USER GUIDE

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Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System

OpenArray® Experiments

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life technologies™

Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide
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Roadmap

BOOKLET 1	**QuantStudio™ 12K Flex OpenArray® Gene Expression Starter Kit**
BOOKLET 2	**QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit**
BOOKLET 3	**QuantStudio™ 12K Flex OpenArray® MicroRNA Starter Kit**
BOOKLET 4	**QuantStudio™ Digital PCR Kit**
BOOKLET 5	**QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes**

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 the "Safety" appendix in this document.

Revision history

Revision	Date	Description
C	April 2014	Updated figures and text to reflect new QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

Purpose

The *Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* functions as both a tutorial for the QuantStudio™ 12K Flex OpenArray® Starter Kits and as a guide for performing your own experiments using the QuantStudio™ 12K Flex TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex System.

Prerequisites

This user guide is intended for users who have been specifically trained by Life Technologies. The manufacturer is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties.

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the internet, and internet-based browsers.

Note: First-time users of the QuantStudio™ 12K Flex System, please read Chapter 1, Introduction in Booklets 1, 2, and 3 thoroughly. The chapter provides general information and instructions that are applicable to all the experiments described in this guide.

How to use this guide

Each booklet in this guide provides instructions for performing experiments on the QuantStudio™ 12K Flex Real-Time PCR System using QuantStudio™ 12K Flex TaqMan® OpenArray® plates. The following booklets are provided:

- *QuantStudio™ 12K Flex OpenArray® Gene Expression Starter Kit* – preparing samples, preparing the sample plate, running and analyzing a Gene Expression experiment.
- *QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit* – preparing samples, preparing the sample plate, running and analyzing a Genotyping experiment.
- *QuantStudio™ 12K Flex OpenArray® microRNA Starter Kit* – preparing samples, preparing the sample plate, running and analyzing a MicroRNA experiment.
- *QuantStudio™ Digital PCR Kit* – preparing samples, preparing the dPCR reactions, and running a Digital PCR experiment.
- *QuantStudio™12K Flex System OpenArray® Experiments - Appendixes* – common information such as ordering information, plate formats, and additional documentation.

The instructions are specific to the QuantStudio™ 12K Flex OpenArray® Starter Kits (listed in Table 1 in Chapter 1 of Booklets 1, 2, 3, and 4). We recommend that you use the starter kits to quickly familiarize yourself with the QuantStudio™ 12K Flex 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.

Note: Instructions specific to the QuantStudio™ Digital PCR Kits are included in Chapter 2 of Booklet 4.

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Booklet 1 - QuantStudio™ 12K Flex OpenArray® Gene Expression Starter Kit

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Contents

1 Introduction

About the OpenArray® Gene Expression starter kit

The QuantStudio™ 12K Flex OpenArray® Gene Expression starter kit:

- Contains all the materials (OpenArray® plates, reagents, and accessories) you need to perform two experiments on the QuantStudio™ 12K Flex System, from sample preparation to data analysis unless otherwise noted in Table 1 on page 8
- Represents a typical setup for two gene expression experiments

Table 1 Starter kit description and contents

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Gene - 20	4469614	• 2X TaqMan® OpenArray® Real-Time PCR Master Mix, 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 (Part no. 4469589)	Contains reagents to conduct two gene expression experiments on the QuantStudio™ 12K Flex System, using the TaqMan® OpenArray® Human Endogenous Control Panel as an example. This kit contains human cDNA control samples.
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit	4469586	• QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (1 or 2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid and tips (6 syringes) • OpenArray® AccuFill™ System Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Contains accessories to assemble QuantStudio™ 12K Flex TaqMan® OpenArray® plates for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

About the plates

The instructions in this document use three types of plates, as described in Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*:

- MicroAmp® Optical 96-Well Reaction Plate (*96-well plate*)
- OpenArray® 384-Well Sample Plate (*384-well plate*)
- TaqMan® OpenArray® Plate (*OpenArray® plate*)

About the data files (how to track your assays and samples)

Overview

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 data files:

- "Sample information file (*.csv)" on page 9 - allows input of Sample IDs
- "Plate setup file (*.tpf)" on page 9 - allows input of Assay IDs and cycling protocol

- "Template file (*.edt)" on page 11 - includes complete setup (samples, assays and cycling protocol) saved as a template
- "Experiment file (*.eds)" on page 11 - complete data file

Note: Additional data files (*.aif, *.txt) are available for selection if you use the Batch Experiment Setup Utility in the QuantStudio™ 12K Flex 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 or gDNA samples. Using a sample information file allows you to:

- Track where samples and controls are located in the 96-well plate (see "Prepare the Samples" on page 15).
- Depending on the TaqMan® OpenArray® plate format being used:
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate (see "Prepare the 384-Well Sample Plate" on page 21).
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each TaqMan® OpenArray® plate (see "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 27).
- Associate information about the samples with the data results in order to normalize data or compute standard curves and calculate concentrations.

IMPORTANT! To ensure accurate results, you need to correctly track sample information from plate to plate.

You can import or manually enter sample information into the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), then export a sample information file (*.csv) in the following formats:

- 384-well plate – Integrate this file with a plate setup file (see below) in the QuantStudio™ OpenArray® AccuFill™ Software (see "Prepare for loading" on page 33).
- OpenArray® plate – Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see "From the QuantStudio™ 12K Flex Software" on page 48), 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)

Using an OpenArray® plate setup file

Plate setup files (*.tpf or *.spf) contain the assay information for individual TaqMan® OpenArray® plates, including the gene symbol, gene name, assay ID, and location of each assay on the plate. You can:

- Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate the sample information from a 384-well plate file (*.csv, see above) with the assay information in the plate setup file (see "Prepare for loading" on page 33).
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (*.eds, see "From the QuantStudio™ 12K Flex Software" on page 48).

Accessing the starter kit plate setup files

To create an experiment for the starter kits:

1. Go to **Download OpenArray® TPF & SPF Plate Files**.
2. Select the plate setup file (*.tpf) for your starter kit from the product drop-down list:

Starter kit	TaqMan® OpenArray® plate	Experiment type
QuantStudio™ 12K Flex OpenArray® Gene Expression Starter Kit	TaqMan® OpenArray® Human Endogenous Control Panel	Gene expression

Downloading your own plate setup files

In order to process TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex System, you need to download the specific plate files that correspond to your plate and experiment type: For gene expression experiments, download and use transcript plate files (*.tpf).

1. Go to the SPF and TPF Plate File Download Options page:
   Download OpenArray® TPF & SPF Plate Files
2. Enter the following for the TaqMan® OpenArray® plate of interest:
   - Sales Order number, as shown on your order invoice

     Note: The Sales Order number is also located in the shipment packing list and in the email confirmation from Life Technologies. If you are unable to locate your order number, please provide Technical Support with the lot number and serial number (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).
   - Lot number and Serial number, as shown on the foil package containing the plates.
   - Serial number, as shown on the TaqMan® OpenArray® plate label.

     Note: The Serial number is also printed on each TaqMan® OpenArray® plate.
3. Download the correct plate file for your TaqMan® OpenArray® plate:

QuantStudio™ 12K Flex TaqMan® OpenArray® plate type	Experiment type	Plate setup file
QuantStudio™ 12K Flex TaqMan® OpenArray® Real-Time PCR Kits	Gene expression	*.tpf

Note: You can either:
1) *(Recommended)* Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate samples with the downloaded *.tpf or *.spf file. Save the file to the OpenArray Plate File Input Folder. The default save location is <*drive*>:\OpenArray\OpenArrayPlates directory. See "Prepare for loading" on page 33 for more information.
Or

2) Use the downloaded *.tpf file directly in the QuantStudio™ 12K Flex Software to start an experiment, then upload samples to the experiment file (*.eds) in the QuantStudio™ 12K Flex Software after the experiment is run (see "Using an OpenArray® plate setup file" on page 49).

Template file (*.edt)

An experiment document template file (*.edt) contains predefined experiment setup information (experiment type, assay names, and run method).

You can use a template to create a new experiment from the:

- QuantStudio™ 12K Flex Software (see page 48)
- QuantStudio™ 12K Flex Instrument Touchscreen (see page 51)

Note: To create and run the starter kit experiments, use the example template files supplied with the QuantStudio™ 12K Flex Software, located at <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Experiment file (*.eds)

An experiment document single file (*.eds) is an electronic record used by the QuantStudio™ 12K Flex Software that contains all of the information about a particular TaqMan® OpenArray® plate run on the QuantStudio™ 12K Flex Instrument, including meta-data (name, barcode, comments), experiment setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.

You can:

- Create and run an experiment using the QuantStudio™ 12K Flex Software (see page 48).

  Note: To create and run the starter kit experiments, use the example template files (*.edt, see page 11) supplied with the QuantStudio™ 12K Flex Software.
- Create and run an experiment using the QuantStudio™ 12K Flex Instrument Touchscreen (see page 51).
- Analyze the experiment results in the QuantStudio™ 12K Flex Software (see page 59).

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

About the Gene Expression starter kit data files

When you perform the Gene Expression starter kit experiment tasks in this guide, you will use example data files supplied with the QuantStudio™ 12K Flex Software and the OpenArray® Sample Tracker Software. Table 2 describes the types of files provided, as well as their file names and installation locations.

Table 2 Starter kit data files referenced in this guide

File type	Description	File name	Location†	Used in
.tpf	Transcript plate file	NA	SPF and TPF Plate File Download Options page	Ch 3
.csv	96-well sample information file	Endogenous Control Plate 96-Well.csv	C:\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples	Ch 3
.edt	Experiment template	TaqMan Gene Expression.edt	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray	Ch 5
.eds	Experiment	Gene Expression Starter Kit Example.eds	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\examples\Gene Expression	Ch 6

† *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software and OpenArray® Sample Tracker Software are installed. The default installation drive for both software programs is the C: drive.

Workflow

Prepare the nucleic acid samples (Chapter 2)
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 (Chapter 3)
Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray® 384-well sample plate.
▼
Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate (Chapter 4)
Prepare for sample transfer using 384-well plates.
▼
Transfer the samples from 384-well plates to TaqMan® OpenArray® plates using the QuantStudio™ OpenArray® AccuFill™ System.
▼
Seal the TaqMan® OpenArray® plates.
▼
Complete sample transfer for the remaining TaqMan® OpenArray® plates.
▼
Perform the instrument run (Chapter 5)
Prepare the QuantStudio™ 12K Flex Software.
▼
Load the OpenArray® plate into the instrument.
▼
Run the OpenArray® plates.
▼
(Optional) Monitor the run.
▼
Unload TaqMan® OpenArray® plates from the instrument.
▼
Transfer the experiments results to the computer.
▼
Analyze the experiment results (Chapter 6)

2 Prepare the Samples

Overview

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

Performing 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 (Table 1 on page 8).

Workflow

Prepare the nucleic acid samples
Obtain human cDNA control samples from starter kit.
▼
(Optional) Transfer the cDNA control samples to a 96-well reaction plate.

Required materials

Item[1]	Source	Part no.
Starting material: Human cDNA control samples (brain, liver, lung, and placenta) from starter kit	Life Technologies	4456328
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813

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

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

To load samples into a TaqMan® OpenArray® plate, you can first pipet the samples into a 96-well reaction plate, then enter sample information for the 96-well reaction plate into the OpenArray® Sample Tracker Software (see "Track the samples" on page 22).

1. Prepare four separate PCR reactions with cDNA and two NTCs in strip cap tubes or in one row of a MicroAmp® Optical 96-Well Reaction Plate as shown below:

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

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

2. Set up four cDNA samples as follows: (excess volume = 10 µL per sample)

Component	µL/subarray/ sample	# Subarrays/ sample	Total Volume (µL)
2X OpenArray® Real-Time Master mix	2.5	8	25.0
Water	1.3	8	13.0
cDNA	1.2 ()	8	12.0
Final volume	5.0	N/A	50.0

3. Set up two NTCs as follows:

Component	µL/subarray/ sample	# Subarrays/ sample	Total Volume (µL)
Master Mix	2.5	8	25.0
Water	2.5	8	25.0
Final volume	5.0	N/A	50.0

Next step

Proceed to Chapter 3, "Prepare the 384-Well Sample Plate" on page 21.

Performing your own experiments with cDNA

When you prepare cDNA samples for your own gene expression experiments, note the following:

Workflow

Prepare the nucleic acid samples
Prepare cDNA samples ("Reverse transcribe the RNA" on page 18).
▼
(Optional) Transfer the prepared cDNA samples to a 96-well reaction plate (page 18).

Required materials

Item[1]	Source	Part no.
Starting material: Total RNA	User-supplied	—
• *(Recommended)* High Capacity cDNA Reverse Transcription Kit • High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor • SuperScript® VILO™ cDNA Synthesis Kit	Life Technologies	• 4368813 • 4374967 • 4453650
RNase-free water	Major laboratory suppliers (MLS)	—
Incubator or thermal cycler	MLS	—
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813
MicroAmp® Clear Adhesive Film	Life Technologies	4306311

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

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 18.

RNA quality

Be sure that the RNA you use for gene expression experiments:

- 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

1. Thaw the High Capacity cDNA Reverse Transcription Kit components and the total RNA on ice.
2. Combine the following components to prepare a 2X reverse transcription mix:

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

3. In the appropriate number of wells of a MicroAmp® Optical 96-Well Reaction Plate, add 10 µL of total RNA to the 2X reverse transcription mix for a total of 20 µL per reaction.
4. Seal the plate using MicroAmp® Clear Adhesive Film and incubate at room temperature for 10 minutes.
5. Incubate at 37°C for 2 hours, place on ice for 5 minutes, then spin down.
6. Incubate at 75°C for 10 minutes, place on ice for 5 minutes, then spin down.

STOPPING POINT If needed, you can store the cDNA at –20°C for up to 2 months.

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

To set up the 96-well sample plate(s):

- Transfer 2.0 µL of the prepared cDNA into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate, depending on the TaqMan® 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 detailed procedures, refer to the OpenArray® Sample Tracker Software *Quick Reference*.

Next step

Proceed to Chapter 3, "Prepare the 384-Well Sample Plate" on page 21.

Performing your own experiments with DNA

When you prepare DNA samples (genomic or plasmid) for your own real-time PCR experiments, note the following:

Workflow

Prepare the nucleic acid samples

Prepare DNA samples (page 19)

▼

Transfer the prepared DNA samples to a 96-well reaction plate (page 19)

Required materials

Item[1]	Source	Part no.
Starting material: gDNA or plasmid DNA	User-supplied	—
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813

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

DNA quality

Be sure that the DNA you use for real-time PCR experiments:

- Is extracted from the raw material 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
- No nucleases present; stabilized for storage
- Has good gel image

DNA quantity

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

- The recommended quantity of starting material (cDNA, gDNA or plasmid DNA) is 10 µL at a concentration of ~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 samples to a 96-well reaction plate

To set up the 96-well sample plate(s):

- Transfer 5.0 µL of the DNA samples into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate, depending on the TaqMan® OpenArray® plate format being used (Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).
- Create a sample information file (*.csv) to track where the samples are in the 96-well sample plate. For detailed procedures, refer to the OpenArray® Sample Tracker Software *Quick Reference*.

Next step

Proceed to Chapter 3, "Prepare the 384-Well Sample Plate" on page 21.

3 Prepare the 384-Well Sample Plate

Overview

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 Plates (see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*). You will 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):

Workflow

Prepare the OpenArray® 384-Well Sample Plate
Track the samples (page 22)
▼
Prepare the PCR mix (page 23)
▼
Transfer the samples to the 384-well sample plate and add the PCR mix (page 24)

Required materials

Item[1]	Source	Part no.[2]
96-well reaction plates, containing prepared cDNA samples	User-supplied (see page 16)	—
2X TaqMan® OpenArray® Real-Time PCR Master Mix, 1.5 mL	Life Technologies	4462159
OpenArray® 384-Well Sample Plates	Life Technologies	4406947
QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals	Life Technologies	4469876
RT-PCR Grade Water	Major Laboratory Suppliers (MLS)	AM9935 (not provided in starter kit)
Fine-tip marker	MLS	—

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

2 Provided in starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For gene expression experiments, we recommend that you use the OpenArray® Sample Tracker Software to track your samples.

Note: This section provides brief procedures for using the OpenArray® Sample Tracker Software. For detailed procedures, refer to the OpenArray® Sample Tracker Software *Quick Reference*.

1. In the OpenArray® Sample Tracker Software Properties window, enter general information about the gene expression experiment:
   a. From the Experiment Type drop-down list, select **Gene Expression**
   b. From the OpenArray® Plate drop-down list, select the appropriate TaqMan® OpenArray® plate format:
      - *(For the starter kit experiment)* **Gene Expression – 56**
      - *(For your own experiments)* **Gene Expression – 18**, **Gene Expression – 56**, **Gene Expression – 112**, **Gene Expression – 168**, or **Gene Expression – 224**
   c. From the Pipettor drop-down list, select **Fixed** or **Adjustable**
   d. If you have added a serial number or barcode to the OpenArray® 384-Well Sample Plate, enter the serial number

2. Enter the sample information:
   - *(For the starter kit experiment)* Navigate to and import sample information from the gene expression starter kit *.csv file into the OpenArray® Sample Tracker Software. The sample information file is located at:

     <drive>:\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples\Endogenous Control Plate 96 well.csv
     where *<drive>* is the computer hard drive on which the OpenArray® Sample Tracker Software is installed. The default installation drive for the software is the C: drive.
   - *(Recommended for your own experiments)* Navigate to and import sample information from a sample information *.csv file into the OpenArray® Sample Tracker Software, if available (see page 16, page 18, or page 19).
   - *(Optional)* Manually enter sample information from the 96-well reaction plates into the OpenArray® Sample Tracker Software.

   The OpenArray® Sample Tracker Software automatically maps the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates and TaqMan® OpenArray® plates.
3. Export the sample information in table format (*.csv):
   a. In the Sample Mapping window, select the 384-well tab, then click **Export** ▸ **Export *.csv**.
   b. Select the plates to export as *.csv files:
      - *(Recommended, and for the starter kit experiment)* **384-well Plate** – Use this file with the QuantStudio™ OpenArray® AccuFill™ Software to create a loaded transcript plate file (*.tpf, see "Prepare for loading" on page 33).
      - *(Optional)* **OpenArray Plate *n*** – Use this *.csv file to import setup information into the QuantStudio™ 12K Flex Software (see "From the QuantStudio™ 12K Flex Software" on page 48).

      All plates are saved to individual *.csv files in the export directory. The OpenArray® Sample Tracker Software automatically assigns the file names.
4. Using a fine-tip marker:
   a. Label the 384-well sample plate with a unique identifier.
   b. Based on the tracking information obtained in steps 1 to 3 above, mark the sections of the 384-well sample plate that you will transfer the samples to from the 96-well reaction plates.

Prepare the PCR mix

1. Mix the 2× TaqMan® OpenArray® Real-Time PCR Master Mix by gently inverting the tube a few times.
2. Combine the following components:

Component	Volume (µL) for 1 area of the 384-well sample plate[1]	Stock concentration	Final concentration	Units
2X TaqMan® OpenArray® Real-Time PCR Master Mix	132.0	2	1	X
RNase-free water	68.6	—	—	—
Final volume of PCR mix	**200.6**	—	—	—

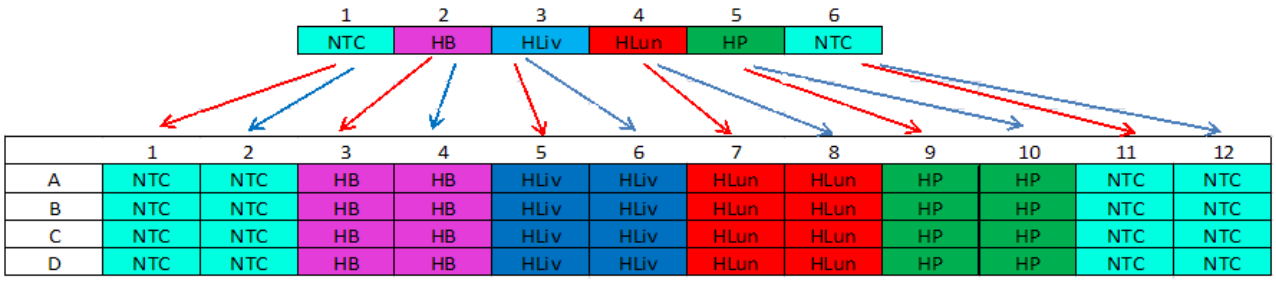
1 One area of a 384-well sample plate corresponds to a single TaqMan® OpenArray® plate (see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).

3. Mix well by pipetting up and down.

Transfer the samples

1. Thaw the cDNA samples at room temperature. Mix the samples by vortexing, then centrifuge for 1 minute @ 1000 rpm.
2. Review the concentration of the normalized samples. The recommended starting concentration for human cDNA, gDNA, and plasmid DNA samples is ~100 ng/µL.

   Note: For optimal results, it is important to normalize all cDNA, gDNA, and plasmid DNA samples in an experiment. For example, if you use 200ng/µL total RNA starting material and assume 100% efficiency in the reverse transcription reaction, you should obtain a human cDNA concentration of ~100 ng/µL equivalent to the total RNA.
3. Based on the Assay Layout you are using (see "Track the samples" on page 22), load the 384-well sample plate:
   a. Add 5 µL of each PCR sample from the microfuge tubes (see page 23) to the 384-well sample plate.
   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 (µL) per 384-well sample plate well[1], when transferring to...	
	Format 56 (starter kit experiment)	**Format 18 (in triplicate) and remaining formats**
Prepared PCR mix	3.8	3.8
Normalized human cDNA, gDNA, or plasmid DNA samples	1.2	1.2
Total volume	**5.0**	**5.0**

1 One well of a 384-well sample plate corresponds to one subarray of an TaqMan® OpenArray® plate. The number of subarrays required depends on the format of the TaqMan® OpenArray® plate. For detailed information about the TaqMan® OpenArray® plates, see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*.

4. Seal the sample plate, vortex gently to mix, then centrifuge for 1 minute @ 2000 rpm to eliminate bubbles.
5. Place the sample plate on ice for up to 1 hour.

Next step

Proceed to Chapter 4, "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 27.

4 Prepare the QuantStudio™ 12K Flex OpenArray® Plate

Overview

In this chapter, you use the QuantStudio™ OpenArray® AccuFill™ System to transfer the nucleic acid samples from the OpenArray® 384–Well Sample Plate to QuantStudio™ 12K Flex TaqMan® OpenArray® plates. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate

Prepare for sample transfer (page 30)

▼

Transfer the samples (page 32)

▼

Seal the plate (page 39)

▼

Complete sample transfer for the remaining TaqMan® OpenArray® plates (page 41)

Required materials

Item[1]	Source	Part no.
QuantStudio™ 12K Flex TaqMan® OpenArray® plates[2]	Life Technologies	See Appendix A in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*
QuantStudio™ OpenArray® AccuFill™ System	Life Technologies	4471021
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit The accessories kit contains: • QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid (6 syringes) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid Tip • OpenArray® AccuFill™ System Loader Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Life Technologies	4469586
QuantStudio™ 12K Flex OpenArray® Plate Press 2.0	Life Technologies	A24945
Foil seals	Major Laboratory Suppliers (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 the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

2 For detailed information about the TaqMan® OpenArray® plates, see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*

Storage conditions

The following materials require special storage conditions:

Item		Storage Conditions
If the QuantStudio™ 12K Flex TaqMan® OpenArray® plate is...	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™ 12K Flex 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 Loader Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Discard unused tips after the expiration date printed on the cardboard box.

Prepare for sample transfer

Guidelines for handling the TaqMan® OpenArray® plate

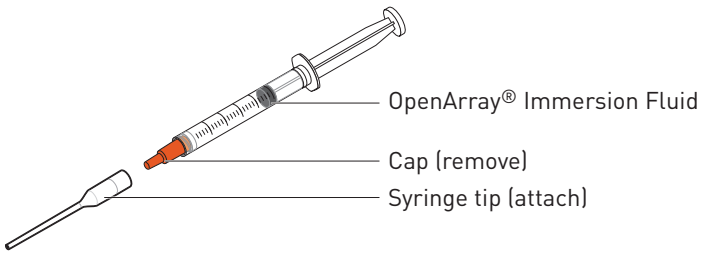
- Hold the OpenArray® case by the edges.
- Do not touch the through-holes of the OpenArray® plate.
- Load and seal an OpenArray® plate within *one hour* after opening the packaging.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray®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

Prepare the equipment and plates

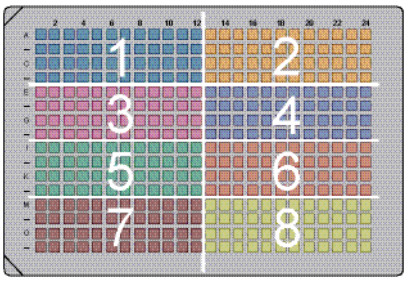
IMPORTANT! Wear powder-free gloves while preparing the OpenArray® plates.

1. Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ System Loader Tips, and plate holder are completely clean and dry. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).
2. Remove an OpenArray® plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).
3. 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.



4. 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

For each OpenArray® plate being prepared, note the following:

- *For the starter kit experiments and recommended for your own experiments*, a plate setup file (*.csv, *.spf, or *.tpf) is needed to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software.

  Note: If no samples are provided with the starter kit, create a *.csv file as you would for your own experiments.

- *For your own gene expression experiments*, the following plate setup files can be used to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software:
  - OpenArray® 384-well sample information file (*.csv, see "Track the samples" on page 22)
  - OpenArray® plate setup file (*.spf or *.tpf, see "Using an OpenArray® plate setup file" on page 9)
- *(Optional)* If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), 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.

Next step

Proceed immediately to "Transfer the samples" on page 32.

Transfer the samples

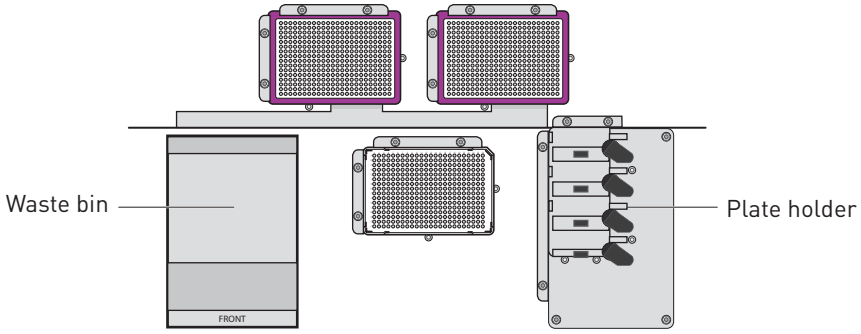
Initialize the system

1. Close the enclosure door, then start the QuantStudio™ OpenArray® AccuFill™ Software. The software checks the computer and connections as the system starts.

   When prompted, clear the deck and empty the waste bin of used tips:

   a. Open the instrument by grasping the enclosure door handle and gently, but firmly, pulling the enclosure door up.

   b. Empty the waste bin and place it back on the deck.



   Note: To safely operate the instrument, it is important to keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

2. Check if there are any OpenArray® plates in the plate holder on the deck. If necessary, remove them.

3. If necessary, replace the tip boxes.

   Note: Tip boxes contain 384 tips, divided into 8 sections. When you click **Load**, the QuantStudio™ OpenArray® AccuFill™ System loads as though a new, full box of tips is on the deck. QuantStudio™ OpenArray® AccuFill™ Software prompts you to verify that tips are in the locations shown in the Setup Deck screen (see "Load the OpenArray® plate" on page 35). Clicking a section in the Setup Deck window confirms that tips are in that section of the tip box.

   a. Place tip boxes on the deck in the two side-by-side recessed rectangular platforms (purple and white locations as shown in the illustration above).

   b. Remove the cover before using the tips for loading.

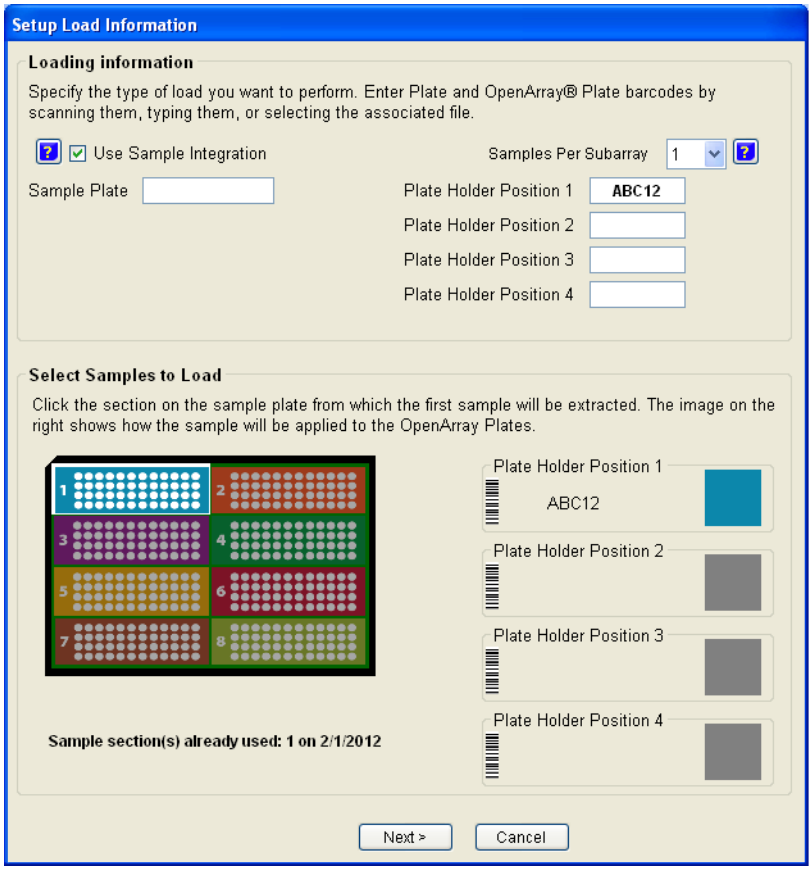
4. Close the door on the instrument.

5. Click **Proceed** to begin the System Self Test. The application performs a number of self tests and is then ready for you to continue.

   Note: System Self Test runs only at start up. The test does not run again unless the system is restarted or a self test is intentionally run. The System Self Test utility is in the Instrument drop-down menu in the QuantStudio™ OpenArray® AccuFill™ Software.

Prepare for loading

1. Click **Setup & Load**, then complete the Setup Load Information window.



2. Do either of the following:
   - Select the **Use Sample Integration** check box, then proceed to step 3.
   - *(Required for the starter kit experiments, recommended for your own experiments)* Proceed to step 5.

     Note: For the starter kit experiments, you will import sample and assay information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 48).

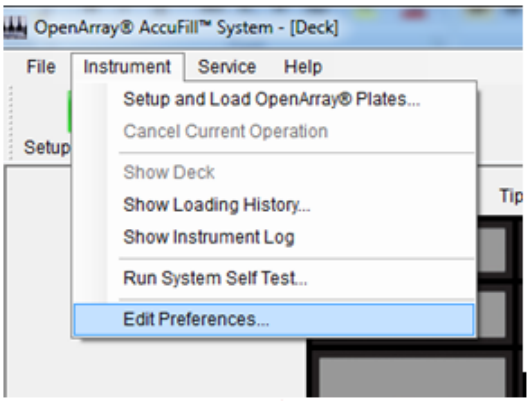
     Note: For the starter kit experiments, you will use an installed *.edt file (with experiment type, assay name, and run method).

3. In the Sample Plate field, browse to and open the *.csv file that contains the 384-well sample plate layout (see "Track the samples" on page 22).

   To set up sample integration in the QuantStudio™ OpenArray® AccuFill™ Software:

   a. Launch QuantStudio™ OpenArray® AccuFill™ Software version 1.1.

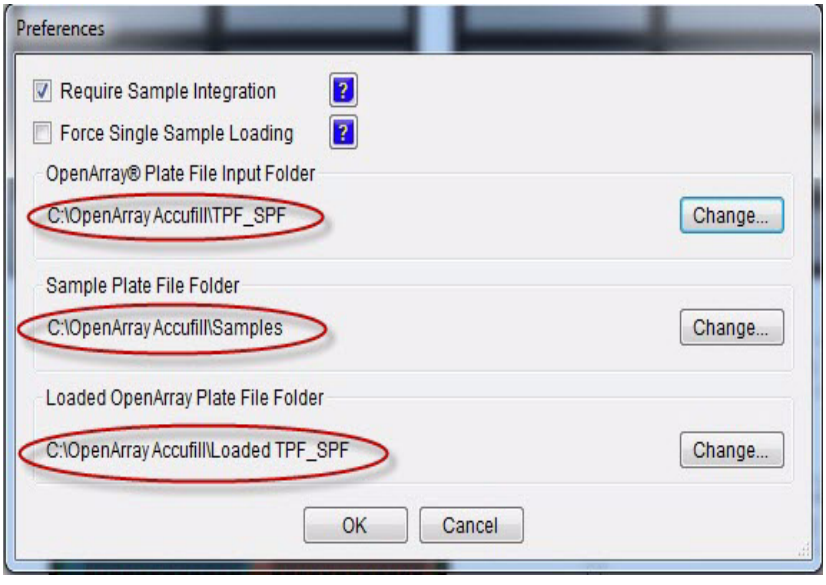
b. Go to **Instrument** ▸ **Edit Preferences**.



c. In the Preferences dialog box, check **Require Sample Integration**.

d. Click **Change** to select another location for the Input, Sample Plate, and Loaded OpenArray Plate folders.

- **Input folder** (<drive>:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF): Contains *.tpf files that are downloaded from the web or CD.
- **Sample Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Samples): Contains sample *.csv files (in the 384 format from Sample Tracker).
- **Loaded OpenArray Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Loaded TPF SPF): Contains integrated *.tpf files with sample names. The QuantStudio™ OpenArray® AccuFill™ Software automatically places the integrated *.tpf file with sample names in this folder (after the QuantStudio™ OpenArray® AccuFill™ Software run). The resulting *.tpf file includes the sample names.



4. Enter the data for the first OpenArray® plate:

a. Select **1** from the Samples Per Subarray drop-down list.

b. In the Plate Holder Position 1 text field, enter the 5-character alphanumeric serial number of the OpenArray® plate you will load into the first position of the plate holder. You can:

- Click **Browse**, then navigate to and open the plate setup file (*.spf or *.tpf) that corresponds to the OpenArray® plate. The software automatically displays the serial number in the Plate Holder Position 1 field.
- Scan the serial number (barcode) located on the OpenArray® plate package.
- Type the serial number.

IMPORTANT! When you integrate a SampleID.csv into a plate setup file and enter the serial number by scanning or typing, the plate setup file must be located in the *<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Sample directory (see "Using an OpenArray® plate setup file" on page 9). Otherwise, the software will not be able to locate the file. *<drive>* is the computer hard drive on which the QuantStudio™ OpenArray® AccuFill™ Software is installed.

Note: The QuantStudio™ OpenArray® AccuFill™ Software uses the serial number to access the appropriate plate setup files. During an instrument run, information in the plate setup files is used to populate the Assays screen in the QuantStudio™ 12K Flex Software. For information on the Assays screen, see "Analyze the Experiment Results" on page 59.

As you enter the serial number, it is reflected in the representation of the OpenArray® plates in the lower section of the window.

5. Repeat step 2 for the remaining OpenArray® plate(s).
6. Click **Next**.

Note: You can also enter sample information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 48).
You can download plate setup files (.tpf/.spf and a new .edt without sample names). You can then add names in the QuantStudio™ 12K Flex Software using the Import or direct edit feature.

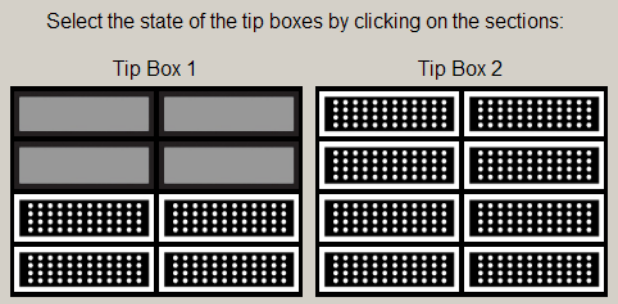
Load the OpenArray® plate

1. Open the enclosure door of the QuantStudio™ OpenArray® AccuFill™ System by grasping the door handle and lifting the door up.
2. Insert the OpenArray® 384-Well Sample Plate with the foil cover still in place. Press on the plate until you hear it snap into place.

   Note: Do not remove the foil from the OpenArray® 384-Well Sample Plate at this time.

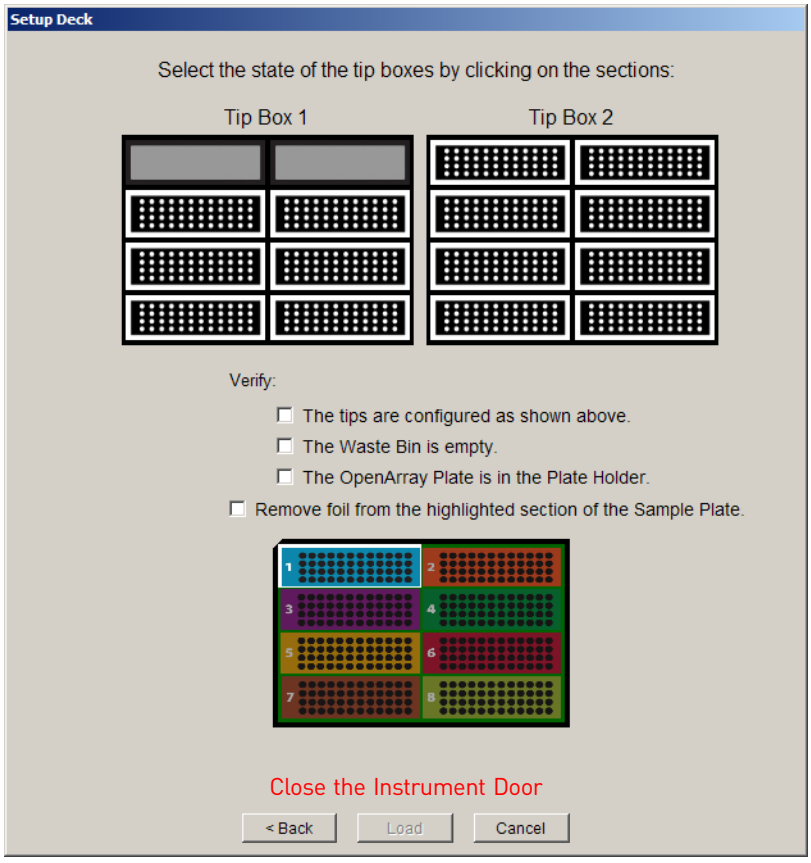
3. Place a thawed OpenArray® plate into the Plate Holder. When handling the OpenArray® plate:
   - Always hold the OpenArray® case by the edges and place it into the Plate Holder with the barcode face up and to the left.
   - If you inadvertently drop a loaded OpenArray® plate, discard it in the sharps waste container.
   - Be sure to load the OpenArray® plate within an hour after you open it.
4. Visually verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that no tips are present.
   - White areas indicate that tips are present.

   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window. For example:



Note: Cover the tip box when not in use. Discard any unused tips after 1 year or after the expiration date printed on the cardboard box.

5. Verify each of the following conditions and, when verified, select its check box:
   - Tips are configured as shown in step 4 above.
   - Waste bin is empty.
   - OpenArray® plate is in the Plate Holder.

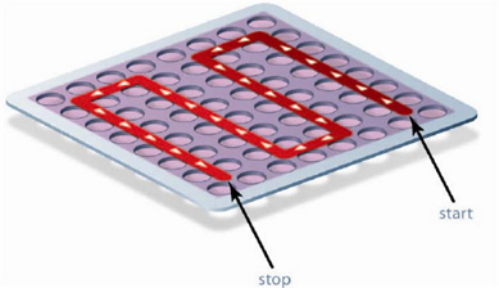


Note: The software will not continue until you select all the check boxes.

6. With forceps, peel off the foil covering the area of the OpenArray® 384-Well Sample Plate containing the samples to be loaded on the OpenArray® plate.
7. Select **Remove foil from the highlighted section of the Sample Plate**.
8. Close the instrument door.
9. Click **Load**.

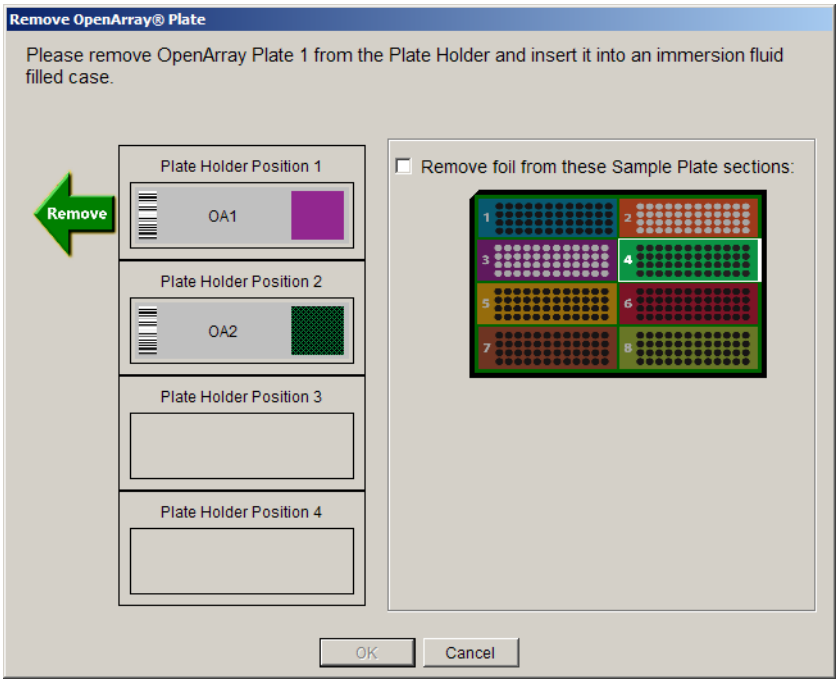
   Note: If the number of OpenArray® plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra OpenArray® plates. Correct the error and continue.

You can follow the progress of the loading on the screen. The samples in each tip are loaded in the OpenArray® plate. Each tip fills the 64 through-holes in one subarray, travelling in the pattern shown (the following illustration shows the load path for only one sample):



10. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate as explained in "Seal the OpenArray® plate" on page 39.

IMPORTANT! Once an OpenArray® plate has been filled, you must seal it within 90 seconds to prevent excessive evaporation.



11. Close the instrument door.

Note: After you load the plate, clean the QuantStudio™ OpenArray® AccuFill™ System according to the Applied Biosystems *QuantStudio™* 12K Flex Real-Time PCR System *Maintenance and Administration Guide* (Part no. 4470689).

Note: *(If Use Sample Integration on page 33 is checked)* You must have the plate setup files (*.spf or *.tpf) in the OpenArrayPlate folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF) and the SampleID.csv file in Sample Plates folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\Samples).

To integrate, click **Browse** next to the blank window under Use Sample Integration and next to Sample Plate, and navigate to the location of the SampleID.csv file. Select the file with the information on the samples that are going to be loaded into a given set of OpenArray® plate(s). Click **Open**.

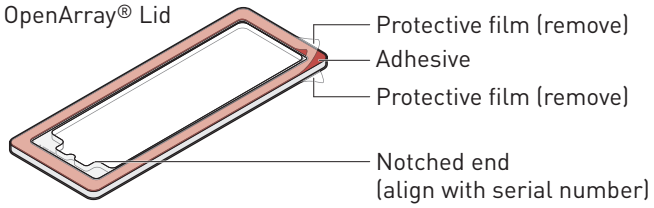
The plate setup file (*.spf or *.tpf) is now integrated with the sample information file (*.csv) and is called *Loaded_<barcode>.tpf*. You can use this file in the QuantStudio™ 12K Flex Software to create and run an OpenArray® experiment (see "Using an OpenArray® plate setup file" on page 49). Proceed with Load the OpenArray plate.

Next step Proceed immediately to "Seal the OpenArray® plate" below.

Seal the OpenArray® plate

1. Remove the protective film from the top *and* bottom of an OpenArray® Case Lid.

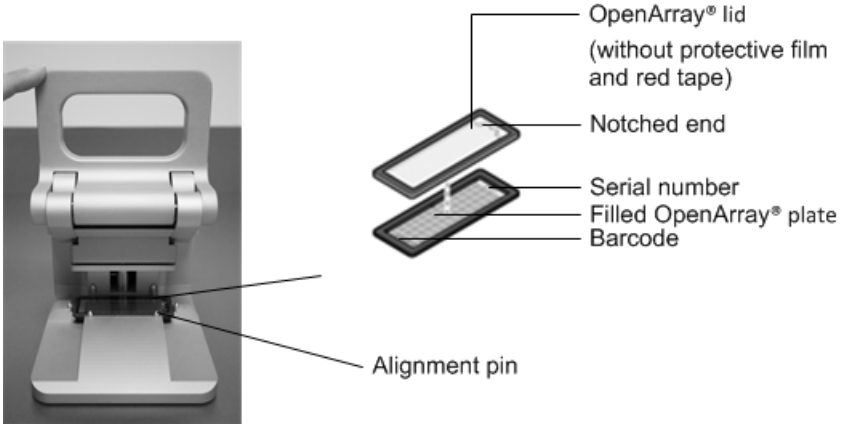
IMPORTANT! The protective film at the bottom of the Case Lid is covered by a red tape that needs to be removed first to access the protective film. Make sure to remove the protective film from *both* sides of the lids.



2. Using the thumb and index finger, grasp the OpenArray® case by the top (nearest the barcode), gently lift the case from the plate holder, then load it into the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

3. Place the Case Lid with red tape and protective film removed (both top and bottom) onto the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the lid must be oriented toward the right side of the Plate Press.



4. Actuate the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0 by pulling down the lever.

5. The status light flashes green for 20 seconds. After 20 seconds, the status light turns solid green indicating that the case is ready.

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

6. Release the lever.

7. Load the OpenArray® case with OpenArray® Immersion Fluid:

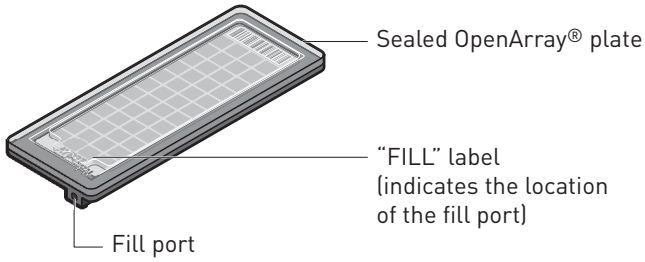
IMPORTANT! Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 seconds.

   a. Remove the sealed plate from the Plate Press, grasping the case on the edges.

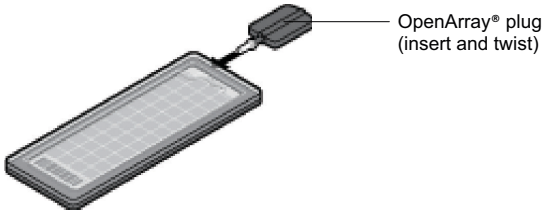
   b. Insert the syringe tip into the loading port at end of the sealed Case, then dispense the fluid completely in one gentle continuous motion.

IMPORTANT! Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through-holes.

Note: Try to minimize creating air bubbles when you dispense the fluid: one small air bubble is acceptable.



c. While holding the OpenArray® plate vertically, seal the loading port by inserting the OpenArray® Plug into the port and twisting the plug clockwise, applying sufficient pressure until the handle breaks off.



d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; be sure to not apply pressure on the OpenArray® plate within the case.

The sealed OpenArray® plate can be loaded into the QuantStudio™ 12K Flex System.

Note: Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.

STOPPING POINT For gene expression, you can store loaded and sealed OpenArray® plates at room temperature, protected from light, for up to 1 hour.

Next step

Proceed to:

- "Complete sample transfer for the remaining plates" below

  or

- Chapter 5, "Perform the Instrument Run" on page 43

Complete sample transfer for the remaining plates

Repeat the following procedures to transfer sample to the remaining OpenArray® plates:

- "Prepare for sample transfer" on page 30 *(if loading > 4* OpenArray® *plates)*
- "Transfer the samples" on page 32
- "Seal the OpenArray® plate" on page 39

Next step Proceed to Chapter 5, "Perform the Instrument Run" on page 43.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (>6) of OpenArray® plates, follow the guidelines below.

- To help avoid mistakes when entering sample information in the QuantStudio™ OpenArray® AccuFill™ Software, load the OpenArray® plates in alphanumeric order (per the OpenArray® plate serial number).
- Seal each OpenArray® plate immediately after loading is completed, while 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 an vertical position.

- Use the QuantStudio™ Carrier to transport up to four loaded OpenArray® plates to the QuantStudio™ 12K Flex Real-Time PCR System.
- After loading is complete, you can use a large bin to properly dispose of any used OpenArray® AccuFill™ System Loader Tips. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

5 Perform the Instrument Run

Overview

In this chapter, you run the QuantStudio™ 12K Flex TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex Real-Time PCR System. During the run, the QuantStudio™ system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Perform the instrument run
Prepare the QuantStudio™ 12K Flex Software (page 45)
▼
Load the TaqMan® OpenArray® plate into the instrument (page 47)
▼
Run the plates (page 48)
▼
(Optional) Monitor the run (page 52)
▼
Unload the instrument (page 55)
▼
Transfer the experiments results to the computer (page 57)

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 OpenArray® experiments.

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

To select OpenArray® experiment preferences:

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. Go to **Tools** ▸ **Preferences** in the QuantStudio™ 12K Flex Software and select the **OpenArray®** tab.
3. Select the following as needed:

Settings	Description
Setup Folder field	Defines the absolute path to the default folder/directory from which the QuantStudio™ 12K Flex Software imports experiment setup files. The Import dialog box opens to the import folder when invoked from the QuantStudio™ 12K Flex Software.
Experiment Folder field	Defines the absolute path to the default folder/directory to which the QuantStudio™ 12K Flex Software reads/writes experiment files. The Open and Save dialog boxes open to the data folder when invoked from the QuantStudio™ 12K Flex Software.
Passive Reference drop-down list	Defines the dye to use as the passive reference. The default is set to None. **Note:** While the QuantStudio™ 12K Flex Software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray® experiments.
Default Browse File Type drop-down list	Defines the file type which the Import, Open, and Save dialog boxes select by default when invoked from the QuantStudio™ 12K Flex Software.
Apply experiment template (EDT) to all OpenArray® experiment check box	If selected, the QuantStudio™ 12K Flex Software applies the Run Method defined in the selected experiment template (*.edt) to all OpenArray® experiments. For more information on OpenArray® experiment templates, see the QuantStudio™ 12K Flex Software *Help*.
Always include Amplification stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds an Amplification stage to the Run Method for all OpenArray® genotyping experiments. If deselected, you need to perform amplification on another instrument. For more information on Run Method settings, see the QuantStudio™ 12K Flex Software *Help*.
Always include Pre-Read stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds a Pre-Read stage to the Run Method for all OpenArray® genotyping experiments. For more information on Run Method settings, see the QuantStudio™ 12K Flex Software *Help*.

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

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

Access the Instrument Console

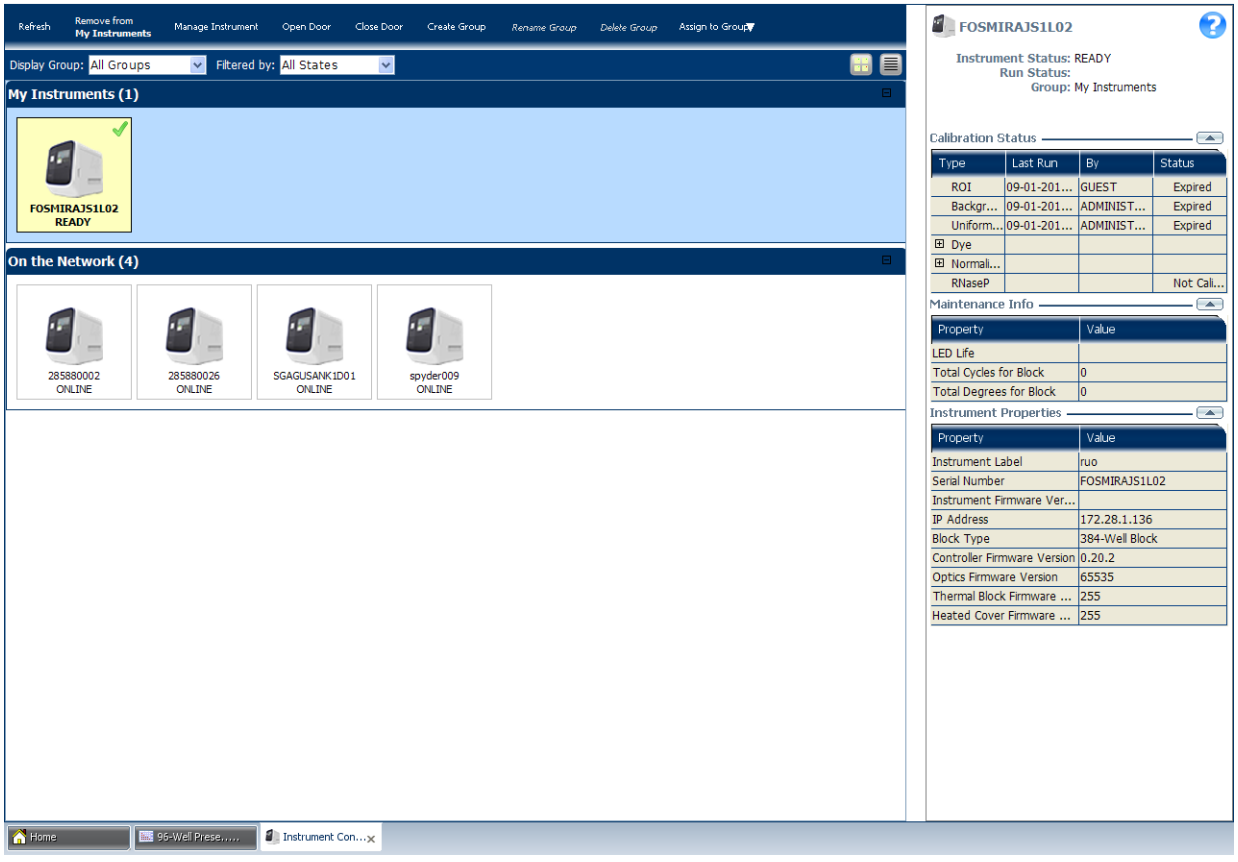
The Instrument Console displays all the QuantStudio™ 12K Flex Instruments discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups:

- **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 QuantStudio™ 12K Flex Software.
2. On the Home tab (), 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 which can be monitored:
   a. Click the instrument of interest, then click **Assign to Group** in the Instrument Console toolbar.
   b. Select the **My Instruments** 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 Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the Notification Settings feature, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

Load the OpenArray® plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

IMPORTANT! Wear powder-free gloves when you handle OpenArray® plates.

IMPORTANT! OpenArray® plates should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to allow the plate adapter to come out from the instrument side.
2. Place the OpenArray® plate(s) on the plate adapter. Make sure that:
   - Each plate is properly aligned in the adapter.
   - The plate barcode is facing up and toward the front of the instrument.
3. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Close Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to retract the plate adapter back into the instrument.

Run the OpenArray® plates

Overview

You can run OpenArray® plates in either of the following two ways:

- "From the QuantStudio™ 12K Flex Software" on page 48
- "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 53

Note: The starter kit experiments in this guide run OpenArray® plates 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 Instrument is in operation.

From the QuantStudio™ 12K Flex Software

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

- For the starter kit experiments:
  - "Using a template file" (*.edt, see below)
- For your own experiments:
  - (Recommended)"Using an OpenArray® plate setup file" (*.spf or *.tpf, see page 49)
  - *(Optional)* "Using a template file" (*.edt, see below)
  - Using the Batch Experiment Setup Utility (see the QuantStudio™ 12K Flex Software *Help*; click or press **F1**)

Using 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(s) before starting the run, or after the run is complete.

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. On the Home tab, select **Create From Template**.
3. Navigate to and select the template file (*.edt) 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 <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.
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) you want to use, then click **Select File**.

     Note: For the gene expression starter kit experiments, use the OpenArray *.csv files you exported from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22).
   - 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 you want to use:

      Gene Expression Source File (*.tpf) – Corresponds to the plate setup file associated with gene expression OpenArray® plates.

      Note: For the gene expression starter kit experiments and for your own experiments, download the appropriate plate setup files from the Life Technologies website. See page 9 for more information.

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

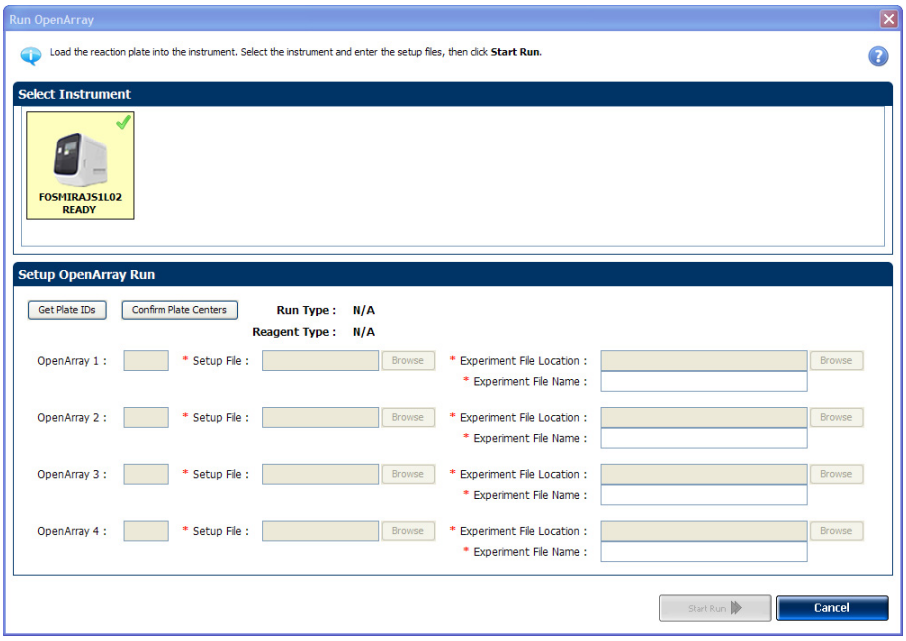
   c. If your experiment already contains plate setup information, the software asks you if you want to replace the plate setup with the data from the file. Click **Yes** to replace the plate setup information.
7. Select **File ▸ Save As...**, enter a file name, select a location for the experiment file (*.eds), then click **Save**.
8. Click **Start Run**.

Using an OpenArray® plate setup file

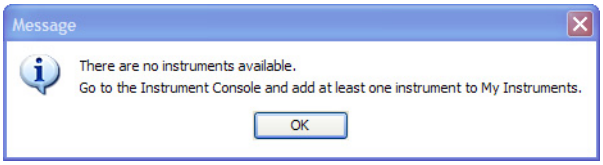
If you exported a 384-well plate file (*.csv) file from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), you can import the sample information in this file into the QuantStudio™ OpenArray® AccuFill™ Software. The QuantStudio™ OpenArray® AccuFill™ Software automatically integrates the sample information into an OpenArray® plate setup file (*.tpf or *.spf). You can save the newly created Loaded_tpf files to the OpenArray Plate File Input Folder you selected in the Preferences dialog box of the QuantStudio™ 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 and run the file.

Note: If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), 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.

1. Click  **OpenArray** from the Run menu on the Home screen of the QuantStudio™ 12K Flex Software.



Note: Be sure to add an instrument to My Instruments in the Instrument Console screen before running an experiment (see "Access the Instrument Console" on page 46). If no instrument is selected, you will receive the following warning.



2. In the Select Instrument pane, select the instrument you want to run the experiment on.

3. In the Setup OpenArray Run pane:
   - Click **Get Plate IDs** to import the barcode of the OpenArray® plates that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray® plates 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 to and select the appropriate OpenArray® plate setup files (*.spf or *.tpf) on your computer.

     Note: Once the setup file is selected, the Experiment File Location and Experiment File Name are automatically populated in the respective fields. 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® plates loaded in the instrument, the barcode of those OpenArray® plates will be populated.

