ABI PRISM® 7000 Sequence Detection System

User Guide

Applied Biosystems

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

Authorized Thermal Cycler

This instrument, Serial No_____, is an Authorized Thermal Cycler. Its purchase price 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.

DISCLAIMER OF LICENSE:

No rights for any application, including any in vitro diagnostic application, are conveyed expressly, by implication or by estoppel under any patent or patent applications claiming homogeneous or real-time detection methods, including patents covering such methods used in conjunction with the PCR process or other amplification processes. The 5' nuclease detection assay and certain other homogeneous or real-time amplification and detection methods are covered by United States Patent Nos. 5,210,015, 5,487,972, 5,804,375 and 5,994,056, owned by Roche Molecular Systems, Inc.; by corresponding patents and patent applications outside the United States, owned by F. Hoffmann-La Roche Ltd; and by United States Patent Nos. 5,538,848 and 6,030,787, and corresponding patents and patent applications outside the United States, owned by PE Corporation (NY). Purchase of this instrument conveys no license or right under the foregoing patents. Use of these and other patented processes in conjunction with the PCR process requires a license. For information on obtaining licenses, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, or The Licensing Department, Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California, 94501.

Part Number 4330228 Rev. B

Contents

Chapter 1 Safety Information

Chapter 2 Getting Started

Chapter 3 Software Overview

Chapter 4 Basic Operation

Chapter 5 Printing Results and Exporting Data

Chapter 6 Analyzing Assays

Chapter 7 Plus/Minus Assays

Chapter 8 System Maintenance

Appendix A Theory of Operation

Appendix B Designing Assays Using TaqMan Probes

Appendix C Consumables and Reagents

Safety Information 1

Introduction

About This Chapter

This chapter provides safety information about using the ABI PRISM® 7000 Sequence Detection System. Make sure you read and understand the safety alerts before using the instrument.

In This Chapter

This chapter contains the following topics:

Safety

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.

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.

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.

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:

1. From the U.S. or Canada, dial **1.800.487.6809**.
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	Dial **1.800.668.6913**, and press **1** for English or **2** for French.
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:

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 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.

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® 7000 Sequence Detection System Site Preparation and Safety Guide* for an explanation of all the safety alert symbols provided in several languages.

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.

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.

Single Power Source

The ABI PRISM 7000 Sequence Detection System is at risk for power surges when it is plugged into a separate power source from the laptop computer. The risk is reduced if both the instrument and the laptop computer are plugged into the same wall outlet, the same power strip, or into a common Universal Power Supply (UPS). Make sure you plug both the instrument and the laptop computer power cords into the same power strip, or plug both the instrument and the computer into the same wall outlet.

If the 7000 system is moved, the power source for both the instrument and the laptop computer must be the same when they are plugged back in.

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.

Getting Started 2

Introduction

About This Chapter

This chapter provides general information about the user guide and its organization. It also provides system requirements, procedures to learn how to start the computer and the instrument, install the software and shut down the system.

In This Chapter

This chapter contains the following topics:

Overview

How to Use This Guide

The *ABI PRISM® 7000 Sequence Detection System User Guide* can be used as a reference or to learn how to operate the 7000 instrument and associated software. It assumes that you know how to use a mouse, keyboard, and the Microsoft® Windows® operating system. If you need further instruction on how to use any of these operations, refer to "Using a Windows Environment" on page D-3.

Menu and Typeface Conventions

Typefaces and Symbols

The following table describes the typefaces that are used to indicate selections on windows and the meaning of symbols in the text in this user guide.

Typeface Conventions

Typeface or Symbol	Meaning	Example
Menu item, **icon**, **or button**	Menu items, icons, or buttons on the windows that activate other processes are shown in bold when you are instructed to select them.	From the Tools menu, select **Detector Manager**.
Italics	Names of manuals or documents are shown in italics.	*ABI PRISM® 7000 Sequence Detection System User Guide*
▸	This arrowhead represents the tab or menu and next item to select on a subtab or submenu.	Select **File** ▸ **New** to open a new document.
Shortcuts	Keyboard shortcuts for window operations are shown in two formats: 1. Commas between commands. When commands are shown in this format, press each key sequentially without holding them down. 2. Plus sign (+) shown between commands. When commands are shown in this format, press and hold the keys together.	Alt, W, T Press **Alt**, then press **W**, then **T** to tile the open windows. Ctrl+N Press and hold **Ctrl** and **N** together to open the New Document dialog box.

Computer Vocabulary and Operations

To use the ABI PRISM 7000 Sequence Detection System, you should be familiar with the following basic computer vocabulary and operations:

Computer Operations

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

If you need to review these operations, refer to “Using a Windows Environment” on page D-3.

Organization of This User Guide

Using This Guide

This user guide contains the following chapters and appendices:

Chapter Descriptions

Chapter/ Appendix	Title	Content
1	Safety Information	Important information on instrument safety.
2	Getting Started	How to use this guide, who should read it, and typeface conventions. System requirements and procedures to start the system, install the software, and shut down the system.
3	Software Overview	Overview of the ABI PRISM 7000 SDS Software. It describes the windows of the software.
4	Basic Operation	Describes how to set up, create, run, and analyze assays. Procedures to use the Dye Manager, Detector Manager, Marker Manager, Well Inspector, analyze and view data.
5	Printing Results and Exporting Data	Describes how to print results from plate documents and export data to another file or to another platform.
6	Analyzing Assays	Describes the various assays used on the ABI PRISM 7000 Sequence Detection System.
7	Plus/Minus Assays	Describes using plus/minus scoring to determine successful PCR amplification.
8	System Maintenance	Explains how to calibrate the system and perform routine maintenance. Describes calibrating ROIs, a background run, and pure dyes.
A	Theory of Operation	Describes the principles behind the operation of the ABI PRISM 7000 Sequence Detection System.
B	Designing Assays Using TaqMan Probes	Describes how to design assays and use precision in designing them.
C	Consumables and Reagents	List of consumables and reagents to use with the system.
D	Using Windows	Descriptions of the Windows environment.
E	Technical Support	Information to get help from technical support and contact Applied Biosystems all over the world.
F	Limited Warranty	Applied Biosystems limited warranty statement.
	Glossary	Terms used in this guide, including acronyms and abbreviations.
	Bibliography	Bibliography of references.
	Index	Index of important references in the user guide.

Intended Audience

Who Should Use This Guide

This user guide provides procedures to operate the 7000 system, presents theory and guidelines for operation, and describes the instrument and software features. It is written for technicians, scientists, and researchers who will use the ABI PRISM 7000 Sequence Detection System instruments.

Background Needed to Use the Instrument

This guide assumes that you are familiar with the following:

- Basic Microsoft Windows 2000 operations such as using the mouse, choosing commands, working with windows, and using the computer hierarchical file system
- A general understanding of hard drives and data files
- An understanding of assay preparation and basic laboratory techniques
- A general understanding of TaqMan® chemistries

For More Information

For information about the topics mentioned above, Applied Biosystems recommends the references listed below:

Title	Part Number
ABI PRISM® 7000 Sequence Detection System Site Preparation and Safety Guide	4330233
ABI PRISM 7000 Sequence Detection System Software Online Help included in the software	—
Microsoft Windows Operating System Online Help	—

Features and Benefits

7000 System Benefits

The 7000 system has the following benefits:

- Easy to use software design
- Absolute quantification using standard curves
- Allelic discrimination assays (SNP detection) using TaqMan MGB probes
- Plus/minus detection using an internal positive control (IPC)
- Dissociation curve analysis
- Primary chemistries using fluorogenic 5´ nuclease assay
  - Ability to detect multiple report dyes in the same tube
  - Double stranded DNA detection using SYBR® Green 1 dye
- On-screen monitoring during data collection for real-time polymerase chain reaction (PCR) and dissociation curve runs
- Detector-centric assay model
- Persistent baseline and threshold settings
- Multi-color detection capability which provides application flexibility

Hardware Benefits

The 7000 system has the following benefits:

- Precision optics combined with a proven multicomponenting algorithm to provide accurate and highly reproducible results. When using the TaqMan RNase P Instrument Verification plate, the system can distinguish between 5,000 and 10,000 template copies with a 99.7% confidence level.
- Small instrument footprint
- Push-push door latch design facilitates plate loading and unloading
- Plate ejector for easy plate removal
- Peltier-based 96-well thermal cycling system
  - Supports standard 96-well plates, eight-strip or individual 0.2 mL tubes
  - 9600 system emulation mode available for easy assay transfer from the 7700 system

System Requirements and Software

Computer Specifications

The ABI PRISM 7000 Sequence Detection System includes a laptop computer with the following components:

- Pentium III 1.0 GHz Processor
- 256 MB RAM (memory)
- 20 GB hard drive (disk space)
- CD-RW (read-write) drive
- 15-inch UXGA color monitor
- Microsoft Windows 2000 Operating System with Service Pack 2 (SP2)

Additional Accessories

The system also comes with the accessories listed below:

- Locking mechanism
- Computer mouse
- Primer Express® Software

Recommended Computer Use

The laptop computer must be connected to the instrument at all times. It must be plugged into the AC current for the duration of the run.

Computer Battery

⚠ CAUTION The instrument cannot be run using battery power because the CPU runs in a slower mode to conserve energy. The battery typically lasts about four hours and a typical run is about two hours. For additional batteries, contact the computer manufacturer.

Computer Locking

There is an integrated lock provided with the computer and it is recommended that the lock be used at all times for security reasons.

Single Power Source

⚠ CAUTION The ABI PRISM 7000 Sequence Detection System is at risk for power surges when it is plugged into a separate power source from the laptop computer. The risk is reduced if both the instrument and the laptop computer are plugged into the same wall outlet, the same power strip, or into a common Universal Power Supply (UPS). Make sure both the instrument and the laptop computer power cords are plugged into the same power strip, or both are plugged into the same wall outlet.

If the 7000 system is moved, the power source for both the instrument and the laptop computer must be the same when they are plugged back in.

Software Revisions

Note: Unreleased or developmental versions of the ABI PRISM 7000 SDS Software are not supported by Applied Biosystems personnel (Technical Support and Field Applications Specialists). Use only released software versions when running or servicing the 7000 system.

Reporting Software Problems

If software problems are discovered:

- Always try to reproduce the problem so that it can be verified.
- Report any software problems to Applied Biosystems Technical Support. Refer to “Technical Support” on page E-1.

