HID Real-Time PCR Analysis Software

Version 1.3

for use with:
7500 Real-Time PCR Instrument
QuantStudio™ 5 Real-Time PCR Instrument (with 0.2-mL 96-Well Block)
Quantifiler™ DNA Quantification Kit

ThermoFisher
SCIENTIFIC

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Revision history: Pub. No. MAN0009819

Revision	Date	Description
E.0	27 August 2018	Updated branding and trademarks, no technical changes.
D.0	8 March 2017	Add support for the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System with 96-well (0.2-mL) sample block. Add Virtual Standard Curve function.
C.0	14 August 2015	Correct quencher listed for Quantifiler™ Duo and Quantifiler™ Male or Human kits. Add reference to evaluating the quality indices determined by the HID Real-Time PCR Analysis Software to determine if highly degraded samples can be better analyzed with the Ion Personal Genome Machine™ (PGM™) System. See the *Quantifiler™ HP and Trio DNA Quantification Kits User Guide*.
B.0	March 2014	Added Chapter 9, "HID Real-Time PCR Analysis Software Validation".
A.0	January 2014	New document for version 1.2 features (support for Quantifiler™ Trio and HP DNA Quantification Kits; Degradation Index). Incorporates all information from the *HID Real-Time PCR Analysis Software v1.1 User Guide* (Pub. no. 4455443)

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About This Guide

Purpose

The 7500 Real-Time PCR System, or the QuantStudio™ 5 Real-Time PCR Instrument, and the HID Real-Time PCR Analysis Software detects and quantifies human and/or male DNA in samples. This guide is intended to help you quickly learn how to use the HID Real-Time PCR Analysis Software to perform analysis of samples prepared with the:

- Quantifiler™ HP DNA Quantification Kit
- Quantifiler™ Trio DNA Quantification Kit
- Quantifiler™ Human DNA Quantification Kit
- Quantifiler™ Duo DNA Quantification Kit
- Quantifiler™ Y Human Male DNA Quantification Kit

This guide assumes that:

- You are familiar with the Microsoft® Windows® operating system, the Internet, and Internet browsers.
- You know how to handle DNA samples and prepare them for PCR.

Use this guide after your plate is prepared and loaded in the 7500 Real-Time PCR System or QuantStudio™ 5 Real-Time PCR Instrument (with 0.2-mL 96-Well Sample Block).

For instructions on preparing a plate, refer to the user guide for the Quantifiler™ Kit you are using.

1   Get Started

This chapter covers:

Software overview

HID Real-Time PCR Analysis Software is designed specifically to assist human identification laboratories performing DNA quantitation, by simplifying assay setup and streamlining data review and dilution and reaction setup for downstream STR analysis. For example, the software automatically selects the appropriate Quantifiler™ Kit target, reporter, quencher, and thermal profile. After a run, the HID Real-Time PCR Analysis Software provides an analysis of each well. The software exports:

• All results
• STR kit setup instructions
• Sample dilutions calculations

HID Real-Time PCR Analysis Software is for use with the 7500 Real-Time PCR Instrument and the QuantStudio™ 5 Real-Time PCR Instrument (with 0.2-mL 96-Well Sample Block).

Applicable HID kits

You can use the HID Real-Time PCR Analysis Software with the following kits:

• Quantifiler™ HP DNA Quantification Kit
• Quantifiler™ Trio DNA Quantification Kit
• Quantifiler™ Human DNA Quantification Kit
• Quantifiler™ Duo DNA Quantification Kit
• Quantifiler™ Human Male DNA Quantification Kit

Custom experiment option

IMPORTANT! The custom assay option is supported for the 7500 system only.

You can also use the HID Real-Time PCR Analysis Software for more complex experiments by selecting the Custom Assay option on the Home screen. If you use the Custom Assay option, refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments* for instructions.

Features in v1.3

HID Real-Time PCR Analysis Software v1.3 includes all of the v1.2 functionality and includes the following new features:

- Virtual Standard Curve support for Quantifiler™ HP, Trio, Duo, and Human DNA Quantification Kits.
- Support for the QuantStudio™ 5 Real-Time PCR Instrument with 0.2-mL 96-Well Sample Block.

Install or upgrade to v1.3

Refer to the *7500/7500 Fast Real-Time PCR System Site Preparation Guide* (Pub. no. 4412843) for system layout, electrical, power, safety, and other site requirements.

Follow the appropriate installation procedure:

Situation	See...
Install the software with a new instrument	page 11
Upgrade from HID Real-Time PCR Analysis Software v1.1 to v1.3	page 12
Upgrade from HID Real-Time PCR Analysis Software v1.2 to v1.3	page 12

Computer and instrument requirements

Component	Requirements
Computer	• Processor (minimum: 2.9 GHz): – (*Recommended*) Intel™ Core™ i7 CPU, 2.9 GHz – Intel™ Core™ i5 Quad Core CPU, 2.9 GHz • 16 GB RAM[1] • One hard drive with at least 10 GB available • 20/48X IDE CD-ROM drive • USB v1.2 • Ethernet network interface adapter (10BASE-T)[2]§ • Microsoft™ Windows™ 7 64-bit/32-bit, Service Pack 1 or later
Software	• Microsoft™ PowerPoint™ software (for direct export of PowerPoint slides) • Microsoft™ Excel™ software (for direct export of data to spreadsheet) IMPORTANT! Do not run antivirus applications while HID Real-Time PCR Analysis Software v1.3 is running.
Monitor	• 1280 × 1024 pixel resolution for full screen display[3] • 16-inch • True Color (32 bit) • UL listed
Instrument	**7500 Real-Time PCR System** Instrument firmware G2.10 (installed on all instruments purchased after 2008) If your instrument was purchased before 2008, check the version of firmware installed by going to *x:/* Applied Biosystems/7500 system/firmware. The example below shows firmware G2.09. Contact Thermo Fisher Scientific if your firmware version is not G2.10.
	QuantStudio™ 5 Real-Time PCR Instrument Instrument firmware 1.3.1 or later To check the firmware version of your instrument, from the Home screen, touch ⚙ **Settings ▸ About Instrument ▸ About Instrument**.

1 The software may experience communication errors if run on computers with less than 1 GB.
2 Required only if you plan to connect the computer to a local area network (LAN).
3 If screen resolution is not set to 1280 X 1024, the Analysis Summary screen may not be properly displayed.

Install with a new instrument

If the HID Real-Time PCR Analysis Software is installed with a new 7500 Real-Time PCR System or QuantStudio™ 5 Real-Time PCR Instrument, both the instrument and the software must be installed by an Thermo Fisher Scientific technical representative.

If you have the instrument but no 7500 Software installed, you can install v1.3 directly. Insert the HID Real-Time PCR Analysis Software CD and follow the Installation Wizard instructions.

Upgrade from v1.1 to v1.3

IMPORTANT! You must have Administrator privileges to perform the upgrade.

1. Uninstall the HID Real-Time PCR Analysis Software v1.1 from your computer.

   IMPORTANT! You must uninstall HID Real-Time PCR Analysis Software v1.1 before you follow this procedure. If v1.1 is present, the installation will fail.

2. Insert the HID Real-Time PCR Analysis Software v1.3 CD.

3. Follow the Installation Wizard instructions.

4. Make sure that all calibrations are up-to-date. See "Calibration procedure" on page 14 for instrument calibration requirements.

Upgrade from v1.2 to v1.3

IMPORTANT! Do not uninstall v1.2 before performing the upgrade. If v1.2 is not present, the upgrade will fail.

IMPORTANT! You must have Administrator privileges to perform this upgrade.

1. Insert the HID Real-Time PCR Analysis Software v1.3 CD.

   The v1.3 upgrade installer automatically backs up the v1.2 calibration, experiments, and log files, then uninstalls v1.2. After the v1.3 software installation is complete, you can restore the backed-up files to the appropriate folders as described in step 4.

2. Follow the Installation Wizard instructions.

   IMPORTANT! If installing on a computer connected to the instrument, enter the same instrument serial number that was entered when v1.2 was installed.

3. Enter the Upgrade Registration Code provided with the installation CD. Do not enter any spaces between the numbers in the registration code.

4. (If installing on a computer connected to the instrument) After installation sucessfully completes, copy the calibration and experiments files from the back-up folders to the appropriate folders:

Calibration files	From	*\<install location\>*\7500\backup\calibration*\<date stamp\>*\eclipse\plugins\ com.apldbio.sds.instrument.sds7500_1.0.0\config\
	To	*\<install location\>*\7500\eclipse\plugins\ com.apldbio.sds.instrument.sds7500_1.0.0\config\
Experiments files	From	*\<install location\>*\7500\backup\experiments*\<date stamp\>*
	To	*\<install location\>*\7500\experiments

Note: During installation, the v1.2 log files are backed up to *\<install location\>*\ 7500\backup\config\logs*\<date stamp\>*. The files do not need to be reinstalled.

5. Make sure that all calibrations are up-to-date. See "Calibration procedure" on page 14 for instrument calibration requirements.

Replace SDS Software v1.2.3

1. Review "Computer and instrument requirements" on page 11 and ensure that your system meets the requirements.

2. Archive all experiment and calibration data.

3. Uninstall the SDS Software v1.2.3.

   IMPORTANT! If the SDS Software v1.2.3 is present, the installation will not run.

4. Insert the HID Real-Time PCR Analysis Software v1.3 CD.

5. Enter the Full Installation Registration Code provided with the installation CD.

6. Follow instructions of the installation wizard.

7. Make sure that all calibrations are up-to-date. See "Calibration procedure" on page 14 for instrument calibration requirements.

7500 Real-Time PCR Instruments purchased before February 2008

Tower and laptop computers of 7500 Real-Time PCR Instruments purchased before February 2008 require a memory upgrade before the computers can install the HID Real-Time PCR Analysis Software. Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR Systems User Bulletin Memory Upgrade Requirements for 7500 Software v2.0* (Pub. no. 4379705) for information.

Calibrate the 7500 Instrument

IMPORTANT! The following procedure is for the 7500 Real-Time PCR Instrument only. If you are using a QuantStudio™ 5 Instrument, see "Calibrate the QuantStudio™ 5 Instrument" on page 16.

If you...	Perform...
Installed HID Real-Time PCR Analysis Software v1.3 with a new instrument	Perform all calibrations and run the RNase P plate
Upgraded from HID Real-Time PCR Analysis Software v1.2	After restoring v1.2 calibration files (see "Upgrade from v1.2 to v1.3" on page 12), perform Custom Dye calibration to calibrate ABY™, JUN™ and Mustang Purple™ (MP) dyes.
Replaced SDS Software v1.2.3	Perform all calibrations and run the RNase P plate

Required materials

Table 1 lists the materials that are required to required to calibrate the instrument.

Table 1   User-supplied materials

If you...	Material	Cat. no.
Replaced SDS Software v1.2.3	7500 Real Time PCR Systems Spectral Calibration Kit I	4349180
	TaqMan™ RNase P Instrument Verification Plate	4350584
Upgraded from HID v1.2 *or* Replaced SDS v.1.2.3	96-Well Spectral Calibration Plate with ABY™ Dye	4461591
	96-Well Spectral Calibration Plate with JUN™ Dye	4461593
	96-Well Spectral Calibration Plate with Mustang Purple™ Dye	4461599

Calibration procedure

The following is an outline of the calibration procedure. See the *Applied Biosystems™ 7500/7500 Fast Real-Time PCR Systems System Maintenance Guide* (Pub. no. 4387777) for complete instructions.

Perform:

- Regions of Interest (ROI) calibration
- Background Calibration
- Optical Calibration
- Dye Calibration:
  - Perform Dye Calibration of the new ABY™, JUN™ and Mustang Purple™ (MP) dyes. Follow the custom dye procedure.
  - Perform Dye Calibration of all system dyes for new instrument installations, or if replacing SDS v.1.2.3
  - Use 60°C as the default temperature for all dye calibration
- RNase P Instrument Verification Plate run

HID Real-Time PCR Analysis Software v1.3 Getting Started Guide

New dye spectra

Figure 1 through Figure 3 show the calibration spectra for ABY™, JUN™ and Mustang Purple™ (MP) dyes.

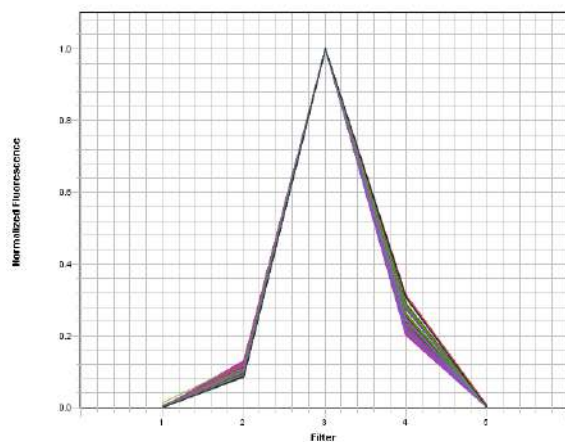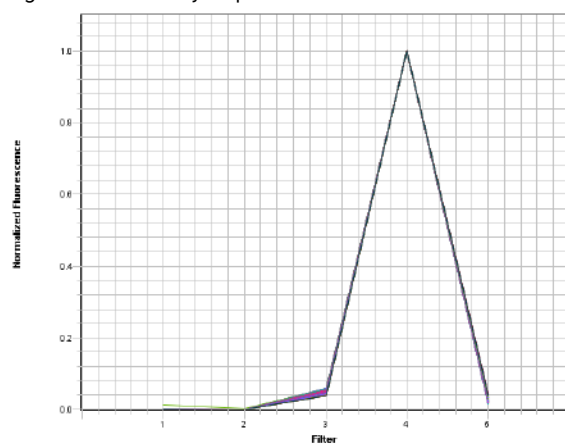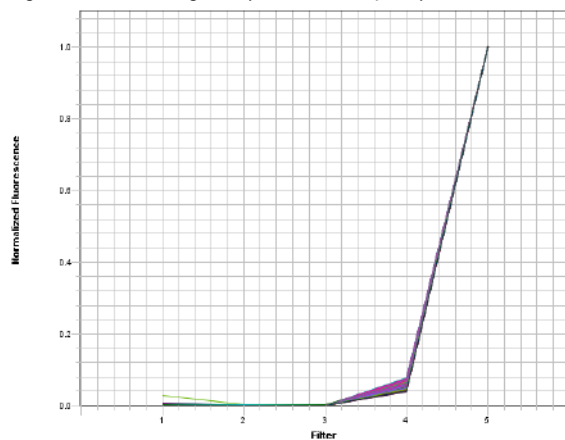Figure 1 ABY™ dye spectra

Figure 2 JUN™ dye spectra

Figure 3 Mustang Purple™ (MP) dye spectra

Calibrate the QuantStudio™ 5 Instrument

IMPORTANT! The following procedure is for the QuantStudio™ 5 Real-Time PCR Instrument only. If you are using a 7500 System, see "Calibrate the 7500 Instrument" on page 14.