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

4. Click **Start Run**.

From the QuantStudio™ 12K Flex Instrument Touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Instrument Touchscreen:

- "From experiments that are already created" below
- "From templates" on page 51
- "From shortcuts" on page 52

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

From experiments that are already created

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.
   Note: If the touchscreen is not at the Main Menu screen, touch  (**Home**).
2. In the Home screen, touch **Run OpenArray Plates**.
   The instrument will retrieve the barcodes and scan 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 QuantStudio™ 12K Flex Instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the QuantStudio™ 12K Flex Instrument Touchscreen. Do not proceed if a barcode is not detected by the QuantStudio™ 12K Flex Instrument.

From templates

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex 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 will retrieve the barcodes and create new experiments based on the template for each plate found.

6. Touch 

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

From shortcuts

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.

   Note: 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 will retrieve the barcodes and create 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 "From the QuantStudio™ 12K Flex Software Run screen" on page 52).
- From the QuantStudio™ 12K Flex Instrument Touchscreen, in the same way that you run the experiment (see "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 53).
- From the Instrument Console of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Instrument Touchscreen) as described in "From the QuantStudio™ 12K Flex Software Instrument Console" on page 52.

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.

From the QuantStudio™ 12K Flex Software Run screen

Click **Amplification Plot** from the Run Experiment Menu to monitor the amplification plot of the experiment you are running.

From the QuantStudio™ 12K Flex Software Instrument Console

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.

2. In the Instrument Console screen, select the icon of the instrument that you are using to run the experiment, then click **Manage Instrument** or double-click on the instrument icon.

   Note: You must add the instrument to a group which can be monitored before you can manage it (see "Access the Instrument Console" on page 46).

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 page 53) available from the QuantStudio™ 12K Flex Software for potential problems.

To...	Action
Stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN**. • In the Stop Run dialog, 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.
View amplification data in real time	Select **Amplification Plot**. See "To monitor the Amplification Plot" below.

To 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 each experiment's Amplification Plot.

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

The Amplification Plot screen 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).

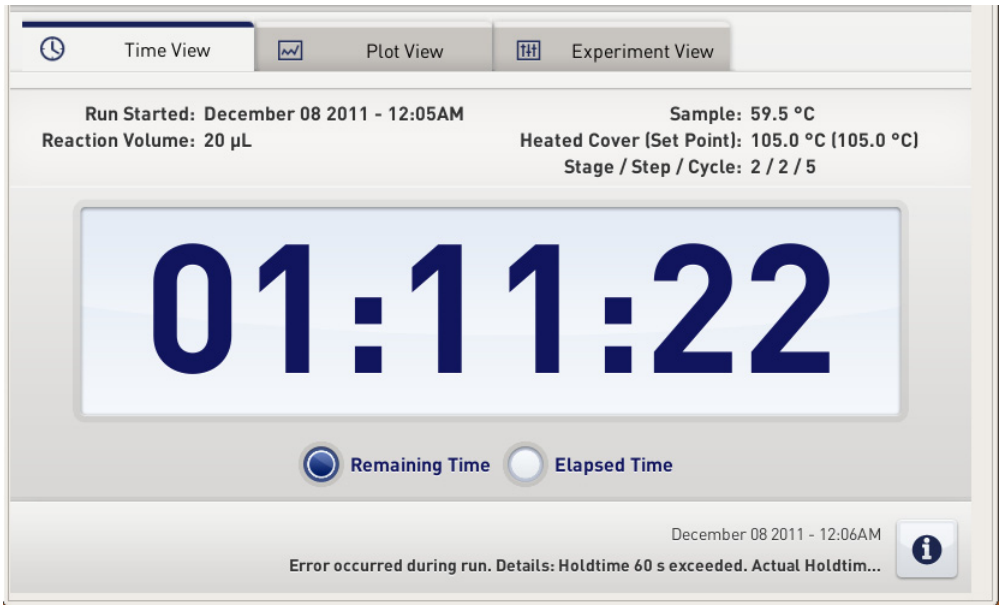
  Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the QuantStudio™ 12K Flex Software *Help* (click or press **F1**).

From the QuantStudio™ 12K Flex Instrument Touchscreen

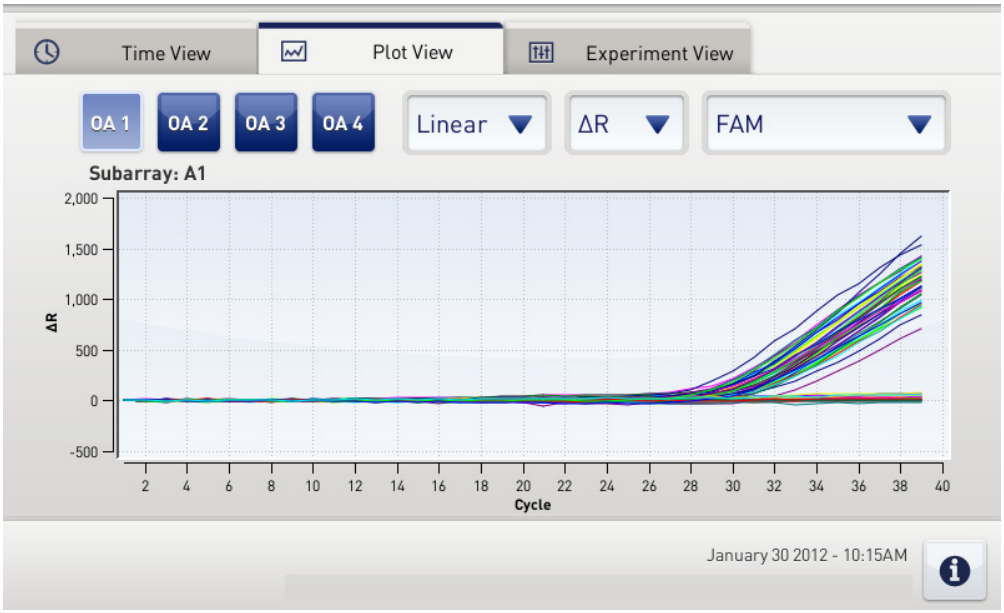
The QuantStudio™ 12K Flex Instrument Touchscreen displays the barcodes (or Plate IDs) of the TaqMan® OpenArray® plates for the run, the date and time at which the run started, the time remaining in the run, and other information.

To...	Action
Display the experiment names in the run	Touch **Experiment View**.
Show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
Display the time elapsed and the time remaining in the run	Touch the **Time View tab**, then touch **Experiment View** tab to return to the previous screen.
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 present below the Plot View tab.

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

Unload the 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 re-open the *.eds file to display the Amplification Plot screen. See "Analyze the Experiment Results" on page 59.
- QuantStudio™ 12K Flex Instrument Touchscreen, see "(Optional) Transfer experiment results" on page 57.

Unload the instrument

When the QuantStudio™ 12K Flex Instrument Touchscreen displays the Home screen, unload the OpenArray® plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

1. Touch  on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen 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 QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate as follows:

   a. Power off the QuantStudio™ 12K Flex Instrument.
   b. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate.
   c. If the instrument does not eject the plate, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door.
   d. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door.

(Optional) Transfer experiment results

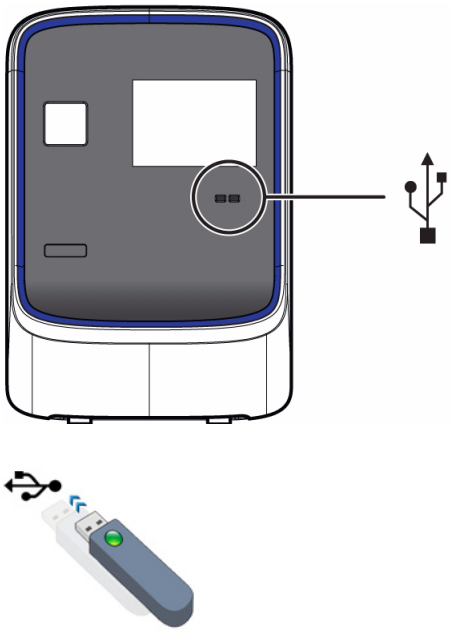
If you started a run from the QuantStudio™ 12K Flex Instrument Touchscreen, transfer the experiment data to the computer for analysis after the run is complete. You can transfer the experiment results in either of the following two ways:

Download the experiment from the QuantStudio™ 12K Flex Instrument over the network

1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. Select the instrument icon of the QuantStudio™ 12K Flex Instrument you just used to run the experiment from the My Instruments list.
3. Click **Manage Instrument** to open the Instrument Manager.
4. In the Instrument Manager, click **Manage Files**.
5. In the Experiments panel, select the experiment to download. Click **Download**.
6. In the Save dialog box, select the folder to hold the experiment results and click **Save**. The experiments folder is located at:
   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where, <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Transfer the experiment from the QuantStudio™ 12K Flex Instrument to the computer via a USB drive

1. If not already connected to the instrument, connect a USB drive to the USB port.



2. Touch the **QuantStudio™ 12K Flex Instrument Touchscreen** to activate it.
3. If the touchscreen is not at the Main Menu screen, 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 your 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:

   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

6 Analyze the Experiment Results

Section 6.1 Analyze the run data

This section includes general information and instructions on how to analyze the gene expression example experiment provided on the QuantStudio™ 12K Flex Software installation CD. For specific instructions see the subsequent section on page 69.

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.

View the results

After an experiment run, you need to close the run and re-open the *.eds file to display the Amplification Plot screen.

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

Setting up the *.eds file

If you run a gene expression experiment using an *.edt file, you will need to 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® format from Sample tracker (*.csv) for the corresponding plate.
- If you use the QuantStudio™ 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 QuantStudio™ OpenArray® AccuFill™ Software.

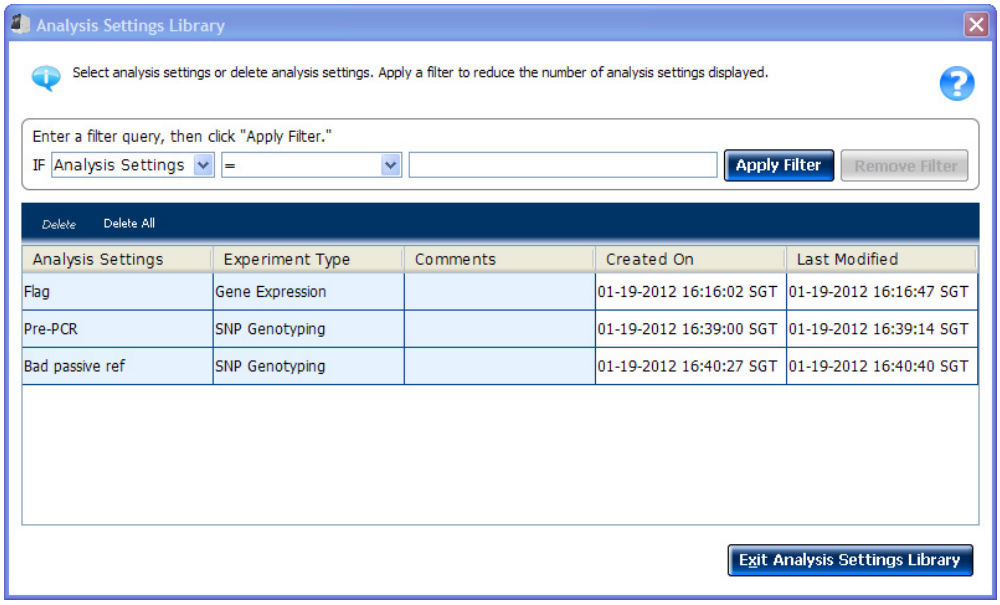
About the Analysis Settings Library

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.

In the Analysis Settings Library dialog box you can apply a filter to reduce the number of setting protocols displayed.

You can access the Analysis Settings Library from the Tools menu.



To change the analysis settings and to save them to the Analysis Settings Library:

1. From the Experiment Menu pane, select **Analysis**.
2. On the Analysis screen, click **Analysis Settings** to open the Analysis Settings dialog box.
3. Change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes you have made to the Analysis Settings Library.

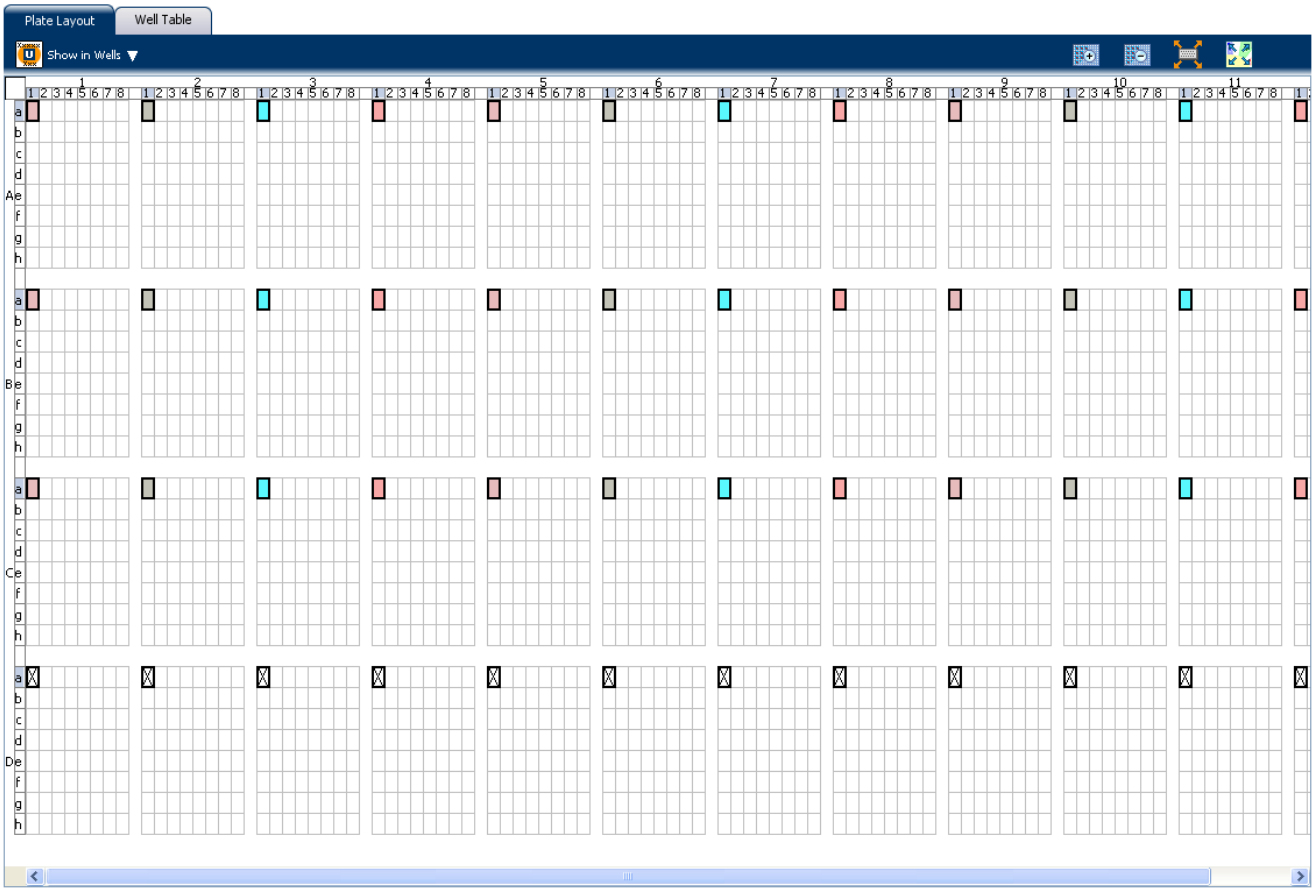
You can import the analysis settings you have previously saved to the Analysis Settings Library, by clicking **Load from Library** in the Analysis Settings dialog box.

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: Select **Sample Color** or **Target Color** for Gene Expression and miRNA experiments. For Genotyping experiments, select **Sample Color** or **Assay Color.**
- To select a single well, click the well in the plate layout.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the plate layout.
- To select all the wells, click the upper left corner of the plate layout.

Plate layout 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) to open the Plot Properties dialog box

2. 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

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

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

(Optional) Export an experiment

About exporting 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

Note: You can also export plate images collected during the run as *.tif files and use them for troubleshooting purposes as needed. To export plate images, first create an export folder on your hard drive. In the Export screen, click **Browse** and navigate to the folder 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/**). Also, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689) for more information on QC Images.

Export procedure

Note: If you choose the Auto Export option before running an experiment, the data is automatically exported to the location you specified. If you did not set the Auto Export option, the analyzed data is not exported automatically.

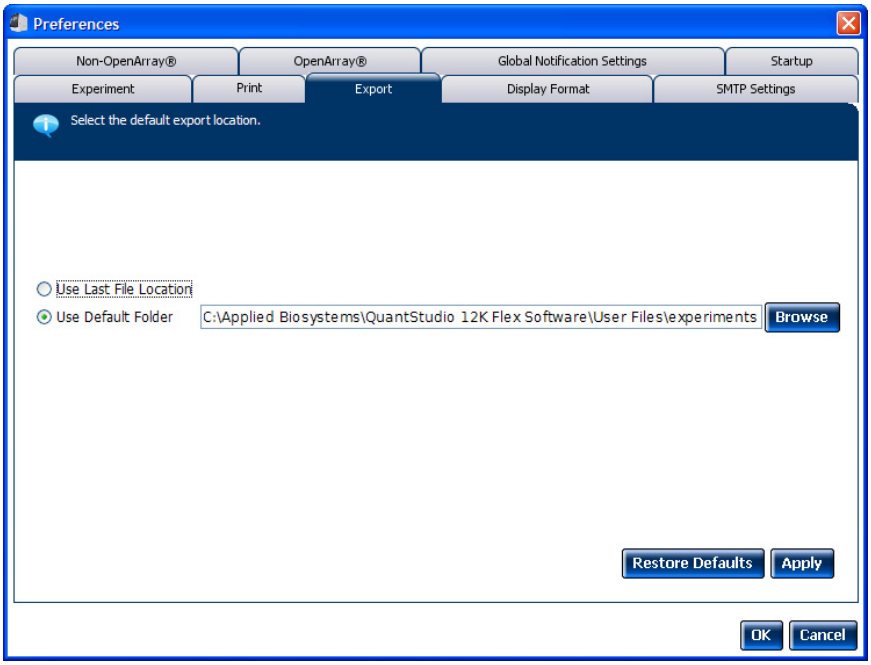
1. Open the experiment file that contains the data to export, and from the Experiment Menu, click  **Export.**
2. Select the format for exported data:
   - **QuantStudio 12k Flex format** (supports .txt and .xlsx data).
   - **RDML format** - Real Time Data Markup Language (supports only .xml type of 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. You can select the export file type (*.xls, *.xlsx or *.txt) to export from the **File Type** drop-down menu.

     Note: You cannot use an exported ***.xls** or an ***.xlsx** file when importing plate setup information.
4. Select **Yes** or **No** to include or exclude bookmarked data, from analysis, in the export set.

   The Filter Bookmark Data feature allows you to include only the data bookmarked during analysis in the export set.
5. *(Optional)* Select the **Open file(s) when export is complet**e check box to automatically open the file when export is complete.
6. Enter a file name and location.
   a. Enter a name for the export file in the **Export File Name** field.

b. Enter the **Export File Location**. 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**, and select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** check box.



7. Select the data to export:

Select...	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

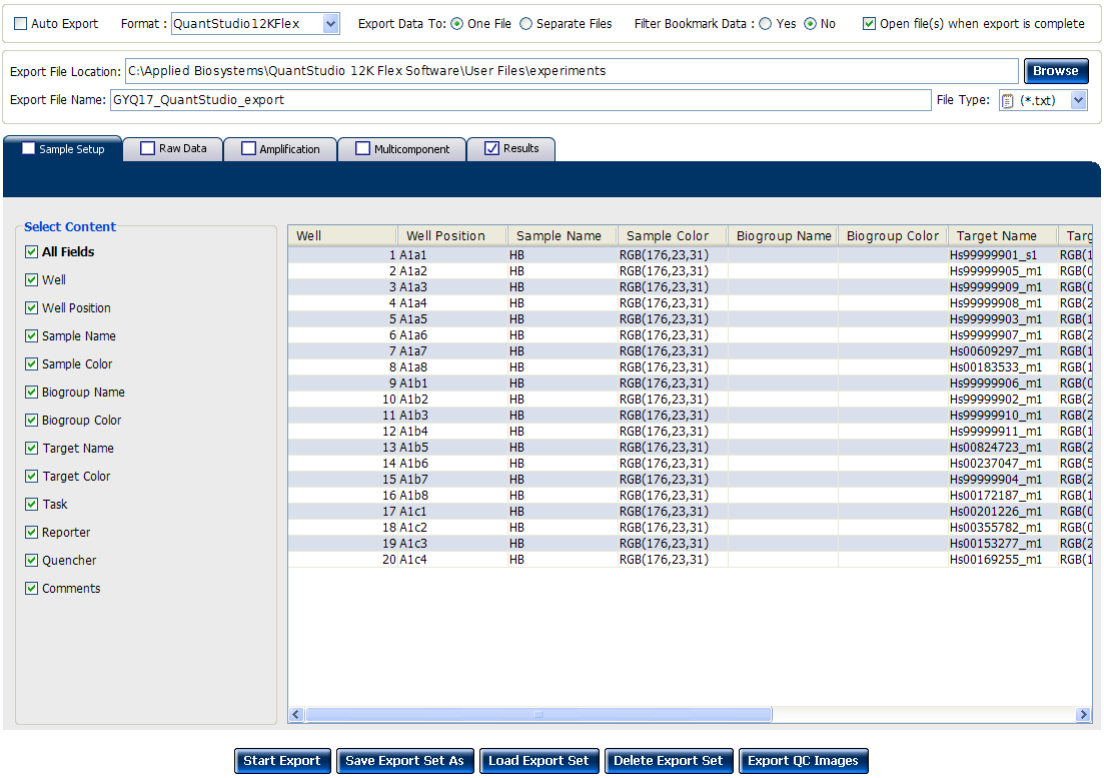
Note: Results data are not available for export until the run status is complete and the data are analyzed.

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.

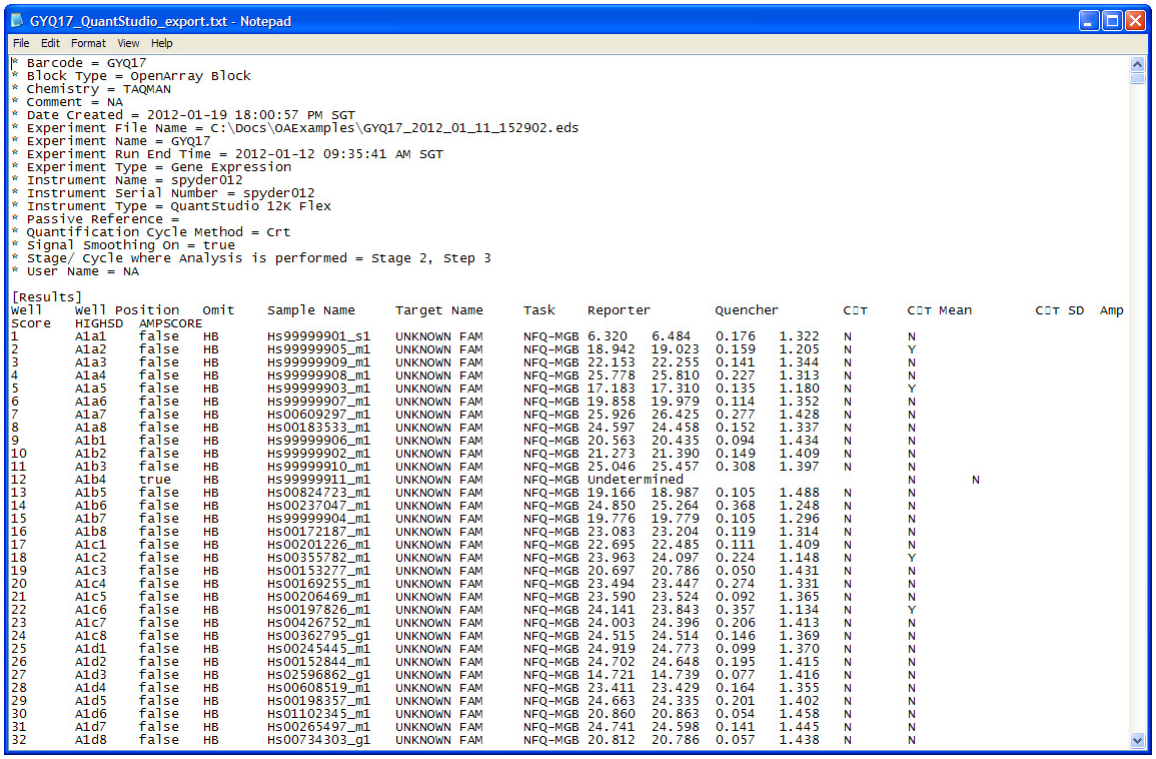
10. Click **Start Export**.

The Export screen for a Gene Expression experiment is shown in the following graphic:



Well	Well Position	Sample Name	Sample Color	Biogroup Name	Biogroup Color	Target Name	Targ
1	A1a1	HB	RGB(176,23,31)			Hs99999901_s1	RGB(1
2	A1a2	HB	RGB(176,23,31)			Hs99999905_m1	RGB(0
3	A1a3	HB	RGB(176,23,31)			Hs99999909_m1	RGB(0
4	A1a4	HB	RGB(176,23,31)			Hs99999908_m1	RGB(2
5	A1a5	HB	RGB(176,23,31)			Hs99999903_m1	RGB(1
6	A1a6	HB	RGB(176,23,31)			Hs99999907_m1	RGB(2
7	A1a7	HB	RGB(176,23,31)			Hs00609297_m1	RGB(1
8	A1a8	HB	RGB(176,23,31)			Hs00183533_m1	RGB(1
9	A1b1	HB	RGB(176,23,31)			Hs99999906_m1	RGB(0
10	A1b2	HB	RGB(176,23,31)			Hs99999902_m1	RGB(2
11	A1b3	HB	RGB(176,23,31)			Hs99999910_m1	RGB(2
12	A1b4	HB	RGB(176,23,31)			Hs99999911_m1	RGB(1
13	A1b5	HB	RGB(176,23,31)			Hs00824723_m1	RGB(2
14	A1b6	HB	RGB(176,23,31)			Hs00237047_m1	RGB(5
15	A1b7	HB	RGB(176,23,31)			Hs99999904_m1	RGB(2
16	A1b8	HB	RGB(176,23,31)			Hs00172187_m1	RGB(1
17	A1c1	HB	RGB(176,23,31)			Hs00201226_m1	RGB(0
18	A1c2	HB	RGB(176,23,31)			Hs00355782_m1	RGB(0
19	A1c3	HB	RGB(176,23,31)			Hs00153277_m1	RGB(2
20	A1c4	HB	RGB(176,23,31)			Hs00169255_m1	RGB(1

The exported file when opened in Notepad appears as shown in the following graphic:



GYQ17_QuantStudio_export.txt - Notepad

File Edit Format View Help

```
* Barcode = GYQ17
* Block Type = OpenArray Block
* Chemistry = TAQMAN
* Comment = NA
* Date Created = 2012-01-19 18:00:57 PM SGT
* Experiment File Name = C:\Docs\OAExamples\GYQ17_2012_01_11_152902.eds
* Experiment Name = GYQ17
* 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        CQT     CQT Mean        CQT 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
```

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

Common features

The ExpressionSuite Software allows you to:

- 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 ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data.

Note: For more information on the ExpressionSuite Software, refer to *Applied Biosystems ExpressionSuite Software User Guide*. The application is available for download from the Life Technologies website. See also, Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*.

Section 6.2 Analyzing Gene Expression Experiments

In this section you use the Gene Expression example experiment files provided on the QuantStudio™ 12K Flex Software installation CD to analyze the experiment results.

Workflow

Analyze the experiment results

Access the example experiment file (page 69)

▼

Assess amplification result using the Amplification Plot (page 70)

▼

Identify well problems using the Well Table (page 72)

▼

Confirm accurate dye signal using the Multicomponent Plot (page 73)

▼

Determine the signal accuracy using the Raw Data Plot (page 75)

▼

View the QC Summary (page 76)

▼

(Optional) Adjust parameters for re-analysis of your own experiments (page 79)

▼

(Optional) Export the analyzed data (page 83)

Open the example experiment file

1. Double-click 

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

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

Setting up the *.eds file

If you ran a gene expression experiment using an *.edt file, you will need to 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® format from Sample tracker (*.csv) for the corresponding plate.
- If you use the QuantStudio™ 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 QuantStudio™ OpenArray® AccuFill™ Software.

Assess amplification results using the Amplification Plot

The Amplification Plot screen displays amplification of all samples in the selected wells. There are three plots 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. You can 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. You can 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. You can use this plot to locate outlying amplification (outliers).

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

Purpose

The purpose of viewing the amplification plot for the example experiment is to:

- Evaluate the quality of the amplification curve
- Check for outliers

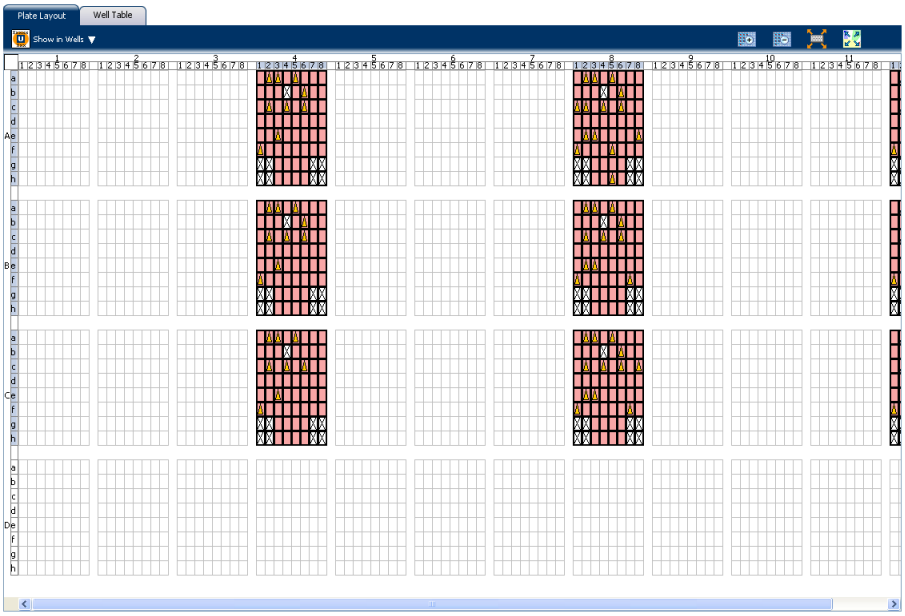
View the Amplification Plot

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

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

2. Display the HPp wells in the Amplification Plot screen:
   a. Click the **Plate Layout** tab.
   b. From the Show in Wells drop-down menu, select **Sample Color**.

The Plate Layout screen should look like this:

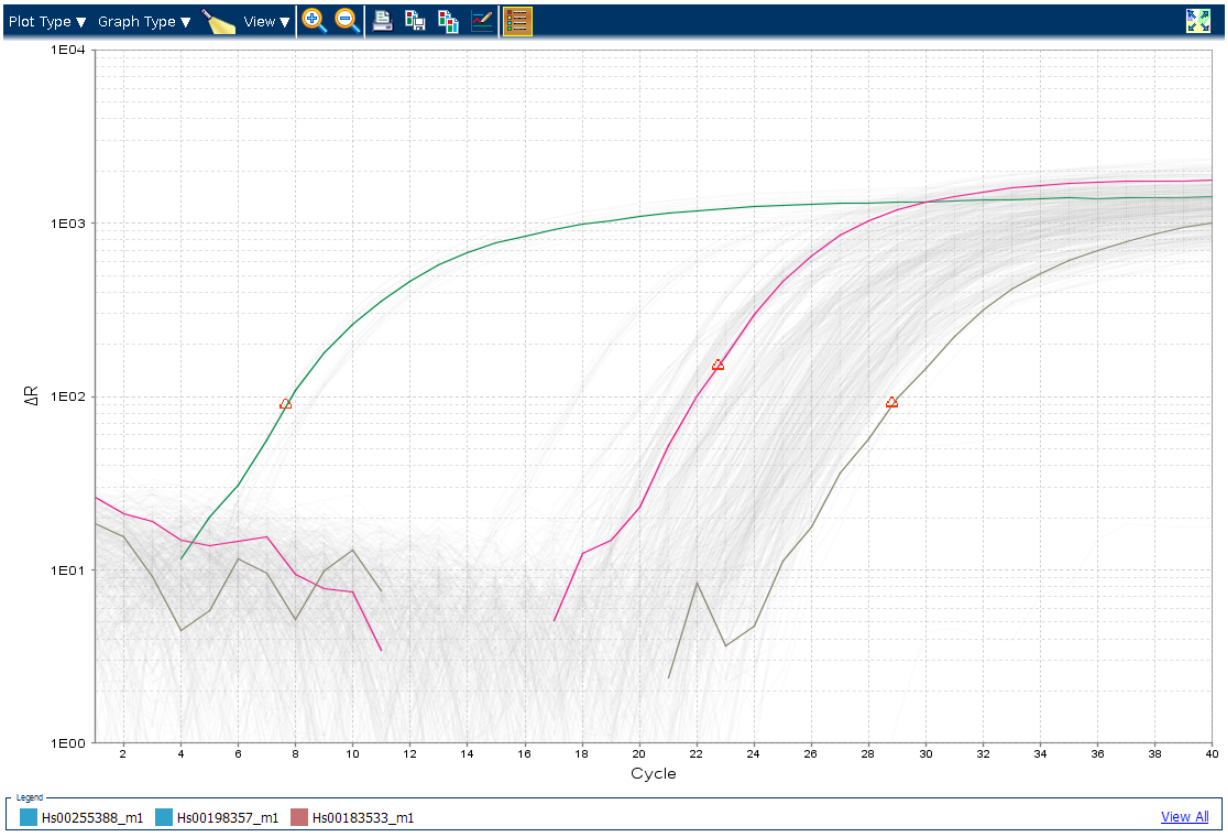


3. In the Amplification Plot screen, select:

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

4. View the C_{RT} values:
   a. From the View drop-down, **Show C_{RT}**.

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



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

Identify well problems using the Well Table

The Well Table displays data for each well in the reaction plate, including:

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

Example experiment values and flags

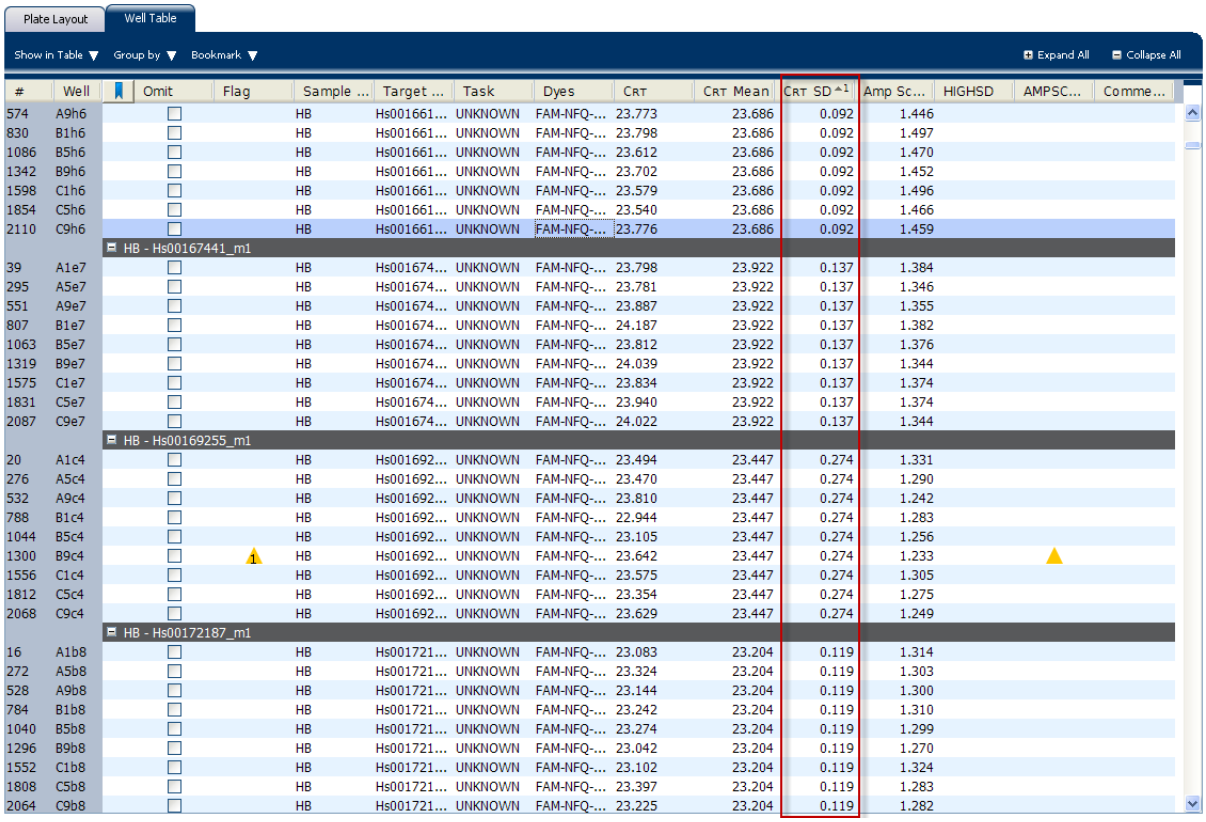
Review the Well Table to evaluate the C_{RT} precision of the replicate groups and AMPSCORE values.

Note: The software produces a flag called AMPSCORE, 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 pane, select **Analysis** ▸ **Amplification Plot**, then click the **Well Table** tab.
2. From the Group By drop-down 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			

Note: To show or hide columns in the Well Table, select or deselect the column name from the Show in Table drop-down menu.

Assessing 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 screen displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

Purpose

In the OpenArray Gene Expression example experiment, you review the Multicomponent Plot screen 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 pane, select **Analysis ▸ Multicomponent Plot**.

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

2. Display the unknown and standard wells one at a time in the Multicomponent Plot screen:

   a. Click the **Plate Layout** tab.

   b. Select one well in the plate layout; the well is shown in the Multicomponent Plot screen.

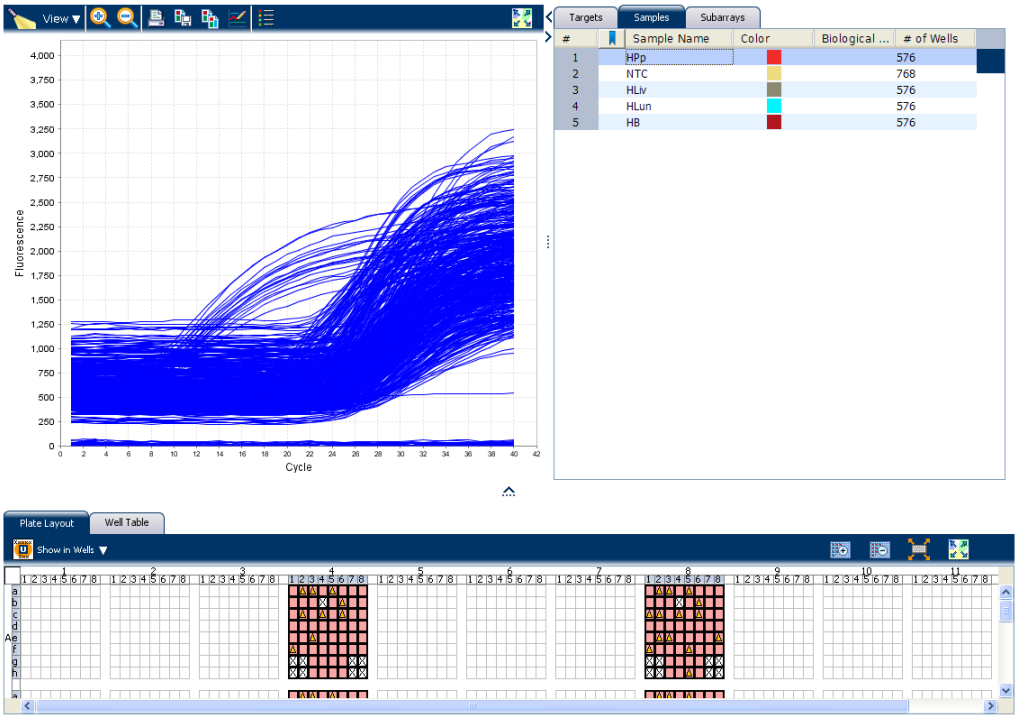
      Note: If you select multiple wells, the Multicomponent Plot screen displays the data for all selected wells simultaneously.

3. From the View drop-down menu, select **Dye Color**.

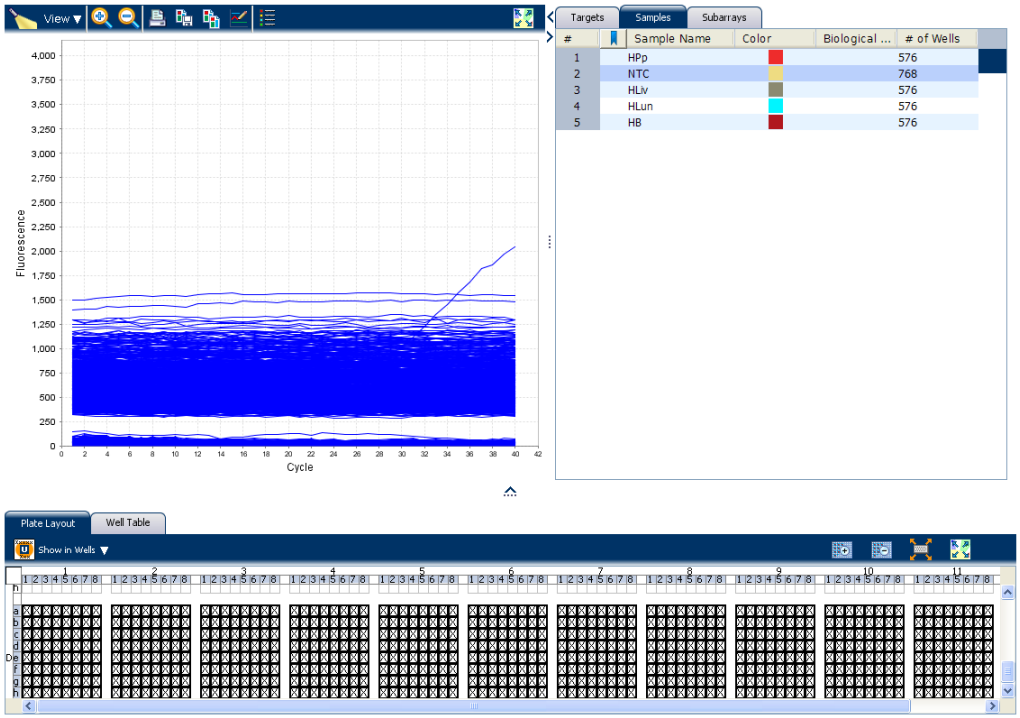
4. Click **Show a legend for the plot** (default).

   Note: 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 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 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 OpenArray Gene Expression experiment, look for:

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

About the example experiment

In the OpenArray Gene Expression 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 pane, select **Analysis ▸ Raw Data Plot**.

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

2. Display all wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the Plate Layout tab.

3. Click **Show a legend for the plot** (default).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

   Note: The legend displays the color code for each row of the reaction plate (see the legend in the Raw Data Plot shown below).

4. Click and drag the Show Cycle pointer from cycle 1 to cycle 40. In the example experiment, there is a stable increase in signal from filter 1, which 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 in each filter:

- Characteristic signal growth
- No abrupt changes or dips

Review the flags in the QC Summary

The QC Summary screen displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment.

Review the QC Summary screen in the OpenArray Gene Expression example experiment for any flags triggered by the experiment data. Wells 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, C12e3 have data that triggered the HIGHSD flag.

Wells 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, C12f1 have data that triggered the AMPSCORE flag.

View the QC Summary

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

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

2. Review the Flags Summary.

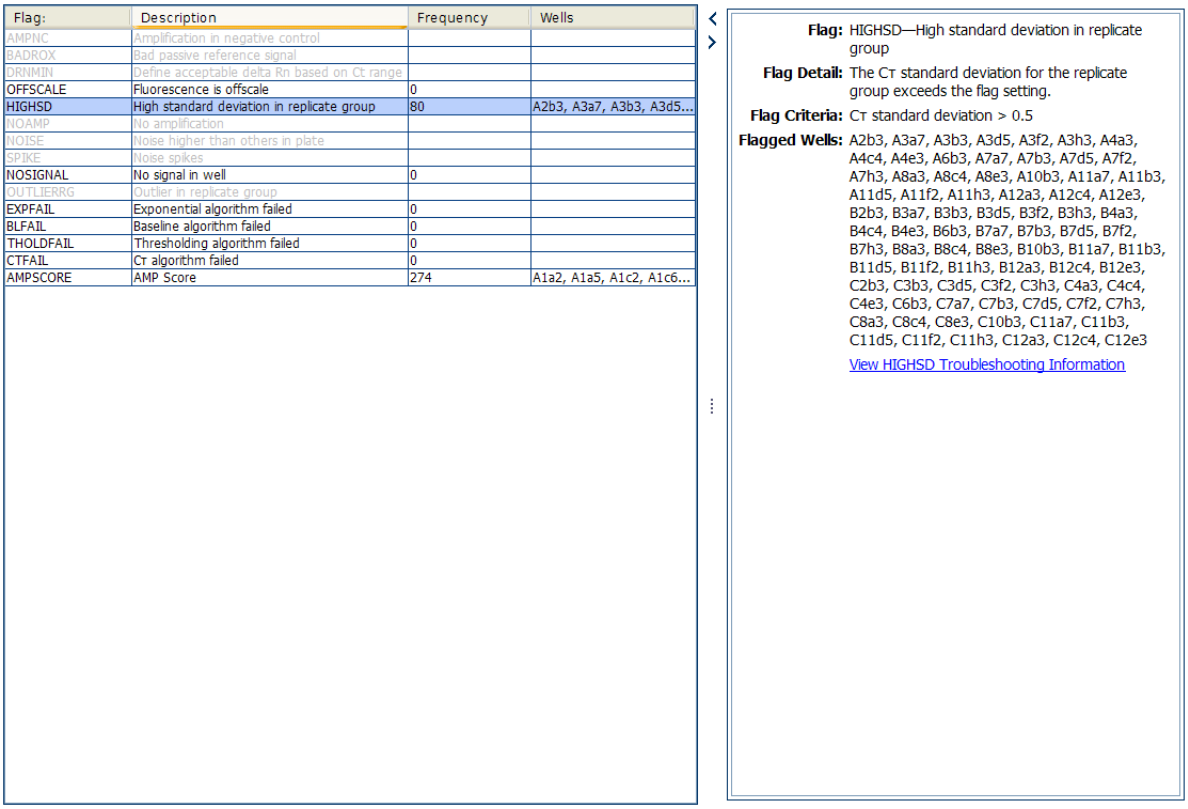
   Note: A 0 displayed in the Frequency column indicates that the flag does not appear in the experiment. If the frequency is > 0, the flag appears somewhere in the experiment; the well position is listed in the Wells column.

   In the example experiment, there are 354 flagged wells.

3. In the Flag Details table, click each flag with a frequency >0 to display 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 frequency >0, click the troubleshooting link to view information on correcting the flag.

The QC Summary for the example experiment looks like this:



Flag:	Description	Frequency	Wells
AMPNC	Amplification in negative control		
BADROX	Bad passive reference signal		
DRNMIN	Define acceptable delta Rn based on Ct range		
OFFSCALE	Fluorescence is offscale	0	
HIGHSD	High standard deviation in replicate group	80	A2b3, A3a7, A3b3, A3d5...
NOAMP	No amplification		
NOISE	Noise higher than others in plate		
SPIKE	Noise spikes		
NOSIGNAL	No signal in well	0	
OUTLIERRG	Outlier in replicate group		
EXPFAIL	Exponential algorithm failed	0	
BLFAIL	Baseline algorithm failed	0	
THOLDFAIL	Thresholding algorithm failed	0	
CTFAIL	Cт algorithm failed	0	
AMPSCORE	AMP Score	274	A1a2, A1a5, A1c2, A1c6...

Possible flags

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

Flag	Description
Pre-processing flag	
OFFSCALE	Fluorescence is offscale
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 certain threshold, corresponding to the score set in the analysis settings

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

Note: The flags AMPNC, BADROX, DRNMIN, NOAMP, NOISE, SPIKE, and OUTLIERRG are, by default, not in use for the Gene Expression experiment.

Note: 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).

Adjust parameters for re-analysis of your own experiments

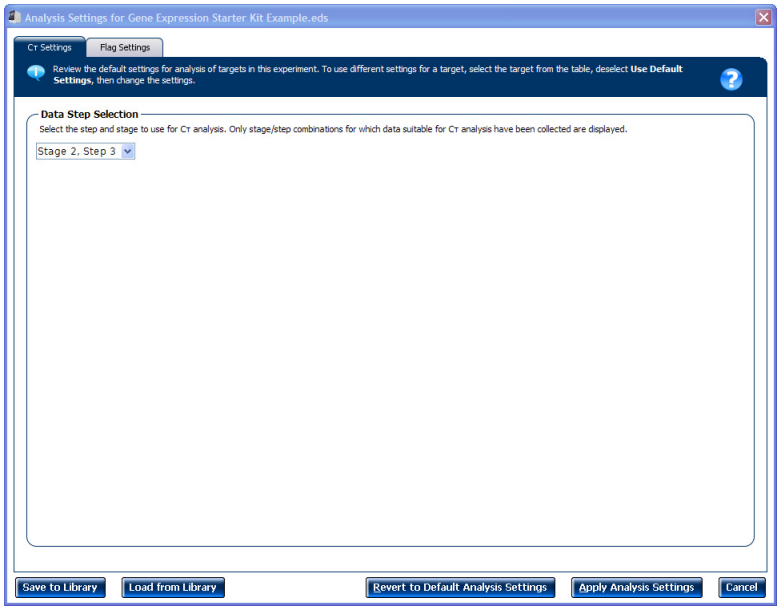
The Analysis Settings dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

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 pane, 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 OpenArray Gene Expression example experiment looks like this:



3. View and, if necessary, change the analysis settings (see "Adjust analysis settings" below).

   Note: You can save the changes to the analysis settings to the Analysis Settings Library for later use. For more information, see "About the Analysis Settings Library" on page 61.

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

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

Adjust analysis settings

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**.**

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.

To adjust the flag settings

1. In the Use column, select the check boxes 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.

   Note: 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 check boxes if you want the software to reject wells with the flag.

   Note: 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** in the Analysis Settings dialog box. 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 ensure C_{RT} precision, omit the outliers from the analysis.

In the OpenArray Gene Expression example experiment, there are 354 outliers. To remove these wells from analysis.

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

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

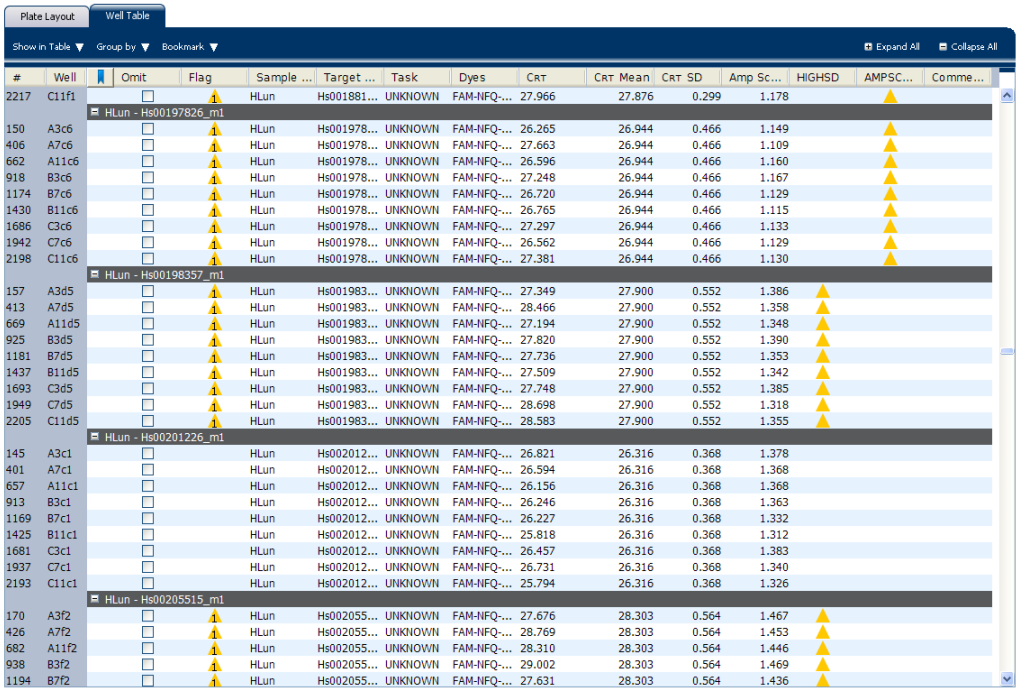
2. In the Amplification Plot screen, select **C_{RT} vs Well** from the Plot Type drop-down menu.

3. Select the **Well Table** tab.

4. In the Well Table, identify outliers:

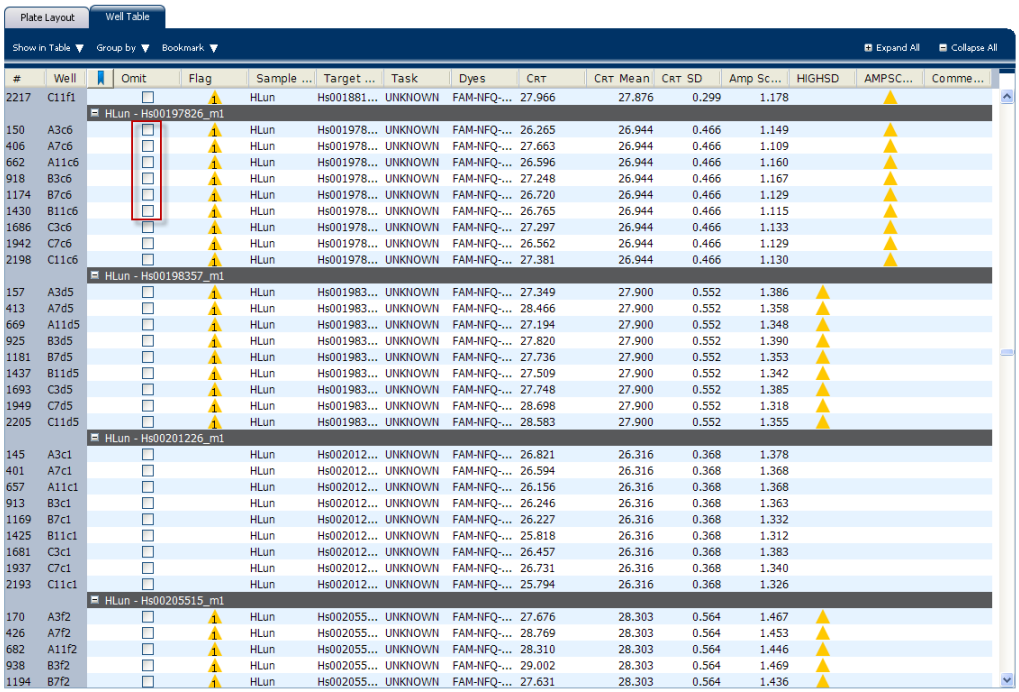
   a. From the Group By drop-down menu, select **Replicate**.

b. Look for outliers in the replicate group (make sure they are flagged).



#	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	▲		

c. Select the **Omit** check box next to outlying well(s).



#	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. Click **Analyze** to reanalyze the experiment data with the outlying well(s) removed from the analysis.

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 and select **Omit**.

