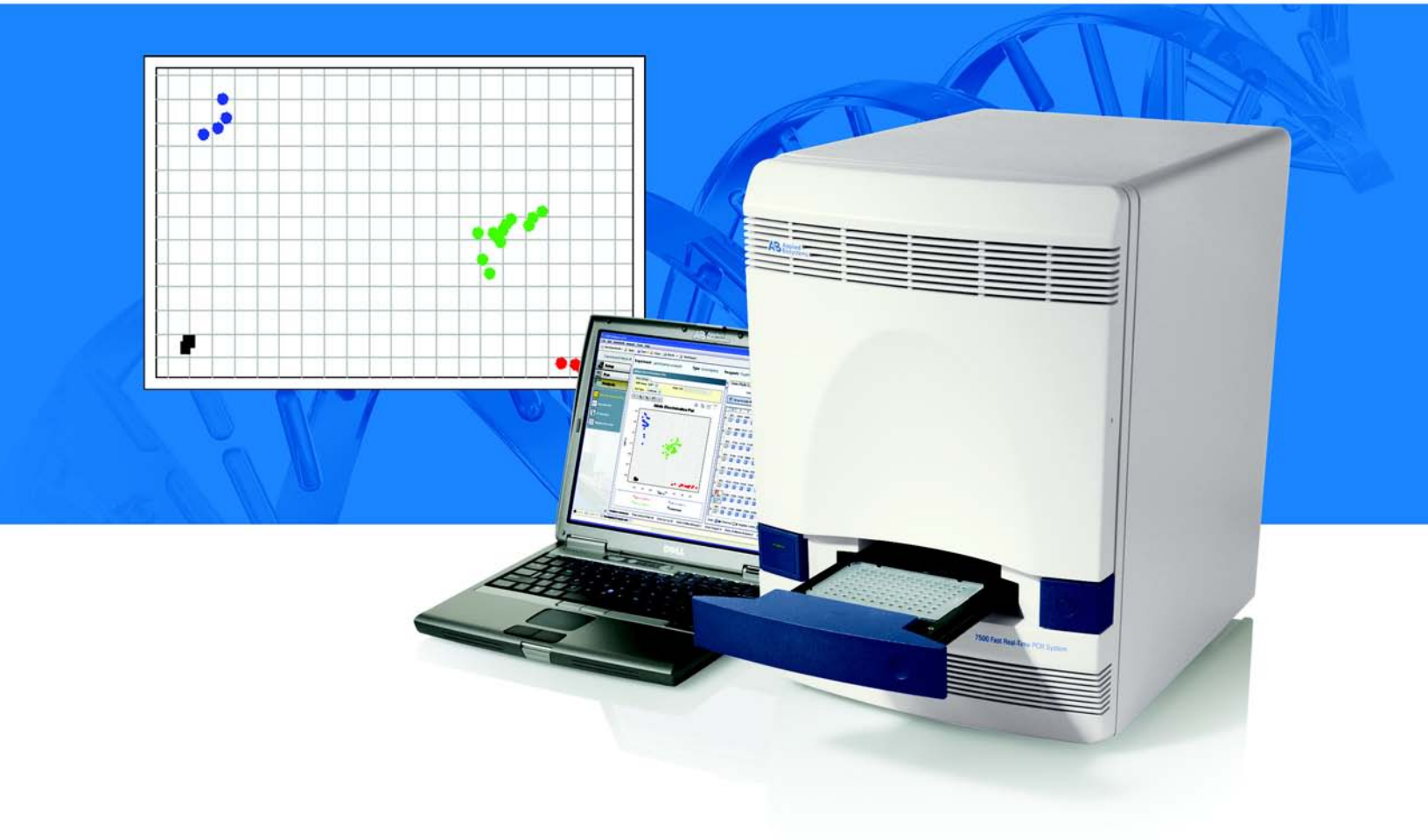
Applied Biosystems

Applied Biosystems 7500/7500 Fast

Real-Time PCR System

Genotyping Experiments



Applied Biosystems

Get Started 1
Design the Experiment 2
Prepare the Reactions 3
Run the Experiment 4
Analyze the Experiment 5

Applied Biosystems 7500/7500 Fast

Real-Time PCR System

Genotyping Experiments

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: Label License

The 7500/7500 Fast Real-Time PCR Systems are covered by US patents, and corresponding claims in their non-US counterparts, owned by Applied Biosystems. No right is conveyed expressly, by implication, or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5 nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

TRADEMARKS:

Applera, Applied Biosystems, AB (Design), MicroAmp, NucPrep, PrepMan, Primer Express, and VIC are registered trademarks and FAM, JOE, NED, and ROX are trademarks of Applied Biosystems or its subsidiaries in the U.S. and/or certain other countries.

AmpErase, AmpliTaq Gold, and TaqMan are registered trademarks of Roche Molecular Systems, Inc.

SYBR is a registered trademark of Molecular Probes, Inc.

Excel, Microsoft and Windows are registered trademarks of Microsoft Corporation.

All other trademarks are the sole property of their respective owners.

Part Number 4387784 Rev. C
06/2010

Contents

Preface . . . . . **v**

How to Use This Guide v
How to Obtain More Information vii
How to Obtain Support ix
Symbols on Instruments xi
Safety Labels on Instruments xii
Chemical Safety xv
Physical Hazard Safety xviii
Biological Hazard Safety xix
Workstation Safety xix

Chapter 1 **Get Started** **1**

About the 7500/7500 Fast System 2
Supported Consumables 4
About Genotyping Experiments 7
How to Use This Guide 11
About the Example Genotyping Experiment 12
Example Experiment Workflow 14

Chapter 2 **Design the Experiment** **15**

Chapter Overview 16
Create a New Experiment 17
Define the Experiment Properties 18
Define the Methods and Materials 20
Set Up the SNP Assays 21
Set Up the Samples and Replicates 23
Set Up the Run Method 25
Review the Reaction Setup 27
Order Materials for the Experiment 29
Finish the Design Wizard 32

Chapter 3 **Prepare the Reactions** **35**

Chapter Overview 36
Prepare the Sample Dilutions 37

Prepare the Reaction Mix 38
Prepare the Reaction Plate 40

Chapter 4 **Run the Experiment 43**

Chapter Overview 44
Prepare for the Run 45
Enable the Notification Settings (Optional) 47
Start the Run 49
Monitor the Run 50
Unload the Instrument 52

Chapter 5 **Analyze the Experiment 53**

Chapter Overview 54

Section 5.1: Review Results 55
Open the Experiment for the Analysis 56
View the Allelic Discrimination Plot 59
View the Plate Layout 64
View the Amplification Plot 66
View the Well Table 73
Publish the Data 76

Section 5.2: Troubleshoot (If Needed) 77
View the Analysis Settings 78
View the QC Summary 81
Omit Wells from the Analysis 83
View the Multicomponent Plot 84
View the Raw Data Plot 87

Appendix A **Alternate Experiment Workflows 89**

Advanced Setup Workflow 90
QuickStart Workflow 92
Template Workflow 94
Export/Import Workflow 96

Bibliography 99

Glossary 101

Index 115

Preface

How to Use This Guide

About the System Documentation

The guides listed below are shipped with the Applied Biosystems 7500/7500 Fast Real-Time PCR System (7500/7500 Fast system).

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments	Explains how to perform experiments on the 7500/7500 Fast system. Each Getting Started Guide functions as both a: • Tutorial, using example experiment data provided with the Applied Biosystems 7500/7500 Fast Real-Time PCR Software (7500 software). • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system.	4387784
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Presence/Absence Experiments		4387785
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments		4387783
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments		4387779
Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide	Explains how to maintain the 7500/7500 Fast system. Intended for laboratory staff responsible for the maintenance of the 7500/7500 Fast system.	4387777
Applied Biosystems 7500/7500 Fast Real-Time PCR System Computer Setup Guide		4387778
RealTime PCR System Reagent Guide	Provides information about the reagents you can use on the 7500/7500 Fast system, including: • An introduction to TaqMan® and SYBR® Green reagents. • Descriptions and design guidelines for the following experiment types: – Quantitation experiments – Genotyping experiments – Presence/absence experiments Intended for laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system.	4387787
Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide	Explains how to prepare your site to receive and install the 7500/7500 Fast system. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the 7500/7500 Fast system.	4387776

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 Help	Explains how to use the 7500 software to: • Set up, run, and analyze experiments using the 7500/7500 Fast system. • Calibrate a 7500/7500 Fast instrument. • Verify the performance of a 7500/7500 Fast instrument with an RNase P run. Intended for: • Laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system. • Laboratory staff responsible for the installation and maintenance of the 7500/7500 Fast system.	NA

Assumptions

This guide assumes that you:

- Are familiar with the Microsoft Windows® operating system.
- Are familiar with the Internet and Internet browsers.
- Know how to handle DNA and/or RNA samples and prepare them for PCR.
- Understand data storage, file transfer, and copying and pasting.
- Have networking experience, if you plan to integrate the 7500/7500 Fast system into your existing laboratory data flow.

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.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open**.

User Attention Words

Two 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 reagent 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, you need a valid user ID.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page x.

How to Obtain More Information

Related Documentation

Documents Related to Genotyping Experiments

Document	PN
Allelic Discrimination Pre-Developed TaqMan® Assay Reagents Quick Reference Card	4312212
Custom TaqMan® Genomic Assays Protocol Submission Guidelines	4367671
Custom TaqMan® SNP Genotyping Assays Protocol	4334431
Ordering TaqMan® SNP Genotyping Assays Quick Reference Card	4374204
Performing a Custom TaqMan® SNP Genotyping Assay for 96-Well Plates Quick Reference Card	4371394
Performing a TaqMan® Drug Metabolism Genotyping Assay for 96-Well Plates Quick Reference Card	4367636
Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol	4312214
TaqMan® Drug Metabolism Genotyping Assays Protocol	4362038
TaqMan® SNP Genotyping Assays Protocol	4332856

Documents Related to Presence/Absence Experiments

Document	PN
DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs Protocol	4343586
NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue Protocol	4333959

Documents Related to Relative Standard Curve and Comparative C_T Experiments

Document	PN
Amplification Efficiency of TaqMan® Gene Expression Assays Application Note	127AP05
Applied Biosystems High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
Custom TaqMan® Gene Expression Assays Protocol	4334429
Primer Express® Software Version 3.0 Getting Started Guide	4362460
TaqMan® Gene Expression Assays Protocol	4333458
User Bulletin #2: Relative Quantitation of Gene Expression	4303859

Documents Related to Standard Curve Experiments

Document	PN
Amplification Efficiency of TaqMan® Gene Expression Assays Application Note	127AP05
Custom TaqMan® Gene Expression Assays Protocol	4334429
Primer Express® Software Version 3.0 Getting Started Guide	4362460
TaqMan® Gene Expression Assays Protocol	4333458
User Bulletin #2: Relative Quantitation of Gene Expression	4303859

Documents Related to the Reagent Guide

Document	PN
Applied Biosystems High-Capacity cDNA Reverse Transcription Kits Protocol	4375575
Custom TaqMan® Gene Expression Assays Protocol	4334429
Custom TaqMan® Genomic Assays Protocol: Submission Guidelines	4367671
Custom TaqMan® SNP Genotyping Assays Protocol	4334431
Power SYBR® Green PCR Master Mix and RT-PCR Protocol	4367218
Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol	4312214
Primer Express® Software Version 3.0 Getting Started Guide	4362460
SYBR® Green PCR and RT-PCR Reagents Protocol	4304965
SYBR® Green PCR Master Mix and RT-PCR Reagents Protocol	4310251
TaqMan® Drug Metabolism Genotyping Assays Protocol	4362038
TaqMan® Exogenous Internal Positive Control Reagents Protocol	4308335
TaqMan® Fast Universal PCR Master Mix (2X) Protocol	4351891
TaqMan® Gene Expression Assays Protocol	4333458
TaqMan® Gene Expression Master Mix Protocol	4371135
TaqMan® Genotyping Master Mix Protocol	4371131
TaqMan® SNP Genotyping Assays Protocol	4332856
TaqMan® Universal PCR Master Mix Protocol	4304449
User Bulletin #2: Relative Quantitation of Gene Expression	4303859
Using TaqMan® Endogenous Control Assays to Select an Endogenous Control for Experimental Studies Application Note	127AP08

Note: For more documentation, see "How to Obtain Support" on page ix.

Obtaining Information from the Software Help

The 7500 Software Help describes how to use each feature of the user interface. Open the Help from within the software by doing one of the following:

- Press **F1**.
- Click in the toolbar.
- Select **Help ▸ 7500 Software Help**.

To find topics of interest in the Help:

- Review the table of contents.
- Search for a specific topic.
- Search an alphabetized 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 only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See the following section, "How to Obtain Support.")

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- 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.

In addition, the Support page provides open to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

IMPORTANT! When directed to do so by this guide, or when you need to schedule maintenance for your 7500/7500 Fast instrument (such as annual planned maintenance or temperature verification/calibration), contact the Applied Biosystems Care Center. To obtain a phone number for or to send an e-mail to the center, go to **http://www.appliedbiosystems.com/support/contact**.

Safety Conventions Used in This Document

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.

– 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.

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

– 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.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems instruments* (see "Safety Symbols" on page xi).

Examples

The following examples show the use of safety alert words:

IMPORTANT! Wear powder-free gloves when you handle the halogen lamp.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. Ethanol is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description	Symbol	Description
	Indicates the **On** position of the main power switch.		Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates the **Off** position of the main power switch.		Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.		Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates the **On/Off** position of a push-push main power switch.		Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety *s*ymbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xii). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Environmental Symbols on Instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **http://www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Français
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.
WARNING This instrument is designed for 12 V, 75 W Halogen lamps only.	**AVERTISSEMENT** Cet instrument est conçu pour des lampes d'halogène de 12 V et 75 W seulement.

Locations of Warnings

The Applied Biosystems 7500/7500 Fast Real-Time PCR System contains warnings at the locations shown below.

General Instrument Safety

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

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

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

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

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page xv.

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

Cleaning or Decontaminating the Instrument

CAUTION Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Chemical Safety

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then click **MSDS Search**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page xv.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Applied Biosystems 7500/7500 Fast Real-Time PCR System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The Applied Biosystems 7500/7500 Fast Real-Time PCR System has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Moving Parts

PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological Hazard Safety

General Biohazard

WARNING **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**).
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Workstation Safety

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

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

To minimize musculoskeletal and repetitive motion risks:

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

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standard
- European Safety and EMC Standards
- Australian EMC Standards

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 61010A-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements" and with standard UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials," and with standard EN 61010-2-081:2002+A1:2003 "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

1 Get Started

This chapter covers:

- About the 7500/7500 Fast System 2
- Supported Consumables 4
- About Genotyping Experiments 7
- How to Use This Guide. 11
- About the Example Genotyping Experiment 12
- Example Experiment Workflow 14

Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

About the 7500/7500 Fast System

The Applied Biosystems 7500/7500 Fast Real-Time PCR System is a 96-well, five-color platform that uses fluorescence-based polymerase chain reaction (PCR) reagents to provide:

- Quantitative detection of target nucleic acid sequences (targets) using real-time analysis.
- Qualitative detection of targets using post-PCR (endpoint) analysis.
- Qualitative analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR).

About Data Collection

The 7500/7500 Fast system collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs:

Run Type		Data Collection Point
Real-time runs	Standard curve	The instrument collects data after each extension step of the PCR.
	Relative standard curve	
	Comparative C_T ($\Delta\Delta C_T$)	
Post-PCR (endpoint) runs	Genotyping	The instrument collects data: • Before the PCR. (For presence/absence experiments, data collection before the PCR is optional but recommended.) • (Optional) During the PCR. The instrument can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting. • After the PCR.
	Presence/absence	

Regardless of the run type, a data collection point or *read* on the 7500/7500 Fast instrument consists of three phases:

1. **Excitation** – The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.
2. **Emission** – The instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.
3. **Collection** – The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The 7500 software stores the raw fluorescence image for analysis.

After a run, the 7500 software uses region of interest (ROI), optical, dye, and background calibrations to determine the location and intensity of the fluorescence in each read, the dye associated with each fluorescent signal, and the significance of the signals.

Notes

About the Filters

The 7500/7500 Fast system uses the following filters:

Filter	1	2	3	4	5
Dye	• FAM™ dye • SYBR® Green dye	• JOE™ dye • VIC® dye	• TAMRA™ dye • NED™ dye • Cy3® dye	• ROX™ dye • Texas Red® dye	Cy5® dye

For More Information

For information on:

- The 7500/7500 Fast system – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR Software Help*.

  Note: To open the Help, select **Help ▸ 7500 Software Help** in the 7500 software.

- Presence/absence experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Presence/Absence Experiments*.
- Relative standard curve and/or comparative C_T ($\Delta\Delta C_T$) experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments*.
- Standard curve experiments – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

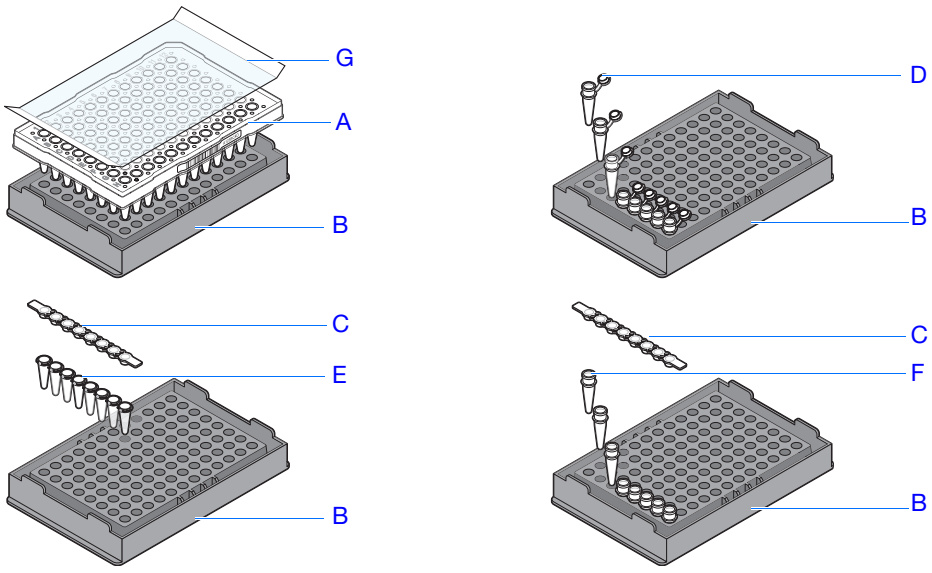
Notes

Supported Consumables

7500 System

The 7500 system supports the consumables listed below.

Consumable	Part Number
• MicroAmp® Optical 96-Well Reaction Plate with Barcode, 0.2-mL • MicroAmp™ Optical Adhesive Film	• 4306737 • 4311971
• MicroAmp™ Optical 8-Tube Strip, 0.2-mL • MicroAmp™ Optical 8-Cap Strip	• 4316567 • 4323032
• MicroAmp® Optical Tube without Cap, 0.2-mL • MicroAmp® Reaction Tube with Cap, 0.2-mL	• N8010933 • N8010540
• MicroAmp® Splash Free Support Base	• N8010531
• MicroAmp™ Adhesive Film Applicator • MicroAmp® Cap Installing Tool (Handle) • MicroAmp™ Multi-Removal Tool	• 4333183 • 4330015 • 4313950



#	Consumable
A	MicroAmp® Optical 96-Well Reaction Plate, 0.2-mL
B	MicroAmp® Splash Free Support Base
C	MicroAmp™ Optical 8-Cap Strip, 0.2-mL
D	MicroAmp® Reaction Tube with Cap, 0.2-mL

#	Consumable
E	MicroAmp™ Optical 8-Tube Strip
F	MicroAmp® Optical Tube without Cap
G	MicroAmp™ Optical Adhesive Film

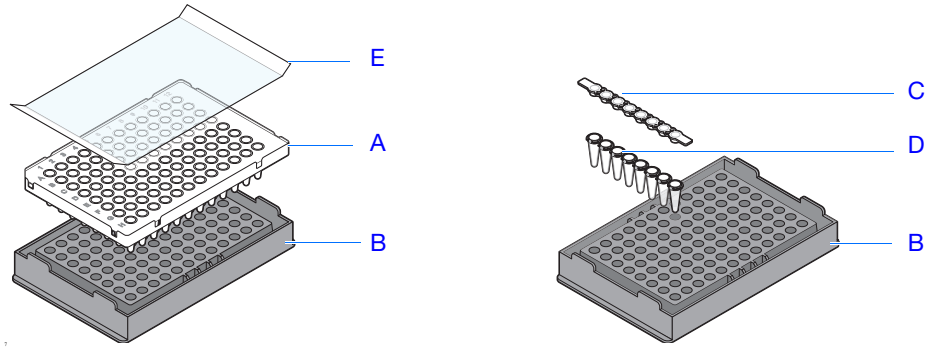
Notes

7500 Fast System

The 7500 Fast system supports the consumables listed below.

IMPORTANT! Use only Fast consumables (reaction plates, tube strips, and tubes) with the 7500 Fast system, even when performing an experiment with standard reagents.

Consumable	Part Number
• MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL • MicroAmp™ Optical Adhesive Film	• 4346906 • 4311971
• MicroAmp™ Fast 8-Tube Strip, 0.1-mL • MicroAmp™ Optical 8-Cap Strip	• 4358293 • 4323032
• MicroAmp® Splash Free Support Base	• N8010531
• MicroAmp™ Adhesive Film Applicator • MicroAmp® Cap Installing Tool (Handle) • MicroAmp™ Multi-Removal Tool	• 4333183 • 4330015 • 4313950



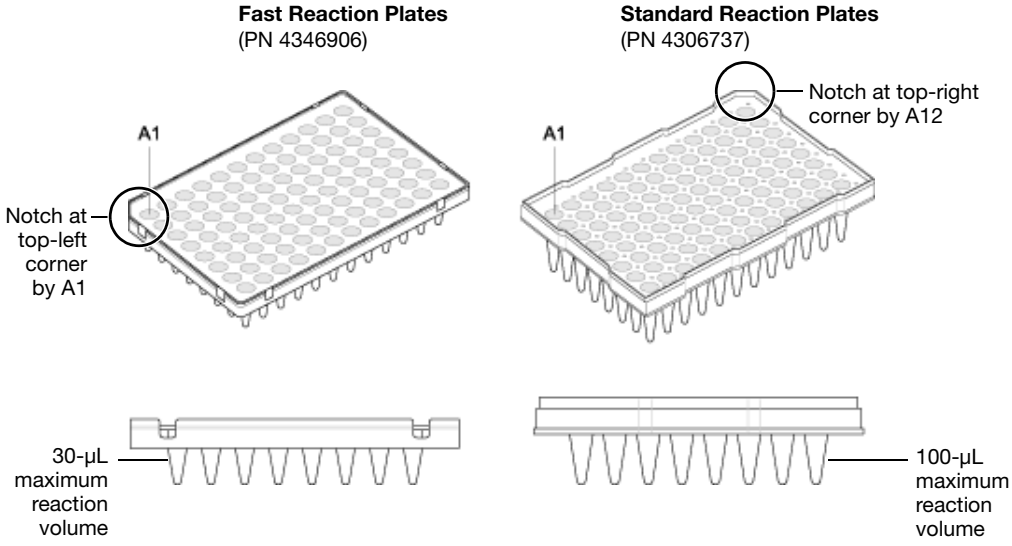
#	Consumable
A	MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1-mL
B	MicroAmp® Splash Free Support Base
C	MicroAmp™ Optical 8-Cap Strip
D	MicroAmp™ Fast 8-Tube Strip
E	MicroAmp™ Optical Adhesive Film

Notes

Standard Versus Fast Reaction Plates and Tubes

Make sure that you use the correct reaction plate for your system:

System	Reaction Plate
7500 system	• MicroAmp® Optical 96-Well Reaction Plate (PN 4306737, also called *standard reaction plates*) • MicroAmp® Optical Tubes without Caps, 0.2-mL (PN N8010933, also called *standard reaction tubes*) • MicroAmp® Reaction Tubes with Caps, 0.2-mL (PN N2070540) • MicroAmp™ Optical 8-Tube Strip, 0.2-mL (PN 4316567, also called *standard reaction tube strips*) **IMPORTANT!** Fast reaction plates and tubes do not fit into the standard sample block correctly and will result in loss of data.
7500 Fast system	• MicroAmp™ Fast Optical 96-Well Reaction Plate, (PN 4346906, also called *Fast reaction plates*) • MicroAmp™ Fast 8-Tube Strip, 0.1-mL (PN 4358293, also called *Fast reaction tube strips*) **IMPORTANT!** Standard reaction plates and tube strips will not function properly and might be crushed when using the Fast sample block.



Notes

About Genotyping Experiments

Endpoint Experiments

Genotyping experiments are endpoint experiments. In endpoint experiments:

- Data are collected at the end of the PCR process.
- Reactions are characterized by the quantity of target sequence accumulated at the end of the PCR.
- The data point is the normalized intensity of the reporter dye, or Rn.

Note: Some endpoint experiments also include pre-PCR data points. If so, the system calculates the delta Rn (ΔRn) value per the following formula:

ΔRn = Rn (post-PCR read) – Rn (pre-PCR read), where Rn = normalized reporter.

Note: In this guide, the term *experiment* refers to the entire process of performing a run using the 7500/7500 Fast system, including setup, run, and analysis.

Real-Time PCR Data for Endpoint Experiments

The 7500 software provides the option of collecting real-time data for both presence/absence and genotyping experiments. In the event that an experiment fails, the real-time data can help you determine the cause of the failure.

About TaqMan® SNP Genotyping Assays

A genotyping assay detects variants of a single nucleic acid sequence, without quantifying the target. The presence of two probes in each reaction allows genotyping of the two possible variants at the single nucleotide polymorphism (SNP) site in a target sequence.

Each TaqMan® SNP Genotyping Assay consists of a single, ready-to-use tube containing:

- Two sequence-specific primers for amplifying the polymorphism of interest
- Two allele-specific TaqMan® MGB probes for detecting the alleles of the specific polymorphism of interest

About TaqMan® MGB Probes

Each allele-specific TaqMan® MGB probe has:

- A reporter dye at its 5′ end
  - VIC® dye is linked to the 5′ end of the Allele 1 probe.
  - FAM™ dye is linked to the 5′ end of the Allele 2 probe.

  The Allele 1 VIC® dye-labeled probe corresponds to the first nucleotide inside the square brackets of the context sequence in the assay information file (AIF) shipped with each order. The Allele 2 FAM™ dye-labeled probe corresponds to the second nucleotide inside the square brackets of the context sequence in the AIF. For the context sequence ATCGATT[G/T]ATCC, the VIC® dye-labeled probe will bind to the G allele, and the FAM™ dye-labeled probe to the T allele.
- A minor groove binder (MGB), which increases the melting temperature (T_m) for a given probe length and allows the design of shorter probes. The use of shorter probes results in greater differences in T_m values between matched and mismatched probes, and more robust genotyping.

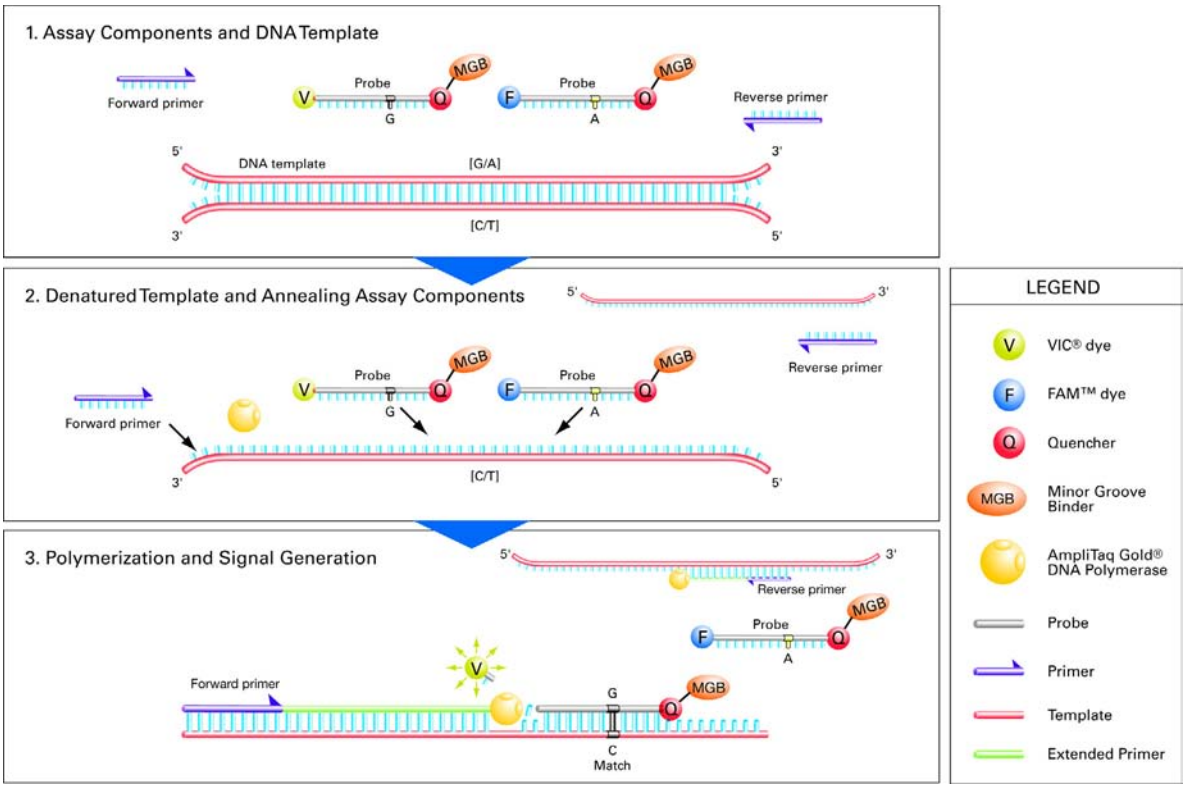
Notes

- A nonfluorescent quencher (NFQ) at its 3′ end, allowing for detection of the reporter dye fluorescence with greater sensitivity than with a fluorescent quencher.

