USER GUIDE

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

OpenArray® Digital PCR Experiments

For use with: DigitalSuite™ Software

Publication Part Number 4471926 Rev. C
Revision Date 22 April 2014

DigitalSuite™
Software v1.0

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About This Guide

Revision history

Revision	Date	Description
C	April 2014	Update in material from OpenArray® Plate Press to QuantStudio™ 12K Flex OpenArray® Plate Press 2.0.

Purpose

The *Applied Biosystems OpenArray® Digital PCR Experiments User Guide* functions as a tutorial for the scientist analyzing experimental studies performed on the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System with DigitalSuite™ Software.

Prerequisites

This user guide is intended for users who have been specifically trained by Life Technologies for using the QuantStudio™ 12K Flex Instrument. Customers may start using DigitalSuite™ Software without specifically being trained on that application. 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 Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System, please see "Documentation and Support" on page 97. The related documentation listed provide resources that detail general instructions applicable to any experiment run on the QuantStudio™ 12K Flex System.

1 Overview of Digital PCR experiments

Introduction

Digital PCR provides sensitive, precise, and absolute quantification of nucleic acids without the use of a standard curve. The following components are required to perform digital PCR on the QuantStudio™ 12K Flex System:

- **QuantStudio™ 12K Flex System** – Instrumentation used to load, cycle and detect targets using digital PCR analysis.
- **QuantStudio™ Digital PCR Plates** – Reaction vessels used to contain the digital PCR reactions for thermal cycling and the subsequent imaging by the QuantStudio™ 12K Flex System.
- **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.
- **DigitalSuite™ Software** – Software used to complete statistical analysis of the digital PCR experiments performed on the QuantStudio™ 12K Flex System.

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

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System consists of the following components:

- **OpenArray® AccuFill™ System** – Loads your samples onto an OpenArray® Digital PCR Plate.
- **QuantStudio™ 12K Flex Instrument** – Performs thermal cycling and imaging of the experiment plates.
- **Computer** – Connects to the QuantStudio™ 12K Flex Instrument. Also has the QuantStudio™ 12K Flex Software installed on it.

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*)

QuantStudio™ OpenArray® 384-Well Sample Plate

The QuantStudio™ OpenArray® 384-Well Sample Plate is a 384-well reaction plate that is provided in the OpenArray® Real-Time Accessories Kit. You combine the TaqMan® OpenArray® Digital PCR Master Mix, TaqMan® assay, and your DNA sample in the sample plate, then use the OpenArray® AccuFill™ System to transfer the mixture from the sample plate to an OpenArray® plate(s).

IMPORTANT! The well dimensions of the QuantStudio™ OpenArray® 384-Well Sample Plates are specifically suited for use with the OpenArray® AccuFill™ System. Life Technologies does 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 3072 reaction through-holes, each of which can accommodate a 33-nL reaction volume. 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 and isolate the reactions from neighboring through-holes. The large number of reaction through-holes along with the precise control of reaction volume provide a robust platform for digital PCR experimentation.

Digital PCR experiments

What is a digital PCR experiment?

Digital PCR is a statistical technique requiring tens to thousands of reaction replicates to accurately quantify the absolute number of starting copies of a target nucleic acid sequence in a genomic or complementary DNA (cDNA) sample without the use of a standard. Digital PCR analysis requires that at least some reactions within the sample replicate group have zero copies (individual PCR reactions will contain either zero, one, or a few target molecules). Amplification is detected in reactions receiving at least one molecule and classified as positive while no amplification is detectable in reactions not receiving target and is conversely classified as negative. Following PCR, the number of positive and negative reactions is counted and fit to a Poisson distribution to estimate the absolute copies of template molecules present in the sample volume.

For concentrated samples, dilution to the single-molecule limit may be required. For detection of rare targets (mutant sequences, pathogens, transgene content) dilution is generally not required.

Applications of digital PCR include, but are not limited to, quantification of low-level pathogens, detection of rare sequences, gene expression in single cells, and low-fold copy number discrimination of genes/targets.

Digital PCR experiments include the following components:

- **Sample** – The genomic or cDNA sample that contains an unknown number of copies of the target nucleic acid sequence.
- **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 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™ dye (or VIC® dye, in case of a duplex experiments) reporter dye linked to the 5′ end of the probe.
- A minor groove binder (MGB) at the 3′ end of the probe.

  MGB increases the melting temperature (Tm) without increasing probe length (Afonina et al., 1997; Kutyavin et al., 1997); enabling the design of shorter, more specific probes.
- A nonfluorescent quencher (NFQ) at the 3′ end of the probe. Because the quencher does not fluoresce, the QuantStudio™ 12K Flex Instrument can measure reporter dye contributions more accurately.

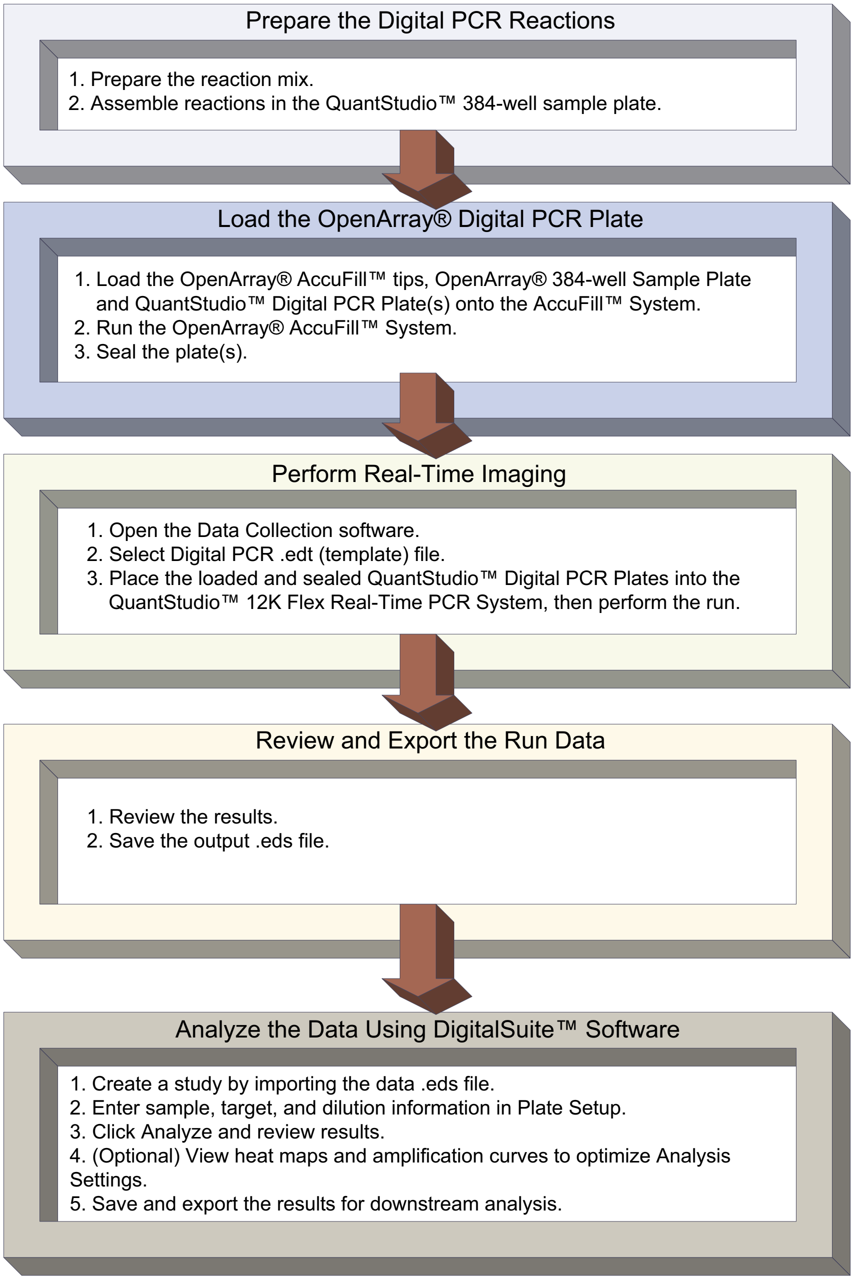
- **Technical replicates** – Through-hole reactions of each subarray that contain identical sample/assay/reaction mix combinations and volumes. Each subarray of the OpenArray® Digital PCR Plate contains a minimum of 64 technical replicates (resulting from a single well of the 384-well sample plate).
- **(Optional) No template controls (NTCs)** – Samples that contain water or buffer instead of template; also known as *negative controls*. NTCs should not amplify.

IMPORTANT! In DigitalSuite™ Software, NTC is not an assigned task. You can identify a negative control in the Software by naming a Sample "NTC" or something similar. The Software 'treats' the NTC-named samples in the same way as the other samples. You need to look out for any amplification in the NTC-named samples as the Software does not produce a flag if amplification occurs in those wells.

About digital PCR experiment setup

In a digital PCR experiment performed on the QuantStudio™ 12K Flex System, dilutions of each gDNA or cDNA sample are loaded into the wells of an QuantStudio™ 384-Well Sample Plate that contain TaqMan® OpenArray® Digital PCR Master Mix and TaqMan® Assay. If the target concentration is high, samples are diluted down to a limiting quantity prior to assembly of the reaction mix in the QuantStudio™ 384-well Sample Plate, such that a portion of the individual PCR reactions receive no target molecules.

Digital PCR experiment workflow



Prepare the Digital PCR Reactions

1. Prepare the reaction mix.
2. Assemble reactions in the QuantStudio™ 384-well sample plate.

Load the OpenArray® Digital PCR Plate

1. Load the OpenArray® AccuFill™ tips, OpenArray® 384-well Sample Plate and QuantStudio™ Digital PCR Plate(s) onto the AccuFill™ System.
2. Run the OpenArray® AccuFill™ System.
3. Seal the plate(s).

Perform Real-Time Imaging

1. Open the Data Collection software.
2. Select Digital PCR .edt (template) file.
3. Place the loaded and sealed QuantStudio™ Digital PCR Plates into the QuantStudio™ 12K Flex Real-Time PCR System, then perform the run.

Review and Export the Run Data

1. Review the results.
2. Save the output .eds file.

Analyze the Data Using DigitalSuite™ Software

1. Create a study by importing the data .eds file.
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 optimize Analysis Settings.
5. Save and export the results for downstream analysis.

DigitalSuite™ Software

DigitalSuite™ Software performs statistical analysis of the digital PCR experiments performed using TaqMan® Assays on the QuantStudio™ 12K Flex System. The DigitalSuite™ Software can be used to detect and measure the absolute number of molecules of specific sequences in a variety of biological samples.

Features

The unique features of the DigitalSuite™ Software include:

- Study-based analysis that can accommodate multiple OpenArrays at a time.
- Accommodates both duplex and singleplex experimental designs.
- Manual and automatic empty well calls.
- Inclusion or omission of individual targets in a well.
- Results generation and re-analysis using an updated dataset.
- Customization of digital PCR settings, flags, and Poisson calculations.
- Export or import of experiment analysis settings.
- Enables viewing of amplification curve groups by target, sample-target and/or sample-target-dilution.
- Provides a histogram plot that helps in distinguishing amplifications.
- Both auto or manual call amplification/non-amplification/undetermined.
- Reporting of results in copies per uL using configurable well volume.
- Individual well-bookmarking enables respective views of the data across the platform.
- Exporting results.
- Print, save to an image file, or export plots.
- Visualization features such as Heat Maps, Scatter Plots and Bar Plots.

Compatible instruments

DigitalSuite™ Software can be used to analyze the results of digital PCR experiments run on the QuantStudio™ 12K Flex System that have been exported as raw amplification curve data (.eds) files.

About the analysis

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 a QuantStudio™ 12K Flex System. Following thermal cycling, the raw amplification curve data from the digital PCR experiment are exported from the data collection software, QuantStudio™ 12K Flex Software. The exported file is then loaded by the DigitalSuite™ Software for analysis.

DigitalSuite™ Software generates calls for all the through-hole reactions. Using the call data, the OpenArray® Digital PCR Software calculates copy number values for all samples present on the plate and, by default, generates 95% confidence intervals according to a Poisson maximum-likelihood algorithm (Fazekas de St. Groth, S, 1982).

DigitalSuite™ Software installation

System requirements

High-level system requirements are as follows:

- 2.4 GHz CPU
- 2 GB RAM

Disk space

Target computer with one hard drive, or no partitions:

- 20 GB of disk space is required.

Target computer with two hard drives, or two partitions:

- 20 GB of disk space is required for the applications drive
- 300 MB of disk space is required for the programs drive

Target computer with three hard drives, or three partitions:

- 20 GB of disk space is required for the applications drive
- 300 MB of disk space is required for the programs drive
- 1 GB of disk space is required for the user files drive

Locate the executable file

Install the software at C:\Program Files\AppliedBiosystems\DigitalSuite Software.

Analysis workflow

The recommended workflow for analyzing results is:

1. Launch DigitalSuite™ Software.
2. Click **Create Study** to begin a new study.
3. Import the data files into the study.

   Note: The Import dialog box opens automatically on clicking Create Study.
4. Assign sample name and target information for all wells.
5. Analyze and view results.

2

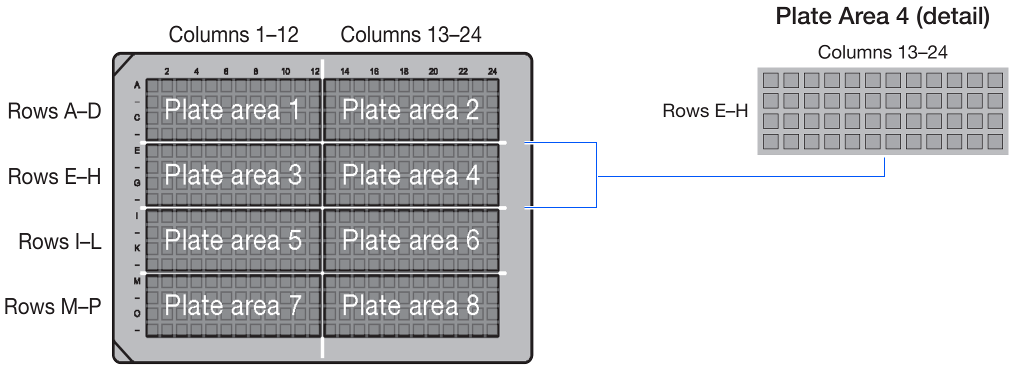
Perform a Digital PCR Experiment

Prepare the OpenArray® 384-Well Sample Plate

Determine layout of the sample plate

About the sample plate

The OpenArray® 384-Well Sample Plate is divided into eight areas; each sample plate area is 12 wells x 4 wells (48 wells). During each load, the OpenArray® AccuFill™ System transfers sample from *one area* of a single sample plate to the respective QuantStudio™ Digital PCR Plate, each well of the sample plate loading an individual subarray on the OpenArray® Plate.



Determine the layout of the sample plate

1. Label the sample plate with a unique identifier.
2. Determine which sections of the sample plate you will load with sample. Each 12-well x 4-well rectangular area corresponds to one QuantStudio™ Digital PCR Plate. You may find it helpful to mark the sample plates with a fine-tip marker.

Prepare the sample plate

A single QuantStudio™ Digital PCR Plate accepts one load from a sample plate. Be sure that the QuantStudio™ OpenArray® 384-Well Sample Plate, OpenArray® AccuFill™ Loader Tips, and Plate Holder are completely clean and dry.

IMPORTANT! Residual water prevents correct loading of the samples into the QuantStudio™ Digital PCR Plates.

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1. Prepare the PCR mix.

   Note: For detailed preparation instructions on making the PCR mix, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide* (Part no. 4470935).

2. Add sample, master mix, and assay(s) to the sample plate.

3. (*Optional*) Store sealed sample plates.

Storage conditions

The following materials require special storage conditions:

Item		Storage Conditions
QuantStudio™ Digital PCR Plate		
If the 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.

Prepare the QuantStudio™ Digital PCR Plate

Required materials

The consumables, materials, and equipment listed are specific to digital experiments.

Item†	Source	Part no.
QuantStudio™ Digital PCR Kit available as 10 Pack or 4 Pack • The 10 Pack includes 10 QuantStudio™ Digital PCR Plates (Part no. 4470197), 2X TaqMan® OpenArray® Digital PCR Master Mix (5 mL, Part no. 4458080), and QuantStudio™ OpenArray® Real-Time PCR Accessories Kit (Part no. 4469576) • The 4 Pack includes four QuantStudio™ Digital PCR Plates (Part no. 4470196) and 2X TaqMan® OpenArray® Digital PCR Master Mix (1.5 mL, Part no. 4458086) **Note:** The QuantStudio™ OpenArray® Accessories Kit (Part no. 4469576) is included with the 10 Pack but must be purchased separately with the 4 Pack.	Life Technologies	4470184 (10 pk) 4470185 (4 pk)
DigitalSuite™ Software	Life Technologies	4472102
DigitalSuite™ Software License Keys (10 pack)	Life Technologies	4472103 (10 pk)

Item†	Source	Part no.
QuantStudio™ OpenArray® AccuFill™ System	Life Technologies	4471021
Foil seals	Major Laboratory Suppliers (MLS)	—
QuantStudio™ OpenArray® Accessories Kit The accessories kit contains: • QuantStudio™ 12K Flex OpenArray® Case • QuantStudio™ 12K Flex OpenArray® Lid • QuantStudio™ 12K Flex OpenArray® Plate Press 2.0	Life Technologies	4469576
Ethanol	MLS	—
Razor blade	MLS	—
Powder-free nitrile gloves	MLS	—
Laboratory-grade wipes	MLS	—
Tweezers (to remove foil from the sample plate)	MLS	—

† For the Safety Data Sheet (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.

Procedure

For detailed instructions on the following prepatory and experimental procedures, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide* (Part no. 4470935):

- Use the QuantStudio™ OpenArray® AccuFill™ System to transfer your DNA samples and assays.
- Seal the QuantStudio™ 12K Flex OpenArray® Case.
- Load the prepared QuantStudio™ Digital PCR Plate into the QuantStudio™ 12K Flex Instrument.

Run the QuantStudio™ Digital PCR Plates

Use the QuantStudio™ 12K Flex Software to run the QuantStudio™ Digital PCR Plate. For Digital PCR experiments, the QuantStudio™ Software uses the Plate template file (*.edt) type of data file.singleplex and duplex experiments require specific *.edt data file. However, the *.edt file used for duplex experiments specifies that both FAM™ dye and VIC® dye should be collected during the run.

Note: For detailed instructions on running the OpenArray® plates, monitoring experiments, and transferring experiment results, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide* (Part no. 4470935):

Analyze the data

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

For detailed analysis and export procedures for example singleplex and duplex experiments, refer to Chapter 3, "Analyze Experiment Results using DigitalSuite™ Software" on page 19.

3

Analyze Experiment Results using DigitalSuite™ Software

Overview

DigitalSuite™ Software is designed for rapid and accurate analysis of QuantStudio™ 12K Flex digital PCR data. In this chapter, you use the example experiment files provided during the DigitalSuite™ Software installation to analyze the digital experiment results. A typical analysis workflow using the DigitalSuite™ Software includes:

1. Launch DigitalSuite™ Software.
2. Create a study.
3. Import one or multiple *.eds file(s).
4. Setup plate layout.
5. Click Analyze.
6. View results.
7. (*Optional*) Conduct further analysis of the digital PCR data for quality by choosing the **Run QC** option.
8. Export the analyzed data.

This chapter is divided into three sections. Section 3.1 provides the information and instructions on the overall analysis workflow. Section 3.2 and Section 3.3 include information specific to singleplex and duplex experiments, respectively.

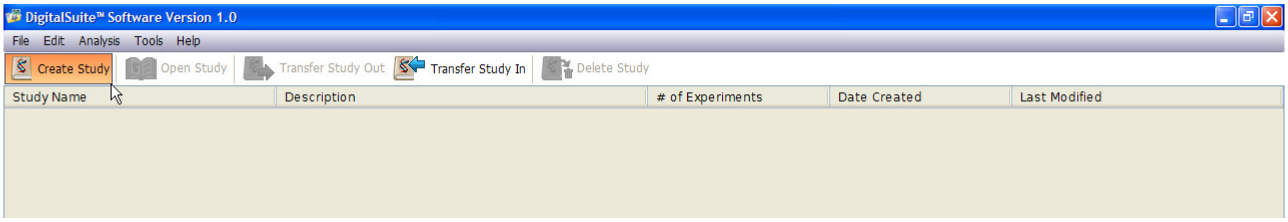
Section 3.1 General Analysis Workflow

Both singleplex and duplex experiments follow the same general analysis procedure. This section describes the general workflow for digital analysis of experiments. The subsequent individual sections describe specific parameters. This section includes:

Create a study

To create a new study, launch DigitalSuite™ Software and on the Home screen, click **Create Study**.

Alternatively you can create a study from the File menu. Go to **File** ▸ **Create Study**.



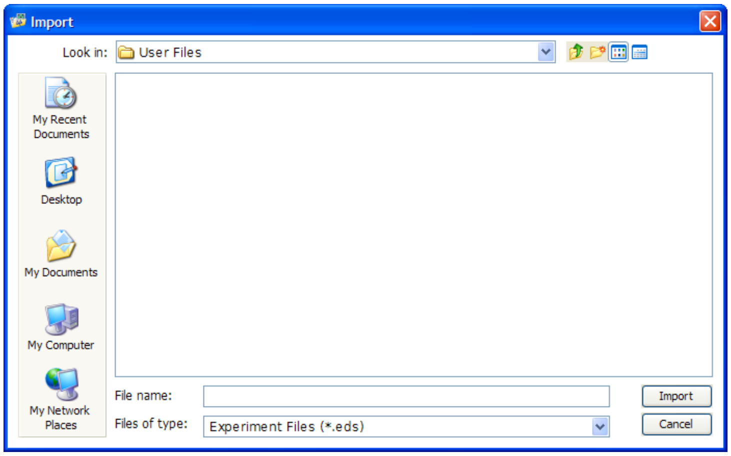
Note: Launch DigitalSuite™ Software from:

- **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **DigitalSuite Software**

  or
- The Analyze menu on the Home screen of the QuantStudio™ 12K Flex Software

Clicking on Create Study takes you to the Plate Setup screen and automatically opens the Import dialog box.

The default location of the *.eds file is C:\Applied Biosystems\DigitalSuite Software\User Files. To change the default location of the *.eds file, go to **Tools** ▸ **Preferences**. For more information on the Preferences dialog box, see "Preferences" on page 62.



Look in: User Files

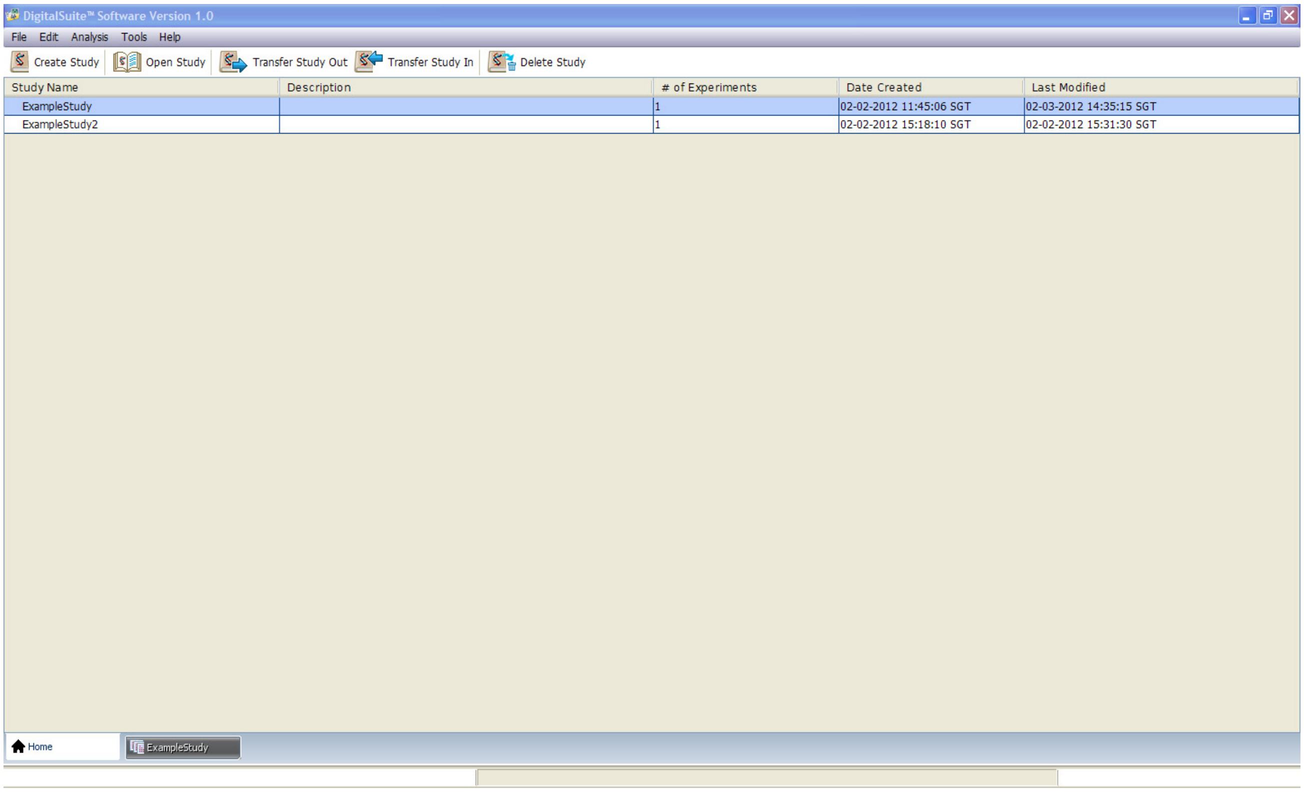
Files of type: Experiment Files (*.eds)

A study can include data from one or more OpenArray® plates.

Note: Life Technologies recommends that you do not mix singleplex and duplex experiments in a single study.

Note: Click **Save** or go to **File** ▸ **Save** to save a study.

Once a study is created and saved, the study name is displayed on the Home screen along with the study properties including the study description, the number of OpenArray® plates included present in the study, the date on which the study was created and the date on which the study was last modified.



Study Name	Description	# of Experiments	Date Created	Last Modified
ExampleStudy		1	02-02-2012 11:45:06 SGT	02-03-2012 14:35:15 SGT
ExampleStudy2		1	02-02-2012 15:18:10 SGT	02-02-2012 15:31:30 SGT

- Click **Open Study** to open a selected study from the study list on the Home screen. Alternatively, you can double-click on a study name from the study list on the Home screen.
- Click **Transfer Study Out** to move one or more selected studies out to another workstation running DigitalSuite™ Software. Use the Transfer Study Out feature to transfer studies into files that you can back up or pass to another user.
- Click **Transfer Study In** to bring in one or more studies from another location. Only files of type *.las can be transferred in to DigitalSuite™ Software.
- Click **Delete Study** to permanently delete a selected study from the study list on the Home screen.

Define study properties

Click **Properties** from the Setup menu located in the Workflow menu on the left to access the Study Properties screen.

1. Enter a unique study name in the Study Name field.
    - Enter a name that is descriptive and easy to remember. You can enter up to 100 characters

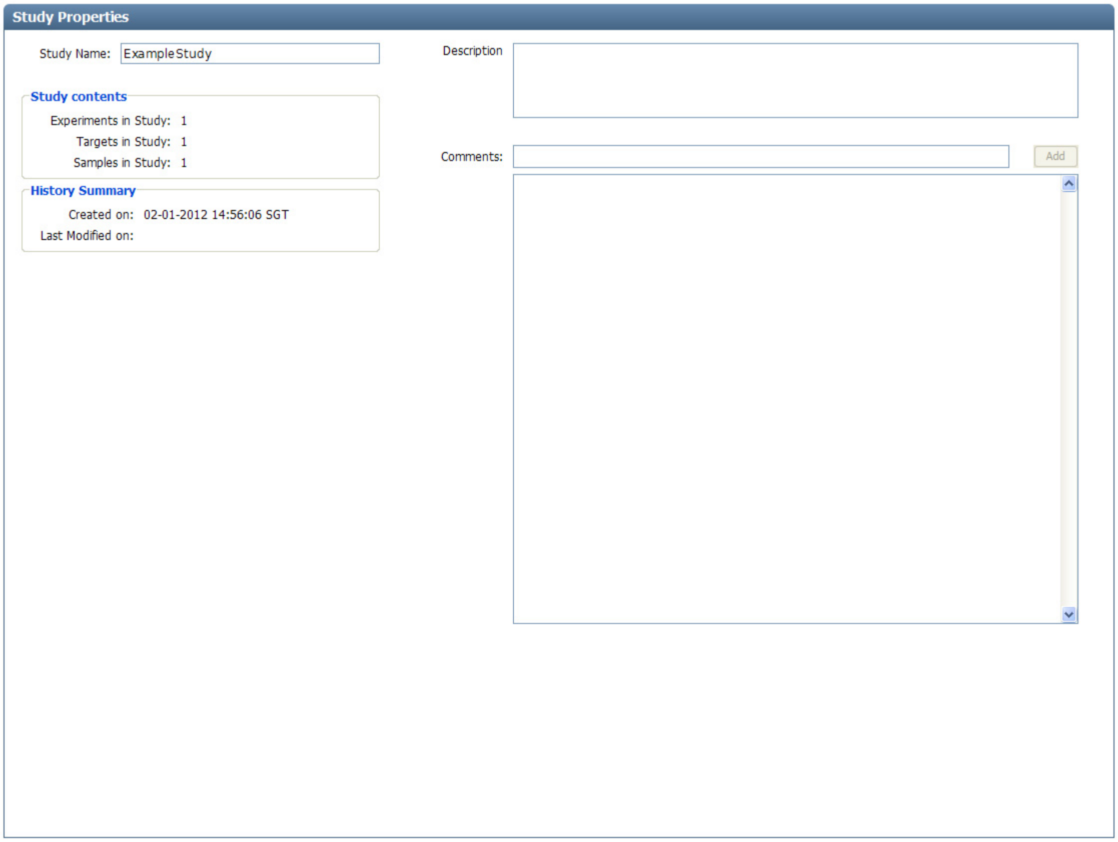
- You can only use the alpha-numeric, period (.), hyphen (-), underscore (_) and spaces ( ) characters

  Note: You cannot use the following characters: % * ? | ; : , ! @ # $ ( ) < > / \ " ' ` ~ [ ] { } = & ^

2. The Study contents box is automatically populated with:
   - The number of experiments in the study
   - The targets in the study
   - The samples in the study
3. The History Summary box is automatically populated with:
   - The date and time stamp when the study was created
   - The date on which the study was last modified
4. *(Optional)* Enter a description for the study in the Description field.
5. *(Optional)* Enter comments in the Comments field. Click **Add** to add the comments to the comments list. You can add multiple comments at different time intervals.

IMPORTANT! Comments added to the Study Properties are time stamped and once added cannot be deleted.

The Study Properties screen for an example study is shown in the following image:



Study Properties

Study Name: ExampleStudy

Study contents
Experiments in Study: 1
Targets in Study: 1
Samples in Study: 1

History Summary
Created on: 02-01-2012 14:56:06 SGT
Last Modified on:

Set up plate layout

Click **Plate Setup** from the Setup menu to access the Plate Setup screen. The Plate Setup screen is divided into two panes: “Manage Experiments” and “Plate Setup for.(Study Name)”.

Manage Experiments

The Manage Experiments view lists all the *.eds files present in the study. The features under Manage Experiments allow you to:

- Import Import one or multiple OpenArray data files (*.eds files) into a study.
- Delete Delete one or multiple experiments from a study.
- Generate Plate Template Generate plate templates (*.xls files), containing setup information (target name and color, sample name and color, dilution and color) to use for OpenArray® plates in a study. The plate template is generated from the OpenArray data file currently selected.
- Load Plate Template Load setup information (target name and color, sample name and color, dilution and color) from plate templates into one or more selected experiment files.

You can view experiment file name, barcode information, the date on which the experiment was added, and comments in the Manage Experiments view.

Note: Comments added to the Plate Setup in the comments field are editable.

Plate Setup for: (Study Name)

The features under the Plate Setup view allow you to assign plate information. The elements of the Plate Setup view are described below.

Assign

Use the Assign feature to assign well contents for a particular sub-array(s) in an experiment.

1. Select one or more sub-arrays in the plate layout.

   Note: You can select multiple sub-arrays by ‘click and drag’ action over the plate layout, the column headings, or the row headings. You can also use Ctrl or Shift to select multiple sub-arrays.

2. Click Assign above the plate layout. Alternatively, right-click on a sub-array or a group of sub-arrays and select **Assign Well Content** from the drop-down menu. If you are assigning contents to a single sub-array, double-click to open the well editor.

   Note: The Assign button remains disabled until you select one or more sub-arrays in the plate layout.

3. In the Assign Well Contents editor:
   a. Select **TaqMan® Assay 1** or enter a name of your choice for the Target (FAM).
   b. *(Optional)* Select a color for the target from the corresponding drop-down menu.

For duplex experiments, select or enter a name of your choice for the Target (VIC). Optionally, select a color for the target from the corresponding drop-down menu.

For singleplex experiments, Target (VIC) is disabled.

c. Select **DNA Sample 1** or enter a name of your choice for the Sample. Optionally, select a color for the sample from the corresponding drop-down menu.

d. Select a dilution from the Dilution drop-down menu or enter a dilution of your choice. Optionally, select a color for the dilution from the corresponding drop-down menu.

Note: When you enter a new name for targets, samples, or dilution, the option gets populated in the Samples/Targets screen.

Note: When you enter a target or sample name of your own choice, Life Technologies recommends that you use short names, especially when you have a large number of Sample or Replicate Groups. Due to the limited screen size, longer names will truncate on the Results Plot in the Digital PCR screen.

