

HID Real-Time PCR Analysis Software Version 1.0 For 7500 Real-Time PCR System



Version 1.0

Applied Biosystems



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Contents

	Preface	vii
	How to use this guide	. vii
	How to obtain more information	viii
	How to obtain support	. ix
Chapter 1	Get Started	. 1
	Software overview	1
	HID Beal-Time PCB Analysis Software v1.0 workflow	2
	How to use your documentation	
	Before vou start	4
	For more information	4
Chapter 2	Select the Experiment and Set Up a Plate	. 5
	Start the software	6
	Start a new experiment	0
	Navigate the software	,
	Specify experiment properties	o
	Define samples and view targets	9
	Assign the targets samples and standards to wells	12
	Save plate lavout as * eds or template	16
	Link your template to a Home screen button	. 16
	For more information	. 17
Chapter 3	Run the Plate	19
	View the run method	20
	Set notifications	. 20
	Start or stop the run	. 22
	Monitor the run	. 24
	Save the results	. 25
	For more information	. 25
		. 20

Chapter 4	Select Analysis Settings and Thresholds	
	Open analysis settings	
	Enter HID settings	
	View/Edit CT settings	
	Enter Flag settings	
	For more information	
Chapter 5	Enhance Data Analysis	35
	View the analysis results	
	Interpret QC flag information	38
	Exclude wells from analysis	
	Change the appearance of, print, and save plots	41
	For more information	41
Chapter 6	Export and Report Results	
	Export data	
	Print a report	
	For more information	47
Chapter 7	Generate Dilution and Reaction Worksheets for STF	<mark>≀ Setup</mark> 49
	Overview	50
	Configure STR Library and default dilution settings	50
	Add kits to an experiment	54
	Select unknown samples for amplification	
	Edit dilution settings for individual samples	55
	View the dilution scheme	56
	Export dilution and reaction worksheets	57
	Save STR Kit information from an experiment into STR Kit Library	57
	For more information	58
Appendix A	Safety Information	59
	Safety alert words	60
	General instrument safety	
	Workstation safety	61
	Index	63

Preface

How to use this guide

Product overview	The 7500 Real-Time PCR System and HID Real-Time PCR Analysis Software v1.0 detects and quantifies human and/or male DNA in samples.
Purpose of this guide	 This guide is intended to help you quickly learn how to use the HID Real-Time PCR Analysis Software v1.0 to perform analysis of samples prepared with the: Quantifiler[®] Human DNA Quantification Kit Quantifiler[®] Y Human Male DNA Quantification Kit Quantifiler[®] Duo DNA Quantification Kit
	Use this guide after your plate is prepared and loaded in the 7500 Real-Time PCR System. For instructions on preparing a plate, refer to the <i>Quantifiler</i> [®] <i>Human DNA Quantification Kit and Quantifiler</i> [®] <i>Y Human Male DNA Quantification Kit User's Manual</i> or the <i>Quantifiler</i> [®] <i>Duo DNA Quantification Kit User's Manual</i> .
Custom experiment option	You can also use the HID Real-Time PCR Analysis Software v1.0 for more complex experiments by selecting the Custom Assay option on the Home screen. If you use the Custom Assay option, refer to the <i>Applied Biosystems</i> 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments for instructions.
Assumptions	 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.
Text conventions	 This guide uses the following conventions: Bold text indicates user action. For example: Type 0, then press Enter for each of the remaining fields. <i>Italic</i> text indicates new or important words and is also used for emphasis. For example: Before analyzing, <i>always</i> prepare fresh matrix. A right arrow symbol () separates successive commands that you select from a drop-down or shortcut menu. For example: Select File > Open > Spot Set. Right-click the sample row, then select View Filter > View All Runs.

User attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

Safety alert Safety alert words also appear in user documentation. For general safety information, see Appendix A, "Safety Information," on page 59.

How to obtain more information

Portable document format (PDF) versions of this guide are available at:

www.appliedbiosystems.com.

For additional documentation, see "How to obtain support" on page ix.

To access the HID Real-Time PCR Analysis Software v1.0 Help system, do one of the following:

- Press F1
- Click ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen
- Select Help > Contents and Index

Send us your comments Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating to documentation only. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to obtain support" below).

How to obtain support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**. At the support page you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

For HID Real-Time PCR Analysis Software v1.0 support, you can email **HIDTechSupport@appliedbiosystems.com**.

In North America, you can also call technical support at 1-888-821-4HID (4443), 5:30 a.m. to 5:00 p.m. PST, Monday through Friday.

Preface

Get Started 1

This chapter covers:

Software overview	1
HID Real-Time PCR Analysis Software v1.0 workflow	2
How to use your documentation.	3
Before you start	4
For more information	4

Software overview

The HID Real-Time PCR Analysis Software v1.0 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[®] target, reporter, quencher, and thermal profile. After a run, the HID Real-Time PCR Analysis Software v1.0 provides an analysis of each well and an analysis summary of all results, STR kit setup instructions, and sample dilutions calculations.

Note: The HID Real-Time PCR Analysis Software v1.0 is for use with the 7500 Real-Time PCR System only.

HID Real-Time PCR Analysis Software v1.0 workflow

	Set up a Quantifiler [®] chemistry kit plate and load in the instrument.	
tware	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 view targets. 5. Assign the targets, samples, and standards to wells. 6. Save the experiment.	
Sof		
<u>.0</u>	Run the plate.	
УS		
k Anal	 Select analysis settings and thresholds: HID settings (includes HID flags) Flag settings 	
U U U		
Time P	Review results: 1. View analysis summary. 2. View quantitation results.	
	+	
Se	Export and print results.	
I	Generate dilution and reaction worksheets for STR set up:	
	1. Configure STR Library and default dilution settings.	
	2. Add kits to an experiment.	
	 Select the unknown samples for amplification. Edit dilution settings for individual samples as 	
	needed.	
	5. Export dilution and reaction worksheets.	
	Perform PGR amplification.	

How to use your documentation

HID Real-Time PCR Analysis Software v1.0 users Refer to the following documents for more information about using HID Real-Time PCR Analysis Software v1.0:

Title	Purpose	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide	Provides information on instrument maintenance.	4412844
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments	Provides further information on system operation and data analysis.	4387779
Quantifiler [®] Human DNA Quantification Kit and Quantifiler [®] Y Human Male DNA Quantification Kit User's Manual	Provides further information on DNA quantification of samples containing human/male DNA.	4344790
Quantifiler [®] Duo DNA Quantification Kit User's Manual	Provides further information on DNA quantification of samples containing mixed human and male DNA.	4391294

Documents for custom experiments

Refer to the following documents for information on performing custom experiments instead of using HID Real-Time PCR Analysis Software v1.0:

Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for:	PN
Genotyping Experiments	4387784
Presence/Absence Experiments	4387785
Relative Standard Curve and Comparative CT Experiments	4387783
Standard Curve Experiments	4387779

Portable document format (PDF) versions of the above documents and this guide are available at **www.appliedbiosystems.com**.