5′ Nuclease Assay

The figure below shows the 5′ nuclease assay. During PCR:

- Each TaqMan® MGB probe anneals specifically to its complementary sequence between the forward and reverse primer sites.
- When the oligonucleotide probe is intact, the proximity of the quencher dye to the reporter dye quenches the reporter signal.
- AmpliTaq Gold® DNA polymerase extends the primers bound to the genomic DNA template.
- AmpliTaq Gold® DNA polymerase (with its 5′ nuclease activity) cleaves probes that are hybridized to the target sequence.
- Cleavage of the probes hybridized to the target sequence separates the quencher dye from the reporter dye, resulting in increased fluorescence by the reporter. The fluorescence generated by PCR amplification indicates which alleles are present in the sample.



Minimizing Non-Specific Fluorescence

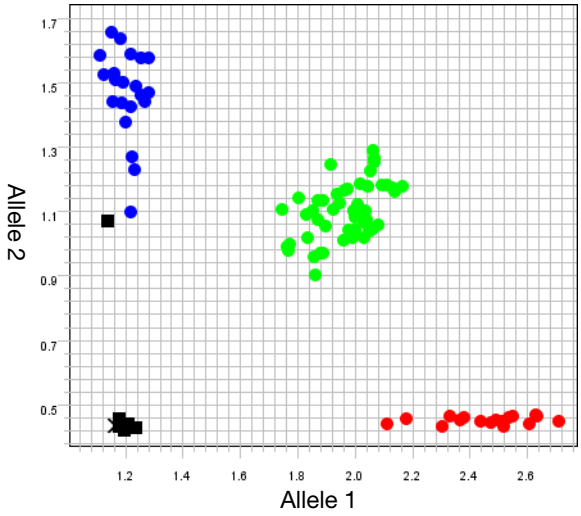
In TaqMan® assays, fluorescence from nonspecifically bound probes is reduced because nucleotide mismatches between a probe and a sequence reduce the chances that the probe will be cleaved. The probe's short length means a one base pair mismatch has larger negative effect on the binding. The mismatched probe does not bind tightly to the allele, allowing the AmpliTaq® Gold DNA polymerase to displace the probe without cleaving the dye.

Notes

Reading and Analyzing the Plates

The 7500 software genotypes the DNA samples from the reaction plate simultaneously. First, the software normalizes the fluorescence of the reporter dyes to the fluorescence of the passive reference dye in each well. Next, the software plots the normalized intensities (Rn) of the reporter dyes in each sample well on an Allelic Discrimination Plot, which contrasts the reporter dye intensities of the allele-specific probes. Finally, the 7500 software algorithmically clusters the sample data, and assigns a genotype call to the samples of each cluster according to its position on the plot.

Note: The 7500 software clustering algorithm does not call genotypes when only one genotype is present in an experiment.



The clustering of data points can vary along the horizontal axis (Allele 1), vertical axis (Allele 2), or diagonal (Allele 1/Allele 2). This variation results from differences in the extent of reporter dye fluorescent intensity after PCR amplification. The table below shows the correlation between fluorescence and sequences in a sample.

A substantial increase in…	Indicates…
VIC® dye-labeled probe fluorescence only	Homozygosity for Allele 1
FAM™ dye-labeled probe fluorescence only	Homozygosity for Allele 2
Both VIC® and FAM™ dye-labeled probes fluorescence	Allele 1-Allele 2 heterozygosity

Notes

Supported Reagents

The 7500/7500 Fast system supports the following reagents for genotyping experiments:

- TaqMan reagents

  Note: If you use TaqMan reagents, the 7500 software automatically calculates reaction volumes in the Reaction Setup screen.

- Other fluorescence-based reagents

  If you use fluorescence-based reagents other than TaqMan® reagents, you must design your experiment using Advanced Setup instead of the Design Wizard (see "Advanced Setup Workflow" on page 90).

IMPORTANT! Fast master mix and Fast protocols do not support genotyping experiments.

Notes

How to Use This Guide

This guide functions as both a tutorial and as a guide for performing your own experiments.

Using This Guide as a Tutorial

By using the example experiment data provided with the 7500 software, you can use this guide as a tutorial for performing a genotyping experiment on a 7500/7500 Fast system. Follow the procedures in Chapters 2 through 5:

Chapter	Procedure
2	Design the experiment using the Design Wizard in the 7500 software.
3	Prepare the experiment, using the reagents and volumes calculated by the Design Wizard in Chapter 2.
4	Run the experiment on a 7500/7500 Fast instrument.
5	Analyze the results of an example experiment.

For more information, see "About the Example Genotyping Experiment" on page 12.

Using This Guide with Your Own Experiments

After completing the tutorial exercises in Chapters 2 through 5, you can use this guide to lead you through your own genotyping experiments. Each procedure in Chapters 2 through 5 includes a set of guidelines that you can use to perform your own experiments.

Additionally, you can use one of the other workflows provided in the 7500 software to perform your experiments. The table below provides a summary of all the workflows available in the 7500 software.

Workflow	Description	See...
Design Wizard	Set up a new experiment with guidance from the 7500 software. The Design Wizard guides you through best practices as you create your own experiment. The Design Wizard is recommended for new users. **Note:** Design options are more limited in the Design Wizard than in Advanced Setup.	Chapter 2
Advanced Setup	Set up a new experiment using advanced options. Advanced Setup allows design flexibility as you create your own experiment. Advanced Setup is recommended for experienced users.	page 90
QuickStart	Run a new experiment with no plate setup information. If desired, you can add design parameters after the run.	page 92
Template	Set up a new experiment using setup information from a template.	page 94
Export/Import	Import experiment designs from ASCII text files that contain experiment setup information.	page 96

Notes

About the Example Genotyping Experiment

To illustrate how to perform genotyping experiments, this guide leads you through the process of designing and analyzing an example experiment. The example experiment represents a typical setup that you can use to quickly familiarize yourself with the 7500/7500 Fast system.

Description

The objective of the example genotyping experiment is to investigate SNP rs8037, where possible genotypes are AA, AG, and GG. In the example, 88 unknown genomic DNA (gDNA) samples were genotyped using TaqMan® Drug Metabolism Genotyping Assay ID C_7571509_1. The reactions were set up so that the PCR primers and probes that target both alleles of SNP rs8037 were present in the same well. The PCR was performed using the TaqMan® Universal PCR Master Mix and run according to the protocol described in the *Performing a TaqMan® Drug Metabolism Genotyping Assay for 96-Well Plates Quick Reference Card*.

Reaction Plate Layout

The 7500 software displays the 96-well reaction plate layout as shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N NTC SNP1	U 14474 SNP1	U 14476 SNP1	U 17102 SNP1	U 17103 SNP1	U 17104 SNP1	U 17106 SNP1	U 17107 SNP1	U 17108 SNP1	U 10859 SNP1	U 17109 SNP1	U 17110 SNP1
B	N NTC SNP1	U 10859 SNP1	U 17111 SNP1	U 17112 SNP1	U 17113 SNP1	U 17114 SNP1	U 17115 SNP1	U 17116 SNP1	U 17117 SNP1	U 17118 SNP1	U 17119 SNP1	U 17120 SNP1
C	N NTC SNP1	U 17121 SNP1	U 17122 SNP1	U 17123 SNP1	U 17124 SNP1	U 17125 SNP1	U 17126 SNP1	U 17127 SNP1	U 17128 SNP1	U 17129 SNP1	U 17130 SNP1	U 17131 SNP1
D	N NTC SNP1	U 17132 SNP1	U 17136 SNP1	U 10859 SNP1	U 17137 SNP1	U 17139 SNP1	U 17140 SNP1	U 17144 SNP1	U 17147 SNP1	U 17149 SNP1	U 17148 SNP1	U 17152 SNP1
E	N NTC SNP1	U 17155 SNP1	U 17188 SNP1	U 17194 SNP1	U 17201 SNP1	U 17202 SNP1	U 17203 SNP1	U 17205 SNP1	U 17207 SNP1	U 17208 SNP1	U 17209 SNP1	U 17211 SNP1
F	N NTC SNP1	U 17212 SNP1	U 17213 SNP1	U 17216 SNP1	U 17217 SNP1	U 17220 SNP1	U 17221 SNP1	U 17222 SNP1	U 17223 SNP1	U 17225 SNP1	U 17226 SNP1	U 17227 SNP1
G	N NTC SNP1	U 17230 SNP1	U 17231 SNP1	U 17232 SNP1	U 17235 SNP1	U 17237 SNP1	U 10859 SNP1	U 17240 SNP1	U 17241 SNP1	U 17242 SNP1	U 17245 SNP1	U 17247 SNP1
H	N NTC SNP1	U 17251 SNP1	U 17252 SNP1	U 10859 SNP1	U 17253 SNP1	U 17254 SNP1	U 17255 SNP1	U 17258 SNP1	U 17259 SNP1	U 17260 SNP1	U 17261 SNP1	U 17263 SNP1

Wells: U **88** Unknown N **8** Negative Control P **0** Positive Control **0** Empty

Notes

About the Example Experiment Data

In this getting started guide you use two files:

- In Chapter 2, you create a genotyping example experiment that contains setup data, then you save the file to your computer.
- In Chapter 5, you view results in a genotyping example experiment file that contains run data. The data file for the example experiment is installed with the 7500 software. You can find the data file for the example experiment on your computer:

  <*drive*>:\Applied Biosystems\<*software name*>\experiments\Genotyping Example.eds

  where:

  – <*drive*> is the computer hard drive on which the 7500 software is installed.

  – <*software name*> is the current version of the 7500 software.

Example Files in the Experiments Folder

The experiments folder of the 7500 software contains several example files that you can reference when analyzing your own data. The following example files install with the 7500 software:

- Comparative Ct Example.eds
- Comparative Ct Study Example.edm
- Comparative Ct Study (Biological Groups).edm
- Genotyping Example.eds
- Presence Absence Example.eds
- Relative Standard Curve Example.eds
- Standard Curve Example.eds

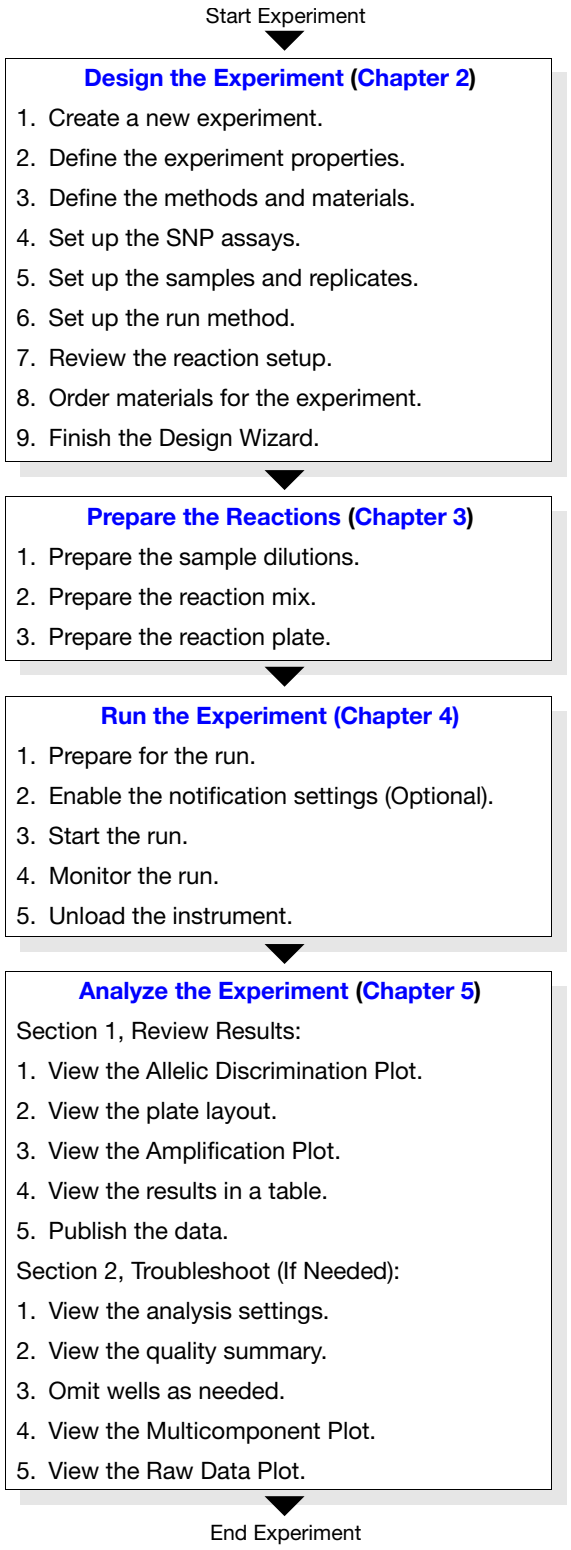
Note: Be sure to use the *Genotyping Example.eds* file when you perform the procedures in this guide.

Notes

Example Experiment Workflow

About the Experiment Workflow

The following figure shows the workflow for the genotyping example experiment.



Notes

2 Design the Experiment

This chapter covers:

- Chapter Overview 16
- Create a New Experiment 17
- Define the Experiment Properties 18
- Define the Methods and Materials 20
- Set Up the SNP Assays 21
- Set Up the Samples and Replicates 23
- Set Up the Run Method 25
- Review the Reaction Setup 27
- Order Materials for the Experiment 29
- Finish the Design Wizard 32

Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

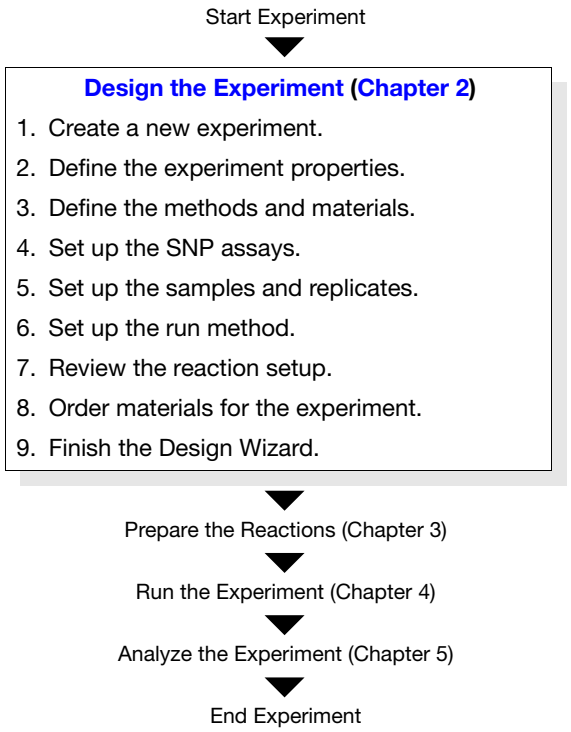
Notes

Chapter Overview

This chapter explains how to use the Design Wizard in the 7500 software to set up the genotyping example experiment. The Design Wizard guides you through Applied Biosystems recommended best practices as you enter design parameters for the example experiment.

Note: When you design your own experiments, you can select alternate workflows (see "Using This Guide with Your Own Experiments" on page 11).

Example Experiment Workflow



Notes

Create a New Experiment

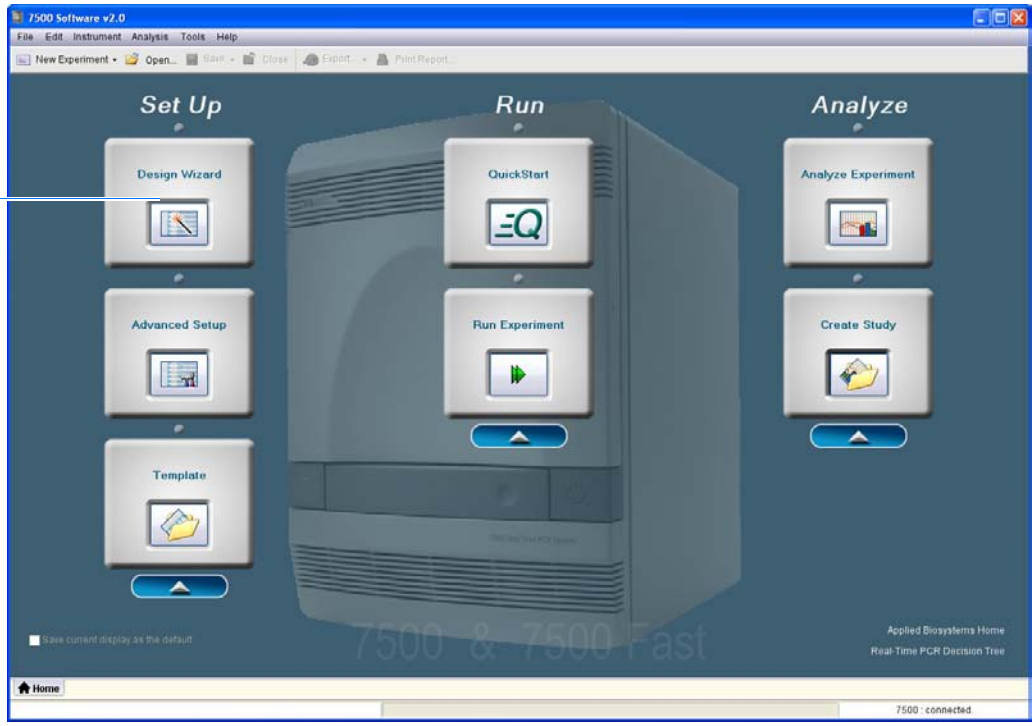
Create a new experiment using the Design Wizard in the 7500 software.

Create an Experiment

1. Double-click (7500 software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***

   where *<software name>* is the current version of the 7500 software.

2. In the Home screen, click **Design Wizard** to open the Design Wizard.



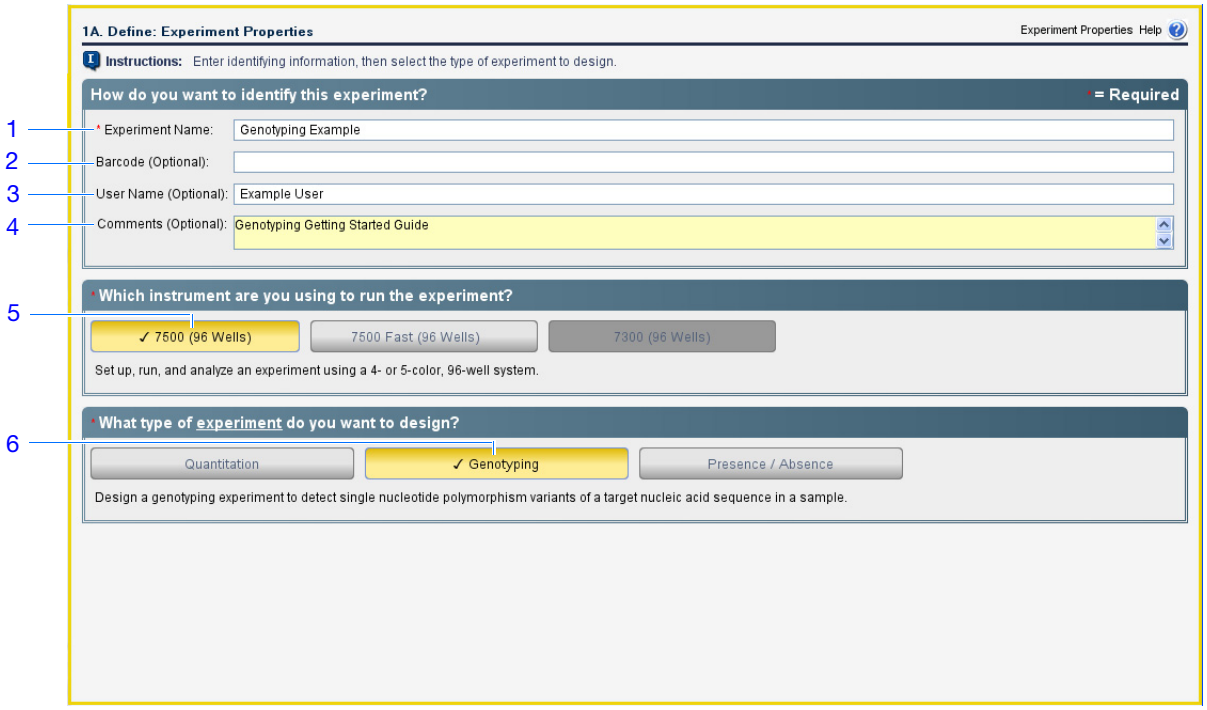
Notes

Define the Experiment Properties

In the Experiment Properties screen, enter identifying information for the experiment, select the instrument type, then select the type of experiment to design.

Complete the Experiment Properties Screen

1. Click the **Experiment Name** field, then enter **Genotyping Example**.
2. Leave the Barcode field empty.
3. Click the **User Name** field, then enter **Example User**.
4. Click the **Comments** field, then enter **Genotyping Getting Started Guide**.
5. Click **7500 (96 Wells)**.
6. Click **Genotyping** for the experiment type.
7. Click **Next**.



Notes

Design Guidelines

When you design your own experiment:

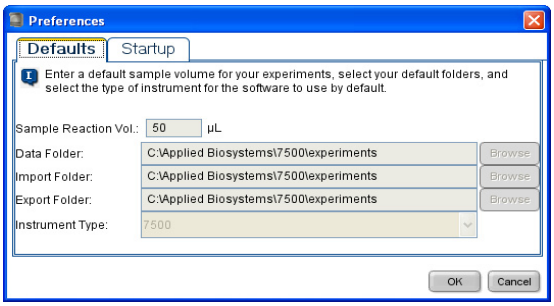
- Enter an experiment name that is descriptive and easy to remember. You can enter up to 100 characters in the Experiment Name field. You cannot use the following characters in the Experiment Name field: / \ > < * ? " | : ;

  Note: The experiment name is used as the default file name.

- (Optional) Enter a barcode of up to 100 characters to identify the reaction plate that you use in the experiment.
- (Optional) Enter a user name to identify the owner of the experiment. You can enter up to 100 characters in the User Name field.
- (Optional) Enter comments to describe the experiment. You can enter up to 1000 characters in the Comments field.
- Select the instrument that you are using to run the experiment:
  - **7500 (96 Wells)**
  - **7500 Fast (96 Wells)**

  Note: You can use 7500 software v2.0 or later to design experiments for the 7500/7500 Fast instrument.

  IMPORTANT! To set the default instrument type, select **Tools** ▸ **Preferences**, then select the **Defaults** tab (default). In the Instrument Type drop-down list, select the appropriate instrument.



For More Information

For more information on:

- Completing the Experiment Properties screen – Open the 7500 Software Help by clicking or pressing **F1**.
- Consumables – See "Supported Consumables" on page 4.
- Quantitation experiments – Refer to the *Real Time PCR System Reagent Guide*.

Notes

Define the Methods and Materials

In the Methods and Materials screen, define the:

- Reagents that you are using to genotype samples
- State (wet or dry) of DNA template that you are genotyping
- Ramp speed best suited for the PCR reactions
- Stages to include in the method

About the Example Design

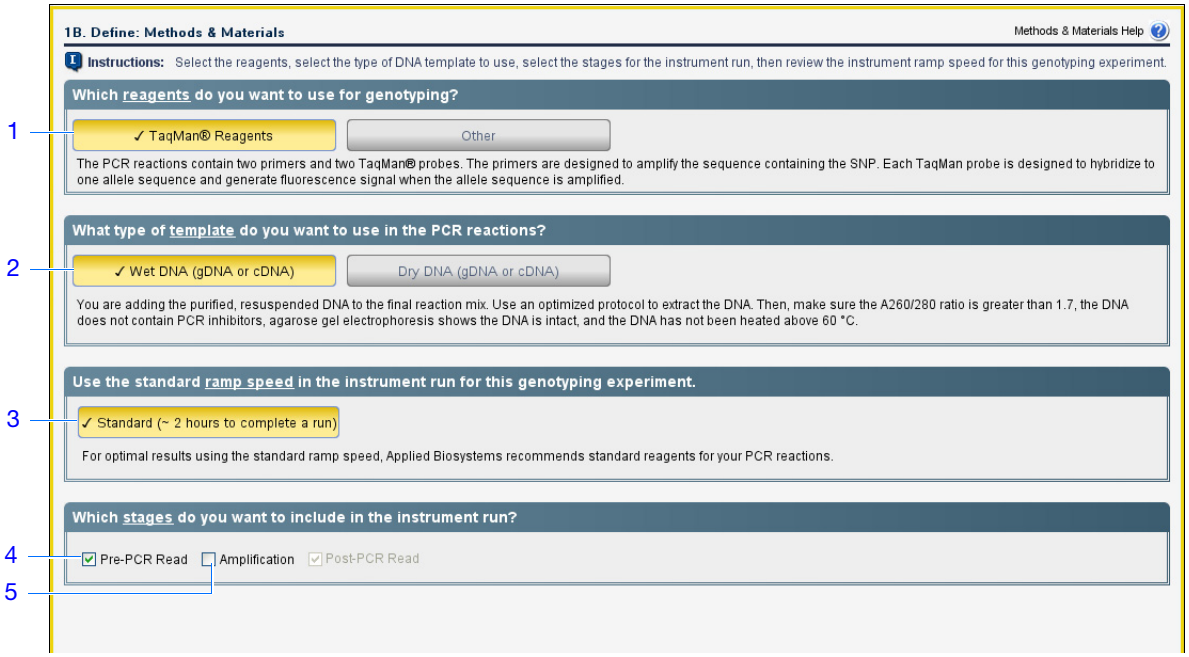
The example experiment uses TaqMan® reagents and wet genomic DNA (gDNA) template in the PCR reactions. Because the reactions do not contain TaqMan® Fast reagents, the 7500/7500 Fast system performs the run using the standard ramp speed. Also, because thermal cycling for this experiment is performed on a dedicated thermal cycler instead of the 7500/7500 Fast system, the run method for the experiment consists of Pre-PCR Read and Post-PCR Read stages only (no Amplification stage).

Complete the Methods and Materials Screen

1. Click **TaqMan® Reagents** for the reagents.
2. Click **Wet DNA (gDNA or cDNA)** for the template type.
3. Click **Standard (~2 hours to complete a run)** for the ramp speed.
4. Select **Pre-PCR Read** to add the stage to the run method.
5. Deselect **Amplification** to exclude the PCR stage from the run method.

   Note: The Post-PCR read is required.

6. Click **Next**.



Notes

Design Guidelines

When you design your own experiment:

- Select **Other** if you are not using TaqMan® reagents to amplify and detect the target sequences in your experiment.

  Note: The Reaction Setup screen is not available if you select Other.

- If you are using a template other than genomic DNA (gDNA) or cDNA, select the option (**Wet DNA** or **Dry DNA**) that describes the state of your samples.
- Select the **Standard** ramp speed for the instrument run.

  IMPORTANT! Applied Biosystems Fast reagents do not support genotyping experiments.

- If you will perform the PCR amplification on a thermal cycler other than a 7500/7500 Fast system, deselect the **Amplification** option.

  Note: The pre-PCR read is optional but recommended. The 7500 software uses the pre-PCR read to normalize the post-PCR data.

For More Information

For more information on:

- The Materials & Methods screen – Open the 7500 Software Help by clicking or pressing **F1**.
- Advanced Setup – See "Advanced Setup Workflow" on page 90.

Set Up the SNP Assays

In the Set Up SNP Assays screen, enter the number of SNP assays in the experiment, then define the properties for each SNP assay.

About the Example Design

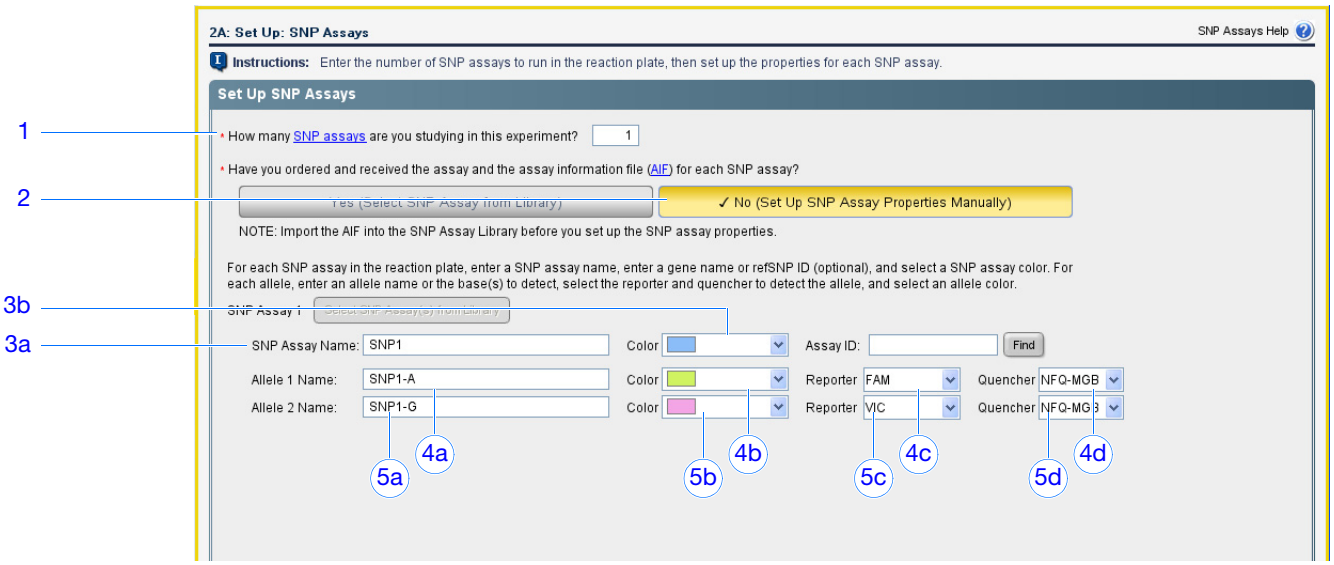
In the example experiment, the target is the keratin 23 gene (histone deacetylase inducible).