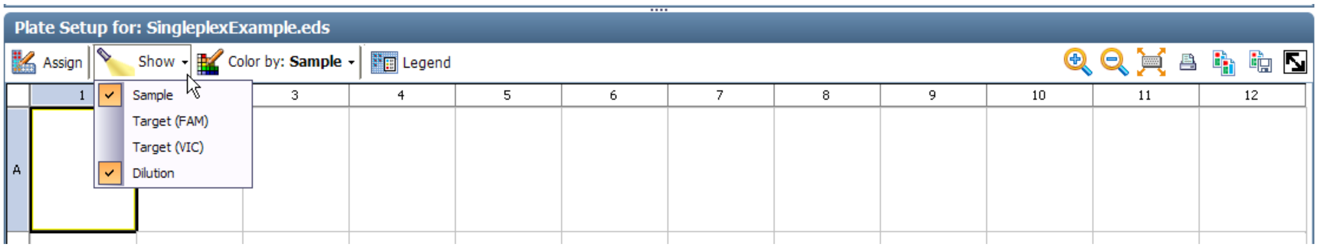
4. Click **Done** after selecting or entering criteria or **Cancel** to exit the Assign Well Contents editor without assigning.

Note: You can also copy well contents from one or multiple sub-arrays to another location using the right-click option. In the plate layout, select one or multiple sub-arrays; right-click and select **Copy Wells** from the drop-down options. Select the sub-array at the top-left corner of the destination location and select **Paste Wells** from the right-click drop-down options to paste.
Select **Clear** from the right-click drop-down options to clear well information from a sub-array.

Show

Use the Show feature to display or hide the well contents in the sub-arrays.

1. Click **Show** above the plate layout.
2. Select the well contents **Sample**, **Target (FAM)**, **Target (VIC)**, **Dilution** to show or hide from the drop-down menu.

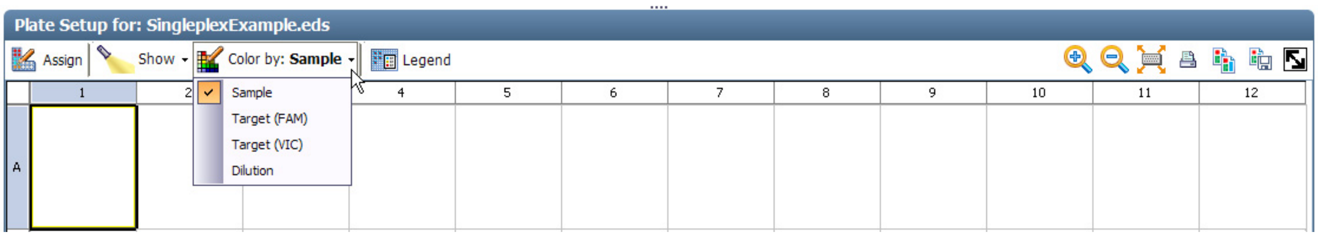


Color by:

Use the Color By feature to display the sub-arrays with the color of the selected well contents.

1. Click **Color by:** above the plate layout.

2. Select from **Sample**, **Target (FAM)**, **Target (VIC)**, **Dilution** in the Color by drop-down menu.



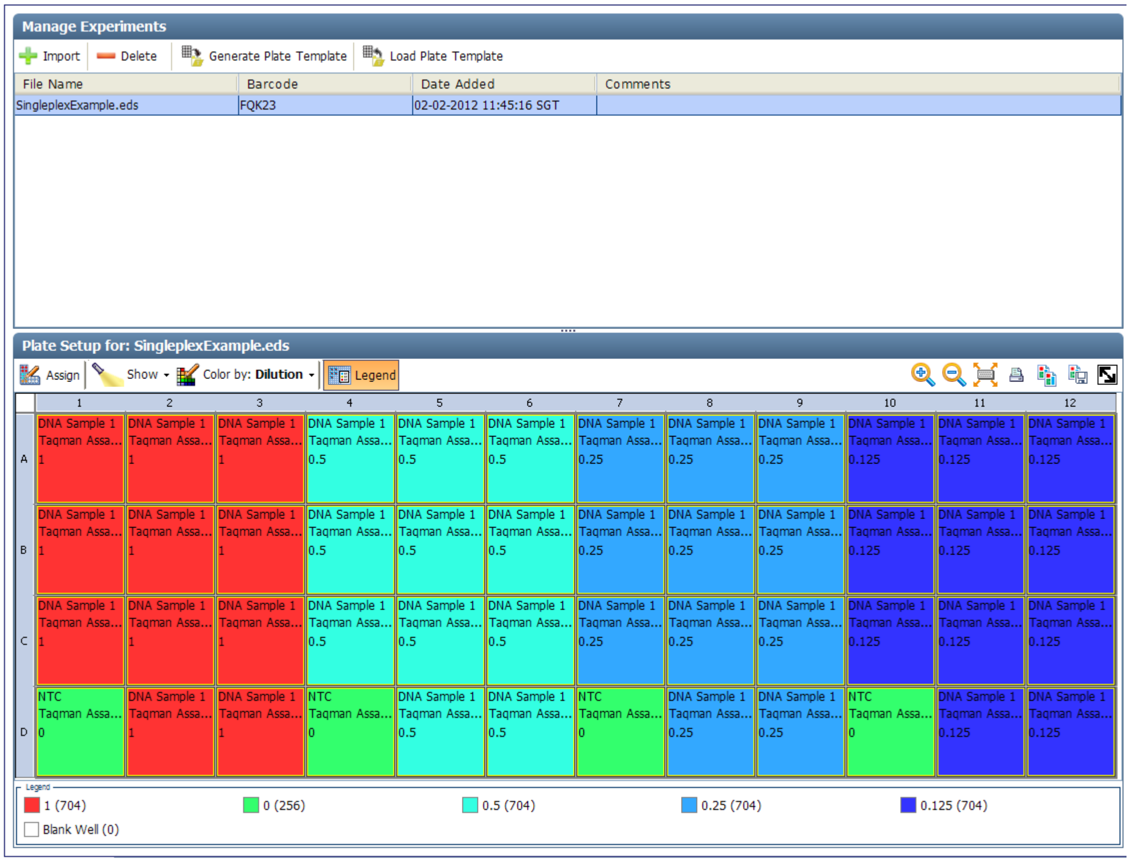
Legend

Click Legend above the plate layout to show or hide the plate layout legend.

In addition, the Plate Setup for: view contains the following tools:

Tool	Function
🔍+	Zoom in to the plate layout
🔍−	Zoom out of the plate layout
⤢	Fit plate layout to window
🖶	Print the current plate layout
⧉	Copy the current plate layout
💾	Save the current plate layout as an image
⤡	View the current plate out in full screen
⤡	Exit full screen view of the plate layout

The Plate Setup screen for an example study is shown in the following image:



File Name	Barcode	Date Added	Comments
SingleplexExample.eds	FQK23	02-02-2012 11:45:16 SGT	

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
B	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
C	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
D	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125

Legend
🟥 1 (704) 🟩 0 (256) 🟦 0.5 (704) 🟦 0.25 (704) 🟦 0.125 (704)
☐ Blank Well (0)

You can prepare the well contents, including samples, targets, and dilutions in the Samples/Targets screen. The samples, targets, and dilutions created in the Samples/Targets screen are populated in the respective drop-down menus of the Well Editor in the Plate Setup screen. You can then select the sample name, target name, and dilutions from the previously-created options. For information on setting up samples, targets, and dilutions in the Samples/Targets screen, see Set up Samples and Targets below.

Set up Samples and Targets

Click **Samples/Targets** from the Setup menu to access the Samples/Targets screen. The Samples/Targets screen is divided into three sections: Manage Samples, Manage Targets, and Manage Dilutions.

Manage Samples

1. Click [Add] to add samples to a study. DNA Sample 1 is the default sample names that appears in the Manage Samples section.
2. *(Optional)* Select a color for the sample from the color drop-down.
3. *(Optional)* Enter comments in the Comments column.
4. Click [Delete] to delete selected samples from a study.

   Note: You cannot delete the sample names that have been assigned to wells in an experiment included in the study.

Note: The number of wells appearing against a sample name is automatically updated as per the assignment in the Plate Setup screen.

Manage Targets

1. Click + Add to add targets to a study. The default target name that appears in the Manage Targets section is TaqMan® Assay 1.
2. Select the Reporter for the target from the Reporter drop-down menu.

   Note: For studies containing singleplex experiments, only FAM™ dye is available in the Reporter drop-down menu. For studies containing duplex experiments, FAM™ dye and VIC® dye are available in the Reporter drop-down menu.
3. *(Optional)* Select a color for the target from the color drop-down.
4. *(Optional)* Enter comments in the Comments column.
5. Click − Delete to delete selected targets from the study. You cannot delete the target names that have been assigned to wells in an experiment included in the study.

Note: The number of wells appearing against a sample name is automatically updated as per the assignment in the Plate Setup screen.

Manage Dilutions

1. Click + Add to add dilutions. The default dilutions that appear in the Manage Dilutions section are 1, 0.1, 0.01, 1E-4, 0.

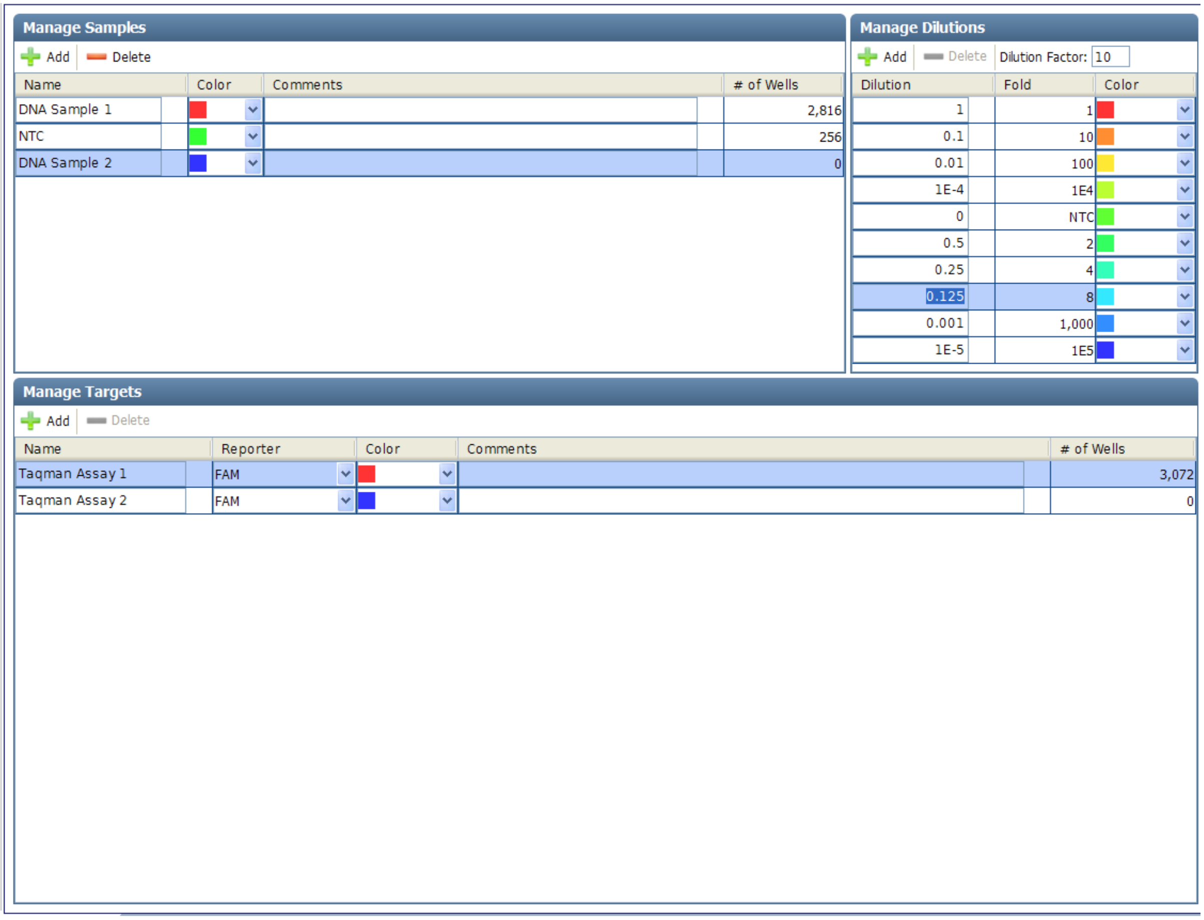
   The DigitalSuite™ Software computes the next dilution based on the dilution factor. Enter a value from 2 to 100. The default dilution factor is 10.

   The corresponding row in the Fold column gets populated with the dilution fold for that particular factor.

   Note: For the dilutions, you can enter numeric values or scientific notations like 1E-3 or 0.5E-4.
2. *(Optional)* Select a color for the dilution from the color drop-down.
3. Click − Delete to delete the selected dilutions from the study.

   Note: You cannot delete the dilutions that have been used in an experiment included in the study.

The Samples/Targets screen for an example study is shown in the following image:



Manage Samples

Name	Color	Comments	# of Wells
DNA Sample 1	🟥		2,816
NTC	🟩		256
DNA Sample 2	🟦		0

Manage Dilutions

Dilution Factor: 10

Dilution	Fold	Color
1	1	🟥
0.1	10	🟧
0.01	100	🟨
1E-4	1E4	🟩
0	NTC	🟩
0.5	2	🟩
0.25	4	🟩
0.125	8	🟦
0.001	1,000	🟦
1E-5	1E5	🟦

Manage Targets

Name	Reporter	Color	Comments	# of Wells
Taqman Assay 1	FAM	🟥		3,072
Taqman Assay 2	FAM	🟦		0

IMPORTANT! You can rename samples, targets, and dilutions after you have analyzed the study. However, the Results, Run QC, and Export options will get disabled until you re-analyze the study.

Analyze a study

You can analyze a study after you have completed plate set up for the experiment(s) in that study. In the absence of plate set up, the Analyze button appears disabled.

Click ▶ Analyze to analyze a study. The DigitalSuite™ Software carries out analysis and displays the analysis results in the Digital PCR screen.

Note: The Results, Run QC, and Export options from the Workflow menu remain disabled until analysis is done.

If the default analysis settings in the DigitalSuite™ Software are not suitable for your own experiment, you can change the settings in the Analysis Settings dialog box, then reanalyze your study. To revert to the default settings, click **Reset to Defaults** at the bottom-left of the Analysis Settings dialog box.

Click Analysis Settings to access the Analysis Settings dialog box.

Analysis settings

Digital PCR settings

Use the Digital PCR Settings tab to change the Confidence Interval, Well Volume, and the Sample Group-Specific Settings.

- **Confidence Interval**: The Confidence Interval is used to calculate the lower and upper confidence values in the Digital PCR screen. For example, for a Confidence Interval value of 95%, the probability of copies/µL falling between the lower and upper confidence values is 95%.
- **Well Volume**: The Well Volume is the actual volume of each through hole in an OpenArray® plate.

  IMPORTANT! This value is provided for reference purpose and should not be changed unless specified.

- **Sample Group-Specific Settings**: This table contains settings for each sample group in the study including the threshold values for the digital call (positive, negative, and undetermined) as well as the threshold values for empty well detection. See "Amplification Plot" on page 40.

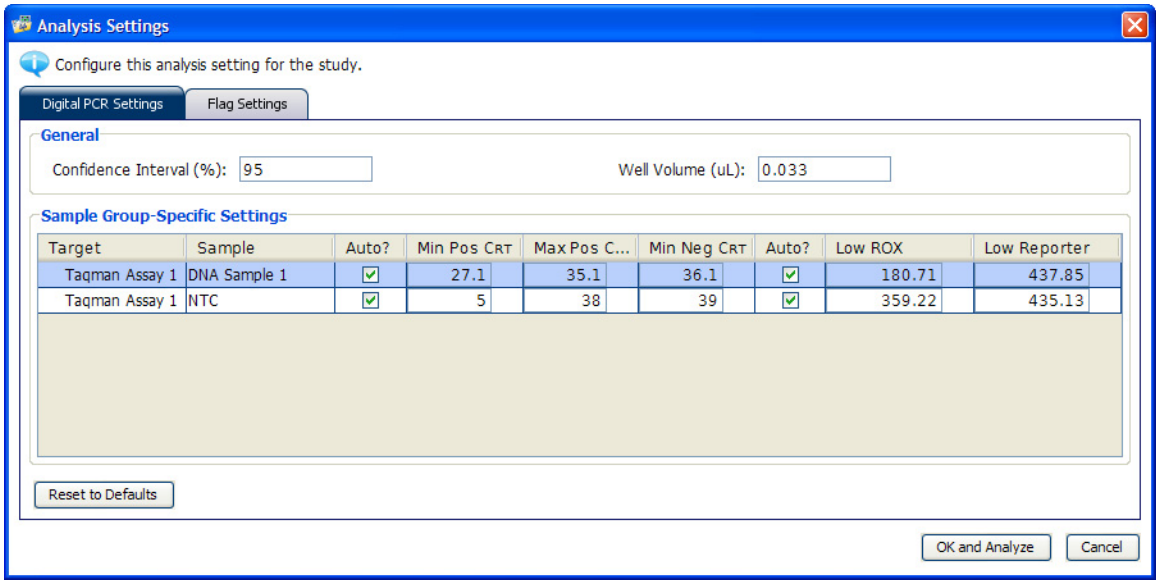
Flag Settings

Use the Flag Settings tab to manually include or exclude a flag and to change the threshold value. The flags present in this table include:

- Low FAM Score
- Low VIC Score

The default threshold value for both the flags is 1.24.

The Analysis Settings dialog box is shown in the following image:



Configure this analysis setting for the study.

General

Confidence Interval (%): 95

Well Volume (uL): 0.033

Sample Group-Specific Settings

Target	Sample	Auto?	Min Pos Crt	Max Pos C...	Min Neg Crt	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.1	35.1	36.1	☑	180.71	437.85
Taqman Assay 1	NTC	☑	5	38	39	☑	359.22	435.13

View the results

The Digital PCR screen displays the analysis results for all the experiments in a study. Irrespective of the experiments, the results are grouped by Samples and Replicates. The screen consists of the Sample Group tab and the Replicate Group tab.

The Replicate Group tab displays results for individual dilutions. The Sample Group tab displays the aggregate results for the entire dilution series.

The Digital PCR screen also displays the Confidence Interval for that study in the title bar of the screen. The default value for the Confidence Interval is 95%. This value can be changed in the Analysis Settings dialog box (see "Analysis settings" on page 31).

Sample Group tab

The Sample Group represents all replicates that are assigned with the same sample and target, regardless of the original plates they belong.

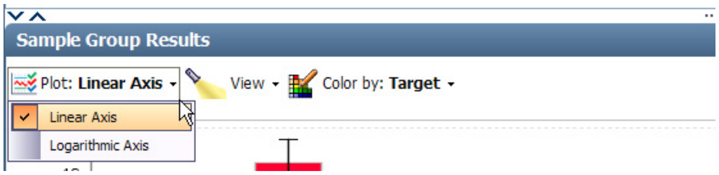
The Sample Group tab table includes the following:

Column	Description
Target	Displays the target name
Sample	Displays the sample name
Copies/µL	Displays the quantity of sample in copies/µL
Lower Confidence Level (Lower Conf)	Displays the lower confidence level for the quantity of sample in copies/µL
Upper Confidence Level (Upper Conf)	Displays the upper confidence level for the quantity of sample in copies/µL
⊕	Displays total number of positive calls **Note:** Positive calls mean the DigitalSuite™ Software determines a well containing at least one copy of the sample.

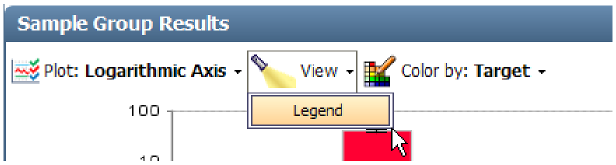
Column	Description
⛔	Displays total number of negative calls **Note:** Negative calls mean the DigitalSuite™ Software determines that a well does not contain any copy of the sample.
❓	Displays total number of undetermined calls. **Note:** Undetermined calls are those that occur between the positive and negative range as well as beyond the positive range on the histogram plot.
# of Positive + Negative (# of Pos+Neg)	Displays the combined number of wells having the positive and negative calls
# of Omitted	Displays the number of wells omitted from the study
# of Empty	Displays the number of empty wells **Note:** Empty wells are those that are flagged with Low ROX, Low FAM, or Low VIC.

On selecting the Sample Group tab, the corresponding Sample Group Results are displayed in the form of a bar graph, with the Sample Group on the X-axis and Copies/µL on the Y-axis, in the lower-half of the Digital PCR screen.

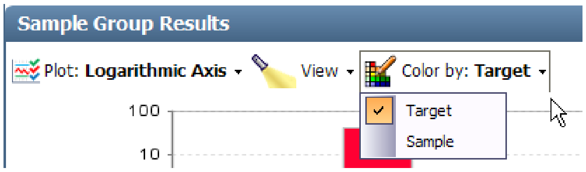
1. To view the plot type, click **Plot:** and select Linear Axis or Logarithmic Axis from the Plot drop-down menu.



2. Click **View** ▸ **Legend** to show or hide the plot legend.



3. Click **Color by:** and select Target or Sample from the Color by: drop-down menu to assign the bars on the graph with the target or sample color.

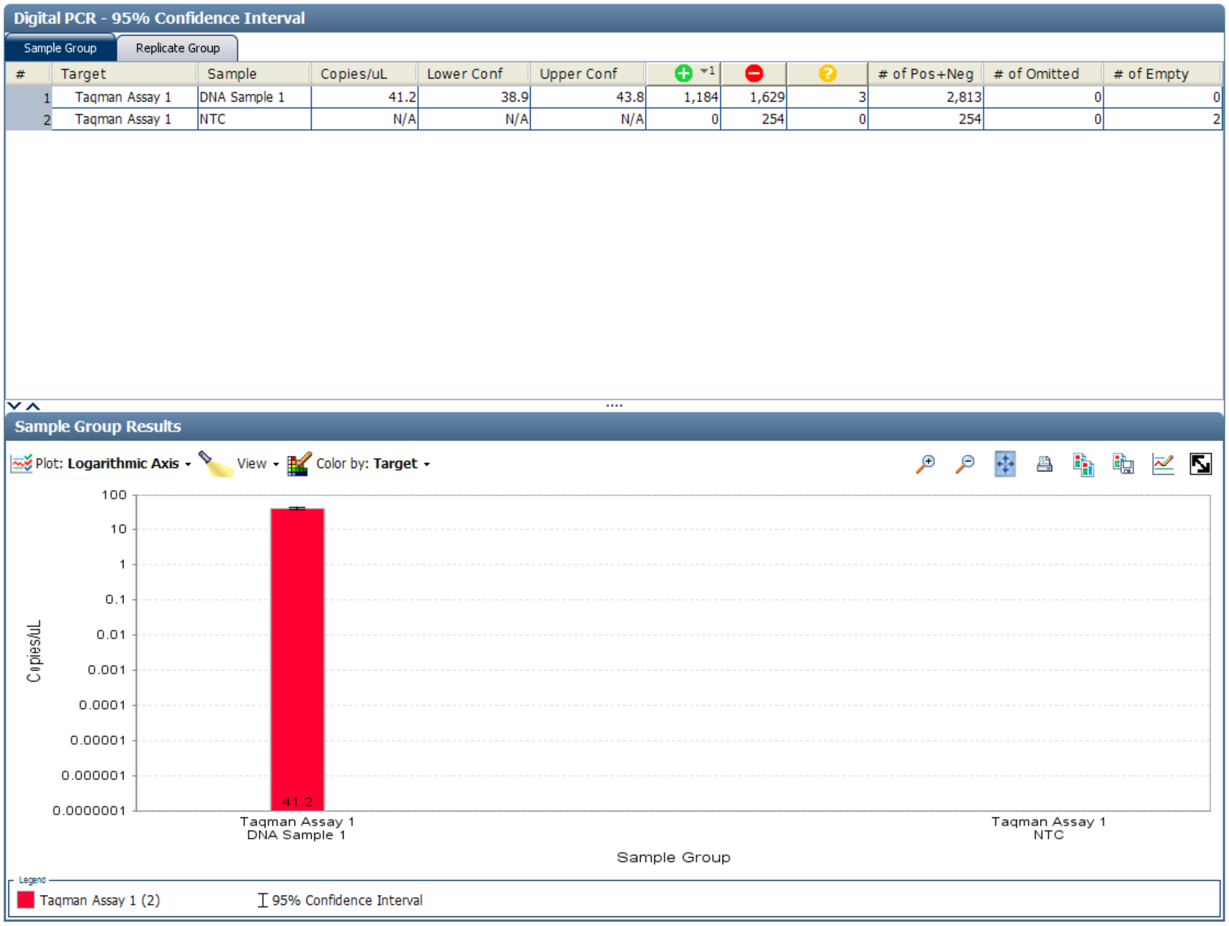


4. Move the cursor over the bars in the graph to view the values for Copies/µL, Lower Confidence, and Upper Confidence.

5. To view only specific bars, select the respective rows in the table in the upper half of the screen.

   Note: To de-select a row, click on that row while holding down the Ctrl key.

The Digital PCR Sample Group screen is shown in the following image:



Digital PCR - 95% Confidence Interval

Sample Group | Replicate Group

#	Target	Sample	Copies/uL	Lower Conf	Upper Conf	⊕ ▾1	⊖	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	41.2	38.9	43.8	1,184	1,629	3	2,813	0	0
2	Taqman Assay 1	NTC	N/A	N/A	N/A	0	254	0	254	0	2

Sample Group Results

Replicate Group tab

The Replicate Group represents all replicates that are assigned with the same sample, target, and dilution regardless of the original plates they belong.

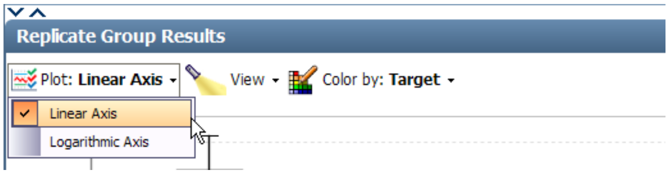
The Replicate Group tab table includes the following:

Column	Description
Target	Displays the target name
Sample	Displays the sample name
Dilution	Displays the dilution for that group
Copies/µL	Displays the number of replicate group copies present per µL
Lower Confidence Level (Lower Conf)	Displays the lower confidence level for the number of copies present per µL
Upper Confidence Level (Upper Conf)	Displays the upper confidence level for the number of copies present per µL
⊕	Displays total number of positive calls **Note:** Positive calls mean the DigitalSuite™ Software determines a well containing at least one copy of the sample.

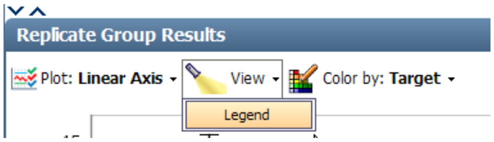
Column	Description
⛔	Displays total number of negative calls **Note:** Negative calls mean the DigitalSuite™ Software determines that a well does not contain any copy of the sample.
❓	Displays total number of undetermined calls. **Note:** Undetermined calls are those that occur between the positive and negative range as well as beyond the positive range on the histogram plot.
# of Positive + Negative (# of Pos+Neg)	Displays the combined number of wells having the positive and negative calls
# of Omitted	Displays the number of wells omitted from the study
# of Empty	Displays the number of empty wells **Note:** Empty wells are those that are flagged with Low ROX, Low FAM, or Low VIC.

On selecting the Replicate Group tab, the corresponding Replicate Group Results are displayed in the form of a bar graph, with the Replicate Group on the X-axis and Copies/µL on the Y-axis, in the lower-half of the Digital PCR screen.

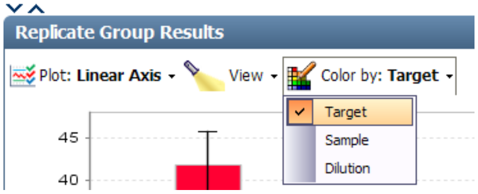
1. To view the plot type, click **Plot:** and select Linear Axis or Logarithmic Axis from the Plot drop-down menu.



2. Click **View** to show or hide the plot legend.



3. Click **Color by:** and select Target, Sample, or Dilution from the Color by: drop-down menu to assign the bar graph with the target, sample, or dilution color.

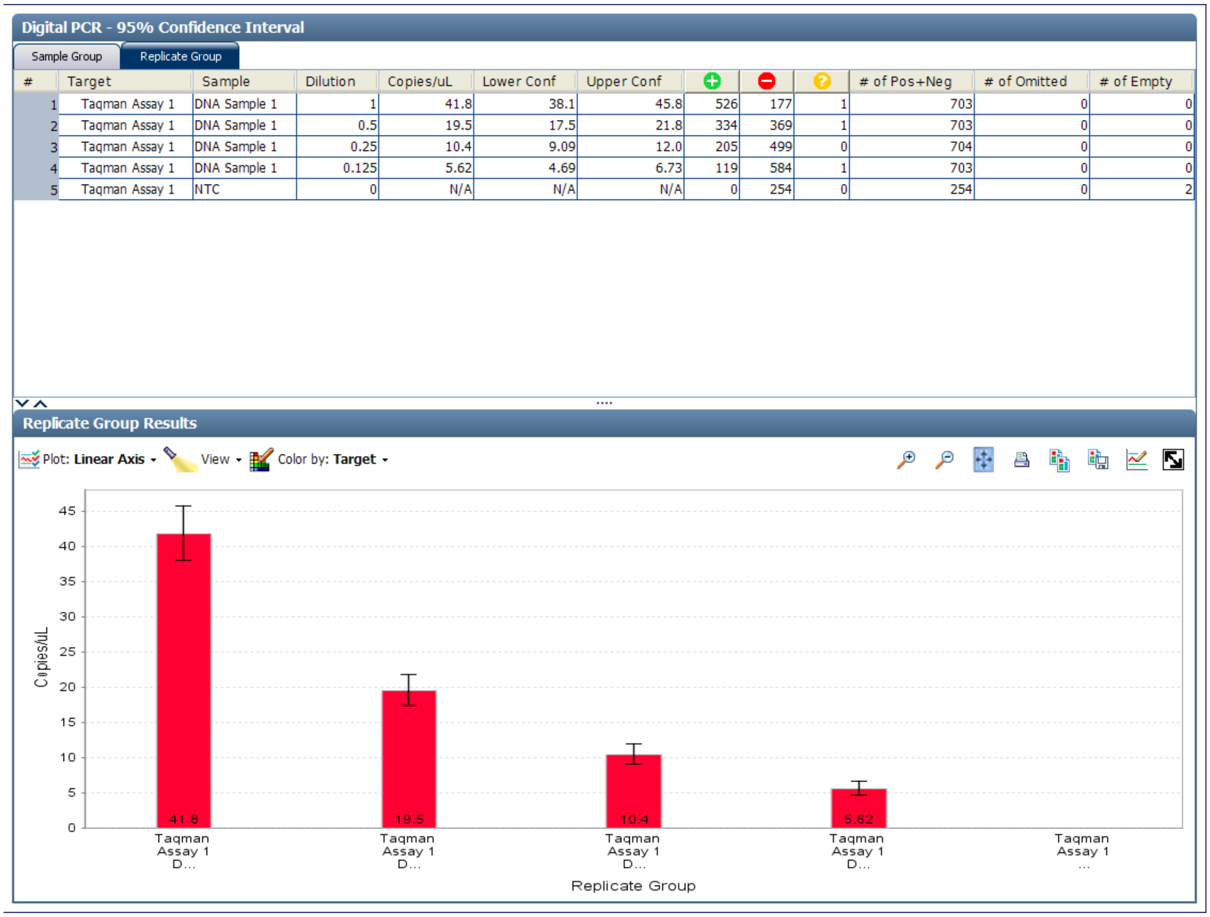


4. Move the cursor over the bars in the graph to view the values for Copies/µL, Lower Confidence, and Upper Confidence.

5. To view only specific bars, select the respective rows in the table in the upper half of the screen.

   Note: To de-select a row, click on that row while holding down the Ctrl key.

The Digital PCR Replicate Group screen is shown in the following image:



#	Target	Sample	Dilution	Copies/uL	Lower Conf	Upper Conf	⊕	⊖	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	1	41.8	38.1	45.8	526	177	1	703	0	0
2	Taqman Assay 1	DNA Sample 1	0.5	19.5	17.5	21.8	334	369	1	703	0	0
3	Taqman Assay 1	DNA Sample 1	0.25	10.4	9.09	12.0	205	499	0	704	0	0
4	Taqman Assay 1	DNA Sample 1	0.125	5.62	4.69	6.73	119	584	1	703	0	0
5	Taqman Assay 1	NTC	0	N/A	N/A	N/A	0	254	0	254	0	2

In addition to the tools mentioned above:

- The Sample Group Results and Replicate Group Results plot view includes , , , and to zoom in, zoom out, to fit data in window, and to view data in full screen respectively.
- Click , , and to print, copy, and save the plot as an image respectively.
- Click to access and edit the Plot Properties such as the font and color of the plot text, and the labels on the X axis and Y Axis. Edit the settings under the General, X Axis, and Y Axis tab.
  - Click the General tab to edit the plot title text, font, or color. You can also select whether to show the plot title.
  - 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.
  - Click OK.

(Optional) Perform QC on results data

You can perform a quality check on the digital PCR data and conduct further analysis, if necessary. Use Heat Maps, Amplification Plots, and Flag Summary from the Run QC Workflow.

Heat Map

The heat map provides you with an overview of all the wells in the same plate. By default, the Heat Map is colored by 'Call'. The green wells represent positive calls while the black wells represent negative calls. The grey wells represent undetermined calls. The heat map allows you to check for:

- The density of the positive and negative calls for quick visual review with respect to the dilution series.
- Amplification in wells labelled as NTC. NTC wells are supposed to be negative.
- Sample filling problems or suspected contamination in the plate through abnormal density patterns.
- Flagged wells. Conduct further investigation of flagged wells in the Amplification Plot screen.

