ABI PRISM® 7900HT

Sequence Detection System

User Guide

Basic Operation and Maintenance

Applied Biosystems

ABI PRISM® 7900HT

Sequence Detection System

User Guide

Applied Biosystems

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Authorized Thermal Cycler

This ABI PRISM® 7900 HT Sequence Detection System Base Unit, Serial No_____, in combination with its immediately attached sample block modules, comprise an Authorized Thermal Cycler. The purchase price of this Base Unit includes the up_front fee component of a license under United States Patent Nos. 4,683,195, 4,683,202 and 4,965,188, owned by Roche Molecular Systems, Inc., and under corresponding claims in patents outside the United States, owned by F. Hoffmann_La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404.

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Contents

Safety 1

In This Chapter This chapter discusses the following topics:

Attention Words and Warning Labels

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

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

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

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

Instrument Safety Labels

Safety labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used
- A message panel, which explains the hazard and any user action required
- A safety alert symbol, which indicates a potential personal safety hazard. See the *ABI PRISM 7900HT Sequence Detection System Site Preparation and Safety Guide* (P/N 4317595) for an explanation of all the safety alert symbols provided in several languages.

Laser Exposure

The components of the ABI PRISM® 7900HT Sequence Detection System contain several lasers including an Argon laser within the 7900HT instrument and a low power laser within each bar code reader (fixed-position and hand-held).

⚠ WARNING **LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Protect others from exposure to the beam.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Chemical Hazards, Waste Profiles, and Disposal

Chemical Hazard Warning

⚠ WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- 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.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* 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 (*e.g.*, 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 Hazard Warning

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

- 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.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* 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 (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- 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.

About Waste Profiles

A waste profile was provided with this instrument and is contained in the *ABI PRISM 7900HT Sequence Detection System Site Preparation and Safety Guide.* Waste profiles list the percentage compositions of the reagents within the waste stream at installation and the waste stream during a typical user application, although this application may not be used in your laboratory. These profiles assist users in planning for instrument waste handling and disposal. Read the waste profiles and all applicable MSDSs before handling or disposing of waste.

IMPORTANT Waste profiles are not a substitute for MSDS information.

About Waste Disposal

As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below.

- 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, or national regulations.

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

Obtaining Material Safety Data Sheets (MSDS)

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current MSDS before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

To order documents by automated telephone service:

Step	Action
1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

To order documents by telephone:

In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	♦ To order in English, dial **1.800.668.6913** and press **1**, then **2**, then **1.** ♦ To order in French, dial **1.800.668.6913** and press **2**, then **2**, then **1.**
From any other country	See the specific region under "To Contact Technical Support by Telephone or Fax (Outside North America)."

To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS.**
3	Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a PDF version of the MSDS.

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Safe Instrument Use

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Read and understood all related MSDSs

⚠ CAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

Safe and Efficient Computer Use

Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.

To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below.

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

- Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures.
  - The bulk of the person's weight should be supported by the buttocks, not the thighs.
  - Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs.
  - Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
  - The proper height to position the forearms horizontally and upper arms vertically.
  - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
  - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
  - Positioning the screen to minimize reflections from ambient light sources.
  - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.

- When considering the user's distance from the screen, the following are useful guidelines:
  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

Product Overview 2

In This Chapter This chapter discusses the following topics:

System Overview

About the 7900HT Instrument

The ABI PRISM® 7900HT Sequence Detection System is a second-generation sequence detection system instrument designed for automated, high-throughput detection of fluorescent PCR-related chemistries. The instrument is capable of real-time, end-point, and dissociation curve analysis of assays arrayed on multiple formats. The 7900HT instrument is optimized for use with Applied Biosystems chemistries including those related to nucleic acid quantification and detection.

Supported Runs and Chemistries

The ABI PRISM 7900HT Sequence Detection System is designed to support several PCR-related chemistries available from Applied Biosystems and affiliated companies. The SDS software features two types of runs to support the variety of applications.

Run/Analysis	Description	Supported Chemistries
End Point (Plate Read)	In an end-point run, the 7900HT instrument collects a single reading after completing a PCR run. After analysis by the SDS software, the resulting multicomponent data is used to assess the presence of target sequences in the unknown samples.	♦ Allelic Discrimination
Real Time	In an real-time run, the 7900HT instrument collects data during each cycle of a pre-programmed PCR run. After analysis by the SDS software, the resulting threshold cycle values (C_T) are used to establish quantitative relationships between the initial template concentrations of the unknowns and those of the controls.	♦ Absolute Quantification ♦ Dissociation Curve Analysis[a]

a. For dissociation curve analysis, the instrument collects data during a pre-programmed temperature ramp and plots the negative of the first derivative for normalized fluorescence over time.

Note For detailed information on any of the chemistries supported by the ABI PRISM 7900HT Sequence Detection System, see the appropriate chapter for the type of run (see Chapter 5, “End-Point Analysis,” and Chapter 6, “Real-Time Analysis,” respectively).

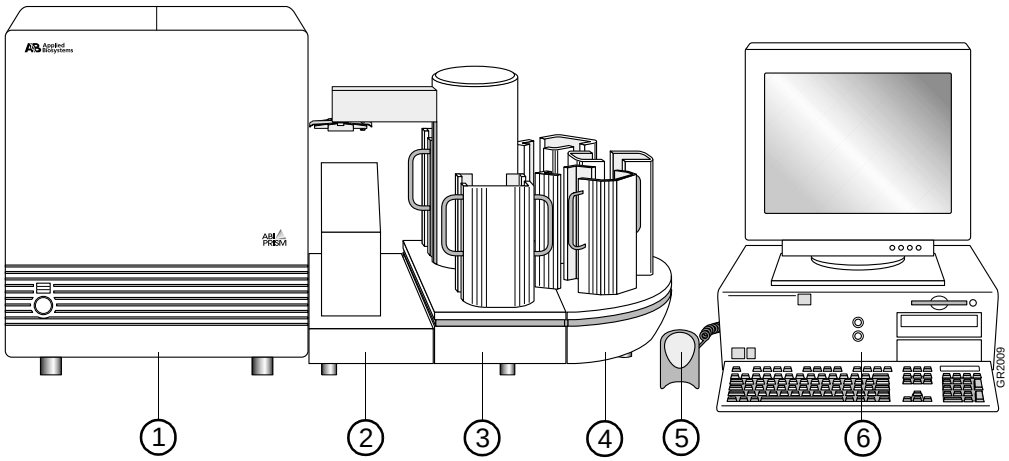
Section: Getting to Know the Hardware

In This Section

This section contains the following information:

Instrument Components

The ABI PRISM 7900HT Sequence Detection System consists of the following components:

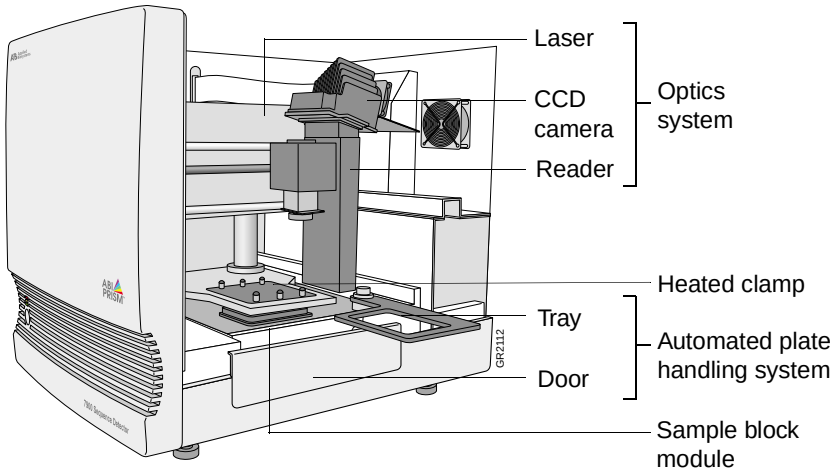


Number	For information on the…		See Page
1	7900HT Instrument		2-4
2	Automation Accessory (optional)	Fixed-Position Bar Code Reader	2-8
3		Zymark® Twister™ Microplate Handler	2-9
4		Extended Capacity Stacks	
5	Hand-Held Bar Code Reader		2-8
6	Microsoft® Windows®-Compatible Computer		2-7

7900HT Instrument

Internal Components

The 7900HT instrument contains the hardware used for thermal cycling and detection of fluorescent chemistries (see page 2-2). The figure below illustrates the major subcomponents of the instrument described in further detail below.



⚠ CAUTION Do not remove the cover to the ABI PRISM 7900HT Sequence Detection System. Only a qualified Applied Biosystems service engineer may repair or adjust the internal components of the 7900HT instrument. Failure to comply can result in serious injury and/or damage to the instrument.

Automated Plate Handling System

⚠ WARNING **PHYSICAL HAZARD**. Keep hands and loose clothing away from the instrument tray and door at all times during instrument operation. The 7900HT instrument contains several internal components that can cause serious physical injury.

The 7900HT instrument features an automated plate handling system to provide easy loading and removal of plates from the instrument. In combination with the automation module, the plate handling system allows unattended operation of the instrument.

Heated Clamp

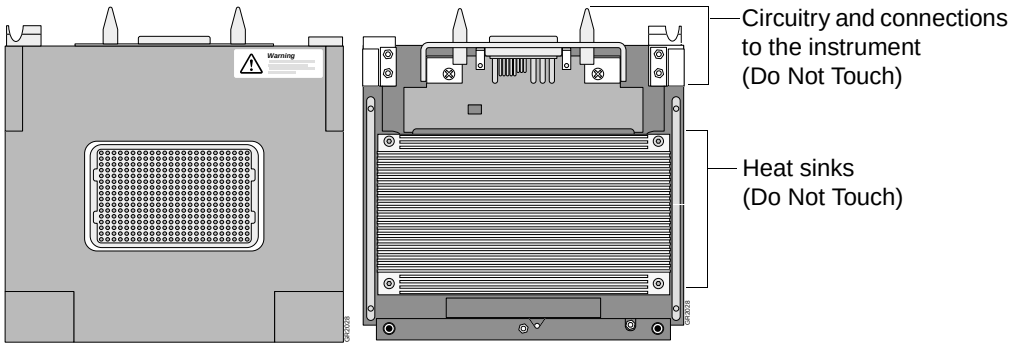
⚠ WARNING **PHYSICAL INJURY HAZARD**. Hot Surface. Use care when working around this area to avoid being burned by hot components.

The 7900HT instrument features a heated clamp that provides optimal heat transfer and uniform heating during thermal cycling. When the instrument tray loads a plate, the clamp applies a downward pressure of 70 lbs (31.8 kg) onto the consumable. During the run, the clamp maintains a constant temperature of 105 °C (± 3 °C) to prevent condensation within the consumable wells.

Interchangeable Thermal Cycler Blocks

⚠ WARNING PHYSICAL INJURY HAZARD. Hot Surface. Use care when working around this area to avoid being burned by hot components.

The 7900HT instrument features a Peltier-based, interchangeable sample block module based on the technology established in the GeneAmp® PCR System 9700 thermal cycler. The internal Peltier heating/cooling unit is housed in the sample block module. The sample block module is made of aluminum to provide the optimal thermal transfer rate.



Top View **Bottom View**

The sample block module provides:

- Wide temperature range: 4–99.9 °C
- Accuracy: ±0.25 °C from 35–99.9 °C
- Heat/cool rate: 1.5 °C per second
- Temperature uniformity: ±0.5 °C (measured 30 sec after the clock starts)
- Long-term stability and high reliability

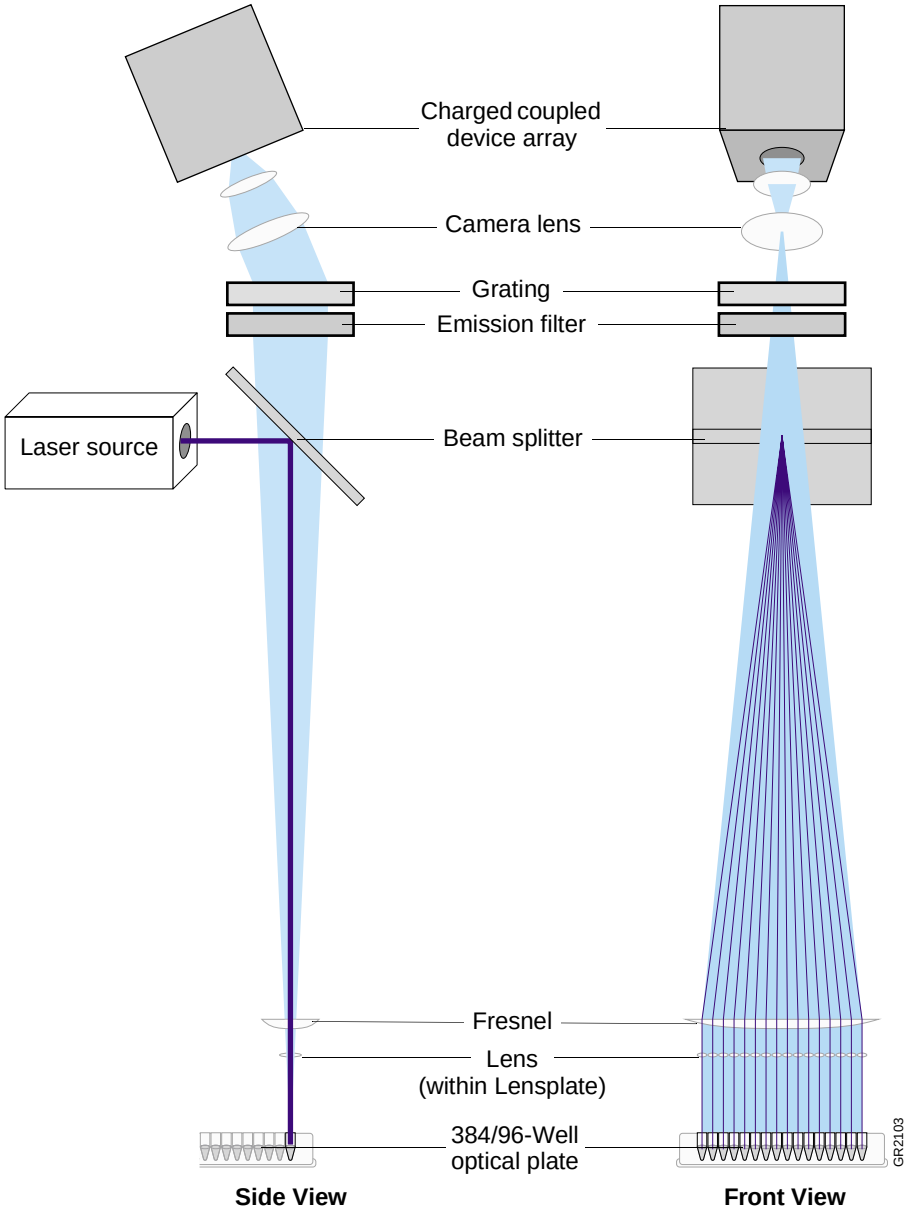
The interchangeable sample block module…	See Page
supports multiple consumable formats.	2-10
provides several different modes of operation (including 9600 mode and programmable temperature ramps).	4-13
reduces instrument downtime by allowing immediate replacement of the block.	7-4
permits easy access to the sample block for troubleshooting and maintenance.	

Optics System

IMPORTANT Do not remove the cover to the 7900HT instrument. Only a qualified Applied Biosystems service engineer may repair or adjust the internal components.

The optical system of the 7900HT instrument is based on the optics system found in the ABI PRISM® 7700 Sequence Detection System. The figure below illustrates the components of the 7900HT optics system.

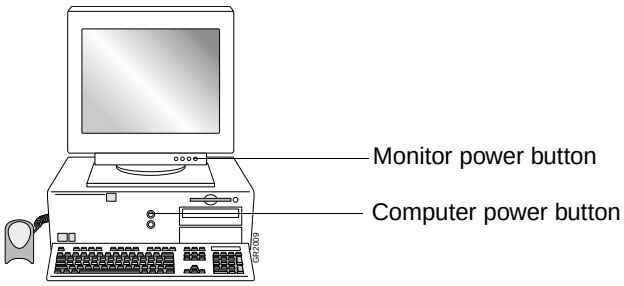
Note For more information about the operation of the optical system of the 7900HT instrument, see Appendix A, “Theory of Operation.”



Computer

Description

The computer coordinates the operation of the Sequence Detection instrument, automation module, and the bar code readers via the SDS software. The figure below illustrates the general configuration of the computer supplied with the ABI PRISM 7900HT Sequence Detection System.



System Requirements

IMPORTANT The following requirements are valid for Version 2.0 of the SDS software and may change with future revisions of the 7900HT instrument software and firmware. Check the release notes accompanying your version of the SDS software for updates.

To run the SDS software and/or to operate the ABI PRISM 7900HT Sequence Detection System, a computer must meet the following minimum requirements:

Component	Minimum Requirement
Processor	Intel Pentium II processor, 400 MHz or faster
Memory	256 MB RAM
Hard Drive	Minimum 25 GB available hard disk space
Additional Drives	CD-ROM drive
Operating Systems	Microsoft Windows NT 4.0 with Service Pack 6A

Hard Drive Partitions

During installation of the 7900HT instrument, the computer hard drive was partitioned to create the following logical drives:

Partition	Size (GB)	Contains
C	2	Operating system files **Note** Applied Biosystems recommends that you do not install programs to the C drive. The computer will boot faster if the C drive contains only the operating system.
D	≥25	♦ SDS 2.0 Software ♦ Automation Controller Software ♦ Additional Third-Party Software ♦ ABI PRISM SDS Plate Document Files

Bar Code Readers

Description

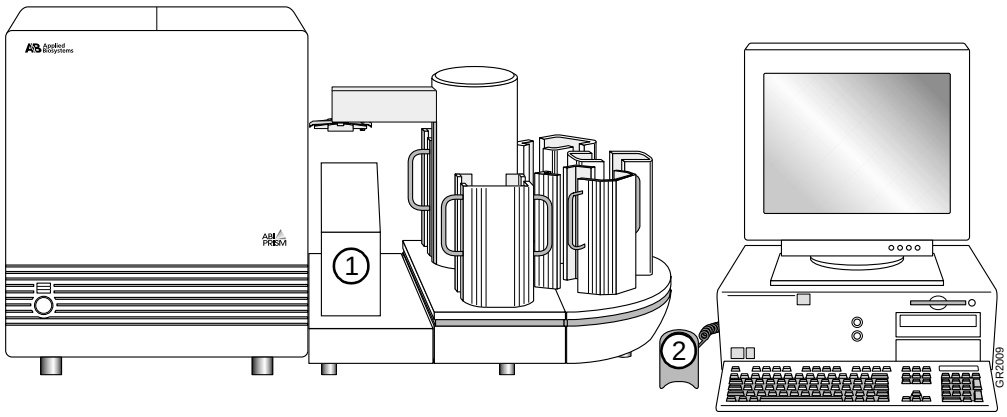
The ABI PRISM 7900HT Sequence Detection System incudes two bar code readers for data entry and plate recognition:

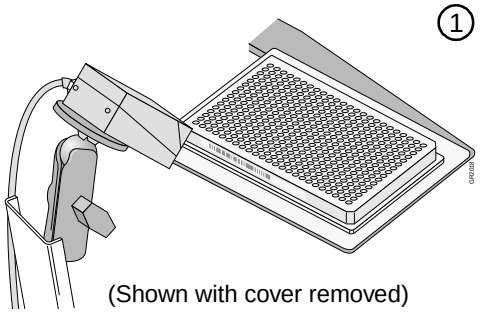
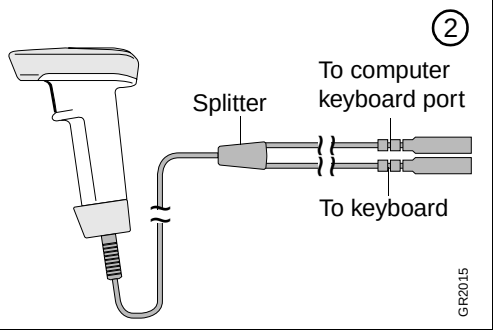
- a hand-held bar code reader for scanning plates manually
- a fixed-position bar code reader for automated scanning of plates as they are loaded into the instrument (available with automation accessory only)

Both bar code readers use a 488 nM laser to scan plates and are capable of reading Code 128 (alpha-numeric), which supports 128 ASCII characters.

Locations of the Bar Code Readers

The following figure illustrates the locations of the bar code readers in the system.



Fixed-Position Bar Code Reader	Hand-Held Bar Code Reader
 (Shown with cover removed)	

Using the Bar Code Readers

⚠ WARNING **LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

For directions on...	See Page
Using the Hand-Held Bar Code Reader	3-19
Aligning the Fixed-Position Bar Code Reader	7-40

Zymark Twister Microplate Handler

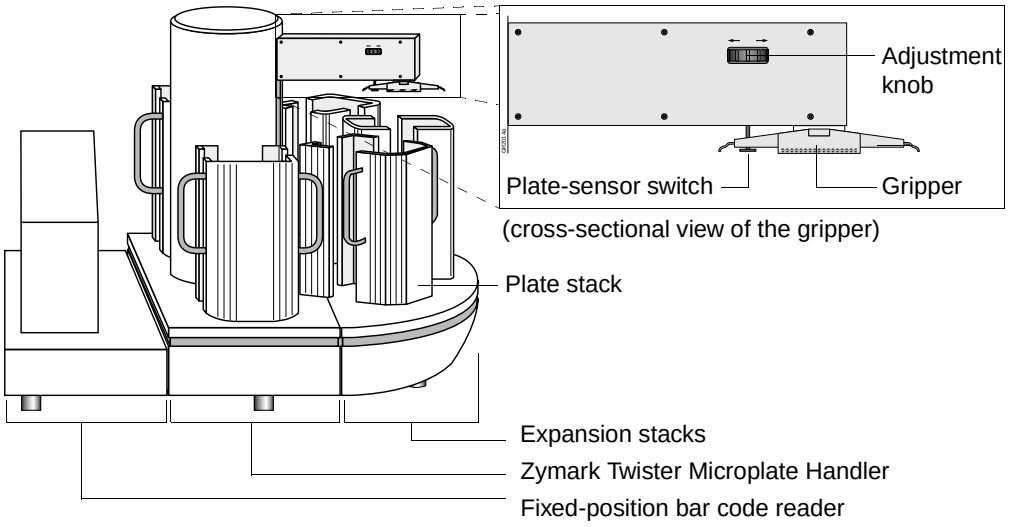
Description

The Zymark Twister Microplate Handler coordinates plate handling for the ABI PRISM 7900HT Sequence Detection System permitting 24-hour unattended operation. The arm features a 310-degree rotational swing that permits access to the 7900HT instrument drawer, up to five plate stacking areas, and the fixed-position bar code reader.

To...	See Page
Turn on the Automation Module	3-5
Align the Automation Module	7-27

Zymark Twister Microplate Handler Components

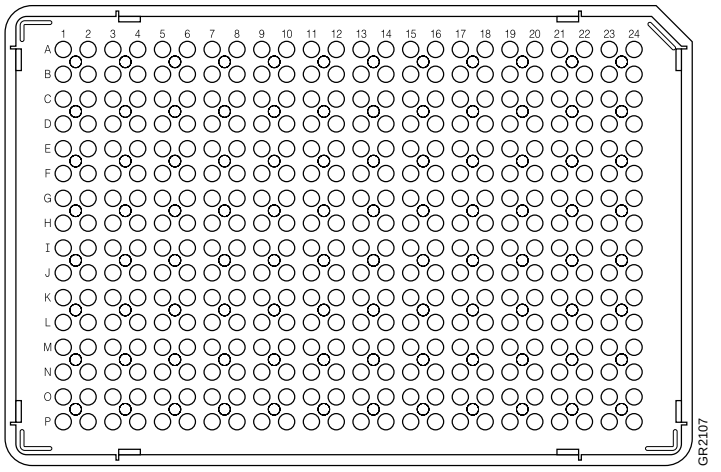
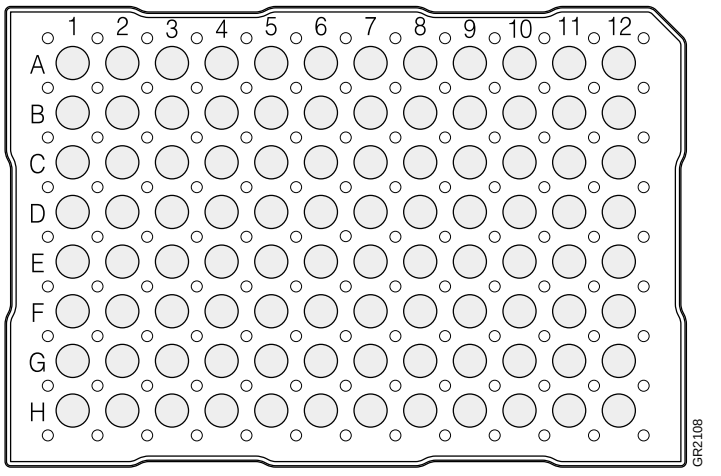
The plate handler consists of the following components:



Compatible Consumables

Consumables for Use with the 7900HT Instrument

The ABI PRISM 7900HT Sequence Detection System can support a variety of consumable formats through the use of interchangeable sample block modules. Applied Biosystems offers sample block modules that support the following consumable formats:

Consumable	Illustration
ABI PRISM™ 384-Well Reaction Plate	
ABI PRISM™ Optical 96-Well Reaction Plate	

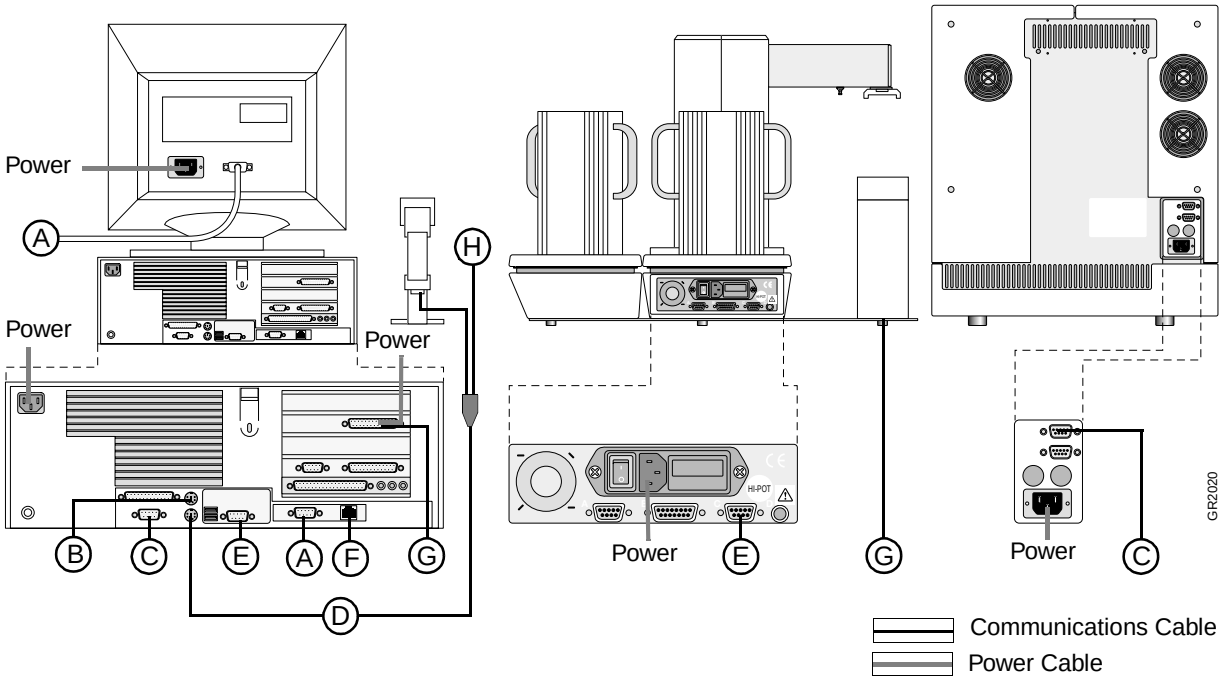
Consumable Requirements

See Appendix D, “Kits, Reagents and Consumables,” for a listing of available consumables, requirements, and purchasing instructions.

Instrument Connections

Electrical Connections

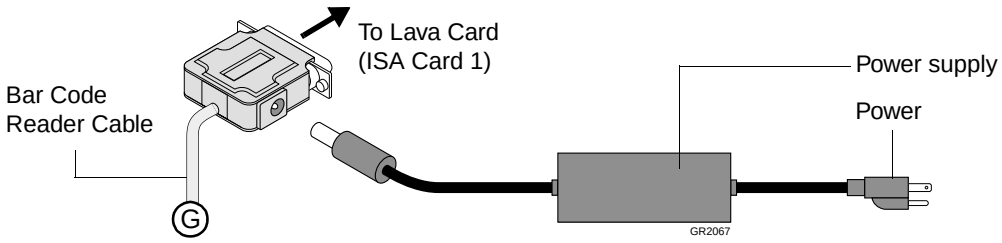
The diagram below illustrates the electrical connections between the components of the ABI PRISM 7900HT Sequence Detection System.



The following table lists the communications cables.

Cable	Type	Connects…	To…
A	Communication	Computer (Monitor Port)	Monitor
B	Comm/Power	Computer (Mouse Port)	Mouse (not shown)
C	Serial	Computer (Serial Port 1)	7900HT Instrument
D	Comm/Power	Computer (Keyboard Port)	Hand-held Bar Code Reader
E	Communication	Computer (Serial Port 2)	Plate Handler (Port C)
F	Ethernet	Network	Computer (Ethernet Port)
G[a]	Comm/Power	Computer (ISA Card 1)	Fixed-Position Bar Code Reader
H	Comm/Power	Bar Code Reader Cable	Keyboard (not shown)

a. See the figure below.



Section: Getting to Know the Software

In This Section This section contains the following information:

Sequence Detection System Software Components and Features

Software Components

The ABI PRISM 7900HT Sequence Detection System uses several software applications to set up, run, and analyze experiments completed on the 7900HT instrument.

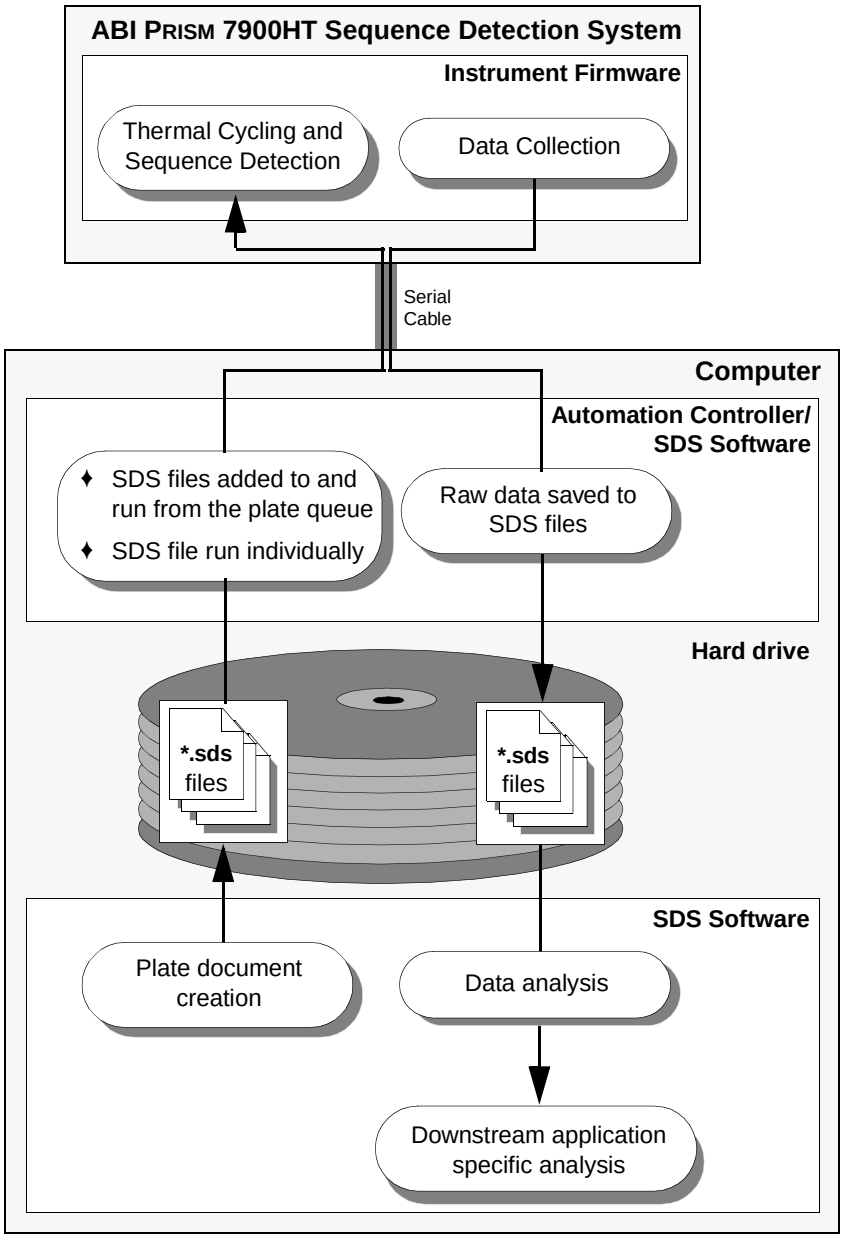
Application	Function
SDS Software	♦ Constructs and edits plate document files (*.sds files) ♦ Performs initial and end analysis of raw data from allelic discrimination and absolute quantification runs ♦ Saves, prints, and exports run data
Automation Controller Software	♦ Controls and coordinates the action of the 7900HT instrument and the automation module ♦ Initiates and controls the sequence detection run ♦ Acquires data during the run
Java® Runtime Environment	It includes additional files and software used to run the SDS software. **IMPORTANT** Do not delete the Java Runtime Environment files. These files are crucial to the operation of the SDS software. If the files are deleted or become corrupt, reinstall the SDS software from the CD as explained on page 7-48.
Zymark Twister Software	Used to calibrate the Zymark Twister Microplate Handler.
LAVA Software	Used to align the fixed-position bar code reader.
Instrument Firmware	♦ Controls the most basic operations of the 7900HT instrument ♦ Controlled by commands sent from the computer ♦ Acts as the link between the software commands and hardware operations

Managing Sequence Detection System Data

7900HT Instrument Dataflow

Data management strategy is a crucial element of successfully integrating the ABI PRISM 7900HT Sequence Detection System into a laboratory workflow. During a single 24-hour period of real-time operation, the 7900HT instrument can produce up to 200 MB of data. To manage the information produced by the 7900HT instrument successfully, it helps to have a basic understanding of how data is collected and processed prior to analysis.

The figure below contains a summary of the 7900HT instrument data flow.



Working with SDS Software Files

Files Used and Created by the SDS Software

IMPORTANT Never move or delete a SDS software file or folder unless specifically directed to do so by an Applied Biosystems representative or documentation.

The SDS software includes many different files and folders. Some of these are created to store run data and calibration data, others are required to run the software.

File Type	Extension	Description
Plate Document Files		
ABI PRISM SDS Single Plate	*.sds	A plate document is a virtual representation of a consumable (plate) used to contain samples and reagents during an sequence detection run. During the run, the software uses the plate document to coordinate the operation of the instrument (thermal cycling, data collection), to organize and store the data gathered during the PCR, and to store the results of the downstream analysis of the run data.
ABI PRISM SDS Template File	*.sdt	Templates contain can be used to create an unlimited number of plate documents. Templates are optional but useful as a time-saving devices for experiments where samples are run on plates with identical assay configurations.
Imported/Exported Files		
Tab-delimited text file	*.txt	The SDS software can export raw or analyzed data in tab-delimited (*.txt) format for all or a select group of wells on a plate document. The exported files are compatible with most spreadsheet applications.
JPEG[a] graphic files	*.jpg	The SDS software can export most panes and plots of the plate document as JPEG graphic files. The JPEG format is compatible with most word processing and spreadsheet applications and can be incorporated directly into HTML documents for viewing by most web browser software.

a. Joint Photographic Experts Group

Average SDS Plate Document File Size

The SDS software can produce SDS files of varying sizes depending on the type of runs for which they are created. The table below lists the average sizes of typical files produced by the SDS software.

Run Type	Average File Size	Compressed File Size[a]
Plate-read	150–180 KB	70–90 KB
Real-time[b]	15–25 MB	10–15 MB

a. Compressed files sizes shown are estimates based on standard compression using the WinZip® utility. For more information, see “Archiving SDS Files” on page 7-46.

b. The maximum file sizes displayed above are nominal for real-time runs (absolute quantification). File size can increase depending on the plate document’s data collection options.

Getting Started 3

In This Chapter This chapter discusses the following topics:

Getting Started

Before You Begin

If this is your first time using the ABI PRISM® 7900HT Sequence Detection System, consider completing the "Basic Software Skills Tutorial" on page 3-11 before continuing. The tutorial will provide you with the fundamental knowledge required to operate the SDS software and will teach you time-saving techniques to allow you to use it quickly and efficiently.

Procedure Quick Reference

The following table contains a list of major procedures described within this manual.

Procedure	See Page
Turning on the ABI PRISM 7900HT Sequence Detection System	3-5
Setting Up and Running SDS Experiments	
Creating an SDS plate document	4-6
Running an individual SDS plate document	4-21
Running batches of SDS plate documents (using the automation accessory)	4-29
Stopping a run in progress	
♦ From the SDS software	4-26
♦ From the Automation Controller Software	4-38
Ejecting a plate	
♦ From the SDS software	4-27
♦ From the Automation Controller Software	4-38
Analyzing Run Data	
Analyzing an allelic discrimination run	5-3
Analyzing an absolute quantification run	6-3
Analyzing a dissociation curve (melting curve) run	6-17
Maintaining the 7900HT Instrument	
Changing the 7900HT Plate Adapter	7-9
Replacing the Sample Block	7-4
Decontaminating the Sample Block	7-11
Performing a Background Run	7-13
Performing a Pure Dye Run	7-17
Adding Custom Dyes to the Pure Dye Set	7-21
Verifying Instrument Performance Using a TaqMan RNase P Plate	7-24
Maintaining the Automation Accessory[a]	
Adjusting the Sensitivity of the Plate Sensor Switch	7-28
Aligning the Plate Handler	7-32
Aligning the Fixed-Position Bar Code Reader	7-40
Cleaning and Replacing Gripper Finger Pads	7-43

a. The automation accessory includes the Zymark® Twister™ Microplate Handler and the fixed-position bar code reader. See "Instrument Components" on page 2-3 for more information.

Using the SDS Software Online Help

The SDS software features an online help system that can guide you through the procedures for setting up, performing, and analyzing runs. To get help at any time, click a Help button located within the dialog box or window in which you are working.

The SDS software provides two ways to access the online help as follows:

To…	Then…
access general help	select **SDS Online Help** from the **Help** menu.
get help for using a specific dialog box, plot, or feature	click a help button (?) located within the dialog box or window in which you are working.

For More Information

For information about the ABI PRISM 7900HT Sequence Detection System or the SDS software, Applied Biosystems recommends the following references:

Title	P/N
ABI PRISM 7900HT Sequence Detection System Site Preparation and Safety Guide	4317595
ABI PRISM 7900HT Sequence Detection System Software Online Help	—
Microsoft Windows Operating System Online Help	—

About This Manual

Intended Audience

This guide is written for technicians, scientists, and researchers who will use ABI PRISM 7900HT Sequence Detection System (SDS) instruments.

Background Needed

This manual assumes that you are familiar with the following:

- Basic Microsoft® Windows® operations such as using the mouse, choosing commands, working with windows, and using the hierarchical file system
- A general understanding of electronic storage devices and data files
- An understanding of assay preparation and basic laboratory techniques

Computer Vocabulary and Operations

To use the ABI PRISM 7900HT Sequence Detection System, you should be familiar with the following basic computer vocabulary and operations:

Vocabulary and Operations	Description
Using the mouse	Clicking and double-clicking, selecting, and dragging.
Choosing commands	Using menus and drop-down lists, dialog boxes, radio buttons, and check boxes.
Working with windows	Opening and closing, resizing and repositioning, scrolling, understanding the active window.
Using the Microsoft Windows hierarchical file system	Finding files and creating folders.

Conventions Used in This Manual

This manual uses the following conventions to convey information:

Convention	Definition	Examples
>	This symbol is used to convey a command or directory path in the Windows operating system.	♦ From the **Start** menu, select **Programs** > **SDS 2.0** > **SDS 2.0**. ♦ Navigate to the **Program Files** > **Applied Biosystems** > **SDS 2.0** > **Templates** directory.
Bold text	Bold text appearing with procedures corresponds to the text as it appears on the screen.	♦ From the **File** menu, select **Save**. ♦ The **Detector Manager** dialog box opens.

How This Manual is Organized

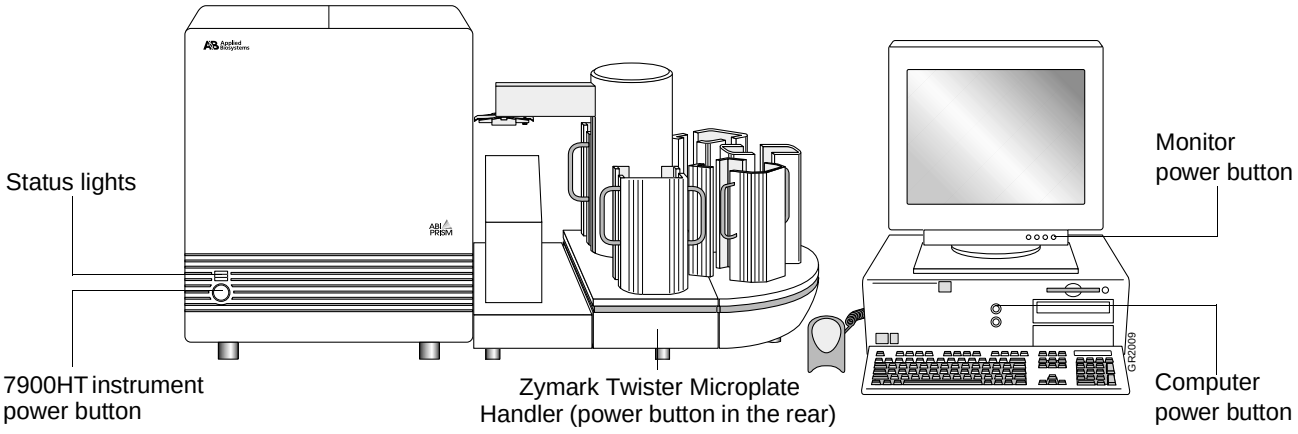
This manual contains the following chapters and supporting appendices:

Chapter/Appendix		Content
1	Safety	Explains information on ABI PRISM 7900HT Sequence Detection System safety
2	Product Overview	Describes the components of the ABI PRISM 7900HT Sequence Detection System and its software
3	Getting Started	Introduces and explains how to use this manual and the SDS software
4	Run Setup and Basic Operation	Explains how to create and run plate documents on the ABI PRISM 7900HT Sequence Detection System
5	End-Point Analysis	Describes how to analyze data from allelic discrimination experiments
6	Real-Time Analysis	Describes how to analyze data from absolute quantification and dissociation curve experiments
7	System Maintenance	Explains how to perform both routine and incidental system maintenance for the components of the ABI PRISM 7900HT Sequence Detection System
8	Troubleshooting	Contains tips for troubleshooting problems with the ABI PRISM 7900HT Sequence Detection System
9	User Bulletins	This chapter is reserved for user bulletins
A	Theory of Operation	Describes the principles behind the operation of the ABI PRISM 7900HT Sequence Detection System
B	Importing and Exporting Plate Document Data	Explains the Import/Export function of the SDS software and also diagrams the structure and annotation of setup table files
C	Designing TaqMan Assays	Contains brief instructions for designing TaqMan probe and Sequence Detection primer sets
D	Kits, Reagents and Consumables	Contains a list of Applied Biosystems kits and consumables for use with the 7900HT instrument
E	References	Contains a bibliography of references for this manual
F	Contacting Technical Support	How to contact Applied Biosystems Technical Support
G	Limited Warranty Statement	The Applied Biosystems limited warranty statement

Turning on the ABI PRISM 7900HT Sequence Detection System

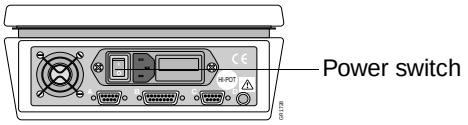
Turning On the 7900HT Instrument

The activation of the ABI PRISM 7900HT Sequence Detection System is sequential, each component must be activated in a specific order for the system to initialize properly. If performed out of sequence, the components may not be able to establish the necessary communication connections.



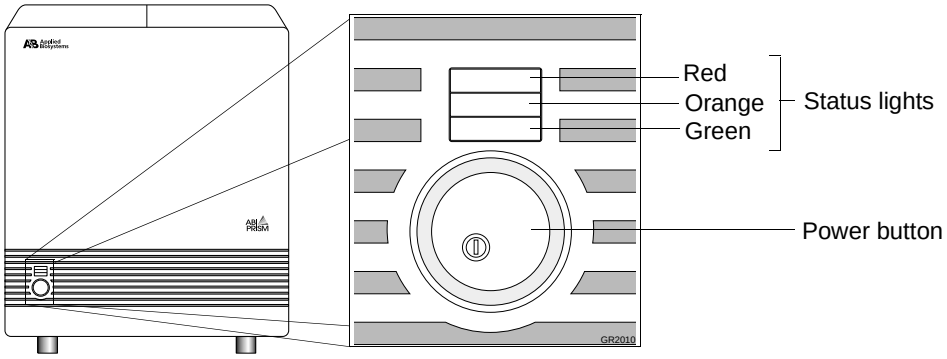
IMPORTANT Turn on the power to the instrument and the plate handler at least 10 min before use. When activated, the instrument heats the sample block cover to 105 °C. If a run is started before the heated cover reaches 105 °C, the instrument will pause until it reaches the optimal temperature before commencing the run.

To activate the components of the ABI PRISM 7900HT Sequence Detection System:

Step	Action
1	Turn on the monitor and computer.
2	Turn on the Zymark Twister Microplate Handler by pressing the power switch located on the back panel of the plate handler (see below). **⚠ CAUTION PHYSICAL HAZARD**. Keep clear of the arm when activating the plate handler. Once activated, the arm automatically moves to its home position.  **Rear Panel of the Twister** If operating normally, the plate handler moves the arm to the home position (over the output stack).
3	Turn on the 7900HT instrument by pressing the power button located below the status lights on the front of the instrument (see the figure at the top of the page). If operating normally, the 7900HT instrument will do the following on startup: ♦ Emit a high-pitched tone signalling that system has been initialized. ♦ Cycle the status lights (Red > Orange > Green) indicating that the 7900HT instrument is active (see “Reading the Instrument Status Lights” on page 3-6 for more information).

Reading the Instrument Status Lights

The 7900HT instrument contains three lights located on the lower-left side of the front panel to indicate the status of the instrument.



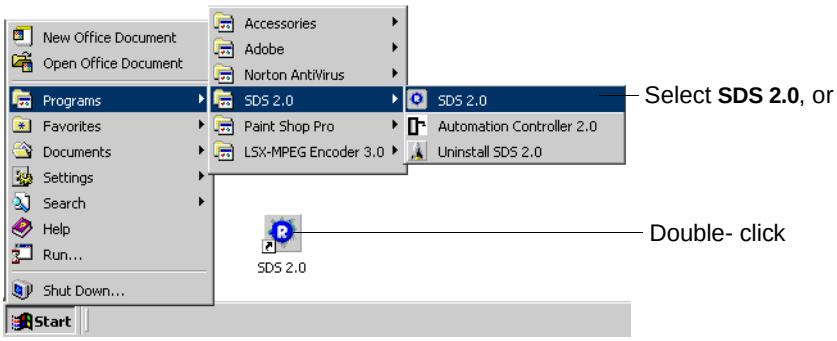
Light/Appearance		Status	Action
Green	Solid	The 7900HT instrument is on and in idle state (ready to run)	None This state indicates normal instrument function.
	Flashing	♦ Interlocks are open and/or the scan head has not reached the safe position. ♦ The instrument door is open.	
Orange	Flashing	The 7900HT instrument is transmitting/receiving data to/from the computer (usually during a run).	None This state indicates normal instrument function.
	Solid	If the light remains on during startup for more than 2 min: ♦ The instrument did not boot properly, or ♦ 7900HT instrument has experienced a system failure	a. Check that the computer is turned on and connected to the instrument. (See page 2-11 for a diagram of instrument connections.) b. If so, turn off the instrument, wait for 30 sec, and then restart as explained on page 3-5.
Red	Solid	The 7900HT instrument has detected a fatal problem.	Turn off the instrument, wait for 30 sec, and then restart.

