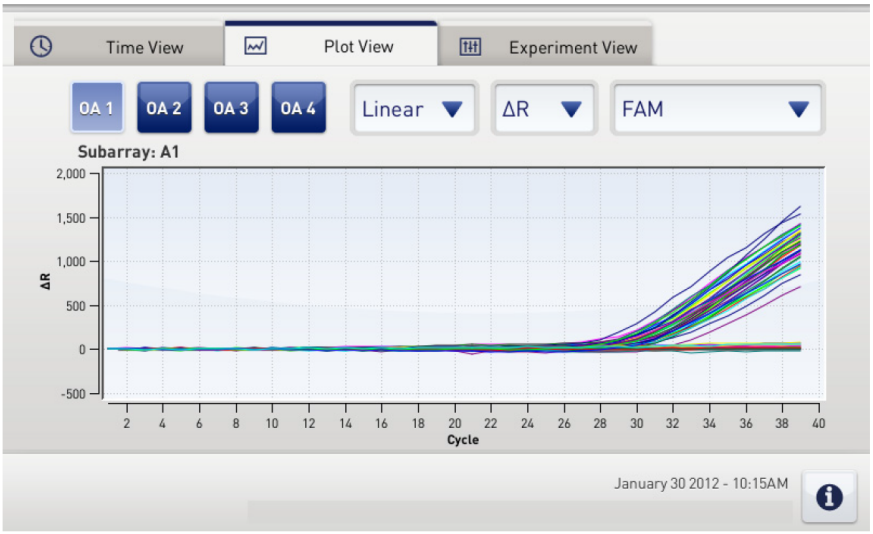
Task	Action
To display the experiment names in the run	Touch **Experiment View**.
To show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
To display the time elapsed and the time remaining in the run	Touch the **Time View** tab, then touch **Experiment View** to return to the previous screen.
To stop the run	Touch **STOP** to stop the run immediately.
View the Events Log	Touch the status bar to display the events log.

Time view



Plot view

The **Plot View** displays the **Amplification Plot** in real time.



You can change the plot using the drop-down menus that are available on the **Plot View** tab.

Touch…	To…
ΔR ▼	Change the data displayed on the y-axis. Select either **R** (reporter) or **ΔR** (baseline-corrected reporter). For OpenArray™ experiments, the data is not normalized.
FAM ▼	Change the reporter dye displayed in the plot. Only the dyes that are used in your experiment are shown.
ℹ	View the run events that occurred during the run. Touch ℹ again to close the event list.

Unload the OpenArray™ Plate from the instrument

Completed runs

After a run is complete, perform the next step.

- If you started the run from the QuantStudio™ 12K Flex Software, close the run and reopen the EDS file to display the **Amplification Plot** (for gene expression experiments) or the **Allelic Discrimination Plot** (for genotyping experiments). See Chapter 18, "Analyze the experiment results".
- If you started the run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, see "(Optional) Transfer experiment results" on page 207.

Unload the instrument

When the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen displays the **Home** screen, unload the TrueMark™ OpenArray™ Plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block temperature can reach 100°C. Allow it to cool to room temperature before handling.

1. Touch on the instrument touchscreen, or click **Open Door** in the **Instrument Console** of the QuantStudio™ 12K Flex Software.
2. Remove the OpenArray™ Plate from the plate adapter.
3. Touch or click **Close Door** to retract the plate adapter back into the instrument.

   If the instrument does not eject the plate, remove the plate.

   a. Power off the instrument.

   b. Wait for 15 minutes, then power on the instrument and eject the plate.

   c. If the instrument does not eject the plate, power off and unplug the instrument, then open the access door.

   d. Wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.

(Optional) Transfer experiment results

If you started a run from the QuantStudio™ 12K Flex Real-Time PCR Instrument touchscreen, transfer the experiment data to the computer for analysis after the run is complete. You can transfer the experiment results two ways.

- "Download the experiment from the instrument over the network" on page 208
- "Transfer the experiment from the instrument to the computer with a USB drive" on page 208

Download the experiment from the instrument over the network

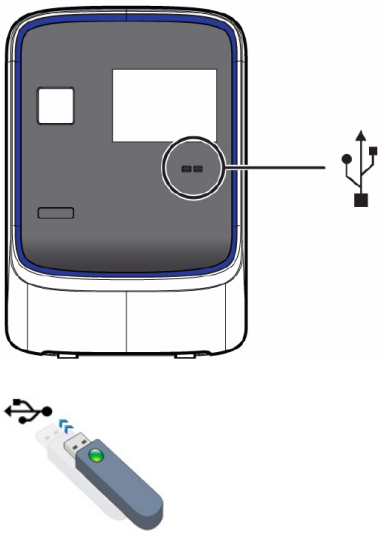
1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. From **My Instruments** list, select the instrument icon of the QuantStudio™ 12K Flex Real-Time PCR Instrument that you just used to run the experiment.
3. Click **Manage Instrument**.
4. In the **Instrument Manager**, click **Manage Files**.
5. In the **Experiments** panel, select the experiment to download, then click **Download**.
6. In the **Save** dialog box, select the folder to hold the experiment results, then click **Save**, then navigate to the experiments folder location.

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

Transfer the experiment from the instrument to the computer with a USB drive

1. If not already connected to the QuantStudio™ 12K Flex Real-Time PCR Instrument, connect a USB drive to the USB port.



2. Touch the instrument touchscreen to activate it.
3. If the touchscreen is not at the **Main Menu**, touch **(Home)**.
4. In the **Main Menu**, touch **(Collect Results)** to save the data to the USB drive.

5. Select one or multiple experiments (by touching them). Then touch **(Save to USB)** to copy selected experiments to the USB drive.

   Note: If your instrument cannot find the USB drive, remove the USB drive, then try again. If the instrument still does not recognize the USB drive, try another USB drive.

6. Touch **(Home)** to return to the **Main Menu**.

7. Remove the USB drive from the instrument, then connect it to one of the USB ports on your computer.

8. In the computer desktop, use the Windows™ Explorer to open the USB drive.

9. Copy the example experiment file to:

   `<...>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\`

   where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.

18 Analyze the experiment results

Analyze the run data

This section includes general information and instructions about how to analyze the example experiments provided with the QuantStudio™ 12K Flex Software. For specific instructions see “Analyze microRNA experiment results” on page 218.

View the data from the EDS file. If the default analysis settings are not suitable for your experiment, you can modify the data. You can also modify the project files, publish data, and export data for downstream analysis using the ExpressionSuite™ Software and TaqMan™ Genotyper Software.

View the results

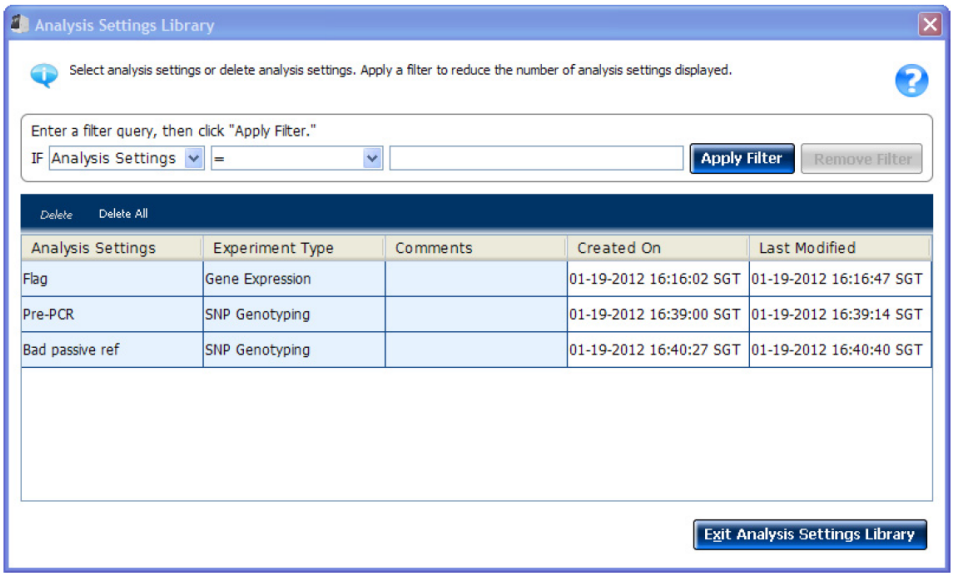
After an experiment run, you close the run and reopen the EDS file to display the **Amplification Plot** (for gene expression or miRNA experiments) or the **Allelic Discrimination Plot** screen (for genotyping experiments).

Note: For auto-analysis of data, after a run, go to **Tools** ▸ **Preferences** ▸ **Experiment** and select the **Auto Analysis** checkbox. By default, **Auto Analysis** is always enabled. To reanalyze the data, select all the wells in the plate layout, then click **Analyze**

Change analysis settings

Analysis settings are different for each experiment type. If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment. You can save the changed analysis settings to the **Analysis Settings Library** to use them in other experiments.

Use the **Analysis Settings Library** dialog box to apply a filter to reduce the number of setting protocols that are displayed. Access the **Analysis Settings Library** from the **Tools** menu.



1. From the **Experiment Menu**, select **Analysis**.
2. On the **Analysis** screen, click **Analysis Settings**.
3. In the **Analysis Settings** dialog box, change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes to the **Analysis Settings Library**.

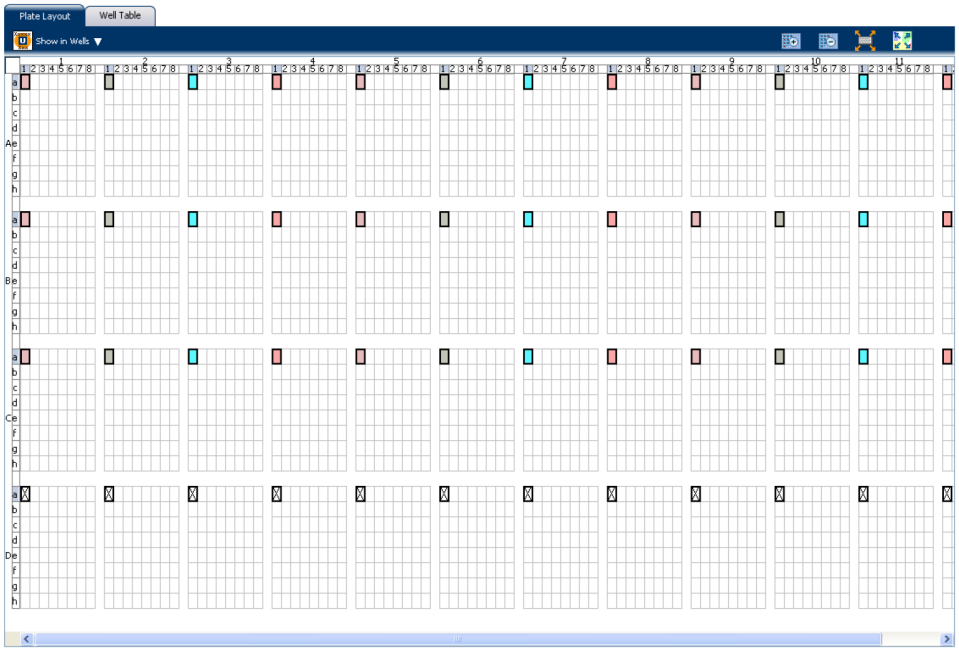
To import the analysis settings that you have previously saved to the **Analysis Settings Library**, in the **Analysis Settings** dialog box, click **Load from Library**.

Display wells

To display specific wells in the analysis plots, select the wells in the **Plate Layout** tab.

- To select specific well type, use the **Show in Wells** drop-down menu.
  - For gene expression and miRNA experiments, select **Sample Color** or **Target Color**.
  - For genotyping experiments, select **Sample Color** or **Assay Color**.
- To select a single well, click the well in the **Plate Layout** tab.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the **Plate Layout** tab.
- To select all wells, click the upper left corner of the **Plate Layout** tab.