  Note: Flagged wells are marked with a △ triangle in the affected well.

View the Heat Map

Go to **Run QC** ▸ **Heat Map** to access the Heat Map screen.

The Heat Map screen includes the Experiments view in the upper half and the Heat Map for view in the lower half of the screen.

The upper half of the Heat Map screen lists the experiments included in the study while the lower half displays the corresponding heat map for the experiment selected in the Experiments (upper half) view.

Experiments

The Experiments view lists all the experiments included in a study. The Experiments view table includes:

Column	Description
Bookmark	Indicates whether there are bookmarked wells in the experiment. **Note:** • To bookmark a well, select one or more wells in the plate layout right-click and select **Add Bookmark** from the drop-down menu • Select **Select all bookmarks** to select all the bookmarked wells in the current plate • Select **Clear selected bookmarks** to clear bookmarks from the selected wells • Select **Clear all bookmarks** to clear all bookmarked wells off the bookmark **IMPORTANT!** Bookmarks are lost once you close a study.
File Name	Displays the file name of an experiment in the study.

Column	Description
Barcode	Displays the barcode of the OpenArray® plate on which the dPCR experiment has been run.
ROX	Displays the number of wells with low ROX signal.
FAM	Displays the number of wells with low FAM™ dye signal.
VIC	Displays the number of wells with low VIC® dye signal (only for duplex experiments). For singleplex experiments, this column displays N/A.
F t	Displays the number of wells with a FAM™ dye score that is lower than the threshold value specified in the Analysis settings.
V t	Displays the number of wells with a VIC® dye score that is lower than the threshold value specified in the Analysis settings (only for duplex experiments). For singleplex experiments, this column displays N/A.

Heat Map for (experiment name):

The Heat Map For: view displays the heat map for the experiment file selected in the Experiments view. The tools on this view include:

Tool	Description
F FAM	Displays the FAM™ dye signal in wells.
V VIC	Displays the VIC® dye signal in wells (only for duplex experiments). This button appears disabled for singleplex experiments.
Show	Drop-down menu that enables you the option to show flags in wells.
Color by: **Call**	Drop-down menu that enables you options to color the cells using different data elements. Select **Call**, **C$_{RT}$**, **Sample**, **Target**, or **Dilution**.
Omission	Drop-down menu that enables you to omit flagged or contaminated wells. You can copy the result from A1 sub-array to other sub-arrays as well as apply to other plates. Select **Copy A1 to all sub-arrays (all dyes)** or **Apply to all plates (all dyes)**
Legend	Toggle button. Shows or hides the plate legend below the heat map.

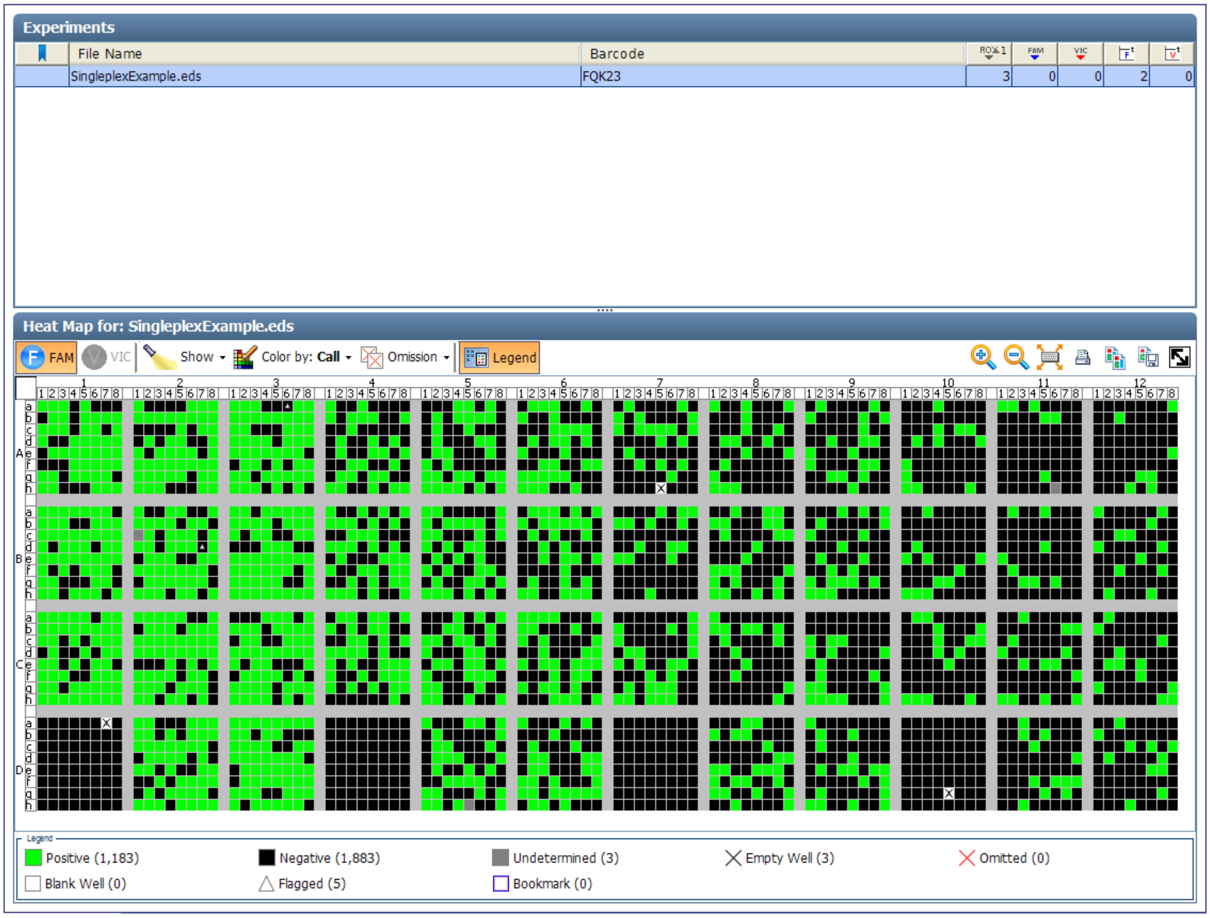
To omit a particular well(s), select the well(s) in the plate layout, right-click on the selection, and select **Omit** from the drop-down menu.

To include an omitted well(s), select the omitted well(s) in the plate layout, right-click on the well(s), and select **Include** from the drop-down menu.

In addition, the Heat Map for: view contains the following tools:

Tool	Function
🔍	Zoom in to the heat map
🔎	Zoom out of the heat map
⛶	Fit heat map to window
🖶	Print the current heat map
⧉	Copy the current heat map
💾	Save the current heat map as an image
⤢	View the current heat map in full screen
⤡	Exit full screen view of the heat map

The Heat Map for an example study is shown in the following image:



Experiments

	File Name	Barcode	ROX 1	FAM	VIC	F	V
	SingleplexExample.eds	FQK23	3	0	0	2	0

Heat Map for: SingleplexExample.eds

Legend

- 🟩 Positive (1,183)
- ■ Negative (1,883)
- ▨ Undetermined (3)
- ✕ Empty Well (3)
- ✕ Omitted (0)
- ☐ Blank Well (0)
- △ Flagged (5)
- ☐ Bookmark (0)

Note: Bookmarks are applied at the Replicate level For duplex experiments, bookmarking wells for one reporter dye does not bookmark the same well with the second reporter dye. To bookmark the same set of wells with the second reporter dye.

1. Go to **Run QC** ▶ **Heat Map**.
2. In the Heat Map, right-click and select **Select all bookmarks** from the menu to select all bookmarked wells.
3. Click the other reporter bye button in the tool bar to switch to the Heat Map for that reporter dye.

   IMPORTANT! Do not left-click anywhere in the heat map at this point as it will clear the well selection.

4. With the wells still selected, right-click on the wells, and select **Add bookmark** from the menu to add the bookmark for the same set of wells for the second reporter dye.

   You can now view the bookmarked wells for the second reporter dye in the well table in the Amplification Plot screen.

Amplification Plot

The Amplification Plot of a study displays the amplification curve for wells grouped by Target, Target and Sample (Sample Group), or Target, Sample, and Dilution (Replicate Group). Two types of amplification plots are available:

- **ΔR vs. Cycle** – ΔR is the baseline adjusted 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 absolute fluorescence signal from the reporter dye. This plot displays R as a function of cycle number. Use this plot to identify and examine irregular amplification.

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

Note: For the Sample Group view, there is an additional plot, ROX vs. Reporter Dye available.

Purpose

The purpose of viewing the amplification plot in a study is to:

- Review analysis results, including C_{RT} value; Amp Score; Positive, Negative or Undetermined calls, and empty wells detection
- Review the Histogram of the C_{RT} values for the selected Sample Group
- Adjust the thresholds for making positive, negative, or undetermined calls for the selected Sample Group
- Evaluate the quality of the amplification curve, especially those wells containing flags and if necessary, omit those
- Manually change calls or empty wells detection results

View the Amplification Plot

The Amplification Plot screen include three views:

- Targets

- Sample Group
- Replicate Group

Target view

The Target table lists the targets in the study. The Targets table includes the following columns:

Column	Description
Bookmark	Indicates whether a target is present in a bookmarked well. To bookmark a well: • On the Heat Map screen, select one or more wells in the plate layout right-click and select **Add Bookmark** from the drop-down menu or • On the Amplification Plot screen, in the Well Table, select **Add Bookmark** from the Bookmark drop-down menu or from the right-click drop-down menu
Target	Displays the target name.
ROX ▾	Displays the number of wells with low ROX signal.
FAM ▾	Displays the number of wells with low FAM™ dye signal.
VIC ▾	Displays the number of wells with low VIC® dye signal (only for duplex experiments). For singleplex experiments, this column displays N/A.
F t	Displays the number of wells with a FAM™ dye score that is lower than the threshold value specified in the Analysis settings.
V t	Displays the number of wells with a VIC® dye score that is lower than the threshold value specified in the Analysis settings (only for duplex experiments). For singleplex experiments, this column displays N/A.

The lower half of the Targets view displays the plot view and well table. Use the >, <, ⌄ , and ⌃ buttons to extend the views horizontally or vertically.

You can select wells in the plot view or the well table. In the plot view, select wells by clicking in the plot and dragging a rubber-band box. In the well table, select wells by selecting the respective rows. Wells selected in the plot view will appear selected in the well table and vice-versa. In the plot view, selected wells will appear colored, while unselected wells will be hidden. To view unselected wells, go to **View ▸ Show Unselected**. The unselected wells will appear in grey color in the plot view. If no wells are selected, the plot view displays all the wells in color.

The Plot View includes the following the tools:

Tool	Description
Plot ▾	Drop-down menu that enables you to select from ΔR vs. Cycle (Linear), ΔR vs. Cycle (Log), R vs. Cycle (Linear), and R vs. Cycle (Log) plot types

Tool	Description
View ▾	Drop-down menu that enables you to show and hide the following: • Legend • Show Unselected • C_{RT} • Show Omitted **Note:** The C_{RT} for a given amplification curve is indicated by a red triangular icon on the curve
Color by: **Call** ▾	Drop-down menu that enables you options to color the plot using different data elements. Select **Bookmarks**, **Targets**, **Wells**, or **Call**

In addition to the tools mentioned above:

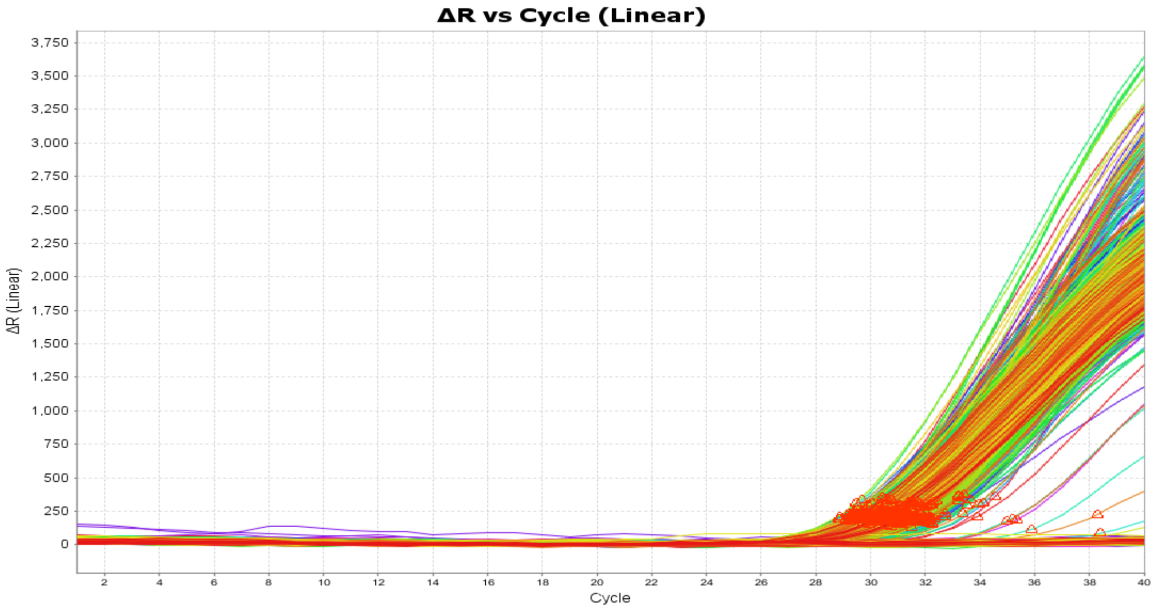
- The Targets plot view includes 🔍, 🔍, ✥, and ⛶ to zoom in, zoom out, to fit data in window, and to view data in full screen respectively.

  Note: To zoom into a particular area of the amplification plot, click at the center of the area of interest then click 🔍.

- Click 🖨, 📋, and 💾 to print, copy, and save the plot as an image respectively.

- Click 📈 to access and edit the Plot Properties.

The Plot view for the Targets tab of an example study is shown in the following image:



The Well Table includes the following tools:

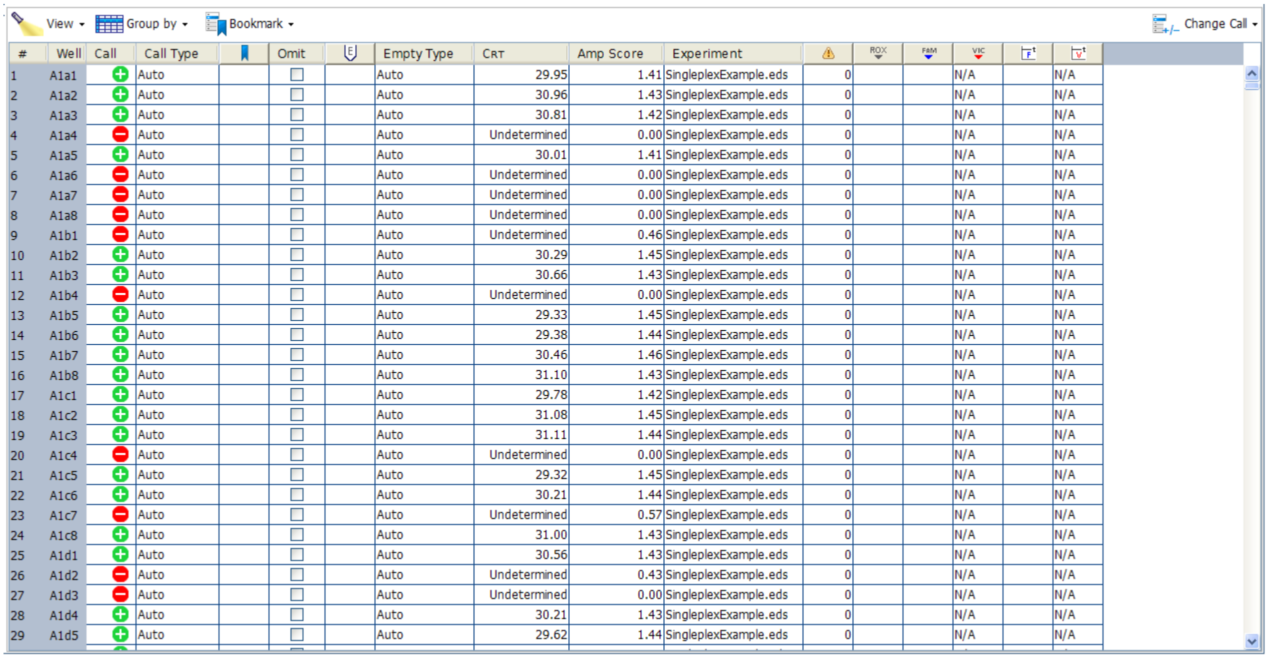
Tool	Description
View	Drop-down menu that allows you to show or hide columns in a table. Select or Deselect the following: • **Call** • **Call Type** • **Bookmark** • **Omit** • **Empty Well Flag** • **Empty Type** • **C_{RT}** • **Amp Score** • **Experiment** • **Flag indicator** • **Flags** – **Low ROX** – **Low FAM** – **Low VIC** – **Low FAM Score** – **Low VIC Score** **Note:** Wells with undetermined calls are not flagged for Low FAM Score or Low VIC Score even if their Amp Score value is less than threshold.
Group by	Drop-down menu that enables you to group the targets by experiments or by none. Select from: • **None** • **Experiment** • **Expand All** • **Collapse All** **Note:** The Expand All and Collapse All options are enabled only when Experiment is selected.
Bookmark	Drop-down menu that enables you add, select, and clear bookmarked wells. Select from **Add Bookmark**, **Select all bookmarks**, **Clear selected bookmark**, **Clear all bookmarks**.
Change Call	Drop-down menu that enables you to manually change the call for a well(s). Select **Positive**, **Negative** or **Undetermined**. You can also use this drop-down menu to Clear Manual Call, Mark as Empty Well, Mark as Non-Empty Well, Auto Empty Detection. **Note:** When you manually change the call for a well(s), the call will remain unchanged even after you re-analyze the study.

The following table provides definitions for the column headings that appear in the tables in the Targets tab:

Column heading	Description
Well	Displays the Well location
Call	Displays the call in the well (Positive, Negative, or Undetermined) **Note:** Positive calls are indicated by ⊕, Negative calls are indicated by ⊖, and Undetermined calls are indicated by ?
Call Type	Whether the call is Auto or has been changed manually
Bookmark	Indicates if the well is bookmarked
Omit	Includes a check box to omit a well
Empty Type	Whether the well has been labelled empty automatically or manually
C_{RT}	C_{RT} value for a well
Amp Score	Amp Score value for a well
Experiment	Name of the experiment to which the target belongs
⚠	Flag Indicator. Displays the number of flags in a well
ROX ▼	The icon displayed if the well is flagged with Low ROX
FAM ▼	The icon displayed if the well is flagged with Low FAM
VIC ▼	The icon displayed if the well is flagged with Low VIC (only for duplex experiments). For singleplex experiments, this column displays N/A
F t	The icon displayed if the well is flagged with Low FAM Score
V t	The icon displayed if the well is flagged with Low VIC Score

Note: You can sort the table columns by clicking once on the column header. You can change the sorting order with a second click. A third click will clear the sorting order. You can sort the table columns at multiple levels. For example, click the Call column to sort the columns by Call, then hold down the Ctrl key and click the C_{RT} column to sort by C_{RT} the wells with the same call.

The Well Table view for the Targets tab of an example study is shown in the following image:



#	Well	Call	Call Type	🔖	Omit	E	Empty Type	CRT	Amp Score	Experiment	⚠	ROX	FAM	VIC	F	V
1	A1a1	⊕	Auto		☐		Auto	29.95	1.41	SingleplexExample.eds	0			N/A		N/A
2	A1a2	⊕	Auto		☐		Auto	30.96	1.43	SingleplexExample.eds	0			N/A		N/A
3	A1a3	⊕	Auto		☐		Auto	30.81	1.42	SingleplexExample.eds	0			N/A		N/A
4	A1a4	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
5	A1a5	⊕	Auto		☐		Auto	30.01	1.41	SingleplexExample.eds	0			N/A		N/A
6	A1a6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
7	A1a7	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
8	A1a8	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
9	A1b1	⊖	Auto		☐		Auto	Undetermined	0.46	SingleplexExample.eds	0			N/A		N/A
10	A1b2	⊕	Auto		☐		Auto	30.29	1.45	SingleplexExample.eds	0			N/A		N/A
11	A1b3	⊕	Auto		☐		Auto	30.66	1.43	SingleplexExample.eds	0			N/A		N/A
12	A1b4	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
13	A1b5	⊕	Auto		☐		Auto	29.33	1.45	SingleplexExample.eds	0			N/A		N/A
14	A1b6	⊕	Auto		☐		Auto	29.38	1.44	SingleplexExample.eds	0			N/A		N/A
15	A1b7	⊕	Auto		☐		Auto	30.46	1.46	SingleplexExample.eds	0			N/A		N/A
16	A1b8	⊕	Auto		☐		Auto	31.10	1.43	SingleplexExample.eds	0			N/A		N/A
17	A1c1	⊕	Auto		☐		Auto	29.78	1.42	SingleplexExample.eds	0			N/A		N/A
18	A1c2	⊕	Auto		☐		Auto	31.08	1.45	SingleplexExample.eds	0			N/A		N/A
19	A1c3	⊕	Auto		☐		Auto	31.11	1.44	SingleplexExample.eds	0			N/A		N/A
20	A1c4	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
21	A1c5	⊕	Auto		☐		Auto	29.32	1.45	SingleplexExample.eds	0			N/A		N/A
22	A1c6	⊕	Auto		☐		Auto	30.21	1.44	SingleplexExample.eds	0			N/A		N/A
23	A1c7	⊖	Auto		☐		Auto	Undetermined	0.57	SingleplexExample.eds	0			N/A		N/A
24	A1c8	⊕	Auto		☐		Auto	31.00	1.43	SingleplexExample.eds	0			N/A		N/A
25	A1d1	⊕	Auto		☐		Auto	30.56	1.43	SingleplexExample.eds	0			N/A		N/A
26	A1d2	⊖	Auto		☐		Auto	Undetermined	0.43	SingleplexExample.eds	0			N/A		N/A
27	A1d3	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
28	A1d4	⊕	Auto		☐		Auto	30.21	1.43	SingleplexExample.eds	0			N/A		N/A
29	A1d5	⊕	Auto		☐		Auto	29.62	1.44	SingleplexExample.eds	0			N/A		N/A

Sample Group view

The Sample Group table lists the sample groups (replicates assigned with the same sample and target) in the study. The Sample Group table includes the following columns:

Column	Description
Bookmark	Indicates whether a target is present in a bookmarked well. To bookmark a well: • On the Heat Map screen, select one or more wells in the plate layout right-click and select **Add Bookmark** from the drop-down menu or • On the Amplification Plot screen, in the Well Table, select **Add Bookmark** from the Bookmark drop-down menu or from the right-click drop-down menu
Target	Displays the target name.
Sample	Displays the sample name.
Copies/µL	Displays the number of copies of the sample group per µL.
⊕	Displays total number of positive calls. **Note:** Positive calls mean the DigitalSuite™ Software determines a well containing at least one copy of the sample.
⊖	Displays total number of negative calls. **Note:** Negative calls mean the DigitalSuite™ Software determines that a well does not contain any copy of the sample.

Column	Description
?	Displays total number of undetermined calls. **Note:** Undetermined calls are those that occur between the positive and negative range as well as beyond the positive range on the histogram plot.
# of Positive + Negative (# of Pos+Neg)	Displays the combined number of wells having the positive and negative calls.
# of Omitted	Displays the number of wells omitted from the study.
# of Empty	Displays the number of empty wells. **Note:** Empty wells are those that are flagged with Low ROX, Low FAM, or Low VIC flags.
ROX ▼	Displays the number of wells with low ROX signal.
FAM ▼	Displays the number of wells with low FAM™ dye signal.
VIC ▼	Displays the number of wells with low VIC® dye signal (only for duplex experiments). For singleplex experiments, this column displays N/A.
F t	Displays the number of wells with a FAM™ dye score that is lower than the threshold value specified in the Analysis settings.
V t	Displays the number of wells with a VIC® dye score that is lower than the threshold value specified in the Analysis settings (only for duplex experiments). For singleplex experiments, this column displays N/A.

The lower half of the Sample Group view displays the plot view and well table. Use the >, <, ⌄ , and ⌃ buttons to extend the views horizontally or vertically.

You can select wells in the plot view or the well table. In the plot view, select wells by clicking in the plot and dragging a rubber-band box. In the well table, select wells by selecting the respective rows. Wells selected in the plot view will appear selected in the well table and vice-versa. In the plot view, selected wells will appear colored, while unselected wells will be hidden. To view unselected wells, go to **View ▸ Show Unselected**. The unselected wells will appear in grey color in the plot view. If no wells are selected, the plot view displays all the wells in color.

The Plot View includes the following tools:

Tool	Description
Plot ▾	Drop-down menu that enables you to select from ΔR vs. Cycle (Linear), ΔR vs. Cycle (Log), R vs. Cycle (Linear), R vs. Cycle (Log), and ROX vs. Reporter Dye plot types.

Tool	Description
View ▾	Drop-down menu that enables you to show and hide the following: • C_{RT} Range **Note:** C_{RT} Range includes two ranges, the positive range and negative range. The positive range is colored in green in the Histogram plot. If the well C_{RT} falls within this range, the well will be called Positive. The negative range is colored red in the Histogram plot. If the well C_{RT} falls within this range, the well will be called Negative. • Histogram **Note:** The Histogram shows the distribution of the C_{RT} values for a given Sample Group. Each bar in the Histogram represents the number of wells whose C_{RT} values falls between a small range of 0.1 such as 30.1-30.2. • No Amplification Bar **Note:** The No Amplification Bar counts the number of wells for undetermined C_{RT}. This option is enabled only when you select the Histogram option. • Legend • Show Unselected • C_{RT} • Show Omitted
Color by: **Call** ▾	Drop-down menu that enables you options to color the plot using different data elements. Select **Bookmarks**, **Targets**, **Wells**, or **Call**.

In addition to the tools mentioned above:

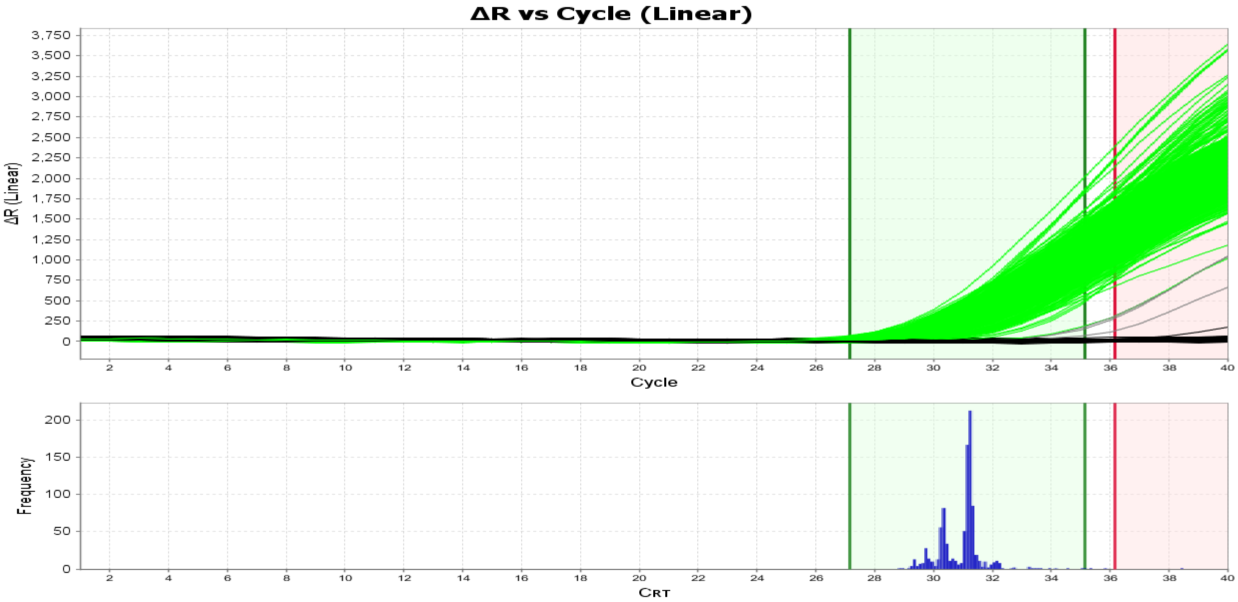
- The Targets plot view includes , , , and to zoom in, zoom out, to fit data in window, and to view data in full screen respectively.

  Note: To zoom into a particular area of the amplification plot, click at the center of the area of interest then click .

- Click , , and to print, copy, and save the plot as an image respectively.

- Click to access and edit the Plot Properties.

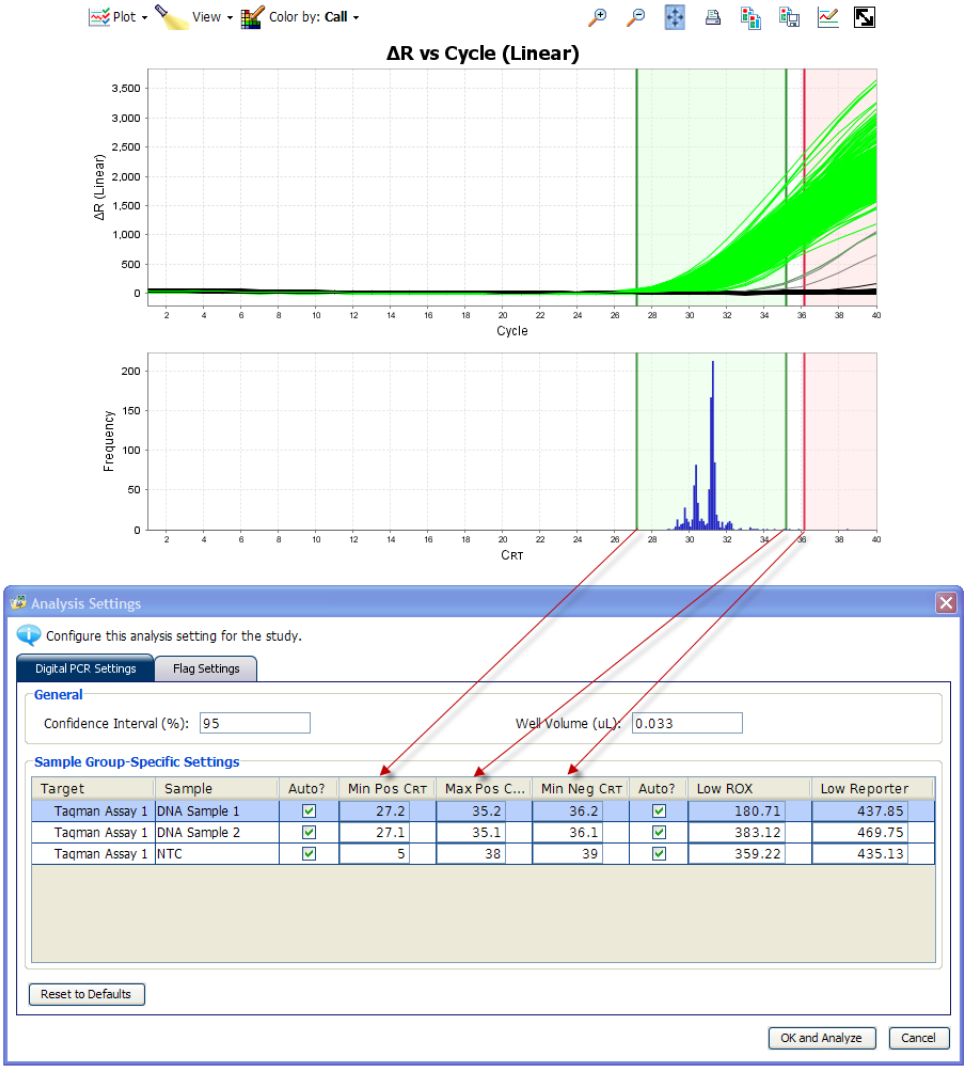
The Plot view for the Sample View tab of an example study is shown in the following image:



Adjusting the C_{RT} range

You can hold the cursor over the bars in the Histogram plot to view the positive and negative C_{RT} range (the green and red shaded regions in the Amplification Plot or Histogram Plot).

The initial C_{RT} range is automatically detected based on the Histogram. However, you can manually change the C_{RT} range by dragging the edge of the shaded region to manually adjust the C_{RT} range. DigitalSuite™ Software does not allow overlapping of the positive and negative C_{RT} range. When you manually change the C_{RT} range, the value gets updated in the Min Pos C_{RT}, Max Pos C_{RT}, and Min Neg C_{RT} columns in the Analysis Settings dialog box.



Configure this analysis setting for the study.

General

Confidence Interval (%): 95

Well Volume (uL): 0.033

Sample Group-Specific Settings

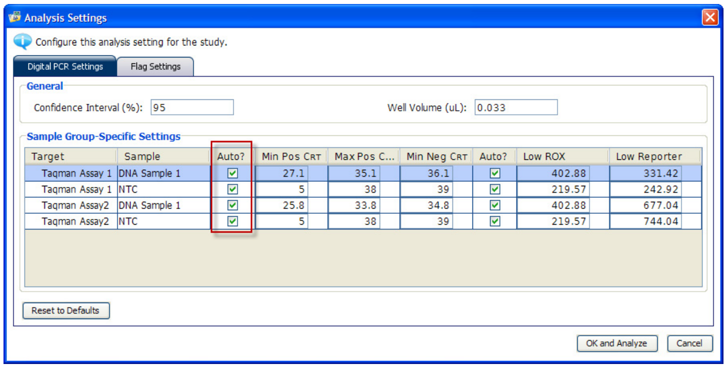
Target	Sample	Auto?	Min Pos CRT	Max Pos C...	Min Neg CRT	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.2	35.2	36.2	☑	180.71	437.85
Taqman Assay 1	DNA Sample 2	☑	27.1	35.1	36.1	☑	383.12	469.75
Taqman Assay 1	NTC	☑	5	38	39	☑	359.22	435.13

Alternatively, the threshold values are also available and can be changed directly in the Analysis settings dialog box (see "Analysis settings" on page 31).