Using the SDS Software Workspace

Launching the Software

To launch the SDS software, either:

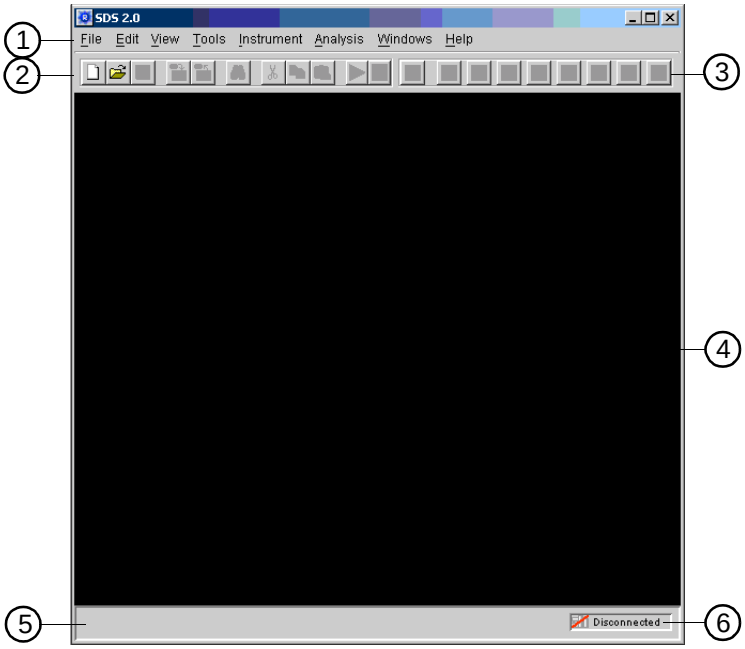
- Select **Start** > **Programs** > **SDS 2.0** > **SDS 2.0**, or
- Double-click the SDS software program icon on the desktop.



The computer launches the SDS software and attempts to establish communication with the 7900HT instrument. If the connection is successful, the software displays the Connected icon (Connected to 'PlateName') in the status bar when a plate document is open. See “About the Status Bar” on page 3-10 for more information.

About the Software Interface

All software operations and displayed information occur within the workspace of the SDS software. The workspace provides quick access to all elements of the software through the menubar and a pair of toolbars. The following figure summarizes the features of the user interface of the SDS software.



The following table describes the elements of the workspace:

Number	Component	Description
1	Menubar	Contains a directory of menus that govern the operation of the software.
2	General Toolbar	Contains clickable icons for controlling the basic functions of the software (file management and basic editorial).
3	Display Toolbar	Contains clickable icons for controlling the display of information within the SDS software workspace.
4	Workspace	Contains all plate documents, dialog boxes, and message boxes used by the SDS software.
5	Message Bar	Displays a variety of messages to indicate the status of the instrument. **Note** See “About the Status Bar” on page 3-10 for a complete description of the message bar.
6	Instrument Connection Icon	Indicates the status of the connection to the 7900HT instrument. **Note** See “About the Status Bar” on page 3-10 for a complete description of the instrument connection icon.

Using the General Toolbar

The following table describes the icons located within the General Toolbar:

Icon	Function
	Creates a new plate document
	Opens an existing plate document
	Saves the current plate document
	Imports data from a text file
	Exports data to a tab-delimited text file
	Opens the SDS software Find utility
	Removes the selected object and places it into memory
	Copies the selected object into memory
	Inserts a cut object into the current selection
	Analyzes the current plate document
	Opens the Analysis Options dialog box for the current plate document

Using the Display Toolbar

The following table describes the icons located within the Display Toolbar:

Icon	Function
	Hides or shows the Well Inspector Panel
	Hides or shows the Plate Grid
	Hides or shows the Table View
	Hides or shows the System Raw Data Plot
	Hides or shows the Multicomponent Plot
	Hides or shows the Amplification Plot
	Hides or shows the Standard Curve Plot
	Hides or shows the Dissociation Plot
	Zooms the plate grid in or out
	Opens the Display Settings dialog box used to modify the appearance of the plate document plate grid, plots, and views

About the Status Bar

The status bar consists of two components: a message bar for indicating the status of software functions and a instrument status icon for indicating the status of the instrument.

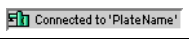
About the Message Bar

The message bar displays a variety of messages to indicate the status of the instrument. The following table summarizes all of the messages displayed in the Message bar.

Message	Then the SDS software is…
Ready	ready and awaiting instructions.
Collecting Data	currently running a plate document.
Reanalyze data	requesting analysis of plate document data. The Analysis Options for the plate document have been changed and the document requires reanalysis for them to take effect.
Analyzing data... + Progress bar	completing the calculations for the current analysis. The metered bar to the right of the message displays the progress of the analysis.
Saving data... + Progress bar	saving the plate document or template to a storage device. The metered bar to the right of the message displays the progress of the action.
Importing data...	importing a file. The metered bar to the right of the message displays the progress of the action.
Exporting data... + Progress bar	exporting the data within the current plate document to a file. The metered bar to the right of the message displays the progress of the action.

About the Instrument Status Icon

Indicates the status of the connection to the 7900HT instrument.

Icon	Instrument Status
	Connected and awaiting a command
	Not connected or turned off

Basic Software Skills Tutorial

About This Tutorial

This tutorial will:

- Teach you to create, save, print, export, and import SDS plate documents
- Familiarize you with the basic components of the SDS software interface
- Explain how to customize and arrange the user interface to suit your needs
- Teach you to use the hand-held bar code reader
- Provide you with time-saving devices to increase your effectiveness on the SDS software

Using the Online Version of the Basic Skills Tutorial

The SDS Online Help features a version of this tutorial. If you prefer to follow the online tutorial open the SDS Online Help as follows:

Step	Action
1	If not already active, launch the SDS software as explained on page 3-7. The SDS software workspace appears.
2	From the **Help** menu, select **SDS Online Help**.
3	When the **SDS Online Help** appears, select **Basic Skills Tutorial** from the list of options.
4	Follow the directions displayed on your screen.

Lesson 1: Using Plate Documents

Every plate run on the ABI PRISM 7900HT Sequence Detection System requires the creation of a plate document within the SDS software. A plate document is a virtual representation of a consumable used to contain samples and reagents during a sequence detection run. The software uses the plate document to coordinate the operation of the instrument (thermal cycling and data collection), to organize and store the data gathered during the PCR, and to analyze the run data.

The SDS software can produce the two types of plate document files described in the table below.

Plate Document File	Extension	Description
ABI PRISM SDS Single Plate	*.sds	SDS Single Plate Documents are the primary file you will use. They are generated for every kind of experiment and are generally used to run plates.
ABI PRISM SDS Template Document	*.sdt	Although optional, templates are useful as time-saving devices for experiments where samples are run on plates with identical assay configurations.

The exercises on the following pages will familiarize you with the use of SDS plate documents.

Exercise 1: Creating a Plate Document

You will need to create a plate document for every plate you run on the 7900HT instrument. The following procedure explains how to create a plate document using the SDS software.

To create a plate document:

Step	Action
1	If not already active, launch the SDS software as explained on page 3-7. The SDS software workspace appears.
2	Choose one of the following options: ♦ Click the New Document button () from the General toolbar, or ♦ From the **File** menu, select **New**. The **New Document** dialog appears. **IMPORTANT** The SDS software can handle multiple documents simultaneously, however the processing speed of your computer will decrease with each open document. For that reason, Applied Biosystems recommends limiting the number of open documents to 10.
3	Configure the **New Document** dialog box with the following settings: **Drop-Down List** / **Select…** Assay / Absolute Quantification Container / 384 Wells Clear Plate Template / Blank Template
4	Click **OK**. The software displays a new plate document with appropriate attributes.

Exercise 2: Saving a Plate Document

The Save command stores any changes to the plate document setup information and display settings. The following procedure explains how to save the open plate document.

To save the plate document:

Step	Action
1	Choose one of the following options: ♦ Click the Save button () from the General toolbar, or ♦ From the **File** menu, select **Save As**.
2	From the **File of type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**.
3	Click the **File name** text field, and type **Practice**.
4	Click **Save**. The software saves the plate document to a file entitled **Practice.sds**. **Note** Do not close the plate document at this time.

Exercise 3: Opening a Plate Document

In this exercise you will be opening a template file that you will use in the following exercises.

To open a plate document:

Step	Action
1	Choose one of the following options: ♦ Click the Open button () from the General toolbar, or ♦ From the **File** menu, select **Open**.
2	From the **Look In** text field of the **Open** dialog box, navigate to **Program Files** > **Applied Biosystems** > **SDS 2.0** > **Templates**.
3	From the **File of type** drop-down list, select **ABI PRISM SDS Template Document (*.sdt)**.
4	From the **Look In** text field, click the file entitled '**384 Well RNaseP Install Plate.sdt**' to select it.
5	Click **Open**. The software opens the plate document file.

Exercise 4: Exporting Data from a Plate Document

In the following exercise, you will export the plate setup so that you can import it (see Exercise 6). The SDS software allows you to export several components of the plate document as tab-delimited text files, a format compatible with most spreadsheet applications.

Note For more information on exporting setup table data using the SDS software, see Appendix B, "Importing and Exporting Plate Document Data."

To export the contents of the template to a setup table file:

Step	Action
1	Choose one of the following options: ♦ Click the Export button () from the General toolbar, or ♦ From the **File** menu, select **Export**.
2	From the **Export** drop-down list of the **Export** dialog box, select **Setup Table**.
3	Select the **All Wells** radio button.
4	Click the **File name** text field, and type **Practice**.
5	Click **Export**. The software saves the plate document setup table information to a tab-delimited text file entitled 'Practice.txt'. **Note** The software also can export the data from most of the analysis plots, graphs, and tables. See Appendix B, "Importing and Exporting Plate Document Data," for more information.

Exercise 5: Closing a Plate Document

When finished viewing or editing a plate document, you will need to close it. If the plate document has been altered since last saving it, the software will prompt you to save the document. In the following procedure, you will close the template document opened in Exercise 3.

To close a plate document:

Step	Action
1	From the **File** menu, select **Close**.
2	If prompted to save the plate document, click **No**. The SDS software closes the file without saving it.

Exercise 6: Importing Setup Table Data into a Plate Document

As a time-saving device, the SDS software allows you to import a setup table information into a plate document from an exported tab-delimited text file. To illustrate this feature, import the plate grid setup information contained in the Practice.txt file (from Exercise 4) into the empty plate document created in Exercise 1.

To import a setup table file into an empty plate document:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>If the plate document from Exercise 1 is still open, go to step 2. Otherwise, create a new plate document to receive the setup table data as follows:
a. From the File menu, select New.
b. Configure the New Document dialog box with settings for the new template.
<table><tr><th>Drop-Down List</th><th>Select…</th></tr><tr><td>Assay</td><td>Absolute Quantification</td></tr><tr><td>Container</td><td>384 Wells Clear Plate</td></tr><tr><td>Template</td><td>Blank Template</td></tr></table>c. Click OK.
The software displays a new plate document with appropriate attributes.</td></tr>
<tr><td>2</td><td>Choose one of the following options:
♦ Click the Import button () from the General toolbar, or
♦ From the File menu, select Import.</td></tr>
<tr><td>3</td><td>From the Look In text field of the Import dialog box, select the Practice.txt file created in Exercise 4.</td></tr>
<tr><td>4</td><td>Click Import.
The software imports the setup information of the Practice.txt file into the plate grid and table of the empty plate document.
Note For more information on importing and exporting setup table data using the SDS software, see Appendix B, “Importing and Exporting Plate Document Data.”</td></tr>
</table>

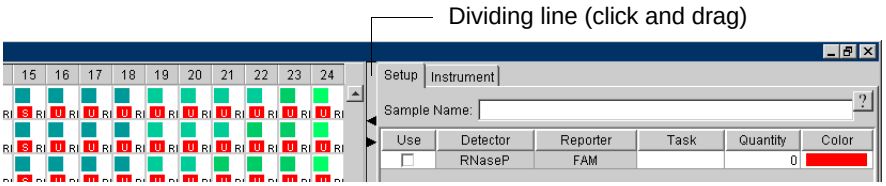
Lesson 2: Viewing and Resizing Panes

Because plate documents can display setup and analysis data in multiple views simultaneously, the SDS software has been designed with several navigational devices to help manage the information. This lesson will teach you to use the different aids to reduce screen clutter and ensure efficient use of the software.

Exercise 1: Resizing Panes, Views, and Plots

You can resize the panes, views, and plots of plate documents by moving the grey lines dividing them horizontally and vertically.

To illustrate the this feature, resize the plate grid pane horizontally as follows:

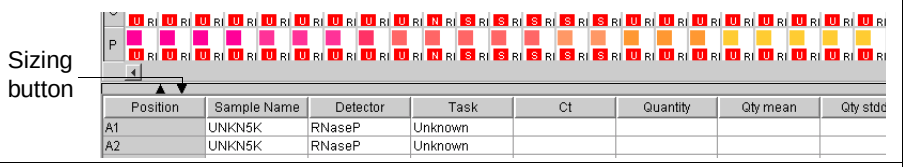
Step	Action
1	Click and drag the grey line dividing the plate grid and well inspector to the right. The software expands the plate grid and table pane to the new width. 
2	Using the grey dividing lines, resize other elements of the plate document until comfortable using the feature.

Exercise 2: Maximizing/Minimizing Panes, Views, and Plots

You can maximize the panes, views, and plots of plate documents by clicking the sizing buttons embedded within the grey dividing lines.

Note Sizing buttons are the arrow-head marks (▲▼) that appear between adjacent elements of the plate document. When clicked, a sizing button hides the element to which it points.

To illustrate the maximize/minimize feature, maximize the plate grid as follows:

Step	Action
1	Click the down-arrow sizing button (▼) in the divider between the plate grid and the table pane to maximize the plate grid vertically. The software maximizes the plate grid by minimizing the table pane. 
2	Click and drag the grey divider at the bottom of the plate document to restore the table pane to its original size (using the action described in Exercise 1 above).
3	Using the sizing buttons, maximize/minimize other elements of the plate document until comfortable using the feature.

Exercise 3: Hiding and Showing Panes, Views, and Plots

You can also toggle the presence of the plate document panes, views, and plots using the icons in the Display toolbar.

To illustrate this feature, hide and show the table pane as follows:

Step	Action
1	From the Display toolbar, click the Show/Hide Table Pane button ( ). The software removes the table pane from the plate document.
2	Click the Show/Hide Table Pane button ( ) again to show the table pane. The software restores the table pane to the plate document. The display toolbar can be particularly useful for manipulating information shown in the plate document. See “Using the Display Toolbar” on page 3-9 for a list of the other icons of the display toolbar and the panes they control.
3	Practice hiding and showing the other plate document elements by clicking other buttons in the Display toolbar until comfortable using the feature.

Lesson 3: Using the Plate Grid

The plate grid (see below) is an important interface tool for the SDS software. The software uses the grid to convey information about the plate and allows you to select specific wells for viewing and analysis. The following exercises will teach you how to use and modify the elements of the plate grid.

Exercise 1: Viewing Well Information

The SDS software provides two methods for viewing the information associated with a well or wells of the plate document.

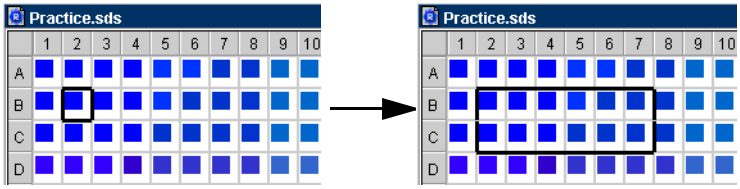
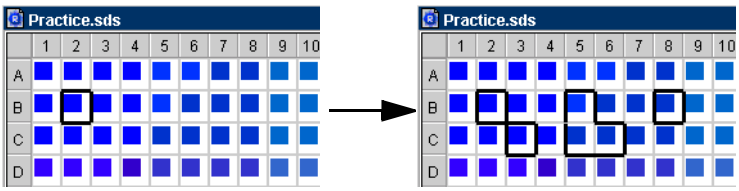
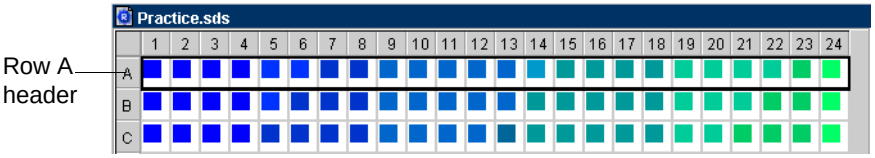
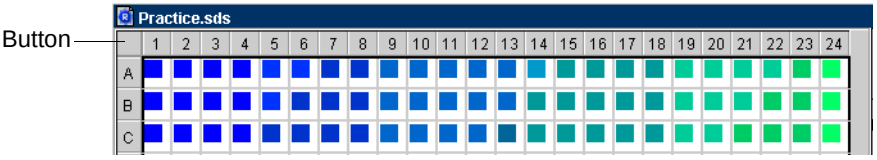
To view the information for a well of the plate document, do one of the following:

Action	Result
Click any well in the plate grid to select it.	When selected, the software outlines the well in black and displays the associated information in the well inspector of the **Setup** tab.
Move the mouse pointer over any well of the plate grid.	After holding position, the software displays the information for the well in a yellow pop-up window.

Exercise 2: Selecting Multiple Wells

The SDS software features several methods for selecting wells from the plate grid. The following exercise will familiarize you with most of them.

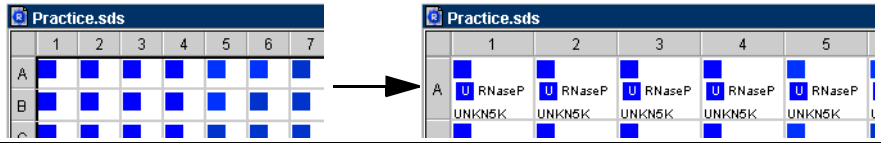
To select groups of wells from the plate grid:

Step	Action
1	Select a block of wells from the plate grid by doing one of the following: ♦ Click and drag the mouse cursor across the block of wells, or ♦ Click the well at the top-left corner of the block, then while holding-down the **Shift** key, click the well at the bottom-right corner of the block. The software outlines the selected wells with a black border. 
2	Select several isolated wells of the plate grid by doing of the following: a. Hold-down the **Ctrl** key, and click individual wells to select them. The software outlines the selected wells with a black border. b. While holding down the **Ctrl** key, de-select wells by clicking individual selected wells. 
3	Select an entire column or row of wells using the headers as follows: a. Click the header for row **A** to select all wells in the row. The software outlines the wells of row A with a black border. b. Press and hold either the **Shift** or **Ctrl** key, then click other columns or row headers to select multiple columns. 
4	Select all wells of the plate grid by clicking the top-left corner of the plate grid. The software outlines all of the wells in the plate document with a black border. 
5	Using the techniques illustrated in steps 1 to 4, practice selecting portions of the plate grid until comfortable using the feature.

Exercise 3: Zooming the Plate Grid

You can zoom the plate grid to display the well information by clicking the Zoom Grid button ( ).

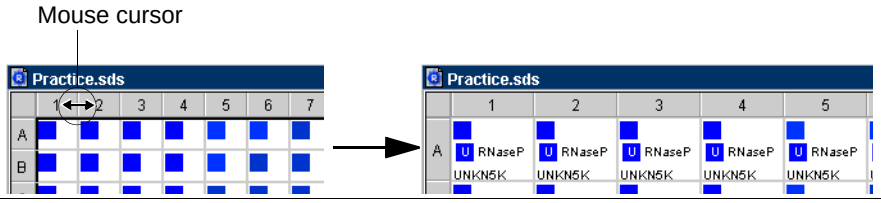
To illustrate this feature:

Step	Action
1	Click the Zoom Grid button ( ) and observe how the grid expands to display the well information. 
2	Click the Zoom Grid button ( ) again to restore the plate grid to the original size.
3	Practice zooming portions of the plate grid until comfortable using the feature.

Exercise 4: Resizing Wells Using the Border Lines

You can also adjust the size of the plate grid wells by moving the lines in the row or column headers.

To illustrate this feature:

Step	Action
1	Move the mouse cursor over a border line in the row or column header. The mouse cursor becomes a double arrow (↔).
2	Click and drag the mouse cursor to adjust the well to a new width. The software resizes all wells in the plate grid to match the new width. 
3	Practice resizing the wells of the plate grid until comfortable using the feature.

Lesson 4: Using the Hand-Held Bar Code Reader

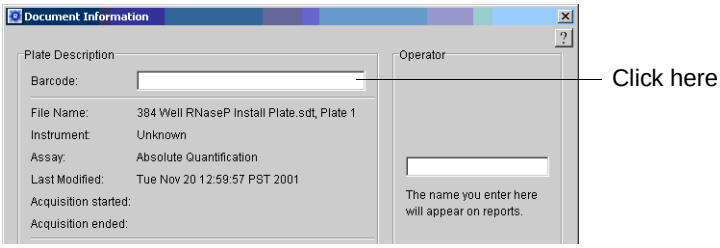
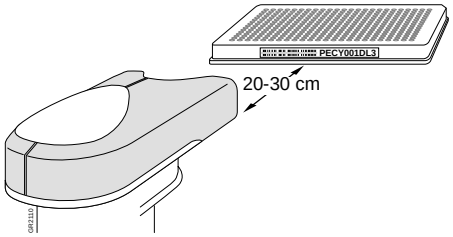
The hand-held bar code reader functions as an extension of the keyboard and can be used to automatically type bar codes into the SDS software. When the reader is used successfully to scan a bar code, it automatically:

- ♦ transmits the alpha-numeric equivalent of the bar code to the software. The software types the bar code text wherever the cursor is active.
- ♦ transmits a carriage-return (the equivalent of pressing the Enter key).

Exercise: Entering Bar Code Information Using the Hand-Held Bar Code Reader

Note The following procedure explains how to enter the bar code number into the plate document from the Document Information dialog box. You can also scan the bar code into the New Document dialog box during plate document creation.

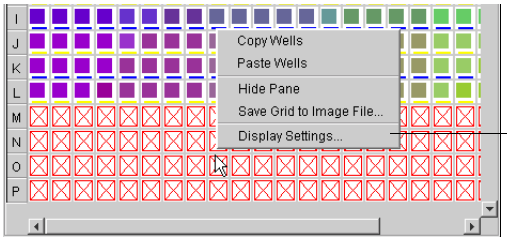
To enter a bar code number using the hand-held bar code reader:

Step	Action
1	From the **Tools** menu, select **Document Information**. The **Document Information** dialog box appears.
2	Click the **Barcode** text field.  The software places the blinking cursor in the text field.
3	While holding the hand-held bar code reader 20 to 30 cm away from a plate, aim at the center of the bar code and press the trigger. The scanner emits a sweeping laser beam that appears as a red line on the plate. Slowly move the scanning beam slowly across the bar code until the scan gun emits a high-pitched tone acknowledging that it has read the code. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.  Once the gun has read the bar code, the software automatically populates the selected text field with the alpha-numeric equivalent of the bar code.
4	Click **OK** to close the **Document Information** dialog box.

Lesson 5: Using Contextual Menus

The SDS software features contextual menus as a time-saving devices that provide access to the commands for an associated view or pane.

To access a contextual menu, move the mouse pointer over a pane or view of interest and click the right mouse button. The menu appears at the location of the pointer.



All contextual menus provide the following common commands:

Command	Result	See Page
Hide <pane or plot>	Hides the pane or view.	3-16
Save <pane or plot> **to Image File**	Opens the **Export Graphic** dialog box for exporting the selected view or pane as a JPEG graphic file.	B-8
Display Settings	Opens the display settings dialog box that allows you to modify the appearance of the view, pane, or plot.	4-17

Lesson 6: Using Keyboard Shortcuts

The SDS software features keyboard shortcuts for invoking the major functions of the software.

Exercise: Closing the Plate Document

To illustrate the use of a keyboard shortcut, close the plate document as follows:

Step	Action
1	Simultaneously press the **Ctrl** and **W** keys (Ctrl+W).
2	When prompted to save the plate document, click **No**.

Note The SDS software online help contains a complete list of the keyboard shortcuts for the SDS software. To view the list, open the online help as explained below.

Lesson 7: Using the SDS Software Online Help

The SDS software features an online help system that can guide you through the procedures for setting up, performing, and analyzing runs. To get help at any time, click a Help button located within the dialog box or window in which you are working.

The SDS software provides two ways to access the online help as follows:

To…	Then…
access general help	select **SDS Online Help** from the **Help** menu.
get help for using a specific dialog box, plot, or feature	click a help button (?) located within the dialog box or window in which you are working.

Using SDS Plate Documents

Using Multiple Plate Documents

The SDS software can handle multiple documents simultaneously, however the processing speed of the computer will decrease with each open document. For that reason, Applied Biosystems recommends limiting the number of open documents to 10.

Elements of a Plate Document

This section will familiarize you with the elements of plate documents. The figure below illustrates a typical plate document. The following pages describe its components.

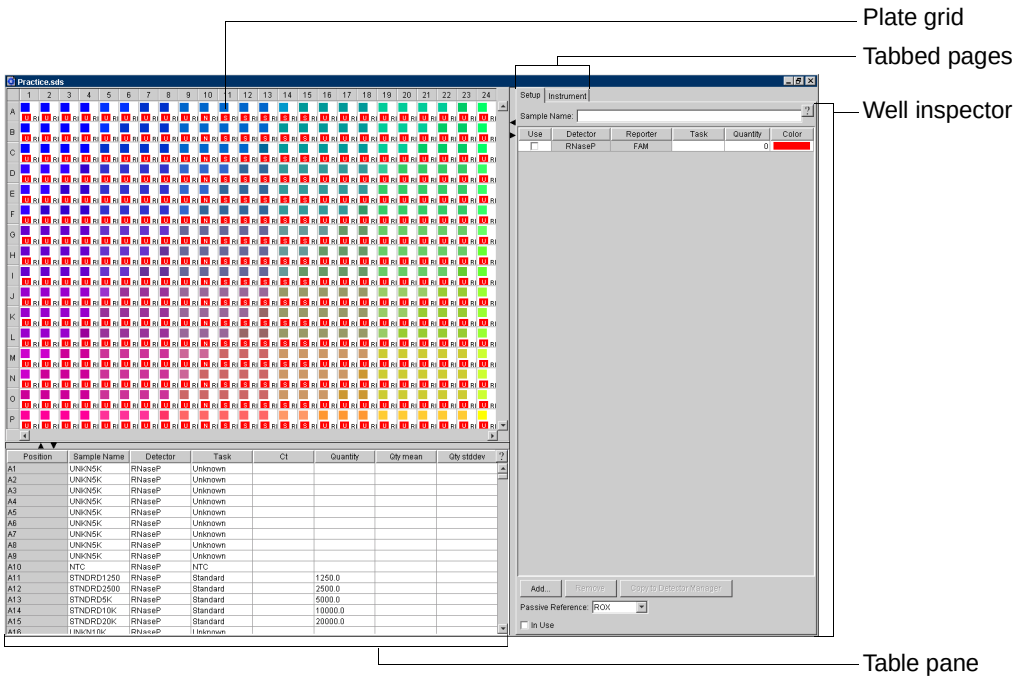


Plate Grid

Each plate document consists of a grid that corresponds to the wells of a reaction device. The grid displays well information depending on the type of plate document. The information displayed within the cells of the grid are determined by the Plate Grid properties settings located within the Display Settings dialog box.

Note For more information on configuring the display settings for the plate grid, click a help button (?).

Table Pane

The table pane displays the setup and analysis properties for the plate document in a tabular format. The table pane can be exported as a tab-delimited text file for use by a spreadsheet application (see Appendix B, “Importing and Exporting Plate Document Data,” for more information).

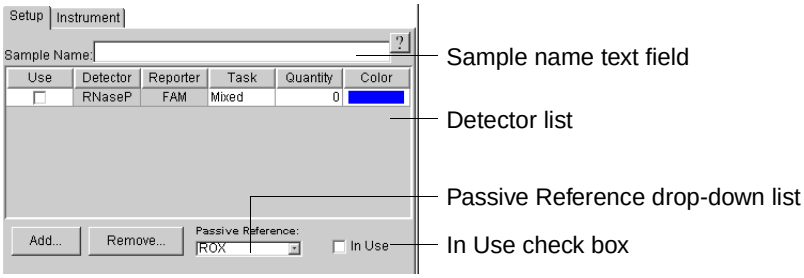
Tabbed Pages

Plate documents can contain up to six tabbed pages depending on their function:

Tab	Used to...
Setup	display well information, and to configure the plate grid with setup information.
Instrument	program the plate document method, and to run the plate document or send it to the Plate Queue.
Raw Data	display the raw fluorescence collected from the sequence detection run.
Calibration Data	display the Background and Pure Spectra calibration data used for the signal normalization and multicomponenting analysis of the current run.
Results	display analyzed run data. The Analysis tab is visible only in plate documents containing analyzed run data.
Dissociation Curve	display analyzed dissociation curve data from a programmed ramp. The Dissociation Curve tab is visible only in plate documents containing analyzed data from a real-time run with a programmed ramp.

Well Inspector

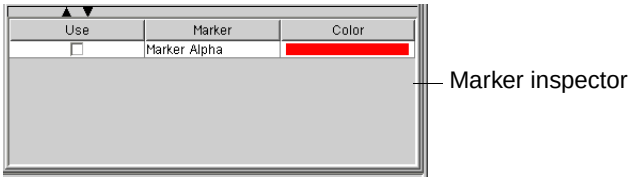
The Well Inspector is used to apply detector and sample information to the wells within the grid pane and to display information from the selected cells in the plate grid.



Component	Description
Sample name text field	An editable text field that displays the sample name applied to the selected well(s) **Note** The Sample name text field will display ***Mixed**** if multiple wells with different sample names are selected.
Detector list	Lists all available detectors copied to the plate document
In Use check box	Toggles the activity of the well. If unchecked, the software eliminates the data from the selected well from all analysis procedures.
Passive Reference drop-down list	Displays the fluorescent dye used as a passive reference

Marker Inspector

Used only for allelic discrimination runs, the Marker Inspector (shown below) appears as the lower half of the Well Inspector and displays all markers available for the plate.



Run Setup and Basic Operation 4

In This Chapter This chapter discusses the following topics:

Before You Begin

Background Information

Chapters 5 and 6 include brief discussions of the experiments that can be performed using the 7900HT instrument. Before beginning, you may want to review the appropriate chapter for your experiment:

Run Type	Experiment	See Page
End-point	Allelic Discrimination	5-3
Real-time	Absolute Quantification	6-3
	Dissociation Curve Analysis	6-17

Getting More Information from the Online Help

The SDS software features an online help system that can guide you through the procedures for setting up, performing, and analyzing runs. To get help at any time, click the ? button located within the dialog box or window in which you are working.

Maximizing Throughput for End-Point Runs

For end-point applications such as allelic discrimination, the throughput of the ABI PRISM® 7900HT Sequence Detection System can be increased by dividing the workload between the 7900HT instrument and several thermal cyclers. Unlike real-time runs, the 7900HT instrument collects data for end-point runs after the completion of the PCR. Consequently, the thermal cycling of end-point plates can be done elsewhere and then transferred to the 7900HT instrument afterwards for data collection and analysis.

IMPORTANT To perform the thermal cycling and the plate read using the 7900HT instrument, run the plate first as a real-time plate document and then again as an allelic discrimination plate document (see “Step 4 – Programming the Plate Document Method” on page 4-13 for the procedure).

Setup Checklists

Experiments/Runs Performed on the 7900HT Instrument

See the appropriate page for the type of experiment or run you want to perform:

Experiment/Run	See Page
Absolute Quantification	4-3
Allelic Discrimination	4-4
Background	7-13
Dissociation Curve (Melting Curve)	4-4
Pure Dye (Spectral Calibration)	7-17
RNase P Instrument Performance Verification	7-24

Absolute Quantification Checklist

To create and set up a plate document for an absolute quantification run:

Done	Step	Description	See Page
☐	**1**	Create an absolute quantification plate document.	4-6
☐	**2**[a]	a. Create detectors for the absolute quantification probes. b. Copy the detectors to the plate document.	4-7 4-8
☐	**3**[a]	a. Configure the plate document with detector tasks (NTC, Standard, and Unknown). b. Assign quantities to the wells of the plate document that contain standards.	4-11 4-12
☐	**4**	a. Program the method for the absolute quantification run. b. If performing an assay in which you would like to collect dissociation data, add a temperature ramp to the thermal profile to perform a dissociation curve analysis.	4-13 4-16
☐	**5**	Choose from the following: **If running…** / **Then…** a single plate / continue to step 7. the first plate in a series of plates with identical assay configurations / Save the plate document as an ABI PRISM SDS Template Document as explained on page 4-17.	
☐	**6**	Create a plate document from the template created in step 5.	4-18
☐	**7**	Configure the document with sample names and plate information.	4-19
☐	**8**	Prepare and run the absolute quantification plate or plates.	4-20
☐	**9**	Analyze the run data.	6-3

a. Steps 2 and 3 can be eliminated by importing the plate document setup information from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Allelic Discrimination Checklist

To create and set up a plate document for an allelic discrimination run:

Done	Step	Description	See Page
☐	**1**	Create an allelic discrimination plate document.	4-6
☐	**2**[a]	a. Create detectors for the allelic discrimination probes. b. Create a marker for each allelic discrimination probe pairing. c. Copy the marker(s) to the plate document.	4-7 4-9 4-10
☐	**3**[a]	Assign detector tasks to the wells of the plate document (NTC and Unknown).	4-11
☐	**4**	If you would like to perform thermal cycling of the allelic discrimination plate on the 7900HT instrument, create a real-time plate document for the plate and program it with the the method for the allelic discrimination run. Otherwise, continue to step 5.	4-13
☐	**5**	Choose from the following: **If running…** / **Then…** a single plate / continue to step 7. the first plate in a series of plates with identical assay configurations / Save the plate document as an ABI PRISM SDS Template Document as explained on page 4-17.	
☐	**6**	Create a plate document from the template created in step 5.	4-18
☐	**7**	Configure the document with sample names and plate information.	4-19
☐	**8**	a. Prepare the allelic discrimination plate or plates and perform thermal cycling on a designated thermal cycler. b. Run the allelic discrimination plate or plates on the 7900HT instrument.	4-20
☐	**9**	Analyze the run data.	5-3

a. Steps 2 and 3 can be eliminated by importing the plate document setup information from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Dissociation (Melting) Curve Checklist

A dissociation curve analysis is performed as part of a real-time PCR run (absolute quantification). To perform a dissociation curve, construct a plate document for absolute quantification as explained on page 4-3 and configure the method with a temperature ramp as explained on page 4-16.

Section: Plate Document Setup

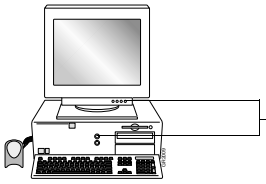
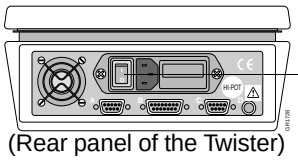
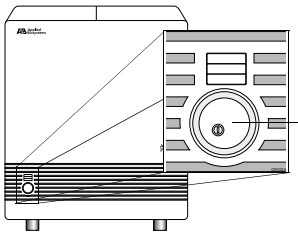
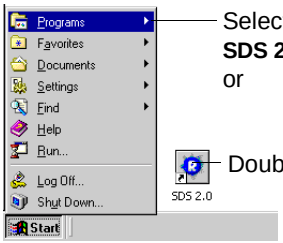
In This Section

This section discusses the following topics:

Quick Review: Turning On the 7900HT Instrument

Note The following table includes a set of abridged procedures for activating the components of the ABI PRISM 7900HT Sequence Detection System. For a complete explanation of the procedure, see "Turning on the ABI Prism 7900HT Sequence Detection System" on page 3-5.

To activate the ABI PRISM 7900HT Sequence Detection System:

Step	Action	Appearance
1	Turn on the monitor and computer.	
2	If using an automation module, turn on the Zymark® Twister™ Microplate Handler. Press the power switch located on the back panel of the plate handler.	 (Rear panel of the Twister)
3	Turn on the 7900HT instrument. Press the power button located on the front panel of the instrument below the LED display. **Note** For a description of the LED display, see "Reading the Instrument Status Lights" on page 3-6.	
4	Launch the SDS 2.0 software by doing one of the following: ♦ Select **Start** > **Programs** > **SDS 2.0** > **SDS 2.0**. ♦ Double-click the **SDS 2.0** icon from the desktop. The computer launches the software.	

Step 1 – Creating a Plate Document

About ABI PRISM Plate Documents

Every plate run on the 7900HT instrument requires the creation of a plate document within the SDS software. Plate documents are virtual representations of the consumables (MicroAmp® 384/96-well plates) used to contain samples and reagents during runs.

Plate documents contain the following information:

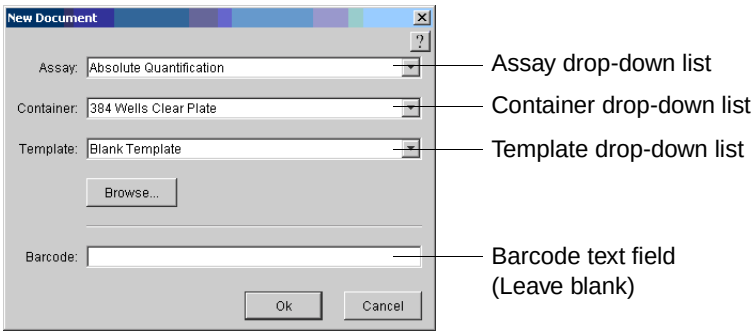
- Detector information and arrangement on the plate
- Marker information and arrangement on the plate (allelic discrimination only)
- Sample information and their arrangement on the plate
- Method parameters for the run (absolute quantification only)

Creating a Plate Document

To create a plate document:

Step	Action
1	If not already open, launch the SDS software as explained on page 4-5.
2	Choose from the following: ♦ From the **File** menu, select **New**, or ♦ From the tool bar, click the New Document button ( ).
3	Configure the **New Document** dialog box with settings for the run as follows:

Drop-Down List	Select…
Assay	the type of assay appropriate for your plate. **Note** If performing a dissociation curve experiment, select **Absolute Quantification** from the **Assay** drop-down list.
Container	the type of plate you intend to run.
Template	**Blank Template**



Note Because this chapter emphasizes the use of a template file, the Barcode text field is left blank. However, if creating a plate document to run a single plate, scan or type the bar code for the plate into the Barcode text field.

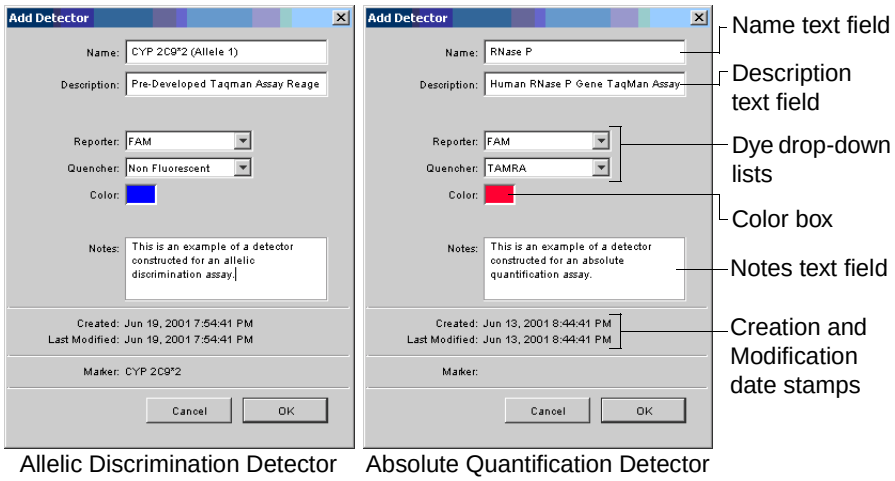
4	Click **OK**. The software displays a new plate document with the appropriate attributes.
5	Create and copy detectors (and markers) to the new plate document as described on page 4-7.

Step 2 – Applying Detectors and Markers to the Plate Document

Creating Detectors

Before a plate document can be used to run a plate, it must be configured with detector information for the experiment (and marker information if performing allelic discrimination). A detector is a virtual representation of: a TaqMan® probe in a master mix used for detection of a single target nucleic acid sequence, or the SYBR® Green Double-Stranded DNA Binding Dye 1 used for the detection of double-stranded DNA. Before using the plate document, you must create and apply detectors for all assays present on the plate.

To create detectors for the plate document:

Step	Action
1	From the **Tools** menu of the SDS software, select **Detector Manager**. The **Detector Manager** dialog box appears.
2	Click **File**, and from the drop-down list select **New**. The **Add Detector** dialog box appears.
3	Click the **Name** text field, and type a name for the detector. **IMPORTANT** The name of the detector must be unique and should reflect the target locus of the assay (such as GAPDH or RNase P).
4	(Optional) Click the **Description** text field, and type a brief, 32-character description of the assay.
5	Click the **Reporter Dye** and **Quencher Dye** drop-down lists and select the appropriate dyes for the assay. **Note** If creating a detector for a SYBR Green 1 assay, set the **Quencher Dye** drop down list to **Non Fluorescent**. **Note** If using a custom dye not manufactured by Applied Biosystems, you must create and run a pure dye plate for the custom dye before it can be applied to a detector (see “Adding Custom Dyes to the Pure Dye Set” on page 7-21).
6	Click the **Color** box, select a color to represent the detector using the **Color Picker** dialog box, and click **OK**.
7	(Optional) Click the **Notes** text field, and type any additional comments for the detector (up to 200 characters).  Allelic Discrimination Detector Absolute Quantification Detector

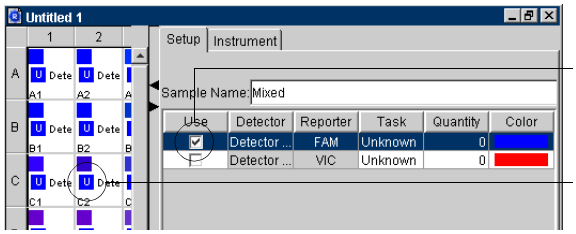
To create detectors for the plate document: *(continued)*

Step	Action
8	Click **OK** to save the detector and return to the **Detector Manager** dialog box. The software saves the new detector and displays it in the detector list.
9	Repeat steps 2 to 8 to create detectors for all remaining assays on the plate. **Note** Click the ? button for information on the features of the **Detector Manager** dialog box or to view the procedures for editing, deleting, or searching for detectors.
10	Choose from the following: **If constructing a plate document for…** — **Then…** absolute quantification — copy the detector(s) to the plate document as explained in the procedure below. allelic discrimination — create markers to the plate document as explained on page 4-9.

Copying and Applying Detectors to the Plate Document

IMPORTANT Once copied to the plate document, a detector is no longer linked to the corresponding entry in the Detector Manager. Therefore, if a detector is modified in the Detector Manager after it has been copied to a plate document, the detector must be removed from the plate and copied again to update the plate document with the changes.

To copy and assign the detectors to the plate document:

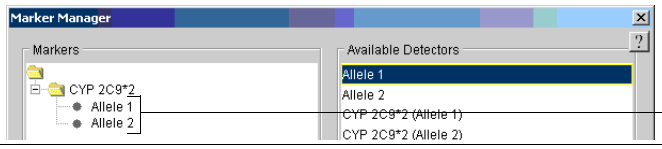
Step	Action
1	From the **Detector Manager** dialog box of the SDS software, copy the detectors to the plate document as follows: a. While pressing and holding the **Ctrl** key, click the detectors you want to apply to the plate document. The software highlights the selected detectors. b. Click **Copy to Plate Document**. The software adds the detectors to the well inspector of the plate document.
2	Click **Done** to close the **Detector Manager** dialog box.
3	From the plate grid, select the wells containing the assay for the first detector. **Note** For easier selection of plate grid wells, use the **Ctrl** and **Shift** keys to select wells individually or in groups. See page 3-16 for more information.
4	Apply detector to the selection by clicking the check box for the detector in the **Use** column of the well inspector. 
5	Repeat steps 3 and 4 to apply the remaining detectors to the plate grid.
6	Configure the plate document with detector tasks as explained on page 4-11.

Creating an Allelic Discrimination Marker

Allelic discrimination plate documents feature the use of 'markers' to aid in organizing and applying detectors based on the loci they target. A marker is a pairing of two detectors representing chemical assays designed to amplify different alleles of a common locus. The SDS software uses marker information during data analysis to organize and compare the processed run data.

IMPORTANT Allelic discrimination plate documents must contain at least one marker.

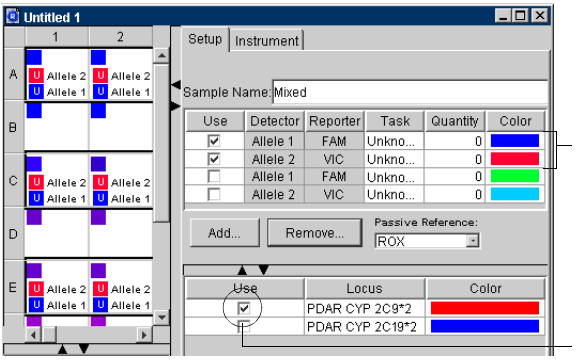
To create a marker for an allelic discrimination plate document:

Step	Action
1	If the **Detector Manager** dialog box is open, click **Done** to close it.
2	From the **Tools** menu of the SDS software, select **Marker Manager**. The **Marker Manager** dialog box appears.
3	Click **Create Marker**. The **Add Marker** dialog box appears.
4	Click the **Enter name of new Marker** text field, type a name for the new marker, and click **OK**. The new marker appears within the **Markers** text field.
5	Apply detectors to the new marker and follows: a. From the **Markers** text field, click the new marker to select it. The software highlights the selected marker. b. From the **Available Detectors** text field, click a detector that you want to add to the marker. The software highlights the selected detector. c. Click **Add Detector**. The software applies the detector to the marker and displays it below the Marker entry. d. Repeat steps b and c for the remaining detector.  Detectors assigned to Marker 'CYP 2C9*2'
6	If evaluating multiple loci, repeat steps 3-5 to create additional markers as needed. **IMPORTANT** A marker must be configured with two detectors before it can be applied to a plate document. **Note** Click the ? button for information on the features of the **Marker Manager** dialog box or to view the procedure for deleting markers from the **Markers** list.
7	Apply the marker(s) to the allelic discrimination plate document as explained in "Copying and Applying Markers to a Plate Document" on page 4-10.

Copying and Applying Markers to a Plate Document

IMPORTANT Once copied to the plate document, a marker is no longer linked to the corresponding entry in the Marker Manager. Therefore, if a marker is modified in the Marker Manager after it has been copied to a plate document, the marker must be removed from the plate and copied again to update the plate document with the changes.

To copy and apply markers to the plate document:

Step	Action
1	From the **Marker Manager** dialog box of the SDS software, copy the allelic discrimination marker to the plate document as follows: a. Click the marker you want to apply to the plate document. The software highlights the selected marker. b. Click **Copy to Plate Document**. The software copies the marker and associated detectors to the plate document. c. Repeat steps a and b to copy additional markers to the allelic discrimination plate document as needed.
2	Click **Done**. The software closes the **Marker Manager** dialog box.
3	Select the wells containing the assays for a marker you configured in the previous procedure. **Note** For easier selection of plate grid wells, use the **Ctrl** and **Shift** keys to select wells individually or in groups. See page 3-16 for more information.
4	From the marker inspector, click the **Use** check box of the marker you want to add to the selected wells. The software labels the selected wells with the marker and its detectors. **Note** The detectors associated with the marker are automatically applied to the selected wells when the marker is placed in **Use**.  Detectors associated with the ‘PDAR CYP 2C9*2’ marker Use check box for the ‘PDAR CYP 2C9*2’ marker (selected)
5	If necessary, repeat steps 3 and 4 to assign additional markers to the plate document.
6	Configure the plate document with detector tasks as explained in “Step 3 – Configuring the Plate Document with Detector Tasks” on page 4-11.

Step 3 – Configuring the Plate Document with Detector Tasks

About Detector Tasks

The detectors applied to each well of the plate document must be assigned a 'task' that defines their specific purpose or function on the plate. The SDS software uses the detector task assignments to determine how to treat the data produced by the wells when analyzing the run data. Detector tasks vary depending on the type of experiment for which the plate document was created.