This example shows the **Plate Layout** tab for a gene expression experiment.



Expand view of a plot or wells

- Click to expand the plot view, on the left side of the screen.
- Click to expand the **Targets**, **Samples**, and **Subarrays** view on the right side of the screen.
- Click to expand the **Plate Layout** or **Well Table** view on the lower half of the screen.
- Click to expand the Plots and **Targets**, **Samples**, and **Subarrays** view on the upper half of the screen.
- Click to expand and collapse the **Plot** or **Plate Layout** view.

Edit plot properties

Use the **Plot Properties** dialog box on the **Analysis** screen to edit plot settings, such as the font and color of the plot text, and the labels on the x-axis and y-axis.

1. Click on the **Analyze** screen (the icon appears above the plot).
2. In the **Plot Properties** dialog box, edit the settings under the **General**, **X Axis**, and **Y Axis** tab.
   - Click the **X Axis** tab to edit the x-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
   - Click the **Y Axis** tab to edit the y-axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display.
3. Click **OK**.

Publish the analyzed data

Task	Click
Save a plot as an image file.	
Print a plot.	
Copy a plot to the clipboard.	
Print a report.	
Export data.	

Task	Go to	Then
Print the plate layout.	**File** ▸ **Print**	Select the background color, then click **Print**.
Create slides.	**File** ▸ **Send to PowerPoint**	Select the slides for your presentation, then click **Create Slides**.
Print a report.	**File** ▸ **Print Report**	Select data for the report, then click **Print Report**.

(Optional) Export an experiment

Use the **Export** feature to export experiment data from the QuantStudio™ 12K Flex Software. You can export experiment data in the QuantStudio™ 12K Flex format (TXT or XLSX) or RDML format (no file selection).

You can export the following experiment data in a comma-separated file format (CSV).

- Sample setup data
- Raw data
- Amplification data
- Multicomponent data
- Results

You can also export plate images collected during the run as TIF files to use them for troubleshooting. To export plate images, first create an export folder on your hard drive. In the **Export** screen, click **Browse**, navigate to the folder that you created, then click **Export QC Images**. You can view the images using a public domain software program such as ImageJ (**http://rsb.info.nih.gov/ij/**). See *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832) for more information on QC Images.

If you selected the **Auto Export** option before running an experiment, the data is automatically exported to the location that you specified. If you did not select the **Auto Export** option, the analyzed data is not exported automatically.

1. Open the experiment file that contains the data to export, then from the **Experiment Menu**, click **Export.**

2. Select the format for exported data.
   - **QuantStudio 12k Flex format**—Supports TXT and XLSX data.
   - **RDML format** (Real Time Data Markup Language)—Supports only XML type of data.

3. Select to export all data in one file or in separate files for each data type.
   - All data types are exported in **one file.**
     - If you select the XLS format, a worksheet is created for each data type.
     - If you select the TXT format, the data are grouped by data type.
   - Each data type is exported in a **separate file**. If you select three different data types (for example, Results, Amplification, and Multicomponent) to export, three separate files are created. From **File Type**, select the export file type (XLS, XLSX, or TXT) to export.

     Note: You cannot use an exported XLS or XLSX file when importing plate setup information.

4. Select **Yes** to include or **No** to exclude bookmarked data from analysis in the export set.

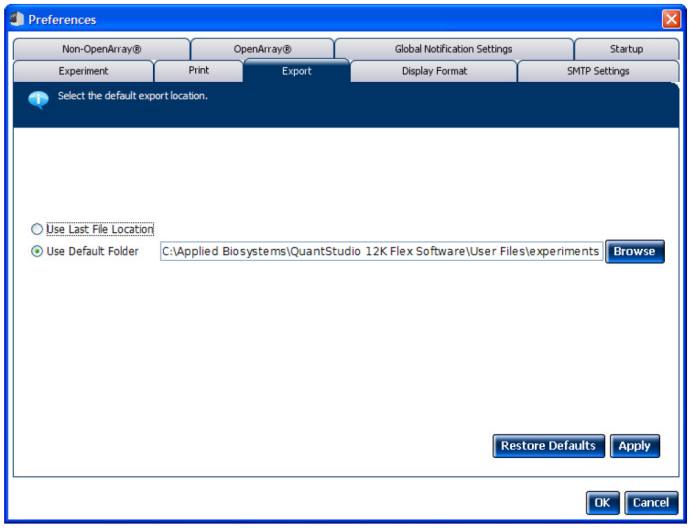
   The **Filter Bookmark Data** feature lets you include only the data bookmarked during analysis in the export set.

5. *(Optional)* Select the **Open file(s) when export is complete** checkbox to open the file when export is complete.

6. Enter a file name and location.
   a. In **Export File Name**, enter a name for the export file.

b. In **Export File Location** accept the default, or click **Browse** if you do not want to save the export file in the default export folder.

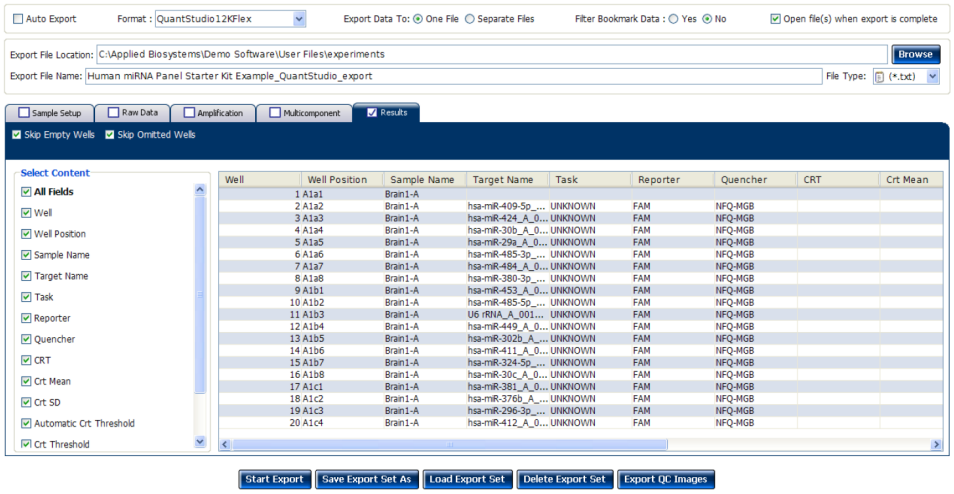
Note: To set up the export file location, go to **Tools** ▸ **Preferences**, then select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** checkbox.



7. Select the type of data to export.

Type of data	Data to export
Sample setup	Well, sample name, sample color, and target name of samples in the plate.
Raw data	Raw fluorescence data for each filter, for each cycle.
Amplification data	Amplification results, such as dC_T values, R, or ΔR.
Multicomponent data	Fluorescence data for each dye, for each cycle.
Results	Results information, such as C_T values, Rn, or calls. Results data are not available for export until the run status is complete and the data are analyzed.

The completed **Export** screen for a microRNA experiment should look like this.



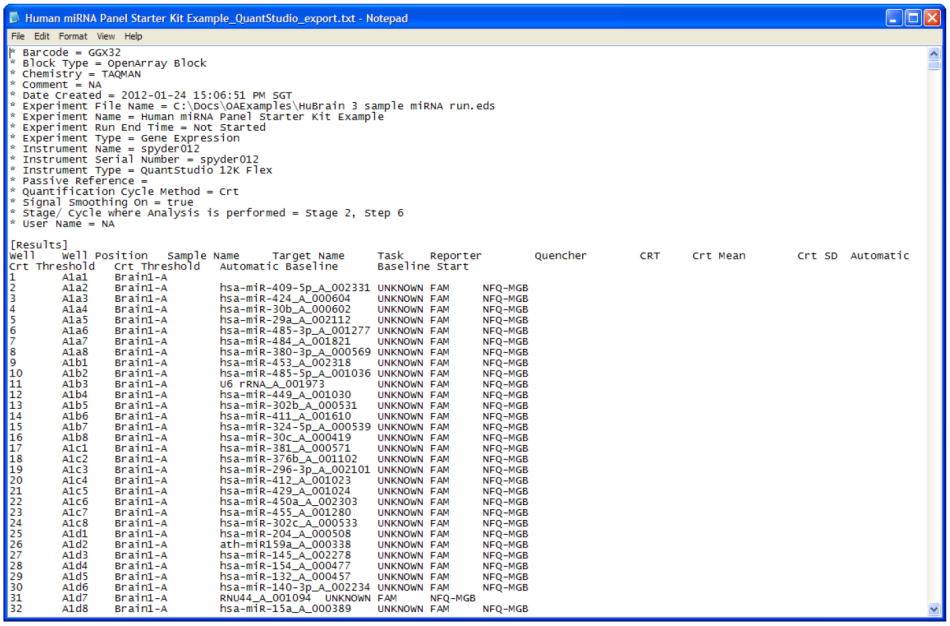
8. *(Optional)* After you have defined the export properties or after moving the table headings order, you can save those export settings as an export set by clicking **Save Export Set As**. Later you can import the heading order into another file by clicking **Load Export Set**. You can also delete export settings by clicking **Delete Export Set**.

9. *(Optional)* Click **Export QC Images** to export quality control (QC) images in experiment files (EDS).

   QC images include calibration images, a barcode image, and images taken during PCR. You can view the images to check sample loading and assay spotting. View PCR images to validate your data.

10. Click **Start Export**.

    The exported file, when opened in Notepad, should look like this.



Perform downstream analysis (secondary analysis)

You can perform downstream analysis of experiments that have been run on any real-time PCR system with the ExpressionSuite™ Software and TaqMan™ Genotyper Software. Use the ExpressionSuite™ Software and TaqMan™ Genotyper Software to efficiently analyze, edit, and conduct a study of a large number of gene expression or miRNA experiments and genotyping experiments, respectively.

- Import data from the QuantStudio™ 12K Flex Software project files, then manage the data in a database.
- Search the database for assays using specific search criteria.
- Easily view data in a variety of ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data.

ExpressionSuite™ Software and TaqMan™ Genotyper Software are available for download from the Thermo Fisher Scientific website.

For more information about the TaqMan™ Genotyper Software, see the *TaqMan™ Genotyper Software Getting Started Guide* (Pub. No. 4448637).

Analyze microRNA experiment results

In this section, you use the example experiment files provided with the QuantStudio™ 12K Flex Software to analyze the experiment results.

Workflow

Analyze microRNA experiment results

- **Access the example experiment file** (page 219)
- **Assess amplification results on the Amplification Plot** (page 219)
- **Identify well problems using the Well Table** (page 221)
- **Confirm accurate dye signal using the Multicomponent Plot** (page 222)
- **Determine signal accuracy using the Raw Data Plot** (page 223)
- **Review the flags in the QC Summary** (page 224)
- **(Optional) Adjust parameters for reanalysis of your own experiments** (page 226)
- **(Optional) Export the analyzed data** (page 229)

Access the example experiment file

1. Double-click (QuantStudio™ 12K Flex Software shortcut) to start the software.
2. In the **Home** tab, in the **Experiment** menu, click **Open**, then browse to the Gene Expression examples folder.

   `<...>:\Program Files (x86)\Applied Biosystems\QuantStudio 12K Flex Software\examples\Gene Expression`, where `<...>` is the installation drive. The default installation drive is `C:` if the software is installed by the customer. The default installation drive is `D:` if the software is installed by a Thermo Fisher Scientific field service engineer.
3. Open the `Human miRNA Panel Starter Kit Example.eds` file.

Assess amplification results on the Amplification Plot

The **Amplification Plot** displays amplification of all samples in the selected wells. View the **Amplification Plot** for the example experiment to evaluate the quality of the amplification curve and to check for outliers.

Three plots are available.