Learning the System

Overview

This section takes you through:

- Starting the computer
- Turning on the instrument
- Installing the software
- Removing a previous version of software, if necessary
- Launching the software
- Turning off the system

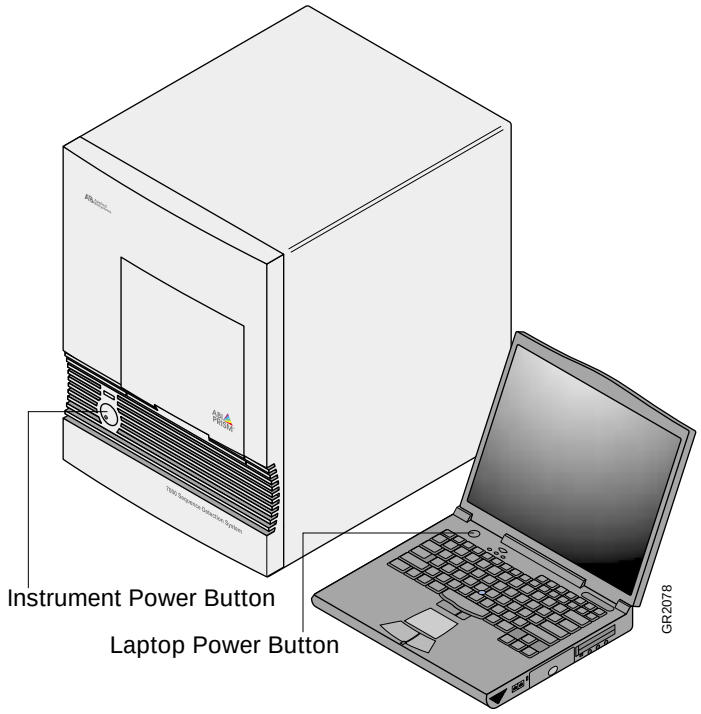
Starting the 7000 System

Starting the Laptop Computer

To start the computer:

1. Open the laptop computer by pushing in the front, center button, holding it, and lifting up the lid.

2. Turn the power on to the laptop computer by pressing the power button above the keyboard.



3. When the login window opens, in the **Enter User name** field, type your name or the user name associated with the computer.

4. If your computer has been set up to require a password, in the **Password** field, enter your password.

While you are waiting for the computer to finish starting up, follow the next procedure to start the instrument.

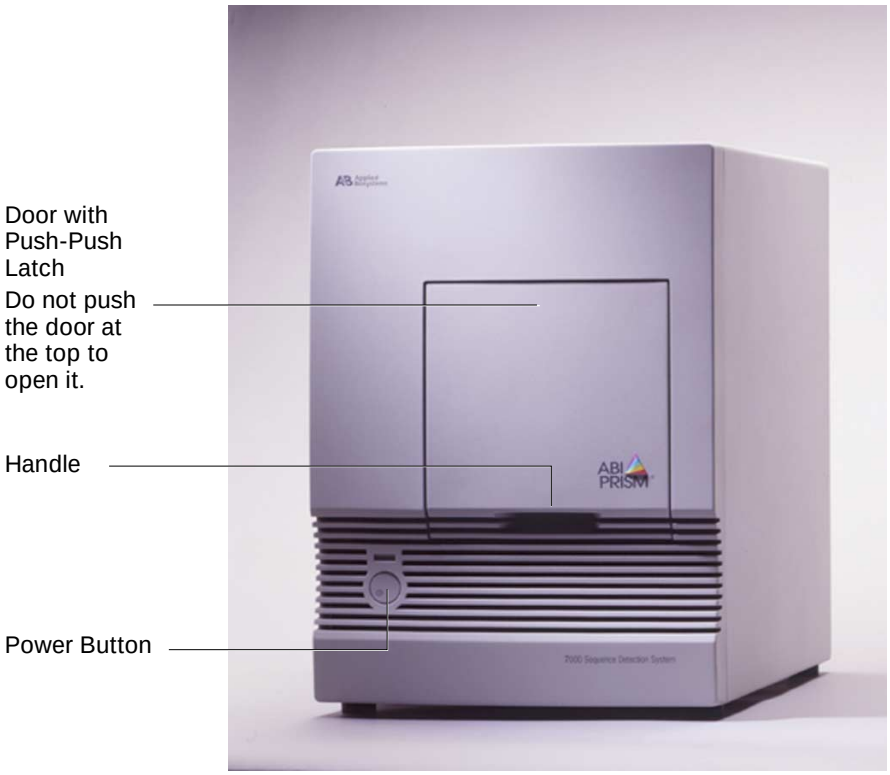
Turning on the Instrument and Loading a Plate

⚠ WARNING **POTENTIAL PINCHING HAZARD**. When loading a plate into the instrument, or pulling the handle of the door, make sure your fingers do not get between any moving parts of the instrument. This could cause your fingers to be pinched.

⚠ CAUTION Do not push the top of the door to open it without lifting the handle first. This will cause serious damage to the door or the door mechanism.

To turn on the instrument and load a plate:

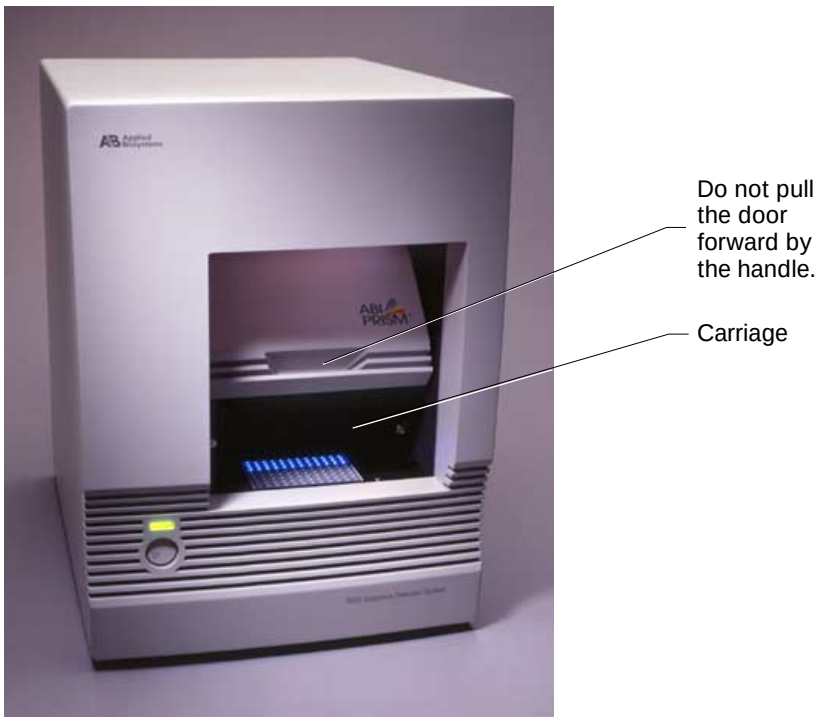
1. Press the power button on the lower left front of the instrument.



2. Lift the handle at the bottom of the door on the front of the instrument until the door has raised completely. Gently push the carriage back until it stops and locks into place.

To turn on the instrument and load a plate: *(continued)*

3. Load a 96-well reaction plate in the sample block so that well A1 is in the upper-left corner.



4. Gently push the carriage back once and release to unlatch it. The carriage will automatically slide forward into position over the sample plate. When the door has moved to the front, pull the handle down into place to close the cover.

   ⚠ CAUTION Do not pull the door handle to move the carriage forward. This may cause serious damage to the door or the door mechanism.

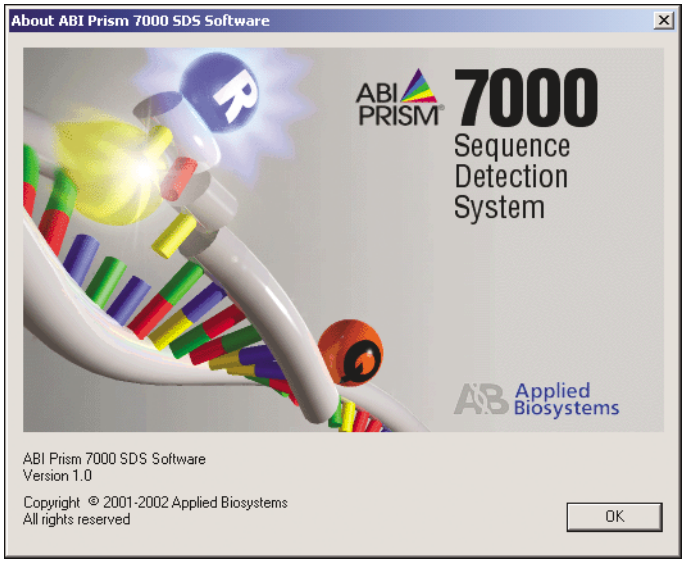
   ⚠ WARNING **PHYSICAL INJURY HAZARD**. During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Before performing the procedure, keep hands away until the heated cover and sample block reach room temperature.

Installing the 7000 Software

Installer Program

Note: The ABI PRISM 7000 SDS Software will be installed on the 7000 system by a service engineer when your system is set up. You will need to install newer versions of the software. The version number is in the Help menu, under About ABI Prism 7000 SDS Software.

You should install the software using the installer program located on the CD that comes with your 7000 system.



What the Software Installs

The installer:

- Installs the appropriate program components and places them in the correct directories
- Updates according to Microsoft Windows specifications
- Sets up the Start menu and desktop with shortcuts to the program

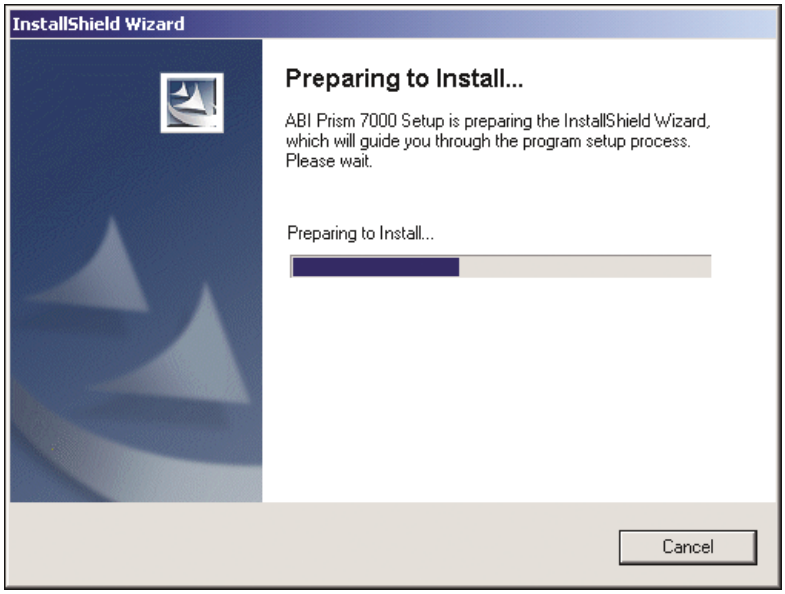
Updating a Previous Version of Software

Note: If there is a previous version of the 7000 software installed on your computer, the installer will update your software.