To revert to automatic C_{RT} range:

1. Click Analysis Settings to access the Analysis Settings dialog box.
2. In Sample Group-Specific Settings find the corresponding Sample Group.

3. Select the check box for the Auto column appearing to the right of the Sample column.



General

Confidence Interval (%): 95

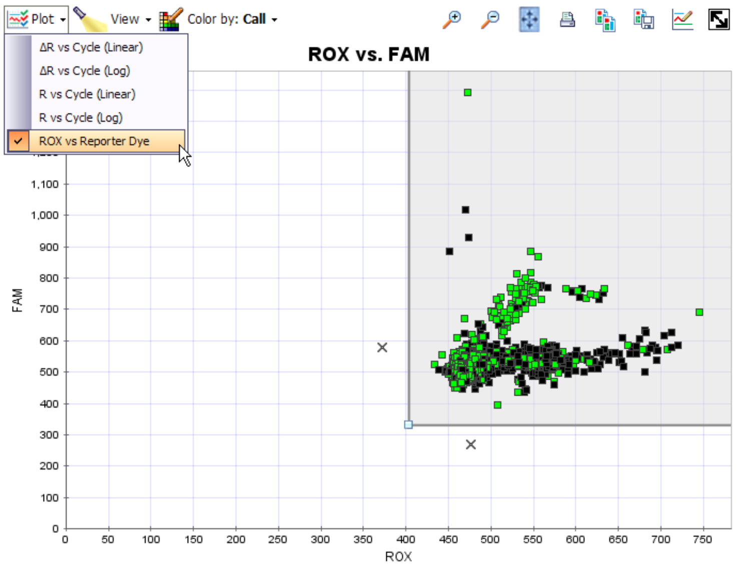
Well Volume (uL): 0.033

Sample Group-Specific Settings

Target	Sample	Auto?	Min Pos CRT	Max Pos C...	Min Neg CRT	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.1	35.1	36.1	☑	402.88	331.42
Taqman Assay 1	NTC	☑	5	38	39	☑	219.57	242.92
Taqman Assay2	DNA Sample 1	☑	25.8	33.8	34.8	☑	402.88	677.04
Taqman Assay2	NTC	☑	5	38	39	☑	219.57	744.04

Adjusting the empty well threshold

You can adjust the empty well threshold in the ROX vs. Reporter Dye plot from the Plot drop-down menu. The ROX vs. Reporter Dye Plot plots the ROX dye signal on the X-axis against the Reporter Dye (FAM™ dye or VIC® dye) signal on the Y-axis. The reporter dye value for a given well is taken from the median value of the first five cycles for that well.



The empty wells are seen as '×' in the ROX vs. Reporter Dye plot, while non-empty wells as seen as colored boxes. By default, the wells are colored by their Calls.

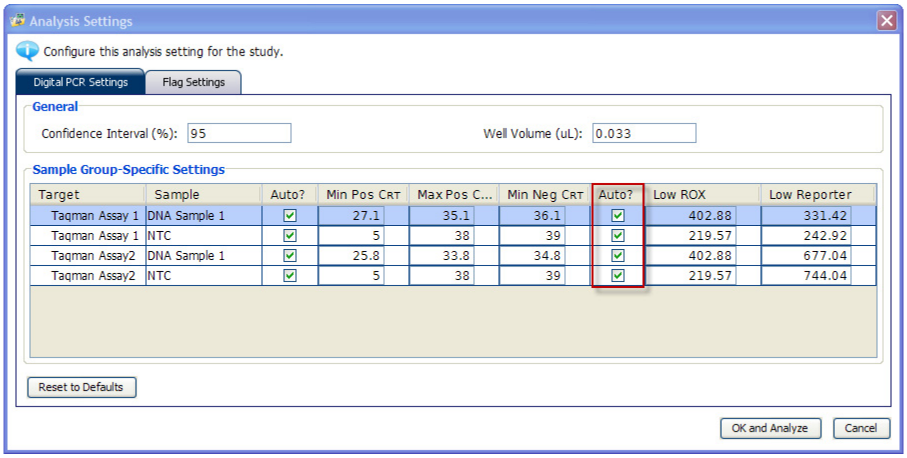
The grey shaded area on this plot indicates the region containing non-empty wells. The left edge of the shaded area is the threshold for Low ROX and the bottom edge of the shaded ares is the threshold for the Reporter Dye.

The initial grey area on the ROX vs. Reporter Dye plot is automatically detected based on the distribution of all the wells on the plot for the given Sample Group. The wells that have ROX or reporter signals significantly lower than other wells, will be flagged as Low ROX, Low FAM or Low VIC accordingly; and subsequently flagged as empty wells. However, you can adjust the area by manually dragging the lower-left corner of the shaded area.

The Threshold values are also available and can be changed in the Analysis settings dialog box (see "Analysis settings" on page 31).

To revert to the initial Low ROX and Low FAM/Low VIC range:

1. Click Analysis Settings to access the Analysis Settings dialog box.
2. In Sample Group-Specific Settings find the corresponding Sample Group.
3. Select the check box for the Auto column appearing to the left of the Low ROX column.



Configure this analysis setting for the study.

General

Confidence Interval (%): 95

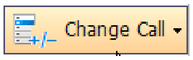
Well Volume (uL): 0.033

Sample Group-Specific Settings

Target	Sample	Auto?	Min Pos CRT	Max Pos C...	Min Neg CRT	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.1	35.1	36.1	☑	402.88	331.42
Taqman Assay 1	NTC	☑	5	38	39	☑	219.57	242.92
Taqman Assay2	DNA Sample 1	☑	25.8	33.8	34.8	☑	402.88	677.04
Taqman Assay2	NTC	☑	5	38	39	☑	219.57	744.04

IMPORTANT! For duplex experiments, there is a possibility to see a '×' within the grey shaded area. The ROX vs. Reporter Dye plot displays only one reporter, either FAM™ dye or VIC® dye. In duplex experiments, the same well appears in two sample groups, one for each reporter dye. Therefore, there is a possibility that the well that appears within the grey shaded area for one reporter dye can appear outside the grey shaded area for the other reporter dye. This well will appear with the '×' for both reporter dyes.

The Well Table includes the following tools:

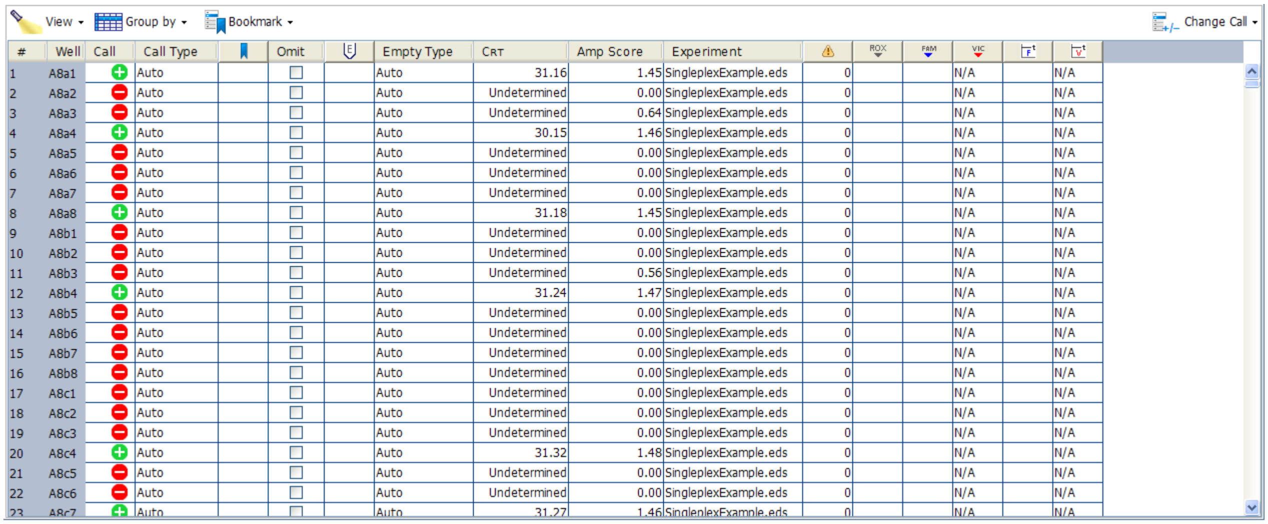
Tool	Description
	Drop-down menu that allows you to show or hide columns in a table. Select or Deselect the following: • **Call** • **Call Type** • **Bookmark** • **Omit** • **Empty Well Flag** • **Empty Type** • **C_{RT}** • **Amp Score** • **Experiment** • **Flag indicator** • **Flags** – **Low ROX** – **Low FAM** – **Low VIC** – **Low FAM Score** – **Low VIC Score** **Note:** Wells with undetermined calls are not flagged for Low FAM Score or Low VIC Score even if their Amp Score value is less than threshold.
	Drop-down menu that enables you to group the targets by experiments or by none. Select from: • **None** • **Experiment** • **Expand All** • **Collapse All** **Note:** The Expand All and Collapse All options are enabled only when Experiment is selected
	Drop-down menu that enables you add, select, and clear bookmarked wells. Select from **Add Bookmark**, **Select all bookmarks**, **Clear selected bookmark**, **Clear all bookmarks**
	Drop-down menu that enables you to manually change the call for a well(s). Select **Positive**, **Negative** or **Undetermined**. You can also use this drop-down menu to Clear Manual Call, Mark as Empty Well, Mark as Non-Empty Well, Auto Empty Detection. **Note:** When you manually change the call for a well(s), the call will remain unchanged even after you re-analyze the study.

The following table provides definitions for the column headings that appear in the well tables in the Sample View tab:

Column heading	Description
Well	Displays the Well location
Call	Displays the call in the well (Positive, Negative, or Undetermined) **Note:** Positive calls are indicated by ⊕, Negative calls are indicated by ⊖, and Undetermined calls are indicated by ?
Call Type	Whether the call is Auto or has been changed manually
Bookmark	Indicates if the well is bookmarked
Omit	Includes a check box to omit a well
Empty Type	Whether the well has been labelled empty automatically or manually
C_{RT}	C_{RT} value for a well
Amp Score	Amp Score value for a well
Experiment	Name of the experiment to which the target belongs
⚠	Flag Indicator. Displays the number of flags in a well
ROX ▼	The icon displayed if the well is flagged with Low ROX
FAM ▼	The icon displayed if the well is flagged with Low FAM
VIC ▼	The icon displayed if the well is flagged with Low VIC (only for duplex experiments). For singleplex experiments, this column displays N/A
F t	The icon displayed if the well is flagged with Low FAM Score
V t	The icon displayed if the well is flagged with Low VIC Score

Note: You can sort the table columns by clicking once on the column header. You can change the sorting order with a second click. A third click will clear the sorting order. You can sort the table columns at multiple levels. For example, click the Call column to sort the columns by Call, then hold down the Ctrl key and click the C_{RT} column to sort by C_{RT} the wells with the same call.

The Well Table view for the Sample Group tab of an example study is shown in the following image:



#	Well	Call	Call Type	🔖	Omit	E	Empty Type	CRT	Amp Score	Experiment	⚠	ROX	FAM	VIC	F	V
1	A8a1	⊕	Auto		☐		Auto	31.16	1.45	SingleplexExample.eds	0			N/A		N/A
2	A8a2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
3	A8a3	⊖	Auto		☐		Auto	Undetermined	0.64	SingleplexExample.eds	0			N/A		N/A
4	A8a4	⊕	Auto		☐		Auto	30.15	1.46	SingleplexExample.eds	0			N/A		N/A
5	A8a5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
6	A8a6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
7	A8a7	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
8	A8a8	⊕	Auto		☐		Auto	31.18	1.45	SingleplexExample.eds	0			N/A		N/A
9	A8b1	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
10	A8b2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
11	A8b3	⊖	Auto		☐		Auto	Undetermined	0.56	SingleplexExample.eds	0			N/A		N/A
12	A8b4	⊕	Auto		☐		Auto	31.24	1.47	SingleplexExample.eds	0			N/A		N/A
13	A8b5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
14	A8b6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
15	A8b7	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
16	A8b8	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
17	A8c1	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
18	A8c2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
19	A8c3	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
20	A8c4	⊕	Auto		☐		Auto	31.32	1.48	SingleplexExample.eds	0			N/A		N/A
21	A8c5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
22	A8c6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
23	A8c7	⊕	Auto		☐		Auto	31.27	1.46	SingleplexExample.eds	0			N/A		N/A

Replicate Group view

The Replicate Group table lists the targets in the study. The Targets table includes the following columns:

Column	Description
Bookmark	Indicates whether a target is present in a bookmarked well To bookmark a well: • On the Heat Map screen, select one or more wells in the plate layout right-click and select **Add Bookmark** from the drop-down menu Or • On the Amplification Plot screen, in the Well Table, select **Add Bookmark** from the Bookmark drop-down menu or from the right-click drop-down menu
Target	Displays the target name.
Sample	Displays the sample name.
Dilution	Displays the dilution for the group.
Copies/µL	Displays the number of copies of the sample group per µL.
⊕	Displays total number of positive calls. **Note:** Positive calls mean the DigitalSuite™ Software determines a well containing at least one copy of the sample.
⊖	Displays total number of negative calls. **Note:** Negative calls mean the DigitalSuite™ Software determines that a well does not contain any copy of the sample.

Column	Description
?	Displays total number of undetermined calls. **Note:** Undetermined calls are those that occur between the positive and negative range as well as beyond the positive range on the histogram plot.
# of Positive + Negative (# of Pos+Neg)	Displays the combined number of wells having the positive and negative calls.
# of Omitted	Displays the number of wells omitted from the study.
# of Empty	Displays the number of empty wells. **Note:** Empty wells are those that are flagged with Low ROX, Low FAM, or Low VIC flags
ROX ▾	Displays the number of wells with low ROX signal.
FAM ▾	Displays the number of wells with low FAM™ dye signal.
VIC ▾	Displays the number of wells with low VIC® dye signal (only for duplex experiments). For singleplex experiments, this column displays N/A.
F t	Displays the number of wells with a FAM™ dye score that is lower than the threshold value (1.41).
V t	Displays the number of wells with a VIC® dye score that is lower than the threshold value (only for duplex experiments). For singleplex experiments, this column displays N/A.

The lower half of the Targets view displays the plot view and well table. Use the ›, ‹, ⌄ , and ⌃ buttons to extend the views horizontally or vertically.

You can select wells in the plot view or the well table. In the plot view, select wells by clicking in the plot and dragging a rubber-band box. In the well table, select wells by selecting the respective rows. Wells selected in the plot view will appear selected in the well table and vice-versa. In the plot view, selected wells will appear colored, while unselected wells will be hidden. To view unselected wells, go to **View** ▸ **Show Unselected**. The unselected wells will appear in grey color in the plot view. If no wells are selected, the plot view displays all the wells in color.

The Plot View includes the following tools:

Tool	Description
Plot ▾	Drop-down menu that allows you to select from ΔR vs. Cycle (Linear), ΔR vs. Cycle (Log), R vs. Cycle (Linear), and R vs. Cycle (Log) plot types.
View ▾	Drop-down menu that enables you to show and hide the following: • Legend • Show Unselected • C_{RT} • Show Omitted

Tool	Description
Color by: Call	Drop-down menu that enables you options to color the plot using different data elements. Select **Bookmarks**, **Targets**, **Wells**, or **Call**.

In addition to the tools mentioned above:

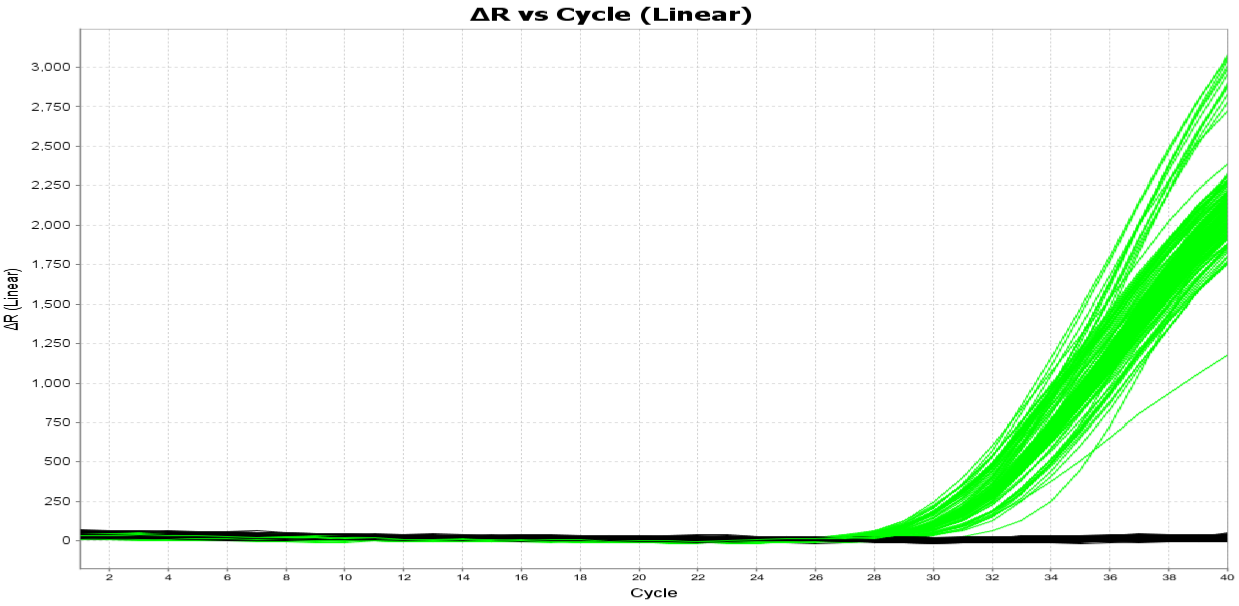
- The Targets plot view includes , , , and to zoom in, zoom out, to fit data in window, and to view data in full screen respectively.

  Note: To zoom into a particular area of the amplification plot, click at the center of the area of interest then click .

- Click , , and to print, copy, and save the plot as an image respectively.

- Click to access and edit the Plot Properties.

The Plot view for the Replicate Group tab of an example study is shown in the following image:



The Well Table includes the following tools:

Tool	Description

View	Drop-down menu that allows you to show or hide columns in a table. Select or Deselect the following:
	- **Call**
	- **Call Type**
	- **Bookmark**
	- **Omit**
	- **Empty Well Flag**
	- **Empty Type**
	- **C_{RT}**
	- **Amp Score**
	- **Experiment**
	- **Flag indicator**
	- **Flags**
	  – **Low ROX**
	  – **Low FAM**
	  – **Low VIC**
	  – **Low FAM Score**
	  – **Low VIC Score**
	Note: Wells with undetermined calls are not flagged for Low FAM Score or Low VIC Score even if their Amp Score value is less than threshold.

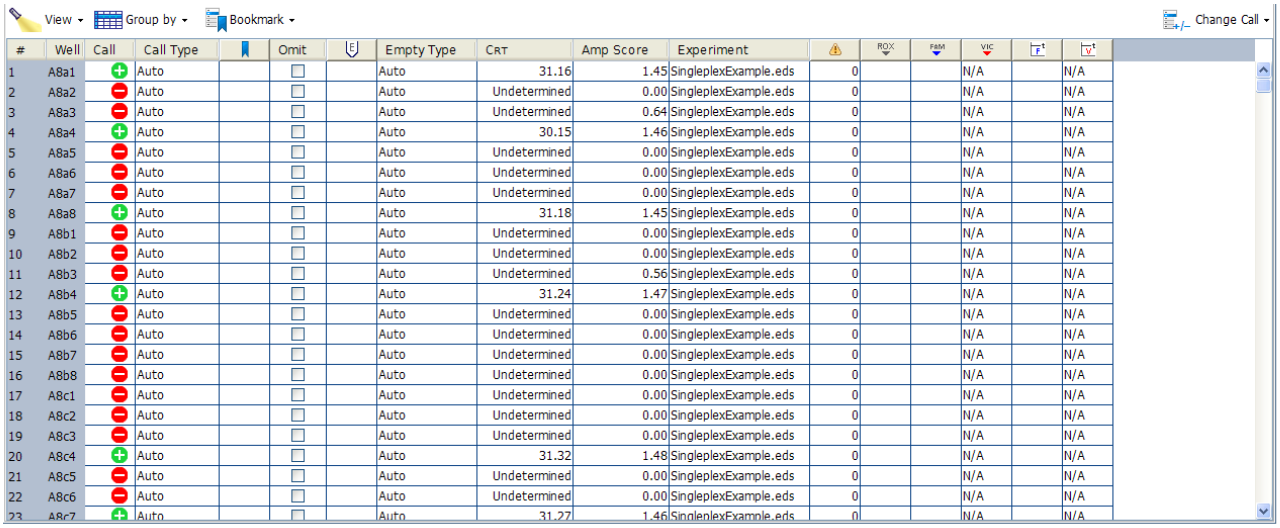
Group by	Drop-down menu that enables you to group the targets by experiments or by none. Select from: - **None** - **Experiment** - **Expand All** - **Collapse All** **Note:** The Expand All and Collapse All options are enabled only when Experiment is selected.
Bookmark	Drop-down menu that enables you add, select, and clear bookmarked wells. Select from **Add Bookmark**, **Select all bookmarks**, **Clear selected bookmark**, **Clear all bookmarks**.
Change Call	Drop-down menu that enables you to manually change the call for a well(s). Select **Positive**, **Negative** or **Undetermined**. You can also use this drop-down menu to Clear Manual Call, Mark as Empty Well, Mark as Non-Empty Well, Auto Empty Detection. **Note:** When you manually change the call for a well(s), the call will remain unchanged even after you re-analyze the study.

The following table provides definitions for the column headings that appear in the tables in the Replicate Group tab:

Column heading	Description
Well	Displays the Well location
Call	Displays the call in the well (Positive, Negative, or Undetermined) **Note:** Positive calls are indicated by ⊕, Negative calls are indicated by ⊖, and Undetermined calls are indicated by ?
Call Type	Whether the call is Auto or has been changed manually
Bookmark	Indicates if the well is bookmarked
Omit	Includes a check box to omit a well
Empty Type	Whether the well has been labelled empty automatically or manually
C_{RT}	C_{RT} value for a well
Amp Score	Amp Score value for a well
Experiment	Name of the experiment to which the target belongs
⚠	Flag Indicator. Displays the number of flags in a well
ROX ▼	The icon displayed if the well is flagged with Low ROX
FAM ▼	The icon displayed if the well is flagged with Low FAM
VIC ▼	The icon displayed if the well is flagged with Low VIC (only for duplex experiments). For singleplex experiments, this column displays N/A
F t	The icon displayed if the well is flagged with Low FAM Score
V t	The icon displayed if the well is flagged with Low VIC Score

Note: You can sort the table columns by clicking once on the column header. You can change the sorting order with a second click. A third click will clear the sorting order. You can sort the table columns at multiple levels. For example, click the Call column to sort the columns by Call, then hold down the Ctrl key and click the C_{RT} column to sort by C_{RT} the wells with the same call.

The Well Table view for the Replicate Group tab of an example study is shown in the following image:

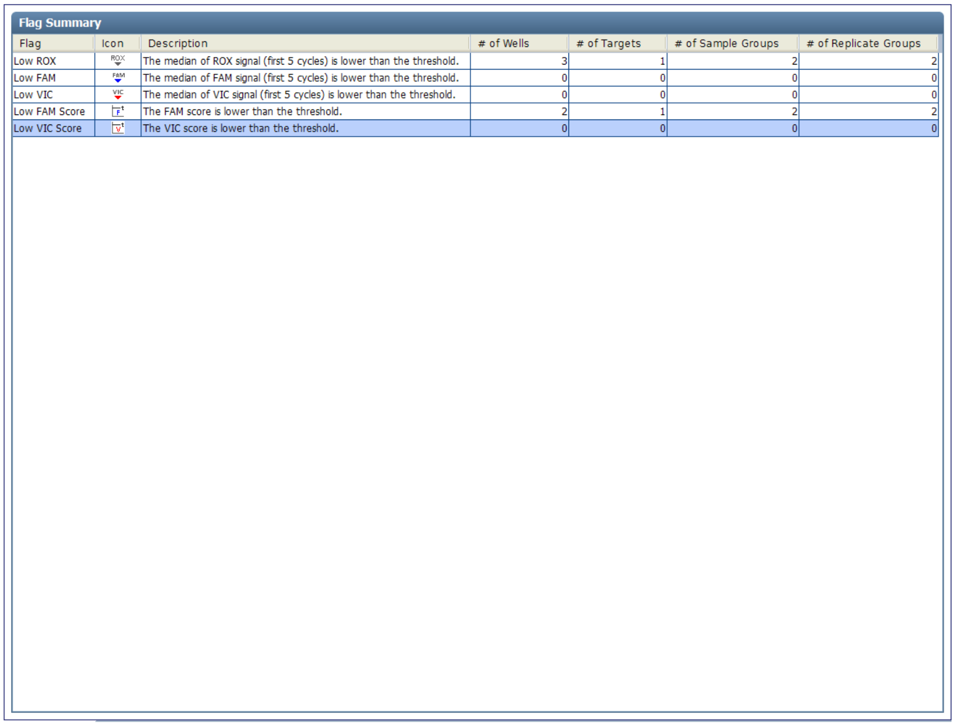


#	Well	Call	Call Type		Omit	E	Empty Type	CRT	Amp Score	Experiment	⚠	ROX	FAM	VIC	F	V
1	A8a1	⊕	Auto		☐		Auto	31.16	1.45	SingleplexExample.eds	0			N/A		N/A
2	A8a2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
3	A8a3	⊖	Auto		☐		Auto	Undetermined	0.64	SingleplexExample.eds	0			N/A		N/A
4	A8a4	⊕	Auto		☐		Auto	30.15	1.46	SingleplexExample.eds	0			N/A		N/A
5	A8a5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
6	A8a6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
7	A8a7	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
8	A8a8	⊕	Auto		☐		Auto	31.18	1.45	SingleplexExample.eds	0			N/A		N/A
9	A8b1	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
10	A8b2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
11	A8b3	⊖	Auto		☐		Auto	Undetermined	0.56	SingleplexExample.eds	0			N/A		N/A
12	A8b4	⊕	Auto		☐		Auto	31.24	1.47	SingleplexExample.eds	0			N/A		N/A
13	A8b5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
14	A8b6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
15	A8b7	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
16	A8b8	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
17	A8c1	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
18	A8c2	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
19	A8c3	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
20	A8c4	⊕	Auto		☐		Auto	31.32	1.48	SingleplexExample.eds	0			N/A		N/A
21	A8c5	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
22	A8c6	⊖	Auto		☐		Auto	Undetermined	0.00	SingleplexExample.eds	0			N/A		N/A
23	A8c7	⊕	Auto		☐		Auto	31.27	1.46	SingleplexExample.eds	0			N/A		N/A

Flag Summary

The Flag Summary screen displays a list of the DigitalSuite™ Software flags; The flag summary table includes the flag description, and the number of wells, targets, sample groups, and replicate groups in which the flag occurs for any experiment added to a study.

The Flag Summary screen for an example study is shown in the following image:



Flag	Icon	Description	# of Wells	# of Targets	# of Sample Groups	# of Replicate Groups
Low ROX	ROX	The median of ROX signal (first 5 cycles) is lower than the threshold.	3	1	2	2
Low FAM	FAM	The median of FAM signal (first 5 cycles) is lower than the threshold.	0	0	0	0
Low VIC	VIC	The median of VIC signal (first 5 cycles) is lower than the threshold.	0	0	0	0
Low FAM Score	F	The FAM score is lower than the threshold.	2	1	2	2
Low VIC Score	V	The VIC score is lower than the threshold.	0	0	0	0

Export the analyzed data

The Export feature of DigitalSuite™ Software enables you to export the analyzed data.

Export procedure

1. Open the study file that contains the data to export, and from the Export Workflow menu, click **Export**.
2. Select to export all data in one file or in separate files for each data type.
   - **One File** — All data types are exported in one file.
     - If you select the *.csv format, a worksheet is created for each data type.
     - If you select the *.txt format, the data are grouped by data type.
   - **Separate Files** — Each data type is exported in a separate file. For example, if you select all three different data types Amplification, Results - Sample Group, Results - Replicate Group to export, three separate files (one each for Amplification, Results - Sample Group, Results - Replicate Group) are created. You can select the type of file (*.csv or *.txt) to export from the File Type drop-down menu.
3. *(Optional)* Select the **Open after export** check box to automatically open the file when export is complete.
4. *(Optional)* Select the **Export Bookmark Data only** check box to only export bookmarked wells, sample groups, or replicate groups.
5. Enter a file name and location.
   a. Enter a name for the export file in the **File Name** field.

      Note: If you choose to export data in separate files, each file must have a different name.

   b. Enter the Directory where you want the exported file to save. 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 **User-defined location:** check box.
6. Select the data to export. Select the check box of the data to be exported.

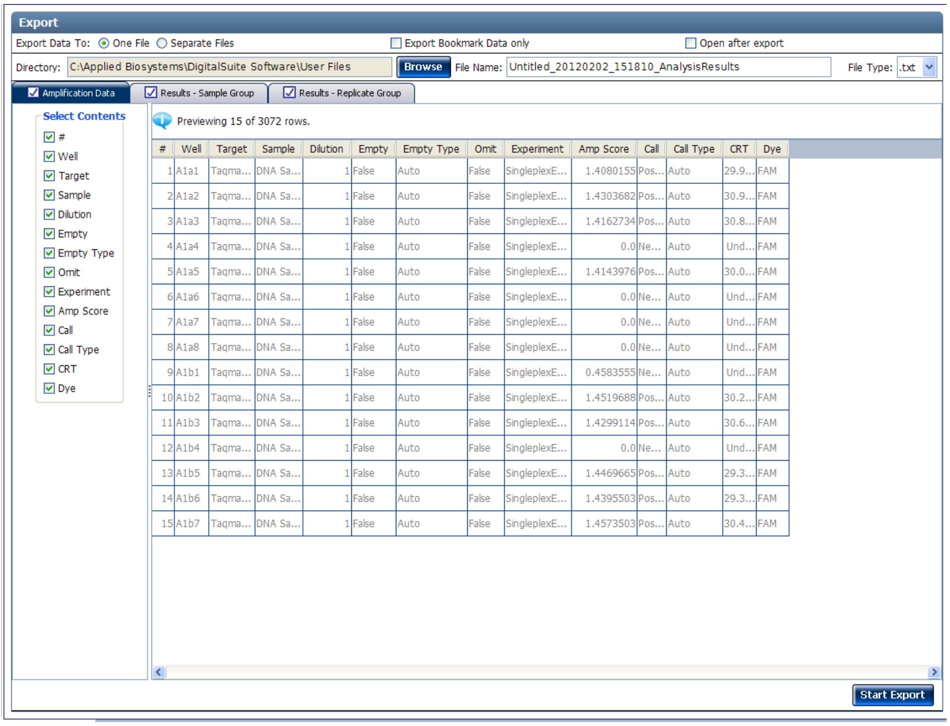
Tool	Function
Amplification data **Note:** Results are reported at a well level	Amplification results, such as C_{RT} values and calls
Results - Sample Group	Results by Sample group
Results - Replicate Group	Results by Replicate group

   Note: Results data are not available for export until the study is analyzed.

   Note: To export Multicomponent Data from digital PCR experiments, use the Export feature in the QuantStudio™ 12K Flex Software.

7. Click **Start Export**.

The Export screen for an example study is shown in the following image:



Export Data To: ● One File ○ Separate Files ☐ Export Bookmark Data only ☐ Open after export

Directory: C:\Applied Biosystems\DigitalSuite Software\User Files

File Name: Untitled_20120202_151810_AnalysisResults

File Type: .txt

☑ Amplification Data ☑ Results - Sample Group ☑ Results - Replicate Group

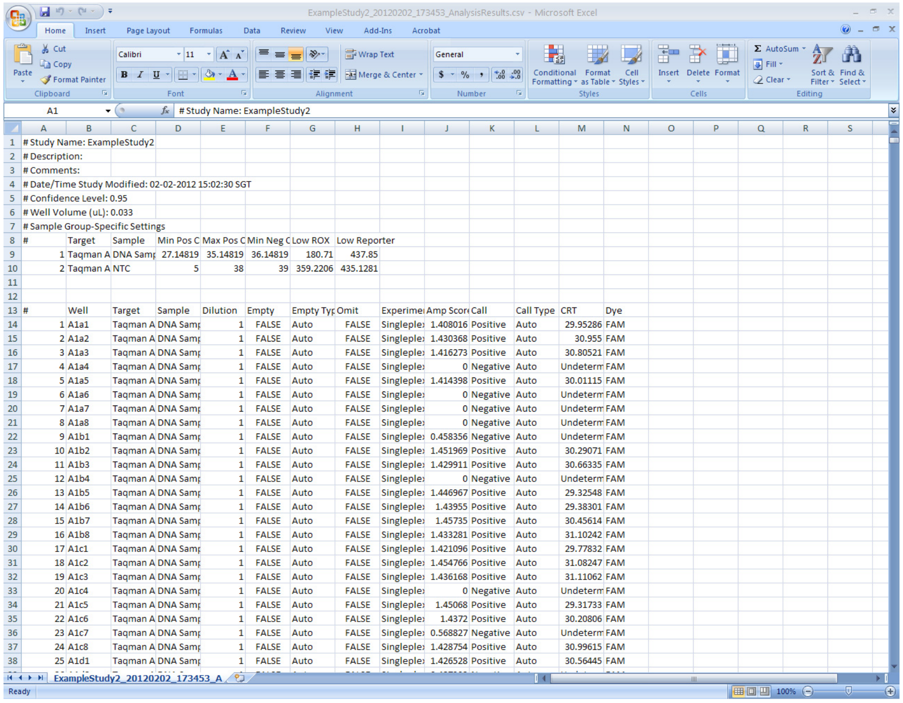
Select Contents
- ☑ #
- ☑ Well
- ☑ Target
- ☑ Sample
- ☑ Dilution
- ☑ Empty
- ☑ Empty Type
- ☑ Omit
- ☑ Experiment
- ☑ Amp Score
- ☑ Call
- ☑ Call Type
- ☑ CRT
- ☑ Dye

Previewing 15 of 3072 rows.