The QuantStudio™ 5 Real-Time PCR Instrument is calibrated during manufacturing; however, you *must* recalibrate the instrument for the dyes that are used for HID analysis before use. If you installed HID Real-Time PCR Analysis Software v1.3 with a new instrument, perform Custom Dye calibrations for the ABY™ and JUN™ HID dyes.

Required materials

Table 2 lists the materials that are required to calibrate the instrument.

Table 2 User-supplied materials

Material	Cat. no.
96-Well Spectral Calibration Plate with ABY™ Dye	4461591
96-Well Spectral Calibration Plate with JUN™ Dye	4461593
TaqMan™ RNase P Instrument Verification Plate	4432382

Calibration procedure

The following is an outline of the calibration procedure. See the *QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide* (Pub. no. MAN0010407) for complete instructions.

Perform:

- Dye Calibration:
  - Perform Dye Calibration of the new ABY™ and JUN™ dyes. Follow the custom dye procedure.
  - Use 60°C as the default temperature for all dye calibrations.

  IMPORTANT! You *must* calibrate the ABY™ Dye as **ABY-HID** and the JUN™ Dye as **JUN-HID**. Calibrating either dye without the "-HID" suffix (as ABY and JUN) overwrites the existing calibrations for the factory-calibrated system dyes. Doing so potentially creates confusion if the instrument is ever calibrated using the QuantStudio™ 3 and 5 Calibration Kit, which lacks the HID versions of the dyes.

- RNase P Instrument Verification Plate run

New dye spectra

Figure 4 through Figure 6 show the calibration spectra for ABY™, JUN™, and Mustang Purple™ (MP) dyes.
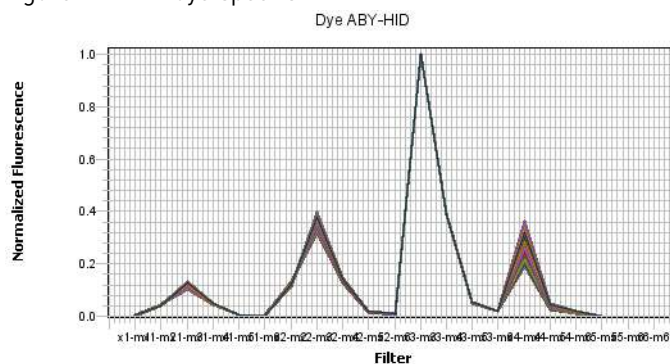
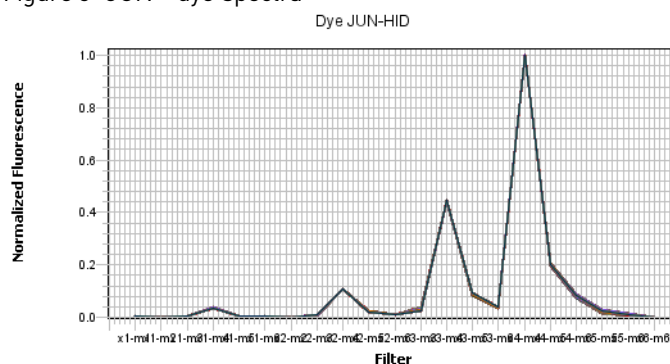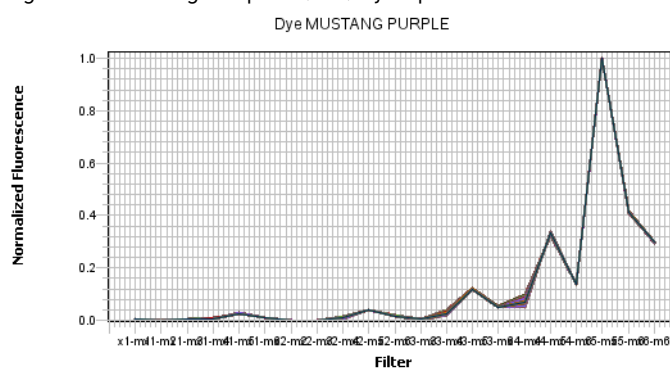Figure 4   ABY™ dye spectra

Figure 5   JUN™ dye spectra

Figure 6   Mustang Purple™ (MP) dye spectra

<div style="text-align: right">

2   Customize the Software

</div>

This chapter covers:

Modify a default experiment template

You can make changes to the experiment templates provided with the software after making a backup copy of the original templates.

Save a copy of the original template

Before you modify a template, save a copy of the original template:

1. Navigate to: `C:\Applied Biosystems\7500\config\templates`

2. Select **Edit ▸ Copy** to copy the `templates` folder.

3. Navigate to a safe location on your computer.

4. Select **Edit ▸ Paste** to insert a copy of the templates folder in the location you selected.

Modify the original template

1. Click the button on the Home screen for the experiment type of interest.

2. Modify the template as needed, including:
   - Moving standards and NTCs to different wells
   - Adding samples and/or extraction blanks
   - Setting plate layout
   - Setting the display defaults for the Amplification plot, plate view, and well table
   - Modifying analysis settings (HID, CT, and Flags)

3. Click the down arrow next to Save in the toolbar, then in the drop-down list, then select **Save as**, then select the name of the original template.

Create an experiment template

1. Set up an experiment with the desired settings, including:
   - Moving standards and NTCs to different wells
   - Adding samples and/or extraction blanks
   - Setting plate layout
   - Setting the display defaults for the Amplification plot, plate view, and well table
   - Modifying analysis settings (HID, CT, and Flags)

2. Click the down arrow next to Save in the toolbar, then select Save as Template.

To use your template instead of a default template, click Open at the top of the Home screen, then select your template instead of clicking a button for an experiment type.

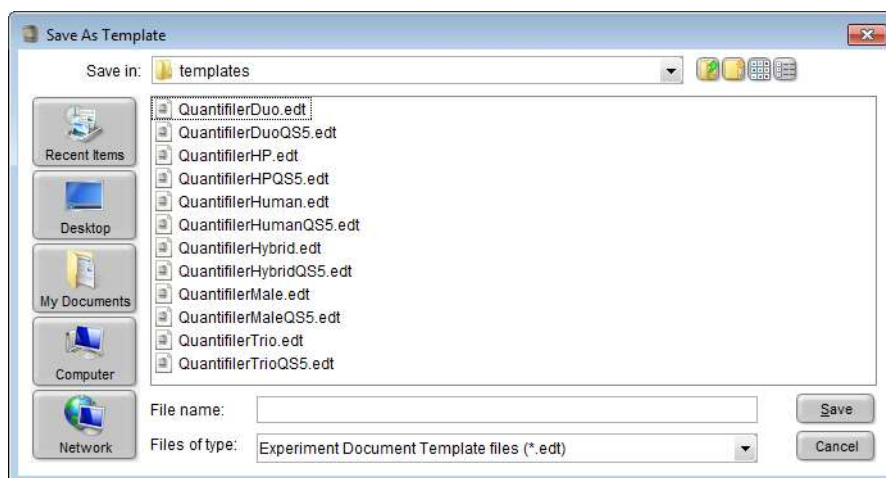# Link your template to a Home screen button

You can link your template to the any of the Quantifiler™ assay icons on the Home screen for:

The software will automatically use the template as the default experiment when you click the corresponding button. You will still be able to use a different template by opening a different experiment.

1. Before you link your template file to a button on the Home screen, save a copy of the original template:

   a. Navigate to: `C:\Applied Biosystems\7500\config\templates`

   b. Select **Edit ▸ Copy** to copy the `templates` folder.

   c. Navigate to a safe location on your computer.

   d. Select **Edit ▸ Paste** to insert a copy of the templates folder in the location you selected.

2. Link your template to a button on the Home screen:

   a. In the toolbar, from the file that you want to link, click the down arrow next to **Save**.

   b. In the drop-down menu, select **Save as Template**.

   c. Navigate to: `C:\Applied Biosystems\7500\config\templates`

d. Select the file corresponding to the assay button that you want to replace.

IMPORTANT! Files that contain the "QS5" suffix are templates used by the QuantStudio™ 5 Instrument. For example, the "QuantifilerTrio.edt" is the template file for the Quantifiler™ Trio Kit used by 7500 instruments, whereas "QuantifilerTrioQS5.edt" is the file used by QuantStudio™ 5 Instruments.

IMPORTANT! Be sure to give the file exactly the same name as the file corresponding to the button that you want to replace.

e. Click **Save**.

Set display defaults

Select information to display in the Plate View

1. Click **Show in Wells** to open the drop-down list.

2. Click the checkbox next to an item of data to select (☑) the item for display or to deselect it (☐).

3. Click **Set as Default** (this button is dimmed before you change a setting, or if you are logged in as Guest).

Specify the information to display in the Well Table

1. Click **Show in Table** to open the drop-down list.

2. Click the checkbox next to an item of data to select (☑) the item for display or to deselect it (☐).

3. Click **Set as Default** (this button is dimmed before you change a setting, or if you are logged in as Guest).

Customize the amplification plot

1. Make changes as described in "Change the appearance of, print, and save plots" on page 57.

2. Click the checkbox next to an item of data to select (☑) the item for display or to deselect it (☐).

3. Click **Save Current Settings as Default** (this button is dimmed before you change a setting, or if you are logged in as Guest).
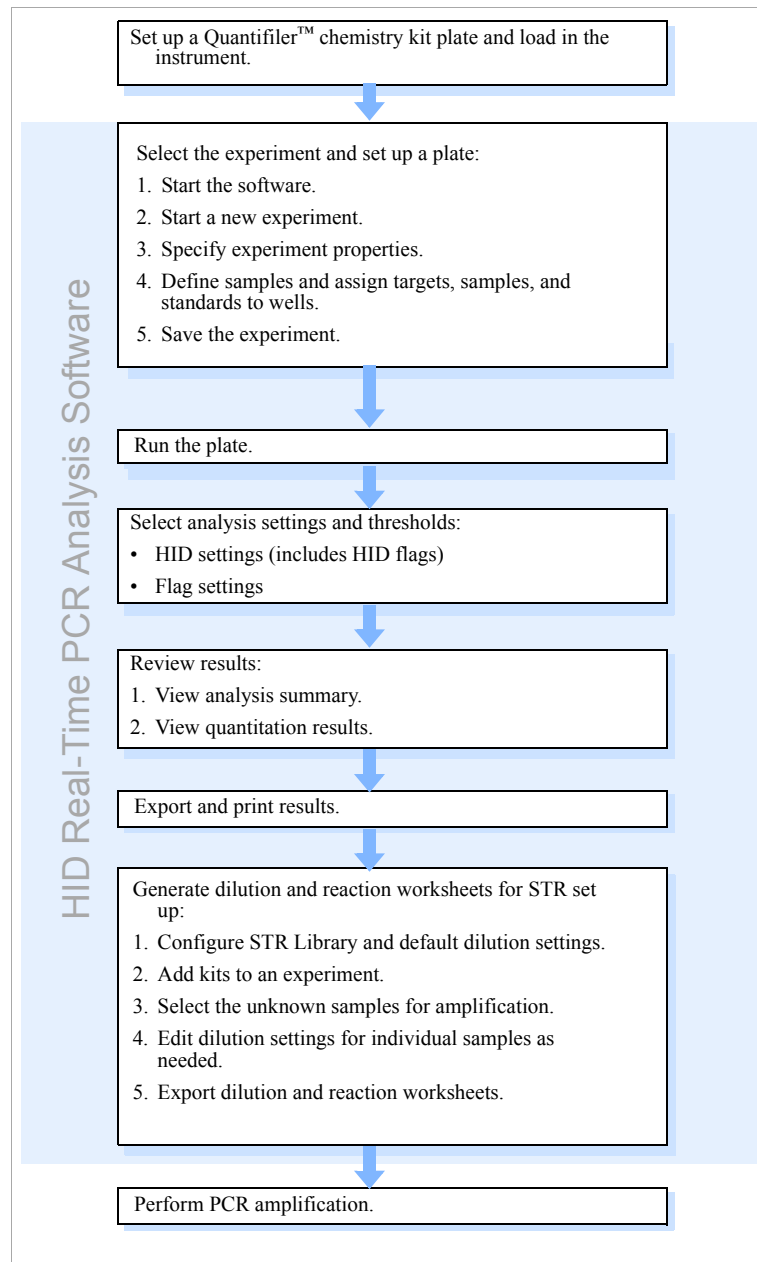
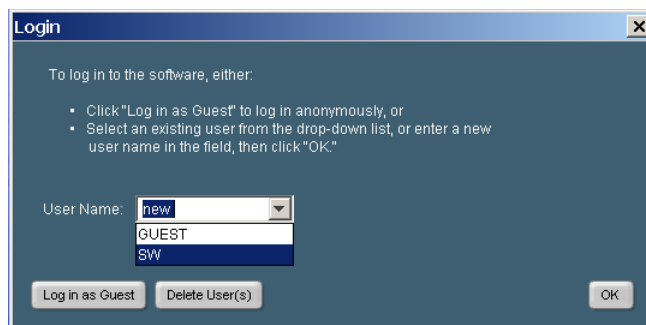# 3　Select the Experiment and Set Up a Plate

This chapter covers:

This chapter assumes that you have prepared a plate according to the instructions in the user guide for the Quantifiler™ Kit you are using.

HID Real-Time PCR Analysis Software workflow

Set up a Quantifiler™ chemistry kit plate and load in the instrument.

Select the experiment and set up a plate:

1. Start the software.
2. Start a new experiment.
3. Specify experiment properties.
4. Define samples and assign targets, samples, and standards to wells.
5. Save the experiment.

Run the plate.

Select analysis settings and thresholds:

• HID settings (includes HID flags)
• Flag settings

Review results:

1. View analysis summary.
2. View quantitation results.

Export and print results.

Generate dilution and reaction worksheets for STR set up:

1. Configure STR Library and default dilution settings.
2. Add kits to an experiment.
3. Select the unknown samples for amplification.
4. Edit dilution settings for individual samples as needed.
5. Export dilution and reaction worksheets.