- **ΔR vs Cycle**—ΔR is the magnitude of fluorescence signal generated by the reporter at each cycle during the PCR amplification. This plot displays ΔR as a function of cycle number. Use this plot to identify and examine irregular amplification and to view C_{RT} values for the run.
- **R vs Cycle**—R is the fluorescence signal from the reporter dye. This plot displays R as a function of cycle number. Use this plot to identify and examine irregular amplification.
- **C_{RT} vs Well**—C_{RT} is the PCR cycle number at which the fluorescence meets the threshold in the amplification plot. This plot displays C_{RT} as a function of well position. Use this plot to locate outlying amplification (outliers).

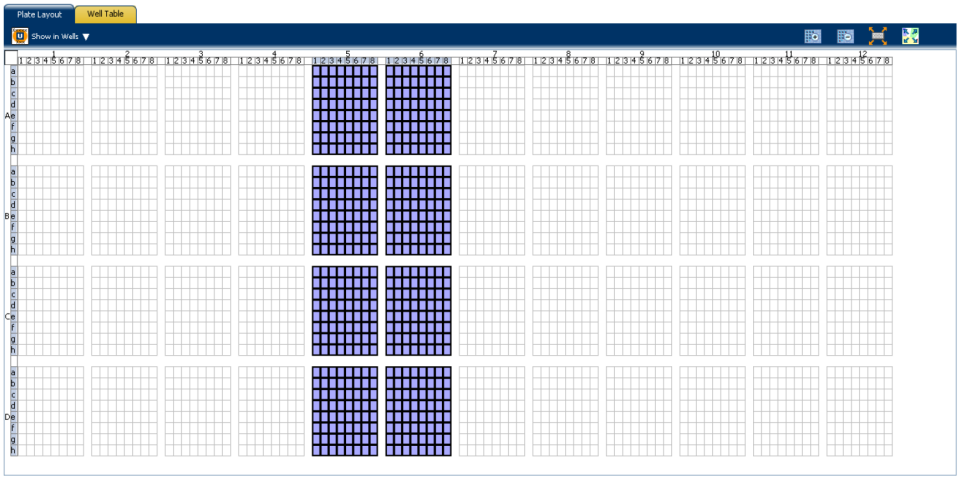
Each plot can be viewed as a linear or log10 graph type.

View the Amplification Plot

1. From the **Experiment Menu**, select **Analysis ▸ Amplification Plot**.

   If no data are displayed, click **Analyze**.
2. Display the Brain2-A wells in the **Amplification Plot**.
   a. Click the **Plate Layout** tab.
   b. Click **Show in Wells ▸ Sample Color**.

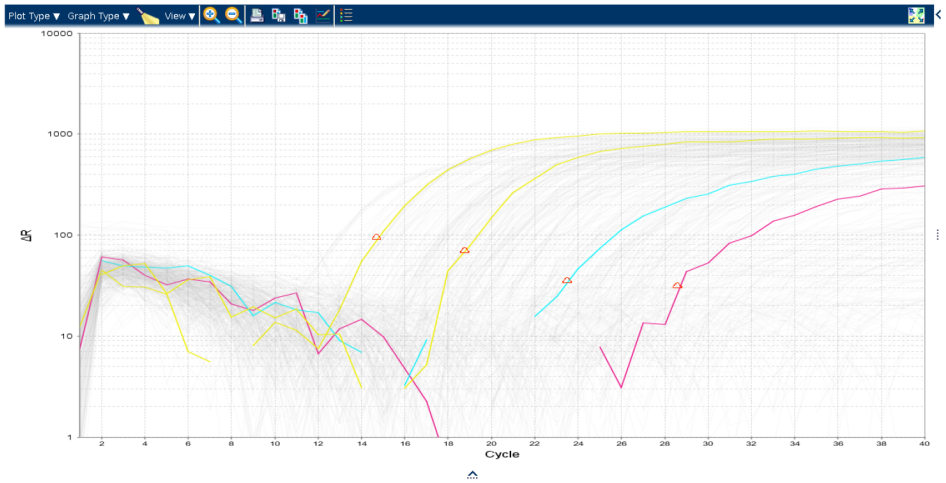
   The **Plate Layout** screen should look like this.



3. Update the **Amplification Plot**.

Menu	Selection
Plot Type	**ΔR vs Cycle** (default)
Graph Type	**Log** (default)
View	**Target Color** (default) **Show Unselection** (default)

4. View the C_{RT} values.
   a. Click **View** ▸ **Show C_{RT}**.
   b. Verify that the C_{RT} value reported matches its occurrence (the triangle icon) on the plot.



5. Repeat from steps 2 for the Brain1-B, Brain3-B, Brain2-B, Brain3-A, and Brain1-A wells.

Identify well problems using the Well Table

The **Well Table** displays the following information for each well in the reaction plate.

- The sample name, target name, task, and dyes.
- The calculated threshold cycle (C_{RT}) and quantity values.
- Flags.

Example experiment values and flags

Review the **Well Table** to evaluate the C_{RT} precision of the replicate groups and AMPSCORE values.

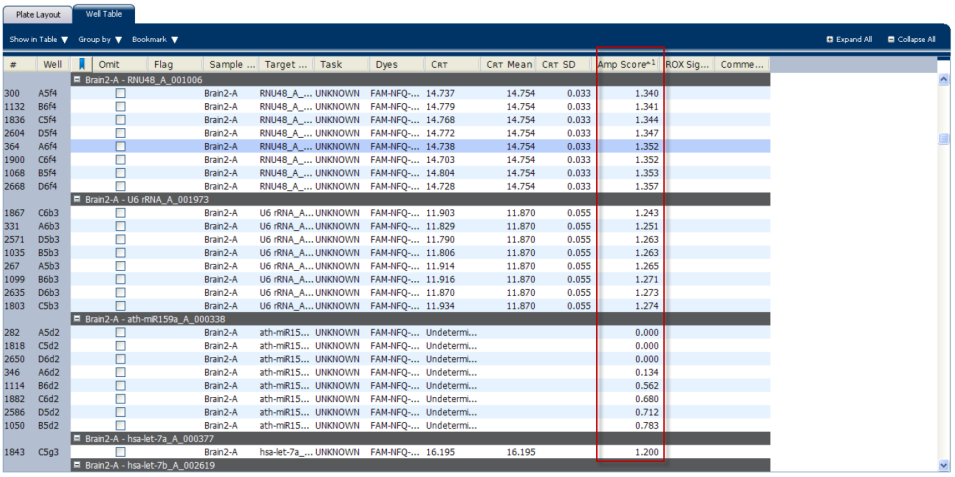
The software produces the AMPSCORE flag if the amplification in the linear region is below a certain threshold, corresponding to the score set in the analysis settings. For robust amplification, AMPSCORE values should be ≥1.24.

View the Well Table

To show or hide columns in the **Well Table**, select or deselect respectively the column name from the **Show in Table** menu.

1. From the **Experiment Menu**, select **Analysis** ▸ **Amplification Plot**, then click the **Well Table** tab.
2. From the **Group By** menu, select **Replicate.**
3. Look at the **Amp Score** column to check for amplification in the linear region of the replicate groups.

   In the example experiment, the **Amp Score** have the expected value of ≥1.24.



Assess the well table in your own experiments

When you analyze your own microRNA experiment, look for **Amp Score** values in the replicate groups (**Amp Score** values). If needed, omit outliers.

Confirm accurate dye signal using the Multicomponent Plot

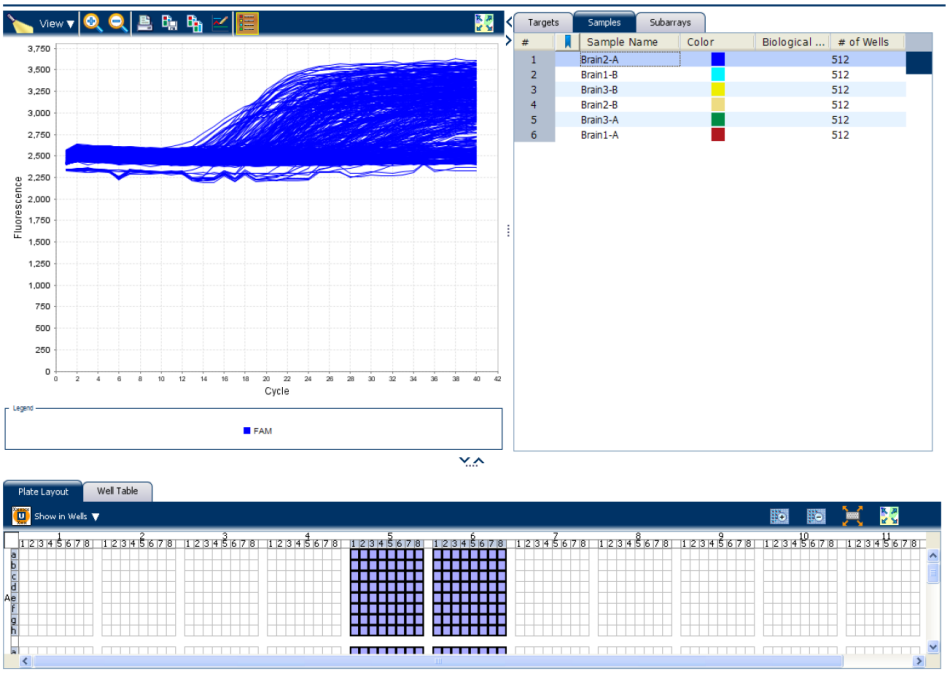
The **Multicomponent Plot** displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

In the microRNA example experiment, you review the **Multicomponent Plot** for:

- FAM™ dye (reporter)
- Spikes, dips, and/or sudden changes
- Amplification in the negative control wells

View the Multicomponent Plot

1. From the **Experiment Menu**, select **Analysis** ▸ **Multicomponent Plot**.
   If no data are displayed, click **Analyze**
2. Display the unknown and standard wells one at a time in the **Multicomponent Plot**.
   a. Click the **Plate Layout** tab.
   b. Select one well in the plate layout; the well is shown in the **Multicomponent Plot**.
      If you select multiple wells, the **Multicomponent Plot** displays the data for all selected wells simultaneously.
3. Click **View** ▸ **Dye Color**.
4. Click **Show a legend for the plot** (default).
   This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.
5. Check the FAM™ dye signals.
   In the microRNA example experiment, the FAM™ dye signal increases throughout the PCR process, indicating normal amplification.



Tips for confirming dye accuracy in your own experiment

When you analyze your own experiment, look for these results.

- Reporter dye—The reporter dye fluorescence level should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- Irregularities in the signal—There should not be any spikes, dips, and/or sudden changes in the fluorescent signal.
- Negative Control wells—There should not be any amplification in the negative control wells.

Determine signal accuracy using the Raw Data Plot

The **Raw Data Plot** displays the raw fluorescence signal (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

In the microRNA example experiment, you review the **Raw Data Plot** screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

View the Raw Data Plot

1. From the **Experiment Menu**, select **Analysis ▸ Raw Data Plot**.

   If no data are displayed, click **Analyze**.

2. To display all wells in the **Raw Data Plot**, click the upper left corner of the plate layout in the **Plate Layout** tab.

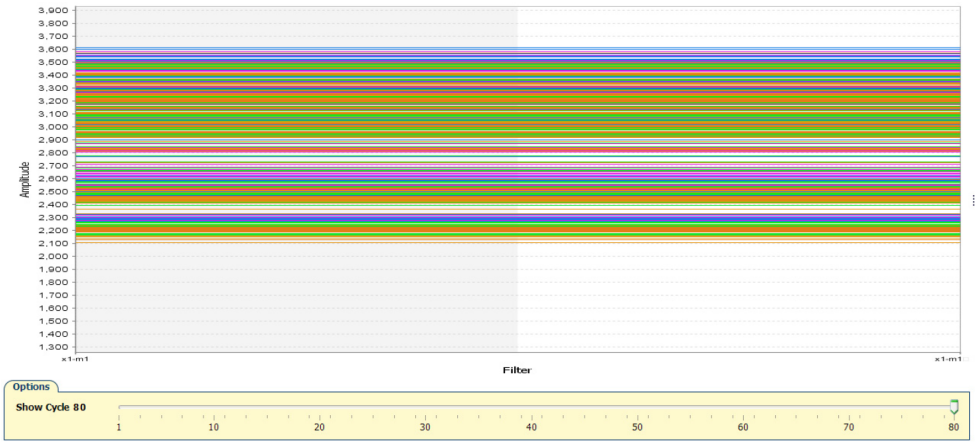
3. Click **Show a legend for the plot** (default).