Applying Detector Tasks

To apply detector tasks to the detectors of the plate document wells:

Step 1

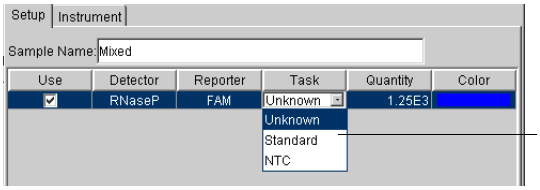
Using the **Ctrl** and **Shift** keys, select the wells of the plate grid containing samples for a particular task described in the table below.

Experiment	Task	Apply to…
Allelic Discrimination	Unknown	all detectors of wells containing PCR reagents and test samples.
	NTC	all detectors of negative control wells containing reagents for the PCR, but lacking samples.
Absolute Quantification	Unknown	all detectors of wells containing PCR reagents and test samples for quantification.
	Standard	the appropriate detectors of wells containing PCR reagents and samples of known quantities.
	NTC	all detectors of negative control wells containing PCR reagents, but lacking samples.

Step 2

From the well inspector, click the text field in the **Task** column for each detector entry, and select the appropriate task from the drop-down list.

The SDS software labels all selected wells with the task.



Step 3

Repeat steps 1 and 2 to apply any remaining tasks to the plate document.

Step 4

Choose from the following options:

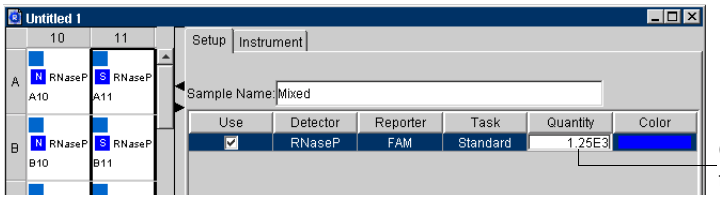
If constructing a plate document for…	Then…
absolute quantification	assign quantities to the standard wells of the plate document as explained on page 4-12. **IMPORTANT** The software will disregard data from all Standard wells that have **Quantity** values of **0**.
allelic discrimination	if necessary, set the passive reference for the plate document as explained page 4-12. Otherwise, continue to "Step 4 – Programming the Plate Document Method" on page 4-13.

Assigning Quantities to Standards for Absolute Quantification

For the SDS software to create a standard curve for quantification of unknown samples, absolute quantification plate documents must contain quantity values for the standards contained on the plate. The software expresses quantity as the number of copies of the target sequence present within an individual well of the plate.

IMPORTANT The SDS software excludes from analysis data from Standard wells assigned a **Quantity** value of **0**.

To apply quantities to the plate document:

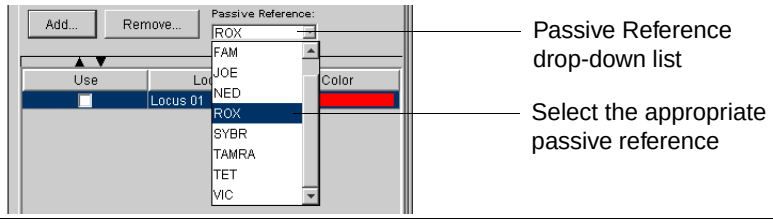
Step	Action
1	From the plate grid of the SDS software, select the replicate wells containing the first standard in the dilution series.
2	From the well inspector, click the text field in the **Quantity** column for the appropriate detector, type the number of copies of the target template the replicate wells contain, and press **Enter**. The software labels the selected standard wells with the specified quantity. 
3	Repeat steps 1 and 2 to configure the other sets of replicate standard wells on the plate with the appropriate quantities. When finished, the plate grid should contain a complete set of replicate wells labeled with the **Standard** task and assigned quantities which the software will use to compute the standard curve for the run.
4	If necessary, set the passive reference for the plate document as explained below. Otherwise, program the method for your run as explained on page 4-13.

Setting the Passive Reference

If using an Applied Biosystems chemistry, use the default Passive Reference setting (Applied Biosystems chemistries use the ROX™ passive reference molecule). If running a custom chemistry, select a dye to use as a passive reference for the run.

Note Applied Biosystems recommends using a passive reference to normalize the signals from the reporter dyes (see page A-6 for more information).

To set the passive reference dye for the plate document:

Step	Action
1	From the **Passive Reference** drop-down list, select the appropriate reference dye. 
2	Program the method for the run as explained on page 4-13.

Step 4 – Programming the Plate Document Method

About SDS Methods

During a run, the SDS software controls the instrument based on the instructions encoded within the method of the plate document. Each new plate document (except allelic discrimination) contains a default method that must be edited for the specifics of the experiment.

Methods contain the:

- Thermal Cycler Conditions
- Auto Increment Values
- Ramp Rates
- Data Collection Options
- Reaction Volume Setting

To create a method for…	Then…
absolute quantification	program the method as explained on page 4-14.
allelic discrimination	see page 4-14.
dissociation curve analysis	Dissociation curves are preformed as part of a real-time PCR run (absolute quantification). Therefore, to perform a dissociation curve analysis do the following: a. Program the method for the absolute quantification experiment as explained on page 4-14. b. Add a temperature ramp to the method for dissociation curve analysis as explained on page 4-16.

Programming Methods for Allelic Discrimination

The SDS software is designed to maximize instrument throughput and therefore does not provide the option to thermal cycle allelic discrimination plate documents. Because allelic discrimination experiments are end-point runs that do not require data collection during the PCR, thermal cycling can be performed on a dedicated thermal cycler and then transferred to the 7900HT instrument for data collection and analysis.

If you want to thermal cycle the allelic discrimination plates on…	Then…
a designated thermal cycler	go on to "Step 5 – Saving the Plate Document as a Template" on page 4-17
the 7900HT instrument	follow the procedure below.

Performing Thermal Cycling of Allelic Discrimination Plates on the 7900HT Instrument

To perform the thermal cycling and the plate read using the 7900HT instrument, run the plate first as a real-time plate document and then again as an allelic discrimination plate document as explained below.

IMPORTANT Follow the procedure below only if you intend to perform the PCR on the 7900HT instrument. Otherwise, perform the PCR on a dedicated thermal cycler and then transfer the plate to the 7900HT instrument for data collection.

To conduct allelic discrimination thermal cycling on the 7900HT instrument:

Step	Action
1	Launch the SDS software.
2	Create a real-time plate document for absolute quantification as described on page 4-6. **Note** It is not necessary to configure the plate document with detectors.
3	Program the plate document method with the thermal cycling times and temperatures for your protocol as described in "Programming the Method for Absolute Quantification," below.
4	Run the plate using the real-time plate document as described on page 4-21. **Note** Although large, the real-time file may be helpful in diagnosing and troubleshooting the experiment later if the data from the allelic discrimination run produces unexpected results.
5	Go on to "Step 5 – Saving the Plate Document as a Template" on page 4-17.

Programming the Method for Absolute Quantification

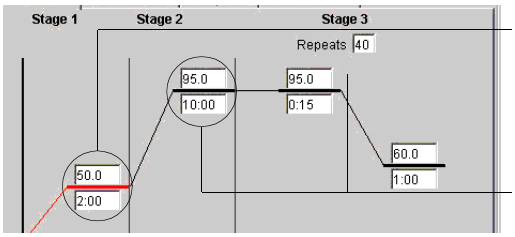
Note The following procedure describes how to configure only the basic features of the method: thermal cycler conditions, sample volume, and data collection options. To further customize the method for the plate document, click the ? button in the Instrument tab and refer to the online help for instructions on configuring the auto increment and ramp rate values.

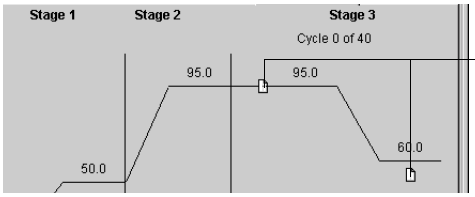
To create a method for the absolute quantification run:

Step	Action
1	From the SDS software, click the **Instrument** tab of the plate document.
2	If necessary, select or de-select the **9600 Emulation** check box. **Note** When the **9600 Emulation** check box is checked, the SDS Software reduces the ramp rate of the 7900HT instrument to match that of the ABI PRISM® 7700 Sequence Detection System instrument.

To create a method for the absolute quantification run: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Modify the default thermal profile for the method as needed:
<table>
<tr><th>To…</th><th>Then…</th></tr>
<tr><td>adjust step parameters (time/temp)</td><td>select a text field value, type a new value, and press Enter.
 

Temperature text field (between 4 and 99.9 °C)
Time text field (between 0:01 and 98:59 minutes)</td></tr>
<tr><td>add a hold, cycle set, or step</td><td>a. Click step to the left of the location you want to place the new stage.
b. Click Add Cycle or Add Hold.
The software inserts the stage into the thermal profile.
Note To add a step to a stage, select the step to the left of the location you want to place the step and click Add Step.
 

Selected step
New step appears here</td></tr>
<tr><td>remove a step</td><td>a. Click the step you want to remove.
The software highlights the selected step.
b. Click Delete Step to remove the step from the profile.</td></tr>
</table></td></tr>
<tr><td>4</td><td>Configure the data collection options for the method as follows:
a. Click the Data Collection tab.
b. Click each plateau or ramp within the cycle stage of the thermal profile to place a data collection icon at each step.
 

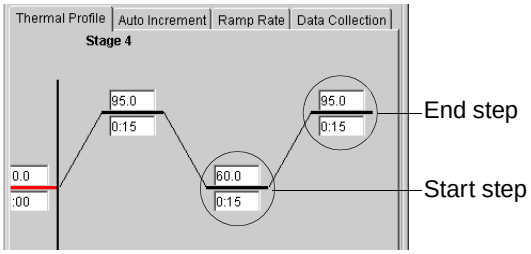
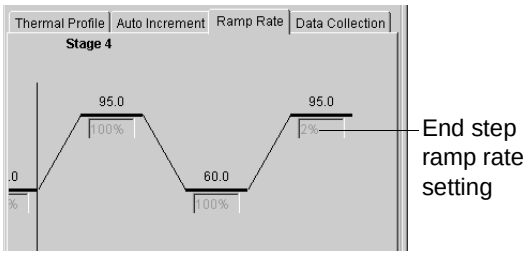
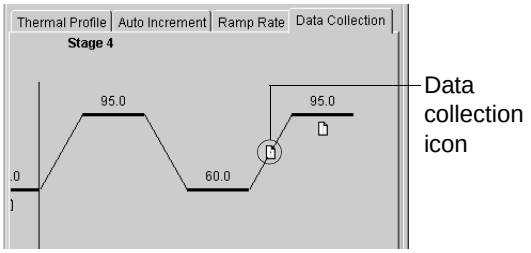
Data collection icons</td></tr>
<tr><td>5</td><td>Click the Sample Volume (µL) text field and type the volume of the reactions to be run on the plate.
Note ‘Sample Volume’ refers to the entire contents of any well, including buffer blank, or any combination of master mix and nucleic acids.
IMPORTANT All wells on one plate must contain the same reaction volume.</td></tr>
<tr><td>6</td><td>Choose from the following options:
<table>
<tr><th>If performing an…</th><th>Then…</th></tr>
<tr><td>absolute quantification run only</td><td>go to “Step 5 – Saving the Plate Document as a Template” on page 4-17.</td></tr>
<tr><td>absolute quantification run with a dissociation curve</td><td>add a temperature ramp to generate dissociation curve data as explained on page 4-16.</td></tr>
</table></td></tr>
</table>

Programming a Temperature Ramp for Dissociation Curve Analysis

To generate the data required to perform a dissociation curve analysis, the 7900HT instrument must be programmed to run a ‘temperature ramp’ in which it slowly elevates the temperature of the samples while collecting fluorescence measurements once every 7-10 seconds (see page 6-18 for a detailed explanation).

To add a temperature ramp to the method for dissociation curve analysis:

Step	Action
1	From the **Instrument** tab of the plate document, click the **Thermal Profile** tab. The software displays the **Thermal Profile** tabbed page.
2	Click **Add Dissociation Stage**. The SDS software inserts a temperature ramp at the end of the thermal profile consisting of a set of default steps. The default temperature ramp can be customized, however Applied Biosystems recommends the following guidelines to ensure the greatest separation of the derivative peaks during the analysis, and therefore the maximum resolution for the run.

Guideline	Example
The Start and End steps of the temperature ramp must: ♦ be separated by a minimum temperature difference of 35 °C. ♦ elapse 15 seconds (0:15) each.	
The ramp rate setting for the End step of the temperature ramp must be 2 %.	
A data collection icon must be placed on the temperature ramp in the thermal profile.	
3	Go to “Step 5 – Saving the Plate Document as a Template” on page 4-17.

Step 5 – Saving the Plate Document as a Template

Adjusting the Display Settings (Optional)

Because plate documents created from the template will retain its display settings, configure the display settings of the template as you would like the child plate documents to be displayed.

To configure the display settings for the template:

Step	Action
1	From the **View** menu of the SDS software, select **Display Settings**. The **Display Settings** dialog box appears.
2	Configure the display settings for the **Results Grid**, and the **Results Table**. For more information on the **Display Settings** dialog box or to view the procedures for configuring the display settings for the template, click the ? button to open the SDS software online help.
3	When finished, click **OK**. The SDS software applies the new display settings to the plate document.
4	Go to "Saving the Plate Document as a Template," below.

Saving the Plate Document as a Template

IMPORTANT Saving the plate document as a template is an optional step and recommended for instances where the document can be used to create duplicate plate documents for a series of plates with identical assay configurations. If you choose not to use your plate document as a template, go to "Step 7 – Applying Sample and Plate Information" on page 4-19.

To save the template file:

Step	Action
1	From the **File** menu of the SDS software, select **Save As**. The **Save As** dialog box appears.
2	From the **Look in** text field, navigate to the **Program Files** > **Applied Biosystems** > **SDS 2.0** > **Templates** directory. **Note** By saving the template file to the Templates directory, it becomes available from the **Template** drop-down list in the **New Document** dialog box.
3	From the **File of type** drop-down list, select **ABI PRISM SDS Template Document (*.sdt)**.
4	Click the **File name** text field, and type a name for the template.
5	Click **Save**. The software saves the template plate document file.
6	From the **File** menu, select **Close**. If the software prompts you to save the plate document, click **No**. The SDS software closes the template file.
7	Create a plate document from the template as explained on page 4-18.

Step 6 – Creating a Plate Document from the Template

Options for Creating Plate Documents from the Template

The SDS software offers two options for creating plate documents from a template file: individually or in batches.

Option	Description	See Page
Create an individual plate document from the template	The procedure below explains how to create a single plate document from a template file for running a plate. By repeating the procedure, you can create as many plate documents as needed.	Follow the procedure below.
Create multiple plate documents using the Template Batch utility	As a faster alternative to the option above, the software includes a Template Batch utility that can simultaneously create multiple plate documents from the template file.	4-32

Creating a Single Plate Document from a Template

To create a plate document from the template file:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>From the File menu of the SDS software, select New.

The New Document dialog box appears.</td></tr>
<tr><td>2</td><td>Configure the New Document dialog box as follows:

<table>
<tr><th>Drop-Down List</th><th>Select…</th></tr>
<tr><td>Assay</td><td>the same assay as the template.</td></tr>
<tr><td>Container</td><td>the same plate type as the template.</td></tr>
<tr><td>Template[a]</td><td>the template file (*.sdt) created on page 4-17.

If the template file does not appear within the Template drop-down list, select the file as explained below:
a. Click Browse.
The Open dialog box appears.
b. From the Look in text field, navigate to and select the template file (*.sdt) created on page 4-17.
c. Click Open.
The Template drop-down list displays the template file.</td></tr>
</table>
a. The Template drop-down list displays all template files contained in the Templates subdirectory of the SDS 2.0 program directory.</td></tr>
<tr><td>3</td><td>(Optional) Click the Barcode text field and either:

♦ Type the bar code number for the plate, or

♦ Scan the plate bar code using the hand-held bar code scanner.</td></tr>
<tr><td>4</td><td>Click OK.

The software creates a new plate document from the template file.</td></tr>
<tr><td>5</td><td>Configure the new plate document with sample and plate information as explained on page 4-19.</td></tr>
</table>

Step 7 – Applying Sample and Plate Information

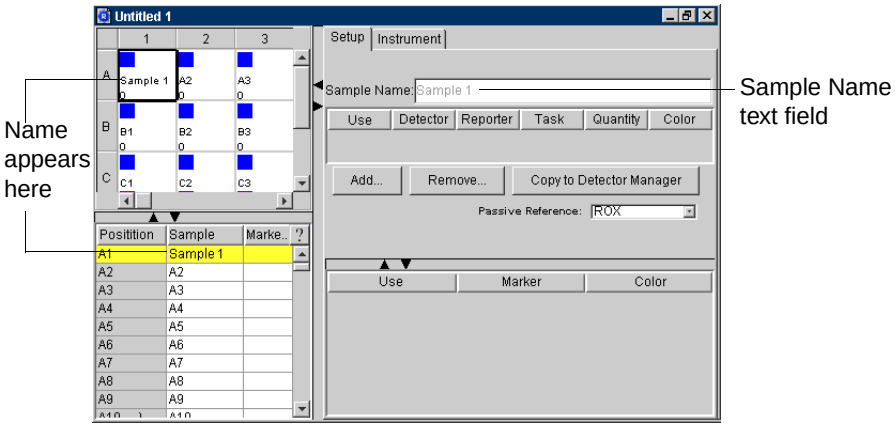
Applying Sample Names to the Plate Document

The plate document must contain sample attributes to effectively organize and analyze data produced from the run. Once applied, the software displays the sample names within the plate grid and table views.

Note You can apply sample names after the plate has been run, but they must be added prior to the analysis of the run data.

Note The SDS software features the ability to import setup table information (detector, detector task, and sample name layouts) into a plate document from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Applying sample names to the plate document:

Step	Action
1	From the plate grid of the plate document, select the wells containing the first sample.
2	Click the **Sample Name** text field, type a name for the sample, and press **Enter**. The software labels the selected wells with the new sample name.
3	Repeat steps 1 and 2 for all remaining samples. 
4	Configure the plate document with plate information as explained below.

Configuring the Plate Document Information (Optional)

To add or edit any plate information (Barcode, Operator setting, or Plate Comments):

Step	Action
1	From the **Tools** menu of the SDS software, select **Document Information**. The **Document Information** dialog box appears.
2	Edit the **Barcode**, **Operator**, or **Plate Comments** information. **Note** For more information on the features of the **Document Information** dialog box or the data it contains, click the ? button to open the contextual online help.
3	When finished, click **OK**.
4	Run the plate document and associated plate as explained on page 4-20.

Step 8 – Running the Plate on the 7900HT Instrument

Options for Running SDS Plates

The 7900HT instrument can run prepared microplates individually or in groups using the Zymark Twister Microplate Handler.

IMPORTANT If you are not using a Zymark Twister Microplate Handler, you must run plates individually.

Choose one of the following options to run the plate:

Option	Description	See Page
Individual Operation	Run the plate individually from the SDS software.	4-22
Automated Operation	Run the plate with others as part of a batch from the Automation Controller Software using the Zymark Twister Microplate Handler. **IMPORTANT** You must have a Zymark Twister Microplate Handler to run plates using this option.	4-31

Section: Running an Individual Plate

In This Section This section discusses the following topics:

Saving the Plate Document

Saving the Plate Document for Single Plate Operation

Before the plate document can be run, you must save it as an ABI PRISM SDS Single Plate (*.sds) file.

To save the plate document:

Step	Action
1	From the **File** menu of the SDS software, select **Save As**. The **Save As** dialog box appears.
2	From the **Look in** text field, navigate to and select a directory for the software to receive the new file.
3	Click the **File name** text field and either: ♦ Type a file name for the plate document file, or ♦ Type or scan the bar code number for the plate into the text field. **Note** The SDS software does not require that the file name match the bar code of the corresponding plate.
4	Click **Save**. The software saves the plate document to the specified directory.
5	Run the plate document and associate plate as explained on page 4-23.

Preparing and Running a Single Plate

Pre-Run Checklist

The following tasks must be complete to run a plate on the 7900HT instrument.

Done	Check	See Page
☐	A background run has been performed in the last month	7-13
☐	A pure dye run has been performed in the 6 months	7-17
☐	The instrument tray does not contain a plate **IMPORTANT** The instrument tray must be empty to begin a run. If the instrument tray contains a plate, eject and remove it before continuing.	4-27

Plate Requirements

See “Consumables and Disposables” on page D-3 for a complete list of ABI PRISM consumables and ordering instructions.

Preparing the Plate

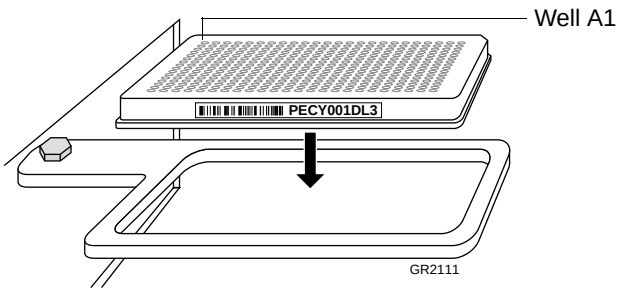
To prepare the plate:

Step	Action
1	Prepare the reactions in an ABI PRISM® Optical Reaction Plate by aliquoting reagents, enzyme, and samples to the appropriate wells of an optical plate. **IMPORTANT** The arrangement of the reactions (samples and assays) on the plate must match the configuration of the corresponding plate document.
2	Seal the ABI PRISM Optical Reaction Plate with an ABI PRISM® Optical Adhesive Cover or ABI PRISM® Optical Flat Cap Strips.
3	Briefly centrifuge the plate to collect the reactions at the bottom of the wells and to eliminate any air bubbles that may be present.
4	Choose from the following:

Experiment	Then…
Absolute Quantification/ Dissociation Curve Analysis	Run the plate as explained in “Running the Plate” on page 4-24.
Allelic Discrimination	a. Load the plate onto a designated thermal cycler and perform the PCR. b. Briefly centrifuge the plate to draw the reactions to the bottom of the wells and to eliminate any air bubbles that may have formed during thermal cycling. c. Run the plate as explained in “Running the Plate” on page 4-24.

Running the Plate

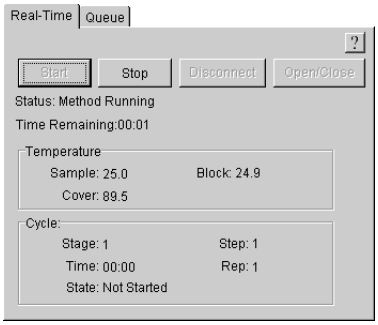
To begin the single plate run:

Step	Action
1	From the SDS software, click the **Instrument** tab of the plate document. The software displays the **Instrument** tabbed page.
2	From the **Real-Time** or **Plate-Read** tab, click **Open/Close**. The instrument tray rotates to the OUT position.
3	Place a plate into the instrument tray as shown below. Before loading the plate onto the instrument tray, make sure that: ♦ The associated plate document is open in the SDS software. ♦ The plate has been sealed using an optical adhesive cover.  **IMPORTANT** The A1 position is located in the top-left side of the instrument.
4	Do one of the following: ♦ If performing a real-time run, click **Start**. ♦ If performing a end-point run, click **Plate Read**. The instrument tray rotates to the IN position and the instrument performs the run or plate-read. **Note** Before starting the run, the instrument may pause (up to 15 minutes) to heat the heated cover to the appropriate temperature. **Note** For more information on the elements of the **Real-Time** and **Plate-Read** tabs, click the ? button and see the SDS software online help. The following options are available during and after the completion of the run:

To…	See Page
Monitor the progress of the run	4-25
Stop the run **IMPORTANT** If you must stop a run in-progress for any reason, carefully read the instructions on page 4-26 before halting the run.	4-26
Open the instrument tray (after the run)	4-27
Analyze the run data after the run is complete	4-27

Monitoring Instrument Progress

The SDS software displays instrument status and run progress in the Real-Time tab (real-time runs) or the Plate Read tab (end-point runs) of the respective plate document. The following figure shows examples of the tabs during operation of the 7900HT instrument.



Real-Time Tab
(Real-Time Runs)

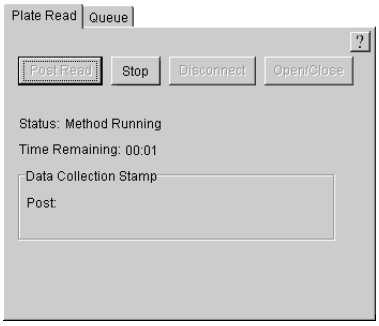


Plate Read Tab
(End-Point Runs)

Report	Displays…
Status	the condition of the 7900HT instrument
Time Remaining	the calculated time remaining in the run
Temperature group box (Real-Time Plate Documents Only)	
Block	the actual temperature of the sample block module
Cover	the actual temperature of the heated cover
Sample	the calculated temperature of the samples
Cycle group box (Real-Time Plate Documents Only)	
Rep	the current cycle repetition
Stage	the current stage of the thermal cycling
State	the current condition of the cycle stage
Step	the current step being run
Time	the calculated time remaining in the current step
Data Collection Stamp group box (End-Point Plate Documents Only)	
Post	the date that the post-read[a] was performed.

a. A ‘post’ read is a plate read performed after a plate has undergone thermal cycling.

Note For more information on the elements of the **Real-Time** and **Plate-Read** tabs, click the ? button and see the SDS software online help.

Stopping the Sequence Detector from the SDS Software

IMPORTANT Read the following directions carefully before stopping a run-in-progress.

Run Type	Action
End-Point Runs ♦ Allelic Discrimination	From the **Plate-Read** tab of the plate document, click **Stop**.
Real-Time Runs ♦ Absolute Quantification ♦ Melting Curve	Has the **Cover** reading in the **Real-Time** tab reached **104 °C**? ♦ **NO** (the heated cover temperature is below 104 °C) From the **Real-Time** tab of the plate document, click **Stop**. ♦ **YES** (the heated cover temperature has reached 104 °C) The instrument has begun to run the plate and is in the process of thermal cycling. Determine a course of action from the options in the following table:

Reason for Stopping the Run	Then…
Forgot to make a change to the plate document such as adding a detector, detector task, or sample name (not including mistakes to the temperature profile)	do not stop the run. Allow the instrument complete the run, then edit the plate document before analyzing the data. The software does not use detector or sample information until the plate document is analyzed.
♦ Forgot to add a reagent to the plate (such as enzyme or master mix) ♦ Programmed the plate document with the wrong thermal profile	a. From the **Real-Time** tab of the plate document, click **Stop**. b. Determine how far into the run the instrument has progressed. c. Based on the state of the run, determine whether the plate can be re-run. d. If necessary, eject the plate and add the missing reaction component. e. If desired, re-run the plate by re-creating the plate document.

After the Run

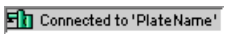
Analyzing the Run Data

The run can be analyzed immediately following the completion of the run:

To analyze data from a plate containing assays for…	See Page
Allelic Discrimination	5-3
Absolute Quantification	6-3
Dissociation Curve Analysis	6-17

Ejecting a Plate (Opening/Closing the Instrument Tray)

The instrument tray must be opened from the plate document that is connected to the 7900HT instrument. View the instrument status icon to determine the plate document to use to eject the plate.

Instrument Status	Action
	a. From the **Window** menu of the SDS software, select the plate document connected to the instrument. b. From the plate document, select the **Instrument** tab c. Click the **Plate-Read** or **Real-Time** tab. d. Click **Open/Close**.
	a. From the **File** menu of the SDS software, select **New**. b. Click **OK**. c. Click the **Instrument** tab. d. Click the **Plate-Read** or **Real-Time** tab. e. Click **Open/Close**.

Disconnecting the Software from the Instrument

The SDS software has the ability to halt all communications with the 7900HT instrument. The ‘disconnect’ option is designed to permit the simultaneous operation of both the SDS software and the Automation Controller Software. Because both programs control the ABI PRISM 7900HT Sequence Detection System, one program must relinquish control of the 7900HT instrument before the other can be used to operate it.

To…	Then…
disconnect the software from the instrument	a. From the SDS software, click the **Instrument** tab of the open plate document. b. Click the **Real-Time** or **Plate-Read** tab. c. Click **Disconnect**. **Note** Once disconnected, the software neither monitors nor controls the 7900HT instrument.
reconnect the software (once disconnected)	to reconnect to an open plate document: a. From the **File** menu, select **Close** to close the plate document. b. From the **File** menu, select **Open**. c. From the **Look In** text field, navigate to and select the plate document of interest. d. Click **Open**, Upon opening the plate document, the software re-establishes the 7900HT instrument connection.
	to reconnect to an new plate document, select **New** from the **File** menu. Upon creation of the plate document, the software re-establishes the connection with the 7900HT instrument.

Section: Running Multiple Plates Using the Automation Controller

In This Section This section discusses the following topics:

Adding Plate Documents to the Plate Queue for Automated Operation

Automation Operation and the Plate Queue

The first step in configuring the ABI PRISM 7900HT Sequence Detection System for automated operation, is to add plate documents to the plate queue. The plate queue is a list of plate document files that the Automation Controller Software uses to identify and run associated plates during automated operation. By adding plate documents to the queue, they automatically become available for use with the Zymark Twister Microplate Handler.

IMPORTANT Once a plate document has been added to the plate queue, the software locks the file preventing any changes from being made to it until the plate document has been run or removed from the queue.

The ABI PRISM 7900HT Sequence Detection System features several options for adding plate documents to the plate queue. Review the options discussed in the following table and choose the method that best suits your needs:

Option	See Page
Add a plate document to the plate queue from the SDS software.	4-31
Using the Template Batch utility, create batches of plate documents from a template file and add them to the plate queue.	4-32
Add or remove individual or multiple plate documents to the plate queue using the Automation Controller Software.	4-34

Adding a Plate Document to the Plate Queue from the SDS Software

Adding the Plate Document to the Plate Queue

IMPORTANT A plate document must contain a bar code before it can be added to the plate queue. See page 4-19 for more information on configuring a plate document with bar code information.

To add the plate document to the plate queue from the SDS software:

Step	Action
1	From the SDS software, send the plate document to the queue as follows: a. Click the **Instrument** tab. The software displays the contents of the **Instrument** tabbed page. b. Click the **Queue** tab. The software displays the contents of the **Queue** tabbed page.
2	Click **Send to Queue**. **If…** / **Then…** the plate document was not saved previously → the **Save As** dialog box appears. a. From the **Look in** text field, navigate to a directory for the software to save the new file. b. From the **Files of type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**. c. Click the **File name** text field and either: – Type a name for the plate document file, or – Type or scan the bar code number of the plate into the text field. d. Click **Save**. The software saves the plate document. the plate document was saved previously → the software automatically saves the plate document.
3	When prompted, click **Yes** to submit the document to the plate queue. Once a plate document has been added to the plate queue, the software locks the file preventing any changes from being made to it until the plate document has been run or removed from the queue. **Note** To release the plate document from the queue, launch the Automation Controller Software and remove the plate document from the queue as explained on page 4-35.
4	Click **OK** to close the dialog confirming that the plate document has been added to the plate queue.
5	From the **File** menu, select **Close**. The SDS software closes the plate document.
6	Repeat the procedures in this chapter to create and add additional plates to the queue as needed.
7	When finished creating plate documents, run the enqueued plates as explained in “Running Plates Using the Automation Controller Software” on page 4-34.

Creating Plate Documents Using the Template Batch Utility

About the Template Batch Utility

The Template Batch utility allows you to quickly create multiple plate documents from a single ABI PRISM SDS Template file (*.sdt). The Template Batch utility can be a useful time-saving device in situations where samples are run on plates with identical assay configurations.

IMPORTANT Plate documents created by the Template Batch utility do not contain sample or plate information. This information must be applied to each plate document individually after the file is run.

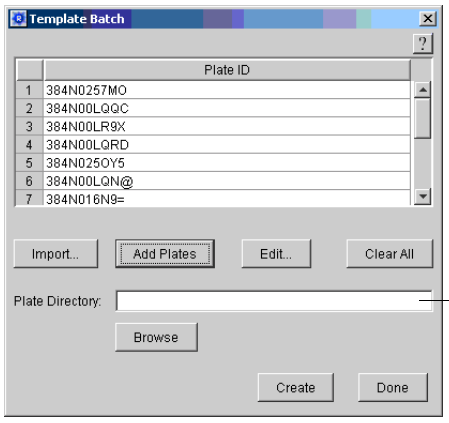
Generating Plate Documents From a Template

Note For more information on the elements of the Template Batch dialog box or to view the procedures for importing or editing Plate IDs, click the ? button and see the SDS software online help.

To create plate documents from a template using the Template Batch Utility:

Step	Action
1	From the SDS software, open a template file as follows: a. From the **File** menu, select **Open**. b. From the **File Type** drop-down list, select **ABI PRISM SDS Template Document (*.sdt)**. c. From the **Look in** text field, navigate to and select the template file. d. Click **Open**. The SDS software displays the template file.
2	Send the plate document to the queue as follows: a. Click the **Instrument** tab. The software displays the contents of the **Instrument** tabbed page. b. Click the **Queue** tab. The software displays the contents of the **Queue** tabbed page.
3	Click **Send to Queue**. The **Template Batch** dialog box appears.
4	Configure the **Template Batch** dialog box with Plate IDs as follows: a. Click **Add Plates**. The **Add Plates** dialog box appears. b. Click the **Plate ID** text field, and scan the bar code of the first plate in the batch using the hand-held bar code scanner. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam. c. Repeat step b for every plate in the batch. d. When finished, click **Done**. The plate bar codes appear within the Plate ID field.

To create plate documents from a template using the Template Batch Utility: *(continued)*

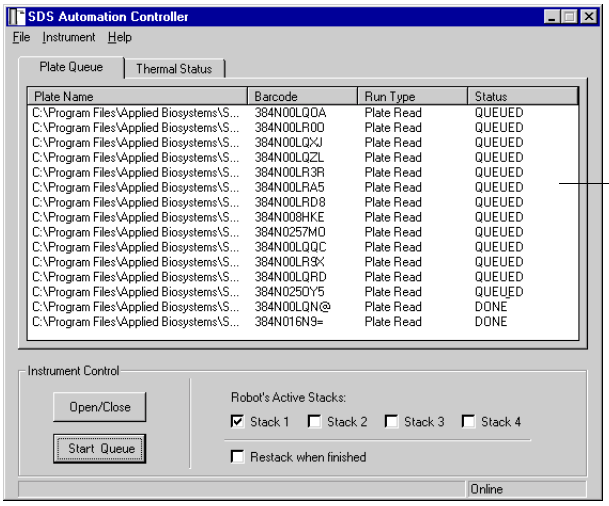
Step	Action
5	Select a destination directory to store the new plate documents as follows: a. Click **Browse**. b. From the **Look in** text field, navigate to and select the directory you want to use to receive the new files. c. Click **Open**. The Template Batch dialog box displays the selected destination directory in the **Plate Directory** text field. 
6	Click **Create**. The software creates plate documents for all entries listed in the Plate ID list, saves them to the destination directory, adds them to the plate queue, and displays a message indicating the number of plate documents it created and sent to the plate queue.
7	Click **OK** to close the message box.
8	Click **Done**. The **Template Batch** dialog box closes.
9	From the **File** menu, select **Close**. The SDS software closes the template document.
10	Repeat the procedures in this chapter to create and add additional plates to the queue as needed. **Note** Once a plate document has been added to the plate queue, the software locks the file preventing any changes from being made to it until the plate document has been run or removed from the queue.
11	When finished creating and adding plate documents to the queue, run the queue as explained in “Running Plates Using the Automation Controller Software” on page 4-34.

Running Plates Using the Automation Controller Software

Launching the Automation Controller Software

The ABI PRISM 7900HT Sequence Detection System employs the Automation Controller Software for automated operation of the 7900HT instrument. The software coordinates the action of the 7900HT instrument, the bar code reader, and the plate handler while acquiring and saving raw data during each run.

To launch the Automation Controller Software as follows:

Step	Action
1	If running the SDS software, do one of the following: ♦ From the **Instrument** menu, select **Disconnect** to discontinue communication between the software and the instrument, or ♦ From the **File** menu, select **Exit** to close the SDS software.
2	Do one of the following: ♦ Double click the Automation Controller Software icon ( ) on the desktop. ♦ From the **Start** menu, select **Programs** > **SDS 2.0** > **Automation Controller 2.0**. The Automation Controller Software launches and displays the main screen.
3	Verify that **Plate Queue** text field contains plate documents for all plates you intend to run. ♦ To add a plate document to the plate queue, see “Adding Plates to the Plate Queue” on page 4-35. ♦ To remove a plate document from the plate queue, see “Removing Plate Documents from the Plate Queue” on page 4-35.  Plate Queue (must contain plate documents for all plates to be run)
4	Prepare and load the plates onto the plate handler as explained on page 4-36.

Adding Plates to the Plate Queue

To add plate documents to the plate queue:

Step	Action
1	From the **File** menu of the Automation Controller Software, select **Add Plates**. The software displays the **Open** dialog box.
2	From the **Look in** text field, navigate to the directory containing the file or files of interest.
3	While pressing and holding the **Ctrl** key, click the plate document file(s) to add to the plate queue. The software highlights selected files. **IMPORTANT** A plate document must contain a bar code before it can be added to the plate queue. See page 4-19 for more information on configuring a plate document with bar code information.
4	Click **Open**. The Automation Controller Software adds the plate document(s) to the Plate Queue. **Note** Once a plate document has been added to the plate queue, the software locks the file preventing any changes from being made to it until the plate document has been run or removed from the queue.

Removing Plate Documents from the Plate Queue

To remove plate documents from the plate queue:

To remove…	Then…
specific plate documents from the plate queue	a. While pressing and holding the **Ctrl** key, click the plate document files to remove. The software highlights selected files. b. From the **File** menu, select **Clear Selected Plate(s)**. The software removes the selected plate documents from the plate queue.
all plate documents from the plate queue	From the **File** menu of the Automation Controller Software, select **Clear All Plates**. The software removes all plate documents from the plate queue.

Loading Plates onto the Automation Module

Pre-Run Checklist

The following tasks must be complete to run plates on the 7900HT instrument. See the associated page number for details on each procedure.

Done	Check	See Page
☐	A background run has been performed in the last month	7-13
☐	A pure dye run has been performed in the 6 months	7-17
☐	The plate queue contains plate documents for all plates to be run	4-6
☐	The instrument tray does not contain a plate **IMPORTANT** The instrument tray must be empty to begin a run. If the instrument tray contains a plate, eject and remove it before continuing.	4-38
☐	The output stack does not contain plates.	–

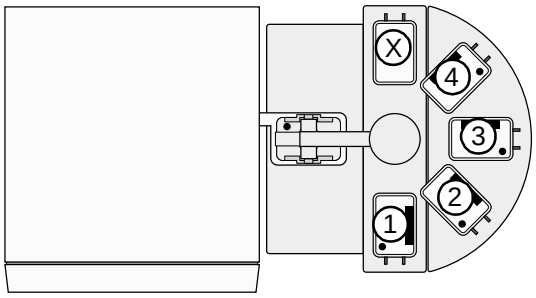
Plate Requirements

See “Consumables and Disposables” on page D-3 for a complete list of ABI PRISM consumables and ordering instructions.

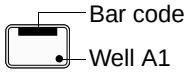
Guidelines

Observe the following guidelines when loading plates onto the plate handler:

- Before loading plates onto the plate handler, make sure that for each plate:
  - the associated plate document has been added to the plate queue
  - the plate has been sealed using an optical adhesive cover.
- Load the plates into the plate handler stacks in any order. The software reads the bar code of each plate before it is run and matches the plate document and method with the plate. The Automation Controller Software can run batches of up to 84 plates in a single session (21 plates/stack).
- Orient the plates within the stacks so that well A1 (•) of each plate corresponds to the locations shown in the illustration below.
- Do not place plates in the output stack (X). The Zymark arm uses the empty stack to store used plates after they are run.

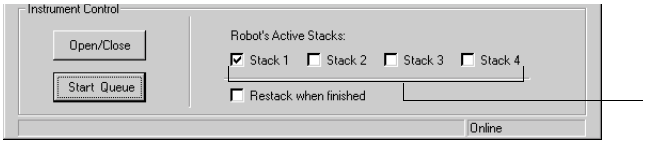


Zymark Twister Microplate Handler
(top view)



Loading Plates

To load plates onto the automation module:

Step	Action
1	Following the guidelines on page 4-36, load the sealed plates into the plate handler stacks.
2	From the Automation Controller Software, select the check boxes for the plate stacks containing plates. **IMPORTANT** If you are not using stack 1 or the Restack option explained below, remove all plates from stack 1 before starting the queue. Under these settings, the plate handler will attempt to stack the run plates from stack 2 in the stack 1 position. If stack 1 contains plates, these settings will cause the plate handler to stop the run.  Plate stack check boxes
3	If you want to retain the location of the plates in the stacks on the plate handler, select the **Restack when finished** check box. When selected, the **Restack** function instructs the arm to replace a stack of used plates to their original stack and in their original order after the stack has been run. If the option is not selected, the arm will place each group of used plates within the next vacant stack in clockwise order beginning with the Output stack. **Note** Restacking plates adds significant operating time when running multiple plates. Use the **Restack** function only when absolutely necessary.
4	Begin the plate queue as explained on page 4-38.

Operating the 7900HT Instrument Using the Automation Controller Software

Starting the Plate Queue

Once all plate documents have been loaded into the Plate Queue and the Instrument Control options are configured you may start the plate queue.

To begin the plate queue, click **Start Queue** from the Plate Queue tabbed page.

The ABI PRISM 7900HT Sequence Detection System loads the first plate, scans the bar code, and the begins the run.

Note Before starting a real-time run, the instrument may pause (up to 15 minutes) to heat the heated cover to the appropriate temperature.

Monitoring Instrument Progress

The Automation Controller Software displays the progress of the current run in the Thermal Status tab. See page 4-25 for an explanation of the Thermal Status tab.

Stopping the Sequence Detector from the Automation Controller Software

To stop the plate queue, click the **Stop** button on the Automation Controller Software dialog at any time.

If the Stop button is clicked...	Then the instrument...
while the plate handler is handling a plate	aborts the run and moves the plate handler to the home position.
after a plate has been loaded into the instrument, but before the run has started.	aborts the current run, ejects the plate, and moves the plate handler to the home position.
after the 7900HT instrument has started a run.	aborts the current run, ejects the plate, and moves the plate handler to the home position. **IMPORTANT** Stopping a run during thermal cycling can affect the chemistry of the reactions within the plate. Before stopping a run, carefully read the guidelines on page 4-26 to determine the best course of action.

Ejecting a Plate (Opening/Closing the Instrument Tray)

To eject a plate following a halted run, click **Open/Close** from the Automation Controller Software window.

After the Run

Analyzing the Run Data

The run can be analyzed immediately following the completion of the run:

To analyze data from a plate containing assays for…	See Page
Allelic Discrimination	5-3
Absolute Quantification	6-3
Dissociation Curve Analysis	6-17

End-Point Analysis

5

In This Chapter

This chapter discusses the following topics:

End-Point Runs on the 7900HT Instrument

End-Point Runs

End-point is the term used to describe the category of sequence detection runs in which the ABI PRISM® 7900HT Sequence Detection System is used to measure the fluorescence of a biological sample after it has undergone thermal cycling. Unlike real-time runs that can yield quantitative measurements, the focus of end-point experiments is typically to produce a qualitative result. End-point analysis is commonly used in combination with TaqMan® chemistry to confirm the presence or absence of specific target nucleic acid sequence in cells, tissues, or fluid samples.

Currently, the SDS software supports one type of end-point analysis: Allelic Discrimination.

Section: Allelic Discrimination

In This Section This section contains the following information:

Overview

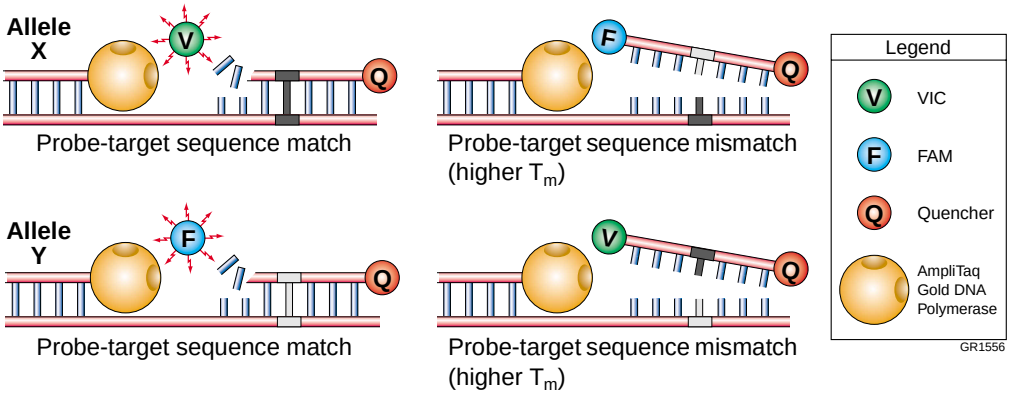
Allelic Discrimination on the 7900HT Instrument

The ABI PRISM 7900HT Sequence Detection System supports allelic discrimination using TaqMan® probes. Allelic discrimination is the process by which two variants of a single nucleic acid sequence are detected in a prepared sample. Allelic discrimination chemistry can be used for single-nucleotide polymorphism (SNP) detection.

Employing the 5´ Nuclease Assay for Allelic Discrimination

Allelic discrimination on the 7900HT instrument is made possible through the use of the fluorogenic 5´ nuclease assay (see page A-2). During the PCR, the fluorogenic probes anneal specifically to complementary sequences between the forward and reverse primer sites on the template DNA. Then during extension, AmpliTaq Gold® DNA polymerase cleaves the probes hybridized to the matching allele sequence(s) present in each sample. The cleavage of each matched probe separates the reporter dye from the quencher dye, which results in increased fluorescence by the reporter. After thermal cycling, the plate is run on the 7900HT instrument which reads the fluorescence generated during the PCR amplification. By quantifying and comparing the fluorescent signals using the SDS software, it is possible to determine the allelic content of each sample on the plate.

Mismatches between a probe and target reduce the efficiency of probe hybridization. Furthermore, AmpliTaq Gold DNA polymerase is more likely to displace the mismatched probe than to cleave it, releasing the reporter dye. By running the extension phase of the PCR at the optimal annealing temperature for the probes, the lower melting temperatures (T_m) for mismatched probes minimizes their cleavage and consequently their fluorescent contribution. The figure below illustrates results from matches and mismatches between target and probe sequences in TaqMan® PDARs for AD assays (Livak *et al.*, 1995; Livak *et al.*, 1999).



The table below shows the correlation between fluorescence signals and sequences present in the sample.

A substantial increase in…	Indicates…
VIC™ fluorescence only	homozygosity for Allele X.
FAM™ fluorescence only	homozygosity for Allele Y.
both fluorescent signals	heterozygosity.

Algorithmic Manipulation of Raw Allelic Discrimination Data

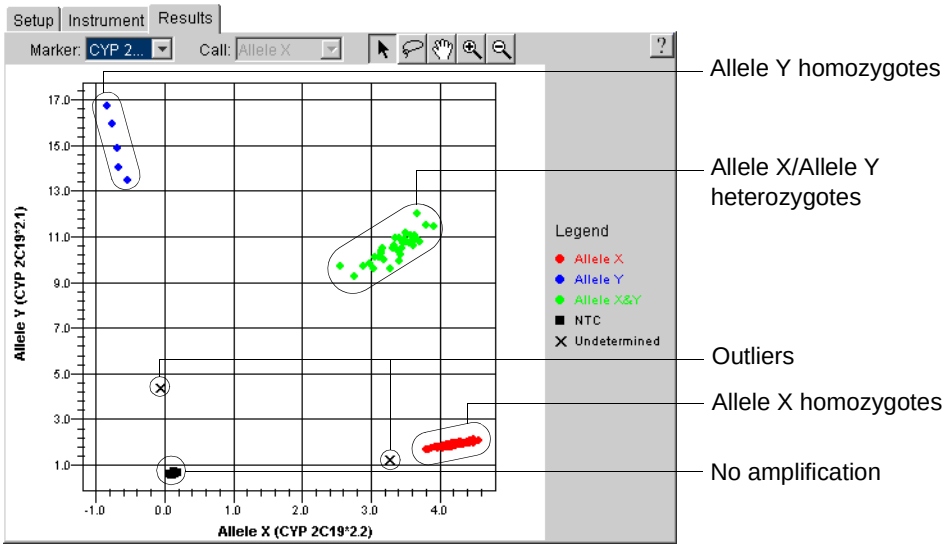
The SDS software can analyze raw data immediately upon completion of an allelic discrimination run. The term ‘raw data’ refers to the spectral data between 500 nm to 660 nm collected by the SDS software during the plate-read. During the analysis, the software employs several mathematical algorithms to generate from the raw data a more direct measure of the relationship between the spectra changes in the unknown samples.