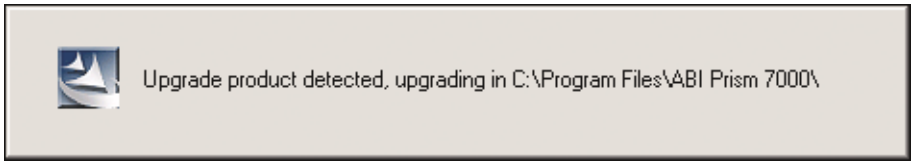
To update the 7000 software:

1. Insert the ABI PRISM 7000 CD into the CD-ROM drive.

2. The InstallShield® Wizard on the CD starts automatically and the Preparing to Install window opens. Wait until the Major Upgrade window opens.

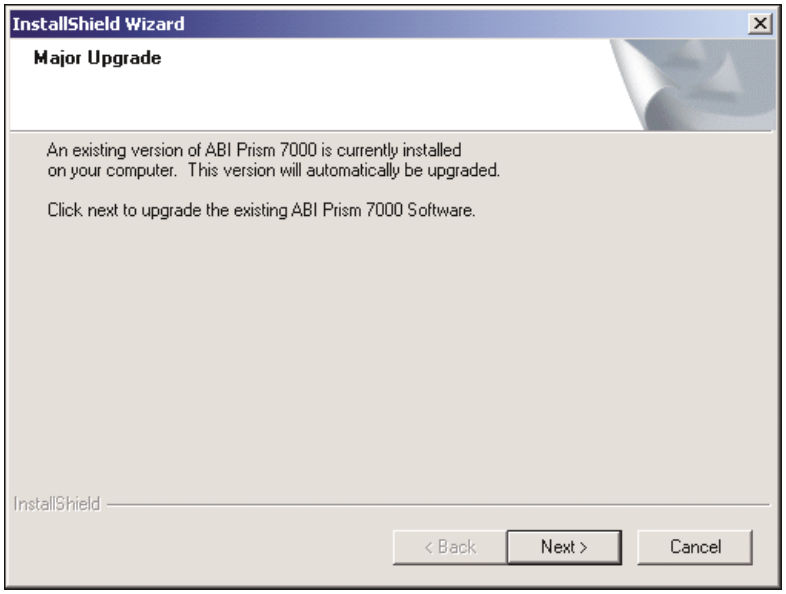


A dialog box will open advising you that it has detected an earlier version of the software.



To update the 7000 software: *(continued)*

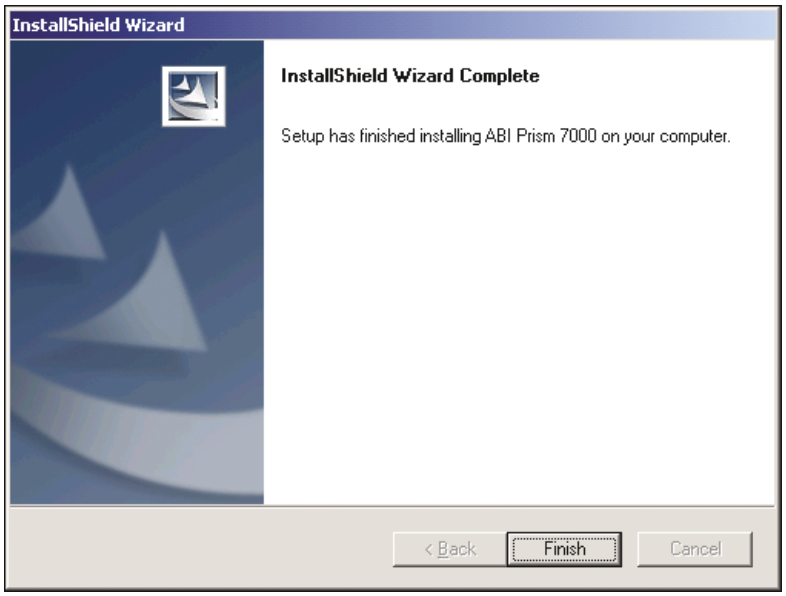
3. When the Major Upgrade window opens, click **Next**.



4. IMPORTANT: Wait until the installation is finished.

   It will remove the old files from your computer, create a new desktop shortcut, and register the product.

5. Click **Finish** when the InstallShield Wizard Complete window opens.



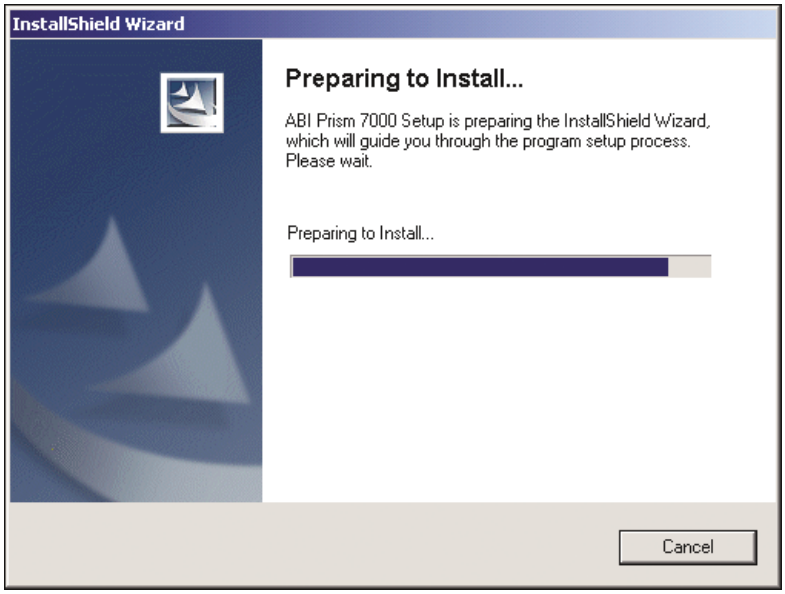
Uninstalling the Software

The installer on the CD will uninstall the 7000 software completely as well as install or update it.

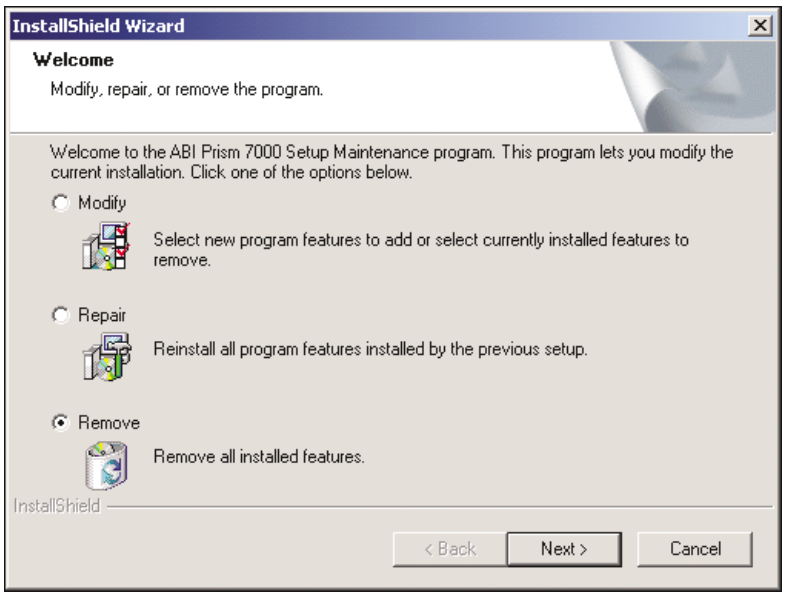
To uninstall the 7000 software:

1. Insert the ABI PRISM 7000 CD into the CD-ROM drive.

2. The installer on the CD starts automatically and the Preparing to Install window opens. Wait until the next window appears.



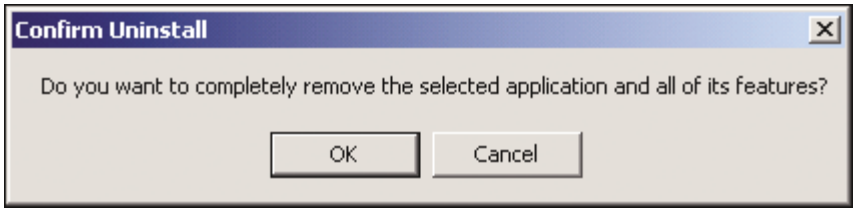
3. When the Welcome window opens, click the **Remove** button to uninstall the software.



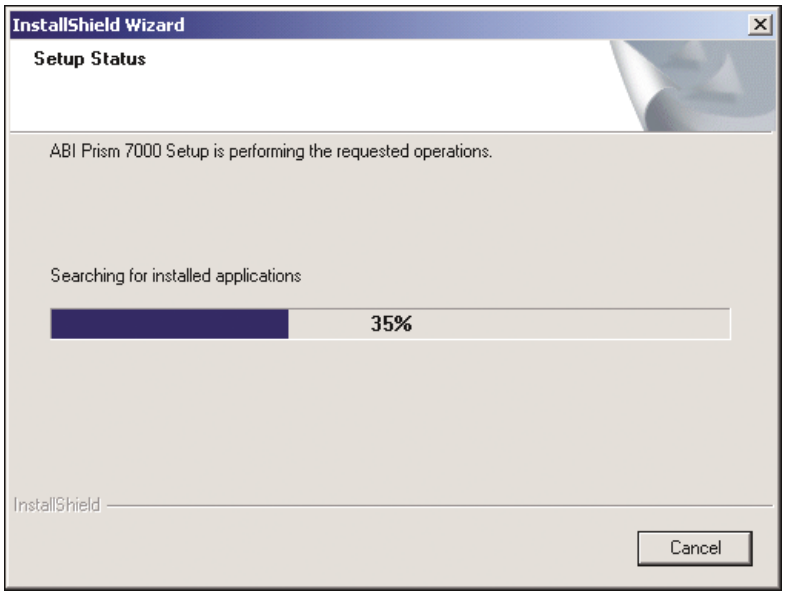
4. Click the **Next** button on the Welcome window.