#	Well	Target	Sample	Dilution	Empty	Empty Type	Omit	Experiment	Amp Score	Call	Call Type	CRT	Dye
1	A1a1	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4080155	Pos...	Auto	29.9...	FAM
2	A1a2	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4303682	Pos...	Auto	30.9...	FAM
3	A1a3	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4162734	Pos...	Auto	30.8...	FAM
4	A1a4	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.0	Ne...	Auto	Und...	FAM
5	A1a5	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4143976	Pos...	Auto	30.0...	FAM
6	A1a6	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.0	Ne...	Auto	Und...	FAM
7	A1a7	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.0	Ne...	Auto	Und...	FAM
8	A1a8	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.0	Ne...	Auto	Und...	FAM
9	A1b1	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.4583555	Ne...	Auto	Und...	FAM
10	A1b2	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4519688	Pos...	Auto	30.2...	FAM
11	A1b3	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4299114	Pos...	Auto	30.6...	FAM
12	A1b4	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	0.0	Ne...	Auto	Und...	FAM
13	A1b5	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4469665	Pos...	Auto	29.3...	FAM
14	A1b6	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4395503	Pos...	Auto	29.3...	FAM
15	A1b7	Taqma...	DNA Sa...	1	False	Auto	False	SingleplexE...	1.4573503	Pos...	Auto	30.4...	FAM

Note: The screenshot displays the export preview. The export preview lists the first 15 rows of data if there are more than that number. Else, all are displayed.

8. The exported file when opened in Microsoft Excel appears as shown below:



A1 # Study Name: ExampleStudy2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	# Study Name: ExampleStudy2													
2	# Description:													
3	# Comments:													
4	# Date/Time Study Modified: 02-02-2012 15:02:30 SGT													
5	# Confidence Level: 0.95													
6	# Well Volume (uL): 0.033													
7	# Sample Group-Specific Settings													
8	#	Target	Sample	Min Pos C	Max Pos C	Min Neg C	Low ROX	Low Reporter						
9	1	Taqman A	DNA Samp	27.14819	35.14819	36.14819	180.71	437.85						
10	2	Taqman A	NTC	5	38	39	359.2206	435.1281						
11														
12														
13	#	Well	Target	Sample	Dilution	Empty	Empty Typ	Omit	Experime	Amp Score	Call	Call Type	CRT	Dye
14	1	A1a1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.408016	Positive	Auto	29.95286	FAM
15	2	A1a2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.430368	Positive	Auto	30.955	FAM
16	3	A1a3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.416273	Positive	Auto	30.80521	FAM
17	4	A1a4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
18	5	A1a5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.414398	Positive	Auto	30.01115	FAM
19	6	A1a6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
20	7	A1a7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
21	8	A1a8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
22	9	A1b1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0.458356	Negative	Auto	Undeterm	FAM
23	10	A1b2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.451969	Positive	Auto	30.29071	FAM
24	11	A1b3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.429911	Positive	Auto	30.66335	FAM
25	12	A1b4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
26	13	A1b5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.446967	Positive	Auto	29.32548	FAM
27	14	A1b6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.43955	Positive	Auto	29.38301	FAM
28	15	A1b7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.45735	Positive	Auto	30.45614	FAM
29	16	A1b8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.433281	Positive	Auto	31.10242	FAM
30	17	A1c1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.421096	Positive	Auto	29.77832	FAM
31	18	A1c2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.454766	Positive	Auto	31.08247	FAM
32	19	A1c3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.436168	Positive	Auto	31.11062	FAM
33	20	A1c4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
34	21	A1c5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.45068	Positive	Auto	29.31733	FAM
35	22	A1c6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.4372	Positive	Auto	30.20806	FAM
36	23	A1c7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0.568827	Negative	Auto	Undeterm	FAM
37	24	A1c8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.428754	Positive	Auto	30.99615	FAM
38	25	A1d1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.426528	Positive	Auto	30.56445	FAM

Supporting features

Preferences

Use the Preferences dialog box to set the default location and additional study properties such as display format.

To access the Preferences dialog box, go to **Tools** ▸ **Preferences**.

Note: You must close all open studies before adjusting the Preferences settings.

In the General tab, change the default location of the *.eds file and display format for date, time, and numeric separator format.

1. Select **User-defined location:**, and browse to the desired location on your computer.
2. To use the default location, select **Last-accessed location**.

IMPORTANT! If you select the Last-accessed location option and use DigitalSuite™ Software to access an external data storage device, make sure the device is connected to your computer until you close the application.

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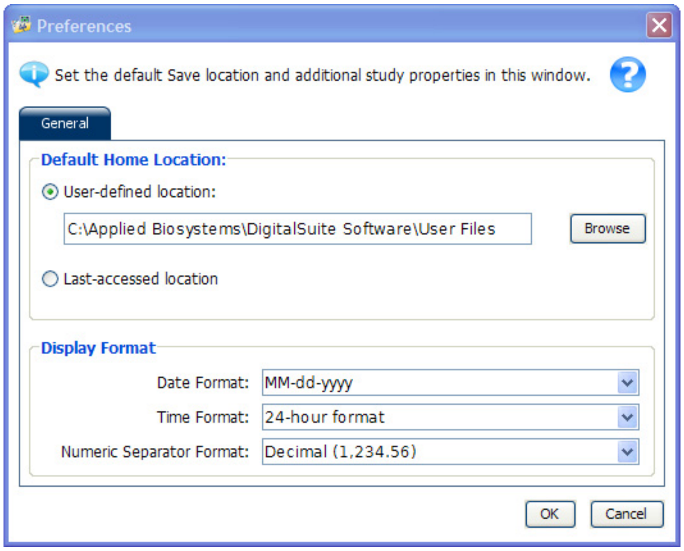
3. Select the date format from the Date Format drop-down menu.
4. Select the time format from the Time Format drop-down menu.
5. Select the numeric separator format from the Numeric Separator Format drop-down menu.
6. Click **OK**.



Preferences

Set the default Save location and additional study properties in this window.

General

Default Home Location:

● User-defined location: C:\Applied Biosystems\DigitalSuite Software\User Files

○ Last-accessed location

Display Format

Date Format: MM-dd-yyyy

Time Format: 24-hour format

Numeric Separator Format: Decimal (1,234.56)

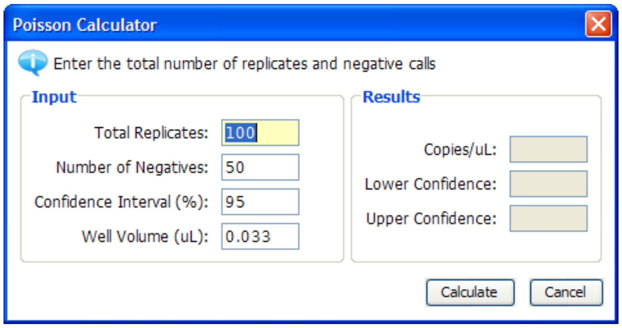
Poisson Calculator

Use the Poisson Calculator dialog box to calculate the copies/µL, lower confidence, and upper confidence for a set of manually entered parameters.

To access the Poisson Calculator dialog box, go to **Tools** ▸ **Poisson Calculator**.

1. Enter the total number of replicates.
2. Enter the number of negatives.

3. Enter the confidence interval in percent.
4. Enter the well volume in µL.
5. Click **Calculate**.



Enter the total number of replicates and negative calls

Total Replicates: 100
Number of Negatives: 50
Confidence Interval (%): 95
Well Volume (uL): 0.033

Section 3.2 Analyzing Singleplex Experiments

In this section, you use the example singleplex experiment file provided with the DigitalSuite™ Software to create a study and analyze the digital experiment results. This section includes:

About Singleplex experiments

Singleplex experiments use FAM™ dye as the only reporter dye.

Setup a singleplex study

Setup a study using the singleplex example experiment from the software installation.

Create a study

1. Launch DigitalSuite™ Software, and on the Home screen click **Create Study**.
2. In the Import dialog box, navigate to C:\Program Files\Applied Biosystems\DigitalSuite Software\examples and select **SingleplexExample.eds**. Click **Import**.

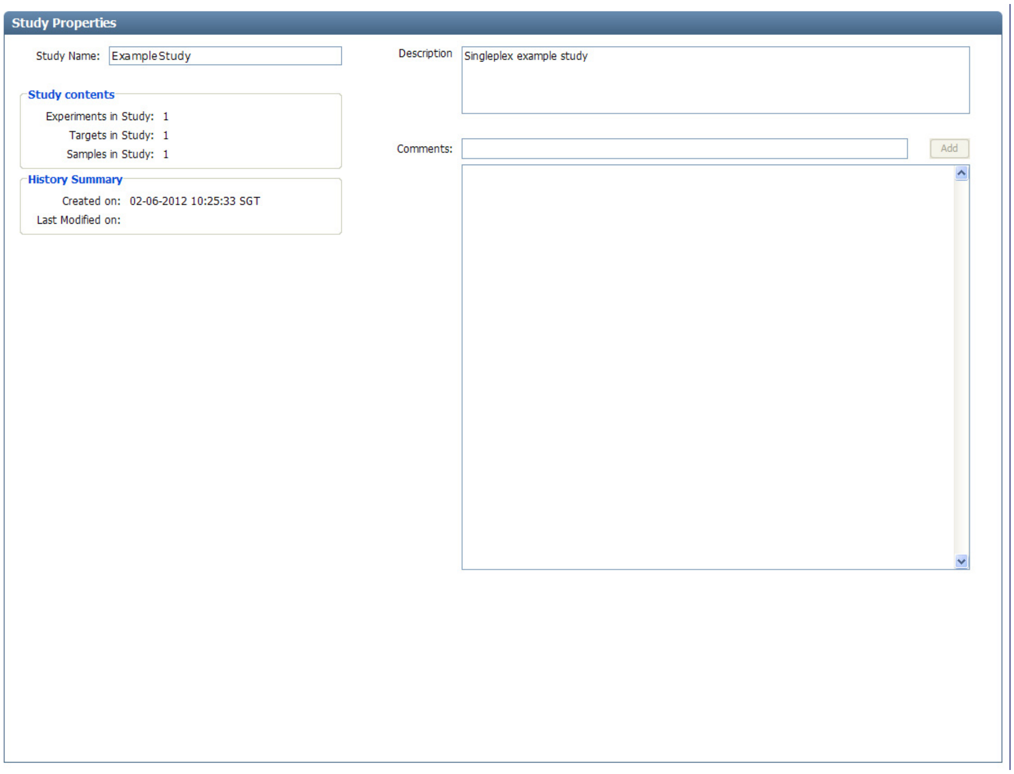
   Note: This is a single experiment singleplex study. While setting up your own singleplex study, you can add multiple singleplex experiments to a study.

Define study properties

1. Click **Properties** from the Setup menu to access the Properties screen.
2. Enter the study name **ExampleStudy**.
3. In the Description field, enter **Singleplex example study**.
4. In the Comments field, enter any comments, then click **Add**.

5. Verify that the Study contents and History Summary match those of your singleplex example study.

The Properties screen of the singleplex example study is shown in the following image:



Study Name: ExampleStudy

Experiments in Study: 1
Targets in Study: 1
Samples in Study: 1

Created on: 02-06-2012 10:25:33 SGT
Last Modified on:

Description: Singleplex example study

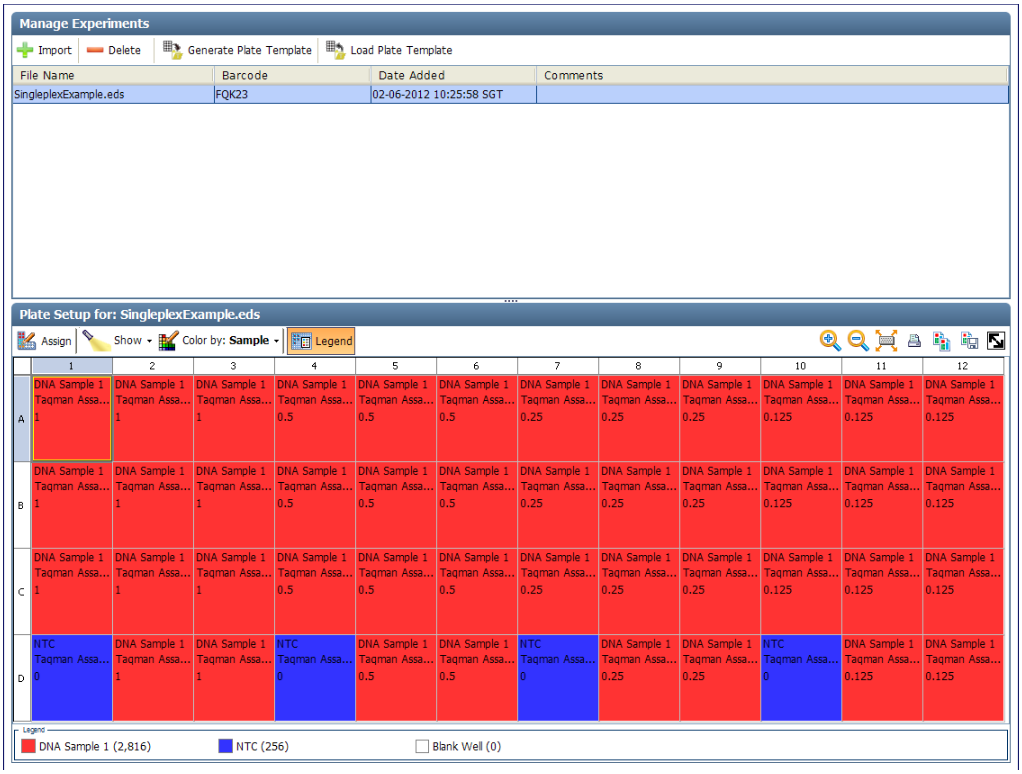
Comments:

Setup plate layout

1. Click **Plate Setup** from the Setup menu to access the Plate Setup screen.
2. Click **Load Plate Template** to load the plate setup contents from the example plate setup file.
3. Browse to C:\Program Files\Applied Biosystems\DigitalSuite Software\examples. Select **SingleplexExamplePlateSetup.csv**.

   Note: For your own singleplex studies, you can setup the plate contents using a previously created plate setup file or click **Assign** to assign the plate contents. For more information on assigning plate contents, see "Set up plate layout" on page 25.
4. Click **Show** to show or hide the data elements in the plate.
5. Click **Color by:** to color the sub-arrays using the different data elements.
6. Click **Legend** to show or hide the plate layout legend.

The Plate Setup screen of the singleplex example study is shown in the following image:



File Name	Barcode	Date Added	Comments
SingleplexExample.eds	FQK23	02-06-2012 10:25:58 SGT	

Color by: **Sample**

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
B	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
C	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125
D	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 1	DNA Sample 1 Taqman Assa... 1	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... 0.5	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... 0.25	NTC Taqman Assa... 0	DNA Sample 1 Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... 0.125

Legend
🟥 DNA Sample 1 (2,816) 🟦 NTC (256) ☐ Blank Well (0)

Setup samples and targets

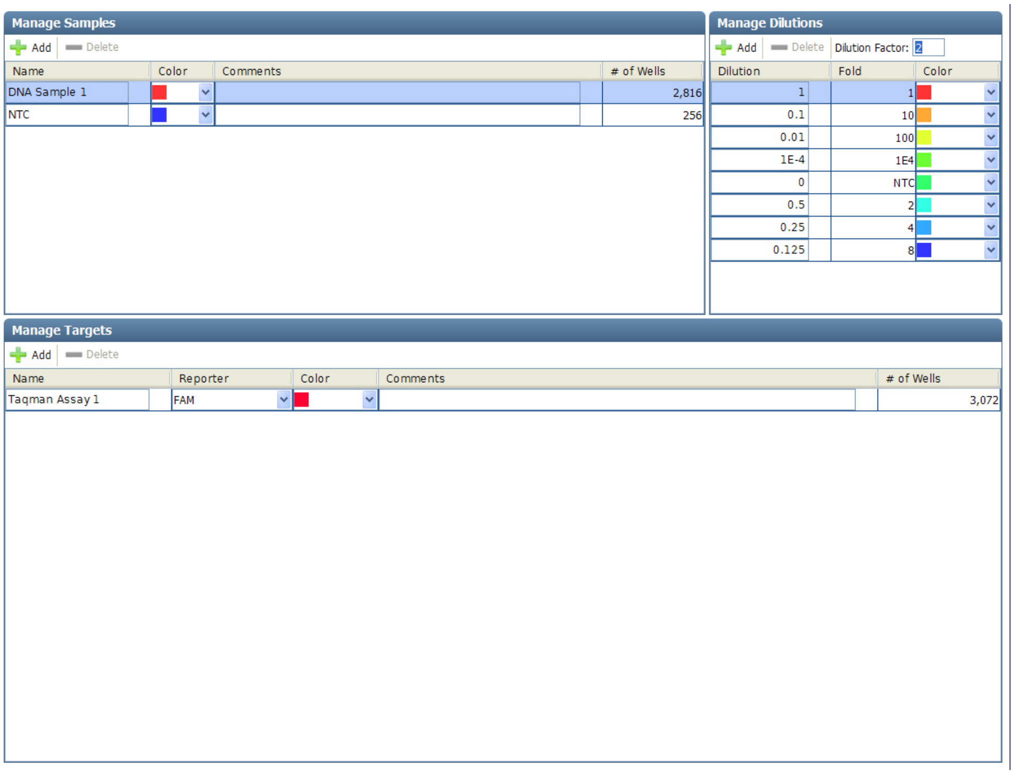
Use this screen to add samples and target names, and dilutions, in addition to the default ones. The names and dilutions will then appear in the Well Editor in the Plate Setup screen and can be used to set up the plate.

1. Click **Samples/Targets** from the Setup menu to access the Samples/Targets screen.
2. Click **Add** to add sample names. You can also click in the Name column to change the default sample name. Optionally, select a color from the Color drop-down menu.

   Note: DigitalSuite™ Software automatically assigns the number of wells for the sample name.
3. Click **Add** to add target names. You can also click in the Name column to change the default target name. Optionally, select a color from the Color drop-down menu.

   Note: DigitalSuite™ Software automatically assigns the number of wells for the target name.
4. Click **Add** to add dilutions. You can also click in the Dilution column to change the default dilutions. Optionally, select a color from the Color drop-down menu.
5. Enter the dilution factor for the dilution.

The Samples/Targets screen of the singleplex example study is shown in the following image:



Manage Samples

Name	Color	Comments	# of Wells
DNA Sample 1	🟥		2,816
NTC	🟦		256

Manage Dilutions

Dilution Factor: 2

Dilution	Fold	Color
1	1	🟥
0.1	10	🟧
0.01	100	🟨
1E-4	1E4	🟩
0	NTC	🟩
0.5	2	🟦
0.25	4	🟦
0.125	8	🟦

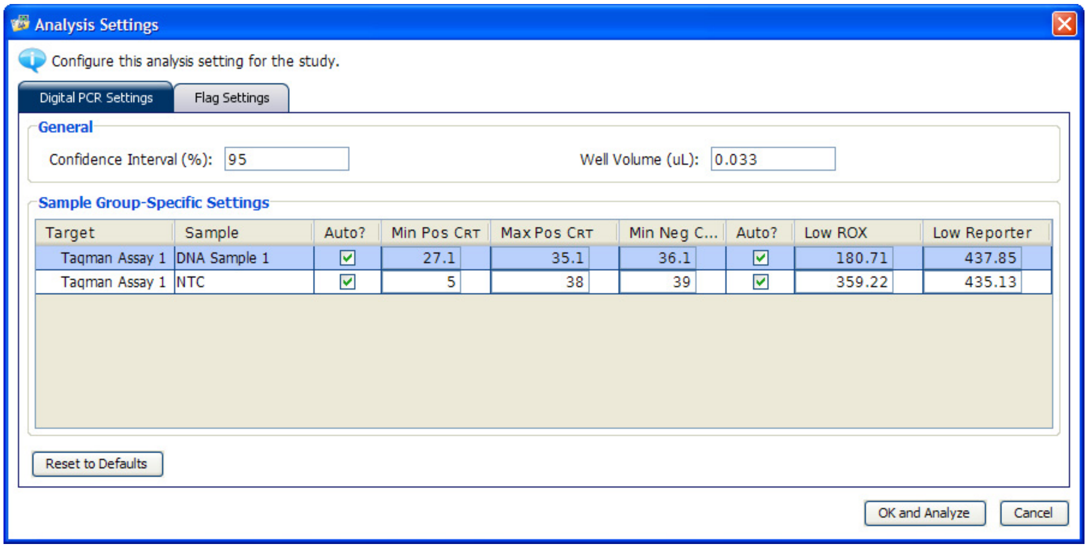
Manage Targets

Name	Reporter	Color	Comments	# of Wells
Taqman Assay 1	FAM	🟥		3,072

Analyze the study

Once you have set up the plate, click **Analyze**. To modify the analysis settings, click **Analysis Settings**.

The Analysis Settings dialog box for a singleplex example study is shown in the following image:



Analysis Settings

Configure this analysis setting for the study.

General

Confidence Interval (%): 95

Well Volume (uL): 0.033

Sample Group-Specific Settings

Target	Sample	Auto?	Min Pos CRT	Max Pos CRT	Min Neg C...	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.1	35.1	36.1	☑	180.71	437.85
Taqman Assay 1	NTC	☑	5	38	39	☑	359.22	435.13

View digital PCR results

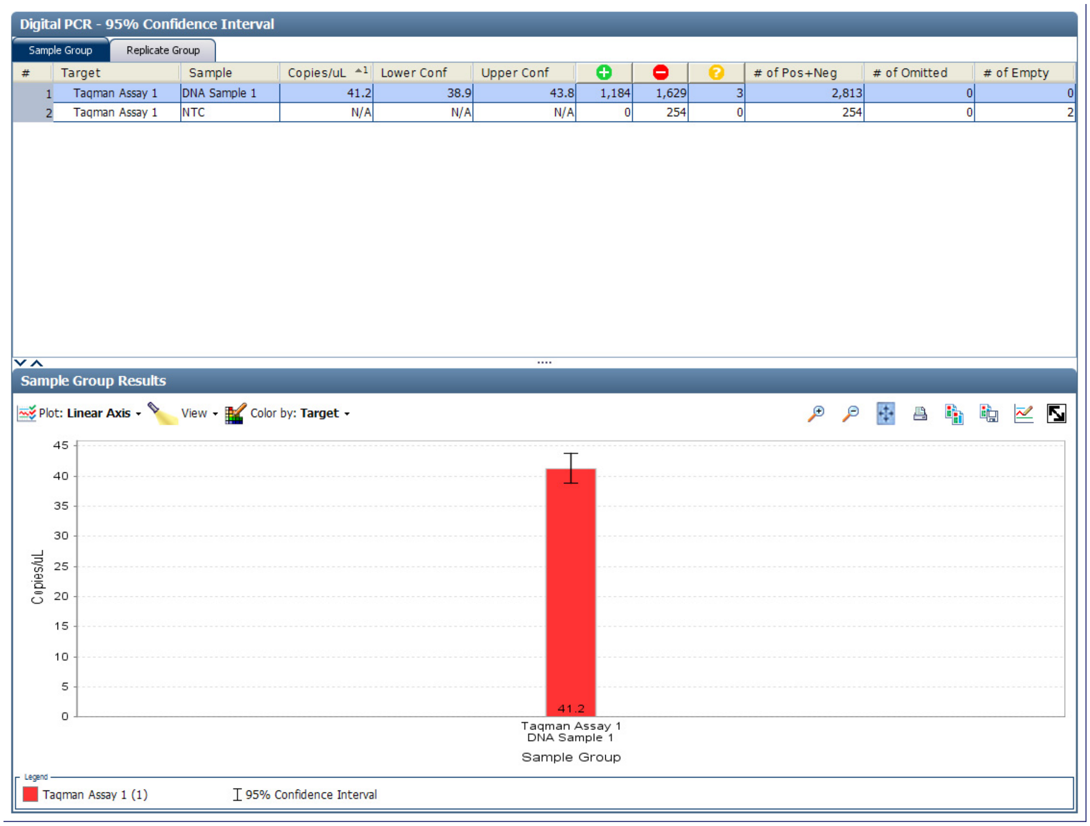
Clicking on the Analysis button automatically takes you to the Digital PCR screen. Alternatively, click **Digital PCR** from the Results Workflow menu to access the screen.

The Confidence Interval value is displayed in the Title Bar of the Digital PCR screen.

Sample Group view

1. Click the **Sample Group** tab to view the sample group results.
   For TaqMan® Assay 1, the Copies/µL is 41.2.
2. View the lower confidence (38.9), upper confidence (43.8) values, number of positive (1,184), negative (1,629), and undetermined (3) calls, total number of positive and negative calls (2,813), number of omitted wells (0), and number of empty wells (0).
3. For the Sample Group, NTC the Copies/µL is NA. This is because the number of positive calls for NTC is 0 and the DigitalSuite™ Software cannot calculate the Copies/µL.
4. View the corresponding plot in the lower half of the screen.
5. From the Plot drop-down menu, select **Linear Axis** (default). The Linear axis plot type displays the Sample Group on the x-axis and Copies/µL on the y-axis.
6. From the View drop-down menu, select **Legend**.
7. From the Color by: drop-down menu, select **Target** (default).

The Sample Group view for the singleplex example study is shown in the following image:



Digital PCR - 95% Confidence Interval

#	Target	Sample	Copies/uL ▲1	Lower Conf	Upper Conf	+	−	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	41.2	38.9	43.8	1,184	1,629	3	2,813	0	0
2	Taqman Assay 1	NTC	N/A	N/A	N/A	0	254	0	254	0	2

Sample Group Results

Plot: **Linear Axis** View Color by: **Target**

Note: For TaqMan® Assay 1, there are 3 undetermined calls. To investigate these 3 calls, go to the Run QC.

Replicate Group view

1. Click the **Replicate Group** tab to view the sample group results.

   For TaqMan® Assay 1, having dilution 1, the Copies/µL is 41.8. The Copies/µL should be roughly proportional to the dilution series. For example, dilution 0.5 is half of dilution 1 then the Copies/µL for dilution 0.5 should be roughly half of that for dilution 1. In the example study, the Copies/µL for dilution 0.5 is 19.5 which is approximately half of 41.8.

   Note: Life Technologies recommends you to check if the Copies/µL results are always roughly proportional to the dilution series. If the ratio of Copies/µL:dilution is significantly different in the Replicate Groups, check the plate setup or re-run the experiment.

2. For TaqMan® Assay 1, having dilution 1, view the lower confidence (38.1) and upper confidence (45.8) values, number of positive (526), negative (177), and undetermined (1) calls, total number of positive and negative calls (703), number of omitted wells (0), and number of empty wells (0).

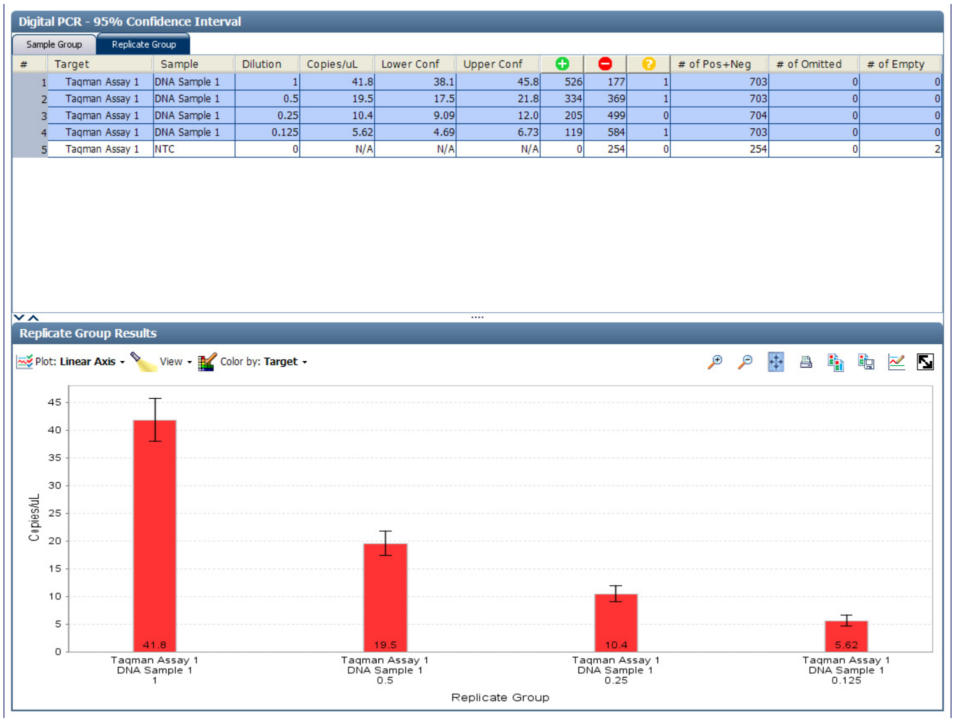
3. View the corresponding plot in the lower half of the screen.

4. From the Plot drop-down menu, select **Linear Axis** (default). The Linear axis plot type displays the Replicate Group on the x-axis and Copies/µL on the y-axis.

5. From the View drop-down menu, select **Legend**.

6. From the Color by: drop-down menu, select **Target** (default).

The Replicate Group view for the singleplex example study is shown in the following image:



#	Target	Sample	Dilution	Copies/uL	Lower Conf	Upper Conf	⊕	⊖	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	1	41.8	38.1	45.8	526	177	1	703	0	0
2	Taqman Assay 1	DNA Sample 1	0.5	19.5	17.5	21.8	334	369	1	703	0	0
3	Taqman Assay 1	DNA Sample 1	0.25	10.4	9.09	12.0	205	499	0	704	0	0
4	Taqman Assay 1	DNA Sample 1	0.125	5.62	4.69	6.73	119	584	1	703	0	0
5	Taqman Assay 1	NTC	0	N/A	N/A	N/A	0	254	0	254	0	2

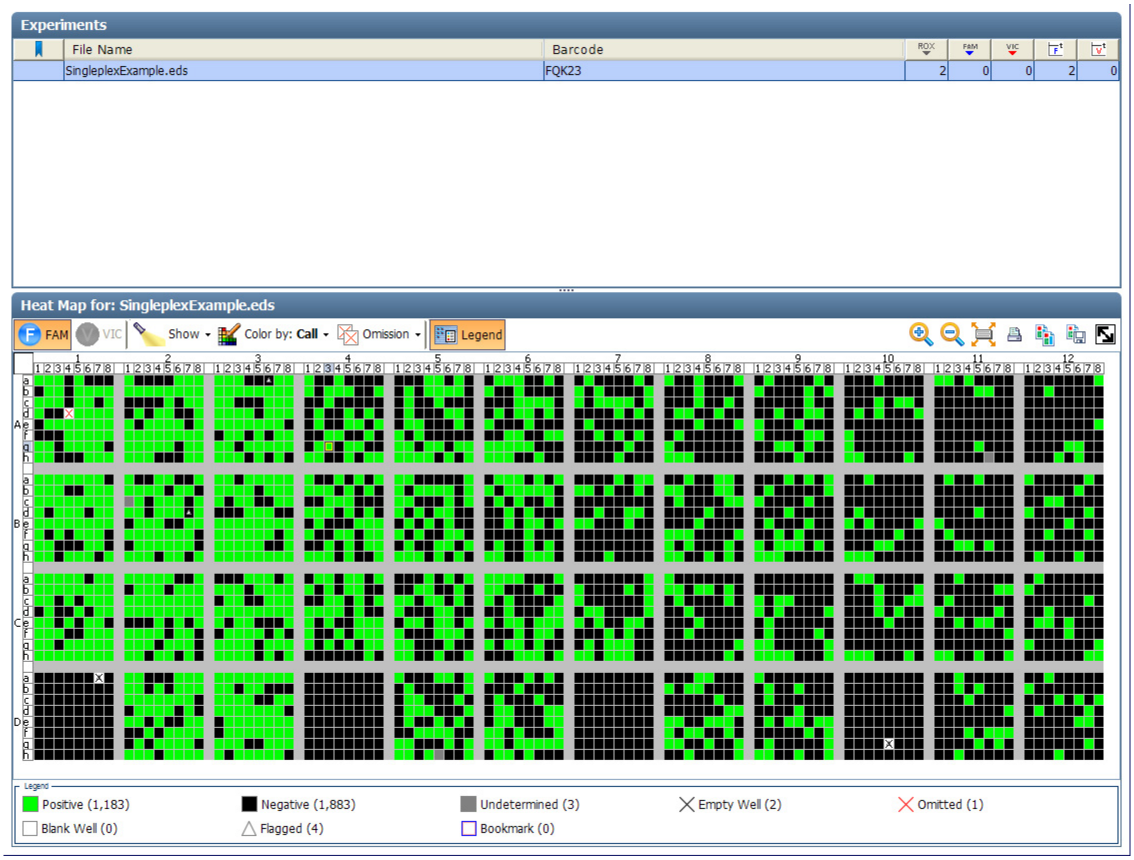
Run QC

Heat Map

1. Go to **Run QC** ▸ **Heat Map** to access the Heat Map screen.
2. In the Experiments view, select **SingleplexExample**.eds.
3. View the corresponding Heat Map in the lower half of the screen.