Before you start

Installation	If the HID Real-Time PCR Analysis Software v1.0 is installed with a new 7500 Real-Time PCR System, both must be installed by an Applied Biosystems technical representative.
	Laboratories already using the 7500 Real-Time PCR System can install the HID Real-Time PCR Analysis Software v1.0 by following the instructions provided with the software CD.
	Refer to the <i>Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide</i> (PN 4412843) for system layout, electrical, power, safety, and other site requirements.
	IMPORTANT! Archive all data from analyses using previous software versions before installing HID Real-Time PCR Analysis Software v1.0. Follow the instructions provided with the software CD.
Systems purchased before February 2008	Tower and laptop computers of Applied Biosystems 7500/7500 Fast Real-Time PCR Systems purchased before February 2008 require a memory upgrade before the computers can install the HID Real-Time PCR Analysis Software v1.0. Refer to the Applied Biosystems 7500/7500 Fast Real-Time PCR Systems User Bulletin <i>Memory Upgrade Requirements for 7500 Software v2.0</i> (PN 4379705) for information.
Applicable HID kits	 You can use the HID Real-Time PCR Analysis Software v1.0 with the following kits: Quantifiler[®] Human DNA Quantification Kit Quantifiler[®] Y Human Male DNA Quantification Kit
	• Quantifier Duo DNA Quantification Kit

For more information

Access the Help system by pressing F1, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screens, or by selecting Help > Contents and Index.

Select the Experiment and Set Up a Plate

This chapter describes how to:

Start the software	. 6
Start a new experiment	. 7
Navigate the software	. 8
Specify experiment properties	. 9
Define samples and view targets	. 9
Assign the targets, samples, and standards to wells	12
Save plate layout as *.eds or template	16
Link your template to a Home screen button	16
For more information	17

This chapter assumes that you have prepared a plate according to the instructions in the *Quantifiler*[®] *Human DNA Quantification Kit and Quantifiler*[®] *Y Human Male DNA Quantification Kit User's Manual* or the *Quantifiler*[®] *Duo DNA Quantification Kit User's Manual*.

2



Start the software

- On your desktop, double-click HID Real-Time PCR Analysis Software v1.0 or select Start > All Programs > Applied Biosystems > HID Real-Time PCR Analysis Software v1.0. To open the Login Screen.
- **2.** In the User Name field, select your user name from the drop-down list. Click **OK** to open the Home screen (Figure 1).



Figure 1 Home screen



Start a new experiment

- **1.** In the Home screen:
 - If the drop-down list of HID experiments is not visible on the Home screen Figure 1), click the down arrow below the Quantifiler[®] Duo button to open the list (Figure 2).



Figure 2 HID Real-Time PCR Analysis Software v1.0 Home Screen

or

• In the toolbar, click New Experiment (Figure 3).





- 2. Select the type of experiment that you want to perform:
 - Quantifiler[®] Duo
 - Quantifiler[®] Male
 - Quantifiler[®] Human

Note: To use a (hybrid) Quantifiler[®] Human and Male plate, select either **Quantifiler[®]** Human or **Quantifiler[®] Male**.



For custom experiments

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

• Custom Assay in 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 *Applied Biosystems* 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments.

Navigate the software

Each HID Real-Time PCR Analysis Software v1.0 experiment screen displays instructions for a step in the experiment. Use the Experiment Menu (Figure 4) at the left of any screen to navigate the software.

Experiment Menu «
Setup
Experiment Properties
Plate Setup
Run Method
www.Run
Amplification Plot
Temperature Plot
Run Method
Notification Settings
Analysis
Amplification Plot
Standard Curve
STR Kit Setup
Multicomponent Plot
Raw Data Plot
QC Summary

Figure 4 Experiment Menu (Showing the fully expanded menu.)

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 v1.0 screens in any sequence.

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



Specify experiment properties

1. In the Experiment Menu, select Setup > Experiment Properties (Figure 5).

Setup	Experiment Properties
Experiment Properties	Enter experiment information.
Plate Setup	How do you want to identify this experiment?
Run Method	* Experiment Name: Untitled Barcode (Optional):
Run	User Name (Optional):
Analysis	Comments (Optional):

Figure 5 Experiment Properties

2. In the "How do you want to identify this experiment?" section, enter in the Experiment Name field the *name of the plate* or experiment information. 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 spreadsheet that correspond to the experiment.

The following parameters are automatically set:

- Instrument: 7500 (96 wells)
- Experiment: Quantitation-Standard Curve (HID Quantitation)
- Reagents: Taqman[®] Reagents
- Ramp Speed: Standard (~2 hours to complete a run)

Define samples and view targets

Note: Targets and an NTC sample are automatically listed and named. Standards dilutions 1 to 8 are listed by default for each Quantifiler[®] Kit. For information about the standard included in the Quantifiler[®] kit, refer to your Quantifiler[®] kit user's manual (see "How to use your documentation" on page 3).

View targets 1. In the Experiment Menu, select Setup > Plate Setup.



2. Select the **Define Targets and Samples** tab to open the Defined Targets area. View the targets list (Figure 6) to verify that you selected the correct experiment in step 2 on page 7.

Define Targe	ets and Samples	Assign Targ	ets and Sam	ples	
Instructions: Define the targets to quantify and the samples to test in the reaction plate.					
Defined Targe	ets				
Target Name		Reporter	Quencher	Color	
[Duo Human	VIC	NFQ-MGB		
	Duo IPC	NED	NFQ-MGB	~	
	Duo Male	FAM	NFQ-MGB	~	

Figure 6 Defined Targets area (example shows Quantifiler[®] Duo experiment targets.)

The quencher for all Quantifiler[®] experiments is NFQ-MGB. The reporter dyes for the Quantifiler[®] Duo and Quantifiler[®] Male or Human kits are different:

- Quantifiler[®] Duo Human: VIC[®], Male: FAM[™], IPC: NED[™]
- Quantifiler[®] Male or Human Human and Male: FAM^{M} , IPC: $VIC^{\mathbb{R}}$

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

- **1.** Click (down arrow) in the Color column.
- 2. Select a color in the drop-down list.

Define samples 1. In the Define Samples area of the Define Targets and Samples tab, specify sample names (Figure 7):

Define Samples							
						_	
Add New Sample	Add Saved Sample	Save Sample	De	lete San	nple		
Sample Name				Co	Sampl	•	
Duo Standard 4				~	Standard		
Duo Standard 5				~	Standard		
Duo Standard 6				~	Standard		
Duo Standard 7				~	Standard		
Duo Standard 8				~	Standard	=	
NTC				~	NTC		
Sample 1				~	UnKnown	~	

Figure 7 Define samples area (New sample added)

• To define a new sample:

- Click Add New Sample. A new line appears in the Sample Name field, *or*

- In the toolbar, select **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 **Save Sample**.

- 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.
- 2. Select the sample type: Standard, NTC, or Unknown.

Note: 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.



3. Repeat step 1 and step 2 for each sample.

IMPORTANT! List each sample individually. For replicates (identical samples), add the sample name only once. To assign the replicate to a well in the plate, in step 3 on page 13, select the well, then select the check box next to the sample name.