Complete the SNP Assays Screen

1. For the number of SNP assays being studied, enter **1**.
2. Click **No (Set Up SNP Assay Properties Manually)**.
3. Set up the SNP assay:
   a. Click the **SNP Assay Name** field, then enter **SNP1**.
   b. In the Color field, leave the default.

Notes

4. Set up the Allele 1 target:
   a. Click the **Allele 1 Name** field, then enter **SNP1-A**.
   b. In the Color field, leave the default.
   c. In the Reporter drop-down list, select **FAM**.
   d. In the Quencher drop-down list, select **NFQ-MGB**.
5. Set up the Allele 2 target:
   a. Click the **Allele 2 Name** field, then enter **SNP1-G**.
   b. In the Color field, leave the default.
   c. In the Reporter drop-down list, select **VIC**.
   d. In the Quencher drop-down list, select **NFQ-MGB**.
6. Select **Next**.



Design Guidelines

When you design your own experiments:

- Applied Biosystems recommends that you evaluate no more than 6 SNPs on a reaction plate.

  Note: The Design Wizard allows no more than two SNPs per plate. Advanced Setup and Quickstart do not restrict the number of SNPs that you can evaluate.

- Identify each SNP assay with a unique name and color.
- If you set up your SNP assays manually, make sure that the reporter dyes that you assign to each allele are correct.

For More Information

For more information on the Targets SNP Assays screen, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Set Up the Samples and Replicates

In the Samples and Replicates screen, enter the number of samples in the experiment, enter the sample names, then enter the number of negative and positive controls.

About the Example Design

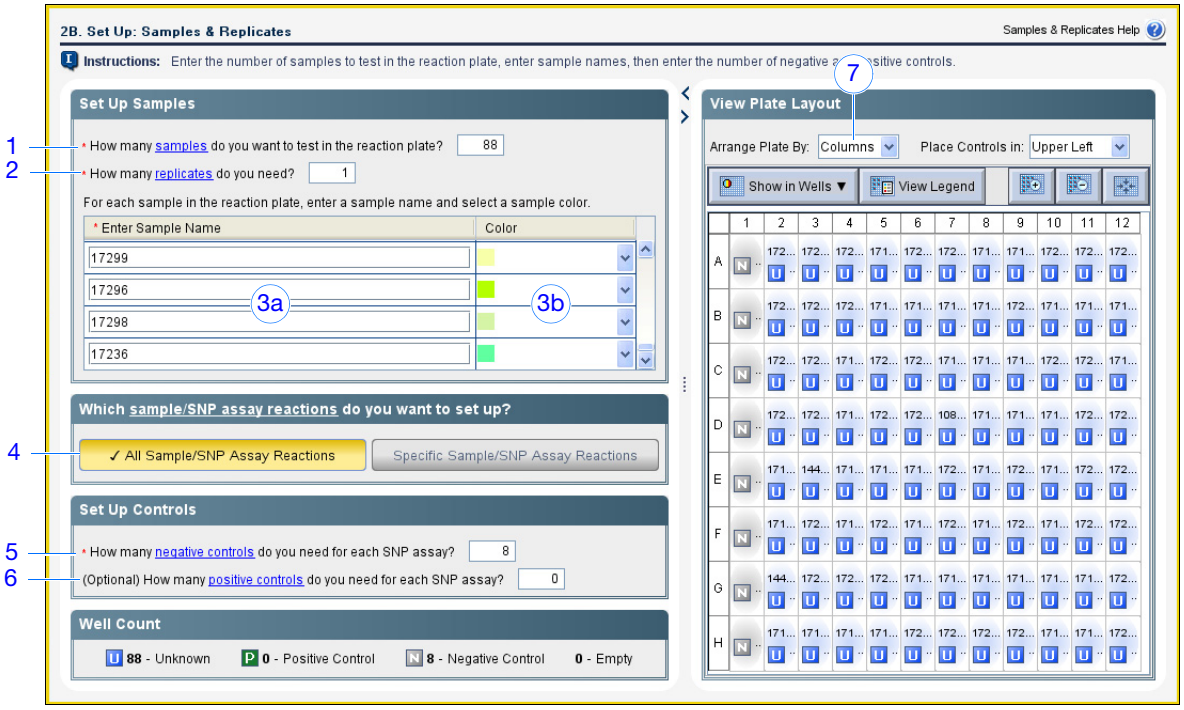
The example experiment evaluates:

- 88 unknown samples
- 8 negative controls

Complete the Samples and Replicates Screen

1. For the number of samples, enter **88**.
2. For the number of replicates, enter **1**.
3. Set up each sample:
   a. Click the **Enter Sample Name** column, then enter the appropriate name (number) for the sample as shown in "Reaction Plate Layout" on page 12.
   b. In the Color field, leave the default.
4. Click **All Sample/SNP Assay Reactions**.
5. For the number of negative controls (wells that contain no template), enter **8**.
6. For the number of positive controls (wells that contain samples of known genotypes), enter **0**.
7. In the Arrange Plate By drop-down list, select **Columns**.
8. Select **Next**.

Notes



Design Guidelines

When you design your own experiment:

- Use between 1 and 96 samples. Give a unique name and color for each sample.
- Applied Biosystems recommends using at least one negative control for each SNP assay.
- Limit the number of total reactions in each experiment to 96 or less. If the number of total reactions required is greater than 96, reduce the number of SNP assays, samples, replicates, or positive and negative controls; or divide the reactions between two or more reaction plates.

For More Information

For more information on the Samples and Replicates screen, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Set Up the Run Method

In the Run Method screen, review the reaction volume and the thermal profile for the default run method. If needed, you can edit the default run method or replace it with one from the Run Method library.

About the Example Design

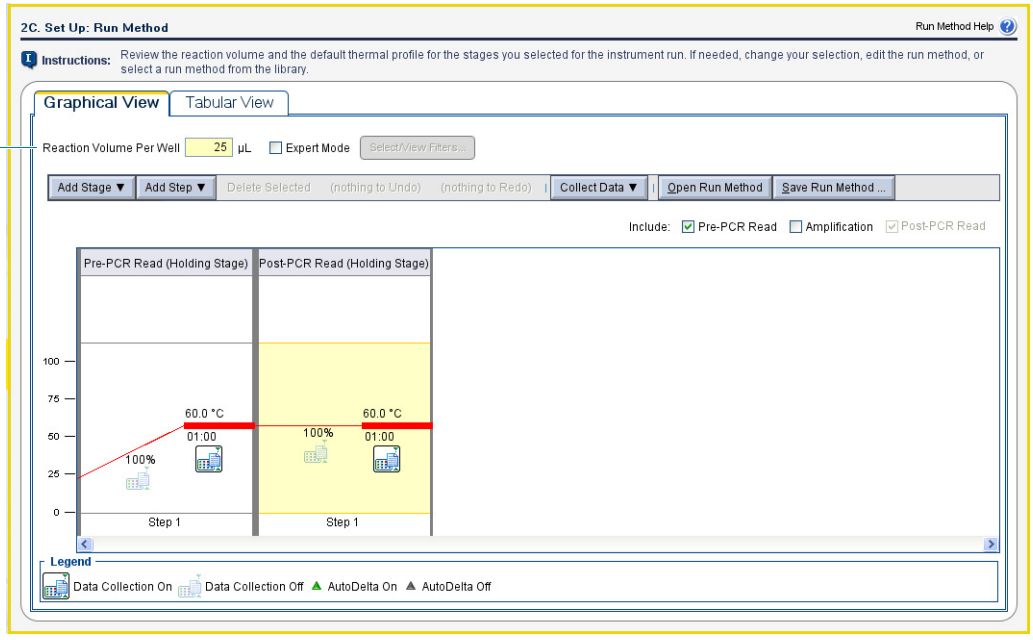
The example experiment runs 25-µL reactions using the TaqMan® Drug Metabolism Genotyping Assay method shown below. For this experiment, thermal cycling is performed on a dedicated thermal cycler instead of the 7500/7500 Fast system.

Stage/Step	Pre-PCR Read	Thermal Cycling (Performed on a dedicated thermal cycler)			Post-PCR Read
	Holding Stage	Holding Stage	Cycling (50 cycles)		Holding Stage
			Denature	Anneal/Extend	
Temperature	60 °C	95 °C	92 °C	60 °C	60 °C
Time (mm:ss)	00:30	10:00	00:15	01:30	00:30
Data Collection	Yes	No	No	Yes‡	Yes

‡ If you choose to use the 7500/7500 Fast system to perform thermal cycling, you can activate data collection for the Anneal/Extend step so that the instrument collects real-time data during the PCR. Although the real-time data are not necessary for genotyping, the data can be useful when troubleshooting a failed PCR.

Review the Run Method Screen

1. In the Graphical View tab, click the **Reaction Volume Per Well** field, then enter **25** µL.
2. Click **Next**.



Notes

Design Guidelines

When you design your own experiment:

- Enter a reaction volume/well. Applied Biosystems recommends a reaction volume of 25 µL for genotyping experiments. The 7500 system supports reaction volumes from 20 to 100 µL. The 7500 Fast system supports reaction volumes from 10 to 30 µL.
- Review the default run method. If your experiment requires different settings, edit the default method as needed.
- Click **Open Run Method** to view a library of run methods. The library may contain the run method for your experiment.
- Consider including amplification in the run method. Real-time data can be useful when troubleshooting a genotyping experiment.

For More Information

For more information on:

- The Run Method Library or on completing the Run Method screen – Open the 7500 Software Help by clicking or pressing **F1**.
- Using Advanced Setup – See "Advanced Setup Workflow" on page 90.

Notes

Review the Reaction Setup

In the Reaction Setup screen, enter the reaction volume and the number of excess reactions to prepare. Review the concentration settings for the PCR master mix, assay mix, diluted sample target, and sample stock(s), and make changes as necessary.

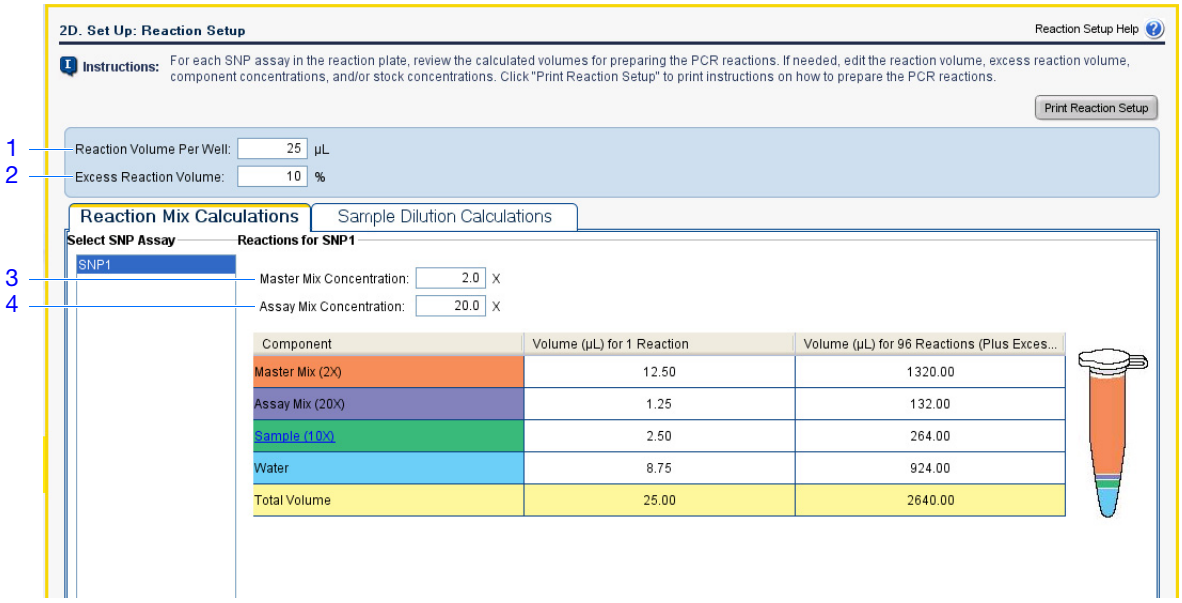
About the Example Design

The example experiment requires sufficient reaction mix for 96 reactions and a 10% excess volume for pipetting error. Each 25-µL reaction consists of the following:

Component	µL/Well
2× TaqMan® Universal PCR Master Mix, No AmpErase® UNG	12.50
20× TaqMan® Drug Metabolism Genotyping Assay	1.25
10× Sample	2.50
Water, DNAse-free	8.75
Total volume	25.00

Complete the Reaction Setup Screen

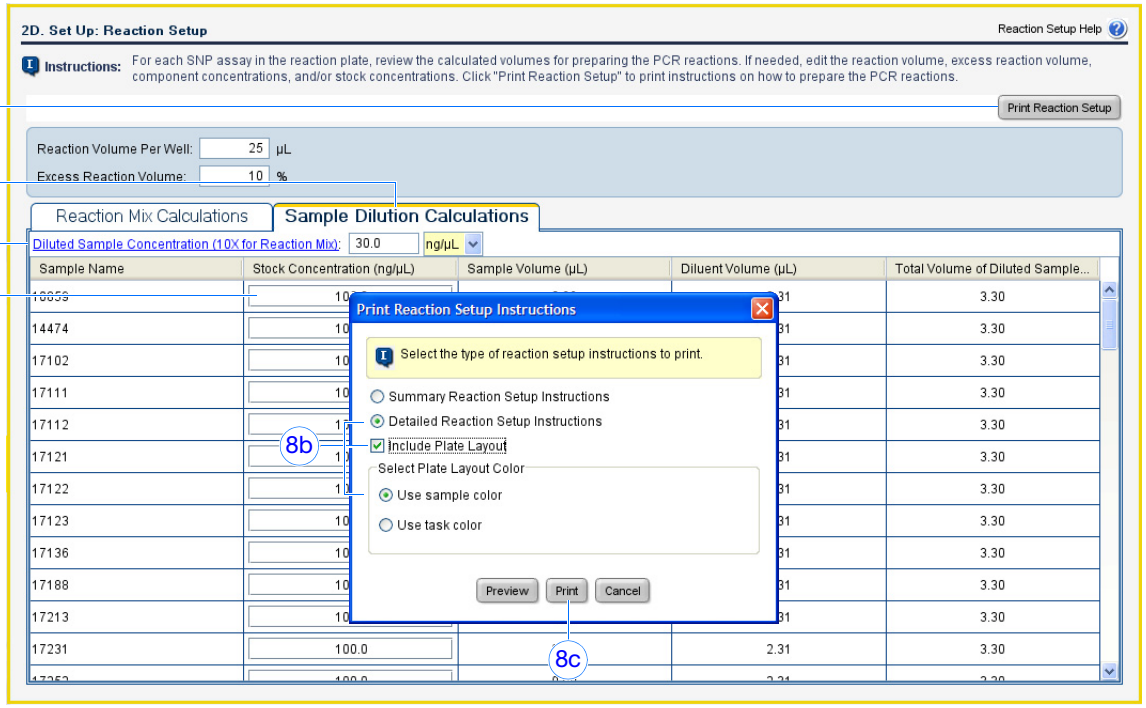
1. Click the **Reaction Volume Per Well** field, then enter **25** µL.
2. Click the **Excess Reaction Volume** field, enter **10**%.
3. Verify that the master mix concentration is **2.0**×.
4. Verify that the assay mix concentration is **20.0**×.



5. Select the **Sample Dilution Calculations** tab.
6. Click the **Diluted Sample Concentration (10× for Reaction Mix)** field, then enter **30** ng/µL.

Notes

7. In the table, verify that the stock concentration of all samples is **100 ng/µL**.
8. Print a report of the plate layout for reference:
   a. Click **Print Reaction Setup**.
   b. In the Print Reaction Setup Instructions dialog box, select:
      - **Detailed Reaction Setup Instructions**
      - **Include Plate Layout**
      - **Use sample color**
   c. Click **Print** to send the reaction setup instructions to your printer.
9. Click **Next**.



Design Guidelines

When you design your own experiment:

- Enter a reaction volume/well. Applied Biosystems recommends a reaction volume of 25 µL for genotyping experiments. The 7500 system supports reaction volumes from 20 to 100 µL. The 7500 Fast system supports reaction volumes from 10 to 30 µL.
- Enter an excess reaction volume of at least 10% to allow for pipetting inaccuracies and other experimental error.
- If you use dry DNA template, the components and volumes are recalculated.

For More Information

For more information on the Reaction Setup screen, open the 7500 Software Help by clicking ? or pressing **F1**.

Notes

Order Materials for the Experiment

In the Materials List screen, review the list of materials recommended to prepare the reaction plate. (Optional) Print the materials list, create a shopping list, then order the recommended materials from the Applied Biosystems Store.

Note: To access the Applied Biosystems Store, you need to have an Internet connection. Product availability and pricing may vary according to your region or country. Online ordering through the Applied Biosystems Store is not available in all countries. Contact your local Applied Biosystems representative for help.

Note: The 7500 software recommends the materials to order based on your experiment design. It is assumed that you will design your experiment, order your materials, then prepare (Chapter 3) and run (Chapter 4) the reaction plate when your materials arrive.

Complete the Ordering Materials Screen

1. In the Enter Gene Name or RS Number field, enter **rs8037**, then click **Find Assay** to get the assay from the Applied Biosystems website
2. In the Find Assay Results dialog box, select the row for Assay ID **C__7571509_1**.
3. Click **Apply Assay Selection**.
4. In the Display drop-down list, select **All Items**, then review the recommended materials. Use the scroll bar to see all items.

   Note: For more information on a specific item, click the part number link. You are connected to the product information page in the Applied Biosystems Store. To access the Applied Biosystems Store, you need to have an Internet connection.

5. (Optional) Click **Print Materials List** to print the materials list.
6. (Optional) Create a shopping list:
   a. Select the check box next to each of the following items:
      - C__7571509_1_ (dbSNP ID rs8037)
      - MicroAmp® Optical 96-Well Reaction Plate
      - MicroAmp™ Optical Adhesive Film
      - MicroAmp® Splash Free Support Base
      - TaqMan® Universal PCR Master Mix, No AmpErase® UNG

      Note: The experiment can be run with other Applied Biosystems genotyping master mixes, such as the TaqMan® Genotyping Master Mix.

   b. Click **Add Selected Items to Shopping List**.

Notes

7. (Optional) Create a shopping basket in the Applied Biosystems Store:

   Note: To access the Applied Biosystems Store, you need to have an Internet connection. Product availability and pricing may vary according to your region or country. Online ordering through the Applied Biosystems Store is not available in all countries. Contact your local Applied Biosystems representative for help.

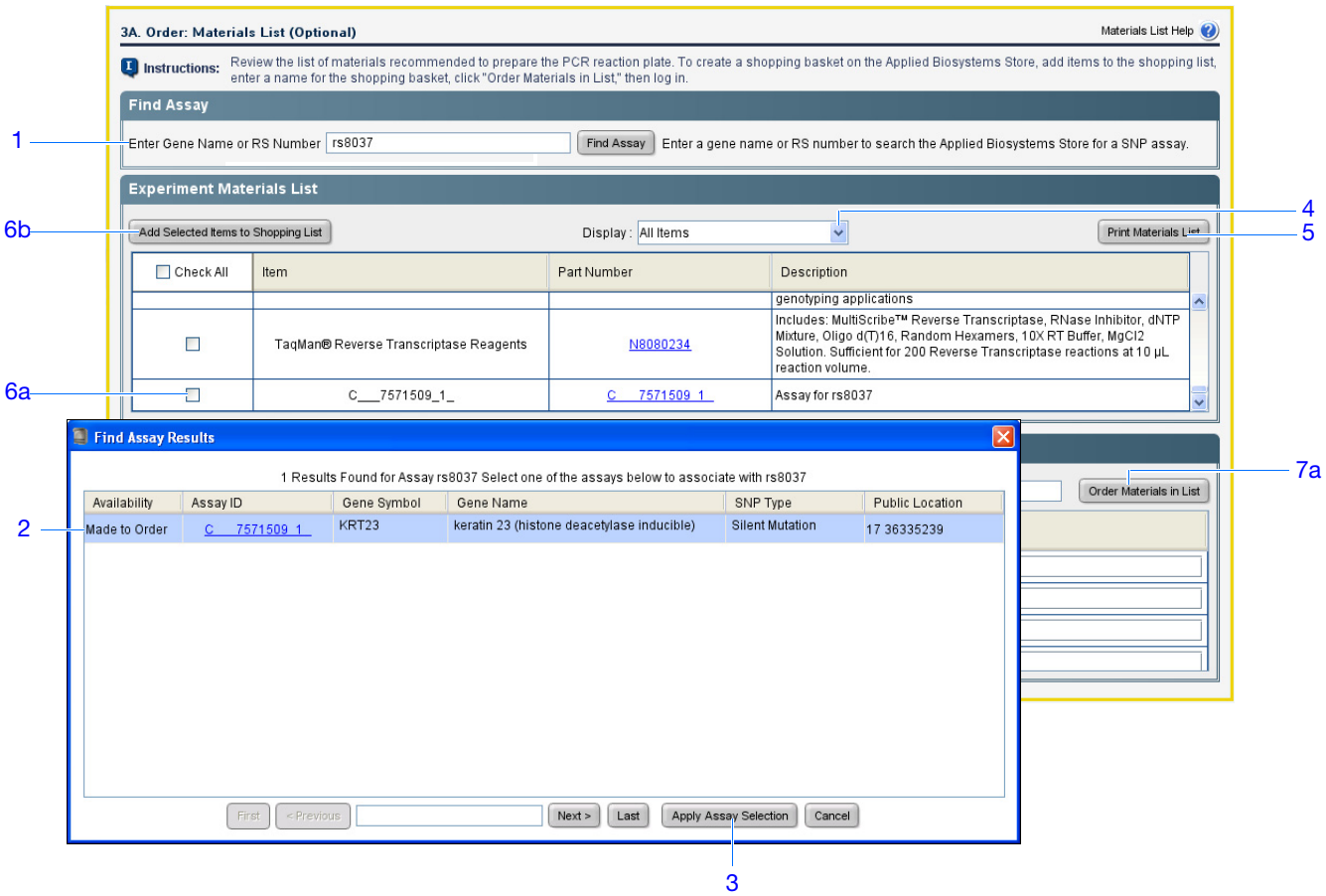
   a. Check that the Experiment Shopping List contains the desired materials and that the quantities are correct, then click **Order Materials in List**.

   b. In the Order Materials - Log In dialog box, enter your user name and password for the Applied Biosystems Store, then click **Log In and Submit**.

      Note: If you do not have an account with the Applied Biosystems Store, click **Register Now** to create an account.

   c. When you are connected to the Applied Biosystems Store, proceed as instructed to complete the order.

8. Go to "Finish the Design Wizard" on page 32.



Notes

Design Guidelines

When you design your own experiment:

- Select all the materials that you require for your experiment, then add them to your shopping list.

  IMPORTANT! The 7500 Fast system runs only Fast consumables (reaction plates, tube strips, and tubes). When performing a genotyping experiment, run the reactions prepared with TaqMan® Universal PCR Master Mix on a Fast consumable using standard PCR conditions.

- To access and use the Applied Biosystems Store:
  - Verify that your computer has an Internet connection.
  - Use the following Applied Biosystems-recommended browsers and versions of Adobe® Acrobat® Reader:

Desktop Operating System	Netscape® Navigator	Microsoft® Internet Explorer	Macintosh® Safari	Adobe® Acrobat® Reader
Windows® 2000/NT/XP/Vista	v6.x or later	v6.x or later	Not applicable	v4.0 or later
Macintosh® OS 9+ or later	Not supported	Not supported	v2.0.4 or later	v4.0 or later

Note: Make sure that cookies and JavaScript are turned on.

For More Information

For more information on the Materials List screen, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Finish the Design Wizard

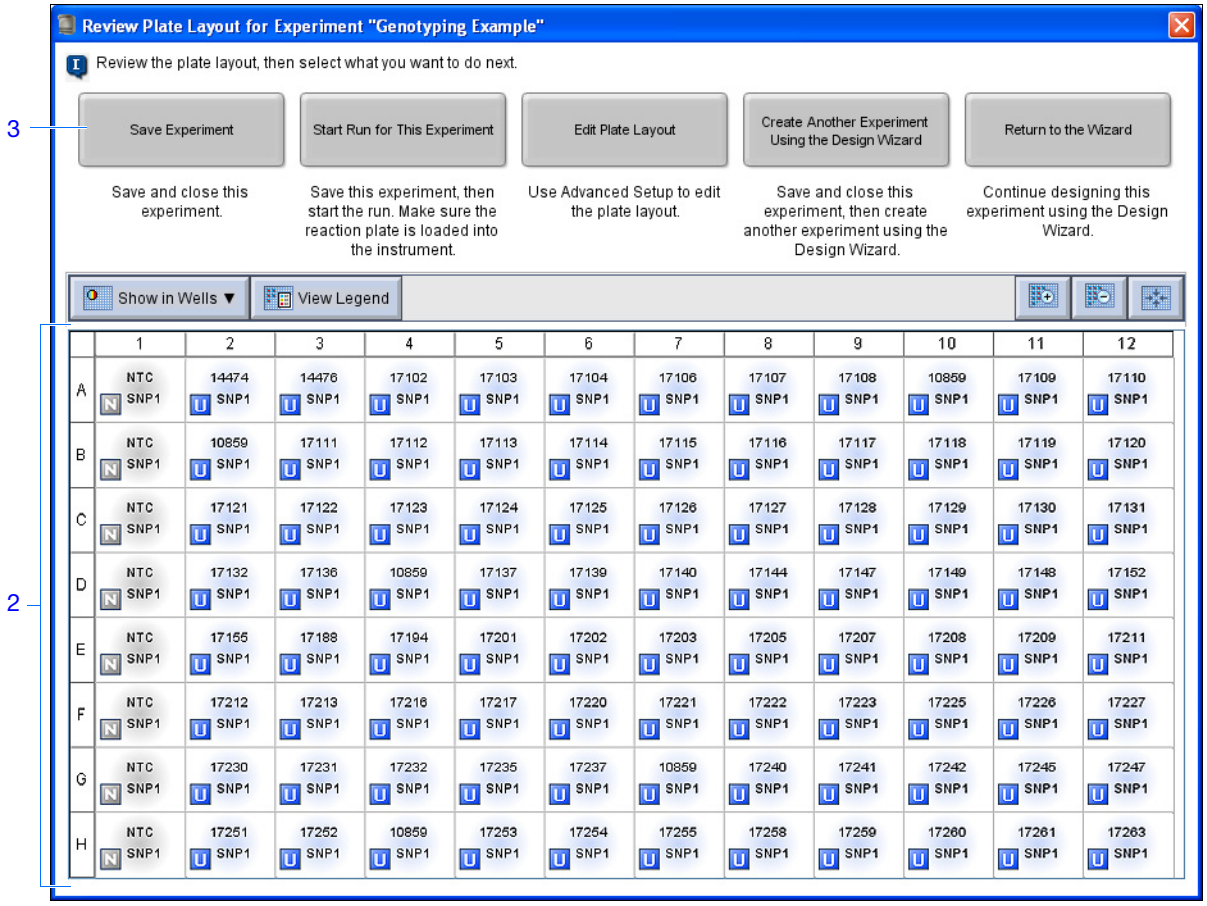
In the Review Plate Layout for Experiment dialog box, review the plate layout, select an option to finish the Design Wizard setup, then start the run.

Finish the Design Wizard

1. At the bottom of the 7500 software screen, click **Finish Designing Experiment**.
2. Review the plate layout. If the plate layout is incorrect, select **Return to the Wizard** and check your entered values.
3. Click **Save Experiment**.
4. In the Save Experiment dialog box, enter **Genotyping Example Setup.eds** in the File name drop-down list, then click **Save**. The example experiment is saved and closed, and you are returned to the Home screen.

IMPORTANT! Do not save the experiment using the default file name. Doing so will overwrite the example experiment already present in the experiments folder.

Note: By default, the example experiment is saved to the *<drive>*:\Applied Biosystems*<software name>*\experiments folder.



Notes

Design Guidelines

When you design your own experiment:

- In the Review Plate Layout for Experiment window, select the appropriate exit option:

Click	To...
Save Experiment	Save and close the experiment without making any further changes or starting the run.
Start Run for This Experiment	Save the experiment and start the run. Make sure the reaction plate is loaded in the instrument.
Edit Plate Layout	Use Advanced Setup to edit the plate layout.
Create Another Experiment Using the Design Wizard	Save and close the current experiment, then create another experiment using the Design Wizard.
Return to the Wizard	Return to the experiment to make changes using the Design Wizard.

- By default, experiments are saved to:

  <*drive*>:\Applied Biosystems\<*software name*>\experiments

  To change the:

  – Save location for a specific experiment – Navigate to the desired location using the Save Experiment dialog box.

  – Default save location – Select **Tools** ▸ **Preferences**, then select the **Default** tab. In the Data Folder field, browse to then select the desired location.

For More Information

For more information on using Advanced Setup, see "Advanced Setup Workflow" on page 90.

Notes

Notes

3 Prepare the Reactions

This chapter covers:

- Chapter Overview 36
- Prepare the Sample Dilutions 37
- Prepare the Reaction Mix 38
- Prepare the Reaction Plate 40

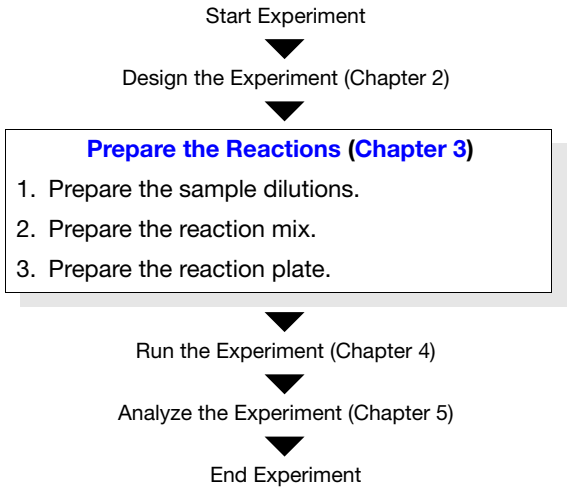
Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Chapter Overview

This chapter explains how to prepare the PCR reactions for the genotyping example experiment.