To uninstall the 7000 software: *(continued)*

5. A dialog box will open and ask you to confirm that you want to uninstall the entire version of the software. Click **OK**.

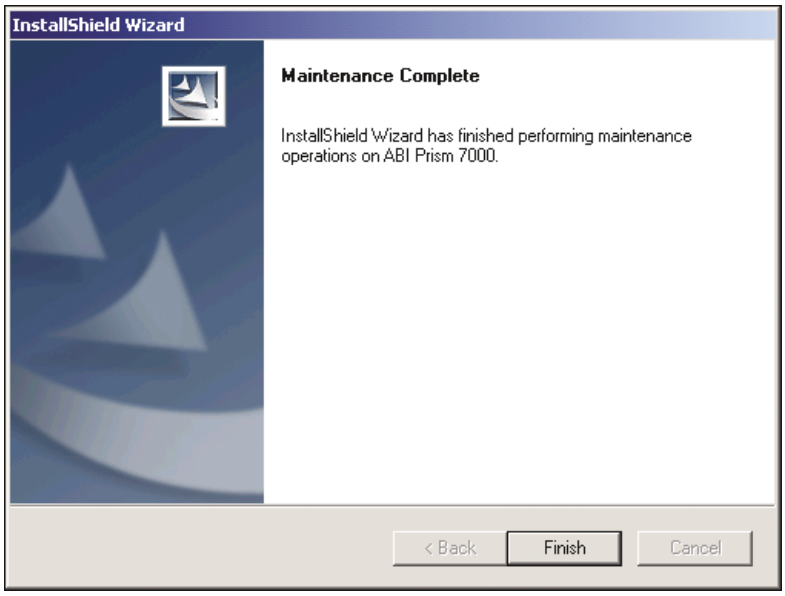


6. The Setup Status window will open to check the files and remove the software application from the computer. Wait until all the files are removed and the next window opens.



To uninstall the 7000 software: *(continued)*

7. When the Maintenance Complete window opens, click **Finish**. All the 7000 software files and the desktop shortcut are removed from the computer. If you want to reinstall the software, follow the procedure under “Installing the Software” on page 2-18.



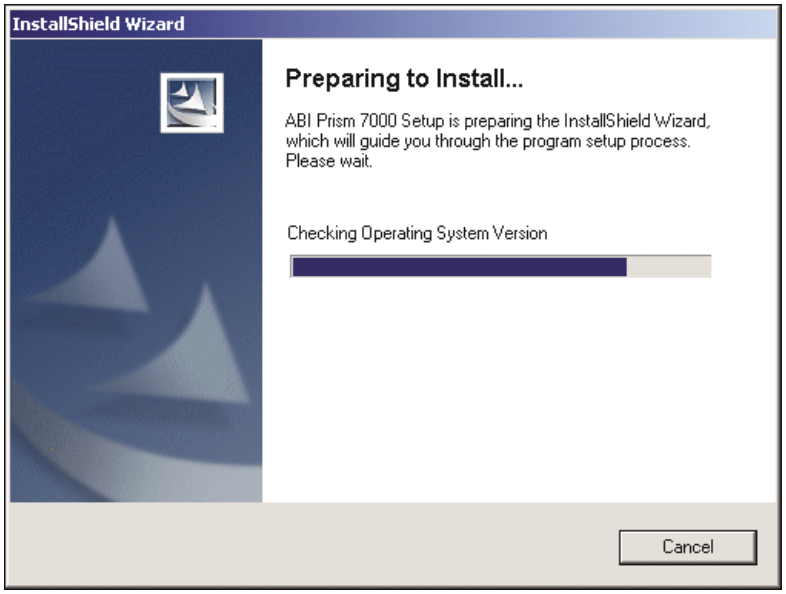
Installing the Software

Note: To install the software, you must have the CD with the current ABI PRISM 7000 SDS Software and all of its associated files.

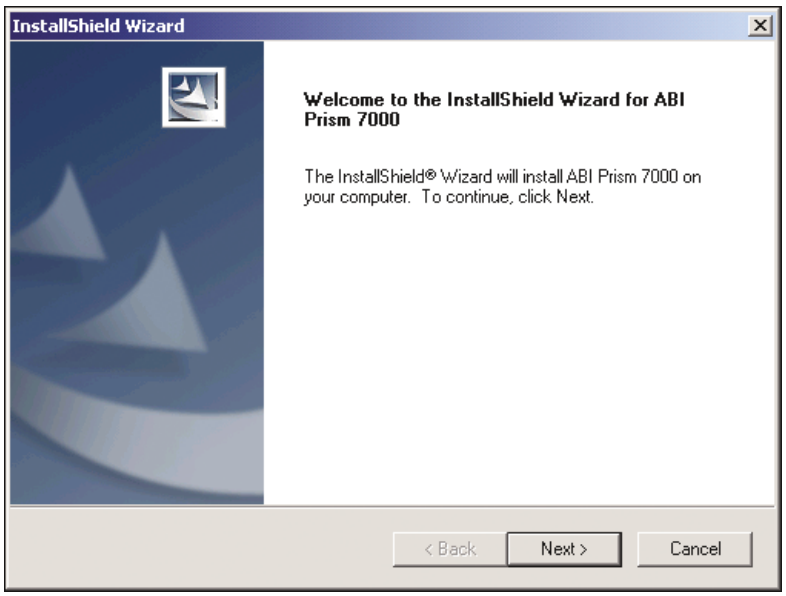
To install the 7000 software:

1. Insert the ABI PRISM 7000 CD into the CD-ROM drive.

2. The installer on the CD starts automatically. Follow the onscreen instructions as the software installs the files.



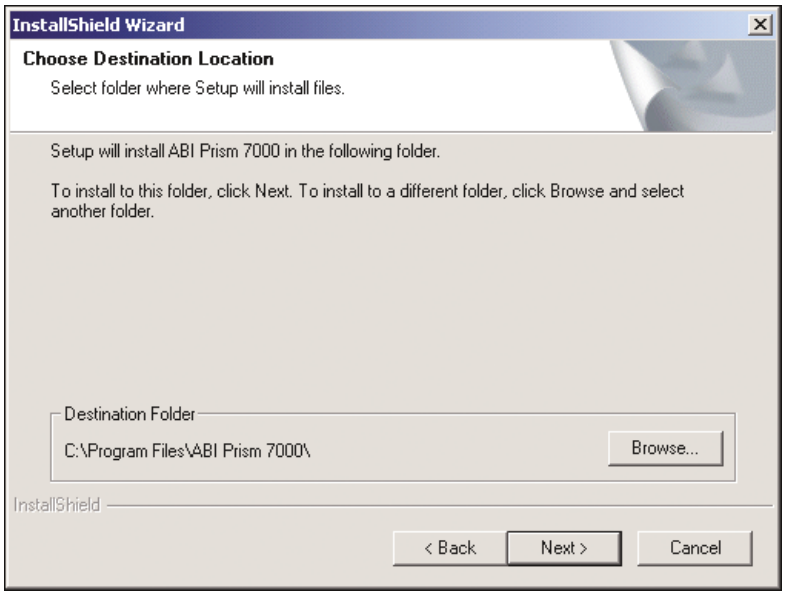
3. When the Welcome window opens, click the **Next** button to install the 7000 system program and files.



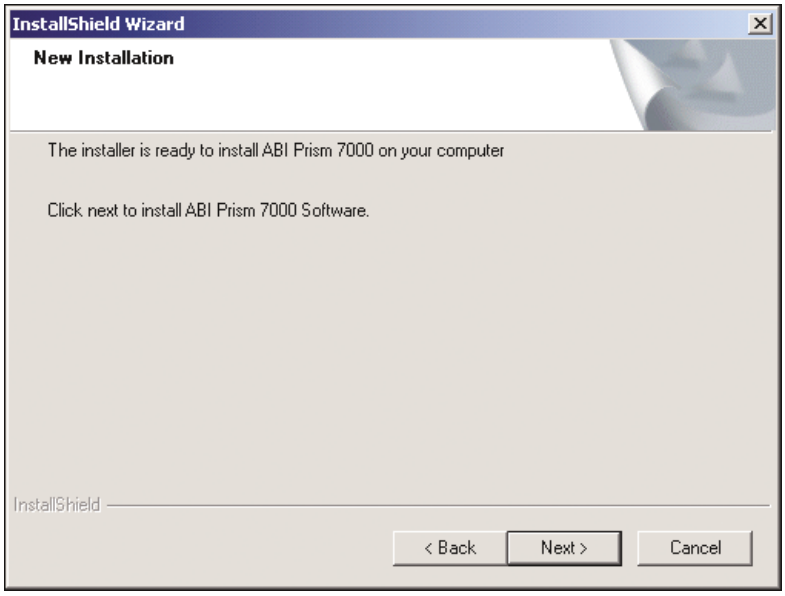
4. Read the software license agreement and select **Yes** to accept it.

To install the 7000 software: *(continued)*

5. When the Choose Destination Location window opens, make sure the software will be installed in the correct drive (C drive). Use the **Browse** button, if necessary, to find the correct folder. Refer to "Location of the Program Files" on page 2-21.



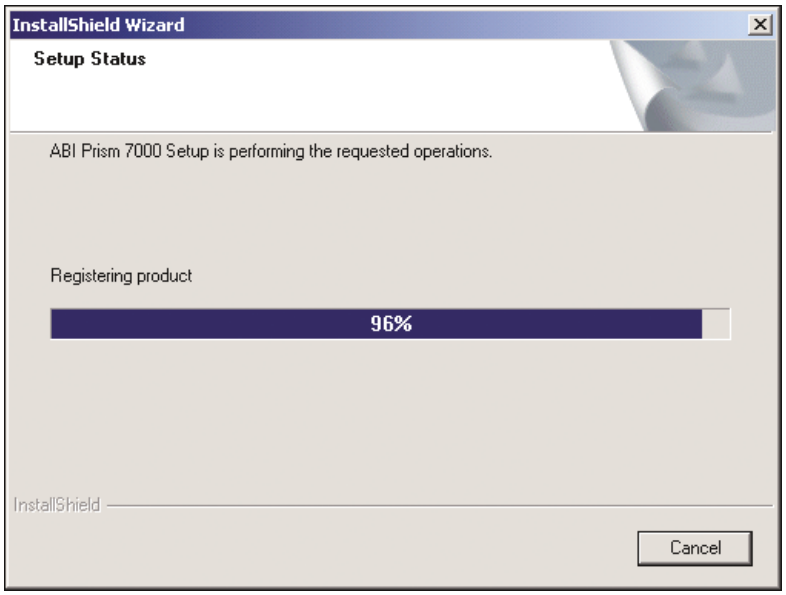
6. Click **Next**. The New Installation window will open to advise you that the installer is ready to perform a new installation.