Assign the targets, samples, and standards to wells

- 1. In the Experiment Menu, select Setup > Plate Setup.
- 2. Select the Assign Targets and Samples tab (Figure 8).

Define Lar	gets and Samp	les Ass	ign Targets	an	d Sam	ples				
Instructions	Standards and N Select wells, ther	ITC are set by d n assign targets	lefault. s if applicable.							
Assign sam	ple(s) to the se	lected wells	. <	V	iew Pla	ate Lay	out	View \	Well Tabl	e
Assign		Sample					S	elect We	IIs With:	Select Ite
	Duo Standard 1		<u> </u>	C	Show	in Wells	v Fe	View L	egend	
	Duo Standard 2									
Г	Duo Standard 3				1	2	3	4	5	6
Γ	Duo Standard 4			A	Duo Hu	Duo Hu	Duo Hu			
	Duo Standard 5				• ···					
 Assign targ	jet(s) to the sele	ected wells.		в	Duo Hu Duo IPC	Duo Hu Duo IPC				
Assign	Target	Task	Quantity		Duo Hu	Duo Hu				
	Duo Hum		50	С	Duo IPC	Duo IPC				
10	Duo IPC				Duo Hu	Duo Hu				
। <u>प</u>	Duo Male 🕕		50	D	Duo IPC					
য় <u>য</u> য য	Duo Male		50	E	Duo IPC Duo Hu Duo IPC Duo IPC	Duo IPC Duo Hu Duo IPC				
	Ouo Male			D E F	Duo IPC Duo Hu Duo IPC Duo IPC Duo IPC	Duo IPC Duo IPC Duo IPC Duo IPC Duo IPC Duo IPC				



Note: The passive reference is ROX^{TM} dye.

Assign Using Plate Layout

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 (Figure 9).

				1								
	Select Wells With: - Select Item - 🔽 - Select Item - 🔽											
Show in Wells ▼ View Legend								*				
	1	2	3	4	5	6	7	8	9	10	11	12
A	Due S Due H Due	Duo H Duo I	 Duo H Duo I									
в	Duo H Duo I	Duo H Duo I										
с	Duo H Duo I	Duo H Duo I										

Figure 9 Plate layout area

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.** 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).

- 4. To change the quantity of standards (optional), enter the quantity in ng/μ Lin the Quantity field.
- **5.** Repeat step 3 and step 4 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.

- 6. Clear all wells not assigned:
 - **a.** Select the well(s) to clear.
 - **b.** Right-click the well(s).
 - c. Select Clear from the drop-down list.

Assign Using Well Table

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

1. Select the View Well Table tab (Figure 10).

ſν	/iew Pla	te Layout 🍸	View Wel	l Table				
Select Wells With: - Select Item - 💉 - Select Item - 🗸								
Sł	Show in Table V Group By V Expand All							
#	Well	Sample	Target	Task	Dyes	Quantity	Comments	
1	A1	Duo Stand	Duo Human	STANDARD	VIC-NFQ-M	50	1	
2	A1	Duo Stand	Duo IPC	UNKNOWN	NED-NFQ			
3	A1	Duo Stand	Duo Male	STANDARD	FAM-NFQ	50	1	
4	A2	Duo Stand	Duo Human	STANDARD	VIC-NFQ-M	50		
5	A2	Duo Stand	Duo IPC	UNKNOWN	NED-NFQ			



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.** 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 Contr+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).

- 4. To change the quantity of standards (optional), enter the quantity in ng/μ Lin the Quantity field. The quantity of samples is set by default.
- **5.** Repeat step 3 and step 4 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.

- **6.** 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.

IMPORTANT! Be sure to close any open template (*.edt) file before you save your (*.eds) file as a template. If a *.edt file is open and you attempt to overwrite it, the *.edt file will be corrupted.

- **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 Quantifiler[®] kit user manual:
 - Applied Biosystems Quantifiler[®] Kits Quantifiler[®] Human DNA Quantification Kit and Quantifiler[®] Y Human Male DNA Quantification Kit User's Manual
 - Applied Biosystems Quantifiler[®] Duo DNA Quantification Kit User's Manual

Link your template to a Home screen button

You can link your template to the Quantifiler[®] Duo assays, Quantifiler[®] Male assays, or Quantifiler[®] Human assays button on the HID Real-Time PCR Analysis Software v1.0 Home screen. 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.

IMPORTANT! Be sure to close any open template (*.edt) file before you save your (*.eds) file as a template. If an *.edt file is open and you attempt to overwrite it, the *.edt file might be corrupted.

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 folder C:\Applied Biosystems\7500\config\ templates.
- 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, select Save as Template.
 - **c.** Navigate to C:\Applied Biosystems\7500\config\templates.
 - d. Select the file corresponding to the Quantifiler[®] Duo assays, Quantifiler[®] Male assays, or Quantifiler[®] Human assays button that you want to replace *QuantifilerDuo.edt*, *QuantifilerMale.edt*, or *QuantifilerHuman.edt*.

IMPORTANT! Be sure to give the file exactly the same name as the file corresponding to the button that you want to replace: *QuantifilerDuo.edt*, *QuantifilerMale.edt*, or *QuantifilerHuman.edt*.

e. Click Save.

For more information

Access the Help system by pressing F1, by clicking @ in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting Help \triangleright Contents and Index.



Run the Plate

This chapter describes how to:

View the run method	20
Set notifications	22
Start or stop the run	24
Monitor the run	25
Save the results	25
For more information	25



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 Quantifiler[®] Duo (Figure 11) or Quantifiler[®] Human and Male (Figure 12).





Figure 12 Quantifiler[®] Human and Male Kit thermal profile

3. To edit the run parameters, select **Add Stage**, **Add Step**, or **Delete Selected**. Click **Undo** to reverse an action and **Redo** to repeat an action. To undo all edits, click **Revert to Defaults**.

64.0	0 0:16
	Max 100.0
	Min 1.0

To change a ramp speed, temperature, or time, click the value you want to change to open the value field.

Use the up or down arrows to adjust the value. The value you select must be between the maximum and minimum values that are in the menu that opens when you click the value.

Note: To change other cycling parameters, perform a custom experiment: either click in the bottom left corner of the screen, then click **Custom assay** or, in the toolbar, select **Assays**, then select **Custom Assays** from the drop-down menu.

4. In either the Graphical View or Tabular View tab (Figure 13), verify that the reaction volume per well is $25 \ \mu$ L.



Figure 13 Reaction Volume Per Well

For more information on run parameters for Quantifiler[®] kits, refer to the *Applied Biosystems Quantifiler*[®] *Kits Quantifiler*[®] *Human DNA Quantification Kit and Quantifiler*[®] *Y Human Male DNA Quantification Kit User's Manual* or the *Applied Biosystems Quantifiler*[®] *Duo DNA Quantification Kit User's Manual*.

Note: A 64% ramp up and a 100% ramp down are set by default for 9600 emulation mode.