The first mathematical algorithm involves the conversion of the raw data, expressed in terms of Fluorescent Signal vs. Wavelength, to pure dye components using the extracted pure dye standards. After the dye components have been identified, the software determines the contribution of each dye in the raw data using the multicomponent algorithm. See “Multicomponenting” on page A-5 for a complete description of the process.

Cluster Variations

The SDS software graphs the results of an allelic discrimination run on a scatter plot contrasting reporter dye fluorescence (Allele X R_n versus Allele Y R_n). The software represents each well of the 384-well plate as a datapoint on the plot. The clustering of these datapoints can vary along the horizontal axis (Allele X), vertical axis (Allele Y), or diagonal (Allele X/Allele Y). This variation is due to differences in the extent of PCR amplification, which could result from differences in initial DNA concentration.

The example below shows the variation in clustering due to differences in the extent of PCR amplification.



Genotypic Segregation of Datapoint Clusters

The figure on the previous page illustrates the concept of genotypic segregation of samples within the allele plot. The plot contains four separate, distinct clusters which represent the No Template Controls and the three possible genotypes (allele X homozygous, allele Y homozygous, and heterozygous). Because of their homogenous genetic compliment, homozygous samples exhibit increased fluorescence along one axis of the plot (depending on the allele they contain). In contrast, heterozygous samples appear within the center of the plot because they contain copies of both alleles, and therefore exhibit increased fluorescence for both reporter dyes.

About Outliers

Samples that did not cluster tightly may:

- Contain rare sequence variations
- Contain sequence duplications
- Not contain a crucial reagent for amplification (the result of a pipetting error)

Before You Begin

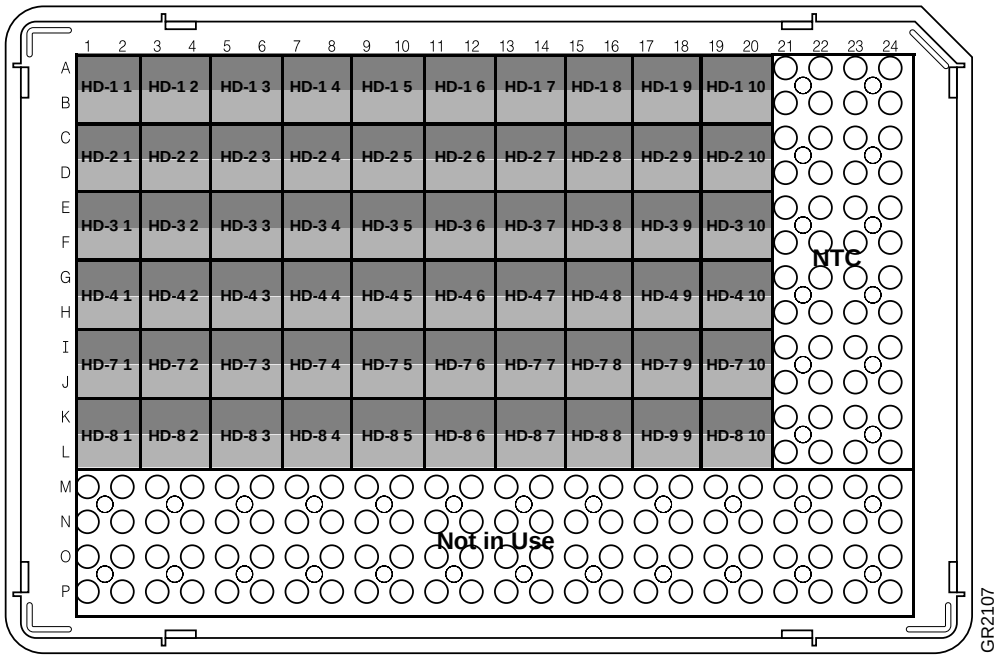
Using SDS Online Help

For specific instructions on any procedure described within this section, refer to the online help accompanying the SDS software. To get help at any time during the procedure, click a help button (?) located within the dialog box or window in which you are working.

Examples in This Section

The illustrations and screenshots that appear within this chapter were created from a plate containing Pre-Developed TaqMan Assays and Reagents for Allelic Discrimination run to screen 6 human genomic DNA samples (HD 1, 2, 3, 4, 7, 8) for 2 targets (CYP 2C9*2 and CYP 2C19*2). Each well of the plate contains 1 µL DNA, 1X TaqMan® Universal PCR Master Mix, forward and reverse primers, and FAM and VIC-labeled TaqMan probes.

The following figure illustrates the arrangement of the assays, unknown samples, and no template control (NTC) wells on the plate.



PDAR CYP 2C9*2

PDAR CYP 2C19*2

Note The probes used in the example experiment were designed using the Primer Express™ Primer Design Software and by following the guidelines explained in “Assay Development Guidelines” on page C-2.

IMPORTANT The SDS software does not require that allelic discrimination plates contain positive controls.

Analysis Checklist

Where You Are in the Procedure

The following checklist illustrates your current position in the overall procedure:

<table>
<tr><th>Done</th><th>Step</th><th>Procedure</th><th>See Page</th></tr>
<tr><td>✓</td><td>1</td><td>Create an allelic discrimination plate document.</td><td>4-6</td></tr>
<tr><td>✓</td><td>2[a]</td><td>a. Create detectors for the allelic discrimination probes.
b. Create a marker for each allelic discrimination probe pairing.
c. Copy the marker(s) to the plate document.</td><td>4-7
4-9
4-10</td></tr>
<tr><td>✓</td><td>3[a]</td><td>Assign detector tasks to the wells of the plate document (NTC and Unknown).</td><td>4-11</td></tr>
<tr><td>✓</td><td>4</td><td>If you would like to perform thermal cycling of the allelic discrimination plate on the 7900HT instrument, create a real-time plate document for the plate and program it with the method for the allelic discrimination run. Otherwise, continue to step 5.</td><td>4-13</td></tr>
<tr><td>✓</td><td>5</td><td>Choose from the following:
<table><tr><th>If running…</th><th>Then…</th></tr><tr><td>a single plate</td><td>continue to step 7.</td></tr><tr><td>the first plate in a series of plates with identical assay configurations</td><td>Save the plate document as an ABI PRISM SDS Template Document as explained on page 4-17.</td></tr></table></td><td></td></tr>
<tr><td>✓</td><td>6</td><td>Create a plate document from the template created in step 5.</td><td>4-18</td></tr>
<tr><td>✓</td><td>7</td><td>Configure the document with sample names and plate information.</td><td>4-19</td></tr>
<tr><td>✓</td><td>8</td><td>a. Prepare the allelic discrimination plate or plates and perform thermal cycling on a designated thermal cycler.
b. Run the allelic discrimination plate or plates on the 7900HT instrument.</td><td>4-20</td></tr>
<tr><td>☐</td><td>9</td><td>Analyze the run data.</td><td>5-9</td></tr>
<tr><td>☐</td><td>10</td><td>View the results of the allelic discrimination run.</td><td>5-10</td></tr>
<tr><td>☐</td><td>11</td><td>Call allele types for each marker.</td><td>5-11</td></tr>
<tr><td>☐</td><td>12</td><td>Scrutinize the allele calls.</td><td>5-13</td></tr>
<tr><td>☐</td><td>13</td><td>Choose from the following post-analysis options:
♦ Reanalyze the run data.
♦ Adjust the display settings for the plate document.
♦ Print elements of the plate document.
♦ Export the plate document results table or plots.</td><td>5-15</td></tr>
</table>

a. Steps 2 and 3 can be eliminated by importing the plate document setup information from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Analyzing a Completed Allelic Discrimination Run

Analyzing the Run

To analyze a completed allelic discrimination run:

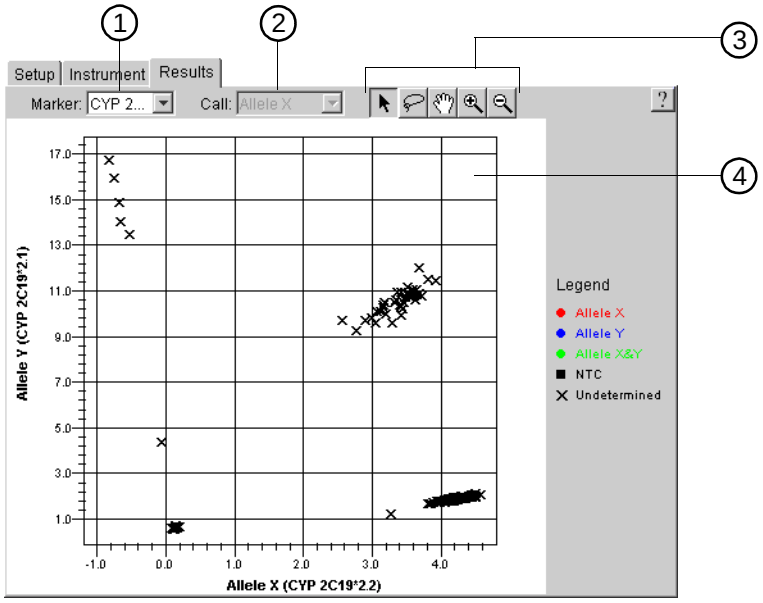
Step	Action
1	If not already open, launch the SDS software as explained on page 4-5.
2	Open the plate document file for analysis as follows: a. From the **File** menu, select **Open**. b. From the **Look in** text field, navigate to and select the plate document file. c. Click **Open**. The SDS software displays the plate document file.
3	Choose one of the following: ♦ From the **Analysis** menu, select **Analyze**. ♦ From the toolbar, click the Analyze button (). The SDS software analyzes the run data and displays the results in the **Results** tab.

Calling and Scrutinizing Allelic Discrimination Data

About the Allelic Discrimination View

The SDS software graphs the results of allelic discrimination runs on a scatter plot contrasting reporter dye fluorescence. After signal normalization and multicomponent analysis, the software graphs the normalized data from each well as a single data point on the plot.

The following figure illustrates the components of the Allelic Discrimination plot.



The following table describes the elements of an SDS plate document:

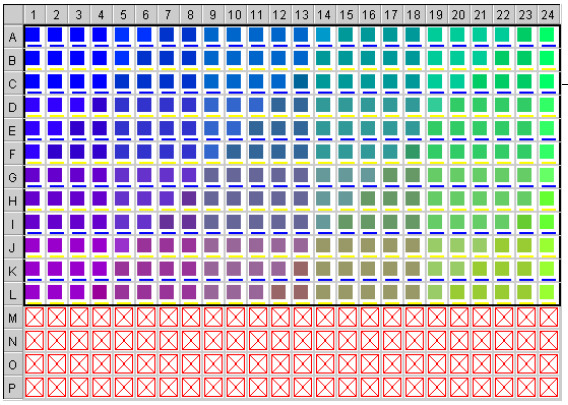
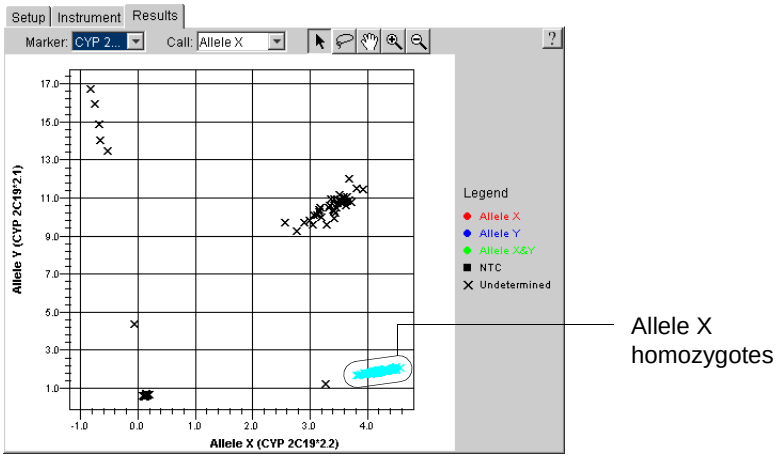
Component		Description
1	**Marker** drop-down list	Determines the marker data that the software displays within the plot.
2	**Call** drop-down list	When a datapoint is selected, this menu allows you to assign an allele call to the datapoint within the scatter plot.
3	Toolbar	Contains the following tools for manipulating the plot.

Icon	Description
	Selects individual data points by clicking or groups of datapoints by clicking and dragging a box across a group of data points.
	Selects groups of datapoints by encircling them with the tool.
	Repositions the view within the plot by clicking and dragging the screen.
	Zooms the plot by clicking the mouse button within the plot or by clicking and dragging a section of the plot to view.
	Zooms out on the plot by clicking the mouse button within the plot.

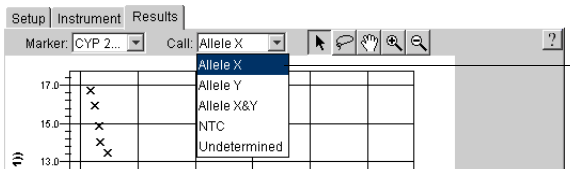
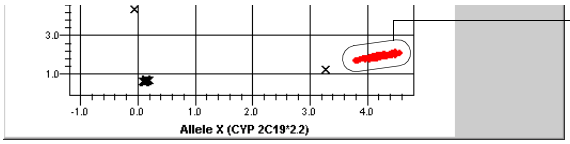
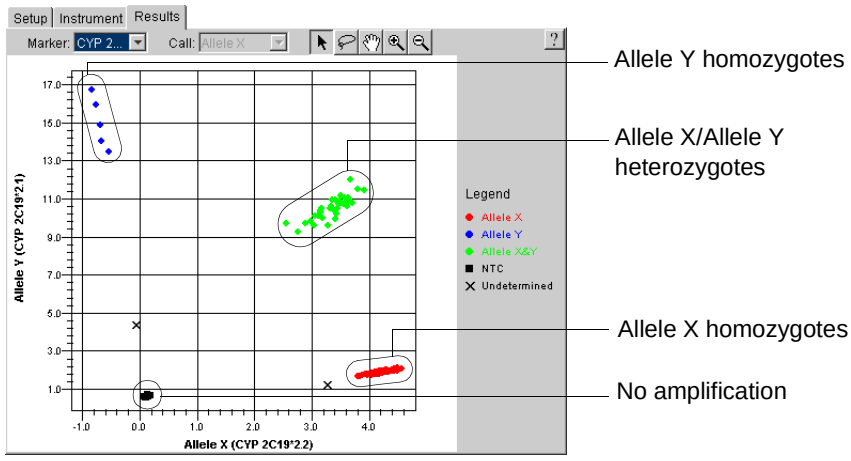
4	Scatter plot	A scatter plot of data points from the run.

Calling Allele Types

To call allele types:

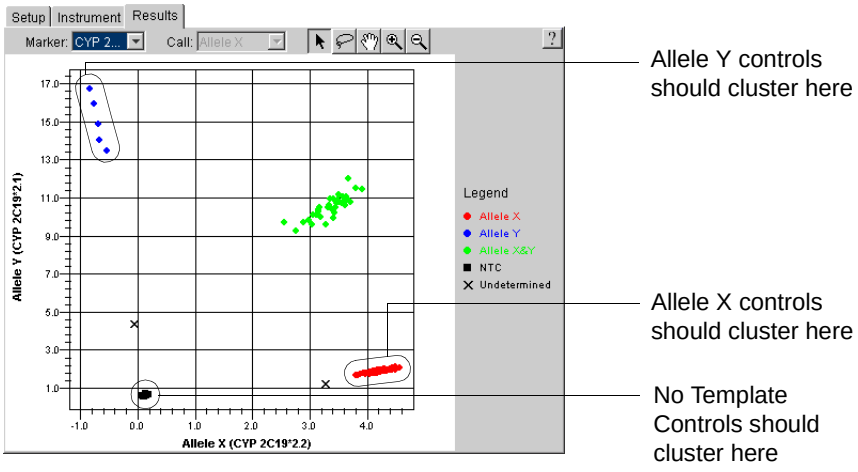
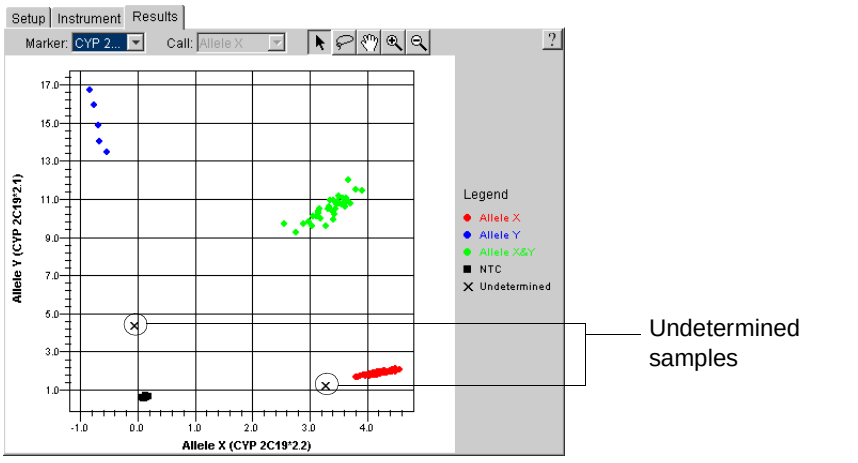
Step	Action
1	Click the **Results** tab. The software displays the **Allelic Discrimination Plot**.
2	Zoom out until all crossmarks are visible in the plot. a. Click the ( ) magnifying glass tool. b. Click the plot to zoom out. c. Click the ( ) lasso tool. d. Select all of the marks within the plot by clicking and dragging the mouse pointer across all datapoints in the plot. The software outlines all selected wells within the grid view. e. Examine the tray pane to confirm that all wells are selected. If not all wells are selected, repeat steps a-d until all wells are visible on the plot. 
3	Select the sample cluster exhibiting amplification of the first probe. 

To call allele types: *(continued)*

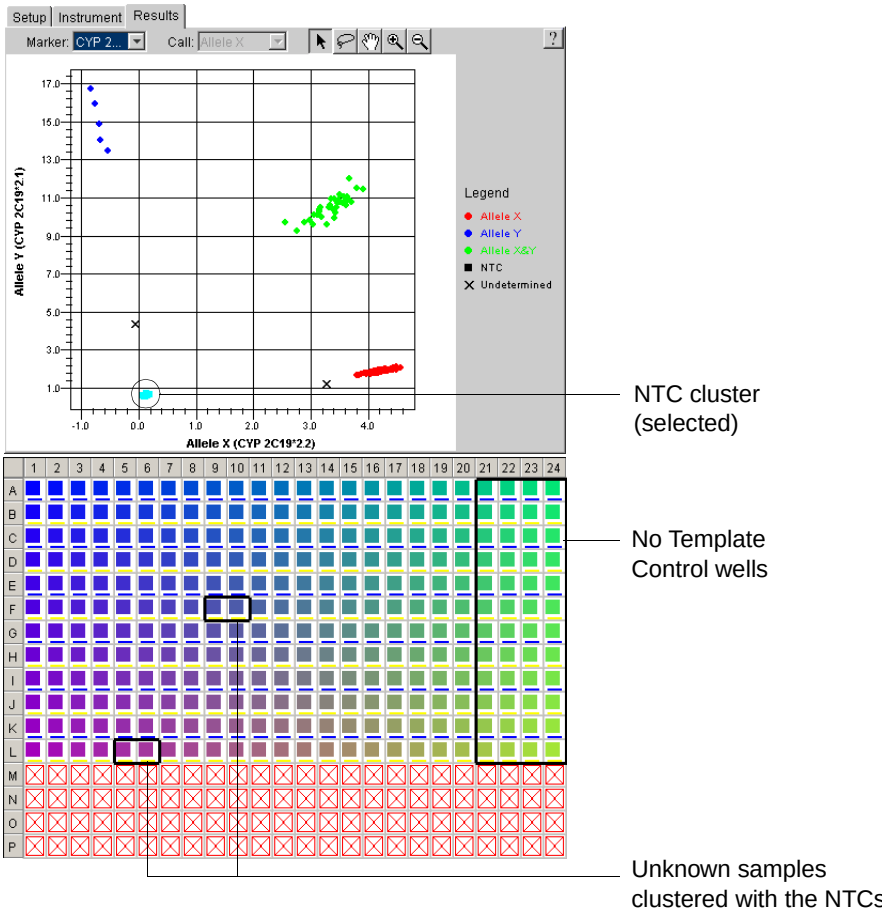
Step	Action
4	From the **Call** drop-down list, select the Allele X call.  The software automatically labels the samples and wells with the **Allele X** call. 
5	Repeat steps 3 and 4 to apply calls to the rest of the samples within the plot. **Call** / **Symbol** / **Definition** Allele X / ● / Homozygous for the allele displayed on the X-axis of the Allelic Discrimination Plot. Allele Y / ● / Homozygous for the allele displayed on the Y-axis of the Allelic Discrimination Plot. Both / ● / Heterozygous (Alleles X and Y) NTC / ■ / No Template Control Undetermined / × / Unknown (Unlabeled) **Note** You can adjust the appearance of the allelic discrimination plot or the data points it contains using the Display Settings dialog box. See the SDS software online help for more information. 
6	If evaluating for multiple markers, do the following: a. From the **Marker** drop-down list, select a different marker. b. Repeat steps 2 to 5 for the new marker. c. Repeat steps a and b until the alleles for each marker have been called.

Scrutinizing the Allele Calls

To analyze allele types:

Step	Action
1	Verify the calls for the **NTC**, **Allele X**, and **Allele Y** controls. a. From the plate grid, select the wells containing the **No Template Control** samples. The software highlights the datapoints within the allele plot. b. Check that the datapoints cluster in the expected position on the plot. c. If using positive controls, repeat steps a and b for the wells containing the **Allele X**, and **Allele Y** controls. 
2	Designate samples that did not cluster tightly as **Undetermined**. Samples that did not cluster tightly may: ♦ Contain rare sequence variations ♦ Contain sequence duplications 

To analyze allele types: *(continued)*

Step	Action
3	Screen for Unknown samples that failed to amplify: a. From the Allelic Discrimination Plot, select the NTC cluster. The SDS software highlights the datapoints within the allele plot and the plate grid. b. From the plate grid, check the wells containing Unknown samples for selected wells that are clustered with the **NTCs**. Samples that clustered with the No Template Control wells may: ♦ Contain no DNA ♦ Contain PCR inhibitors ♦ Be homozygous for a sequence deletion 
4	Retest any samples that did not cluster tightly or clustered with **NTCs** to confirm the results.
5	If evaluating for multiple markers, do the following: a. From the **Marker** drop-down list, select a different marker. b. Repeat steps 1 to 4 for the new marker. c. Repeat steps a and b until the calls for each marker have been verified.

After the Analysis

Changing the Plate Document Display Settings

Before printing or exporting the analyzed data, the software allows you to reconfigure the appearance of several elements of the plate document including the results table, plate grid, and most plots (Allelic Discrimination, Raw Data, and Background plots).

To configure the display settings for the plate document:

Step	Action
1	From the **View** menu, select **Display Settings**.
2	From the **Display Settings** dialog box, click the help button (?) for further instructions on modifying the display settings.

Saving the Plate Document

The software allows you to save any changes to the appearance of the plate document, however it does not save the calls made during the analysis. To save the plate document, select Save from the File menu.

Printing a Report

The SDS software can print a report of the analyzed data containing individual or multiple elements of the plate document.

To print a report of the plate document data:

Step	Action
1	From the **File** menu, select **Print Report**.
2	From the **Print Report** dialog box, click the help button (?) for instructions on setting up, previewing, and printing the report.

Exporting Plate Document Data

Exporting Plate Document Data as a Tab-Delimited Text File

The SDS software can export raw or analyzed data in tab-delimited (*.txt) format for all or a select group of wells on a plate document. The exported files are compatible with most spreadsheet applications and programs that can read tab-delimited text.

To export run data as a tab-delimited text file, choose one of the following for further instructions:

- ♦ See “Exporting Plate Document Data” on page B-9.
- ♦ Click the help button (?) within the table view.

Exporting Plots as Graphics

The SDS software can export most panes and plots of the plate document as JPEG (Joint Photographic Experts Group) graphic files. The JPEG file format is compatible with most word processing and spreadsheet applications and can be incorporated directly into HTML documents for viewing by most web browser software.

To export a plot as a graphic file, see “Exporting Graphics” on page B-8 or click the help button (?) within the plot of interest for further instructions.

Real-Time Analysis 6

In This Chapter This chapter discusses the following topics:

Real-Time Runs on the 7900HT Instrument

Real-Time Runs

Real-time is the term used to describe the category of sequence detection runs in which the ABI PRISM® 7900HT Sequence Detection System is used to measure the fluorescence of a biological sample during thermal cycling. In contrast to end-point runs, real-time experiments can be used to achieve both qualitative and quantitative measurements. Real-time analysis can be used in combination with either TaqMan® or SYBR® Green 1 double-stranded DNA binding dye chemistry for a variety of purposes including quantitative PCR and dissociation curve analysis.

Quantitative RT-PCR

Quantitative RT-PCR is a method used to measure small quantities of ribonucleic acid sequences isolated from biological samples. Typical biological samples include cells, tissues, and fluids. During the RT step, reverse transcription of target RNA produces corresponding complementary DNA (cDNA) sequences. During the subsequent PCR, the initial concentration of target cDNA is quantified by amplifying it to a detectable level.

There are two types of quantitative RT-PCR:

- Absolute quantification
- Relative quantification

Section: Absolute Quantification

In This Section This section contains the following information:

Overview

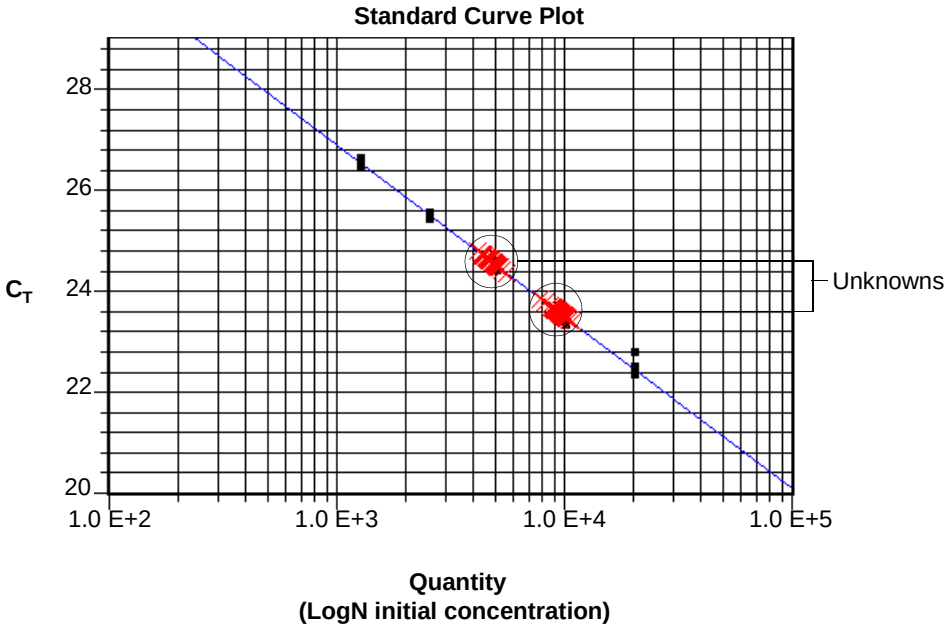
About Absolute Quantification

The ABI PRISM 7900HT Sequence Detection System supports real-time absolute quantification of nucleic acids using a standard curve method. The objective of absolute quantification is to accurately determine the absolute quantity of a single nucleic acid target sequence within an unknown sample. The results of an absolute quantification experiment are reported in the same unit measure of the standard used to make them.

Employing the 5´ Nuclease Assay

Absolute quantification on the 7900HT instrument is accomplished through the use of the polymerase chain reaction and the fluorogenic 5´ nuclease assay (see page A-2). During setup, standards diluted over several orders of magnitude and unknown samples are loaded onto an ABI PRISM® Optical Reaction Plate containing master mix and TaqMan assays targeting a specific nucleic acid sequence. The plate is then loaded into a 7900HT instrument which has been configured to perform a real-time run. During the thermal cycling, the instrument records the emission resulting from the cleavage of TaqMan® probes in the presence of the target sequence. After the run, the SDS software processes the raw fluorescence data to produce threshold cycle (C_T) values for each sample (see page A-10). The software then computes a standard curve from the C_T values of the diluted standards and extrapolates absolute quantities for the unknown samples based on their C_T values (see below).

Note See Appendix A, “Theory of Operation,” for more information on the fluorogenic 5´ nuclease assay, real-time data collection, or the mathematical transformations of sequence detection data.



The figure above illustrates a standard curve generated from a standard RNase P Installation Plate. The arrangement of the samples and standards on the plate are shown in “Examples in This Chapter” on page 6-6.

Algorithmic Manipulation of Raw Data

The SDS software can analyze raw data immediately upon completion of absolute quantification run. The term raw data refers to the spectral data between 500 nm to 660 nm collected by the Automation Controller Software during the plate-read. During the analysis, the software automatically applies several mathematical transformations to the raw data to generate a more direct measure of the relationship between the spectral changes in the unknown samples.

Multicomponenting

The first mathematical transformation involves the conversion of the raw data, expressed in terms of Fluorescent Signal vs. Wavelength, to pure dye components using the extracted pure dye standards. After the dye components have been identified, the software determines the contribution of each dye in the raw data using the multicomponent algorithm. See “Multicomponenting” on page A-5 for a complete description.

Setting the Threshold and Calling C_Ts

After multicomponenting, the baseline and threshold values must be set for the run (see “Kinetic Analysis/ Quantitative PCR” on page A-7 for more information). The results of the experiment can be visualized in the Standard Curve graph of the Results tab. The graph consists of a scatter plot of standard and unknown samples graphed on a linear-scale plot of Threshold Cycle (C_T) versus Starting copy number.

Before You Begin

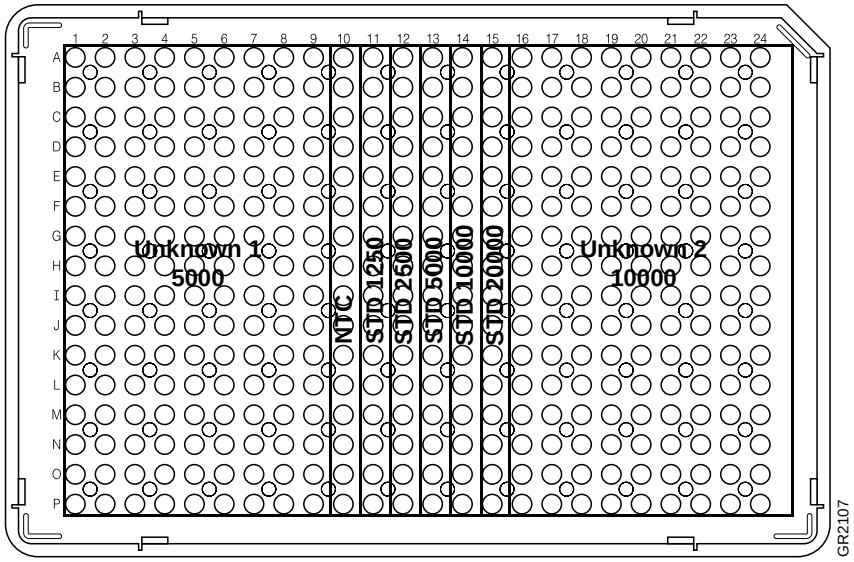
Using SDS Online Help

For specific instructions on any procedure described within this section, refer to the online help accompanying the SDS software. To get help at any time during the procedure, click a help button (?) located within the dialog box or window in which you are working.

Examples in This Chapter

The illustrations and screenshots that appear within this chapter were created for a TaqMan® RNase P Instrument Verification Plate, an experiment run during the installation of the 7900HT instrument to verify its performance. The sealed plate is pre-loaded with the reagents necessary for the detection and quantification of genomic copies of the human RNase P gene (a single-copy gene encoding the moiety of the RNase P enzyme). Each well contains pre-loaded reaction mix (1X TaqMan® Universal PCR Master Mix, RNase P primers, and FAM™-labeled probe) and template.

The following figure illustrates the arrangement of standards and samples on the RNase P plate. As shown below, the RNase P plate consists of 5 columns of template standards (1250, 2500, 5000, 10,000, and 20,000 copies) and two unknown populations (5000 and 10,000 copies).



Analysis Checklist

Where You Are in the Procedure

The following checklist illustrates your current position in the overall procedure:

<table>
<tr><th>Done</th><th>Step</th><th>Procedure</th><th>See Page</th></tr>
<tr><td>✓</td><td>1</td><td>Create an absolute quantification plate document.</td><td>4-6</td></tr>
<tr><td>✓</td><td>2[a]</td><td>a. Create detectors for the absolute quantification probes.
b. Copy the detectors to the plate document.</td><td>4-7
4-8</td></tr>
<tr><td>✓</td><td>3[a]</td><td>a. Configure the plate document with detector tasks (NTC, Standard, and Unknown).
b. Assign quantities to the wells of the plate document that contain standards.</td><td>4-11
4-12</td></tr>
<tr><td>✓</td><td>4</td><td>a. Program the method for the absolute quantification run.
b. If performing an assay in which you would like to collect dissociation data, add a temperature ramp to the thermal profile to perform a dissociation curve analysis.</td><td>4-13
4-16</td></tr>
<tr><td>✓</td><td>5</td><td>Choose from the following:
<table><tr><th>If running…</th><th>Then…</th></tr><tr><td>a single plate</td><td>continue to step 7.</td></tr><tr><td>the first plate in a series of plates with identical assay configurations</td><td>Save the plate document as an ABI PRISM SDS Template Document as explained on page 4-17.</td></tr></table></td><td></td></tr>
<tr><td>✓</td><td>6</td><td>Create a plate document from the template created in step 5.</td><td>4-18</td></tr>
<tr><td>✓</td><td>7</td><td>Configure the document with sample names and plate information.</td><td>4-19</td></tr>
<tr><td>✓</td><td>8</td><td>Prepare and run the absolute quantification plate or plates.</td><td>4-20</td></tr>
<tr><td>☐</td><td>9</td><td>Configure the analysis options for the run.</td><td>6-8</td></tr>
<tr><td>☐</td><td>10</td><td>Analyze the run data.</td><td>6-9</td></tr>
<tr><td>☐</td><td>11</td><td>Set the baseline and threshold values for each detector.</td><td>6-10</td></tr>
<tr><td>☐</td><td>12</td><td>Visualize outliers and eliminate any outlying amplification from the run data.</td><td>6-12</td></tr>
<tr><td>☐</td><td>13</td><td>View the results of the absolute quantification run.</td><td>6-13</td></tr>
<tr><td>☐</td><td>14</td><td>Choose from the following post-analysis options:
♦ Reanalyze the run data.
♦ Adjust the display settings for the results table, plate grid, and plate document plots.
♦ Print elements of the plate document.
♦ Export the plate document results table or plots.</td><td>6-15</td></tr>
</table>

a. Steps 2 and 3 can be eliminated by importing the plate document setup information from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Analyzing the Run Data

Configuring the Analysis Options (Optional)

Before analyzing the data collected from the complete absolute quantification run, decide whether to configure the options for the software analysis. The analysis options allow you to pre-configure the threshold and baseline settings applied to the plate document data during the analysis. If you choose not to configure the analysis options, the SDS software uses the default baseline range and automatically calculates the threshold value for the run.

To configure the analysis options for the absolute quantification run:

Step	Action
1	If not already open, launch the SDS software as explained on page 4-5.
2	Open the plate document file for analysis as follows: a. From the **File** menu, select **Open**. b. From the **File Type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**. c. From the **Look in** text field, navigate to and select the plate document file. d. Click **Open**. The SDS software displays the plate document file.
3	From the **Analysis** menu, select **Analysis Options**. The **Analysis Options - Absolute Quantification** dialog box appears.
4	If desired, click the **Threshold** text field, and type an initial threshold value to use for the analysis of the plate document. For more information about the threshold setting, see “Calculating Threshold Cycles” on page A-10. **Note** If no threshold is specified, the SDS software will automatically assign a threshold value during the analysis.
5	If desired, set a default baseline for the analysis: a. Click the **Baseline Start** text field, and type or dial an initial baseline to use for the analysis of the plate document. b. Click the **Stop** text field, and type or dial an stop baseline to use for the analysis of the plate document. **Note** If no baseline is specified, the SDS software will use the default baseline range of cycles **3**-**15** for the analysis.
6	Set the baseline and threshold values for any remaining detectors present on the plate as follows: a. From the **Detector** drop-down list, select another detector. b. Repeat steps 4 and 5 until the baseline and threshold values have been set for each detector.
7	Click **OK**. The software closes the dialog box and configures the analysis with new settings.

Analyzing the Run

Once you have configured the analysis options, you can analyze the run data. During the analysis, the software mathematically transforms the raw data to establish a comparative relationship between the spectral changes in the passive reference dye and those of the reporter dye. Based on that comparison, the software calculates a cycle threshold (C_T) for each reaction (standard and unknown). The software then generates a standard curve for the run by plotting the standard samples on a graph of C_T versus initial copy number.

Note See Appendix A, "Theory of Operation," for a detailed description of the SDS software mathematical transformation of real-time run data.

To analyze the run:

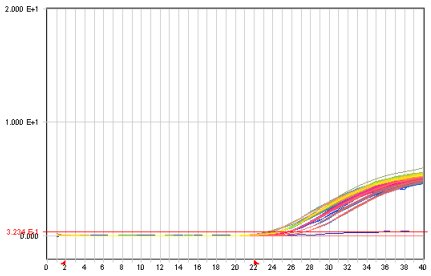
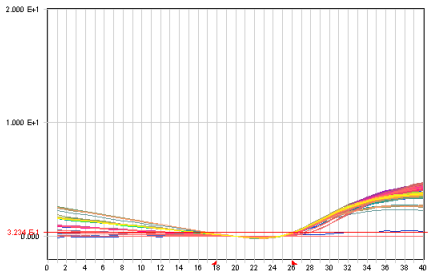
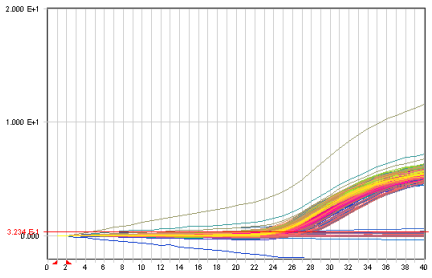
Step	Action
1	Select all wells in the plate grid. The software outlines the selected wells with a black line.
2	Choose one of the following: ♦ From the **Analysis** menu, select **Analyze**. ♦ From the toolbar, click the Analyze button (). The SDS software analyzes the run data and displays the results in the **Results** tab.
3	Set the baseline and threshold values for each detector on the plate as explained on page 6-10.

Setting the Baseline and Threshold Values for the Run

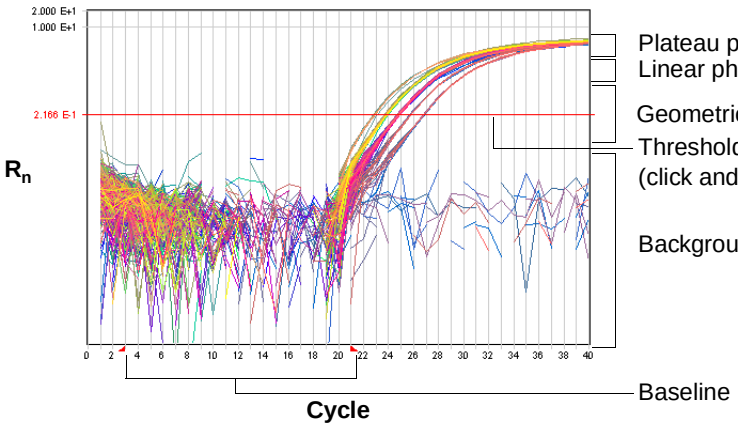
Before calculating absolute quantification values, the baseline and threshold values must be set for all detectors present on the plate.

To set the baseline and threshold values for the run:

Step	Action
1	Click the **Results** tab. The software displays the contents of the **Results** tabbed page.
2	If the Amplification Plot is not visible, click the Amplification Plot button ( ). The software displays the results of the sequence detection run in an amplification plot of normalized reporter fluorescence (R_n) versus threshold cycle (C_T).
3	From the **Plot** drop-down list, select **Rn vs. Cycle**. The software plots the analyzed data in the graph of reporter fluorescence versus cycle number.
4	Identify the components of the linear scale amplification plot and set the baseline so that the amplification curve growth begins at a cycle number greater than the Stop baseline cycle. **IMPORTANT** Do not adjust the default baseline if the amplification curve growth begins after cycle 15.

If the amplification plot looks like...	Then...
	the amplification curve begins after the maximum baseline (cycle 15). Do not adjust the baseline.
	the maximum baseline is set too high. Decrease the Stop baseline value by clicking and dragging the right carrot to an earlier cycle.
	the maximum baseline is set too low. Increase the Stop baseline value by clicking and dragging the right carrot to a later cycle.

To set the baseline and threshold values for the run: *(continued)*

Step	Action
5	From the **Plot** drop-down list, select **ΔRn vs. Cycle**. The software plots the analyzed data in the graph of normalized reporter fluorescence (ΔR_n) versus cycle number.
6	Identify the components of the amplification curve and set the threshold so that it is: ♦ Above the background ♦ Below the plateaued and linear regions of the amplification curve ♦ Within in the geometric phase of the amplification curve 
7	Set the baseline and threshold for any remaining detectors present on the plate as follows: a. From the **Detector** drop-down list, and select another detector. b. Repeat steps 3 to 6 until the baseline and threshold values have been set for each detector.
8	Eliminate outliers from the analyzed run data as explained on page 6-12.

Eliminating Outliers

For any PCR, experimental error may cause some wells to amplify insufficiently or not at all. These wells typically produce C_T values that differ significantly from the average for the associated replicate wells. If included in the absolute quantification calculations, these outliers can potentially result in erroneous measurements.

Visualizing Outliers

To ensure precise absolute quantification, replicate groups must be carefully scrutinized for outlying wells. The C_T vs. Well Position view of the Amplification Plot allows you to examine each set of replicate wells for outliers.

To visualize the replicate groups for outlying amplification:

Step	Action
1	From the **Plot** drop-down list, select **Ct vs. Well Position**. The SDS software displays the results data in a Well versus Threshold Cycle plot.
2	Verify the uniformity of each replicate population by comparing the groupings of C_T values for the wells that comprise the set.

Are outliers present?	Then...
Yes	a. Determine and record the well numbers of all outlying wells. b. Go to the next step.
No	go to the next step.

Step	Action
3	Check for remaining detectors present on the plate for outliers: a. Click the **Detector** drop-down list, and select another detector. b. Repeat step 2 until each detector has been checked for outliers.
4	If outliers are present in your data, eliminate them as explained below. Otherwise, go on to “Viewing Results” on page 6-13.

Eliminating Wells from the Analysis

IMPORTANT If one or more wells are removed from use before a plate document has been run, the SDS software will not collect data for those wells.

If you identified any outliers in the previous procedure, you must eliminate them from the standard curve calculation before viewing the results.

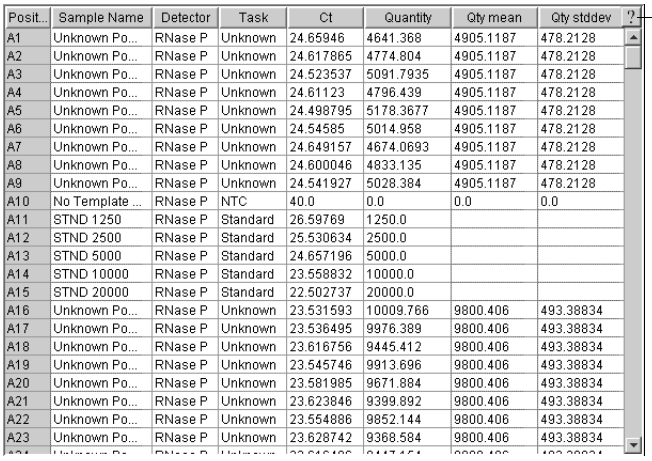
To eliminate outliers from the calculations:

Step	Action
1	While pressing and holding the **Ctrl** key, click each well in the plate grid that you identified as an outlier in the previous procedure. The SDS software creates a black boarder around each cell as it is selected.
2	Click the **Setup** tab.
3	From the well inspector of the **Setup** tabbed page, uncheck the **In Use** check box.
4	From the **Analysis** menu, select **Analyze**.
5	Reapply the baseline and threshold values determined on page 6-10.

Viewing Results

Viewing the Analysis Table

Displays the results of the absolute quantification calculation in the results table of the plate document. The figure below shows an example of the results table containing the data from a TaqMan RNase P Instrument Verification Plate.



Posit...	Sample Name	Detector	Task	Ct	Quantity	Qty mean	Qty stddev
A1	Unknown Po...	RNase P	Unknown	24.65946	4641.368	4905.1187	478.2128
A2	Unknown Po...	RNase P	Unknown	24.617865	4774.804	4905.1187	478.2128
A3	Unknown Po...	RNase P	Unknown	24.523537	5091.7935	4905.1187	478.2128
A4	Unknown Po...	RNase P	Unknown	24.61123	4796.439	4905.1187	478.2128
A5	Unknown Po...	RNase P	Unknown	24.498795	5178.3677	4905.1187	478.2128
A6	Unknown Po...	RNase P	Unknown	24.54585	5014.958	4905.1187	478.2128
A7	Unknown Po...	RNase P	Unknown	24.649157	4674.0693	4905.1187	478.2128
A8	Unknown Po...	RNase P	Unknown	24.600046	4833.135	4905.1187	478.2128
A9	Unknown Po...	RNase P	Unknown	24.541927	5028.384	4905.1187	478.2128
A10	No Template ...	RNase P	NTC	40.0	0.0	0.0	0.0
A11	STND 1250	RNase P	Standard	26.59769	1250.0		
A12	STND 2500	RNase P	Standard	25.530634	2500.0		
A13	STND 5000	RNase P	Standard	24.657196	5000.0		
A14	STND 10000	RNase P	Standard	23.558832	10000.0		
A15	STND 20000	RNase P	Standard	22.502737	20000.0		
A16	Unknown Po...	RNase P	Unknown	23.531593	10009.766	9800.406	493.38834
A17	Unknown Po...	RNase P	Unknown	23.536495	9976.389	9800.406	493.38834
A18	Unknown Po...	RNase P	Unknown	23.616756	9445.412	9800.406	493.38834
A19	Unknown Po...	RNase P	Unknown	23.545746	9913.696	9800.406	493.38834
A20	Unknown Po...	RNase P	Unknown	23.581985	9671.884	9800.406	493.38834
A21	Unknown Po...	RNase P	Unknown	23.623846	9399.892	9800.406	493.38834
A22	Unknown Po...	RNase P	Unknown	23.554886	9852.144	9800.406	493.38834
A23	Unknown Po...	RNase P	Unknown	23.628742	9368.584	9800.406	493.38834

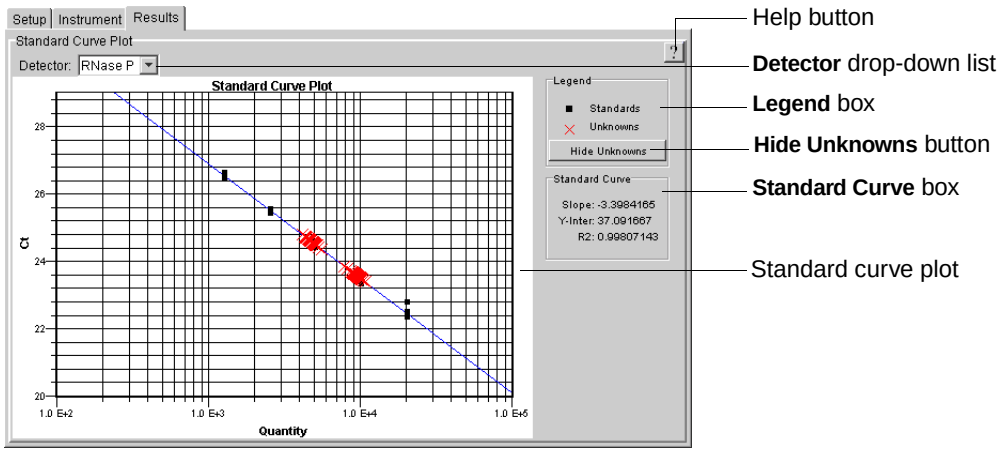
Table elements:

Column	Displays
Position	The coordinate position of the well on the plate.
Sample Name	The sample name applied to the well. **Note** See page 4-19 for information on applying sample names to the plate document.
Detector	The name of the detector assigned to the well. **Note** See page 4-7 for information on applying detectors to the plate document.
Task	The task (**NTC**, **Standard**, or **Unknown**) assigned to the well. **Note** See page 4-11 for information on applying detector tasks to the plate document.
C_T	The threshold cycle generated by the well during the PCR.
Quantity	♦ For wells containing **Unknown** samples, this column displays the starting copy number for the well calculated by the software from the standard curve. ♦ For wells containing **Standard**, this column displays the quantity assigned to the well. **Note** See page 4-11 for information on applying quantities to standard wells of the plate document.
The following two columns contain data only if a well is run as part of a replicate group.	
Qty Mean	The arithmetic mean for the quantity values of the replicate group associated with the well.
Qty stddev	The standard deviation of the quantity values of the replicate group associated with the well.