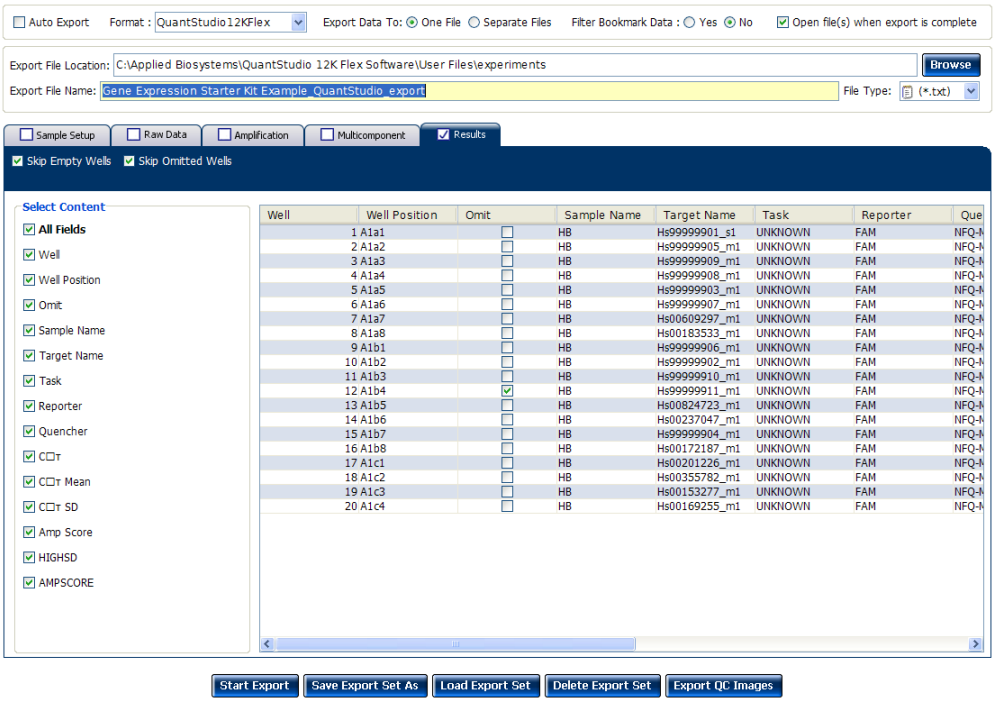
Export the analyzed data

1. Open the OpenArray Gene Expression example experiment file that you analyzed in Chapter 5.
2. In the Experiment Menu, click  **Export.**

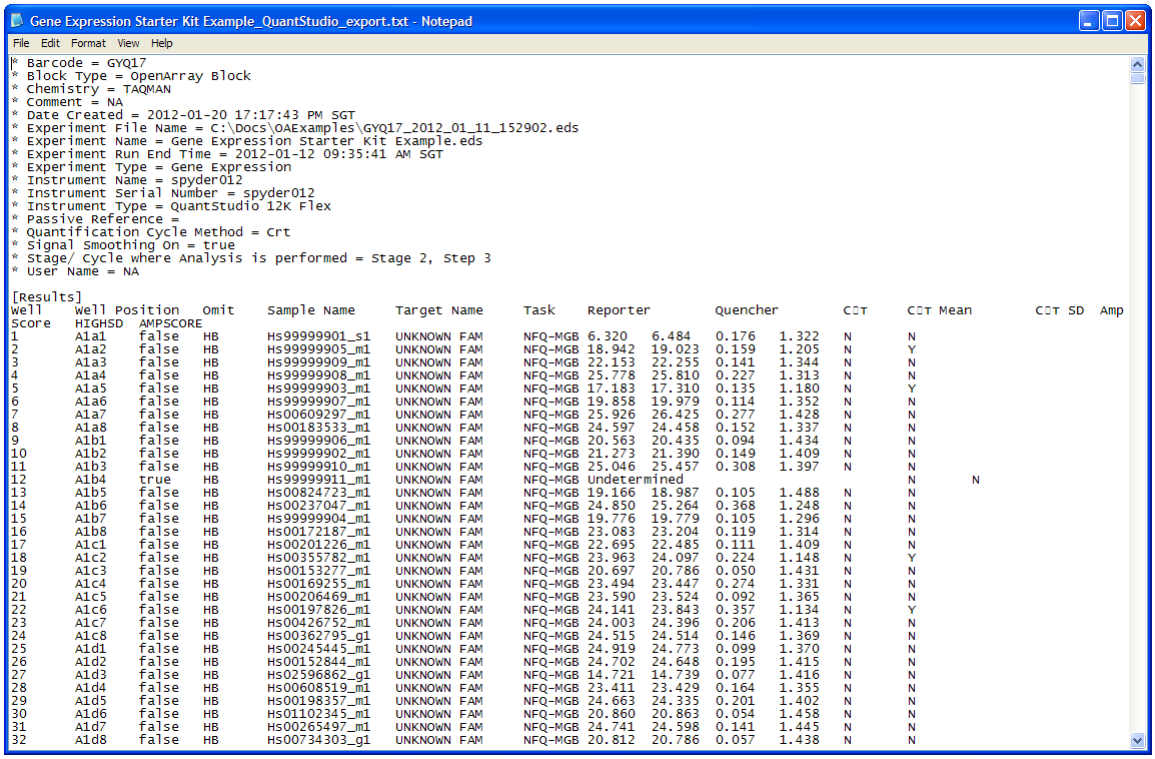
   Note: To export data automatically after analysis, select the **Auto Export** check box during experiment setup or before running the experiment. Auto export is unchecked for the example experiment.
3. Select **QuantStudio™ 12K Flex format**.
4. Complete the Export dialog box as shown below:

Field or Selection	Entry
Select Data to export/ Select Content	Results
Export Data To	One File
Export File Name	Gene Expression Starter Kit Example_QuantStudio_export
Filter Bookmark Data	No
File Type	*.txt
Export File Location	<drive>:\Applied Biosystems\QuantStudio 12K Flex Software\experiments

Your Export screen should look like this:



Your exported file when opened in Notepad 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        CT      CT Mean         CT 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
```

Symbols

Numerics

A

B

C

D

E

P

Q

R

S

T

U

W

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USER GUIDE

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Booklet 2 - QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit

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Contents

1 Introduction

About the OpenArray® Genotyping Starter Kit

Each QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit:

- Contains all the materials (OpenArray® plates, reagents, and accessories) you need to perform two experiments on the QuantStudio™ 12K Flex System, from sample preparation to data analysis unless otherwise noted in Table 1 on page 8
- Represents a typical setup for two genotyping experiments

Table 1 Starter kit description and contents

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit	4469605	• 2X TaqMan® OpenArray® Genotyping Master Mix • TaqMan® OpenArray® Genotyping Training Plates (2 plates) • QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit (Part no. 4469589)	Contains reagents to conduct two real-time genotyping experiments on the QuantStudio™ 12K Flex System, using the TaqMan® OpenArray® Genotyping Training Plate as an example. This kit does not contain samples.
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit	4469586	• QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (1 or 2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid and tips (6 syringes) • OpenArray® AccuFill™ System Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Contains accessories to assemble QuantStudio™ 12K Flex TaqMan® OpenArray® plates for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

About the plates

The instructions in this document use three types of plates, as described in Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*:

- MicroAmp® Optical 96-Well Reaction Plate (*96-well plate*)
- OpenArray® 384-Well Sample Plate (*384-well plate*)
- TaqMan® OpenArray® Plate (*OpenArray® plate*)

About the data files (how to track your assays and samples)

Overview

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 data files:

- "Sample information file (*.csv)" on page 9 - allows input of Sample IDs
- "Plate setup file (*.spf)" on page 9 - allows input of Assay IDs and cycling protocol

- "Template file (*.edt)" on page 11 - includes complete setup (samples, assays and cycling protocol) saved as a template
- "Experiment file (*.eds)" on page 11 - complete data file

Note: Additional data files (*.aif, *.txt) are available for selection if you use the Batch Experiment Setup Utility in the QuantStudio™ 12K Flex Software to create and run your own experiments (see the *QuantStudio™ 12K Flex Software Help*; click or press **F1**).

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. Using a sample information file allows you to:

- Track where samples and controls are located in the 96-well plate (see "Prepare the Samples" on page 15).
- Depending on the TaqMan® OpenArray® plate format being used:
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate (see "Prepare the 384-Well Sample Plate" on page 19).
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each TaqMan® OpenArray® plate (see "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 23).
- Associate information about the samples with the data results in order to normalize data or compute standard curves and calculate concentrations.

IMPORTANT! To ensure accurate results, you need to correctly track sample information from plate to plate.

You can import or manually enter sample information into the OpenArray® Sample Tracker Software (see "Track the samples" on page 20), then export a sample information file (*.csv) in the following formats:

- 384-well plate – Integrate this file with a plate setup file (see below) in the QuantStudio™ OpenArray® AccuFill™ Software (see "Prepare for loading" on page 29).
- OpenArray® plate – Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see "From the QuantStudio™ 12K Flex Software" on page 44), 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)

Using an OpenArray® plate setup file

Plate setup files (*.tpf or *.spf) contain the assay information for individual TaqMan® OpenArray® plates, including the gene symbol, gene name, assay ID, and location of each assay on the plate. You can:

- Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate the sample information from a 384-well plate file (*.csv, see above) with the assay information in the plate setup file (see "Prepare for loading" on page 29).
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (*.eds, see "From the QuantStudio™ 12K Flex Software" on page 44).

Accessing the starter kit plate setup files

To create an experiment for the starter kits:

1. Go to **Download OpenArray® TPF & SPF Plate Files**.
2. Select the plate setup file (*.spf) for your starter kit from the product drop-down list:

Starter kit	TaqMan® OpenArray® plate	Experiment type
QuantStudio™ 12K Flex OpenArray® Genotyping Starter Kit	TaqMan® OpenArray® Genotyping Training Plate	Genotyping

Downloading your own plate setup files

In order to process TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex System, you need to download the specific plate files that correspond to your plate and experiment type: For genotyping, download and use SNP plate files (*.spf).

1. Go to the SPF and TPF Plate File Download Options page:
   Download OpenArray® TPF & SPF Plate Files
2. Enter the following for the TaqMan® OpenArray® plate of interest:
   - Sales Order #, as shown on your order invoice

     Note: The Sales Order number is also located in the shipment packing list and in the email confirmation from Life Technologies. If you are unable to locate your order number, please provide Technical Support with the lot number and serial number (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).
   - Lot number and Serial number, as shown on the foil package containing the plates.
   - Serial number, as shown on the TaqMan® OpenArray® plate label.

     Note: The Serial number is also printed on each TaqMan® OpenArray® plate.
3. Download the correct plate file for your TaqMan® OpenArray® plate:

QuantStudio™ 12K Flex TaqMan® OpenArray® plate type	Experiment type	Plate setup file
QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Kits	Genotyping	*.spf

Note: You can either:
1) *(Recommended)* Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate samples with the downloaded *.tpf or *.spf file. Save the file to the OpenArray Plate File Input Folder. The default save location is *<drive>*:\OpenArray\OpenArrayPlates directory. See "Prepare for loading" on page 29 for more information.
Or

2) Use the downloaded *.tpf file directly in the QuantStudio™ 12K Flex Software to start an experiment, then upload samples to the experiment file (*.eds) in the QuantStudio™ 12K Flex Software after the experiment is run (see "Using an OpenArray® plate setup file" on page 45).

Template file (*.edt)

An experiment document template file (*.edt) contains predefined experiment setup information (experiment type, assay names, and run method).

You can use a template to create a new experiment from the:

- QuantStudio™ 12K Flex Software (see page 44)
- QuantStudio™ 12K Flex Instrument Touchscreen (see page 47)

Note: To create and run the starter kit experiments, use the example template files supplied with the QuantStudio™ 12K Flex Software, located at <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Experiment file (*.eds)

An experiment document single file (*.eds) is an electronic record used by the QuantStudio™ 12K Flex Software that contains all of the information about a particular TaqMan® OpenArray® plate run on the QuantStudio™ 12K Flex Instrument, including meta-data (name, barcode, comments), experiment setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.

You can:

- Create and run an experiment using the QuantStudio™ 12K Flex Software (see page 44).

  Note: To create and run the starter kit experiments, use the example template files (*.edt, see page 11) supplied with the QuantStudio™ 12K Flex Software.
- Create and run an experiment using the QuantStudio™ 12K Flex Instrument Touchscreen (see page 47).
- Analyze the experiment results in the QuantStudio™ 12K Flex Software (see page 57).

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

About the OpenArray® Genotyping Starter Kit data files

When you perform the Genotyping starter kit experiment tasks in this guide, you will use example data files supplied with the QuantStudio™ 12K Flex Software and the OpenArray® Sample Tracker Software. Table 2 describes the types of files provided, as well as their file names and installation locations.

Table 2 Starter kit data files referenced in this guide

File type	Description	File name	Location†	Used in
.spf	SNP plate file	NA	SPF and TPF Plate File Download Options page	Ch 3
.csv	96-well sample information file	GT Training Plate DNA 96-Well.csv	C:\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples	Ch 3
.edt	Experiment template	Genotyping.edt	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray	Ch 4
.eds	Experiment	Genotyping Starter Kit Example.eds	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\examples\Genotyping	Ch 6

† *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software and OpenArray® Sample Tracker Software are installed. The default installation drive for both software programs is the C: drive.

General workflow

Prepare the nucleic acid samples (Chapter 2)

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 384-well sample plate (Chapter 3)

Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray® 384-well sample plate.

▼

Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate (Chapter 4)

Prepare for sample transfer using 384-well plates.

▼

Transfer the samples from 384-well plates to TaqMan® OpenArray® plates using the using the QuantStudio™ OpenArray® AccuFill™ System.

▼

Seal the TaqMan® OpenArray® plates.

▼

Complete sample transfer for the remaining TaqMan® OpenArray® plates.

▼

Perform the instrument run (Chapter 5)

Prepare the QuantStudio™ 12K Flex Software.

▼

Load the OpenArray® plate into the instrument.

▼

Run the OpenArray® plates.

▼

(Optional) Monitor the run.

▼

Unload TaqMan® OpenArray® plates from the instrument.

▼

Transfer the experiments results to the computer.

▼

Analyze the experiment results (Chapter 6)

2 Prepare the Samples

Overview

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

Workflow

When you prepare gDNA samples for the real-time genotyping starter kit experiment or for your own genotyping experiments, use the following workflow:

Prepare the nucleic acid samples
Measure the gDNA quality and quantity (page 16)
▼
Transfer the prepared gDNA samples to a 96-well reaction plate (page 17)

Required materials

Item[1]	Source	Part no.
Starting material: human gDNA	User-supplied	—
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813
DNase-free, sterile-filtered water	Major laboratory suppliers (MLS)	—

Item[1]	Source	Part no.
Dual Flat Block GeneAmp® PCR System 9700	Life Technologies	4428234

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

DNA quality

Be sure that the DNA you use with genotyping experiments:

- Is extracted from the raw material 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 not been heated above 60°C; temperatures above 60°C can cause degradation

DNA quantity

Recommended template amount

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

Note that:

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

  Note: 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.

Quantify the DNA

To quantify the amount of gDNA in your human DNA samples, do any of the following:

- Generate an A_{260} reading using a UV spectrophotometer.
- Generate a Qubit® dsDNA HS assay reading using the Qubit® Fluorometer.

Note: Refer to the appropriate instrument or chemistry kit user guide for detailed instructions on performing the DNA quantitation (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).

Transfer the samples to a 96-well reaction plate

To set up the 96-well sample plate(s):

- Transfer 5.0 µL of the gDNA samples and controls (see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*) into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate, based on the OpenArray® plate format being used.

  Note: For the starter kit experiment, the Format 64 OpenArray® genotyping plate format (see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*) is used to test a maximum of 48 samples against the TaqMan® OpenArray® Genotyping Training Plate, a fixed content panel containing 64 TaqMan® SNP Genotyping Assays.

- *(Recommended)* Create a sample information file (*.csv) to track where the samples are in the 96-well sample plate. For detailed procedures, refer to the OpenArray® Sample Tracker Software *Quick Reference*.

Next step

Proceed to Chapter 3, "Prepare the 384-Well Sample Plate" on page 19.

Performing 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's 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 **www.genomesize.com** or **http://data.kew.org/cvalues/**.

- Transfer 5.0 µL of the gDNA samples and controls (Appendix B in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*) into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate, based on the OpenArray® genotyping plate format being used (Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*).

3 Prepare the 384-Well Sample Plate

Overview

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 Plates (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*). You will 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):

Workflow

Prepare the OpenArray® 384-Well Sample Plate
Track the samples (page 20)
▼
Transfer the samples to the 384-well sample plate and add master mix (page 21)

Required materials

Item[1]	Source	Part no.[2]
96-well reaction plates, containing prepared gDNA samples	User supplied (see page 15)	—
2X TaqMan® OpenArray® Genotyping Master Mix	Life Technologies	4404846
OpenArray® 384-Well Sample Plates	Life Technologies	4406947
QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals	Life Technologies	4469876
Fine-tip marker	Major Laboratory Suppliers (MLS)	—

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

2 Provided in starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For genotyping experiments, we recommend that you use the OpenArray® Sample Tracker Software to track your samples.

Note: This section provides brief procedures for using the OpenArray® Sample Tracker Software. For detailed procedures, refer to the OpenArray® *Sample Tracker Software Quick Reference*.

1. In the Sample Tracker Software Properties window, enter general information about the genotyping experiment:
   a. From the Experiment Type drop-down list, select **Genotyping**.
   b. From the Assay Layout drop-down list, select the appropriate TaqMan® OpenArray® plate format:
      - *(For the starter kit experiment)* **Genotyping – 64**
      - *(For your own experiments)* **Genotyping – 16**, **Genotyping – 32**, **Genotyping – 64**, **Genotyping – 128**, **Genotyping – 192**, or **Genotyping – 256**
      - From the Pipettor drop-down list, select **Fixed** or **Adjustable**
      - If you have added a serial number or barcode to the OpenArray® 384-Well Sample Plate, enter the serial number

2. Enter the sample information:
   - *(Recommended)* Navigate to and import sample information from a sample information *.csv file into the OpenArray® Sample Tracker Software, if created (see page 17).
   - Modify the installed <drive>\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples\96-Well Sample Plate 1.csv
   - *(Optional)* Manually enter sample information from the 96-well reaction plates into the OpenArray® Sample Tracker Software.

   The OpenArray® Sample Tracker Software automatically maps the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates and TaqMan® OpenArray® plates.

3. Export the sample information in table format (*.csv):
   a. In the Sample Mapping window, select the 384-well tab, then click **Export** ▸ **Export *.csv**.
   b. Select the plates to export as *.csv files:
      - *(Recommended, and for the starter kit experiment)* **384-well Plate** – Use this file with the QuantStudio™ OpenArray® AccuFill™ Software to create an integrated SNP plate file (*.spf, see "Prepare for loading" on page 29).
      - *(Optional)* **OpenArray Plate *n*** – Use this *.csv file to import setup information into the QuantStudio™ 12K Flex Software (see "From the QuantStudio™ 12K Flex Software" on page 44).

        Note: You must select this option when using the Format 16 or Format 32 OpenArray® genotyping plate formats (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*) for your own experiments.

      All plates are saved to individual *.csv files in the export directory. The OpenArray® Sample Tracker Software automatically assigns the file names.

4. Using a fine-tip marker:
   a. Label the 384-well sample plate with a unique identifier.
   b. Based on the tracking information obtained in steps 1 to 3 above, mark the sections of the 384-well sample plate that you will transfer the samples to from the 96-well reaction plates.

Transfer the samples

1. Thaw the 96-well reaction plate containing prepared gDNA samples at room temperature. Mix the gDNA samples by vortexing, then spin for 1 minute @ 1000 rpm.

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

   Note: For optimal results, it is important to normalize all gDNA samples in an experiment. For human gDNA, make sure 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 2X TaqMan® OpenArray® Genotyping Master Mix by gently inverting the bottle 10 times.

4. Based on the layout you determined (see page 20), load the 384-well sample plate:

   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 (µL) per well[1], when transferring to...		
	Format 64 *(starter kit experiment)* and larger formats	Format 16	Format 32
2X TaqMan® OpenArray® Genotyping Master Mix	2.5	1.5	2.0
Normalized gDNA sample *(human gDNA starting concentration = 50 ng/µL)*	2.5	1.5	2.0
Total volume	**5.0**	**3.0**	**4.0**

1 One well of a 384-well sample plate corresponds to one subarray of a TaqMan® OpenArray® plate (see Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*). The component volumes vary, depending on the format of the TaqMan® OpenArray® plate that you will later transfer the samples to.

5. Cover the sample plate with foil, vortex gently to mix, then centrifuge for 1 minute @ 1000 rpm to eliminate bubbles.

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

Next step

Proceed to Chapter 4, "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 23.

4 Prepare the QuantStudio™ 12K Flex OpenArray® Plate

Overview

In this chapter, you use the QuantStudio™ OpenArray® AccuFill™ System to transfer the nucleic acid samples from the OpenArray® 384–Well Sample Plate to QuantStudio™ 12K Flex TaqMan® OpenArray® plates. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate

Prepare for sample transfer (page 26)

▼

Transfer the samples (page 28)

▼

Seal the plate (page 35)

▼

Complete sample transfer for the remaining TaqMan® OpenArray® plates (page 37)

Required materials

Item[1]	Source	Part no.
QuantStudio™ 12K Flex TaqMan® OpenArray® plates[2]	Life Technologies	See Appendix A in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*
QuantStudio™ OpenArray® AccuFill™ System	Life Technologies	4471021
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit The accessories kit contains: • QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid (6 syringes) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid Tip • OpenArray® AccuFill™ System Loader Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Life Technologies	4469586
QuantStudio™ 12K Flex OpenArray® Plate Press 2.0	Life Technologies	A24945
Foil seals	Major Laboratory Suppliers (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 the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

2 For detailed information about the TaqMan® OpenArray® plates, see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*

Storage conditions

The following materials require special storage conditions:

Item		Storage Conditions
If the QuantStudio™ 12K Flex TaqMan® OpenArray® plate is...	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.
QuantStudio™ 12K Flex 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 Loader Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Discard unused tips after the expiration date printed on the cardboard box.

Prepare for sample transfer

Guidelines for handling the TaqMan® OpenArray® plate

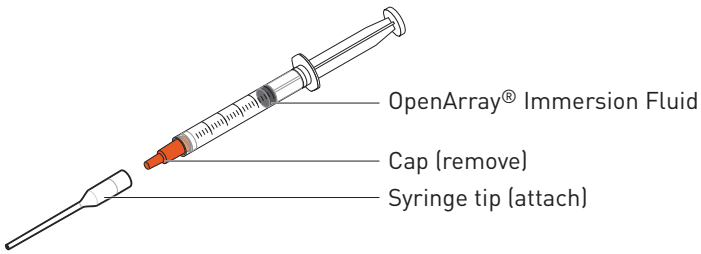
- Hold the OpenArray® case by the edges.
- Do not touch the through-holes of the OpenArray® plate.
- Load and seal an OpenArray® plate within *one hour* after opening the packaging.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray®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

Prepare the equipment and plates

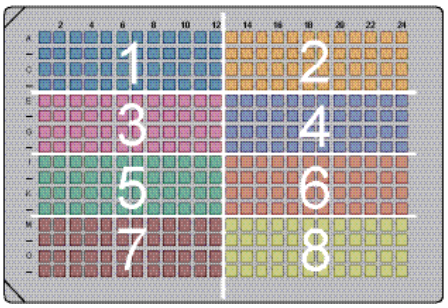
IMPORTANT! Wear powder-free gloves while preparing the OpenArray® plates.

1. Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ System Loader Tips, and plate holder are completely clean and dry. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).
2. Remove an OpenArray® plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).
3. 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.



4. 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

For each OpenArray® plate being prepared, note the following:

- *For the starter kit experiments and recommended for your own experiments*, a plate setup file (*.csv, *.spf, or *.tpf) is needed to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software.

  Note: If no samples are provided with the starter kit, create a *.csv file as you would for your own experiments.

- *For your own genotyping experiments*, the following plate setup files can be used to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software:
  - OpenArray® 384-well sample information file (*.csv, see "Track the samples" on page 20)
  - OpenArray® plate setup file (*.spf or *.tpf, see "Using an OpenArray® plate setup file" on page 9)
- *(Optional)* If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 20), 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.

Next step

Proceed immediately to "Transfer the samples" on page 28.

Transfer the samples

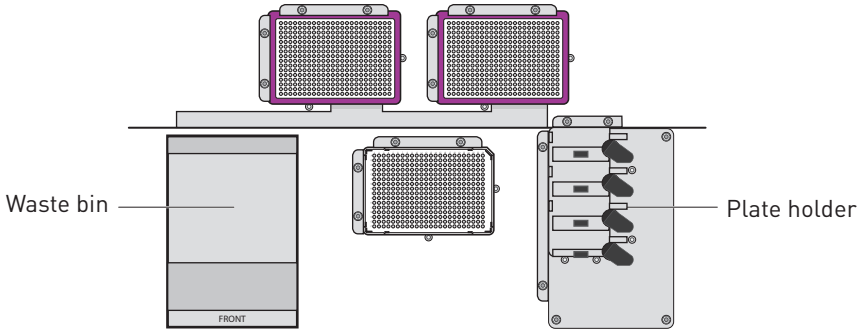
Initialize the system

1. Close the enclosure door, then start the QuantStudio™ OpenArray® AccuFill™ Software. The software checks the computer and connections as the system starts.

   When prompted, clear the deck and empty the waste bin of used tips:

   a. Open the instrument by grasping the enclosure door handle and gently, but firmly, pulling the enclosure door up.

   b. Empty the waste bin and place it back on the deck.



   Note: To safely operate the instrument, it is important to keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

2. Check if there are any OpenArray® plates in the plate holder on the deck. If necessary, remove them.

3. If necessary, replace the tip boxes.

   Note: Tip boxes contain 384 tips, divided into 8 sections. When you click **Load**, the QuantStudio™ OpenArray® AccuFill™ System loads as though a new, full box of tips is on the deck. QuantStudio™ OpenArray® AccuFill™ Software prompts you to verify that tips are in the locations shown in the Setup Deck screen (see "Load the OpenArray® plate" on page 31). Clicking a section in the Setup Deck window confirms that tips are in that section of the tip box.

   a. Place tip boxes on the deck in the two side-by-side recessed rectangular platforms (purple and white locations as shown in the illustration above).

   b. Remove the cover before using the tips for loading.

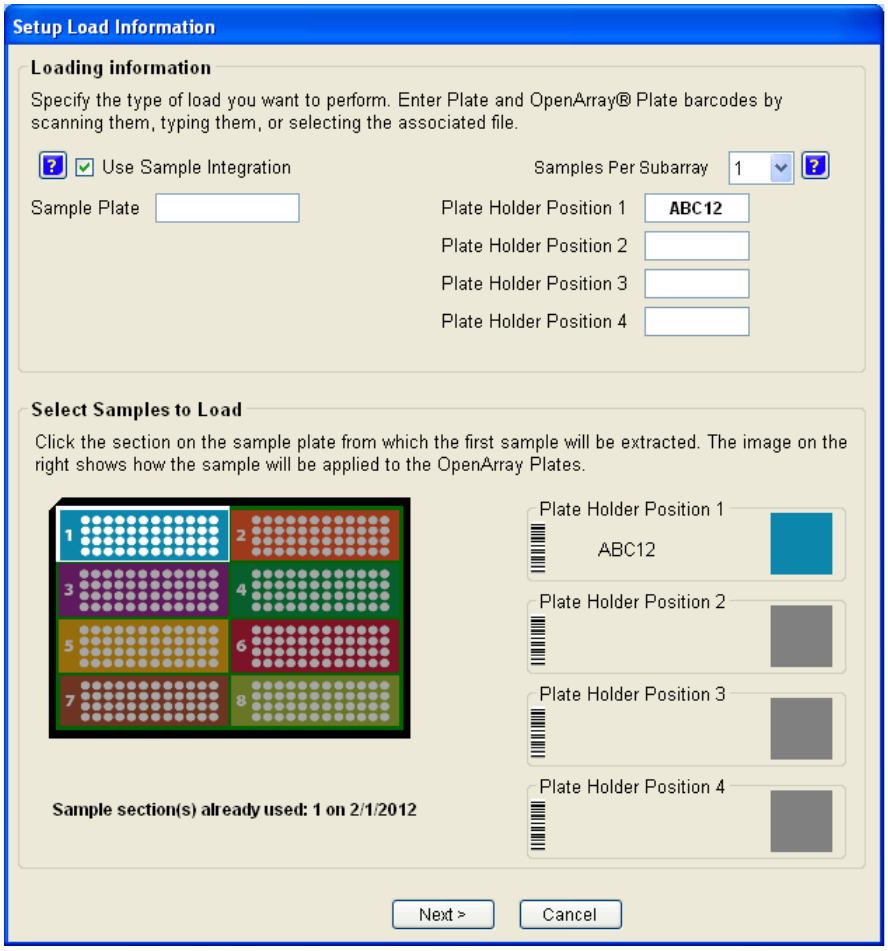
4. Close the door on the instrument.

5. Click **Proceed** to begin the System Self Test. The application performs a number of self tests and is then ready for you to continue.

   Note: System Self Test runs only at start up. The test does not run again unless the system is restarted or a self test is intentionally run. The System Self Test utility is in the Instrument drop-down menu in the QuantStudio™ OpenArray® AccuFill™ Software.

Prepare for loading

1. Click **Setup & Load**, then complete the Setup Load Information window.



2. Do either of the following:
   - Select the **Use Sample Integration** check box, then proceed to step 3.
   - *(Required for the starter kit experiments, recommended for your own experiments)* Proceed to step 5.

     Note: For the starter kit experiments, you will import sample and assay information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 44).

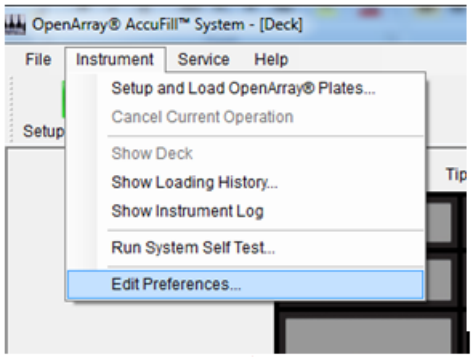
     Note: For the starter kit experiments, you will use an installed *.edt file (with experiment type, assay name, and run method).

3. In the Sample Plate field, browse to and open the *.csv file that contains the 384-well sample plate layout (see "Track the samples" on page 20).

   To set up sample integration in the QuantStudio™ OpenArray® AccuFill™ Software:

   a. Launch QuantStudio™ OpenArray® AccuFill™ Software version 1.1.

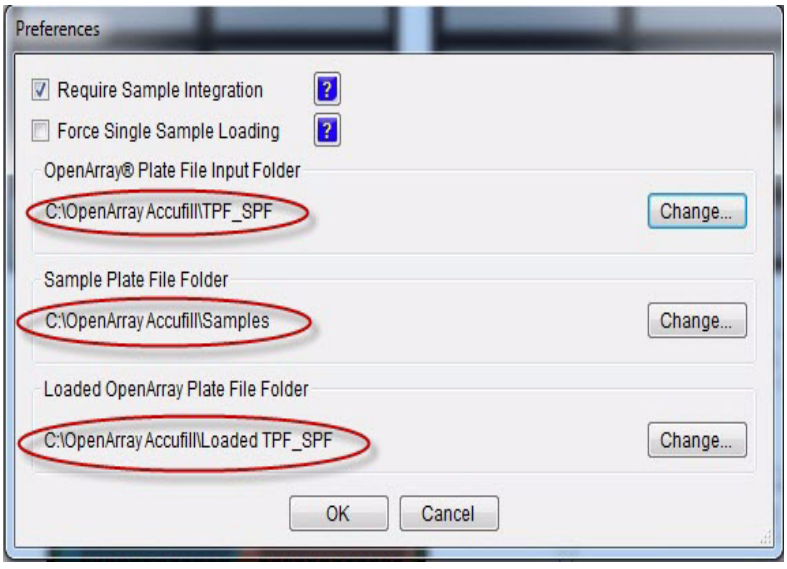
b. Go to **Instrument** ▸ **Edit Preferences**.



c. In the Preferences dialog box, check **Require Sample Integration**.

d. Click **Change** to select another location for the Input, Sample Plate, and Loaded OpenArray Plate folders.

- **Input folder** (<drive>:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF): Contains *.tpf files that are downloaded from the web or CD.
- **Sample Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Samples): Contains sample *.csv files (in the 384 format from Sample Tracker).
- **Loaded OpenArray Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Loaded TPF SPF): Contains integrated *.spf files with sample names. The QuantStudio™ OpenArray® AccuFill™ Software automatically places the integrated *.spf file with sample names in this folder (after the QuantStudio™ OpenArray® AccuFill™ Software run). The resulting *.spf file includes the sample names.



4. Enter the data for the first OpenArray® plate:

a. Select **1** from the Samples Per Subarray drop-down list.

b. In the Plate Holder Position 1 text field, enter the 5-character alphanumeric serial number of the OpenArray® plate you will load into the first position of the plate holder. You can:

- Click **Browse**, then navigate to and open the plate setup file (*.spf or *.tpf) that corresponds to the OpenArray® plate. The software automatically displays the serial number in the Plate Holder Position 1 field.
- Scan the serial number (barcode) located on the OpenArray® plate package.
- Type the serial number.

IMPORTANT! When you integrate a SampleID.csv into a plate setup file and enter the serial number by scanning or typing, the plate setup file must be located in the *<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Sample directory (see "Using an OpenArray® plate setup file" on page 9). Otherwise, the software will not be able to locate the file. *<drive>* is the computer hard drive on which the QuantStudio™ OpenArray® AccuFill™ Software is installed.

Note: The QuantStudio™ OpenArray® AccuFill™ Software uses the serial number to access the appropriate plate setup files. During an instrument run, information in the plate setup files is used to populate the Assays screen in the QuantStudio™ 12K Flex Software. For information on the Assays screen, see "Analyze the Experiment Results" on page 57.

As you enter the serial number, it is reflected in the representation of the OpenArray® plates in the lower section of the window.

5. Repeat step 2 for the remaining OpenArray® plate(s).
6. Click **Next**.

Note: You can also enter sample information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 44).
You can download plate setup files (.tpf/.spf and a new .edt without sample names). You can then add names in the QuantStudio™ 12K Flex Software using the Import or direct edit feature.

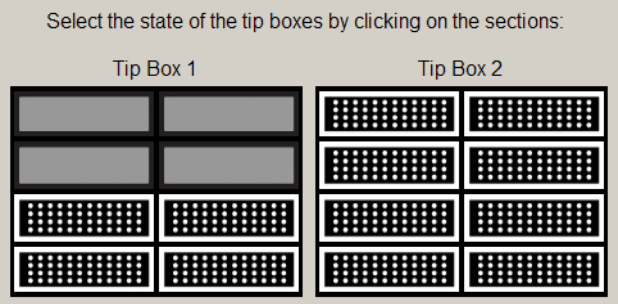
Load the OpenArray® plate

1. Open the enclosure door of the QuantStudio™ OpenArray® AccuFill™ System by grasping the door handle and lifting the door up.
2. Insert the OpenArray® 384-Well Sample Plate with the foil cover still in place. Press on the plate until you hear it snap into place.

   Note: Do not remove the foil from the OpenArray® 384-Well Sample Plate at this time.

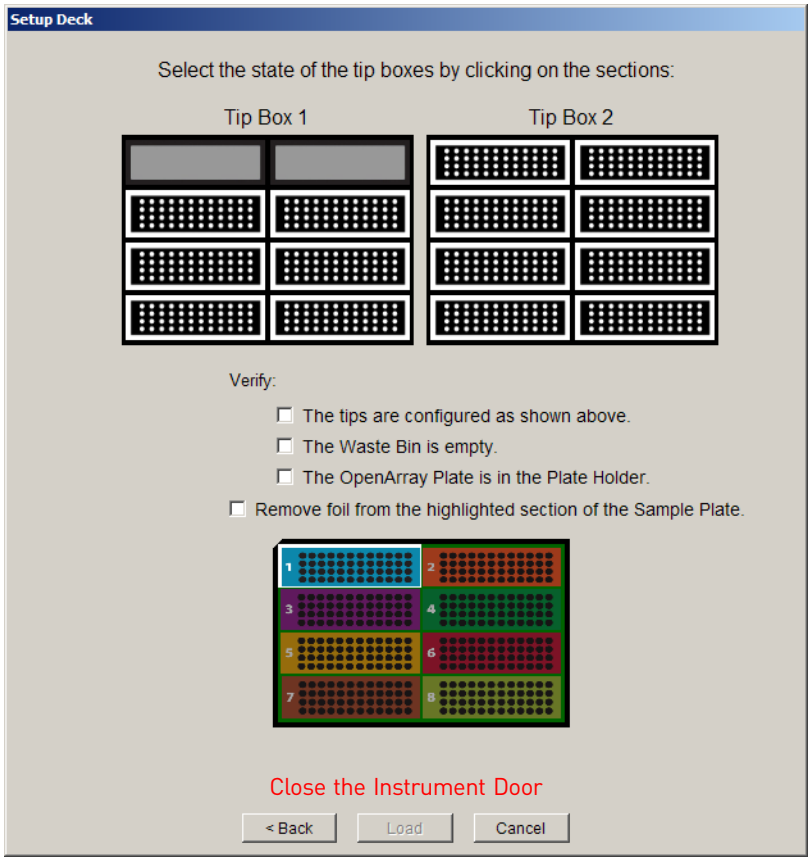
3. Place a thawed OpenArray® plate into the Plate Holder. When handling the OpenArray® plate:
   - Always hold the OpenArray® case by the edges and place it into the Plate Holder with the barcode face up and to the left.
   - If you inadvertently drop a loaded OpenArray® plate, discard it in the sharps waste container.
   - Be sure to load the OpenArray® plate within an hour after you open it.
4. Visually verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that no tips are present.
   - White areas indicate that tips are present.

   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window. For example:



Note: Cover the tip box when not in use. Discard any unused tips after 1 year or after the expiration date printed on the cardboard box.

5. Verify each of the following conditions and, when verified, select its check box:
   - Tips are configured as shown in step 4 above.
   - Waste bin is empty.
   - OpenArray® plate is in the Plate Holder.

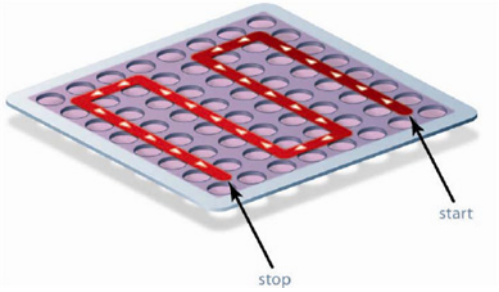


Note: The software will not continue until you select all the check boxes.

6. With forceps, peel off the foil covering the area of the OpenArray® 384-Well Sample Plate containing the samples to be loaded on the OpenArray® plate.
7. Select **Remove foil from the highlighted section of the Sample Plate**.
8. Close the instrument door.
9. Click **Load**.

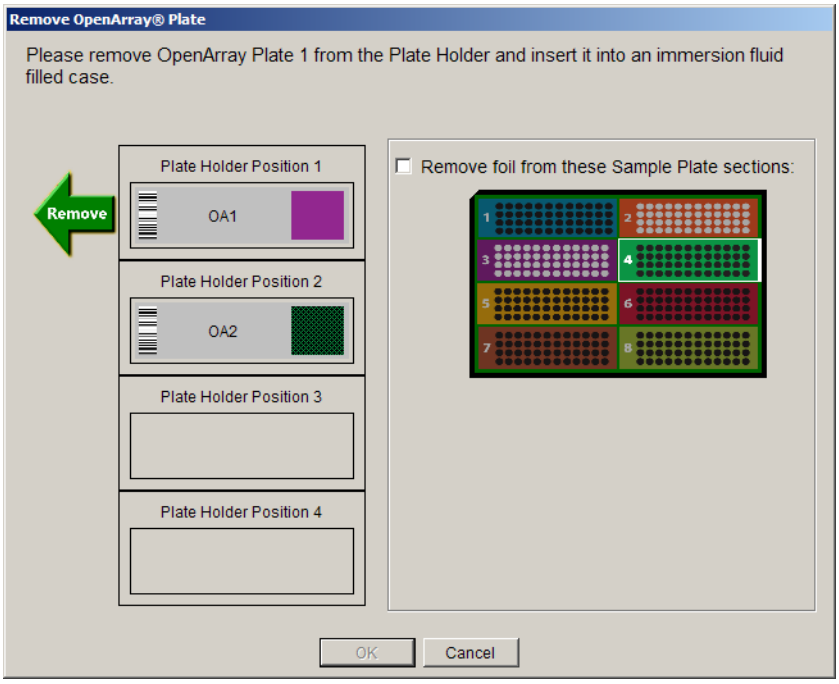
   Note: If the number of OpenArray® plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra OpenArray® plates. Correct the error and continue.

You can follow the progress of the loading on the screen. The samples in each tip are loaded in the OpenArray® plate. Each tip fills the 64 through-holes in one subarray, travelling in the pattern shown (the following illustration shows the load path for only one sample):



10. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate as explained in "Seal the OpenArray® plate" on page 35.

IMPORTANT! Once an OpenArray® plate has been filled, you must seal it within 90 seconds to prevent excessive evaporation.



11. Close the instrument door.

Note: After you load the plate, clean the QuantStudio™ OpenArray® AccuFill™ System according to the Applied Biosystems *QuantStudio™* 12K Flex Real-Time PCR System *Maintenance and Administration Guide* (Part no. 4470689).

Note: *(If Use Sample Integration on page 29 is checked)* You must have the plate setup files (*.spf or *.tpf) in the OpenArrayPlate folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF) and the SampleID.csv file in Sample Plates folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\Samples).

To integrate, click **Browse** next to the blank window under Use Sample Integration and next to Sample Plate, and navigate to the location of the SampleID.csv file. Select the file with the information on the samples that are going to be loaded into a given set of OpenArray® plate(s). Click **Open**.

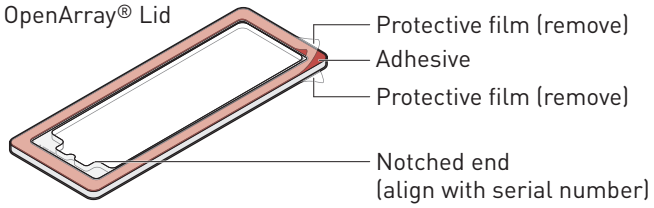
The plate setup file (*.spf or *.tpf) is now integrated with the sample information file (*.csv) and is called *Loaded_<barcode>.spf*. You can use this file in the QuantStudio™ 12K Flex Software to create and run an OpenArray® experiment (see "Using an OpenArray® plate setup file" on page 49). Proceed with Load the OpenArray plate.

Next step Proceed immediately to "Seal the OpenArray® plate" below.

Seal the OpenArray® plate

1. Remove the protective film from the top *and* bottom of an OpenArray® Case Lid.

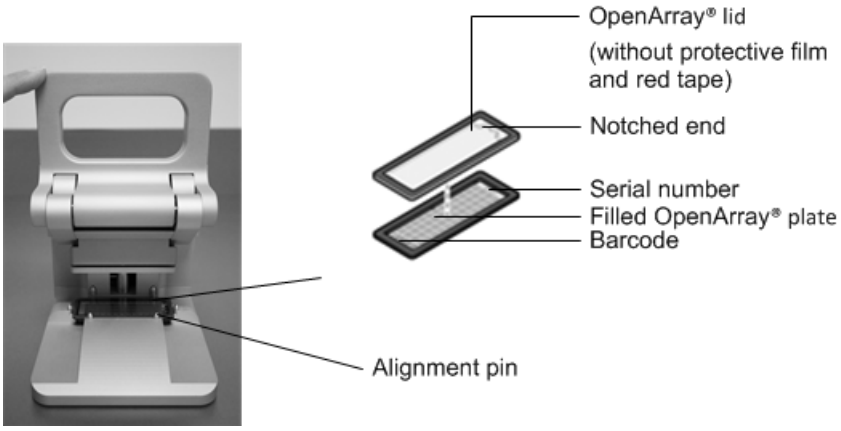
IMPORTANT! The protective film at the bottom of the Case Lid is covered by a red tape that needs to be removed first to access the protective film. Make sure to remove the protective film from *both* sides of the lids.



2. Using the thumb and index finger, grasp the OpenArray® case by the top (nearest the barcode), gently lift the case from the plate holder, then load it into the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

3. Place the Case Lid with red tape and protective film removed (both top and bottom) onto the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the lid must be oriented toward the right side of the Plate Press.



4. Actuate the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0 by pulling down the lever.

5. The status light flashes green for 20 seconds. After 20 seconds, the status light turns solid green indicating that the case is ready.

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

6. Release the lever.

7. Load the OpenArray® case with OpenArray® Immersion Fluid:

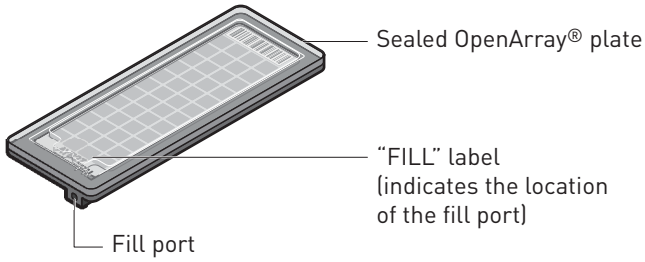
IMPORTANT! Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 seconds.

   a. Remove the sealed plate from the Plate Press, grasping the case on the edges.

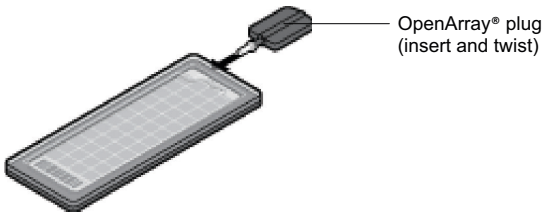
   b. Insert the syringe tip into the loading port at end of the sealed Case, then dispense the fluid completely in one gentle continuous motion.

IMPORTANT! Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through-holes.

Note: Try to minimize creating air bubbles when you dispense the fluid: one small air bubble is acceptable.



c. While holding the OpenArray® plate vertically, seal the loading port by inserting the OpenArray® Plug into the port and twisting the plug clockwise, applying sufficient pressure until the handle breaks off.



d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; be sure to not apply pressure on the OpenArray® plate within the case.

The sealed OpenArray® plate can be loaded into the QuantStudio™ 12K Flex System.

Note: Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.

STOPPING POINT For genotyping experiments, you can store loaded and sealed OpenArray® plates at room temperature, protected from light, for up to 72 hours.

Next step

Proceed to:

- "Complete sample transfer for the remaining plates" below

  or

- Chapter 5, "Perform the Instrument Run" on page 39

Complete sample transfer for the remaining plates

Repeat the following procedures to transfer sample to the remaining OpenArray® plates:

- "Prepare for sample transfer" on page 26 *(if loading > 4* OpenArray® *plates)*
- "Transfer the samples" on page 28
- "Seal the OpenArray® plate" on page 35

Next step Proceed to Chapter 5, "Perform the Instrument Run" on page 39.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (>6) of OpenArray® plates, follow the guidelines below.

- To help avoid mistakes when entering sample information in the QuantStudio™ OpenArray® AccuFill™ Software, load the OpenArray® plates in alphanumeric order (per the OpenArray® plate serial number).
- Seal each OpenArray® plate immediately after loading is completed, while 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 an vertical position.

- Use the QuantStudio™ Carrier to transport up to four loaded OpenArray® plates to the QuantStudio™ 12K Flex Real-Time PCR System.
- After loading is complete, you can use a large bin to properly dispose of any used OpenArray® AccuFill™ System Loader Tips. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

5 Perform the Instrument Run

Overview

In this chapter, you run the QuantStudio™ 12K Flex TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex Real-Time PCR System. During the run, the QuantStudio™ system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Perform the instrument run

Prepare the QuantStudio™ 12K Flex Software (page 41)

▼

Load the TaqMan® OpenArray® plate into the instrument (page 43)

▼

Run the plates (page 44)

▼

(Optional) Monitor the run (page 49)

▼

Unload the instrument (page 52)

▼

Transfer the experiments results to the computer (page 54)

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 OpenArray® experiments.

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

To select OpenArray® experiment preferences:

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. Go to **Tools** ▸ **Preferences** in the QuantStudio™ 12K Flex Software and select the **OpenArray®** tab.
3. Select the following as needed:

Settings	Description
Setup Folder field	Defines the absolute path to the default folder/directory from which the QuantStudio™ 12K Flex Software imports experiment setup files. The Import dialog box opens to the import folder when invoked from the QuantStudio™ 12K Flex Software.
Experiment Folder field	Defines the absolute path to the default folder/directory to which the QuantStudio™ 12K Flex Software reads/writes experiment files. The Open and Save dialog boxes open to the data folder when invoked from the QuantStudio™ 12K Flex Software.
Passive Reference drop-down list	Defines the dye to use as the passive reference. The default is set to None. **Note:** While the QuantStudio™ 12K Flex Software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray® experiments.
Default Browse File Type drop-down list	Defines the file type which the Import, Open, and Save dialog boxes select by default when invoked from the QuantStudio™ 12K Flex Software.
Apply experiment template (EDT) to all OpenArray® experiment check box	If selected, the QuantStudio™ 12K Flex Software applies the Run Method defined in the selected experiment template (*.edt) to all OpenArray® experiments. For more information on OpenArray® experiment templates, see the *QuantStudio™ 12K Flex Software Help*.
Always include Amplification stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds an Amplification stage to the Run Method for all OpenArray® genotyping experiments. If deselected, you need to perform amplification on another instrument. For more information on Run Method settings, see the *QuantStudio™ 12K Flex Software Help*.
Always include Pre-Read stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds a Pre-Read stage to the Run Method for all OpenArray® genotyping experiments. For more information on Run Method settings, see the *QuantStudio™ 12K Flex Software Help*.

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

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

Access the Instrument Console

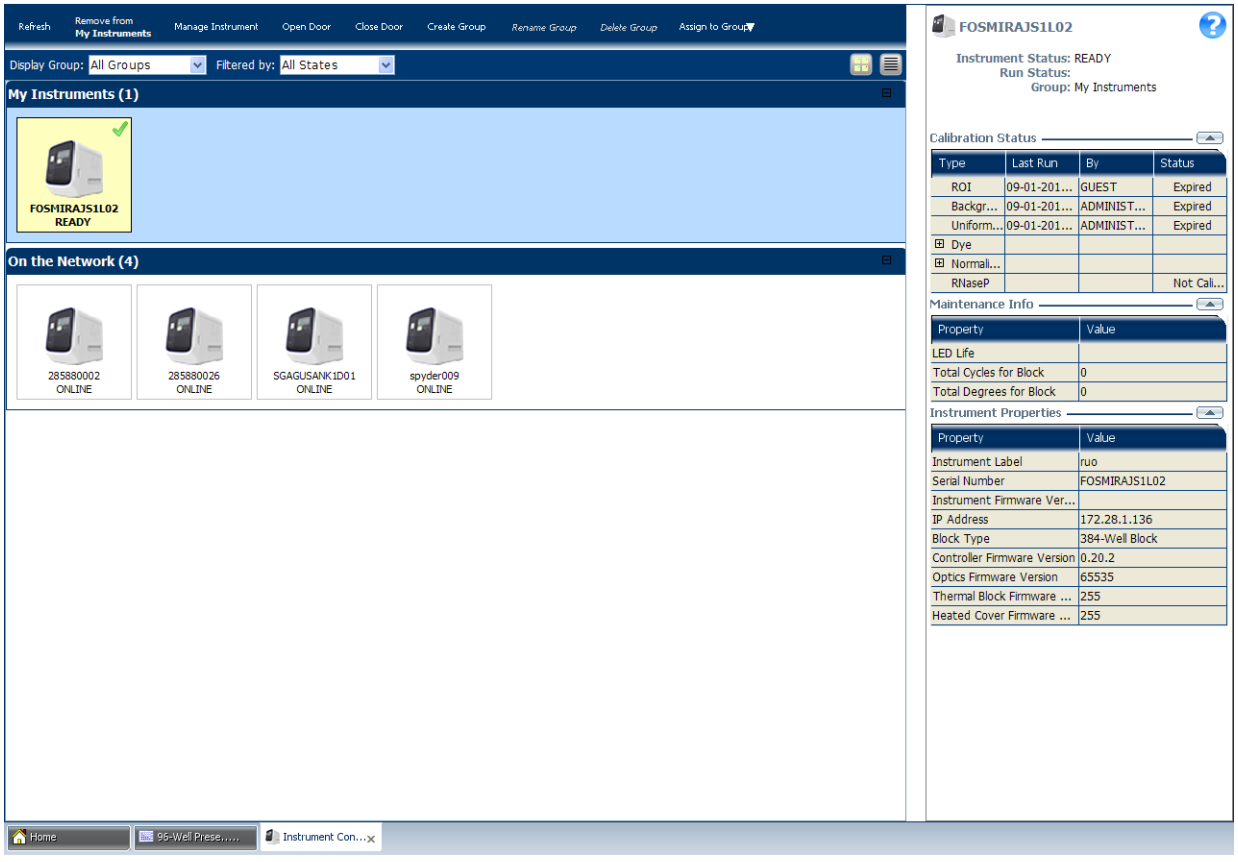
The Instrument Console displays all the QuantStudio™ 12K Flex Instruments discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups:

- **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 QuantStudio™ 12K Flex Software.
2. On the Home tab (), 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 which can be monitored:

a. Click the instrument of interest, then click **Assign to Group** in the Instrument Console toolbar.

b. Select the **My Instruments** 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 Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the Notification Settings feature, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

Load the OpenArray® plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

IMPORTANT! Wear powder-free gloves when you handle OpenArray® plates.

IMPORTANT! OpenArray® plates should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to allow the plate adapter to come out from the instrument side.
2. Place the OpenArray® plate(s) on the plate adapter. Make sure that:
   - Each plate is properly aligned in the adapter.
   - The plate barcode is facing up and toward the front of the instrument.
3. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Close Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to retract the plate adapter back into the instrument.

Run the OpenArray® plates

Overview

You can run OpenArray® plates in either of the following two ways:

- "From the QuantStudio™ 12K Flex Software" on page 44
- "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 51

Note: The starter kit experiments in this guide run OpenArray® plates 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 Instrument is in operation.

From the QuantStudio™ 12K Flex Software

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

- For the starter kit experiments:
  - "Using a template file" (*.edt, see below)
- For your own experiments:
  - *(Recommended)* "Using a template file" (*.edt, see below)
  - "Using an OpenArray® plate setup file" (*.spf or *.tpf, see page 45)
  - Using the Batch Experiment Setup Utility (see the *QuantStudio™ 12K Flex Software Help*; click or press **F1**)

Using 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(s) before starting the run, or after the run is complete.

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. On the Home tab, select **Create From Template**.
3. Navigate to and select the template file (*.edt) 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 <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.
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) you want to use, then click **Select File**.

     Note: For the genotyping starter kit experiments, use the OpenArray *.csv files you exported from the OpenArray® Sample Tracker Software (see "Track the samples" on page 20).
   - 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 you want to use:

      Genotyping Source File (*.spf) – Corresponds to the plate setup file associated with genotyping OpenArray® plates.

      Note: For the genotyping starter kit experiments and for your own experiments, download the appropriate plate setup files from the Life Technologies website. See page 9 for more information.

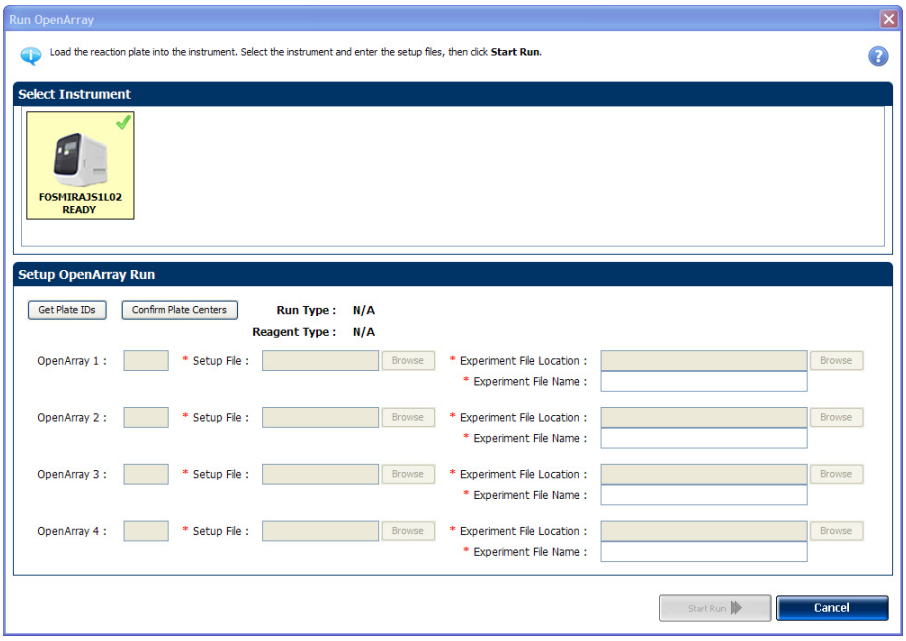
   b. Click **Select**, then click **Start Import**.
   c. If your experiment already contains plate setup information, the software asks you if you want to replace the plate setup with the data from the file. Click **Yes** to replace the plate setup information.
7. Select **File ▸ Save As...**, enter a file name, select a location for the experiment file (*.eds), then click **Save**.
8. Click **Start Run**.

Using an OpenArray® plate setup file

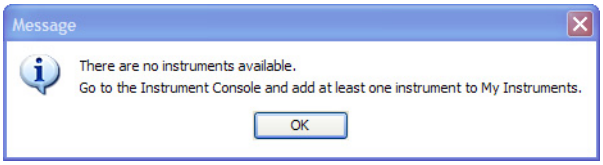
If you exported a 384-well plate file (*.csv) file from the OpenArray® Sample Tracker Software (see "Track the samples" on page 20), you can import the sample information in this file into the QuantStudio™ OpenArray® AccuFill™ Software. The QuantStudio™ OpenArray® AccuFill™ Software automatically integrates the sample information into an OpenArray® plate setup file (*.tpf or *.spf). You can save the newly created Loaded_tpf files to the OpenArray Plate File Input Folder you selected in the Preferences dialog box of the QuantStudio™ 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 and run the file.

Note: If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 20), 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.

1. Click  **OpenArray** from the Run menu on the Home screen of the QuantStudio™ 12K Flex Software.



Note: Be sure to add an instrument to My Instruments in the Instrument Console screen before running an experiment (see "Access the Instrument Console" on page 42). If no instrument is selected, you will receive the following warning.



2. In the Select Instrument pane, select the instrument you want to run the experiment on.

3. In the Setup OpenArray Run pane:
   - Click **Get Plate IDs** to import the barcode of the OpenArray® plates that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray® plates 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 to and select the appropriate OpenArray® plate setup files (*.spf or *.tpf) on your computer.

     Note: Once the setup file is selected, the Experiment File Location and Experiment File Name are automatically populated in the respective fields. 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® plates loaded in the instrument, the barcode of those OpenArray® plates will be populated.

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

4. Click **Start Run**.

From the QuantStudio™ 12K Flex Instrument Touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Instrument Touchscreen:

- "From experiments that are already created" below
- "From templates" on page 47
- "From shortcuts" on page 48

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

From experiments that are already created

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.