Example Experiment Workflow



For More Information

For more information on preparing TaqMan® SNP Genotyping Assays, see the:

- *Custom TaqMan® SNP Genotyping Assays Protocol*
- *Custom TaqMan® Genomic Assays Protocol*
- *TaqMan® SNP Genotyping Assays Protocol*
- *TaqMan® Drug Metabolism Genotyping Assays Protocol*
- *Performing a Custom TaqMan® SNP Genotyping Assay for 96-Well Plates Quick Reference Card*
- *Performing a TaqMan® Drug Metabolism Genotyping Assay for 96-Well Plates Quick Reference Card*
- *Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol*
- *Allelic Discrimination Pre-Developed TaqMan® Assay Reagents Quick Reference Card*

Note: The procedures in this chapter focus on the use of wet DNA samples. If you are using dried DNA, consult the chemistry protocol accompanying your PCR kit for details on reconstituting and plating your samples for use.

Notes

Prepare the Sample Dilutions

Dilute the sample stock to working concentrations using the volumes calculated by the 7500 software (see "Review the Reaction Setup" on page 27).

About the Example Experiment

For the genotyping example experiment:

- Sample dilutions are necessary because the sample volume is limited to 10% of the total reaction volume in the 7500 software. Because the total reaction volume is 25 µL/reaction, the sample volume must be 2.5 µL/reaction.
- The sample concentration (stock) is 100 ng/µL. After diluting the sample according to the Sample Dilutions Calculations table, the sample has a concentration of 30 ng/µL. Adding 2.5 µL to the final reaction mix volume of 25 µL, yields a 1× concentration in the final reaction.
- The volumes calculated in the software are:

Sample Name	Sample Stock Concentration (ng/µL)	Sample Volume (µL)	Diluent Volume (µL)	Total Volume of Diluted Sample (µL)
17217	100.0	1.0	2.33	3.33
17240	100.0	1.0	2.33	3.33
…				
17144	100.0	1.0	2.33	3.30

Required Materials

- DNAse-free water (to dilute the sample)
- Microcentrifuge tubes
- Pipettors and pipette tips
- Sample stock
- Vortexer
- Centrifuge

Prepare the Samples

1. Label a separate microcentrifuge tube for each sample: **17217**, **17240**,… through **17144**.
2. Add 2.33 µL DNAse-free water to each empty tube.
3. Add 1.00 µL of the appropriate sample stock to each tube.
4. Vortex each diluted sample for 3 to 5 sec, then briefly centrifuge the tube(s).

Preparation Guidelines

When you prepare the samples for your own experiment:

- Use DNAse-free water to dilute the samples.
- Use the same quantity of DNA per well for each experiment.
- Do not heat the DNA samples.

Notes

For More Information

For more information on preparing TaqMan® SNP Genotyping Assays, refer to the protocol appropriate for the reagents that you are using in the PCR reactions (see "For More Information" on page 36).

Prepare the Reaction Mix

Prepare the reaction mix for the experiment using the volumes calculated by the 7500 software. The 7500 software determines which reaction mix components to use based on the selections made in the Methods and Materials screen (see "Review the Reaction Setup" on page 27). For genotyping experiments, the reaction mix contains all components *except* sample, buffer, or positive control.

Required Materials

- TaqMan® Universal PCR Master Mix, No AmpErase® UNG

  Note: The experiment can be run with other Applied Biosystems genotyping master mixes, such as the TaqMan® Genotyping Master Mix.

- TaqMan® Drug Metabolism Genotyping Assay (20×)
- DNAse-free water
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Centrifuge

Prepare the Reaction Mix

CAUTION CHEMICAL HAZARD. TaqMan® 2× Universal PCR Master Mix, No AmpErase® UNG may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. For the SNP assay, add the required volumes of each component to an appropriately sized tube:

Component	Reaction Volume	
	Per Well (µL)	96 Rxns Including 10% Excess (µL)
TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2×)	12.50	1320.0
SNP Assay Mix (20×)	1.25	132.0
Water, DNase-free	8.75	924.0
Total Reaction Mix Volume	**13.75**	**2376.0**

2. Gently pipette the reaction mix up and down, then cap the tube.

Notes

3. Centrifuge the tube briefly.

Preparation Guidelines

When you prepare the reaction mix for your own experiment, make sure you:

- Prepare the reactions for each SNP separately.
- Include excess volume in your calculations to provide excess volume for the loss that occurs during reagent transfers.
- Include all required components.
- Prepare the reagents according to the manufacturer's instructions.
- Keep the assay mix protected from light in the freezer until you are ready to use it. Excessive exposure to light may affect the fluorescent probes.

Prior to use:

- Mix the master mix thoroughly by swirling the bottle.
- Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
- Thaw any frozen samples by placing them on ice. When they are thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.

For More Information

For more information on how to prepare the reaction mix, refer to the protocol appropriate for the reagents you are using in the PCR reactions (see "For More Information" on page 36).

Notes

Prepare the Reaction Plate

Load the reaction plate with the reaction mix from page 38 and the dilute samples from page 37. The reactions are added to wells according to the plate layout generated in the 7500 software (see "Set Up the Samples and Replicates" on page 23).

Required Materials

- Centrifuge
- MicroAmp® Optical 96-Well Reaction Plate
- MicroAmp™ Optical Adhesive Film
- Pipettors and pipette tips

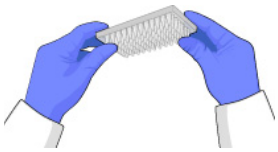
Prepare the Reaction Plate: Wet gDNA

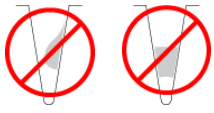
1. Into each well of the reaction plate, pipette one control or sample aliquot of the volume appropriate for the reaction plate type:
   a. For each unknown reaction, add 2.5 µL of the diluted sample to the appropriate well (see "Reaction Plate Layout" on page 12).
   b. For each negative control reaction, add 2.5 µL of DNAse-free water to the appropriate wells (see "Reaction Plate Layout" on page 12).
   c. For each positive control reaction, add 2.5 µL of the positive control to the appropriate wells. The example experiment does not use positive controls.

   IMPORTANT! Make sure the genotype for the positive control template that you add matches the genotype assigned to the well.

2. Transfer 22.5 µL of reaction mix to the appropriate wells.
3. Seal the reaction plate with optical adhesive film.
4. Vortex the reaction plate for 3 to 5 sec.
5. Centrifuge the reaction plate briefly.

IMPORTANT! Do not allow the bottom of the reaction plate to become dirty. Fluids and other contaminants that adhere to the bottom of the reaction plate can contaminate the sample block and cause an abnormally high background signal.



Correct	Incorrect
 Liquid is at the bottom of the well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

Notes

Preparation Guidelines

When you prepare the reaction plate for your own experiment:

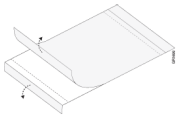
- Make sure that you use the appropriate consumables.
- Make sure that the reaction locations match the plate layout in the 7500 software.
- If you are running less than 40 reactions, use tube strips rather than a reaction plate.
- Load 3 to 20 ng of purified genomic DNA per reaction.
- All wells belonging to the same genotyping assay must contain the same quantity of sample or control.
- Multiple assays can be run on one reaction plate, but must be analyzed separately.
- If you use optical adhesive film, seal each reaction plate as follows:

a. Place the reaction plate onto the center of the 96-well base.

b. Load the reaction plate as desired.

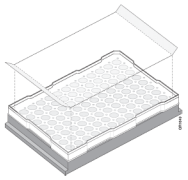
c. Remove a single optical adhesive film (film) from the box. Fold back one of the end-tabs. Hold the film with its backing side up.

d. In one continuous movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.

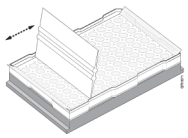


IMPORTANT! Improper peeling of the optical adhesive film may result in haziness, but does not affect results. Haziness disappears when the film comes into contact with the heated cover in the instrument.

e. While holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure the film completely covers all wells of the reaction plate.

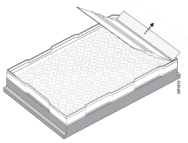


f. While applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.



g. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat for the other end-tab.

h. Repeat step f to ensure a tight, evaporation-free seal. While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film.



Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight seal.

i. Inspect the reaction plate to be sure all wells are sealed. You should see an imprint of all wells on the surface of the film.

Notes

For More Information

For more information on how to prepare the reaction plate, refer to the protocol appropriate for the reagents that you are using in the PCR reactions (see "For More Information" on page 36).

Notes

4 Run the Experiment

This chapter covers:

- Chapter Overview 44
- Prepare for the Run 45
- Enable the Notification Settings (Optional) 47
- Start the Run 49
- Monitor the Run 50
- Unload the Instrument 52

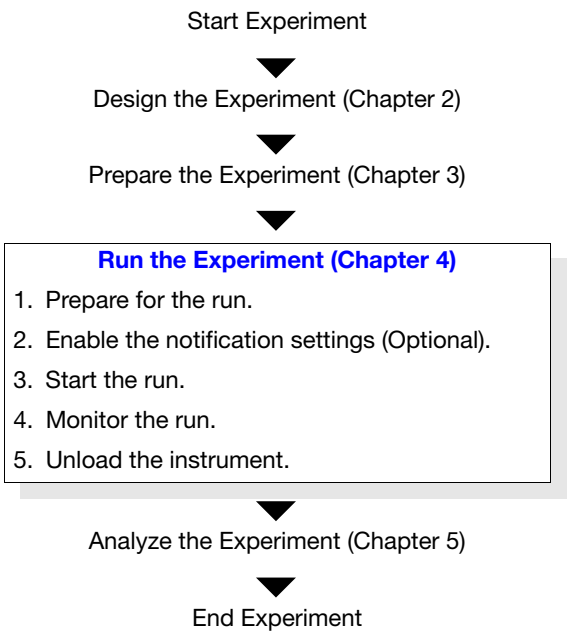
Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Chapter Overview

This chapter explains how to perform a run on the Applied Biosystems 7500/7500 Fast Real-Time PCR System.

Example Experiment Workflow



Notes

Prepare for the Run

Prepare for the run by opening the example experiment file you created in Chapter 2, then loading the sealed reaction plate into the 7500/7500 Fast instrument.

Open the Example Experiment

1. Double-click (7500 software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

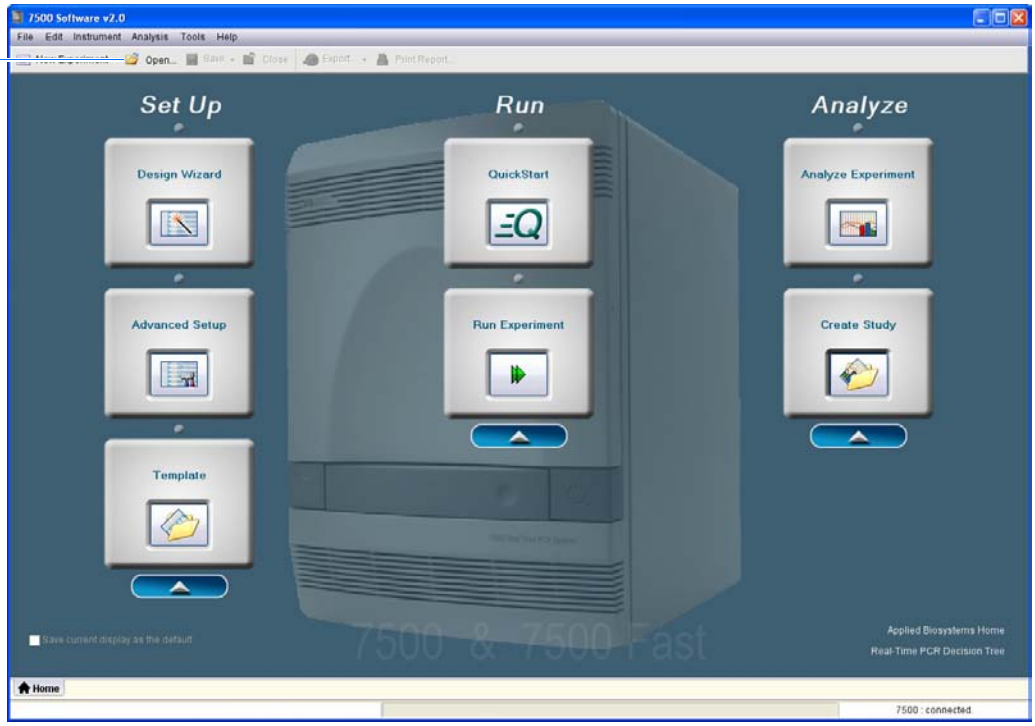
   where *<software name>* is the current version of the 7500 software.

2. In the Home screen, click **Open**.

3. In the Open dialog box, navigate to the **experiments** folder:

   <drive>:\Applied Biosystems*<software name>*\experiments

4. Double-click the example experiment file that you created in Chapter 2.



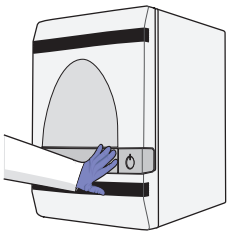
Notes

Load the Reaction Plate into the Instrument

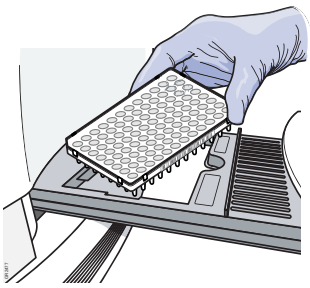
WARNING **PHYSICAL INJURY HAZARD.** During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

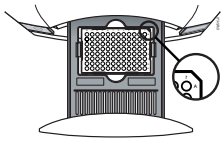
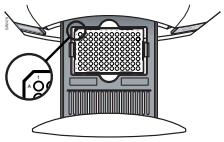
IMPORTANT! Wear powder-free gloves when you handle the reaction plate.

1. Push the tray door to open it.



2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



7500 system	7500 Fast system
Load standard plates with the notched A12 position at the top-right of the tray.	Load Fast plates with the notched A1 position at the top-left of the tray.
	

3. Place the reactions in the precision plate holder (PPH). If you use:
   - **A reaction plate** – Place the reaction plate in the PPH with well A1 at the back-left corner.
   - **Reaction tube strips** – Place the tube strips in the PPH for tube strips.

   Note: Fast Reaction 8-tube strips can be used only on the 7500 Fast system. For the 7500 system, use MicroAmp® Optical 8-Tube Strips.

Notes

- **Reaction tubes** – Place the tubes in the PPH.

Note: MicroAmp® Fast Reaction Tubes (PN 4358297) cannot be used in the 7500 Fast system.

IMPORTANT! For optimal performance with partial loads:

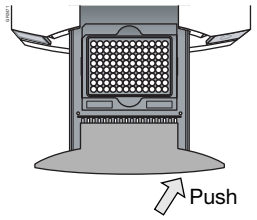
For a 7500 Instrument

Load at least 16 tubes, arranging them first in the center columns of the instrument system (columns 6 and 7). Move outward towards columns 1 and 12 as you add more tubes.

For a 7500 Fast Instrument

- Place empty tube strips in columns 1 and 12 to prevent crushing of tubes containing samples.
- Place tube strips with samples in the PPH vertically, starting in columns 6 and 7 and moving outward.
- A maximum of 6 tube strips can be used in the 7500 Fast instrument. Leave columns 2, 3, 10, and 11 empty.

4. Close the tray. Apply pressure to the right side of the tray and at an angle.



Enable the Notification Settings (Optional)

Enable the notification settings so that the 7500 software alerts you by e-mail when the 7500/7500 Fast instrument begins and/or completes a run, or if an error occurs during a run. Enabling the notifications settings feature is optional and does not affect the performance of the 7500/7500 Fast system or the duration of the run.

IMPORTANT! The notification settings feature is available only if the computer that you are using is running the 7500/7500 Fast instrument *and* is connected to an Ethernet network.

About the Example Experiment

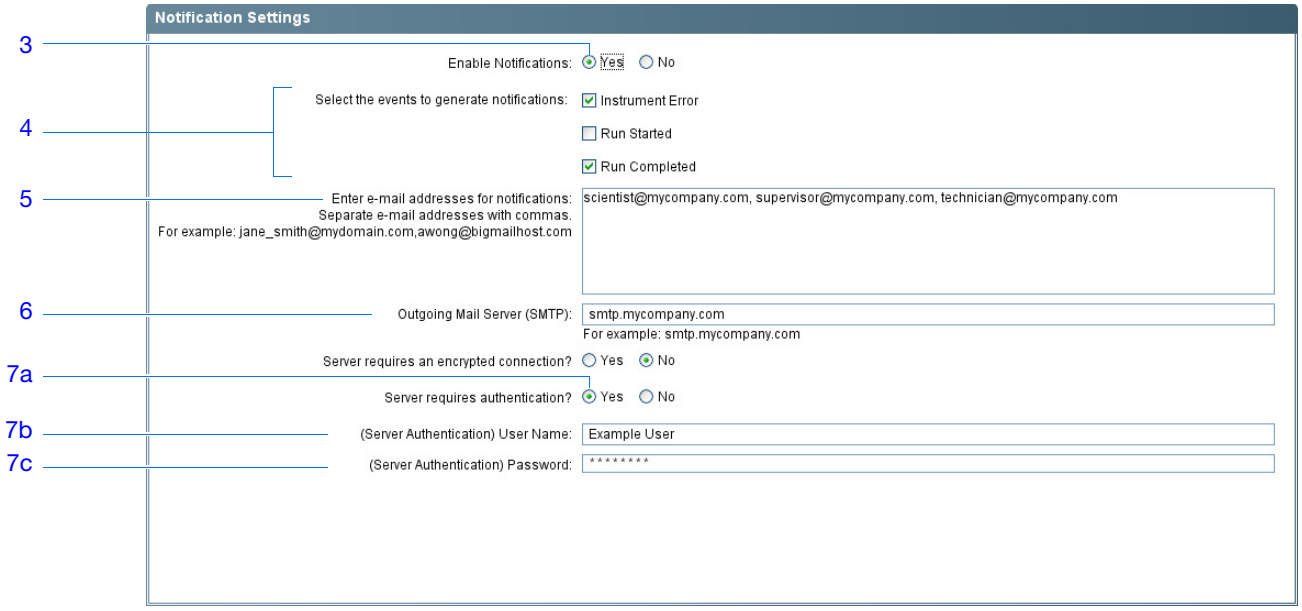
In the example experiment:

- The 7500 software is set up to send notifications to three users (scientist, supervisor, and technician at mycompany.com) when the 7500/7500 Fast system ends the run and if it encounters any errors during operation.
- The example SMTP server (smtp.mycompany.com) is set up for secure sockets layer (SSL) encryption and requires authentication for use.

Notes

Set Up the Notification Settings

1. In the 7500 software, click **Run** in the navigation pane.
2. Click **Notification Settings**.
3. Select **Yes** for Enable Notifications.
4. Select the events that will generate notifications:
   a. Select **Instrument Error**.
   b. Select **Run Completed**.
5. In the Enter e-mail addresses for notifications field, enter: **scientist@mycompany.com, supervisor@mycompany.com, technician@mycompany.com**.
6. In the Outgoing Mail Server (SMTP) field, enter **smtp.mycompany.com**.
7. Set the authentication settings:
   a. Select **Yes** for Server requires authentication.
   b. In the User Name field, enter **Example User**.
   c. In the Password field, enter **password**.



Notes

Run Guidelines

When you set up the 7500/7500 Fast system for automatic notification:

- Your system must be set up for network use. Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- Select the events for which you want to receive e-mail notifications. Select:
  - **Instrument Error** – To notify recipients by email of all errors encountered by the instrument during each run.
  - **Run Started** – To notify recipients by email when the instrument starts a run.
  - **Run Completed** – To notify recipients by email when the instrument completes a run.
- Obtain e-mail addresses to receive notifications.

  IMPORTANT! Separate addresses with a comma (,).

- Contact your systems administrator or information technology department if you need:
  - E-mail addresses for users who will receive notifications
  - A network address for a simple mail transfer protocol (SMTP) server on the LAN
  - A user name and password for the server, if required for access
  - The secure sockets layer (SSL) setting of the server (on or off)

Start the Run

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation mode. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

To start your 7500/7500 Fast instrument:

1. In the 7500 software, click  **Run** in the navigation pane.
2. Click **START RUN** .

Notes

Monitor the Run

You can view the progress of the run in real time as described below. During the run, periodically view all three available plots from the 7500 software for potential problems.

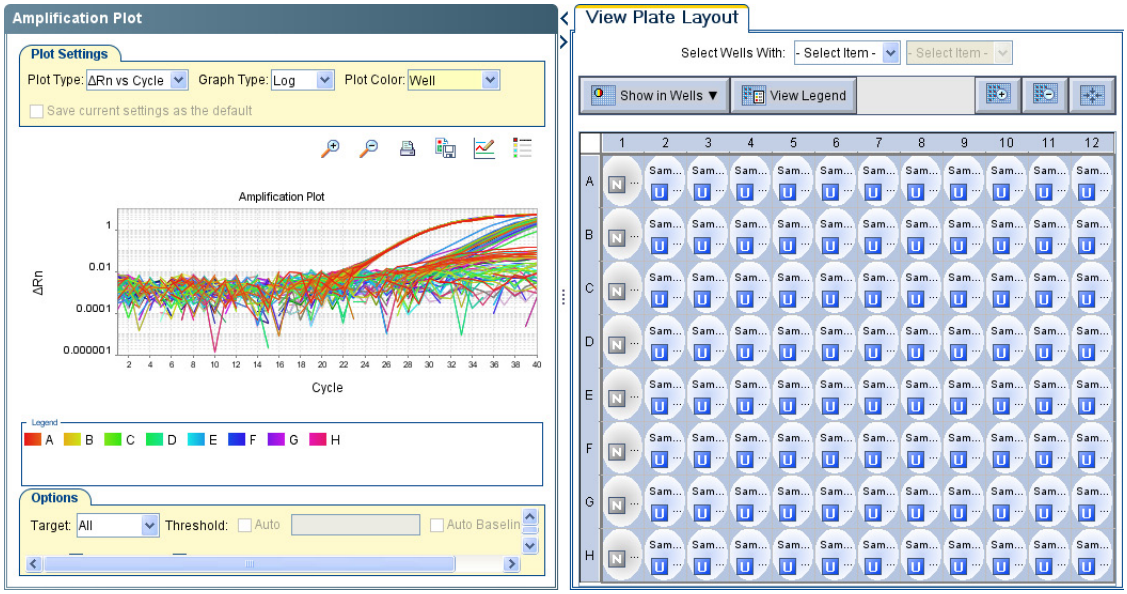
To…	Action
Stop the run	1. In the 7500 software, click **STOP RUN**. 2. In the Stop Run dialog box, click one of the following: – **Stop Immediately** to stop the run immediately. – **Stop after Current Cycle/Hold** to stop the run after the current cycle or hold. – **Cancel** to continue the run.
View amplification data in real time	Select **Amplification Plot**. See "About the Amplification Plot Screen" on page 50.
View progress of the run in the Run Method screen	Select **Run Method**. See "About the Run Method Screen" on page 51.
Enable/disable the Notification Settings	Select or deselect **Enable Notifications**. See "Enable the Notification Settings (Optional)" on page 47.

About the Amplification Plot Screen

The screen displays sample amplification as your instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the Amplification Plot screen displays the data for the wells selected in the View Plate Layout tab. The plot displays normalized dye fluorescence (ΔRn) as a function of cycle number. The figure below shows the Amplification Plot screen as it appears during a run.

To view data in the Amplification Plot screen, select the wells that you want to view in the View Plate Layout tab.

Notes



The Amplification Plot screen is useful for identifying and examining abnormal amplification. Abnormal amplification can include:

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

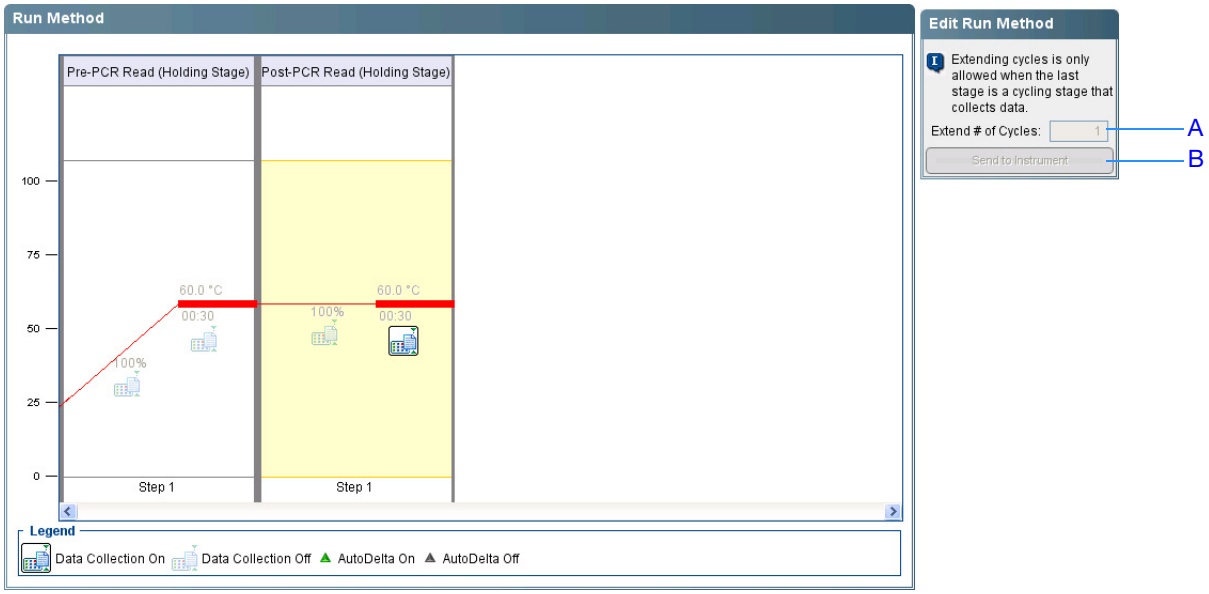
If you notice abnormal amplification or a complete absence of florescence, troubleshoot the error as explained in the 7500 Software Help (click or press **F1**).

About the Run Method Screen

The screen displays the method for the run in progress. The software updates the screen throughout the run. The figure below shows the Run Method screen as it appears in the example experiment.

	To…	Action
A	Change the number of cycles	In the Extend # of Cycles field, enter a number.
B	Apply your changes	Click **Send to Instrument**.

Notes



If an alert is displayed, click the error for more information, then troubleshoot the problem as explained in the 7500 Software Help (click or press **F1**).

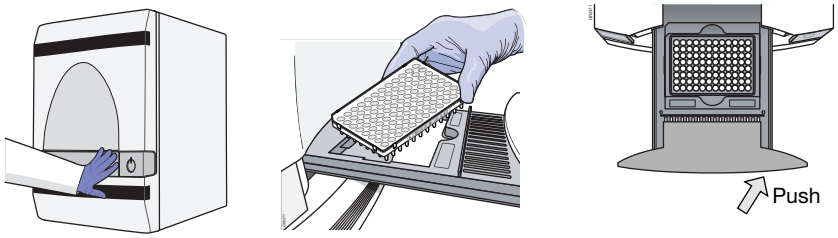
Unload the Instrument

When your 7500/7500 Fast system displays the Run Complete message, unload the reaction plate from the instrument.

Unload the Reaction Plate

WARNING **PHYSICAL INJURY HAZARD.** During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Remove the reaction plate.
3. Push the tray door to close it.



Notes

5 Analyze the Experiment

This chapter covers:

- Chapter Overview 54

Section 5.1 Review Results 55

- Open the Experiment for the Analysis 56
- View the Allelic Discrimination Plot 59
- View the Plate Layout 64
- View the Amplification Plot 66
- View the Well Table 73
- Publish the Data 76

Section 5.2 Troubleshoot (If Needed) 77

- View the Analysis Settings 78
- View the QC Summary 81
- Omit Wells from the Analysis 83
- View the Multicomponent Plot 84
- View the Raw Data Plot 87

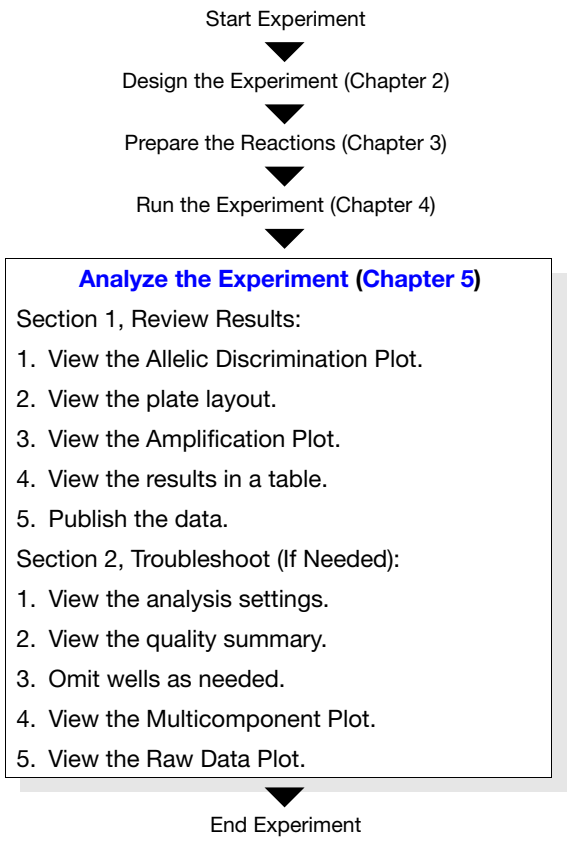
Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Chapter Overview

This chapter explains how to view, analyze, and publish the analyzed experiment.