Viewing the Standard Curve

The software displays the standard curve generated from the run data within the Results tab of the plate document. The standard curve plot displays the unknown samples on a graph of C_T (threshold cycle) vs. initial quantity (LogN).

The following figure illustrates the components of the standard curve plot.



The following table describes the elements of an SDS plate document:

Component	Description
Help button	Launches the SDS software online help.
Detector menu	Toggles the data displayed within the plot based on detector name.
Legend box	Displays a symbol key for the datapoints appearing in the plot.
Hide Unknowns button	Toggles the presence of data from unknown samples in the plot.
Standard Curve box	Contains statistical data describing the standard curve.

Item	Definition
Slope	The slope of the standard curve. The slope of the standard curve is useful for assessing the efficiency of the assay. At 100% efficiency, a reaction should achieve a slope of -3.33 since every 10-fold difference in quantity translates to a difference of 3.33 C_Ts.
Y Inter	The Y-axis intercept of the standard curve.
R2	The R Square value for the standard curve that describes the correlation between threshold cycles (C_T) and the log of the starting copy number for the samples that comprise the standard curve plot. The calculation yields a value between 1 and 0, where values closer to 1 indicate better correlation between C_T and the log of the starting copy number. **Note** The software calculates the R Square value by taking the square of the Pearson Coefficient of Correlation (also known as the *r* value) calculated for the data points that comprise the plot. The software calculates the R2 value only for the standards that make up the curve.

Standard curve plot	A scatterplot of datapoints from the absolute quantification run.

After the Analysis

Changing the Plate Document Display Settings

Before printing or exporting the analyzed data, the software allows you to reconfigure the appearance of several elements of the plate document including the results table, plate grid, and most plots.

To configure the display settings for the plate document:

Step	Action
1	From the **View** menu, select **Display Settings**.
2	From the **Display Settings** dialog box, click the help button (?) for further instructions on modifying the display settings.

Saving the Plate Document

The software allows you to save any changes made the display settings and/or analysis settings (baseline and threshold values) of the plate document.

To save the analyzed plate document, select Save from the File menu.

IMPORTANT The Save command saves does not save the results of the analysis, only the analysis settings. Saved plate documents must be re-analyzed each time they are opened.

Printing a Report

The SDS software can print a report of the analyzed data containing individual or multiple elements of the plate document.

To print a report of the plate document data:

Step	Action
1	From the **File** menu, select **Print Report**.
2	From the **Print Report** dialog box, click the help button (?) for instructions on setting up, previewing, and printing the report.

Exporting Plate Document Data

Exporting Plate Document Data as a Tab-Delimited Text File

The SDS software can export raw or analyzed data in tab-delimited (*.txt) format for all or a select group of wells on a plate document. The exported files are compatible with most spreadsheet applications and programs that can read tab-delimited text.

To export run data as a tab-delimited text file, choose one of the following for further instructions:

- See “Exporting Plate Document Data” on page B-9.
- Click the help button (?) within the table view.

Exporting Plots as Graphics

The SDS software can export most panes and plots of the plate document as JPEG (Joint Photographic Experts Group) graphic files. The JPEG file format is compatible with most word processing and spreadsheet applications and can be incorporated directly into HTML documents for viewing by most web browser software.

To export a plot as a graphic file, see “Exporting Graphics” on page B-8 or click the help button (?) within the plot of interest for further instructions.

Section: Dissociation Curve Analysis

In This Section This section contains the following information:

Overview

About Dissociation Curve Analysis

The ABI PRISM 7900HT Sequence Detection System supports dissociation curve analysis of nucleic acids using SYBR® Green 1 double-stranded DNA binding dye chemistry. The objective of dissociation curve analysis is to accurately determine the melting temperature (T_m) of a single target nucleic acid sequence within an unknown PCR sample. Typical uses of dissociation curves include detection of non-specific products and primer concentration optimization.

Employing the SYBR Green 1 Dye

Dissociation curve analysis on the 7900HT instrument is made possible through the use of the fluorogenic SYBR Green 1 double-stranded DNA binding dye chemistry (see page A-3). Dissociation curves are commonly performed following the PCR stage of a SYBR Green experiment to screen for non-specific products. To generate the data needed to create a curve, the 7900HT instrument performs a programmed temperature 'ramp' in which it slowly elevates the temperature of the plate over several minutes. The specific binding characteristic of the SYBR Green 1 Dye permits the 7900HT instrument to monitor the hybridization activity of the nucleic acids present in the sample. During the run, the instrument records the decrease in SYBR Green fluorescence resulting from the dissociation of dsDNA.

Mathematical Transformations

After the run, the SDS software processes the raw fluorescence data from the SYBR Green 1 Dye to generate a more meaningful representation of the relationship between spectral change and temperature for the dissociation curve run.

Multicomponenting and Normalization

The first mathematical transformation involves the conversion of the raw data, expressed in terms of Fluorescent Signal vs. Wavelength, using the extracted pure dye standards, to pure dye components. After the dye components have been identified, the software determines the contribution of each dye in the normalized data using the multicomponent algorithm (see "Multicomponenting" on page A-5 for a complete description of the process). Finally, the software normalizes the data using the component of the passive reference dye as shown below.

$$R_n = \frac{R_{(SYBR)}}{R_{(PassiveReference)}}$$

Derivation of Dissociation Curve Data

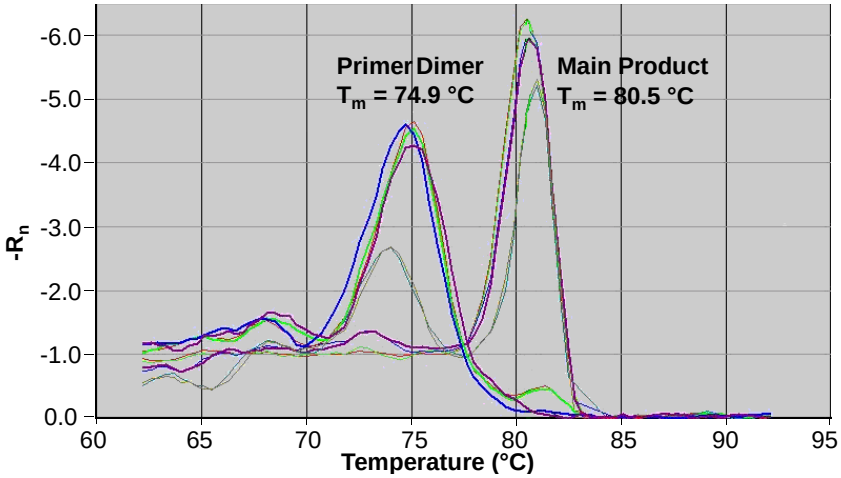
The SDS software then computes the first derivative of the normalized data (R_n) for each reading taken by the 7900HT instrument during the temperature ramp. The resulting derivative data (R_n') is the rate of change in fluorescence as a function of temperature (see below).

$$R_n' = \frac{dR_n}{dT}$$

The software plots the negative of the resulting derivative data on graph of $-R_n'$ versus temperature (T) that visualizes the change in fluorescence at each temperature interval. The T_m for the target nucleic acid can be determined from the graph by identifying the maximum for the rate of change (displayed as a peak) for the appropriate amplification curve.

Example Results

The following figure illustrates a typical dissociation curve from an experiment run to detect non-specific amplification in cDNA samples.



The plot above displays the dual amplification peaks typical of primer-dimer formation. The amplification from the specific product is displayed with a T_m of 80.5 °C, while the primer-dimer product has a characteristically lower T_m of 74.9 °C.

Before You Begin

Using SDS Online Help

For specific instructions on any procedure described within this section, refer to the online help accompanying the SDS software. To get help at any time during the procedure, click a help button (?) located within the dialog box or window in which you are working.

Examples in This Chapter

The illustrations and screenshots that appear within this chapter were created from a plate run to determine the purity of a β-actin amplification in unknown samples. Each well of the plate contains SYBR Green 1 dye, forward and reverse primers, and genomic DNA known to contain complimentary binding sites.

Analysis Checklist

Where You Are in the Procedure

The following checklist illustrates your current position in the overall procedure:

<table>
<tr><th>Done</th><th>Step</th><th>Procedure</th><th>See Page</th></tr>
<tr><td>✓</td><td>1</td><td>Create an absolute quantification plate document.</td><td>4-6</td></tr>
<tr><td>✓</td><td>2[a]</td><td>a. Create detectors for the absolute quantification probes.
b. Copy the detectors to the plate document.</td><td>4-7
4-8</td></tr>
<tr><td>✓</td><td>3[a]</td><td>a. Configure the plate document with detector tasks (NTC, Standard, and Unknown).
b. Assign quantities to the wells of the plate document that contain standards.</td><td>4-11
4-12</td></tr>
<tr><td>✓</td><td>4</td><td>a. Program the method for the absolute quantification run.
b. Add a temperature ramp to the thermal profile.</td><td>4-13
4-16</td></tr>
<tr><td>✓</td><td>5</td><td>Choose from the following:
<table>
<tr><th>If running…</th><th>Then…</th></tr>
<tr><td>a single plate</td><td>continue to step 7.</td></tr>
<tr><td>the first plate in a series of plates with identical assay configurations</td><td>Save the plate document as an ABI PRISM SDS Template Document as explained on page 4-17.</td></tr>
</table></td><td></td></tr>
<tr><td>✓</td><td>6</td><td>Create a plate document from the template created in step 5.</td><td>4-18</td></tr>
<tr><td>✓</td><td>7</td><td>Configure the document with sample names and plate information.</td><td>4-19</td></tr>
<tr><td>✓</td><td>8</td><td>Prepare and run the dissociation curve plate or plates.</td><td>4-20</td></tr>
<tr><td>☐</td><td>9</td><td>Analyze the run data.</td><td>6-21</td></tr>
<tr><td>☐</td><td>10</td><td>View the results of the dissociation curve analysis.</td><td>6-22</td></tr>
<tr><td>☐</td><td>11</td><td>Determine melting temperature (T_m) values for the derivative peaks.</td><td>6-23</td></tr>
<tr><td>☐</td><td>12</td><td>Choose from the following post-analysis options:
♦ Reanalyze the run data.
♦ Adjust the display settings for the results table, plate grid, and plate document plots.
♦ Print elements of the plate document.
♦ Export the plate document results table or plots.</td><td>6-24</td></tr>
</table>

a. Steps 2 and 3 can be eliminated by importing the plate document setup information from a tab-delimited text file. See “Importing Plate Document Setup Table Files” on page B-2 for more information.

Analyzing the Run Data

Analyzing the Run

The run data from a temperature ramp can be analyzed immediately following the completion of the run. For an explanation of how the software manipulates the raw data, see “Algorithmic Manipulation of Raw Data” on page 6-5.

To analyze the run:

Step	Action
1	If not already open, launch the SDS software as explained on page 4-5.
2	Open the plate document file for analysis as follows: a. From the **File** menu, select **Open**. b. From the **File Type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**. c. From the **Look in** text field, navigate to and select the plate document file. d. Click **Open**. The SDS software displays the plate document file.
3	Select all wells in the plate grid. The software outlines the selected wells with a black line.
4	Choose one of the following: ♦ From the **Analysis** menu, select **Analyze**. ♦ From the toolbar, click the Analyze button (). The software analyzes the run data and displays the results in the **Dissociation Curve** tab.
5	Determine the T_m for the dissociation curves displayed within the **Dissociation Curve** tabbed page as explained on page 6-23.

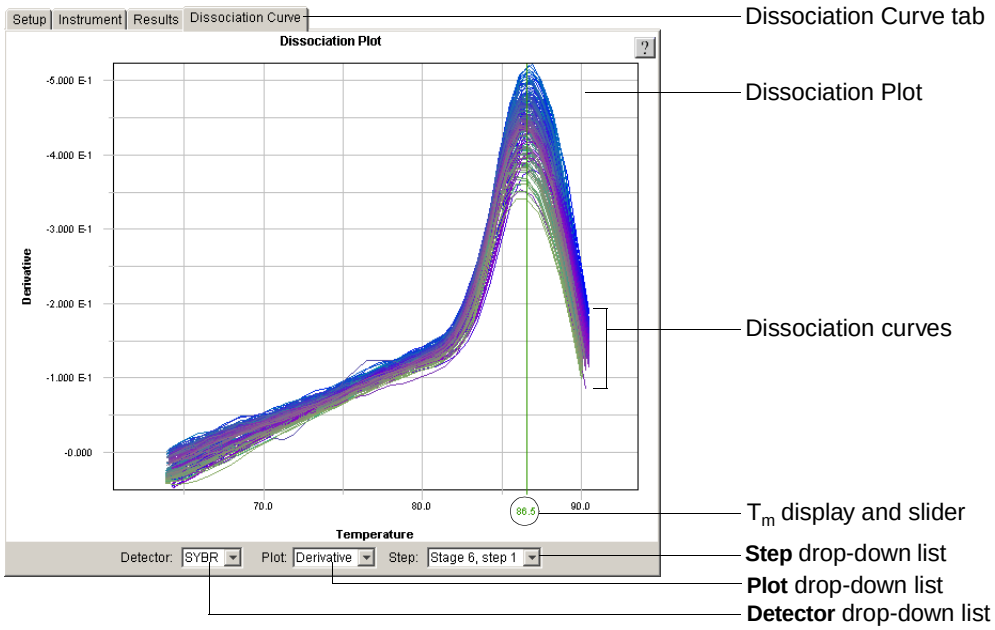
Determining T_m Values for the Analyzed Run

Viewing Analyzed Dissociation Curve Data

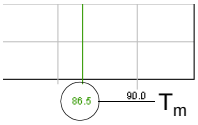
The SDS software displays the results of the dissociation curve analysis within the Dissociation Curve tab of the plate document. The tab displays the analyzed data in a graph of the negative of the derivative ($-R_n'$) versus temperature (T) that visualizes the change in fluorescence at each temperature interval during the ramp.

Note The plot displays data from the selected wells of the plate grid. If you do not see dissociation curve data, select the wells of the plate grid containing the SYBR Green reactions.

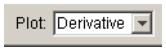
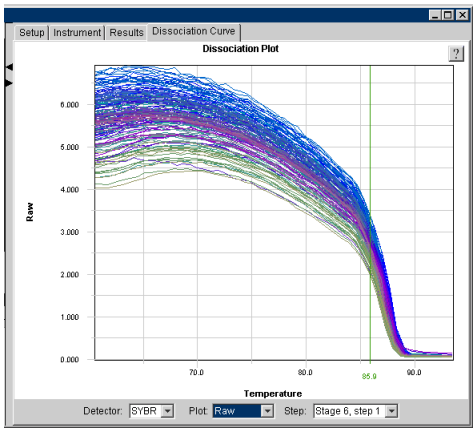
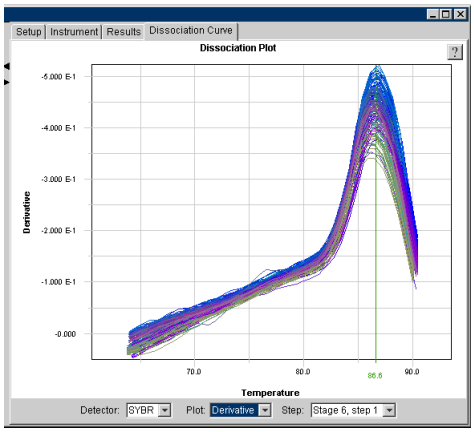
The following figure illustrates the components of the Dissociation Plot.



The following table describes the elements of the Dissociation Plot:

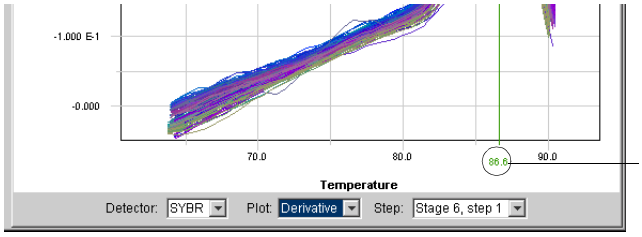
Component	Description
Dissociation Plot	The plot displays data from the selected wells in the plate grid. **Note** The properties of the Dissociation Plot are adjustable. For more information on adjusting the appearance of the plot, click the help button (?) and see the SDS software online help.
Step drop-down list	Chooses the data displayed within the plot based on the ramp. If a plate document contains data from more than one temperature ramp, the **Step** drop-down list allows you to displays the data from each by selecting the position of the ramp in the thermal profile.
T_m display and slider 	The SDS software displays the T_m below the green slider (see above). There are two definitions for the T_m value: ♦ The chemical definition is the temperature at which 50% of the DNA is in a double-stranded configuration. ♦ The mathematical definition is the maximum value for the first derivative curve within a specific peak.

The following table describes the elements of the Dissociation Plot: *(continued)*

Component	Description
Plot drop-down list 	Chooses the data displayed within the plot based on the derivative calculation. The list offers the following selections: ♦ **Raw** – When selected, this option plots the normalized reporter fluorescence data (R_n) on a graph of fluorescence *vs.* temperature (see below left). ♦ **Derivative** – When selected, this option plots derivative data (R_n') on a graph of the derivative *vs.* temperature (see below right). The derivative data is the negative of the rate of change in fluorescence as a function of temperature.
The following figures show the plots accessible from the **Plot** drop-down list.  Raw Plot  Derivative Plot	
Detector drop-down list	Chooses the data displayed within the plot based on detector name.

Determining T_m Values

To determine the T_m value of a melting curve within the Dissociation Plot:

Step	Action
1	Move the mouse pointer over the green T_m line located on the Y-axis line of the plot. The mouse pointer becomes a hand.
2	Click and drag the T_m line to the maximum point of the derivative plot of interest. The SDS software displays the T_m for the curve below the T_m line.  T_m display and slider **Note** The apex of the curvature of represents the maximum rate of change in normalized fluorescence.

After the Analysis

Changing the Plate Document Display Settings

Before printing or exporting the analyzed data, the software allows you to reconfigure the appearance of several elements of the plate document including the results table, plate grid, and most plots.

To configure the display settings for the plate document:

Step	Action
1	From the **View** menu, select **Display Settings**.
2	From the **Display Settings** dialog box, click the help button (?) for further instructions on modifying the display settings.

Saving the Plate Document

The software allows you to save any changes to the appearance of the plate document, however it does not save the threshold or baseline values made during the analysis. To save the plate document, select Save from the File menu.

Printing a Report

The SDS software can print a report of the analyzed data containing individual or multiple elements of the plate document.

To print a report of the plate document data:

Step	Action
1	From the **File** menu, select **Print Report**.
2	From the **Print Report** dialog box, click the help button (?) for instructions on setting up, previewing, and printing the report.

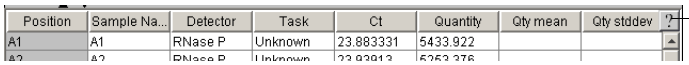
Exporting Plate Document Data

Exporting Plate Document Data as a Tab-Delimited Text File

The SDS software can export raw or analyzed data in tab-delimited (*.txt) format for all or a select group of wells on a plate document. The exported files are compatible with most spreadsheet applications and programs that can read tab-delimited text.

To export run data as a tab-delimited text file, choose one of the following for further instructions:

- See “Exporting Plate Document Data” on page B-9.
- Click the help button (?) within the table view.



Exporting Plots as Graphics

The SDS software can export most panes and plots of the plate document as JPEG (Joint Photographic Experts Group) graphic files. The JPEG file format is compatible with most word processing and spreadsheet applications and can be incorporated directly into HTML documents for viewing by most web browser software.

To export a plot as a graphic file, see “Exporting Graphics” on page B-8 or click the help button (?) within the plot of interest for further instructions.

System Maintenance 7

In This Chapter This chapter discusses the following topics:

Recommended Maintenance Schedule

Maintenance Schedule

The following table includes a list of tasks that should be performed on the ABI PRISM® 7900HT Sequence Detection System regularly.

Interval	Task	See Page
Weekly	Archive SDS Files	7-46
	Perform a Background Run	7-13
Monthly	Check (and if necessary replace) Gripper Finger Pads	7-43
	Defragment the Computer Hard Drive	7-47
Semi-Annually	Perform a Pure Dye Run[a]	7-17
	Check Applied Biosystems Web Site for Software Updates	7-48

a. Perform a background run prior to each Pure Dye Run.

Section: Maintaining the 7900HT Instrument

In This Section This section contains the following information:

Replacing the Sample Block

When to Perform

IMPORTANT Before changing the sample block, perform all required upgrades to the SDS software and instrument firmware. Failure to update the software can render the instrument inoperable or result in damage to instrument components.

You will need to remove the 7900HT instrument sample block, when:

- Decontaminating the wells of the sample block (see page 7-11)
- Changing sample block formats

IMPORTANT Always run a background plate after installing the sample block.

Sample Block Installation Checklist

Unless instructed to do otherwise, adhere to the following guidelines when exchanging sample block modules of different formats:

To install a sample block module:

Step	Procedure	See Page
1	Perform all required software and firmware upgrades to the ABI PRISM 7900HT Sequence Detection System.	7-48
2	Remove the existing sample block.	7-5
3	Install the new sample block.	7-7
4	Change the plate adapter.	7-9
5	Run a background plate to check the sample block for contamination.	7-13
If changing block formats[a] or if installing a new block, also perform the following…		
6	Run a pure dye plate to create the pure spectra calibration values for the new format.	7-17
7	Run an TaqMan® RNase P Instrument Verification Plate to confirm the proper operation of the sample block.	7-24
If using an automation accessory, also perform the following…		
8	If changing consumable formats[a], adjust the plate sensor switch on the plate handler arm for the new plates.	7-28
9	Align the Zymark® Twister™ Microplate Handler fixed-position bar code reader for the new plate format. **Note** It is necessary to align the plate handler to only the Instrument position (Zymark position 2) as explained on pages 7-32 to 7-35.	7-32
10	Align the fixed-position bar code reader for the new plate format.	7-40

a. For example, when replacing a 384-well sample block with a 96-well block

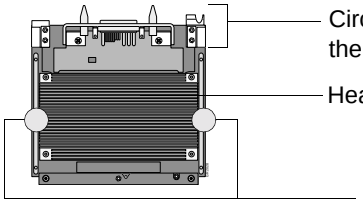
Materials Required

The procedure below requires the use of the following materials:

Material	Part Number
Replacement Sample Block (if replacing the sample block)	—
5/32 inch Hex key (necessary only for certain instruments)	—
5/16 inch Hex key (some instruments may require a crescent wrench)	—

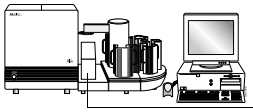
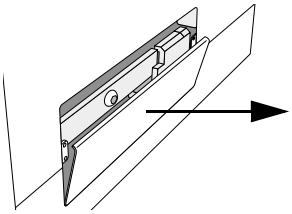
Handling the Sample Block

The interchangeable sample blocks are delicate pieces of equipment containing several fragile components that can break if handled improperly. The figure below illustrates the correct locations for handling the interchangeable sample block module.

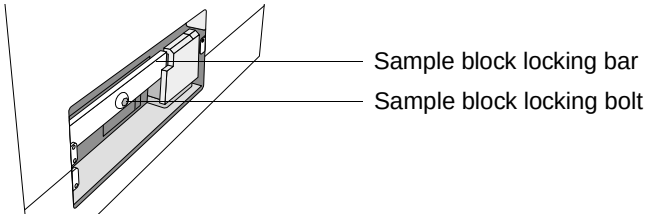
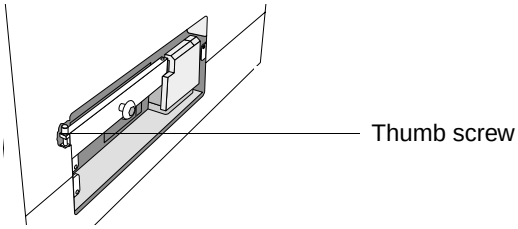
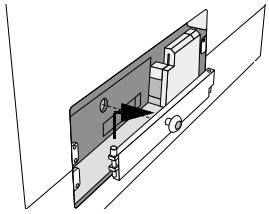
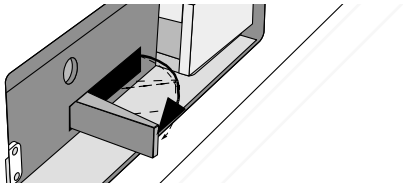
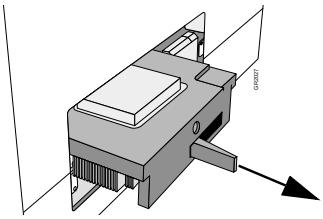


Removing the Sample Block

To remove the sample block:

Step	Action
1	Launch the Automation Controller Software and perform the following tasks: a. Click the **Thermal Status** tab, and confirm the function of the current module. The module is operating normally if the software is receiving a temperature reading. b. Click **Open/Close** to rotate the instrument tray to the OUT position.
2	From the **File** menu, select **Exit** to close the Automation Controller Software.
3	Turn off and unplug the 7900HT instrument. **⚠ WARNING PHYSICAL HAZARD**. The instrument must be unplugged and turned off at all times during the following procedure. Failure to comply can result in serious physical injury to the user or damage to the instrument.
4	Wait 20–30 min for the heated cover to cool. **⚠ CAUTION PHYSICAL HAZARD**. During instrument operation, the temperature of the sample block can be as high as 100 °C. Before performing this procedure, wait until the sample block reaches room temperature.
5	If the instrument tray is in the OUT position (outside of the instrument), push it into the instrument to provide an open work space.
6	If using a Zymark Twister Microplate Handler remove the covers for the fixed-position bar code reader and the underlying platform.  Fixed-position bar code reader and underlying platform covers
7	Push the instrument tray within the instrument, and remove the thermal cycler access cover to permit access to the sample block. **Note** The thermal cycler access cover is secured to the instrument by non-locking pins and may require force to remove it (no tools are required). 

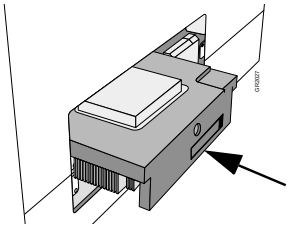
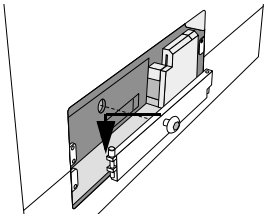
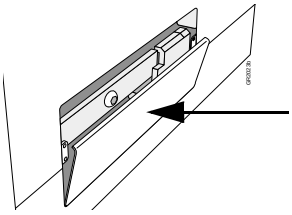
To remove the sample block: *(continued)*

Step	Action
8	Using a 5/16 inch Hex key, turn the sample block locking bolt counter-clockwise until it is very loose, but still attached to the sample block locking bar. **Note** Some instruments may require the use of an adjustable crescent wrench to loosen the sample block locking bolt. 
9	Loosen the thumb screw securing the sample block locking bar to the instrument chassis (may be a 5/32 Hex bolt on some instruments). 
10	Lift the sample block locking bar up and out of the instrument. 
11	Remove the sample block from the instrument as follows: a. Rotate the release lever at the base of the sample block 90 degrees.  b. Being careful of the cooling fins on the bottom of the sample block, slide the sample block out of the instrument and place it on a clean, level surface. 

Replacing the Sample Block

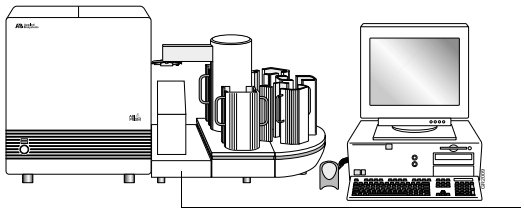
IMPORTANT Before changing the sample block, perform all required upgrades to the SDS software and instrument firmware. Failure to upgrade the software can render the instrument inoperable or result in damage to instrument components.

To replace the sample block:

Step	Action
1	Load the sample block into the instrument compartment as follows: a. Being careful of the heat sinks on the bottom of the sample block, rest the sample block on the metal runners on either side of the instrument bay. b. Carefully slide the sample block into the instrument until the front of the block is flush with the rear of the locking bar.  c. Once seated, firmly press on the sample block to ensure a good connection.
2	Replace the sample block locking bar. 
3	Tighten the thumb screw (from step 9 on page 7-6) to secure the sample block locking bar to the instrument chassis (may be a 5/32 Hex bolt).
4	Using the 5/16 Hex key, turn the sample block locking bolt clockwise until it is flush with the locking bar.
5	Again, press on the right and left sides of the front surface of the sample block to ensure that it is seated securely.
6	Replace the thermal cycler access cover as follows: a. Fit the lip at the bottom of the access cover over the lower edge of the bay. b. Push the cover towards the instrument until it snaps into place. 

To replace the sample block: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>7</td><td>If using a plate handler, replace the covers for the fixed-position bar code reader and the underlying platform (removed in step 6 on page 7-5).

 
Fixed-position bar code reader and underlying platform covers</td></tr>
<tr><td>8</td><td>Plug in and turn on the 7900HT instrument.</td></tr>
<tr><td>9</td><td>Confirm the function of the installed sample block module as follows:
a. Launch the Automation Controller Software.
b. Click the Thermal Status tab.

<table>
<tr><th>Does the software display temperatures?</th><th>Then…</th></tr>
<tr><td>Yes</td><td>the installation is successful.

The presence of temperature readings confirm that the 7900HT instrument successfully established the connection to the new sample block.</td></tr>
<tr><td>No</td><td>the 7900HT instrument is unable to establish communication with the new sample block.

To troubleshoot the problem:
a. Turn off and unplug the 7900HT instrument.
b. Remove the thermal cycler access cover.
c. Press on the right and left sides of the front plate of the sample block to ensure that it is seated securely.
d. Reinstall the thermal cycler access cover.
e. Repeat step 8 until you hear a high-pitched tone confirming communication between the instrument and sample block.</td></tr>
</table>
</td></tr>
<tr><td>10</td><td>Once the sample block is loaded into the instrument do the following:
a. Perform a background run (see page 7-13) to verify that the sample block:
– Is connected and working properly
– Contains no contaminants that will interfere with fluorescent detection
b. If changing sample block formats, perform any remaining tasks outlined in the “Sample Block Installation Checklist” on page 7-4.</td></tr>
</table>

Changing the 7900HT Plate Adapter

When to Perform

Remove and replace the 7900HT instrument plate adapter after changing the sample block module format (for example, replacing a 384-well sample block module with a 96-well version).

Note The sample block must be used with the corresponding plate adapter of the same plate format.

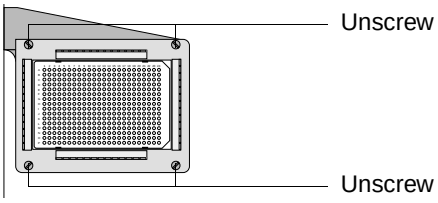
Materials Required

The procedure below requires the use of the following materials:

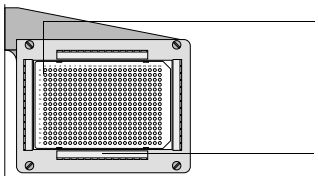
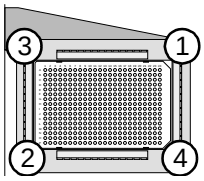
Material	Part Number
One of the following: ♦ 384-Well Plate Adapter ♦ 96-Well Plate Adapter	See page D-3
3/32 inch Hex key	—

Changing the Plate Adapter

To replace the 7900HT instrument plate adapter:

Step	Action
1	If the instrument tray is inside the 7900HT instrument, move the instrument tray to the OUT position as follows: a. Launch the SDS software. b. From the **File** menu, select **New**. The **New Document** dialog box appears. c. Click **OK**. The software generates a plate document. d. Click the **Instrument** tab. e. From the **Real-Time** tab of the **Instrument** tabbed page, click **Open/Close**. The instrument tray rotates to the OUT position. f. From the **File** menu, select **Exit**. The SDS software exits.
2	Remove the four screws attaching the plate holder to the plate arm. 
3	Remove the plate adapter from the instrument tray. **Note** If changing sample block formats (for example, replacing a 384-well sample block with a 96-well version), store the plate adapter with the sample block module of the same format.

To replace the 7900HT instrument plate adapter: *(continued)*

Step	Action
4	Place the new plate adapter into the instrument tray with the **A1** label in the rear-left corner (see below). **IMPORTANT** Make sure to install the correct version of the plate adapter (384- or 96-well) for the plate format you intend to use. The plate adapters are labeled for the consumable format they support. 
5	Replace and tighten the four screws in the order shown below:  **IMPORTANT** The order in which the screws are tightened is important to ensure proper alignment of the plate to the sample block within the 7900HT instrument.

Decontaminating the Sample Block

When to Perform

The following procedure describes how to decontaminate the wells of a sample block module. The procedure will eliminate residual PCR related products, including fluorescent labeled TaqMan® probes. Clean the sample block as often as needed.

IMPORTANT If preforming a cleaning or decontamination method other than the one in this manual, check with Applied Biosystems first to ensure that the method will not damage the sample block module or the 7900HT instrument.

Materials Required

The cleaning procedure requires the following materials:

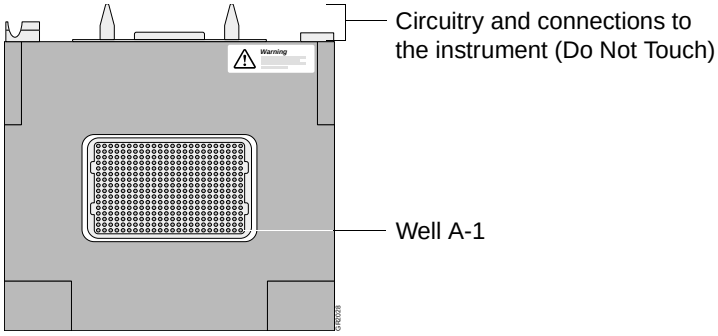
Material	Part Number
Cotton swabs	—
10% Sodium hypochlorite (bleach) solution	—
Isopropanol, 100 percent pure	—
5/32 in Hex key	—

⚠ WARNING **CHEMICAL HAZARD**. **Sodium hypochlorite (bleach)** is a liquid disinfectant that can be corrosive to the skin and can cause skin depigmentation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ WARNING **CHEMICAL HAZARD**. **Isopropanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Cleaning the Sample Block Wells

To clean the sample block wells:

Step	Action
1	Identify the contaminated wells as follows: a. If not already run, perform a background run as explained on page 7-13. b. Identify the contaminated wells on the sample block by following the procedure, "Isolating Sample Block Contamination" on page 8-8.
2	Remove the sample block from the 7900HT instrument as explained in "Removing the Sample Block" on page 7-5.
3	Using the following figure as a guide, locate the suspected contaminated wells on the sample block. 
4	Pipet the appropriate volume of 10% bleach solution into each suspected contaminated well of the sample block module. ♦ For a 96-well sample block, pipet 150 µL bleach solution to each well. ♦ For a 384-well sample block, pipet 40 µL bleach solution to each well.
5	Allow the sample block to sit for 3-5 min.
6	Using a pipet, remove the bleach solution from the wells of the sample block.
7	Rinse (pipet and remove) each contaminated well with 3 treatments of deionized water at the appropriate volume for the sample block. ♦ For a 96-well sample block, rinse affected wells with 150 µL deionized water. ♦ For a 384-well sample block, rinse affected wells with 40 µL deionized water. **Note** Absolute isopropanol can be substituted for water in the third treatment.
8	Remove any remaining isopropanol or water from the wells of the sample block module.
9	Replace the sample block as explained in "Replacing the Sample Block" on page 7-7.
10	Run a background plate to confirm that the contamination has been removed.

Performing a Background Run

When to Perform

Applied Biosystems recommends running a background plate weekly or as often as necessary depending on instrument use.

Purpose of Background Runs

A background run measures the ambient fluorescence in a background plate containing deionized water. During the run, the 7900HT instrument conducts a continuous scan of the plate for 2 minutes at 60 °C. Afterwards, the SDS software averages the spectrum recorded during the run and is used to extract the resulting spectral component to a calibration file. The software uses the calibration file during subsequent runs to remove the background signal from the run data.

Because the background signal can change with instrument age, Applied Biosystems recommends regenerating the Background component calibration every month.

Note Background runs can also be used to detect and troubleshoot sample block contamination.

About the Background Component

Fluorescence collected by the ABI PRISM 7900HT Sequence Detection System includes a “background” component, a fluorescent signal that is inherent to the system. The background component is a composite signal found in all spectral data that consists of fluorescence from several sources including: the background electronic signal, the sample block, the water within the consumable, and the plastic consumable itself. Because the background signal can interfere with the precision of SDS data, the 7900HT instrument has been engineered to minimize the background signal. Additionally, the SDS software also algorithmically eliminates the background signal from each fluorescent sample to maximize the instrument’s sensitivity (see page A-5).

Materials Required

The following materials are required to perform a background run:

Material	Part Number
One of the following: ♦ Background Plate included in a ABI PRISM Sequence Detection Systems Spectral Calibration Kit, or ♦ Make a background plate (requires the following) – ABI PRISM® Optical 394- or 96-Well Reaction Plate – ABI PRISM® Optical Adhesive Cover or ABI PRISM Optical Flat Caps – Pipettor, 100-µL (with pipet tips)	See page D-4
Centrifuge, with plate adapter	—

Prepare a Background Plate Document

To prepare a plate document for the background run:

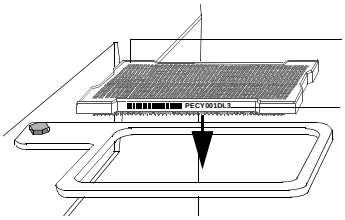
Step	Action
1	Launch the SDS software.
2	From the **File** menu, select **New**. The **New Document** dialog appears.
3	Configure the **New Document** dialog box as follows: **Drop-Down List** / **Select** Assay / Background Container / <the appropriate plate format> Template / Blank Template
4	If the background plate is labelled with a bar code, click the **Barcode** text field and scan the bar code number using the hand-held bar code reader.
5	Click **OK**. The software creates a plate document with the attributes for a background run. **Note** Do not modify the background plate document. The method for a Background run is hard-coded into the SDS software and consists of a single hold at 60 °C for 2 min. Because the plate contains only deionized water, the plate document does not require sample or detector labels.
6	Save the plate document as follows: a. From the **File** menu, select **Save**. The **Save** dialog appears. b. Click the **File name** text field, and type **Background**_<date in MMDDYY format>. For example, the file name for a background plate run on May 31, 2001 would be: **Background_053101** c. Click **Save**. The software saves the plate document. The software is now configured for the Background run.
7	Prepare and run the plate as explained on page 7-15.

Preparing and Running a Background Plate

To conduct the background run:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Choose from the following:

<table>
<tr><th>If using…</th><th>Then…</th></tr>
<tr><td>a background plate from a Spectral Calibration Kit</td><td>Remove the plate from the freezer and allow it to thaw to room temperature.</td></tr>
<tr><td>an ABI PRISM Optical Reaction Plate</td><td>a. Pipet deionized water to each well of the plate.
– If using a 384-well plate, add 20 µL per well.
– If using a 96-well plate, add 50 µL per well.
b. Seal the plate using an optical adhesive cover or optical flat caps.</td></tr>
</table></td></tr>
<tr><td>2</td><td>Briefly centrifuge the background plate.</td></tr>
<tr><td>3</td><td>Load the background plate into the 7900HT instrument as follows:
a. From the plate document in the SDS software, click the Instrument tab.
b. From the lower portion tab, click the Real-Time tab.
c. From the Real-Time tab of the Instrument tabbed page, click Open/Close.
d. Place the background plate into the instrument tray as shown below.

 
Well A1

Position the plate so that the bar code faces towards the front of the instrument

Note The A1 position is located in the top-left side of the instrument tray.</td></tr>
<tr><td>4</td><td>Click Start.

The 7900HT instrument begins the background run.

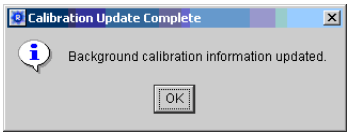
Note Before starting the run, the instrument may pause (up to 15 min) to heat the heated cover to the appropriate temperature.</td></tr>
<tr><td>5</td><td>When the background run is complete and the Run Complete dialog box appears:
a. Click OK to close the dialog box.
b. Click Open/Close, and remove the background plate from the instrument tray.
c. Extract the background component as explained on page 7-16.</td></tr>
</table>

Extracting the Background

In this procedure you will extract the calibration values from the background plate document. Once extracted, the SDS software stores the data as part of the calibration file located in the Calibration subdirectory of the SDS 2.0 directory.

To extract the background component from the run data:

Step	Action
1	From the **Analysis** menu, select **Extract Background**. The software attempts to extract the background signal and displays the success of the extraction in a dialog box.

Dialog Box	Then...
	the run is successful The raw spectra read from the Background plate conform to acceptable limits. Proceed to step 3.
	the run is unsuccessful. The software has stopped the extraction because one or more raw spectra exceed 2500 FSU.

Troubleshoot and decontaminate the sample block as explained in “Background Runs” on page 8-8.

2	From the **File** menu, select **Save**. The software saves the plate document.
3	From the **File** menu, select **Close**. The software closes the plate document.

Performing a Pure Dye Run

When to Perform

Applied Biosystems recommends performing spectral calibration:

- Every 6 months depending on instrument use
- After changing sample block formats (see page 7-4)

IMPORTANT Always run a background plate before performing a Pure Dye calibration.

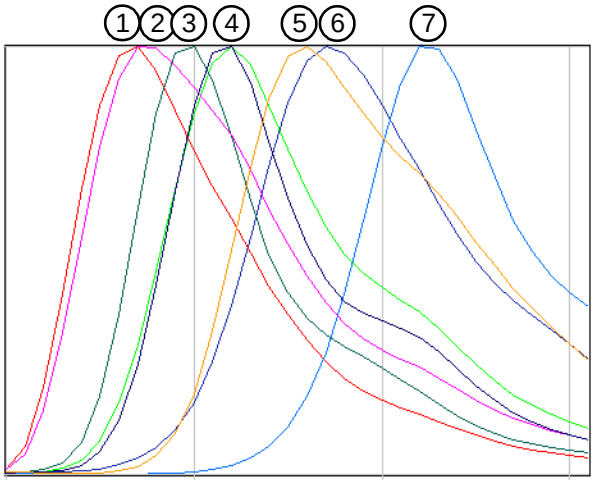
Purpose of Pure Dye Runs

Pure dye data is generated from the results of a pure dye run in which the SDS software collects spectral data from a set of dye standards during a 2-min hold at 60 °C. The software stores the spectral information for the pure dye standards within a calibration file located in the SDS directory. After the run, the software extracts each component dye spectrum from the collected data in the pure spectra run file.

IMPORTANT Because the age and use of instrument components can affect pure spectra readings, Applied Biosystems recommends updating the pure spectra data files once or twice annually depending on instrument use.

Components of the Pure Dye Spectra

The ABI PRISM 7900HT Sequence Detection System monitors fluorescent signals generated by several dyes FAM™, NED™, ROX™, SYBR®, TAMRA™, TET™, and VIC™. The figure below compares the pure spectra for each dye.



Dye		Peak (nm)
1	FAM	~520
2	SYBR	~520
3	TET	~540
4	VIC	~550
	JOE	~550
5	NED	~570
6	TAMRA	~580
7	ROX	~610

Note The 7900HT instrument supports the detection of custom pure dyes (dyes other than those provided by Applied Biosystems). To add custom pure dyes to the Pure Dye set for your instrument, see “Adding Custom Dyes to the Pure Dye Set” on page 7-21.

After a run, the SDS software receives run data in the form of a raw spectra signal for each reading. To make sense of the raw data, the software must determine the contribution of each fluorescent dye used in the sample through a process called multicomponenting (see page A-5). The software accomplishes the separation by comparing the raw spectra with a set of pure dye standards contained within a calibration file. When a plate document is saved after analysis, the software stores the pure spectra information with the collected fluorescent data for that experiment.

Materials Required

The following materials are required to perform a pure dye run:

Material	Part Number
Sequence Detection Systems Spectral Calibration Kit	
384-Well Version 96-Well Version	See page D-3
Pure Dye Plate[a,b]	—
Product Insert[b]	—
Centrifuge, with plate adapter	—

a. The 96-Well version of the Spectral Calibration Kit contains 2 Pure Dye plates.

b. Included with the Sequence Detection Systems Spectral Calibration Kit.

Preparing a Pure Dye Plate Document

IMPORTANT A background run must be performed prior to running a pure dye plate. See "Performing a Background Run" on page 7-13 for more information.

To prepare a plate document for the pure dye run:

Step	Action
1	Remove the pure dye plate from the freezer, place it aside to thaw to room temperature, and return to the computer.
2	Launch the SDS software.
3	From the **File** menu, select **New**. The **New Document** dialog appears.
4	Select the following options from the menus within the **New Document** dialog box. **From…** / **Select…** Assay / Pure Dyes Container / <Select the appropriate plate format> Template / ♦ For a 384-Well Pure Dye Run, select **384 Well Pure Dyes Plate.sdt**. ♦ For 96-Well Pure Dye Runs, select the template matching the Pure Dye plate you intend to run. – To run Plate 1 (containing FAM, JOE, NED, and ROX), select **96 Well Pure Dyes Plate 1.sdt**. – To run Plate 2 (containing SYBR, TAMRA, TET, and VIC), select **96 Well Pure Dyes Plate 2.sdt**. **Note** If no templates are available, construct a Pure Dye plate document using the product insert from the Sequence Detection Systems Spectral Calibration Kit and the procedure on page 7-22.
5	Remove the pure dye plate from its packaging.
6	Click the **Barcode** text field, and scan the bar code number using the hand-held bar code reader.
7	Click **OK**. The software displays a plate document with the attributes for a pure dye run.

To prepare a plate document for the pure dye run: *(continued)*

Step	Action
8	Save the Pure Dye plate document as follows: a. From the **File** menu, select **Save**. b. From the **Files of type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**. c. From the **Save** dialog, click the **File name** text field, and choose from the following: **Plate Format** / **Type…** 384-Well: **PureDye**_<date in MMDDYY format> For example, the file name for a plate run on May 31, 2001 would be: **PureDye_053101**. 96-Well: **PureDye_Plate**<plate number>_<date in MMDDYY format> For example, the file name for a Pure Dye Plate 1 run on May 31, 2001 would be: **PureDye_Plate1_053101**. d. Click **Save**. The software saves the plate document.
9	Prepare and run the pure dye plate as explained below.

Preparing and Running the Pure Dye Plate

IMPORTANT A background run must be performed prior to running a pure dye plate. See "Performing a Background Run" on page 7-13 for more information.