   Note: If the touchscreen is not at the Main Menu screen, touch  (**Home**).
2. In the Home screen, touch **Run OpenArray Plates**.
   The instrument will retrieve the barcodes and scan 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 QuantStudio™ 12K Flex Instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the QuantStudio™ 12K Flex Instrument Touchscreen. Do not proceed if a barcode is not detected by the QuantStudio™ 12K Flex Instrument.

From templates

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex 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 will retrieve the barcodes and create new experiments based on the template for each plate found.

6. Touch 

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

From shortcuts

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.

   Note: 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 will retrieve the barcodes and create new experiments based on the template for each plate found.

3. Touch 

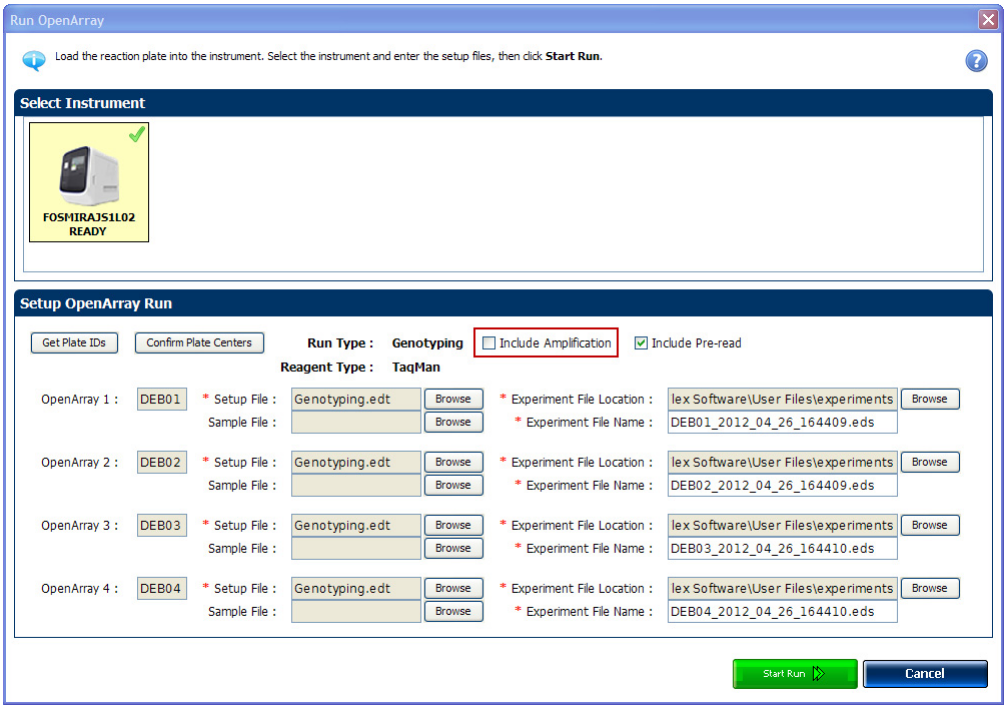
(**Start Run Now**) to start the run.

(Optional) Offline cycling of genotyping experiments

You can cycle QuantStudio™ OpenArray® plates on theApplied Biosystems GeneAMP 9700 Flat Block Cycler. To perform offline genotyping, you must use the QuantStudio™ 9700 Flatblock Adaptor. See Booklet 5, Appendix A *Ordering Information* to purchase the QuantStudio™ 9700 Flatblock Adaptor. The thermal protocol to be used for offline cycling is as follows:

Stage	Step	Temp (°C)	Time (min:sec)	Ramp rate (%)	Repetitions
Pre-PCR hold	1	93.0	10:00	100	—
PCR cycles	1	95.0	0:45	84	50 cycles
	2	94.0	0:13	100	—
	3	53.5	2:14	44	—
Pre-PCR hold	1	25.0	2:00	100	—

Note: To image plates on the QuantStudio™ 12K Flex Instrument, be sure to uncheck the Include Amplfication check box on the Run screen of the QuantStudio™ 12K Flex Software.



(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 "From the QuantStudio™ 12K Flex Software Run screen" on page 49).
- From the QuantStudio™ 12K Flex Instrument Touchscreen, in the same way that you run the experiment (see "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 51).
- From the Instrument Console of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Instrument Touchscreen) as described in "From the QuantStudio™ 12K Flex Software Instrument Console" on page 50.

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.

From the QuantStudio™ 12K Flex Software Run screen

Click **Amplification Plot** from the Run Experiment Menu to monitor the amplification plot of the experiment you are running.

From the QuantStudio™ 12K Flex Software Instrument Console

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

   Note: You must add the instrument to a group which can be monitored before you can manage it (see "Access the Instrument Console" on page 42).
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 page 50) available from the QuantStudio™ 12K Flex Software for potential problems.

To...	Action
Stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN**. • In the Stop Run dialog, 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.
View amplification data in real time	Select **Amplification Plot**. See "To monitor the Amplification Plot" below.

To 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 each experiment's Amplification Plot.

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

The Amplification Plot screen 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).

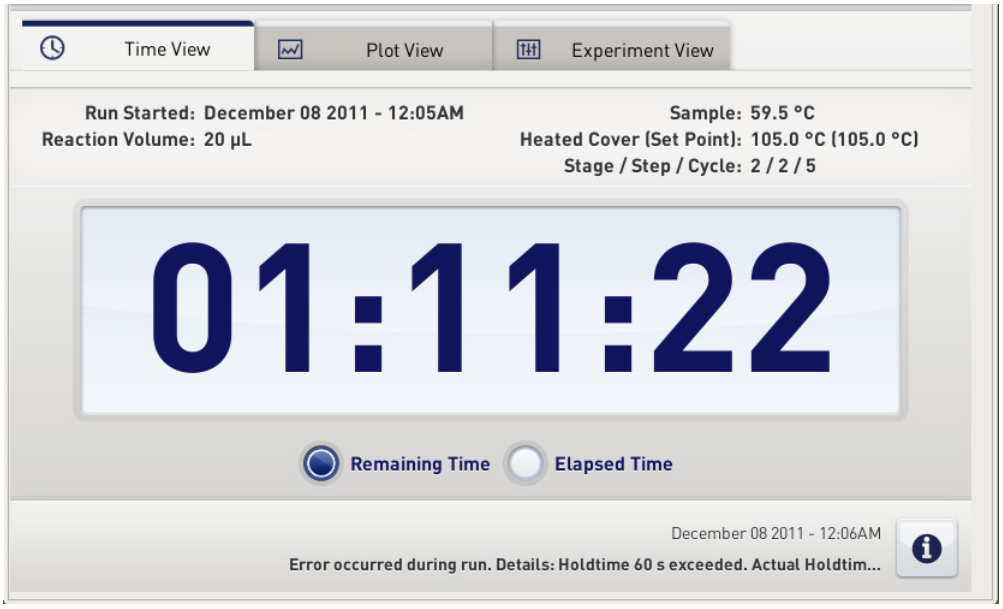
  Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the *QuantStudio™ 12K Flex Software Help* (click or press **F1**).

From the QuantStudio™ 12K Flex Instrument Touchscreen

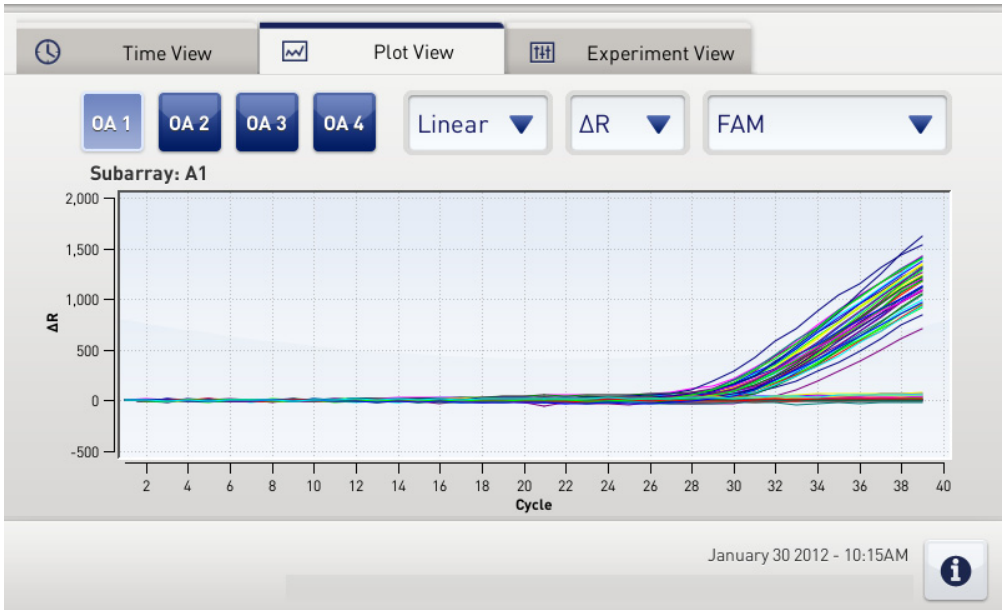
The QuantStudio™ 12K Flex Instrument Touchscreen displays the barcodes (or Plate IDs) of the TaqMan® OpenArray® plates for the run, the date and time at which the run started, the time remaining in the run, and other information.

To...	Action
Display the experiment names in the run	Touch **Experiment View**.
Show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
Display the time elapsed and the time remaining in the run	Touch the **Time View tab**, then touch **Experiment View** tab to return to the previous screen.
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 present below the Plot View tab.

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

Unload the 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 re-open the *.eds file to display the Allelic Discrimination Plot screen (for genotyping experiments). See "Analyze the Experiment Results" on page 57.
- QuantStudio™ 12K Flex Instrument Touchscreen, see "(Optional) Transfer experiment results" on page 54.

Unload the instrument

When the QuantStudio™ 12K Flex Instrument Touchscreen displays the Home screen, unload the OpenArray® plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen 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 QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate as follows:

   a. Power off the QuantStudio™ 12K Flex Instrument.
   b. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate.
   c. If the instrument does not eject the plate, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door.
   d. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex 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 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:

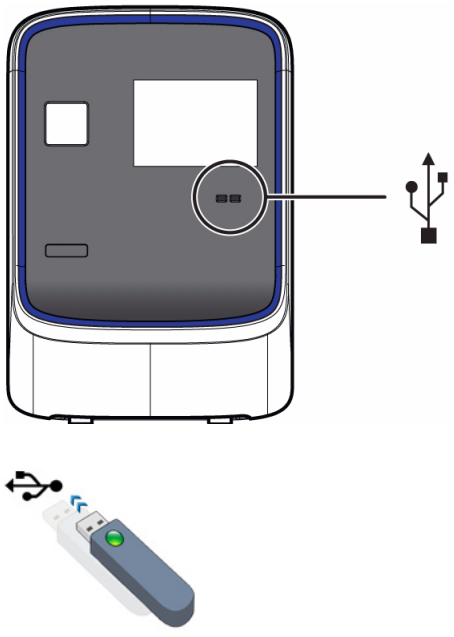
Download the experiment from the QuantStudio™ 12K Flex Instrument over the network

1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. Select the instrument icon of the QuantStudio™ 12K Flex Instrument you just used to run the experiment from the My Instruments list.
3. Click **Manage Instrument** to open the Instrument Manager.
4. In the Instrument Manager, click **Manage Files**.
5. In the Experiments panel, select the experiment to download. Click **Download**.
6. In the Save dialog box, select the folder to hold the experiment results and click **Save**. The experiments folder is located at:

   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where, <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Transfer the experiment from the QuantStudio™ 12K Flex Instrument to the computer via a USB drive

1. If not already connected to the instrument, connect a USB drive to the USB port.



2. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.
3. If the touchscreen is not at the Main Menu screen, 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 your 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:

   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

6 Analyze the Experiment Results

Section 6.1 Analyze the run data

This section includes general information and instructions on how to analyze the example experiments provided on the QuantStudio™ 12K Flex Software installation CD. For specific instructions see page 67.

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 need to close the run and re-open the *.eds file to display 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** check box. By default, Auto Analysis is always enabled. To reanalyze the data, select all the wells in the plate layout, then click **Analyze**.

Setting up the *.eds file

If you run a genotyping experiment using an *.edt file, you will need to 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® format from Sample tracker (*.csv) for the corresponding plate.
- If you use the QuantStudio™ 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 QuantStudio™ OpenArray® AccuFill™ Software.

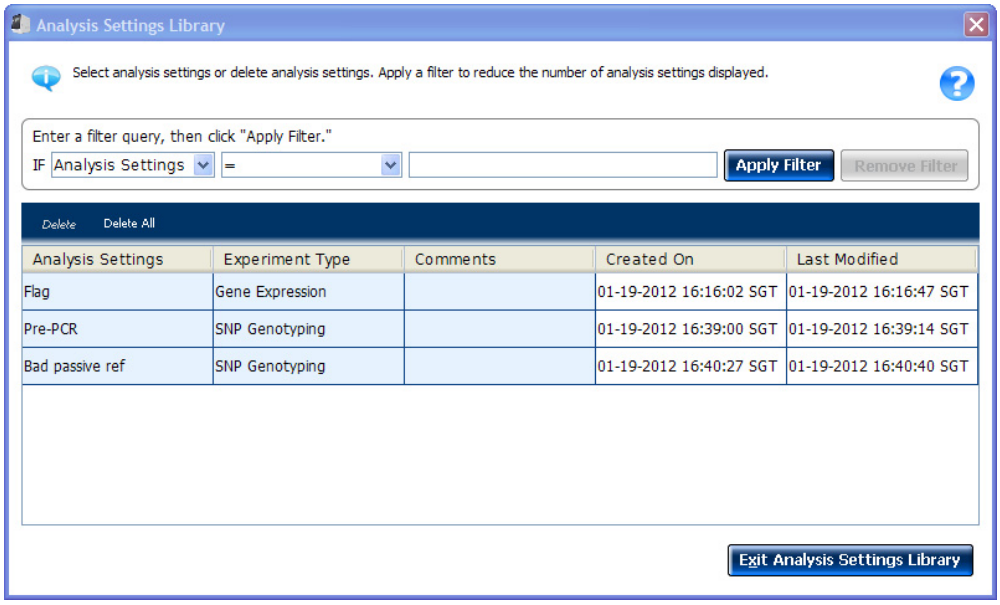
About the Analysis Settings Library

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.

In the Analysis Settings Library dialog box you can apply a filter to reduce the number of setting protocols displayed.

You can access the Analysis Settings Library from the Tools menu.



To change the analysis settings and to save them to the Analysis Settings Library:

1. From the Experiment Menu pane, select **Analysis**.
2. On the Analysis screen, click **Analysis Settings** to open the Analysis Settings dialog box.
3. Change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes you have made to the Analysis Settings Library.

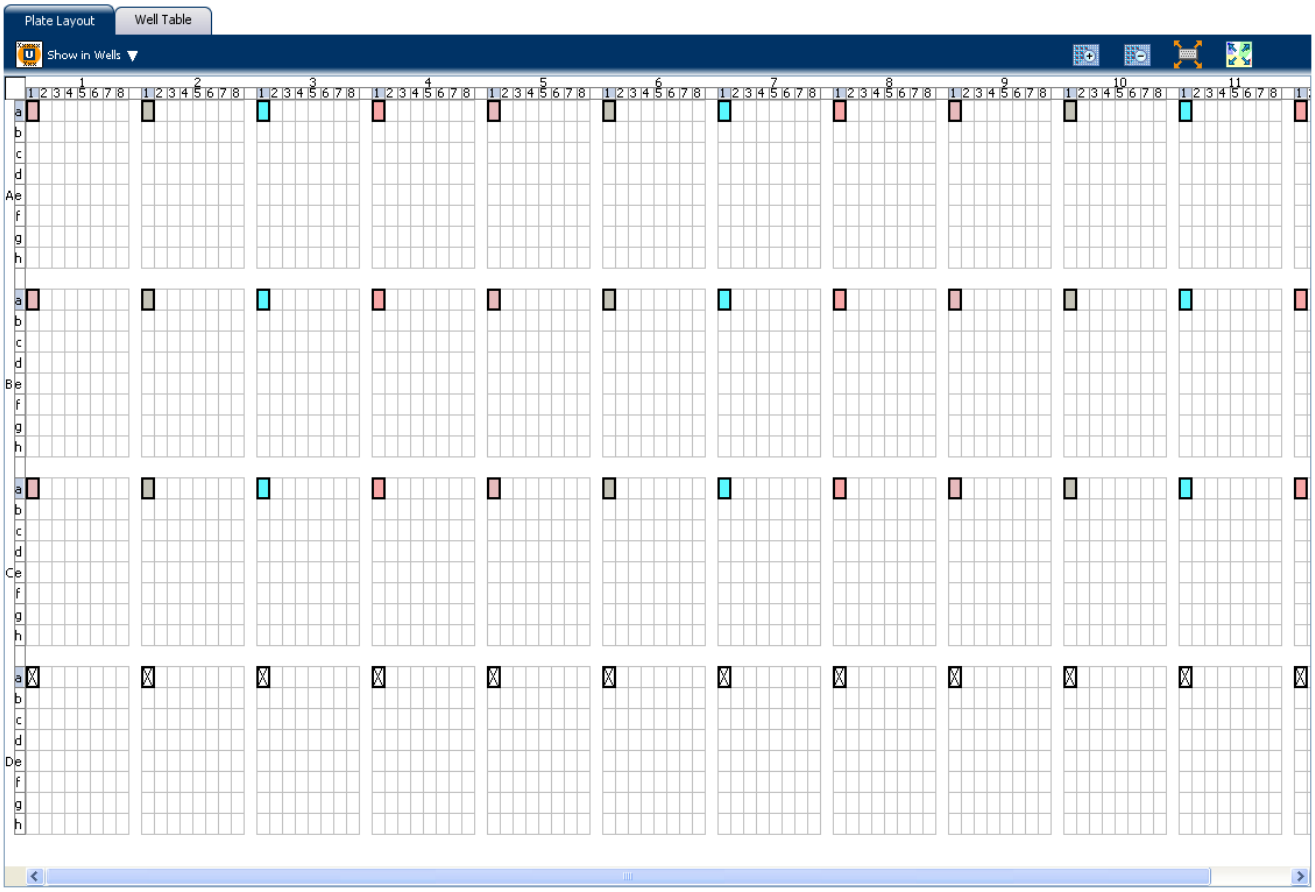
You can import the analysis settings you have previously saved to the Analysis Settings Library, by clicking **Load from Library** in the Analysis Settings dialog box.

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 Genotyping experiments, select **Sample Color** or **Assay Color.**
- To select a single well, click the well in the plate layout.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the plate layout.
- To select all the wells, click the upper left corner of the plate layout.

Plate layout 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) to open the Plot Properties dialog box

2. 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

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

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

(Optional) Export an experiment

About exporting 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

Note: You can also export plate images collected during the run as *.tif files and use them for troubleshooting purposes as needed. To export plate images, first create an export folder on your hard drive. In the Export screen, click **Browse** and navigate to the folder 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/**). Also, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689) for more information on QC Images.

Export procedure

Note: If you choose the Auto Export option before running an experiment, the data is automatically exported to the location you specified. If you did not set the Auto Export option, the analyzed data is not exported automatically.

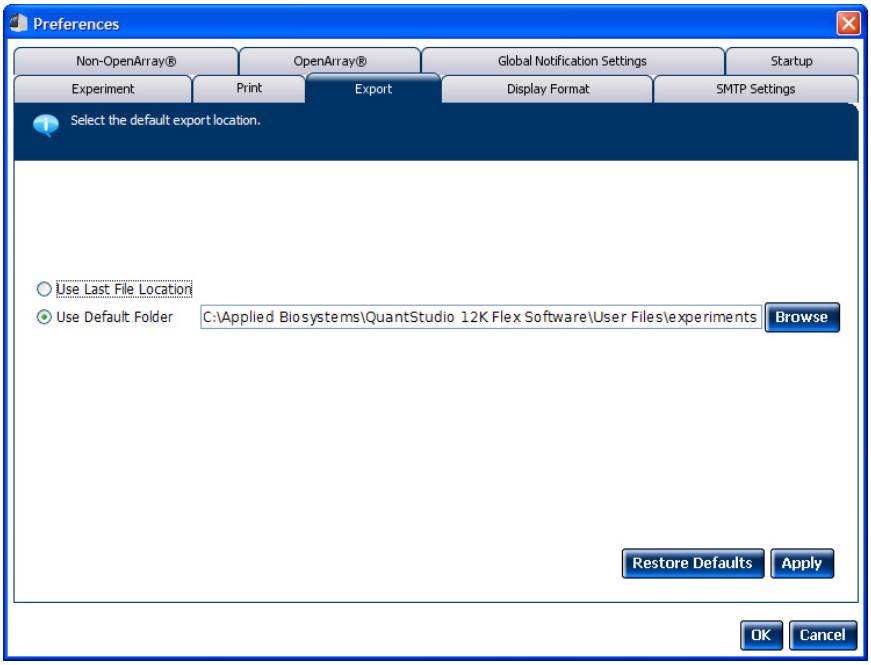
1. Open the experiment file that contains the data to export, and from the Experiment Menu, click  **Export.**
2. Select the format for exported data:
   - **QuantStudio 12k Flex format** (supports .txt and .xlsx data).
   - **RDML format** - Real Time Data Markup Language (supports only .xml type of 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. You can select the export file type (*.xls, *.xlsx or *.txt) to export from the **File Type** drop-down menu.

     Note: You cannot use an exported ***.xls** or an ***.xlsx** file when importing plate setup information.
4. Select **Yes** or **No** to include or exclude bookmarked data, from analysis, in the export set.

   The Filter Bookmark Data feature allows you to include only the data bookmarked during analysis in the export set.
5. *(Optional)* Select the **Open file(s) when export is complet**e check box to automatically open the file when export is complete.
6. Enter a file name and location.
   a. Enter a name for the export file in the **Export File Name** field.

b. Enter the **Export File Location**. 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**, and select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** check box.



7. Select the data to export:

Select...	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

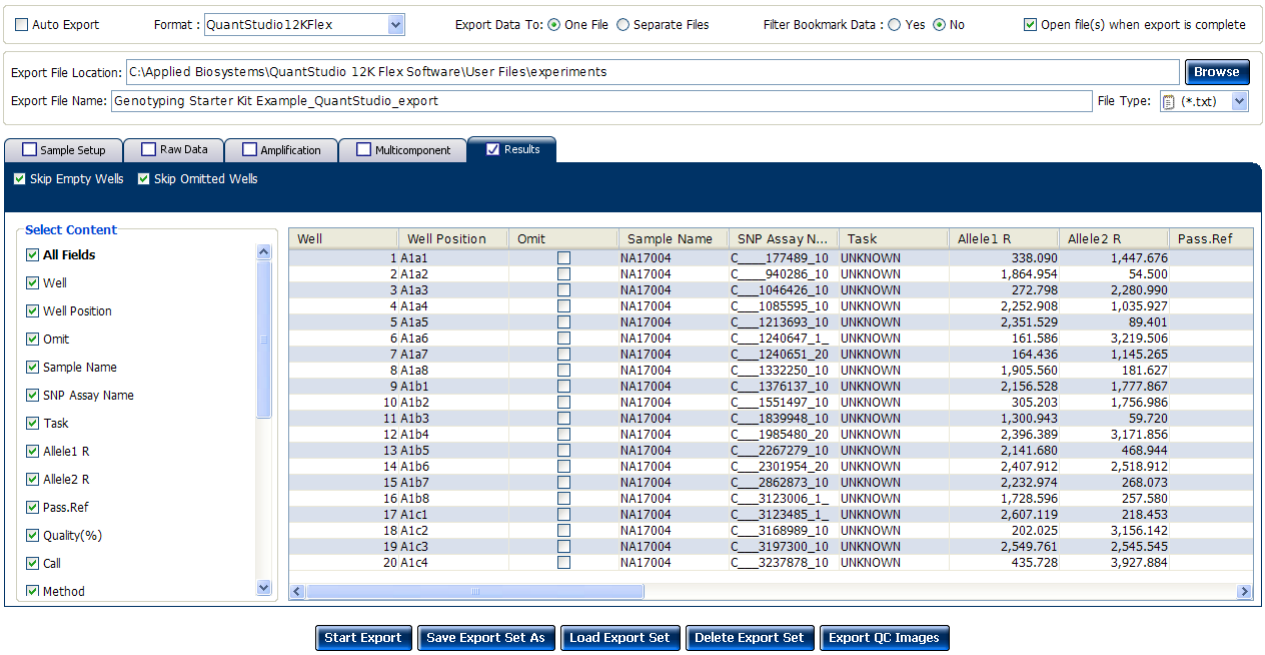
Note: Results data are not available for export until the run status is complete and the data are analyzed.

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.

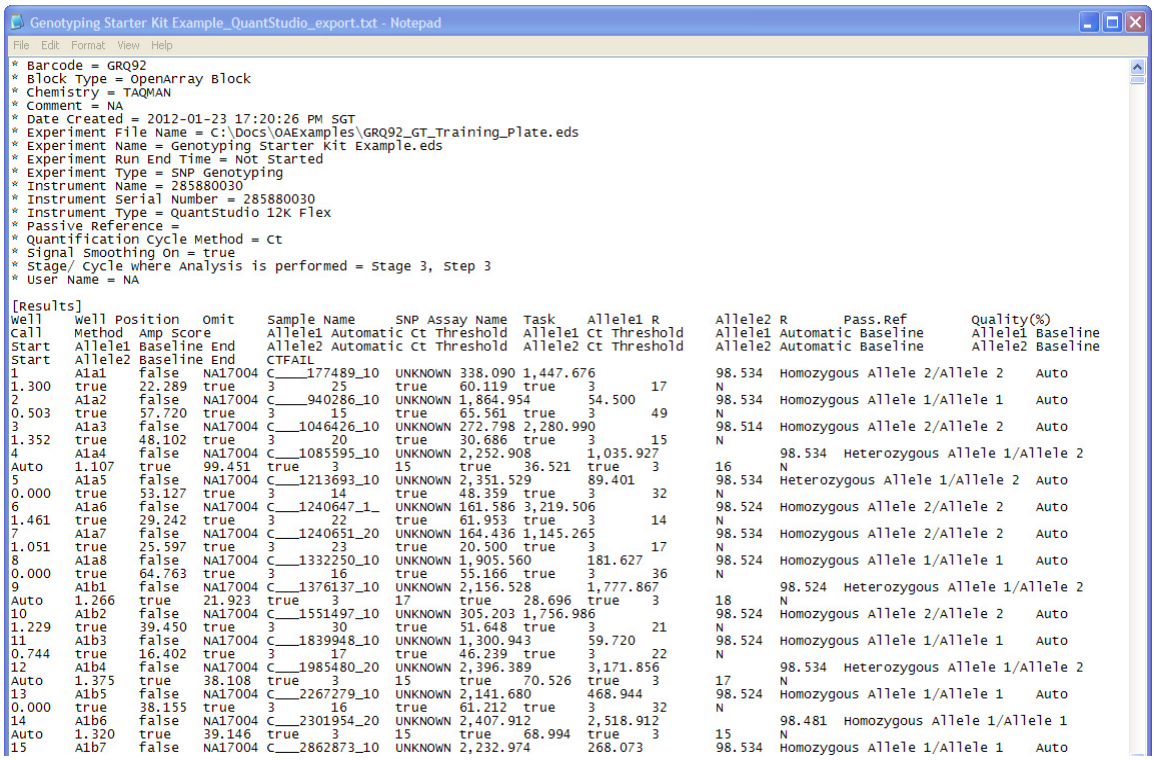
10. Click **Start Export**.

The Export screen for a Genotyping experiment is shown in the following graphic:



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	

The exported file when opened in Notepad appears as shown in the following graphic:



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.

Common features

The ExpressionSuite Software and TaqMan® Genotyper Software allow you to:

- 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 ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data.

Note: For more information on the ExpressionSuite Software and the TaqMan® Genotyper Software, refer to *Applied Biosystems ExpressionSuite Software User Guide* and *Applied Biosystems TaqMan® Genotyper Software Getting Started Guide* respectively. Both the applications are available for download from the Life Technologies website. See also, Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*.

Section 6.2 Analyzing Genotyping Experiments

In this section, you use the example experiment files provided on the QuantStudio™ 12K Flex Software installation CD to analyze the experiment results.

Workflow

Analyze the experiment results

Access the example experiment file (page 67)

▼

View clusters in the Allelic Discrimination Plot (page 68)

▼

Confirm setup accuracy using Plate Layout (page 71)

▼

Assess amplification results using the Amplification Plot (page 72)

▼

Identify well problems using the Well Table (page 74)

▼

Confirm accurate dye signal using the Multicomponent Plot (page 78)

▼

Determine the signal accuracy using the Raw Data Plot (page 79)

▼

View the QC Summary (page 80)

▼

(Optional) Adjust parameters for re-analysis of your own experiments (page 83)

▼

(Optional) Export the analyzed data (page 86)

Open the example experiment file

1. Double-click 

(QuantStudio™ 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. From the Home screen, click **Open**, then browse to the **experiments** folder:
<*drive*>:Program Files\Applied Biosystems\QuantStudio 12K Flex Software\examples\Genotyping
3. Open the Genotyping Starter Kit Example.eds file.

Setting up the *.eds file

If you ran a genotyping experiment using an *.edt file, you will need to 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® format from Sample tracker (*.csv) for the corresponding plate.
- If you use the QuantStudio™ 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 QuantStudio™ OpenArray® AccuFill™ Software.

View 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 for the three possible genotypes (Allele 1 homozygous, Allele 2 homozygous, and Allele 1/2 heterozygous).
- A cluster for the no template controls.

To view and assess the Aallelic Discrimination Plot

1. From the Experiment menu pane, select **Analysis ▸ Allelic Discrimination Plot.**
2. Click the **Plate Layout** tab, then click any empty well to select it.

   Note: 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. (To enable or disable Autocalling, go to Analysis Settings. By default, Autocaller is enabled but you can edit the default call settings and uncheck the Autocalling Enabled check box).

   The Allelic Discrimination Plot displays allele symbols for each sample evaluated for the selected SNP. The samples are grouped on the plot as follows:

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

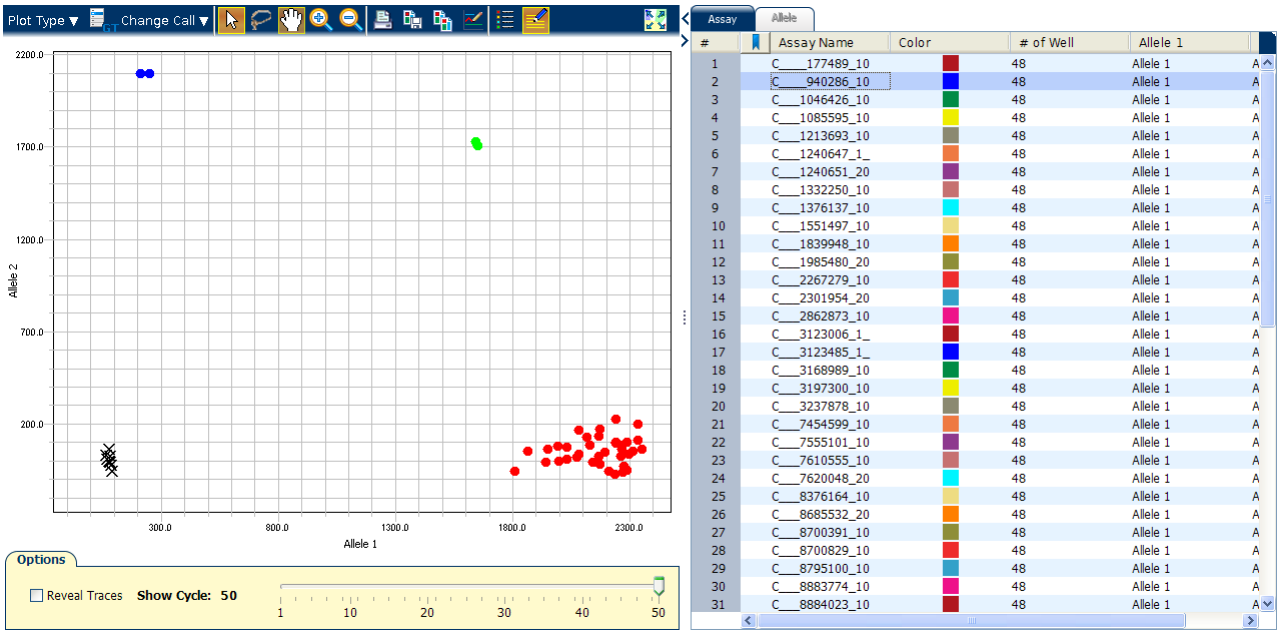
Note: If the Autocaller is not enabled, the Allelic Discrimination Plot displays a crossmark (X – Undetermined) for each sample.

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.
   d. The table below describes the elements of the Allelic Discrimination Plot.

Element	Description
Assay tab	Located on the top-right side of the screen. Determines the assay data that the QuantStudio™ 12K Flex Software displays in the plot.
Plot Type drop-down menu	Determines the type of plot (Cartesian or Polar) that the QuantStudio™ 12K Flex Software uses to display the data.
Change Call drop-down menu	When a datapoint is selected, this menu allows you to assign an allele call to the datapoint within the scatterplot.
Toolbar	Contains tools for manipulating the scatterplot: •  – Selection tool. •  – Selection tool. •  – Repositioning tool. •  – Zooms in. •  – Zooms out.
Legend	Explains the symbols in the scatterplot.
Show Options	The Reveal Traces option allows you to trace the clusters throughout the PCR process. This option is not activated for the example experiment. To activate the feature, see "Adjust analysis settings" on page 84.

The Allelic Discrimination plot for the OpenArray Genotyping example experiment looks like this:



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 Allelic Discrimination Plot.

Confirm setup accuracy using 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 on 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:

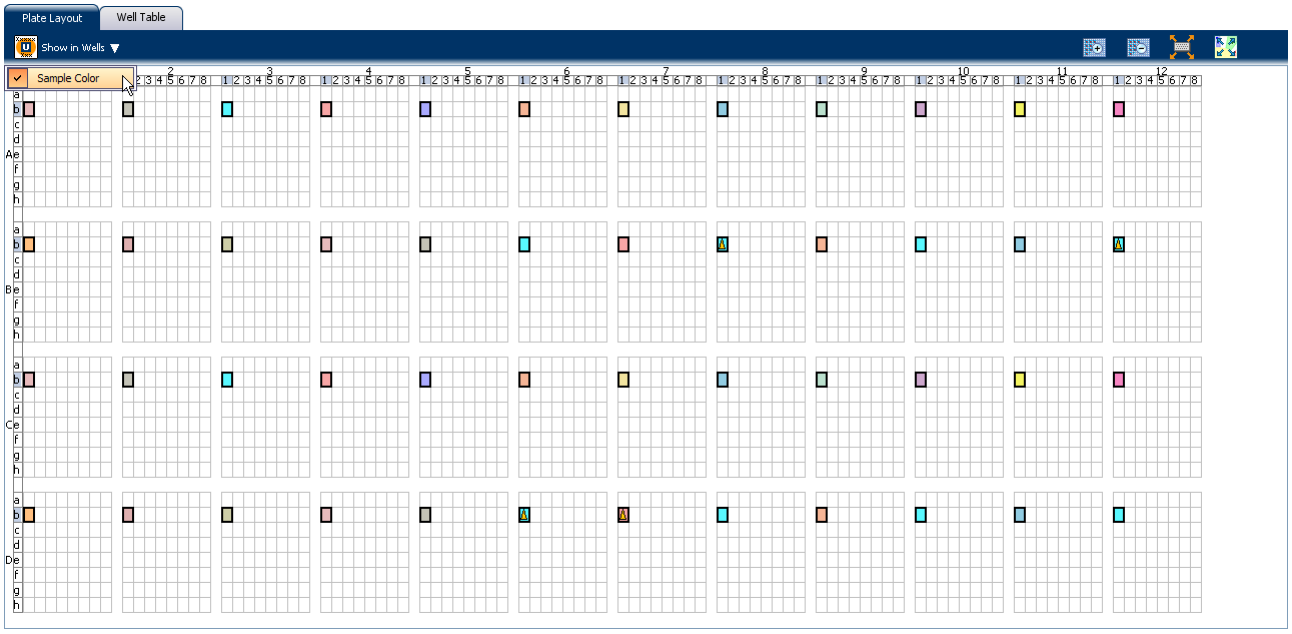
- 2 samples as Allele 1 homozygous (●)
- 34 samples as Allele 2 homozygous (●)
- 4 samples as heterozygous (●)
- 8 samples as undetermined (X)

Confirm that no wells of the reaction plate triggered QC flags (▲). The example experiment displays 3 flags.

View the layout

1. Click the  and  icons beside and below the Allelic Discrimination Plot, respectively, to maximize 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 your 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.
- You can 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 tab 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 using the Amplification Plot

IMPORTANT! Amplification plots are not used to make SNP calls. Examine the plots to help with troubleshooting and quality control.

The Amplification Plot screen displays amplification of all samples in the selected wells. There are three plots 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. You can 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. You can 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. You can 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 pane, select **Analysis ▸ Amplification Plot**.

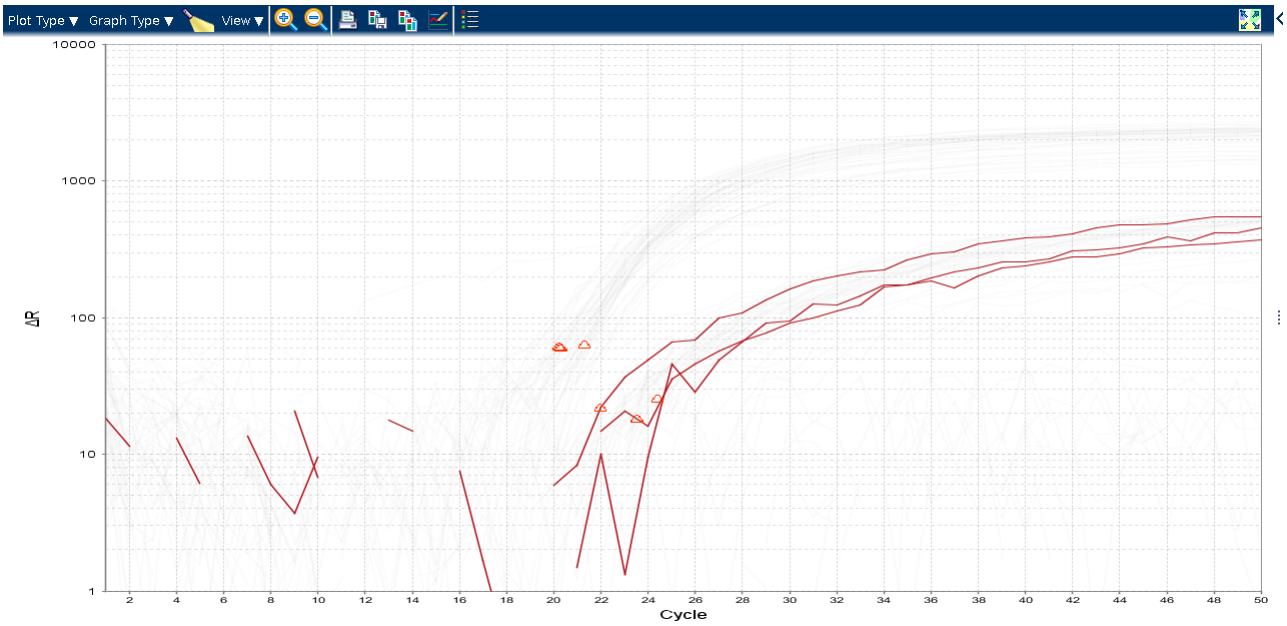
   Note: If no data are displayed, click **Analyze**.

2. In the Amplification Plot screen, select:

Menu	Selection
Plot Type	ΔR vs Cycle (default)
Graph Type	Log (default)
View	SNP Allele Color and (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 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. Select the **Well Table** tab.
2. Click the **Flag** column header 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 figure below shows the well table of the OpenArray Genotyping example experiment.

#	Well	Omit	Flag	Sample ...	SNP As...	Assay ID	Task	Allele 1	Allele 2	Allele 1 ...	Allele 2 ...	Allele 1 ...	Allele 2 ...	ROX Sig...	Call	Quality(...	M
1	A1a1	☐		NA17004	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	338.090	1,447.676		Homozygo...	98.534	Aut
65	A2a1	☐		NA17057	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	414.040	1,812.982		Homozygo...	98.534	Aut
129	A3a1	☐		NA17005	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	1,369.277	1,575.605		Heterozyg...	98.534	Aut
193	A4a1	☐		NA17059	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	504.790	2,014.206		Homozygo...	98.534	Aut
257	A5a1	☐		NA17034	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	576.798	2,080.472		Homozygo...	98.534	Aut
321	A6a1	☐		NA17060	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	611.732	2,223.806		Homozygo...	98.534	Aut
385	A7a1	☐		NA17051	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	594.411	2,174.000		Homozygo...	98.534	Aut
449	A8a1	☐		NA17104	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	1,497.123	1,928.546		Heterozyg...	98.534	Aut
513	A9a1	☐		NA17053	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	573.567	2,359.790		Homozygo...	98.534	Aut
577	A10a1	☐		NA17105	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	537.917	2,115.444		Homozygo...	98.534	Aut
641	A11a1	☐		NA17055	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	525.499	2,210.420		Homozygo...	98.534	Aut
705	A12a1	☐		NA17108	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	512.175	2,196.327		Homozygo...	98.534	Aut
769	B1a1	☐		NA17109	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	441.100	2,013.071		Homozygo...	98.534	Aut
833	B2a1	☐		NA17208	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	504.539	2,159.505		Homozygo...	98.534	Aut
897	B3a1	☐		NA17125	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	459.415	2,116.520		Homozygo...	98.534	Aut
961	B4a1	☐		NA04671	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	1,663.670	295.365		Homozygo...	97.040	Aut
1025	B5a1	☐		NA17201	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	580.349	2,165.138		Homozygo...	98.534	Aut
1089	B6a1	☐	1	NTC	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	33.294	-25.671		Undetermi...	100.000	Aut
1153	B7a1	☐		NA17202	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	587.505	2,212.112		Homozygo...	98.534	Aut
1217	B8a1	☐		NTC	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	40.205	3.613		Undetermi...	100.000	Aut
1281	B9a1	☐		NA17203	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	531.179	2,296.937		Homozygo...	98.534	Aut
1345	B10a1	☐		NTC	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	53.797	-45.074		Undetermi...	100.000	Aut
1409	B11a1	☐		NA17205	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	547.880	2,253.406		Homozygo...	98.534	Aut
1473	B12a1	☐		NTC	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	55.325	-35.682		Undetermi...	100.000	Aut
1537	C1a1	☐	1	NA17004	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	423.932	1,935.394		Homozygo...	98.534	Aut
1601	C2a1	☐		NA17057	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	483.336	2,113.948		Homozygo...	98.534	Aut
1665	C3a1	☐		NA17005	C____177...	C____177...	Unknown	Allele 1	Allele 2	VIC-NFQ-M...	FAM-NFQ-...	1,486.921	1,878.511		Heterozyg...	98.534	Aut

The following table gives the names and description of the columns in the well table:

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 (▲) 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 $_{Rn}$	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.

Column	Description
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 • ✕ 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).
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 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 **defined in the analysis settings**.

Flag	Description
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 certain 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(s) triggered by the associated well.
- Select areas of the table or wells of a specified type by:
  - Left-clicking the mouse and dragging across the area you want to select an area of the table.
  - Selecting **Sample**, **SNP Assay**, or **Task** from the Select Wells menu in the Well Table tab to select wells of a specific type using the well-selection tool.
- Group the rows of the plate layout by selecting an option from the Group By menu. You can then collapse or expand the lists either by clicking the **+/-** icon next to individual lists, or by clicking **Collapse All** or **Expand All**.
- Omit a well from the analysis by selecting the **Omit** check box for that well. To include the well in the analysis, deselect the **Omit** check box.

  Note: You must reanalyze the experiment each time you omit or include a well.

Confirm accurate dye signal using the Multicomponent Plot

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

Purpose

In the OpenArray Genotyping example experiment, you review the Multicomponent Plot screen 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 pane, select **Analysis** ▸ **Multicomponent Plot**.

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

2. Display the unknown wells one at a time in the Multicomponent Plot screen:
   a. Click the **Plate Layout** tab.
   b. Select one well in the plate layout; the well is shown in the Multicomponent Plot screen.

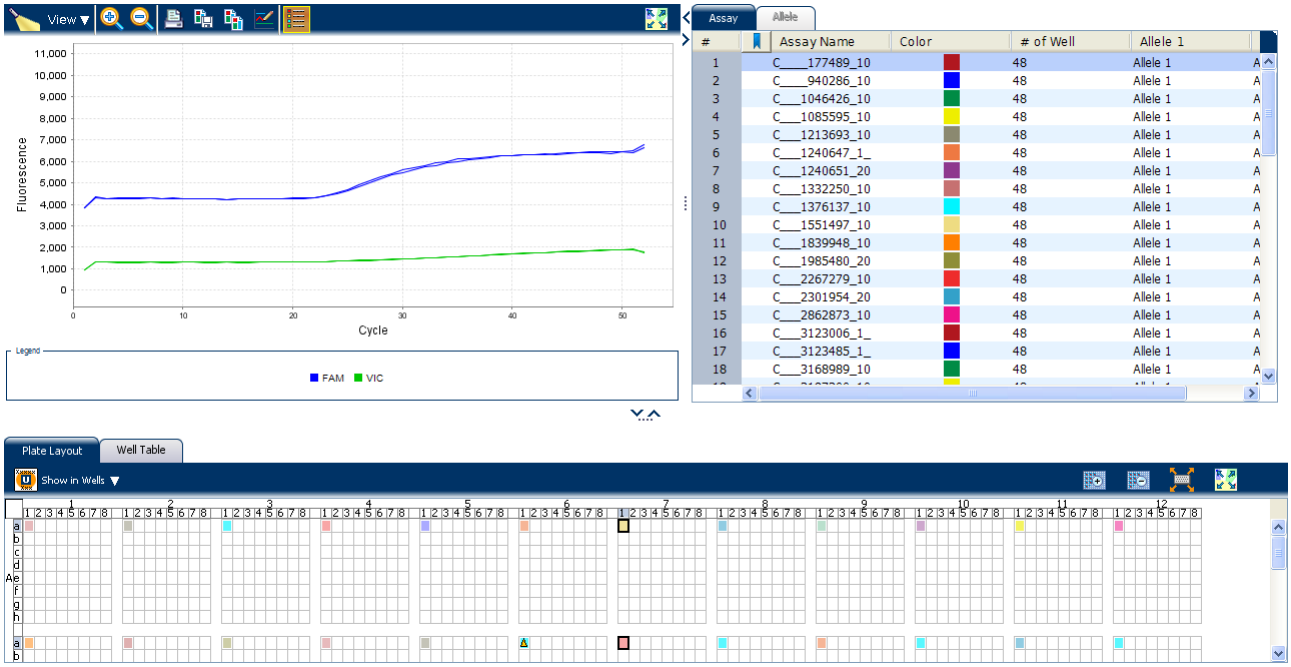
      Note: If you select multiple wells, the Multicomponent Plot screen displays the data for all selected wells simultaneously.

3. From the View drop-down menu, select **SNP Allele Color**.

4. Click  **Show a legend for the plot** (default).

   Note: 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 OpenArray 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 OpenArray Genotyping experiment, look for:

- **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.
- **No Template Control wells** – There should not be any amplification in the no template control wells.

Determine signal accuracy using the Raw Data Plot

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

About the example experiment

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 pane, select **Analysis** ▸ **Raw Data Plot**.

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

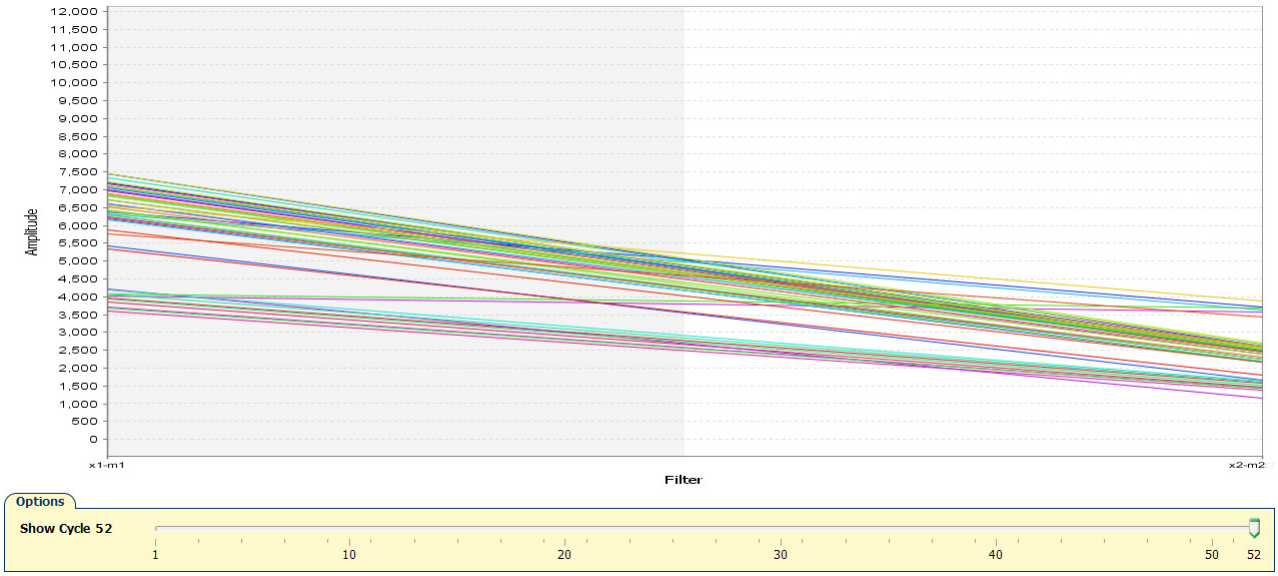
2. Display all wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the Plate Layout tab.

3. Click **Show a legend for the plot** (default).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

   Note: The legend displays the color code for each row of the reaction plate (see the legend in the Raw Data Plot shown below).

4. Click and drag the Show Cycle pointer from cycle 1 to cycle 52. In the example experiment, there is a stable increase in signal from filter 1, which 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 in each filter:

- Characteristic signal growth
- No abrupt changes or dips

Review the flags in the QC Summary

The QC Summary screen displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment.

Review the QC Summary screen in the OpenArray Genotyping example experiment for any flags triggered by the experiment data. Wells A1a1, 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 have data that triggered the SMCLUSTER flag.

Wells 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 have data that triggered the CTFAIL flag.

View the QC Summary

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

   Note: 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.

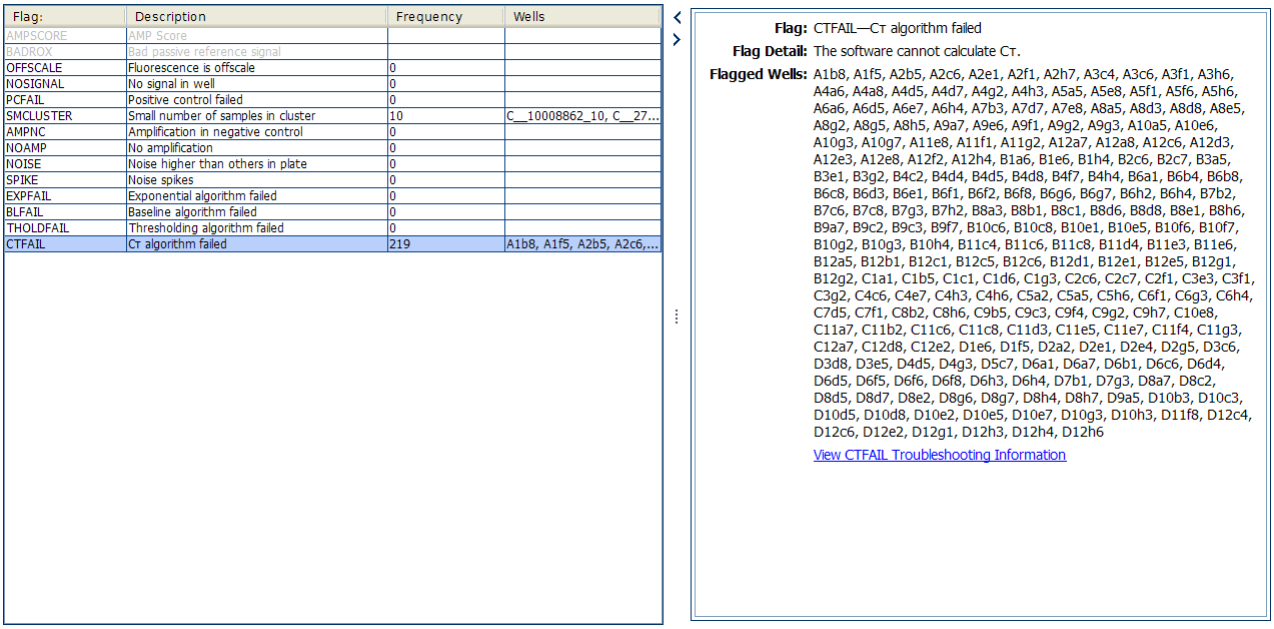
   In the example experiment, there are 635 flagged wells.

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.

   Note: 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 of the samples only have one allele present and therefore display a large number of CTFAIL flags since there is no amplification plot for the second allele.

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

   The QC Summary for the OpenArray Genotyping example experiment looks like this:



Flag:	Description	Frequency	Wells
AMPSCORE	AMP Score		
BADROX	Bad passive reference signal		
OFFSCALE	Fluorescence is offscale	0	
NOSIGNAL	No signal in well	0	
PCFAIL	Positive control failed	0	
SMCLUSTER	Small number of samples in cluster	10	C__10008862_10, C__27...
AMPNC	Amplification in negative control	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
SPIKE	Noise spikes	0	
EXPFAIL	Exponential algorithm failed	0	
BLFAIL	Baseline algorithm failed	0	
THOLDFAIL	Thresholding algorithm failed	0	
CTFAIL	Ct algorithm failed	219	A1b8, A1f5, A2b5, A2c6,...

Possible flags

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

Flag	Description
Pre-processing flag	
OFFSCALE	Fluorescence is offscale
Primary analysis flags	
BADROX	Bad passive reference signal
NOAMP	No amplification

Flag	Description
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 certain 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

Note: The flags BADROX and AMPSCORE are, by default, not in use for the Genotyping experiment.

Note: 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).

Adjust parameters for re-analysis of your own experiments

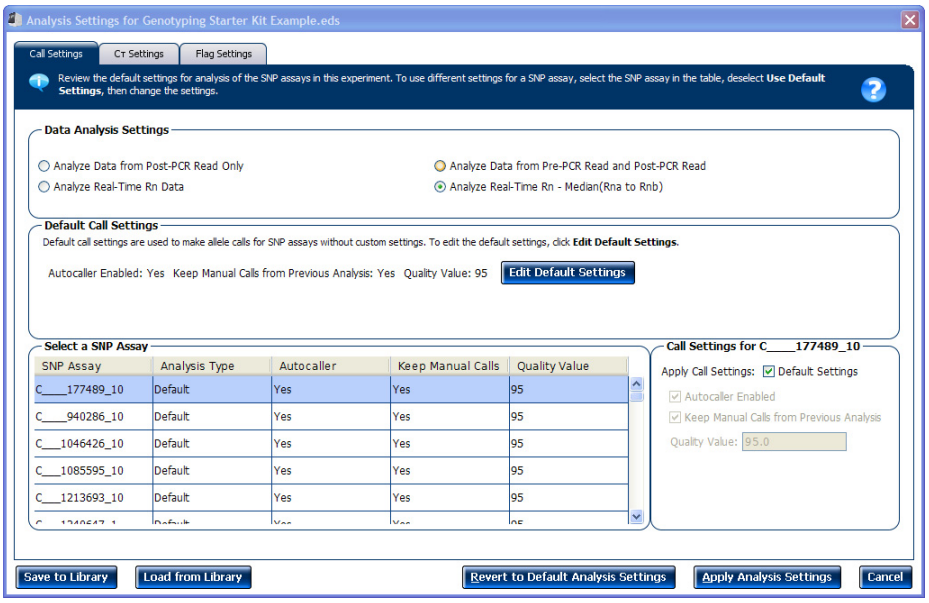
The Analysis Settings dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

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 pane, 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" below).

   Note: You can save the changes to the analysis settings to the Analysis Settings Library for later use. For more information, see "About the Analysis Settings Library" on page 59.

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

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

Adjust analysis settings

Call Settings

Use the Call Settings tab to:

- Change the default data analysis settings. You can select from:
  - Analyze data from Post-PCR Read only - Select 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 if you want 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 if you want to use the normalized reporter (Rn) data from the cycling stage to determine genotype calls.
  - Analyze data from Rn - Avg (Rna to Rnb) - If you included amplification in the run, select if you want 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 scree, 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 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 a SNP assay.
  - Select one or more SNP assays in the table, then deselect the **Default Settings** check box.
  - **Define the custom call settings**.

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**.**

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.

To adjust the flag settings

1. In the Use column, select the check boxes 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.

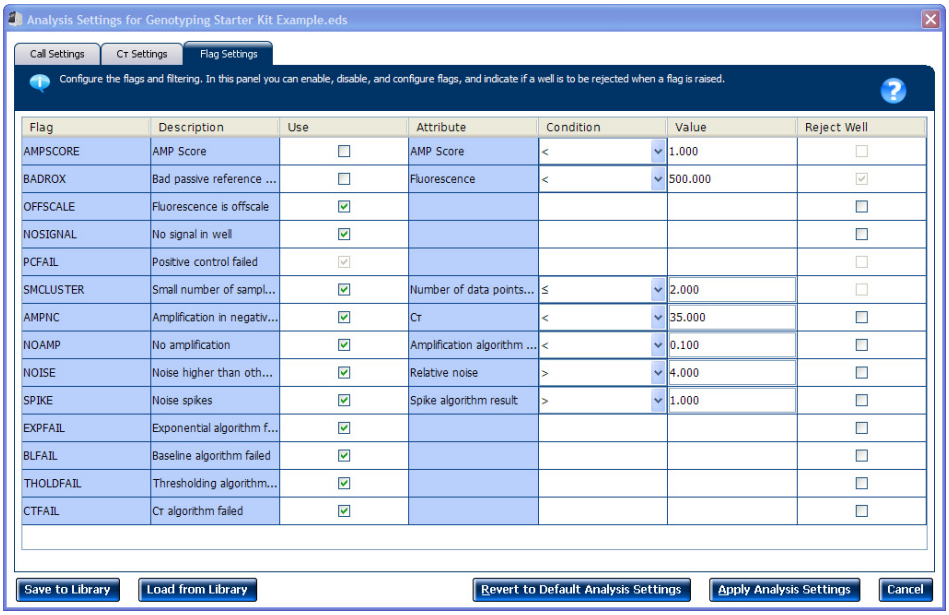
   Note: If you choose to adjust the setting for applying a flag, make minor adjustments as you evaluate the appropriate setting.

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

   Note: 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** in the Analysis Settings dialog box. If the run status is complete, the data are reanalyzed.

   The Flag Settings tab looks like this:



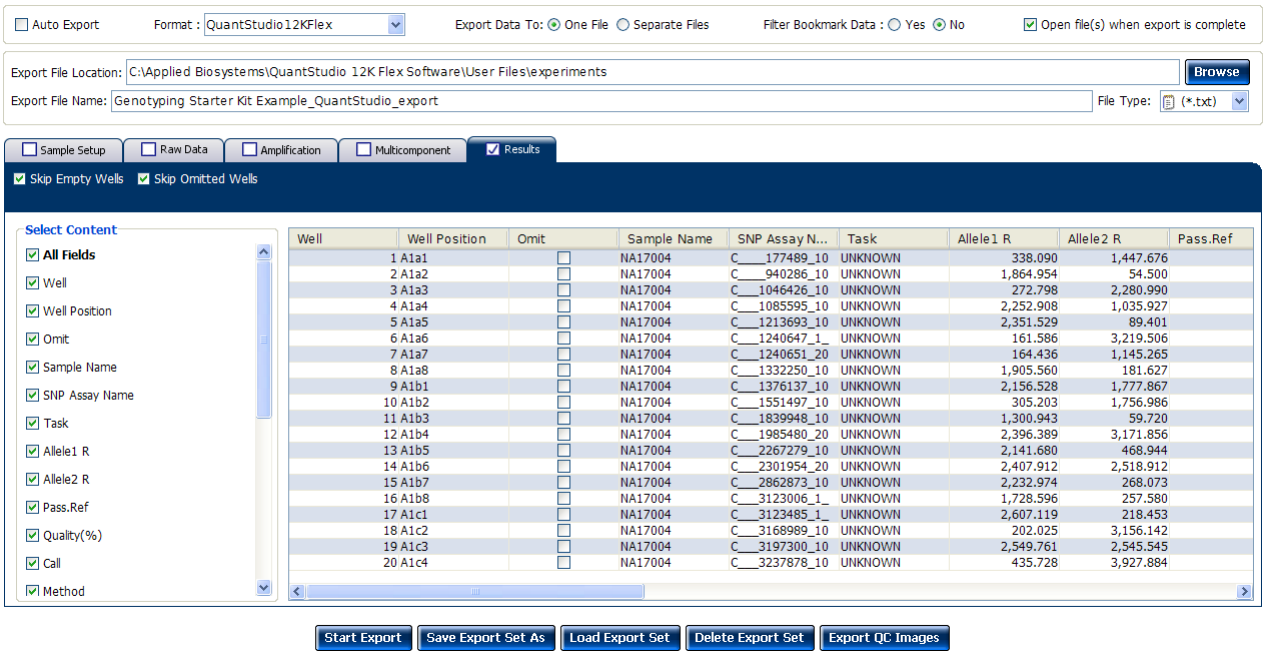
Export the analyzed data

1. Open the OpenArray Genotyping example experiment file that you analyzed in Chapter 5.
2. In the Experiment Menu, click  **Export.**

   Note: To export data automatically after analysis, select the **Auto Export** check box during experiment setup or before running the experiment. Auto export is unchecked for the example experiment.
3. Select **QuantStudio™ 12K Flex format**.
4. Complete the Export dialog box as shown below:

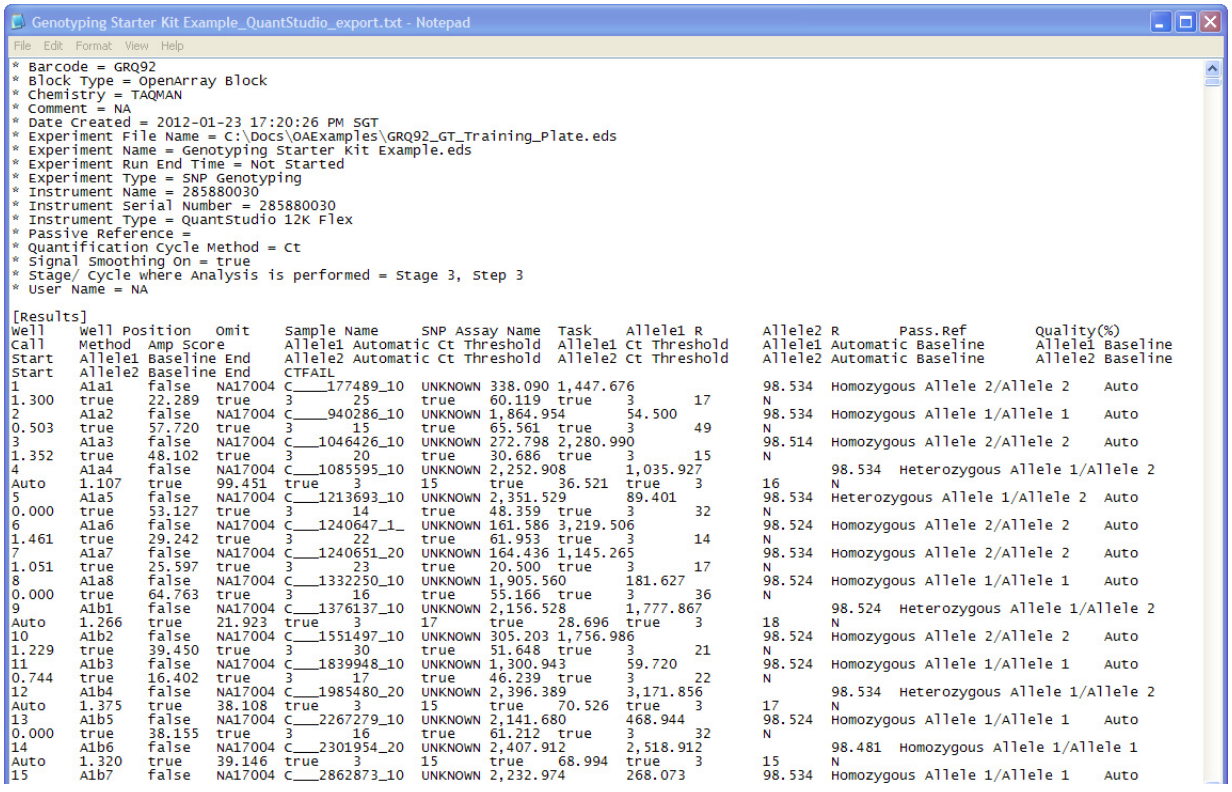
Field or Selection	Entry
Select Data to export/ Select Content	Results
Export Data To	One File
Export File Name	Genotyping Starter Kit Example_QuantStudio_export
Filter Bookmark Data	No
File Type	*.txt
Export File Location	<drive>:\Applied Biosystems\QuantStudio 12K Flex Software\experiments

Your Export screen 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	

Your exported file when opened in Notepad should look like this:



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USER GUIDE

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Booklet 3 - QuantStudio™ 12K Flex OpenArray® microRNA Starter Kit

Publication Part Number 4470935 Rev. C
Revision Date 22 April 2014

life technologies™

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Contents

1 Introduction

About the OpenArray® MicroRNA Starter Kits

Each QuantStudio™ 12K Flex OpenArray® MicroRNA Starter Kit:

- Contains all the materials (OpenArray® plates, reagents, and accessories) you need to perform two experiments on the QuantStudio™ 12K Flex System, from sample preparation to data analysis unless otherwise noted in Table 1 on page 7
- Represents a typical setup for two microRNA experiments

Table 1 Starter kit description and contents

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Human miRNA Starter Kit	4469606	• TaqMan® MicroRNA Reverse Transcription Kit (200 reactions) • Megaplex™ RT Primers, Human Pool A v2.1 • Megaplex™ PreAmp Primers, Human Pool A v2.1 • 2X TaqMan® PreAmp Master Mix Kit • Human brain and lung total RNA, 100 µg (1 mg/mL) each • 2X TaqMan® OpenArray® Real-Time PCR Master Mix, 1.5 mL • TaqMan® OpenArray® Human MicroRNA Panels (2 plates) • QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit (Part no. 4469589)	Contains reagents to conduct a single microRNA (miRNA) experiment on the QuantStudio™ 12K Flex System, using the TaqMan® OpenArray® Human MicroRNA Panel as an example. This kit contains human brain and lung total RNA samples.

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit	4469586	• QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (1 or 2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid and tips (6 syringes) • OpenArray® AccuFill™ System Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Contains accessories to assemble QuantStudio™ 12K Flex TaqMan® OpenArray® plates for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

About the plates

The instructions in this document use three types of plates, as described in Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*:

- MicroAmp® Optical 96-Well Reaction Plate (*96-well plate*)
- OpenArray® 384-Well Sample Plate (*384-well plate*)
- TaqMan® OpenArray® Plate (*OpenArray® plate*)

About the data files (how to track your assays and samples)

Overview

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 data files:

- "Sample information file (*.csv)" on page 9 - allows input of Sample IDs
- "Plate setup file (*.tpf)" on page 9 - allows input of Assay IDs and cycling protocol
- "Template file (*.edt)" on page 10 - includes complete setup (samples, assays and cycling protocol) saved as a template
- "Experiment file (*.eds)" on page 10 - complete data file

Note: Additional data files (*.aif, *.txt) are available for selection if you use the Batch Experiment Setup Utility in the QuantStudio™ 12K Flex 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 or gDNA samples. Using a sample information file allows you to:

- Track where samples and controls are located in the 96-well plate (see "Prepare the Samples" on page 13.
- Depending on the TaqMan® OpenArray® plate format being used:
  - Map the sample locations from the 96-well plate to the appropriate locations in the 384-well plate (see "Prepare the 384-Well Sample Plate" on page 21).
  - Map the sample locations from the 384-well plate areas to the appropriate locations in each TaqMan® OpenArray® plate (see "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 25).
- Associate information about the samples with the data results in order to normalize data or compute standard curves and calculate concentrations.

IMPORTANT! To ensure accurate results, you need to correctly track sample information from plate to plate.

You can import or manually enter sample information into the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), then export a sample information file (*.csv) in the following formats:

- 384-well plate – Integrate this file with a plate setup file (see below) in the QuantStudio™ OpenArray® AccuFill™ Software (see "Prepare for loading" on page 31).
- OpenArray® plate – Import this file directly into the QuantStudio™ 12K Flex Software before starting a run (see "From the QuantStudio™ 12K Flex Software" on page 46), 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)

(Optional) Using an OpenArray® plate setup file

Plate setup files (*.tpf) contain the assay information for individual TaqMan® OpenArray® plates, including the gene symbol, gene name, assay ID, and location of each assay on the plate. You can:

- Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate the sample information from a 384-well plate file (*.csv, see above) with the assay information in the plate setup file (see "Prepare for loading" on page 31).
- Upload the assay information in the plate setup file directly into the QuantStudio™ 12K Flex Software to create and run an experiment (*.eds, see "From the QuantStudio™ 12K Flex Software" on page 46).

Accessing the starter kit plate setup files

There is no plate setup file For microRNA experiments. Use template files (*.edt) to create a new experiment. See "Template file (*.edt)" on page 10 for information on creating a new experiment from an *.edt file.

Downloading your own plate setup files

For miRNA experiments, use template files (*.edt) supplied with the QuantStudio™ 12K Flex Software, located at <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive. The miRNA *.edt files located at C:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray include:

- miRNA_Human.edt
- miRNA_Rodent.edt

Note: You can either:
1) *(Recommended)* Use the QuantStudio™ OpenArray® AccuFill™ Software to integrate samples with the *.edt file. If you will integrate samples, save the file to the OpenArray Plate File Input Folder you selected in the Preferences dialog box of the QuantStudio™ OpenArray® AccuFill™ Software. The default save location is <*drive*>:\OpenArray\OpenArrayPlates directory. See "Prepare for loading" on page 31 for more information.
Or
2) 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 QuantStudio™ 12K Flex Software after the experiment is run (see "Using an OpenArray® plate setup file" on page 47).

Template file (*.edt)

An experiment document template file (*.edt) contains predefined experiment setup information (experiment type, assay names, and run method).

You can use a template to create a new experiment from the:

- QuantStudio™ 12K Flex Software (see page 46)
- QuantStudio™ 12K Flex Instrument Touchscreen (see page 49)

Note: To create and run the starter kit experiments, use the example template files supplied with the QuantStudio™ 12K Flex Software, located at <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Experiment file (*.eds)

An experiment document single file (*.eds) is an electronic record used by the QuantStudio™ 12K Flex Software that contains all of the information about a particular TaqMan® OpenArray® plate run on the QuantStudio™ 12K Flex Instrument, including meta-data (name, barcode, comments), experiment setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.

You can:

- Create and run an experiment using the QuantStudio™ 12K Flex Software (see page 46).

  Note: To create and run the starter kit experiments, use the example template files (*.edt, see page 10) supplied with the QuantStudio™ 12K Flex Software.

- Create and run an experiment using the QuantStudio™ 12K Flex Instrument Touchscreen (see page 49).
- Analyze the experiment results in the QuantStudio™ 12K Flex Software (see page 57).

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

About the starter kit data files

When you perform the starter kit experiment tasks in this guide, you will use example data files supplied with the QuantStudio™ 12K Flex Software and the OpenArray® Sample Tracker Software. Table 2 describes the types of files provided, as well as their file names and installation locations.

Table 2 Starter kit data files referenced in this guide

File type	Description	File name	Location†	Used in
.tpf	Transcript plate file	NA	**Download OpenArray® TPF & SPF Plate Files**	Ch 3
.csv	OA format sample information file	Human miRNA OA Plate Example.csv	C:\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples	Ch 3
.edt	Experiment template	• miRNA_Human.edt • miRNA_Rodent.edt	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray	Ch 5
.eds	Experiment	Human miRNA Panel Starter Kit Example.eds	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\examples\Gene Expression	Ch 6

† *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software and OpenArray® Sample Tracker Software are installed. The default installation drive for both software programs is the C: drive.

General workflow

Prepare the nucleic acid samples (Chapter 2)

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 384-well sample plate (Chapter 3)

Track the samples, prepare the PCR mix, and transfer the samples to the OpenArray® 384-well sample plate.

▼

Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate (Chapter 4)

Prepare for sample transfer using 384-well plates.

▼

Transfer the samples from 384-well plates to TaqMan® OpenArray® plates using the QuantStudio™ OpenArray® AccuFill™ System.

▼

Seal the TaqMan® OpenArray® plates.

▼

Complete sample transfer for the remaining TaqMan® OpenArray® plates.

▼

Perform the instrument run (Chapter 5)

Prepare the QuantStudio™ 12K Flex Software.

▼

Load the OpenArray® plate into the instrument.

▼

Run the OpenArray® plates.

▼

(Optional) Monitor the run.

▼

Unload TaqMan® OpenArray® plates from the instrument.

▼

Transfer the experiments results to the computer.

▼

Analyze the experiment results (Chapter 6)

2 Prepare the Samples

Overview

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

The TaqMan® OpenArray® Human MicroRNA Panels enable simultaneous running of hundreds of TaqMan® Human MicroRNA Assays in a plate format on the QuantStudio™ 12K Flex System. The TaqMan® OpenArray® Human MicroRNA Panels also require the use of matching Megaplex™ Primer Pools (composed of Megaplex™ RT Primers and Megaplex™ PreAmp Primers) for microRNA (miRNA) reverse transcription and preamplification prior to real-time PCR. See "About the Megaplex™ Primer Pools" on page 15 for more information.

Workflow

Prepare the nucleic acid samples
Obtain human brain and lung total RNA control samples from starter kit (page 15) *or* Isolate the RNA (page 15)
▼
Reverse transcribe the RNA (page 15)
▼
Preamplify the cDNA (page 17)

Required materials

Item[1]	Source	Part no.
• *For the starter kit experiment*: – Human brain total RNA control samples – Human lung total RNA control samples • *For your own experiments:* *mir*Vana™ miRNA Isolation Kit	Ambion	• *Provided in starter kit:* – AM7962 – AM7968 • AM1560
Reverse transcribe the RNA and preamplify the cDNA:		
One set of Megaplex™ Primer Pools for your selected microRNA panel	Life Technologies	See "About the Megaplex™ Primer Pools" on page 15
TaqMan® MicroRNA Reverse Transcription Kit, 200 reactions	Life Technologies	4366596 *(provided in starter kit)*
TaqMan® PreAmp Master Mix Kit	Life Technologies	4384266 *(provided in starter kit)*
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813
• MicroAmp® Clear Adhesive Film *or* • MicroAmp® Optical 8-Cap Strips	Life Technologies	• 4306311 *or* • 4323032
Thermal Cycler	Life Technologies	—
0.1× TE pH 8.0	Major laboratory suppliers (MLS)	—

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

Starting material

(For the starter kit experiment) Obtain the RNA

When you perform the miRNA starter kit experiment, use the human brain and lung total RNA control samples provided in the QuantStudio™ 12K Flex OpenArray® Human miRNA Starter Kit (Table 1 on page 7).

(For your own experiments) Isolate the RNA

To prepare RNA samples for your own miRNA experiments, we recommend the *mir*Vana™ miRNA Isolation Kit 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 files to run the miRNA experiment.

Reverse transcribe the RNA with Megaplex™ RT Primers

About the Megaplex™ Primer Pools

The Megaplex™ Primer Pools include content-matched pools of Megaplex™ RT Primers and Megaplex™ PreAmp Primers. Be sure to select the correct Megaplex™ Primer Pools set for your microRNA panel:

For...	Use...		
	Megaplex™ Primer Pools	**Contents**	**Storage**
Starter kit experiment:			
TaqMan® OpenArray® Human MicroRNA Panel	Megaplex™ Primer Pools, Human Pool A v3.0 (Part no. 4444750)	• Megaplex™ RT Primers Human Pool A v2.1 • Megaplex™ PreAmp Primers Pool A v2.1	–15 to –25°C
Your own experiments:			
TaqMan® OpenArray® Human MicroRNA Panel	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	–15 to –25°C
TaqMan® OpenArray® Rodent MicroRNA Panel	Megaplex™ Primer Pools, Rodent Pools Set v3.0 (Part no. 4444766)		

About 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 Pools A and 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

Set up the RT reactions

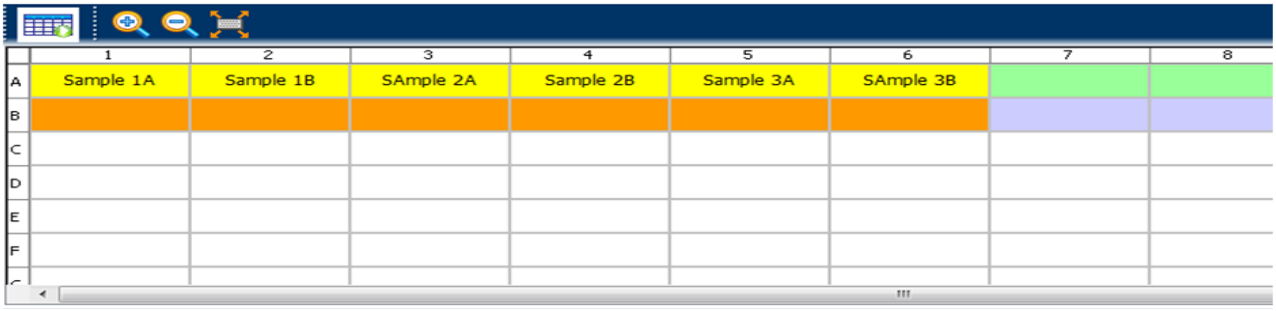
1. Thaw the following on ice:
   - Megaplex™ RT Primers
   - TaqMan® MicroRNA Reverse Transcription Kit components (do not vortex the MultiScribe™ Reverse Transcriptase)
   - $MgCl_2$ (supplied with the Megaplex™ RT Primers)
2. Combine the following in each of two 1.5-mL microcentrifuge tubes (one for Pool A, the other for Pool B):

RT Reaction Mix Components	Volume per reaction	Volume for 3 reactions[1]
Megaplex™ RT Primers (10X), 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
10X 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
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 centrifuge the tubes briefly.
4. Transfer 4.5 µL of the RT Reaction Mix into the appropriate number of wells of a 96-well MicroAmp® Optical Reaction Plate, as shown in Figure 1 below.

Figure 1 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. Thus 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.

   Note: 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 Strip Caps, then invert the plate a few times.

   Note: Do not use MicroAmp® *Optical* Adhesive Film to seal the plate as this film may be difficult to remove once run in the thermal cycler.
7. Spin 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: **9700** 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	Temp	Time
Cycle (40 cycles)	16°C	2 min
	42°C	1 min
	50°C	1 sec
Hold	85°C	5 min
Hold	4°C	∞

2. Load, then run the plate.

STOPPING POINT If needed, you can store the RT product (cDNA) at -15 to -25°C for up to 1 month.

Preamplify the cDNA with Megaplex™ PreAmp Primers

About the preamplification reaction

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 17