Perform PCR amplification.
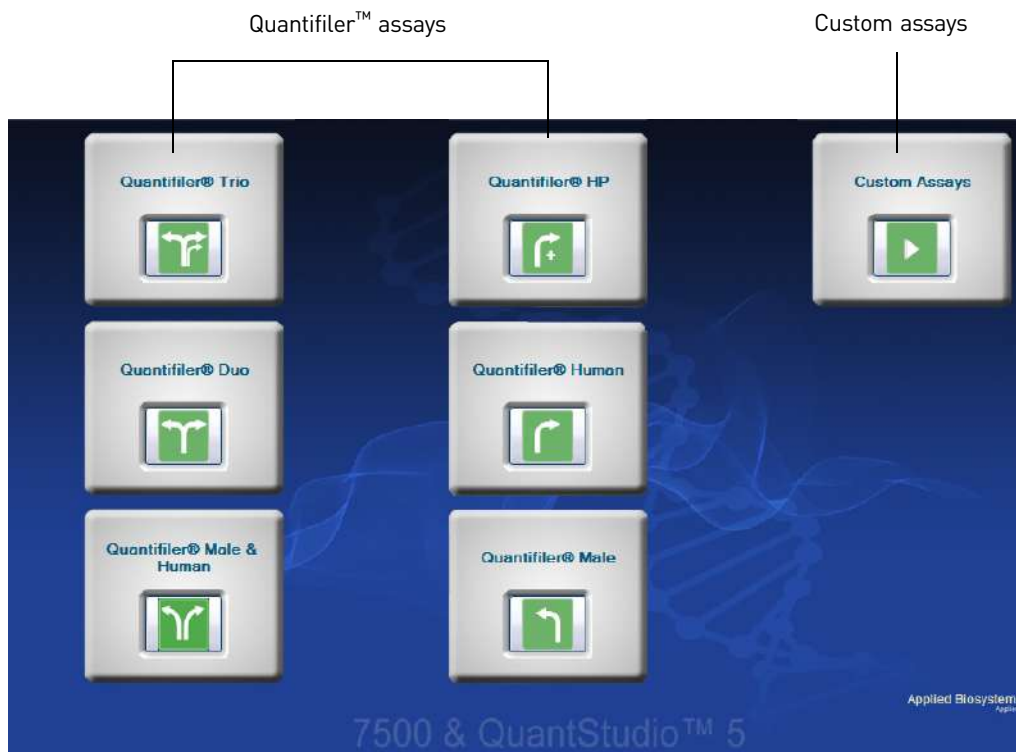
HID Real-Time PCR Analysis Software

Start the software and select an experiment

1. On your desktop, double-click 📕 or select **Start ▸ All Programs ▸ Applied Biosystems ▸ HID Real-Time PCR Analysis Software ▸** HID Real-Time PCR Analysis Software. The Login Screen should open within 1 minute.

2. In the User Name field, enter your user name or select it from the drop-down list. You can log in as a guest, but only users logged in with a user name can:

   - Edit the names of folders for experiment information import, information export, or data.
   - Enable or disable the requirement to enter a user name to start the software.
   - Set a plate layout as the default layout (See "Link your template to a Home screen button" on page 35).
   - Configure how data is displayed (see Chapter 2, "Customize the Software").

3. Click **OK** to open the Home screen with icons for HID and Custom Assays as shown.

4. Choose an HID experiment:

- Click one of the HID template icons:
    - **Quantifiler™ HP**
    - **Quantifiler™ Trio**
    - **Quantifiler™ Duo**
    - **Quantifiler™ Male**
    - **Quantifiler™ Human**
    - **Quantifiler™ Male & Human** (hybrid plate)

    or

- In the toolbar, click the down arrow next to **New Experiment** to open the drop-down list and select the appropriate experiment.

For custom experiments

IMPORTANT! The custom experiments feature is supported for the 7500 system only.

To perform a non-HID experiment, or a modified experiment, click:

- **Custom Assay** on the right side of the Home screen.

    or

- **Assays** in the toolbar, then select **Custom Assays** in the drop-down list.

For information on running custom experiments, refer to the *7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

Navigate the software

Each HID Real-Time PCR Analysis Software experiment screen displays instructions for a step in the experiment. Use the Experiment Menu at the left of any screen to navigate the software.
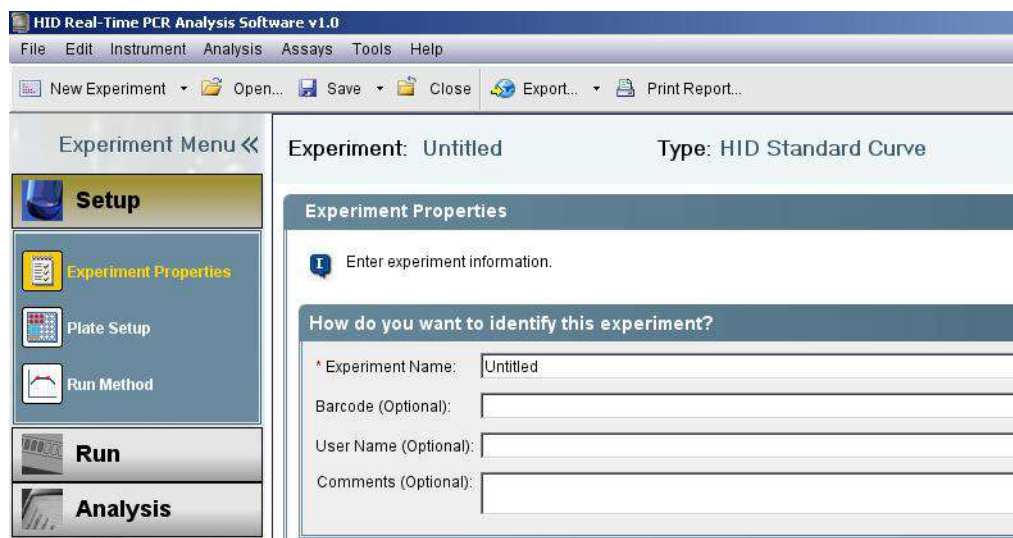
Click **»** (Expand) to expand the Experiment Menu.

Click **«** (Collapse) to collapse the Experiment Menu.

Click **Setup**, **Run**, or **Analysis**, to display screens used in the corresponding process.

You can access HID Real-Time PCR Analysis Software screens in any sequence.

To return to the Home screen at any time, click ⌂ (Home) at the bottom left of any screen.

Specify experiment properties

1. In the Experiment Menu, select **Setup ▶ Experiment Properties**.

2. In the "How do you want to identify this experiment?" section, enter the name of the plate or experiment information in the Experiment Name field. Entries in the other fields are optional.

Note: The name you enter in the Experiment Name field appears on the data report and on *.xls spreadsheets of data that you export. If you do not enter a name, "Untitled" appears on the report and in the exported spreadsheet.

The following parameters are automatically set:

- Experiment Name: *Untitled*
- Instrument:
    – 7500 (96 wells)
    – QuantStudio 5 (96 wells)
- Experiment Type: Quantitation-HID Standard Curve
- Reagents: TaqMan™ Reagents
- Ramp Speed: Standard (~1 hour to complete a run for Quantifiler™ HP and Quantifiler™ Trio kits, and ~2 hours for all other Quantifiler™ kits)

Define samples and view targets

Note: Targets are automatically listed and named. Standards dilutions and an NTC sample are listed by default for each Quantifiler™ Kit. For information about the standard included in the Quantifiler™ Kit, refer to your Quantifiler™ Kit user guide (see "How to use your documentation" on page 77).

Define samples

1. In the Experiment Menu, click **Setup ▸ Plate Setup**. Select the **Define Targets and Samples** tab.

2. In the Define Samples area on the right side of the pane, specify sample names.

- To define a new sample:
  - Click **Add New Sample**. A new line appears in the Sample Name field, *or*
  - In the toolbar, click **Tools ▸ Sample Library** to open the Sample Library screen, then click **New**.

  The default name for the new sample is Sample X (where X=1 or the highest listed Sample # + 1). You can enter a new name for the sample. To save the name of the sample for future experiments, click **OK**.
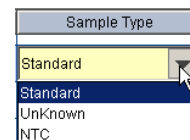
- To use a sample from your sample library:

  a. In the Define Samples pane, select **Add Saved Sample**.

  b. Select the sample(s) to use, then click **Add Selected Sample(s)**.

Note: You can also add a sample to a single well in the Plate Setup screen. See "Assign a new sample to a well" on page 32.

3. Select the sample type: **Standard, NTC,** or **Unknown**.

Unknown is the default sample type for new samples.

When you assign the sample type, the software automatically assigns the appropriate task to each target.

4. Repeat steps 2 and 3 for each sample.

IMPORTANT! List each sample individually. For replicates (identical samples), add the sample name only once. To assign a replicate to a well in the plate, in step 4 on page 31, select the well, then select the checkbox next to the sample name.

View targets

1. In the Experiment Menu, select **Setup ▸ Plate Setup**.

2. Select the **Define Targets and Samples** tab.

3. In the Defined Targets area on the left side of the pane, view the targets list to verify that you selected the correct experiment in step 4 on page 26.

Kit	Reporter dyes	Quencher
Quantifiler™ Trio	Small autosomal: VIC™ dye	NFQ-MGB
	Male (Y): FAM™ dye	NFQ-MGB
	Large autosomal: ABY™ dye	QSY7
	IPC: JUN™ dye	QSY7
Quantifiler™ HP	Small autosomal: VIC™ dye	NFQ-MGB
	Large autosomal: ABY™ dye	QSY7
	IPC: JUN™ dye	QSY7
Quantifiler™ Duo	Human: VIC™ dye	NFQ-MGB
	Male: FAM™ dye	NFQ-MGB
	IPC: NED™ dye	NFQ-MGB
Quantifiler™ Male or Human	Human: FAM™ dye	NFQ-MGB
	Male: FAM™ dye	NFQ-MGB
	IPC: VIC™ dye	NFQ-MGB
Quantifiler™ Human	Human: FAM™ dye	NFQ-MGB
	IPC: VIC™ dye	NFQ-MGB
Quantifiler™ Male	Male: FAM™ dye	NFQ-MGB
	IPC: VIC™ dye	NFQ-MGB

Change color designation

To change the color that represents a target in the data analysis:

1. Click [icon] (down arrow) in the Color column.

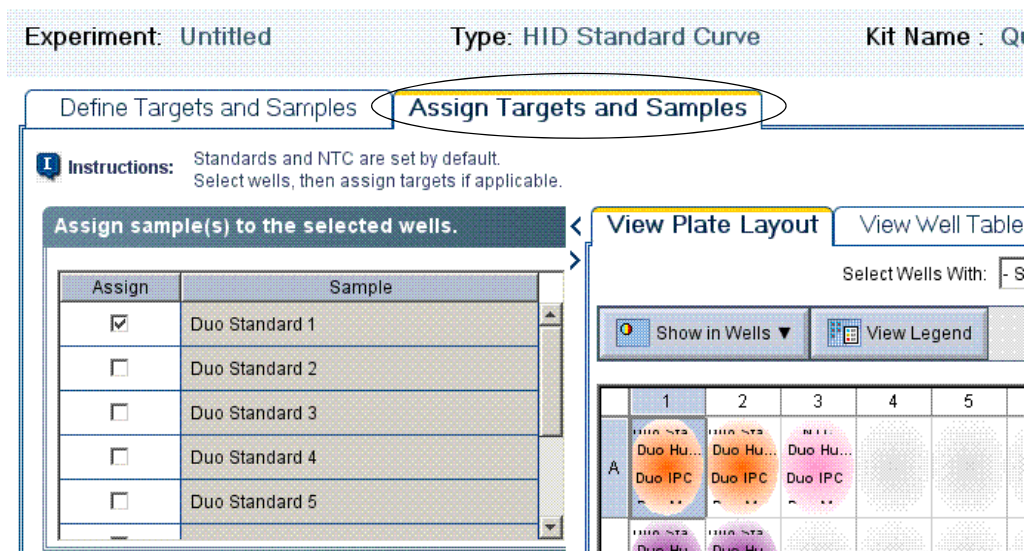2. Select a color in the drop-down list.

Assign the targets, samples, and standards to wells
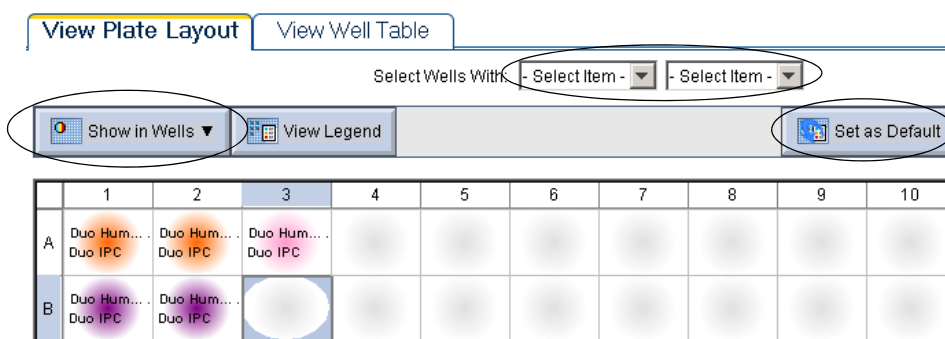
Go to the **Assign Targets and Samples** tab.

- In the Define Targets and Samples tab, click **Assign Targets and Samples** beneath the Define Samples area.

  or

- In the Experiment Menu, select **Setup ▸ Plate Setup**, then select the **Assign Targets and Samples** tab.
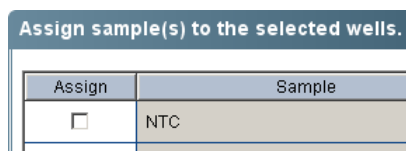

Assign Using Plate Layout

Assign samples, standards, and NTCs to wells

To assign samples, standards, and NTCs using the View Plate Layout tab:

1. Select the **View Plate Layout** tab in the pane on the right of the screen.

   To select wells with specific characteristics:

   a. Click the left **Select Wells With** button above the layout diagram.

   b. Select **Sample, Target,** or **Task** in the drop-down list.

   c. Click the right **Select Wells With** button.

   d. Select a specific sample, target, or task.

2. Specify the information to display in the wells:

    a. Click **Show in Wells** to open the drop-down list. Items that are marked with a check (✔) are selected for display.

    b. Click an item to select or deselect it for display.

3. (Optional) To save your selections as default settings, click **Set as Default** at the top right of the View Plate Layout toolbar.

4. Assign standards, NTCs, and unknown samples to well(s).

    a. To select:

- **Well** – Click the well
- **Row of wells** – Click a letter on the side of the layout
- **Column of wells** – Click a number at the top of a column
- **More than one well, row, or column** – Drag the pointer over the wells, letters, or columns to select

    b. In the Assign Sample(s) to the Selected Wells section to the left of the plate layout, select the check box in the Assign column corresponding to the unknown, standard, or NTC sample in the well(s). The target for each sample is set by default.