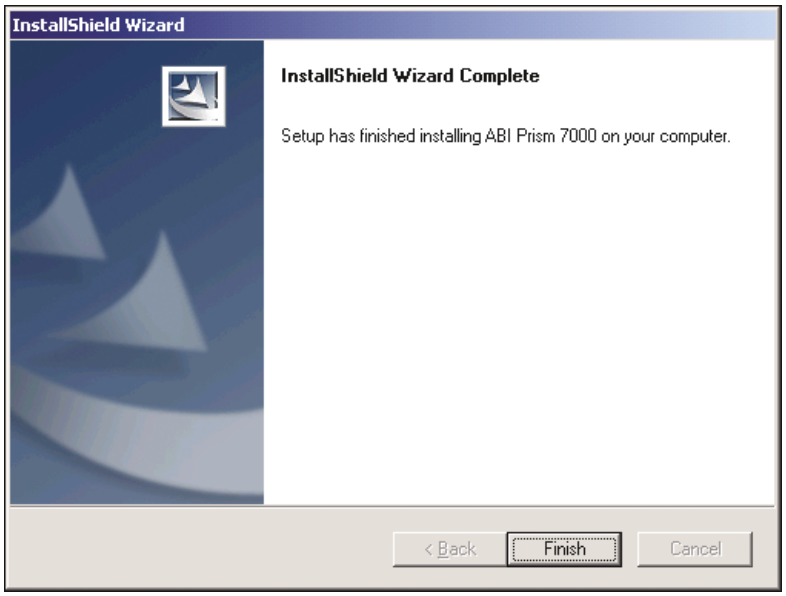
7. Click **Next** again.

To install the 7000 software: *(continued)*

8. The Wizard will load all the necessary files, create the ABI Prism 7000 SDS Software shortcut on the desktop, and register your software. Wait until this process has finished. The progress bar will show the status of the installation.



9. When the installation is complete, click **Finish**.



10. Remove the CD, place it in its holder, and store it in a safe place.

Location of the Program Files

After the software is installed, check that the program files are in the locations shown below:

- The default location for the 7000 software program should be:

  C:\Program Files\ABI Prism 7000\[ProgramName]

- The Start menu location for the 7000 software shortcut should be:

  Start\Programs\ABI Prism 7000\[ProgramName]

Shortcut to the 7000 Software

The installer program creates a link to the ABI PRISM 7000 SDS Software by placing a shortcut on the desktop during the installation. This enables you to open the program from the desktop without having to go through the Start menu.

7000 Software Directory Elements

⚠ CAUTION Do not modify, change, move, or delete any of these files. Any of these changes could make your software inoperable.

Directory Descriptions

Software	Description
ABI Prism 7000	This is the ABI PRISM 7000 SDS Software application program.
Plug Ins\ Instrument Assays	This folder (and named subfolders) contain the instrument and assay plug-ins.
Templates	This folder contains the .sdt files that are templates installed with the software and files that you create and save with the .sdt extension for future use.
Calibration folder	This folder contains the background and pure dyes calibration data.
Detectors folder	This folder contains the master list of detectors and markers: detectors.xxx markers.xxx

After the software is loaded, you will be able to start the software and look at its features when you load a plate. To do this, continue to the next chapter.

To shut down the instrument, follow the next procedure. Make sure you understand the following shutdown procedure to safely shut off the computer.

Shutting Down the System

Shutting Down the Software and Instrument

When you have finished using the instrument, you may want to close the software and shut down the instrument. Make sure you understand how to shut down the computer and the instrument by the time you are ready to shut off the system. Do not turn off the computer without going through the following safe shutdown procedure.

To shut down the 7000 system:

1. Click the **Close** button ( ) on the upper-right corner on any of the documents that are open and save them, if necessary.
2. Select **File ▸ Exit** on the 7000 software window.
3. Click the **Start** button located on the taskbar, and select **Shut Down**.
4. Select **Shut down** again in the Shut Down Windows dialog box and click **OK**.
5. Press the power button on the front of the instrument to turn it off.

Software Overview 3

Introduction

About This Chapter

This chapter is an overview of the ABI PRISM® 7000 SDS Software windows and their descriptions. There is a procedure to start the software, then an overview of the plate document and software.

In This Chapter

This chapter contains the following topics:

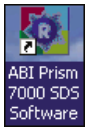
Starting the 7000 Software

Launching the Software

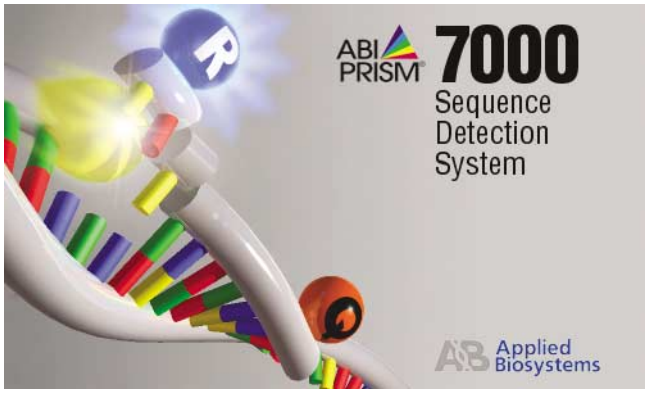
Launch the 7000 software by selecting the shortcut on the desktop.

To start the program:

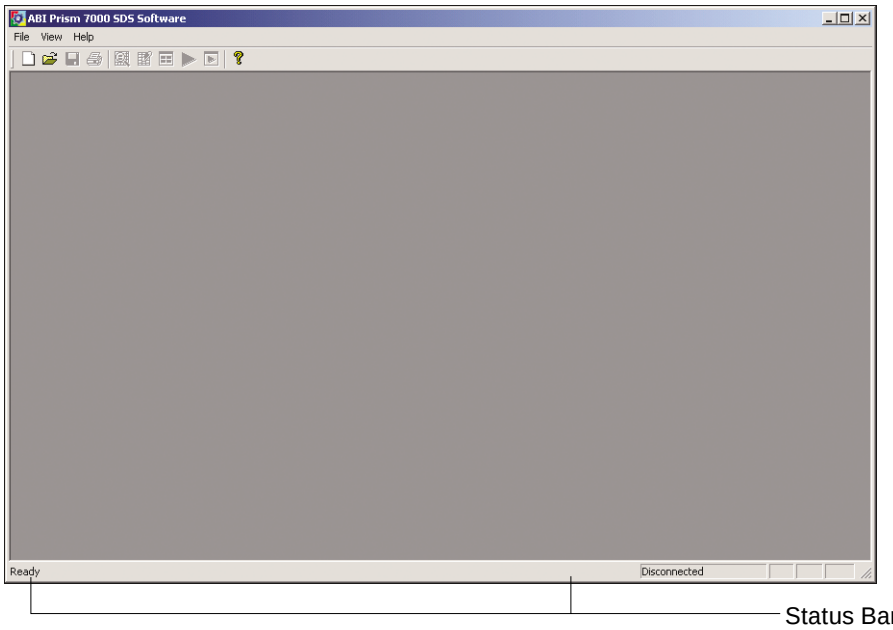
1. Double-click the **ABI Prism 7000 SDS Software** shortcut on the desktop.



A splash screen appears with the 7000 system logo. Then there will be an initializing message displayed until the software loads.



A blank 7000 software window will open like the one shown below. The status bar shows the instrument as Ready on the left and Disconnected on the right.



To start the program: *(continued)*

2. Select **File ▸ New**, and click **OK** to accept the default parameters in the New Document dialog box.

   This opens a new plate document. There will not be any data in the document until you actually set up and run an experiment.

Once you have selected a new plate document, the connection to the instrument is established. The instrument is connected when a document without data is opened, not when the program loads. The message in the lower right of the window will show "Connected" to indicate that the instrument is connected to the software.

The following section describes the elements of the 7000 software.

7000 Software Overview

What the Software Does

The 7000 software manages all communication with the instrument to perform the following tasks during runs:

- Calibrate ROIs and pure dye spectra
- Set up sample and enter experimental information
- Operate the 7000 system
- Collect and analyze fluorescence data
- Display data in graphic charts
- Export data and print reports of the results

Description

The 7000 software provides the following features:

- Absolute quantification assay
- Allelic discrimination assay
- Plus/minus assay

Window Elements

Overview

The 7000 software has a window interface described below:

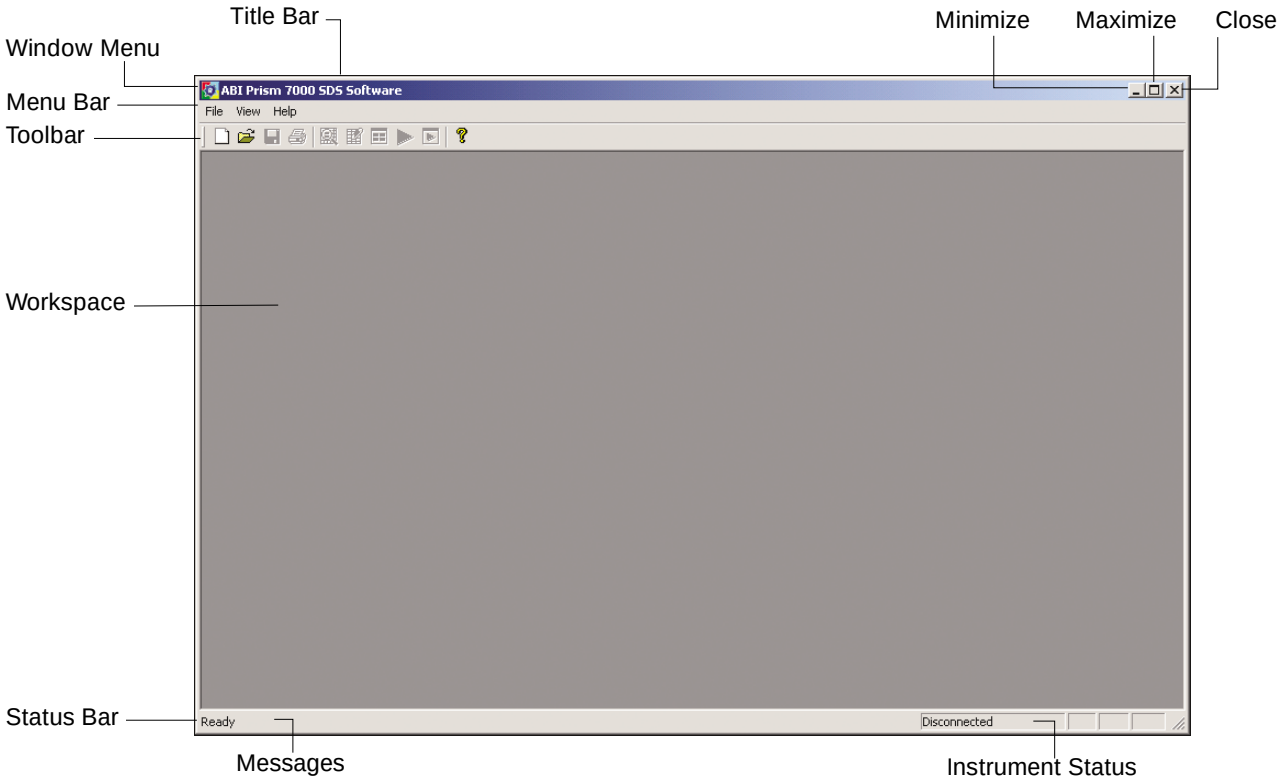
- Only one instance of the program may be open at any time.
- Multiple documents may be open within the program simultaneously. Only one document can be connected to the instrument at a time.
- The program may be opened without an instrument connection for off-line work.