Set notifications

You can specify that the software 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 (Figure 14).

START RUN 🏷		Instrument Status: 🔌 Connected
Run Status:	Not Started	Enable Notifications
Notification Setting	js	
	Enable Notifications:	⊙ Yes 🔘 No
	Select the events to generate notifications:	Instrument Error
		Run Started
		Run Completed
For example: jane_smit	Enter e-mail addresses for notifications: Separate e-mail addresses with commas. th@mydomain.com,awong@bigmailhost.com	
	Outgoing Mail Server (SMTP):	
		For example: smtp.mycompany.com
	Server requires an encrypted connection?	VYES ONO
	Server requires authentication?	
	(Server Authentication) User Name:	
	(Server Authentication) Fassword.	
II.	Figure 14 Notification	n settings screen
	2. To send notifications:	
	a. In the Run Status	area, select the Enable Notifications check box.
	b. In the Notification not want the system	as Settings area, select Yes for Enable Notifications. If you do m to send notifications, select No .
	IMPORTANT! Notifications on an e-mail network.	cannot be sent unless the computer that performs the run is
Sele notificatio events and ent address	 For "Select the events that you want to generated in that you want to generated in the selection of the selection of the selection. 	to generate notifications," select the check boxes for events the e-mails. You can select: \mathbf{r} – Notifies addressees that the run stopped before run potifies addressees that the run began
	Run Completed -	- Notifies addressees that the run is finished

Notes_

3

2. 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.

Define the outgoing server (SMTP) If you need information about the outgoing server to perform the steps listed below, contact your network system administrator.

- **1.** In the Outgoing Server (SMTP) field, enter the name of the outgoing server. For example: **smtp.mycompany.com**
- 2. 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.
- **3.** 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

Note: You can set analysis parameters before or after you run a plate. If you prefer to set parameters before you run a plate, see Chapter 4, "Select Analysis Settings and Thresholds," for information on setting analysis parameters.

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

Start To start a run:

• .In the Experiment Menu, select **Setup**, then select any screen, then click **Start Run** at the top right corner

or

• In the Experiment Menu, select **Run**, select any screen, then click **Start Run** at the top left corner (Figure 15).



Figure 15 Start Run button

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.

Monitor the run

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 v1.0 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 v1.0 prompts you to save the results.

After the run, see Chapter 5, "Enhance Data Analysis," to view and manage the results.

For more information

Access the Help system by pressing F1, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting Help → Contents and Index. 3



Chapter 3 Run the Plate For more information

Select Analysis Settings and Thresholds

This chapter describes how to:

Open analysis settings	28
Enter HID settings	29
View/Edit CT settings	32
Enter Flag settings	33
For more information	34


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 must set values for the analysis parameters:

- HID flag thresholds
- C_T threshold, baseline start cycle and end cycle
- 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 55 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
 - Raw Data Plot
 - QC Summary
 - Multicomponent Plot

Click **Analysis Settings** in the top right corner of the screen to display the Analysis Settings screen (Figure 16).



Figure 16 Analysis Settings button



4

Enter HID settings

1. Select the **HID Settings** tab (Figure 17) to view the Dilution Scheme area, the HID Flags area, and the HID Flag Settings area.

HID Setting	IS CT Settings Flag Se	ttings	ſ		
Ind Setting		sturiys			
— Dilution Sch	eme	— Dilut	tion Method		Displa
Pipetting Over Minimum Pipe Maximum Sar	rage 10.0 % etting Volume 1.0 µL mple Volume 10.0 µL	⊙ ⊙ ○ s	ne Step Dilu ystem Selec Max. Allowec	ition C t I Diluti	only to Fem (1:X) if comportatio (X than or than or
— HID Flags —	27 03/49/ 13				HID Flag Settings
Select an HID fla	ag to specify its settings		Lico		Quantifiler Duo Kit:
IPCCT	Internal PCR Control CT value				Human DNA quantity is greater than (
NTCCT	Non-Template Control sample am	plificati	✓		Male DNA quantity is greater than or e
LOWQT	Low Quantity of DNA		✓		
нібнат	High Quantity of DNA		V		Quantifiler Human Kit
SLOPE	Non-optimal slope of the Standard	curve	✓		Human DNA quantity is greater than o
R²	Low Standard curve R² value		✓	1	
YINT	Y-Intercept				Quantifiler Male Kit
MTFR	Ratio of Male to Female DNA quant	tities	~		Male DNA quantity is greater than or e



Note: See "Edit dilution settings for individual samples" on page 55 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.

Note: 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 "Exclude wells from analysis" on page 39 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 step a and step b 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 24.)

- 4. To analyze the data with new settings, click Apply Analysis Settings.
- **HIGHQT** The HIGHQT flag indicates that the well exhibits quantities above a threshold that you set.
 - **IPCCT** The IPCCT flag indicates an unknown sample that has an IPC (Internal PCR Control) C_T value greater than the average of the IPC C_T values for all the standards plus the threshold 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.

Applied Biosystems strongly recommends that you base the threshold setting on validation data produced by your laboratory. For the Quantifiler[®] Duo DNA Quantification kit, IPC C_T values for all standards are very similar. In contrast, for the 50 ng/ μ L Standard of Quantifiler[®] Human and Human Male DNA Quantification kits, the IPC C_T value tends to be more than the value for the other quantification standards. The higher IPC C_T value of this standard can increase the average for all standards.

During validation of the HID Real-Time PCR Analysis Software v1.0 by your laboratory, Applied Biosystems recommends that you evaluate the 50 ng/ μ L standard IPC C_T value. You can set a C_T threshold that is higher to account for the higher IPC C_T value of the 50 ng/ μ L standard. As a result, the software flags only samples that have potential PCR inhibition or that do not amplify as expected.

- **LOWQT** The LOWQT flag indicates that the well exhibits quantities 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/ μ L of male DNA and more than 55 ng/ μ L of human DNA generates an MTFR flag. The flag for this condition is a yellow triangle (\checkmark) in the Plate Layout or Well Table tab, and a red octagon () in the Analysis Summary (see Chapter 5, 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 $1:\geq 1$. 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.

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

 Table 1
 Results of example M:F and MTFR settings

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.

R2 This flag indicates the regression coefficient calculated from the regression line of the standard curve. The R^2 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.

View/Edit C_T settings

Select the C_T Settings tab to view and edit the settings for C_T (Figure 18).

A	nalysis Settings	for Untitled				
	HID Settings	Ст Se <u>t</u> tings	<u>F</u> lag Setting	s		
	Review the def different setting Default CT Setting efault CT settings	ault settings for anal gs for a target, select s are used to calculate	ysis of targets in this the target from the ta the Cτ for targets wit	experiment. To edit th ble, deselect "Use De hout custom settings	ne defi efault . To e	ault settings, click "Edit Default Settings." T Settings," then change the settings that are dit the default settings, click "Edit Default S
	Threshold: 0.2 B	aseline Start Cycle: 3	Baseline End Cycl	e:15 Edit Default S	ettings	
	Select a Target —					CT Settings for Duo Human
Т	arget	Threshold	Baseline Start	Baseline End		Ст Settings to Use: 🔲 Use Default Setti
Du	io Human	0.2	3	15	^	
		Figure 18 (C ₊ settings area			

The default 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**.