   The legend displays the color code for each row of the reaction plate (see the legend in the **Raw Data Plot**).

This is a toggle button. When the legend is displayed, the button changes to **Hide the plot legend**.

4. Click and drag the **Show Cycle** pointer from cycle 1 to cycle 80.

   In the example experiment, the stable increase in signal from filter 1 corresponds to the FAM™ dye filter.



Tips for determining signal accuracy in your own experiment

When you analyze your own microRNA experiment, look for the following conditions in each filter.

- Characteristic signal growth.
- No abrupt changes or dips.

Review the flags in the QC Summary

The **QC Summary** displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment.

Review the **QC Summary** in the microRNA example experiment for any flags triggered by the experiment data. There are no flags in the example experiment.

1. From the **Experiment Menu**, select **Analysis ▸ QC Summary**.

   If no data are displayed, click **Analyze**.

2. Review the **Flags Summary**.

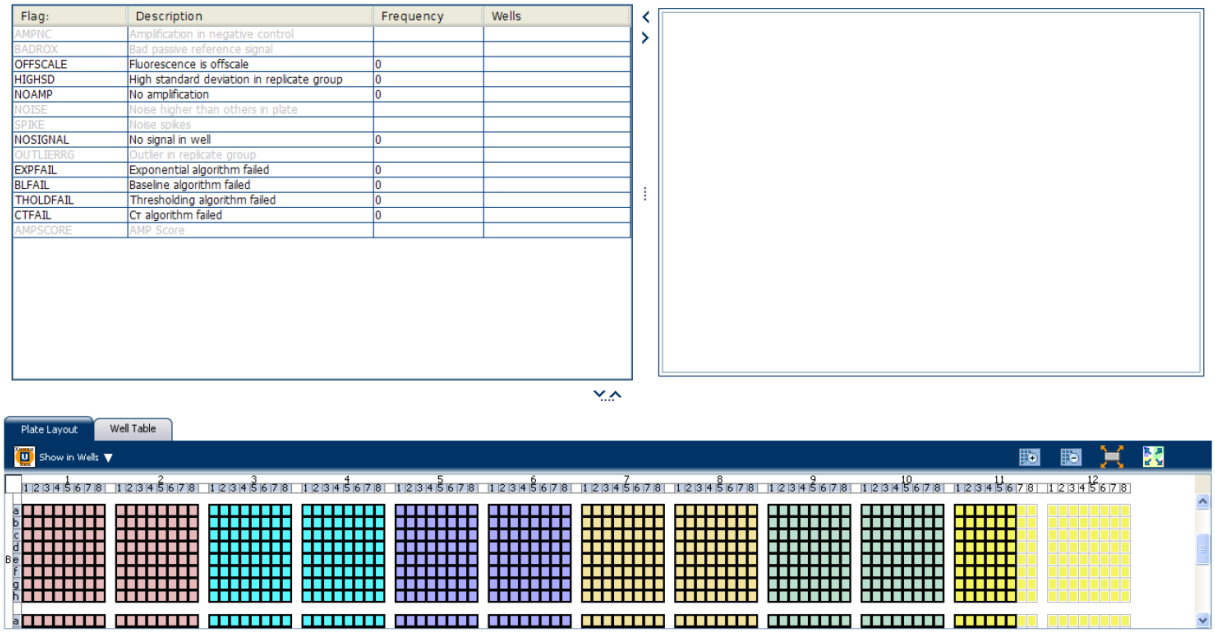
   A 0 displayed in the **Frequency** column indicates that the flag does not appear in the experiment. If the frequency is >0, the flag appears somewhere in the experiment. The well position is listed in the **Wells** column.

   In the example experiment, there are 0 flagged wells.

3. In the **Flag Details** table, click each flag with a frequency >0 to display detailed information about the flag.

4. *(Optional)* For those flags with frequency >0, click the troubleshooting link to view information about correcting the flag.

The **QC Summary** for the microRNA example experiment looks like this.



Possible flags

The flags listed below may be triggered by the microRNA experiment data.

Flag	Description
Preprocessing flag	
OFFSCALE	Fluorescence is off scale.
Primary analysis flags	
BADROX	Bad passive reference signal.
NOAMP	No amplification.
NOISE	Noise higher than others in plate.
SPIKE	Noise spikes.
NOSIGNAL	No signal in well.
EXPFAIL	Exponential algorithm failed.
BLFAIL	Baseline algorithm failed.
THOLDFAIL	Thresholding algorithm failed.
CTFAIL	C_T algorithm failed.
AMPSCORE	Amplification in the linear region is below a specific threshold, corresponding to the score set in the analysis settings.

(continued)

Flag	Description
Secondary analysis flags	
OUTLIERRG	Outlier in replicate group.
AMPNC	Amplification in the negative control.
HIGHSD	High standard deviation in replicate group.

The AMPNC, BADROX, NOISE, SPIKE, OUTLIERRG, and AMPSCORE flags, by default, are not in use for the microRNA experiment.

For the **Relative Threshold** algorithm, the EXPFAIL, BLFAIL, THOLDFAIL, and CTFAIL flags are not reported, but they appear in the **QC Summary** (by default, a 0 is displayed in the **Frequency** column for each flag).

(Optional) Adjust parameters for reanalysis of your own experiments

The **Analysis Settings** dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

If the default analysis settings in the QuantStudio™ 12K Flex Software are not suitable for your own experiment, you can change the settings in the **Analysis Settings** dialog box, then reanalyze your experiment.

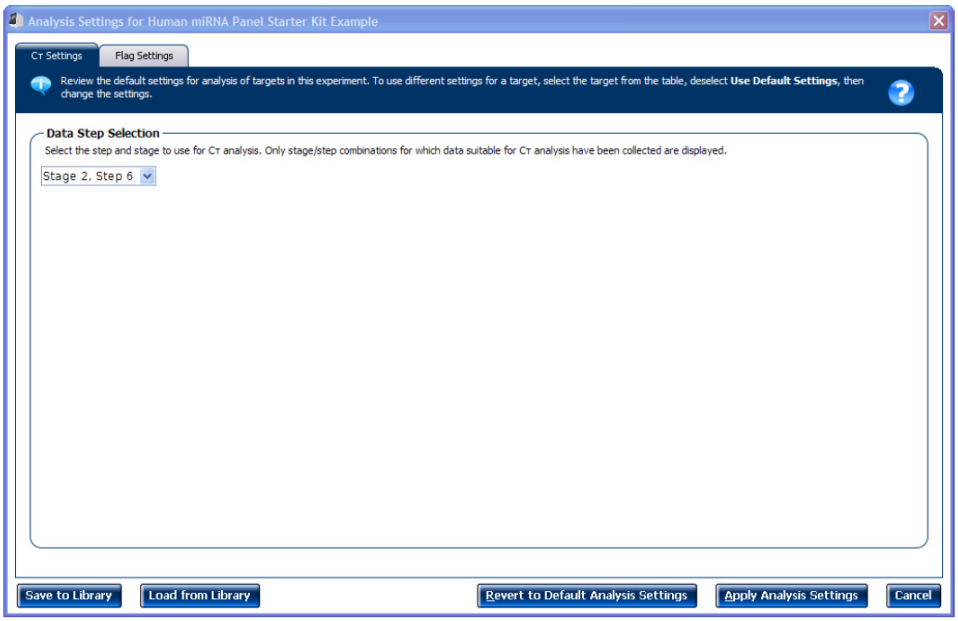
View the analysis settings

1. From the **Experiment Menu**, select **Analysis**.
2. Click **Analysis** ▸ **Analysis Settings** to open the **Analysis Settings** dialog box.

   In the example experiment, the default analysis settings are used for each tab.

   - **C_T Settings**
   - **Flag Settings**

   The **Analysis Settings** dialog box for the microRNA example experiment looks like this.



3. View and, if necessary, change the analysis settings.

   See “Adjust analysis settings” on page 227.

   You can save the changes to the analysis settings to the **Analysis Settings Library** for later use. See “Change analysis settings” on page 210.

4. Click **Apply Analysis Settings** to apply the current analysis settings.

   If needed, click **Revert to Default Analysis Settings** to return to the default analysis settings.

Adjust analysis settings

Adjust C_T settings

Use the **Data Step Selection** feature to select one stage/step combination for C_T analysis when there is more than one data collection point in the run method.

Adjust flag settings

Use the **Flag Settings** tab to adjust flag settings.

- Adjust the sensitivity so that more wells or fewer wells are flagged.
- Change the flags that are applied by the QuantStudio™ 12K Flex Software.

1. In the **Flag Settings** tab, in the **Use** column, select the checkboxes for flags to apply during analysis.

2. *(Optional)* If an attribute, condition, and value are listed for a flag, specify the setting for applying the flag.

   If you choose to adjust the setting for applying a flag, make minor adjustments as you evaluate the appropriate setting.

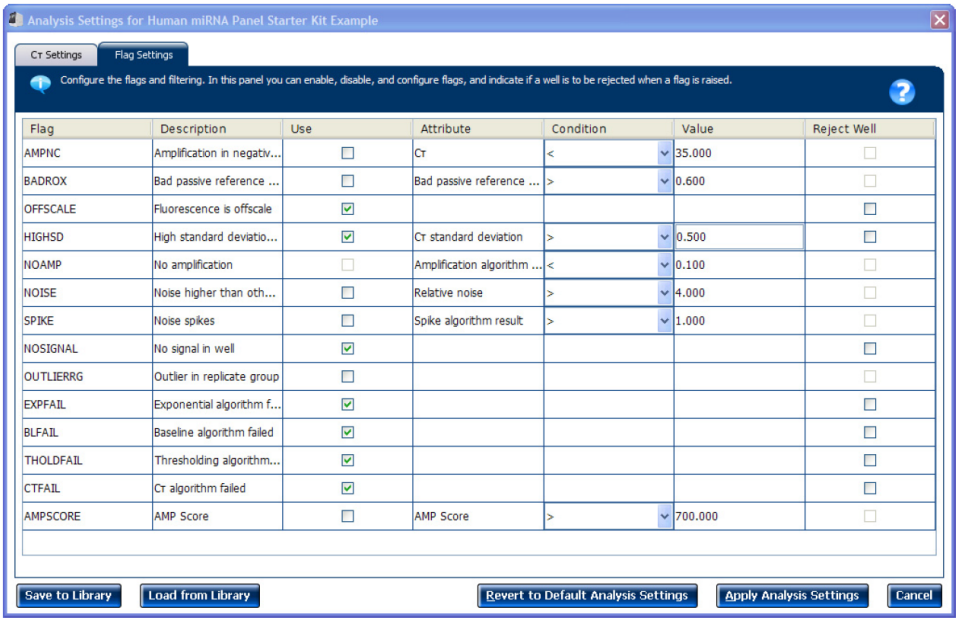
3. In the **Reject Well** column, select the checkboxes if you want the software to reject wells with the flag.

   After you have rejected the flagged wells, analysis results depend on factors such as the experiment type and flag type. For example, rejecting wells flagged by HIGHSD in experiments using the Standard Deviation calculations may change the result of C_{RT} SD. For some flags, analysis results calculated before the well is rejected are maintained.

4. Click **Apply Analysis Settings**.

   If the run status is complete, the data are reanalyzed.

   The **Flag Settings** tab looks like this:



Improve C_{RT} precision by omitting wells

Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_{RT} values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outliers can result in erroneous measurements. To enable C_{RT} precision, omit the outliers from the analysis.

In the OpenArray™ gene expression example experiment, there are 0 outliers. Use these steps to remove these wells from analysis.