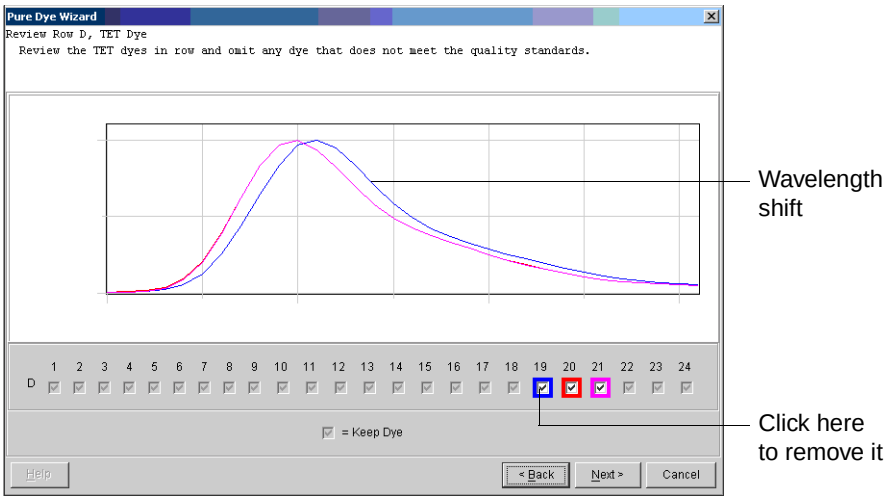
To prepare the pure dye run:

Step	Action
1	Briefly centrifuge the pure dye plate.
2	Load the pure dye plate into the 7900HT instrument as follows: a. From the plate document in the SDS software, click the **Instrument** tab. b. From the **Real-Time** tab in the **Instrument** tabbed page, click **Open/Close**. The instrument tray rotates to the OUT position. c. Place the pure dye plate into the instrument tray. **Note** The A1 position is located in the top-left side of the instrument tray.
3	Click **Start**. The 7900HT instrument begins the pure dye run. The method for a pure dye run is hard-coded into the software and consists of a single 2-min hold at 60 °C. **Note** Before starting the run, the instrument may pause (up to 15 min) to heat the heated cover to the appropriate temperature.
4	When the pure dye run is complete and the **Run Complete** dialog box appears: a. Click **OK** to close the dialog box. b. Click **Open/Close**, and remove the pure dye plate from the instrument tray. c. Extract the pure dye calibration information as explained on page 7-20.

Extracting Pure Dye Information from the Analyzed Run

The purpose of viewing the data in the Pure Dye Wizard is to eliminate irregular pure dye peaks from the data set. The wizard presents the spectral data from the pure dye plate in sets of three wells, each containing the same pure dye. Because the wells displayed by the wizard contain the pure dye at an identical concentration, the signal peaks for the set should be identical. Occasionally, pipetting inaccuracies or contamination can cause a well signal to shift slightly. While viewing the data, the outlying peaks must be eliminated.

To extract the pure dye information from the run data:

Step	Action
1	From the **Analysis** menu, select **Extract Pure Dye Wizard**. The **Extract Pure Dye Wizard** dialog appears.
2	Follow the instructions as explained by the **Extract Pure Dye Wizard** to extract the pure dye spectra. When presented with each screen, do the following: a. Inspect the spectra for shifts in peak location.  b. If the data set contains a outlying peak, eliminate it by clicking check box of the associated well. **Note** Dye spectra are generally acceptable if they peak at the same location as their group but diverge slightly at other wavelengths. c. Click **Next** to view the next three wells. d. Repeat steps a to c for all remaining wells until prompted with a message reporting the extraction of the pure dyes. The software extracts the pure spectra and stores the data as a component of the calibration file.
3	From the **File** menu, select **Save**. The software saves the plate document.
4	From the **File** menu, select **Close**. The software closes the plate document.
5	If performing spectral calibration of a 96-well block module, repeat the previous procedures on pages 7-18, 7-19, and 7-20 to run the second Pure Dye plate.

Adding Custom Dyes to the Pure Dye Set

When to Perform

The ABI PRISM 7900HT Sequence Detection System can be used to run assays designed with custom dyes (dyes not manufactured by Applied Biosystems). However, before using custom dyes with the 7900HT instrument, you must create and run a custom pure dye plate.

Materials Required

The following materials are required to perform a pure dye run:

Material	Part Number
ABI PRISM Optical 394- or 96-Well Reaction Plate[a]	See page D-3
ABI PRISM Optical Adhesive Cover or ABI PRISM Optical Flat Caps	
Custom Dye(s)	—
Pipettor, 100-µL (with pipet tips)[a]	—
Centrifuge, with plate adaptor	—

a. Used to add custom dyes to the Dye Set.

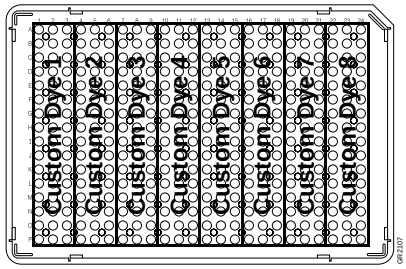
Creating a Custom Pure Dye Plate

Note The purpose of the custom pure dye plate is identical to that of an ABI PRISM Pure Dye Plate. The SDS software uses the custom plate to create a spectral standard for multicomponenting the custom dye.

To create a pure dye plate for custom dyes:

Step	Action
1	Prepare a microplate with a dilution series of the custom dye.
2	Launch the SDS software.
3	Create an allelic discrimination plate document and run the dilution series plate. **Note** It is not necessary to configure detector, sample, and method information for the dilution series plate document. The purpose of the run is to establish the correct working concentration for the dye by viewing the intensity of the raw spectra produced by the wells in the dilution series.
4	From the **Analysis** menu, select **Analyze**. The software analyzes the raw run data,
5	Click the Show Raw Data Plot button () from the Display toolbar. The software displays the Raw Data Plot.
6	From the raw spectra, determine the highest concentration of dye that does not produce a saturated signal, and record it for future use. **Note** Saturated signals are characterized by their high peaks that rise beyond detectable levels (> 65,000 fluorescent units) and appear as plateaus on the **Raw Data** plot. The concentration of the custom dye that yields the highest possible signal but does not saturate is the maximum concentration for use with the 7900HT instrument.
7	Repeat steps 1 to 6 for any additional custom dyes.

To create a pure dye plate for custom dyes: *(continued)*

Step	Action
8	Create a pure dye plate for the custom dye(s) by pipetting each custom dye to at least three columns of an ABI PRISM® Optical Reaction Plate at the concentrations determined in step 7.  **IMPORTANT** The optical configuration of the 7900HT instrument requires that each pure dye occupy at least 3 columns of the Pure Dye plate to permit adequate data collection.
9	Seal the plate using an optical adhesive cover or optical flat caps.
10	Create a template document for the custom pure dye plate as explained below.

Constructing a Custom Pure Dye Plate Document Template

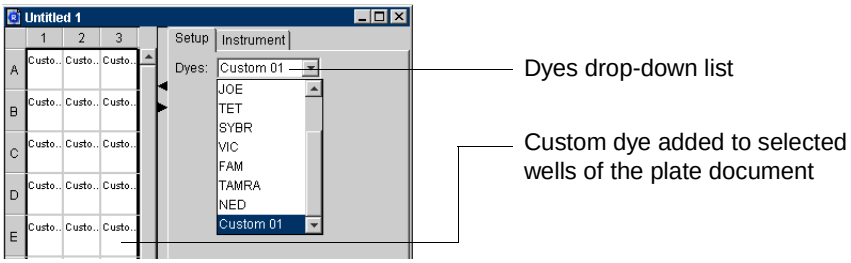
To create a template for running the custom pure dye plate:

Step	Action
1	Launch the SDS software.
2	Add the new dye to the software using the **Dye Manager** as follows: a. From the **Tools** menu, select **Dye Manager**. The **Dye Manager** dialog box appears. b. Click **Add**. The **Add Dye** dialog box appears. c. Type a name for the custom dye, and click **OK**. The software adds the new dye to the **Custom** dye list. d. Repeat steps 2 and 3 to add any additional custom dyes to the **Dye Manager**. e. Click **Done**. The SDS software makes the new dyes available to pure dye plate documents.
3	Create a custom pure dye plate document for the run as follows: a. From the **File** menu, select **New**. The **New Document** dialog box appears. b. Configure the drop-down lists with the following options:

Drop-Down List	Select
Assay	Pure Dyes
Container	<the appropriate plate format>
Template	Blank Template

c. Click **OK**.
The software creates a new plate document.

To create a template for running the custom pure dye plate: *(continued)*

Step	Action
4	Apply pure dyes to the custom plate document as follows: a. Select the wells containing the custom dye. b. From the **Setup** tabbed page, click the **Dyes** drop-down list, and select the appropriate dye. The software applies the dye to the selected wells. c. Repeat steps 6 and 7 to configure the plate document with any additional custom dyes. 
5	Save the custom pure dye plate document as a template file as follows: a. From the **File** menu, select **Save**. The **Save** dialog appears. b. Navigate to **Program Files** > **Applied Biosystems** > **SDS 2.0** > **Templates**. The **Templates** directory appears within the **Look in** text field. By saving the template to the Templates folder, it becomes available from the **Template** drop-down list in the **New Document** dialog box. c. From the **Files of type** drop-down list, select **ABI PRISM SDS Template Document**. d. Click the **File name** text field, and type a name for the template document. e. Click **Save**. The software saves the plate document as a template file (*.sdt).
6	Run the custom pure dye plate as explained in “Preparing and Running the Pure Dye Plate” on page 7-19.

Verifying Instrument Performance Using a TaqMan RNase P Plate

When to Perform

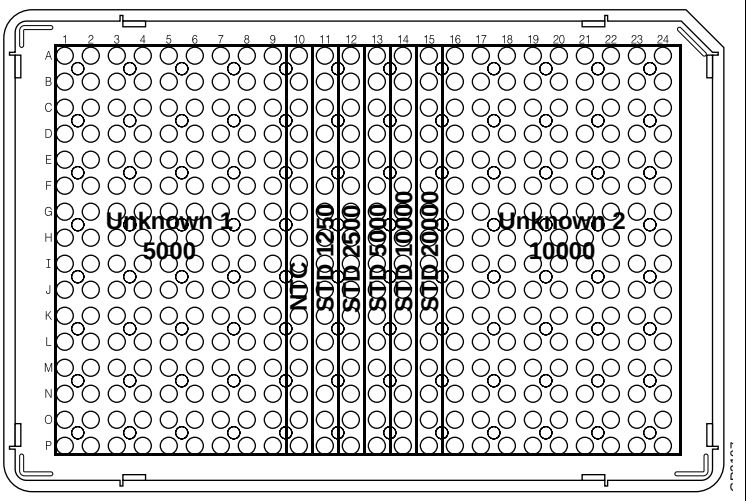
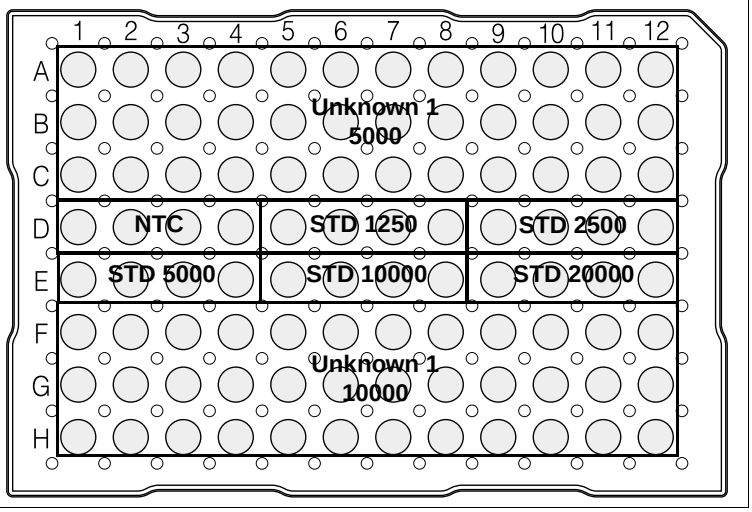
Applied Biosystems recommends running a TaqMan® RNase P Instrument Verification Plate:

- ♦ When changing sample block formats for the first time
- ♦ As needed to verify the function of the 7900HT instrument

Purpose of RNase P Runs

TaqMan RNase P Instrument Verification Plate is an experiment run to verify the performance of the 7900HT instrument. The sealed plate is pre-loaded with the reagents necessary for the detection and quantification of genomic copies of the human RNase P gene (a single-copy gene encoding the moiety of the RNase P enzyme). Each well contains pre-loaded reaction mix (1X TaqMan® Universal PCR Master Mix, RNase P primers, and FAM-labeled probe) and template.

The following figures illustrate the arrangement of standards and samples on the RNase P plate. As shown below, the RNase P plate consists of 5 columns of template standards (1250, 2500, 5000, 10,000, and 20,000 copies) and two unknown populations (5000 and 10,000 copies).

RNase P Plate	Sample Configuration
384-Well	
96-Well	

Materials Required

The following materials are required to perform the RNase P run:

Material	Part Number
TaqMan RNase P Instrument Verification Plate	See page D-4
Centrifuge, with plate adaptor	

Preparing a RNase P Plate Document

To prepare a plate document for the RNase P plate:

Step	Action
1	Remove the TaqMan RNase P Instrument Verification Plate from the freezer and allow it to thaw to room temperature.
2	Launch the SDS software.
3	From the **File** menu, select **New**. The **New Document** dialog appears.
4	Configure the **New Document** dialog box as follows. **Drop-Down List** / **Select** Assay / Absolute Quantification Container / <the appropriate plate format> Template / <the template file of the appropriate plate format>
5	If desired, enter the bar code information into the plate document as follows: a. Click the **Barcode** text field. b. Remove the RNase P plate from the packaging and scan its bar code using the hand-held bar code reader.
6	Click **OK**. The software creates a plate document. **Note** Do not modify the RNase P plate document. The template is pre-programmed with detector and method information for the run.
7	Save the plate document as follows: a. From the **File** menu, select **Save**. The **Save** dialog appears. b. Click the **Barcode** text field and either: – Type a name or bar code number for the plate, and click **Save**. – Using the hand-held bar code reader, scan the bar code number. c. From the **Files of type** drop-down list, select **ABI PRISM SDS Single Plate (*.sds)**. d. Click **Save**. The software saves the plate document. The software is now configured for the RNase P run.
8	Prepare and run the RNase P plate as explained on page 7-26.

Preparing and Running an RNase P Plate

To run the RNase P plate:

Step	Action
1	Briefly centrifuge the TaqMan RNase P Instrument Verification Plate.
2	From the plate document in the SDS software, click the **Instrument** tab. The software displays the Instrument tabbed page.
3	From the lower portion of the **Instrument** tab, click the **Real-Time** tab. The software displays the Real-Time tabbed page.
4	If the instrument tray is within the 7900HT instrument, click **Open/Close**. The instrument tray rotates to the OUT position.
5	Place the RNase P plate into the instrument tray. **Note** The A1 position is located in the top-left corner of the instrument tray.
6	Click **Start**. The 7900HT instrument begins the run. **Note** Before starting the PCR run, the instrument may pause (up to 15 min) to heat the heated cover to the appropriate temperature.
7	When the run is complete: a. Analyze the run data as explained on page 6-9. a. Set the baseline and threshold values for the analyzed data as explained on page 6-10. b. Verify the performance of the 7900HT instrument as explained below.

Verifying Instrument Performance

The install specification of the ABI PRISM 7900HT Sequence Detection System demonstrates the ability to distinguish between 5,000 and 10,000 genome equivalents with a 99.7% confidence level for a subsequent sample run in a single well. The following equation verifies the 7900HT install specifications:

$$(\mathrm{CopyUnk}_1) - 3(\sigma_{\mathrm{CopyUnk1}})] > [(\mathrm{CopyUnk}_2) - 3(\sigma_{\mathrm{CopyUnk2}})]$$

where:

$\mathrm{CopyUnk}_1$[a]	=	Average Copy Number of Unknown #1 (10,000 replicate population)
$\sigma_{\mathrm{CopyUnk1}}$[a]	=	Standard Deviation of Unknown #1 (10,000 replicate population)
$\mathrm{CopyUnk}_1$[a]	=	Average Copy Number of Unknown #2 (5000 replicate population)
$\sigma_{\mathrm{CopyUnk2}}$[a]	=	Standard Deviation of Unknown #2 (5000 replicate population)

a. These values can easily be obtained from the experimental report window.

Note Up to 6 wells from each replicate group in a 96-well TaqMan RNase P Instrument Verification Plate can be ignored to meet specification.

Note Up to 10 wells from each replicate group in a 384-well TaqMan RNase P Instrument Verification Plate can be ignored to meet specification.

Section: Maintaining the Plate Handler

In This Section

This section contains the following information:

Automation Module Components

Refer to the figure below for the components discussed in this section.

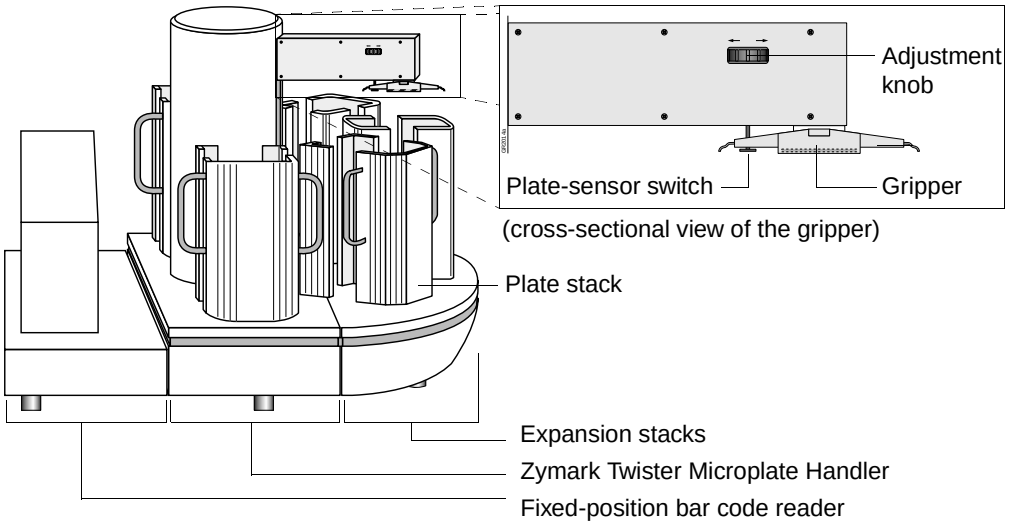
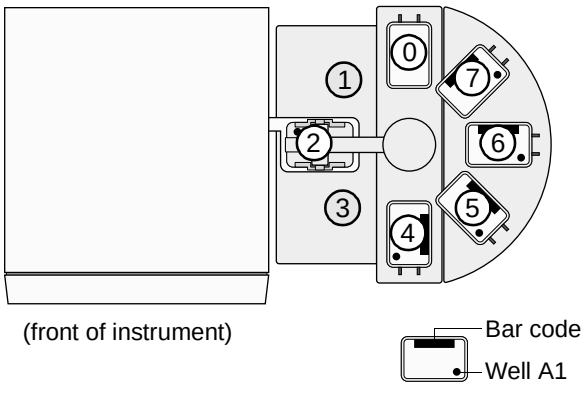


Plate Stack Positions

The Zymark Twister Microplate Handler alignment is performed using the Zymark® Twister Software. The software refers to the positions of the plate stacks differently than the Automation Controller Software. The following diagram lists the positions defined by the Zymark Twister Software and the Automation Controller equivalents.



Zymark Twister Software	Automation Controller
Position 0	Output
Position 1	(unused)
Position 2	Instrument
Position 3	(unused)
Position 4	Stack 1
Position 5	Stack 2
Position 6	Stack 3
Position 7	Stack 4

Adjusting the Sensitivity of the Plate Sensor Switch

When to Perform

The plate sensor switch located underneath the arm of the Zymark Twister Microplate Handler requires adjustment under the following circumstances:

- ♦ When changing sample block module formats
- ♦ If the plate handler is having difficulty sensing plates

Materials Required

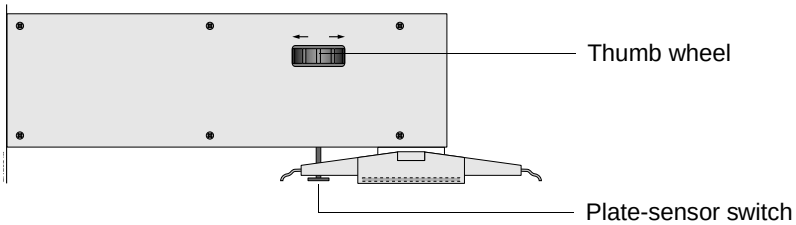
The following materials are required to adjust the plate sensor switch:

Material	Part Number
ABI PRISM Optical Reaction Plate (of the current sample block format)	See page D-1

Adjusting the Switch

The dimensions of different plate formats can place different requirements on how the plate handler grips plates. To ensure smooth operation of the automation accessory, adjust the plate sensor switch when changing consumable formats.

To adjust the plate sensor switch:

Step	Action
1	Turn off the Zymark Twister Microplate Handler. **⚠ WARNING PHYSICAL HAZARD**. The Zymark Twister Microplate Handler must be turned off at all times during the following procedure. Failure to comply can result in physical injury to the user or damage to the plate handler.
2	Clear the switch position by turning the thumb wheel all the way to the Up extreme (as indicated on the side panel). 
3	Begin the adjustment of the sensor switch as follows: a. Grasp a 96- or 384-well ABI PRISM Optical Reaction Plate by the sides making sure not to place pressure in the center of the plate to deform it. b. Place the plate between the fingers of the gripper assembly and align it to the middle of the centering device. c. While holding the plate in position, slowly turn the thumb wheel to lower the switch onto the reaction plate until the switch: – Contacts the top of the plate, and – Emits a soft, audible “clicking” noise **IMPORTANT** The sound emitted by the sensor switch is very faint and may be difficult to hear. To make the adjustment easier, place your ear close to the sensor switch while making the adjustment and listen for the switch to engage.

To adjust the plate sensor switch: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>Remove the plate and listen for the plate-sensor switch to disengage.

<table>
<tr><th>Did you hear the switch disengage?</th><th>Then…</th></tr>
<tr><td>No</td><td>a. Move the switch Down a few steps by turning the thumb wheel in the direction indicated on the arm.
b. Replace the plate within the gripper and listen for the switch to engage:
– If you do not hear the switch engage, then remove the plate and repeat steps a and b above.
– If you hear the switch engage, remove the plate and continue to step a below.</td></tr>
<tr><td>Yes</td><td>a. Move the switch Up by turning the thumb wheel one step in the direction indicated on the arm.
b. Replace the plate and listen for the switch to engage:
– If you hear the switch engage, remove the plate and repeat steps a and b.
– If you do not hear the switch engage, then you have successfully identified the zero point of the plate-sensor switch.

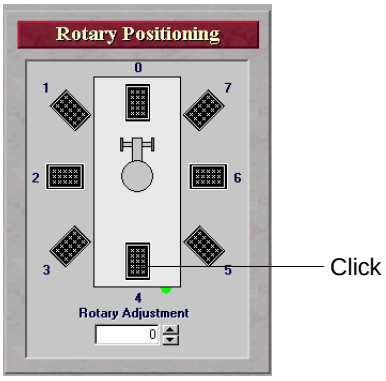
Note At the zero point, one step of the thumb wheel in the Down direction causes the switch to engage.</td></tr>
</table>
</td></tr>
<tr><td>5</td><td>Once the zero point is established, carefully turn the thumb wheel in the Down direction the number of steps appropriate for your plate format as indicated below:

<table>
<tr><th>Plate Format</th><th>Turn the thumb wheel in the Down direction…</th></tr>
<tr><td>96-Well</td><td>20 steps</td></tr>
<tr><td>384-Well</td><td>15 steps</td></tr>
</table>

Note If you lose count, begin again from step 4 and identify the zero point for the switch.</td></tr>
<tr><td>6</td><td>Test the adjustment as explained on page 7-30.</td></tr>
</table>

Testing the Adjustment

To test the sensitivity of the plate sensor switch:

Step	Action
1	Place the reaction plate in the input stack 1 of the plate handler.
2	Turn on the 7900HT instrument, the plate handler, and the computer.
3	From the **Start** menu, select **Programs** > **Zymark Twister Plate Handler** > **Twister**. The Zymark Twister Software launches.
4	Click **Manual Control**. The software displays the **Manual Control** dialog box.
5	Click stack **4**.  The plate handler arm moves over the input stack.
6	Click **Find Plate**. If the adjustment was successful, the plate handler arm will lower upon the plate until the plate detector switch engages confirming the presence of the plate. If plate handler arm emits a grinding sound, adjust the plate sensor switch as follows: a. From the Zymark Twister Software, click **Vertical Home** to raise the plate handler arm. b. Turn the thumb screw in the Down direction 10 steps. c. Repeat step 6 until the plate handler arm successfully detects the plate.
7	Click **Close Gripper**, then click **Vertical Home**. If the adjustment was successful, the plate handler arm will grasp the plate and remove it from the plate stack. If plate handler arm stops before the gripper fingers are able to contact the plate and fails to grasp or pick up the plate, adjust the plate sensor switch as follows: a. Turn the thumb screw in the Up direction 10 steps. b. Grasp the plate with one hand and, from the Zymark Twister Software, click **Open Gripper** to release the plate. c. Replace the reaction plate into input stack 1 of the plate handler. d. Repeat steps 6 and 7 until the plate handler arm successfully retrieves the plate.
8	Grasp the plate with one hand and, from the Zymark Twister Software, click **Open Gripper** to release the plate.

To test the sensitivity of the plate sensor switch: *(continued)*

Step	Action
9	Exit the Zymark Twister Software. a. Click **Main Menu**. The software displays the main menu. b. Click **Quit Application**. The software closes.
10	**Note** A bug in the Zymark Twister Software can cause portions of the program to persist in memory even after the software has been closed. Because the Zymark Twister Software conflicts with the SDS software, the residual elements of the software must be closed within the Windows Task Manager before continuing. Confirm that the stack has closed by viewing the Task Manager. a. Press the **Crtl** + **Alt** + **Del** keys in unison. The Windows Security dialog box appears. b. Click **Task Manager**. The **Task Manager** dialog box appears. c. Confirm that the software has closed by looking for the Zymark Twister Software entry in the **Task** list. If the software is still running, click the software entry and click Close to exit the remaining software. d. From the **File** menu, select **Exit**.

Aligning the Plate Handler

When to Perform

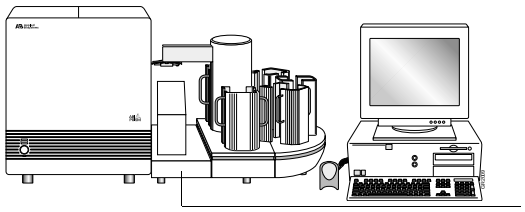
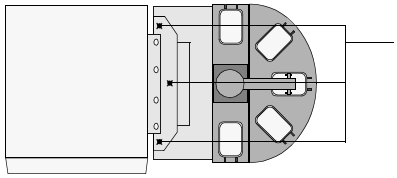
Perform the following procedure if the ABI PRISM 7900HT Sequence Detection System is moved or the Zymark Twister Microplate Handler becomes mis-aligned.

Symptoms that the plate handler is out of alignment include:

- Excessive downward movement of the plate handler arm (the arm grinds when grasping or releasing plates)
- The plate handler arm collides with the plate stacks
- The plate handler arm releases plates above the bottom of the plate stacks
- Reaction plates tip or tilt when placed into the instrument tray by the arm

Preparing the Instrument for the Alignment

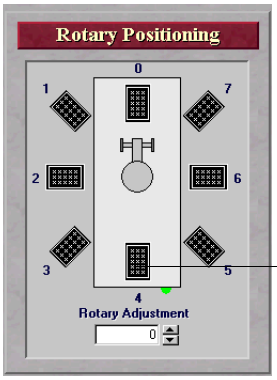
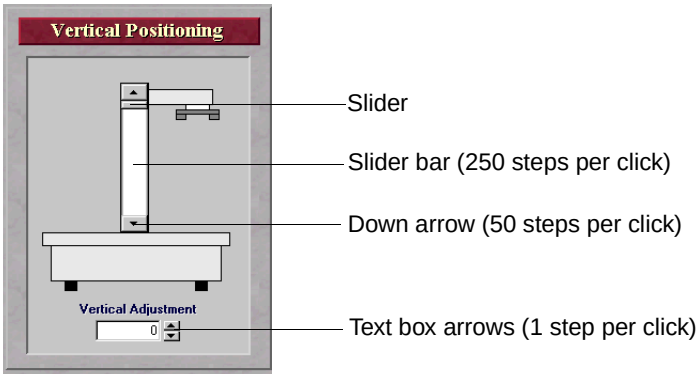
To move the instrument tray to the OUT position:

Step	Action
1	Remove the covers for the fixed-position bar code reader and the underlying platform.  Fixed-position bar code reader and underlying platform covers
2	Loosen the three black thumb screws on the platform connecting the 7900HT instrument and the plate handler base.  Black thumb screws
3	Move the instrument tray to the out position. a. Launch the Automation Controller Software. If an error dialog appears reading, '**Machine calibration values are not valid. Please refer to documentation for calibration process**,' click **OK**. b. Click **Open/Close** (Open/Close). The 7900HT instrument moves the instrument tray to the out position, perpendicular to the instrument. c. From the **File** menu, select **Exit**. The software quits the Automation Controller Software.
4	From the **Start** menu, select **Programs** > **Zymark Twister Plate Handler** > **Twister**. The Zymark Twister Software launches.
5	Click **Manual Control**. The software displays the **Manual Control** dialog box.

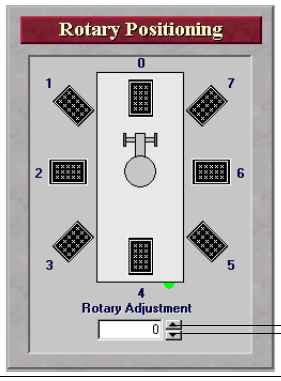
Aligning Input Stack 1 (Zymark Position 4)

The alignment of input stack 1 (position 4 in the Zymark Twister Software) is the first step in the alignment procedure. This alignment provides the basis for aligning all subsequent stacks on the plate handler.

To align the input stack 1 (Zymark position 4):

Step	Action
1	Place an empty plate into input stack 1(Zymark position 4).
2	From the Zymark Twister Software, click position **4**.  The plate handler arm moves over the input stack.
3	Using the **Vertical Positioning** commands, lower the plate handler arm until it is just above the stack. The **Vertical Positioning** box offers four ways to move the plate handler arm: ♦ Move the slider for large increments. ♦ Click inside the slider bar to move the arm in 250 step increments. ♦ Click the lower arrow on the bar to move the arm in 50 step increments. ♦ Click the up or down arrows in the **Vertical Adjustment** text box to move the arm in 1 step increments. ♦ Click the **Vertical Adjustment** text box, type a value, and press **Enter** to move the arm into a specific location. 
4	Check the rotary position of the plate handler arm to confirm that the gripper: ♦ is centered over the stack ♦ will not contact the sides of the stack when lowered

To align the input stack 1 (Zymark position 4): *(continued)*

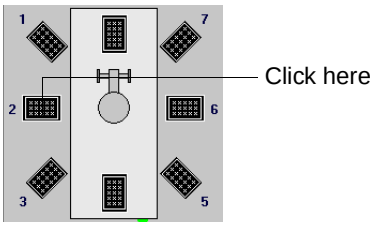
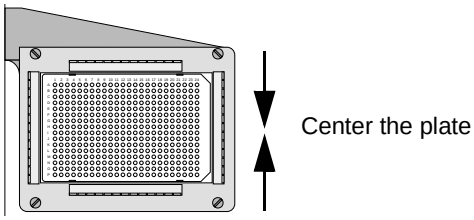
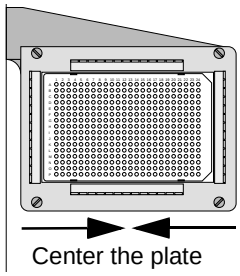
Step	Action
5	Using the **Rotary Adjustment** arrows, adjust the rotational position of the gripper so that it is centered over the input stack and will not contact the sides when lowered. ♦ To move the plate handler arm clockwise, click the up arrow. ♦ To move the plate handler arm counter-clockwise, click the down arrow. 
6	Using the **Vertical Positioning** commands, carefully lower the plate handler arm into the stack. Adjust the **Rotary Adjustment** value as needed to center the gripper within the stack.
7	Once the gripper is centered within the stack, click **Find Plate**. The plate handler arm lowers upon the plate. Confirm the following: ♦ the plate is in the middle of the gripper span ♦ the plate sensor switch is contacting the plate ♦ the gripper or plate do not contact the side of the stack
8	Click **Close Gripper.** The gripper grips the plate between its fingers.
9	Select **Vertical Home**. The plate handler raises the arm to its highest position. If the plate contacts the sides of the stack, re-adjust the rotary position of the plate handler arm until the plate moves freely in the stack. **Note** Contact between the plate and the stack or all stacks may be unavoidable. However, try to minimize the contact as much as possible.
10	Using the **Vertical Positioning** commands, raise and lower the plate handler arm several times to check the alignment.
11	Lower the plate handler arm to the bottom of the plate stack, click **Rotary Offset**, and click **Yes**. The software records the rotary position for the Zymark position 4 (input stack 1).
12	Click **Open Gripper.** The gripper releases the plate.

Aligning the Plate Handler to the Instrument

The next step is to align the plate handler arm to the instrument tray (Zymark position 2). This alignment will ensure a smooth exchange between the plate handler arm and the instrument tray during operation of the instrument.

Note The following procedure requires you to position the plate handler relative to the 7900HT instrument, Before moving the plate handler, loosen the three black thumb screws on the platform connecting the 7900HT instrument and the plate handler.

To adjust the plate handler relative to the 7900HT instrument:

Step	Action
1	If not already present, place an empty plate into input stack 1(Zymark position 4) and pick it up with the plate handler arm as follows: a. From the Zymark Twister Software, click position **4**. b. Click **Find Plate**. c. Click **Close Gripper**.
2	Click position **2**.  The plate handler arm moves over the instrument tray.
3	Use **Vertical Positioning** to lower the plate handler arm until it is approximately 1 cm above the instrument tray.
4	Using the **Rotary Adjustment** arrows, center the gripper and plate along the Y-axis of the instrument tray. 
5	Center the gripper and plate along the X-axis of the instrument tray by sliding the plate handler and base towards or away from the 7900HT instrument. 
6	Again, using the software to move the plate handler arm, center the gripper and plate along the Y-axis of the instrument tray as explained in step 4.

To adjust the plate handler relative to the 7900HT instrument: *(continued)*

Step	Action
7	Using the **Vertical Positioning** commands, carefully lower the plate handler arm onto the instrument tray and confirm that the plate rests squarely within it.
8	Tighten the three black thumb screws on the platform connecting the 7900HT instrument and the plate handler.
9	Release the plate from the plate handler arm. a. Click **Open Gripper**. b. Click **Vertical Home**.
10	Click **Find Plate**. The plate handler arm lowers onto the plate.
11	Save the rotary and vertical offset information: a. Click **Rotary Offset**, and click **Yes**. The software records the rotary position for the plate drawer (Zymark position 2). b. Click **Vertical Offset**, and click **Yes**. The software records the vertical position for the plate drawer.

Re-checking the Input Stack 1

Now that the positions of the plate handler and instrument are fixed, the plate handler stacks can be aligned and the positional values recorded.

To re-check the position of input stack 1 (Zymark position 4):

Step	Action
1	Place an empty plate into input stack 1.
2	From the Zymark Twister Software, click the icon for stack **4**. The plate handler arm moves over the input stack.
3	Using the **Vertical Positioning** commands, lower the plate handler arm until it is 1 cm above the stack and verify that it is centered on the stack. If necessary, center the stack using the **Rotary Adjustment** arrows.
4	Carefully lower the plate handler arm into the stack. Center the gripper as it moves down the stack by adjusting the **Rotary Adjustment** arrows if needed.
5	Once the plate handler arm is centered within the stack, click **Find Plate**. The plate handler arm lowers upon the plate. Confirm the following: ♦ The plate is in the middle of the gripper span. ♦ The plate sensor switch is contacting the plate. ♦ The gripper does not contact the side of the stack.
6	Click **Close Gripper**.
7	Click **Vertical Home**. The plate handler raises the arm to its highest position. If the plate contacts the sides of the stack, re-adjust the rotary position of the plate handler arm until the plate moves freely within the stack. **Note** Contact between the plate and the stack or all stacks may be unavoidable. However, try to minimize the contact as much as possible.

To re-check the position of input stack 1 (Zymark position 4): *(continued)*

Step	Action
8	Click **Rotary Offset**, and click **Yes**. The software re-records the rotary position for the input stack 1(Zymark position 4).
9	While holding the plate, click **Open Gripper** and remove the plate.

Defining the Bottom of the Stack

The Automation Controller Software requires a bottom position value for all stacks. This value is used to prevent the plate handler arm from colliding or grinding as it moves to the bottom of each stack.

To find the bottom of the stack:

Step	Action
1	Remove all plates from the instrument and the plate handler arm.
2	Place an empty plate into the output stack (Zymark position 0).
3	From the Zymark Twister Software, click position **0**. The plate handler arm moves over the output stack.
4	Using the **Vertical Positioning** commands, lower the plate handler arm until it is just above the stack.
5	Check the rotary position of the plate handler arm to confirm that the gripper: ♦ is centered over the stack ♦ will not contact the sides of the stack when lowered
6	Using the **Rotary Adjustment** arrows, adjust the rotational position of the gripper so that it is centered over the input stack and will not contact the sides when lowered.
7	Using the **Vertical Positioning** commands, carefully lower the plate handler arm into the stack. Adjust the **Rotary Adjustment** value as needed to center the gripper within the stack.
8	Once the gripper is centered within the stack, click **Find Plate**. The plate handler arm lowers upon the plate. Confirm the following: ♦ The plate is in the middle of the gripper span ♦ The plate sensor switch is contacting the plate ♦ The gripper does not contact the side of the stack
9	Click **Close Gripper.** The gripper grips the plate between its fingers.
10	Select **Vertical Home**. The plate handler raises the arm to its highest position. If the plate contacts the sides of the stack, re-adjust the rotary position of the plate handler arm until the plate moves freely in the stack. **Note** Contact between the plate and the stack or all stacks may be unavoidable. However, try to minimize the contact as much as possible.
11	Using the **Vertical Positioning** commands, raise and lower plate handler arm several times to check the alignment.
12	Lower the plate handler arm and click **Rotary Offset**, and click **Yes**. The software records the rotary position for position 0 (the output stack).

To find the bottom of the stack: *(continued)*

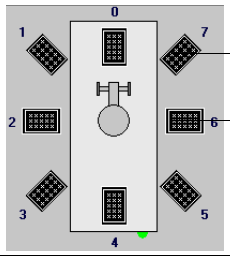
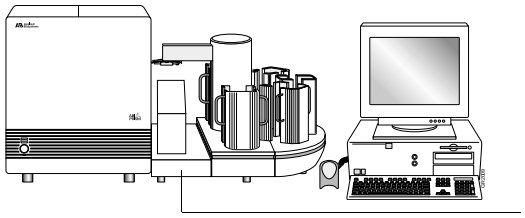
Step	Action
13	Click the **Vertical Home**.
14	While holding the plate, click **Open Gripper** and remove the plate.
15	Click the **Vertical Adjustment** text field, type **–3200**, and press **Enter**. The plate handler lowers the arm to a position near the base of the output stack.
16	Carefully lower the plate handler arm until it is approximately 1–2 mm from the bottom of the stack.
17	Click **Vertical Offset**, click **Yes**, and record the number in the **Vertical Adjustment** text field. The software records the vertical position for position 0 (the output stack).
18	Click **Vertical Home**. The plate handler raises the plate handler arm to its highest position.
19	Click the **Vertical Adjustment** text field, type the **Vertical Offset** value determined in step 5, and press **Enter**. The plate handler lowers the plate handler arm to a **Vertical Offset** position.
20	If necessary, readjust the **Vertical Offset** value and repeat steps 6 and 7 until satisfied with the setting.

Defining the Positions of the Remaining Stacks

To define the positions of the remaining stacks:

Step	Action
1	Place an empty plate into input stack 2 (Zymark position 5).
2	From the Zymark Twister Software, click position **5**. The plate handler arm moves over the input stack.
3	Using the **Vertical Positioning** commands, lower the plate handler arm until it is approximately 1 cm above the stack and center it using the **Rotary Adjustment** arrows.
4	Carefully lower the plate handler arm into the stack. Center the gripper as it moves down the stack by adjusting the **Rotary Adjustment** arrows as needed.
5	Once the plate handler arm is centered within the stack, click **Find Plate**. The plate handler arm lowers upon the plate. Confirm the following: ♦ The plate is in the middle of the gripper span. ♦ The plate sensor switch is contacting the plate. ♦ The gripper does not contact the side of the stack.
6	Click **Close Gripper**.
7	Click **Vertical Home**. The plate handler arm raises to its highest position. If the plate contacts the sides of the stack, re-adjust the rotary position of the plate handler arm until the plate moves freely in the stack. **Note** Contact between the plate and the stack or all stacks may be unavoidable. However, try to minimize the contact as much as possible.

To define the positions of the remaining stacks: *(continued)*

Step	Action
8	Using the **Vertical Positioning** commands, raise and lower plate handler arm several times to check the alignment.
9	Click **Rotary Offset**, and click **Yes**. The software records the rotary position for the Zymark position 5 (input stack 2).
10	Repeat steps 1–8 for input stacks **3** and **4** to define Rotary Offset values for the remaining positions **6** and **7**:  Zymark position **7** (input stack 4) Zymark position **6** (input stack 3)
11	Exit the Zymark Twister Software. a. Click **Main Menu**. The software displays the main menu. b. Click **Exit**. The software closes.
12	**Note** A bug in the Zymark Twister Software can cause portions of the program to persist in memory even after the software has been closed. Because the Zymark Twister Software conflicts with the SDS software, these residual elements must be closed within the Windows Task Manager before continuing. Confirm that the Zymark Twister Software has closed by viewing the Task Manager. a. Press the **Ctrl** + **Alt** + **Del** keys in unison. The Windows Security dialog box appears. b. Click **Task Manager**. The **Task Manager** dialog box appears. c. Confirm that the Twister software has closed by looking for the **Twister** software entry in the **Task** list. If the software is still running, click the software entry and click **End Task** to exit the software. d. From the **File** menu, select **Exit** to quit the Task Manager.
13	Replace the covers for the fixed-position bar code reader and the underlying platform (removed in step 1 on page 7-32).  Fixed-position bar code reader and underlying platform covers

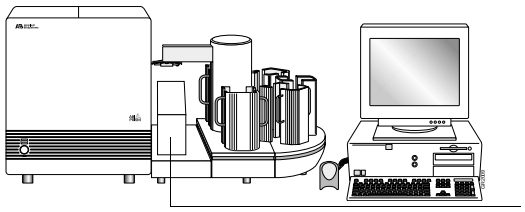
Aligning the Fixed-Position Bar Code Reader

Description

The fixed-position bar code reader must be set so that it automatically scans the plate's bar code as the plate is placed into the instrument tray by the plate handler.

Preparing the Instrument for the Alignment

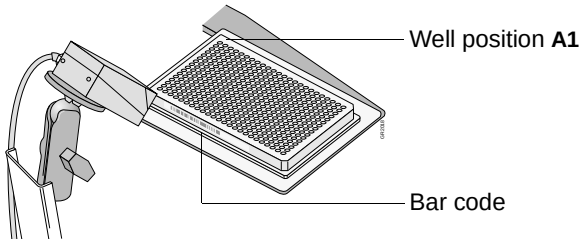
To move the instrument tray to the Out position:

Step	Action
1	Remove the cover for the fixed-position bar code reader.  Fixed-position bar code reader cover
2	Turn on the computer.
3	Launch the Automation Controller Software.
4	Click **Open/Close** (Open/Close). The 7900HT instrument moves the instrument tray to the out position, perpendicular to the instrument.
5	From the **File** menu, select **Exit**. The software quits the Automation Controller Software.

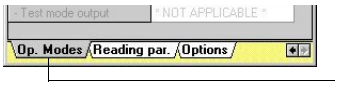
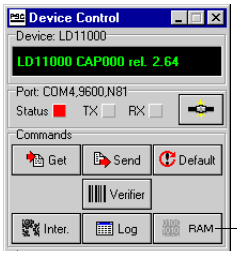
Positioning the Fixed-Position Bar Code Reader

IMPORTANT The instrument tray must be in the OUT position to align the bar code reader.

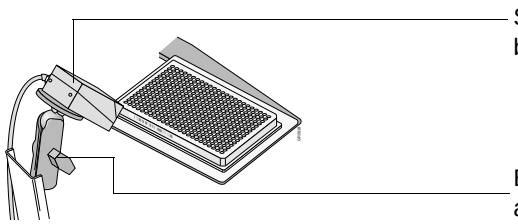
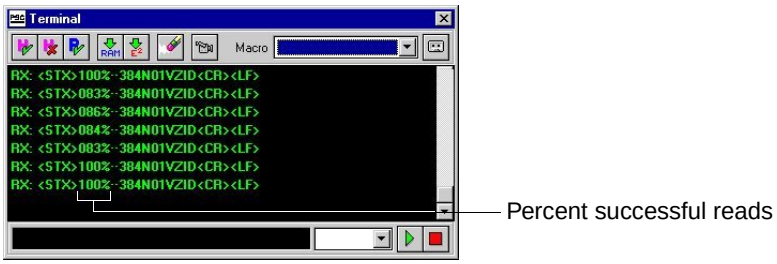
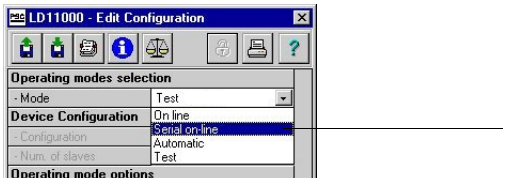
To position the fixed-position bar code reader:

Step	Action
1	Place a plate with bar code onto the instrument tray. **IMPORTANT** Orient the plate so that well **A1** aligns to the **A1** position of the instrument tray and that the bar code faces the fixed-position bar code reader.  Well position **A1** Bar code
2	Select **Start** > **Programs** > **PSC Laser Data** > **LDHOST**. The LDHOST software launches and displays the **LDHOST** window.

To position the fixed-position bar code reader: *(continued)*

Step	Action
3	Establish communication with the fixed-position bar code reader as follows: a. Click the **Edit** button ( ). b. Click the **Terminal** button ( ). The software opens the **Edit Configuration** and **Terminal** dialog boxes. c. From the **Device Control** dialog box, click the **Connect to Device** button ( ). The terminal window displays the fixed-position bar code reader response. d. Click **OK** to close the **Information** dialog box. The LD Host program communicates with the bar code reader and updates the **Edit Configuration** dialog box with the current configuration settings.
4	Configure the software for the alignment as follows: a. From the bottom of the **Edit Configuration** dialog box, locate and click the **Op. Modes** tab.  Op. Modes tab **Note** You may need to use the arrows located in the bottom of the dialog box to locate the Op. Modes tab. b. From the **Operating modes selection** group of the **Edit Configuration** dialog box, click the arrow to the right of the **Mode** heading and select **Test** from the drop-down list.  Mode drop-down list c. From the **Device Control** dialog box, click **RAM** to toggle to **EEPROM** mode.  RAM button d. From the **Device Control** dialog box, click **Send**. e. From the **Confirm** dialog box, click **YES** to save to EEPROM. The fixed-position bar code reader begins a continuous repeating scan of the bar code. The software updates the **Terminal** dialog box every 0.5 sec indicating the percentage of accurate reads completed during the 0.5 sec interval.

To position the fixed-position bar code reader: *(continued)*

Step	Action
5	Loosen the black positional adjustment knob on the fixed-position bar code reader, and position the scan head of the reader as far as possible from the plate while maintaining the orientation towards the bar code on the plate (see below) 
6	While watching the **Terminal** dialog box, slowly adjust the orientation of the fixed-position bar code reader until the percent successful reading displays the highest number possible.  **Note** It may be helpful to briefly place a sheet of white paper in front of the plate bar code to view the area scanned by the laser.
7	When satisfied with the alignment, tighten the black positional adjustment knob on the fixed-position bar code reader.
8	Restore the fixed-position bar code reader to normal operation: a. From the **Edit Configuration** dialog box, change from **Test** back to **Serial on Line**.  b. From the **Device Control** dialog box, confirm that **EEPROM** is still selected, and click **Send**. c. From the **New Decision** dialog box, click **YES** to save to EEPROM. The bar code reader stops scanning the plate bar code and resumes normal operation.
9	Click **Exit** ( ) to quit the **LDHOST** window. The **LDHOST** window closes.
10	Replace the cover for the fixed-position bar code reader (from step 1 on page 7-40).

Cleaning and Replacing Gripper Finger Pads

When to Perform

The adhesive used to affix bar code labels to certain brands of microplates can build up on the gripper pads of the Zymark Twister Microplate Handler. Over time, the residue can cause the gripper pads to stick to the microplates while handling them, causing misfeeds. To prevent buildup, inspect the gripper pads monthly and clean or replace the pads as needed.

Materials Required

The following materials are required to replace the finger pads:

Material	Part Number
Finger Pad Replacement Kit, containing 10 finger pads	4315472
Flat-blade screwdriver, small	—
Phillips head screwdriver, small	—
Isopropanol in a squeeze bottle	—

⚠ WARNING **CHEMICAL HAZARD**. **Isopropanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Cleaning the Finger Pads

To clean the finger pads, wipe each pad thoroughly with Isopropanol until the residue has been resolved. If the pads appear rough or the adhesive cannot be removed, replace the pads as described below.