Note: <Sample 1> is automatically assigned to all wells that are not assigned as standard(s) or NTC(s).

5. (Optional) To change the quantity of standards, enter the quantity in ng/µL in the Quantity field in the Assign Targets to the Selected Wells area. The quantity of standard samples is set by default.

6. Repeat steps 4 and 5 until you assign samples, standards, and NTCs to all wells that you use in the experiment. You can delete empty wells after data analysis.

Note: If you delete the samples/standards/NTCs in a well and then restore them, you must reenter the well information.

The task for each target/sample combination is set automatically.

7. Clear all wells that do not contain samples or targets:

    a. Select the well(s) to clear.

    b. Right-click the well(s).

    c. Select **Clear** from the drop-down list.
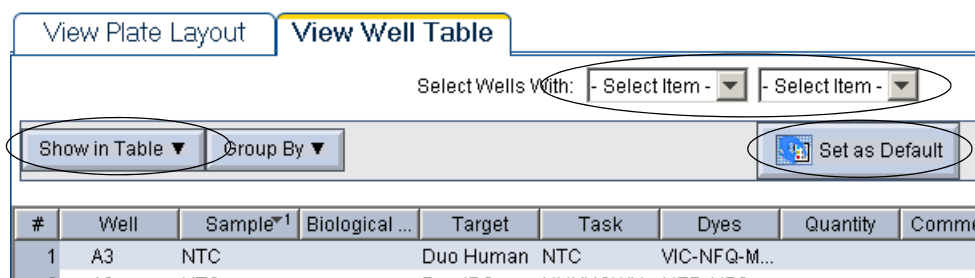
Assign a new sample to a well

To add a new sample to a well:

1. Double-click the well to open the Add New Sample dialog box.

2. Click **Add New Sample**.

3. Target and task are set by default according to sample type. To change the sample type, click the down arrow in the Sample column header and select the appropriate sample type from the drop-down list.

4. To change the sample quantity setting for standard samples, perform step 5 on page 31.
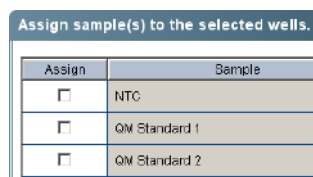
Move samples, standards, and NTCs

1. Select the wells for the samples, standards or NTCs you want to move.

2. Deselect (☐) the items in the Assign Sample(s) to the Selected Wells pane, or right-click the wells and select **Clear**.

3. One at a time, select the new wells for an item you are moving, then select (☑) the items in the Assign Sample(s) to the Selected Wells pane.

Assign Using Well Table

To assign samples, standards, and NTCs using the View Well Table tab:

1. Select the **View Well Table** tab.

    Each row in the table represents one well. To group the rows by a characteristic, click the column header. For example, click **Task** to group rows by task.

    To select wells with specific characteristics:

    a. Click the left **Select Wells With** button above the layout diagram.

    b. Select **Sample, Target,** or **Task** in the drop-down list.

    c. Click the right **Select Wells With** button.

    d. Select a specific sample, target, or task.

2. Specify the information to display in the table:

    a. Click **Show in table** to open the drop-down list. Items that are checked in the check box (☑) are selected for display.

    b. Click an item to select or deselect it for display.

3. (Optional) To save your selections as default settings, click **Set as Default** at the top right of the View Plate Layout toolbar.

4. Assign samples, standards, and NTCs to well(s):

    a. Select the well(s). To select:

    - **Well** – Click under one of the column headings in the row next to the well location (for example, to select well A6, click in row A6 under Sample).
    - **More than one well** – Drag the pointer over the wells that you want to select, or **Ctrl**+Click the wells that you want to select.

    b. In the Assign Sample(s) to the Selected Wells section, select the check box in the Assign column corresponding to the unknown, standards, or NTC sample in the well(s). The target for each sample is set by default.

    Note: <Sample 1> is automatically assigned to all wells that are not assigned as standard(s) or NTC(s).

5. (Optional) To change the quantity of standards, enter the quantity (in ng/μL) in the Quantity field in the Assign Targets to the Selected Wells area. The quantity of samples is set by default.

6. Repeat steps 1 and 5 until you assign samples, standards, and NTCs to all wells that you use in the experiment. You can delete empty wells after data analysis.

   Note: If you delete the samples, standards, or NTCs in a well and then restore them, you must reenter the well information.

   The task for each target/sample combination is set automatically.

7. Clear all wells not assigned:

   a. Click the left **Select Wells With** button at the top of the table.

   b. Select **Sample** from the drop-down list.

   c. In the well table, select the sample name(s) of the well(s) to clear.

   d. In the Assign samples to the selected wells area, deselect the checkbox in the Assign column beside the sample name.

Save plate layout as *.eds or template

IMPORTANT! Do not save the experiment to the network folder until the plate run is completed.
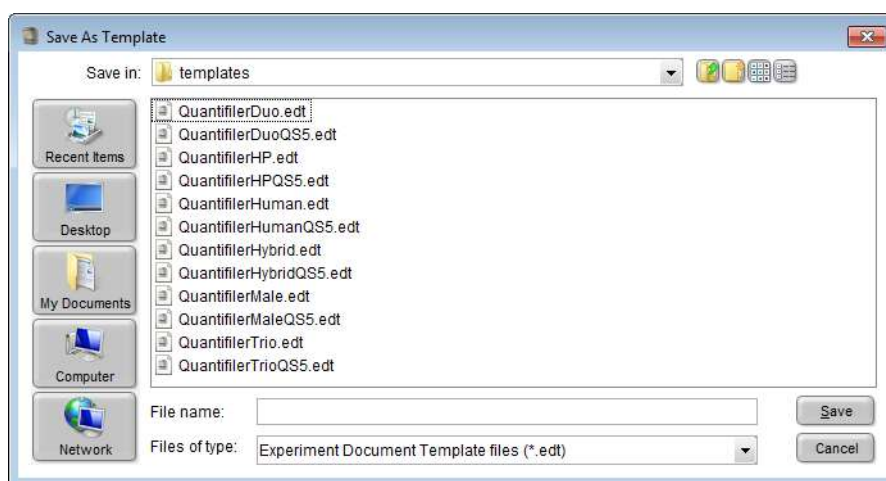
1. To save your plate layout, in the toolbar, click the down arrow next to Save, then in the drop-down list, select:
   - **Save** – to save the plate layout as an Experiment Document Single (*.eds) file
   - **Save as** – to save the plate layout as a *.eds file with a different name
     or
   - **Save as Template** – to save the experiment file as a template for future experiments

2. If you want to save the file with a different name, enter the new name in the File Name field.

3. Click **Save**.

4. Before you start the run, verify that the plate is loaded in the instrument, as described in the user guide for the Quantifiler™ Kit you are using.

Link your template to a Home screen button

You can link your template to the any of the Quantifiler™ assay icons on the Home screen for:

The software will automatically use the template as the default experiment when you click the corresponding button. You will still be able to use a different template by opening a different experiment.

1. Before you link your template file to a button on the Home screen, save a copy of the original template:

    a. Navigate to: `C:\Applied Biosystems\7500\config\templates`

    b. Select **Edit ▸ Copy** to copy the *templates* folder.

    c. Navigate to a safe location on your computer.

    d. Select **Edit ▸ Paste** to insert a copy of the templates folder in the location you select.

2. Link your template to a button on the Home screen:

    a. In the toolbar, from the file that you want to link, click the down arrow next to **Save**.

    b. In the drop-down menu, then select **Save as Template**.

    c. Navigate to: `C:\Applied Biosystems\7500\config\templates`

    d. Select the file corresponding to the assay button that you want to replace. IMPORTANT! Files that contain the "QS5" suffix are templates used by the QuantStudio™ 5 Instrument. For example, the "QuantifilerTrio.edt" is the template file for the Quantifiler™ Trio Kit used by 7500 instruments, whereas "QuantifilerTrioQS5.edt" is the file used by QuantStudio™ 5 Instruments.

    IMPORTANT! Be sure to give the file exactly the same name as the file corresponding to the button that you want to replace.
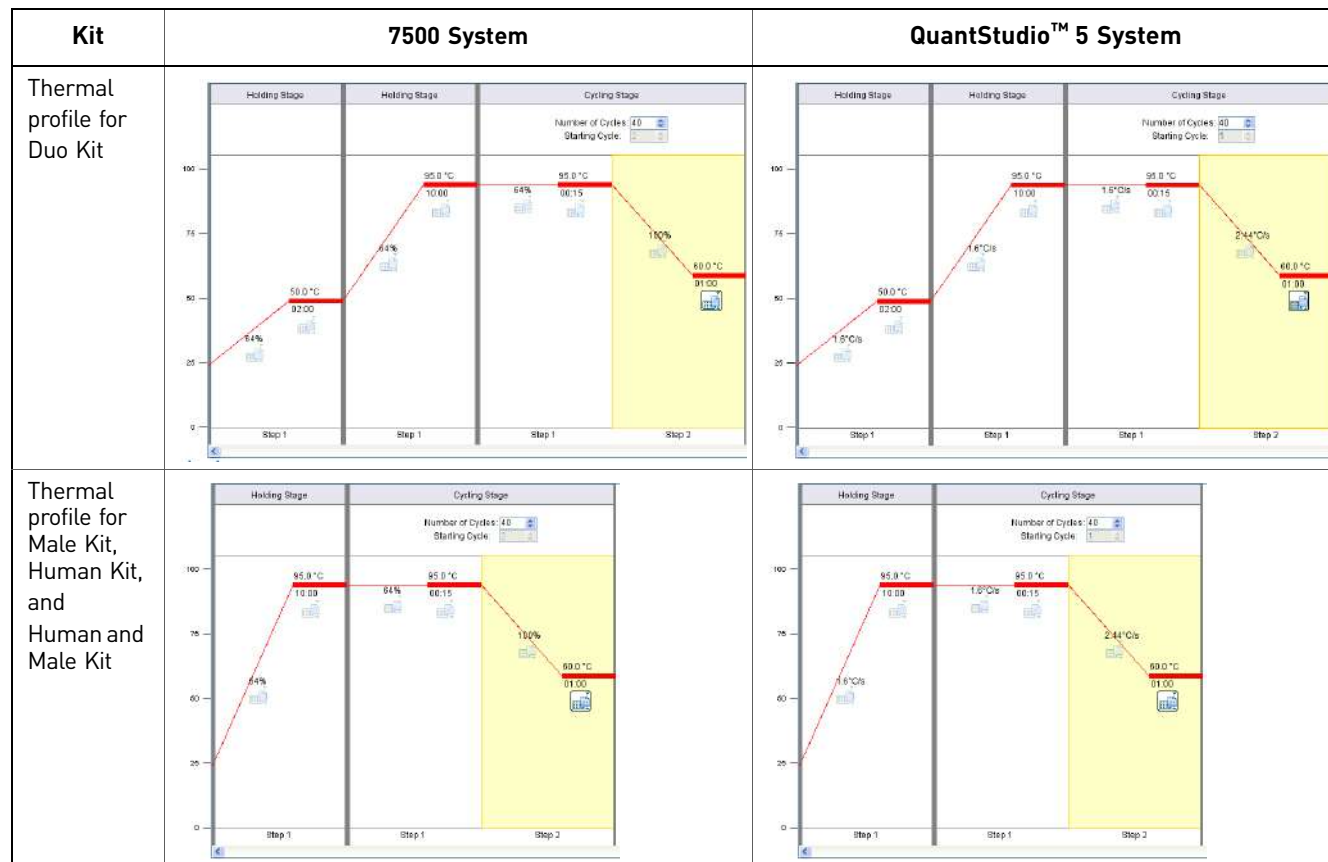
    e. Click **Save**.

4   Run the Plate

This chapter covers:

View the run method

1. In the Experiment Menu, select **Setup ▸ Run Method** to open the Run Method screen.

2. Select the **Graphical View** tab to open the thermal profile for the assay.

   Note: The Graphical View tab displays the Run Method ramp rate as a percentage when using a 7500 System and in degrees Celsius (°C) when using a QuantStudio™ 5 Instrument.

Kit	7500 System	QuantStudio™ 5 System
Thermal profile for Duo Kit		
Thermal profile for Male Kit, Human Kit, and Human and Male Kit		

3. Verify that the value in the reaction volume field is:
   - 25 μL for Quantifiler™ Human, Human Male, and Duo Kits
   - 20 μL for Quantifiler™ HP and Trio Kits

   For more information on run parameters, refer to the user guide for the Quantifiler™ Kit you are using.
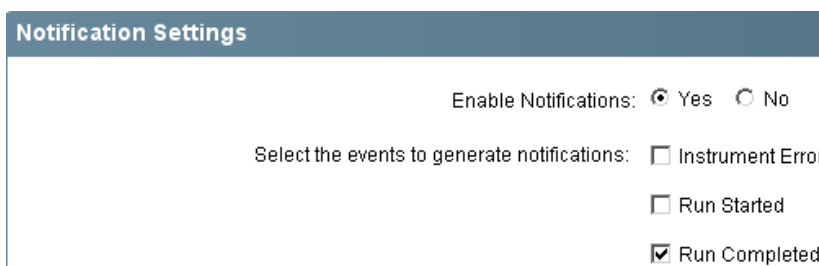
Set notifications (7500 System Only)

IMPORTANT! The following procedure is for the 7500 Real-Time PCR Instrument only and not available for the QuantStudio™ 5 Real-Time PCR Instrument.

You can set the software to send e-mail notification of selected events to e-mail addresses that you specify.

1. In the Experiment Menu, select **Run ▸ Notification Settings** to open the screen.

2. To send notifications:

    - In the Run Status area, select the **Enable Notifications** check box.

      or

    - In the Notifications Settings area, select **Yes** for Enable Notifications. If you do not want the system to send notifications, select **No**.

    IMPORTANT! Notifications cannot be sent unless the computer that performs the run is on an e-mail network.

3. For "Select the events to generate notifications," select the check boxes for events that you want to generate e-mails. You can select:

    - **Instrument Error** – Notifies addressees that the run stopped before completion of the run
    - **Run Started** – Notifies addressees that the run began
    - **Run Completed** – Notifies addressees that the run is finished

4. In the "Enter email addresses for notifications" field, enter the e-mail address(es) (including you) to which notifications are sent. Use the format shown on the screen. Enter a comma between addresses.

5. Define the outgoing server. If you need information about the server, contact your network system administrator.

    a. In the Outgoing Server (SMTP) field, enter the name of the outgoing server. For example: smtp.mycompany.com

    b. Select **Yes** next to "Server requires an encrypted connection?" if the outgoing server requires an encrypted connection. If no encrypted connection is required, select **No**.

    c. If the outgoing server requires authentication to receive the e-mail from the instrument, select **Yes** next to "Server requires authentication?" Enter the authentication user name and password in the dialog box.