1. From the **Experiment Menu**, select **Analysis ▸ Amplification Plot**.

   If no data are displayed, click **Analyze**.

2. In the **Amplification Plot**, select **Plot Type ▸ C_{RT} vs Well**.

3. Select the **Well Table** tab.

4. In the **Well Table**, identify outliers.

   a. Select **Group By ▸ Replicate.**.

b. Look for outliers in the replicate group (ensure that they are flagged).

c. Select the **Omit** checkbox next to outlying well or wells.

5. With the outlying well or wells removed from the analysis, click **Analyze** to reanalyze the experiment data.

Note: You can also omit undesirable wells in an experiment from the **Plate Layout** screen. To omit a well from the **Plate Layout** screen, right-click the well, then select **Omit**.

(Optional) Export the analyzed data

1. Open the microRNA example experiment file that you analyzed in Chapter 17, “Perform the instrument run”.

2. In the **Experiment Menu**, click **Export.**

Note: To export data automatically after analysis, select the **Auto Export** checkbox during experiment setup or before running the experiment. Auto export is unchecked for the example experiment.

3. In the top of the screen, select **QuantStudio 12K Flex format.**

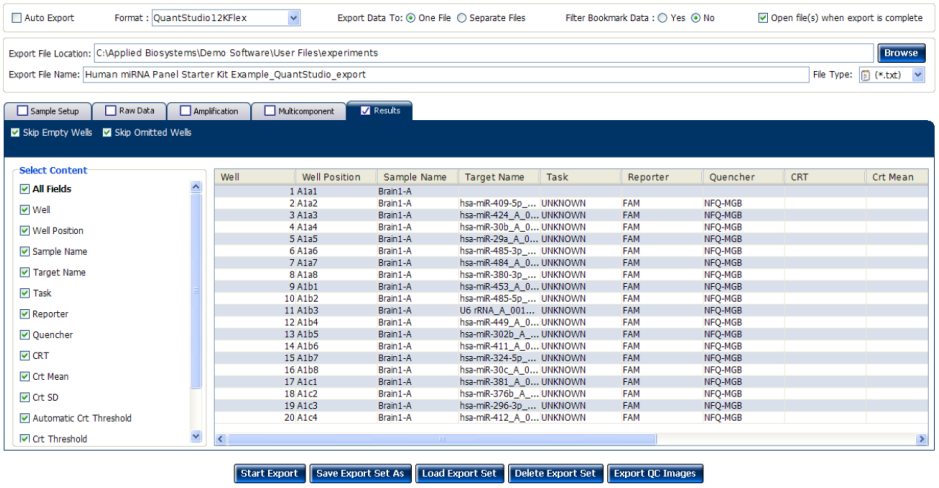
4. Complete the **Export** screen.

Field or selection	Entry
Export Data To	**One File**
Filter Bookmark Data	**No**
Export File Location	`<...>:\Applied Biosystems\QuantStudio 12K Flex Software\experiments`
Export File Name	***Human miRNA Panel Starter Kit Example_QuantStudio_export***

(continued)

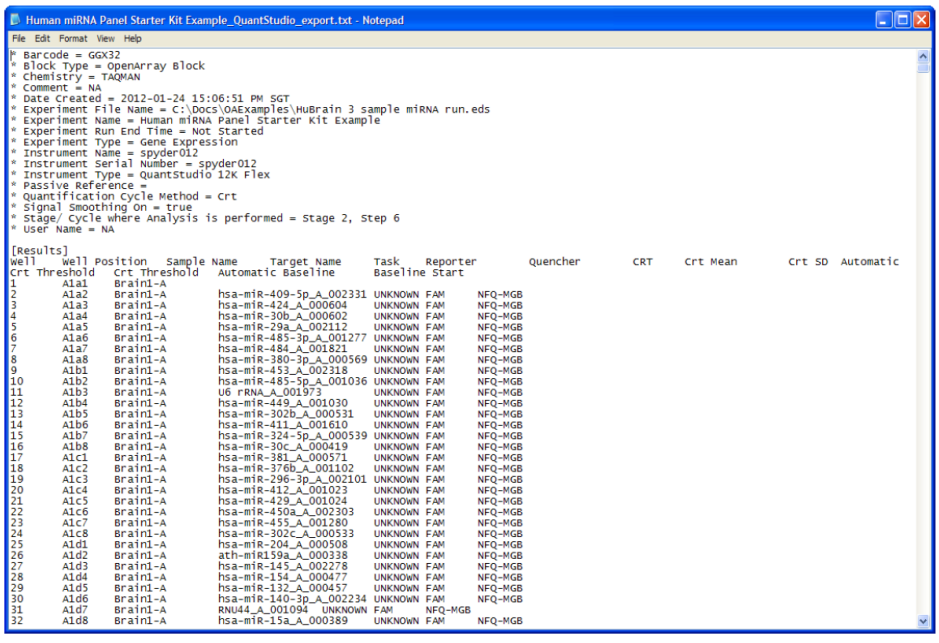
Field or selection	Entry
File Type	**(*.txt)**
Select Data to export/ Select Content	Select the **All Fields** checkbox or select a specific set of checkboxes.

The completed **Export** screen should look like this.



5. Click **Start Export**.

When opened in Notepad, the exported file should look like this.



A Ordering information

How to order

Order materials and accessories at **thermofisher.com**.

Product availability and pricing might vary according to your region or country. Online ordering through the Thermo Fisher Scientific website is not available in all countries. Contact your local Thermo Fisher Scientific representative for help.

Starter kits and other kits

Cat. No.	Kit	Storage conditions
Starter kits[1]		
4469604	QuantStudio™ 12K Flex OpenArray™ Gene Expression Starter Kit	Upon receipt, store the frozen, unopened kits at –20°C.
4469605	QuantStudio™ 12K Flex OpenArray™ Genotyping Starter Kit	
Other kits		
4469620	QuantStudio™ 12K Flex OpenArray™ Practice Kit	–20°C or ambient temperature

[1] A QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit, Part no. 4469586, is included with each experiment starter kit order.

General equipment and reagents for starter kits

Item	Source
ProFlex™ 2 × Flat PCR System	4484078
Qubit™ 2.0 Fluorometer	Q32866
Powder-free gloves	MLS
DNase-free, sterile-filtered water	MLS
RNase-free water	MLS
0.1× TE pH 8.0	MLS
Bleach (10%)	MLS
Ethanol	MLS
Lint-free wipes	MLS
Fine-tip marker	MLS
Foil seals	MLS
Razor blade	MLS
Safety glasses	MLS
Tweezers or forceps	MLS
Disposable transfer pipettes	MLS
Pipettes, P10 to P1000	MLS
Pipette tips, 10 to 1000 µL	MLS
Incubator	MLS
Centrifuge with plate adaptor	MLS
Vortexer	MLS

OpenArray™ Plate formats

Note: A QuantStudio™ 12K Flex OpenArray™ Accessories Kit (Cat. No. 4469576) is included with 10-pack array orders.

OpenArray™ Plate	Cat. No.	Storage
TaqMan™ OpenArray™ Real-Time PCR Plate with Genotyping Assays	4471113 Custom Format 16 4471114 Custom Format 32 4471115 Custom Format 64 4471116 Custom Format 128 4471117 Custom Format 192 4471118 Custom Format 256	Upon receipt, store the frozen, unopened plates at –20°C.
TaqMan™ OpenArray™ Real-Time PCR Plate with Gene Expression Assays	4471119 Custom Format 18 4471120 Custom Format 56 4471121 Custom Format 112 4471122 Custom Format 168 4471123 Custom Format 224	
TaqMan™ OpenArray™ Real-Time PCR Plate with Inventoried Gene Expression Assays	4471124 Format 18 4471125 Format 56 4471126 Format 112 4471127 Format 168 4471128 Format 224	
TaqMan™ OpenArray™ Loading Plate, QuantStudio™ 12K Flex	4471227	

Reagents

Note: For reagent shelf-life expiration date, see the package label.

Use the following reagents with the QuantStudio™ 12K Flex Real–Time PCR System.

QuantStudio™ 12K Flex reagent		Source
TaqMan™ OpenArray™ Real-Time PCR Master Mix	5 mL	4462164
	1.5 mL	4462159
TaqMan™ OpenArray™ Genotyping Master Mix	1 x 5.0 mL for 10 arrays	4404846

Consumables (accessories)

Note: For consumable shelf-life expiration date, see the package label.

Use the following consumables with the QuantStudio™ 12K Flex Real–Time PCR System.

QuantStudio™ 12K Flex consumable		Part number
OpenArray™ AccuFill™ System Tips	Box of 384	4457246
	10 boxes of 384	4458107
OpenArray™ 384-well Sample Plate	10 plates	4406947
OpenArray™ 384-Well Sample Plates, Barcoded	10 plates	4453929
OpenArray™ 384-Well Plate Seals	10 seals	4469876

B

Plate information

IMPORTANT! Be sure to track where the samples are in each sample plate. For each sample plate, we recommend creating a sample information file (CSV) in the OpenArray™ Sample Tracker Software.

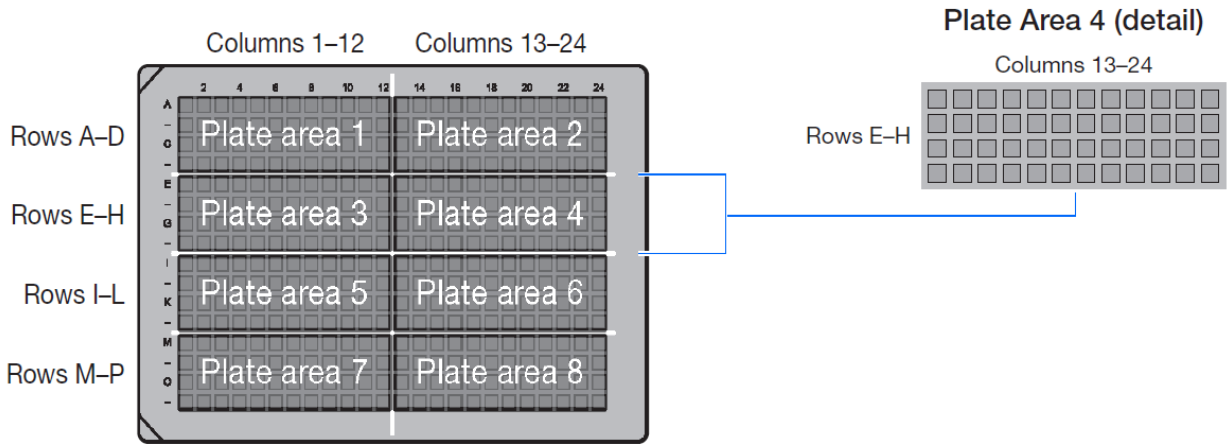
MicroAmp™ Optical 96-Well Reaction Plate

The MicroAmp™ Optical 96-Well Reaction Plate is a 96-well reaction plate for thermocycling. The 96-well reaction plates are used for nucleic acid sample preparation and to transfer the nucleic acid samples to an OpenArray™ 384-well Sample Plate.

A non-optical 96-well reaction plate can also be used.

OpenArray™ 384-well Sample Plate

The OpenArray™ 384-well Sample Plate is divided into eight areas. Each area is 12 wells × 4 wells (48 wells). During each load, the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System transfers samples from one area of the OpenArray™ 384-well Sample Plate to a single OpenArray™ Plate (see “OpenArray™ Plate” on page 236).