Replacing the Finger Pads

To replace the finger pad(s):

Step	Action
1	Using the Phillips-head screwdriver, remove the two small Phillips-head screws from the fingers on each side of the gripper, then remove the fingers. **Note** Move the plate handler arm into any position where it is easy to access the screws.
2	Using a small flat-blade screwdriver, pry the worn finger pads off the fingers. **Note** The manufacturer recommends replacing all finger pads at the same time.
3	Clean any residual adhesive off the fingers using isopropanol.
4	Remove a replacement finger pad from the paper backing, and place the finger pad on the appropriate finger position.
5	Repeat for the remaining finger pads.
6	Install the fingers with the fingers pointing down and the finger pads toward the center of the gripper.
7	Insert the screws into the fingers and tighten. **Note** The screws do not automatically align the grippers. Make sure that the finger pads are making good contact with the plate when the arm grips a plate.

Section: Maintaining the Computer and SDS Software

In This Section This section contains the following information:

Note The SDS software is a multicomponent system that must be maintained to ensure optimal operation of the ABI PRISM 7900HT Sequence Detection System. Although, most of the maintenance will be completed by an Applied Biosystems service engineer, this section discusses important issues that you should understand.

General Computer Maintenance

Maintenance Schedule

The computer connected to the 7900HT instrument requires regular maintenance to ensure reliable operation of the ABI PRISM 7900HT Sequence Detection System components. Applied Biosystems recommends the following tasks as part of routine maintenance of the computer system:

Maintenance Task	Perform
Archive or Remove Old SDS Files	Weekly
Defragmenting the Hard Drive	Monthly or before fragmentation reaches 10%
Upgrading the Operating System Software	When available/advisable
Upgrading the 7900HT SDS Software	When available

Developing a Data Management Strategy

Applied Biosystems recommends developing a strategy for dealing with the files produced by the SDS software. During a single day of real-time operation, the ABI PRISM 7900HT Sequence Detection System can generate over 200 MB of data. Without a strategy for distributing and archiving SDS-related files, the 7900HT instrument can easily fill the hard drive of the computer within just a few weeks of operation. See “Managing Sequence Detection System Data” on page 2-15 for a discussion of management strategies.

Archiving SDS Files

To conserve space on the computer hard drive, SDS files can be archived using a data compression utility. The compression utility archives files by encoding them in a compressed form, thereby reducing the size of a file. SDS files can be compressed and decompressed many times.

Several commercially available compression utilities are available. PKZIP and *.arc are archive formats common to the Microsoft Windows operating system.

Defragmenting the Hard Drive

Applied Biosystems recommends defragmenting the hard drive of the computer attached to the instrument at least once every week or before fragmentation reaches 10%. As the ABI PRISM 7900HT Sequence Detection System is used and files are deleted and created, the free space on the computer hard drive eventually is split into increasingly smaller blocks (called “clusters”). Consequently, as the SDS software creates new files and extends old ones, the computer cannot store each file in a single block. Instead, the system will ‘fragment’ the files by scattering their component pieces across different sectors of the hard drive.

The fragmentation of SDS files decreases the performance of both the SDS software and the computer operating system. As the hard drive becomes fragmented, programs take greater time to access files because they must perform multiple seek operations to access the fragments.

Several commercially available software utilities are available for repairing fragmented file systems. The software utility defragments broken files by combining their component pieces at a single location on the hard drive, thereby optimizing system performance.

Upgrading the Operating System Software

Do not upgrade the operating system of the computer connected to the 7900HT instrument unless instructed to do otherwise by an Applied Biosystems service engineer. New versions of the Microsoft Windows operating system can be incompatible with the SDS software and render it and the instrument inoperable.

The Applied Biosystems service engineer maintains the operating system software as part of planned maintenance visits. During the visit, the engineer will update the computer operating system as upgrades become available and are validated by Applied Biosystems.

Maintaining the SDS software

Administration Privileges

IMPORTANT You must have administrator privileges on the computer to install and/or upgrade the SDS software.

Upgrading the 7900HT SDS Software

Applied Biosystems continually develops the SDS software to provide increased functionality and reliability of the ABI PRISM 7900HT Sequence Detection System. As updates become available, Applied Biosystems sends notifications of the upgrades to all ABI PRISM 7900HT Sequence Detection System customers. If an upgrade is user-installable, it can be found on the Applied Biosystems company Web site (see Appendix F, “Contacting Technical Support,” to visit the Applied Biosystems Web Site).

Note Applied Biosystems service engineers perform regular updates the SDS software during planned maintenance visits.

Reinstalling the Software

On rare occasions, when a piece of the SDS software becomes corrupt, it may be necessary to re-install the software. In the event that the software must be re-installed, observe the following guidelines to re-install or upgrade the software.

- Unless instructed to do otherwise, remove the SDS software using the uninstall utility. Do not delete the program folder from the Program Files directory.
- Install the SDS software under a user login that has administrator privileges on the computer.
- Unless instructed to do otherwise, re-install the SDS software to the same directory as the previous installation.
- Review all documentation accompanying the new software (such as installation notes or user bulletin). The updated version of the software may contain new features that require special consideration.

8 Troubleshooting

In This Chapter

This chapter discusses the following topics:

Troubleshooting Table

Overview

The following table is designed to help you troubleshoot most of the problems you may encounter while using the ABI PRISM® 7900HT Sequence Detection System.

The information in the table is arranged by category as follows:

- Chemistry problems
- Run problems
- Instrument and Automation Module Problems

Each category contains subcategories, followed by a brief description of the symptoms you might encounter.

To use this table, look for the category and the symptom you are experiencing. The page number in the right-hand column corresponds to a description of the possible cause(s) and recommended action(s) for that particular problem.

Table 8-1 Troubleshooting Table

Category	Symptom	Page
Chemistry and Run Problems		
Chemistry	Low Precision	8-4
	Irreproducibility	
Run Problems		
Background Runs	Software will not extract background data	8-8
	Background is too high (greater than 2500)	
Pure Dye Runs	Software will not extract pure dye data	8-10
	Raw data from pure dye run appears strange	
	Signals plateau (saturation)	
	Signal is too low (< 10,000 FSU)	
	More than two outliers per dye in a single row	
Real-Time Runs (Quantitative PCR and Dissociation Curves)		8-11
End-Point Runs (Allelic Discrimination)		8-13

Table 8-1 Troubleshooting Table *(continued)*

Category	Symptom	Page
Instrument and Automation Module Problems		
Software and 7900HT Instrument	SDS software will not launch	8-14
	Software crashes/freezes the computer or displays an error message	
	Communication error	
	Thermal cycler errors	
	Automation Controller Software cannot find a plate document file	
	Computer and/or software displays the **Run Completed Successfully** dialog box but will not respond and appears to be frozen	
	Run will not start	
	Computer is slow when analyzing data, opening or closing dialog boxes, and other software processes.	
	The computer will not logon to the Windows Operating System.	
	The computer will not boot up at all.	
Zymark Twister Microplate Handler and Fixed-Position Bar Code Reader	Plate handler emits grinding noise when picking up or putting down plates	8-17
	Plate handler arm contacts racks when retrieving or stacking plates	
	Plate handler arm releases plates awkwardly into the plate stack	
	Reaction plates tip or tilt when placed into the instrument tray by the plate handler arm	
	Plate handler fails to sense or grasp plates	
	Plates stick to the gripper fingers of the plate handler arm	
	Plate handler does not restack plates in original locations	
	Fixed-position bar code reader not reading plate bar codes	

Low Precision or Irreproducibility

Overview

There are many reasons why an assay run with the ABI PRISM 7900HT Sequence Detection System can have less than optimal precision. Factors that can affect precision are described in detail below.

Factor	See Page
Improper Threshold Setting	8-4
Imprecise Pipetting	8-5
Non-Optimized Chemistry	8-5
Incomplete Mixing	8-5
Air Bubbles	8-5
Splashing PCR Reagents	8-5
Drops	8-6
Writing on the Reaction Plates	8-6
Fluorescent Contamination on the Plates	8-6
Errors	8-6
Contaminated Sample Block	8-7
Improper or Damaged Plastics	8-7
Low Copy Templates	8-7
Use of Non-Applied Biosystems PCR Reagents	8-7

Improper Threshold Setting

The key to high-precision quantitative PCR is accurate detection of the geometric phase. The ABI PRISM 7900HT Sequence Detection System typically delivers sufficient sensitivity so that at least 3 cycles of the geometric phase are visible, assuming reasonably optimized PCR conditions. The SDS software calculates a fixed signal intensity, called a threshold, which each signal generated from PCR amplification must reach before it is recognized as actual amplification. The calculated threshold is an approximation, and should be examined and modified as needed.

Modifying the Threshold

In a real-time document of the SDS software, the threshold can be modified via the Amplification Plot view following analysis of the run data. See “Setting the Baseline and Threshold Values for the Run” on page 6-10 for more information.

Imprecise Pipetting

The calculated quantities of target nucleic acid are directly affected by how precisely the template volumes are added to the reaction mixes. Other individually added reagents are also affected by pipetting precision (such as, variable magnesium affects amplification efficiency).

Using Master Mixes

For this reason, Applied Biosystems highly recommends using a master mix. All common components to a set of reactions should be mixed together and then dispensed to the wells of the plate. Sub-master mixes can be used to further improve the precision of identical replicates. For example, instead of pipetting 5 µL of the same template into four replicate wells, pipette 20 µL of the template into a sub-master mix, then divide the sub-master mix into four equal parts for amplification. When making each master mix, add 5–10% additional volume to compensate for pipetting losses.

Using Pipettors

Pipetting precision is also improved by:

- Calibrating and servicing the pipettors regularly
- Pipetting larger volumes
- Reducing the number of pipetting steps whenever possible
- Increasing the consistency of the pipetting method

Consult the manufacturer about the correct method of dispensing liquid volumes accurately from the pipettor. For example, some pipettors are designed to deliver the designated volume at the first plunger stop, so 'blowing out' the residue may cause error. Also, before using a new pipettor tip to serially dispense a master mix, wet the tip once by drawing up some of the master mix and dispensing it back into the mix again.

Non-Optimized Chemistry

Chemistries that have not been optimized may be susceptible to inconsistencies. To maximize precision and reaction efficiency, optimize the primer and probe concentrations of each individual assay used. Refer to the *TaqMan Universal PCR Master Mix Protocol* (P/N 4304449) for specific information about optimizing probe and primer concentrations for TaqMan-related chemistries.

Incomplete Mixing

For maximum precision, the PCR master mix must be mixed to uniformity. Once all reaction components are added to master mix, it should be vortexed for 4–5 seconds before aliquotting it to the wells of the plate. Any dilutions performed during the assay should also be vortexed.

Air Bubbles

Air bubbles in the wells can refract and distort the fluorescent signals. Ideally, the reagents would be applied to the wells using a pipetting technique that does not form air bubbles. However, if a plate does contain air bubbles, they can usually be removed by swinging, tapping, or briefly centrifuging the reaction plate.

Splashing PCR Reagents

If PCR reagents splash the undersides of the optical adhesive covers, the heat from the lid may bake the liquid to the cover and may distort the signal. If splashing occurs, briefly centrifuge the reaction plate to remove all traces of liquid from the caps.

Drops

Drops of reagents that cling to the sides of the wells may not contact the thermal cycler sample block and consequently may not amplify. If the drop slides into the mix during PCR, then the amplified products will become diluted and the final result will be less than replicate wells that did not have drops. Therefore, carefully monitor the reaction plate as it is being transferred into the thermal cycler or 7900HT instrument. If you observe any drops, take steps to remove them, such as centrifugation.

Writing on the Reaction Plates

Do not write on any surface of the Optical 384/96-Well Reaction Plates or the Optical Adhesive Covers. The fluorescent properties of the ink can potentially affect the fluorescence emission from the plate and alter the results. Instead, note the contents of each well on a sheet of paper, or on a printout of the sample setup.

Fluorescent Contamination on the Plates

Many compounds found in laboratories are fluorescent. If they come in contact with certain optical surfaces, such as the optical adhesive covers, the fluorescent results may be affected. For example, it has been noted that the powder used to lubricate the insides of plastic gloves often contains fluorescent compounds. Use only powder-free gloves and do not needlessly touch the reaction plates or optical adhesive seals.

Errors

Human errors from time to time are inevitable, such as pipetting into the wrong well, or making a dilution mistake.

Human error can be reduced in the following ways:

- Perform the assay in a systematic fashion. For example, the pattern of sample positions should be simple (such as avoid putting gaps in the rows).
- When pipetting the master mix, look directly down into the reaction plate so that you can verify the transfer of the solution.
- If adding a small-volume reagent, such as template, place the drop of liquid on the side of the well. Briefly tap or centrifuge the plate afterwards to bring the droplet down into the well.
- After all pipetting is complete, visually inspect all the wells to confirm the presence of the reagent drops. Tapping or centrifuging the reaction plate will cause all the drops to slide down into the wells simultaneously.
- When making serial dilutions, be sure to change the pipet tip after each dilution step.
- Visually inspect the liquid volumes being pipetted to verify that the volume is approximately correct. A common mistake is using the wrong pipettor volume setting (such as setting 20 µL instead of 2.0 µL).
- Visually inspect the volumes of the completed reactions, looking for any wells that have volumes that do not match those of the other wells.

Contaminated Sample Block

Any material contaminating the sample block can affect the results. For example, mineral oil reduces thermal transfer. Residue from writing on reaction plates darkens the wells, absorbing light.

The sample blocks should be periodically inspected for cleanliness. Sample block contamination can be visualized by running a background plate and inspecting the resulting background signal for aberrant peaks above 2500 FSU (see page 7-13). See page 7-11 for instructions on decontaminating the sample block.

Improper or Damaged Plastics

Only ABI PRISM optical grade reaction plates, optical adhesive covers, and ABI PRISM® optical flat caps should be used with the ABI PRISM 7900HT Sequence Detection System. The plastics that comprise the optical parts undergo special testing for the absence of fluorescent impurities. Optical reaction plates are frosted to improve the degree and precision of light reflection. Bent, creased, or damaged plastics may adversely affect the transmission of fluorescent signal or prevent proper sealing of a well resulting in evaporation, change in sample volume, and altered PCR chemistry. Make sure to use the correct plastics and visually inspect each reaction plate before use.

Note See Appendix D, “Kits, Reagents and Consumables,” for a list of compatible consumables and reagents.

Low Copy Templates

When amplifying samples that contain very low quantities of nucleic acid (generally less than 100 molecules), expect lowered precision due to the Poisson distribution and biochemical effects related to binding probabilities. Low copy templates are also more susceptible to losses due to non-specific adhesion to plastic wells, pipettor tips, etc. The addition of carrier to the sample, such as yeast tRNA or glycogen, can help prevent these losses, increasing the precision and sensitivity of the assay.

Use of Non-Applied Biosystems PCR Reagents

The Applied Biosystems buffer contains an internal passive reference molecule (ROX™), which acts as a normalization factor for fluorescent emissions detected in the samples (see page A-6).

IMPORTANT Non-Applied Biosystems PCR buffers may not contain the ROX passive reference. If running non-Applied Biosystems chemistry, be sure to set the passive reference for your experiment as explained on “Setting the Passive Reference” on page 4-12.

Background Runs

Background Troubleshooting Table

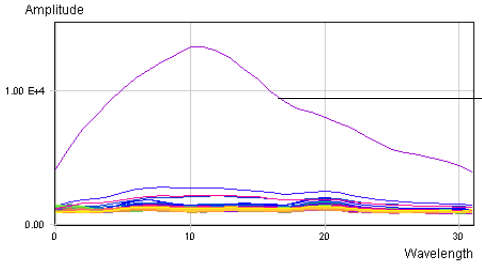
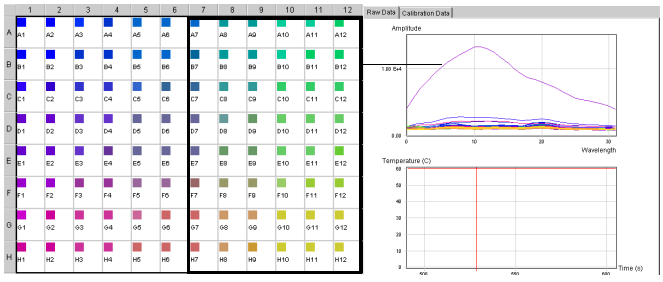
Observation	Possible Cause	Recommended Action
Software will not extract background data	During setup, the wrong plate type was assigned to the plate document	Run a new background plate document with the proper plate type setting.
	Background is too high (≥ 2500 FSU[a])	See below.
Background is too high (=>2500)	Sample block contamination	a. Construct and run a new background plate. b. See "Isolating Sample Block Contamination" below.
	Background plate contamination	

a. Fluorescent standard units – The measure of amplitude displayed along the Y-axis of the Background Plot.

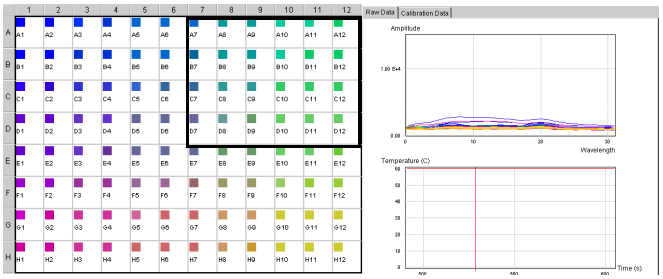
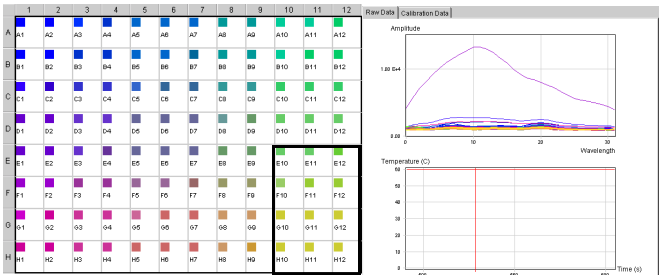
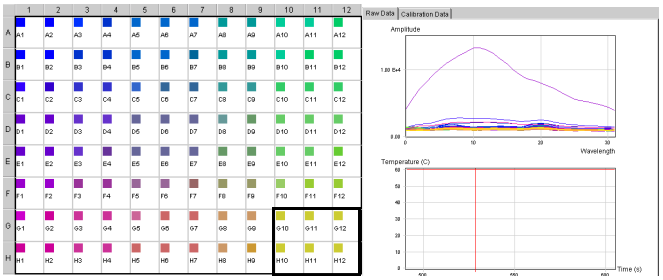
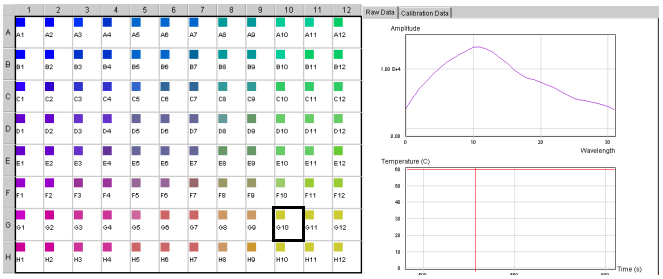
Isolating Sample Block Contamination

Signals exceeding 2500 FSU are considered outside the limit of normal background fluorescence and indicates that the either the background plate or the sample block module may be contaminated.

To determine the location of the contamination on the sample block:

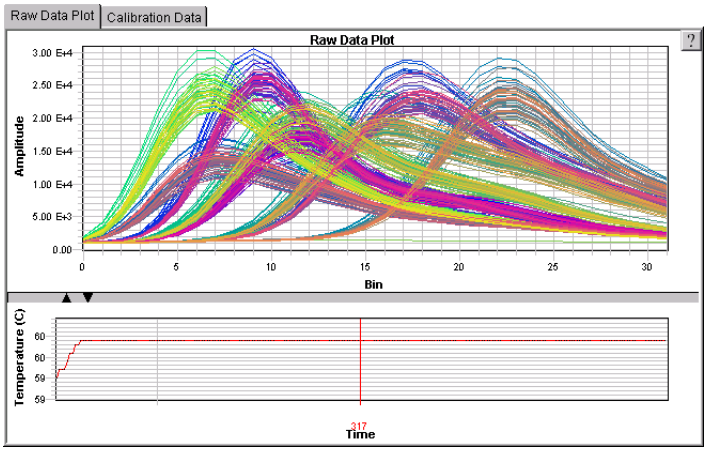
Step	Action
1	If not already open, open the plate document for the background run.
2	From the toolbar, click the **Hide/Show System Raw Data Pane** button ( ). The SDS software displays the raw data pane for the background run.
3	Select all wells in the plate document.
4	Inspect the raw background data for an aberrant spectral peak or peaks. Wells producing raw spectra that exceed 2500 FSU are considered irregular and could be contaminated. The following figure illustrates the raw data produced by a run on a sample block module containing a contaminated well. 
5	Identify the location(s) of the contaminated well(s) on the sample block by selecting increasingly smaller regions of the plate document (see below).  a. The raw data from the selected wells contains the peak. The contaminated well must be in columns 7-12.

To determine the location of the contamination on the sample block: *(continued)*

Step	Action
	Example: *(continued)*  b. The raw data from the selected wells does not contain the peak. The contaminated well must be in the last four wells of columns 7-12.  c. The raw data from the selected wells contains the peak. The contaminated well must be in the last four wells of columns 10-12.  d. The raw data from the selected wells contains the peak. The contaminated well must be in the last two wells of columns 10-12.  e. By selecting each of the wells from the last two wells of columns 10-12, the location of the contaminated well (G10) is determined.
6	Repeat step 4 until you identify the location of each contaminated well.
7	Decontaminate the sample block as explained in “Decontaminating the Sample Block” on page 7-11.
8	Run a background plate to confirm that the contaminants have been removed. If the contamination is present after running the background plate for a second time, the background plate is likely to be the source of contamination.

Pure Dye Runs

Pure Dye Troubleshooting Table

Observation	Possible Cause	Recommended Action
Software will not extract pure dye data	During plate setup, the wrong plate type was assigned to the plate document	Create and run a new pure dye plate document with the proper plate type setting
	A background plate was not run before the pure dye plate	Run a background plate, then run the pure dye plate again
Raw data from pure dye run appears strange (see below)	Pure dye plate was loaded backwards	a. Verify the pure dye wavelengths are as expected. b. Rerun the pure dye plate.
		
Signals plateau (saturation)	Intensity is set too high/low	Call Applied Biosystems Technical Support.
Signal is too low (< 10,000 FSU)		
More than two outliers per dye in a single row	♦ Evaporation ♦ Contamination	Rerun the pure dye plate. If the problem persists, discard the pure dye plate and run a new one.

Real-Time Runs (Quantitative PCR and Dissociation Curves)

Troubleshooting Analyzed Data from a Real-Time Run

When faced with irregular data, you can use the SDS software to diagnose some chemistry- and instrument-related problems. The following table contains a summary of checks for verifying the integrity of your run data and to help you begin troubleshooting potential problems.

Troubleshooting Analyzed Real-Time Run Data

Analysis View/Description	What to look for...
Raw Data Plot Displays the raw reporter fluorescence signal (not normalized) for the selected wells during each cycle of the real-time PCR.	Signal tightness and uniformity – Do the raw spectra signals from replicate groups and controls exhibit similar spectral 'profiles'? If not, the plate or sample block could be contaminated.
	Characteristic signal shape – Do the samples peak at the expected wavelengths? For example, samples containing only FAM™- labeled TaqMan® probes should not produce raw fluorescence in the wavelength of a VIC™ dye component. A signal present in wells that do not contain the dye could indicate that the sample, master mix, or well contains contaminants.
	Characteristic signal growth – As you drag the bar through the PCR cycles, do you observe growth as expected? Absent growth curves may indicate a pipetting error (well lacks template).
	Signal Plateaus – Do any of the signals plateau? Signal plateaus or saturation can be an indication that a well contains too much template or fluorescent signal.
Multicomponent Plot Displays a plot of normalized multicomponent data from a single well of a real-time run. The plot displays the component dye signals that contribute to the composite signal for the well.	Correct dyes displayed – Does the plot display all dyes as expected? The presence of an unexpected dye may be the result of an error in detector setup, such as assigning the wrong reporter or quencher dye.
	ROX fluorescence level – Does the ROX signal fluoresce below the reporter dyes? If not, the lack of reporter fluorescence may be caused by an absence of probe in the well (a pipetting error).
	Background fluorescence – Do all dyes fluoresce above the background? The Background signal is a measure of ambient fluorescence. If a dye fails to fluoresce above the background, it is a strong indication that the well is missing probes labeled with the dye (well does not contain probe, PCR master mix, or both).
	MSE Level – The MSE (mean squared error) is a mathematical representation of how accurately the multicomponented data fits the raw data. The higher the MSE value, the greater the deviation between the multicomponented data and the raw data.

Troubleshooting Analyzed Real-Time Run Data *(continued)*

Analysis View/Description	What to look for...
Amplification Plot Displays data from real-time runs after signal normalization and Multicomponent analysis. It contains the tools for setting the baseline and threshold cycle (C_T) values for the run.	Correct baseline and threshold settings – Are the baseline and threshold values set correctly? Identify the components of the amplification curve and set the baseline so that the amplification curve growth begins at a cycle number that is greater than the highest baseline number. **IMPORTANT** Do not adjust the default baseline if the amplification curve growth begins after cycle 15. Identify the components of the amplification curve and set the threshold so that it is: ♦ Above the background ♦ Below the plateaued and linear regions ♦ Within in the geometric phase of the amplification curve
	Irregular amplification – Do all samples appear to have amplified normally? The three phases of the amplification curve should be clearly visible in each signal.
	Outlying amplification – When the run data is viewed in the C_T vs. Well Position plot, do replicate wells amplify comparably? Wells producing C_T values that differ significantly from the average for the associated replicate wells may be considered outliers. If a plate produces non-uniformity between replicates, some samples on the plate could have evaporated. Check the seal of the optical adhesive cover for leaks.

End-Point Runs (Allelic Discrimination)

Troubleshooting Analyzed Data from an End-Point Run

When faced with irregular data, you can use the SDS software to diagnose some chemistry- and instrument-related problems. The following table contains a summary of checks for verifying the integrity of your run data and to help you begin troubleshooting potential problems.

Troubleshooting Analyzed End-Point Run Data

Analysis View/Description	What to look for...
Raw Data Displays the raw reporter fluorescence signal (not normalized) for the selected wells during each cycle of the PCR.	♦ Signal tightness and uniformity – Do the raw spectra signals from replicate groups and controls exhibit similar spectral 'profiles'? If not, the plate or sample block could be contaminated. ♦ Characteristic signal shape – Do the samples peak at the expected wavelengths? For example, samples containing only FAM- labeled TaqMan probes should not produce raw fluorescence in the peak wavelength of the VIC dye component. A signal present in wells that do not contain the dye could indicate that the sample, master mix, or well contains contaminants. ♦ Signal Plateaus – Do any of the signals plateau? Signal plateaus or saturation can be an indication that a well contains too much template or fluorescent signal.

Software and 7900HT Instrument

Troubleshooting Software and Computer Problems

Troubleshooting Software and Computer Problems

Observation	Possible Cause	Recommended Action
SDS software will not launch The software crashes/freezes the computer or displays an error message	♦ Incorrect start-up sequence ♦ Corrupted software ♦ Computer hardware failure ♦ Operating System (OS) corruption ♦ Loose bar code reader cable	Follow the solutions listed until the symptom goes away.
		1
		a. Turn off the 7900HT instrument. b. Check cable connections. c. Restart the computer and logon to the computer. d. Turn on the 7900HT instrument. e. Launch the SDS software.
		2
		a. Restart the computer and logon to your computer. b. Reinstall the SDS software. c. Launch the SDS software.
		3
		Contact Applied Biosystems Service for OS problems or if the computer will not boot up at all. You may have to reload the OS from the CDs.
		4
		Contact Dell for troubleshooting the computer hardware.
Communication error	Cables are connected incorrectly	Check cable connections and COM port setup. See “Instrument Connections” on page 2-11.
Thermal cycler errors	Sample block module not fully engaged	Reseat the sample block module as explained “Replacing the Sample Block” on page 7-4.
Automation Controller Software cannot find a plate document file	File not in correct location	Remove file entry from plate queue and add the file to the plate queue again.
Dialog box does not respond to mouse clicks or key strokes	Java Runtime Error	Click the close box of the dialog box to close it.

Troubleshooting Software and Computer Problems *(continued)*

Observation	Possible Cause	Recommended Action
Run will not start	No calibration file	Perform background and pure dye runs. See “Performing a Background Run” on page 7-13 and “Performing a Pure Dye Run” on page 7-17.
	No background data in calibration file (background run has not been performed)	
	No pure dye data in calibration file (pure dye run has not been performed)	
	Calibration file does not contain pure dye data for a dye used on the plate document	
	Calibration file was created on another instrument	
	Disk drive containing the plate document has less than 50 MB of free space	Check the capacity of the destination drive. If less than 50 MB of free space remains, remove or archive existing data files (see page 7-46).
	Heated cover cannot reach running temperature because no plate loaded	Open the instrument tray and check that the instrument contains a plate.
	Instrument tray contains a plate	
	Output stack contains a plate or plates	Remove all plates from the output stack of the plate handler before starting the queue.
Computer is slow when analyzing data, opening or closing dialog boxes, and other software processes	Hard drive is fragmented	Defragment the hard drive as explained on “Defragmenting the Hard Drive” on page 7-47.
	Hard drive is almost full	Remove or archive existing data files as explained on “Archiving SDS Files” on page 7-46.
The computer will not logon to the Windows Operating System	**Logon** window does not appear	Restart the computer and logon to your computer.
	You are not logged on as the **Administrator**	a. Logoff of your computer. b. Logon again as the **Administrator**.
	After the above solutions have been tried, the problem is still not fixed	Contact Dell for troubleshooting the computer hardware or OS.

Troubleshooting Software and Computer Problems *(continued)*

Observation	Possible Cause	Recommended Action
The computer will not boot up at all	Cables are not connected or are not seated properly	Check the cables.
		The boot disk is corrupted. a. Boot directly off of the NT Installation CD. b. Boot off of the emergency disk. c. Reload the Windows NT Operating System from the CD.
	After the above solution has been tried, the problem is still not fixed	Contact Dell for troubleshooting the computer hardware.

Zymark Twister Microplate Handler and Fixed-Position Bar Code Reader

Automation Accessory Troubleshooting Table

Observation	Possible Cause	Recommended Action
Plate handler emits grinding noise when picking up or putting down plates	Vertical offset too low	Re-align the plate handler as explained in “Aligning the Plate Handler” on page 7-32.
	Plate detector switch set too high	
Plate handler arm contacts racks when retrieving or stacking plates	Plate handler rotary offset is incorrect or vertical offset is too low	
The plate handler arm releases plates awkwardly into the plate racks		
Reaction plates tip or tilt when placed into the instrument tray by the plate handler arm		
Plate handler fails to sense or grasp plates	Plate sensor switch not adjusted properly	Adjust the plate sensor switch as explained in “Adjusting the Sensitivity of the Plate Sensor Switch” on page 7-28.
	Gripper pads on the fingers of the plate handler arm are worn or dirty	Change the gripper pads as explained in “Cleaning and Replacing Gripper Finger Pads” on page 7-43.
Plates stick to the gripper fingers of the plate handler arm	Gripper pads are worn or dirty	Change the gripper pads as explained in “Cleaning and Replacing Gripper Finger Pads” on page 7-43.
Plate handler does not restack plates in original locations	**Restack when finished** option not selected	Configure the Automation Controller Software to restack the plates as explained in page 4-37.
Fixed-position bar code reader not reading plate bar codes	Bar code reader is mis-aligned	Re-align the fixed-position bar code reader as explained in “Aligning the Fixed-Position Bar Code Reader” on page 7-40.
	Bar code reader is broken	

User Bulletins

9

About This Chapter

A user bulletin is an advisory issued by Applied Biosystems. User bulletins contain new information, advances, or procedures that may immediately influence your use of Applied Biosystems instruments.

This section of the user guide is intended as a storage space for any user bulletins you may receive regarding your ABI PRISM® 7900HT Sequence Detection System.

Theory of Operation

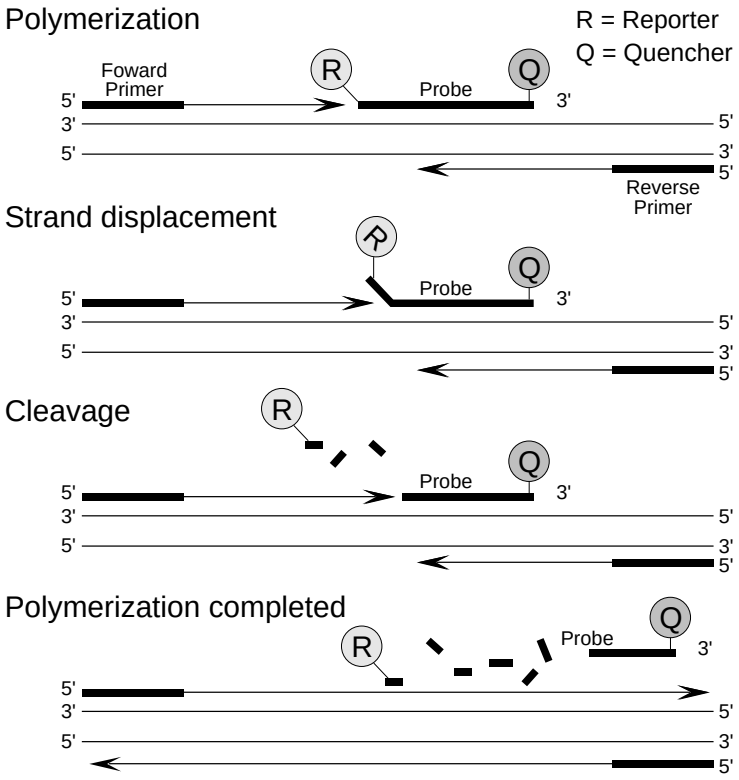
A

In This Appendix This appendix discusses the following topics:

Fluorescent-Based Chemistries

Fundamentals of the 5´ Nuclease Assay

The PCR reaction exploits the 5´ nuclease activity of AmpliTaq Gold® DNA Polymerase to cleave a TaqMan® probe during PCR. The TaqMan probe contains a reporter dye at the 5´ end of the probe and a quencher dye at the 3´ end of the probe. During the reaction, cleavage of the probe separates the reporter dye and the quencher dye, which results in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. The figure below shows the forklike-structure-dependent, polymerization-associated 5´–3´ nuclease activity of AmpliTaq Gold DNA Polymerase during PCR.



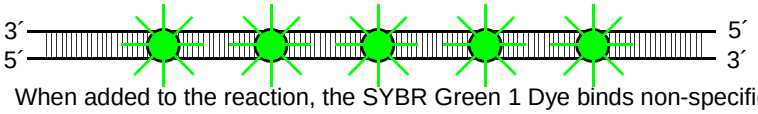
When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Förster-type energy transfer (Förster, 1948; Lakowicz, 1983). During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites.

The 5´–3´ nucleolytic activity of the AmpliTaq Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3´ end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product.

The increase in fluorescence signal is detected only if the target sequence is complementary to the probe and is amplified during PCR. Because of these requirements, any nonspecific amplification is not detected.

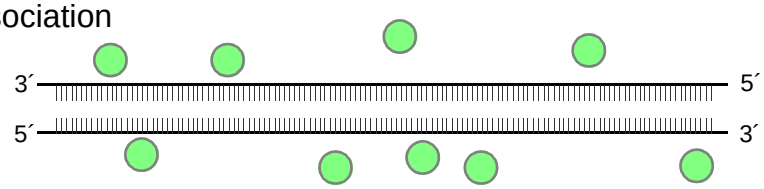
Basics of SYBR Green Chemistry

The SYBR® Green 1 Double-Stranded Binding Dye is used for the fluorescent detection of double-stranded DNA (dsDNA) generated during PCR. The SYBR Green 1 Dye binds non-specifically to dsDNA and generates an excitation-emission profile similar to that of the FAM™ reporter dye. When used in combination with a passive reference, the SYBR Green 1 Dye can be employed to perform several SDS-related experiments including quantitative PCR and dissociation cure analysis. The following figure illustrates the action of the SYBR Green 1 dye during a single cycle of a PCR.



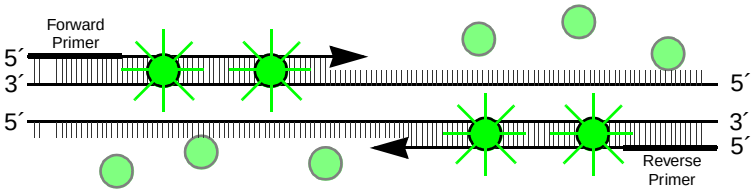
When added to the reaction, the SYBR Green 1 Dye binds non-specifically to the hybridized dsDNA and fluoresces

Dissociation



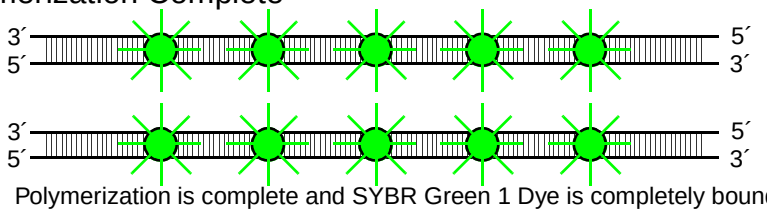
Denaturation complete, the SYBR Green 1 Dye dissociates from the strand, resulting in decreased fluorescence

Polymerization



During the extension phase, the SYBR Green 1 Dye begins binding to the PCR product

Polymerization Complete



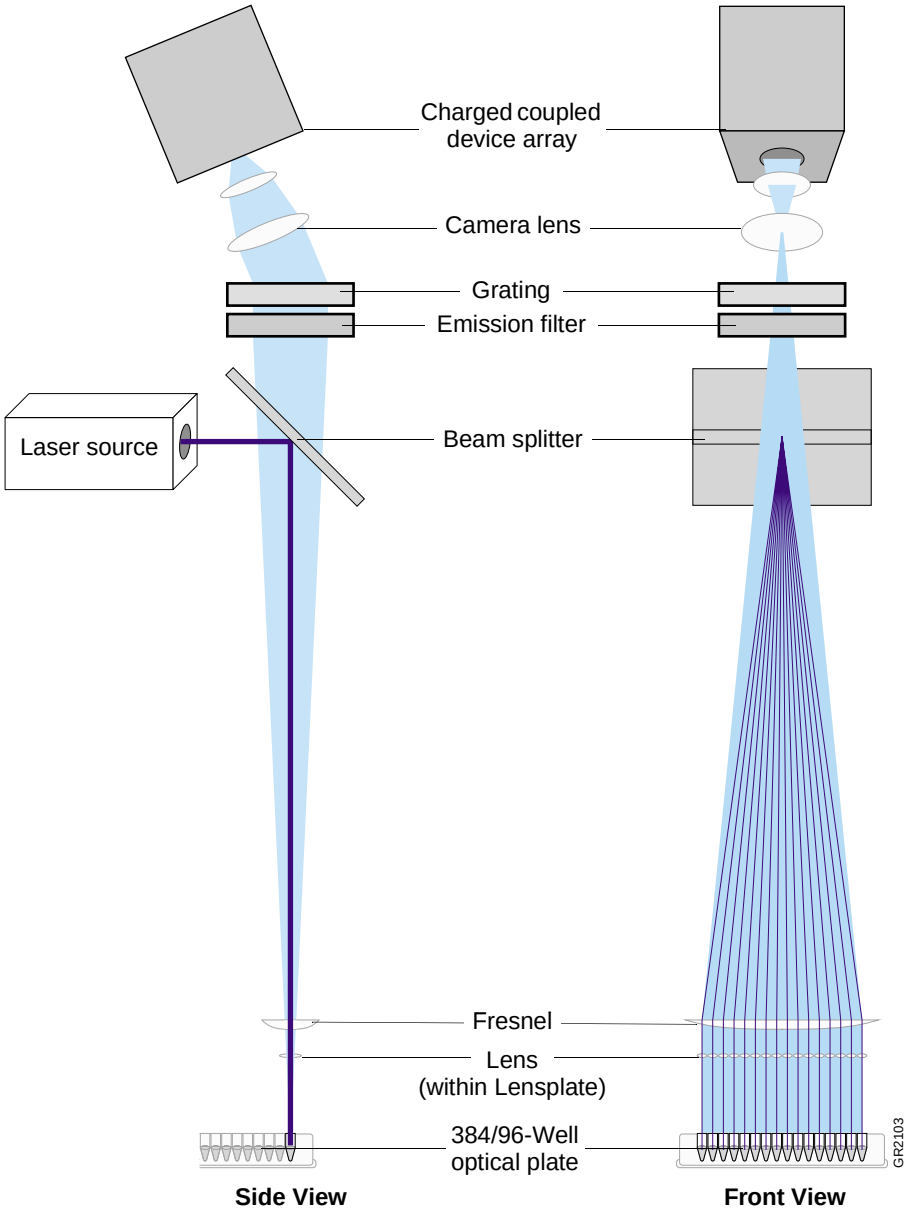
Polymerization is complete and SYBR Green 1 Dye is completely bound, resulting in a net increase in fluorescence

Fluorescence Detection and Data Collection

Fluorescent Sequence Detection

During PCR, light from an argon ion laser is sequentially directed to each well on the microplate. The light passes through the ABI PRISM™ Optical Adhesive Cover and the laser excites the fluorescent dyes present in each well of the consumable. The resulting fluorescence emission between 500 nm and 660 nm is collected from each well, with a complete collection of data from all wells approximately once every 7–10 seconds.

A system of lenses, filters, and a dichroic mirror focus the fluorescence emission into a grating. The grating separates the light (based on wavelength) into a predictably spaced pattern across a charge-coupled device (CCD) camera. The SDS software collects the fluorescent signals from the CCD camera and applies data analysis algorithms.



Mathematical Transformations

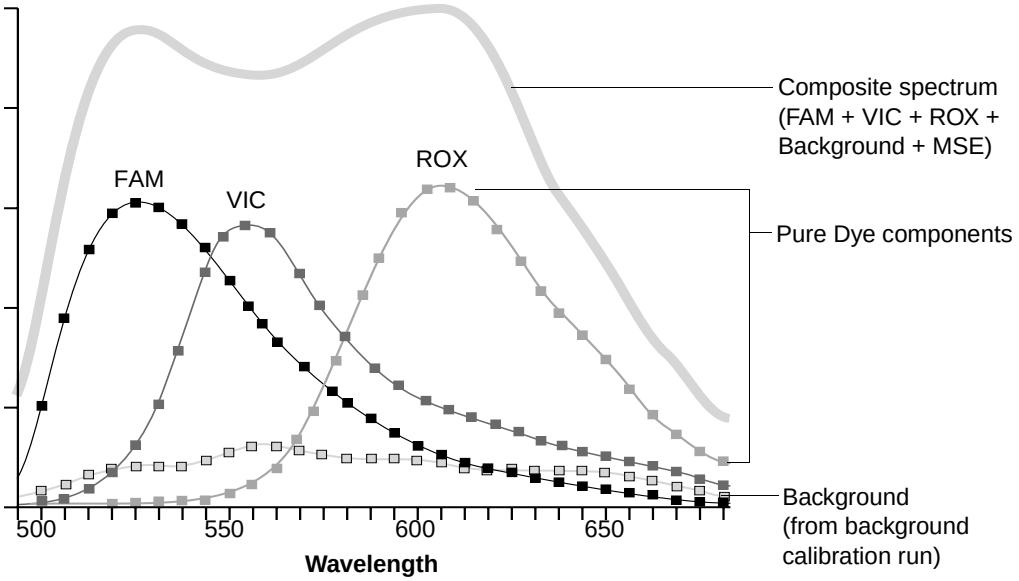
Overview

The SDS software performs a series of mathematical transformations on the raw data during an analysis of all end-point and real-time runs. The term raw data refers to the spectral data between 500 nm to 660 nm collected by the SDS software during the sequence detection run. The following section describes the fundamental analysis of raw run data performed on both real-time and end-point run by the SDS software.

Multicomponenting

Multicomponenting is the term used for distinguishing the contribution each individual dye and background component makes to the fluorescent spectra detected by the 7900HT instrument. During the multicomponent transformation, the SDS software employs several algorithms to separate the composite spectra from the raw spectrum and then to determine the contribution of each dye in the raw data.

First, the algorithm eliminates the contribution of background fluorescence in the raw data, by subtracting the background component stored within the background calibration file (see page 7-13). Next, the software employs the extracted pure dye standards (see page 7-17) to express the composite spectrum in terms of the pure dye components. The figure below shows one composite spectrum that represents a fluorescent reading from a single well that contains the passive reference and two fluorogenic probes, labeled with the FAM and VIC™ reporter dyes and a non-fluorescent quencher. The example spectra demonstrate how the overlapping component dye spectra contribute to the composite spectrum. The SDS software multicomponenting algorithm applies matrix calculations to determine the contributions of each component dye spectra.



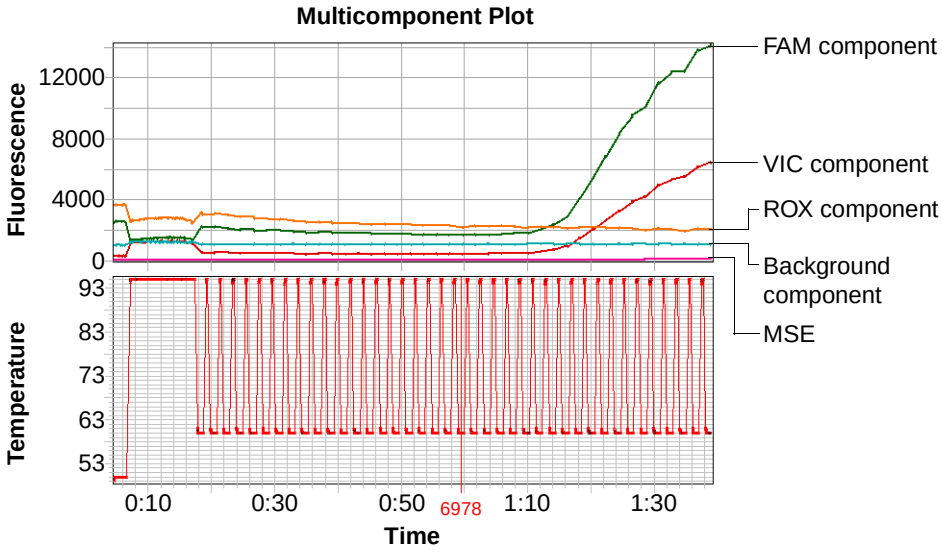
The software uses the pure dye spectra, generated as part of instrument calibration (see page 7-17), to solve for coefficients a, b, and c in the following equation:

$$\text{Measured spectrum} = a(\text{FAM}) + b(\text{VIC}) + c(\text{ROX}) + d(\text{Background}) + \text{MSE}$$

where the coefficients a, b, and c represent each dye component's contribution to the composite spectrum.

Note The example calculation above assumes that pure dye components exist for three dyes (FAM, VIC, and ROX™) and for the instrument background.

After solving for a, b, c, and d, the algorithm calculates the mean squared error (MSE), which measures how closely the collective multicomponent spectrum conforms to the raw spectra. The figure below shows a typical display of the contribution of each component spectra for one well.



Normalization of Reporter Signals

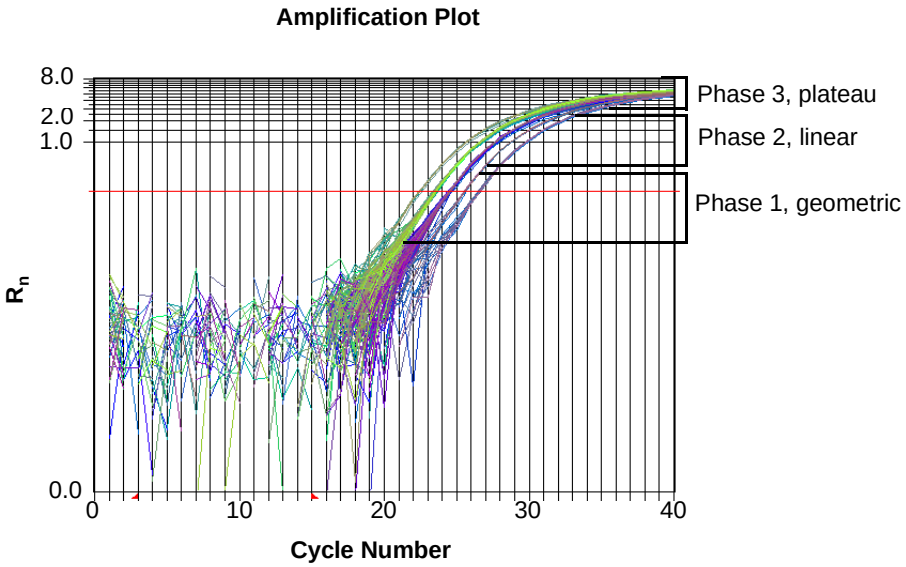
While multicomponenting illustrates absolute change in emission intensity, the SDS software displays cycle-by-cycle changes in normalized reporter signal (R_n). The SDS software normalizes each reporter signal by dividing it by the fluorescent signal of the passive reference dye. Because the passive reference is a component of the PCR master mix, it is present at the same concentration in all wells of the plate. By normalizing the data using the passive reference, the software can account for minor variations in signal strength caused by pipetting inaccuracies and make better well-to-well comparisons of reporter dye signal.