Start or stop the run

IMPORTANT! If the computer that performs the run is on a network, avoid excess use of the network during a run.

Note: You can set analysis parameters before or after you run a plate. To set parameters before you run a plate, see Chapter 5, "Select Analysis Settings and Thresholds".

Start

To start a run:

Experiment: Untitled

Run Status

START RUN ▷

Run Status: Not Started

- In the Experiment Menu, select **Setup**, select any screen, then click **Start Run** at the top right corner.
  or
- In the Experiment Menu, select **Run**, select any screen, then carefully click **Start Run** at the top left corner.

  Note: If you double-click the Start Run button, it may not become a Stop Run button, but the run proceeds normally.

Stop

When you start a run, the green Start Run button becomes a red Stop Run button. Click the **Stop Run** button to stop the run immediately.

STOP RUN ⊗

Monitor the run (7500 System Only)

IMPORTANT! The following procedure is for the 7500 Real-Time PCR Instrument only and not available for the QuantStudio™ 5 Real-Time PCR Instrument.

During a run, you can access the amplification plot, temperature plot, and run method.

In the Experiment Menu, select **Run**, then click:

- **Amplification Plot** – To view amplification plots of reactions
- **Temperature Plot** – To view temperature plots of reactions
- **Run Method** – To view and edit the run method during the run

Save the results

After a run is complete, HID Real-Time PCR Analysis Software automatically performs analysis and saves the initial results file. If you modify the plate (for example, if you remove a well from analysis and reanalyze the results), the software does not automatically save the changes. After reanalysis, the HID Real-Time PCR Analysis Software prompts you to save the results.

After the run, see Chapter 6, "Enhance Data Analysis," to view and manage the results.

5 Select Analysis Settings and Thresholds

This chapter covers:

IMPORTANT! All default settings shown in this guide and in the software screens are for illustration only. For your experiments, set the parameters and thresholds according to your laboratory protocol.

Before analyzing data from a completed run, you can edit values for the analysis parameters:

- C$_T$ threshold, baseline start cycle, and end cycle
- HID flag thresholds
- QC flag thresholds

The Analysis Settings screen also contains the area where you set the parameters for the Dilution Calculation tool to use in calculating a dilution scheme for downstream amplification.

Note: See "Edit dilution settings for individual samples" on page 65 for more information about settings in the Dilution Scheme area.

Open analysis settings

1. In the Experiment Menu, select **Analysis**, then select any one of the following data displays:
    - **Amplification Plot**
    - **Standard Curve**
    - **Virtual Standard Curve**
    - **Multicomponent Plot**
    - **Raw Data Plot**
    - **QC Summary**

2. Click the **Analysis Settings** button in the top right corner of the screen to display the Analysis Settings dialog box.
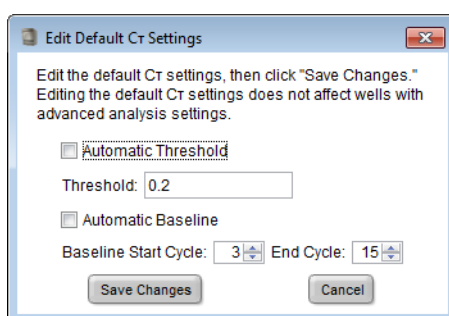
View/Edit C_T settings

Select the **C_T Settings** tab to view the settings for C_T. The recommended C_T settings for each Quantifiler™ kit are included in the experiment templates provided with the software and in the user guide for the associated Quantifiler™ kit. The recommended settings are those which were used in the validation experiments performed for each kit by Thermo Fisher Scientific.
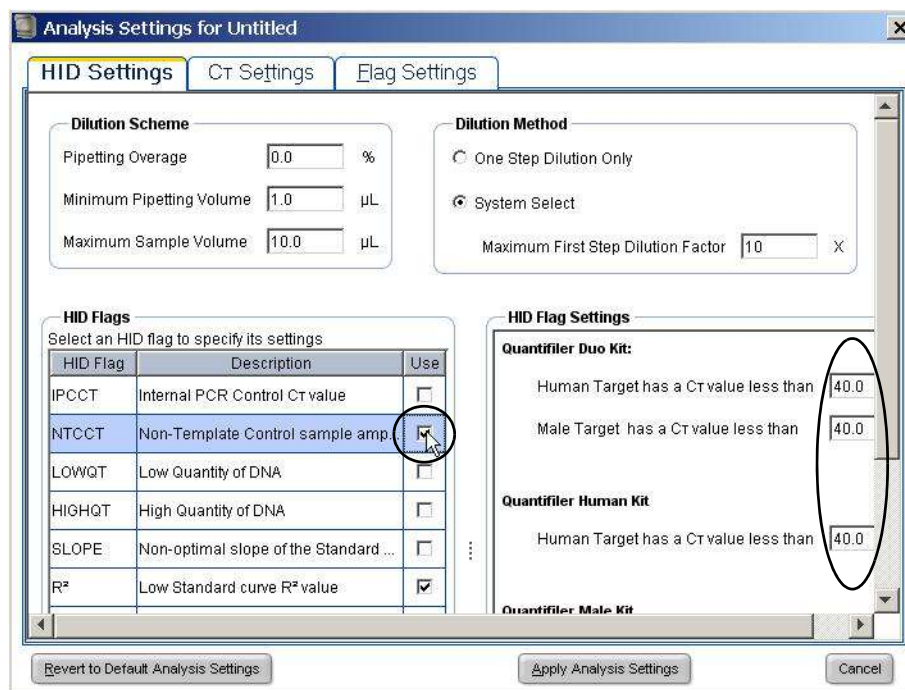
The default system settings are:

- Manual C_T Threshold = 0.2
- Manual Baseline Start Cycle = 3
- Manual Baseline End Cycle = 15

To change these settings, click **Edit Default Settings**, then enter the new values.

To analyze the data with new settings, click **Apply Analysis Settings** at the bottom of the Analysis Settings dialog box.

Enter HID settings

1. Select the **HID Settings** tab to view the Dilution Scheme, HID Flags, and HID Flag settings.

   See "Edit dilution settings for individual samples" on page 65 for more information about settings in the Dilution Scheme area.

2. In the Use column in the HID Flags table, select the check box for each flag that you want to include in the analysis.

   You can use a flag to identify quality issues and help to interpret results for wells. Flags can indicate samples that may require further attention. You can exclude wells from data analysis. See "Omit wells from analysis" on page 55 for instructions on excluding wells from analysis.

3. Enter threshold settings for the flag(s) that you select:

   a. In the HID Flags table, select the flag of interest.

   b. In the HID Flag Settings area, enter in the corresponding fields the value(s) that you want to use.

   Repeat steps 2 and 3 until you enter settings (or view the default settings), for all the flags that you select.

   Note: To save your HID flag settings for future use, save the experiment as a template before you start the run (see "Start or stop the run" on page 40).

4. To analyze the data with new settings, click **Apply Analysis Settings** at the bottom of the Analysis Settings dialog box.

HIGHQT

The HIGHQT flag indicates that the quantity, or mean quantity of sample replicates, is above a threshold that you set.

IPCCT

The IPCCT flag indicates one of the following:

Well contents	Cause	Comment
Unknown sample	The IPC (Internal PCR Control) C_T value is greater than the average of the IPC C_T values for all the standards plus the threshold that you set.	We strongly recommend that you base the threshold setting on validation data produced by your laboratory. We have observed the following: • For information on interpreting the IPCCT flag for Quantifiler™ Kit experiments, refer to the user guide for the kit you are using.
Standard or NTC	The IPC (Internal PCR Control) C_T value is above or below the maximum or minimum, respectively, that you set.	In Quantifiler™ Kit experiments, IPC target amplification should be within an expected range. Low or no IPC amplification can indicate the presence of PCR inhibitors, incorrect experiment setup, or reagent or instrument failure.

LOWQT

The LOWQT flag indicates that the quantity, or mean quantity of sample replicates, is below a threshold that you set.

NTCCT

This flag refers to the C_T value of the NTC (non-template control). No amplification of human and/or male target(s) should occur in NTC wells.

MTFR flag and M:F ratio display

The MTFR (Male to Female Ratio) is expressed as 1:X. A well is flagged if X is greater than the threshold that you set. For example, if you set the MTFR flag threshold at 1:10, then a sample containing 5 ng/μL of male DNA and more than 55 ng/μL of human DNA generates an MTFR flag. The flag for this condition is a yellow triangle (⚠) in the Plate Layout or Well Table tab, and a red octagon (⬤) in the Analysis Summary (see Chapter 6, "Enhance Data Analysis").

Samples that generate the MTFR flag are labeled "Thresholds Not Met" in the Analysis Summary area of the QC Summary tab. The MTFR flag indicates samples that might require Yfiler™ Kit amplification due to low quantities of male DNA relative to female DNA. Autosomal amplification of these samples may result in partial to no profile for the secondary (male) contributor.

In contrast, the M:F ratio display does not have an associated flag. The M:F ratio is also expressed as 1:X and is displayed in the M:F ratio column of the well table only if X is greater than or equal to the threshold that you set for the M:F ratio display.

The M:F ratio display threshold is expressed as 1:X where X must be less than or equal to the X value for the MTFR flag. For example, if you set the M:F ratio display to 1:1, then the MTFR flag must be set to 1:>1. Samples with ratios greater than the MTFR flag display the MTFR flag and display the calculated M:F ratio. The M:F Ratio Display function alerts you to male and female mixtures before STR analysis.

Table 3 Results of example M:F and MTFR settings

| Male DNA | Female DNA | Male:Female ratio | HID setting | | M:F ratio display? | MTFR flag? |
			M:F Ratio display (1:X) X =	MTFR flag (1:X) X =		
1 ng/μL	1 ng/μL	1:1	1	1	Yes	No
1 ng/μL	2 ng/μL	1:2	1	1	Yes	Yes
1 ng/μL	1 ng/μL	1:1	1	2	Yes	No

SLOPE

Indicates the PCR amplification efficiency for the experiment. The amplification efficiency is calculated using the slope of the regression line in the standard curve. The standard wells are flagged if the slope is not between the minimum and maximum values that you set.

The standard curve is derived from a serial dilution set of standards containing a range of known quantities. Results from amplifications of these standards are used to generate a curve.

A slope of -3.3 indicates 100% amplification efficiency. Refer to the *Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual* and the *Quantifiler™ Duo DNA Quantification Kit User's Manual* for more information on the standard curve and slope.
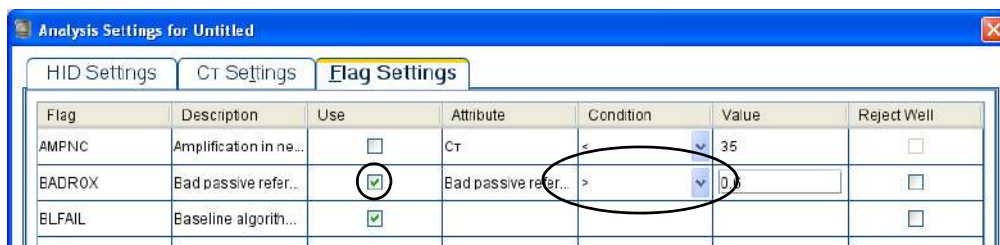
R^2

This flag indicates the regression coefficient calculated from the regression line of the standard curve. The R2 value indicates the closeness of fit between the standard curve regression line and individual C_T data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.

YINT

The Y-intercept value of the standard curve indicates the expected C_T value for a sample with a quantity of 1 (for example, 1 ng/μL). The YINT flag can assist in evaluating standard performance and serial dilution preparation. Your laboratory can perform validation studies to determine a range for the Y-intercept and you can set the HID Flag values for each Quantifiler™ kit and the HID Flag values for each target (human and male) in the Quantifiler™ Duo assay. A YINT flag may indicate incorrectly prepared standard concentrations, degraded standard, or other preparation errors.

Enter Flag settings

1. Select the **Flag Settings** tab to view and define instrument, sample, and data collection flags. Flags not used in the analysis are gray. Table 4 explains the flags.

2. In the Use column, select each flag that you want to include in the analysis.

3. Select the condition (< > =) in the Condition column drop-down lists and enter the corresponding values in the Value column to specify the conditions that generate a flag.

4. To omit the wells that have a flag from the analysis, select the corresponding **Reject Well** check boxes.

5. To analyze the data with new settings, click **Apply Analysis Settings**.

Table 4 QC flags

Flag	Description
AMPNC	Amplification in non-template control
BADROX	Bad passive reference signal
BLFAIL	Baseline algorithm failed
CTFAIL	C_T algorithm failed
DRNMIN	Define acceptable delta Rn based on Ct range
EXPFAIL	Exponential algorithm failed
OFFSCALE	Fluorescence is offscale
HIGHSD	High standard deviation in replicate group
PRFLOW	Low passive reference signal
NOAMP	No amplification
NOISE	Noise higher than others in plate
SPIKE	Noise spikes
NOSIGNAL	No signal in well
OUTLIERRG	Outlier in replicate group
PRFDROP	Passive reference signal changes near C_T
THOLDFAIL	Thresholding algorithm failed

Add a virtual standard curve to the experiment

If you are using a virtual standard curve to analyze experiments, use the software to create the virtual standard curve, then assign it to your experiments as needed or export it for further use.

Guidelines for using virtual standard curves

- The software will *not* analyze an experiment using a virtual standard curve if:
  - The plate layout of the experiment contains wells that are configured with the "standard" task type.
  - The Expiration Date specified for the virtual standard curve has expired.
  - The Quantifiler Kit specified for the experiment and the curve do not match.
- When analyzing an experiment using a virtual standard curve, all "unknown" samples generates the IPCCT (Internal PCR Control Ct) flag by default.
- Laboratories should perform internal validation studies to ensure that implementation of a virtual standard curve is appropriate and generates reliable downstream data. For optimal results, virtual standard curves should be evaluated independently for each real-time PCR instrument. We recommend the re-evaluation of virtual standard curves with each new lot of quantification kit.

Create a virtual standard curve

IMPORTANT! To create the virtual standard curve, you must know the slopes and y-intercepts of the targets for the kit that you are using.

1. In the Experiment Menu, select **Analysis**, then select **Virtual Standard Curve**.

2. Click the **Add Standard Curve to Experiment** button in the top left corner of the screen to display the Virtual Standard Curve Library dialog box.