The 7000 software window supports standard Microsoft® Windows® functions, such as minimize/maximize and resizing.

The main elements of the 7000 software window are shown in the following table and figure:

7000 Software Window Elements

Element	Description
Title Bar	Contains the software icon, software name, document name, and window Minimize/Maximize/Close buttons.
Menu Bar	A fixed menu bar containing the main menus you will use.
Toolbar	Icons containing shortcuts to system processes and tools.
Workspace	Main area of the window where the plate documents and analyses are displayed.
Status Bar	Area at the bottom of the window that displays status information in two areas: lower-left corner shows application status and user messages, lower-right corner shows instrument and document connection status messages.



Menu Bar

When a file is open, the menu bar of the 7000 software has the menus shown below. These menus are described in detail in the following tables.

7000 Software Icon

The menu under the 7000 software icon  has selections that control the window operations that appear in the upper-right corner of the window. This menu is under both the icon on the title bar and the menu bar of the window, depending on what mode you have previously chosen.

Window Shortcuts

Note: The shortcuts are command sequences to use from the keyboard. The plus sign (+) means that you must press the keys together. The comma indicates that you press and release each key sequentially.

Window Operation Descriptions

Menu Command	Description	Shortcut	Title Bar Button
Restore	Restores the window to the size of the plate document.	Alt, spacebar, R	
Move	Changes to a four-headed pointer to move the window.	Alt, spacebar, M	
Size	Changes to a double-headed pointer to resize the window by dragging the lower right corner.	Alt, spacebar, S	
Minimize	Minimizes the window to a label in the bottom of the Microsoft window (taskbar).	Alt, spacebar, N	
Maximize	Sizes the plate document to the size of the whole screen.	Alt, spacebar, X	
Close	Closes the open windows and program.	Alt+F4	
Next	Only appears on the menu bar menu.	Ctrl+F6	—

File Menu

The following table shows the selections on the File menu, their descriptions, shortcuts, and their associated icons on the toolbar, if they exist.

File Menu

Menu Command	Description	Shortcut	Toolbar Button
New	Creates a new document.	Ctrl+N	
Open	Opens an existing document.	Ctrl+O	
Close	Closes the topmost document window.	Ctrl+W	
Save	Saves the topmost document.	Ctrl+S	
Save As	Saves the topmost document with a name you give it.	Alt, F, A	—
Import Sample Setup	Imports data into the plate document from another file.	Ctrl+I	—
Export	Exports a plate document sample setup of an SDS file into a text format. You can also export calibration data, spectra, components, R_n values, C_T values, dissociation data, and results data from the run shown on the Report window.	Ctrl+E	—
Page Setup	Opens the Page Setup dialog box.	Alt, F, U	—
Print Preview	Opens the Print Preview window when a document is open to see how it will look when it is printed.	Alt, F, R	—
Print	Opens the Print dialog box.	Ctrl+P	
Recent Files	Lists the last four plate documents opened.	—	—
Exit	Exits the application.	Alt, F, X	

View Menu

The following table shows the selections on the View menu, their descriptions, shortcuts, and their associated icons on the toolbar, if they exist.

View Menu

Menu Command	Description	Shortcut	Toolbar Button
Toolbar	Shows/hides the toolbar with icons.	Alt, V, T	—
Status Bar	Shows/hides the Status Bar.	Alt, V, S	—
Well Inspector	Shows/hides the Well Inspector dialog box.	Ctrl+1	

Tools Menu

The following table shows the selections on the Tools menu, their descriptions, shortcuts, and their associated icons on the toolbar, if they exist.

Tools Menu

Menu Command	Description	Shortcut	Toolbar Button
Detector Manager	Opens the Detector Manager dialog box.	Ctrl+T	
Marker Manager	Opens the Marker Manager which contains the list of marker elements for allelic discrimination.	—	—
Dye Manager	Opens the Dye Manager dialog box.	Ctrl+U	—
Report Settings	Opens the Report Settings dialog box.	Alt, T, R	

Instrument Menu

The following table shows the selections on the Instrument menu, the shortcuts, and their descriptions.

Instrument Menu

Menu Command	Description	Shortcut
Start	Starts the run (available in absolute quantification).	Ctrl+R
Stop	Stops the current run (available in absolute quantification). Data collection and analysis may be incomplete.	Ctrl+Y
Pre-Read	Runs an allelic discrimination pre-read of a plate document (available in allelic discrimination and plus/minus assays).	
Post-Read	Runs an allelic discrimination post-read of a plate document (available in allelic discrimination and plus/minus assays).	
Disconnect	Disconnects the document from the instrument.	Alt, I, D
Calibrate	Opens the ROI Inspector calibration window.	Alt I, C
Function Test	Opens a window to test the system hardware.	Alt I, U

Analysis Menu

The contents of the Analysis menu change depending on the assay selected for the document.

Analysis Menu

Menu Command	Description	Shortcut	Toolbar Button
Analyze	Analyzes data after the plate document has been run.	Alt, A, A	
Analysis Settings	Provides settings for the analysis process.	Alt, A, N	
Display	When Results/Plate is selected, the selections change the values in the wells.	Alt, A, D	—
Quantity	Displays the quantity.	Alt, A, D, Q	
Delta Rn	Displays the ΔR_n values.	Alt, A, D, R	
Ct	Displays the C_T values.	Alt, A, D, C	
Delta Rn and +/- Calls	Available for plus/minus runs only. When Results/Plate is selected, the menu selections show either the ΔR_n values or +/- calls in the wells.	Alt, A, D, R Alt, A, D, C	
Delta Rn and AD Calls	Available for allelic discrimination runs only. When Results/Plate is selected, the menu selections show the ΔR_n values or AD calls in the wells.	Alt, A, D, R Alt, A, D, C	

Window Menu

The Window menu items allow you to arrange the way you view the windows.

Window Menu

Menu Command	Description	Shortcut
Cascade	Arranges the open windows in a cascade pattern so that each window opens on top and to the right of the one behind it.	Alt, W, C
Tile Horizontal	Arranges the open windows in a tile pattern so that each window opens on top and below the one behind it.	Alt, W, T
Tile Vertical	Arranges the open windows in a tile pattern so that each window opens to the right of the one before it.	
List of documents	Lists all documents open during the current session. The top window shows a check mark next to it. To change the order, click the document on the menu.	Alt, W, number

Help Menu

The following table shows the selections on the Help menu, their descriptions, shortcuts, and their associated icons on the toolbar, if they exist.

Help Menu

Menu Command	Description	Toolbar Button
Contents and Index	Opens the online help windows that explain the 7000 software, show shortcut commands, and procedures to use the software.	
About 7000 Software	Opens the splash screen that shows the software name, version, and copyright date.	—

Toolbar

The toolbar is a series of icons that provide a shortcut to some of the windows described in the previous section.

Status Bar

The status bar at the bottom of the 7000 software window displays two regions of information: messages for the user, and instrument and document status information.

Elements of the Status Bar

The messages for the user (left side of the status bar) provide information on messages and the instrument connection as described below.

Message Region

Status Bar Display	Meaning
Description of the function of the icon when the pointer rolls over it	Click the icon to start the process described.
Ready	The instrument is idle.

Instrument Connection Status Region

The messages on the right side of the status bar display the connection state of the instrument.

Status Messages

Status Bar Message	Meaning
Connected: [documentname.sds]	The instrument is connected to the specified document.
Disconnected	The instrument is not connected.
Running: [documentname.sds]	The instrument is connected and running, and displays the name of the document being run.

Plate Documents

Overview

The 7000 plate document appears as a three-tabbed pane in the 7000 software window. Each tab relates to a step in the analysis process: Setup, Instrument, and Results. The Results tab has several sub-tabs for the various viewers associated with an assay.

Plate Document Characteristics

The plate documents have the following characteristics:

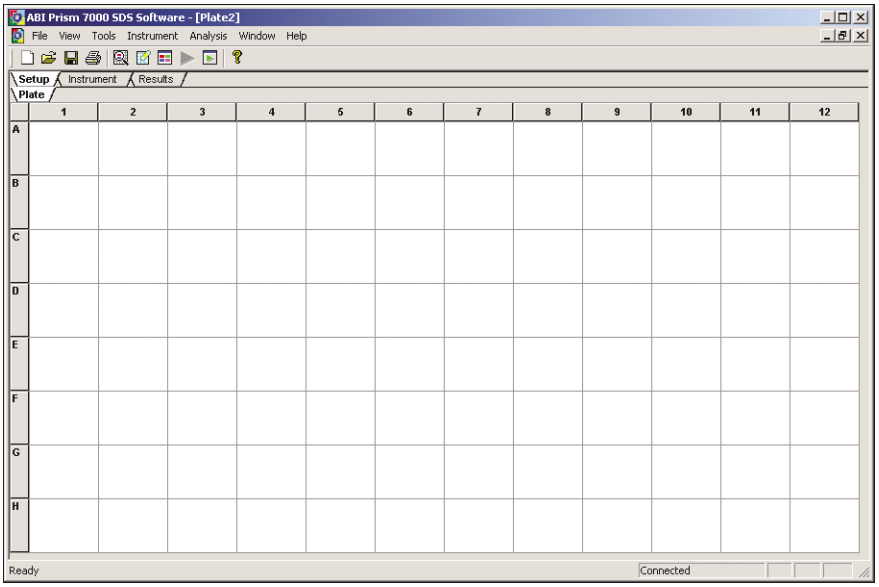
- The document may be resized, minimized, and maximized.
- Data viewers (grids, graphs) are dynamically synchronized. A selection in one view selects related elements in the other views.
- The views and displays for a document are dependent on the assay specified when the document is created. Tabs, views, and panes may vary.
- You may connect only one document to the instrument at a time. Connection is made when you start a run. You must close or disconnect a connected document before you can connect another document to the instrument.
- Documents with run data have the Setup, Instrument, and Results tabs enabled. However, documents with data may not be run again – the system must disable instrument controls. The exception is allelic discrimination, which can be run both as pre-read and post-read documents.