Note For the example above, the resulting data from the normalizing is displayed as FAM R_n and VIC R_n.

Real-Time Data Analysis

Kinetic Analysis/ Quantitative PCR

The 7900HT instrument can be used to determine the absolute or relative quantity of a target nucleic acid sequence in a test sample by analyzing the cycle-to-cycle change in fluorescence signal as a result of amplification during a PCR. This form of quantitative PCR analysis, called “kinetic analysis,” was first described using a non-sequence-specific fluorescent dye, ethidium bromide, to detect PCR product (Higuchi *et al.*, 1992; Higuchi *et al.*, 1993). The use of TaqMan probes and reagents further enhances the method by providing sequence-specific amplification of multiple targets for ‘comparative’ or ‘relative’ quantification. The fewer cycles it takes to reach a detectable level of fluorescence, the greater the initial copy number of the target nucleic acid.



When graphed in real-time on a linear scale, normal amplification of PCR product generates a curve similar to the one shown in the figure above. This ‘amplification’ curve consists of three distinct regions that characterize the progression of the PCR.

Phase 1: Geometric (Exponential)

Detection of the high-precision geometric phase is the key to high-precision quantitative PCR. The geometric phase is a cycle range of high precision during which is characterized by a high and constant amplification efficiency. It occurs between the first detectable rise in fluorescence and before the beginning of the Linear phase. When plotted on a log scale of DNA vs. cycle number, the curve generated by the geometric phase should approximate a straight line with a slope. The 7900HT instrument typically delivers sufficient sensitivity to detect at least 3 cycles in the geometric phase, assuming reasonably optimized PCR conditions.

Phase 2: Linear

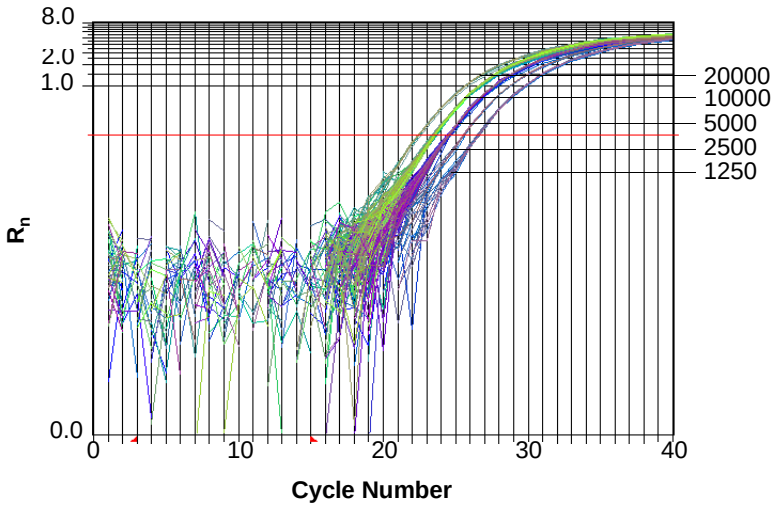
The linear phase is characterized by a leveling effect where the slope of the amplification curve decreases steadily. At this point, one or more components have fallen below a critical concentration and the amplification efficiency has begun to decrease. This phase is termed linear, because amplification approximates an arithmetic progression, rather than a geometric increase. Because the amplification efficiency is continually decreasing during the Linear phase, it exhibits low precision.

Phase 3: Plateau

Finally, the amplification curve achieves the plateau phase at which time the PCR stops and the R_n signal remains relatively constant.

Determining Initial Template Concentration and Cycle Number

At any given cycle within the geometric phase of PCR, the amount of product is proportional to the initial number of template copies. When one template is diluted several times, as with the RNase P target in the TaqMan® RNase P Instrument Verification Plate (see Appendix D), the ratio of template concentration to detectable signal is preserved within the exponential phase for all dilutions (see below). This relationship appears to change as rate of amplification approaches a plateau.



Fluorescence vs. Amplified Product

When using TaqMan fluorogenic probes with the 7900HT instrument, fluorescence emission increases in direct proportion to the amount of specific amplified product. As the figure on page A-8 demonstrates, the graph of normalized reporter (R_n) vs. cycle number during PCR appears to have three stages. Initially, R_n appears as a flat line because the fluorescent signal is below the detection limit of the Sequence Detector. In the second stage, the signal can be detected as it continues to increase in direct proportion to the increase in the products of PCR. As PCR product continues to increase, the ratio of AmpliTaq Gold polymerase to PCR product decreases. When template concentration reaches 10^{-8} M, PCR product ceases to grow exponentially. This signals the third stage of R_n change, which is roughly linear and finally reaches a plateau at about 10^{-7} M (Martens and Naes, 1989).

The progressive cleavage of TaqMan fluorescent probes during the PCR makes possible the correlation between initial template concentration and the rise in fluorescence. As the concentration of amplified product increases in a sample, so does the R_n value. During the exponential growth stage (the geometric phase), the relationship of amplified PCR product to initial template can be shown in the following equation:

$$N_c = N(1 + E)^c$$

where N_c is the concentration of amplified product at any cycle, N is the initial concentration of target template, E is the efficiency of the system, and *c* is the cycle number.

Calculating Threshold Cycles

The ABI PRISM 7900HT Sequence Detection System creates quantifiable relationships between test samples based on the number of cycles elapsed before achieving detectable levels of fluorescence. Test samples containing a greater initial template number cross the detection threshold at a lower cycle than samples containing lower initial template. The SDS software uses a Threshold setting to define the level of detectable fluorescence.

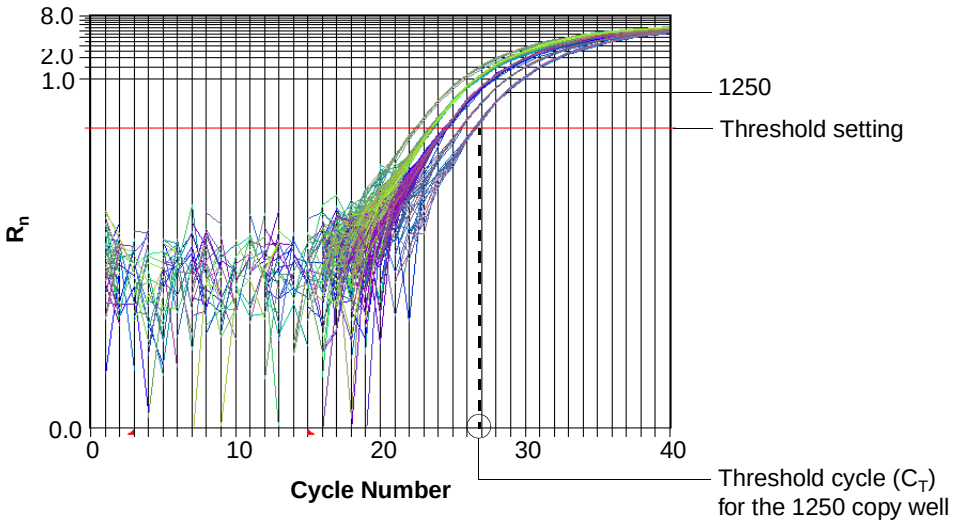
The threshold cycle (C_T) for a given amplification curve occurs at the point that the fluorescent signal grows beyond the value of the threshold setting. The C_T represents a detection threshold for the 7900HT instrument and is dependent on two factors:

- Starting template copy number
- Efficiency of DNA amplification the PCR system

How the SDS Software Determines C_Ts

To determine the C_T for an amplification plot, the SDS software uses data collected data from a predefined range of PCR cycles called the 'baseline' (the default baseline occurs between cycles 3 and 15). First, the software calculates a mathematical trend based on the baseline cycles' R_n values to generate a baseline subtracted amplification plot of ΔR_n versus cycle number. Next, an algorithm searches for the point on the amplification plot at which the ΔR_n value crosses the threshold setting (the default threshold setting is 0.2). The fractional cycle at which the intersection occurs is defined as the threshold cycle (C_T) for the plot.

Note It may be necessary to adjust the baseline and threshold settings to obtain accurate and precise data. For further information on resetting the baseline and threshold settings, see "Setting the Baseline and Threshold Values for the Run" on page 6-10.



Significance of Threshold Cycles

Beginning with the equation describing the exponential amplification of the PCR:

$$X_n = X_m(1 + E_X)^{n-m}$$

where:

X_n	=	number of target molecules at cycle n (so that $n \geq m$)
X_m	=	number of target molecules at cycle m (so that m ″ n)
E_X	=	efficiency of target amplification (between 0–1)
n - m	=	number of cycles elapsed between cycle m and cycle n

Amplicons designed and optimized according to Applied Biosystems guidelines (amplicon size <150 bp) have amplification efficiencies that approach 100 percent. Therefore E_X=1 so that:

$$X_n = X_m(1 + 1)^{n-m}$$
$$= X_m(2)^{n-m}$$

To define the significance in amplified product of one thermal cycle, set n - m = 1 so that:

$$X_1 = X_0(2)^1$$
$$= 2X_0$$

Therefore, each cycle in the PCR reaction corresponds to a two-fold increase in product. Likewise, a change in threshold cycle number of one must equate to a two-fold difference in initial template concentration.

Importing and Exporting Plate Document Data

B

In This Appendix

This appendix discusses the following topics:

Importing Plate Document Setup Table Files

About the Import Function

The SDS software features the ability to import setup table information (detector, detector task, marker, and sample name layouts) into a plate document from a tab-delimited text file. The import feature is designed to be a time-saving device that facilitates the exchange of setup information between other programs and the SDS software. Instead of setting up plate documents individually, a third-party program can be used to construct setup table files which can then be imported into plate documents for use.

To guarantee a successful incorporation of setup information from a text file to the plate document, the file must:

- Be saved in a tab-delimited text format
- Conform to the setup table file formats described on page B-4

Creating and Importing Setup Table Data into a Plate Document

Importation of setup table data into a plate document is accomplished in three major steps.

Creating an Empty Setup Table File

The first step in the procedure is to export a setup table file from a blank plate document.

Note The blank setup table file can be created using a secondary application (such as Microsoft Excel or a text editor) so long as it is saved in tab-delimited format and is configured according to the file structure explained on page B-4.

To export a blank setup table file using the SDS software:

Step	Action
1	Launch the SDS software.
2	From the **File** menu, select **New**.
3	Configure the **New Document** dialog box with the correct assay type and plate format for your experiment, and click **OK**.
4	From the **File** menu, select **Export**.
5	From the **Look In** text field of the **Export** dialog box, navigate to the directory you would like to receive the exported file.
6	From the **Export** drop-down list, select **Setup Table**.
7	Select the **All Wells** radio button.
8	Click the **File name** text box, and type a name for the file.
9	Click **Export**. The software exports the setup table data for the empty plate document as a tab-delimited text file.
10	Configure the setup table file with plate document information (detector, task, marker, and sample data) as explained on page B-3.

Configuring the Setup Table File with Plate Document Information

The second step in the procedure is to import the setup table file into a secondary application, configure it with sample and detector information, and then save the completed setup table file in tab-delimited format.

To configure the setup table file with information:

Step	Action
1	Launch the application that you want to use to edit the setup table file.
2	Import the setup table file from the previous procedure as tab-delimited text. If using a spreadsheet application to edit the setup table file, the application automatically parses the tab-delimited information into the cells of a spreadsheet.
3	Configure the setup table file with sample and detector information according to the file structure explained on page B-5.
4	Save the setup table file in tab-delimited format.
5	Import the completed setup table file into an empty plate document as explained below.

Importing the Completed Setup Table File into a Plate Document

The final step in the procedure is to import the completed setup table tab-delimited file into an empty plate document.

To import setup information from a tab-delimited text file to a plate document:

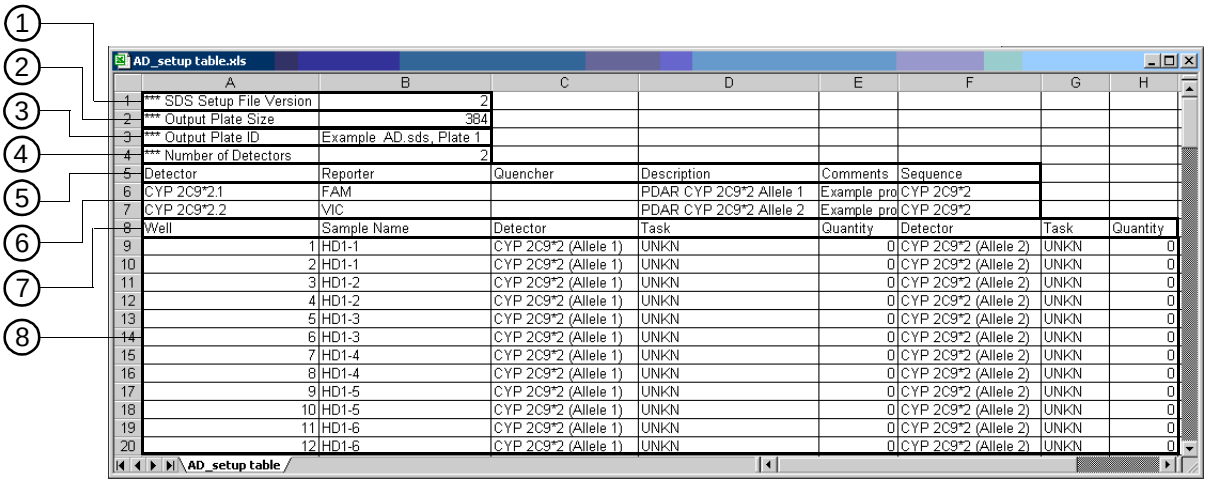
Step	Action
1	If the plate document created in “Creating an Empty Setup Table File” on page B-2 is still open in the SDS software, continue to step 3. Otherwise, create a plate document to receive the setup table data as follows: a. Launch the SDS software. b. Create or open a plate document to receive the information from the text file.
2	Choose one of the following options: ♦ From the toolbar, click the Import button (). ♦ From the **File** menu, select **Import**.
3	From the **Look In** text field of the **Import** dialog box, navigate to and select the completed tab-delimited setup table file from step 4 in the previous procedure.
4	Click **Import**. The software imports the setup table information from the text file and automatically configures the plate document plate grid and setup table with detector, detector task, marker, and sample data.

Setup Table File Format

Example Setup Table Files

To guarantee a successful importation of setup table data into a plate document, the imported setup table file must be configured in the correct format for the assay type. The following figures illustrate the orientation of information in tab-delimited setup table files as viewed in a Microsoft® Excel spreadsheet document. The numbered elements of the setup table files are explained on page B-5.

Example Setup Table File from an Allelic Discrimination Run

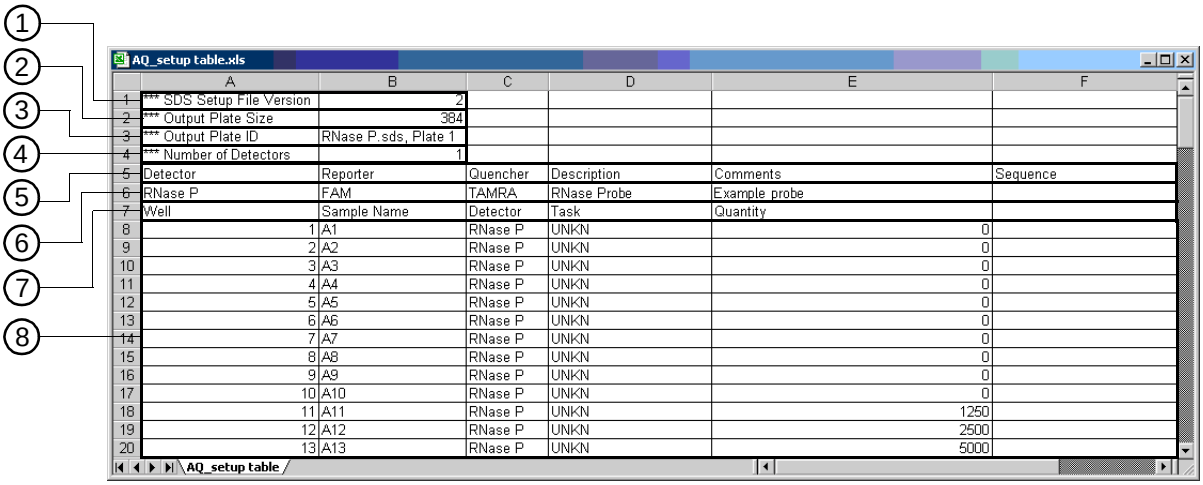


AD_setup table.xls

	A	B	C	D	E	F	G	H
1	*** SDS Setup File Version	2						
2	*** Output Plate Size	384						
3	*** Output Plate ID	Example_AD.sds, Plate 1						
4	*** Number of Detectors	2						
5	Detector	Reporter	Quencher	Description	Comments	Sequence		
6	CYP 2C9*2.1	FAM		PDAR CYP 2C9*2 Allele 1	Example pro	CYP 2C9*2		
7	CYP 2C9*2.2	VIC		PDAR CYP 2C9*2 Allele 2	Example pro	CYP 2C9*2		
8	Well	Sample Name	Detector	Task	Quantity	Detector	Task	Quantity
9	1	HD1-1	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
10	2	HD1-1	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
11	3	HD1-2	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
12	4	HD1-2	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
13	5	HD1-3	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
14	6	HD1-3	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
15	7	HD1-4	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
16	8	HD1-4	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
17	9	HD1-5	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
18	10	HD1-5	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
19	11	HD1-6	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0
20	12	HD1-6	CYP 2C9*2 (Allele 1)	UNKN	0	CYP 2C9*2 (Allele 2)	UNKN	0

AD_setup table

Example Setup Table File from an Absolute Quantification Run



AQ_setup table.xls

	A	B	C	D	E	F
1	*** SDS Setup File Version	2				
2	*** Output Plate Size	384				
3	*** Output Plate ID	RNase P.sds, Plate 1				
4	*** Number of Detectors	1				
5	Detector	Reporter	Quencher	Description	Comments	Sequence
6	RNase P	FAM	TAMRA	RNase Probe	Example probe	
7	Well	Sample Name	Detector	Task	Quantity	
8	1	A1	RNase P	UNKN	0	
9	2	A2	RNase P	UNKN	0	
10	3	A3	RNase P	UNKN	0	
11	4	A4	RNase P	UNKN	0	
12	5	A5	RNase P	UNKN	0	
13	6	A6	RNase P	UNKN	0	
14	7	A7	RNase P	UNKN	0	
15	8	A8	RNase P	UNKN	0	
16	9	A9	RNase P	UNKN	0	
17	10	A10	RNase P	UNKN	0	
18	11	A11	RNase P	UNKN	1250	
19	12	A12	RNase P	UNKN	2500	
20	13	A13	RNase P	UNKN	5000	

AQ_setup table

About the Setup Table File Format

This section explains the elements of setup table files shown on the previous page.

The following table describes the conventions used in the rest of this section.

Format/Symbol	Definition
courier	Text appearing in bolded courier font must be applied to a setup table file exactly as appears in this document.
italic	Text appearing in italic courier font must be substituted with custom values when applied to a setup table file.
[*required text*]	Text appearing between brackets is required information in setup table files. All information within the brackets must be present in the setup table file for the SDS software to import it.
{ *required text* }	Text appearing between braces is optional in setup table files.
<tab>	The tab character (the equivalent of pressing the **Tab** key)
<cr>	The carriage-return character (the equivalent of pressing the **Enter** key)

IMPORTANT To guarantee a successful importation of the setup table file into a plate document, the file can must contain all of the sections in the following table in the order that they appear in this document.

Setup Table Elements

Number	Contents	Description
1	File Version	This line defines the version of SDS Assay Plate File format used to generate the document.
	Format: [***** SDS Setup File Version** <tab> *version number* <cr>] Example: *** SDS Setup File Version 2	
2	Plate Size	This line defines the number of wells in the plate modeled by the file (384 or 96).
	Format: [***** Output Plate Size** <tab> *number of wells* <cr>] Example: *** Output Plate Size 384	
3	Plate ID	This line defines the ID of the Assay Plate. Normally this will be a bar code that is printed on the plate.
	Format: [***** Output Plate ID** <tab> *plate id* <cr>] Example: *** Output Plate ID 384N75822034	

Setup Table Elements *(continued)*

Number	Contents	Description
Detector Definitions		Element numbers 4 to 6 define the detectors that will appear in the well descriptions that follow in a later section. The detector definition consists of three sections: the declaration of the number of detectors, the detector list header, and the detector list.
4	Number of Detectors	This line defines the total number of detectors on the plate.
	Format: [****** Number of Detectors*** <tab> *number of detectors* <cr>] Example: *** Number of Detectors 5	
5	Detectors List Header	This line contains the column headings for the Detector Definitions section of the setup table file that make the file easier to edit using a program such as Microsoft Excel.
	Format: [**Detector** <tab> **Reporter** <tab> **Quencher** <tab> **Description** <tab> **Comments** <tab> **Sequence** <cr>] Example: Detector ReporterQuencher Description Comments Sequence	
6	Detectors List	The detector list consists of one or more lines displaying the information for each detector used on the plate document. ♦ The Detectors List section must contain one line (or definition) for each detector present on the plate. ♦ The number of lines in the Detectors List section must be equal to the number defined in the Number of Detectors section (see number 4 above). ♦ Leave blank the Quencher Dye entry for detectors created for the SYBR Green I Dye or probes labeled with a non-fluorescent quencher. ♦ The Sequence text value contains the name of the Allelic Discrimination marker associated with the detector. Because markers are used exclusively in allelic discrimination plate documents, assign Sequence text values to detector definitions of allelic discrimination setup table files only.
	Format for a single detector: [*detector name* <tab> *reporter dye* <tab> *quencher dye* <tab> *description* <tab> *comments* <tab> *sequence* <cr>] Example for an allelic discrimination setup table file: CYP 2C9*2.1 FAM PDAR CYP 2C9*2 Allele 1Example ProbeCYP 2C9*2 CYP 2C9*2.2 VIC PDAR CYP 2C9*2 Allele 2Example ProbeCYP 2C9*2 Example for an absolute quantification setup table file: GAPDH VIC GAPDH Probe Example Probe SYBR Green SYBR SYBR Green I Example Probe RNase P FAM TAMRA RNase P Probe Example Probe	

Setup Table Elements *(continued)*

<table>
<tr><th>Number</th><th>Contents</th><th>Description</th></tr>
<tr><td colspan="2">Assay Plate Wells</td><td>Element numbers 7 and 8 define the contents of the wells on the plate. The Assay Plate Wells definition consists of two sections: the Well List Header and the Well Definition List.</td></tr>
<tr><td rowspan="2">7</td><td>Well List Header</td><td>This line contains the column headings for the Assay Plate Wells section of the setup table file that make the file easier to edit using a program such as Microsoft Excel.</td></tr>
<tr><td colspan="2">Format:

[Well <tab> Sample Name <tab> Detector <tab> Task <tab> Quantity] { ... <tab> Detector <tab> Task <tab> Quantity } [<cr>]

Example:

Well Sample Name Detector Task Quantity ...DetectorTask Quantity</td></tr>
<tr><td rowspan="3">8</td><td>Well Definition List</td><td>This section defines the contents of the plate wells. The setup table file must contain a definition for each well used on the plate. Each well definition list consists of one string of characters terminated by a <cr>. The definition can be of three main functional divisions:

♦ Well number – The first tab-delimited text block defines the number of the well on the plate. Well numbers start at 1 for well A-1 (upper-left corner of the plate) and increases from left to right and from top to bottom. The wells must be listed in order (1,2,3,…).

♦ Sample name – The second tab-delimited text block defines the name of the sample assigned to the well.

♦ Detector assignments – The remaining tab-delimited text blocks for the well definition define the detectors assigned to the well. Each detector is represented by three text blocks that define the following information:

– The name of the detector

– The task assignment of the detector for the well (UNKN - Unknown, STND - Standard, NTC - No Template Control)

– The quantity assignment of the detector for the well (For wells containing standards, assign the quantity for the standard sample in initial copy number. For all other wells, assign the quantity value as 0.)

To assign more than one detector to a well, then repeat the detector definition text blocks for each detector. There is no limit to the number of detectors that can appear in a well.

IMPORTANT All detectors that appear in this section must have been previously defined in the Detector Definitions section (elements 4–6).</td></tr>
<tr><td colspan="2">Format for a single Well:

[Well number <tab> SDS Sample Name <tab> Detector name <tab> Detector task <tab> Detector quantity]{ <tab> Detector name <tab> Detector task <tab> Detector quantity ... <tab> Detector name <tab> Detector task <tab> Detector quantity }[<cr>]

Example for allelic discrimination setup table files:

1 Sample 1 CYP 2C9*2.1 UNKN 0 CYP 2C9*2.2 UNKN 0
2 Sample 2 CYP 2C9*2.1 UNKN 0 CYP 2C9*2.2 UNKN 0
3 ...

Example for absolute quantification setup table files:

1 Sample 1 GAPDH UNKN 0
2 Sample 2 GAPDH UNKN 0
3 Sample 3 GAPDH STND 20000
4 Sample 4 GAPDH STND 15000
5 Sample 5 GAPDH NTC 0</td></tr>
</table>

Exporting Graphics

Exporting a Plot as a JPEG Graphic File

The SDS software can export most panes and plots of the plate document as JPEG (Joint Photographic Experts Group) graphic files. The JPEG file format is compatible with most word processing and spreadsheet applications and can be incorporated directly into HTML documents for viewing by most web browser software.

To export an element of a plate document as a graphic:

Step	Action
1	Click the plot or grid you want to export.
2	Choose from the following: ♦ If exporting a plot, adjust its dimensions (length and width) as you want them to appear in the exported graphic file. The exported graphic file retains the dimensions of the original screen element. ♦ If exporting the plate grid, do not adjust the size of the wells. The software captures the whole grid regardless of the size of the view.
3	Right-click the plot or grid, and select **Save Plot/Grid to Image File** from the contextual menu. **Note** If a pane cannot be exported as a graphic, the contextual menu will not contain the **Save Plot/Grid to Image File** option.
4	From the **Save As** dialog box, navigate to the directory you want to receive the exported graphic file.
5	Click the **File name** text field, and type a name for the new file.
6	Click **Save**. The software saves the plot or grid as a JPEG graphic in the designated directory.

Exporting Plate Document Data

Exporting Data from a Plate Document

The SDS software can export raw or analyzed data in tab-delimited (*.txt) format for all or a select group of wells on a plate document. The exported files are compatible with most spreadsheet applications.

To export an element of a plate document:

Step	Action
1	Click the plate document to select it. **Note** The plate document must be the top-most object in the workspace.
2	From the **File** menu, select **Export**.
3	Navigate to the directory you would like to receive the exported file(s).
4	Click the **Export** drop-down list, and select the type of data you would like the software to export.

Data Type	The exported file contains…
Background Spectra	Fluorescence readings for each well from the background component used to analyze the run
Clipped	Average R_n and ΔR_n of the last three data points collected during the extension phase of each cycle repetition for each well
Dissociation Curve	♦ Temperature Data – For each well in use on the plate, the file displays the calculated temperature of the wells during each data collection reading of the temperature ramp. ♦ Raw Data – For each well in use on the plate, the file displays the R_n of the well during each data collection reading of the temperature ramp. ♦ Derivative Data – For each well in use on the plate, the file displays the first derivative data during each data collection reading of the temperature ramp.
Multicomponent	♦ Calculated amounts the dye components in a single well throughout all stages of the PCR that were labeled with data collection icons ♦ Pure spectra component data ♦ Calculated inverse matrix ♦ Singular values of the inverse matrix
Pure Spectra	Fluorescence readings for each well from the pure spectra calibration component used to analyze the run
Raw Spectra	Unmodified fluorescence readings taken for each spectral bin during the course of the run When exported, the software creates a directory and saves each the raw spectra data for each well in a separate *.txt file.
Results Table	The contents of the results table of an analyzed plate document **Note** The contents of the exported data varies depending on the type of plate document.
Setup Table	The contents of the results table of a plate document prior to analysis The contents of the exported data varies depending on the type of plate document used to produce it. See page B-4 for a detailed description of the Setup Table file.

To export an element of a plate document: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Select the appropriate well radio button as follows:

<table><tr><th>To export data from…</th><th>Select the…</th></tr><tr><td>all wells on the plate document</td><td>All Wells radio button</td></tr><tr><td>selected wells of the plate grid only</td><td>Selected Wells radio button</td></tr></table></td></tr>
<tr><td>6</td><td>From the files of type drop-down list, select the appropriate format for the exported data.</td></tr>
<tr><td>7</td><td>Click the File name text box, and type a name for the exported file.</td></tr>
<tr><td>8</td><td>Click Export.

The software saves the exported data to the designated location.</td></tr>
</table>

Designing TaqMan Assays

C

In This Appendix This appendix discusses the following topics:

Assay Development Guidelines

TaqMan Assay Development Program

To develop custom TaqMan 5´ nuclease assays:

Step	Action	See Page
1	Identify target sequence(s).	C-2
2	Design the TaqMan® probes and the forward and reverse primers.	C-3
3	Order reagents.	C-4
4	Quantitate the concentrations of the probes and primers.	C-4
5	Prepare the master mix.	C-4
6	Optimize the primer concentrations.	C-4
7	Run the assay.	C-4

Identify Target Sequence(s)

A target template is a DNA, cDNA, RNA, or plasmid containing the nucleotide sequence of interest. For optimal results, the target template should meet the following requirements:

- The target nucleotide sequence must contain binding sites for both primers (forward and reverse) and the fluorogenic probe.
- Short amplicons work best. Amplicons ranging from 50–150 bp typically yield the most consistent results.
- If designing assays for quantitative PCR, see “Design Tips for Quantitative PCR Assays” on page C-6 for additional recommendations.

Design Probes and Primers

The following sections contain general guidelines for designing primers and probes. For specific design tips, refer to the appropriate section: for Allelic Discrimination see page C-5 and for Quantitative PCR see page C-6.

Design Probe(s) for the Assay

Adhere to the following guidelines when designing TaqMan probes:

- Keep the G-C content in the range of 30–80%.
- Avoid runs of an identical nucleotide (especially guanine, where runs of four or more Gs should be avoided).
- No G on 5´ end.
- Keep the melting temperature (T_m) in the range of 68-70 °C for quantitative PCR and 65-67 °C for allelic discrimination (using the Primer Express™ software).
- Select the strand that gives the probe with more Cs than Gs.
- For allelic discrimination (see page C-5):
  - Adjust probe length so that both probes have the same T_m.
  - Position the polymorphism site approximately in the center of each probe.
- For multiplex PCR applications (involving multiple probes), design the probes with different fluorescent reporter dyes as explained below:

Application	Reporter Dye[a]	
	First Probe	Second Probe
Allelic Discrimination	FAM™	VIC™

a. The use of the FAM and VIC reporter dyes for multiplex applications provides the greatest degree of spectral separation.

Design Primers for the Assay

Adhere to the following guidelines when designing primers for 5´-nuclease assays:

- Keep the G-C content in the range of 30–80%.
- Avoid runs of an identical nucleotide (especially guanine, where runs of four or more bases should be avoided).
- Keep the T_m in the range of 58-60 °C (using the Primer Express™ software).
- Limit the G and/or C bases on the 3´ end. The five nucleotides at the 3´ end should have no more than two G and/or C bases.
- Place the forward and reverse primers as close as possible to the probe without overlapping the it.
- Use an annealing temperature of 60 °C for quantitative PCR, and 62 °C for allelic discrimination (except for TaqMan® PDARs for Allelic Discrimination).

Order Reagents

Note Because part numbers can change as new and improved products are introduced. Contact your Applied Biosystems sales representative for specific ordering information.

You will need the following reagents and equipment to create your own applications:

- Custom Synthesized TaqMan Probes
- Sequence Detection Primers
- TaqMan® Universal PCR Master Mix (optimized for TaqMan reactions containing AmpliTaq® Gold DNA Polymerase, AmpErase® UNG, dNTPs with dUTP, ROX™ Passive Reference I, and optimized buffer components)

IMPORTANT PCR master mix used with the 7900HT instrument must contain a passive reference dye. The SDS software uses the signal from the passive reference to normalize the reporter fluorescence making well-to-well comparisons possible. All Applied Biosystems master mix products contain an optimal concentration of the Passive Reference I.

- ABI Prism® Optical Reaction Plates
- ABI Prism® Optical Adhesive Covers or ABI PRISM® Optical Flat Cap Strips
- TaqMan Spectral Calibration Reagents
- Centrifuge with 384- or 96-well plate adapter
- Deionized water or Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Disposable Gloves

Quantitate the Probes and Primers

Use a spectrophotometric method to determine the concentrations of the probes and primers received. See the *TaqMan Universal PCR Master Mix Protocol* (P/N 4304449) for specific information about primer and probe quantification.

Prepare Master Mix

Refer to the *TaqMan Universal PCR Master Mix Protocol* (P/N 4304449) for specific information about preparing the master mix for use.

IMPORTANT PCR master mix used with the 7900HT instrument must contain a passive reference dye. The SDS software uses the signal from the passive reference to normalize the reporter fluorescence making well-to-well comparisons possible. All Applied Biosystems master mix products contain an optimal concentration of the ROX passive reference dye.

Note Applied Biosystems protocols are available on the Applied Biosystems Company Web Site, see Appendix F, “Contacting Technical Support,” for more information.

Optimize Primer/Probe Concentrations

Refer to the *TaqMan Universal PCR Master Mix Protocol* (P/N 4304449) for specific information about preparing the master mix for use.

Note Applied Biosystems protocols are available on the Applied Biosystems Company Web Site, see Appendix F, “Contacting Technical Support,” for more information.

Run Your Custom Assay

Run your experiment.

Note If conducting a quantitative PCR experiment, consider the use of replicate assays to enhance the precision of you data.

Design Tips for Allelic Discrimination Assays

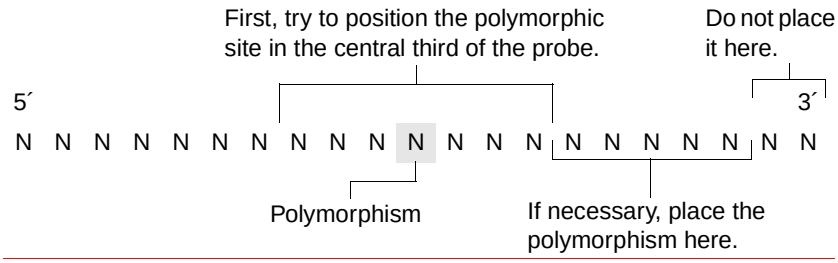
Discrimination by Multiple Probes

By using different reporter dyes, cleavage of multiple probes can be detected in a single PCR. One application of this multi-probe capability is to use allele-specific probes to distinguish genetic polymorphisms (Bloch, 1991, Lee *et al.*, 1993). Probes that differ by as little as a single nucleotide will exhibit allele-specific cleavage. This is true even for probes with a reporter on the 5' end and the non-fluorescent quencher on the 3´ end (Bloch, 1991).

TaqMan Probe Design Guidelines

IMPORTANT When designing probes, it is important to consider probes from both strands.

Follow the guidelines in the table below for designing TaqMan MGB probes:

Priority	Guideline
1	Avoid probes with a guanine residue at the 5´ end of the probe. A guanine residue adjacent to the reporter dye will quench the reporter fluorescence, even after cleavage.
2	Select probes with a Primer Express software–estimated T_m of 65–67 °C.
3	Make the TaqMan MGB probes as short as possible, but no fewer than 13 nucleotides in length.
4	Avoid runs of an identical nucleotide. This is especially true for guanine, where runs of four or more should be avoided.
5	Position the polymorphic site in the central third of the probe. **Note** The polymorphic site can be shifted toward the 3´ end to meet the above guidelines, however, the site must be located more than two nucleotides upstream from the 3´ terminus. The following figure illustrates the placement of a polymorphism in an example probe (N = Nucleotide). 

Design Tips for Quantitative PCR Assays

Selecting an Amplicon Site for Gene Expression Assays

Selecting a good amplicon site ensures amplification of the target mRNA without co-amplifying the genomic sequence, pseudogenes, and related genes. Applied Biosystems recommends the following guidelines when selecting an amplicon site for quantification assays:

- Primers and probes must be designed following the "Assay Development Guidelines" on page C-2.
- The amplicon should span one or more introns to avoid amplification of the target gene in genomic DNA.
- The primer pair has to be specific to the target gene and does not amplify pseudogenes or other related genes.
- Test amplicons and select those that have the highest signal-to-noise ratio (such as those yielding low C_Ts with cDNA and no amplification with no template control or genomic DNA).
- If no good sequence is found, it may be necessary to examine the sequence and redesign the amplicon or simply screen for more sites.

If the gene you are studying does not have introns, then you cannot design an amplicon that will amplify the mRNA sequence without amplifying the genomic sequence. In this case, it may be necessary to run RT minus controls.

Selecting and Preparing Standards for Absolute Quantification

To ensure accurate results, the standards used for absolute quantification must be carefully engineered, validated, and quantified before use. Consider the following critical points for the proper use of absolute standard curves:

- The DNA or RNA used must be a single, pure species. For example, plasmid DNA prepared from E. coli often is contaminated with RNA, which increases the A_{260} measurement and inflates the copy number determined for the plasmid.
- In general, DNA cannot be used as a standard for absolute quantification of RNA because there is no control for the efficiency of the reverse transcription step.
- Absolute quantities of the standard must be known by some independent means. Plasmid DNA or *in vitro* transcribed RNA are commonly used to prepare absolute standards. Concentration is measured by A_{260} and converted to the number of copies using the molecular weight of the DNA or RNA.
- Consider the stability of the diluted standards, especially for RNA. Divide diluted standards into small aliquots, store at -80 °C, and thaw only once before use. An example of the effort required to generate trustworthy standards is provided by Collins *et al.* (Anal. Biochem. 226:120-129, 1995), who reported on the steps they used in developing an absolute RNA standard for viral quantification.
- Pipetting must be accurate because the standards must be diluted over several orders of magnitude. Plasmid DNA or *in vitro* transcribed RNA must be concentrated in order to measure an accurate A_{260} value. The concentrated DNA or RNA must then be diluted 10^6 –10^{12} -fold to be at a concentration similar to the target in biological samples.

Kits, Reagents and Consumables

D

In This Appendix This appendix discusses the following topics:

Note Part numbers listed within this appendix are for customers within the United States. Contact your Regional Sales Office for local Part numbers and prices (see Appendix F, “Contacting Technical Support,” for a list of telephone and Fax numbers).

Interchangeable Sample Block Modules and Accessories

The 7900HT instrument features a Peltier-based, interchangeable sample block module based on the technology established in the GeneAmp® PCR System 9700 thermal cycler.

The use of an interchangeable sample block module:

- Reduces instrument downtime by allowing immediate replacement of the block.
- Permits easy access to the sample block for troubleshooting and maintenance (see page 7-11).
- Supports multiple consumable formats.
- Provides several different modes of operation (including Max mode and programmable temperature ramps).

Part No.	Description	Quantity
[a]	384-Well Interchangeable Sample Block Module for the ABI PRISM® 7900HT Sequence Detection System Includes a 384-Well Sample Block Module, a 384-well plate adapter, and a Sequence Detection Systems 384-Well Spectral Calibration Kit (P/N 4323977)	1 kit
[a]	96-Well Interchangeable Sample Block Module for the ABI PRISM® 7900HT Sequence Detection System Includes a 96-Well Sample Block Module, a 96-well plate adapter, and an ABI PRISM™ 7900HT Sequence Detection Systems 96-Well Spectral Calibration Kit (P/N 4328639)	1 kit

a. contact your local Applied Biosystems Sales and Service Office for information.

Consumables and Disposables

The ABI PRISM 7900HT Sequence Detection System can run both:

- ABI PRISM™ Optical 384-Well Reaction Plates sealed with ABI PRISM™ Optical Adhesive Covers
- ABI PRISM™ 96-Well Reaction Plates sealed with ABI PRISM Optical Adhesive Covers or ABI PRISM® Optical Caps (flat cap strips only)

IMPORTANT Do not use MicroAmp® Optical Caps or MicroAmp® Optical Tubes with the 7900HT instrument. The instrument is not designed to run MicroAmp consumables which may damage its internal components if used.

Note ABI PRISM Optical Reaction Plates are designed specifically for fluorescence-based PCR chemistries and are frosted to minimize external fluorescent contamination. Before running prepared ABI PRISM Optical Reaction Plates on the 7900HT instrument, each plate must be sealed with an ABI PRISM Optical Adhesive Cover. Applied Biosystems' optical adhesive covers are specifically designed to permit the transmission of light to and from the wells of the optical plate.

Part No.	Description	Quantity
ABI PRISM™ Optical Adhesive Covers		
4313663	ABI PRISM™ Optical Adhesive Cover Starter Kit Includes 20 ABI PRISM Optical Adhesive Covers, an Applicator, and a ABI PRISM Optical Cover Compression Pad.	20 Covers
4311971	ABI PRISM™ Optical Adhesive Covers	100 Covers
4323032	ABI PRISM™ Optical Caps, 8 Caps/Strip	300 Strips/ Pkg 2400 Caps/ Pkg
384-Well Optical Reaction Plates		
4309849	ABI PRISM™ 384-Well Clear Optical Reaction Plate with Barcode (code 128)	50 Plates
4326270	10-Pack, ABI PRISM™ 384-Well Clear Optical Reaction Plate with Barcode (code 128) Includes 10 ABI PRISM 384-Well Clear Optical Reaction Plate with Barcode (P/N 4309849).	500 Plates
96-Well Optical Reaction Plates		
4306737	ABI PRISM™ 96-Well Optical Reaction Plate with Barcode (code 128)	20 Plates
4326659	25-Pack, ABI PRISM™ 96-Well Optical Reaction Plate with Barcode (code 128) Includes 25 ABI PRISM 96-Well Optical Reaction Plate with Barcode (P/N 4306737).	500 Plates
4314320	ABI PRISM™ 96-Well Optical Reaction Plate with Barcode (code 128) and ABI PRISM™ Optical Adhesive Covers Includes 100 ABI PRISM Optical Adhesive Covers (P/N 4311971) and 5 ABI PRISM 96-Well Optical Reaction Plate with Barcode packages (P/N 4306737).	100 Plates 100 Covers
Miscellaneous		
4312063	MicroAmp® Splash Free Support Base for 96-Well Reaction Plates	10 Bases

Instrument Maintenance and Verification

The following sequence detection kits and reagents are used to perform routine maintenance on and verify the function of the ABI PRISM 7900HT Sequence Detection System. For more information about the use of the kits below, see Chapter 7, "System Maintenance."

Part Number	Description	Quantity
Sequence Detection Systems Spectral Calibration Kits		
4328639	ABI PRISM™ 7900HT Sequence Detection Systems 96-Well Spectral Calibration Kit Includes three ABI PRISM Optical 384-Well Reaction Plates: one preloaded and sealed Background plate, and two preloaded and sealed Spectral Calibration plates containing eight separate dye standards (FAM™, JOE™, NED™, ROX™, SYBR® Green, TAMRA™, TET™, VIC™).	3 x 96-Well Plates
4323977	Sequence Detection Systems 384-Well Spectral Calibration Kit Includes two ABI PRISM Optical 384-Well Reaction Plates: one preloaded and sealed Background plate, and one preloaded and sealed Spectral Calibration plate containing eight separate dye standards (FAM, JOE, NED, ROX, SYBR Green, TAMRA, TET, VIC).	2 x 384-Well Plates
TaqMan RNase P Instrument Verification Plates		
4310982	TaqMan® RNase P Instrument Verification Plate Includes one ABI PRISM Optical 96-well Reaction Plate pre-loaded and sealed with Sequence Detection primers and TaqMan® probe to detect and quantitate genomic copies of the human RNase P gene.	1 x 96-Well Plate
4323306	TaqMan® RNase P 384-Well Instrument Verification Plate Includes one ABI PRISM Optical 384-well Reaction Plate preloaded and sealed with complete Sequence Detection primers and TaqMan® probe to detect and quantitate genomic copies of the human RNase P gene.	1 x 384-Well Plate

TaqMan Pre-Developed Assays and Reagents

For the latest information on TaqMan PDARs covering gene expression quantification and allelic discrimination, visit the TaqMan PDAR list on the Applied Biosystems web site at:

- www.appliedbiosystems.com/pdarlist

Custom Oligonucleotide Synthesis

To order custom oligonucleotides:

- Visit the Applied Biosystems Online Store (http://store.appliedbiosystems.com), or
- Email Applied Biosystems with your order (OligosUS@appliedbiosystems.com)

Part Number	Description
TaqMan® MGB Probes (5'-Fluorescent label: 6-FAM, VIC or TET)	
4316034	5,000-6,000 pmols
4316033	15,000-25,000 pmols
4316032	50,000-100,000 pmols
TaqMan® Probes (5'-Fluorescent label: 6-FAM, VIC or TET; 3'-label: TAMRA)	
450025	5,000-6,000 pmols
450024	15,000-25,000 pmols
450003	50,000-100,000 pmols
Sequence Detection Primers	
4304970	Minimum 4,000 pmols purified for sequence detection
4304971	Minimum 40,000 pmols purified for sequence detection
4304972	Minimum 130,000 pmols purified for sequence detection

References

E

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Livak, K.J., Flood, S.J.A., Marmaro, J., and Mullah, K.B., inventors; Applied Biosystems (Foster City, CA), assignee. 2 Mar. 1999. Hybridization assay using self-quenching fluorescence probe. United States patent 5,876,930.

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Martens, H. and Naes, T., 1989. In: Multivariate Calibration, John Wiley & Sons, Chichester.

Contacting Technical Support

F

Services and Support

Applied Biosystems Web Site

To access the Applied Biosystems Web site, go to:

http://www.appliedbiosystems.com

At the Applied Biosystems Web site, 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 Applied Biosystems Web site provides a list of telephone and fax numbers that can be used to contact Technical Support.

Limited Warranty Statement

G

Warranty Statement

PE Corporation (NY), through its Applied Biosystems Group (“Applied Biosystems“) warrants to the customer that, for a period ending on the earlier of one year from the completion of installation or fifteen (15) months from the date of shipment to the customer (the “Warranty Period”), the ABI PRISM® 7900 HT Sequence Detection System purchased by the customer (the “Instrument”) will be free from defects in material and workmanship, and will perform in accordance with the installation specifications set forth in the system specifications sheet which accompanies the instrument or which is otherwise available from an Applied Biosystems sales representative.

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems' expense. However, if the thermal cycling module becomes damaged or contaminated, or if the chemical performance of the Instrument otherwise deteriorates due to solvents and/or reagents other than those supplied or expressly recommended by Applied Biosystems, Applied Biosystems will return the Instrument to Specification at the customer's request and at the customer's expense. After this service is performed, coverage of the parts repaired or replaced will be restored thereafter for the remainder of the original Warranty Period.

This Warranty does not extend to any Instrument or part which has been (a) the subject of an accident, misuse, or neglect, (b) modified or repaired by a party other than Applied Biosystems, or (c) used in a manner not in accordance with the instructions contained in the Instrument User's Manual. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual. Those items are covered by their own warranties.

Applied Biosystems' obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct those failures of the Instrument to meet the Specifications of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by Applied Biosystems on site at the Customer's location at Applied Biosystems's sole expense.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the Instrument that is not contained in Applied Biosystems's printed product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

Applied Biosystems shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the purchase or use of the Instrument. Applied Biosystems makes no warranty whatsoever with regard to products or parts furnished by third parties.

This Warranty is limited to the original location of installation and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND IS IN LIEU OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

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Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 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 or to the Technical Support and Training appendix in this document.

www.appliedbiosystems.com

Applied Biosystems

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ABI PRISM® 7900HT Sequence Detection System

User Guide

Applied Biosystems