3. In the Virtual Standard Curve Library dialog box, click **New** to create a new virtual standard curve.

4. Specify the settings for the virtual standard curve:
   a. Enter a name for the curve.

   b. (*Optional*) Select **Is Standard Curve Default?** to analyze all new experiments of the same selected kit type (see substep 4d) using the virtual curve.

   c. Select the date on which the curve expires. When the curve expires, the software can no longer use it to analyze data.

    d. Select the kit to which the virtual standard curve applies.

    e. Enter the **Slope** and **Y-Intercept** for each target of the selected kit.

    f. Enter any comments for the virtual standard curve, then click **OK** to save it to the library.

Apply a virtual standard curve to an experiment

Before you apply the curve

The software cannot use a virtual standard curve to analyze an experiment that already contains wells that are assigned the Standard task. Therefore, before applying a virtual standard curve, you *must* either omit or reassign the task of any well on the plate layout that is configured as a standard.

To...	See...
Omit wells from the analysis	"Omit wells from analysis" on page 55
Reassign the task assignment of a well	"Assign samples, standards, and NTCs to wells" on page 30

Apply a standard curve

IMPORTANT! Before you apply a virtual standard curve, you *must* either omit or reassign the task of any well configured as a standard on the plate layout.

To apply a virtual standard curve to the open experiment:

1. In the Experiment Menu, select **Analysis**, then select **Virtual Standard Curve**.

2. Click the **Add Standard Curve to Experiment** button in the top left corner of the screen.

3. From the Virtual Standard Curve Library dialog box, click **Add selected Virtual Standard Curve**.

Note: When analyzing an experiment using a virtual standard curve, all "unknown" samples generate the IPCCT (Internal PCR Control Ct) flag by default.

Automatic analysis using a default virtual standard curve

If you select **Is Standard Curve Default?** in the settings of a virtual standard curve, then the HID Software automatically analyzes all new experiments using that default virtual standard curve unless:

Is Standard Curve Default ? ☑

Expiration Date * Jan 24, 2017 ▼

- The plate layout of a new experiment contains wells that are configured as standards.
- The Expiration Date setting for the default virtual standard curve has passed.
- You select the **Is Standard Curve Default?** option for another virtual standard curve (or the option is deselected for the existing virtual standard curve).

<div style="float:left">

6

</div>

Enhance Data Analysis

This chapter covers:

View the analysis results

Flagged wells

To view the results of the data analysis:

1. In the Experiment Menu, select **Analysis ▸ QC Summary** to open the QC Summary screen.

   Note: If screen resolution is not set to 1280 X 1024, the Analysis Summary may not be properly displayed.

2. In the QC Summary area, select the **Analysis Summary** tab to display areas that list the HID-specific flags that you selected to include in the data analysis and indicate the number of wells that meet/do not meet the threshold that you set. The table below shows the meaning of the symbols.

Location	Symbol	Meaning
Standard Curve bar	Green square (▣)	A value for Slope, R2, or Y-Intercept meets the threshold
	Red octagon (⬤)	A value for Slope, R2, or Y-Intercept does not meet the threshold
All Thresholds Met column of: • Standard bar • NTC bar • Unknown bar	Hyperlinked numbers	The number of wells that meet the thresholds for a flag value
All Thresholds Not Met column of: • Standard bar • NTC bar • Unknown bar	Hyperlinked numbers	The number of wells that do not meet the thresholds for a flag value

Standard curve   The Standard Curve bar contains the SLOPE, R2, and Y-Intercept flags. Click the
                              column heading for a red octagon (●) to highlight in the plate layout the wells
                              represented in the standard curve. This graphical view simplifies the identification of
                              wells that require further analysis using your laboratory protocol.

Standard   The Standard bar reports the IPCCT flags for all the wells on the plate that you
                              designated as sample type Standard. Click the number in the Thresholds Not Met
                              column to view the well(s) that do not meet the IPCCT threshold in the plate layout or
                              well table format. You can use the amplification, multi-component, or the raw data
                              plot(s) to troubleshoot the data for these wells. You can examine the wells that meet the
                              threshold by clicking the number in the All Threshold Met column.

NTC (non-template   The NTC bar reports the IPCCT and NTCCT flags for all the wells on the plate that you
control)   designated as sample type NTC (non-template control). Click the number in the
                              Thresholds Not Met column to view the well(s) that do not meet the IPCCT or NTCCT
                              threshold in the plate layout or well table format. You can use the amplification, multi-
                              component, or raw data plot(s) to troubleshoot the data for these wells. You can
                              examine the wells that meet the threshold by clicking the number in the All Threshold
                              Met column.

Unknown   The Unknown bar reports the IPCCT, HIGHQT, LOWQT, and MTFR flags for all the
                              wells on the plate that you designated as sample type Unknown (note that the MTFR
                              flag is not available in Human, HP, or Human Male kit experiments). The HIGHQT,
                              LOWQT, and MTFR (male to female ratio) flags indicate that the quantity of DNA or
                              ratios of male to female DNA in unknown samples might require additional attention.
                              Numbers below the flag indicate the number of wells that do not meet the threshold.

                              Click the number in the Thresholds Not Met column to view the well(s) that do not
                              meet a threshold in plate layout or well table format. You can use the amplification,
                              multi-component, or raw data plot(s) to troubleshoot the data for these wells. You can
                              examine the wells that meet the threshold by clicking the number in the All Threshold
                              Met column.

Instrument-related   In addition to the flags listed above, a message might be displayed to indicate that one
flags   or more of the instrument-related flags is generated by a potential problem with the
                              instrument. The message prompts you to select the **QC Flags Details** tab to view the
                              flags.

Well(s) automatically omitted

In certain rare instances, such as assignment of targets to empty wells, HID Real-Time PCR Analysis Software may automatically omit certain wells of a Quantifiler™ Kit run.

⚠ Automatically Omitted : 1

The software automatically omits wells that may prevent the completion of data analysis, so that analysis can continue for the rest of the wells in the plate. These wells are indicated by a red exclamation point above the Analysis Summary tables. You can examine the automatically omitted wells by clicking the number next to the exclamation point.

Interpret QC flag information

QC Flags Detail

1. In the QC Summary screen, select the **QC Flags Detail** tab to view all QC flags (both general and HID).

2. Click a flag to select all affected wells in the plate layout, and to open a brief description of the flag and wells in a box below the list.

Also in the QC Flags Details description box is a hyperlink to online Help for troubleshooting the flag and the criteria used for analysis (see Chapter 5, "Select Analysis Settings and Thresholds," for more information about these flags).

For more information about how to view and edit the information about samples, see "Change the appearance of a plot" on page 57.

Omit wells from analysis

You can omit wells from analysis. To view data from individual wells on the Amplification analysis plot, in the Experiment Menu, select one of the following screens:

- **Amplification** – Amplification vs. cycle and amplification vs. well
- **Standard curve** – C_T vs. quantity of standards, flagged samples, and unflagged samples
- **Multicomponent plot** – Fluorescence vs. cycle of all reaction components
- **Raw data plot** – Amplitude vs. filter
- **Multiple plots view** – Amplification, Standard curve, Multicomponent, and Raw data plots in one pane

1. In the Experiment Menu, select **Analysis**. Click any Analysis screen. If no data are displayed, click **Analyze**.

2. Omit wells using the well table or plate layout:

    To use the well table, select the **View Well Table** tab, then select the **Omit** check boxes corresponding to the wells to exclude from the analysis.

    Note: If the Omit Well column is not visible in the table, click **Show in Table**, then select **Omit Well** to show the column.
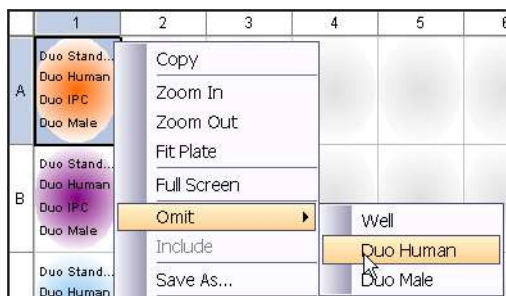

    or

    To use the plate layout, select the **View Plate Layout** tab. Right-click the well(s) to omit, then select **Omit ▸ Well.**

3. Click **Analyze** to reanalyze the experiment data with the omitted well(s) excluded from the analysis.

4. Review the data that are analyzed without the omitted well(s).

Omit targets in an experiment well

For Duo, HP, and Trio experiments, you can omit one of the standard targets in a well from analysis (shown for a Duo experiment in the example below).

1. Right-click a well with a standard target that you want to omit.

   📝 Note: You can omit only one target from one well at a time.

2. Select **Omit** from the drop-down list, then select:
   - **Well** – to omit all targets from the well. The ⊠ (well omitted) icon appears in the well.
   - Individual Target (for example, **Duo Human**) – to omit a specific target from the well, select the name of the target. The individual target omitted icon (for example, ⊞ for Duo Human omitted) appears in the well.

3. Click **Analyze** to reanalyze the experiment data with the omitted target(s) excluded from the analysis.

Examine the Degradation Index

Degradation Index refers to the data observed when a sample may be degraded: a decrease in measured amount for large DNA fragments compared to small DNA fragments. While DNA degradation is not the only theoretically possible mechanism for a decrease in amount, it is the predominant mechanism in the absence of inhibitors. The Degradation Index is for use as a general indicator of whether large DNA fragments may perform more poorly in STR reactions. Evaluate Degradation Index in conjunction with the IPC C_T.

The Degradation Index is automatically calculated by the HID Real-Time PCR Software using the following formula:

$$\frac{\text{Small autosomal target DNA conc. (ng/}\mu\text{L)}}{\text{Large autosomal target DNA conc. (ng/}\mu\text{L)}}$$
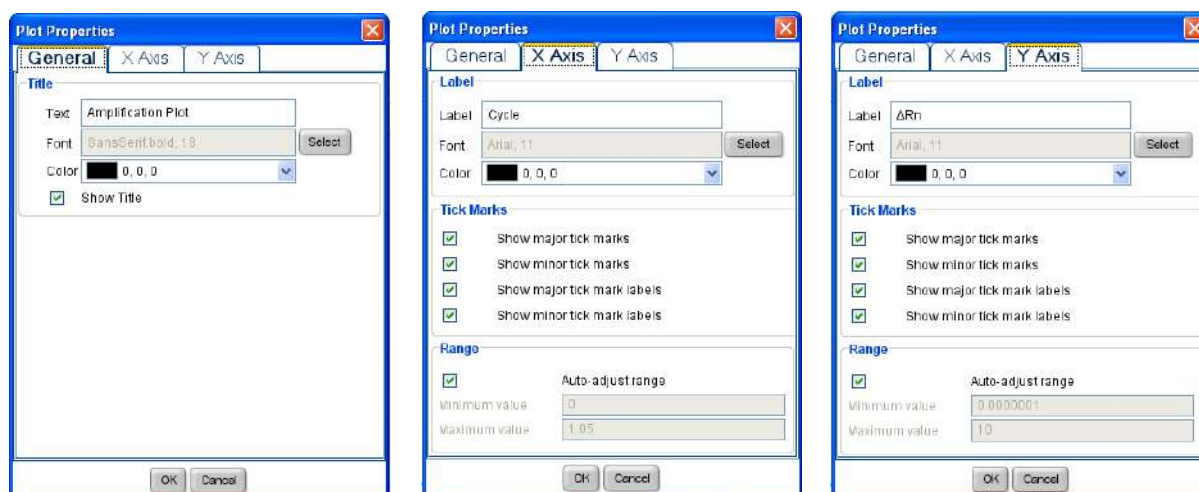
The Degradation Index value is displayed in the Well Table view in any of the analysis screens (you may have to scroll to the right to display it.)

For more information on Degradation Index or evaluating the quality indices determined by the HID Real-Time PCR Software to determine if highly degraded samples can be better analyzed with the Ion Personal Genome Machine™ (PGM™) System, refer to the *Quantifiler™ HP and Trio DNA Quantification Kits User Guide*.

Change the appearance of, print, and save plots

Change the appearance of a plot

1. In the Experiment Menu, select **Analysis**, then click the name of a plot of interest.

2. In the plot screen, locate the icon bar above the plot.

3. Click (**Hide**) to hide the plot legend.

4. To change the appearance of a plot, click (**Edit Plot Properties**) to open the Plot Properties dialog box. Three tabs are displayed.

5. Select the appropriate tab to enter the values you want to use to plot the data.

6. Click **OK** to apply the changes.

Specify wells to report

You can specify which wells to include in the amplifications plots and results table of reports:

1. In the Experiment Menu, select any Analysis screen.

2. Select the well(s) to include, using either the View Plate Layout tab (see step 4 on page 31) or the View Well Table tab (see step 4 on page 33).

Print or save a plot

Click (**Print**) to print the plot.

Click (**Save**) to save the plot as a *.jpg file.

Printed plots and *.jpg files include the slope, Y-intercept, and R2.

<div style="float:left">**7**</div>

Export and Report Results

This chapter covers:

Overview

After the HID Real-Time PCR Analysis Software completes analysis and after you review the data, you can generate a customized report in *.pdf files, then save or print the report.
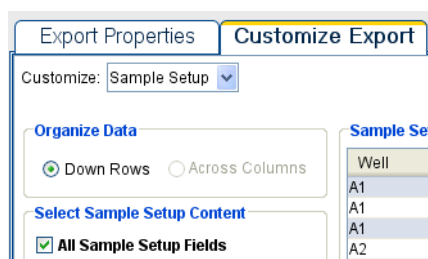
You can also export and save data in these formats:

- Excel™ (*.xls)
- Powerpoint™ (*.ppt)
- Text (*.txt)

Export data

1. In the Experiment Menu, select **Analysis**. Click any Analysis screen, then click either **View Plate Layout** or **View Well Table**.
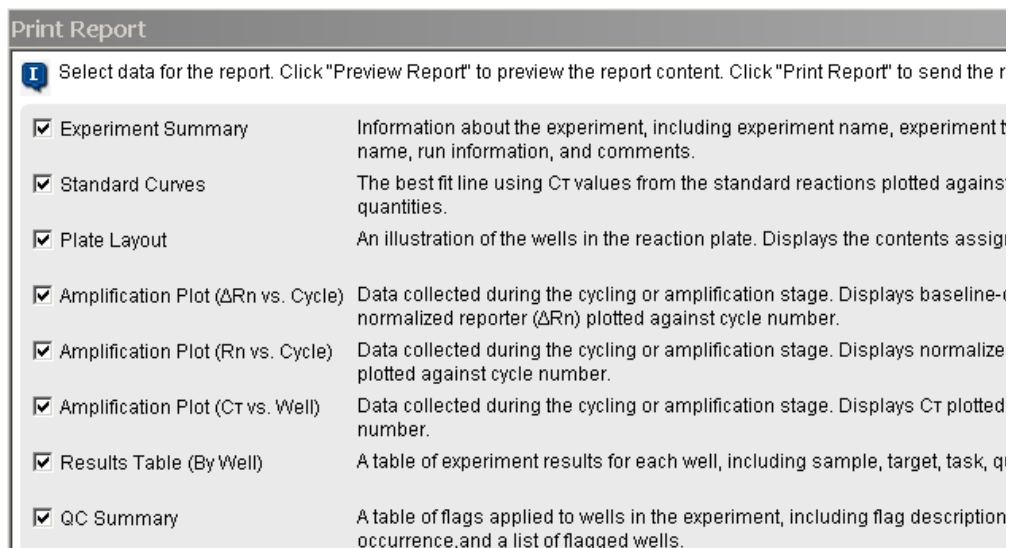
2. Highlight the wells to export.