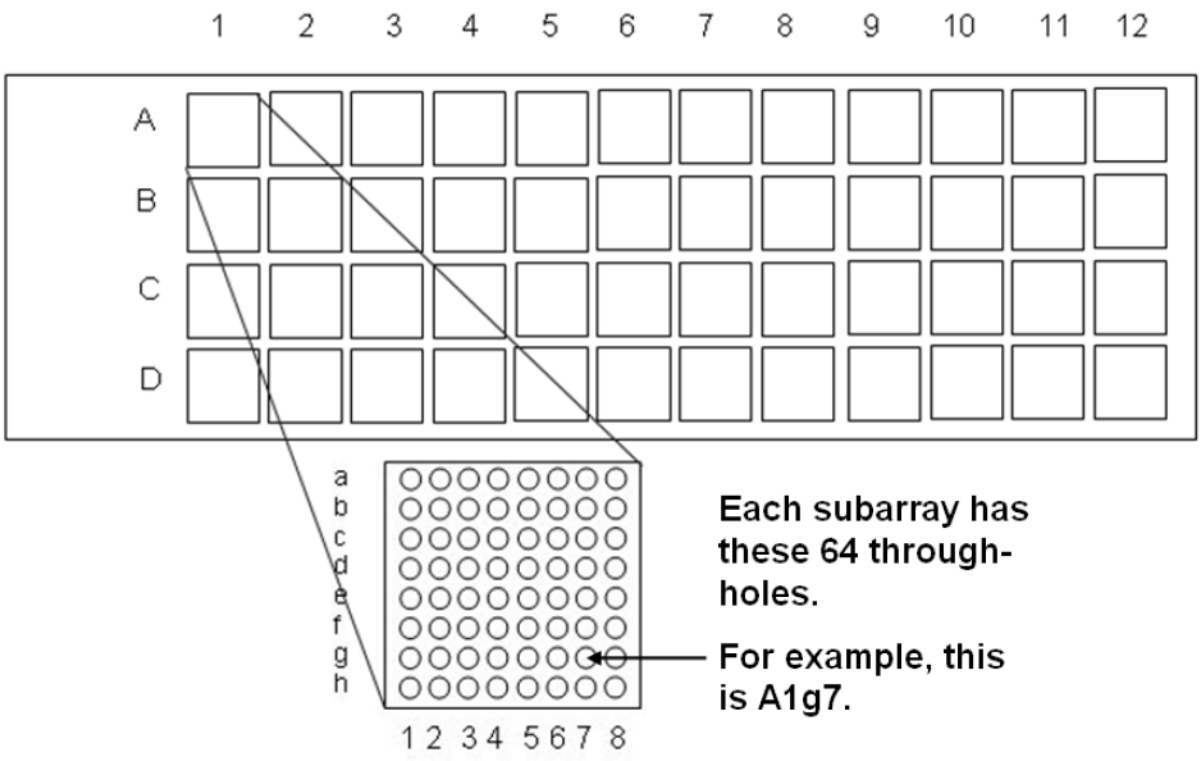


IMPORTANT! The way that you set up the 384-well sample plates depends on the format of the OpenArray™ Plate that you will be transferring the samples to. For more information on each format, see “Available plate formats for gene expression experiments” on page 238, “Available formats for genotyping experiments” on page 240, and “Panels for microRNA profiling experiments” on page 245.

OpenArray™ Plate

An OpenArray™ Plate is a 63-mm × 19-mm mid-density reaction plate. There are 3072 reaction through-holes in the plate. Individual through-holes can accommodate a 33-nL reaction volume. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes.

As shown in the following figure, the OpenArray™ Plate is divided into 48 subarrays. Each subarray consists of 64 through-holes. Depending on the OpenArray™ Plate format being used, an entire subarray is loaded from one or more wells in the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.



Available OpenArray™ Plate formats

Each through-hole in an OpenArray™ Plate contains a single assay. The number of assays in the OpenArray™ Plate and the number of samples that you can load in the plate depend on the format that you select. For more information on each format, see “Available plate formats for gene expression experiments” on page 238, “Available formats for genotyping experiments” on page 240, and “Panels for microRNA profiling experiments” on page 245.

Include no template controls

We strongly recommend that you include at least one no template control (NTC) for each OpenArray™ Plate, especially when preparing plates for genotyping experiments. NTCs serve as negative controls, and are also useful in data analysis. When adding NTCs to the 96-well sample plate, place one NTC in each section of the sample plate to ensure that the NTCs are plated in the correct location in the OpenArray™ Plate. Also follow this procedure for any positive controls, for example, CEPH DNA.

OpenArray™ Plate formats for gene expression experiments

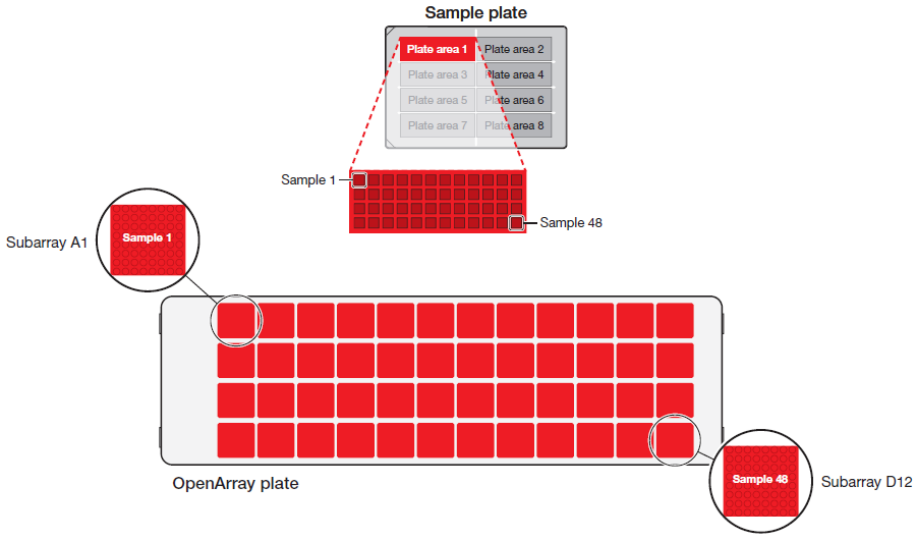
Available plate formats for gene expression experiments

The table provides a list of OpenArray™ Plate formats that are available for use in gene expression experiments.

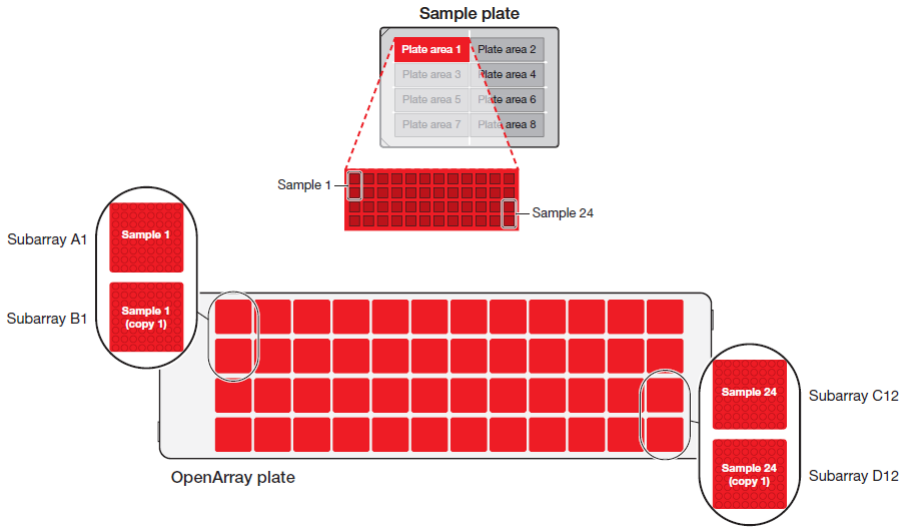
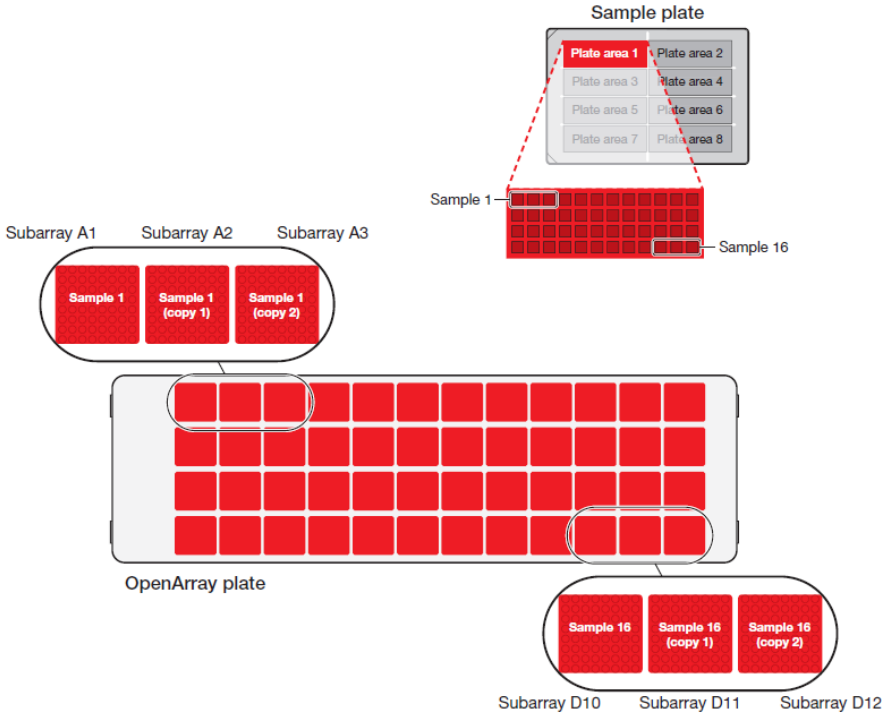
OpenArray™ Plate format	Part no.	No. of preloaded assays	Maximum no. of samples
Format 18	4471124	18 (in triplicate)	48
Format 56	4471125	56	48
Format 112	4471126	112	24
Format 168	4471127	168	16
Format 224	4471128	224	12

Recommended arrangements

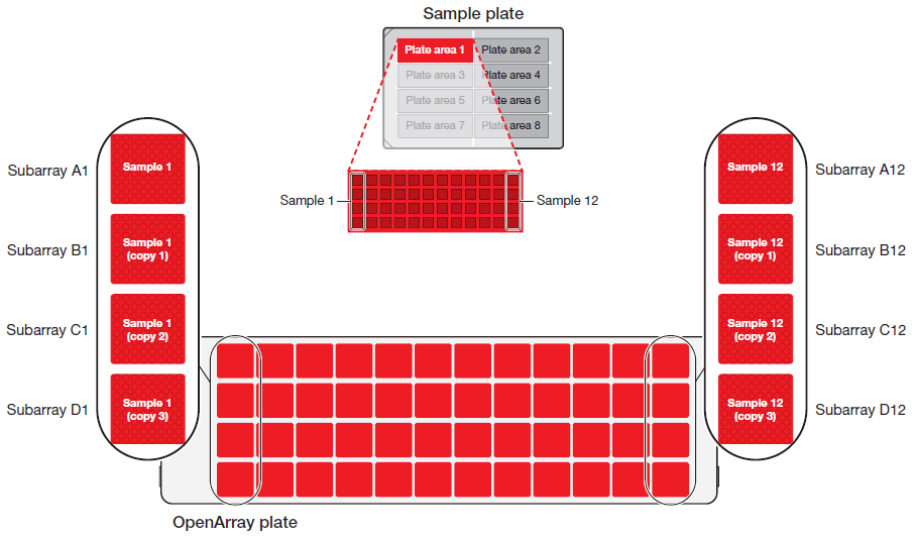
When loading the 384-well sample plate for gene expression experiments, we recommend the following arrangements.

OpenArray™ Plate	Recommended loading
Format 18 and Format 56	Load samples 1 to 48 in one area of the sample plate. 

(continued)

OpenArray™ Plate	Recommended loading
Format 112	Load samples 1 to 24 in one area of the sample plate, in duplicate. 
Format 168	Load samples 1 to 16 in one area of the sample plate, in triplicate. 

(continued)

OpenArray™ Plate	Recommended loading
Format 224	Load samples 1 to 12 in one area of the sample plate, in quadruplicate. 