Example Experiment Workflow



How to Evaluate the Results

Review of the results occurs in three steps:

1. Review the Allelic Discrimination Plot (see page 59), the plate layout (see page 64), the amplification data (see page 66), and the well table (see page 73) to evaluate the genotype calls made by the 7500 software.
2. If necessary, define the analysis settings (see page 78) or modify the calls manually (see page 63).
3. Review of the QC Summary (see page 81) and the raw data (see page 87) to evaluate the samples that generated QC flags.

After evaluating the results, you can publish the results as explained in "Publish the Data" on page 76.

Notes

Section 5.1 Review Results

This section covers:

- Open the Experiment for the Analysis 56
- View the Allelic Discrimination Plot 59
- View the Plate Layout 64
- View the Amplification Plot 66
- View the Well Table 73
- Publish the Data 76

Notes

Open the Experiment for the Analysis

Prepare for the analysis by opening the experiment.

About the Example Experiment

For the genotyping example experiment, use the data file that is installed with the 7500 software. The data file was created with the same design parameters provided in Chapter 2, then run and analyzed on a 7500/7500 Fast instrument.

The data file for the example experiment is on your computer:

<drive>:\Applied Biosystems*<software name>*\experiments\Genotyping Example.eds

where:

- *<drive>* is the computer hard drive on which the 7500 software is installed.
- *<software name>* is the current version of the 7500 software.

Open the Experiment

1. Double-click (7500 software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***

   where *<software name>* is the current version of the 7500 software.

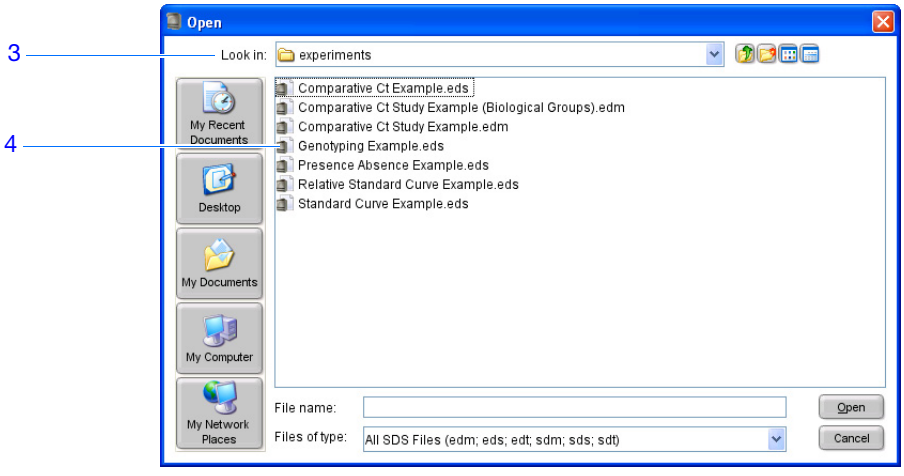
2. In the Home screen, click **Open**.

3. In the Open dialog box, navigate to the **experiments** folder at:

   <drive>:\Applied Biosystems*<software name>*\experiments

4. Double-click **Genotyping Example.eds** to open the example experiment data file.

Note: The experiments folder contains several data files; be sure to select **Genotyping Example.eds**.



Notes

Guidelines

When you analyze your own genotyping experiment:

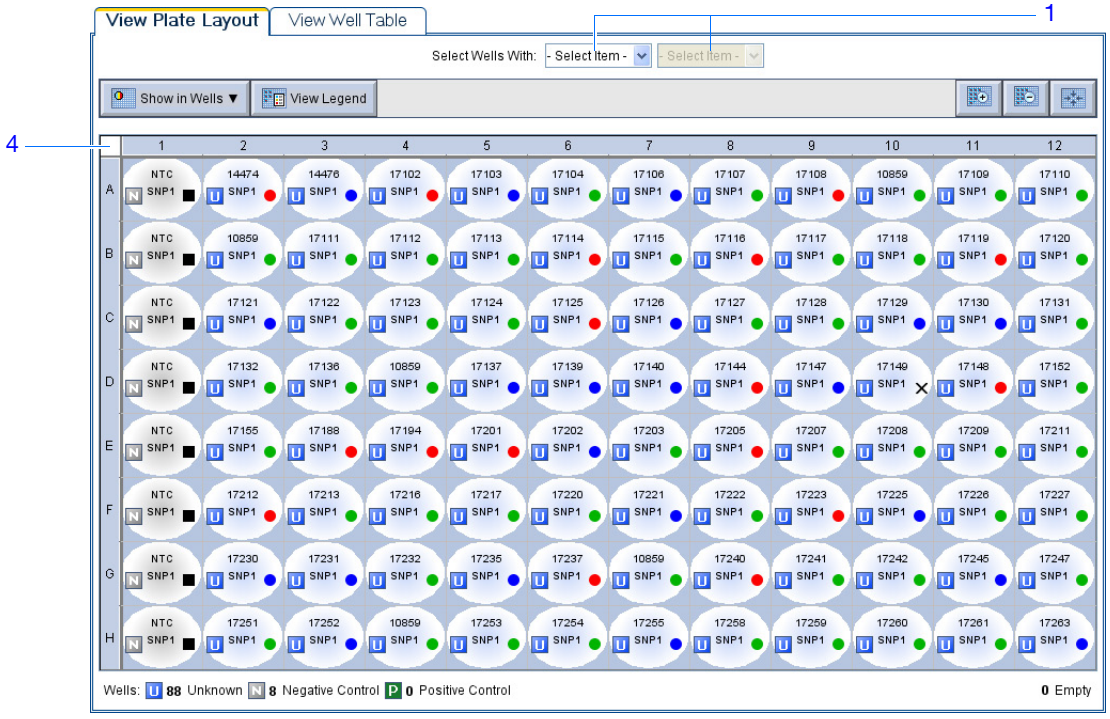
- Immediately after a run, the 7500 software automatically analyzes the data using the default analysis settings, then displays the Allelic Discrimination Plot screen on your computer.
- To reanalyze the data, select all the wells in the plate layout, then click **Analyze**.

Navigation Tips

How to Select Wells

To display specific wells in the analysis screens, select the wells in the View Plate Layout tab as follows:

1. To select wells of a specific type, use the Select Wells With drop-down lists, select **Sample**, **SNP Assay**, or **Task**, then select the sample, SNP assay, or task name.
2. To select a single well, click the well in the plate layout.
3. To select multiple wells, click-drag over the desired wells, or **CTRL**+**click**, or **Shift**+**click** the desired wells in the plate layout.
4. To select all 96 wells, click the upper left corner of the plate layout.

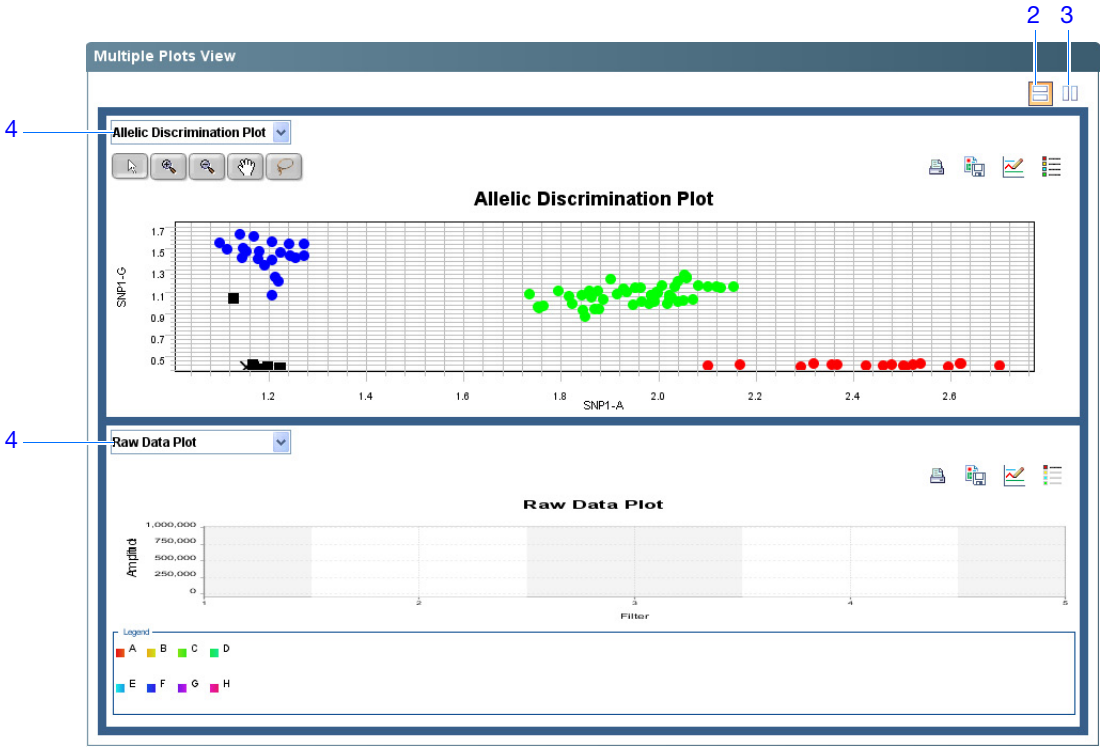


Notes

How to Display Multiple Plots

Use the Multiple Plots screen to display two plots simultaneously. To navigate within the Multiple Plots screen:

1. In the navigation pane, select **Analysis** ▸ **Multiple Plots View**.
2. To display two plots in rows, click (Show plots in two rows).
3. To display two plots in columns, click (Show plots in two columns).
4. To display a specific plot, select the plot in the drop-down list above each plot display.



For More Information For more information, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Allelic Discrimination Plot

Perform an initial review of the experiment results in the Allelic Discrimination Plot, which contrasts the normalized reporter dye fluorescence (R_n) for the allele-specific probes of the SNP assay. See "Reading and Analyzing the Plates" on page 9 for a complete description of the plot.

About the Example Data

For the example experiment, verify that the Allelic Discrimination Plot displays:

- Clusters for the three possible genotypes (Allele 1 homozygous, Allele 2 homozygous, and Allele 1/2 heterozygous)
- A cluster for the negative controls

View the Plot

1. In the navigation pane, select **Analysis** ▸ **Allelic Discrimination Plot**.

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

2. Select the **View Plate Layout** tab, then click any well to select it.

   Note: In the Allelic Discrimination Plot, the software highlights all wells that are selected in the View Plate Layout tab. If the plot displays a single color for all wells, then all wells in the plate layout are selected.

3. In the Plot Settings tab, select **SNP1** in the SNP Assay drop-down list.
   - If the Autocaller is enabled, the Allelic Discrimination Plot displays allele symbols for each sample evaluated for the selected SNP.

     The samples are grouped on the plot as follows:

Symbol	Are grouped along the...	The genotypes of the samples are...
● (red)	X-axis of the plot	Homozygous for Allele 1 of the selected SNP assay.
● (blue)	Y-axis of the plot	Homozygous for Allele 2 of the selected SNP assay.
● (green)	Midway between the homozygote clusters	Heterozygous for both alleles of the selected SNP assay (Allele 1 and Allele 2).
■ (black)	Bottom-left corner of the plot	Negative controls.
× (black)	Anywhere on plot	Undetermined.

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

4. For each cluster in the plot:
   a. Click-drag a box around the cluster to select the associated wells in the plate layout and well table.

Notes

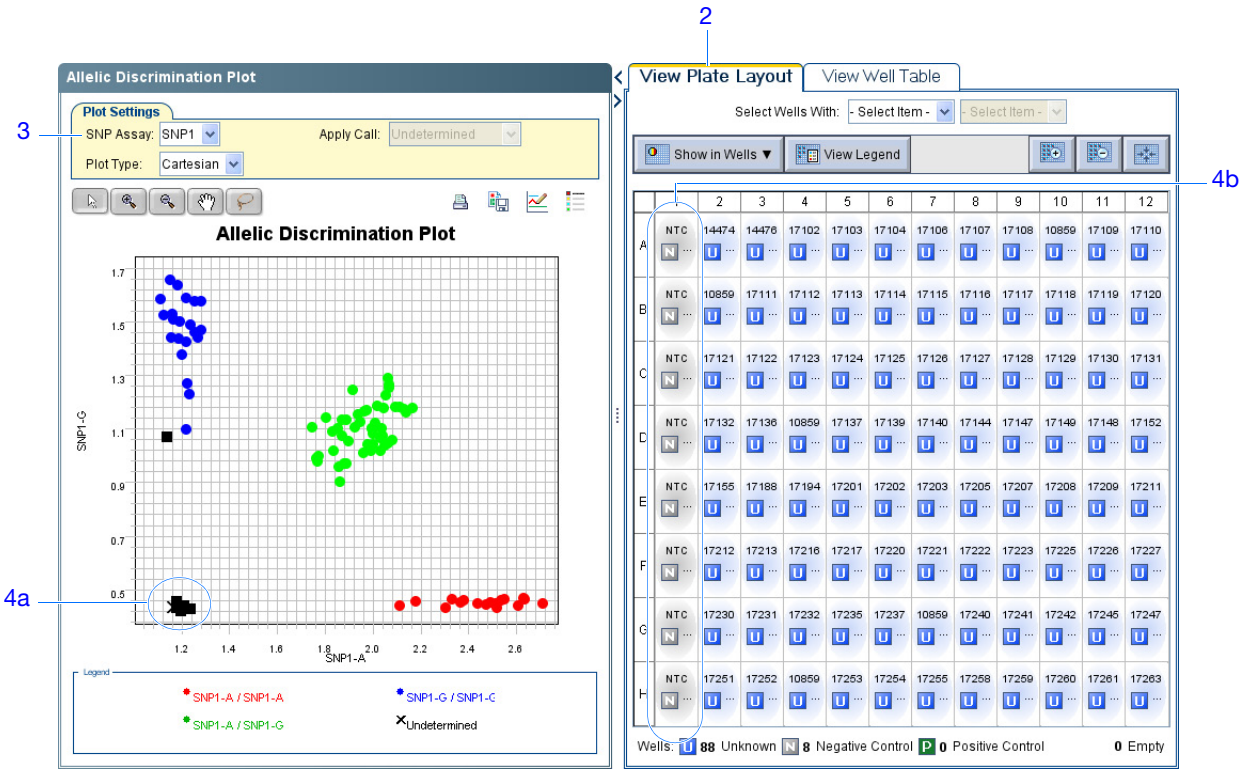
b. Verify that the expected wells are selected in the well table.

For example, if you select the cluster at the bottom-left corner of the plot, only the negative controls should be selected. The presence of an unknown among the negative controls may indicate that the sample failed to amplify.

c. Repeat steps 4a and 4b for all other clusters in the plot.

Element	Description
SNP Assay drop-down list	Determines the SNP assay data that the 7500 software displays in the plot.
Plot Type drop-down list	Determines the type of plot (Cartesian or Polar) that the 7500 software uses to display the data.
Apply Call drop-down list	When a data point is selected, this list enables you to assign an allele call to the data point within the scatterplot.
Toolbar	Contains tools for manipulating the scatterplot: • – Selects data points by clicking individual data points, or by click-dragging a box around a group of data points. • – Selects data points by encircling them. • – Repositions the scatterplot. • – Magnifies the scatterplot. • – Zooms out the scatterplot.
Legend	An explanation of the symbols in the scatterplot.

The figure below shows the Allelic Discrimination Plot of the example experiment.



Notes

Analysis Guidelines

When you analyze your own experiment:

- Verify that all controls have the correct genotype.
- If using positive controls, verify the calls for the positive controls:
  a. In the plate layout, select the wells containing a positive control to highlight the corresponding data points in the Allelic Discrimination Plot.
  b. Check that the data points for the positive controls cluster along the expected axis of the plot. For example, if you select the Positive Control Allele 1/Allele 1, then the controls should cluster along the X-axis.
  c. Repeat steps a and b for the wells containing the other positive controls.
- Screen the negative control cluster for unknown samples that failed to amplify:
  a. Select the data points of the cluster in the lower left corner of the Allelic Discrimination Plot to select the corresponding wells in the plate layout.
  b. Check that the selected wells in the plate layout are negative controls, and not unknown samples.
- Samples that cluster with the negative controls may:
  – Contain no DNA
  – Contain PCR inhibitors
  – Be homozygous for a sequence deletion
- Verify that the results of the samples that did not cluster tightly or are clustered with negative controls by retesting them.
- If you choose to run replicate reactions, carefully review your data set for outliers to ensure the accuracy of the genotype calls. If outliers are present, verify the results of the associated samples by retesting them.
- Observe the number of clusters in the plot. If the Allelic Discrimination Plot contains less than the three representative genotype clusters (heterozygous, homozygous allele 1, and homozygous allele 2), then the 7500 software may not be able to genotype the samples until you enable the 2-Cluster Calling feature.

  If the plot contains less than three clusters:

  a. Click **Analysis Settings**.
  b. Click **Edit Default SNP Assay Settings**.
  c. Select **2-Cluster Calling Enabled**, then click **Save Changes**.
  d. Click **Apply Analysis Settings**.
  e. Click **Analyze** to reanalyze the experiment using 2-Cluster Calling.

Note: The results displays are synchronized. For example, selecting a well in the plate layout selects the corresponding data in the well table and Allelic Discrimination Plot.

Notes

How to Assign Calls Automatically

1. In the experiment, click **Analysis Settings**.
2. Select the desired SNP Assay from the Select a SNP Assay table.
3. Click **Edit Default Settings**.
4. If you have made manual calls, select **Keep Manual Calls from Previous Analysis**.
5. Select **Autocaller Enabled** to activate automatic analysis.
6. If you expect the analyzed data to consist of only two clusters, select **2-Cluster Calling Enabled**.
7. In the Quality Value field, enter a percentage value to apply as the quality interval for auto-calling samples. (The greater the value, the more stringent the allele calling.)
8. Click **Save Changes** to save your settings.
9. (Optional) Assign flags:
   a. Select the **Flag Settings** tab.
   b. Assign flags as desired.

      Note: When you assign flags, you can flag conditions and omit wells when certain criteria are met (for example, when a well has missing data). For more information, see the *7500 Software Help*.

   c. Click **Apply Analysis Settings** to close the Analysis Settings dialog box.
10. Click **Analyze** to reanalyze the data using the new settings.

Notes

How to Assign Calls Manually

1. In the experiment, click **Analysis Settings**.
2. Select the desired SNP Assay from the Select a SNP Assay table.
3. Set the SNP assay analysis settings:
   a. Click **Edit Default Settings**.
   b. Deselect the **Autocaller Enabled**.
   c. Click **Save Changes** to save your settings.
4. (Optional) Assign flags:
   a. Select the **Flag Settings** tab.
   b. Assign flags as desired.

   Note: When you assign flags, you can flag conditions and omit wells when certain criteria are met (for example, when a well has missing data). For more information, see the *7500 Software Help*.

   c. Click **Apply Analysis Settings**.
5. Click **Analyze** to reanalyze the data using the new settings.
6. In the navigation column, select **Allelic Discrimination Plot**.
7. Select an assay in the SNP Assay drop-down list. Crossmarks (× – Undetermined) representing the data points for the selected SNP assay are displayed in the Allelic Discrimination Plot.
8. To assign calls:
   a. Click (selection tool).
   b. Click-drag a box around the desired data points in the plot.
   c. In the Apply Call drop-down list, select the desired call.
   d. Repeat steps 8b and 8c to apply calls to the rest of the data points.
9. If you are assigning calls for multiple SNPs, select a different assay in the SNP assay drop-down list, then repeat step 8.

For More Information

For more information on the Allelic Discrimination Plot, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Plate Layout

Review the experiment results in the plate layout. The plate layout displays the assay-specific setup and analysis properties for the experiment in a well format corresponding to the type of reaction plate used for the run.

About the Example Data

For the example experiment, verify that the 7500 software called:

- 17 samples as SNP1-A homozygous (●)
- 21 samples as SNP1-G homozygous (●)
- 49 samples as SNP1-A/SNP1-G heterozygous (●)
- 8 samples as negative controls (■)
- 1 sample as unknown (✕)

Verify that no wells of the reaction plate generated QC flags (▲).

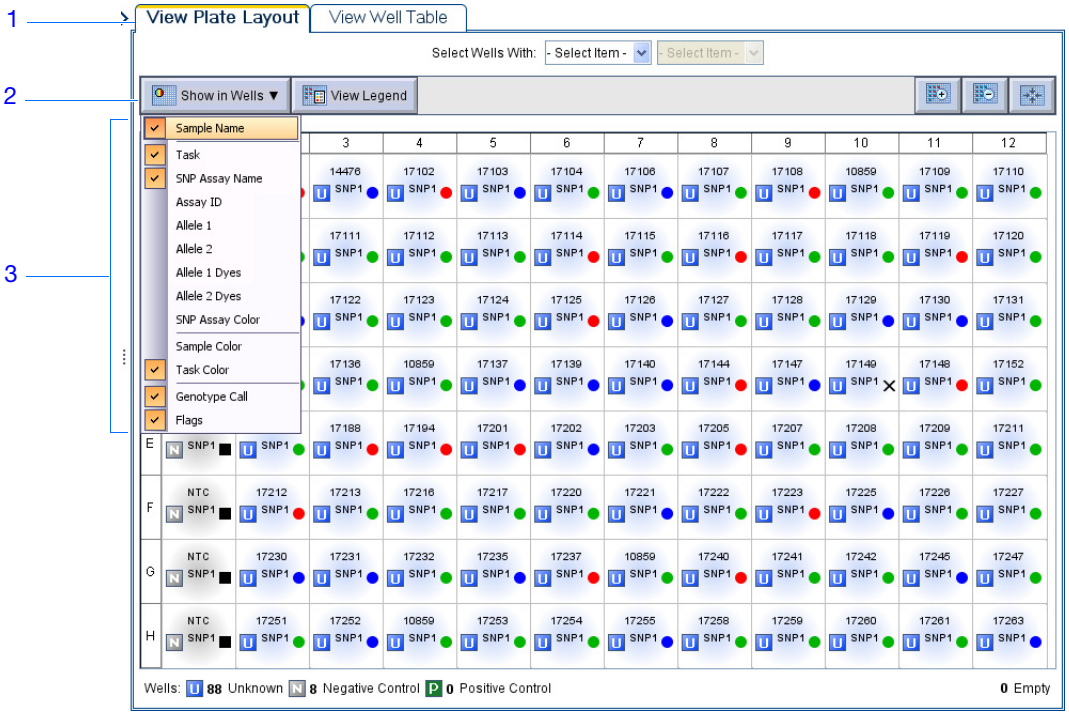
View the Layout

1. Click ‹ at the top right of the Allelic Discrimination Plot to maximize the plate layout.
2. Click **Show in Wells**, then select (or deselect) a parameter that you want the wells to display (or not display).
3. Repeat step 2 until the plate layout contains all the desired parameters.

Parameter	Description
Sample Name	The name of the sample applied to the well.
Task	The task assigned to the well: • U – Unknown • N – Negative Control • P – Positive Control
SNP Assay Name	The name of the SNP evaluated by the well.
Assay ID	The Assay ID number of the SNP evaluated by the well.
Allele 1 / Allele 2	The name of the associated allele for the SNP evaluated by the well
Allele 1 Dyes / Allele 2 Dyes	The name of the reporter and quencher dyes of the associated allele for the SNP evaluated by the well
SNP Assay Color	The color of the SNP evaluated by the well.
Sample Color / Task Color	The color of the sample or task applied to the well.
Genotype Call	The allele call assigned to the sample: • ● Homozygous SNP1-A • ● Homozygous SNP1-G • ● Heterozygous SNP1-A/SNP1-G • ■ Negative Control • ✕ Undetermined
Flag	The number of QC flags the well generated as listed in the ▲ symbol.

Notes

The following figure shows the plate layout of the example experiment.



Analysis Guidelines

When you analyze your own experiment:

- The 7500 software displays ☒ in the top-left corner of each well omitted by a user; it displays ☐ in the corner of each well omitted by the QC flag settings.
- Note the location of any samples that generate QC flags (▲). Understanding the position of errors can aid in diagnosing any failures that may occur.
- You can select the entire reaction plate, areas of the reaction plate, or specific wells:
  - Click the upper left corner of the reaction plate to select all 96 wells.
  - Click-drag across an area to select it.
  - Select **Sample**, **SNP Assay**, or **Task** in the Select Items drop-down list in the View Plate tab. Then select the sample, SNP assay, or task name in the second Select Items drop-down list to select wells of a specific type.
- You can adjust the plate layout:
  - Click (Zoom In), (Zoom Out), and (Fit All) buttons to increase or decrease the number of wells that are displayed.
  - Click ❮ to expand the plate layout to cover the entire screen.

For More Information

For more information on the plate layout, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Amplification Plot

If you collected real-time data for your experiment, review the amplification data to evaluate the flags that were generated by the experiment data.

The Amplification Plot screen displays amplification of all samples in the selected wells. Three plots are available:

- **ΔRn vs Cycle** – ΔRn is the difference in normalized fluorescence generated by the reporter between the pre-PCR read and the post-PCR read. This plot displays ΔR_n as a function of cycle number. You can use this plot to identify and examine irregular amplification and to view threshold and baseline values for the run.
- **Rn vs Cycle** – Rn is the fluorescence from the reporter dye normalized to the fluorescence from the passive reference. This plot displays R_n as a function of cycle number. You can use this plot to identify and examine irregular amplification.
- **C_T vs Well** – C_T is the PCR cycle number at which the fluorescence equals the threshold in the amplification plot. This plot displays C_T as a function of well position. You can use this plot to locate outlying amplification (outliers).

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

Note: For more information about the Amplification Plots, refer to the *Real Time PCR System Reagent Guide* or the 7500 Software Help.

About the Example Data

Because the example experiment does not contain real-time data, the 7500 software does not display the Amplification plot. If the example experiment contained real-time data, you would review the Amplification Plot for:

- Correct baseline and threshold values
- Outliers

View the Plot

Note: The following procedure is provided as an example. Because the example experiment does not contain real-time data, the 7500 software does not display the Amplification Plot.

1. In the navigation column, select **Amplification Plot**.
2. In the Amplification Plot:
   a. In the Plot Type drop-down list, select **ΔRn vs Cycle**.
   b. In the Plot Color drop-down list, select **Well**.
   c. Click (Show a legend for the plot).

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

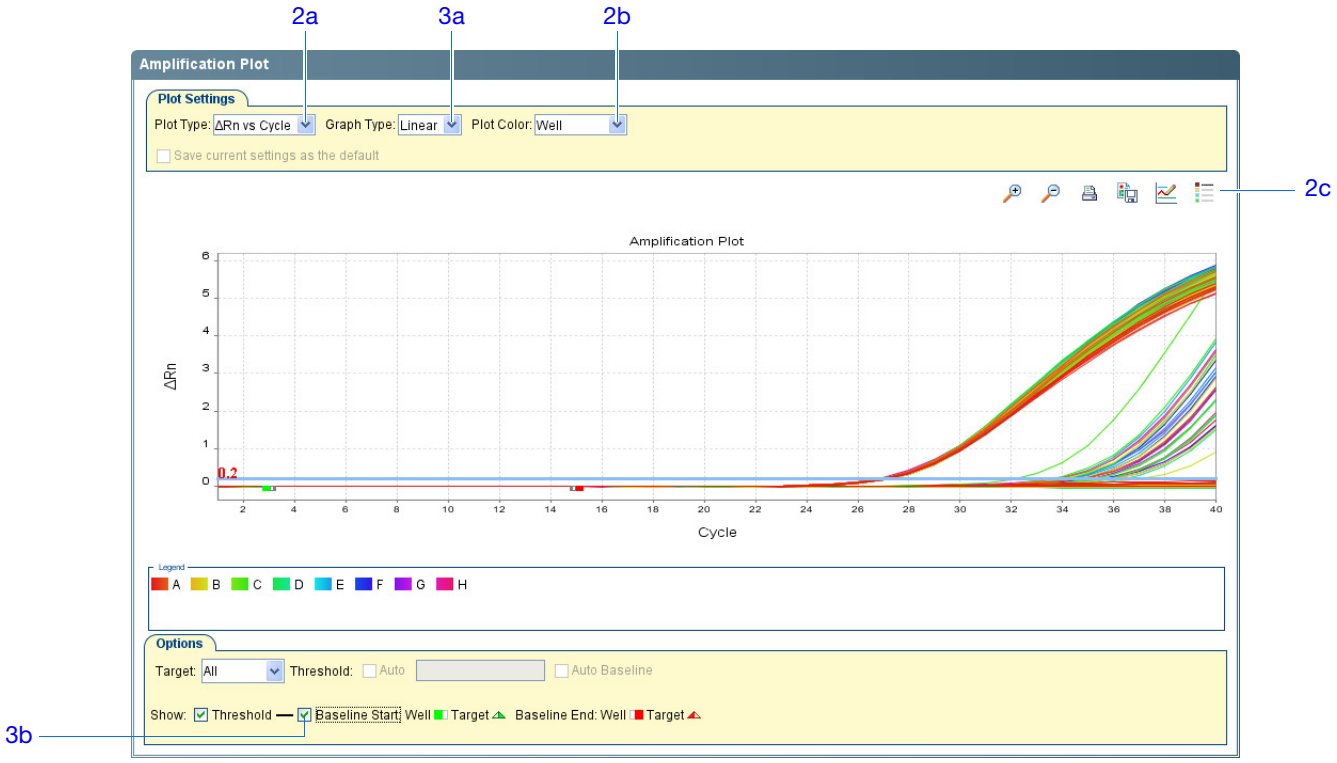
Notes

3. View the baseline values:

   a. In the Graph Type drop-down list, select **Linear**.