Enter Flag settings

1. Select the **Flag Settings** tab to view and define instrument, sample, and data collection flags (Figure 19). Flags not used in the analysis are gray.

1	Analysis Settings (for Untitled					
	HID Settings	Ст Se <u>t</u> tings	Elag Setting	js			
	Flag	Description	Use	Attribute	Condition	Value	Reject Well
	AMPNC	Amplification in ne		Ст	< 🗸	35	
	BADROX	Bad passive refer	✓	Bad passive refer	> *	0.6	
	BLFAIL	Baseline algorith	✓				
	CTFAIL	Ст algorithm failed					
	EXPFAIL	Exponential algorit					
	OFFSCALE	Fluorescence is of	✓				
	HIGHSD	High standard dev		Ст standard deviat	> •	0.5	
	NOAMP	No amplification		Amplification algor	< 🗸	0.1	
	NOISE	Noise higher than	✓	Relative noise	> •	4	
	SPIKE	Noise spikes	✓	Spike algorithm re	> *	1	
	NOSIGNAL	No signal in well					
		1.152 million (1997) and	1 (<u>111</u>)				

Figure 19 Flag Settings tab

- 2. In the Use column, select each flag that you want to include in the analysis.
- **3.** Select the condition (<, >, or =) 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 from the analysis the wells that have a flag, select the corresponding **Reject** Well check boxes.
- 5. To analyze the data with new settings, click Apply Analysis Settings.

Table 2 explains the flags.

Table 2 QC flags

Flag	Description
AMPNC	Amplification in non-template control
BADROX	Bad passive reference signal
BLFAIL	Baseline algorithm failed
CTFAIL	C _T algorithm failed
EXPFAIL	Exponential algorithm failed

Table 2 QC flags (continued)

Flag (continued)	Description
OFFSCALE	Fluorescence is offscale
HIGHSD	High standard deviation in replicate group
NOAMP	No amplification
NOISE	Noise higher than others in plate
SPIKE	Noise spikes
NOSIGNAL	No signal in well
OUTLIERRG	Outlier in replicate group
THOLDFAIL	Thresholding algorithm failed

For more information

Access the Help system by pressing F1, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting Help → Contents and Index.

5

Enhance Data Analysis

This chapter describes how to:

View the analysis results	36
Interpret QC flag information	38
Exclude wells from analysis	39
Change the appearance of, print, and save plots	41
For more information	41



View 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.
- **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 (Figure 20).

Flag Summary) Fotal Wells: 96 1 Wells Set 17 1	Processed Wel 17 Mani Flagged Wells: 0 Anal	ually Omitted Wel 0 Tar ysis Omitted Wells: 0 Sar	gets Used: 3 nples Us 9
Analysis Su	mmary QC Flags	Detail	
Standard	Thresholds Met	🧑 Thresholds Not Met	
Standard	Thresholds Met	Thresholds Not Met 0	
Standard IPCCT	Thresholds Met	O Thresholds Not Met	
Standard IPCCT NTC	Thresholds Met <u>16</u> Thresholds Met	Thresholds Not Met 0 Thresholds Not Met	
Standard IPCCT NTC	Thresholds Met <u>16</u> Thresholds Met <u>1</u>	Thresholds Not Met 0 Thresholds Not Met 0	

Figure 20 Analysis Summary tab

In the Standard Curve bar, a green square (\square) indicates that a value for Slope, R², or Y-Intercept meets the threshold. A red octagon (\bigcirc) indicates a value that does not meet the threshold. In the Standard Curve, Standard, NTC, and Unknown bars, hyperlinked numbers in the All Threshold Met or Thresholds Not Met columns indicate the number of wells that meet/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 display the standard curve(s). 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 control)

The NTC bar reports the IPCCT and NTCCT flags for all the wells on the plate that you 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 available only in Quantifiler[®] Duo 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, multicomponent, 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 flags

In addition to the areas listed above, a message might be displayed to indicate that one 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.



Interpret QC flag information

QC Flags Detail

1. In the QC Summary screen, select the QC Flags Detail tab (Figure 21) to view all QC flags (both general and HID)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.

otal Wells: /ells Set	96 Processed Wel., 17 Manua	Ily Omitted We	I 0 Targets	Used
vens det	. If Thagged Wells. O Analys		is. 0 Sample	5 05
Analysi	s Summary OC Flags)etail		
, analysi				
lag Deta	alls			
Flag:	Name	Frequ	Wells	
UT SUAL		U .		
HIGHSD	High standard deviation in replicat	te		
NOAMP	No amplification			
	No signal in well	0		
SPIKE	Noise spikes	0		
	Outlier in replicate group			
THOLDF	Thresholding algorithm failed			
PCCT	Internal PCR Control CT value	0		
NTCCT	Non-Template Control sample an	n 0		
LOWQT	Low Quantity of DNA	0		
HIGHQT	High Quantity of DNA	0		
SLOPE	Non-optimal slope of the Standard	i 0		
R²	Low Standard curve R ² value	0		
YINT	Y-Intercept			
MTFR	Ratio of Male to Female DNA quar	nti O		•
	Flag: HIGHQT—High Q	uantity of DN	A	
	Flag Detail: The DNA Quantity	of the Unkn	own sample	is
	greater than the f	ag setting		
	an Critoria: Quantifiliar Dua	Lit.		
FI	ag onteria. Quantimer Duo Human DNA au	nii. 50.0	ng/ul	
	Male DNA quar	anuuy ≥ 50.0 http:> 50.0 ∞	ng/µi a/ul	
		nacy ≥ 50.0 Nj an Kit:	grµi	
	Human DNA au	an NL	ng/ul	
	Ouantifiliar Mak	anuty≥ 50.0 ⊾k/i+•	ng/µi	
	Male DNA guer	= NIL.	a/ul	
	Male DNA quar	$acy \ge 50.0$ h	g/µi	
Flag	ged Wells: None			
	View LIQUOT Tes	ubloobooting	Information	

Figure 21 QC Flags detail tab

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 4, "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 41.

Exclude wells from analysis

You can exclude 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

Exclude wells from analysis

- 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 (Figure 22).

		_							
	>		iew Plate	Layout	View Wel	l Table	Dilution Se	etup 📃	
							Select	Wells With:	Select Item -
		Sh	ow in Table '	Group I	By ▼ Edit D	ilutions			
٦									
		#	Well	Omit	Flag	Sample	Target N	Task	Dyes
		2	A1	Image: A start and a start		Duo Stand	Duo IPC	UNKNOWN	NED-NFQ
		3	A1			Duo Stand	Duo Male	STANDARD	FAM-NFQ
		4	A2	_k		Duo Stand	Duo Human	STANDARD	VIC-NFQ-M
		5	A2			Duo Stand	Duo IPC	UNKNOWN	NED-NFQ
		6	A2			Duo Stand	Duo Male	STANDARD	FAM-NFQ
	1 1	_		_		· ·			



or

• To use the plate layout, select the View Plate Layout tab (Figure 23).