Template File

A template is a document that contains setup data that you create once and then can open later without having to recreate the plate document. When you open a template document (*.sdt), a plate document opens containing the setup data you entered when you created it.

Setup Tab

The Setup tab allows you to manage and change tasks related to the contents of a plate prior to thermal cycling and analysis. The plate document has 12 columns of eight rows designated as A to H.



Selecting Wells

Selected wells have a gray background, as shown in the graphic below, and unselected wells have a white background, as shown in the graphic under “Data Displayed in Wells” on page 3-12.

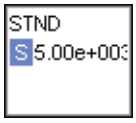
The wells can be selected in the following ways:

- Single-clicking a well selects the well.
- Single-clicking a column label selects all wells in that column.
- Single-clicking a row label selects all wells in that row.
- Single-clicking the upper-left corner of the row and column labels selects all wells.
- Shift-clicking wells selects a continuous range of wells.
- Control-clicking wells selects a discontinuous range of wells.
- Double-clicking a well opens the Well Inspector.

Data Displayed in Wells

Each well displays the setup data for the well, presented as follows.

- Line 1 shows the sample name.
- Lines 2 through 5 show colored boxes that represent detectors assigned to the well, and the quantity of any standard. Each box has a single-letter abbreviation identifying the task of the detector (first letter of the task).



Note: The maximum number of detector labels per well is four.

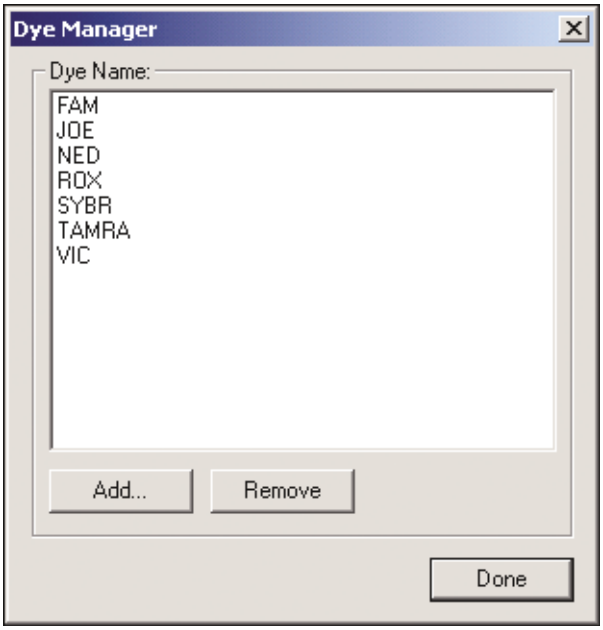
Instrument Tab

The Instrument tab is discussed later. Refer to “Instrument Tab” on page 3-19.

Dye Manager

About the Dye Manager

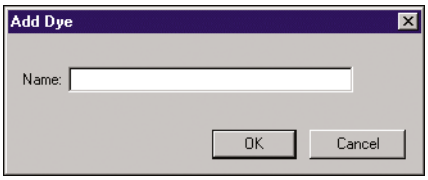
The Dye Manager maintains the list of dyes associated with the installed application. It also manages the creation and deletion of dyes. You can open the Dye Manager from the Tools menu. Dyes installed with the application cannot be deleted.



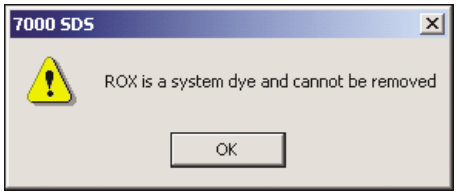
Elements of the Dye Manager

The Dye Manager has several components described below.

Dye Manager

Element	Description
Dye Name	Contains the names of dyes. When you select a dye, the Remove button becomes available.
Add button	Displays the Add Dye dialog box. Sets the name of the dye and adds it to the dye list. 

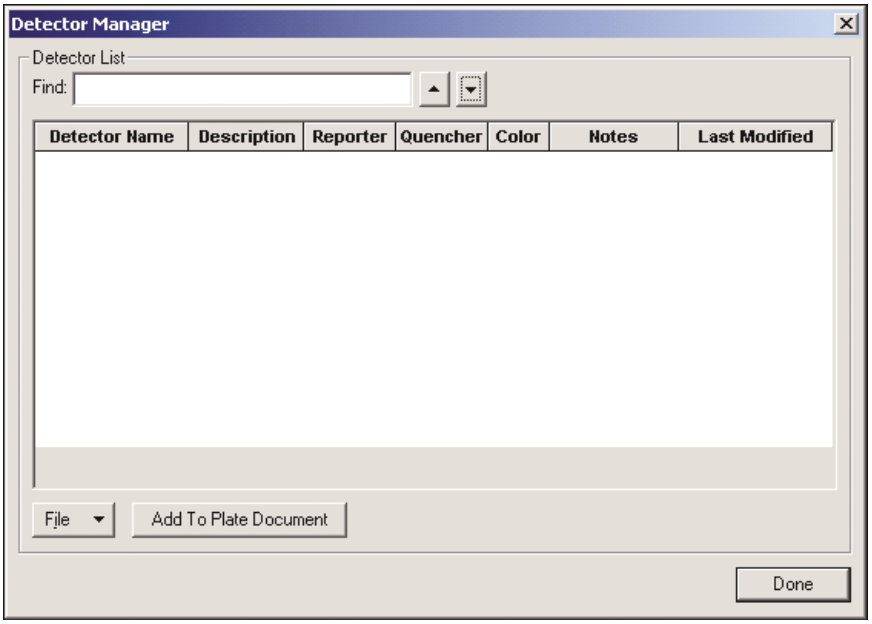
Dye Manager *(continued)*

Element	Description
Remove button	This allows you to remove a custom dye. You will receive a message if the dye is being used by one or more detectors. You can remove the dye from the detectors before removing the dye from the Dye Manager. If you try to remove a system-installed dye, there will be a message that you cannot remove it. 
Done button	Saves changes and closes the Dye Manager window.

Detector Manager

Overview

The Detector Manager maintains the list of detectors associated with the installed application. It also manages the creation, editing, and deletion of detectors.



Elements of the Detector Manager

The Detector Manager has several components described below.

Detector Manager

Element	Description
Find field and buttons	Allows you to enter text into the Find field, then use the up and down arrow buttons to find the previous and next instance of the string in the list. Find operates on all columns.
Detector List	Contains the list of detectors and their properties. Data in columns cannot be edited. Clicking a row selects the detector in that row. Columns include: • **Detector Name** – Name of the detector. Detector names must be unique. • **Description** – Description of the detector. • **Reporter** – Name of the Reporter dye. • **Quencher** – Name of the Quencher dye. • **Color** – Color associated with the detector. • **Notes** – Notes about the detector. • **Last Modified** – The last date the detector was edited. Shift-click to select continuous detectors and Ctrl-click to select discontinuous detectors. Double-click a detector to open the Detector Properties dialog box.

Detector Manager *(continued)*

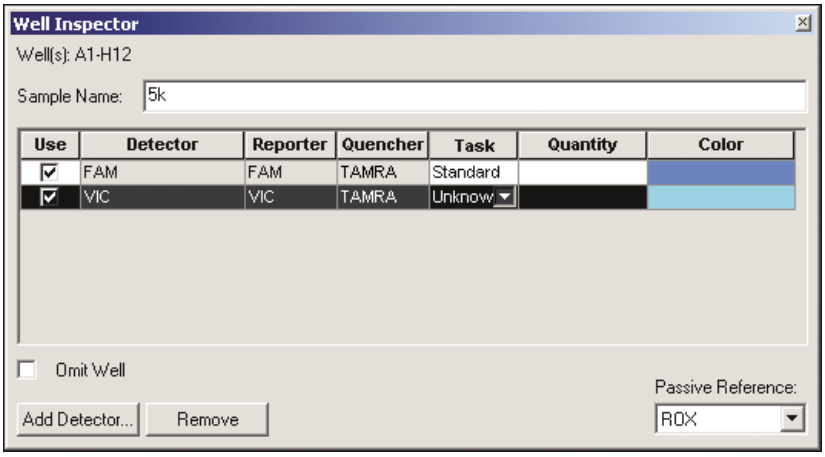
Element	Description
File button	Opens a menu containing these items: • **New** – Opens the New Detector dialog box. • **Duplicate** – Opens the Duplicate Detector dialog box to duplicate a highlighted detector. • **Add To Plate Document** – Adds the selected detector to the Well Inspector and the plate document. • **Import** – Opens the Import dialog box. • **Export** – Opens the Export dialog box. • **Clear** – Clears the selected detector. You cannot undo this action once you click Yes. • **Clear All** – Clears all the detectors. You cannot undo this action once you click Yes. • **Properties** – Opens the Detector Properties dialog box.
Add To Plate Document button	Adds the selected detector to the Well Inspector and the plate document.
Done button	Saves changes and closes the Detector Manager dialog box.

Well Inspector

About the Well Inspector

After defining dyes, detectors, and markers for allelic discrimination, you can set up a plate. Each well in the plate grid represents a sample and its associated detectors or markers. To associate a sample with a well, use the Well Inspector to specify a sample name and the detectors associated with the well. It shows the properties (contents) of a selected well or range of wells. You can hide and show the Well Inspector by:

- Clicking the Well Inspector button on the toolbar
- Selecting the View menu, then the Well Inspector
- Double-clicking a well



Elements of the Well Inspector

The Well Inspector has the components described below.

Well Inspector

Element	Description
Well(s)	This field cannot be edited. It displays the selected range of wells. For example: • Single well: A1 • Continuous well range: A1-A4 • Discontinuous well range: A1-A4, B1-B4, B5, H12
Sample Name	This field specifies the sample name you enter for the selected wells. • If wells contain the same sample name, the name is displayed. • If wells contain different sample names, then (mixed) is displayed in the field. Note: Replicates are defined as samples having the same sample name.