   Note: The VIC button appears disabled for singleplex experiments.
4. Roll-over a sub-array. The tool-tip displays the well contents.
5. From the Show drop-down menu, select **Flag** to display flags in the heat map.
   a. Look out for flagged wells or empty wells.
   b. Check the location of these wells in the plate. If the location does not appear to form any pattern, check the amplification curve in the amplification plot. Based on these assessments, you can decide to manually change the call or omit the well.
   c. Click **Analyze** to re-analyze the study.
6. From the Color by: drop-down menu, select **Call** to display the wells by the color of the Call.
7. Click **Legend** to show or hide the plate legend.

The Heat Map for the singleplex example study is shown in the following image:



Experiments

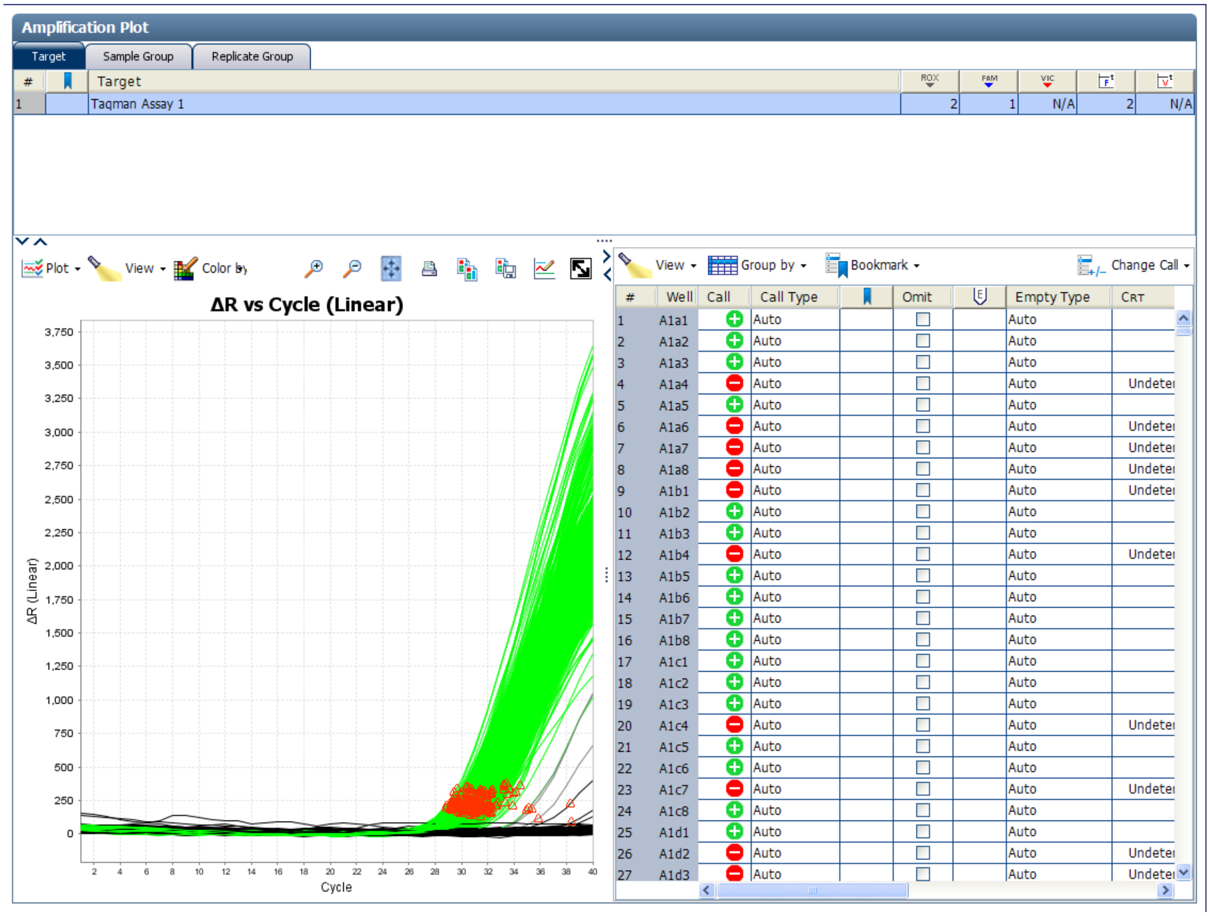
	File Name	Barcode	ROX	FAM	VIC	F	V
	SingleplexExample.eds	FQK23	2	0	0	2	0

Heat Map for: SingleplexExample.eds

Amplification Plot

1. Go to **Run QC** ▸ **Amplification Plot** to access the Amplification Plot screen.
2. In the Targets view, select **TaqMan® Assay 1**.
3. View the corresponding Amplification Plot in the lower half of the screen.
4. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).
5. From the View drop-down menu, select **C_{RT}** to display the C_{RT} icons on the plot.
6. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Targets tab for the singleplex example study is shown in the following image:

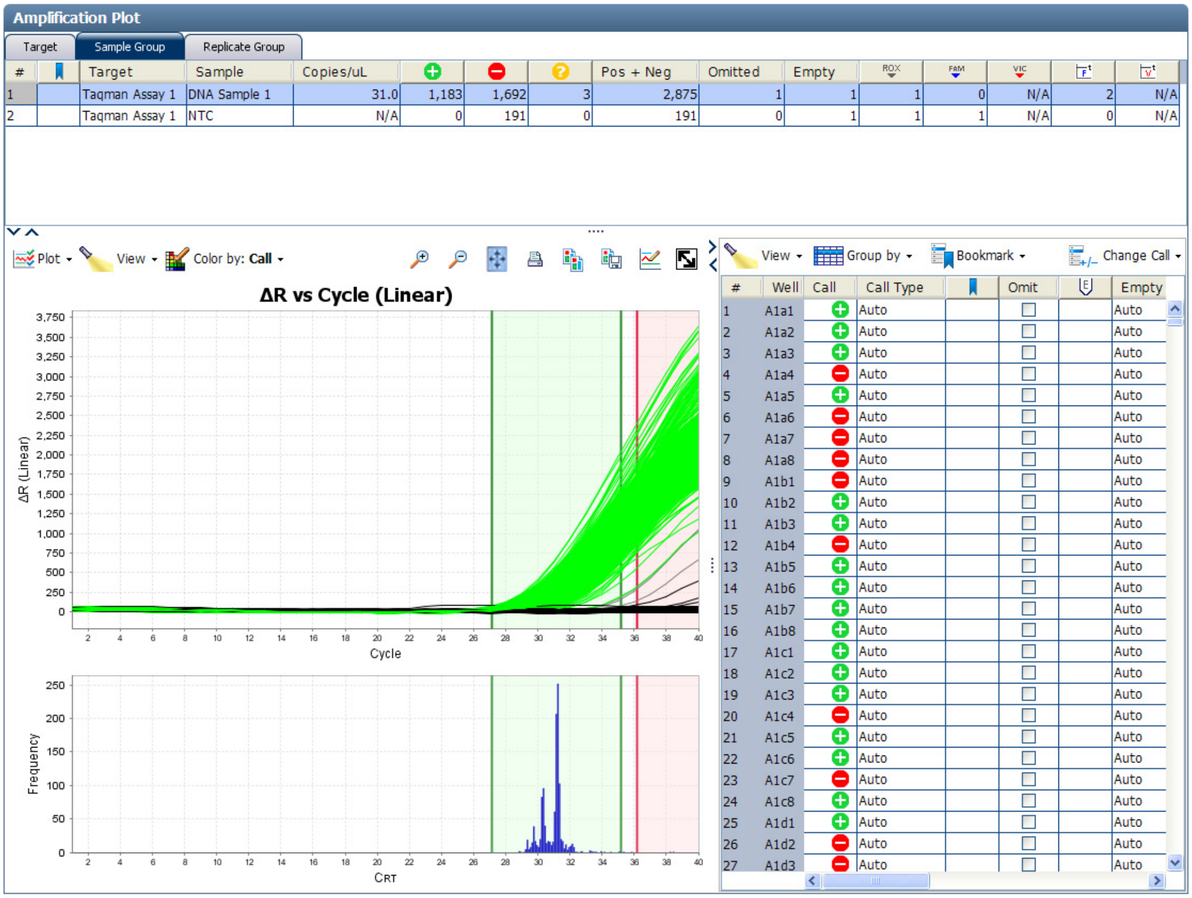


#		Target	ROX	FAM	VIC	F	V
1		Taqman Assay 1	2	1	N/A	2	N/A

#	Well	Call	Call Type		Omit	E	Empty Type	CRT
1	A1a1	⊕	Auto		☐		Auto	
2	A1a2	⊕	Auto		☐		Auto	
3	A1a3	⊕	Auto		☐		Auto	
4	A1a4	⊖	Auto		☐		Auto	Undeter
5	A1a5	⊕	Auto		☐		Auto	
6	A1a6	⊖	Auto		☐		Auto	Undeter
7	A1a7	⊖	Auto		☐		Auto	Undeter
8	A1a8	⊖	Auto		☐		Auto	Undeter
9	A1b1	⊖	Auto		☐		Auto	Undeter
10	A1b2	⊕	Auto		☐		Auto	
11	A1b3	⊕	Auto		☐		Auto	
12	A1b4	⊖	Auto		☐		Auto	Undeter
13	A1b5	⊕	Auto		☐		Auto	
14	A1b6	⊕	Auto		☐		Auto	
15	A1b7	⊕	Auto		☐		Auto	
16	A1b8	⊕	Auto		☐		Auto	
17	A1c1	⊕	Auto		☐		Auto	
18	A1c2	⊕	Auto		☐		Auto	
19	A1c3	⊕	Auto		☐		Auto	
20	A1c4	⊖	Auto		☐		Auto	Undeter
21	A1c5	⊕	Auto		☐		Auto	
22	A1c6	⊕	Auto		☐		Auto	
23	A1c7	⊖	Auto		☐		Auto	Undeter
24	A1c8	⊕	Auto		☐		Auto	
25	A1d1	⊕	Auto		☐		Auto	
26	A1d2	⊖	Auto		☐		Auto	Undeter
27	A1d3	⊖	Auto		☐		Auto	Undeter

7. In the Sample Group view, select **TaqMan® Assay 1**.
8. View the corresponding Amplification Plot in the lower half of the screen.
9. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).
10. From the View drop-down menu, select **C_{RT} Range** and **Histogram** to display the C_{RT} range and the Histogram on the plot.
11. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Sample Group tab for the singleplex example study is shown in the following image:

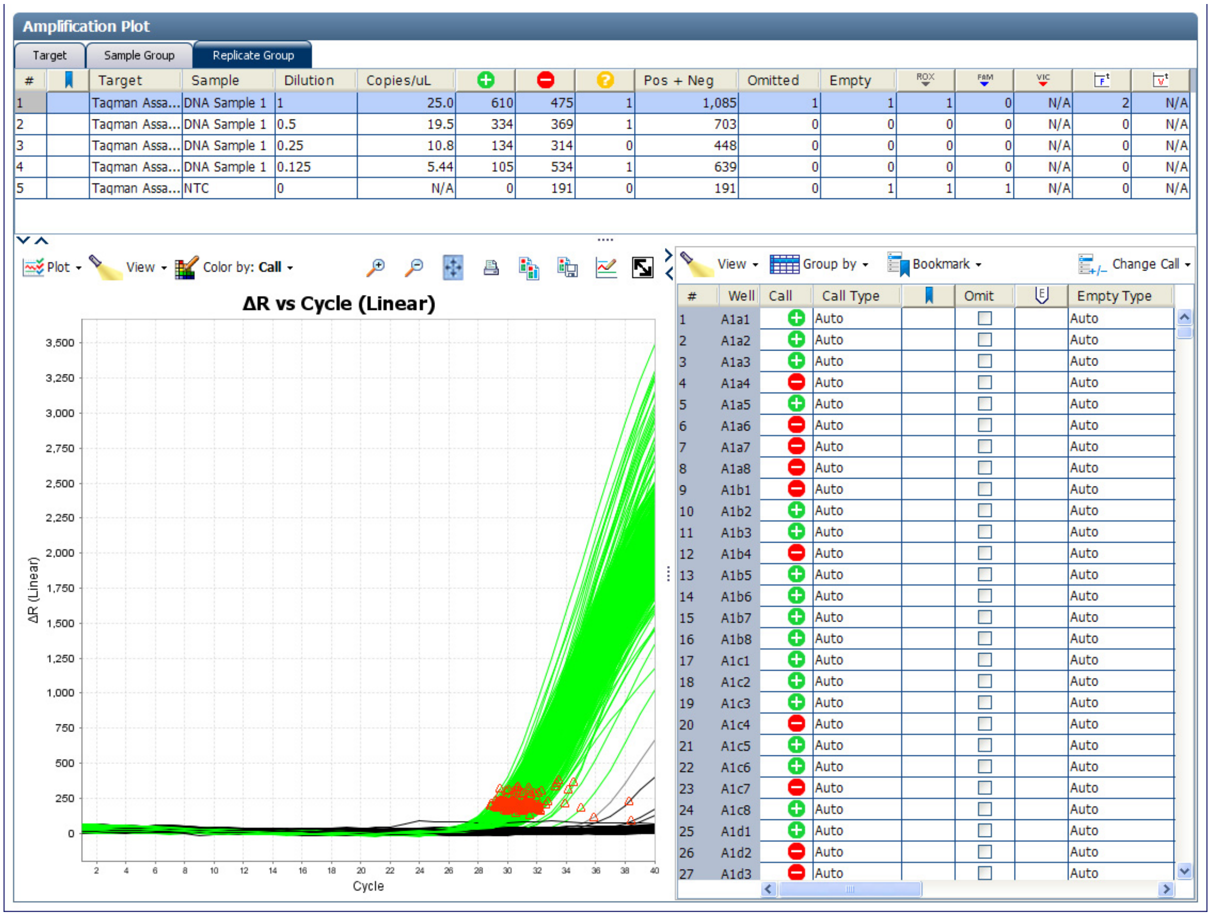


#		Target	Sample	Copies/uL	⊕	⊖	?	Pos + Neg	Omitted	Empty	ROX	FAM	VIC	F	V
1		Taqman Assay 1	DNA Sample 1	31.0	1,183	1,692	3	2,875	1	1	1	0	N/A	2	N/A
2		Taqman Assay 1	NTC	N/A	0	191	0	191	0	1	1	1	N/A	0	N/A

#	Well	Call	Call Type		Omit	E	Empty
1	A1a1	⊕	Auto		☐		Auto
2	A1a2	⊕	Auto		☐		Auto
3	A1a3	⊕	Auto		☐		Auto
4	A1a4	⊖	Auto		☐		Auto
5	A1a5	⊕	Auto		☐		Auto
6	A1a6	⊖	Auto		☐		Auto
7	A1a7	⊖	Auto		☐		Auto
8	A1a8	⊖	Auto		☐		Auto
9	A1b1	⊖	Auto		☐		Auto
10	A1b2	⊕	Auto		☐		Auto
11	A1b3	⊕	Auto		☐		Auto
12	A1b4	⊖	Auto		☐		Auto
13	A1b5	⊕	Auto		☐		Auto
14	A1b6	⊕	Auto		☐		Auto
15	A1b7	⊕	Auto		☐		Auto
16	A1b8	⊕	Auto		☐		Auto
17	A1c1	⊕	Auto		☐		Auto
18	A1c2	⊕	Auto		☐		Auto
19	A1c3	⊕	Auto		☐		Auto
20	A1c4	⊖	Auto		☐		Auto
21	A1c5	⊕	Auto		☐		Auto
22	A1c6	⊕	Auto		☐		Auto
23	A1c7	⊖	Auto		☐		Auto
24	A1c8	⊕	Auto		☐		Auto
25	A1d1	⊕	Auto		☐		Auto
26	A1d2	⊖	Auto		☐		Auto
27	A1d3	⊖	Auto		☐		Auto

12. In the Replicate Group view, select **TaqMan® Assay 1**.
13. View the corresponding Amplification Plot in the lower half of the screen.
14. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).
15. From the View drop-down menu, select **C_{RT}** to display the C_{RT} icons on the plot.
16. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Replicate Group tab for the singleplex example study is shown in the following image:



#		Target	Sample	Dilution	Copies/uL	⊕	⊖	?	Pos + Neg	Omitted	Empty	ROX	FAM	VIC	F	V
1		Taqman Assa...	DNA Sample 1	1	25.0	610	475	1	1,085	1	1	1	0	N/A	2	N/A
2		Taqman Assa...	DNA Sample 1	0.5	19.5	334	369	1	703	0	0	0	0	N/A	0	N/A
3		Taqman Assa...	DNA Sample 1	0.25	10.8	134	314	0	448	0	0	0	0	N/A	0	N/A
4		Taqman Assa...	DNA Sample 1	0.125	5.44	105	534	1	639	0	0	0	0	N/A	0	N/A
5		Taqman Assa...	NTC	0	N/A	0	191	0	191	0	1	1	1	N/A	0	N/A

#	Well	Call	Call Type		Omit	E	Empty Type
1	A1a1	⊕	Auto		☐		Auto
2	A1a2	⊕	Auto		☐		Auto
3	A1a3	⊕	Auto		☐		Auto
4	A1a4	⊖	Auto		☐		Auto
5	A1a5	⊕	Auto		☐		Auto
6	A1a6	⊖	Auto		☐		Auto
7	A1a7	⊖	Auto		☐		Auto
8	A1a8	⊖	Auto		☐		Auto
9	A1b1	⊖	Auto		☐		Auto
10	A1b2	⊕	Auto		☐		Auto
11	A1b3	⊕	Auto		☐		Auto
12	A1b4	⊖	Auto		☐		Auto
13	A1b5	⊕	Auto		☐		Auto
14	A1b6	⊕	Auto		☐		Auto
15	A1b7	⊕	Auto		☐		Auto
16	A1b8	⊕	Auto		☐		Auto
17	A1c1	⊕	Auto		☐		Auto
18	A1c2	⊕	Auto		☐		Auto
19	A1c3	⊕	Auto		☐		Auto
20	A1c4	⊖	Auto		☐		Auto
21	A1c5	⊕	Auto		☐		Auto
22	A1c6	⊕	Auto		☐		Auto
23	A1c7	⊖	Auto		☐		Auto
24	A1c8	⊕	Auto		☐		Auto
25	A1d1	⊕	Auto		☐		Auto
26	A1d2	⊖	Auto		☐		Auto
27	A1d3	⊖	Auto		☐		Auto

In the singleplex example study, there are 3 undetermined wells when the study is analyzed with the default analysis settings. You can review the amplification curve for these three wells and after assessing the amplification curves, you can decide whether you want to adjust the C_{RT} range, manually change the call, or omit the well altogether.

Tips for viewing the Amplification Plot for your own study

- Sort the undetermined calls by clicking on the Call column header in the Well Table so that you can select all the undetermined calls quickly and review the respective amplification curves.
- Sort the flagged wells by clicking on the ⚠ column header or the individual flag column header in the Well Table so that you can select flagged wells quickly and review their amplification curves.
- After assessing the individual amplification curves, you can decide whether you want to adjust the C_{RT} range, manually change the call, or omit the well altogether.
- To view the updated results, you must re-analyze the study after making any of the above changes.

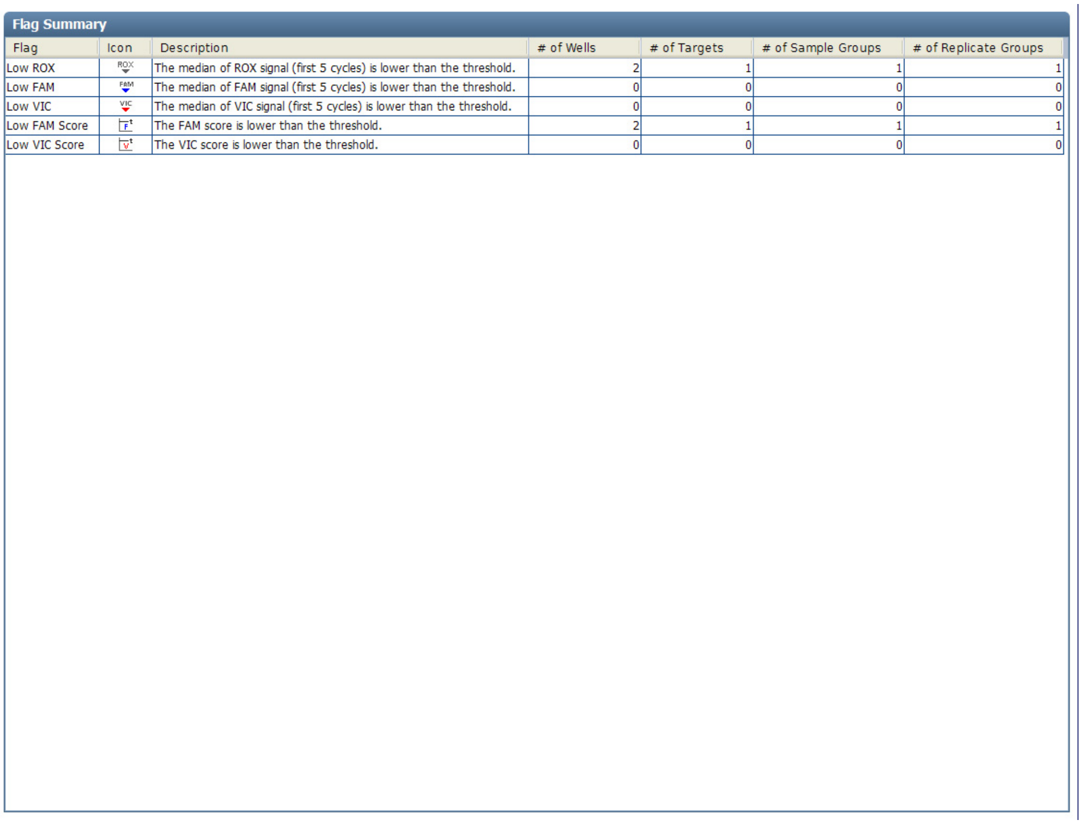
Flag Summary

1. Go to **Run QC** ▸ **Flag Summary** to access the Flag Summary screen.
2. View the Flag Summary table.

3. View the number of wells in which the flags Low ROX, Low FAM, Low VIC, Low FAM Score, and Low VIC Score appear.
4. In the singleplex example study, the Low ROX and Low FAM score flags occur in two wells each. To view the location of the flagged wells, go to **Run QC ▸ Heat Map**.

   The flagged wells are displayed with a triangle in the well. For the example study, the flagged wells are also empty wells. Therefore the wells also contain a '×' along with the triangle.

The Flag Summary for the singleplex example study is shown in the following image:



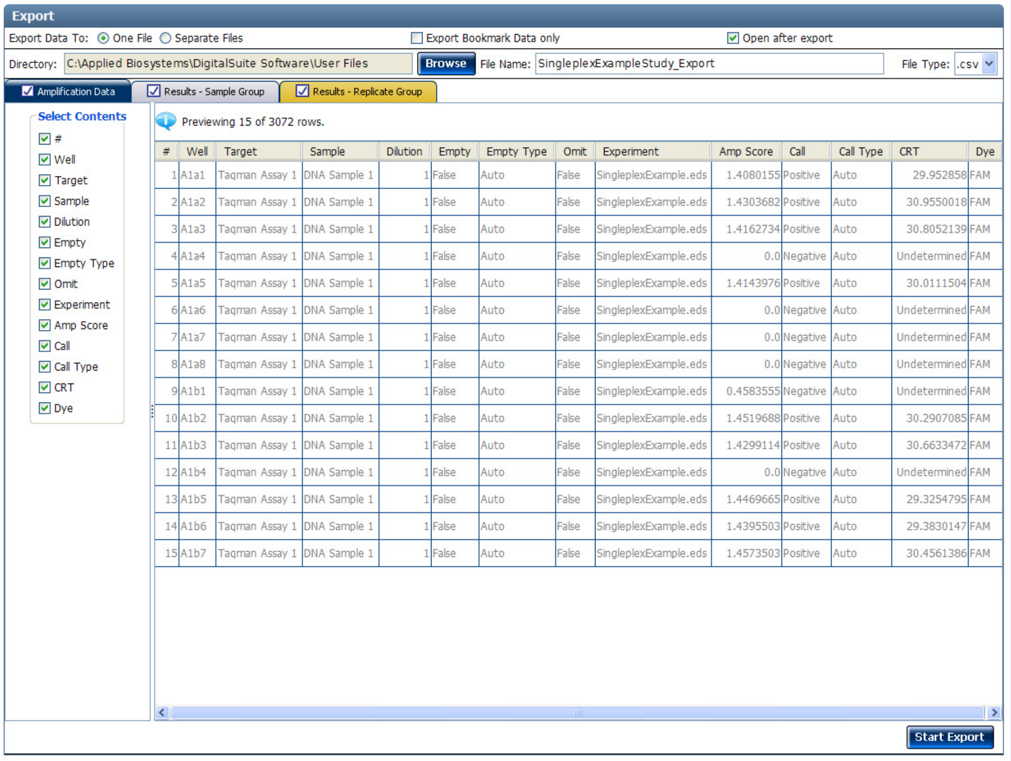
Flag Summary

Flag	Icon	Description	# of Wells	# of Targets	# of Sample Groups	# of Replicate Groups
Low ROX	ROX	The median of ROX signal (first 5 cycles) is lower than the threshold.	2	1	1	1
Low FAM	FAM	The median of FAM signal (first 5 cycles) is lower than the threshold.	0	0	0	0
Low VIC	VIC	The median of VIC signal (first 5 cycles) is lower than the threshold.	0	0	0	0
Low FAM Score	F	The FAM score is lower than the threshold.	2	1	1	1
Low VIC Score	V	The VIC score is lower than the threshold.	0	0	0	0

Export the Study

1. From the Export Workflow menu, click **Export**.
2. Select **One file**.
3. Select the **Open after export** check box.
4. Click **Browse** to select the directory and location to save the exported file.
5. Enter the file name **SingleplexExampleStudy_Export** for the exported file.
6. Select .**csv** from the File Type drop-down menu.
7. Select the **Results - Sample Group** tab to export the Sample Group results.
8. Click **Start Export**.

The Export screen for the singleplex example study is shown in the following image:



Export Data To: ● One File ○ Separate Files ☐ Export Bookmark Data only ☑ Open after export

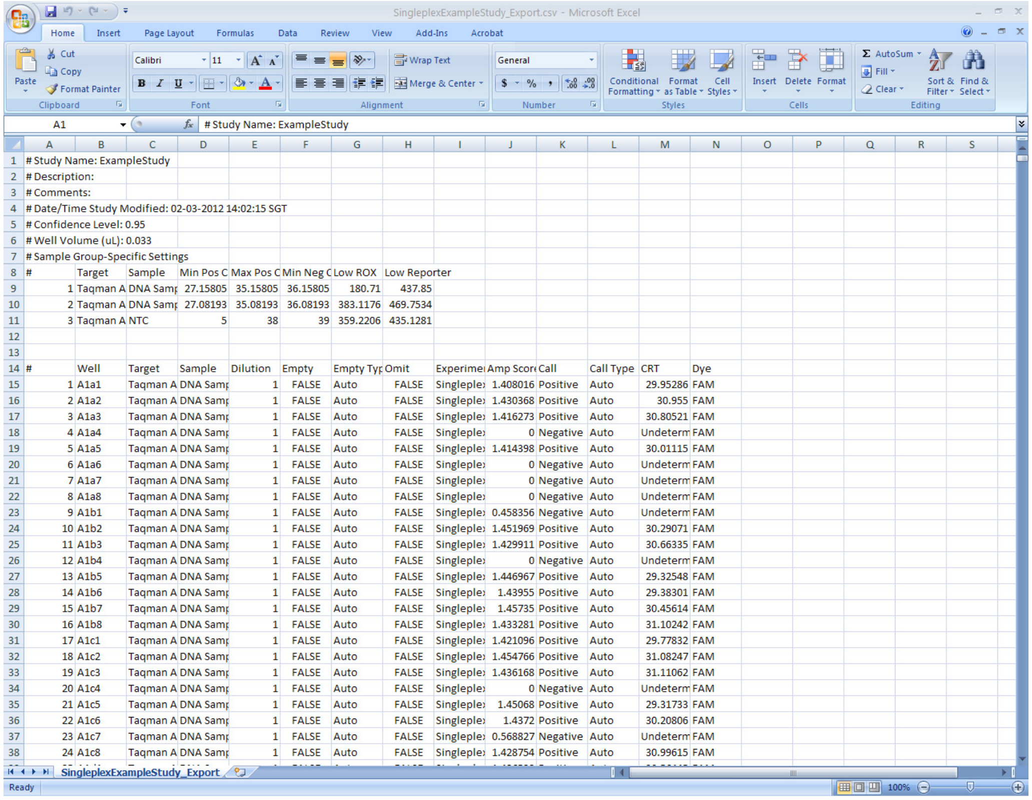
Directory: C:\Applied Biosystems\DigitalSuite Software\User Files File Name: SinglepIexExampleStudy_Export File Type: .csv

☑ Amplification Data ☑ Results - Sample Group ☑ Results - Replicate Group

Previewing 15 of 3072 rows.

#	Well	Target	Sample	Dilution	Empty	Empty Type	Omit	Experiment	Amp Score	Call	Call Type	CRT	Dye
1	A1a1	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4080155	Positive	Auto	29.952858	FAM
2	A1a2	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4303682	Positive	Auto	30.9550018	FAM
3	A1a3	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4162734	Positive	Auto	30.8052139	FAM
4	A1a4	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
5	A1a5	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4143976	Positive	Auto	30.0111504	FAM
6	A1a6	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
7	A1a7	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
8	A1a8	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
9	A1b1	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.4583555	Negative	Auto	Undetermined	FAM
10	A1b2	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4519688	Positive	Auto	30.2907085	FAM
11	A1b3	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4299114	Positive	Auto	30.6633472	FAM
12	A1b4	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
13	A1b5	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4469665	Positive	Auto	29.3254795	FAM
14	A1b6	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4395503	Positive	Auto	29.3830147	FAM
15	A1b7	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	SingleplexExample.eds	1.4573503	Positive	Auto	30.4561386	FAM

The exported file when opened in Microsoft Excel is shown in the following image:



A1 # Study Name: ExampleStudy

1 # Study Name: ExampleStudy
2 # Description:
3 # Comments:
4 # Date/Time Study Modified: 02-03-2012 14:02:15 SGT
5 # Confidence Level: 0.95
6 # Well Volume (uL): 0.033
7 # Sample Group-Specific Settings

	A	B	C	D	E	F	G	H
8	#	Target	Sample	Min Pos C	Max Pos C	Min Neg C	Low ROX	Low Reporter
9	1	Taqman A	DNA Samp	27.15805	35.15805	36.15805	180.71	437.85
10	2	Taqman A	DNA Samp	27.08193	35.08193	36.08193	383.1176	469.7534
11	3	Taqman A	NTC	5	38	39	359.2206	435.1281
12								
13								

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
14	#	Well	Target	Sample	Dilution	Empty	Empty Typ	Omit	Experime	Amp Score	Call	Call Type	CRT	Dye
15	1	A1a1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.408016	Positive	Auto	29.95286	FAM
16	2	A1a2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.430368	Positive	Auto	30.955	FAM
17	3	A1a3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.416273	Positive	Auto	30.80521	FAM
18	4	A1a4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
19	5	A1a5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.414398	Positive	Auto	30.01115	FAM
20	6	A1a6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
21	7	A1a7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
22	8	A1a8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
23	9	A1b1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0.458356	Negative	Auto	Undeterm	FAM
24	10	A1b2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.451969	Positive	Auto	30.29071	FAM
25	11	A1b3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.429911	Positive	Auto	30.66335	FAM
26	12	A1b4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
27	13	A1b5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.446967	Positive	Auto	29.32548	FAM
28	14	A1b6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.43955	Positive	Auto	29.38301	FAM
29	15	A1b7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.45735	Positive	Auto	30.45614	FAM
30	16	A1b8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.433281	Positive	Auto	31.10242	FAM
31	17	A1c1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.421096	Positive	Auto	29.77832	FAM
32	18	A1c2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.454766	Positive	Auto	31.08247	FAM
33	19	A1c3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.436168	Positive	Auto	31.11062	FAM
34	20	A1c4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0	Negative	Auto	Undeterm	FAM
35	21	A1c5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.45068	Positive	Auto	29.31733	FAM
36	22	A1c6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.4372	Positive	Auto	30.20806	FAM
37	23	A1c7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	0.568827	Negative	Auto	Undeterm	FAM
38	24	A1c8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	Singleplex	1.428754	Positive	Auto	30.99615	FAM

Section 3.3 Analyzing Duplex Experiments

In this section, you use the example duplex experiment file provided with the DigitalSuite™ Software to create a study and analyze the digital experiment results. This section includes:

About Duplex experiments

Duplex experiments include two TaqMan® Assays using FAM™ dye and VIC® dye as the reporter dyes respectively.

Setup a duplex study

Setup a study using the duplex example experiment from the software installation.

Create a study

1. Launch DigitalSuite™ Software, and on the Home screen click **Create Study**.
2. In the Import dialog box, navigate to C:\Program Files\Applied Biosystems\DigitalSuite Software\examples and select **DuplexExample.eds**. Click **Import**.