ſ	V	iew P	late I	ayou	nt 🗋	√iew \	Vell T	able	Di	lution	Setup		
			ę	Select W	/ells Wi	th: - S	elect Ite	m - 🔽	- Sele	ct Item	*		
	C	Sho	w in We	ells v		View Le	gend				+	Ð	-22
		1	2	3	4	5	6	7	8	9	10	11	12
	A	Duo H Duo H Duo Duo	Duo Duo H., Duo Duo	NTC Duo H Duo									
	в	Duo H Duo H Duo	Duo H Duo H Duo										
	с	Duo H Duo H Duo	Duo Duo H Duo										
	D	Duo H Duo H Duo	Duo H Duo H Duo										



Right-click the well(s) to omit, then select **Omit** (Figure 24).

C	Show in We	lls ▼	E V	iew Legend
	1 Duo Standard 1 Duo Human	Duo St Duo Hu	2 andard 1 man	3 NTC Duo Human
A	Duo IPC Duo Male	Duo IP Du	Copy	Duo IPC
в	Duo Standard 2 Duo Human Duo IPC Duo Male	Du Du Du	Zoom I Zoom (Fit Plat	in Dut :e
с	Duo Standard 3 Duo Human Duo IPC Duo Male	Du Du Du	Full Sci Omit	reen
	Duo Standard 4	Du	Save A	ls

Figure 24 Omit wells using the plate layout

- **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).

Change the appearance of, print, and save plots

Change the appearance of a plot You can change the appearance of, print, and save any of the analysis plots.

- 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 (Figure 25):

🔎 🔎 🛔 牘 📈 🛅

Figure 25 Plot modification icons

Click 📘 (Hide) to hide the plot legend.

3. To change the appearance of a plot, click ≥ (Edit Plot Properties) to open the Plot Properties dialog box. Three tabs are displayed (Figure 26).

Plot Properties	Plot Properties	Plot Properties
Plot Properties Ceneral XAvis YAvis Title Text Amplification Plot Font SansSerif bold, 18 Color 0, 0, 0 ♥ Show Title	Plot Properties General X Axis Label Label Color 0.0.0 Tick Marks Show major tick marks Show major tick mark labels Show major tick mark labels Show major tick mark labels Auto-adjust range	Plot Properties Xavis General XAvis Label Xavis Label Rn Label General Font Ariai, 11 Color 0, 0, 0 Tick Marks Xavis Y Show major tick marks Y Show major tick marks Y Show minor tick mark labels Y Show minor tick mark labels P Auto-adjust range Minimum value 0.000001
OK Cancel	Minimum value 0 Maximum value 1.05	Maximum value 10 OK Cancel

Figure 26 Plot Properties areas

- 4. Select the appropriate tab to enter the values you want to use to plot the data.
- 5. Click **OK** to apply the changes.

Print or save a
plotClick (Print) to print the plot.
Click (Save) to save the plot as a *.jpg file.

For more information

For more information on:

- Analysis methodology, refer to the *Applied Biosystems* 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments.
- Troubleshooting data and standard curve metrics, refer to the *Applied Biosystems Quantifiler*[®] *Kits Quantifiler*[®] *Human DNA Quantification Kit and Quantifiler*[®] *Y Human Male DNA Quantification Kit User's Manual* or the *Applied Biosystems Quantifiler*[®] *Duo DNA Quantification Kit User's Manual*.



Access the Help system by pressing **F1**, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help → Contents and Index**.

6

Export and Report Results

This chapter describes how to:

Export data	44
Print a report.	46
For more information	47

6



Overview After the HID Real-Time PCR Analysis Software v1.0 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:

- Excel (*.xls)
- Powerpoint (*.ppt)
- text (*.txt)

Export data

1. In the toolbar, click 🛷 (Export) to open the Export Data screen, then select the **Export Properties** tab (Figure 27).

🗐 Export Data	×
Select the type of data to export, select whether to export one file or separate files, then enter export file properties. (Optional) Click "Customize Export" to change the export format and to select fields to export. Click "Start Export" to export your data.	?
Export Properties Customize Export	
Sample Setup	
Raw Data Raw Data	
Amplification Data STR Dilution Setup	
STR Reaction Setup	
2. Select one file or separate files: One File Select to export all data in one file or in separate files for each data type	
3. Enter export file properties:	
Export File Name: Untitled_data File Type: 💐 (*.xls) 💌	
Export File Location: C:\Applied Biosystems\7500\experiments Browse	
Open file(s) when export is complete	
Figure 27 Export Properties tab	I

- **2.** Select the type of data to export:
 - **Sample Setup** Setup information such as well, sample name, and sample color
 - Raw Data Raw fluorescence data for each filter, for each cycle
 - Multicomponent Data Fluorescence data for each dye, for each cycle
 - Amplification Data Data that was collected during the cycling or amplification stage
 - STR Dilution Setup Sample dilution worksheet to prepare samples for amplification. For more information, see Chapter 7, "Generate Dilution and Reaction Worksheets for STR Setup."



- **Results** Results of the analysis
- STR Reaction Setup STR reaction setup worksheet to prepare samples for amplification. For more information, see Chapter 7, "Generate Dilution and Reaction Worksheets for STR Setup."
- 3. Select Separate Files or One File in the drop-down list.
- 4. Enter the export file properties. For:
 - 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 slides *.ppt slides.
 - **Export File Location** Enter the filepath to the location where you want to store the report.
- **5.** To customize the data:
 - a. Select the Customize Export tab (Figure 28).

	Export Properties Custor	nize Export
	Customize: Sample Setup 👻	
	Organize Data	Sample Set
	Down Rows Across Columns	Well
		A1
	Select Sample Setup Content	A1
		A1
	All Sample Setup Fields	A2
	Mall Not	A2
	VVen	A2
	Sample Name	A3
		A3
	Sample Color	A3
		A4
	🔽 Target Name	A5
Figure 28	Customize Export tab	

b. Select the information that you want to export.

Note: Sample setup should be exported as a .txt file only.

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

6



Print a report

1. In the toolbar, click **Print Report** to display the Print Report screen (Figure 29).

Drint Report	
Frinkkepon	
Select data for the report. Click "P	review Report" to preview the report content. Click "Print Report" to send the report to
Experiment Summary	Information about the experiment, including experiment name, experiment type, file name, run information, and comments.
Results Summary	A table of experiment results for each target, including sample, quantity (mean), qu dev), Cτ (mean) and Cτ (std dev).
Amplification Plot (ΔRn vs. Cycle)	Data collected during the cycling or amplification stage. Displays baseline-correcte normalized reporter (ΔRn) plotted against cycle number.
Amplification Plot (Rn vs. Cycle)	Data collected during the cycling or amplification stage. Displays normalized report plotted against cycle number.
Amplification Plot (CT vs. Well)	Data collected during the cycling or amplification stage. Displays CT plotted agains number.
Results Table (By Well)	A table of experiment results for each well, including sample, target, task, quantity, a
🔽 QC Summary	A table of flags applied to wells in the experiment, including flag description, freque occurrence, and a list of flagged wells.
Figur	e 29 Print Report screen
2. Selec the re	t the check box corresponding to each data topic that you want to include in port.
Note: tab, tl click printe print.	To print the plate layout, select Analysis , then select the View Plate Layout then click Show in Wells . Select items in the drop-down list to display. Right- the plate layout, then select Print Preview to view the data that display in the ed document. If you select many report items, not all items may display and
3. Click	Print Preview or Print Report at the bottom of the screen.
IMPC print	PRTANT! To save the report to a file, you must click Print Preview before you the report.
4. Selec	t 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."