OpenArray™ plates for genotyping experiments

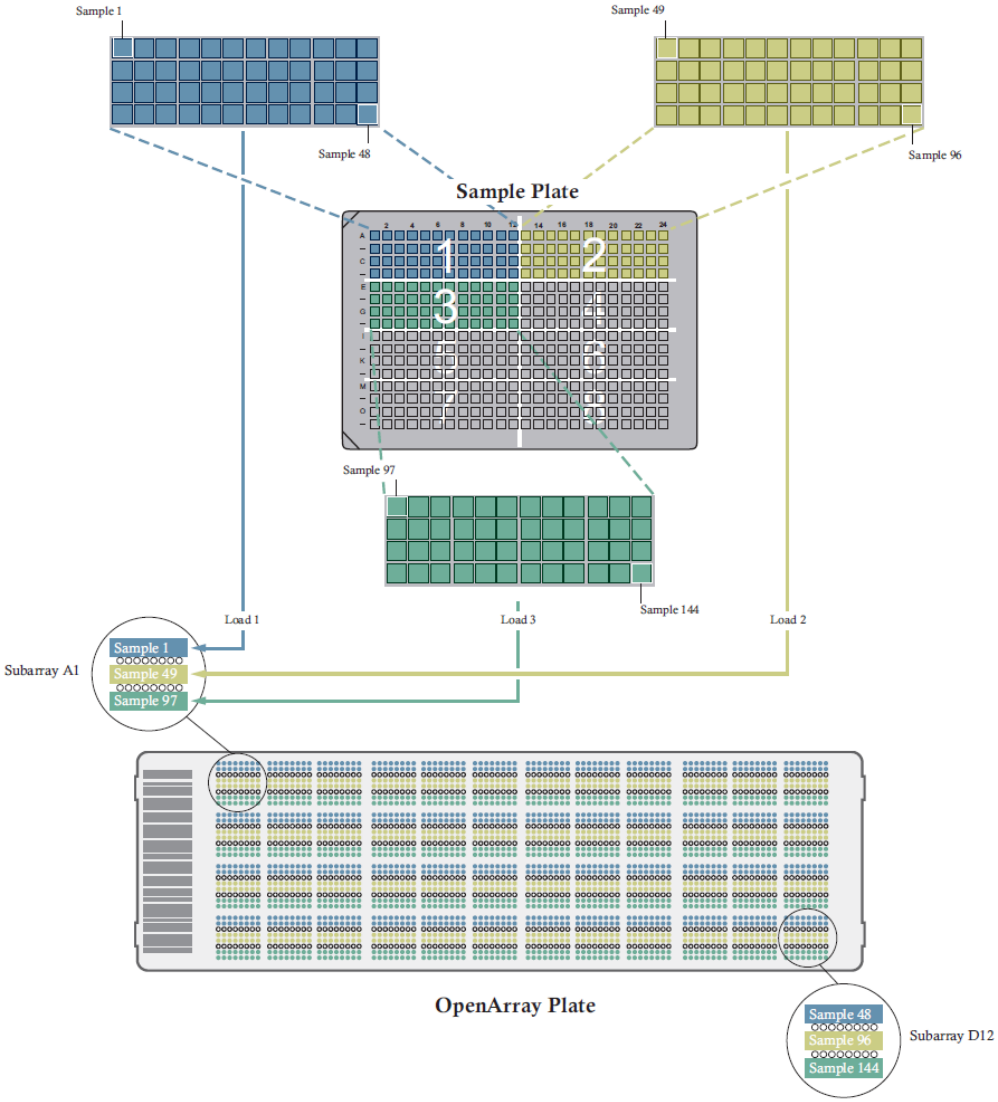
Available formats for genotyping experiments

The table provides a list OpenArray™ genotyping plate formats that are available for use in real-time genotyping experiments.

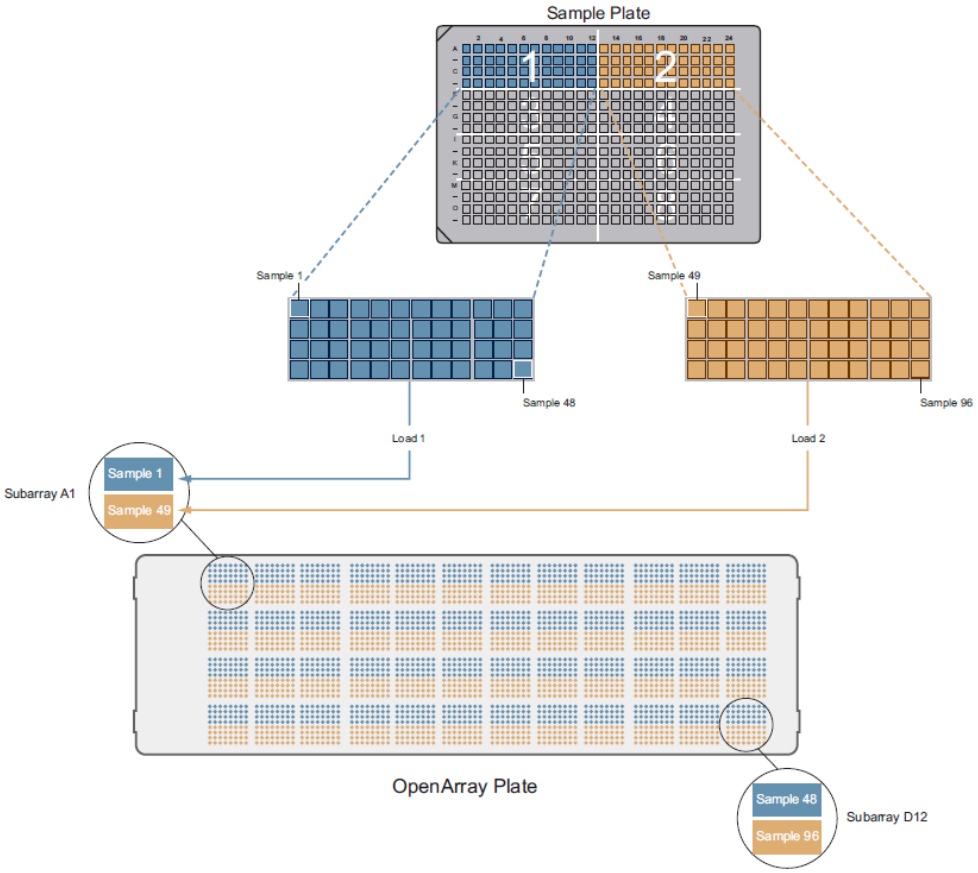
OpenArray™ Plate	Part no.	No. of preloaded assays	Maximum no. of samples
Format 16	4471113	16	144
Format 32	4471114	32	96
Format 64	4471115	64	48
Format 128	4471116	128	24
Format 192	4471117	192	16
Format 256	4471118	256	12

Recommended arrangements

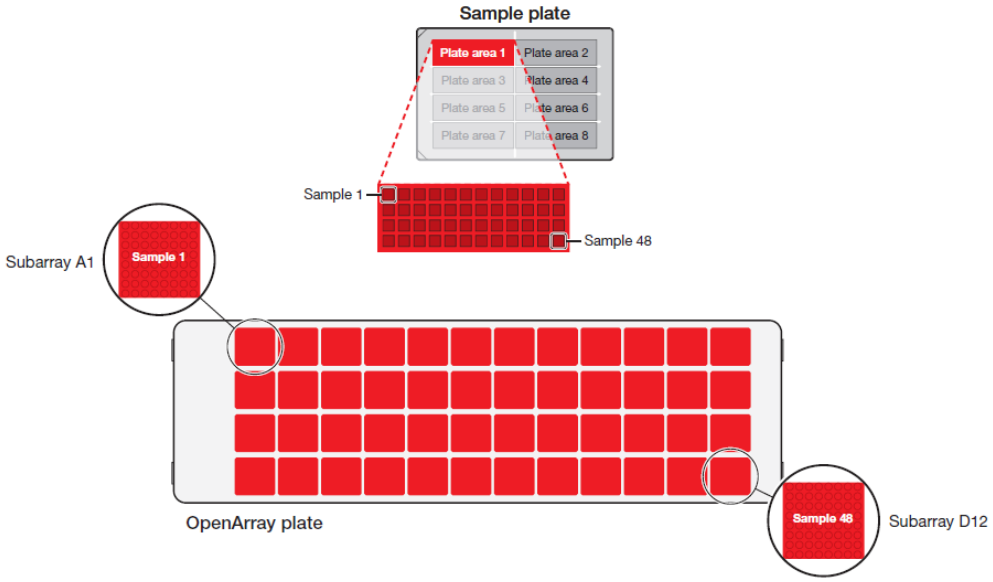
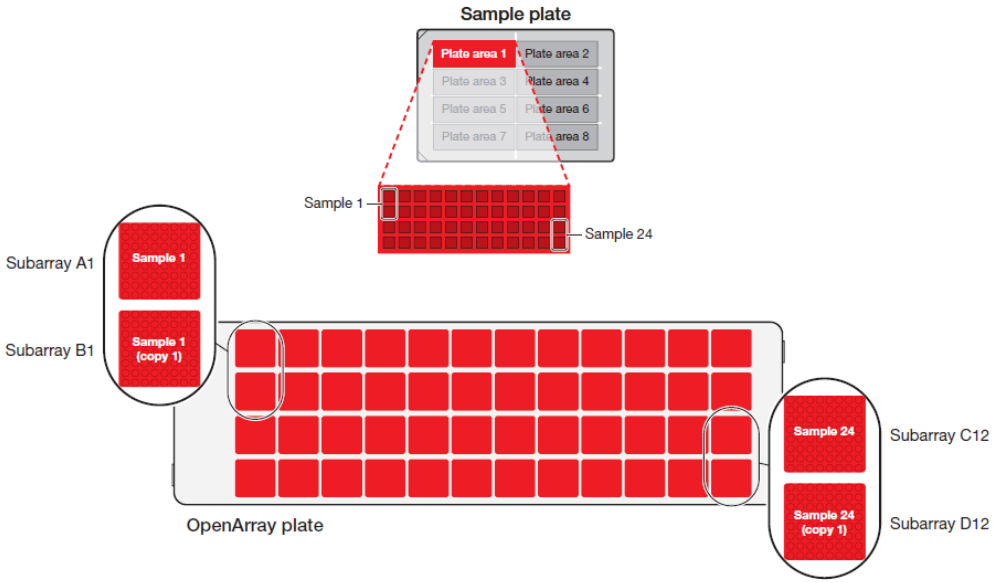
When loading the 384-well sample plate for genotyping experiments, we recommend the following arrangements.

OpenArray™ Plate	Recommended loading
Format 16	• For sample load 1, load samples 1 to 48 in one area (section 1) of the sample plate. • For sample load 2, load samples 49 to 96 in one area (section 2) of the sample plate. • For sample load 3, load samples 97 to 144 in one area (section 3) of the sample plate. 