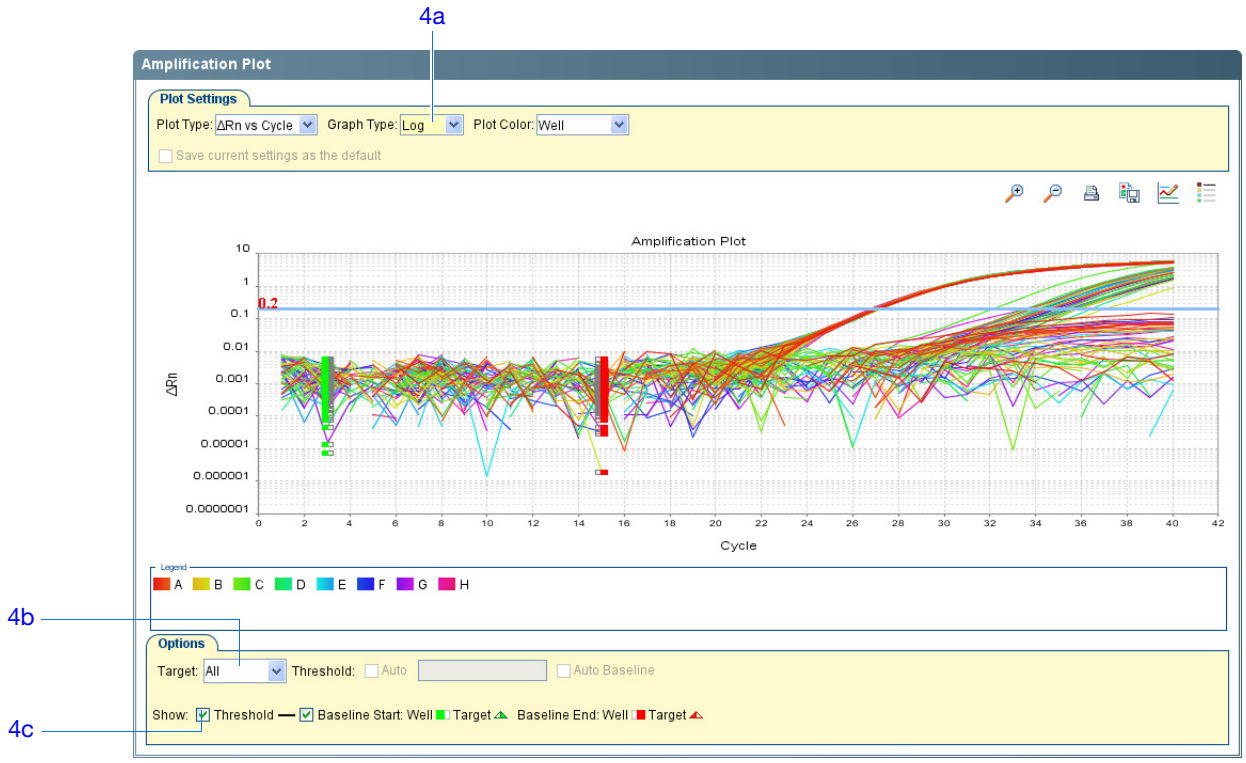
   b. Select **Baseline** to show the start cycle and end cycle.

   c. Verify that the baseline is set correctly: The end cycle should be set a few cycles before the cycle number where significant fluorescence is detected. In the example below, the baseline is set correctly.



Notes

4. View the threshold values:
   a. In the Graph Type drop-down list, select **Log**.
   b. In the Target drop-down list, select a SNP assay.
   c. Select **Threshold** to show the threshold.
   d. Verify that the threshold is set correctly. In the example below, the threshold is in the exponential phase.
   e. Repeat steps 4a through 4d for the other SNP assays.



5. Locate any outliers:
   a. In the Plot Type drop-down list, select **C_T vs Well**.
   b. Verify that replicate wells have achieved similar amplification. The example experiment does not use replicate wells.

Notes

Analysis Guidelines

When you analyze your own experiment, look for:

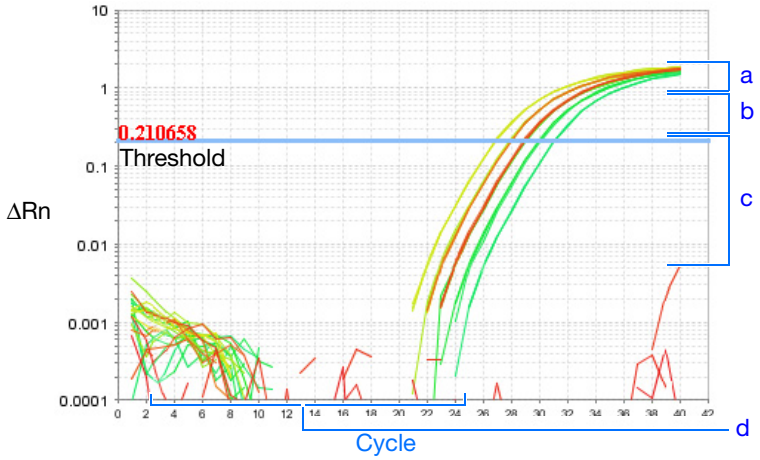
- Outliers
- A typical amplification plot – The 7500 software automatically calculates baseline and threshold values based on the assumption that the data exhibit a *typical* amplification plot. A typical amplification plot has four distinct sections:

  a. Plateau phase

  b. Linear phase

  c. Exponential (geometric phase)

  d. Baseline

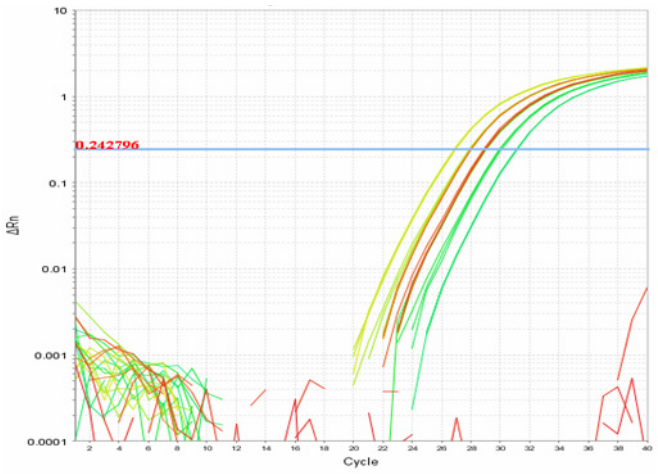
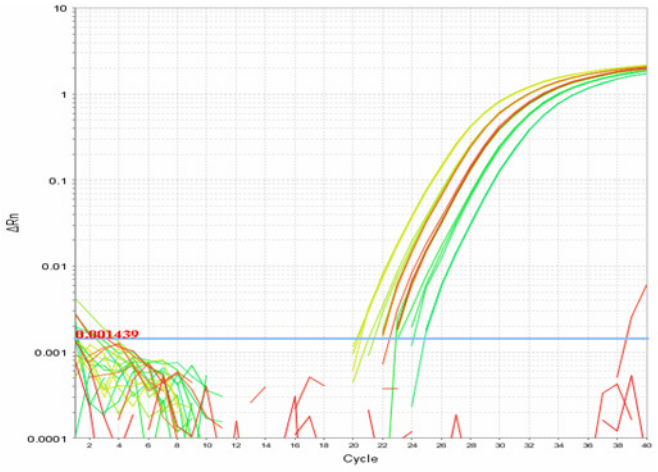
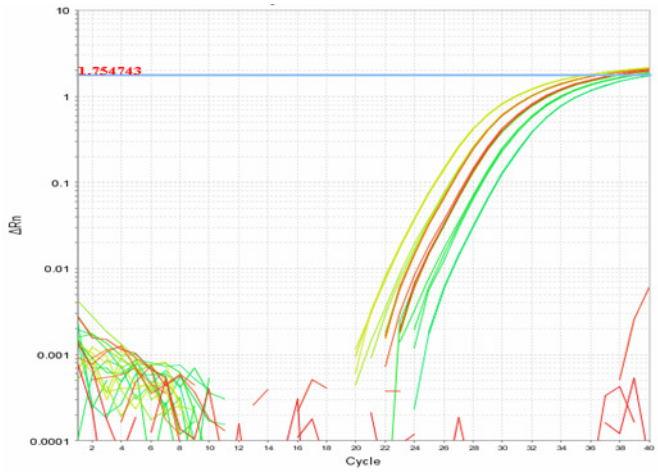


IMPORTANT! Experimental error (such as contamination or pipetting errors) can produce atypical amplification curves that can result in incorrect baseline and threshold value calculations by the 7500 software. Therefore, Applied Biosystems recommends that you examine the Amplification Plot screen and review the assigned baseline and threshold values for each well after analysis completes. For more information, see the *7500 Software Help*.

- Correct baseline and threshold values – See "Threshold Examples" on page 70 and the "Baseline Examples" on page 71.

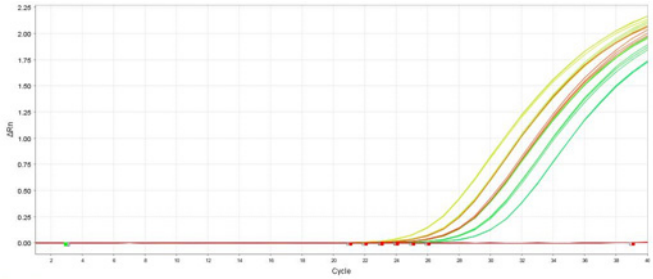
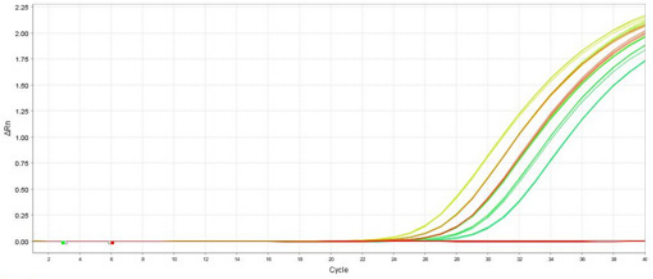
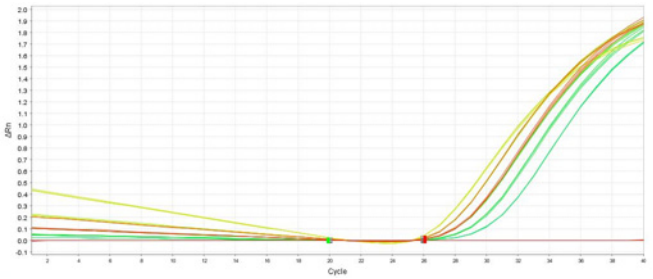
Notes

Threshold Examples

Threshold Set Correctly The threshold is set in the exponential phase of the amplification curve. Threshold settings above or below the optimum increase the standard deviation of the replicate groups.	
Threshold Set Too Low The threshold is set below the exponential phase of the amplification curve. The standard deviation is significantly higher than that for a plot where the threshold is set correctly. Drag the threshold bar up into the exponential phase of the curve.	
Threshold Set Too High The threshold is set above the exponential phase of the amplification curve. The standard deviation is significantly higher than that for a plot where the threshold is set correctly. Drag the threshold bar down into the exponential phase of the curve.	

Notes

Baseline Examples

Baseline Set Correctly The amplification curve begins after the maximum baseline.	
Baseline Set Too Low The amplification curve begins too far to the right of the maximum baseline. Increase the End Cycle value.	
Baseline Set Too High The amplification curve begins before the maximum baseline. Decrease the End Cycle value.	

If your experiment does not meet the guidelines above:

- Manually adjust the baseline and/or threshold (see the 7500 Software Help).

  or

- Omit a well by right-clicking the well in the plate layout, then selecting **Omit**.

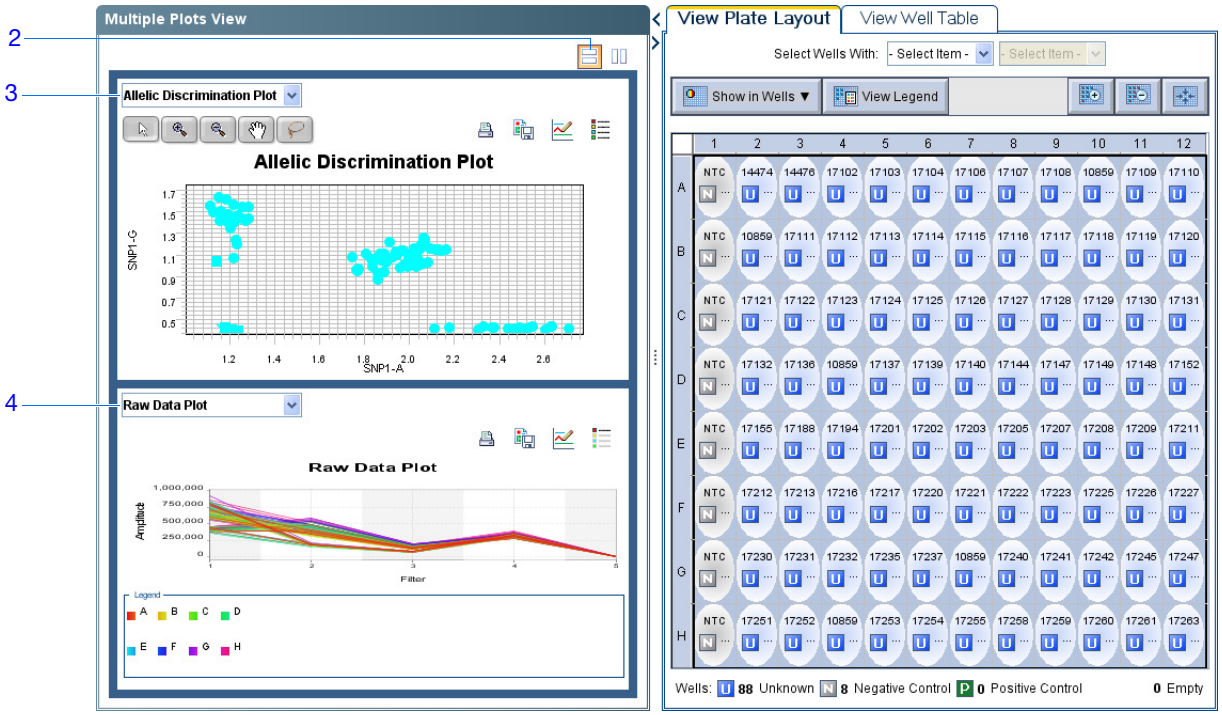
Notes

How to View Multiple Plots Simultaneously

The Multiple Plots View displays two plots for simultaneous analysis. You can view each plot individually, or two plots as rows or columns.

To review the results in multiple plots:

1. In the navigation column, select **Multiple Plots View**.
2. Click to display two plots in parallel.
3. In the top plot, select **Allelic Discrimination Plot** to view results of the run.
4. In the bottom plot, select **Raw Data Plot** to view the raw data for each well selected in the plate layout.



For More Information For more information on the Amplification Plot or on the Multiple Plot View, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Well Table

Review the details of the experiment results in the well table and identify any flagged wells. The well table displays the assay-specific setup and analysis properties for the experiment in a table format.

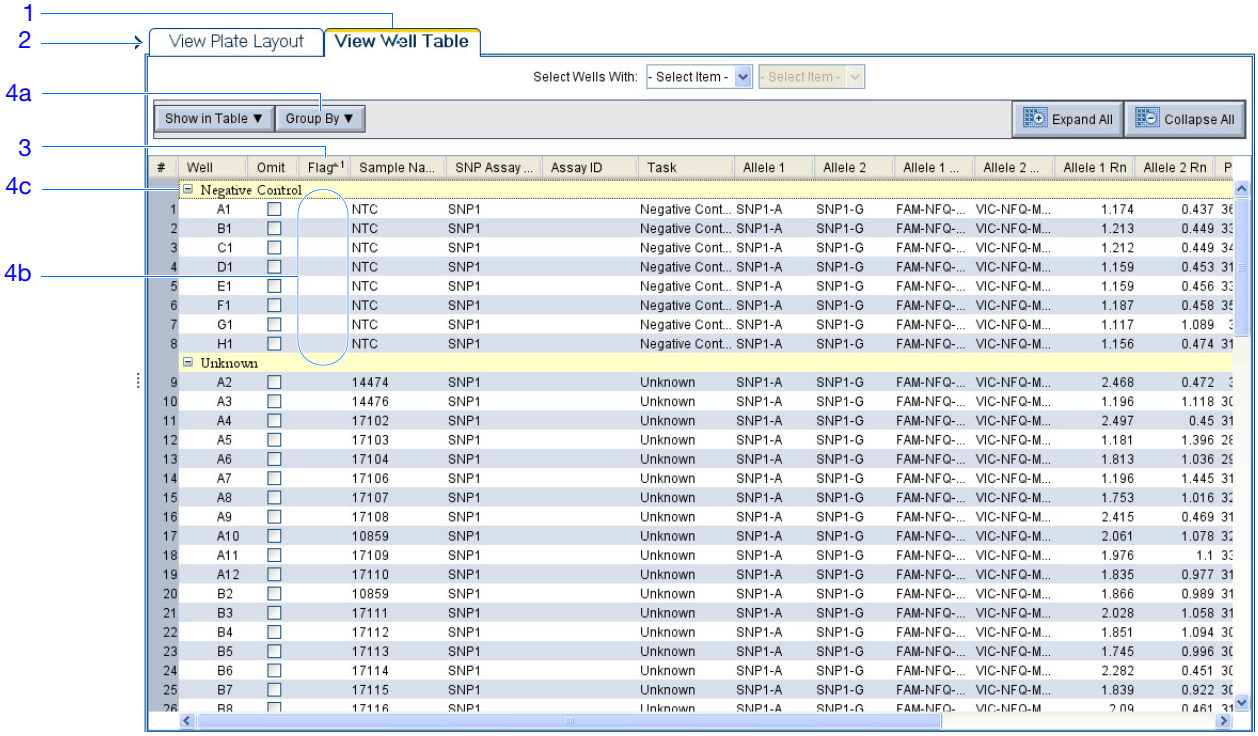
About the Example Data

For the example experiment, verify that no wells of the reaction plate generated QC flags (▲).

View the Table

1. Select the **View Well Table** tab.
2. Click ❮ at the top left of the View Well Table tab.
3. Click the **Flag** column header to sort the data so that the wells that generated flags appear at the top of the table.
4. Verify the integrity of the controls:
   a. In the Group By drop-down list, select **Task** to organize the table rows by their function on the reaction plate.
   b. Verify that the controls do not display flags (⚠).
   c. Click the – icons to collapse the negative controls.
5. Click ❯ at the top left of the View Well Table tab to simultaneously display the Allelic Discrimination Plot and the well table.

   The figure below shows the well table of the example genotyping experiment.



Notes

Well Table Columns

Column	Description
Well	The position of the well on the reaction plate.
Omit	A check mark indicates that the well has been removed from the analysis.
Flag	A ⚠1 indicates that the well generated the number of flags listed inside the symbol.
Sample Name	The name of the sample.
SNP Assay Name	The name of the SNP assay that was evaluated by the well.
Assay ID	The Assay ID number of the SNP that was evaluated by the well.
Task	The task assigned to the well (Unknown, Negative Control, or Positive Control).
Allele 1 / 2	The name of the associated allele for the SNP that was evaluated by the well.
Allele 1 / 2 Dyes	The name of the reporter and quencher dyes of the associated allele for the SNP that was evaluated by the well.
Allele 1 / 2 R_n	Normalized signal (R_n) of the reporter dye of the associated allele for the SNP that was evaluated by the well.
Pass Ref	The signal of the passive reference dye for the well.
Call	The allele call assigned to the sample, where possible calls are: • ● Homozygous 1/1 - Homozygous for SNP1-A • ● Homozygous 2/2 - Homozygous for SNP1-G • ● Heterozygous 1/2 - Heterozygous • ■ Negative Control • ✕ Undetermined
Quality (%)	The quality value that was calculated for the genotype call.
Method	The method used to assign the call to the sample (Auto, if assigned by the 7500 software, or Manual, if applied by a user).
Comments	Comments entered for the associated sample well.
Allele 1 / 2 C_T	Threshold cycle (C_T) of the sample for the associated allele for the SNP that was evaluated by the well.
QC Flag Columns	The well table displays columns for QC flags that are generated by the experimental data. If the experiment data do not trigger a QC flag, then the 7500 software does not display a corresponding column for the flag. A ▲ in one of the columns indicates that the associated well generated the flag. See "Possible Flags" on page 82 for a description of the flags.

Notes

Analysis Guidelines

When you analyze your own experiment:

- If you are using positive controls, verify the integrity of the positive controls:
  a. In the Group By drop-down list, select **Task** to organize the table rows by their function on the reaction plate
  b. Verify that the positive controls do not display flags (▲) and that their normalized reporter dye fluorescence (R_n) is appropriate for the genotype (for example, if evaluating the Positive Control Allele 1/Allele 1, you would expect to see significant increase in R_n for the Allele 1 probe and very little increase for the Allele 2 probe).
  c. Repeat step b for the each positive control.
- Review the data for the Unknown samples. For each row that displays ▲ in the Flag column, note the data and the flag(s) generated by the associated well.
- Select areas of the table or wells of a specified type by:
  – Click-dragging across the area of the table that you want to select.
  – Selecting **Sample**, **SNP Assay**, or **Task** in the Select Items drop-down list in the View Table tab, then selecting the sample, SNP assay, or task name in the second Select Items drop-down list to select wells of a specific type.
- Group the rows of the plate layout by selecting an option in the Group By drop-down list. You can then collapse or expand the lists either by clicking +/- next to individual lists, or by clicking (Collapse All) or (Expand All).
- Omit a well from the analysis by selecting the **Omit** check box for that well. To include the well in the analysis, deselect the **Omit** check box.

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

For More Information

For more information on the well table, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Publish the Data

You can publish the experiment data in several ways:

- Save the plot as an image file
- Print the plot
- Print the plate layout
- Create slides
- Print a report
- Export data

For More Information

For more information on publishing data, open the 7500 Software Help by clicking  or pressing **F1**.

Notes

Section 5.2 Troubleshoot (If Needed)

- View the Analysis Settings 78
- View the QC Summary 81
- Omit Wells from the Analysis 83
- View the Multicomponent Plot 84
- View the Raw Data Plot 87

Notes

View the Analysis Settings

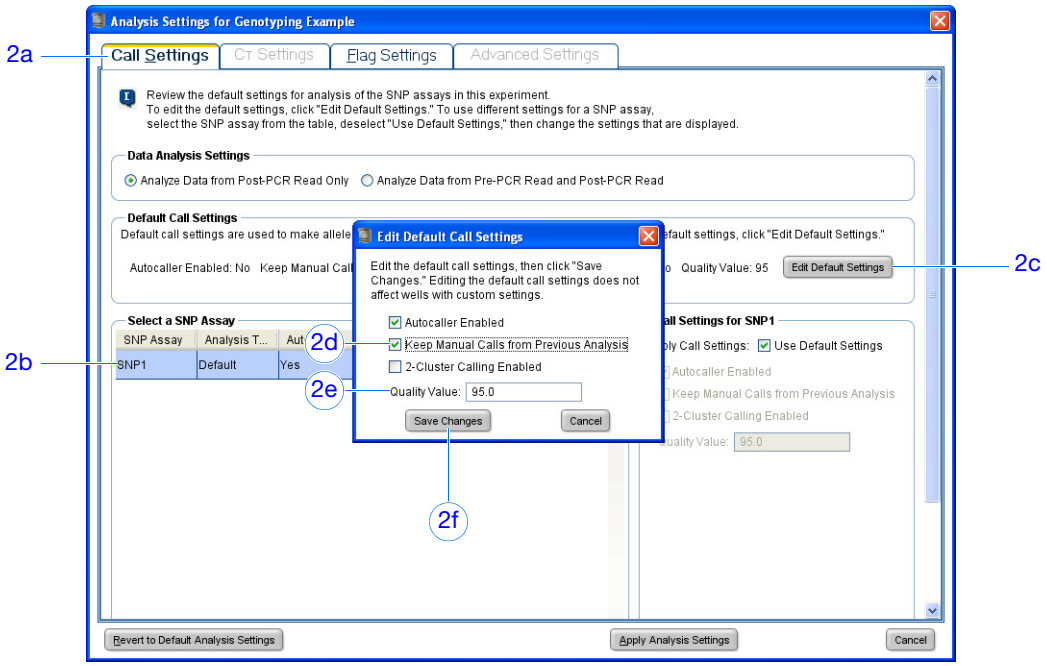
If you are dissatisfied with how the 7500 software is calling genotypes or the thresholds of the QC flags, review and adjust the analysis settings and/or calls as needed.

About the Example Data

In the example experiment, review and adjust the analysis settings as desired to learn how the call, C_T, and flag settings contribute to the analysis of the genotyping data.

Modify the Settings

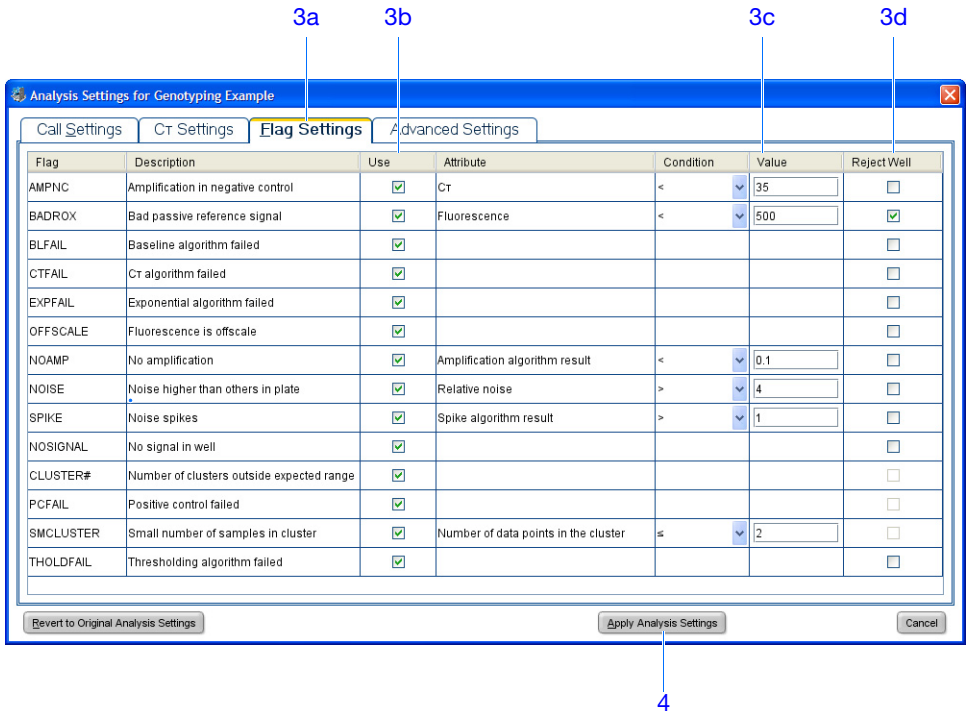
1. In the experiment, click **Analysis Settings**.
2. Adjust the call settings:
   a. Select the **Call Settings** tab.
   b. Select the **SNP1** in the Select a SNP Assay table.
   c. Click **Edit Default Settings**.
   d. If you have made manual calls, select **Keep Manual Calls from Previous Analysis** in the Edit Default Call Settings dialog box.
   e. In the Quality Value field, enter a percentage value to apply as the quality interval for autocalling samples. The greater the value, the more stringent is the allele calling.
   f. Click **Save Changes** to save your settings.



Notes

3. Adjust the flag settings:
   a. Select the **Flag Settings** tab.
   b. In the Use column, select the check box of each flag that you want to enable.
   c. Adjust the value(s) for the enabled flags as needed.
   d. If you want an enabled QC flag to automatically omit wells that test positive for the condition that the flag defines, select the **Reject Well** check box for the flag.

Note: The QC flags allow you to flag conditions and omit wells when certain criteria are met (for example, when a well has missing data). For more information, see the 7500 Software Help.



4. Click **Apply Analysis Settings**.
5. Click **Analyze** to reanalyze the data using the new settings.

Notes

Analysis Guidelines

When you analyze your own experiment:

- If your experiment consists of only two clusters, activate the 2-Cluster Calling algorithm by selecting **2-Cluster Calling Enabled** in the Edit Default SNP Assay Settings dialog box.
- The C_T Settings are available only for experiments that include amplification data. Experiments that consist only of Pre- and Post-PCR reads do not use the threshold cycle (C_T) system for analysis. To adjust the C_T settings:

  a. Select the **C_T Settings** tab.

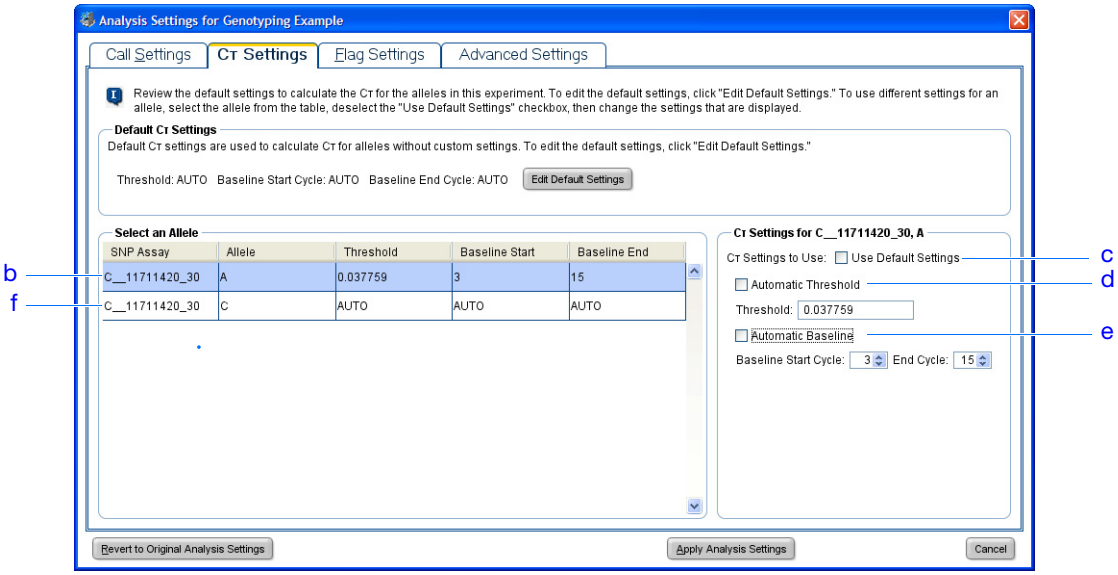
  b. Select an allele in the Select an Allele table.

  c. Deselect **Use Default Settings**.

  d. Deselect **Automatic Threshold**, then enter a new threshold value.

  e. Deselect **Automatic Baseline**, then enter new baseline values.

  f. Repeat steps b through e for the remaining allele.