For more information

Access the Help system by pressing **F1**, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting **Help → Contents and Index**.

Notes

6



Chapter 6 Export and Report Results For more information

7

Generate Dilution and Reaction Worksheets for STR Setup

This chapter describes how to:

Overview	50
Configure STR Library and default dilution settings	50
Add kits to an experiment	54
Select unknown samples for amplification	55
Edit dilution settings for individual samples	55
View the dilution scheme	56
Export dilution and reaction worksheets	57
Save STR Kit information from an experiment into STR Kit Library	57
For more information	58



Overview

After a run is complete, you can use the HID Real-Time PCR Analysis Software v1.0 to generate dilution and reaction worksheets for STR set up.

The software uses the AmpF*l*STR kit information you enter in the STR Kit Library and the default dilution settings you specify in Analysis Settings to generate dilution and reaction setup worksheets to perform calculations for the kit(s) that you select.

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 kit information:

1. In the toolbar, select Tools → AmpFℓSTR Kit Library to open the Kit Dilutions Library screen (Figure 30).

🗐 Kit Dilutions Library				
Add new kits, edit existing displayed.	kits, delete kits, imp	ort kits, or export kits. Apply a filter to	reduce the number of kits	0
Enter a filter query, then click "Apply Filter." To enter multiple filter queries, click "Advanced Filter." Advanced Filter IF Kit Name = V Apply Filter Remove Filter				
New Edit Delete	Delete All			
Kit Name	Comments	Created On	Last Modified	
AmpF l STR® Identifiler®		Jul 31, 2008	Sep 9, 2008	<u>^</u>
AmpF&STR® SGM Plus®		Jul 31, 2008	Sep 9, 2008	
AmpF&STR® Profiler Plus®		Jul 31, 2008	Sep 9, 2008	=
AmpFlSTR® COfiler®		Jul 31, 2008	Sep 9, 2008	
AmpFℓSTR® MiniFiler™		Jul 31, 2008	Sep 9, 2008	

Figure 30 Kit library

2. Click New or Edit to open the Create New STR Kit screen (Figure 31).

Note: To remove an amplification kit from the list, select the kit, then select Delete.

iter all the information for the new STR Kit, the	en click OK to save.	r= Requ
STR Kit Name STR Kit 1		
Target Conc. (ng/µL) 0.1		
STR Reaction		
PCR Master Mix	0.0	µL/reaction
Sample	0.0	µ⊔/reaction
Additional # of reactions and/or Amplificati	ion Controls	
PCR Master Mix		
Component 1 Name	Volume	0.0 µL
Component 2 Name	Volume	0.0 µL
Component 3 Name	Volume	0.0 µL
Component 4 Name	Volume	0.0 µL
Component 5 Name	Volume	0.0 µL
Comments		

Figure 31 Create new STR kit

- **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 sum of the Master Mix volume and the sample volume must equal the total volume of the STR reaction. Example:

Sample (µL)	PCR Master Mix (µL)	Reaction volume (µL)
10	15	25
20	30	50

• 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 58) 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 STR Kit information from an experiment into STR Kit Library" on page 57.

Set default 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 (Figure 32).

HID Settings CT Settings Elag Settings Dilution Scheme Dilution Method Pipetting Overage 10.0 % Minimum Pipetting Volume 1.0 µL Maximum Sample Volume 10.0 µL	
Dilution Scheme Pipetting Overage 10.0 % Minimum Pipetting Volume 1.0 µL Maximum Sample Volume 10.0 µL Max. Allowed Dilution Factor 10 X	Settings
	Dilution Method Display Image: One Step Dilution Only Display Image: System Select System Select Image: Max. Allowed Dilution Factor 10 X
HID Flags Select an HID flag to specify its settings HID Flag Quantifiler Duo Kit:	Use HID Flag Settings
IPCCT Internal PCR Control C⊤ value IPCCT Internal PCR Control C⊤ value	Human DNA quantity is greater than o
NTCCT Non-Template Control sample amplificati 🔽 Male DNA quantity is greater that	amplificati 🔽 Male DNA quantity is greater than or e
LOWQT Low Quantity of DNA	
HIGHQT High Quantity of DNA Quantifiler Human Kit	Quantifiler Human Kit
SLOPE Non-optimal slope of the Standard curve . Human DNA quantity is greater	Iard curve III . Human DNA quantity is greater than or
R ² Low Standard curve R ² value	
YINT Y-Intercept Quantifiler Male Kit	Quantifiler Male Kit
MTFR Ratio of Male to Female DNA quantities	uantities 🛛 🗹 Male DNA quantity is greater than or e

Figure 32 HID Settings tab – Default dilution settings

- **3.** In the Dilution Method area:
 - **a.** Select a dilution method:
 - One Step Dilution Use a single dilution in all instances.
 - System Select Use a dilution scheme that depends on your preferences, with a maximum of two dilutions.
 - Enter in the Dilution Scheme area 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.

Notes

7



- **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**.

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.

Add kits to an experiment

Add kits for the 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 (Figure 33).



Figure 33 Add a kit to the experiment

- **4.** Select the kit(s) to use in the experiment.
- 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.
- **2.** Select the check box corresponding to the unknown sample to use and the STR kit with which to use the sample.

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** (Figure 34) to open the Edit Target Dilution Details screen (Figure 35).



Figure 34 Edit Dilutions button



Edit <#6> Target Dilution D	etails			
Settings				
Sample Concentration: 6.53	3 ng/µl			
Min Pipetting Vol. 1.0	µi Max Sample Vol.	10.0 µl Dilution Facto	r 🔟 X	
Kits				
Kit	Target Conc. (ng/µl)	# Replicates	DNA to D1	Diluent to D1
AmpF&STR® Identifiler® 💌	0.1	1	1	9
	-			



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 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 (Figures 36 and 37).

View Plate Layout	View Well	Table	Dil	ution \$	Setup		
STR Kit	Sample Name	Quantity N	/lean	IPC Ct	STR Targ	jet Co	STR Input Amount (ng)
AmpFℓSTR® Identifiler®	#6	6.5333690	06	26.254	0.1		1.00
AmpF{STR®Identifiler®	740	3.9434428	32	26.926	0.1		1.00

Figure 36 Dilution Setup tab (left)

STR Input Amount (ng)	DNA to D1	Diluent to D1	D1 to D2	Diluent to D2	# of STR Rxn.
1.00	1.0	64.3	10.0	0.0	1
1.00	1.0	38.4	10.0	0.0	1

Figure 37 Dilution Setup tab (right)

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 44.