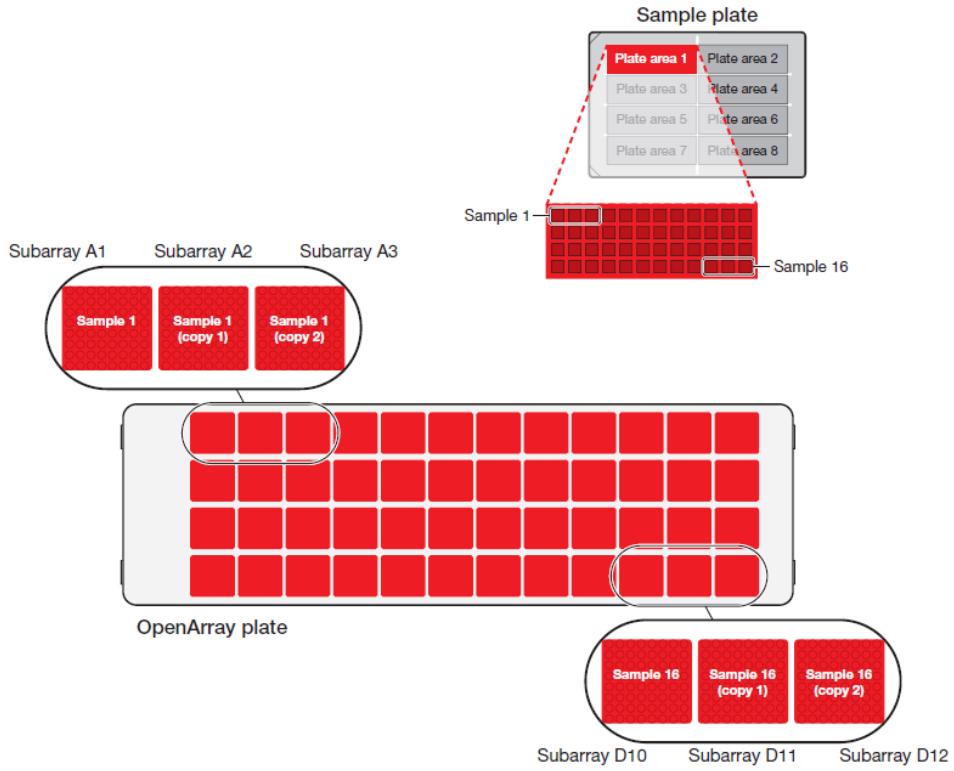
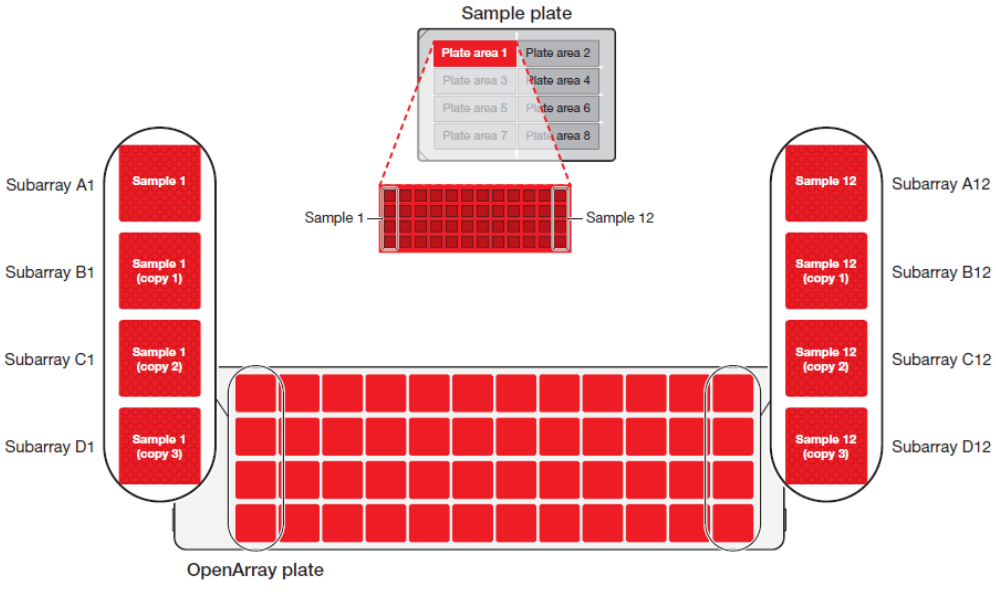
(continued)

OpenArray™ Plate	Recommended loading
Format 32	• For sample load 1, load samples 1 to 48 in one area (section 1) of the sample plate. • For sample load 2, load samples 49 to 96 in one area (section 2) of the sample plate. 

(continued)

OpenArray™ Plate	Recommended loading
Format 64	Load samples 1 to 48 in one area of the sample plate. 
Format 128	Load samples 1 to 24 in one area of the sample plate, in duplicate. 

(continued)

OpenArray™ Plate	Recommended loading
Format 192	Load samples 1 to 16 in one area of the sample plate, in triplicate. 
Format 256	Load samples 1 to 12 in one area of the sample plate, in quadruplicate. 

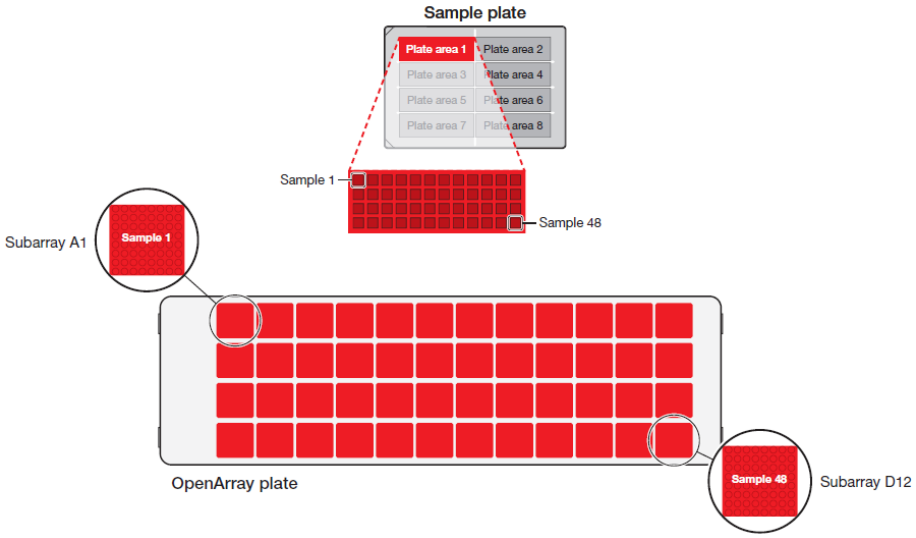
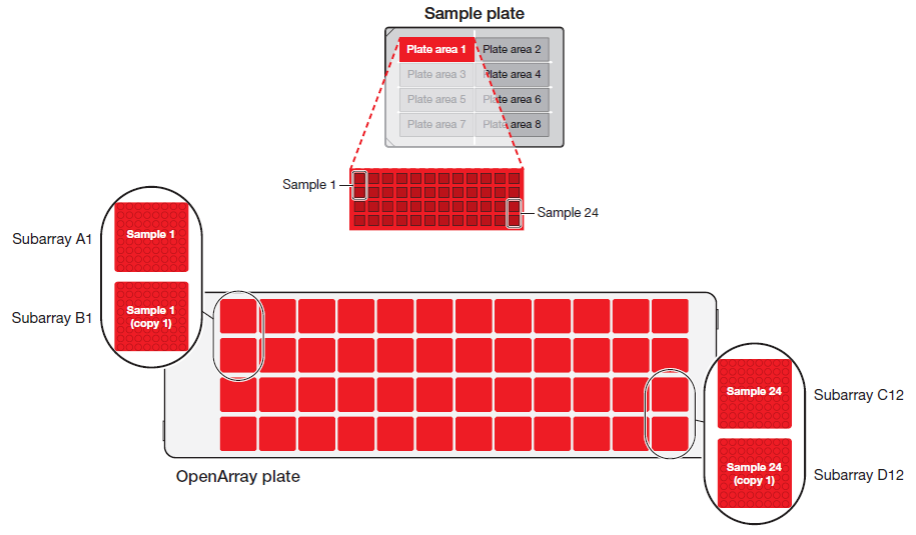
Custom miRNA OpenArray™ panels

Panels for microRNA profiling experiments

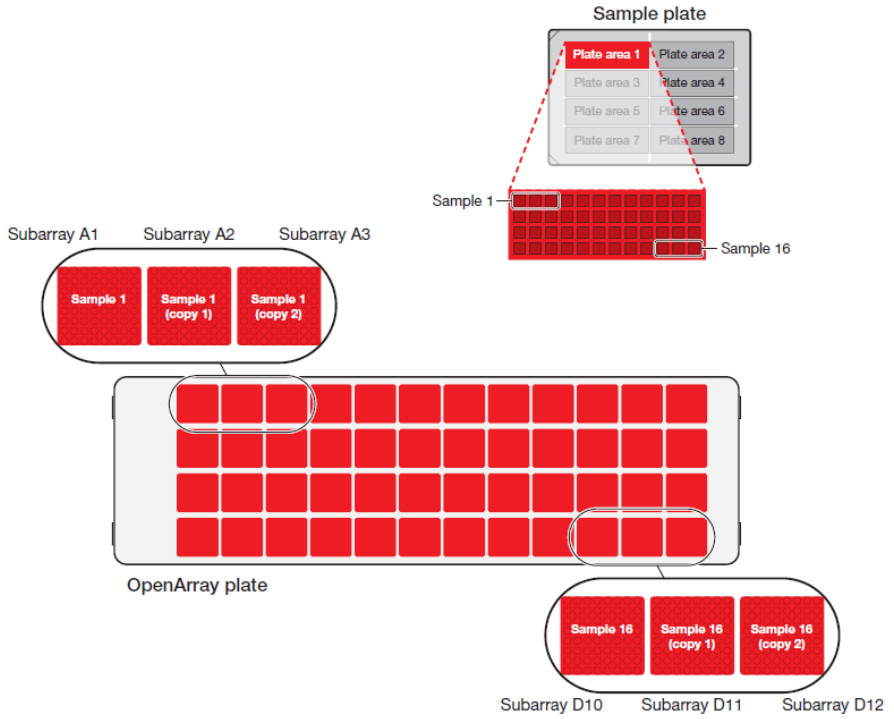
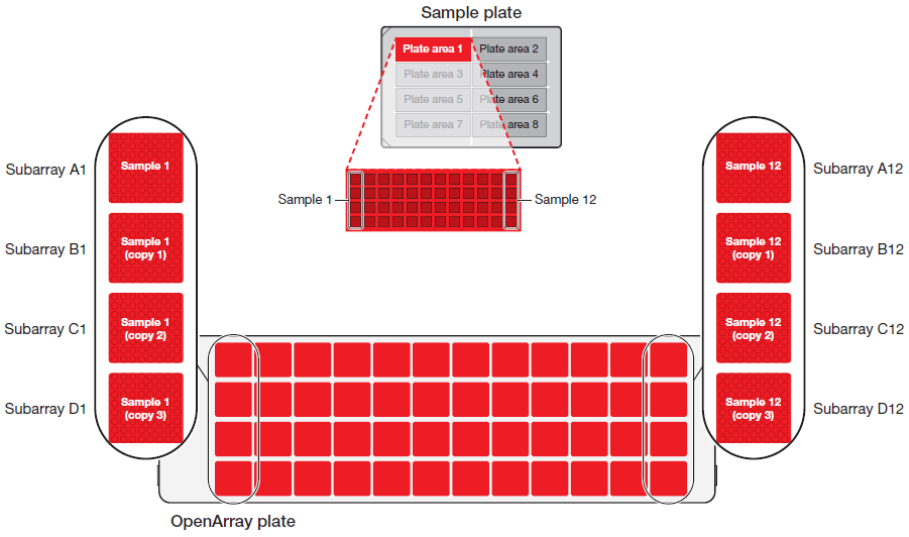
Panels for microRNA profiling are ordered as custom miRNA OpenArray™ panels.

Recommended arrangements

When loading the 384-well sample plate for microRNA experiments, we recommend the following arrangements.

OpenArray™ Plate	Recommended loading
Format 18 and Format 56	Load samples 1 to 48 in one area of the sample plate. 
Format 112	Load samples 1 to 24 in one area of the sample plate, in duplicate. 

(continued)

OpenArray™ Plate	Recommended loading
Format 168	Load samples 1 to 16 in one area of the sample plate, in triplicate. 
Format 224	Load samples 1 to 12 in one area of the sample plate, in quadruplicate. 

C PCR good laboratory practices

Follow good laboratory practices when preparing samples for PCR amplification.

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for these activities.
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Try not to splash or spray PCR samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Clean laboratory benches and equipment periodically with 10% bleach solution.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate Quick Reference	4478673
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide	MAN0025669

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- 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/or its affiliate(s) 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 any questions, please contact Life Technologies at **www.thermofisher.com/support**.