Note: For more information on setting the threshold cycles for genotyping runs, see the 7500 Software Help.

- You can call sample data:
  - Automatically, using the Autocaller (see page 62)
  - Manually, using the toolbar and scatterplot (see page 63)

For More Information

For more information on the Analysis Settings, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the QC Summary

Review the summary of QC flags generated by the experiment data and troubleshoot them. The QC summary displays the frequency and location of all QC flags. If a flag does not appear in the experiment, its frequency is 0. If the frequency is not 0, the flag was generated by the well(s) listed in the location column. Clicking a flag displays the flag details, including a list of all flagged wells.

About the Example Data

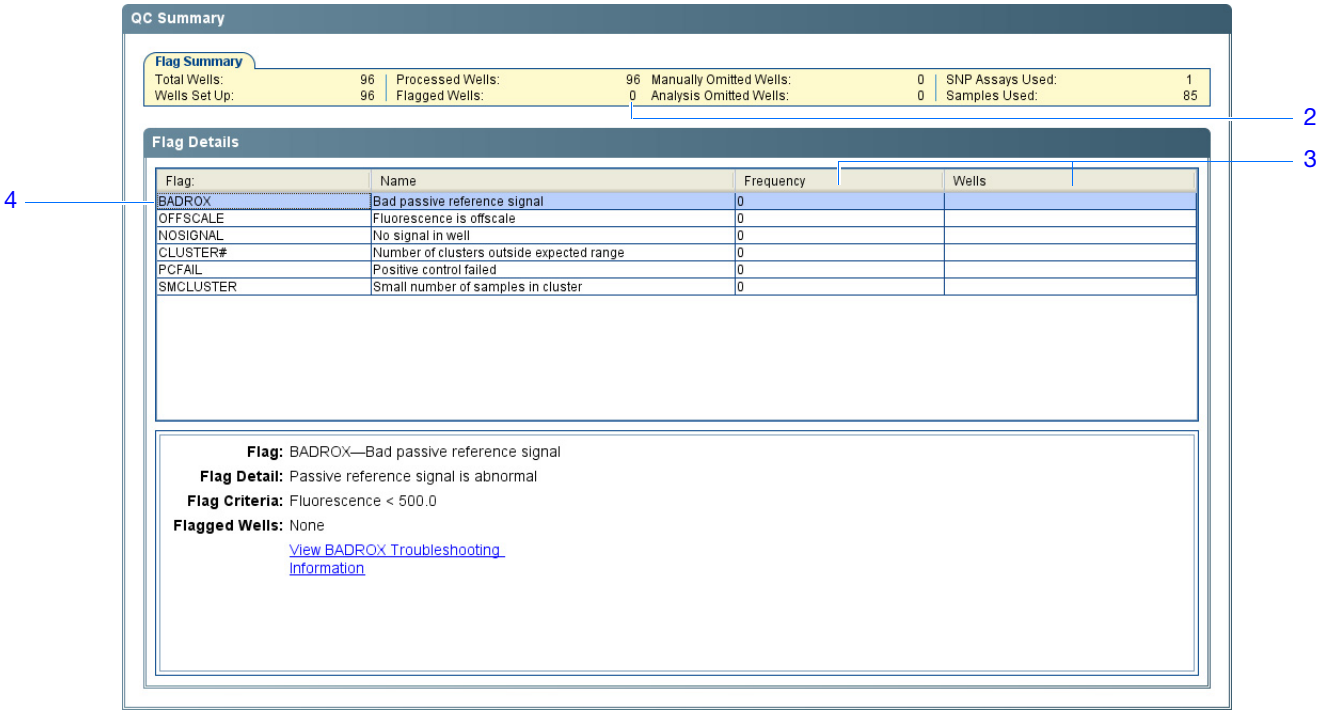
In the example experiment, you review the QC Summary screen for any flags that are generated by the experiment data; however, no flags have been generated.

View the Summary

1. In the navigation column, select **QC Summary**.
2. Review the Flag Summary. In the example experiment, there are 0 flagged wells.
3. In the Flag Details table, look in the Frequency and Wells columns to determine the flags that appear in the experiment. In the example experiment, the Frequency column displays 0 for all flags.

   Note: A 0 displayed in the Frequency column indicates that the flag does not appear in the experiment.

4. (Optional) Click each flag row to display details about the flag.



Notes

Possible Flags

For genotyping experiments, the flags listed below may be generated by the experiment data.

If a flag does not appear in the experiment, its frequency is 0. If the frequency is >0, the flag appears somewhere in the experiment; the well position is listed in the Wells column.

Flag	Description
BADROX	The well produced a passive reference signal greater than the limit defined in the analysis settings.
OFFSCALE	The well produced a level of fluorescence greater than the 7500/7500 Fast system can measure.
NOSIGNAL	The well did not produce a detectable level of fluorescence.
CLUSTER#	For the SNP evaluated by the well, the number of clusters that were generated from the experiment data is greater than the limit defined in the analysis settings.
PCFAIL	The positive control did not produce an R_n for the associated allele greater than the limit defined in the analysis settings, indicating that the control may have failed to amplify.
SMCLUSTER	The number of data points in the associated cluster is less than the limit defined in the analysis settings.
AMPNC	The negative control produced a R_n greater than the limit defined in the analysis settings, indicating possible amplification.
NOAMP	The well did not produce an R_n for either allele that is greater than the limit defined in the analysis settings, indicating that the well may have failed to amplify.
NOISE	The background fluorescence (noise) produced by the well is greater than that of the other wells on the reaction plate by a factor greater than the limit defined in the analysis settings.
SPIKE	The amplification plot for the well contains one or more data points that are not consistent with the other points in the plot.
EXPFAIL	The software cannot identify the exponential region of the amplification plot for the well.
BLFAIL	The software cannot calculate the best fit baseline for the data for the well.
THOLDFAIL	The software cannot calculate a threshold for the associated well.
CTFAIL	The software cannot calculate a threshold cycle (C_T) for the associated well.

Analysis Guidelines

When you analyze your own experiment, select each QC flag in the Flag Details table that has a frequency greater than 0, review the frequency and location of the wells that generated the QC flag, then click the link for troubleshooting the flag.

Note: When you select a flag in the Flag Details table, the wells that generated the flag are highlighted in the well table and plate layout.

For More Information

For more information on the QC Flags, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Omit Wells from the Analysis

You can omit one or more wells from analysis if you do not want to consider data generated by the well.

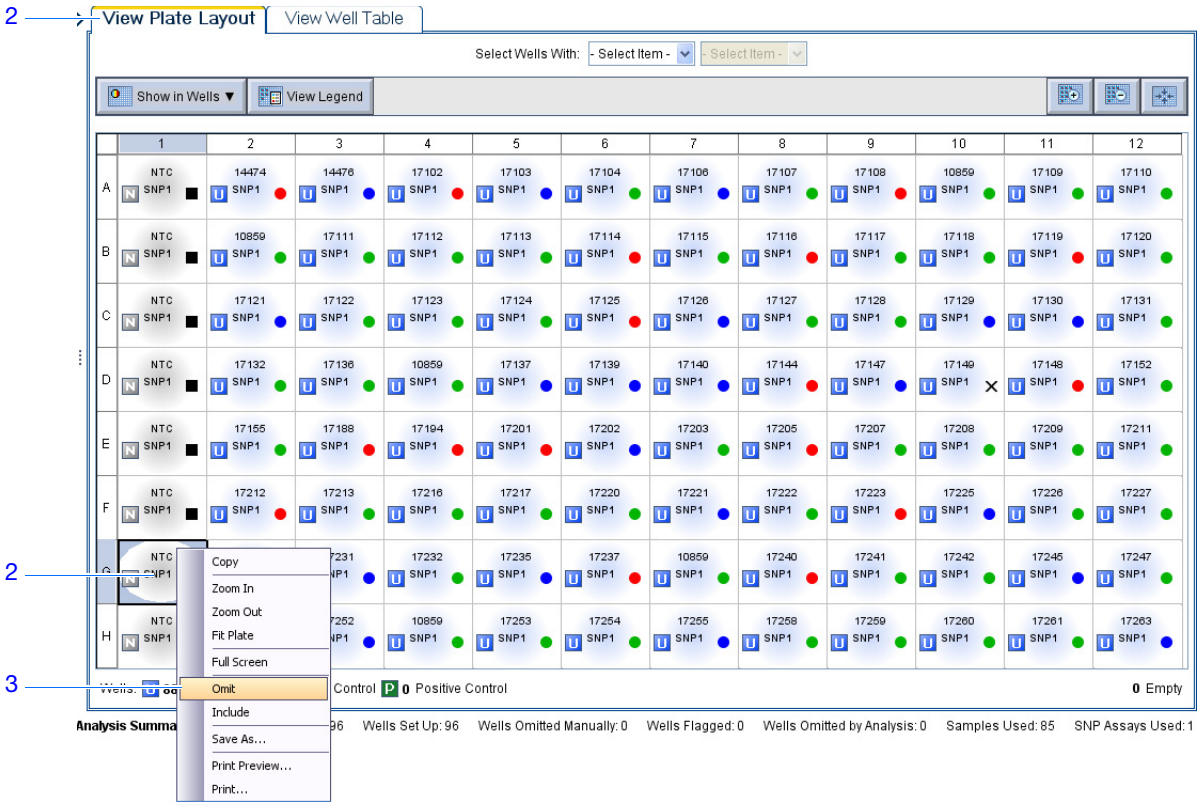
About the Example Experiment

In the example experiment, negative control well G1 exhibits fluorescence and should be removed from the analysis.

Note: The fluorescence from negative control well G1 indicates possible contamination of the well.

Omit Wells

1. Select the **View Plate Layout** tab.
2. Select well **G1** to omit it from analysis.
3. Right-click well **G1**, then select **Omit**. Results for the well are removed.



4. Click **Analyze** to reanalyze the data without the omitted wells.

Notes

Analysis Guidelines

When you analyze your own genotyping experiment, carefully consider which wells to omit from analysis. If needed, remove outliers manually using the well table.

1. In the navigation pane, select **Analysis** ▸  **Amplification Plot**.

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

2. In the Amplification Plot screen, select **C_T vs Well** in the Plot Type drop-down list.
3. Select the **View Well Table** tab.
4. Review the well table:
   a. Look for any outliers.
   b. Select the Omit check box next to the outlying well(s).
5. Click **Analyze** to reanalyze the experiment data with the outlying well(s) removed from the analysis.

For More Information

For more information on omitting wells from the analysis, open the 7500 Software Help by clicking ? or pressing **F1**, then:

1. Select the **Search** tab.
2. Enter **omit well**.
3. Click **List Topics**.
4. Double-click the topics you want to review.

View the Multicomponent Plot

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

About the Example Data

Because the example experiment does not contain real-time data, the 7500 software does not display the Multicomponent plot. If the example experiment contained real-time data, you would review the Multicomponent Plot for:

- ROX™ dye signal (passive reference)
- FAM™ and VIC® dye signals (reporter)
- Spikes, dips, and/or sudden changes
- Amplification in the negative control wells

Notes

View the Plot

Note: The following procedure is provided as an example. Because the example experiment does not contain real-time data, the 7500 software does not display the Multicomponent plot.

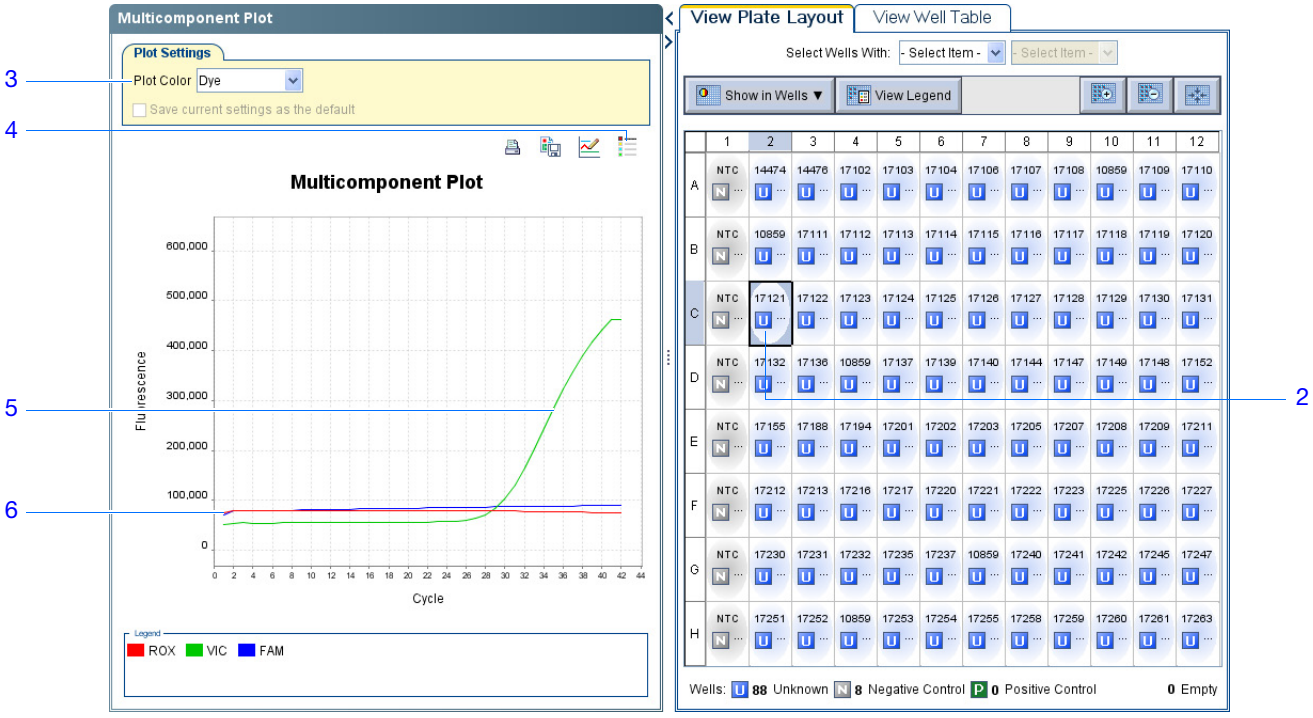
1. In the navigation column, select **Multicomponent Plot**.
2. Select an unknown well in the plate layout to display the corresponding data in the Multicomponent Plot.

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

3. In the Plot Color drop-down list, select **Dye**.
4. Click (Show a legend for the plot).

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

5. Check the FAM™ and VIC® dye signals. In the example below, the VIC® dye signal increases throughout the PCR, indicating normal amplification.
6. Check the ROX® dye signal. In the example below, the ROX dye signal remains constant throughout the PCR process, which indicates typical data.
7. Select the negative control wells one at a time and check for amplification. There should be no amplification in the negative control wells.



Notes

Analysis Guidelines

When you analyze your own experiment, look for:

- Passive reference – The fluorescence of the passive reference dye should remain relatively constant throughout the PCR process.
- Reporter dyes – The fluorescence of the reporter dyes should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- Any irregularities in the signal – There should be no spikes, dips, and/or sudden changes in the fluorescence.
- Negative control wells – There should be no amplification in the negative control wells.

For More Information

For more information on the Multicomponent Plot, open the 7500 Software Help by clicking or pressing **F1**.

Notes

View the Raw Data Plot

If necessary, review the Raw Data Plot for irregularities in the raw spectra collected by the 7500/7500 Fast instrument. The Raw Data Plot displays the amplitude of the raw fluorescence collected in each channel (1 through 5) during the run cycle (indicated by the Show Cycle slider). The plot displays the raw spectra for the wells selected in the plate layout or the well table.

About the Example Data

In the example experiment, review the Raw Data Plot screen for fluorescent signal in the filters that correspond to the dyes used in the experiment (filters 1, 2, and 4).

View the Plot

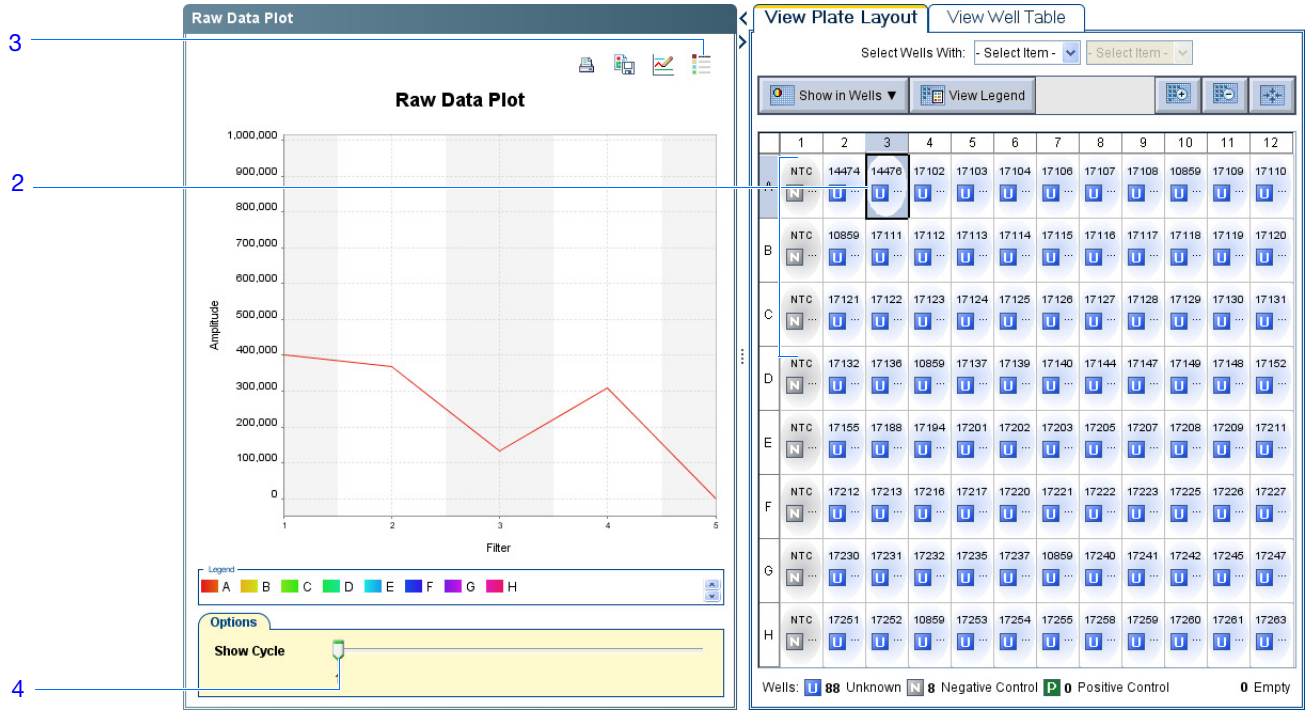
1. In the navigation pane, select **Raw Data Plot**.
2. In the well table, select the wells that you want to inspect.
3. Click (Show a legend for the plot).

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

   Note: The legend displays the color code for each row of the reaction plate.

4. Drag the Show Cycle slider to view temporal changes in each filter of the raw data profile. The example experiment contains only one reading (post-read data).

The figure below shows raw data from the example genotyping experiment.



Notes

Analysis Guidelines

When you analyze your own experiment, look for the following in each filter:

- Characteristic signal growth
- No abrupt changes or dips

If you are reviewing an experiment that contains real-time data, review the Raw Data Plot screen for stable increases in signal (no abrupt changes or dips) in the appropriate filters.

The filters for 7500/7500 Fast instruments are:

Filter	1	2	3	4	5
Dye	• FAM™ dye • SYBR® Green dye	• JOE™ dye • VIC® dye	• TAMRA™ dye • NED™ dye • Cy3® dye	• ROX™ dye • Texas Red® dye	Cy5® dye

For More Information

For more information on the Raw Data Plot, open the 7500 Software Help by clicking or pressing **F1**.

Notes

Alternate Experiment Workflows

This appendix covers:

- Advanced Setup Workflow 90
- QuickStart Workflow 92
- Template Workflow 94
- Export/Import Workflow 96

Note: For more information about any of the topics discussed in this guide, open the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Advanced Setup Workflow

When you create an experiment using Advanced Setup in the 7500 software, you can set up the experiment according to your own design.

1. Double-click (7500 software) or select **Start ▸ All Programs ▸ Applied Biosystems ▸ 7500 Software ▸ *<software name>***

   where *<software name>* is the current version of the 7500 software.

2. In the Home screen, click **Advanced Setup**.

   Note: If you do not see the Advanced Setup icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

3. To set up a new experiment:

   a. Click **Experiment Properties** (default), enter the experiment name, then select the experiment properties.

   b. Click **Plate Setup**:

Experiment Type	Action
Genotyping	Define the SNP assays, then assign them to wells in the reaction plate.
All other experiments	Define the targets, then assign them to wells in the reaction plate.

   c. Click **Run Method**, review the reaction volume and thermal profile, then edit as needed.

   d. Click **Reaction Setup**, review the components and calculated volumes for the PCR reactions, then edit as needed.

   e. (Optional) Click **Materials List**, review the list of materials, then order the materials you need to prepare the reaction plate.

4. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

Notes

5. Run the experiment:

   IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

   a. Load the reaction plate into the instrument.
   b. Start the run.
   c. (Optional) Monitor the run.
   d. Unload the reaction plate from the instrument.

6. Analyze the data:

   a. Open the experiment in the 7500 software.
   b. In the navigation pane, click **Analysis**.
   c. If the data are not analyzed, click **Analyze**.
   d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

QuickStart Workflow

When you create an experiment using QuickStart, you can run the reactions on the instrument with no reaction plate setup information.

1. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

2. QuickStart the experiment:
   a. Double-click  (7500 software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***
      where *<software name>* is the current version of the 7500 software.
   b. In the Home screen, click  **QuickStart**.
   c. Select the **Experiment Properties** tab (default), enter the experiment name, then select the experiment properties.
   d. Select the **Run Method** tab, review the reaction volume and thermal profile, then edit as needed.
3. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

   a. Load the reaction plate into the instrument.
   b. Start the run.
   c. (Optional) Monitor the run.
   d. Unload the reaction plate from the instrument.

Notes

4. In the 7500 software, complete the plate setup:

Experiment Type	Select, then complete the…
Genotyping	a. **Define SNP Assays and Samples** tab. b. **Assign SNP Assays and Samples** tab.
All other experiments	a. **Define Targets and Samples** tab. b. **Assign Targets and Samples** tab.

5. Analyze the data:

   a. Open the experiment in the 7500 software.

   b. In the navigation pane, click **Analysis**.

   c. If the data are not analyzed, click **Analyze**.

   d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

Template Workflow

You can use a template to create a new experiment. Templates allow you to create many experiments with the same setup information.

Create a Template

1. Double-click (7500 software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where *<software name>* is the current version of the 7500 software.

2. Open an existing experiment, or create a new experiment.

   Note: You can create a new experiment using the Design Wizard (see Chapter 2) or Advanced Setup (see page 90).

3. Select **File** ▸ **Save As Template**.
4. Enter a file name, select a location for the template, then click **Save**.
5. Click **Close**.

Create an Experiment with a Template

1. In the Home screen, click **Template**.

   Note: If you do not see the Template icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

2. Locate and select the template you created above, then click **Open**. A new experiment is created using the setup information from the template:
   - Experiment properties
   - Plate setup
   - Run method
   - Reaction setup
3. (Optional) If you want to modify the experiment, use Advanced Setup (see page 90).
4. Click **Save**, enter a file name, then click **Save** to save the experiment.

Notes

5. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

6. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

   a. Load the reaction plate into the instrument.

   b. Start the run.

   c. (Optional) Monitor the run.

   d. Unload the reaction plate from the instrument.

7. Analyze the data:

   a. Open the experiment in the 7500 software.

   b. In the navigation pane, click **Analysis**.

   c. If the data are not analyzed, click **Analyze**.

   d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

Export/Import Workflow

Use the Export/Import workflow to set up a new experiment using setup data exported from another experiment. Only reaction plate setup data are exported and imported.

Export Setup Data

1. Double-click (7500 software) or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **7500 Software** ▸ ***<software name>***

   where *<software name>* is the current version of the 7500 software.

2. Open an existing experiment, or create a new experiment.

   Note: You can create a new experiment using the Design Wizard (see Chapter 2) or Advanced Setup (see page 90).

3. Select **File** ▸ **Export**.

4. Select the **Export Properties** tab (default), then:

   a. Select **Setup**.

   b. Select **One File** in the drop-down list.

   c. Enter a name, then select a location for the export file.

   d. Select **(*.txt)** in the File Type drop-down list.

   IMPORTANT! You cannot export *.xml files.

5. (Optional) Select the **Customize Export** tab, then select the appropriate options.

6. Click **Start Export**.

7. When prompted, click **Close Export Tool**.

Create an Experiment with an Exported Text File

You can import plate setup data from an exported text file (*.txt) to complete the reaction plate setup data for your experiment.

IMPORTANT! Be sure that the exported text file that you select contains only reaction plate setup data and that the experiment types match.

1. Import the reaction plate setup data from the exported text file:

   a. Using a spreadsheet application (such as Microsoft® Excel software), open an exported text file.

   b. Replace the parameters of the text file as needed, then save the file as a tab-delimited text file.

Notes

c. In the Home screen, click  **Advanced Setup**.

Note: If you do not see the Advanced Setup icon, click the arrow beneath the Design Wizard icon to expand the Set Up menu.

d. Create a new experiment or open an existing experiment.

e. Select **File ▸ Import**.

f. Click **Browse**, locate and select the text file (*.txt), then click **Select**.

g. Click **Start Import**. The setup data from the exported text file is imported into the open experiment.

Note: If your experiment already contains plate setup information, the software prompts you to replace the plate setup with the data from the text file. Click **Yes** to replace the plate setup.

2. Use Advanced Setup to finish setting up your experiment (see page 90).

3. Prepare the PCR reactions:

Experiment Type	Prepare the…
Relative standard curve	a. Template. b. Sample dilutions. c. Standard dilution series. d. Reaction mix. e. Reaction plate.
Standard curve	
Comparative C_T	a. Template. b. Sample dilutions. c. Reaction mix. d. Reaction plate.
Genotyping	
Presence/absence	

4. Run the experiment:

IMPORTANT! While the 7500/7500 Fast instrument is performing a run, do not create experiments, perform maintenance, or allow the computer to run antivirus software or to enter hibernation. Performing such activities while the instrument is running an experiment will cause gaps in data collection.

a. Select **Sample Setup**.

b. Start the run.

c. (Optional) Monitor the run.

d. Unload the reaction plate from the instrument.

Notes

5. Analyze the data:
    a. Open the experiment in the 7500 software.
    b. In the navigation pane, click **Analysis**.
    c. If the data are not analyzed, click **Analyze**.
    d. In the navigation pane, select an analysis screen to view the data (for example, select **QC Summary** to view a quality summary of the data).

Notes

Bibliography

Afonina, I., Zivarts, M., Kutyavin, I., *et al.,* **1997**. Efficient priming of PCR with short oligonucleotides conjugated to a minor groove binder. *Nucleic Acids Res.* 25:2657–2660.

Kutyavin, I.V., Lukhtanov, E.A., Gamper, H.B., and Meyer, R.B. **1997**. Oligonucleotides with conjugated dihydropyrroloindole tripeptides: base composition and backbone effects on hybridization. *Nucleic Acids Res.* 25:3718–3723.

Kwok, S. and Higuchi, R. **1989**. Avoiding false positives with PCR. *Nature* 339:237–238.

Lakowicz, J.R. **1983**. Energy Transfer. In Principles of Fluorescence Spectroscopy, New York: Plenum Press 303–339.

Lee, L. G., Connell, C. R., and Block, W. **1993**. Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Res.* 21:3761–3766.

Livak, K.J., Marmaro, J., and Todd, J.A. **1995**. Towards fully automated genome-wide polymorphism screening [letter]. *Nat. Genet.* 9:341–342.

Longo, M.C., Berninger, M.S., and Hartley, J.L. **1990**. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* 93:125–128.

Mullis, K.B. and Faloona, F.A. **1987**. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155:335–350.

Saiki, R.K., Scharf, S., Faloona, F., *et al.,* **1985**. Enzymatic amplification of ß-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350–1354.

Glossary

Advanced Setup — In the 7500 software, a feature that allows you to set up your experiment according to your experiment design. Advanced Setup provides you with maximum flexibility in the design and setup of your experiment.

AIF — See assay information file (AIF).

allele — For a given target, any of the different sequences that occurs in the population.

allelic discrimination plot — Display of data collected during the post-PCR read. The allelic discrimination plot is a graph of the normalized reporter signal from the allele 1 probe plotted against the normalized reporter signal from the allele 2 probe.

amplicon — A segment of DNA amplified during PCR.

amplification — Part of the instrument run in which PCR produces amplification of the target. For quantitation experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data are used to calculate results. For genotyping or presence/absence experiments, fluorescence data collected during amplification are displayed in an amplification plot, and the data can be used for troubleshooting.

amplification efficiency (EFF%) — Calculation of efficiency of the PCR amplification. The amplification efficiency is calculated using the slope of the regression line in the standard curve. A slope close to −3.32 indicates optimal, 100% PCR amplification efficiency. Factors that affect amplification efficiency:

- **Range of standard quantities** – To increase the accuracy and precision of the efficiency measurement, use a broad range of standard quantities, 5 to 6 logs (10^5 to 10^6 fold).
- **Number of standard replicates** – To increase the precision of the standard quantities and decrease the effects of pipetting inaccuracies, include replicates.
- **PCR inhibitors** – PCR inhibitors in the reaction can reduce amplification and alter measurements of the efficiency.

amplification plot — Display of data collected during the cycling stage of PCR amplification. Can be viewed as:

- Baseline-corrected normalized reporter (ΔRn) vs. cycle
- Normalized reporter (Rn) vs. cycle
- Threshold cycle (C_T) vs. well

amplification stage Part of the instrument run in which PCR produces amplification of the target. The amplification stage is called a cycling stage in the thermal profile and consists of denaturing, primer annealing, and polymerization steps that are repeated.