Save 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.

To save kit information from an experiment to the library:

- **1.** Open the experiment.
- 2. In the STR Kit Setup screen, select the kit to save.
- 3. Click Save Kit to Library.

7



For more information

For more information on analysis methodology, refer to the *Applied Biosystems* 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments.

For more information on AmpFlSTR PCR amplification kits, refer to the:

- AmpF STR[®] Identifiler[®] PCR Amplification Kit User's Manual
- AmpF STR[®] MiniFiler[™] PCR Amplification Kit User's Manual
- AmpF STR[®] Profiler Plus[®] PCR Amplification Kit User's Manual (includes information about the COfiler[®] amplification kit)
- AmpF STR[®] SEfiler Plus[™] PCR Amplification Kit User's Manual
- AmpF STR[®] SGM Plus[®] PCR Amplification Kit User's Manual
- AmpF STR[®] Yfiler[®] PCR Amplification Kit User's Manual

Access the Help system by pressing F1, by clicking ② in the toolbar of the HID Real-Time PCR Analysis Software v1.0 screen, or by selecting Help → Contents and Index.



Safety Information

This appendix covers:

Safety alert words	60
General instrument safety	61
Workstation safety	61



Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Examples

The following examples show the use of safety alert words:

IMPORTANT! The sample name, run folder name, and path name, *combined*, can contain no more than 250 characters.

CAUTION MUSCULOSKELETAL AND REPETITIVE MOTION

HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.



Α

General instrument safety

WARNING PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting stand-alone computers and monitors **WARNING** Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION MUSCULOSKELETAL AND REPETITIVE MOTION

HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.



Appendix A Safety Information Workstation safety

Index

Numerics

7500 Real-Time PCR System vii

Α

Amplification Plot screen (analysis) 39 Amplification screen (analysis) 39 Amplification screen (run) 25 AMPNC flag 33 analysis flagged wells 36 flags 29 NTC 37 omit wells 39 QC 36 standard curve 36 standards 37 unknown 37 analysis settings CT 32 flags 33 HID 29 Analysis Settings screen 28 analysis summary 36 **Applied Biosystems** contacting ix Technical Support ix

В

BADROX flag 33 BLFAIL flag 33

С

CAUTION, description 60 color, change 10 concentration DNA 51 STR kit dilutions 55 configure analysis settings 28 conventions for describing menu commands vii IMPORTANTS! viii Notes viii text vii user attention words viii CT analysis settings 32 CTFAIL flag 33 custom experiment documents for 3 selecting 8

D

DANGER, description 60 define samples 11 targets 9 delete wells 39 dilutions library, sample 51 samples, individual 55 STR 55 DNA amount 51 documentation 3 Duo, Quantifiler experiment 7 dyes, reporter 10

E

enable notifications 23 ergonomics, safety 61 exclude wells from analysis 39 Experiment Properties screen 9 experiment workflow 2 experiments custom 3 Quantifiler 7 EXPFAIL flag 33 export *.pdf 44 *.ppt 44 *.txt 44 *.xls 44

F

Flag settings 33 flagged wells 44 flags AMPNC 33
analysis 36 BADROX 33 BLFAIL 33 CTFAIL 33 EXPFAIL 33 HIGHQT 30 HIGHSD 34 instrument-related 37 IPCCT 30 LOWQT 30 MTFR, MF ratio 30 NOAMP 34 NOISE 34 NOSIGNAL 34 NTCCT 30 OFFSCALE 34 OUTLIERRG 34 R2 31 SLOPE 31 SPIKE 34 THOLDFAIL 34 YINT 32 flags, QC, detail 38

G

graphs. modify, print, save 41

Η

hazard symbols. *See* safety symbols, on instruments HID flags 29 HID settings 29 HIGHQT flag 30 HIGHSD flag 34 Home screen 6 Human, Quantifiler experiment 7 hybrid plate 7

IMPORTANT, description 60 installation, software 4 instrument-related flags 37 IPCCT flag 30

K

kits for HID assays 4

L

LOWQT flag 30

Μ

Male, Quantifiler experiment 7 method, run 20 MF ratio 30 monitor run 25 moving and lifting, safety 61 MSDSs, obtaining ix MTFR flag 30 MTFR flag vs. MF ratio 30 Multicomponent Plot screen 39 Multiple Plots screen 39

Ν

NOAMP flag 34 NOISE flag 34 NOSIGNAL flag 34 Notification Settings screen 23 notifications email addresses 24 enable 23 SMTP 24 NTC, analysis 37 NTCCT flag 30

0

OFFSCALE flag 34 omit wells from analysis 39 outgoing server 24 OUTLIERRG flag 34

Ρ

parameters, run 20 passive reference 12 plate layout save 16 save as template 16 Plate Setup screen 9 plate, hybrid 7 plots. modify, print and save 41 print plot 41 report 46

Q

QC analysis flags detail 38 instrument-related flags 37 NTC 37 standard curve 36 standards 37 QC Summary screen 36 Quantifiler experiments 7 quantity, DNA 51 quencher 10

R

R2 flag 31 ratio, MF 30 Raw Data screen 39 remove wells 39 repetitive motion, safety 61 report export 44 print 46 reporter dyes 10 run method 20 monitor 25 preparation 16 start 24 stop 24 Run Method screen (run) 25 Run Method screen (setup) 20

S

safety before operating the instrument 61 ergonomic 61 moving/lifting 61 repetitive motion 61 workstation 61 sample assign to wells 12 samples, define 11 save plots 41 sample name 11 screens Amplification (analysis) 39 Amplification (run) 25 Amplification Plot (analysis) 39 Analysis Settings 28 Experiment Properties 9 Home 6 Multicomponent Plot 39 Multiple Plots 39 Notification Settings 23 Plate Setup 9 QC Summary 36

Raw Data 39 Run Method (run) 25 Run Method (setup) 20 Standard Curve 39 STR Kit Setup 54 Temperature Plot (run) 25 server, outgoing 24 settings dilution 55 flag 29, 33 HID 29 notifications 23 SLOPE, detail 31 SMTP 24 software installation 4 start 6 SPIKE flag 34 standard curve analysis 36 Standard Curve screen 39 standards analysis 37 assign to wells 12 start run 24 software 6 stop run 24 STR 55 kit library 50 reaction volumes 51, 52 STR Kit Setup screen 54

T

Target 51 targets assign to wells 12 concentration 51 define 9 Technical Support, contacting ix Temperature Plot screen (run) 25 templates creating 16 link to home screen 16 thermal profile 20 THOLDFAIL flag 34 thresholds, setting 28 training, information on ix

U

unknown, analysis 37

user attention words, described viii

W

WARNING, description 60 wells assign standards, targets, samples 12 workflow 2 workstation safety 61

Υ

YINT flag 32

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