3. In the toolbar, click (**Export**) to open the Export Data screen, then select the **Export Properties** tab.

4. Select the type of data to export:

   • **Amplification Data** – Data that was collected during the cycling or amplification stage.

   • **Multicomponent Data** – Fluorescence data for each dye, for each cycle.

   • **Raw Data** – Raw fluorescence data for each filter, for each cycle.

   • **Results** – Results of the analysis.

   • **Sample Setup** – Setup information such as well, sample name, and sample color.

   • **STR Dilution Setup** – Sample dilution worksheet to prepare samples for amplification. For more information, see Chapter 8, "Generate Dilution and Reaction Worksheets for STR Setup."

   • **STR Reaction Setup** – STR reaction setup worksheet to prepare samples for amplification. For more information, see Chapter 8, "Generate Dilution and Reaction Worksheets for STR Setup."

5. Select **Separate Files** or **One File** in the drop-down list.

6. Enter the export file properties. For:

   • **Export File Name** – Enter the name of the report.

   • **File Type** – Select the type of file to which you want to send the data. Refer to the online Help for information on creating *.ppt slides.

   • **Export File Location** – Enter the filepath to the location where you want to store the report.

7. To customize the data:

   a. Select the **Customize Export** tab.

   b. Select the information that you want to export.

   ![note icon] Note: Sample setup should be exported as a .txt file only.

   c. To sort data in the export by column, click the column header (for example, click **Well** to sort the data by well).

8. Click **Start Export** to export the data to the file(s) that you selected.

Print a report

1. Click **Plate Setup ▸ Assign Targets and Samples**, then click either **View Plate Layout** or **View Well Table**.

2. Highlight the wells to include in the report.

3. In the toolbar, click 🖨 **(Print Report)** to display the Print Report screen.

4. Select the check box corresponding to each data topic that you want to include in the report.

    ✑ Note: Exported standard curves do not include unknown data points.

5. Click **Print Preview** or **Print Report** at the bottom of the screen.

    IMPORTANT! To save the report to a file, you must click **Print Preview** before you print the report.

6. Select **Save** to save the report, or select **Print** to print the report.

Note: If you do not enter a name in the Experiment Name field of the Experiment Properties screen, the experiment name on the report is "Untitled."

8 Generate Dilution and Reaction Worksheets for STR Setup
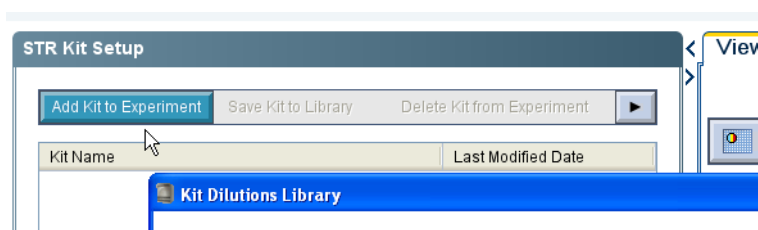
This chapter covers:

Overview

After a run is complete, you can use the HID Real-Time PCR Analysis Software to generate dilution and reaction worksheets for STR set up.

The software generate dilution and reaction setup worksheets to perform calculations for the kit(s) you select from the STR Kit Library, and the kit information and default dilution settings you specify.

See Appendix A, "Configure STR Library and Default Dilution Settings" to:

• Enter, edit, or delete kit information in the STR Kit Library
• Set default dilution settings for the calculations

Add kits to an experiment

Before exporting worksheets, add kits to an experiment:

1. Open the experiment of interest.

2. In the Experiment Menu, select **STR Kit Setup**.

3. In the STR Kit Setup area, click **Add Kit to Experiment** to open the Kit Dilutions Library.

4. Select the kit(s) to use in the experiment. To edit kit information, see "Configure the STR Kit Library" on page 73.

Kit Name	Last Mo
AmpF*l*STR® Identifiler®	Oct 6, 2008
AmpF*l*STR® Profiler Plus®	Oct 8, 2008
AmpF*l*STR® COfiler®	Sep 9, 2008

STR Kit Name   AmpF*l*STR® Identifiler®

Target Conc. (ng/µL)   0.1

5. Repeat steps 2 through 4 until you select all the kits to use in the experiment.

6. To delete a kit from the experiment (not from the Kit Library), select the kit to delete, then click **Delete Kit from Experiment**.

Select unknown samples for amplification

After adding kits to an experiment, select the unknown samples for amplification and associate samples with kits:

1. In the Experiment Menu, select any analysis screen, then select the **View Well Table** tab.

   Note: If the Well Table does not display a column for the selected STR kit, click **Show in Table**, then select the kit name from the list of available columns.

2. Select the check box corresponding to the unknown sample to use and the STR kit with which to use the sample. If a sample is not for amplification (for example a standard), it is not available for selection.
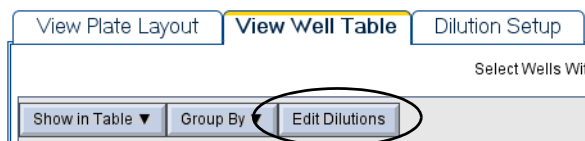
   To select all of the samples for a kit, select the check box beside the kit name at the top of the column.

   Note: The software automatically assigns the same kit for replicates.

3. Select the **Dilution Setup** tab to view the dilution scheme and the STR kit(s) that you selected for each sample.

4. Repeat steps 2 and 3 for each unknown sample and kit(s).

Note: You cannot select an STR kit for standard or NTC sample types. Dilution calculations apply only to the unknown sample (Human or Male) target in the well(s), not to standards or NTCs.

Edit dilution settings for individual samples

If needed, edit the default dilution settings for samples:

1. Select the **View Well Table** tab.

2. Select the sample of interest.

3. In the toolbar at the top of the well table, click **Edit Dilutions** to open the Edit Target Dilution Details screen.

Note: If you quantify replicates, this screen displays the sample concentration or the mean sample concentration.

4. View or edit:
   - **Min. Pipetting Vol.** – The minimum quantity to pipette.
   - **Max. Sample Vol.** – The maximum volume of available sample.
   - **Dilution Factor** – For example, enter 10 for 10-fold dilutions.
   - **Target Conc.** – The amount of target DNA that you want to use divided by the total sample volume per STR reaction.
   - # **Replicates** – The number of identical reactions.

Note: The software displays target sample concentration based on maximum sample volume, number of replicates, sample volume per STR reaction, and pipetting overage that you set if the desired target concentration cannot be reached.

View the dilution scheme

View Plate Layout	View Well Table	Dilution Setup							

STR Kit	Sample Name	Quantity Mean	IPC Ct	STR Target Co...	STR Input Amount (ng)	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2	# of STR Rxn.
AmpFℓSTR® Identifiler®	#6	6.53336906...	26.254...	0.1	1.00	1.0	64.3	10.0	0.0	1
AmpFℓSTR® Identifiler®	740	3.94344282...	26.926...	0.1	1.00	1.0	38.4	10.0	0.0	1
AmpFℓSTR® MiniFiler™	#6	6.53336906...	26.254...	0.1	0.50	1.0	129.7	10.0	0.0	1
AmpFℓSTR® MiniFiler™	740	3.94344282...	26.926...	0.1	0.50	1.0	77.9	10.0	0.0	1
AmpFℓSTR® Yfiler®	#6	6.62583065...	27.185...	0.1	1.00	1.0	65.3	10.0	0.0	1

View the dilution scheme to ensure settings are appropriate for the experiment:

1. In the Experiment Menu, select **Analysis**.

2. Click any plot to open a plot screen.

3. Select the **Dilution Setup** tab to open the Dilution Setup screen.

4. Review the dilution setup settings for downstream reactions.

Export dilution and reaction worksheets

Export the STR Dilution Setup worksheet and the STR Reaction Setup worksheet as described in "Export data" on page 59.

Save new STR Kit information from an experiment into STR Kit Library

You can save a kit from an experiment into the library (for example, if you import an experiment from a system with a different library setup).

Note: If the STR kit name you are saving from the experiment is already listed in the library, rename or delete the kit from the library before saving the kit information from the experiment.

1. Open the experiment.

2. In the STR Kit Setup screen, select the kit to save.

3. Click **Save Kit to Library**.

9 HID Real-Time PCR Analysis Software Validation

This chapter covers:

Introduction

The HID Real-Time PCR Analysis Software v1.3 is designed specifically for the Quantifiler™ DNA Quantification Kits and the Applied Biosystems™ 7500 Real-Time PCR System or the QuantStudio™ 5 Real-Time PCR System with 0.2-mL 96-well sample block. The software enables streamlined quantification run setup, data analysis, and STR reaction setup by providing Quantifiler™-specific templates and quality flags as well as STR sample normalization (dilution) and reaction setup tools. HID Real-Time PCR Analysis Software v1.3 contains the same functionality as the version 1.2 software in addition to new features that support the use of virtual standard curves and the use of the QuantStudio™ 5 system. See "Features in v1.3" on page 10 for more information.

This chapter describes the results of experiments that Thermo Fisher Scientific performed to validate the HID Real-Time PCR Analysis Software v1.3. Data was collected using versions 1.2 and 1.3 of the HID Real-Time PCR Analysis Software, the 7500 Real-Time PCR System and the QuantStudio™ 5 Real-Time PCR System, and the Quantifiler™ HP, Trio, Duo, and Human DNA Quantification Kits.

The data collected from both the 7500 and QuantStudio™ 5 instruments were analyzed to verify:

• The HID Real-Time PCR Analysis Software v1.3 performs as designed to analyze data generated on the 7500 Real-Time PCR System and the QuantStudio™ 5 Real-Time PCR System.

• The new features do not adversely affect either the quantification assays or the software functionality carried over from the HID Real-Time PCR Analysis Software v 1.2.

• Data generated using the 7500 Real-Time PCR System and the QuantStudio™ 5 Real-Time PCR System when analyzed using the HID Software v1.3 demonstrate reproducible performance for the respective instrument models.

For validation experiments and results for the Quantifiler™ Trio, Duo, HP, and Human kits, see the Quantifiler™ *HP and Trio DNA Quantification Kits User Guide* (Pub. no. 4485354), the Quantifiler™ *Duo DNA Quantification Kit User Guide* (Pub. no. 4391294), and the Quantifiler™ *Human and Y Human Male DNA Quantification Kits User Guide* (Pub. no. 4344790).

Materials and methods

- Instrument, computer, and software configuration
  - Applied Biosystems™ 7500 Real-Time PCR System, firmware v2.10 (3)
  - QuantStudio™ 5 Real-Time PCR System, 0.2-mL 96-well sample block (3)

	Instrument 1	**Instrument 2**	**Instrument 3**	**Instrument 4**	**Instrument 5**	**Instrument 6**
Model	QuantStudio 5 System	QuantStudio 5 System	QuantStudio 5 System	7500 System	7500 System	7500 System
Computer	Laptop	Laptop	Desktop	Laptop	Laptop	Desktop
Microsoft™ OS	Windows™ 7 64-bit				Windows™ 7 32-bit	
HID Real-Time PCR Analysis Software	v1.3			v1.2 and v1.3	v1.2	

- Chemistries and consumables

Item	**Cat. No.**
Quantifiler™ Trio DNA Quantification Kit, 14 kits (from single lot)	4482910
Quantifiler™ HP DNA Quantification Kit, 5 kits (from single lot)	4482911
Quantifiler™ Duo DNA Quantification Kit, 5 kits (from single lot)	4387746
Quantifiler™ Human DNA Quantification Kit, 5 kits (from single lot)	4343895
MicroAmp™ Optical 96-Well Reaction Plate, 10 plates	N8010560
Modified TE Buffer (10/0.1)	300675
MicroAmp™ Optical Adhesive Film, 100 covers	4311971
7500 Real-Time PCR Systems Spectral Calibration Kit I	4349180
ABY™ Dye Spectral Calibration Plate, 96-well	4461591
JUN™ Dye Spectral Calibration Plate, 96-well	4461593
TaqMan™ RNase P Instrument Verification Plate, 96-well	4350584
AmpFℓSTR™ Control DNA 007, Male	N/A
AmpFℓSTR™ Control DNA 9947A, Female	N/A

The following test cases were performed for each chemistry kit:

Quantifiler™ Kit	**Plates per instrument for the experiment**				
	Precision/ linearity	**Accuracy/ reproducibility**	**Sensitivity**	**Mixture**	**Inhibition**
Trio Kit	3 plates	1 plate	1 plate	1 plate	1 plate
Duo Kit	3 plates	1 plate	1 plate	1 plate	1 plate
HP Kit	1 plate	1 plate	—	—	—
Human Kit	1 plate	1 plate	—	—	—

- Samples

Experiment	Sample	Replicates per plate
Precision and linearity	Quantifiler™ Trio, Duo, HP, and Human Kit DNA Standards	6 standard curve 12 dilution series
Accuracy and reproducibility	One male DNA, 1 ng/µL	96 replicates
Sensitivity	• **Quantifiler™ Trio Kit** – One male and one female DNA diluted to 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 ng/µL • **Quantifiler™ Duo Kit** – One male DNA diluted to 50, 5, 0.5, 0.05, and 0.005 ng/µL	• 5 dilution series • 5 dilution series
Mixture analysis	• **Quantifiler™ Trio Kit** – One set of male/female DNA mixture at 1:0, 1:1, 1:10, 1:100, 1:1000, 1:4000, 0:1 ratios • **Quantifiler™ Duo Kit** – One set of male/female DNA mixture at 1:0, 1:1, 1:10, 1:100, 1:500, 1:1000, 0:1 ratios	• 3 mixture series • 3 mixture series
Inhibition	• **Quantifiler™ Trio Kit** – One male 007 DNA (0.1 ng/µL) with hematin (550 µM) • **Quantifiler™ Duo Kit** – One male 007 DNA (0.1 ng/µL) with hematin (80 µM)	• 96 replicates • 96 replicates

- Data collection and analysis
  - Run Method and analysis settings were configured as outlined in the respective Quantifiler™ kit user manuals. The design and execution of all workflows (including instrument calibration, run setup, data analysis, run methods, HID quality flags, dilution calculations import/export and reporting) were identical for both versions of the software.
    Note: The 7500 Systems were calibrated as explained in the *Applied Biosystems™ 7500/7500 Fast Real-Time PCR Systems System Maintenance Guide* (Pub. no. 4387777).
    Note: The QuantStudio™ 5 Systems were calibrated as explained in the *QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide* (Pub. no. MAN0010407). The instruments were calibrated for the ABY-HID and JUN-HID dyes using the ABY™ Dye Spectral Calibration Plate (Cat. no. 4461591) and the JUN™ Dye Spectral Calibration Plate (Cat. no. 4461593).