- 22.5 µL of PreAmp Reaction Mix, containing Megaplex™ PreAmp Primers Pool A or Pool B and 2× TaqMan® PreAmp Master Mix

  Use Megaplex™ PreAmp Primers Pools A or B corresponding to the Megaplex™ RT Primers Pool used for reverse transcription.

Set up the preamplification reactions

1. Thaw the Megaplex™ PreAmp Primers on ice and mix by inverting a few times. Spin 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 PreAmp Reaction Mix, one for Pool A and one for Pool B, by combining the following in each of two 1.5-mL microcentrifuge tubes:

PreAmp Reaction Mix components	Volume for 1 reaction	Volume for 3 reactions[2]
2X TaqMan® PreAmp Master Mix	12.5 µL	42.4 µL
Megaplex™ PreAmp Primers (10X), Pool A or Pool B[1]	2.5 µL	8.4 µL
Nuclease-free water	7.5 µL	25.3 µL
Total	22.5 µL	76.1 µL

1 Use Pool A in one tube, and Pool B in the other.
2 Includes 12.5% excess for volume loss from pipetting.

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 22.5 µL 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 Strip Caps.
     or
   - Seal the plate using MicroAmp® Clear Adhesive Film or MicroAmp® Optical Strip Caps, then invert the plate a few times.
8. Spin 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: **9700** 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	∞

1 Required for enzyme inactivation.

Dilute the preamplification products

1. Remove the 96-well plate from the thermal cycler and 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 Strip Caps, then invert the plate a few times.
5. Spin the plate briefly to collect the contents at the bottom of the wells, then place the plate on ice.

STOPPING POINT If needed, you can store the preamplified product (diluted or undiluted) at 4°C for up to 12 hours, or at –15 to –25°C for up to 1 week.

Next step

Proceed to Chapter 3, "Prepare the 384-Well Sample Plate" on page 21.

3 Prepare the 384-Well Sample Plate

Overview

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 Plates (see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*). You will 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):

Workflow

Prepare the OpenArray® 384-Well Sample Plate

Track the samples (page 22)

▼

Prepare PCR reaction mix A and B (page 23)

▼

Transfer the PCR reaction mix to the 384-well sample plate (page 24)

Required materials

Item[1]	Source	Part no.[2]
96-well reaction plates, containing preamplified cDNA samples	User supplied (see page 14)	—
2X TaqMan® OpenArray® Real-Time PCR Master Mix, 1.5 mL	Life Technologies	4462159
MicroAmp® Optical 96-Well Reaction Plate	Life Technologies	4316813[3]
MicroAmp® Clear Adhesive Film	Life Technologies	4306311[4]
OpenArray® 384-Well Sample Plates	Life Technologies	4406947
QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals	Life Technologies	4469876
Fine-tip marker	Major Laboratory Suppliers (MLS)	—

1 For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.
2 Provided in starter kit.
3 Not provided in starter kit.
4 Not provided in starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates.

1. *(For the starter kit experiment)* Edit the sample information in the microRNA starter kit *.csv file:
   a. Navigate to and double-click the file to open in it Microsoft® Excel® Software.

      Note: The sample information file is located at: <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\examples\User Sample Files\Human miRNA OA Plate Example.csv, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

   b. In the SampleID column, enter the sample name for each sample in the miRNA starter kit: **Brain 1**, **Brain 2**, and **Brain 3** and **Lung 1**, **Lung 2**, and **Lung 3**.

   c. Select **File** ▸ **Save As** and save the spreadsheet as a *.csv file.

      Note: You can import the sample information from this *.csv file directly into the QuantStudio™ 12K Flex Software before starting the run (see Chapter 5 on page 41).

      For the starter kit experiment, you can also use an installed .edt file with sample and assay names, and edit the names in the QuantStudio™ 12K Flex Software directly.

2. *(For your own experiments)* Proceed to transfer the samples as shown in Figure 2. You do not need to enter sample information from the 96-well sample plate(s) at this time.

   Note: You can edit the sample information directly in the QuantStudio™ 12K Flex Software before starting the run (see Chapter 5 on page 41). For the starter kit experiments, you can download an unloaded .tpf & a new .edt (without sample names). You can then add the sample names in the QuantStudio™ OpenArray® AccuFill™ Software or QuantStudio™ 12K Flex Software using the Import or direct edit feature.

3. Using a fine-tip marker:
   a. Label the 384-well sample plate with a unique identifier.
   b. Based on the tracking information obtained in step 1, mark the sections of the 384-well sample plate that you will transfer the samples to from the 96-well reaction plate(s).

Prepare PCR reaction mix A and B

Note: All volumes include 12.5% excess volume to accommodate the loss that occurs during pipetting.

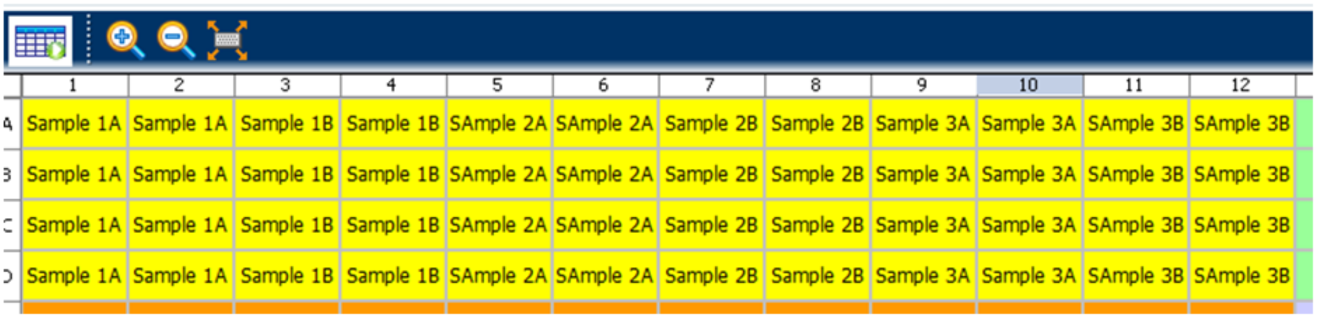
1. If the diluted preamplification products were stored frozen, thaw the 96-well reaction plate completely on ice. Mix by inverting the sealed plate a few times or by gently vortexing, then centrifuge the plate briefly.
2. Mix the 2× TaqMan® OpenArray® Real-Time PCR Master Mix by swirling the bottle.
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.

STOPPING POINT If needed, you can store the sealed 96-well reaction plate at 4°C for up to 12 hours.

Transfer the PCR reaction mix

As shown in Figure 2 below:

Figure 2 384-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 @ 1000 rpm to eliminate bubbles.
4. *(Optional)* Place the sample plate on ice, in the dark, for up to 1 hour.

Next step

Proceed to Chapter 4, "Prepare the QuantStudio™ 12K Flex OpenArray® Plate" on page 25.

4 Prepare the QuantStudio™ 12K Flex OpenArray® Plate

Overview

In this chapter, you use the QuantStudio™ OpenArray® AccuFill™ System to transfer the nucleic acid samples from the OpenArray® 384–Well Sample Plate to QuantStudio™ 12K Flex TaqMan® OpenArray® plates. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Prepare the QuantStudio™ 12K Flex TaqMan® OpenArray® plate

Prepare for sample transfer (page 28)

▼

Transfer the samples (page 30)

▼

Seal the plate (page 37)

▼

Complete sample transfer for the remaining TaqMan® OpenArray® plates (page 39)

Required materials

Item[1]	Source	Part no.
QuantStudio™ 12K Flex TaqMan® OpenArray® plates[2]	Life Technologies	See Appendix A in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*
QuantStudio™ OpenArray® AccuFill™ System	Life Technologies	4471021
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit The accessories kit contains: • QuantStudio™ 12K Flex OpenArray® Lids (6 lids) • QuantStudio™ 12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™ 12K Flex OpenArray® Carriers (2 carriers) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid (6 syringes) • QuantStudio™ 12K Flex OpenArray® Immersion Fluid Tip • OpenArray® AccuFill™ System Loader Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Life Technologies	4469586
QuantStudio™ 12K Flex OpenArray® Plate Press 2.0	Life Technologies	A24945
Foil seals	Major Laboratory Suppliers (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 the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

2 For detailed information about the TaqMan® OpenArray® plates, see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*

Storage conditions

The following materials require special storage conditions:

Item		Storage Conditions
If the QuantStudio™ 12K Flex TaqMan® OpenArray® plate is...	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™ 12K Flex 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 Loader Tips	Unopened	Store at room temperature until the expiration date printed on the cardboard box.
	Opened	Store at room temperature. Discard unused tips after the expiration date printed on the cardboard box.

Prepare for sample transfer

Guidelines for handling the TaqMan® OpenArray® plate

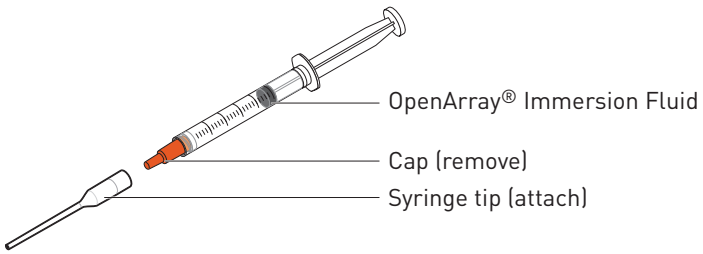
- Hold the OpenArray® case by the edges.
- Do not touch the through-holes of the OpenArray® plate.
- Load and seal an OpenArray® plate within *one hour* after opening the packaging.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.
- Do not reinsert an OpenArray®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

Prepare the equipment and plates

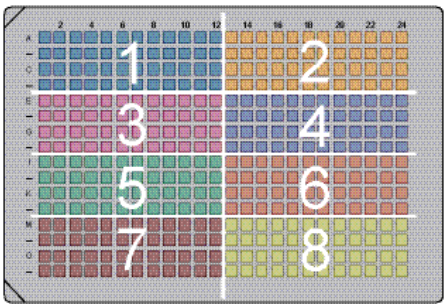
IMPORTANT! Wear powder-free gloves while preparing the OpenArray® plates.

1. Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ System Loader Tips, and plate holder are completely clean and dry. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).
2. Remove an OpenArray® plate from the freezer, *but do not open the packaging*. Allow the plate to thaw at room temperature (approximately 15 minutes).
3. 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.



4. 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

For each OpenArray® plate being prepared, note the following:

- *For the starter kit experiments and recommended for your own experiments*, a plate setup file (*.csv, *.spf, or *.tpf) is needed to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software.

  Note: If no samples are provided with the starter kit, create a *.csv file as you would for your own experiments.

- *For your own gene expression and genotyping experiments*, the following plate setup files can be used to transfer samples using the QuantStudio™ OpenArray® AccuFill™ Software:
  - OpenArray® 384-well sample information file (*.csv, see "Track the samples" on page 22)
  - OpenArray® plate setup file (*.spf or *.tpf, see "(Optional) Using an OpenArray® plate setup file" on page 9)
- *(Optional)* If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), 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.

Next step

Proceed immediately to "Transfer the samples" on page 30.

Transfer the samples

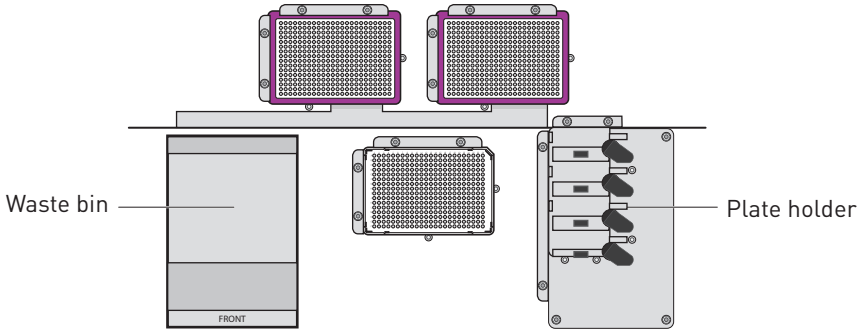
Initialize the system

1. Close the enclosure door, then start the QuantStudio™ OpenArray® AccuFill™ Software. The software checks the computer and connections as the system starts.

   When prompted, clear the deck and empty the waste bin of used tips:

   a. Open the instrument by grasping the enclosure door handle and gently, but firmly, pulling the enclosure door up.

   b. Empty the waste bin and place it back on the deck.



   Note: To safely operate the instrument, it is important to keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

2. Check if there are any OpenArray® plates in the plate holder on the deck. If necessary, remove them.

3. If necessary, replace the tip boxes.

   Note: Tip boxes contain 384 tips, divided into 8 sections. When you click **Load**, the QuantStudio™ OpenArray® AccuFill™ System loads as though a new, full box of tips is on the deck. QuantStudio™ OpenArray® AccuFill™ Software prompts you to verify that tips are in the locations shown in the Setup Deck screen (see "Load the OpenArray® plate" on page 33). Clicking a section in the Setup Deck window confirms that tips are in that section of the tip box.

   a. Place tip boxes on the deck in the two side-by-side recessed rectangular platforms (purple and white locations as shown in the illustration above).

   b. Remove the cover before using the tips for loading.

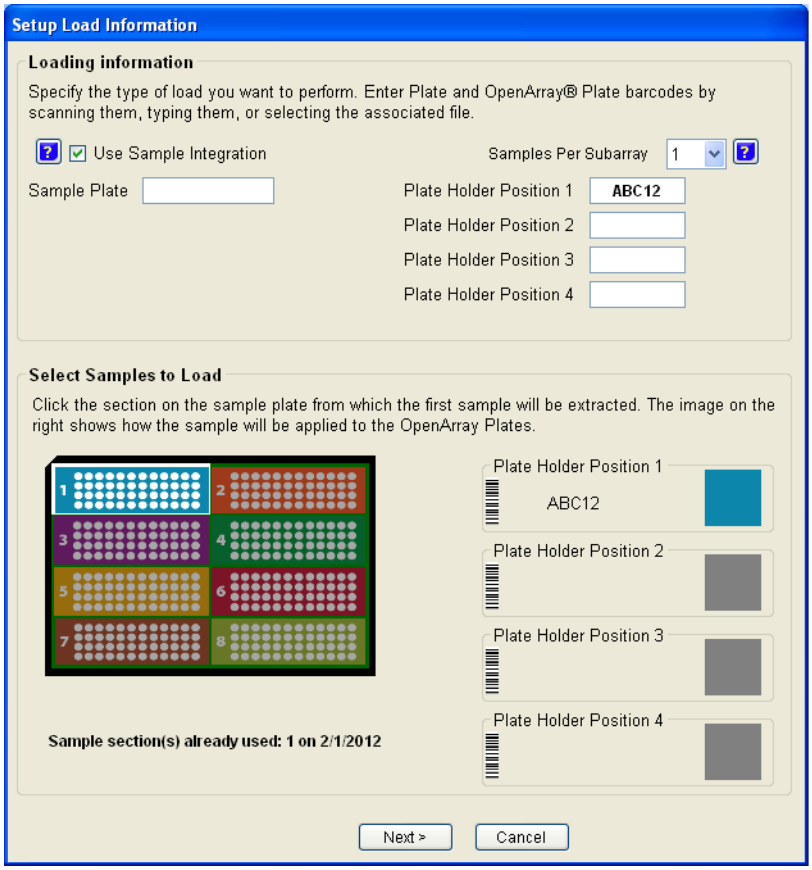
4. Close the door on the instrument.

5. Click **Proceed** to begin the System Self Test. The application performs a number of self tests and is then ready for you to continue.

   Note: System Self Test runs only at start up. The test does not run again unless the system is restarted or a self test is intentionally run. The System Self Test utility is in the Instrument drop-down menu in the QuantStudio™ OpenArray® AccuFill™ Software.

Prepare for loading

1. Click **Setup & Load**, then complete the Setup Load Information window.



2. Do either of the following:
   - Select the **Use Sample Integration** check box, then proceed to step 3.
   - *(Required for the starter kit experiments, recommended for your own experiments)* Proceed to step 5.

     Note: For the starter kit experiments, you will import sample and assay information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 46).

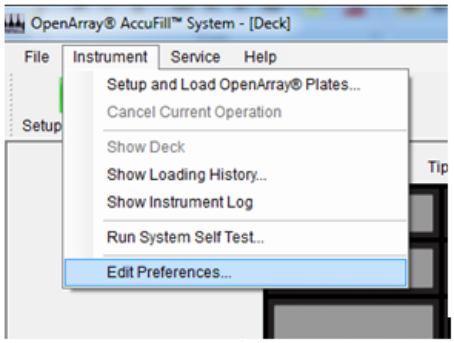
     Note: For the starter kit experiments, you will use an installed *.edt file (with experiment type, assay name, and run method).

3. In the Sample Plate field, browse to and open the *.csv file that contains the 384-well sample plate layout (see "Track the samples" on page 22).

   To set up sample integration in the QuantStudio™ OpenArray® AccuFill™ Software:

   a. Launch QuantStudio™ OpenArray® AccuFill™ Software version 1.1.

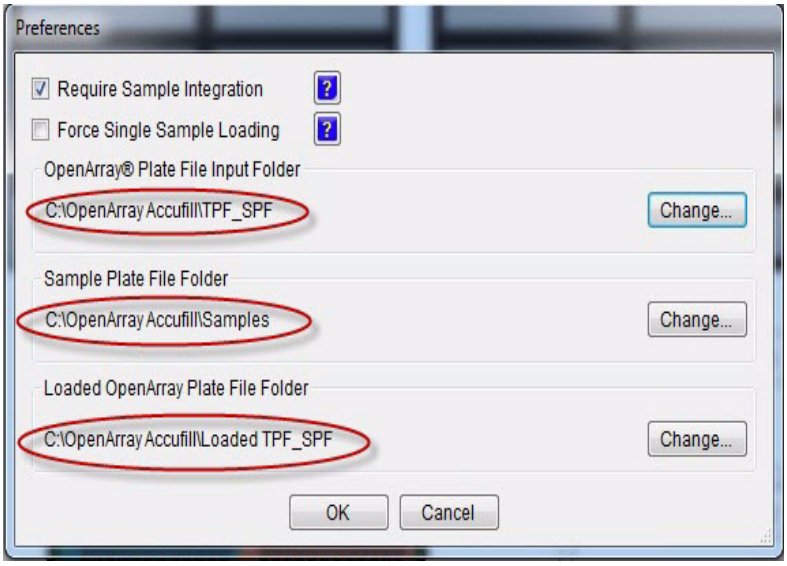
b. Go to **Instrument** ▸ **Edit Preferences**.



c. In the Preferences dialog box, check **Require Sample Integration**.

d. Click **Change** to select another location for the Input, Sample Plate, and Loaded OpenArray Plate folders.

- **Input folder** (<drive>:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF): Contains *.tpf files that are downloaded from the web or CD.
- **Sample Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Samples): Contains sample *.csv files (in the 384 format from Sample Tracker).
- **Loaded OpenArray Plate folder** (*<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Loaded TPF SPF): Contains integrated *.tpf files with sample names. The QuantStudio™ OpenArray® AccuFill™ Software automatically places the integrated *.tpf file with sample names in this folder (after the QuantStudio™ OpenArray® AccuFill™ Software run). The resulting *.tpf file includes the sample names.



4. Enter the data for the first OpenArray® plate:

a. Select **1** from the Samples Per Subarray drop-down list.

b. In the Plate Holder Position 1 text field, enter the 5-character alphanumeric serial number of the OpenArray® plate you will load into the first position of the plate holder. You can:

- Click **Browse**, then navigate to and open the plate setup file (*.spf or *.tpf) that corresponds to the OpenArray® plate. The software automatically displays the serial number in the Plate Holder Position 1 field.
- Scan the serial number (barcode) located on the OpenArray® plate package.
- Type the serial number.

IMPORTANT! When you integrate a SampleID.csv into a plate setup file and enter the serial number by scanning or typing, the plate setup file must be located in the *<drive>*\Program Files\Applied Biosystems\OpenArray AccuFill\Sample directory (see "Using an OpenArray® plate setup file" on page 9). Otherwise, the software will not be able to locate the file. *<drive>* is the computer hard drive on which the QuantStudio™ OpenArray® AccuFill™ Software is installed.

Note: The QuantStudio™ OpenArray® AccuFill™ Software uses the serial number to access the appropriate plate setup files. During an instrument run, information in the plate setup files is used to populate the Assays screen in the QuantStudio™ 12K Flex Software. For information on the Assays screen, see "Analyze the Experiment Results" on page 57.

As you enter the serial number, it is reflected in the representation of the OpenArray® plates in the lower section of the window.

5. Repeat step 2 for the remaining OpenArray® plate(s).
6. Click **Next**.

Note: You can also enter sample information directly in the QuantStudio™ 12K Flex Software before starting the run (see page 46).
You can download plate setup files (.tpf/.spf and a new .edt without sample names). You can then add names in the QuantStudio™ 12K Flex Software using the Import or direct edit feature.

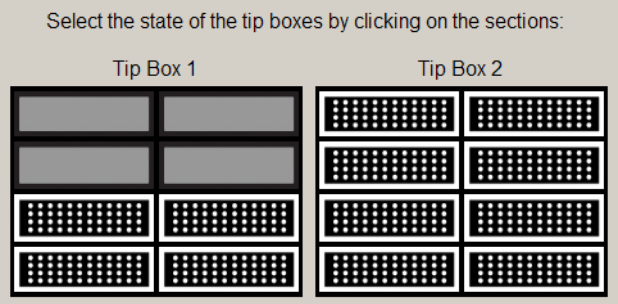
Load the OpenArray® plate

1. Open the enclosure door of the QuantStudio™ OpenArray® AccuFill™ System by grasping the door handle and lifting the door up.
2. Insert the OpenArray® 384-Well Sample Plate with the foil cover still in place. Press on the plate until you hear it snap into place.

   Note: Do not remove the foil from the OpenArray® 384-Well Sample Plate at this time.

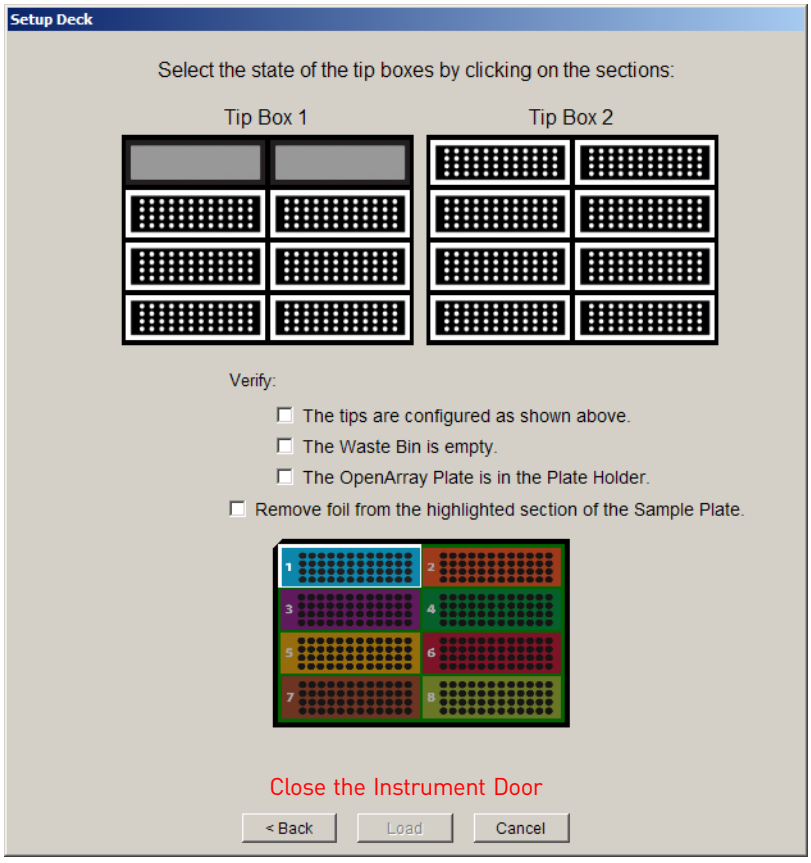
3. Place a thawed OpenArray® plate into the Plate Holder. When handling the OpenArray® plate:
   - Always hold the OpenArray® case by the edges and place it into the Plate Holder with the barcode face up and to the left.
   - If you inadvertently drop a loaded OpenArray® plate, discard it in the sharps waste container.
   - Be sure to load the OpenArray® plate within an hour after you open it.
4. Visually verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that no tips are present.
   - White areas indicate that tips are present.

   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window. For example:



Note: Cover the tip box when not in use. Discard any unused tips after 1 year or after the expiration date printed on the cardboard box.

5. Verify each of the following conditions and, when verified, select its check box:
   - Tips are configured as shown in step 4 above.
   - Waste bin is empty.
   - OpenArray® plate is in the Plate Holder.

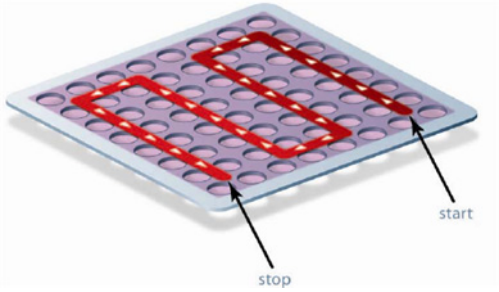


Note: The software will not continue until you select all the check boxes.

6. With forceps, peel off the foil covering the area of the OpenArray® 384-Well Sample Plate containing the samples to be loaded on the OpenArray® plate.
7. Select **Remove foil from the highlighted section of the Sample Plate**.
8. Close the instrument door.
9. Click **Load**.

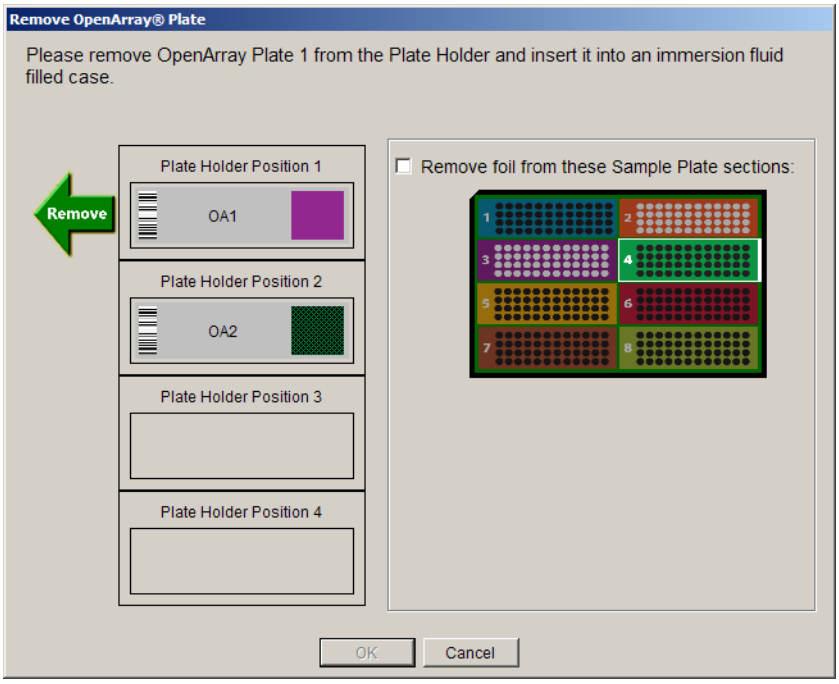
   Note: If the number of OpenArray® plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra OpenArray® plates. Correct the error and continue.

You can follow the progress of the loading on the screen. The samples in each tip are loaded in the OpenArray® plate. Each tip fills the 64 through-holes in one subarray, travelling in the pattern shown (the following illustration shows the load path for only one sample):



10. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate as explained in "Seal the OpenArray® plate" on page 37.

IMPORTANT! Once an OpenArray® plate has been filled, you must seal it within 90 seconds to prevent excessive evaporation.



11. Close the instrument door.

Note: After you load the plate, clean the QuantStudio™ OpenArray® AccuFill™ System according to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

Note: *(If Use Sample Integration on page 31 is checked)* You must have the plate setup files (*.spf or *.tpf) in the OpenArrayPlate folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\TPF SPF) and the SampleID.csv file in Sample Plates folder (C:\Program Files\Applied Biosystems\OpenArray AccuFill\Samples).

To integrate, click **Browse** next to the blank window under Use Sample Integration and next to Sample Plate, and navigate to the location of the SampleID.csv file. Select the file with the information on the samples that are going to be loaded into a given set of OpenArray® plate(s). Click **Open**.

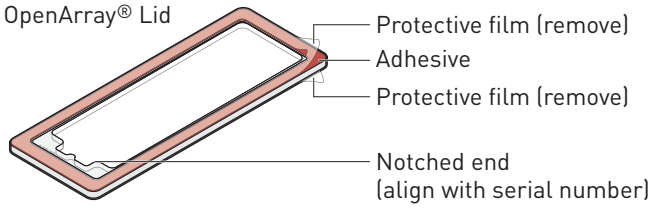
The plate setup file (*.spf or *.tpf) is now integrated with the sample information file (*.csv) and is called *Loaded_<barcode>.tpf*. You can use this file in the QuantStudio™ 12K Flex Software to create and run an OpenArray® experiment (see "Using an OpenArray® plate setup file" on page 49). Proceed with Load the OpenArray plate.

Next step Proceed immediately to "Seal the OpenArray® plate" below.

Seal the OpenArray® plate

1. Remove the protective film from the top *and* bottom of an OpenArray® Case Lid.

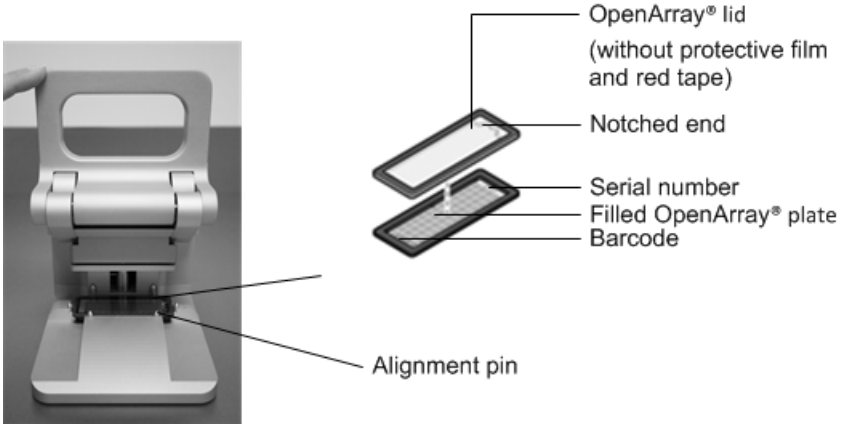
IMPORTANT! The protective film at the bottom of the Case Lid is covered by a red tape that needs to be removed first to access the protective film. Make sure to remove the protective film from *both* sides of the lids.



2. Using the thumb and index finger, grasp the OpenArray® case by the top (nearest the barcode), gently lift the case from the plate holder, then load it into the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

3. Place the Case Lid with red tape and protective film removed (both top and bottom) onto the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the lid must be oriented toward the right side of the Plate Press.



4. Actuate the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0 by pulling down the lever.

5. The status light flashes green for 20 seconds. After 20 seconds, the status light turns solid green indicating that the case is ready.

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

6. Release the lever.

7. Load the OpenArray® case with OpenArray® Immersion Fluid:

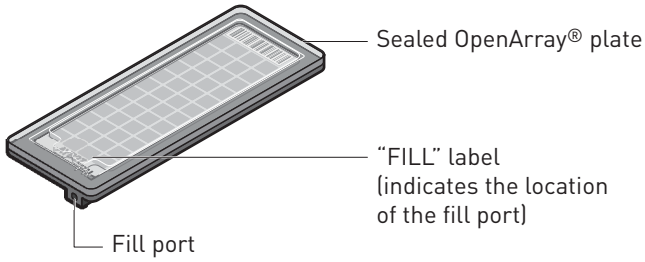
IMPORTANT! Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 seconds.

   a. Remove the sealed plate from the Plate Press, grasping the case on the edges.

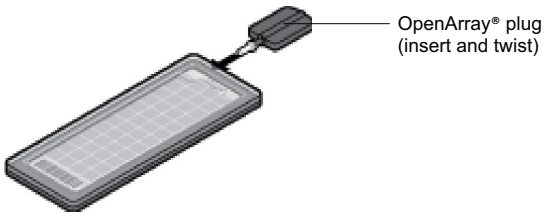
   b. Insert the syringe tip into the loading port at end of the sealed Case, then dispense the fluid completely in one gentle continuous motion.

IMPORTANT! Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through-holes.

Note: Try to minimize creating air bubbles when you dispense the fluid: one small air bubble is acceptable.



c. While holding the OpenArray® plate vertically, seal the loading port by inserting the OpenArray® Plug into the port and twisting the plug clockwise, applying sufficient pressure until the handle breaks off.



d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; be sure to not apply pressure on the OpenArray® plate within the case.

The sealed OpenArray® plate can be loaded into the QuantStudio™ 12K Flex System.

Note: Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.

STOPPING POINT For microRNA experiments, you can store loaded and sealed OpenArray® plates at room temperature, protected from light, for up to 1 hour.

Next step

Proceed to:

- "Complete sample transfer for the remaining plates" below

  or

- Chapter 5, "Perform the Instrument Run" on page 41

Complete sample transfer for the remaining plates

Repeat the following procedures to transfer sample to the remaining OpenArray® plates:

- "Prepare for sample transfer" on page 28 *(if loading > 4* OpenArray® *plates)*
- "Transfer the samples" on page 30
- "Seal the OpenArray® plate" on page 37

Next step Proceed to Chapter 5, "Perform the Instrument Run" on page 41.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (>6) of OpenArray® plates, follow the guidelines below.

- To help avoid mistakes when entering sample information in the QuantStudio™ OpenArray® AccuFill™ Software, load the OpenArray® plates in alphanumeric order (per the OpenArray® plate serial number).
- Seal each OpenArray® plate immediately after loading is completed, while 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 an vertical position.

- Use the QuantStudio™ Carrier to transport up to four loaded OpenArray® plates to the QuantStudio™ 12K Flex Real-Time PCR System.
- After loading is complete, you can use a large bin to properly dispose of any used OpenArray® AccuFill™ System Loader Tips. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

5 Perform the Instrument Run

Overview

In this chapter, you run the QuantStudio™ 12K Flex TaqMan® OpenArray® plates on the QuantStudio™ 12K Flex Real-Time PCR System. During the run, the QuantStudio™ system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Perform the instrument run

Prepare the QuantStudio™ 12K Flex Software (page 43)

▼

Load the TaqMan® OpenArray® plate into the instrument (page 45)

▼

Run the plates (page 46)

▼

(Optional) Monitor the run (page 50)

▼

Unload the instrument (page 53)

▼

Transfer the experiments results to the computer (page 55)

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 OpenArray® experiments.

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

To select OpenArray® experiment preferences:

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. Go to **Tools** ▸ **Preferences** in the QuantStudio™ 12K Flex Software and select the **OpenArray®** tab.
3. Select the following as needed:

Settings	Description
Setup Folder field	Defines the absolute path to the default folder/directory from which the QuantStudio™ 12K Flex Software imports experiment setup files. The Import dialog box opens to the import folder when invoked from the QuantStudio™ 12K Flex Software.
Experiment Folder field	Defines the absolute path to the default folder/directory to which the QuantStudio™ 12K Flex Software reads/writes experiment files. The Open and Save dialog boxes open to the data folder when invoked from the QuantStudio™ 12K Flex Software.
Passive Reference drop-down list	Defines the dye to use as the passive reference. The default is set to None. **Note:** While the QuantStudio™ 12K Flex Software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray® experiments.
Default Browse File Type drop-down list	Defines the file type which the Import, Open, and Save dialog boxes select by default when invoked from the QuantStudio™ 12K Flex Software.
Apply experiment template (EDT) to all OpenArray® experiment check box	If selected, the QuantStudio™ 12K Flex Software applies the Run Method defined in the selected experiment template (*.edt) to all OpenArray® experiments. For more information on OpenArray® experiment templates, see the *QuantStudio™ 12K Flex Software Help*.
Always include Amplification stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds an Amplification stage to the Run Method for all OpenArray® genotyping experiments. If deselected, you need to perform amplification on another instrument. For more information on Run Method settings, see the *QuantStudio™ 12K Flex Software Help*.
Always include Pre-Read stage for Genotyping experiment check box	*(Genotyping experiments only)* If selected, the QuantStudio™ 12K Flex Software adds a Pre-Read stage to the Run Method for all OpenArray® genotyping experiments. For more information on Run Method settings, see the *QuantStudio™ 12K Flex Software Help*.

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

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

Access the Instrument Console

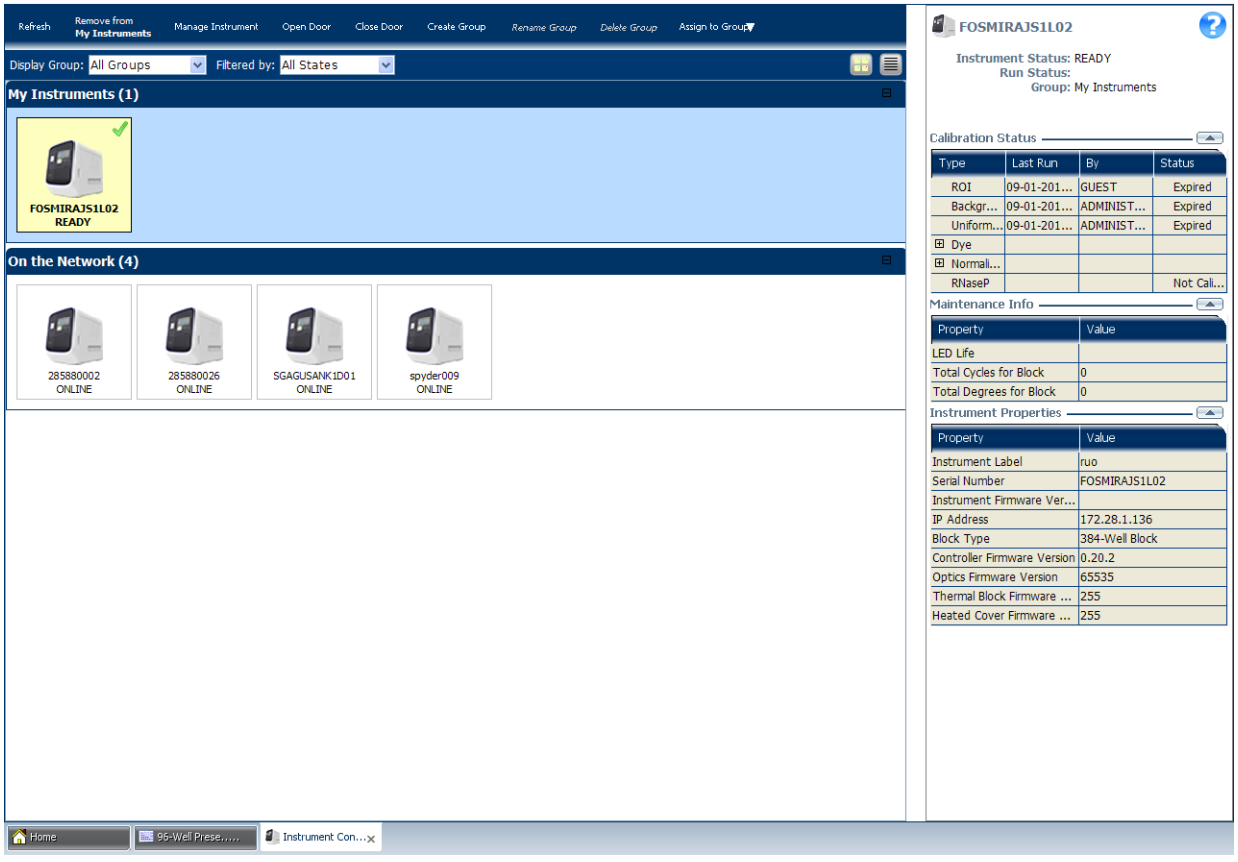
The Instrument Console displays all the QuantStudio™ 12K Flex Instruments discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups:

- **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 QuantStudio™ 12K Flex Software.
2. On the Home tab (), 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 which can be monitored:

a. Click the instrument of interest, then click **Assign to Group** in the Instrument Console toolbar.

b. Select the **My Instruments** 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 Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the Notification Settings feature, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

Load the OpenArray® plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

IMPORTANT! Wear powder-free gloves when you handle OpenArray® plates.

IMPORTANT! OpenArray® plates should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to allow the plate adapter to come out from the instrument side.
2. Place the OpenArray® plate(s) on the plate adapter. Make sure that:
   - Each plate is properly aligned in the adapter.
   - The plate barcode is facing up and toward the front of the instrument.
3. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Close Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to retract the plate adapter back into the instrument.

Run the OpenArray® plates

Overview

You can run OpenArray® plates in either of the following two ways:

- "From the QuantStudio™ 12K Flex Software" on page 46
- "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 51

Note: The starter kit experiments in this guide run OpenArray® plates 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 Instrument is in operation.

From the QuantStudio™ 12K Flex Software

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

- For the starter kit experiments:
  - "Using a template file" (*.edt, see below)
- For your own experiments:
  - *(Recommended)* "Using a template file" (*.edt, see below)
  - "Using an OpenArray® plate setup file" (*.spf or *.tpf, see page 47)
  - Using the Batch Experiment Setup Utility (see the *QuantStudio™ 12K Flex Software Help*; click or press **F1**)

Using 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(s) before starting the run, or after the run is complete.

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. On the Home tab, select **Create From Template**.
3. Navigate to and select the template file (*.edt) 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 *<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates, where *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.
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) 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.

     Note: For the microRNA starter kit experiment, click **New** (in the toolbar above the sample table) twice, then enter **Brain 1**, **Brain 2**, and **Brain 3** as the new sample names in the OpenArray® plate.
6. From the open experiment, select **File ▸ Import Plate Setup.**
   a. Click **Browse**, navigate to and select the plate setup file you want to use:

      MicroRNA Source File (*.tpf) – Corresponds to the plate setup file associated with miRNA OpenArray® plates.

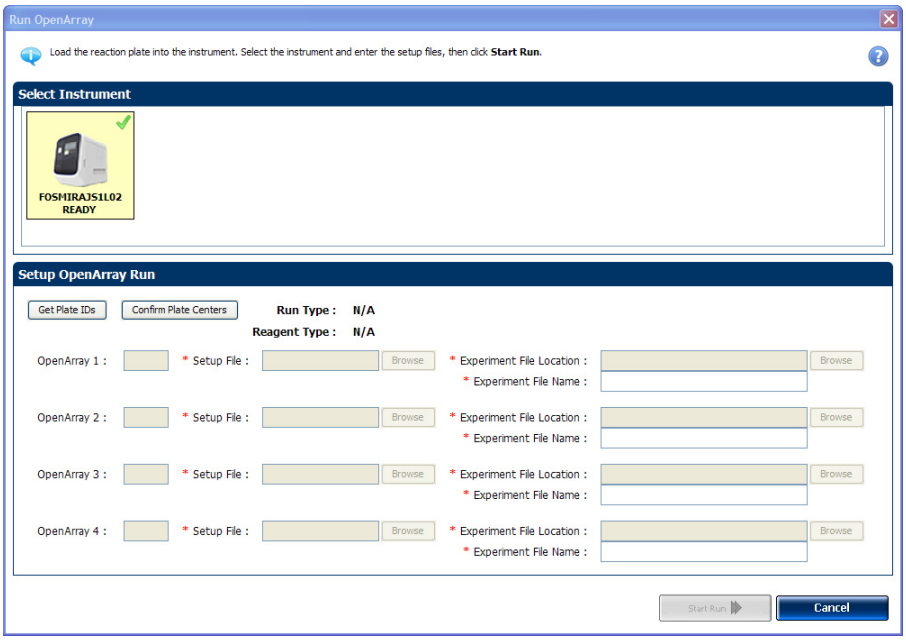
      Note: For the microRNA starter kit experiments and for your own experiments, use the *.edt template file supplied with the QuantStudio™ 12K Flex Software. See page 9 for more information.
   b. Click **Select**, then click **Start Import**.
   c. If your experiment already contains plate setup information, the software asks you if you want 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**.

Using an OpenArray® plate setup file

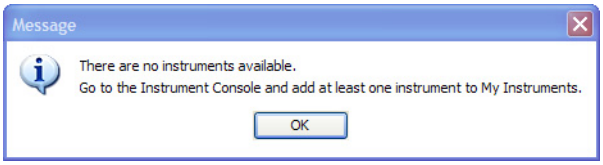
If you exported a 384-well plate file (*.csv) file from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), you can import the sample information in this file into the QuantStudio™ OpenArray® AccuFill™ Software. The QuantStudio™ OpenArray® AccuFill™ Software automatically integrates the sample information into an OpenArray® plate setup file (*.tpf or *.spf). You can save the newly created Loaded_tpf files to the OpenArray Plate File Input Folder you selected in the Preferences dialog box of the QuantStudio™ 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 and run the file.

Note: If you exported an OpenArray® plate file (*.csv) from the OpenArray® Sample Tracker Software (see "Track the samples" on page 22), 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.

1. Click **OpenArray** from the Run menu on the Home screen of the QuantStudio™ 12K Flex Software.



Note: Be sure to add an instrument to My Instruments in the Instrument Console screen before running an experiment (see "Access the Instrument Console" on page 44). If no instrument is selected, you will receive the following warning.



2. In the Select Instrument pane, select the instrument you want to run the experiment on.

3. In the Setup OpenArray Run pane:
   - Click **Get Plate IDs** to import the barcode of the OpenArray® plates that you want to run.
   - *(Optional)* Click **Confirm Plate Centers** to view the center of the OpenArray® plates 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 to and select the appropriate OpenArray® plate setup files (*.spf or *.tpf) on your computer.

     Note: Once the setup file is selected, the Experiment File Location and Experiment File Name are automatically populated in the respective fields. 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® plates loaded in the instrument, the barcode of those OpenArray® plates will be populated.

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

4. Click **Start Run**.

From the QuantStudio™ 12K Flex Instrument Touchscreen

There are three ways to start a run from the QuantStudio™ 12K Flex Instrument Touchscreen:

- "From experiments that are already created" below
- "From templates" on page 49
- "From shortcuts" on page 50

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

From experiments that are already created

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.
   Note: If the touchscreen is not at the Main Menu screen, touch  (**Home**).
2. In the Home screen, touch **Run OpenArray Plates**.
   The instrument will retrieve the barcodes and scan 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 QuantStudio™ 12K Flex Instrument does not detect a barcode, repeat the barcode read. If the barcode is detected incorrectly, type the correct barcode number on the QuantStudio™ 12K Flex Instrument Touchscreen. Do not proceed if a barcode is not detected by the QuantStudio™ 12K Flex Instrument.

From templates

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex 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 will retrieve the barcodes and create new experiments based on the template for each plate found.

6. Touch 

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

From shortcuts

From the QuantStudio™ 12K Flex Instrument Touchscreen:

1. Touch the QuantStudio™ 12K Flex Instrument Touchscreen to activate it.

   Note: 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 will retrieve the barcodes and create 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 "From the QuantStudio™ 12K Flex Software Run screen" on page 50).
- From the QuantStudio™ 12K Flex Instrument Touchscreen, in the same way that you run the experiment (see "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 51).
- From the Instrument Console of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Instrument Touchscreen) as described in "From the QuantStudio™ 12K Flex Software Instrument Console" on page 50.

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.

From the QuantStudio™ 12K Flex Software Run screen

Click **Amplification Plot** from the Run Experiment Menu to monitor the amplification plot of the experiment you are running.

From the QuantStudio™ 12K Flex Software Instrument Console

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.

2. In the Instrument Console screen, select the icon of the instrument that you are using to run the experiment, then click **Manage Instrument** or double-click on the instrument icon.

   Note: You must add the instrument to a group which can be monitored before you can manage it (see "Access the Instrument Console" on page 44).

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 page 51) available from the QuantStudio™ 12K Flex Software for potential problems.

To...	Action
Stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN**. • In the Stop Run dialog, 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.
View amplification data in real time	Select **Amplification Plot**. See "To monitor the Amplification Plot" below.

To 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 each experiment's Amplification Plot.

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

The Amplification Plot screen 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).

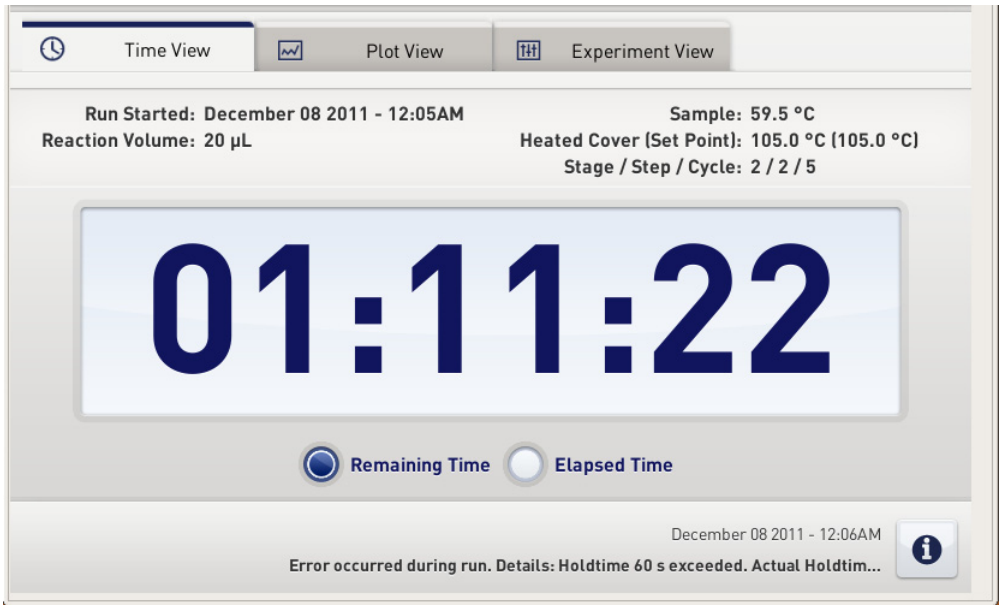
  Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the *QuantStudio™ 12K Flex Software Help* (click or press **F1**).

From the QuantStudio™ 12K Flex Instrument Touchscreen

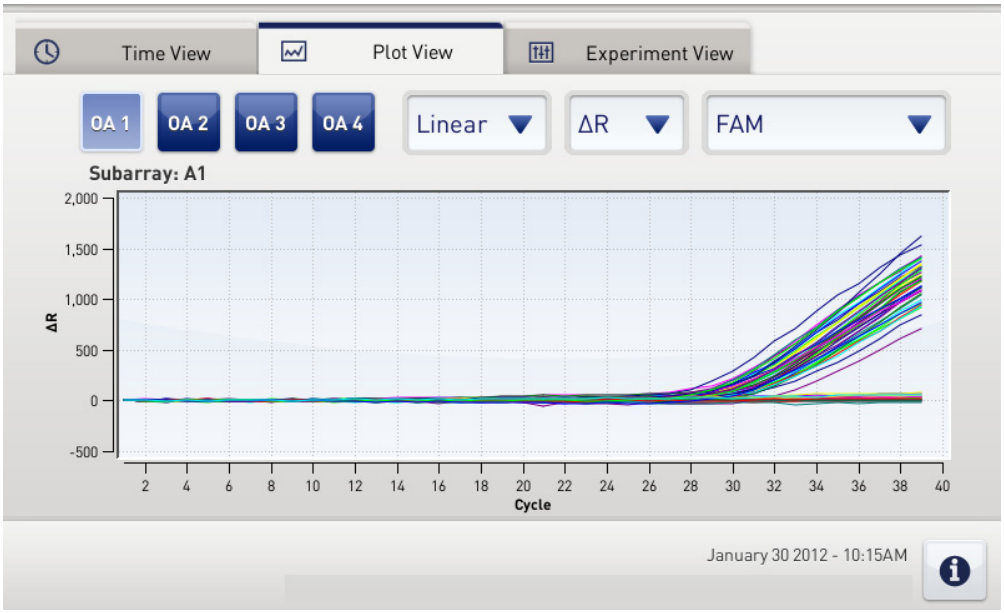
The QuantStudio™ 12K Flex Instrument Touchscreen displays the barcodes (or Plate IDs) of the TaqMan® OpenArray® plates for the run, the date and time at which the run started, the time remaining in the run, and other information.

To...	Action
Display the experiment names in the run	Touch **Experiment View**.
Show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
Display the time elapsed and the time remaining in the run	Touch the **Time View tab**, then touch **Experiment View** tab to return to the previous screen.
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 present below the Plot View tab.

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

Unload the 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 re-open the *.eds file to display the Amplification Plot screen (for gene expression experiments) or the Allelic Discrimination Plot screen (for genotyping experiments). See "Analyze the Experiment Results" on page 57.
- QuantStudio™ 12K Flex Instrument Touchscreen, see "(Optional) Transfer experiment results" on page 55.

Unload the instrument

When the QuantStudio™ 12K Flex Instrument Touchscreen displays the Home screen, unload the OpenArray® plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100 °C. Do not touch the sample block until it reaches room temperature.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen 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 QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate as follows:

   a. Power off the QuantStudio™ 12K Flex Instrument.
   b. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate.
   c. If the instrument does not eject the plate, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door.
   d. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door.

(Optional) Transfer experiment results

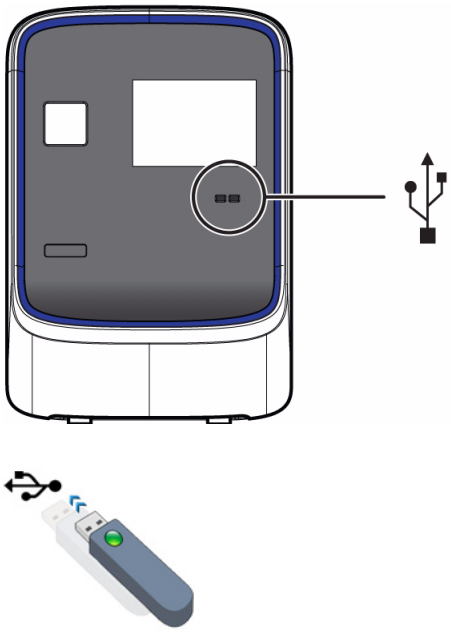
If you started a run from the QuantStudio™ 12K Flex Instrument Touchscreen, transfer the experiment data to the computer for analysis after the run is complete. You can transfer the experiment results in either of the following two ways:

Download the experiment from the QuantStudio™ 12K Flex Instrument over the network

1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. Select the instrument icon of the QuantStudio™ 12K Flex Instrument you just used to run the experiment from the My Instruments list.
3. Click **Manage Instrument** to open the Instrument Manager.
4. In the Instrument Manager, click **Manage Files**.
5. In the Experiments panel, select the experiment to download. Click **Download**.
6. In the Save dialog box, select the folder to hold the experiment results and click **Save**. The experiments folder is located at:
   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where, <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Transfer the experiment from the QuantStudio™ 12K Flex Instrument to the computer via a USB drive

1. If not already connected to the instrument, connect a USB drive to the USB port.



2. Touch the **QuantStudio™ 12K Flex Instrument Touchscreen** to activate it.
3. If the touchscreen is not at the Main Menu screen, 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 your 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:

   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

6 Analyze the Experiment Results

Section 6.1 Analyze the run data

This section includes general information and instructions on how to analyze the example experiments provided on the QuantStudio™ 12K Flex Software installation CD. For specific instructions see page 67.

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 need to close the run and re-open the *.eds file to display the Amplification Plot screen (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** check box. By default, Auto Analysis is always enabled. To reanalyze the data, select all the wells in the plate layout, then click **Analyze**.

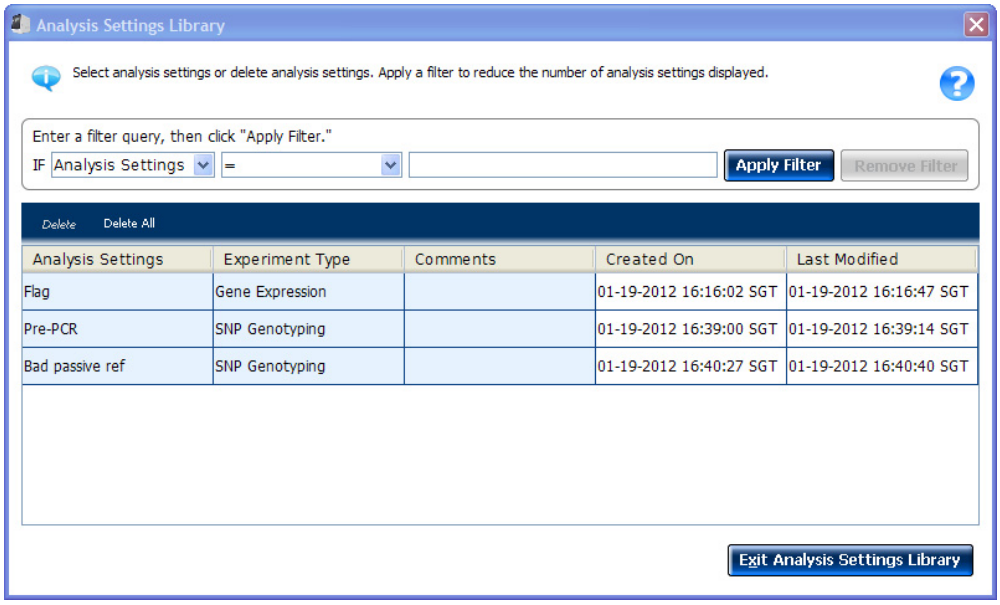
About the Analysis Settings Library

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.

In the Analysis Settings Library dialog box you can apply a filter to reduce the number of setting protocols displayed.

You can access the Analysis Settings Library from the Tools menu.



To change the analysis settings and to save them to the Analysis Settings Library:

1. From the Experiment Menu pane, select **Analysis**.
2. On the Analysis screen, click **Analysis Settings** to open the Analysis Settings dialog box.
3. Change the analysis settings according to your requirement.
4. Click **Save to Library** to save the changes you have made to the Analysis Settings Library.

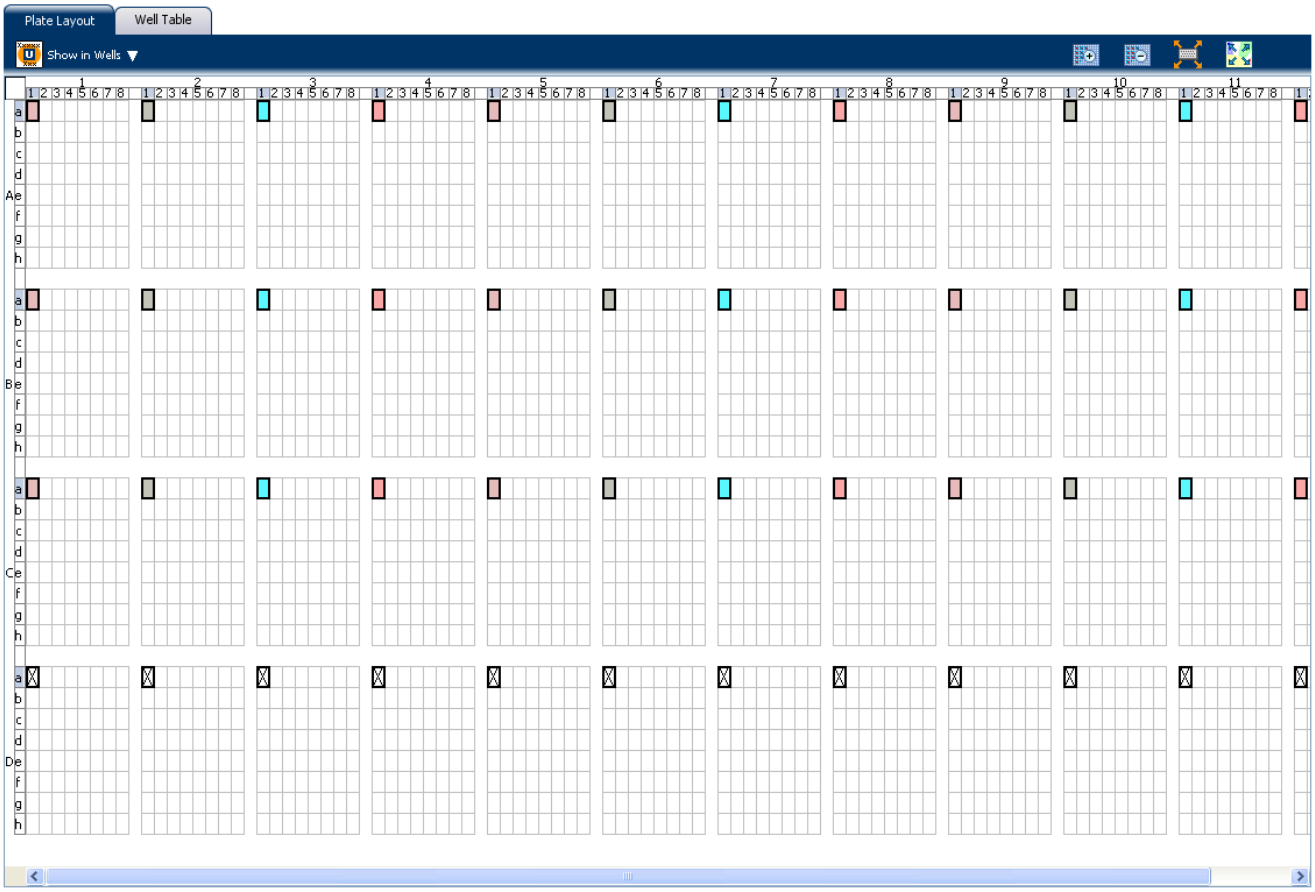
You can import the analysis settings you have previously saved to the Analysis Settings Library, by clicking **Load from Library** in the Analysis Settings dialog box.

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: Select **Sample Color** or **Target Color** for Gene Expression and miRNA experiments. For Genotyping experiments, select **Sample Color** or **Assay Color.**
- To select a single well, click the well in the plate layout.
- To select multiple wells, click and drag over the desired wells, press **Ctrl-click**, or press **Shift-click** in the plate layout.
- To select all the wells, click the upper left corner of the plate layout.

Plate layout 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) to open the Plot Properties dialog box

2. 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

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

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

(Optional) Export an experiment

About exporting 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

Note: You can also export plate images collected during the run as *.tif files and use them for troubleshooting purposes as needed. To export plate images, first create an export folder on your hard drive. In the Export screen, click **Browse** and navigate to the folder 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/**). Also, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689) for more information on QC Images.

Export procedure

Note: If you choose the Auto Export option before running an experiment, the data is automatically exported to the location you specified. If you did not set the Auto Export option, the analyzed data is not exported automatically.

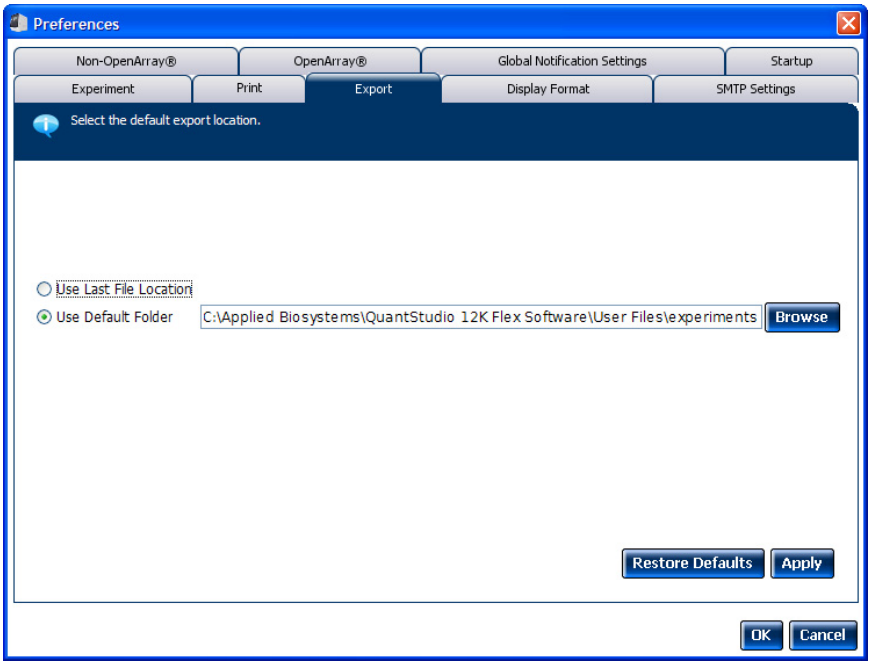
1. Open the experiment file that contains the data to export, and from the Experiment Menu, click  **Export.**
2. Select the format for exported data:
   - **QuantStudio 12k Flex format** (supports .txt and .xlsx data).
   - **RDML format** - Real Time Data Markup Language (supports only .xml type of 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. You can select the export file type (*.xls, *.xlsx or *.txt) to export from the **File Type** drop-down menu.

     Note: You cannot use an exported ***.xls** or an ***.xlsx** file when importing plate setup information.
4. Select **Yes** or **No** to include or exclude bookmarked data, from analysis, in the export set.

   The Filter Bookmark Data feature allows you to include only the data bookmarked during analysis in the export set.
5. *(Optional)* Select the **Open file(s) when export is complet**e check box to automatically open the file when export is complete.
6. Enter a file name and location.
   a. Enter a name for the export file in the **Export File Name** field.

b. Enter the **Export File Location**. 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**, and select the **Export** tab. You can select the **Use Last File Location** or **Use Default Folder** check box.



7. Select the data to export:

Select...	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

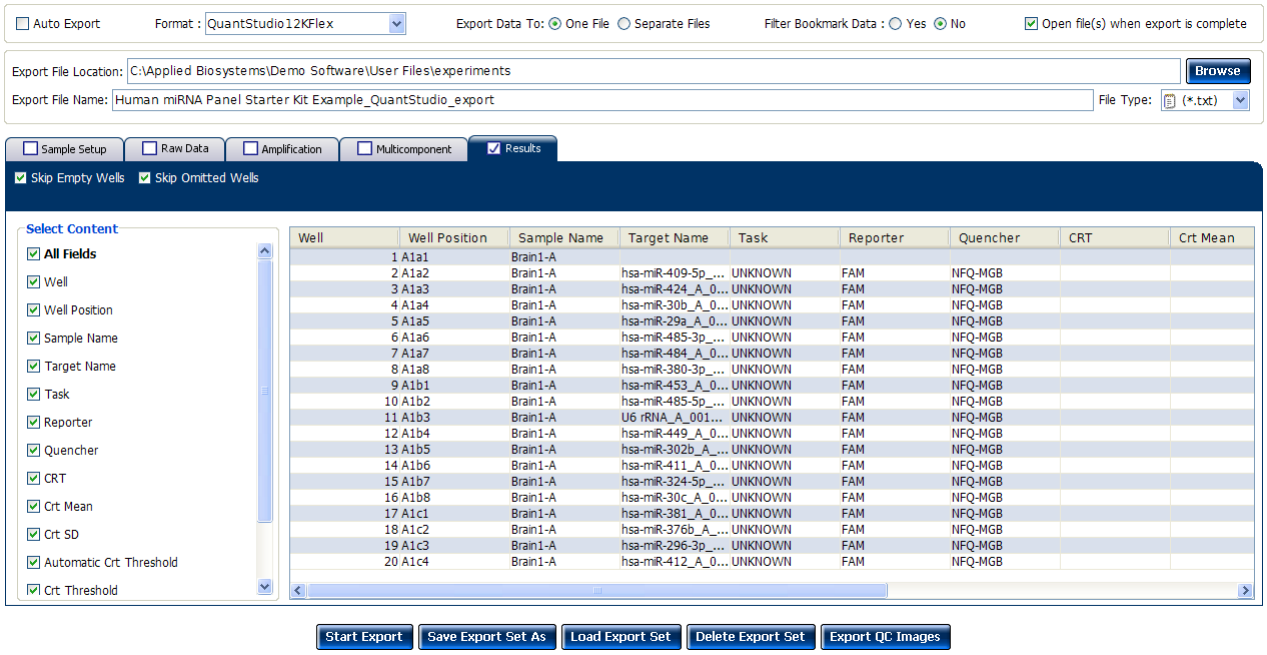
Note: Results data are not available for export until the run status is complete and the data are analyzed.

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.

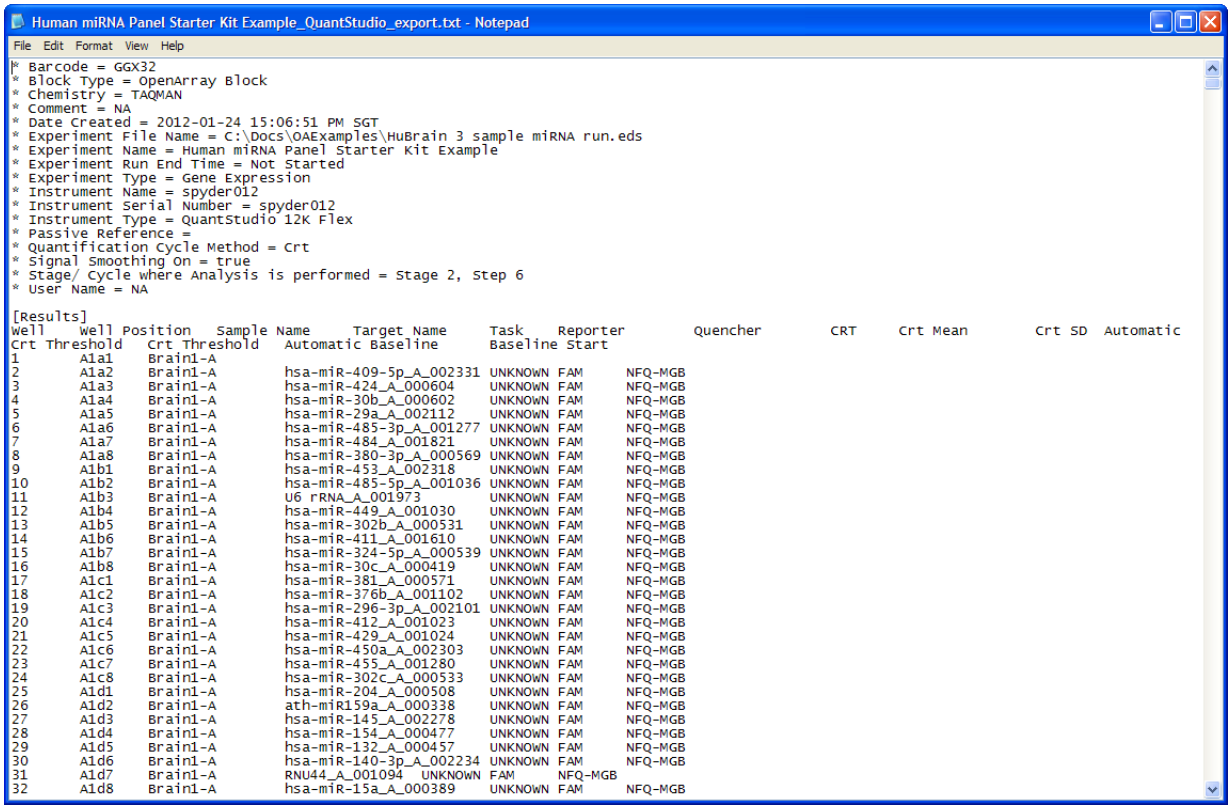
10. Click **Start Export**.

The Export screen for a microRNA experiment is shown in the following graphic:



Well	Well Position	Sample Name	Target Name	Task	Reporter	Quencher	CRT	Crt Mean
1	A1a1	Brain1-A						
2	A1a2	Brain1-A	hsa-miR-409-5p_...	UNKNOWN	FAM	NFQ-MGB		
3	A1a3	Brain1-A	hsa-miR-424_A_0...	UNKNOWN	FAM	NFQ-MGB		
4	A1a4	Brain1-A	hsa-miR-30b_A_0...	UNKNOWN	FAM	NFQ-MGB		
5	A1a5	Brain1-A	hsa-miR-29a_A_0...	UNKNOWN	FAM	NFQ-MGB		
6	A1a6	Brain1-A	hsa-miR-485-3p_...	UNKNOWN	FAM	NFQ-MGB		
7	A1a7	Brain1-A	hsa-miR-484_A_0...	UNKNOWN	FAM	NFQ-MGB		
8	A1a8	Brain1-A	hsa-miR-380-3p_...	UNKNOWN	FAM	NFQ-MGB		
9	A1b1	Brain1-A	hsa-miR-453_A_0...	UNKNOWN	FAM	NFQ-MGB		
10	A1b2	Brain1-A	hsa-miR-485-5p_...	UNKNOWN	FAM	NFQ-MGB		
11	A1b3	Brain1-A	U6 rRNA_A_001...	UNKNOWN	FAM	NFQ-MGB		
12	A1b4	Brain1-A	hsa-miR-449_A_0...	UNKNOWN	FAM	NFQ-MGB		
13	A1b5	Brain1-A	hsa-miR-302b_A_...	UNKNOWN	FAM	NFQ-MGB		
14	A1b6	Brain1-A	hsa-miR-411_A_0...	UNKNOWN	FAM	NFQ-MGB		
15	A1b7	Brain1-A	hsa-miR-324-5p_...	UNKNOWN	FAM	NFQ-MGB		
16	A1b8	Brain1-A	hsa-miR-30c_A_0...	UNKNOWN	FAM	NFQ-MGB		
17	A1c1	Brain1-A	hsa-miR-381_A_0...	UNKNOWN	FAM	NFQ-MGB		
18	A1c2	Brain1-A	hsa-miR-376b_A_...	UNKNOWN	FAM	NFQ-MGB		
19	A1c3	Brain1-A	hsa-miR-296-3p_...	UNKNOWN	FAM	NFQ-MGB		
20	A1c4	Brain1-A	hsa-miR-412_A_0...	UNKNOWN	FAM	NFQ-MGB		

The exported file when opened in Notepad appears as shown in the following graphic:




```
* Barcode = GGX32
* Block Type = OpenArray Block
* Chemistry = TAQMAN
* Comment = NA
* Date Created = 2012-01-24 15:06:51 PM SGT
* Experiment File Name = C:\Docs\OAExamples\HuBrain 3 sample miRNA run.eds
* Experiment Name = Human miRNA Panel Starter Kit Example
* Experiment Run End Time = Not Started
* 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 6
* User Name = NA

[Results]
Well    Well Position   Sample Name     Target Name     Task    Reporter        Quencher        CRT     Crt Mean        Crt SD  Automatic
Crt Threshold   Crt Threshold   Automatic Baseline      Baseline Start
1       A1a1    Brain1-A
2       A1a2    Brain1-A        hsa-miR-409-5p_A_002331 UNKNOWN FAM     NFQ-MGB
3       A1a3    Brain1-A        hsa-miR-424_A_000604    UNKNOWN FAM     NFQ-MGB
4       A1a4    Brain1-A        hsa-miR-30b_A_000602    UNKNOWN FAM     NFQ-MGB
5       A1a5    Brain1-A        hsa-miR-29a_A_002112    UNKNOWN FAM     NFQ-MGB
6       A1a6    Brain1-A        hsa-miR-485-3p_A_001277 UNKNOWN FAM     NFQ-MGB
7       A1a7    Brain1-A        hsa-miR-484_A_001821    UNKNOWN FAM     NFQ-MGB
8       A1a8    Brain1-A        hsa-miR-380-3p_A_000569 UNKNOWN FAM     NFQ-MGB
9       A1b1    Brain1-A        hsa-miR-453_A_002318    UNKNOWN FAM     NFQ-MGB
10      A1b2    Brain1-A        hsa-miR-485-5p_A_001036 UNKNOWN FAM     NFQ-MGB
11      A1b3    Brain1-A        U6 rRNA_A_001973        UNKNOWN FAM     NFQ-MGB
12      A1b4    Brain1-A        hsa-miR-449_A_001030    UNKNOWN FAM     NFQ-MGB
13      A1b5    Brain1-A        hsa-miR-302b_A_000531   UNKNOWN FAM     NFQ-MGB
14      A1b6    Brain1-A        hsa-miR-411_A_001610    UNKNOWN FAM     NFQ-MGB
15      A1b7    Brain1-A        hsa-miR-324-5p_A_000539 UNKNOWN FAM     NFQ-MGB
16      A1b8    Brain1-A        hsa-miR-30c_A_000419    UNKNOWN FAM     NFQ-MGB
17      A1c1    Brain1-A        hsa-miR-381_A_000571    UNKNOWN FAM     NFQ-MGB
18      A1c2    Brain1-A        hsa-miR-376b_A_001102   UNKNOWN FAM     NFQ-MGB
19      A1c3    Brain1-A        hsa-miR-296-3p_A_002101 UNKNOWN FAM     NFQ-MGB
20      A1c4    Brain1-A        hsa-miR-412_A_001023    UNKNOWN FAM     NFQ-MGB
21      A1c5    Brain1-A        hsa-miR-429_A_001024    UNKNOWN FAM     NFQ-MGB
22      A1c6    Brain1-A        hsa-miR-450a_A_002303   UNKNOWN FAM     NFQ-MGB
23      A1c7    Brain1-A        hsa-miR-455_A_001280    UNKNOWN FAM     NFQ-MGB
24      A1c8    Brain1-A        hsa-miR-302c_A_000533   UNKNOWN FAM     NFQ-MGB
25      A1d1    Brain1-A        hsa-miR-204_A_000508    UNKNOWN FAM     NFQ-MGB
26      A1d2    Brain1-A        ath-miR159a_A_000338    UNKNOWN FAM     NFQ-MGB
27      A1d3    Brain1-A        hsa-miR-145_A_002278    UNKNOWN FAM     NFQ-MGB
28      A1d4    Brain1-A        hsa-miR-154_A_000477    UNKNOWN FAM     NFQ-MGB
29      A1d5    Brain1-A        hsa-miR-132_A_000457    UNKNOWN FAM     NFQ-MGB
30      A1d6    Brain1-A        hsa-miR-140-3p_A_002234 UNKNOWN FAM     NFQ-MGB
31      A1d7    Brain1-A        RNU44_A_001094  UNKNOWN FAM     NFQ-MGB
32      A1d8    Brain1-A        hsa-miR-15a_A_000389    UNKNOWN FAM     NFQ-MGB
```

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.

Common features

The ExpressionSuite Software and TaqMan® Genotyper Software allow you to:

- 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 ways (plots, statistics, status codes, and so on).
- Edit data (your edits are saved to the database).
- Overlay data from multiple plates.
- Export data.

Note: For more information on the ExpressionSuite Software and the TaqMan® Genotyper Software, refer to *Applied Biosystems ExpressionSuite Software User Guide* and *Applied Biosystems TaqMan® Genotyper Software Getting Started Guide* respectively. Both the applications are available for download from the Life Technologies website. See also, Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes*.

Section 6.2 Analyzing microRNA Experiments

In this section, you use the example experiment files provided on the QuantStudio™ 12K Flex Software installation CD to analyze the experiment results.

Workflow

Analyze the experiment results

Access the example experiment file (page 67)

▼

Assess amplification result using the Amplification Plot (page 68)

▼

Identify well problems using the Well Table (page 69)

▼

Confirm accurate dye signal using the Multicomponent Plot (page 70)

▼

Determine the signal accuracy using the Raw Data Plot (page 72)

▼

View the QC Summary (page 73)

▼

(Optional) Adjust parameters for re-analysis of your own experiments (page 75)

▼

(Optional) Export the analyzed data (page 78)

Open the example experiment file

1. Double-click 

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

   <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12KFlex Software\examples\Gene Expression
3. Open the Human miRNA Panel Starter Kit Example.eds file.

Assess amplification results using the Amplification Plot

The Amplification Plot screen displays amplification of all samples in the selected wells. There are three plots 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. You can 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. You can 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. You can use this plot to locate outlying amplification (outliers).

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

Purpose

The purpose of viewing the amplification plot for the example experiment is to:

- Evaluate the quality of the amplification curve
- Check for outliers

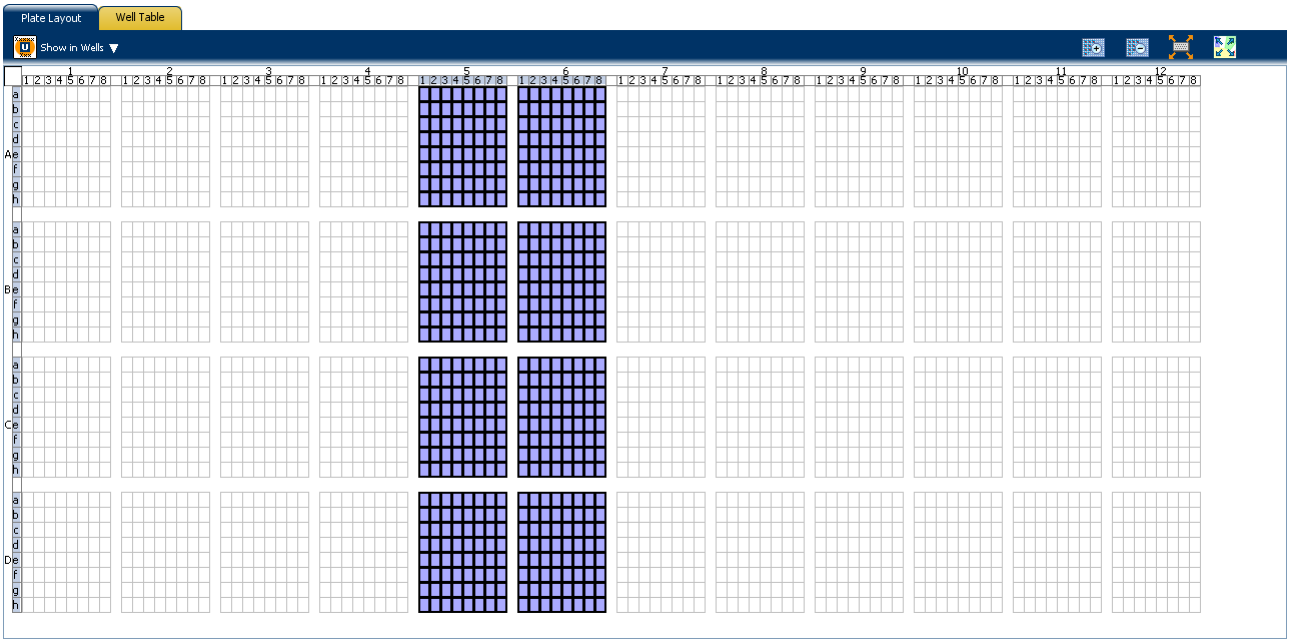
View the Amplification Plot

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

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

2. Display the Brain2-A wells in the Amplification Plot screen:
   a. Click the **Plate Layout** tab.
   b. From the Show in Wells drop-down menu, select **Sample Color**.

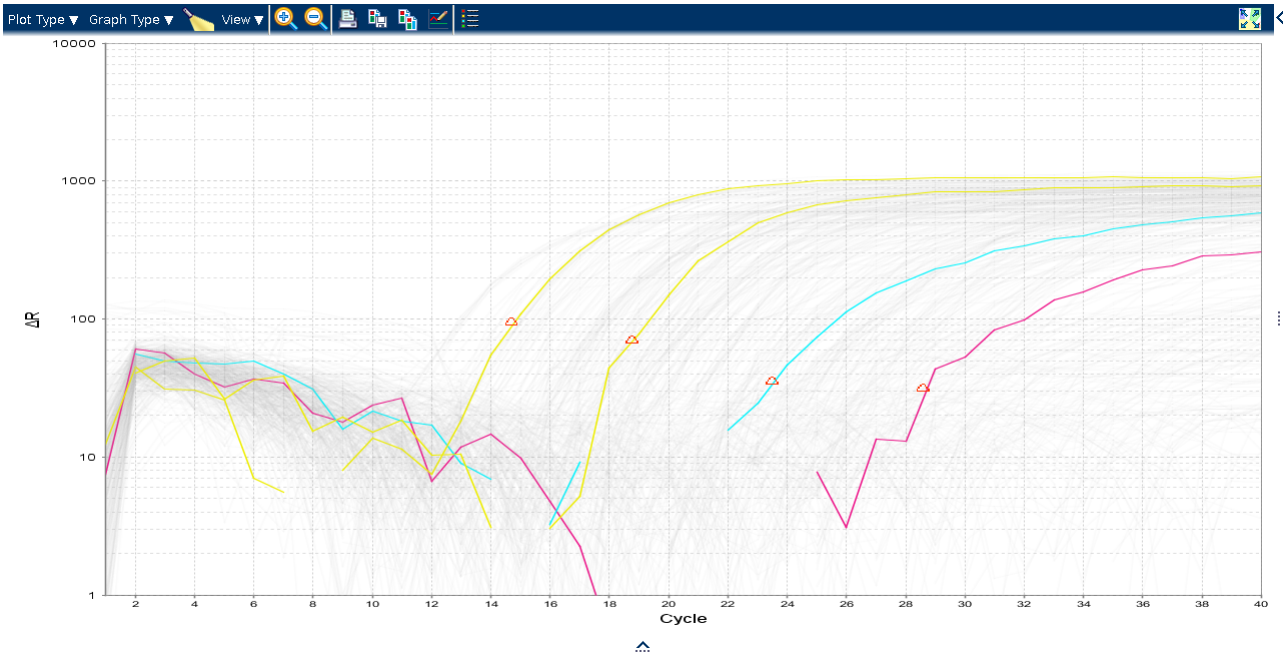
   The Plate Layout screen should look like this:



3. In the Amplification Plot screen, select:

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

4. View the C_{RT} values:
   a. From the View drop-down, **Show C_{RT}**.
   b. Verify that the C_{RT} value reported matches its occurrence (the triangle icon) on the plot.



5. Repeat steps 2 through 4 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 data for each well in the reaction plate, including:

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

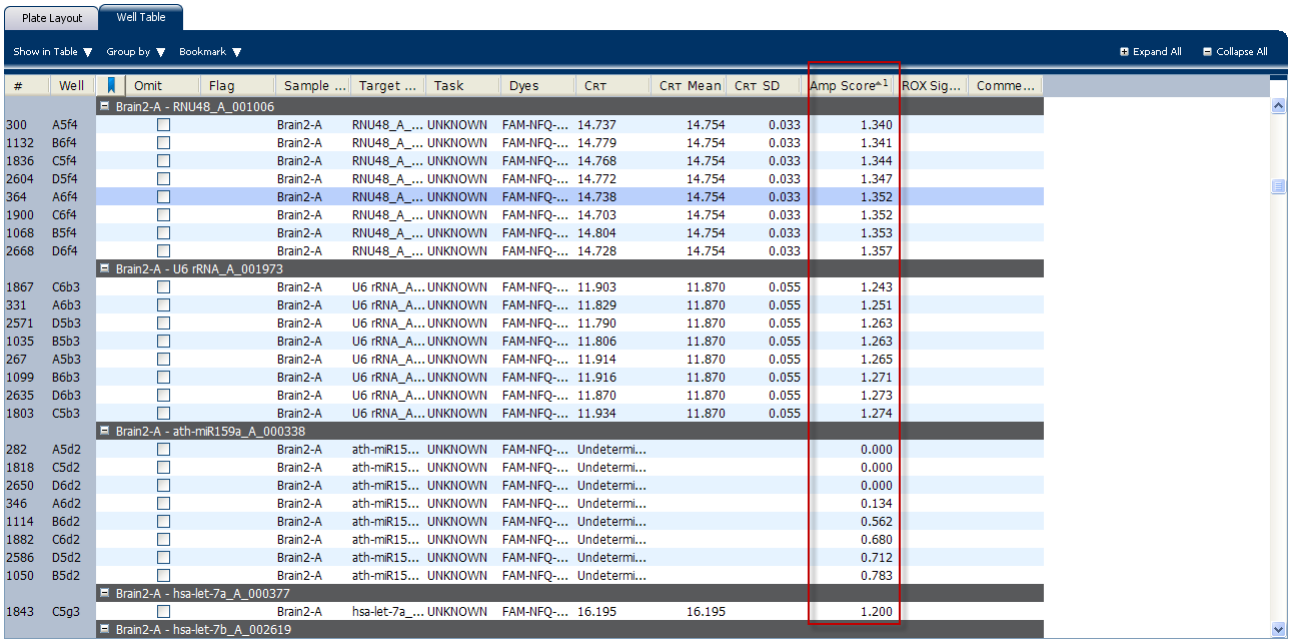
Example experiment values and flags

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

Note: The software will produce a flag, called AMPSCORE, 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 pane, select **Analysis** ▸ **Amplification Plot**, then click the **Well Table** tab.
2. From the Group By drop-down 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.



#	Well	Omit	Flag	Sample ...	Target ...	Task	Dyes	CRT	CRT Mean	CRT SD	Amp Score[1]	ROX Sig...	Comme...
Brain2-A - RNU48_A_001006													
300	A5f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.737	14.754	0.033	1.340		
1132	B6f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.779	14.754	0.033	1.341		
1836	C5f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.768	14.754	0.033	1.344		
2604	D5f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.772	14.754	0.033	1.347		
364	A6f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.738	14.754	0.033	1.352		
1900	C6f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.703	14.754	0.033	1.352		
1068	B5f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.804	14.754	0.033	1.353		
2668	D6f4	☐		Brain2-A	RNU48_A_...	UNKNOWN	FAM-NFQ-...	14.728	14.754	0.033	1.357		
Brain2-A - U6 rRNA_A_001973													
1867	C6b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.903	11.870	0.055	1.243		
331	A6b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.829	11.870	0.055	1.251		
2571	D5b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.790	11.870	0.055	1.263		
1035	B5b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.806	11.870	0.055	1.263		
267	A5b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.914	11.870	0.055	1.265		
1099	B6b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.916	11.870	0.055	1.271		
2635	D6b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.870	11.870	0.055	1.273		
1803	C5b3	☐		Brain2-A	U6 rRNA_A...	UNKNOWN	FAM-NFQ-...	11.934	11.870	0.055	1.274		
Brain2-A - ath-miR159a_A_000338													
282	A5d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.000		
1818	C5d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.000		
2650	D6d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.000		
346	A6d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.134		
1114	B6d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.562		
1882	C6d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.680		
2586	D5d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.712		
1050	B5d2	☐		Brain2-A	ath-miR15...	UNKNOWN	FAM-NFQ-...	Undetermi...			0.783		
Brain2-A - hsa-let-7a_A_000377													
1843	C5g3	☐		Brain2-A	hsa-let-7a_...	UNKNOWN	FAM-NFQ-...	16.195	16.195		1.200		
Brain2-A - hsa-let-7b_A_002619													

Note: To show or hide columns in the Well Table, select or deselect respectively the column name from the Show in Table drop-down menu.

Assessing 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 screen displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run.

Purpose

In the microRNA example experiment, you review the Multicomponent Plot screen 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 pane, select **Analysis** ▸ **Multicomponent Plot**.

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

2. Display the unknown and standard wells one at a time in the Multicomponent Plot screen:

   a. Click the **Plate Layout** tab.

   b. Select one well in the plate layout; the well is shown in the Multicomponent Plot screen.

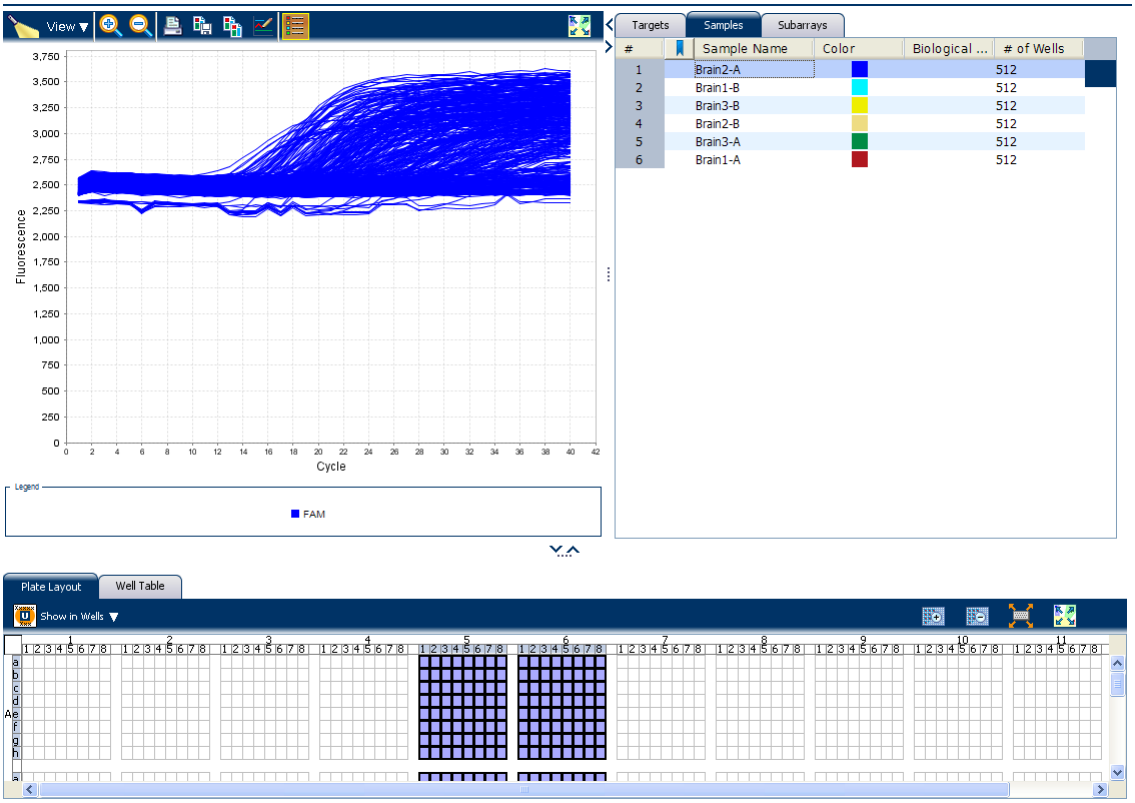
      Note: If you select multiple wells, the Multicomponent Plot screen displays the data for all selected wells simultaneously.

3. From the View drop-down menu, select **Dye Color**.

4. Click  **Show a legend for the plot** (default).

   Note: 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 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 microRNA experiment, look for:

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

About the example experiment

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 pane, select **Analysis ▸ Raw Data Plot**.

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

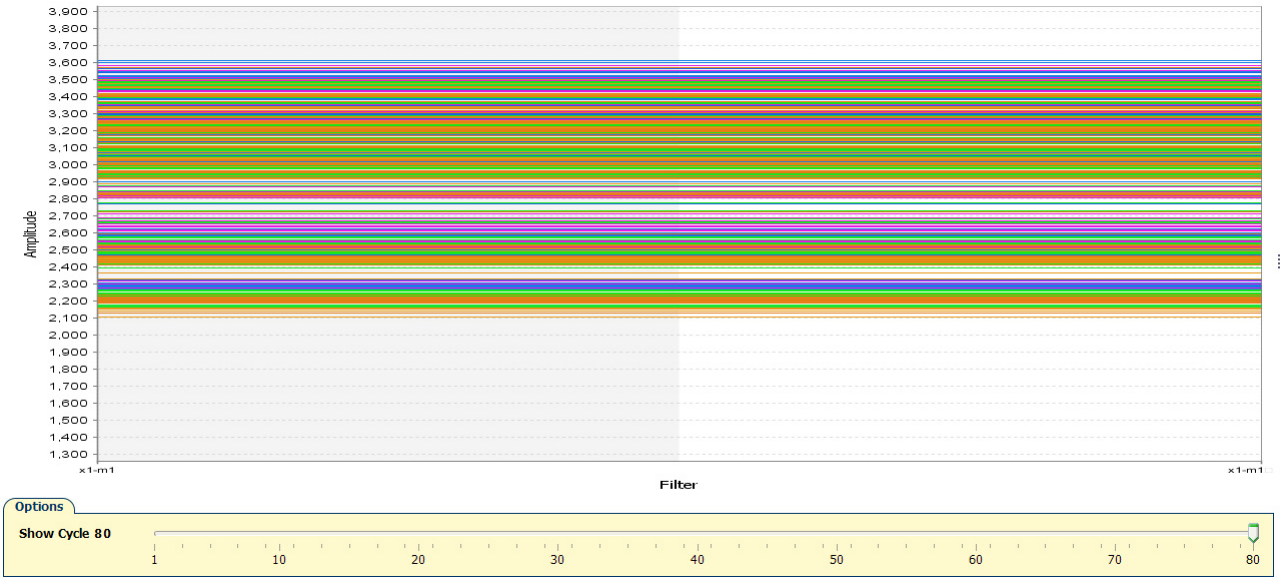
2. Display all wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the Plate Layout tab.

3. Click  **Show a legend for the plot** (default).

   Note: This is a toggle button. When the legend is displayed, the button changes to Hide the plot legend.

   Note: The legend displays the color code for each row of the reaction plate (see the legend in the Raw Data Plot shown below).

4. Click and drag the Show Cycle pointer from cycle 1 to cycle 80. In the example experiment, there is a stable increase in signal from filter 1, which 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 in each filter:

- Characteristic signal growth
- No abrupt changes or dips

Review the flags in the QC Summary

The QC Summary screen displays a list of the QuantStudio™ 12K Flex Software flags, including the flag frequency and location for the open experiment.

Review the QC Summary screen in the microRNA example experiment for any flags triggered by the experiment data. There are no flags in the example experiment.

View the QC Summary

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

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

2. Review the Flags Summary.

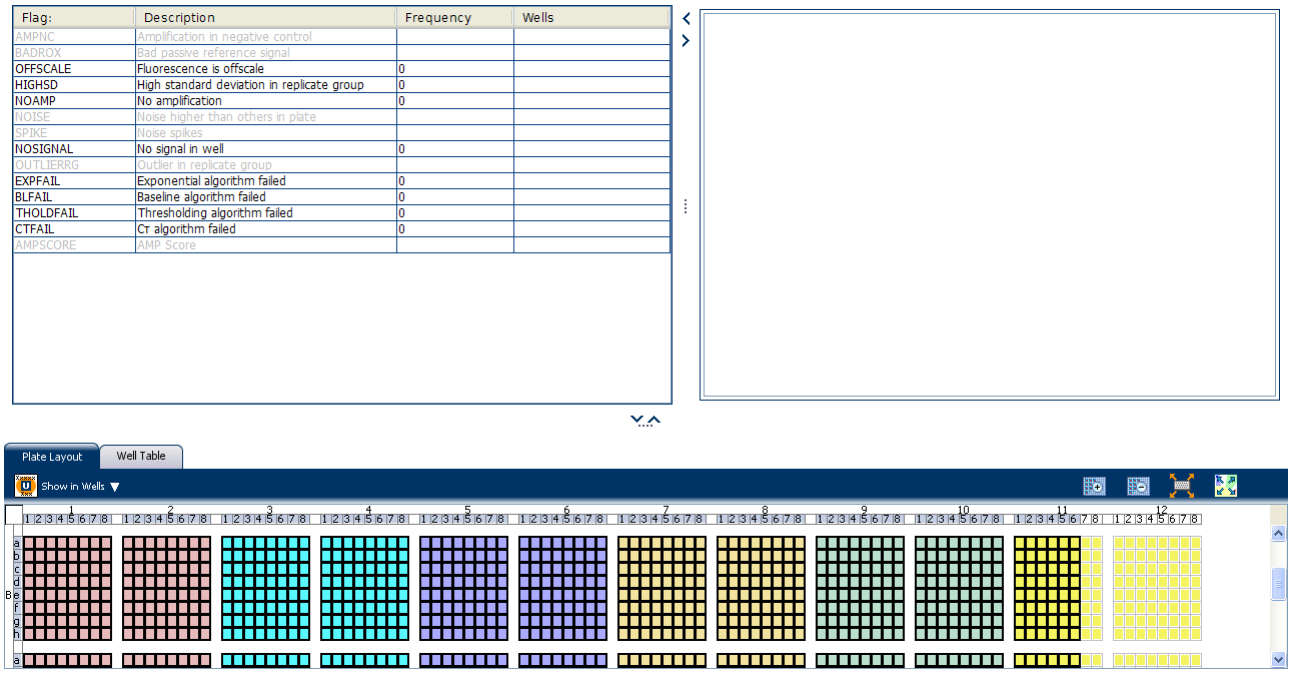
   Note: A 0 displayed in the Frequency column indicates that the flag does not appear in the experiment. If the frequency is > 0, the flag appears somewhere in the experiment; the well position is listed in the Wells column.

   In the example experiment, there are 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 on 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 experiment data.

Flag	Description
Pre-processing flag	
OFFSCALE	Fluorescence is offscale
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 certain threshold, corresponding to the score set in the analysis settings
Secondary analysis flags	
OUTLIERRG	Outlier in replicate group
AMPNC	Amplification in the negative control

Flag	Description
HIGHSD	High standard deviation in replicate group

Note: The flags AMPNC, BADROX, NOISE, SPIKE, OUTLIERRG, and AMPSCORE are, by default, not in use for the Gene Expression experiment.

Note: 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).

Adjust parameters for re-analysis of your own experiments

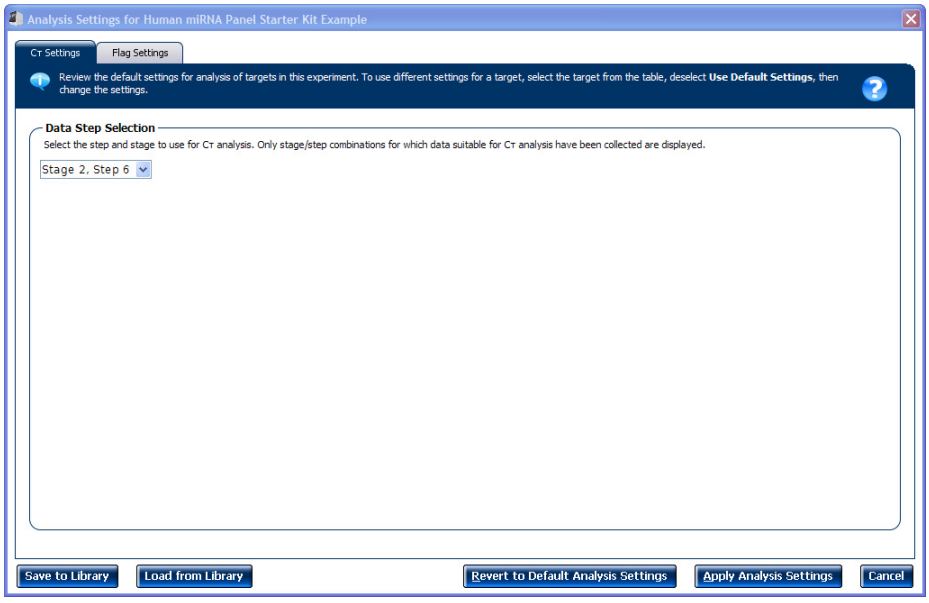
The Analysis Settings dialog box displays the analysis settings for the threshold cycle (C_{RT}), and flags options.

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 pane, 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" below).

   Note: You can save the changes to the analysis settings to the Analysis Settings Library for later use. For more information, see "About the Analysis Settings Library" on page 59.

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

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

Adjust analysis settings

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**.**

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.

To adjust the flag settings

1. In the Use column, select the check boxes 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.

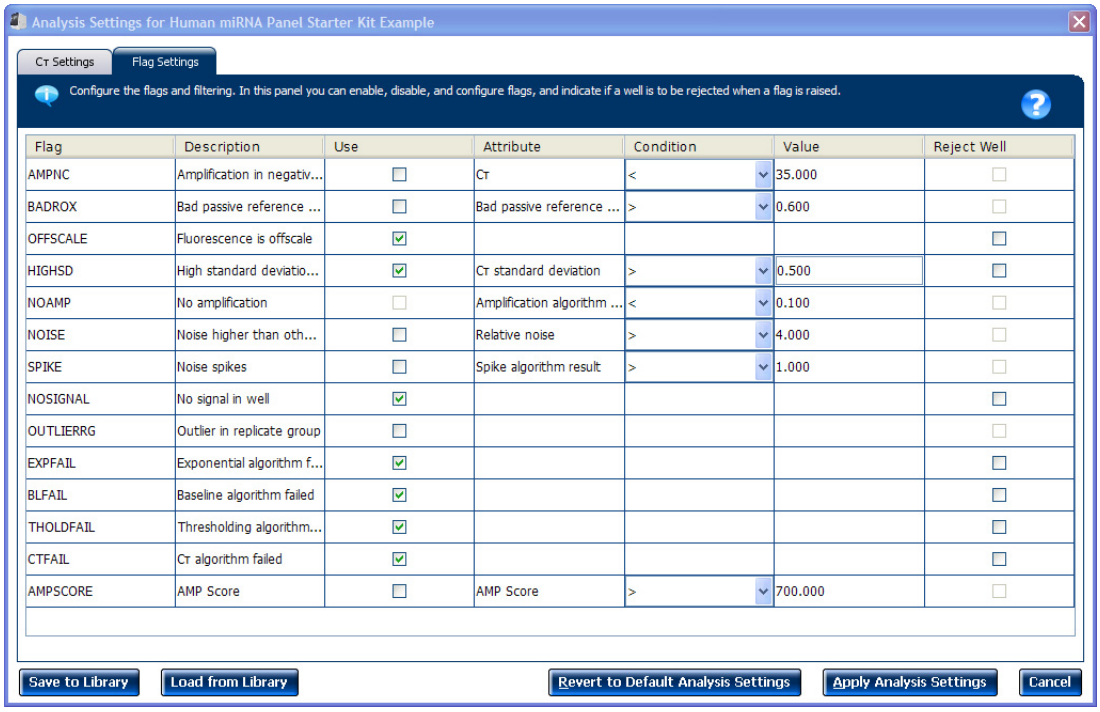
   Note: If you choose to adjust the setting for applying a flag, make minor adjustments as you evaluate the appropriate setting.

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

   Note: 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** in the Analysis Settings dialog box. 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 ensure C_{RT} precision, omit the outliers from the analysis.

In the OpenArray Gene Expression example experiment, there are 0 outliers. To remove these wells from analysis.

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

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

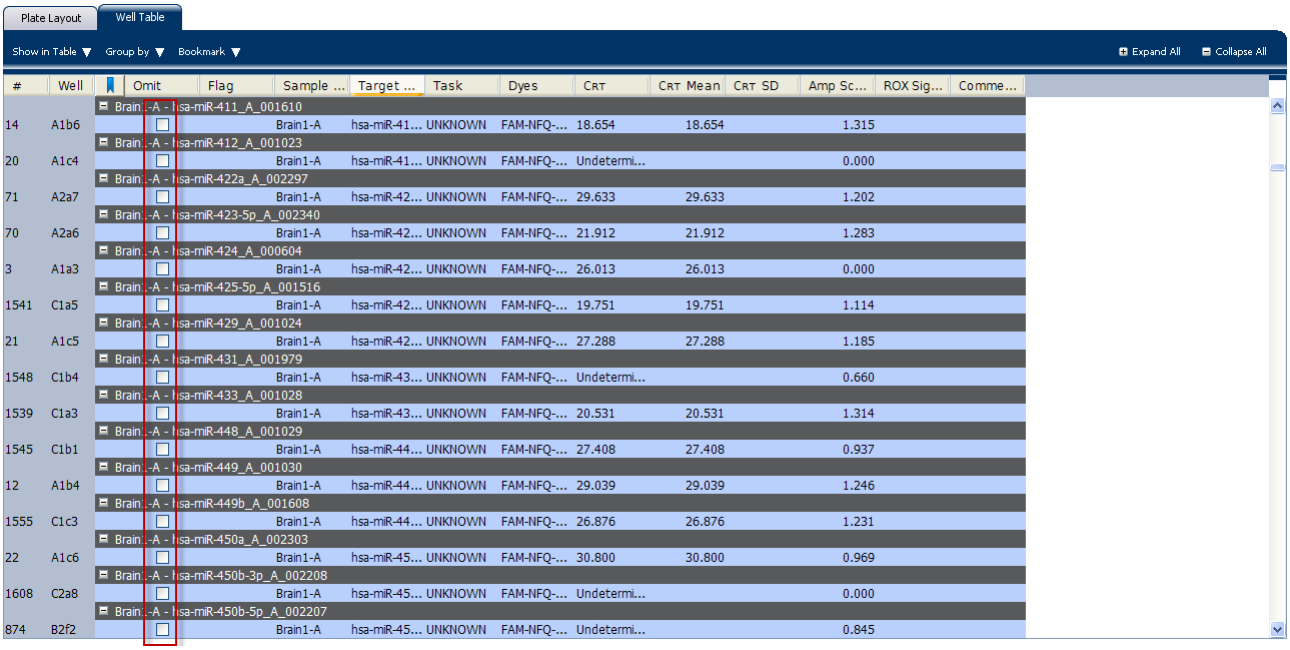
2. In the Amplification Plot screen, select **C_{RT} vs Well** from the Plot Type drop-down menu.

3. Select the **Well Table** tab.

4. In the Well Table, identify outliers:

   a. From the Group By drop-down menu, select **Replicate**.

b. Look for outliers in the replicate group (make sure they are flagged). Select the **Omit** check box next to outlying well(s).



5. Click **Analyze** to reanalyze the experiment data with the outlying well(s) removed from the analysis.

   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 and select **Omit**.

Export the analyzed data

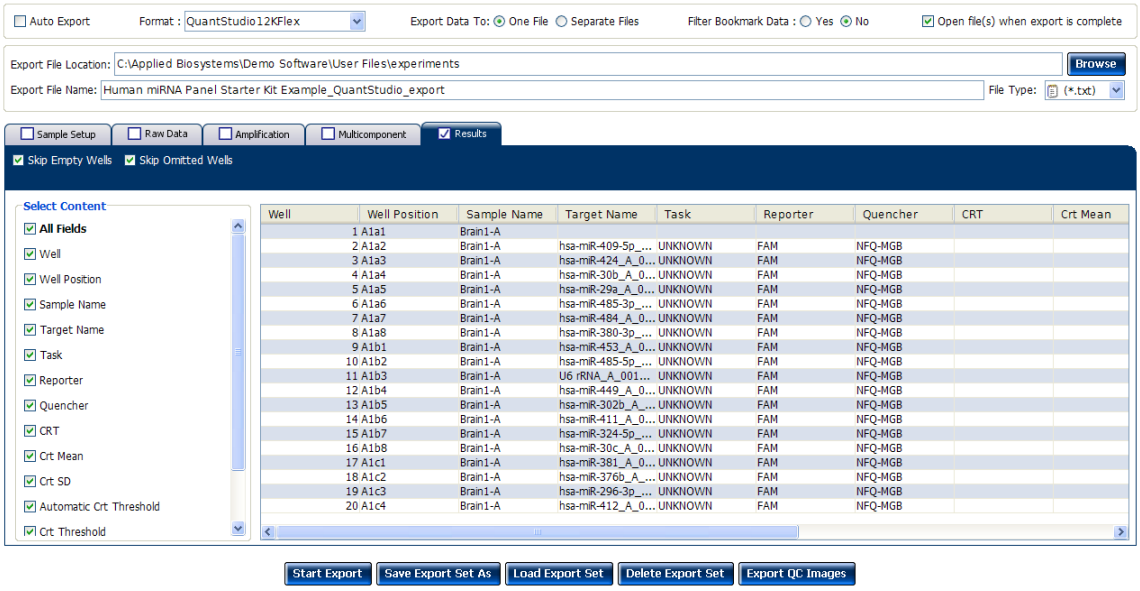
1. Open the OpenArray Gene Expression example experiment file that you analyzed in Chapter 5.
2. In the Experiment Menu, click  **Export.**

   Note: To export data automatically after analysis, select the **Auto Export** check box during experiment setup or before running the experiment. Auto export is unchecked for the example experiment.
3. Select **QuantStudio™ 12K Flex format**.
4. Complete the Export dialog box as shown below:

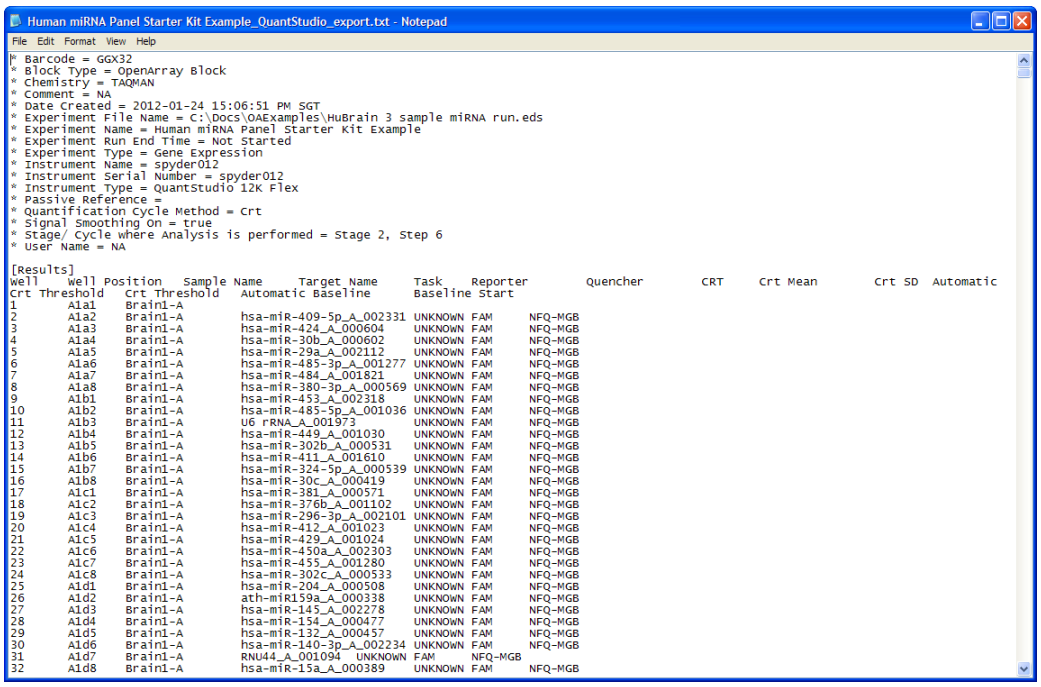
Field or Selection	Entry
Select Data to export/ Select Content	Results
Export Data To	One File
Export File Name	Human miRNA Panel Starter Kit Example_QuantStudio_export

Field or Selection	Entry
Filter Bookmark Data	No
File Type	*.txt
Export File Location	<drive>:\Applied Biosystems\QuantStudio 12K Flex Software\experiments

Your Export screen should look like this:



Your exported file when opened in Notepad should look like this:



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USER GUIDE

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Booklet 4 - QuantStudio™ Digital PCR Kit

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Contents

1 Introduction

About the QuantStudio™ 12K Flex OpenArray® Digital PCR Starter Kit

The DigitalSuite™ Software QuantStudio™ 12K Flex OpenArray® Digital PCR Starter Kit:

- Contains all the materials (OpenArray® plates, reagents, and accessories) you need to perform two experiments on the QuantStudio™ 12K Flex System, from sample preparation to data analysis unless otherwise noted in Table 1 below
- Represents a typical setup for two digital PCR experiments

Table 1 Starter kit description and contents

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Digital PCR Starter Kit – 15°C to –20°C	4469650	• 2X TaqMan® OpenArray® Digital Master Mix, 1.5 mL • 20X Primer and TaqMan® OpenArray® Digital Master Mix, 1.5 mL • QuantStudio™ TaqMan® Probe (TaqMan® Assay), 120 µL • Human Male DNA, 100 µL	Contains reagents to conduct two digital PCR experiments on the QuantStudio™ 12K Flex System.

Starter kit (2-20 components)	Part no.	Kit contents	Description
QuantStudio™ 12K Flex OpenArray® Accessories Starter Kit	4469586	• QuantStudio™12K Flex OpenArray® Lids (6 lids) • QuantStudio™12K Flex OpenArray® Plugs (6 plugs) • QuantStudio™12K Flex OpenArray® Carriers (1 or 2 carriers) • QuantStudio™12K Flex OpenArray® Immersion Fluid and tips (6 syringes) • OpenArray® AccuFill™ System Tips (1 box of 384 tips) • OpenArray® 384-Well Sample Plates (10 plates) • QuantStudio™12K Flex OpenArray® 384-Well Plate Seals (10 seals)	Contains accessories to assemble QuantStudio™12K Flex TaqMan® OpenArray® plates for a single experiment starter kit. Each experiment starter kit contains this accessories starter kit. This kit does not contain samples.

Table 2 QuantStudio™ Digital PCR kit description and contents

QuantStudio™ Digital PCR kit (10 or 4 pack)	Part no.	Kit contents	Description
QuantStudio™ Digital PCR Kit - 10 pack	4470184	• 2X TaqMan® OpenArray® Digital PCR Master Mix, 5 mL • QuantStudio™ Digital PCR Plates (10 plates) • QuantStudio™ OpenArray® Real-Time PCR Accessories Kit (Part no. 4469589)	Contains reagents to run up to 30,720 reactions on the QuantStudio™ 12K Flex System.
QuantStudio™ Digital PCR Kit - 4 pack	4470185	• 2X TaqMan® OpenArray® Digital PCR Master Mix, 1.5 mL • QuantStudio™ Digital PCR Plates (4 plates)	Contains reagents to up to 12,288 digital PCR reactions on the QuantStudio™ 12K Flex System.

About Digital PCR experiments

The QuantStudio™ 12K Flex Real-Time PCR System can be used to perform and analyze digital PCR experiments for accurate and sensitive quantitation of nucleic acid targets. The following components are required to perform digital PCR on the OpenArray® System:

- **QuantStudio™ 12K Flex Real-Time PCR System** – Instrumentation used to load and thermal cycle QuantStudio™ digital PCR Plates for quantitative detection of targets using digital PCR analysis (see the following section).
- **QuantStudio™ Digital PCR Plates** – OpenArray® Plates used to load and contain the digital PCR reactions for thermal cycling and the subsequent imaging by the QuantStudio™ 12K Flex Real-Time PCR System (see page 8 for more information).

- **TaqMan® OpenArray® Digital PCR Master Mix and Assays** – Fluorescence-based polymerase chain reaction (PCR) reagents used to amplify and detect nucleic acid targets for digital PCR analysis (see page 8 for more information).
- **DigitalSuite™ Software** – Software used to calculate copy number through Poisson statistical analysis of the digital PCR data generated on the QuantStudio™ 12K Flex Real-Time PCR System (see page 9 for more information).

QuantStudio™ 12K Flex Real-Time PCR System

The QuantStudio™ 12K Flex System consists of the following components:

- **QuantStudio™ 12K Flex AccuFill™ System** – Loads your samples onto an QuantStudio™ Digital PCR Plate.
- **QuantStudio™ 12K Flex OpenArray® Plate Press 2.0** – Seals the QuantStudio™ Digital PCR Plate Cases.
- **QuantStudio™ 12K Flex Real-Time PCR System** – Performs thermal cycling and imaging of the experiment plates.
- **Computer** – Connects to the QuantStudio™ 12K Flex Real-Time PCR System.

About data collection

The QuantStudio™ 12K Flex Real-Time PCR System collects raw fluorescence data during thermal cycling (PCR amplification). A data collection point (*data point*) on the QuantStudio™ 12K Flex System consists of three phases:

1. **Excitation** – The QuantStudio™ 12K Flex Instrument illuminates all through-holes of the experiment plate, exciting the fluorophores in each reaction.
2. **Emission** – The QuantStudio™ 12K Flex Instrument optics collect the residual fluorescence emitted from the through-holes of the experiment plate. The resulting image consists only of light that corresponds to the range of emission wavelengths.
3. **Collection** – The QuantStudio™ 12K Flex Instrument assembles a digital image representation of the residual fluorescence collected over a fixed time interval, then stores the raw fluorescence image for analysis.

After a run, the QuantStudio™ 12K Flex Software uses regions of interest (ROI), optical, dye, and background calibration data to determine the location and intensity of the fluorescence signals in each read, the dye associated with each fluorescence signal, and the significance of the signal.

QuantStudio™ OpenArray® Plates

The QuantStudio™ 12K Flex System requires two plate types:

- QuantStudio™ OpenArray® 384-Well Sample Plate (*sample plate*)
- QuantStudio™ Digital PCR Plate (*experiment plate*)

OpenArray® 384-Well Sample Plate

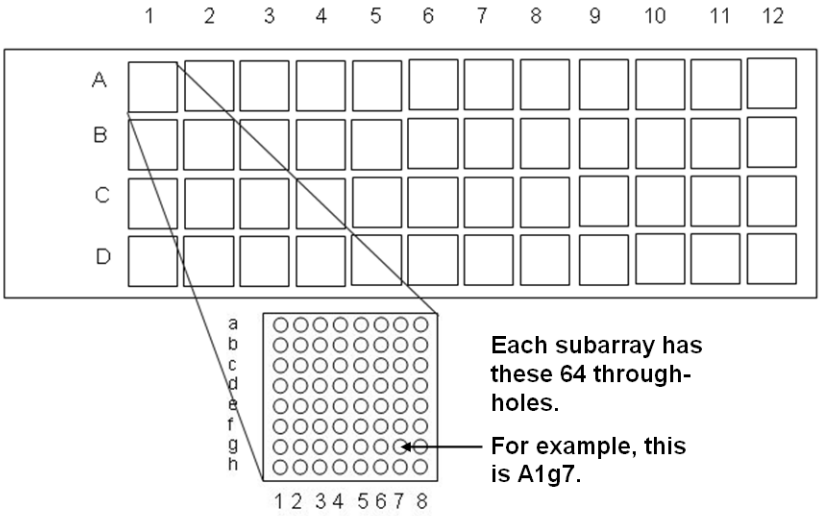
The OpenArray® 384-Well Sample Plate is a 384-well reaction plate. You combine the TaqMan® OpenArray® Digital PCR Master Mix, TaqMan® assay, and your DNA sample in the sample plate, then use the QuantStudio™ 12K Flex AccuFill™ System to transfer the mixture from the sample plate to an experiment plate(s).

IMPORTANT! The well dimensions of the OpenArray® 384-Well Sample Plates are specifically suited for use with the QuantStudio™ 12K Flex AccuFill™ System. We do not recommend the use of other microtiter plates with this system.

QuantStudio™ Digital PCR Plate

The QuantStudio™ Digital PCR Plate is a 63-mm × 19-mm mid-density reaction plate. Each plate contains 3,072 reaction through-holes, each of which accommodates a 33 nL reaction volume.

As shown in the following figure, the QuantStudio™ Digital PCR Plate is divided into 48 subarrays, where each subarray consists of 64 through-holes. Hydrophilic and hydrophobic coatings allow reagents to be held within the through-holes.



Digital PCR experiments

What is a digital PCR experiment?

Digital PCR is a biochemical technique used to quantify the number of starting copies of a target nucleic acid sequence in a genomic or complementary DNA sample.

Digital PCR experiments include the following components:

- **Sample** – The genomic or complementary DNA sample that contains an unknown number of copies of the target nucleic acid sequence. In a digital PCR experiment samples are diluted down to a limiting quantity, such that on average, individual PCR reactions contain a single target molecule.

  Note: Digital PCR experiments can be performed without knowing that most wells have either zero or one target molecules, provided that some reactions within a sample group will have 0 copies.

- **TaqMan® OpenArray® Digital PCR Master Mix** – An optimized mixture of dNTP, salt, buffer, AmpliTaq® DNA Polymerase, and ROX™ dye passive reference designed for use with TaqMan® assays and the QuantStudio™ 12K Flex Real-Time PCR System.
- **TaqMan® Assay** – Includes forward and reverse primers and a specific fluorescent-dye-labeled probe for the target nucleic acid sequence.

  The probe contains:
  - A FAM™ reporter dye linked to the 5′ end of the probe.
  - A minor groove binder (MGB) at the 3′ end of the probe.

    MGBs increase the melting temperature (T_m) without increasing probe length (Afonina et al., 1997; Kutyavin et al., 1997); they also enable design of shorter, and thus more specific, probes.
  - A nonfluorescent quencher (NFQ) at the 3′ end of the probe.

    Because the quencher does not fluoresce, QuantStudio™ 12K Flex Systems can measure reporter dye contributions more accurately.
- **Technical replicates** – Through-hole reactions of each subarray that contain identical sample/assay/reaction mix combinations and volumes. Each subarray of the QuantStudio™ Digital PCR Plate contains 64 technical replicates (resulting from a single well of the 384-well sample plate). See Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes* for a complete discussion of replicates.
- ***(Optional)*** **No template controls (NTCs)** – Samples that contain water or buffer instead of template; also known as *negative controls*. NTCs should not amplify. See Appendix B in Booklet 5, *QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes* for a complete discussion of no template controls.

About digital PCR experiment setup

In a digital PCR experiment performed on an QuantStudio™ 12K Flex System, dilutions of each gDNA or cDNA sample are loaded into the wells of an OpenArray® 384-Well Sample Plate that contain TaqMan® OpenArray® Digital PCR Master Mix and TaqMan® assay. The samples are diluted down to a limiting quantity, such that most individual PCR reactions contain either zero or one target molecule.

DigitalSuite™ Software

DigitalSuite™ Software performs statistical analysis of the digital PCR experiments performed using TaqMan® assays on an QuantStudio™ 12K Flex Real-Time PCR System. The DigitalSuite™ Software can be used to calculate and output the absolute number of molecules of specific sequences in a variety of biological samples. The error of the measurement is also output as a 95% confidence interval.

Features

The DigitalSuite™ Software can:

- Organize data for a study-based analysis that can accommodate multiple OpenArrays at a time.
- Accommodate both duplex and singleplex experimental designs.
- Automatically make empty well calls (manual empty well override is also possible).
- Include or omit individual targets in a well.

- Generate results and support re-analysis using an updated dataset.
- Enable customization of digital PCR settings, flags, and Poisson calculations.
- Export or import experiment analysis settings.
- Enable viewing of amplification curve groups by target, sample-target and/or sample-target-dilution.
- Provide a histogram plot that helps in distinguishing amplifications.
- Support both, auto or manual call amplification/non-amplification/undetermined.
- Report results in copies per uL.
- Enable well-bookmarking in respective views of the data across the platform.
- Export results.
- Print, save to an image file, or export plots.
- Visualize data with tools such as Heat Maps, Scatter Plots, and Bar Plots.

Compatible instruments

The DigitalSuite™ Software can be used to analyze the results of digital PCR experiments run on the QuantStudio™ 12K Flex Real-Time PCR System that have been saved as .eds files.

Note: Data generated on the OpenArray® NT Cycler Instrument is not compatible with the DigitalSuite™ Software.

About the analysis

The DigitalSuite™ Software generates copy number data from fluorescence data collected from TaqMan® reactions that have been loaded onto a QuantStudio™ Digital PCR Plate and run on an QuantStudio™ 12K Flex Real-Time PCR System. Following thermal cycling, the raw amplification curve data are imported directly into DigitalSuite™ Software for analysis.

The DigitalSuite™ Software employs one of four methods to generate calls for all through-hole reactions, where reactions that exhibit amplification are assigned positive calls, and those without amplification are assigned negative calls. Using the call data, the DigitalSuite™ Software calculates copy number values for all samples present on the plate and generates 95% confidence intervals according to a Poisson maximum-likelihood algorithm (Fazekas de St. Groth, S, 1982).

About the QuantStudio™ Digital PCR Kit data files

When you perform the QuantStudio™ Digital PCR Kit experiment tasks in this guide, you will use example data files supplied with the QuantStudio™ 12K Flex Software and the OpenArray® Sample Tracker Software. Table 3 describes the types of files provided, as well as their file names and installation locations.

Table 3 Starter kit data files referenced in this guide

File type	Description	File name	Location†	Used in
.edt	Experiment template. Two .edt files (simplex.edt and duplex.edt) are provided for the digital PCR application to support singleplex and duplex experiments respectively.	Digital PCR Multiplex.edt and Digital PCR Singleplex.edt	*<drive>*:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates\OpenArray	Ch 3
.eds	Experiment	DuplexExample.eds and SingleplexExample.eds	*<drive>*:\Program Files\Applied Biosystems\DigitalSuite Software\examples	Ch 3

† *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software and OpenArray® Sample Tracker Software are installed. The default installation drive for both software programs is the C: drive.

Workflow

Prepare the digital PCR reactions (Chapter 2)

1. Prepare the reaction mix.
2. Load the reaction mix and samples into the OpenArray® Sample Plate.

▼

Load the QuantStudio™ Digital PCR Plate (Chapter 2)

1. Prepare for loading.
2. Place a QuantStudio™ Digital PCR Plate into the QuantStudio™ OpenArray® AccuFill™ System.
3. Load the OpenArray® AccuFill™ Tips.
4. Run the QuantStudio™ OpenArray® AccuFill™ System.
5. Seal the QuantStudio™ Digital PCR Plate.
6. Perform thermal cycling.

▼

Perform real-time imaging (Chapter 3)

1. Load the QuantStudio™ Digital PCR Plate in the QuantStudio™ 12K Flex Instrument.
2. Select either **Simplex.edt** or **Duplex.edt** file as appropriate in the QuantStudio™ 12K Flex Software.
3. Click **Run**.

▼

Analyze the data using the DigitalSuite™ Software

1. Create a study by importing the data .eds files created during the data collection phase.
2. Enter sample, target, and dilution information in Plate Setup.
3. Click **Analyze** and review results.
4. *(Optional)* View heat maps and amplification curves to QC Data.
5. Save and export the results for downstream analysis.

Note: For detailed instructions on using DigitalSuite™ Software, please refer to the *Applied Biosystems OpenArray® Digital PCR Experiments User Guide* (Part no. 4471926).

2 Prepare and Perform the Digital PCR Experiments

Overview

In this chapter, you prepare the DNA samples and digital PCR reactions for your digital PCR experiment using the QuantStudio™ Digital PCR Kit. In this chapter, you also load the reactions into the QuantStudio™ Digital PCR Plate using the QuantStudio™ OpenArray® AccuFill™ System.

Prepare the DNA samples

Life Technologies recommends the following best practices for the preparation of DNA template for use in digital PCR experiments. Because digital PCR experiment strategy and methodology can vary significantly, sample preparation and template quality must be assessed on an individual basis.

Quality of DNA

Make sure that the DNA you use for experiments:

- 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 $A_{260/230}$ and $A_{260/280}$ ratios between 1.7 and 1.9
- Is intact as visualized by gel electrophoresis
- Has not been heated above 60°C; temperatures above 60°C can cause degradation

Quantity of DNA

The quantity of sample added to a digital PCR reaction depends on the:

- Concentration of genomic or complementary DNA (gDNA or cDNA) present in each sample
- Number of copies of the target sequence present in the genome or total RNA of your samples

Quantitation methods

Before performing digital PCR experiments, consider quantifying the amount of gDNA or cDNA in each sample.

We recommend the following methods of quantitation:

- Quant-iT™ assay nucleic acid quantitation using the Qubit™ Quantitation Platform.

  or

- Use spectrophotometer to determine nucleic acid concentration

Sample dilution

Should a target be present at a sufficiently high concentration in the sample of interest, it is possible that all reaction replicates will be positive thus preventing the digital calculation of the target concentration. In this case, the sample must first be diluted prior to running the digital PCR experiment.

Determine the optimal dilution when the target is known

In a digital PCR experiment performed on an OpenArray® System, gDNA samples are diluted down to a limiting quantity, such that most individual PCR reactions contain either zero or one target molecule. The procedure for determining the optimal dilution for a sample differs depending on whether or not the target copy number per genome is known.

If the target copy number per genome of your samples is *known*, dilute the samples so that, when aliquotted to a subarray, each through-hole reaction will contain approximately 0.6 to 1.6 copies of the target sequence. For example, assuming 3.3 pg/copy of a given gene are present per genome and a 33-nL through-hole volume, the stock gDNA in a given sample would be diluted down to 60 pg/µL (0.06 ng/µL) in the final reaction to give 0.6 copies per through-hole.

Copies/hole	Copies/µL	ng/µL
0.6	18.18	0.06

How to determine the target copy number per genome

To help determine copy number per genome, collect the following information:

1. If the source or species of the gDNA is known but the genome size of the organism of interest is unknown, refer to **http://www.cbs.dtu.dk/databases/DOGS/index.html** to determine the size of the genome in question.
2. Once the size of the genome is known, determine the mass of the genome using the following formula:

   $m = (n) (1.096 \times 10^{-21}$ g/bp)

   where m is the genome mass in grams, and n is the genome size in base pairs.

The following example calculates the mass of the human genome using the Celera Genomics estimate of 3.0×10^9 bp (haploid):

$m = (3.0 \times 10^9$ bp) $(1.096 \times 10^{-21}$ g/bp)

$m = 3.3 \times 10^{-12}$ g or 3.3 pg

The example is relevant to any gene that is present at the "normal" rate of two copies per diploid genome, such as RNase P, and provides a basis to perform a digital screening experiment to determine the optimal digital range.

Determine the optimal dilution when the target is unknown

If the target copy number per genome is *unknown*, say for a locus of unknown copies per genome or RNA of unknown expression level, we recommend that you determine the optimal dilution by loading a QuantStudio™ Digital PCR Plate with a three- or four-fold dilution series of the each sample at the expected digital range. By assaying three to four data points above and below the expected digital range, you ensure that one of the data points is within the optimal digital range. Should real-time data be available for the assay and sample being used, this can guide the starting and ends point of the dilution series.

Prepare the digital PCR experiment

Preparation of digital PCR experiments involves the following steps that must be completed prior to running the QuantStudio™ Digital PCR Plates:

Choose an experiment layout

▼

Prepare reaction mix and sample plate

▼

Load the QuantStudio™ Digital PCR Plates

Choose an experiment layout

Before you perform digital PCR experiments on the QuantStudio™ 12K Flex Real-Time PCR System, you must choose an experiment layout for your QuantStudio™ Digital PCR Plates. The QuantStudio™ 12K Flex does not restrict the placement of TaqMan® Assays and samples on the QuantStudio™ Digital PCR Plates. The number and arrangement of TaqMan® assays and samples that you can load in a QuantStudio™ 12K Flex Real-Time PCR System can vary based on the experiment layout that you select. For more information on choosing a layout, see Appendix B in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*.

Required materials

Storage

Part number	Part	Storage conditions
4470197	QuantStudio™ Digital PCR Plates, 10 pack	15 to 25°C
4470196	QuantStudio™ Digital PCR Plates, 4 pack	
4458080	2X TaqMan® OpenArray® Digital PCR Master Mix, 5 mL	15 to 25°C until first use, then 2 to 8°C
4458086	2X TaqMan® OpenArray® Digital PCR Master Mix, 1.5 mL	

Store the OpenArray® and TaqMan® materials and reagents according to the labels on the packaging.

Compatible reagents

Note: Where noted, products are available from major laboratory suppliers (MLS).

Table 4 OpenArray® Sample Plate set up and loading

Product		Part number	Source
Corning® 96 Well Microplate Aluminum Sealing Tape, Nonsterile		6570	Corning Life Sciences
Finnpipette Multichannel Digital Pipettor, 5 to 50 µL		4452470	Life Technologies
OpenArray® 384-Well Sample Plates		4406947	
QuantStudio™ OpenArray® AccuFill™ System		4471021	
QuantStudio™ 12K Flex OpenArray® Plate Press 2.0		A24945	
QuantStudio™ Digital PCR Plates	4 plates	4470196	
	10 plates	4470197	
TaqMan® OpenArray® Digital PCR Master Mix, 2X	1.5 mL	4458086	
	5.0 mL	4458080	
(Optional) Fine-tip marker		—	MLS

Table 5 QuantStudio™ Digital PCR Plate sealing

Product	Part number	Source
TaqMan® OpenArray® Accessories Kit: • TaqMan® OpenArray® Case • OpenArray® Sealing Glue • OpenArray® Immersion Fluid	4453975	Life Technologies
QuantStudio™ 12K Flex OpenArray® Plate Press 2.0	A24945	
25 Slide Holder	4407056	
Ethanol	—	MLS
Razor blade	—	MLS

Table 6 General use

Product	Part number	Source
Non-Stick RNase-free Microfuge Tubes, 0.5-mL (500 tubes)	AM12350	Life Technologies
Non-Stick RNase-free Microfuge Tubes, 1.5-mL (250 tubes)	AM12450	
Non-Stick RNase-free Microfuge Tubes, 2.0-mL (250 tubes)	AM12475	

Product	Part number	Source
Centrifuge with plate adaptor	—	MLS
Disposable transfer pipettes	—	
Forceps	—	
Gloves, powder-free, nitrile	—	
Laboratory-grade wipes	—	
Lint-free wipes	—	
Pipette tips, 10 to 100 µL	—	
Pipettes, P10 to P1000	—	
Plastic bins (3), medium to large[1]	—	
TE Buffer, 1X Molecular Biology Grade	—	
Vortexer	—	
Water, DNase-free, sterile-filtered	—	
(Optional) Filtered 100% compressed nitrogen gas or residue-free compressed air canister[2]	—	

1 For washing the tip blocks and plate holders.
2 For drying the plate holder, tip blocks, and plate guides.

Prepare reaction mix and sample plate

For the following hazard, see the complete safety alert description in Appendix D in Booklet 5, *QuantStudio™ 12K Flex System* OpenArray® *Experiments - Appendixes*.

CAUTION! CHEMICAL HAZARD. TaqMan® OpenArray® Digital PCR Master Mix (2×) and TaqMan® Assay

1. Remove the following from the freezer and allow them to thaw at room temperature:
   - TaqMan® OpenArray® Digital PCR Master Mix
   - TaqMan® Assay(s)
2. Vortex, then centrifuge the DNA samples for 1 minute at 1,000 rpm.
3. Review the concentration of your DNA samples, and prepare a dilution if deemed necessary:

Material	Volume (µL)
Stock gDNA (10 ng/µL)	7.5
TE Buffer, 0.1×	492.5
Total	**500**

   See "Quantity of DNA" on page 14 for information on the recommended starting concentration for gDNA and cDNA samples.
4. Gently invert the tube of TaqMan® OpenArray® Digital PCR Master Mix 10 times.

5. Transfer the master mix, TaqMan® assay, and DNA samples to the OpenArray® 384-Well Sample Plate.

IMPORTANT! The component amounts vary, depending your experiment layout.

Note: When preparing master mix for multiple QuantStudio™ Digital PCR Plates, adjust the volumes accordingly.

Material	Volume (µL)		Stock	Final
	Subarray	Plate[1]		
TaqMan® OpenArray® Digital PCR Master Mix, 2×	2.5	156	2×	1×
TaqMan® Assay, 20× (primer/probe mix)	0.25	15.6	20×	1×
Diluted DNA	2	124.8	0.15 ng/µL	0.06 ng/µL
Water	0.25	15.6	—	—
Total volume	**5.0**	**312**	**—**	**—**

1 Per OpenArray® Digital PCR Plate; volumes include 30% excess for volume loss from pipetting.

6. Mix well by gently pipetting up and down.
7. Cover the OpenArray® Sample Plate with sealing tape.
8. Centrifuge the OpenArray® Sample Plate for 1 minute at 1,000 rpm to eliminate bubbles from the wells.

IMPORTANT! For optimal results, we recommend that you load QuantStudio™ Digital PCR Plates within an hour of setting up the OpenArray® Sample Plates.

Load the QuantStudio™ Digital PCR Plates

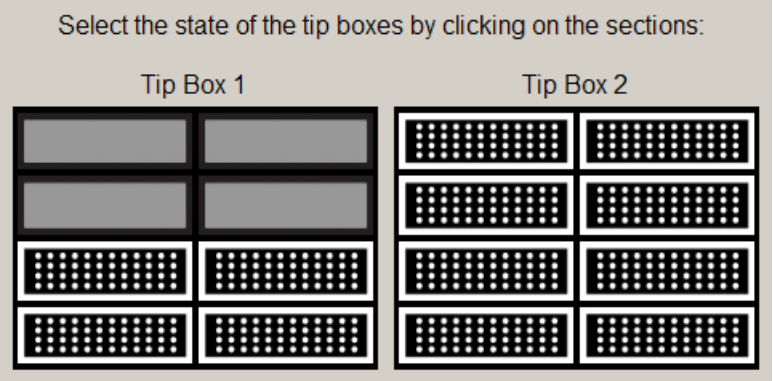
Use the QuantStudio™ OpenArray® AccuFill™ System to transfer the reactions from the OpenArray® 384-Well Sample Plate to an QuantStudio™ Digital PCR Plate.

1. Open the enclosure door of the QuantStudio™ OpenArray® AccuFill™ System by grasping the door handle and lifting the door up.
2. Insert the OpenArray® 384-Well Sample Plate with the foil cover still in place. Press on the plate until you hear it snap into place.

   Note: Do not remove the foil from the OpenArray® 384-Well Sample Plate at this time.

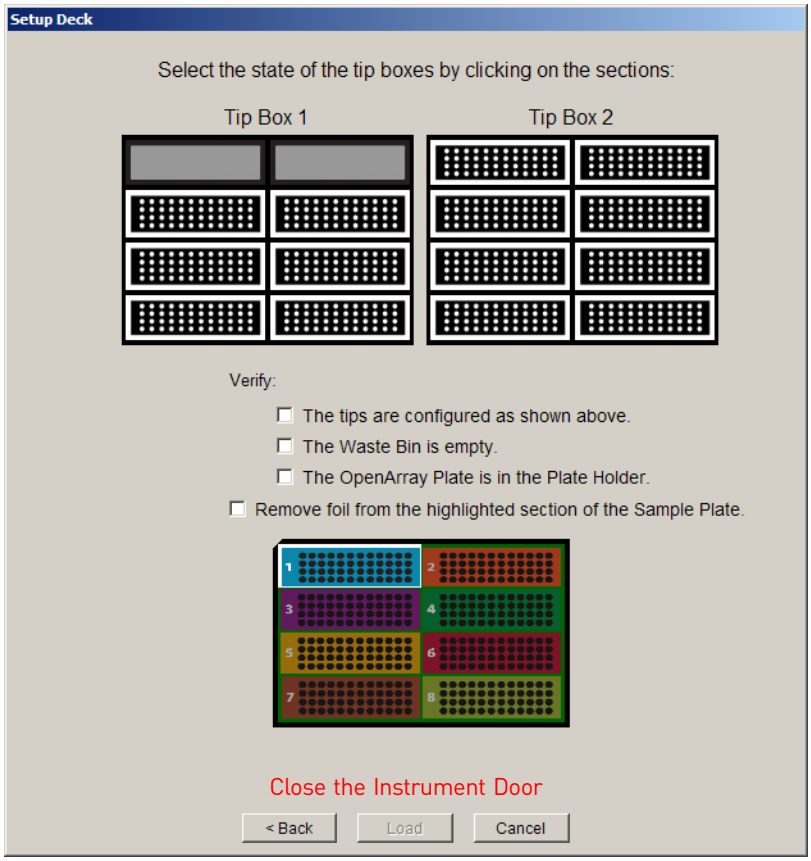
3. Place a thawed QuantStudio™ Digital PCR Plate plate into the Plate Holder. When handling the QuantStudio™ Digital PCR Plate plate:
   - Always hold the OpenArray® case by the edges and place it into the Plate Holder with the barcode face up and to the left.
   - If you inadvertently drop a loaded QuantStudio™ Digital PCR Plate plate, discard it in the sharps waste container.
   - Be sure to load the QuantStudio™ Digital PCR Plate plate within an hour after you open it.
4. Visually verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that no tips are present.
   - White areas indicate that tips are present.

   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window. For example:



Note: Cover the tip box when not in use. Discard any unused tips after 1 year or after the expiration date printed on the cardboard box.

5. Verify each of the following conditions and, when verified, select its check box:
   - Tips are configured as shown in step 4 above.
   - Waste bin is empty.
   - OpenArray® plate is in the Plate Holder.

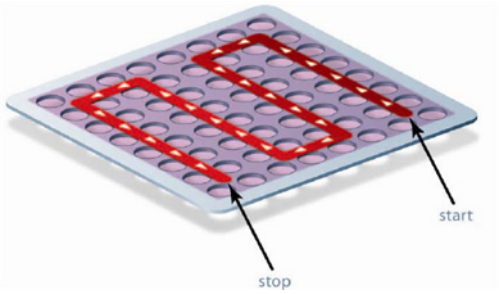


Note: The software will not continue until you select all the check boxes.

6. With forceps, peel off the foil covering the area of the OpenArray® 384-Well Sample Plate containing the samples to be loaded on the QuantStudio™ Digital PCR Plate plate.
7. Select **Remove foil from the highlighted section of the Sample Plate**.
8. Close the instrument door.
9. Click **Load**.

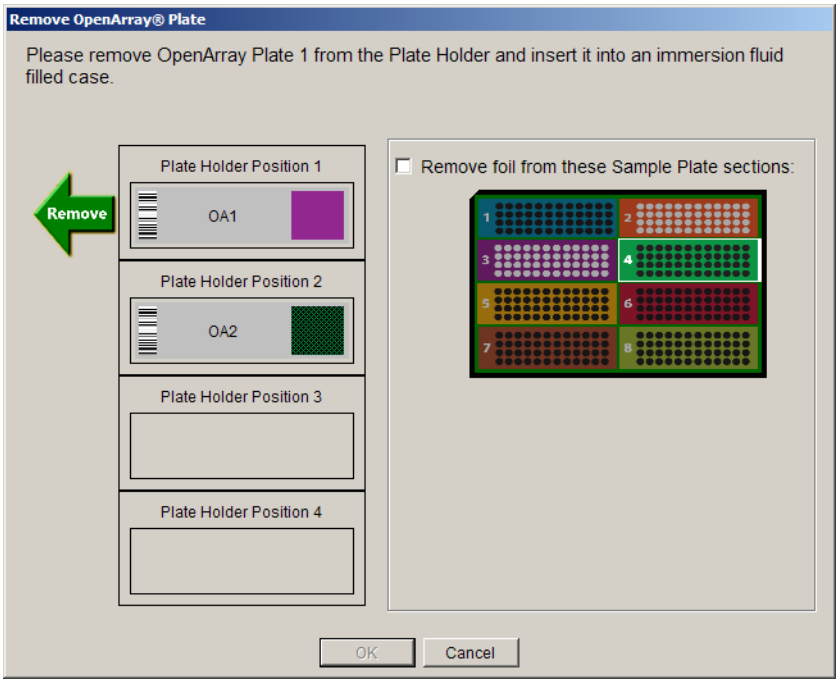
   Note: If the number of OpenArray® Plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra QuantStudio™ Digital PCR Plates. Correct the error and continue.

You can follow the progress of the loading on the screen. The samples in each tip are loaded in the QuantStudio™ Digital PCR Plate. Each tip fills the 64 through-holes in one subarray, travelling in the pattern shown (the following illustration shows the load path for only one sample):



10. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated QuantStudio™ Digital PCR Plate, then immediately seal the plate as explained in "Seal the QuantStudio™ Digital PCR Plate" on page 23.

IMPORTANT! Once an QuantStudio™ Digital PCR Plate has been filled, you must seal it within 90 seconds to prevent excessive evaporation.



11. Close the instrument door.

Note: After you load the plate, clean the QuantStudio™ OpenArray® AccuFill™ System according to the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System *Maintenance and Administration Guide* (Part no. 4470689).

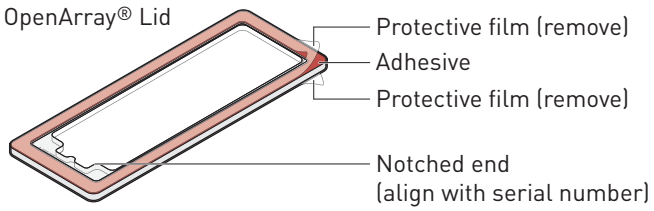
Next step

Proceed immediately to "Seal the QuantStudio™ Digital PCR Plate" below.

Seal the QuantStudio™ Digital PCR Plate

1. Remove the protective film from the top *and* bottom of an OpenArray® Case Lid.

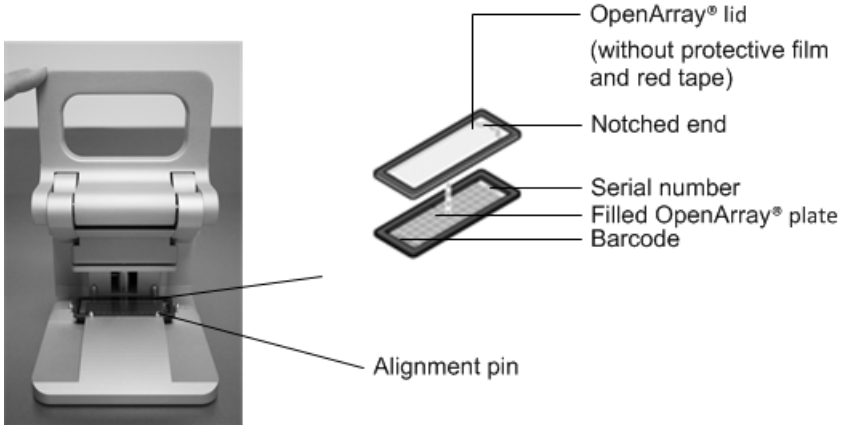
IMPORTANT! The protective film at the bottom of the Case Lid is covered by a red tape that needs to be removed first to access the protective film. Make sure to remove the protective film from *both* sides of the lids.



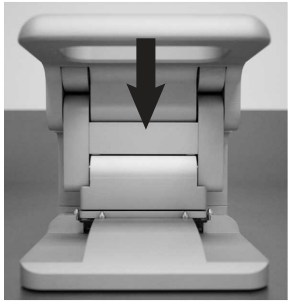
2. Using the thumb and index finger, grasp the OpenArray® case by the top (nearest the barcode), gently lift the case from the plate holder, then load it into the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

3. Place the Case Lid with red tape and protective film removed (both top and bottom) onto the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the lid must be oriented toward the right side of the Plate Press.



4. Actuate the QuantStudio™ 12K Flex OpenArray® Plate Press 2.0 by pulling down the lever.



5. The status light flashes green for 20 seconds. After 20 seconds, the status light turns solid green indicating that the case is ready.

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

6. Release the lever.

7. Load the OpenArray® case with OpenArray® Immersion Fluid:

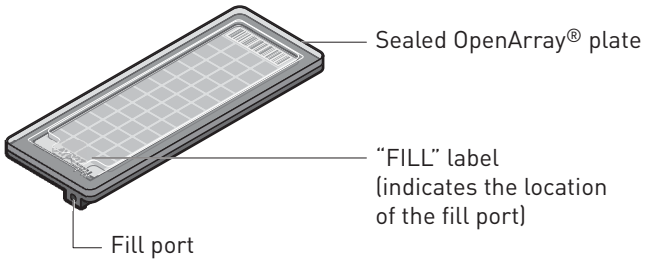
   IMPORTANT! Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 seconds.

   a. Remove the sealed plate from the Plate Press, grasping the case on the edges.

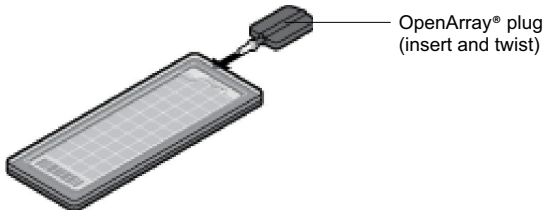
   b. Insert the syringe tip into the loading port at end of the sealed Case, then dispense the fluid completely in one gentle continuous motion.

      IMPORTANT! Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through-holes.

      Note: Try to minimize creating air bubbles when you dispense the fluid: one small air bubble is acceptable.



   c. While holding the OpenArray® plate vertically, seal the loading port by inserting the OpenArray® Plug into the port and twisting the plug clockwise, applying sufficient pressure until the handle breaks off.



   d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; be sure to not apply pressure on the OpenArray® plate within the case.

The sealed OpenArray® plate can be loaded into the QuantStudio™ 12K Flex System.

Note: Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.

STOPPING POINT For digital PCR experiments, you can store loaded and sealed OpenArray® plates at room temperature, protected from light, for up to 1 hour.

Next step

Proceed to Chapter 3, "Perform the Instrument Run" on page 27.

Guidelines for high-throughput loading

For optimal efficiency during and after loading large numbers (>6) of OpenArray® plates, follow the guidelines below.

- To help avoid mistakes when entering sample information in the QuantStudio™ OpenArray® AccuFill™ Software, load the OpenArray® plates in alphanumeric order (per the OpenArray® plate serial number).
- Seal each OpenArray® plate immediately after loading is completed, while 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 an vertical position.

- Use the QuantStudio™ Carrier to transport up to four loaded OpenArray® plates to the QuantStudio™ 12K Flex Real-Time PCR System.
- After loading is complete, you can use a large bin to properly dispose of any used OpenArray® AccuFill™ System Loader Tips. For cleaning procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

3 Perform the Instrument Run

Overview

In this chapter, you run the QuantStudio™ Digital PCR plates on the QuantStudio™ 12K Flex Real-Time PCR System. During the run, the QuantStudio™ system performs thermal cycling (if the experiment includes amplification) and collects fluorescence data. The workflow is the same for all of the TaqMan® OpenArray® plates, and is provided below.

Workflow

Perform the instrument run
Prepare the QuantStudio™ 12K Flex Software (page 29)
▼
Load the TaqMan® OpenArray® plate into the instrument (page 31)
▼
Run the plates (page 31)
▼
(Optional) Monitor the run (page 32)
▼
Unload the instrument (page 35)
▼
Transfer the experiments results to the computer (page 36)

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 OpenArray® experiments.

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

To select OpenArray® experiment preferences:

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. Go to **Tools** ▸ **Preferences** in the QuantStudio™ 12K Flex Software and select the **OpenArray®** tab.
3. Select the following as needed:

Settings	Description
Experiment Folder field	Defines the absolute path to the default folder/directory to which the QuantStudio™ 12K Flex Software reads/writes experiment files. The Open and Save dialog boxes open to the data folder when invoked from the QuantStudio™ 12K Flex Software.
Passive Reference drop-down list	Defines the dye to use as the passive reference. **Note:** While the QuantStudio™ 12K Flex Software requires a selection, a passive reference dye is not used to normalize fluorescence signals collected during OpenArray® experiments.
Default Browse File Type drop-down list	Defines the file type which the Import, Open, and Save dialog boxes select by default when invoked from the QuantStudio™ 12K Flex Software.
Apply experiment template (EDT) to all OpenArray® experiment check box	If selected, the QuantStudio™ 12K Flex Software applies the Run Method defined in the selected experiment template (*.edt) to all OpenArray® experiments. For more information on OpenArray® experiment templates, see the QuantStudio™ 12K Flex Software *Help*.

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

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

Access the Instrument Console

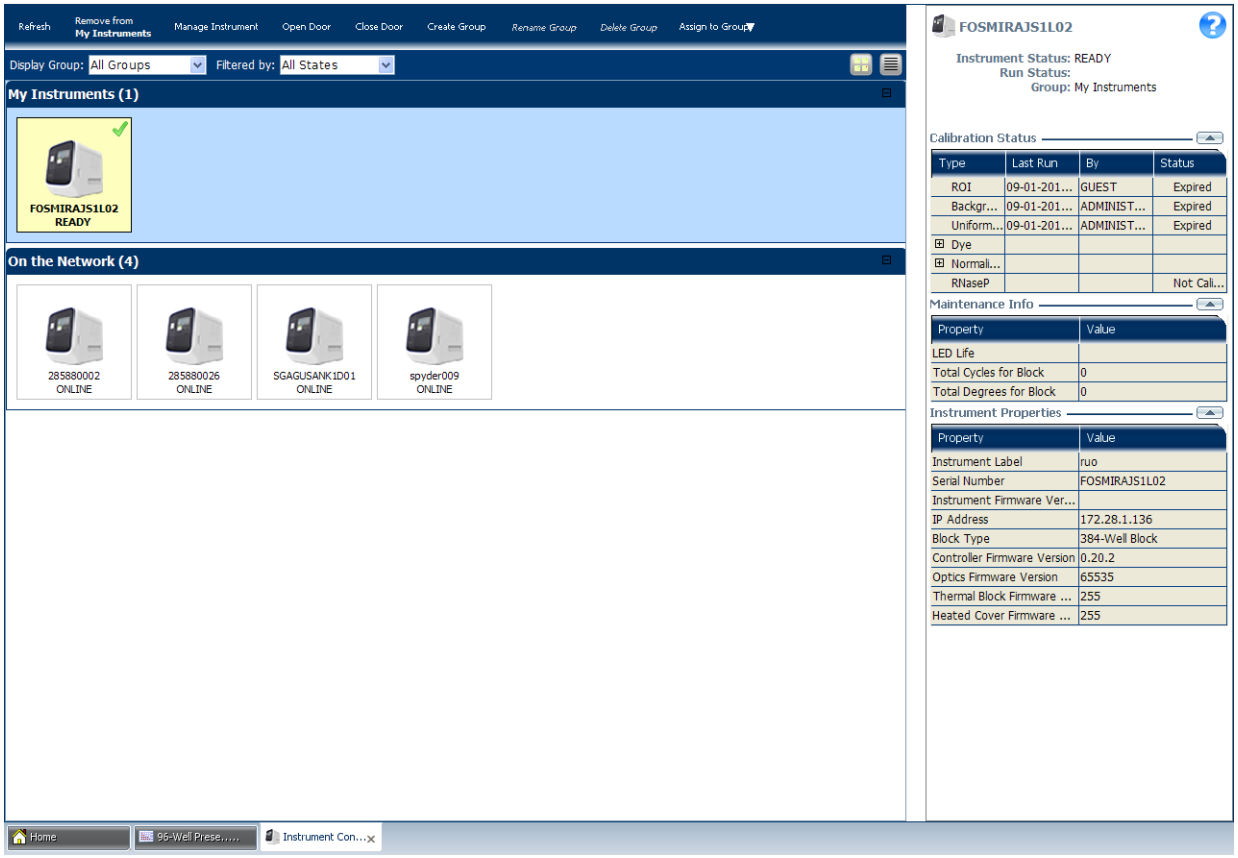
The Instrument Console displays all the QuantStudio™ 12K Flex Instruments discovered on a network, divided into groups. A group is a way to organize your instruments. By default, there are two groups:

- **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 QuantStudio™ 12K Flex Software.
2. On the Home tab (), 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 which can be monitored:
   a. Click the instrument of interest, then click **Assign to Group** in the Instrument Console toolbar.
   b. Select the **My Instruments** 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 Instrument begins and completes a run, or if an error occurs during a run.

Note: For details on using the Notification Settings feature, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

Load the QuantStudio™ Digital PCR Plate into the instrument

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100°C. Do not touch the sample block until it reaches room temperature.