For quantitation experiments, fluorescence data collected during the amplification stage are displayed in an amplification plot, and the data are used to calculate results. For genotyping or presence/absence experiments, fluorescence data collected during the amplification stage are displayed in an amplification plot, and the data can be used for troubleshooting. See also cycling stage.

assay In the 7500/7500 Fast system, a PCR reaction mix that contains primers to amplify a target and a reagent to detect the amplified target.

Assay ID Identifier assigned by Applied Biosystems to TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays.

assay information file (AIF) Data file on a CD shipped with each assay order. The file name includes the number from the barcode on the plate. The information in the AIF is provided in a tab-delimited format.

assay mix PCR reaction component in Applied Biosystems TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays. The assay mix contains primers designed to amplify a target and a TaqMan® probe designed to detect amplification of the target.

AutoDelta In the run method, a setting to increase or decrease the temperature and/or time for a step with each subsequent cycle in a cycling stage. When AutoDelta is enabled for a cycling stage, the settings are indicated by an icon in the thermal profile:

- AutoDelta on: ▲
- AutoDelta off: ▲

automatic baseline An analysis setting in which the software calculates the baseline start and end values for the amplification plot. You can apply the automatic baseline setting to specific wells in the reaction plate. See also baseline.

automatic C_T An analysis setting in which the software calculates the baseline start and end values and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle (C_T). See also threshold cycle (CT).

baseline In the amplification plot, a line fit to the fluorescence levels during the initial stages of PCR, when there is little change in fluorescence.

baseline-corrected normalized reporter (ΔRn)

The magnitude of normalized fluorescence generated by the reporter:

1. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence generated by the reporter at each cycle during the PCR amplification. In the ΔRn vs. Cycle amplification plot, ΔRn is calculated at each cycle as:

   ΔRn (cycle) = Rn (cycle) − Rn (baseline), where Rn = normalized reporter

2. In genotyping experiments and presence/absence experiments, the difference in normalized fluorescence generated by the reporter between the pre-PCR read and the post-PCR read. In the allelic discrimination plot (genotyping experiments) and the presence/absence plot (presence/absence experiments), ΔRn is calculated as:

   ΔRn = Rn (post-PCR read) − Rn (pre-PCR read), where Rn = normalized reporter

See also normalized reporter (Rn).

biological replicates

Reactions that contain identical components and volumes, but evaluate separate samples of the same biological source (for example, samples from three different mice of the same strain, or separate extractions of the same cell line or tissue sample).

See also technical replicates.

blocked IPC

In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the 7500 software, the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells.

C_T

See threshold cycle (CT).

calibrator

See reference sample.

chemistry

See reagents.

comparative C_T ($\Delta\Delta C_T$) method

Method for determining relative target quantity in samples. With the comparative C_T ($\Delta\Delta C_T$) method, the 7500 software measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. The software determines the relative quantity of target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample.

custom dye

Dye that is not supplied by Applied Biosystems. Custom dyes may be adapted for use in experiments on the 7500/7500 Fast system. When using custom dyes, the custom dye should be added to the Dye Library and a custom dye calibration performed.

cycle threshold

See threshold cycle (CT).

cycling stage

In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. For cycling stages, you can enable AutoDelta settings. See also amplification stage.

data collection

A process during the instrument run in which an instrument component detects fluorescence data from each well of the reaction plate. The instrument transforms the signal to electronic data, and the data are saved in the experiment file. In the 7500 software, a data collection point is indicated by an icon in the thermal profile:

- Data collection on: 
- Data collection off: 

delta Rn (ΔRn)

See baseline-corrected normalized reporter (DRn).

derivative reporter (–Rn′)

The negative first-derivative of the normalized fluorescence generated by the reporter during PCR amplification. In the derivative reporter (–Rn′) vs. temperature melt curve, the derivative reporter signal is displayed in the y-axis.

Design Wizard

A feature in the 7500 software that helps you set up your experiment by guiding you through best practices as you enter your experiment design.

diluent

A reagent used to dilute a sample or standard before adding it to the PCR reaction. The diluent can be water or buffer.

Diluted Sample Concentration (10× for Reaction Mix)

In the 7500 software, a field displayed on the Sample Dilution Calculations tab of the Reaction Setup screen. For this field, enter the sample concentration you want to use to add to the reaction mix for all samples in the experiment. "10× for Reaction Mix" indicates that the software assumes the sample or standard component of the reaction mix is at a 10× concentration. For example, if the diluted sample concentration is 50.0 ng/µL (10×), the final sample concentration in the reaction is 5 ng/µL (1×).

dilution factor

See serial factor.

dissociation curve

See melt curve.

EFF%

See amplification efficiency (EFF%).

endogenous control

A target or gene that should be expressed at similar levels in all samples you are testing. Endogenous controls are used in relative standard curve and comparative C_T ($\Delta\Delta C_T$) experiments to normalize fluorescence for the target you are quantifying. Housekeeping genes can be used as endogenous controls. See also housekeeping gene.

endpoint read

See post-PCR read.

experiment

Refers to the entire process of performing a run using the 7500/7500 Fast system, including setup, run, and analysis. The types of experiments you can perform using the 7500/7500 Fast systems:

- Quantitation - standard curve
- Quantitation - relative standard curve
- Quantitation - comparative C_T ($\Delta\Delta C_T$)
- Melt curve
- Genotyping
- Presence/absence

experiment name Entered during experiment setup, the name that is used to identify the experiment. Experiment names cannot exceed 100 characters and cannot include any of the following characters: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (|), colon (:), or semicolon (;).

experiment type The type of experiment you are performing using the 7500/7500 Fast system:

- Standard curve
- Comparative C_T ($\Delta\Delta C_T$)
- Relative standard curve
- Melt curve (not available in the Design Wizard)
- Genotyping
- Presence/absence

The experiment type you select affects the setup, run, and analysis.

forward primer Oligonucleotide that flanks the 5′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

holding stage In the thermal profile, a stage that includes one or more steps. You can add a holding stage to the thermal profile to activate enzymes, to inactivate enzymes, or to incubate a reaction.

housekeeping gene A gene that is involved in basic cellular functions and is constitutively expressed. Housekeeping genes can be used as endogenous controls. See also endogenous control.

internal positive control (IPC) In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. You can use the IPC to distinguish between true negative results (that is, the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.

inventoried assays TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays that have been previously manufactured, passed quality control specifications, and stored in inventory.

IPC In presence/absence experiments, abbreviation for internal positive control (IPC). In the 7500 software, the task for the IPC target in wells that contain the IPC and do not contain IPC blocking agent. See also internal positive control (IPC).

IPC blocking agent Reagent added to PCR reactions to block amplification of the internal positive control (IPC).

IPC+ See negative control-IPC wells.

made-to-order assays TaqMan® Gene Expression Assays or TaqMan® SNP Genotyping Assays that are manufactured at the time of order. Only assays that pass manufacturing quality control specifications are shipped.

manual baseline An analysis setting in which you enter the baseline start and end values for the amplification plot. You can apply the manual baseline setting to specific wells in the reaction plate.

manual C_T An analysis setting in which you enter the threshold value and select whether to use automatic baseline or manual baseline values. The software uses the baseline and the threshold values to calculate the threshold cycle (C_T).

melt curve	A plot of data collected during the melt curve stage. Peaks in the melt curve can indicate the melting temperature (Tm) of the target or can identify nonspecific PCR amplification. In the 7500 software, you can view the melt curve as normalized reporter (Rn) vs. temperature or as derivative reporter (−Rn′) vs. temperature. Also called dissociation curve.
melt curve stage	In the thermal profile, a stage with a temperature increment to generate a melt curve.
melting temperature (Tm)	In melt curve experiments, the temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. The Tm is displayed in the melt curve.
multicomponent plot	A plot of the complete spectral contribution of each dye for the selected well(s) over the duration of the PCR run.
negative control (NC)	In the 7500 software, the task for targets or SNP assays in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Previously called no template control (NTC).
negative control-blocked IPC wells	In presence/absence experiments, wells that contain IPC blocking agent instead of sample in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample and amplification of the IPC is blocked. Previously called no amplification control (NAC).
negative control-IPC wells	In presence/absence experiments, wells that contain IPC template and buffer or water instead of sample. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample. Previously called IPC+.
no amplification control (NAC)	See negative control-blocked IPC wells.
no template control (NTC)	See negative control (NC).
nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3′ end of TaqMan® probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantitation. The minor groove binder (MGB) increases the melting temperature (Tm) without increasing probe length. It also allows the design of shorter probes.
normalized quantity	Quantity of target divided by the quantity of endogenous control.
normalized reporter (Rn)	Fluorescence from the reporter dye normalized to the fluorescence of the passive reference.
omit well	An action that you perform before reanalysis to omit one or more wells from analysis. Because no algorithms are applied to omitted wells, omitted wells contain no results.
outlier	For a set of data, a data point that is significantly smaller or larger than the others.

passive reference A dye that produces fluorescence. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in concentrations or volume. Normalization to the passive reference signal allows for high data precision.

plate layout An illustration of the grid of wells and assigned content in the reaction plate. In the 7500/7500 Fast system, the grid contains 8 rows and 12 columns.

In the 7500 software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.

point One standard in a standard curve. The standard quantity for each point in the standard curve is calculated based on the starting quantity and serial factor.

positive control In genotyping experiments, a DNA sample with a known genotype, homozygous or heterozygous. In the 7500 software, the task for the SNP assay in wells that contain a sample with a known genotype.

post-PCR read Used in genotyping and presence/absence experiments, the part of the instrument run that occurs after amplification. In genotyping experiments, fluorescence data collected during the post-PCR read are displayed in the allelic discrimination plot and used to make allele calls. In presence/absence experiments, fluorescence data collected during the post-PCR read are displayed in the presence/absence plot and used to make detection calls. Also called endpoint read.

pre-PCR read Used in genotyping and presence/absence experiments, the part of the instrument run that occurs before amplification. The pre-PCR read is optional but recommended. Fluorescence data collected during the pre-PCR read can be used to normalize fluorescence data collected during the post-PCR read.

primer mix PCR reaction component that contains the forward primer and reverse primer designed to amplify the target.

primer/probe mix PCR reaction component that contains the primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.

pure dye See custom dye and system dye.

quantitation method In quantitation experiments, the method used to determine the quantity of target in the samples. In 7500/7500 Fast systems, there are three types of quantitation methods: standard curve, relative standard curve, and comparative C_T ($\Delta\Delta C_T$).

quantity In quantitation experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.

quencher A molecule attached to the 3′ end of TaqMan® probes to prevent the reporter from emitting fluorescence while the probe is intact. With TaqMan® reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher. With SYBR® Green reagents, no quencher is used.

QuickStart A feature in 7500/7500 Fast systems that allows you to run an experiment without entering plate setup information.

R^2 value Regression coefficient calculated from the regression line in the standard curve. The R^2 value indicates the closeness of fit between the standard curve regression line and the 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.

ramp The rate at which the temperature changes during the instrument run. For the melt curve step, the ramp can also be defined as a temperature increment. In the graphical view of the thermal profile, the ramp is indicated by a diagonal line.

ramp speed Speed at which the temperature ramp occurs during the instrument run. Available ramp speeds include fast and standard.

- For optimal results using the fast ramp speed, Applied Biosystems recommends using TaqMan® Fast reagents in your PCR reactions.
- For optimal results using the standard ramp speed, Applied Biosystems recommends using standard reagents in your PCR reactions.

IMPORTANT! TaqMan Fast reagents are not supported for genotyping or presence/absence experiments.

raw data plot A plot of raw fluorescence (not normalized) for each optical filter.

reaction mix A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control).

reagents The PCR reaction components you are using to amplify the target and to detect amplification. Types of reagents used on the 7500/7500 Fast systems:

- TaqMan® reagents
- SYBR® Green reagents
- Other reagents

real-time PCR Process of collecting fluorescence data during PCR. Data from the real-time PCR are used to calculate results for quantitation experiments or to troubleshoot results for genotyping or presence/absence experiments.

reference sample In relative standard curve and comparative C_T ($\Delta\Delta C_T$) experiments, the sample used as the basis for relative quantitation results. Also called the calibrator.

refSNP ID Identifies the reference SNP (refSNP) cluster ID. Generated by the Single Nucleotide Polymorphism Database of Nucleotide Sequence Variation (dbSNP) at the National Center for Biotechnology Information (NCBI). The refSNP ID can be used to search the Applied Biosystems Store for an Applied Biosystems SNP Genotyping Assay. Also called an rs number.

regression coefficients Values calculated from the regression line in standard curves, including the R^2 value, slope, and y-intercept. You can use the regression coefficients to evaluate the quality of results from the standards. See also standard curve.

regression line — In standard curve and relative standard curve experiments, the best-fit line from the standard curve. Regression line formula:

$C_T = m [\log (Qty)] + b$

where m is the slope, b is the y-intercept, and Qty is the standard quantity.

See also regression coefficients.

reject well — An action that the software performs during analysis to remove one or more wells from further analysis if a specific flag is applied to the well. Rejected wells contain results calculated up to the point of rejection.

relative standard curve method — Method for determining relative target quantity in samples. With the relative standard curve method, the 7500 software measures amplification of the target and of the endogenous control in samples, in a reference sample, and in a standard dilution series. Measurements are normalized using the endogenous control. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates target quantity in the samples and in the reference sample. The software determines the relative quantity of target in each sample by comparing target quantity in each sample to target quantity in the reference sample.

replicate group — A set of identical reactions in an experiment.

replicates — See technical replicates or biological replicates.

reporter — Fluorescent dye used to detect amplification. If you are using TaqMan® reagents, the reporter dye is attached to the 5′ end. If you are using SYBR® Green reagents, the reporter dye is SYBR® Green dye.

reverse primer — An oligonucleotide that flanks the 3′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

reverse transcriptase — An enzyme that converts RNA to cDNA. Reverse transcriptase is added to the PCR reaction to perform 1-step RT-PCR.

Rn — See normalized reporter (Rn).

ROX™ dye — A dye supplied by Applied Biosystems and precalibrated on the 7500/7500 Fast systems. ROX dye is used as the passive reference.

rs number — See refSNP ID.

run method — Definition of the reaction volume and the thermal profile for the 7500/7500 Fast instrument run.

sample — The template that you are testing.

Sample DNA (10×) — In the 7500 software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample DNA is added to the reaction mix at a 10× concentration. For example, if the reaction volume is 20 µL, the calculated volume of sample for 1 reaction is 2 µL.

Sample Library — In the 7500 software, a collection of samples. The Sample Library contains the sample name and the sample color.

Sample or Standard (10×) — In the 7500 software, a reaction component displayed on the Reaction Mix Calculations tab of the Reaction Setup screen. The software assumes the sample or standard is added to the reaction mix at a 10× concentration. For example, if the reaction volume is 20 µL, the calculated volume of sample or standard for 1 reaction is 2 µL.

sample/SNP assay reaction — In genotyping experiments, the combination of which sample to test and which SNP assay to perform in one PCR reaction. Each PCR reaction can contain only one sample and one SNP assay.

sample/target reaction — In quantitation experiments, the combination of which sample to test and which target to detect and quantify in one PCR reaction. In the Design Wizard, you can detect and quantify only one target in one PCR reaction. Use Advanced Setup to detect and quantify more than one target in one PCR reaction.

serial factor — In the 7500 software, a numerical value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10×, the difference between any 2 adjacent points in the curve is 10-fold.

series — See standard dilution series.

slope — Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of −3.32 indicates 100% amplification efficiency. See also amplification efficiency (EFF%) and regression line.

SNP — Abbreviation for single nucleotide polymorphism. The SNP can consist of a base difference or an insertion or deletion of one base.

SNP assay — Used in genotyping experiments, a PCR reaction that contains primers to amplify the SNP and two probes to detect different alleles.

SNP Assay Library — In the 7500 software, a collection of SNP assays to add to genotyping experiments. The SNP assays in the library contain the SNP assay name, SNP assay color, and for each allele, the allele name or base(s), reporter, quencher, and allele colors. The SNP assays in the library may also contain the assay ID and comments about the SNP assay.

region of interest (ROI) calibration — Type of 7500/7500 Fast system calibration in which the system maps the positions of the wells in the sample block. ROI calibration data are used so that the software can associate increases in fluorescence during a run with specific wells in the reaction plate.

stage — In the thermal profile, a group of one or more steps. There are three types of stages: holding stage (including pre-PCR read and post-PCR read), cycling stage (also called amplification stage), and melt curve stage.

standard — Sample that contains known standard quantities. Standard reactions are used in quantitation experiments to generate standard curves. See also standard curve and standard dilution series.

standard curve

In standard curve and relative standard curve experiments:

- The best-fit line in a plot of the C_T values from the standard reactions plotted against standard quantities. See also regression line.
- A set of standards containing a range of known quantities. Results from the standard curve reactions are used to generate the standard curve. The standard curve is defined by the number of points in the dilution series, the number of standard replicates, the starting quantity, and the serial factor. See also standard dilution series.

standard curve method

Method for determining absolute target quantity in samples. With the standard curve method, the 7500 software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples. See also standard and standard curve.

standard dilution series

In standard curve and relative standard curve experiments, a set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards. For example, the standard stock is used to prepare the first dilution point, the first dilution point is used to prepare the second dilution point, and so on. In the 7500 software, the volumes needed to prepare a standard dilution series are calculated by the number of dilution points, the number of standard replicates, the starting quantity, the serial factor, and the standard concentration in the stock. See also standard curve.

standard quantity

A known quantity in the PCR reaction.

- In standard curve experiments, the quantity of target in the standard. In the 7500 software, the units for standard quantity can be for mass, copy number, viral load, or other units for measuring the quantity of target.
- In relative standard curve experiments, a known quantity in the standard. Standard quantity can refer to the quantity of cDNA or the quantity of standard stock in the PCR reaction. The units are not relevant for relative standard curve experiments because they cancel out in the calculations.

starting quantity

When defining a standard curve in the 7500 software, corresponds to the highest or lowest quantity.

step

A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt curve steps), hold temperature, hold time (duration), and you can turn data collection on or off for the ramp or the hold parts of the step. For cycling stages, a step is also defined by the AutoDelta status.

SYBR® Green reagents

PCR reaction components that consist of two primers designed to amplify the target and SYBR® Green dye to detect double-stranded DNA.

system dye

Dye supplied by Applied Biosystems and precalibrated on the 7500/7500 Fast system. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Maintenance Manager.

System dyes of the 7500/7500 Fast system include:

- FAM™ dye
- JOE™ dye
- NED™ dye
- ROX™ dye
- SYBR® Green dye
- TAMRA™ dye
- VIC® dye
- CY3 dye
- CY5 dye
- TEXAS RED® dye

TaqMan® reagents

PCR reaction components that consist of primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.

target

The nucleic acid sequence that you want to amplify and detect.

target color

In the 7500 software, a color assigned to a target to identify the target in the plate layout and analysis plots.

Target Library

In the 7500 software, a collection of targets to add to experiments. The targets in the library contain the target name, reporter, quencher, and target color. The target in the library may also contain comments about the target.

task

In the 7500 software, the type of reaction performed in the well for the target or SNP assay. Available tasks:

- Unknown
- Negative Control
- Standard (standard curve and relative standard curve experiments)
- Positive control (genotyping experiments)
- IPC (presence/absence experiments)
- Blocked IPC (presence/absence experiments)

technical replicates

Identical reactions that contain identical components and volumes and evaluate the same sample.

See also biological replicates.

template

In the Design Wizard of the 7500 software (and in QuickStart for quantitation experiments), the type of nucleic acid to add to the PCR reaction. The recommended template varies according to experiment type:

- Quantitation experiments (standard curve, relative standard curve, and comparative C_T) – cDNA (complementary cDNA), RNA, or gDNA (genomic DNA)

  For quantitation experiments, the template type selection affects the run method, reaction setup, and materials list.
- Genotyping experiments – Wet DNA (gDNA or cDNA) or dry DNA (gDNA or cDNA)

  For genotyping experiments, the template type selection affects the reaction setup.
- Presence/absence experiments - DNA

  For presence/absence experiments, Applied Biosystems recommends adding DNA templates to the PCR reactions.

thermal profile

Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the 7500/7500 Fast instrument run.

threshold

1. In amplification plots, the level of fluorescence above the baseline and within the exponential growth region The threshold can be determined automatically (see automatic CT) or can be set manually (see manual CT).
2. In presence/absence experiments, the level of fluorescence above which the 7500 software assigns a presence call.

threshold cycle (C_T)

The PCR cycle number at which the fluorescence meets the threshold in the amplification plot.

Tm

See melting temperature (Tm).

unknown

In the 7500 software, the task for the target or SNP assay in wells that contain the sample you are testing:

- In quantitation experiments, the task for the target in wells that contain a sample with unknown target quantities.
- In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype.
- In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known.

unknown-IPC wells

In presence/absence experiments, wells that contain a sample and internal positive control (IPC).

y-intercept

In the standard curve, the value of y where the regression line crosses the y-axis. The y-intercept indicates the expected threshold cycle (C_T) for a sample with quantity equal to 1.

Index

Numerics

5′ Nuclease Assay 8
7500/7500 Fast system
  consumables 4
  data collection 2
  filters 3, 88
  reagents 7

A

Advanced Setup 10, 11, 90
allele calling
  automatic 62
  manual 63
Allelic Discrimination Plot 9, 59
  assign calls manually 63
  evaluate the results 59–63
alternate experiment workflows. See workflows.
amplification
  NOAMP flag 82
  view after a run 66–71
Amplification Plot 66
  typical 69
  view after a run 66–72
Amplification Plot screen
  monitor during a run 50
AMPNC flag 82
analysis guidelines 61, 65, 69, 75, 80, 82, 86, 88
analysis screens
  Allelic Discrimination Plot 59–63
  Amplification Plot 66–71
  Multicomponent Plot 84–86
  Multiple Plots screen 58
  Multiple Plots View 72
  navigation tips 57
  plate layout 64–65
  QC Summary screen 81–82
  Raw Data Plot 87–88
  well table 73–75
analysis settings 78–80
analyze experiment. Also see *troubleshooting*.
  analyze 54
  export data 76
  guidelines 57, 61, 65, 69, 75, 80, 82, 86, 88
  print data 76
  view Allelic Discrimination Plot 59
  view Amplification Plot 66
  view analysis settings 78
  view Multicomponent Plot 84
  view Multiple Plots screen 58
  view plate layout 64
  view QC summary 81
  view Raw Data Plot 87
  view well table 73
  workflow 54
Applied Biosystems
  contacting ix
  customer feedback on documentation ix
  Technical Support ix
Applied Biosystems 7500/7500 Fast Real-Time PCR System. See 7500/7500 Fast system.
assay information file (AIF) 21
assay mix 38
assumptions for using this guide vi
automatic allele calling 62

B

BADROX flag 81, 82
baseline
  about 69
  adjusting 78
  correct values 69
  examples 71
biohazardous waste, handling xvii
biological hazard guidelines xix
BLFAIL flag 82

C

CAUTION, description x
chemical safety xv, xvi
chemical waste safety xvii
CLUSTER flag 82
consumables 29, 37, 38, 40
  See also materials required
  supported 4
control
  negative 23, 24, 61, 64, 86
  positive 23, 24, 61
conventions
  safety x
conventions used in this guide vi
create an experiment
  example experiment 17
  export 76
  print 28, 76
CTFAIL flag 82

customer feedback, on Applied Biosystems documents ix

D

DANGER, description x
data
  about data collection 2
  example experiment 11, 56
design experiment
  create new 17
  define experiment properties 18
  define methods and materials 20
  finish design wizard 32
  for more information 33
  guidelines 19, 21, 22, 24, 26, 28, 31, 33
  order materials 29
  set up reactions 27
  set up run method 25
  set up samples and replicates 23
  set up SNP assays 21
  workflow 16
design wizard 14
  Experiment Properties screen 18
  finish 32
  Materials List screen 29
  Methods and Materials screen 20
  Reaction Setup screen 27
  Run Method screen 25
  Samples and Replicates screen 23
  SNP Assays screen 21
deviation, standard 70
documentation, related vii

E

electrical safety xviii
electromagnetic compatibility standards. *See* EMC standards
EMC standards xx
endpoint experiment 7
ergonomics, safety xix
example experiment
  analyze 54
  data 59, 64, 66, 73, 78, 81, 84, 87
  description 12
  design 16, 20, 21, 23, 25, 27
  plate layout 12
  prepare 36
  prepare reactions 36
  run 44
  workflow 14
Experiment Properties screen 18
EXPFAIL flag 82
export data 76
Export/Import 11, 96

F

flag
  AMPNC 82
  BADROX 82
  BLFAIL 82
  CLUSTER 82
  CTFAIL 82
  EXPFAIL 82
  NOAMP 82
  NOISE 82
  NOSIGNAL 82
  OFFSCALE 82
  PCFAIL 82
  SMCLUSTER 82
  SPIKE 82
  THOLDFAIL 82
flags 74, 81
  in comparative CT experiments 82
fluorescence
  NOISE flag 82
  NOSIGNAL flag 82
  OFFSCALE flag 82
  SPIKE flag 82

G

guidelines
  analysis 57, 61, 65, 69, 75, 80, 82, 86, 88
  chemical safety xvi
  chemical waste disposal xvii
  chemical waste safety xvii
  design 19, 21, 22, 24, 26, 28, 31, 33
  preparation 37, 39, 41
  run 49

H

hazard icons. *See* safety symbols, on instruments
hazard symbols. *See* safety symbols, on instruments
hazards. *See* safety
Help system, opening ix

I

IMPORTANT, description x
installation category xviii
instrument operation, safety xiv

L

load reaction plate 40, 46

M

manual allele calling 63
Materials List screen 29
materials required 29, 37, 38, 40
Methods and Materials screen 20

monitor run
  Amplification Plot screen 50
  Run Method screen 51
moving and lifting, safety xiv
moving parts, safety xviii
MSDSs
  description xv
  obtaining ix, xv
Multicomponent Plot 84
Multiple Plots screen 58
multiple plots, viewing 72

N

navigation tips
  display multiple plots 58
  select wells 57
negative control 64, 86
  AMPNC flag 82
  apply 23, 24
  confirm 61, 73
no signal flag 82
NOAMP flag 82
NOISE flag 82
non-specific fluorescence 8
NOSIGNAL flag 82
notification settings 47

O

OFFSCALE flag 82
omit wells 74, 75, 83
online Help. *See* Help system
other fluorescent-based reagents 10
overvoltage category (rating) xviii

P

passive reference
  BADROX flag 82
PCFAIL flag 82
physical hazard safety xviii
plate layout 64
positive control
  apply 23, 24
  confirm 61, 73, 75
  PCFAIL flag 82
preparation guidelines 37, 39, 41
prepare experiment
  for more information 36
  guidelines 37, 39, 41
  reaction mix 38
  reaction plate 40
  sample dilutions 37
prepare for run 36, 45
print data 76

Q

QC Summary screen 81
QuickStart 11, 92

R

radioactive waste, handling xvii
ramp speed 21
Raw Data Plot 87
reaction mix
  order 29
  prepare 38
  set up 27
reaction plate
  layout 12
  load 46
  order 29
  prepare 40
  standard versus Fast 6
  unload from the instrument 52
Reaction Setup screen 27
reagents
  order 29
  other fluorescent-based 10
  supported 10
  TaqMan® reagents 7–9, 20
repetitive motion, safety xix
results
  assigning calls automatically 62
  assigning calls manually 63
  interpreting 54
run experiment
  alerts 52
  enable notification settings 47
  for more information 52
  guidelines 49
  monitor 50
  prepare for 45
  start 49
  workflow 44
run method
  set up 25
Run Method screen 25
  monitor during a run 51

S

safety
  before operating the instrument xiv
  biological hazards xix
  chemical xv
  chemical waste xvii
  conventions x
  electrical xviii
  ergonomic xix
  guidelines xvi, xvii
  instrument operation xiv

moving and lifting instrument xiv
moving parts xviii
moving/lifting xiv
physical hazard xviii
repetitive motion xix
standards xx
workstation xix
safety labels, on instruments xii
safety standards xx
safety symbols, on instruments xi
sample dilutions, calculated volumes 37
Samples and Replicates screen 23
select wells 57, 65
single-nucleotide polymorphism 7
SMCLUSTER flag 82
SNP assay 7
SNP Assays screen 21
SPIKE flag 82
standard deviation, effect of threshold on 70
standard vs. fast plates 6
standards
EMC xx
safety xx
SYBR Green reagents 3, 88
symbols, safety xi

T

TaqMan reagents 3, 88
TaqMan® MGB Probes 7
TaqMan® SNP Genotyping Assays 7
Technical Support, contacting ix
Template 11, 94
template, preparation 37
text conventions vi
THOLDFAIL flag 82
threshold
adjusting 78
correct values 69
examples 70
training, information on ix
troubleshooting
adjust baseline 78
adjust threshold 78
flags 74, 82
omit wells 83
view analysis settings 78
view Multicomponent Plot 85
view QC Summary 81
view Raw Data Plot 87

U

unknown samples 23, 64
unload instrument 52
user attention words, described vi
using this guide
as a tutorial 11
with your own experiments 11

W

WARNING, description x
waste disposal, guidelines xvii
well table 73
wells
negative control 23, 64
omitting 83
plate layout 64
positive control 23, 64
selecting 57, 65
unknown 64
unknowns 23
well table 73
workflows
Advanced Setup 90
Design Wizard 14
Export/Import 11, 96
QuickStart 11, 92
Template 11, 94
workstation safety xix

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

Applied Biosystems is committed to providing the world's leading technology and information for life scientists.

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

06/2010

Applied Biosystems

Part Number 4387784 Rev. C