  - Calibration and experiment (.eds) data files that were previously generated using HID Real-Time PCR Analysis Software v1.2 were imported and reanalyzed using HID Real-Time PCR Analysis Software v1.3. The results from v1.2 and v1.3 were then compared for differences in data output.

Experiments and results

Instrument performance

Test Case	Description	Passing Criteria	Results
Precision and linearity	Using each instrument, set up and run: • Three plates of the standard curve dilution series using the Quantifiler™ Trio and Duo Kits, standard curve dilution series. • One plate using the Quantifiler™ HP and Human Kits, standard curve dilution series. Produce a standard curve from each pair of dilution series, so that six curves are generated per plate on each instrument. Statistically evaluate the C_T and R^2 values for variation within an instrument.	For each respective system (7500 or QuantStudio™ 5): • When running standard curves using the Quantifiler™ Trio, HP, DUO, and Human Assays, the C_T values shall have a coefficient of variation (CV) $\leq$ 20% within an instrument. • When running standard curves using the Quantifiler™ Trio, HP, DUO, and Human Assays, the system shall have a standard curve R^2 value $\geq$ 0.98.	Pass
Accuracy and reproducibility	Using each instrument, set up and run one plate of 007 DNA with 1 ng/µL input using each Quantifiler™ Kit, 96 replicates per plate. Statistically evaluate the C_T values and DNA quantity for variation within an instrument.	When running the Quantifiler™ Trio, HP, DUO, and Human Assays, the within-instrument C_T and quantity values shall have a CV $\leq$ 20% within an instrument.	Pass
Sensitivity	Using each instrument, set up and run: • One plate containing seven dilutions of 007 and 9947a DNA (from 0.0001 ng/µL to 100 ng/µL) prepared using the Quantifiler™ Trio Kit. • One plate containing five dilutions of 007 DNA (from 0.005 ng/µL to 50 ng/µL) prepared using the Quantifiler™ Duo Kit. Run five dilution series per sample per plate, providing a total of five replicates for each sample dilution. Statistically evaluate the C_T values and DNA quantity data for variation within an instrument.	When running Quantifiler™ Trio Assays using 0.01 ng/µL to 10 ng/µL DNA input and when running Quantifiler™ Duo Assays using 0.05 ng/µL to 5 ng/µL DNA input, the in-plate C_T and quantity values shall have a CV $\leq$ 20% within an instrument.	Pass
Mixture analysis	Using each instrument, set up and run: • One set of male and female DNA mixture samples consisting of seven mixture ratios (1:0, 1:1, 1:10, 1:100, 1:1000, 1:4000, and 0:1) using the Quantifiler™ Trio Kit. • One set of male and female DNA mixture samples with seven mixture ratios (1:0, 1:1, 1:10, 1:100, 1:500, 1:1000, and 0:1) using the Quantifiler™ Duo Kit. Run three replicates for each mixture sample. Statistically evaluate the C_T values and DNA quantity data for variation within an instrument.	When running Quantifiler™ Trio and DUO Assays using mixtures where the male DNA input is 0.02 ng/µL and female DNA input is between 0.02 and 20 ng/µL, the C_T and quantity values shall have a CV $\leq$ 20% within an instrument when results are within range on the standard curve.	Pass

Test Case	Description	Passing Criteria	Results
Inhibition	Using each instrument, set up and run: • One plate of 0.1 ng/µL 007 DNA with 550 µM hematin using the Quantifiler™ Trio Kit. • One plate of 0.1 ng/µL 007 DNA with 80 µM hematin using the Quantifiler™ Duo Kit. Run 96 replicates per plate. Statistically evaluate the C_T values and quantity data for variation within an instrument.	When running Quantifiler™ Trio and DUO Assays, the generated C_T and quantity values shall have a CV ≤ 20% within an instrument.	Pass Exception conditions [1,2]

1 When tested using the Quantifiler™ DUO Kit and the QuantStudio™ 5 Instrument, samples with extreme Hematin concentrations (>40 µM) can produce a biphasic curve that may result in overestimation of DNA concentrations or quantity value CV significantly >20%.

2 At extreme Hematin concentrations (approximately 550 µM), more variation (quantity value CV significantly >20%) may be observed in the large autosomal and Y targets on both 7500 and QuantStudio™ 5 systems.

Software performance

Test Case	Description	Passing Criteria	Results
Custom standard curve	Using one 7500 System and one QuantStudio™ 5 System, set up a virtual standard curve using estimated slope and y-intercept values. Perform a Quantifiler™ Trio run without standard curve samples.	The software shall successfully collect the data and automatically apply the virtual standard curve. The generated DNA quantities shall be 100% concordant with the manual calculation.	Pass
	Using three 7500 Systems and three QuantStudio™ 5 Systems, evaluate the virtual standard curve function using the sensitivity of a collected run. Create a virtual standard curve using the parameters (slope and y-intercept) of the standard curve generated from the run. Analyze the data using both the actual standard curve and the virtual standard curve, then compare the DNA quantity values.	For each respective system (7500 or QuantStudio™ 5), analyzing the same experiment using the actual and virtual standard curves, the software shall calculate quantification values that match to the third decimal point.	Pass
Workflow and user interface	Using one 7500 System computer and one QuantStudio™ 5 System computer, confirm that the settings match for all run/analysis methods and flag thresholds.	When comparing the v1.2 and v1.3 software, all values for the run/analysis methods and flag thresholds shall be same.	Pass
	Using both 7500 and QuantStudio™ 5 Systems, run Quantifiler™ Trio and Quantifiler™ HP Assays by following the standard workflow and using the necessary software functions.	The software functions necessary for the use of the Quantifiler™ Assays shall perform without error.	Pass
Backward compatibility	Use the v1.3 software to reanalyze an experiment (EDS) file generated using a 7500 System and the v1.2 software, then compare the quantification values calculated by both versions of the software.	When analyzing the EDS file using the v1.2 and v1.3 software, the calculated quantification values shall match to the third decimal point.	Pass

Conclusions

Based on the validation of the HID Real-Time PCR Analysis Software v1.3 and the precision and linearity, accuracy and reproducibility, sensitivity, mixture analysis, and inhibition validation experiments performed using the Quantifiler™ Trio, Duo, HP, and Human Kits [see the *Quantifiler™ HP and Trio DNA Quantification Kits User Guide* (Pub. no. 4485354), Quantifiler™ *Duo DNA Quantification Kit User Guide* (Pub. no. 4391294), and Quantifiler™ *Human and Y Human Male DNA Quantification Kits User Guide* (Pub. no. 4344790)]:

- All updates to v1.3 were successfully and correctly implemented without negative effects on functionality carried over to v1.3 from v1.2.
- The HID Real-Time PCR Analysis Software v1.3 successfully controlled both the 7500 Real-Time PCR Systems and QuantStudio™ 5 Real-Time PCR Systems, and reliably and reproducibly set up and collected quantification data using the Quantifiler™ kits.
- The software provided accurate results when used to process Quantifiler™ kits for the analysis of genomic DNA samples.
- The user interface and HID workflows in v1.3 software performed as expected for both instruments when using the Windows™ 7 operating systems.
- The coefficient of variation (CV) for the average values of DNA quantity data and standard curve parameters (C_T and R^2 values) where tested varied less than 20% within each instrument (7500 and QuantStudio™ 5 system) using both HID Real-Time PCR Software versions 1.2 and 1.3.

Laboratories should determine the appropriate level of testing required before implementation, based on the nature of the changes made to the software, how the software pertains to their laboratory workflow, their internal software validation guidelines, and those of the appropriate governing agencies.

A   Configure STR Library and Default Dilution Settings

Configure the STR Kit Library

Most AmpF*l*STR Kits are listed in the STR Kit Library by default. To add or modify amplification kit information:

1. In the toolbar, select **Tools ▸ AmpF*l*STR Kit Library** to open the Kit Dilutions Library screen.

2. To:
   - **Add a kit** – click **New**. The Create New STR Kit dialog box opens.
   - **Configure a kit** – select the kit, then click **Edit**. The Create New STR Kit dialog box opens.
   - **Remove a kit** – select the kit, then select **Delete**.

3. Enter settings:

- **STR Kit Name** – The name of the kit that you are adding to the list.

  Note: Kit names must be unique. To use the same kit with different sample types or different input amounts of DNA, add the kit with a different name, such as Identifiler_1.5 ng.

- **Target Conc.** – The amount of DNA that you want to use divided by the total sample volume per reaction. Examples:

Total DNA (ng)	Volume/reaction (µL)	Target Conc. (ng/µL)
0.5	10	0.05
1.0	10	0.1
1.0	20	0.05
2.0	20	0.1

- **STR Reaction**
  - **PCR Master Mix** – Enter appropriate volumes (µL)
  - **Sample** – Enter appropriate volumes (µL)

    The volume of the Master Mix volume and the volume of the sample must equal the total volume of the STR reaction:

    Sample (µL) + PCR Master Mix (µL) = Reaction Volume (µL)

- **Additional # of Reactions and/or Amplification Controls** – Enter the number of additional STR reactions per amplifications to allow for pipetting overage.

  IMPORTANT! Because not all kits allow for pipetting overage, you might need to enter more Additional Reactions to compensate for volume losses that occur during pipetting. Refer to your kit user manual (see page 78) for information about pipetting overage.

- **PCR Master Mix** – List each component of the STR Reaction Master Mix. Refer to your kit user's manual for more information.

4. Click **OK.**

5. Repeat steps 2 through 4 for all needed kits.

6. Verify that the kits to be used in the downstream STR reactions are listed, with correct information.

   Note: You can also save a kit from an experiment into the library (for example, if you import an experiment from a system with a different library setup). See "Save new STR Kit information from an experiment into STR Kit Library" on page 66.

Set default dilution settings

In Analysis Settings, you can specify default dilutions settings to apply to all samples (You can edit individual sample dilution settings after you associate an STR kit with an experiment).

1. In the Experiment Menu, select any analysis screen, then click **Analysis Settings.**

2. Select the **HID Settings** tab.

3. In the Dilution Method area, select:
   - **One Step Dilution** – To use a single dilution in all instances.

     or
   - **System Select** – To use a dilution scheme that depends on your preferences, with a maximum of two dilutions.

   The software displays target sample concentration based on maximum sample volume, number of replicates, sample volume per STR reaction, and pipetting overage that you set if the desired target concentration cannot be reached.

4. In the Dilution Scheme area, enter dilution scheme parameters according to your preferences or laboratory protocol. Enter the:
   - **Pipetting overage** – The percent to add to compensate for error in pipetting. If the sample concentration is less than the target concentration and the sample volume is limited, set the pipetting overage to zero to maximize the amount of DNA in the STR reaction.
   - **Minimum Pipetting Volume** – The minimum volume that you want to pipette.
   - **Maximum Sample Volume** – The maximum quantity of sample that you want to use.
   - **Dilution Factor** – The maximum first dilution that you want to perform with the available DNA. For example, for 10-fold first dilutions, enter **10**.

Documentation and Support

How to use your documentation

Portable document format (PDF) versions of this guide and the documents listed in this section are available at **http://www.thermofisher.com**.

Note: To open the user documentation available from the Life Technologies web site, use the Adobe™Acrobat™ Reader™ software available from **www.adobe.com**.

HID Real-Time PCR Analysis Software users

Refer to the following documents for more information about using HID Real-Time PCR Analysis Software.

Product	Title	Purpose	Pub. no.
Quantifiler™ Kits	*Quantifiler™ HP and Trio DNA Quantification Kit User Guide*	Provides further information on DNA quantification of samples containing human and male DNA using multiple-copy target loci for improved detection sensitivity.	4485354
	Quantifiler™ Duo DNA Quantification Kit User Guide	Provides further information on DNA quantification of samples containing mixed human and male DNA	4391294
	Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User Guide	Provides further information on DNA quantification of samples containing human/male DNA	4344790
7500/7500 Fast Real-Time PCR Systems	*7500/7500 Fast Real-Time PCR Systems Maintenance Guide*	Provides information on instrument maintenance	4412844
	7500/7500 Fast Real-Time PCR System Getting Started Guide for Absolute Quantification Experiments	Provides further information on system operation and data analysis	4378658
	7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments	Provides further information on system operation and data analysis	4387779
QuantStudio™ 5 Real-Time PCR Systems	*QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide*	Provides information on preparing, using, maintaining, and troubleshooting the system	MAN0010407
	QuantStudio™ Design and Analysis Desktop Software User Guide	Provides instructions for performing standard curve experiments on the QuantStudio™ Design and Analysis desktop Software	MAN0010408

Documents for custom experiments

Refer to the following documents for information on performing custom experiments instead of using HID Real-Time PCR Analysis Software:

7500/7500 Fast Real-Time PCR System Getting Started Guide for…	**Part Number**
Genotyping Experiments	4387784
Presence/Absence Experiments	4387785
Relative Standard Curve and Comparative CT Experiments	4387783
Standard Curve Experiments	4387779
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Absolute Quantification Getting Started Guide	4378658

Obtaining related documentation

For more information on analysis methodology, refer to the *7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments* or the *QuantStudio™ Design and Analysis Desktop Software User Guide*.

For more information on PCR amplification kits, go to **www.thermofisher.com**.

Obtaining information from the Help system

The HID Real-Time PCR Analysis Software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

• Click ❓ in the toolbar of the HID Real-Time PCR Analysis Software window.
• Select **Help ▸ Contents and Index**.
• Press **F1**.

You can use the Help system to find topics of interest by:

• Reviewing the table of contents
• Searching for a specific topic
• Searching an alphabetized index

Customer and technical support

For HID support:

- **In North America**—Send an email to **HIDTechSupport@lifetech.com**, or call **888-821-4443** option **1**.
- **Outside North America**—Contact your local support office.
- For latest services and support information for all locations, go to **thermofisher.com/support**.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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