IMPORTANT! Wear powder-free gloves when you handle OpenArray® plates.

IMPORTANT! QuantStudio™ Digital PCR Plates should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to allow the plate adapter to come out from the instrument side.
2. Place the OpenArray® plate(s) on the plate adapter. Make sure that:
   - Each plate is properly aligned in the adapter.
   - The plate barcode is facing up and toward the front of the instrument.
3. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Close Door** in the Instrument Console screen of the QuantStudio™ 12K Flex Software to retract the plate adapter back into the instrument.

Run the QuantStudio™ Digital PCR Plates

Overview

You can run OpenArray® plates from the QuantStudio™ 12K Flex Software as shown below.

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

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

- For the starter kit experiments:
  - "Using a template file" (*.edt, see below)
- For your own experiments:
  - *(Optional)* "Using a template file" (*.edt, see below)
  - Using the Batch Experiment Setup Utility (see the QuantStudio™ 12K Flex Software *Help*; click or press **F1**)

Using 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(s) before starting the run, or after the run is complete.

1. Double-click (QuantStudio 12K Flex Software shortcut) to start the QuantStudio™ 12K Flex Software.
2. On the Home tab, select **Create From Template**.
3. Navigate to and select the template file (*.edt) 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 <*drive*>:\Program Files\Applied Biosystems\QuantStudio 12K Flex Software\templates, where <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.
4. In the Experiment Properties screen, scan the OpenArray® plate barcode or type the OpenArray® plate serial number.
5. Select **File** ▸ **Save As...**, enter a file name, select a location for the experiment file (*.eds), then click **Save**.
6. Click **Start 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 "From the QuantStudio™ 12K Flex Software Run screen" on page 33).
- From the QuantStudio™ 12K Flex Instrument Touchscreen, in the same way that you run the experiment (see "From the QuantStudio™ 12K Flex Instrument Touchscreen" on page 34).
- From the Instrument Console of the QuantStudio™ 12K Flex Software (to monitor an experiment started from another computer or from the QuantStudio™ 12K Flex Instrument Touchscreen) as described in "From the QuantStudio™ 12K Flex Software Instrument Console" on page 33.

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.

From the QuantStudio™ 12K Flex Software Run screen

Click **Amplification Plot** from the Run Experiment Menu to monitor the amplification plot of the experiment you are running.

From the QuantStudio™ 12K Flex Software Instrument Console

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

   Note: You must add the instrument to a group which can be monitored before you can manage it (see "Access the Instrument Console" on page 29).
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 page 33) available from the QuantStudio™ 12K Flex Software for potential problems.

To...	Action
Stop the run	• In the QuantStudio™ 12K Flex Software, click **STOP RUN**. • In the Stop Run dialog, 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.
View amplification data in real time	Select **Amplification Plot**. See "To monitor the Amplification Plot" below.

To 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 each experiment's Amplification Plot.

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

The Amplification Plot screen 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).

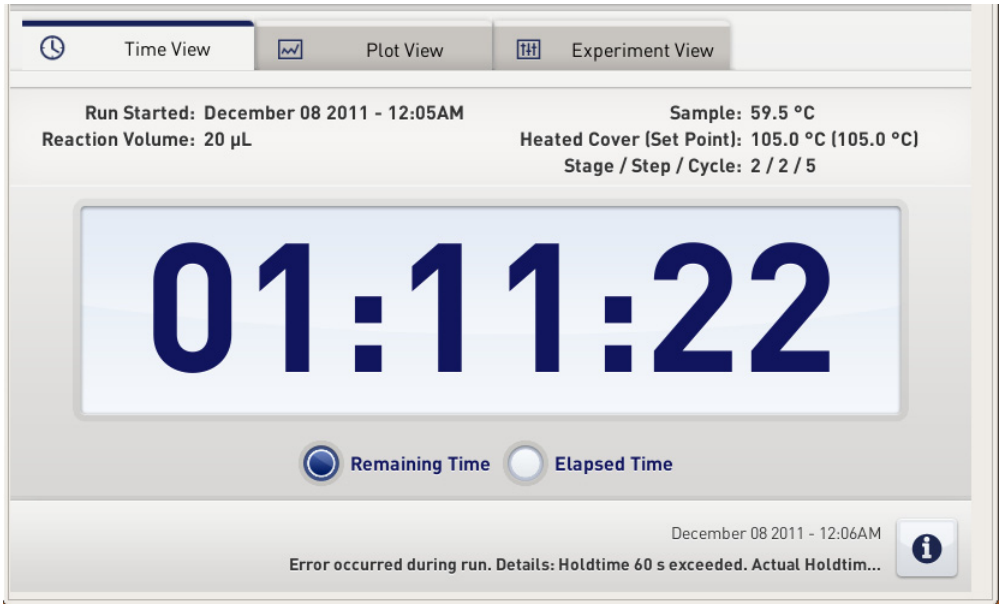
  Note: If you notice abnormal amplification or a complete absence of signal, troubleshoot the error as explained in the QuantStudio™ 12K Flex Software *Help* (click or press **F1**).

From the QuantStudio™ 12K Flex Instrument Touchscreen

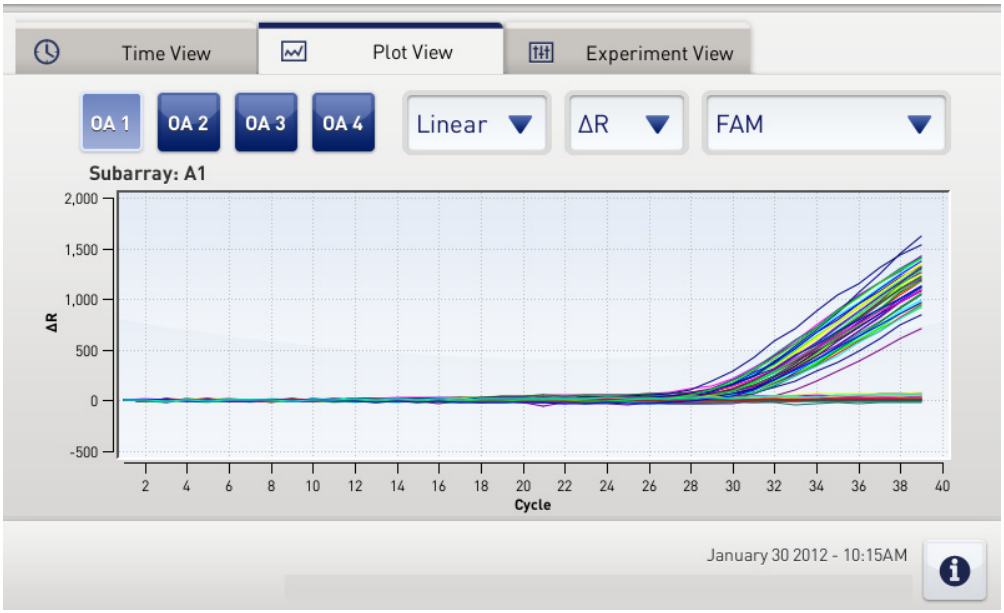
The QuantStudio™ 12K Flex Instrument Touchscreen displays the barcodes (or Plate IDs) of the TaqMan® OpenArray® plates for the run, the date and time at which the run started, the time remaining in the run, and other information.

To...	Action
Display the experiment names in the run	Touch **Experiment View**.
Show the Amplification Plot for the run	Touch the **Plot View**, then touch **Experiment View** to return to the previous screen.
Display the time elapsed and the time remaining in the run	Touch the **Time View tab**, then touch **Experiment View** tab to return to the previous screen.
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 present below the Plot View tab.

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

Unload the QuantStudio™ Digital PCR 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 re-open the *.eds file to display the Amplification Plot screen. See "Analyze the results using the DigitalSuite™ Software" on page 37.
- QuantStudio™ 12K Flex Instrument Touchscreen, see "(Optional) Transfer experiment results" on page 36.

Unload the instrument

When the QuantStudio™ 12K Flex Instrument Touchscreen displays the Home screen, unload the OpenArray® plate from the instrument.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the temperature of the sample block can exceed 100°C. Do not touch the sample block until it reaches room temperature.

1. Touch on the QuantStudio™ 12K Flex Instrument Touchscreen or click **Open Door** in the Instrument Console screen 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 QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate as follows:

   a. Power off the QuantStudio™ 12K Flex Instrument.
   b. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate.
   c. If the instrument does not eject the plate, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door.
   d. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex 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 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:

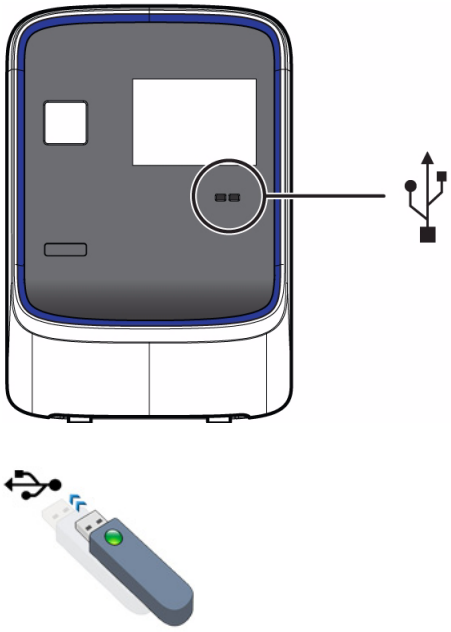
Download the experiment from the QuantStudio™ 12K Flex Instrument over the network

1. In the QuantStudio™ 12K Flex Software, select **Instrument** ▸ **Instrument Console**.
2. Select the instrument icon of the QuantStudio™ 12K Flex Instrument you just used to run the experiment from the My Instruments list.
3. Click **Manage Instrument** to open the Instrument Manager.
4. In the Instrument Manager, click **Manage Files**.
5. In the Experiments panel, select the experiment to download. Click **Download**.
6. In the Save dialog box, select the folder to hold the experiment results and click **Save**. The experiments folder is located at:

   <*drive*>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where, <*drive*> is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Transfer the experiment from the QuantStudio™ 12K Flex Instrument to the computer via a USB drive

1. If not already connected to the instrument, connect a USB drive to the USB port.



2. Touch the **QuantStudio™ 12K Flex Instrument Touchscreen** to activate it.
3. If the touchscreen is not at the Main Menu screen, 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 your 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:

   <drive>:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments\, where *<drive>* is the computer hard drive on which the QuantStudio™ 12K Flex Software is installed. The default installation drive for the software is the C: drive.

Analyze the results using the DigitalSuite™ Software

DigitalSuite™ Software is designed for rapid and accurate analysis of QuantStudio™ 12K Flex digital PCR data. A typical analysis workflow using the DigitalSuite™ Software includes:

1. Launch DigitalSuite™ Software.

2. Create a study by importing the data *.eds files created during the data collection phase.
3. Enter sample, target, and dilution information in Plate Setup.
4. Click **Analyze** and review results.
5. *(Optional)* View heat maps and amplification curves to optimize Analysis Settings
6. Save and export the results for downstream analysis.

Note: For more information and detailed instructions on analyzing experiment results using DigitalSuite™ Software, please refer to the *Applied Biosystems OpenArray® Digital PCR Experiments User Guide* (Part no. 4471926).

4 Troubleshooting

The TaqMan® Assays are optimized for use with OpenArray® digital PCR reagents, OpenArray® instruments, and thermal-cycling conditions. If you experience problems with assay performance, be sure that you have followed our protocols, then check the following troubleshooting table.

Observation	Possible cause	Recommended solution
High fluorescence signal in the NTCs.	Non-specific probe cleavage.	Perform proper bioinformatics on the sequence, evaluate the assay design, and consider redesigning the assay.
	The NTC is contaminated.	Examine other assays for high fluorescence signal in the NTCs. Consider replacing the water used for the NTCs (the water may be a possible source of contamination).
	The probe is degraded.	Store the assays correctly.
	Unfilled through-hole.	1. In the DigitalSuite™ Software, select the **Heatmap** tab. 2. From the Data to Display drop-down list, select **+/- Amplification**, then use the heatmap to determine the location of the unfilled through-hole(s). 3. Visually inspect the array to confirm the existence of the unfilled through-holes.
Amplification curve shows abnormal plot.	The baseline was set improperly (some samples have C_T values lower than the baseline stop value).	Refer to your real-time PCR system user guide for procedures on setting the baseline. Switch from manual to automatic baselining, or move the baseline stop value to a lower C_T (2 cycles before the amplification curve for the sample crosses the threshold).
	An amplification signal is detected in the early cycles (no baseline can be set because the signal is detected too early).	Dilute the sample to increase the C_T value.
		Follow the recommended sample preparation procedures for digital PCR (see "Prepare the DNA samples" on page 14).
Amplification curve shows a rising baseline.	Primer and probe interaction.	• Adjust the threshold manually. • Select another assay from the same gene.
Amplification curve shows low ROX™ dye (passive reference dye).	Inaccurate pipetting. Little or no TaqMan® OpenArray® Digital PCR Master Mix.	Follow accurate pipetting practices.

Observation	Possible cause	Recommended solution
More than the expected number of samples failed to properly amplify.	Degraded DNA. Degraded DNA may not amplify as efficiently as high-quality DNA, so fluorescence intensities vary.	Perform a gel analysis to visualize the DNA quality. Re-extract samples that are degraded or remove them from the analysis. See "Prepare the DNA samples" on page 14.
	Genomic DNA is not properly quantitated. Samples with differing concentrations result in varied fluorescence intensities. Samples with lower starting quantities exponentially amplify lower yields compared to samples with higher starting quantities.	Use a high-quality spectrophotometer or perform an RNase P quantitation assay to determine the concentration of each sample. Normalize as needed. Refer to the *User Bulletin: Human DNA Sample Quantification Protocol Using the RNase P Kit* (available from the Life Technologies website.
	Pipetting errors. Poorly calibrated pipettes, incorrect pipette tips, or inefficient technique result in varied volumes pipetted into the sample plate, and in varied genomic DNA concentrations.	• Ensure that all pipettes are calibrated on a routine basis and use the recommended pipette tips. Consult the pipette manufacturer for proper testing and maintenance. • Check the ROX™ dye levels. Variation in the ROX dye levels may indicate pipetting errors.
	Expired reagents.	Replace with fresh reagents.
	Evaporation has occurred prior to loading the plate in the case.	Check the ROX™ dye levels after imaging. Variation in the ROX dye levels may indicate evaporation.
	PCR inhibitors, ranging from organics to non-organics, can cause samples to fail amplification.	Examine the purity of the DNA by checking the: • A_{260}/A_{280} ratio, which should be between 1.7 and 1.9. A ratio <1.7 indicates protein contamination. • A_{260}/A_{230} ratio, which should be similar to the A_{260}/A_{280} ratio. A ratio <1.7 indicates salts, solvents, and alcohols may be present. Evaluate the current DNA extraction method and consider an alternative protocol.
Noisy signal above the threshold.	Empty through-hole due to inaccurate loading.	• Visually inspect the array for the empty through-hole. • Pipet more than 5 µL of sample and reaction mix to the OpenArray™ 384-Well Sample Plate when loading.

Observation	Possible cause	Recommended solution
All samples failed to amplify.	Numerous problems can cause complete failure of an assay. In addition to the previously mentioned issues in this table, consider the following:	
	A phenol/chloroform DNA extraction method was used.	• Use molecular biology-grade phenol/chloroform, and remove all traces of phenol. • Consider a bead-based or column-based extraction method.
	The DNA sample contains impurities.	Dilute the DNA sample 1:10 to dilute impurities.
	The DNA sample was not properly prepared.	Use an Life Technologies TaqMan® Control Genomic DNA (Part no. 4312660) to determine if the problem arises from the sample preparation.
	Lower-grade reagents were used.	Lower-grade reagents may contain PCR inhibitors. Use molecular biology-grade reagents in all assay-related experiments, including DNA preparation.
	Heparin was used as an anti-coagulant.	If your sample DNA is extracted from blood, do not use Heparin as an anti-coagulant as it can inhibit PCR. Use EDTA as an alternative.
	Samples failed to amplify on the OpenArray® System.	Contact Life Technologies Technical Support. • Perform proper bioinformatics on the sequence, evaluate the assay design, and consider redesigning the assay. • Verify the presence of the outlier. Examine the performance of the sample in other assays to rule out problems caused by this particular sample, such as sample impurity or degradation. Search the public databases to see if the additional copies have been discovered. Perform comparative sequencing on the subjects to identify any undocumented copies present under the primer or probe.
	The reaction may not have enough copies of the target gene. (Sample is too dilute.)	Perform a dilution series of the sample, by increasing the quantity of input DNA added to the first point of the series.
Amplification occurs in the no RT controls.	gDNA contamination.	• Perform bioinformatics: Design the assay to span an exon-exon junction. Refer to the: – *Custom TaqMan® Genomics Assays Protocol: Submission Guidelines* (Part no. 4367671) – *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (from **www.lifetechnologies.com**) • Improve sample extraction methods to eliminate gDNA. See "Prepare the DNA samples" on page 14. • Treat the sample with DNase.
	Template or amplicon contamination.	Follow established PCR good laboratory practices.

Observation	Possible cause	Recommended solution
Amplification curve shows weak amplification.	Sequence mismatches between target and assay sequences.	Perform bioinformatics. For more information, refer to the: • *Custom TaqMan® Genomics Assays Protocol: Submission Guidelines* (Part no. 4367671) • *Bioinformatic Evaluation of a Sequence for Custom TaqMan® Gene Expression Assays Tutorial* (from **www.lifetechnologies.com**)
	Degraded reagents and/or probe.	• Check the expiration date of the reagents. • Verify that you followed the correct handling and storage conditions. • Avoid excessive freeze-thaw cycles. (Consider diluting the 60X TaqMan® assay to a 20X working stock.)
	Degraded or contaminated template.	• Improve the sample integrity (extraction methods). See "Prepare the DNA samples" on page 14. • Check each template preparation by agarose gel electrophoresis or bioanalyzer to determine the: – Purity (only one product should be formed) – Level of degradation • Use RNase-free, sterile, filtered water.
	Inhibitors present in the reaction.	• Verify the presence of an inhibitor: **a.** Create a serial dilution of your sample. **b.** Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected C_T values. (High concentration means more inhibition because the sample is not diluted.) **c.** Rerun the assay with purified template. • Improve sample integrity (extraction methods). See "Prepare the DNA samples" on page 14.
	Poor reverse transcription (RT) conversion to cDNA.	• Check the RNA sample for degradation. • Input RNA could be too concentrated or too dilute. Verify the concentration by optical density (OD), make new serial dilutions of template RNA from original stock, then repeat the RT-PCR. • Ensure that the RT-PCR setup is performed under the appropriate conditions to avoid premature cDNA synthesis. • Check the RT reagents for contamination and/or degradation.
	Primer-dimer formation and residual polymerase activity.	For optimal results, run the reaction plate as soon as possible after completing the reaction setup. If you cannot run a reaction plate within 2 hours after completing the reaction setup, refrigerate or freeze the reaction plate until you can run it.

Observation	Possible cause	Recommended solution
Amplification curve shows no amplification of the sample (C_T = 40) across all assays or in an unusually large number of assays.	One or more reaction components were not added.	Verify that the cDNA and TaqMan® OpenArray® Digital PCR Master Mix were added to the reaction plate. (If the master mix is missing, the passive reference fails.)
	Incorrect dye components were selected.	Check the dye components settings and reanalyze the data.
	The annealing temperature on the thermal cycler was too high for the primers and/or probe.	Verify that the thermal cycler is set to the correct annealing and extension temperatures. Ensure that the thermal cycler is calibrated and maintained regularly.
	Inappropriate reaction conditions were used.	Troubleshoot the RT-PCR optimization.
	Degraded template.	• Determine the quality of the template. • Rerun the assay with fresh template. • Use RNase-free reagents. • Use an RNase inhibitor.
	Inhibitors present in the reaction.	Verify the presence of an inhibitor: 1. Create a serial dilution of your sample. 2. Run the serial dilution with an expressing assay (for example, an endogenous control). If an inhibitor is present, low concentrations yield higher-than-expected C_T values. (High concentration means more inhibition because the sample is not diluted.) 3. Rerun the assay with purified template.
	The baseline and/or threshold was improperly set.	Refer to your real-time PCR system user guide for procedures on setting the baseline and threshold: • Switch from automatic to manual baselining, or from manual to automatic. • Lower the threshold value to within the appropriate range.
	Assay design or synthesis failure. The wrong sequence was submitted to Life Technologies.	• Verify that the sequence you submitted is correct. • Check for an alternative transcript or a splice variant.
	Assay is designed in a variable region of the gene transcript.	Verify that the location targeted by the assay is not within the 5′ untranslated region (UTR), which can be highly variable between transcripts. If the assay is designed within the 5′ UTR, select a different assay that is within the coding region of the transcript. Otherwise, select an assay for an alternative transcript or splice variant.
	cDNA conversion failed.	• Check the RNA integrity and concentration. • Check for RNase activity. • Follow the recommended thermal profile. • Repeat the RT step using new reagents.

Observation	Possible cause	Recommended solution
Amplification curve shows no amplification of the sample (C_T = 40) in the target assay.	One or more of the reaction components was not added.	Check your pipetting equipment and/or technique.
	Incorrect dye components were selected.	Check the settings of the dye components before data analysis.
	The gene is not expressed in the tested sample.	• Verify by: – Rerunning the sample using the same assay – Running the sample using an alternative assay for the same gene • Verify the known expression of the gene in the sample type. **Note:** If the recommended actions do not resolve the problem, the result may be correct.
	The reaction may not have enough copies of the target gene. Sample is too dilute.	Verify by: • Rerunning the sample using the same assay • Rerunning the assay using more sample • Running the sample using an alternative assay for the same gene **Note:** If the recommended actions do not resolve the problem, the result may be correct.
		Perform a dilution series of the sample, by increasing the quantity of input DNA added to the first point of the series.
Decrease in ROX™ dye fluorescence (passive reference dye).	Precipitation in the TaqMan® buffers.	• When using the TaqMan® PCR Core Reagents Kit, be sure to mix the tubes well. • Use TaqMan® OpenArray® Digital PCR Master Mix. Be sure to mix thoroughly to produce a homogenous solution.
	Degraded TaqMan® buffers.	Verify that the kits have been stored according to the instructions on the packaging and have not expired.
Simultaneous increase in fluorescence from both the: • Passive reference (ROX™) dye • Reporter dye(s)	Evaporation.	Check the seal of the optical adhesive cover for leaks.
No template control (NTC) shows amplification.	Contaminated reagents (contaminated with gDNA, amplicon, or plasmid clones).	• Rerun the assay using new reagents. • Be sure your workspace and equipment are cleaned properly. • Use AmpErase® UNG. • Run no-reverse-transcription controls to rule out genomic DNA contamination. • (*gDNA contamination only*) Design an assay that spans an exon-exon boundary.

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USER GUIDE

applied biosystems® by life technologies™

Booklet 5 - QuantStudio™ 12K Flex System OpenArray® Experiments - Appendixes

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Revision Date 22 April 2014

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

Contents

A Ordering Information

How to order

You can order materials and accessories from **www.lifetechnologies.com**.

Note: Product availability and pricing may vary according to your region or country. Online ordering through the Life Technologies website is not available in all countries. Contact your local Life Technologies representative for help.

To order through the website or the QuantStudio™ 12K Flex Software:

- Confirm that your computer has an internet connection.
- We recommend the following browsers and Adobe® Acrobat® Reader® versions to use the Life Technologies website:

Operating system	Microsoft® Internet Explorer®	Apple® Safari®	Mozilla® Firefox®	Adobe® Acrobat® Reader®
Microsoft® Windows®	v7.x or later	None†	v2.x or later	v4.0 or later
Macintosh®	None†	v2.0.4 or later		

† Browser not available for this platform.

Note: Confirm that cookies and Javascript are turned on for the website to function correctly.

IMPORTANT! For Digital PCR experiments, for details on how to order a TaqMan® assay, refer to the TaqMan® assays products page at **www.allgenes.com**, the *TaqMan® Gene Expression Assays Protocol* (Part no. 4333458), or the *TaqMan® Copy Number Assays Protocol* (Part no. 4397425).

Ordering from the Life Technologies Website

To order...	Procedure
Assays and reagents	1. Go to **www.lifetechnologies.com** 2. Under "I Want to Buy," select the product of interest.
Instrument parts and accessories	1. Go to **www.lifetechnologies.com/quantstudio** 2. Click **Parts and accessories**. 3. Select the desired components, the complete the order as instructed. See "Reagents" on page 10 for a complete list of compatible instrument parts, accessories, and kits.

QuantStudio™ 12K Flex OpenArray® starter kits and other kits

Part Number	QuantStudio™ 12K Flex OpenArray® Kits	Storage Conditions
QuantStudio™ 12K Flex OpenArray® Starter Kits†		
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	
4469606	QuantStudio™ 12K Flex OpenArray® Human miRNA Starter Kit	
4469650	QuantStudio™12K Flex OpenArray® Digital PCR Starter Kit	–15°C to –20°C
4470184	QuantStudio™ Digital PCR Kit (10 pack)	15 to 20°C
4470185	QuantStudio™ Digital PCR Kit (4 pack)	
Other QuantStudio™ 12K Flex OpenArray® Kits		
4469620	QuantStudio™ 12K Flex OpenArray® Practice Kit	–20°C or ambient

† 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	Part no.
Dual Flat Block GeneAmp® PCR System 9700	Life Technologies	4428234
Dual Flat Block GeneAmp® PCR System 9700, Sample Module Only	Life Technologies	4425757
QuantStudio™ 12K Flex Adapter for 9700 Flat block		4472306
Qubit® 2.0 Fluorometer	Life Technologies	Q32866
Powder-free gloves	Major Laboratory Supplier (MLS)	—
DNase-free, sterile-filtered water	Life Technologies	—
RNase-free water	MLS	—
0.1× TE pH 8.0	MLS	—
Bleach (10%)	MLS	—
Ethanol	MLS	—
Lint-free (Laboratory-grade?) 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	—

QuantStudio™ 12K Flex TaqMan® OpenArray® plates

Part Number	QuantStudio™ 12K Flex TaqMan® OpenArray® Plates	Storage Conditions
4470187	QuantStudio™ 12K Flex TaqMan® OpenArray® Human MicroRNA Panel	Upon receipt, store the frozen, unopened plates at –20°C.
4470188	QuantStudio™ 12K Flex TaqMan® OpenArray® Rodent MicroRNA Panel	
4471113	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 16	
4471114	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 32	
4471115	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 64	
4471116	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 128	
4471117	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 192	
4471118	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Plate, Custom Format 256	
4471119	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Custom Format 18	
4471120	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Custom Format 56	
4471121	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Custom Format 112	
4471122	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Custom Format 168	
4471123	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Custom Format 224	
4471124	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Inventoried Format 18	
4471125	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Inventoried Format 56	
4471126	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Inventoried Format 112	
4471127	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Inventoried Format 168	
4471128	QuantStudio™ 12K Flex TaqMan® OpenArray® RT PCR Inventoried Format 224	
4471225	QuantStudio™ 12K Flex TaqMan® OpenArray® Genotyping Training Plate	
4471226	QuantStudio™ 12K Flex TaqMan® OpenArray® HS Endogenous Control Panel	
4471227	QuantStudio™ 12K Flex TaqMan® OpenArray® Loading Plate	
4470197	QuantStudio™ Digital PCR Plates, 10 pack	15 to 25°C
4470196	QuantStudio™ Digital PCR Plates, 4 pack	

Note: A QuantStudio™ 12K Flex OpenArray® PCR Accessories Kit, Part no. 4469576, is included with 10-pack array orders.

Reagents

Note: For reagent shelf-life expiration date, see the package label.

The following reagents are to be used with the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

QuantStudio™ 12K Flex Reagent		Part number
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
2X TaqMan® OpenArray® Digital PCR Master Mix	5 mL	4458080
	1.5 mL	4458086

Consumables (Accessories)

Note: For consumable shelf-life expiration date, see the package label.

The following consumables are to be used with the Applied Biosystems 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 Plates	10 plates	4406947
OpenArray® 384-Well Sample Plates, Barcoded	10 plates	4453929
QuantStudio™ 12K Flex OpenArray® 384-Well Plate Seals	10 seals	4469876

B Plate Information

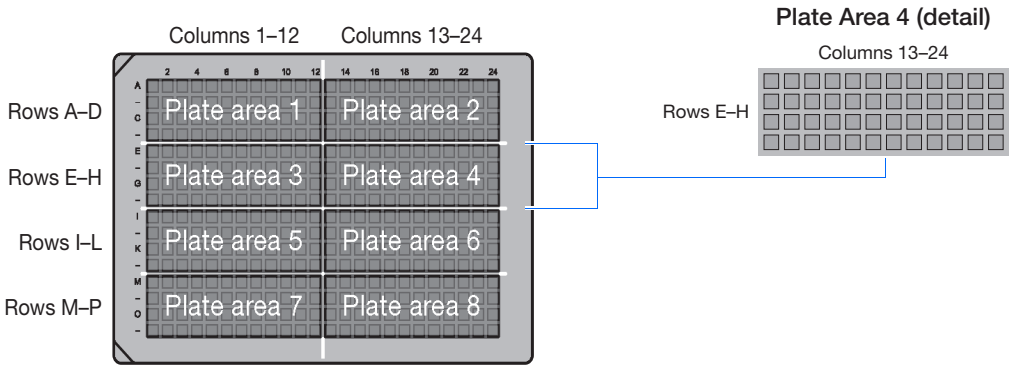
IMPORTANT! Be sure to track where the samples are in each sample plate. For each sample plate, we recommend creating a sample information file (*.csv) in the OpenArray® Sample Tracker Software.

MicroAmp® Optical 96-Well Reaction Plate

This is a 96-well thermocycling reaction plate. 96-well reaction plates are used for nucleic acid sample preparation (Booklets 1, 2 and 3, Chapter 2 Prepare the Samples) and to transfer the nucleic acid samples to OpenArray® 384–Well Sample Plates (Booklets 1, 2, and 3, Chapter 3 Prepare the 384-Well Sample Plate).

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™ OpenArray® AccuFill™ System transfers sample from *one area* of the 384-well sample plate to a single OpenArray® plate (see "OpenArray® plate overview" on page 14).

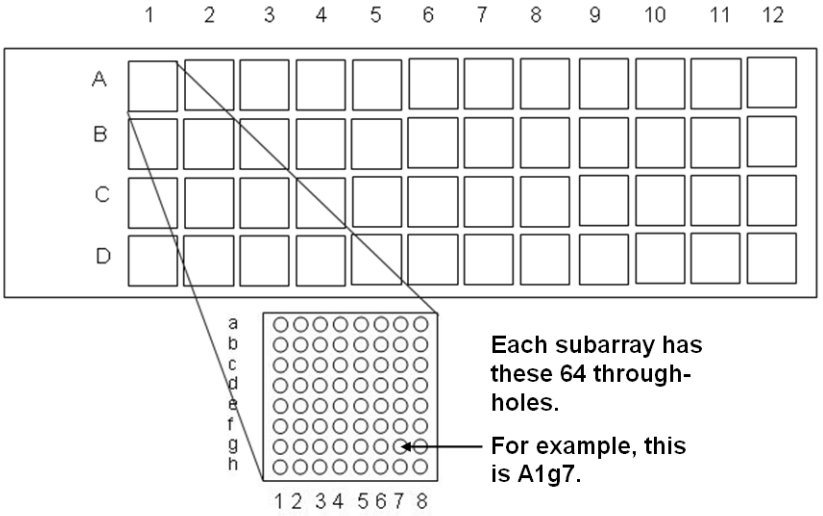


IMPORTANT! The way that you set up the 384-well sample plates depends on the format of the OpenArray® plate that you will be transferring the samples to in Booklets 1, 2, and 3. For more information on each format, see pages 15, 17, and 20.

OpenArray® plate overview

The QuantStudio™ 12K Flex TaqMan® OpenArray® plate (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 OpenArray® 384-Well Sample Plate using the QuantStudio™ OpenArray® AccuFill™ System.



Available formats

Each through-hole in an OpenArray® plate contains a single TaqMan® assay. The number of assays in the OpenArray® plate and the number of samples you can load in the plate depend on the format you select. For more information on each format, see pages 15, 17, and 20.

Include no template controls

We strongly recommend that you include at least one no template control (NTC) per OpenArray® plate, especially when preparing OpenArray® 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® plates for gene expression experiments

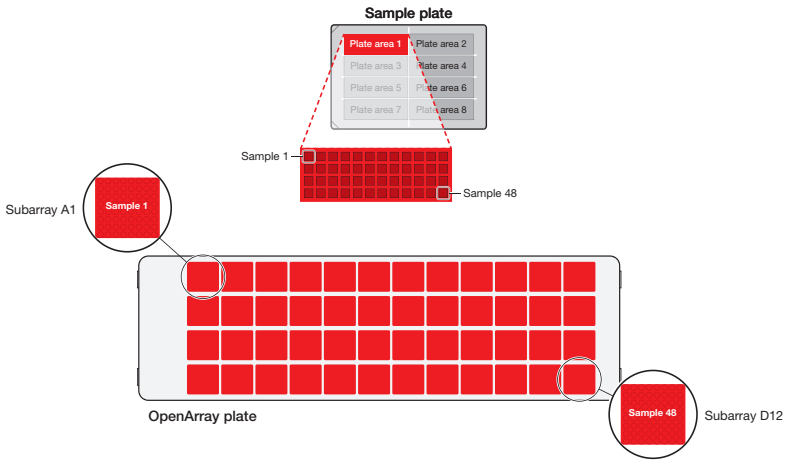
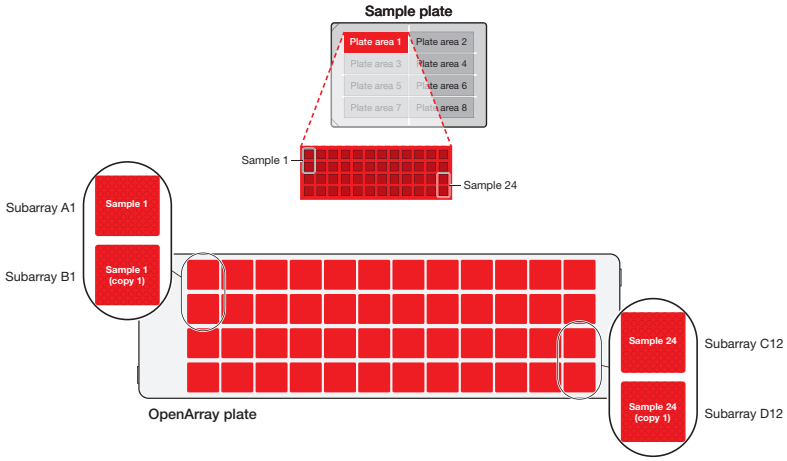
Available formats

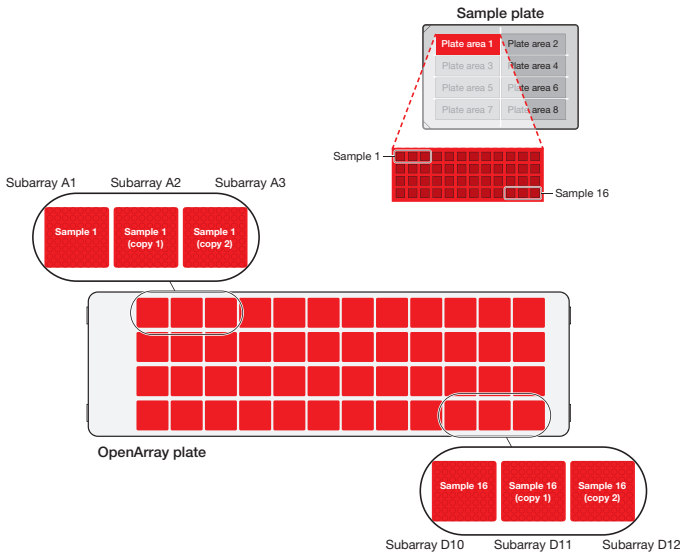
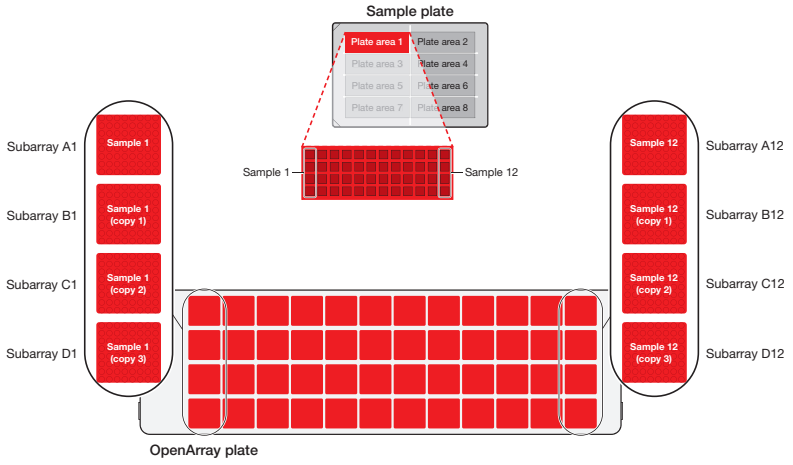
The table below provides a list of available formats for the OpenArray® real-time PCR plates, for use in gene expression experiments.

OpenArray® plate format	QuantStudio™ 12K Flex System 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, we recommend the following arrangements:

OpenArray plate	Recommended loading	Example
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.	

OpenArray plate	Recommended loading	Example
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.	

OpenArray® plates for genotyping experiments

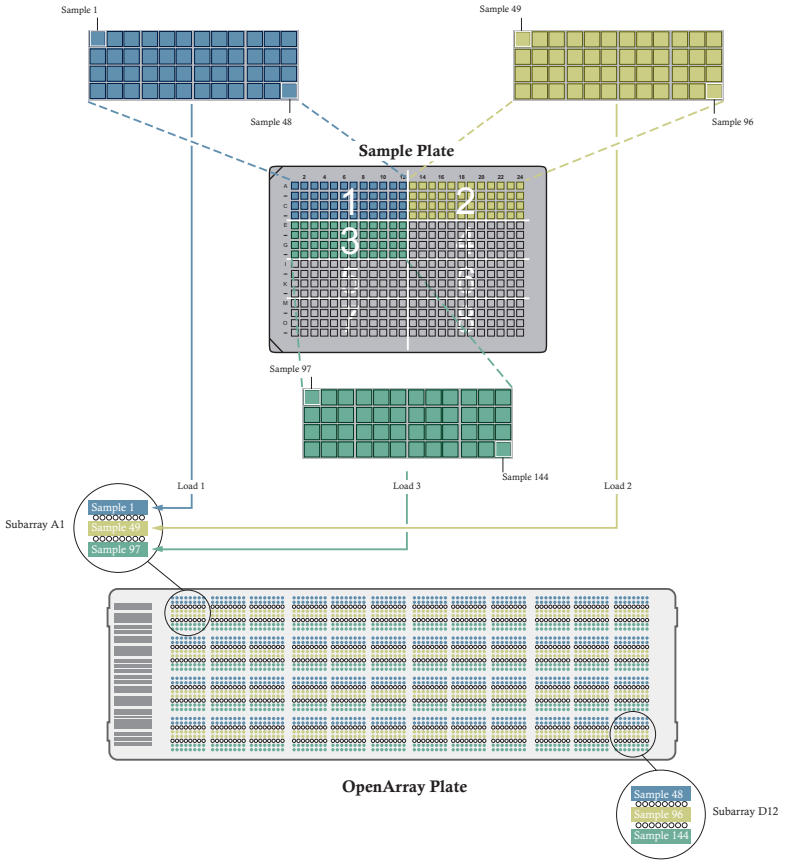
Available formats

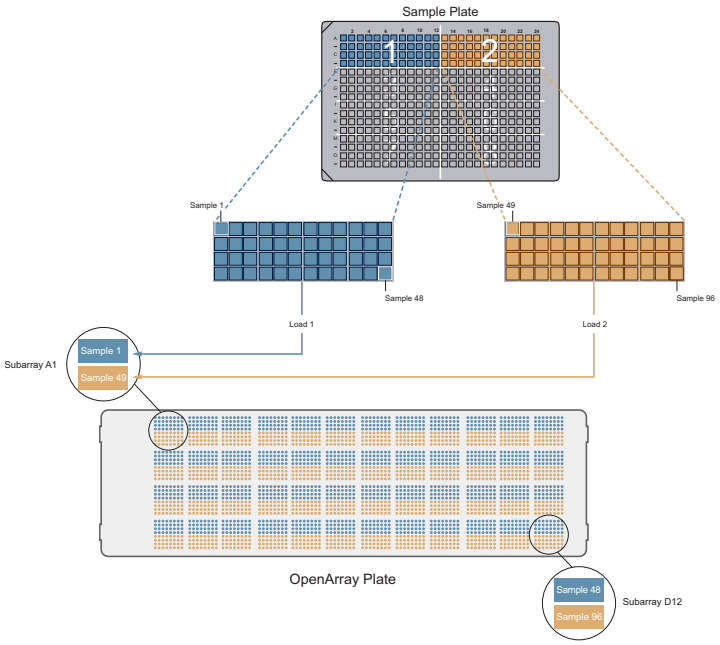
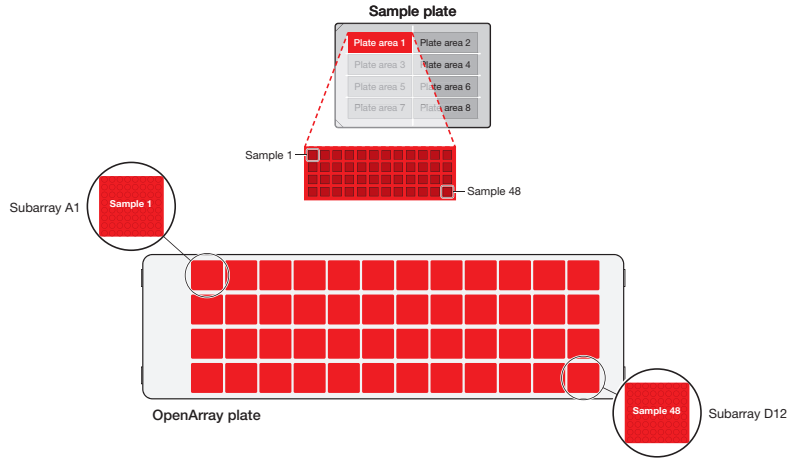
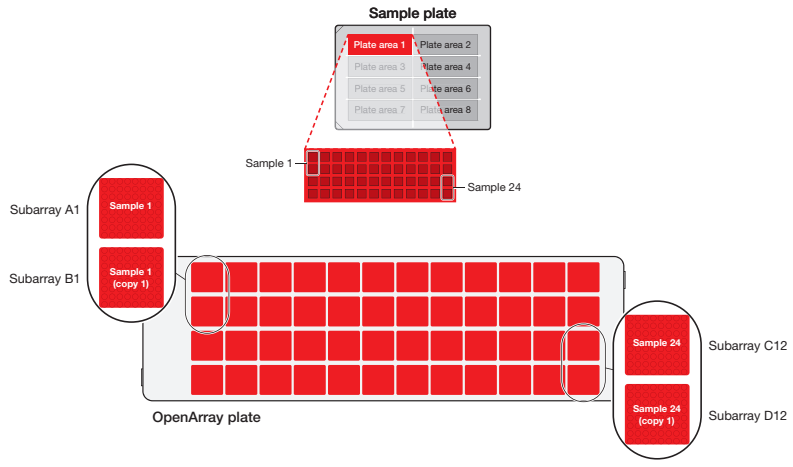
The table below provides a list of available formats for the OpenArray® genotyping plates, for use in real-time genotyping experiments.

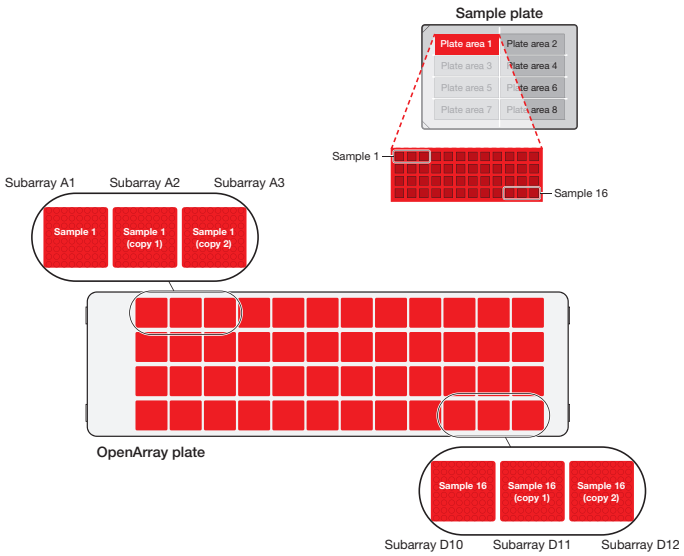
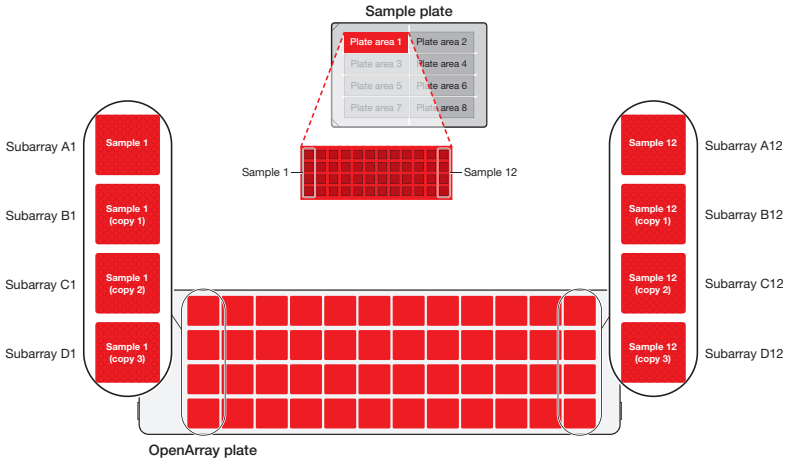
OpenArray® plate format	QuantStudio™ 12K Flex System Part no.	No. of preloaded assays	Maximum no. of samples
Format 16	4471113	16	144
Format 32	4471114	32	96
Format 64	4471115	64	48
Format 128	4471116	128	24
Format 192	4471117	192	16
Format 256	4471118	256	12

Recommended arrangements

When loading the 384-well sample plate, we recommend the following arrangements:

OpenArray plate	Recommended loading	Example
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.	

OpenArray plate	Recommended loading	Example
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.	
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.	

OpenArray plate	Recommended loading	Example
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.	

OpenArray® panels for microRNA experiments

Available panels

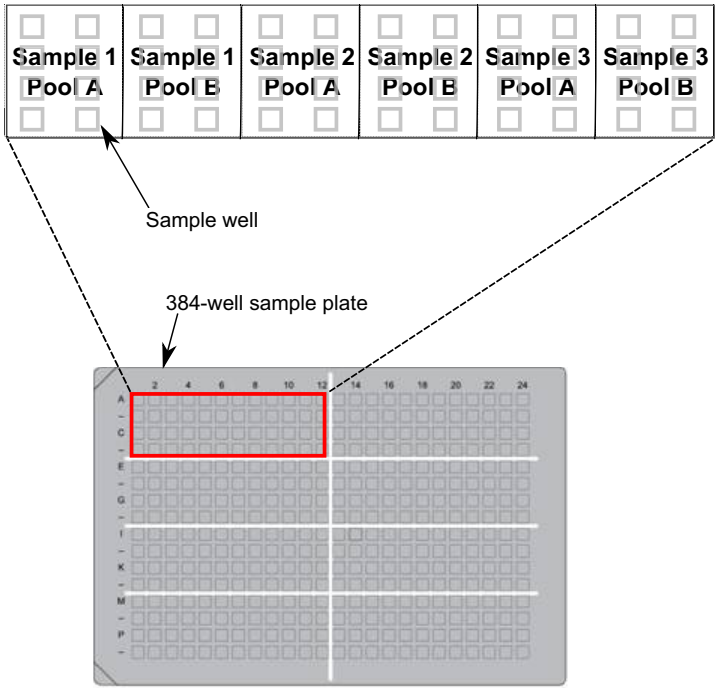
The table below provides a list of available OpenArray® microRNA panels, for use in microRNA profiling experiments.

OpenArray® microRNA panel	QuantStudio™ 12K Flex System Part no.	No. of preloaded assays†	Maximum no. of samples
Human microRNA panel	4470189	754	3 (or 1 in triplicate)
Rodent microRNA panel	4470190	754 (mouse and rat)	

† Each panel also includes a total of four controls.

Recommended arrangements

When loading the 384-well sample plate, we recommend the following arrangements:

OpenArray plate	Recommended loading	Example
Human or Rodent microRNA panel	Load samples 1 to 3 (or one sample in triplicate) in one area of the sample plate, 8 wells per sample-pool combination (Megaplex™ Primer Pools A and B).	

OpenArray® panels for digital PCR experiments

Digital PCR experiment setup

Guidelines

- Load a maximum of 48 samples per OpenArray® Digital PCR Plate.

  Note: Loading one sample per subarray does not provide sufficient confidence intervals for quantitative digital PCR; however, the single replicate setup can be used in experiments that screen candidate assays or optimal dilutions.

- Use a minimum of 64 technical replicates (1 subarray) for each gDNA/cDNA sample.
- Apply sample names and assay labels to the digital PCR experiment either:
  - Prior to the run using the OpenArray™ System Software, or
  - After the run using the QuantStudio™ 12K Flex.
- Apply the identical sample name to the subarrays of each group of technical replicates. The QuantStudio™ 12K Flex combines data of replicate subarrays only if they share the same sample name. If the replicate wells are named differently (for example, smpl012a and smpl012b), the software analyzes the wells as different samples.
- Apply unique assay names to the subarrays of plates that contain multiple TaqMan® assays. When a plate contains more than one assay, label the wells according to the assay(s) that they contain. The QuantStudio™ 12K Flex can separate the data from multiple assays only if the associated wells are labeled with unique assay names.

No template controls

We strongly recommend that you include at least one no template control (NTC) on each OpenArray® Digital PCR Plate. NTCs serve as negative controls that can be useful in data analysis. When adding NTCs to the OpenArray® 384-Well Sample Plate, place one NTC in each section of the stock plate to ensure that the NTCs are plated in the correct location in the OpenArray® Digital PCR Plate.

Note: The NTC rate for assays of interest can be characterized on the first few OpenArray® Digital PCR Plates of an experiment and excluded from the remaining plates. The nature of digital PCR places constraints on the number of NTCs per run.

Technical replicates

We recommend the use of technical replicates to provide optimal confidence intervals for the digital PCR analysis. By default, an OpenArray® Digital PCR Plate provides 64 technical replicates per loaded sample/assay combination (resulting from the loading of a single subarray). Increasing the number of replicate subarrays loaded with the same sample/assay combination provides increasingly narrower confidence intervals.

When selecting the number of replicates to use per sample/assay combination in a digital PCR experiment, select the number of replicates at which the benefit of adding more falls below a target percentage.

Example plate layout 1

This section describes an example plate layout for a digital PCR experiment that evaluates a broad dilution range of two samples for a single nucleic acid target.

Description

In this example:

- Dilutions of two samples (S01 and S02) are evaluated for one nucleic acid target using a single TaqMan® assay (A01).
- A ten-fold, six-point dilution series of each sample is evaluated for the nucleic acid target. The dilutions are such that each through-hole reaction contains either 1.0e4, 1.0e3, 100, 10, 1, or 0.1 dilution of the stock solution.
- The samples are arrayed in replicate so that the plate evaluates each sample/dilution/assay combination in quadruplicate. The plate contains a total of 12 replicate groups, where each group consists of 256 replicate reactions (4 subarrays × 64 through-holes = 256 reactions).

Experiment layout

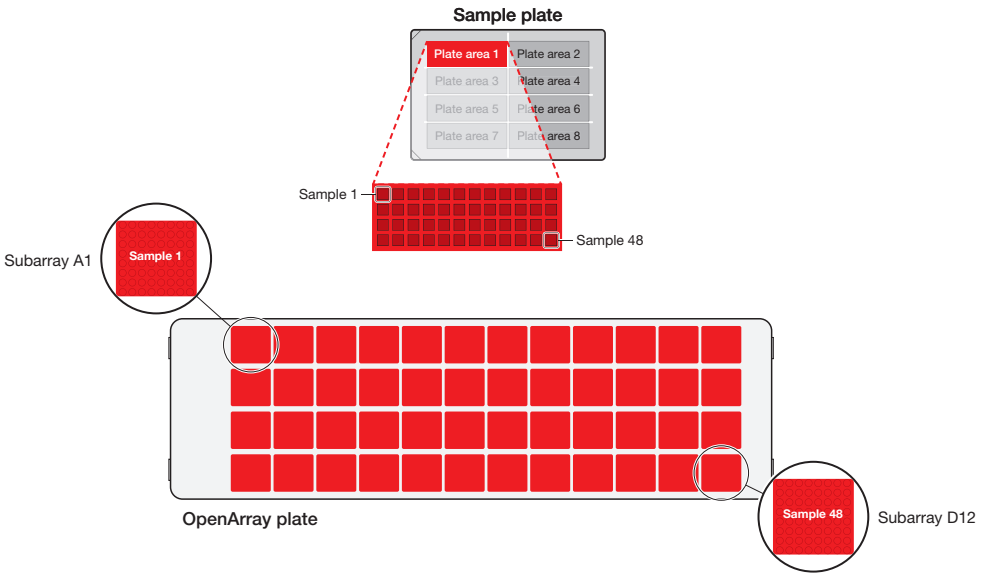
The following figure illustrates the OpenArray® 384-well sample plate layout for the example experiment:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S01 1.0e4 A01	S01 1.0e3 A01	S01 100 A01	S01 10 A01	S01 1 A01	S01 0.1 A01	S02 1.0e4 A01	S02 1.0e3 A01	S02 100 A01	S02 10 A01	S02 1 A01	S02 0.1 A01
B	S01 1.0e4 A01	S01 1.0e3 A01	S01 100 A01	S01 10 A01	S01 1 A01	S01 0.1 A01	S02 1.0e4 A01	S02 1.0e3 A01	S02 100 A01	S02 10 A01	S02 1 A01	S02 0.1 A01
C	S01 1.0e4 A01	S01 1.0e3 A01	S01 100 A01	S01 10 A01	S01 1 A01	S01 0.1 A01	S02 1.0e4 A01	S02 1.0e3 A01	S02 100 A01	S02 10 A01	S02 1 A01	S02 0.1 A01
D	S01 1.0e4 A01	S01 1.0e3 A01	S01 100 A01	S01 10 A01	S01 1 A01	S01 0.1 A01	S02 1.0e4 A01	S02 1.0e3 A01	S02 100 A01	S02 10 A01	S02 1 A01	S02 0.1 A01

Sample plate setup

Sample dilutions and assays are loaded to the 384-well sample plate as follows:

Sample	Dilution	Assay	Load to wells...
Sample 1	1.0e4	Assay 1	A1, B1, C1, D1
Sample 1	1.0e3	Assay 1	A2, B2, C2, D2
Sample 1	100	Assay 1	A3, B3, C3, D3
Sample 1	10	Assay 1	A4, B4, C4, D4
Sample 1	1.0	Assay 1	A5, B5, C5, D5
Sample 1	0.1	Assay 1	A6, B6, C6, D6
Sample 2	1.0e4	Assay 1	A7, B7, C7, D7
Sample 2	1.0e3	Assay 1	A8, B8, C8, D8
Sample 2	100	Assay 1	A9, B9, C9, D9
Sample 2	10	Assay 1	A10, B10, C10, D10
Sample 2	1.0	Assay 1	A11, B11, C11, D11
Sample 2	0.1	Assay 1	A12, B12, C12, D12



Subarray locations

When you transfer the samples from the sample plate to the OpenArray® Digital PCR Plate, program the OpenArray® Accufill™ System or the AutoLoader System to perform one load. The system transfers the samples to the following locations of the OpenArray® Digital PCR Plate:

Sample plate	Load	QuantStudio™ 12K Flex subarray locations
1	1	Through-holes A1 through H8

Example plate layout 2

This section describes an example plate layout for a digital PCR experiment that evaluates a short dilution range of two samples for two nucleic acid targets.

Description

In this example:

- Dilutions of two samples (S01 and S02) are evaluated for two nucleic acid targets using a pair of TaqMan® assays (A01 and A02).
- A two-fold, three-point dilution series of each sample is evaluated for the nucleic acid targets. The dilutions are such that each through-hole reaction contains either a 0.5, 1.0, or 2.0 dilution of the stock solution.
- The samples are arrayed in replicate so that the plate evaluates each sample/dilution/assay combination in quadruplicate. The plate contains a total of 12 replicate groups, where each group consists of 256 replicate reactions (4 subarrays × 64 through-holes = 256 reactions).

Experiment layout

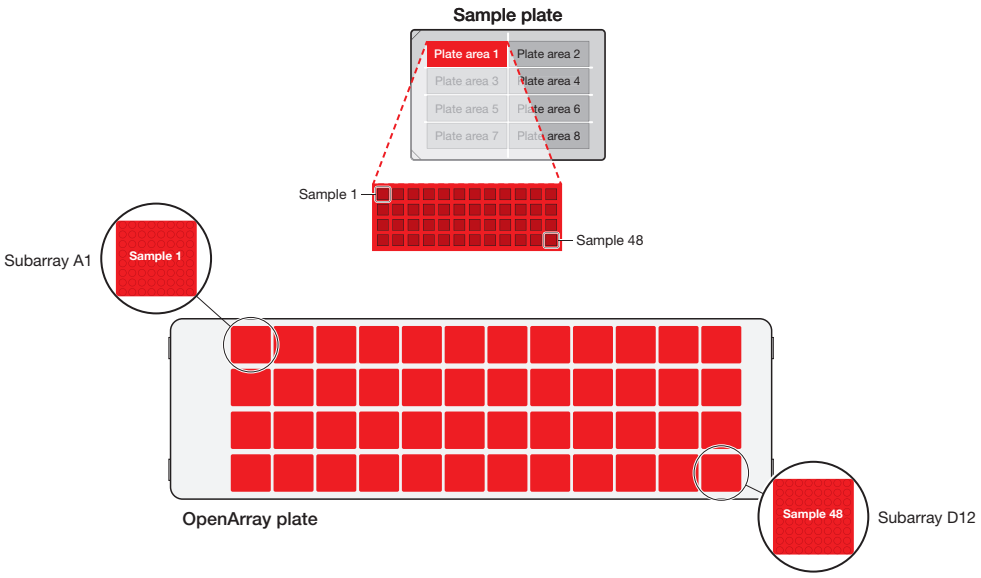
The following figure illustrates the plate layout for the example digital PCR experiment.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S01 2 A01	S01 2 A01	S01 1 A01	S01 1 A01	S01 0.5 A01	S01 0.5 A01	S02 2 A01	S02 2 A01	S02 1 A01	S02 1 A01	S02 0.5 A01	S02 0.5 A01
B	S01 2 A01	S01 2 A01	S01 1 A01	S01 1 A01	S01 0.5 A01	S01 0.5 A01	S02 2 A01	S02 2 A01	S02 1 A01	S02 1 A01	S02 0.5 A01	S02 0.5 A01
C	S01 2 A02	S01 2 A02	S01 1 A02	S01 1 A02	S01 0.5 A02	S01 0.5 A02	S02 2 A02	S02 2 A02	S02 1 A02	S02 1 A02	S02 0.5 A02	S02 0.5 A02
D	S01 2 A02	S01 2 A02	S01 1 A02	S01 1 A02	S01 0.5 A02	S01 0.5 A02	S02 2 A02	S02 2 A02	S02 1 A02	S02 1 A02	S02 0.5 A02	S02 0.5 A02

Sample plate setup

Sample dilutions and assays are loaded to the 384-well sample plate as follows:

Sample	Dilution	Assay	Load to wells...
Sample 1	2.0	Assay 1	A1, A2, B1, B2
Sample 1	1.0	Assay 1	A3, A4, B3, B4
Sample 1	0.5	Assay 1	A5, A6, B5, B6
Sample 2	2.0	Assay 1	A7, A8, B7, B8
Sample 2	1.0	Assay 1	A9, A10, B9, B10
Sample 2	0.5	Assay 1	A11, A12, B11, B12
Sample 1	2.0	Assay 1	C1, C2, D1, D2
Sample 1	1.0	Assay 1	C3, C4, D3, D4
Sample 1	0.5	Assay 1	C5, C6, D5, D6
Sample 2	2.0	Assay 1	C7, C8, D7, D8
Sample 2	1.0	Assay 1	C9, C10, D9, D10
Sample 2	0.5	Assay 1	C11, C12, D11, D12



Subarray locations

When you transfer the samples from the sample plate to the OpenArray® Digital PCR Plate, program the OpenArray® Accufill™ System or AutoLoader System to perform one load. The system transfers the samples to the following locations of the OpenArray® Digital PCR Plate:

Sample plate	Load	OpenArray® Digital PCR Plate subarray locations
1	1	Through-holes A1 through H8

C

Prevent Contamination

Note: This information in this appendix is applicable only to Digital PCR experiments.

General guidelines

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

Note: After a OpenArray® Digital PCR Plate has been sealed in a TaqMan® OpenArray® Case, it is less likely to spread contamination than other types of reaction plates.

PCR 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:
  - 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 lab benches and equipment periodically with 10% bleach solution.

D

Safety

This appendix covers:

Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Life Technologies instruments.

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

Safety symbols

The following table describes the safety symbols that may be displayed on Life Technologies devices. Each symbol may appear by itself or with text that explains the relevant hazard. These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should proceed with appropriate caution and consult the product insert for further information. If a product insert does not exist, or if the product insert does not contain the symbol or the required information, consult the user manual.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

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

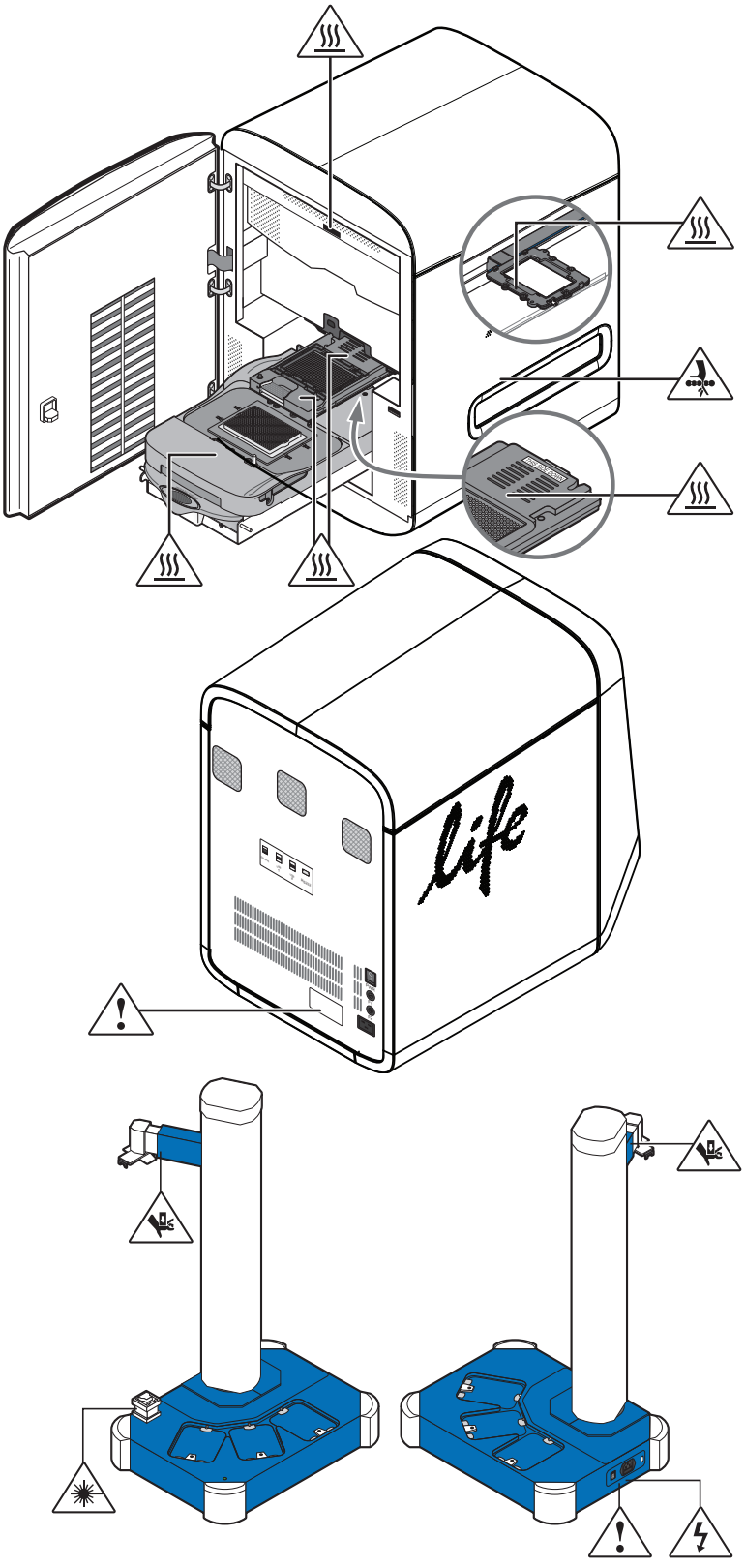
Environmental symbols on instruments

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

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

Locations of safety labels on instruments

The QuantStudio™ 12K Flex Instrument contains warnings at the locations shown below:



General instrument safety

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

Moving and lifting the instrument

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

Moving and lifting stand-alone computers and monitors

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

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

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

Operating the instrument

Ensure that anyone who operates the instrument has:

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

Cleaning or decontaminating the instrument

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

Physical hazard safety

Ultraviolet light

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

Moving parts

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

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the QuantStudio™ 12K Flex Instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

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

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

WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The QuantStudio™ 12K Flex System has an installation (overvoltage) category of II, and is classified as portable equipment.

Bar code scanner laser safety

Laser classification

The bar code scanners included with the QuantStudio™ 12K Flex Instrument are categorized as Class 2 (II) lasers.

Laser safety requirements

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

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

Workstation safety

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

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

To minimize musculoskeletal and repetitive motion risks:

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

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australia and New Zealand EMC standards

U.S. and Canadian safety standards

The instrument has been tested to and complies with standard:

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

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

Canadian EMC standard

This instrument has been tested to and complies with standard:

ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."
Cet appareil numerique de la classe B est conforme a la norme NMB-001 du Canada.

European safety and EMC standards

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards:

EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010:2003, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081:2002+A1:2003, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR Instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC).

EN 61326-1:2006 "Electrical equipment for measurement, control and laboratory use-Part 1 General EMC requirements." (Group 1, Class B)

Australia and New Zealand EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning

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

WARNING! CHEMICAL HAZARD. All chemicals in the instrument are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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

Chemical safety guidelines

To minimize the hazards of chemicals:

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

SDSs

About SDSs

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

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

Obtaining SDSs

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

1. Go to **www.lifetechnologies.com/sds**, then select **MSDS & other Support Documents**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

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

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

Biological hazard safety

General biohazard

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

In the U.S.:

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

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition **http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Safety alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

General alerts for instrumentation

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

WARNING! This instrument is designed for 12 V, 75 W halogen LED only.

Specific alerts for instrumentation

CAUTION! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the instrument.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least two people are required to lift the instrument.

CAUTION! PHYSICAL INJURY HAZARD. Do not remove the instrument cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact an Life Technologies Service Representative.

WARNING! PHYSICAL INJURY HAZARD. The QuantStudio™ 12K Flex System and LED are hot! The LED can become very hot while in use. Allow the LED to cool for 15 minutes and put on protective, powder-free gloves before handling it.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

CAUTION! PHYSICAL INJURY HAZARD. Wear disposable, powder-free gloves when handling the LED to prevent burns and to prevent shortening the life of the replacement LED.

Documentation and Support

Related documentation

The following related documents are available for use with the system:

Document	Part number	Description
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide	4470689	Describes the QuantStudio™ 12K Flex System hardware and software and provides information on preparing, maintaining, and troubleshooting the system.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide	4470935	Provides brief, step-by-step procedures for performing experiments using the OpenArray® sample block on the QuantStudio™ 12K Flex System. It is designed to help you quickly learn to use the QuantStudio™ 12K Flex System.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050	Provides brief, step-by-step procedures for performing experiments using the 96-well, Fast 96-well, 384-well plates and Array Card sample blocks on the QuantStudio™ 12K Flex System. It is designed to help you quickly learn to use the QuantStudio™ 12K Flex System.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments Quick Reference Guide	4470688	Provides experienced customers with sufficient instructions to use the QuantStudio™ 12K Flex System to run multi-well plate or array card experiments, or other information that is frequently referenced to use the system.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments Quick Reference Guide	4478673	Provides experienced customers with sufficient instructions to use the QuantStudio™ 12K Flex System to run OpenArray® experiments, or other information that is frequently referenced to use the system.
Applied Biosystems OpenArray® Sample Tracker Software Quick Reference	4460657	Provides experienced customers with sufficient instructions to use the OpenArray® Sample Tracker Software or other information that is frequently referenced to use the software.
Applied Biosystems OpenArray® Digital PCR Experiments User Guide	4471926	Provides information and instructions for using the DigitalSuite™ Software to analyze Digital PCR experiments that have been performed on the QuantStudio™ 12K Flex System.
QuantStudio™ 12K Flex Software Help	4470695	Describes the QuantStudio™ 12K Flex System software and provides procedures for common tasks.

Portable document format (PDF) versions of the above guides are available at:
www.lifetechnologies.com/quantstudio

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

Note: For additional documentation or if you cannot access the user documentation, see "Obtaining support" on page 44.

Obtaining information from the Help system

The QuantStudio™ 12K Flex Software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the QuantStudio™ 12K Flex Software window.

Select Help ▸ QuantStudio™ 12K Flex Software Help.

- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

Obtaining support

For service and technical support, call toll-free in US: 1.800.955.6288, or contact your local Life Technologies representative.

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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 found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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