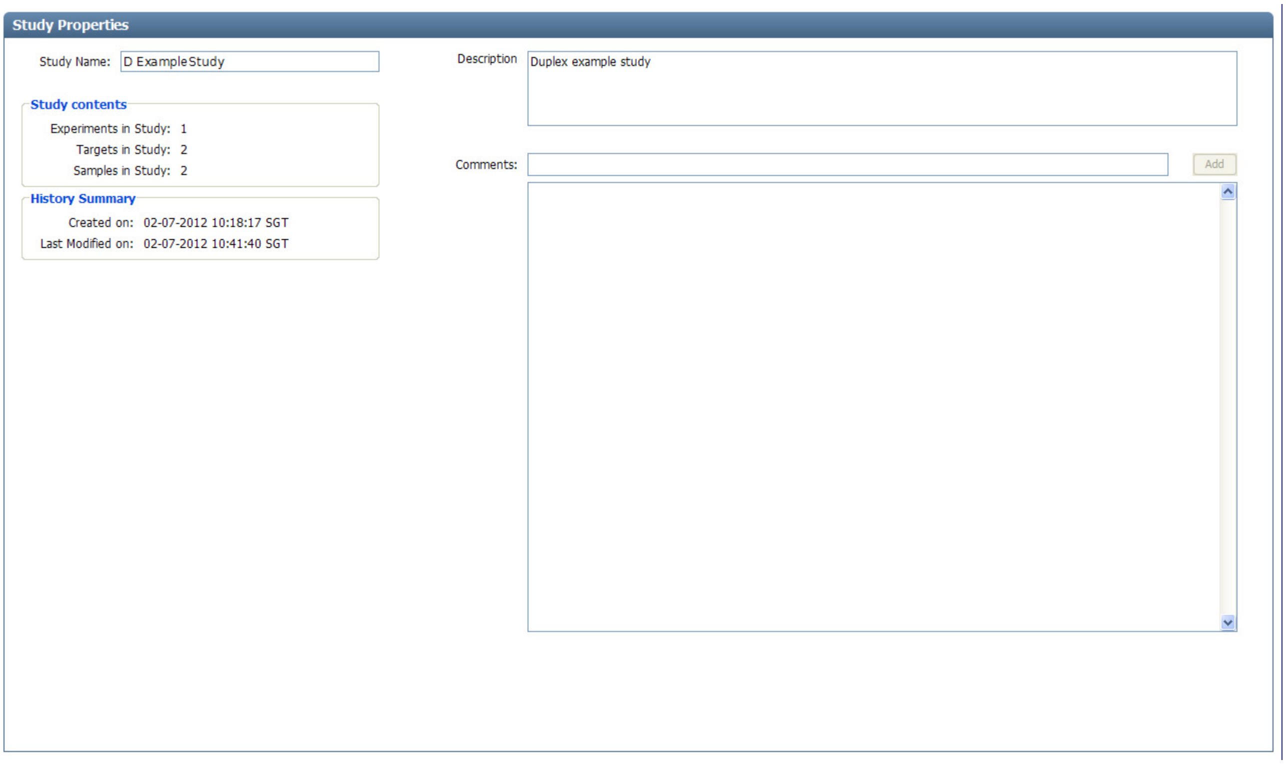
   Note: This contains duplex data from a single OpenArray® plate. When setting up your own Duplex study, you can add duplex data from multiple OpenArray® plates to the study.

Define study properties

1. Click **Properties** from the Setup menu to access the Properties screen.
2. Enter the study name **D ExampleStudy**.

3. In the Description field, enter **Duplex example study**.
4. In the Comments field, enter any comments, then click **Add**.
5. Verify that the Study contents and History Summary match those of your duplex example study.

The Properties screen of the duplex example study is shown in the following image:



Study Name: D ExampleStudy

Description: Duplex example study

Comments:

Study contents
Experiments in Study: 1
Targets in Study: 2
Samples in Study: 2

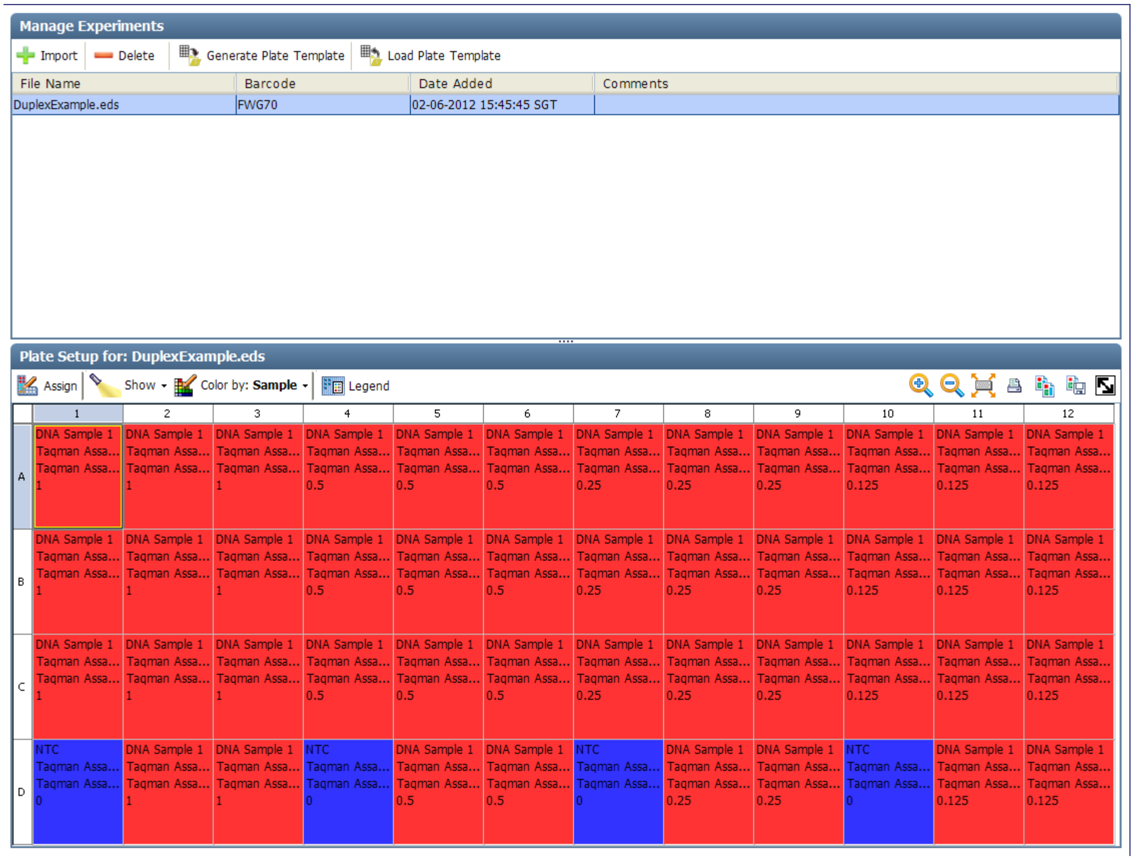
History Summary
Created on: 02-07-2012 10:18:17 SGT
Last Modified on: 02-07-2012 10:41:40 SGT

Setup plate layout

1. Click **Plate Setup** from the Setup menu to access the Plate Setup screen.
2. Click **Load Plate Template** to load the plate setup contents from the example plate setup file.
3. Browse to C:\Program Files\Applied Biosystems\DigitalSuite Software\examples. Select **DuplexExamplePlateSetup.csv**.

   Note: For your own duplex studies, you can setup the plate contents using a previously created plate setup file or click **Assign** to assign the plate contents. For more information on assigning plate contents, see "Set up plate layout" on page 25.
4. Click **Show** to show or hide the data elements in the plate.
5. Click **Color by:** to color the sub-arrays using the different data elements.
6. Click **Legend** to show or hide the plate layout legend.

The Plate Setup screen of the duplex example study is shown in the following image:



Manage Experiments

File Name	Barcode	Date Added	Comments
DuplexExample.eds	FWG70	02-06-2012 15:45:45 SGT	

Plate Setup for: DuplexExample.eds

	1	2	3	4	5	6
A	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5
B	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5
C	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5
D	NTC Taqman Assa... Taqman Assa... 0	DNA Sample 1 Taqman Assa... Taqman Assa... 1	DNA Sample 1 Taqman Assa... Taqman Assa... 1	NTC Taqman Assa... Taqman Assa... 0	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5	DNA Sample 1 Taqman Assa... Taqman Assa... 0.5

	7	8	9	10	11	12
A	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125
B	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125
C	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125
D	NTC Taqman Assa... Taqman Assa... 0	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	DNA Sample 1 Taqman Assa... Taqman Assa... 0.25	NTC Taqman Assa... Taqman Assa... 0	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125	DNA Sample 1 Taqman Assa... Taqman Assa... 0.125

Setup samples and targets

Use this screen to add samples and target names, and dilutions, in addition to the default ones. The names and dilutions will then appear in the Well Editor in the Plate Setup screen and can be used to set up the plate.

1. Click **Samples/Targets** from the Setup menu to access the Samples/Targets screen.
2. Click **Add** to add sample names. You can also click in the Name column to change the default sample name. Optionally, select a color from the Color drop-down menu.

   Note: DigitalSuite™ Software automatically assigns the number of wells for the sample name.

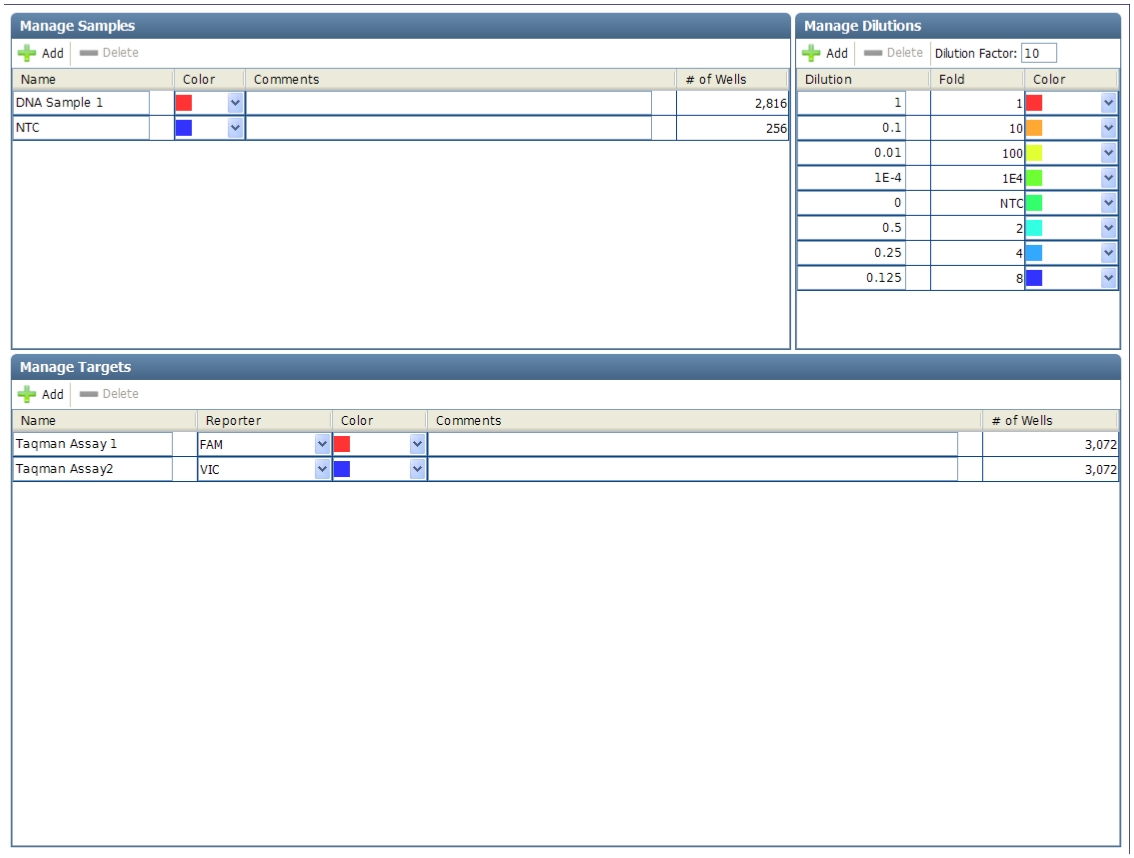
3. Click **Add** to add target names. You can also click in the Name column to change the default target name. Optionally, select a color from the Color drop-down menu.

   Note: DigitalSuite™ Software automatically assigns the number of wells for the target name.

4. Click **Add** to add dilutions. You can also click in the Dilution column to change the default dilutions. Optionally, select a color from the Color drop-down menu.
5. Enter the dilution factor for the dilution.

In the duplex example study, TaqMan® Assay 1 has FAM™ dye as the reporter dye while TaqMan® Assay 2 has VIC® dye as the reporter dye.

The Samples/Targets screen of the duplex example study is shown in the following image:



Manage Samples

Name	Color	Comments	# of Wells
DNA Sample 1	🟥		2,816
NTC	🟦		256

Manage Dilutions

Dilution Factor: 10

Dilution	Fold	Color
1	1	🟥
0.1	10	🟧
0.01	100	🟨
1E-4	1E4	🟩
0	NTC	🟩
0.5	2	🟦
0.25	4	🟦
0.125	8	🟦

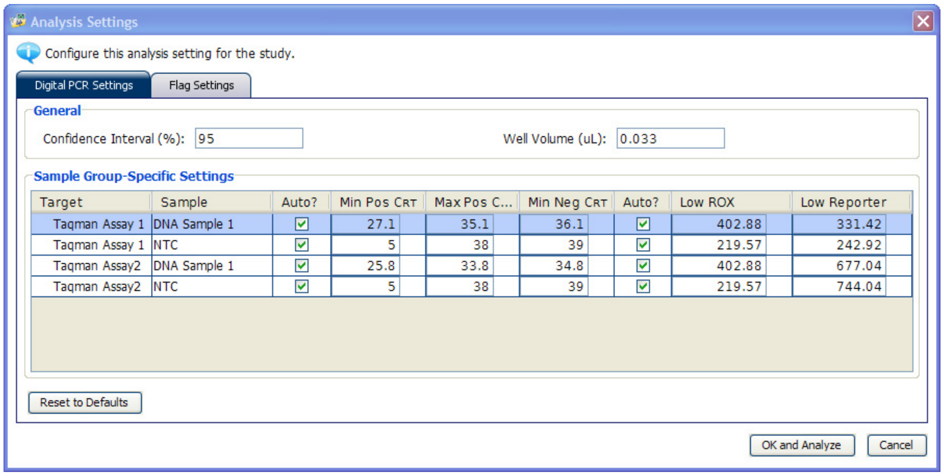
Manage Targets

Name	Reporter	Color	Comments	# of Wells
Taqman Assay 1	FAM	🟥		3,072
Taqman Assay2	VIC	🟦		3,072

Analyze the study

Once you have set up the plate, click **Analyze**. To modify the analysis settings, click **Analysis Settings**.

The Analysis Settings dialog box for a duplex example study is shown in the following image:



Analysis Settings

Configure this analysis setting for the study.

General

Confidence Interval (%): 95

Well Volume (uL): 0.033

Sample Group-Specific Settings

Target	Sample	Auto?	Min Pos Crt	Max Pos C...	Min Neg Crt	Auto?	Low ROX	Low Reporter
Taqman Assay 1	DNA Sample 1	☑	27.1	35.1	36.1	☑	402.88	331.42
Taqman Assay 1	NTC	☑	5	38	39	☑	219.57	242.92
Taqman Assay2	DNA Sample 1	☑	25.8	33.8	34.8	☑	402.88	677.04
Taqman Assay2	NTC	☑	5	38	39	☑	219.57	744.04

View digital PCR results

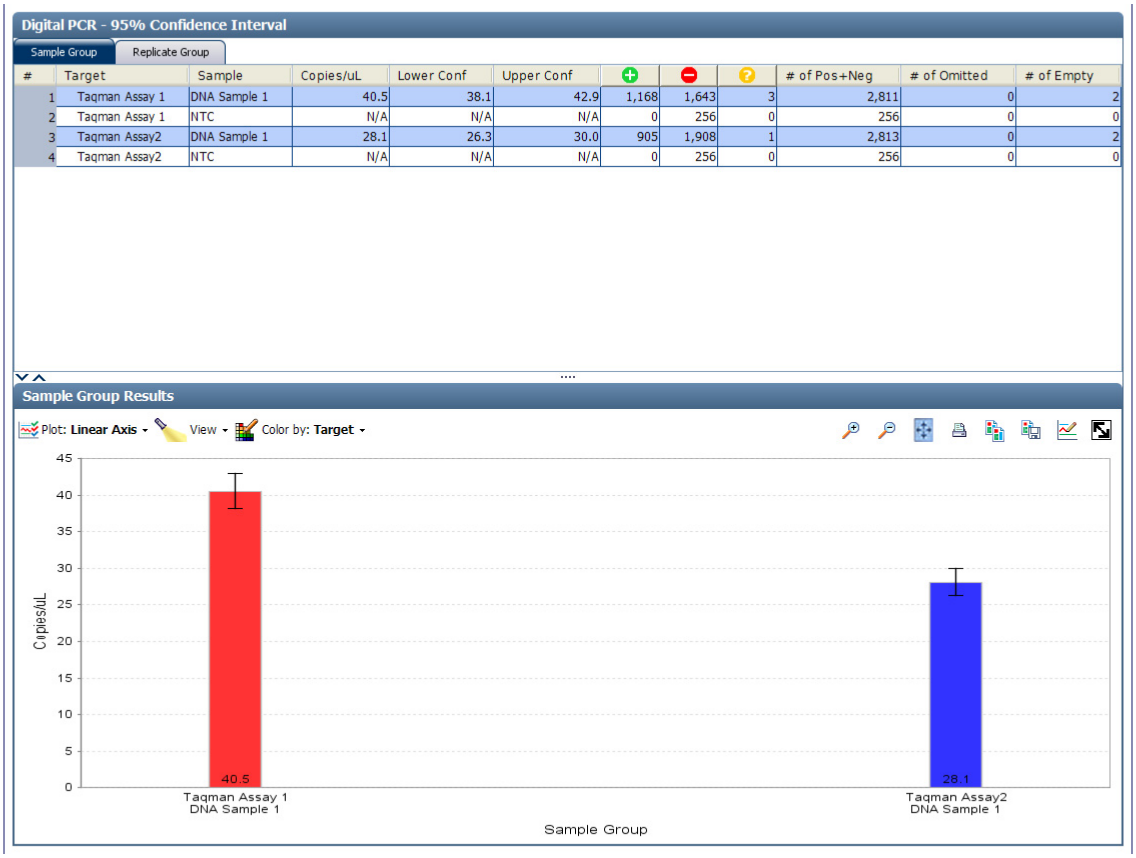
Clicking on the Analysis button automatically takes you to the Digital PCR screen. Alternatively, click **Digital PCR** from the Results Workflow menu to access the screen.

The Confidence Interval value is displayed in the Title Bar of the Digital PCR screen.

Sample Group view

1. Click the **Sample Group** tab to view the sample group results.
   For TaqMan® Assay 1, the Copies/µL is 40.5.
   For TaqMan® Assay 2, the Copies/µL is 28.1.
2. For TaqMan® Assay 1, view the lower confidence (38.1), upper confidence (42.9) values, number of positive (1,168), negative (1,643), and undetermined (3) calls, total number of positive and negative calls (2,811), number of omitted wells (0), and number of empty wells (0).
3. For TaqMan® Assay 1, for the Sample NTC the Copies/µL is NA. This is because the number of positive calls for NTC is 0 and the DigitalSuite™ Software cannot calculate the Copies/µL.
4. For TaqMan® Assay 2, view the lower confidence (26.3), upper confidence (30.0) values, number of positive (905), negative (1,908), and undetermined (1) calls, total number of positive and negative calls (2,813), number of omitted wells (0), and number of empty wells (0).
5. For TaqMan® Assay 2, for the Sample NTC the Copies/µL is NA. This is because the number of positive calls for NTC is 0 and the DigitalSuite™ Software cannot calculate the Copies/µL.
6. View the corresponding plot in the lower half of the screen.
7. From the Plot drop-down menu, select **Linear Axis** (default). The Linear axis plot type displays the Sample Group on the x-axis and Copies/µL on the y-axis.
8. From the View drop-down menu, select **Legend**.
9. From the Color by: drop-down menu, select **Target** (default).

The Sample Group view for the duplex example study is shown in the following image:



Digital PCR - 95% Confidence Interval

#	Target	Sample	Copies/uL	Lower Conf	Upper Conf	⊕	⊖	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	40.5	38.1	42.9	1,168	1,643	3	2,811	0	2
2	Taqman Assay 1	NTC	N/A	N/A	N/A	0	256	0	256	0	0
3	Taqman Assay2	DNA Sample 1	28.1	26.3	30.0	905	1,908	1	2,813	0	2
4	Taqman Assay2	NTC	N/A	N/A	N/A	0	256	0	256	0	0

Replicate Group view

1. Click the **Replicate Group** tab to view the sample group results.

   For TaqMan® Assay 1, having dilution 1, the Copies/µL is 40.5. The Copies/µL should be roughly proportional to the dilution series. For example, dilution 0.5 is half of dilution 1 then the Copies/µL for dilution 0.5 should be roughly half of that for dilution 1. In the example study, the Copies/µL for dilution 0.5 is 20 which is approximately half of 40.5.

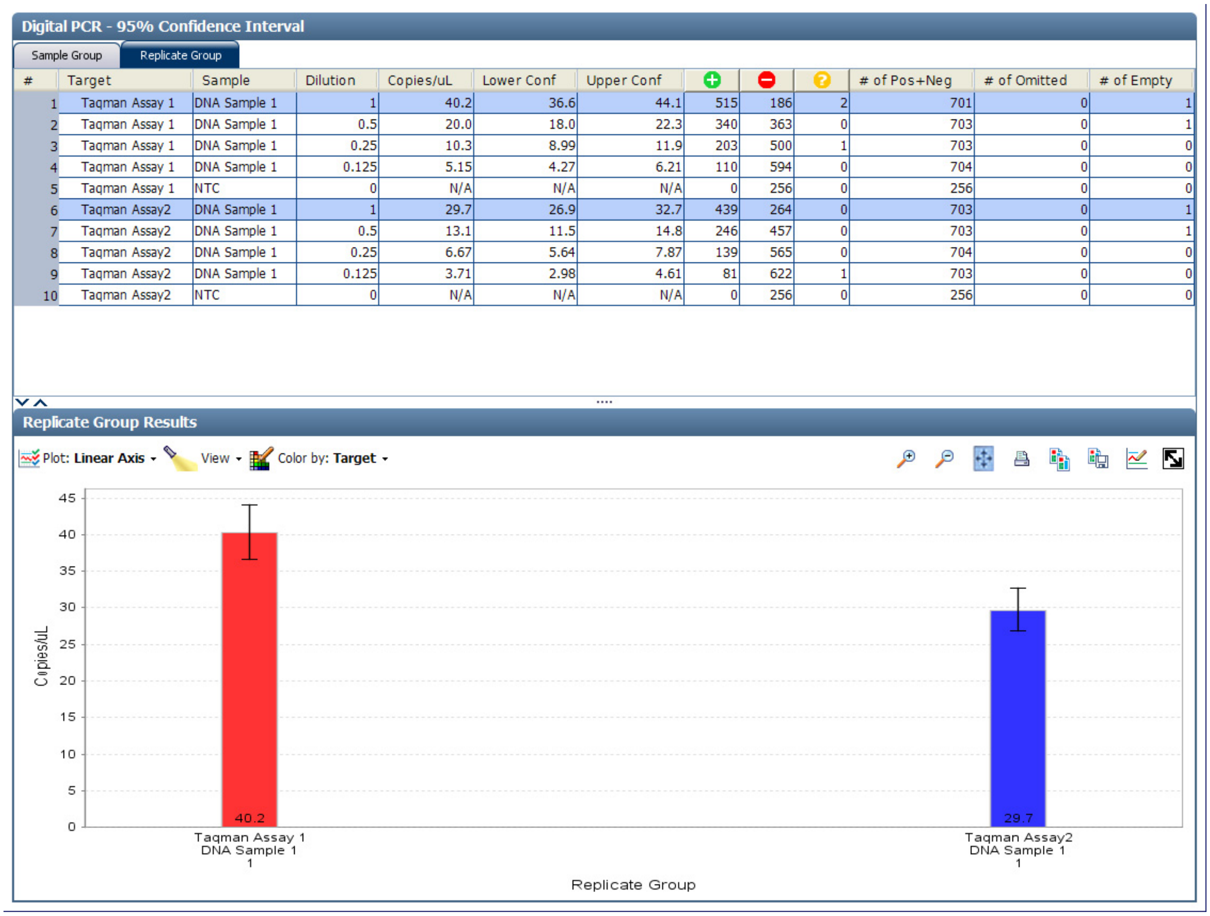
   For TaqMan® Assay 2, having dilution 1, the Copies/µL is 29.7. The Copies/µL should be roughly proportional to the dilution series. For example, dilution 0.5 is half of dilution 1 then the Copies/µL for dilution 0.5 should be roughly half of that for dilution 1. In the example study, the Copies/µL for dilution 0.5 is 13.1 which is approximately half of 29.7.

   Note: Life Technologies recommends you to check if the Copies/µL results are always roughly proportional to the dilution series. If the ratio of Copies/µL:dilution is significantly different in the Replicate Groups, check the plate setup or re-run the experiment.

2. For TaqMan® Assay 1, having dilution 1, view the lower confidence (36.6) and upper confidence (44.1) values, number of positive (515), negative (186), and undetermined (2) calls, total number of positive and negative calls (701), number of omitted wells (0), and number of empty wells (1).

3. For TaqMan® Assay 2, having dilution 1, view the lower confidence (26.9) and upper confidence (32.7) values, number of positive (439), negative (264), and undetermined (0) calls, total number of positive and negative calls (703), number of omitted wells (0), and number of empty wells (1).
4. View the corresponding plot in the lower half of the screen.
5. From the Plot drop-down menu, select **Linear Axis** (default). The Linear axis plot type displays the Replicate Group on the x-axis and Copies/µL on the y-axis.
6. From the View drop-down menu, select **Legend**.
7. From the Color by: drop-down menu, select **Target** (default).

The Replicate Group view for the duplex example study is shown in the following image:



Digital PCR - 95% Confidence Interval

Sample Group | Replicate Group

#	Target	Sample	Dilution	Copies/uL	Lower Conf	Upper Conf	⊕	⊖	?	# of Pos+Neg	# of Omitted	# of Empty
1	Taqman Assay 1	DNA Sample 1	1	40.2	36.6	44.1	515	186	2	701	0	1
2	Taqman Assay 1	DNA Sample 1	0.5	20.0	18.0	22.3	340	363	0	703	0	1
3	Taqman Assay 1	DNA Sample 1	0.25	10.3	8.99	11.9	203	500	1	703	0	0
4	Taqman Assay 1	DNA Sample 1	0.125	5.15	4.27	6.21	110	594	0	704	0	0
5	Taqman Assay 1	NTC	0	N/A	N/A	N/A	0	256	0	256	0	0
6	Taqman Assay2	DNA Sample 1	1	29.7	26.9	32.7	439	264	0	703	0	1
7	Taqman Assay2	DNA Sample 1	0.5	13.1	11.5	14.8	246	457	0	703	0	1
8	Taqman Assay2	DNA Sample 1	0.25	6.67	5.64	7.87	139	565	0	704	0	0
9	Taqman Assay2	DNA Sample 1	0.125	3.71	2.98	4.61	81	622	1	703	0	0
10	Taqman Assay2	NTC	0	N/A	N/A	N/A	0	256	0	256	0	0

Replicate Group Results

Plot: **Linear Axis** · View · Color by: **Target**

Run QC

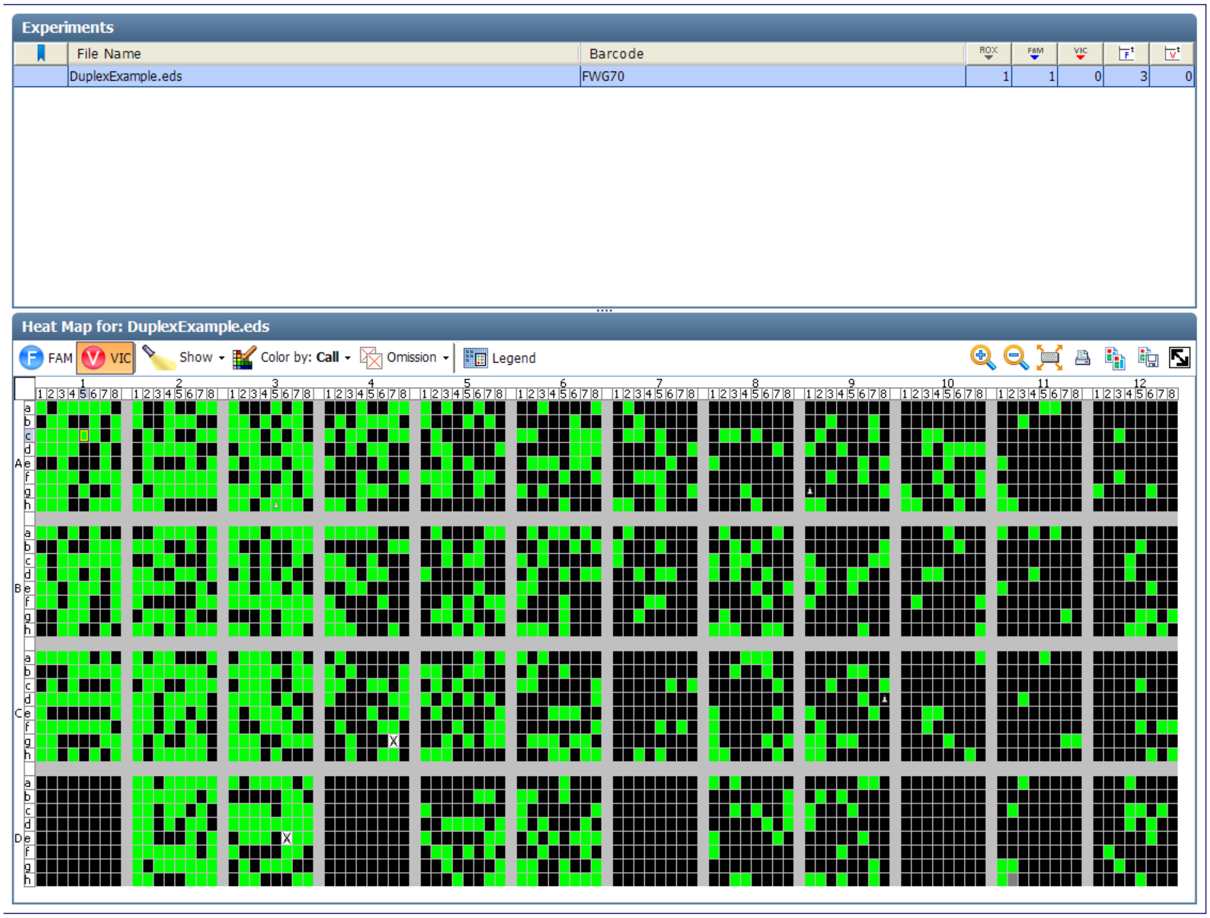
Heat Map

1. Go to **Run QC** ▸ **Heat Map** to access the Heat Map screen.
2. In the Experiments view, select **DuplexExample**.eds.
3. View the corresponding Heat Map in the lower half of the screen.

Note: The VIC button appears enabled for duplex experiments. Click **VIC** to view the Heat Map for VIC® dye.

4. Roll-over a sub-array. The tool-tip displays the well contents.
5. From the Show drop-down menu, select **Flag** to display flags in the heat map.
    a. Look out for flagged wells or empty wells.
    b. Check the location of these wells in the plate. If the location does not appear to form any pattern, check the amplification curve in the amplification plot. Based on these assessments, you can decide to manually change the call or omit the well.
    c. Click **Analyze** to re-analyze the study.
6. From the Color by: drop-down menu, select **Call** to display the wells by the color of the Call.
7. Click **Legend** to show or hide the plate legend.

The Heat Map for the duplex example study is shown in the following image:



Experiments

	File Name	Barcode	ROX	FAM	VIC	F	V
	DuplexExample.eds	FWG70	1	1	0	3	0

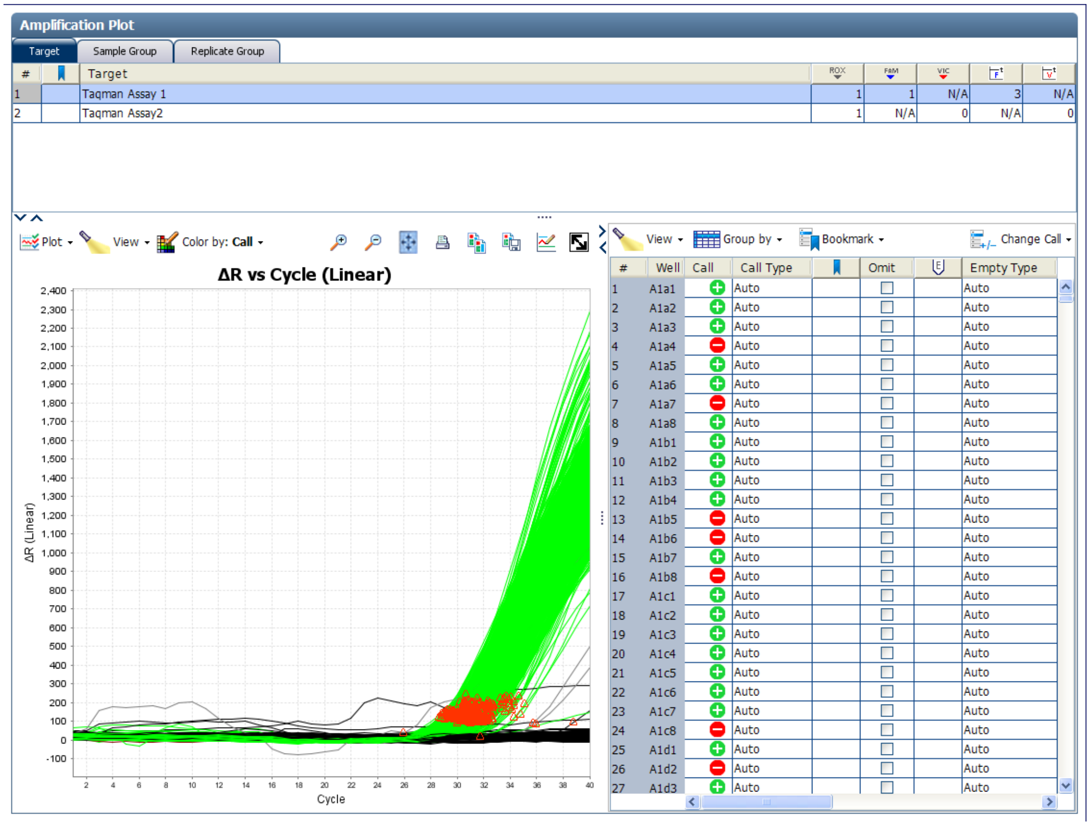
Heat Map for: DuplexExample.eds

Amplification Plot

1. Go to **Run QC** ▸ **Amplification Plot** to access the Amplification Plot screen.
2. In the Targets view, select **TaqMan® Assay 1**.
3. View the corresponding Amplification Plot in the lower half of the screen.

4. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).
5. From the View drop-down menu, select **C_{RT}** to display the C_{RT} icons on the plot.
6. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Targets tab for TaqMan® Assay 1 in the duplex example study is shown in the following image:

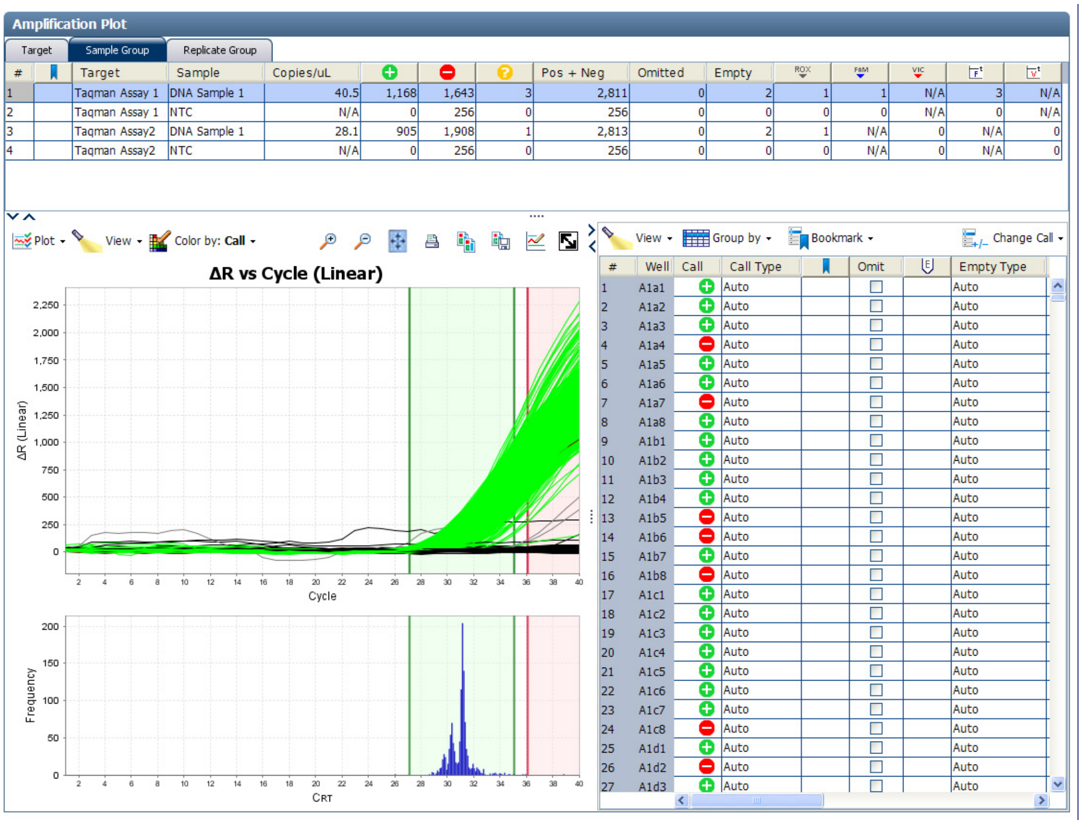


#		Target	ROX	FAM	VIC	F	V
1		Taqman Assay 1	1	1	N/A	3	N/A
2		Taqman Assay2	1	N/A	0	N/A	0

#	Well	Call	Call Type		Omit	E	Empty Type
1	A1a1	⊕	Auto		☐		Auto
2	A1a2	⊕	Auto		☐		Auto
3	A1a3	⊕	Auto		☐		Auto
4	A1a4	⊖	Auto		☐		Auto
5	A1a5	⊕	Auto		☐		Auto
6	A1a6	⊕	Auto		☐		Auto
7	A1a7	⊖	Auto		☐		Auto
8	A1a8	⊕	Auto		☐		Auto
9	A1b1	⊕	Auto		☐		Auto
10	A1b2	⊕	Auto		☐		Auto
11	A1b3	⊕	Auto		☐		Auto
12	A1b4	⊕	Auto		☐		Auto
13	A1b5	⊖	Auto		☐		Auto
14	A1b6	⊖	Auto		☐		Auto
15	A1b7	⊕	Auto		☐		Auto
16	A1b8	⊖	Auto		☐		Auto
17	A1c1	⊕	Auto		☐		Auto
18	A1c2	⊕	Auto		☐		Auto
19	A1c3	⊕	Auto		☐		Auto
20	A1c4	⊕	Auto		☐		Auto
21	A1c5	⊕	Auto		☐		Auto
22	A1c6	⊕	Auto		☐		Auto
23	A1c7	⊕	Auto		☐		Auto
24	A1c8	⊖	Auto		☐		Auto
25	A1d1	⊕	Auto		☐		Auto
26	A1d2	⊖	Auto		☐		Auto
27	A1d3	⊕	Auto		☐		Auto

7. Perform step 2. through 6. to observe the Amplification Plot for the target TaqMan® Assay 2.
8. In the Sample Group view, select **TaqMan® Assay 1**.
9. View the corresponding Amplification Plot in the lower half of the screen.
10. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).
11. From the View drop-down menu, select **C_{RT} Range** and **Histogram** to display the C_{RT} range and the Histogram on the plot.
12. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Sample Group tab for TaqMan® Assay 1 in the duplex example study is shown in the following image:



#	🔖	Target	Sample	Copies/uL	⊕	⊖	?	Pos + Neg	Omitted	Empty	ROX	FAM	VIC	F	V
1		Taqman Assay 1	DNA Sample 1	40.5	1,168	1,643	3	2,811	0	2	1	1	N/A	3	N/A
2		Taqman Assay 1	NTC	N/A	0	256	0	256	0	0	0	0	N/A	0	N/A
3		Taqman Assay2	DNA Sample 1	28.1	905	1,908	1	2,813	0	2	1	N/A	0	N/A	0
4		Taqman Assay2	NTC	N/A	0	256	0	256	0	0	0	N/A	0	N/A	0

#	Well	Call	Call Type	🔖	Omit	E	Empty Type
1	A1a1	⊕	Auto		☐		Auto
2	A1a2	⊕	Auto		☐		Auto
3	A1a3	⊕	Auto		☐		Auto
4	A1a4	⊖	Auto		☐		Auto
5	A1a5	⊕	Auto		☐		Auto
6	A1a6	⊕	Auto		☐		Auto
7	A1a7	⊖	Auto		☐		Auto
8	A1a8	⊕	Auto		☐		Auto
9	A1b1	⊕	Auto		☐		Auto
10	A1b2	⊕	Auto		☐		Auto
11	A1b3	⊕	Auto		☐		Auto
12	A1b4	⊕	Auto		☐		Auto
13	A1b5	⊖	Auto		☐		Auto
14	A1b6	⊖	Auto		☐		Auto
15	A1b7	⊕	Auto		☐		Auto
16	A1b8	⊖	Auto		☐		Auto
17	A1c1	⊕	Auto		☐		Auto
18	A1c2	⊕	Auto		☐		Auto
19	A1c3	⊕	Auto		☐		Auto
20	A1c4	⊕	Auto		☐		Auto
21	A1c5	⊕	Auto		☐		Auto
22	A1c6	⊕	Auto		☐		Auto
23	A1c7	⊕	Auto		☐		Auto
24	A1c8	⊖	Auto		☐		Auto
25	A1d1	⊕	Auto		☐		Auto
26	A1d2	⊖	Auto		☐		Auto
27	A1d3	⊕	Auto		☐		Auto

For TaqMan® Assay 1, DNA Sample 1, there are 3 undetermined wells when the study is analyzed with the default analysis settings. You can review the amplification curve for these three wells and after assessing the amplification curves, you can decide whether you want to adjust the C_{RT} range, manually change the call, or omit the well altogether.

13. Perform step 8. through 12. to observe the Amplification Plot for the target TaqMan® Assay 2.

14. In the Replicate Group view, select **TaqMan® Assay 1**.

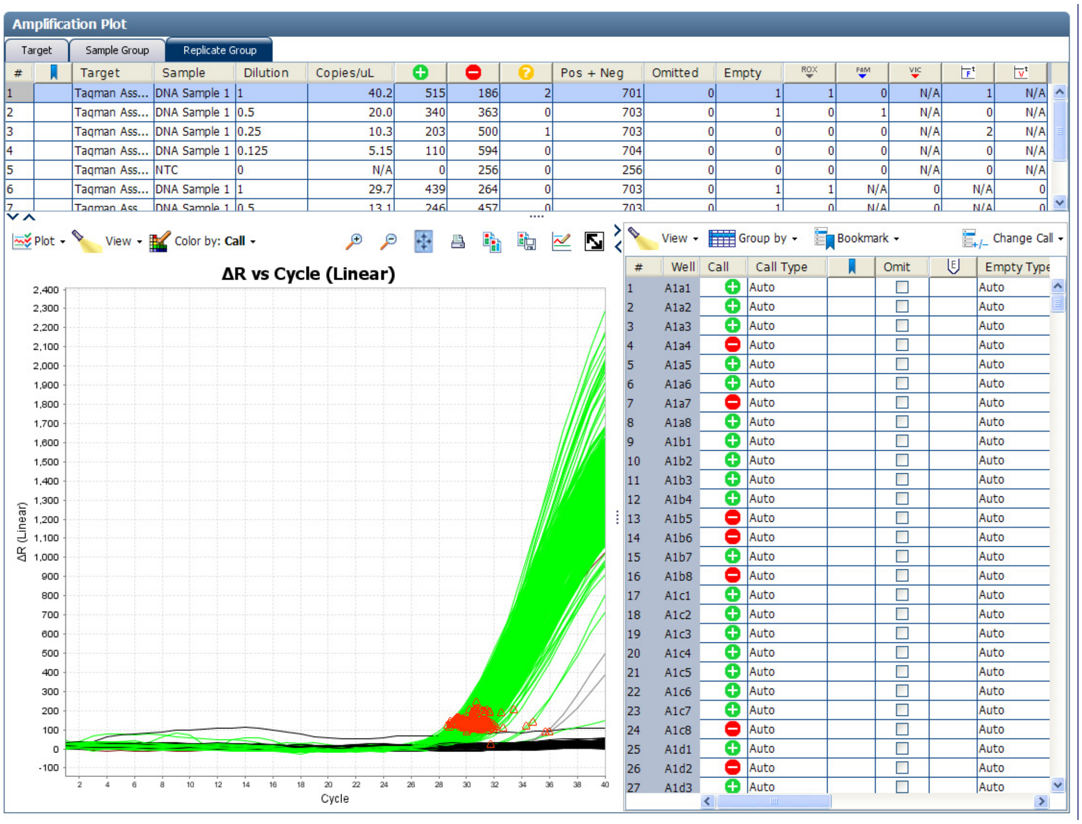
15. View the corresponding Amplification Plot in the lower half of the screen.

16. From the Plot drop-down menu, select **ΔR vs Cycle (Linear)** (default).

17. From the View drop-down menu, select **C_{RT}** to display the C_{RT} icons on the plot.

18. From the Color by: drop-down menu, select **Call** to display the plot by the color of the Call.

The Amplification Plot - Replicate Group tab for the duplex example study is shown in the following image:



#		Target	Sample	Dilution	Copies/uL	⊕	⊖	?	Pos + Neg	Omitted	Empty	ROX	FAM	VIC	F	V
1		Taqman Ass...	DNA Sample 1	1	40.2	515	186	2	701	0	1	1	0	N/A	1	N/A
2		Taqman Ass...	DNA Sample 1	0.5	20.0	340	363	0	703	0	1	0	1	N/A	0	N/A
3		Taqman Ass...	DNA Sample 1	0.25	10.3	203	500	1	703	0	0	0	0	N/A	2	N/A
4		Taqman Ass...	DNA Sample 1	0.125	5.15	110	594	0	704	0	0	0	0	N/A	0	N/A
5		Taqman Ass...	NTC	0	N/A	0	256	0	256	0	0	0	0	N/A	0	N/A
6		Taqman Ass...	DNA Sample 1	1	29.7	439	264	0	703	0	1	1	N/A	0	N/A	0
7		Taqman Ass	DNA Sample 1	0.5	13.1	246	457	0	703	0	1	0	N/A	0	N/A	0

#	Well	Call	Call Type		Omit		Empty Type
1	A1a1	⊕	Auto		☐		Auto
2	A1a2	⊕	Auto		☐		Auto
3	A1a3	⊕	Auto		☐		Auto
4	A1a4	⊖	Auto		☐		Auto
5	A1a5	⊕	Auto		☐		Auto
6	A1a6	⊕	Auto		☐		Auto
7	A1a7	⊖	Auto		☐		Auto
8	A1a8	⊕	Auto		☐		Auto
9	A1b1	⊕	Auto		☐		Auto
10	A1b2	⊕	Auto		☐		Auto
11	A1b3	⊕	Auto		☐		Auto
12	A1b4	⊕	Auto		☐		Auto
13	A1b5	⊖	Auto		☐		Auto
14	A1b6	⊖	Auto		☐		Auto
15	A1b7	⊕	Auto		☐		Auto
16	A1b8	⊖	Auto		☐		Auto
17	A1c1	⊕	Auto		☐		Auto
18	A1c2	⊕	Auto		☐		Auto
19	A1c3	⊕	Auto		☐		Auto
20	A1c4	⊕	Auto		☐		Auto
21	A1c5	⊕	Auto		☐		Auto
22	A1c6	⊕	Auto		☐		Auto
23	A1c7	⊕	Auto		☐		Auto
24	A1c8	⊖	Auto		☐		Auto
25	A1d1	⊕	Auto		☐		Auto
26	A1d2	⊖	Auto		☐		Auto
27	A1d3	⊕	Auto		☐		Auto

For TaqMan® Assay 1, Dilution 1, there are 2 undetermined wells when the study is analyzed with the default analysis settings. You can review the amplification curve for these three wells and after assessing the amplification curves, you can decide whether you want to adjust the C_{RT} range, manually change the call, or omit the well altogether.

19. Perform step 13. through 18. to observe the Amplification Plot for the target TaqMan® Assay 2 at other dilutions.

Tips for viewing the Amplification Plot for your own study

- Sort the undetermined calls by clicking on the Call column header in the Well Table so that you can select all the undetermined calls quickly and review the respective amplification curves.
- Sort the flagged wells by clicking on the ⚠ column header or the individual flag column header in the Well Table so that you can select flagged wells quickly and review their amplification curves.
- After assessing the individual amplification curves, you can decide whether you want to adjust the C_{RT} range, manually change the call, or omit the well altogether.
- To view the updated results, you must re-analyze the study after making any of the above changes.

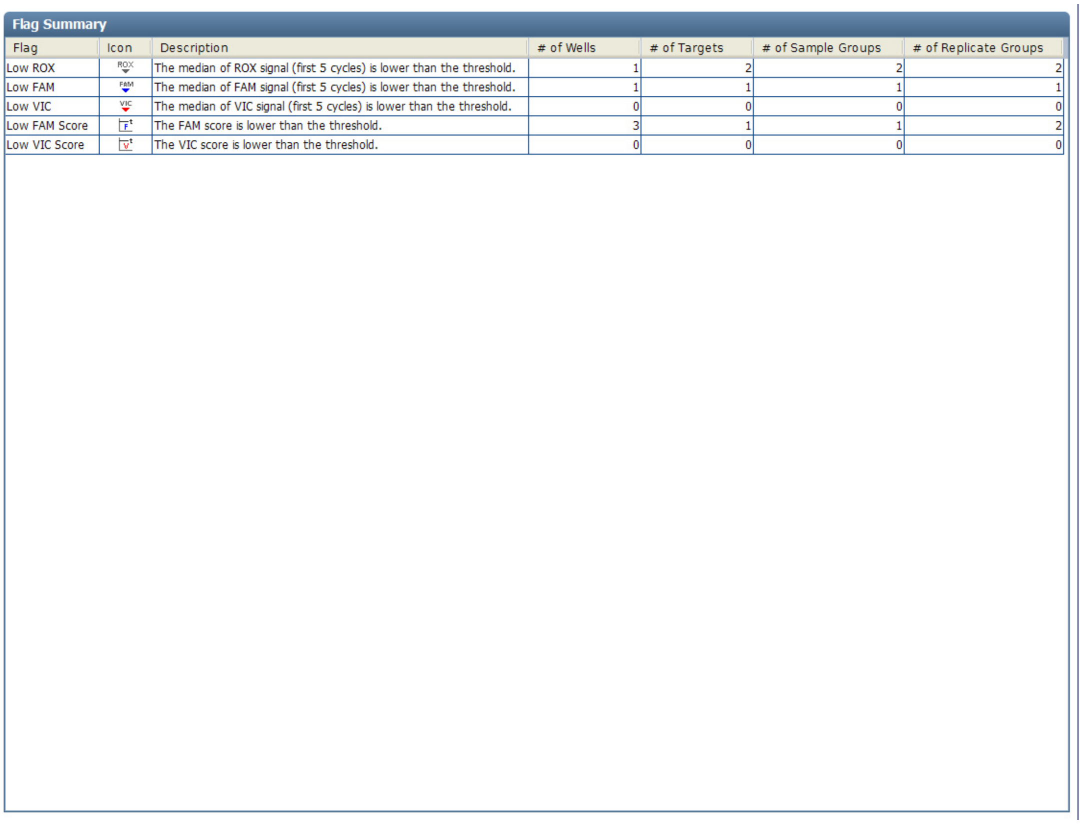
Flag Summary

1. Go to **Run QC** ▸ **Flag Summary** to access the Flag Summary screen.
2. View the Flag Summary table.

3. View the number of wells in which the flags Low ROX, Low FAM, Low VIC, Low FAM Score, and Low VIC Score appear.
4. In the duplex example study, the Low ROX and Low FAM flags occur in one well each, while the Low FAM Score flag occurs in three wells. To view the location of the flagged wells, go to **Run QC** ▸ **Heat Map**.

   The flagged wells are displayed with a triangle in the well. For the example study, the wells flagged with Low ROX and Low FAM are also empty wells. Therefore the wells also contain a '×' along with the triangle.

The Flag Summary for the duplex example study is shown in the following image:



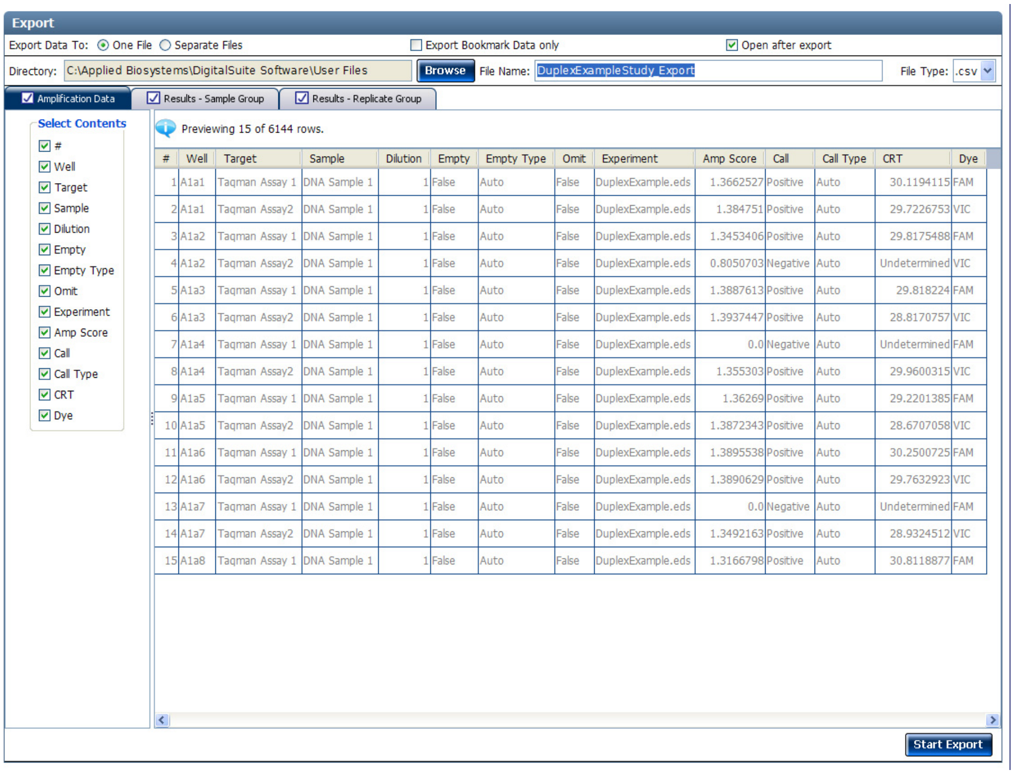
Flag Summary

Flag	Icon	Description	# of Wells	# of Targets	# of Sample Groups	# of Replicate Groups
Low ROX	ROX▼	The median of ROX signal (first 5 cycles) is lower than the threshold.	1	2	2	2
Low FAM	FAM▼	The median of FAM signal (first 5 cycles) is lower than the threshold.	1	1	1	1
Low VIC	VIC▼	The median of VIC signal (first 5 cycles) is lower than the threshold.	0	0	0	0
Low FAM Score	F	The FAM score is lower than the threshold.	3	1	1	2
Low VIC Score	V	The VIC score is lower than the threshold.	0	0	0	0

Export the Study

1. From the Export Workflow menu, click **Export**.
2. Select **One file**.
3. Select the **Open after export** check box.
4. Click **Browse** to select the directory and location to save the exported file.
5. Enter the file name **DuplexExampleStudy_Export** for the exported file.
6. Select **.csv** from the File Type drop-down menu.
7. Select the **Results - Sample Group** tab to export the Sample Group results.
8. Click **Start Export**.

The Export screen for the duplex example study is shown in the following image:



Export Data To: ● One File ○ Separate Files ☐ Export Bookmark Data only ☑ Open after export

Directory: C:\Applied Biosystems\DigitalSuite Software\User Files

File Name: DuplexExampleStudy_Export

File Type: .csv

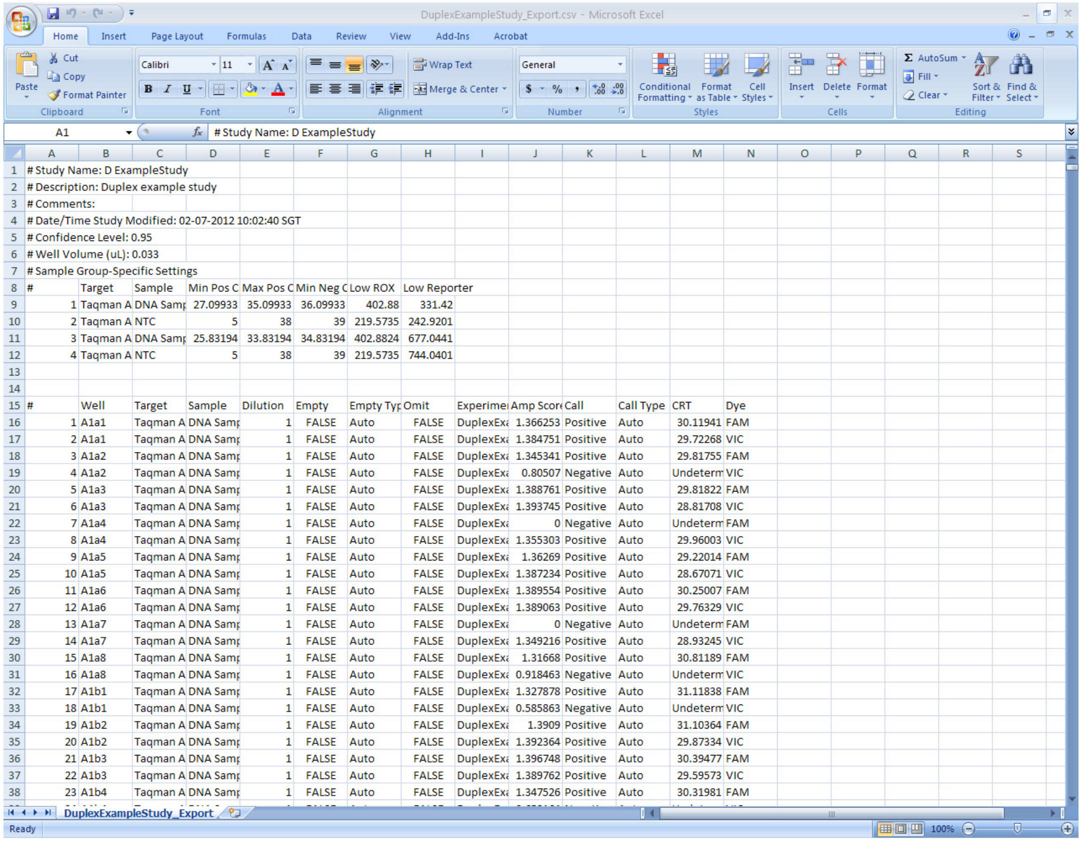
☑ Amplification Data ☑ Results - Sample Group ☑ Results - Replicate Group

Select Contents:
- ☑ #
- ☑ Well
- ☑ Target
- ☑ Sample
- ☑ Dilution
- ☑ Empty
- ☑ Empty Type
- ☑ Omit
- ☑ Experiment
- ☑ Amp Score
- ☑ Call
- ☑ Call Type
- ☑ CRT
- ☑ Dye

Previewing 15 of 6144 rows.

#	Well	Target	Sample	Dilution	Empty	Empty Type	Omit	Experiment	Amp Score	Call	Call Type	CRT	Dye
1	A1a1	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3662527	Positive	Auto	30.1194115	FAM
2	A1a1	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.384751	Positive	Auto	29.7226753	VIC
3	A1a2	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3453406	Positive	Auto	29.8175488	FAM
4	A1a2	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	0.8050703	Negative	Auto	Undetermined	VIC
5	A1a3	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3887613	Positive	Auto	29.818224	FAM
6	A1a3	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3937447	Positive	Auto	28.8170757	VIC
7	A1a4	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
8	A1a4	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.355303	Positive	Auto	29.9600315	VIC
9	A1a5	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.36269	Positive	Auto	29.2201385	FAM
10	A1a5	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3872343	Positive	Auto	28.6707058	VIC
11	A1a6	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3895538	Positive	Auto	30.2500725	FAM
12	A1a6	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3890629	Positive	Auto	29.7632923	VIC
13	A1a7	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	0.0	Negative	Auto	Undetermined	FAM
14	A1a7	Taqman Assay2	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3492163	Positive	Auto	28.9324512	VIC
15	A1a8	Taqman Assay 1	DNA Sample 1	1	False	Auto	False	DuplexExample.eds	1.3166798	Positive	Auto	30.8118877	FAM

The exported file when opened in Microsoft Excel is shown in the following image:



A1 # Study Name: D ExampleStudy

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	# Study Name: D ExampleStudy													
2	# Description: Duplex example study													
3	# Comments:													
4	# Date/Time Study Modified: 02-07-2012 10:02:40 SGT													
5	# Confidence Level: 0.95													
6	# Well Volume (uL): 0.033													
7	# Sample Group-Specific Settings													
8	#	Target	Sample	Min Pos C	Max Pos C	Min Neg C	Low ROX	Low Reporter						
9	1	Taqman A	DNA Samp	27.09933	35.09933	36.09933	402.88	331.42						
10	2	Taqman A	NTC	5	38	39	219.5735	242.9201						
11	3	Taqman A	DNA Samp	25.83194	33.83194	34.83194	402.8824	677.0441						
12	4	Taqman A	NTC	5	38	39	219.5735	744.0401						
13														
14														
15	#	Well	Target	Sample	Dilution	Empty	Empty Typ	Omit	Experime	Amp Scor	Call	Call Type	CRT	Dye
16	1	A1a1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.366253	Positive	Auto	30.11941	FAM
17	2	A1a1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.384751	Positive	Auto	29.72268	VIC
18	3	A1a2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.345341	Positive	Auto	29.81755	FAM
19	4	A1a2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	0.80507	Negative	Auto	Undeterm	VIC
20	5	A1a3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.388761	Positive	Auto	29.81822	FAM
21	6	A1a3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.393745	Positive	Auto	28.81708	VIC
22	7	A1a4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	0	Negative	Auto	Undeterm	FAM
23	8	A1a4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.355303	Positive	Auto	29.96003	VIC
24	9	A1a5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.36269	Positive	Auto	29.22014	FAM
25	10	A1a5	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.387234	Positive	Auto	28.67071	VIC
26	11	A1a6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.389554	Positive	Auto	30.25007	FAM
27	12	A1a6	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.389063	Positive	Auto	29.76329	VIC
28	13	A1a7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	0	Negative	Auto	Undeterm	FAM
29	14	A1a7	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.349216	Positive	Auto	28.93245	VIC
30	15	A1a8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.31668	Positive	Auto	30.81189	FAM
31	16	A1a8	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	0.918463	Negative	Auto	Undeterm	VIC
32	17	A1b1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.327878	Positive	Auto	31.11838	FAM
33	18	A1b1	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	0.585863	Negative	Auto	Undeterm	VIC
34	19	A1b2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.3909	Positive	Auto	31.10364	FAM
35	20	A1b2	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.392364	Positive	Auto	29.87334	VIC
36	21	A1b3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.396748	Positive	Auto	30.39477	FAM
37	22	A1b3	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.389762	Positive	Auto	29.59573	VIC
38	23	A1b4	Taqman A	DNA Samp	1	FALSE	Auto	FALSE	DuplexExa	1.347526	Positive	Auto	30.31981	FAM

A

Ordering Information

Note: A QuantStudio™ OpenArray® Accessories Kit, Part no. 4469576, is included with 10-pack array orders.

QuantStudio™ Digital PCR Kits

Item	Part number	Storage Conditions
QuantStudio™ Digital PCR Kit (10 Pack)	4470184	Upon receipt, store the frozen, unopened plates at –20°C.
QuantStudio™ Digital PCR Kit (4 Pack)	4470185	

Description of components

Item	Part number	Description
QuantStudio™ Digital PCR Plates	4470196 (4 Plates) 4470197 (10 Plates)	4 plates or 10 plates, depending on the order
2X TaqMan® OpenArray® Digital PCR Master Mix	4458086 (1.5 mL) 4458080 (5 mL)	1.5 mL or 5 mL, depending on the order
QuantStudio™ OpenArray® Accessories Kit	4469576	1 kit, enough for 10 arrays (not included with Part no. 4470185)

Documentation and Support

Related documentation

The following related documents are shipped 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 384-well, 96-well, Fast 96-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 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 97.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

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**.

Glossary

amplification curve	General term for time series data of PCR fluorescence vs. cycle number; amplification curves are often normalized by a passive reference and usually referred to as Rn vs. Cycle data. Curves that have been baseline compensated (see baselined data) are typically referred to as Rn vs. Cycle data.
amp score	A value that reflects the level of amplification in a well. In general, a low value indicates lesser amplification in that well.
baselined data	Amplification curves where the baseline has been removed by either subtraction or division of a line computed by a linear regression through the baseline region.
blank well	Wells in the sub-array that do not contain any setup information.
confidence interval	The Confidence Interval is used to calculate the lower and upper confidence values in the Digital PCR screen. For example, for a Confidence Interval value of 95%, the actual possibility of copies/µL falling between the lower and upper confidence values is 95%.
C_{RT}	Result obtained on applying the Relative Threshold algorithm on the amplification curve. The Relative Threshold algorithm is a well-based analysis based on the PCR reaction efficiency and fitted to the Amplification curve. This setting is ideal for a single sample across genes with no dependence on targets, thereby reducing variability.
dilution	The concentration of diluted sample in a well with a value of 0.0 ~ 1.0 normalized against the original concentration of the sample.
empty well	Empty wells are those that are flagged with Low ROX, Low FAM, or Low VIC flags.
flagged well	Wells containing one or more flags.
negative well	Wells with negative calls. Negative calls mean the DigitalSuite™ Software determines that a well does not contain any copy of the sample.
omitted well	Wells manually omitted by a user
Poisson distribution	In probability theory and statistics, the Poisson distribution (or Poisson law of small numbers) is a discrete probability distribution that expresses the probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.
positive well	Wells with positive calls. Positive calls mean the DigitalSuite™ Software determines that a well contains at least one copy of the sample.

replicate group	A group of replicates that share the same sample, target and dilution.
replicate	A combination of one sample and one target in a well.
sample group	A group of replicates that share the same sample and target.
undetermined well	A well is undetermined when a user: • Cannot tell if a well is amplified or not. • Sees a spurious signal called with a C_T value. • Does not want to include low efficiency wells in calculations.
well	A well in a conventional plate, or a through hole in an OpenArray plate.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

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22 April 2014