Well Inspector *(continued)*

Element	Description
Detector List	Contains the list of detectors set up in the Detector Manager that can be added to the plate document. When a new plate document is created, this list is empty. Columns may be resized by using the vertical column separator. The columns in the Detector List are the following: • **Use** is a check box that adds a detector to the selected wells. It shows a mixed state when two or more selected wells contain different detectors. • **Marker** contains markers set up in the Marker Manager and shows up only for allelic discrimination. • **Detector** contains the name of the detector. • **Reporter** contains the name of the detector reporter. • **Quencher** contains the name of the detector quencher. • **Task** specifies the task for the detector (Unknown, Standard, NTC, IPC, or IPC+). The default task is always displayed when the detector is added (the default is fixed, based on the type of assay). It displays (mixed) when two or more selected wells contain different tasks. Contents of the list are based on the assay being run.  • **Quantity** can be edited only when the task is Standard. It displays (mixed) when two or more selected wells contain a different quantity. • **Color** is the color of the detector you defined in the Detector Manager.
Omit Well check box	This toggles a well on and off. It displays a mixed state (checked with gray background) when two or more selected wells have been omitted. The well information remains, but the software does not use the well in its calculations. Text of an omitted well is shown in light gray.
Add Detector button	Opens the Detector Manager dialog box which enables you to add detectors to the document from the master list of detectors.
Remove button	Removes a selected detector from the list. This will clear the detector from any wells where it is specified. You cannot undo this operation.
Passive Reference	This specifies the passive reference that you can apply to all samples in the plate. Contents of the list come from the Dye Manager dialog box.

Instrument Tab

About the Instrument Tab

To open the panes in the Instrument tab, click it from the plate document. The Instrument tab opens a page to set up the thermal cycler, run plates, and monitor run status.

The Instrument window contains the following tabs:

- Thermal Profile
- Auto Increment

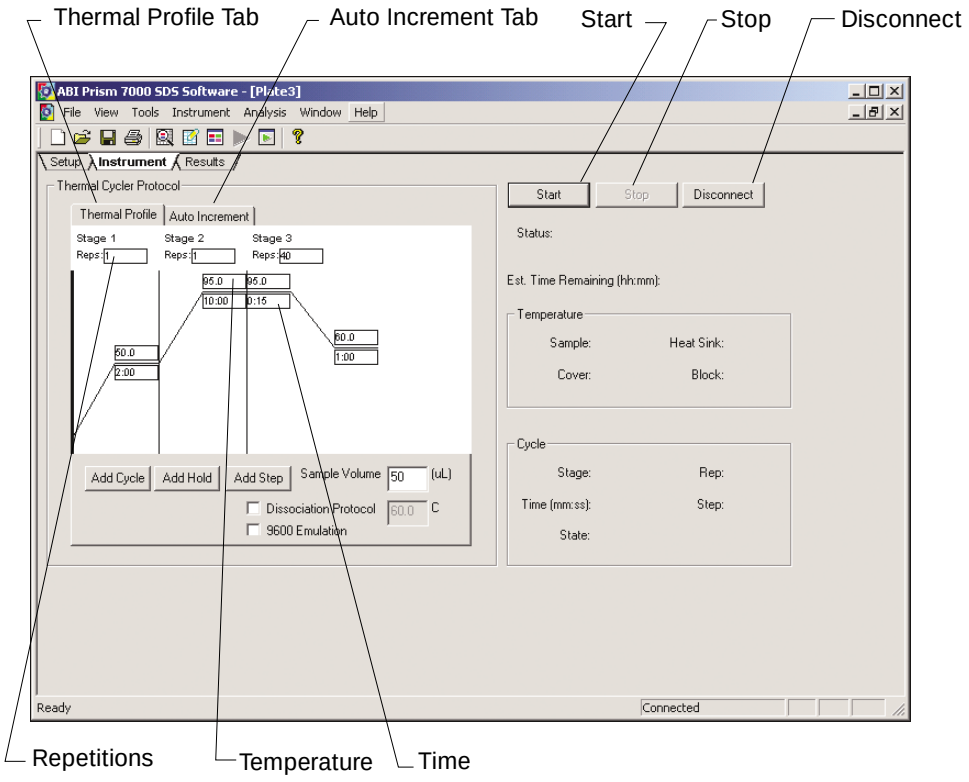
Thermal Profile Tab

To open the Thermal Profile pane if it is not already open, click the Instrument tab, then the Thermal Profile tab.

The Thermal Profile pane displays the primary view of the cycles showing the number of cycles with time and temperatures for each step and hold. This pane does not display in real time. You can edit the time and temperatures of these cycles by clicking inside the small boxes in each stage and typing a new repetition, temperature, or time.

The Thermal Profile pane allows you to:

- Create holds and cycles (segments)
- Create up to six temperatures within a cycle
- Set the last time on last hold temperature to infinity
- Set the number of cycles for a PCR segment
- Select a dissociation protocol



Text Boxes Under Stage Labels

The Stage labels in the Thermal Profile pane are listed below:

- Reps text box shows the number of repetitions the run will cycle for each stage. The range is from 0 to 99.
- The text box under Reps is the temperature in °C of the hold or cycle ramp. The range is 4.0 to 99.9 °C.
- The bottom text box is the duration in minutes of the cycle or hold. The range is 0:00 to 89:59 minutes.

Thermal Cycler Protocol Buttons

Sections of the Stages can be selected to add cycles, holds, or steps. When you click on the Thermal Profile pane, a heavy vertical black line indicates that the section to the right of the black line is selected before a ramp or a cool down. The pointer appears between steps and indicates the current selection that can be edited in the thermal profile. Press the Shift key and click to select entire steps or a range of steps.The buttons in the Thermal Cycler Protocol pane have the following actions:

Thermal Cycler Protocol

Button	Description
Add Cycle	When you click the position in the pane where you want to add a cycle: • If the insertion point is to the left of a Stage, the cycle is added before the Stage. • If the insertion point is to the right of a Stage, the cycle is added after the Stage. • If the insertion point is in the middle of a Stage, the cycle is added after the Stage.
Add Hold	When you click the position in the pane between steps where you want to add a hold: • If the insertion point is to the left of a Stage, the hold is added before the Stage. • If the insertion point is to the right of a Stage, the hold is added after the Stage. • If the insertion point is in the middle of a Stage, the hold is added after the Stage.
Add Step	When you click the position in the pane between steps where you want to add a step, it adds it to the right of the black selection line.
Delete Step	To delete a step: • Select the bar to the left of the step you want to delete. • Hold down the Shift key and click inside the step you want to delete. • Press the Delete key or the Backspace key. If all steps of a Stage are deleted, the Stage is deleted.

During background, pure spectra, and allelic discrimination assays, the thermal cycler has only one stage and one step. The only field that can be edited is the temperature field.

Instrument Buttons

The buttons on the top of the Instrument page (Start, Stop, Disconnect) control the actions of the run once it has been set up.

Instrument Buttons

Button	Description
Start	Starts the run when all the stages are set up. Note: If the plate document is not saved, another document is connected, or the software cannot establish a connection to the instrument, there will be an error message of this condition.
Stop	Stops the run before it has finished.
Disconnect	Disconnects the instrument from the plate document.

Other Parameters

Additional parameters in the Instrument page are listed below.

Other Instrument Parameters

Parameter	Action
Sample Volume field	The amount of sample (in µL) in all the wells. This is used to calculate the sample temperature.
Dissociation Protocol check box	Use this check box to add a dissociation protocol to the thermal profile. This sets the starting temperature for the protocol. The range is 20 to 60 °C.
9600 Emulation check box	Use this check box when you want to slow the thermal cycler down to emulate the GeneAmp® PCR 9600 thermal cycler.
Status area	Shows the status of the run as it goes through the stages: running, holding, heating.
Est. Time Remaining (hh:mm)	Shows the remaining time of the run in hours and minutes.
Temperature	Shows the calculated temperature of the sample, cover, heat sink, and block in °C during the progress of the runs while the thermal cycler ramps, holds, and cools. The range is 4.0 to 99.9.
Cycle	A series of steps that are repeated *n* times during each stage of the run. • **Stage** – Shows the current cycle and hold of each run in the Thermal Profile diagram. • **Rep** – Specifies the number of repetitions in the stage it is running. The range is from 0 to 99. • **Time (mm:ss)** – Shows the minutes and seconds of temperature holds in the cycle. The range is 0:00 to 89:59. • **Step** – A temperature plateau preceded by a ramp. • **State** – Shows the state of the run.

Other Instrument Parameters *(continued)*

Parameter	Action
Infinite Hold	The temperature the instrument will hold until it is manually stopped. It is an option at the end of the run. This option is represented by the infinity symbol (∞). This symbol automatically appears when 99:59 is in the last hold field. Note: Applied Biosystems does not recommend infinite holds at the end of a run at any temperature.

Auto Increment Tab

The Auto Increment pane displays fields on the thermal profile associated with auto-incrementing and decrementing the time and temperature during a PCR cycle. This pane does not display in real time. It automatically adds an increment that you set up to the temperature or the time of each cycle. This process is particularly useful at the end of the cycle.

The descriptions of all other buttons and parameters of the Auto Increment tab are the same as the Thermal Profile pane described in "Thermal Profile Tab" on page 3-19.

Analyzing Data

Analyzing Data After a Run

After a run is complete, analyze the data whenever there are pending changes to the analysis settings. Whenever you change the threshold, you must analyze the data to update the file. Analysis settings are stored in the plate document. You do not have to reanalyze data when you open a plate document.

Analysis Steps

The steps for general data analysis are listed below.

Analysis Steps

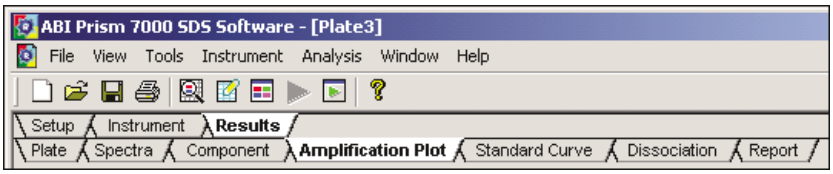
Step	Description
Data collection from the instrument is complete.	The software automatically saves the collected data. Then you must analyze the data using default analysis settings. The results are immediately available in the Results tab.
Change or modify analysis settings. For example: • Drag the threshold bar in the amplification plot to a different position. • Change a parameter in the Analysis Settings dialog box.	The Analyze item in the menus and the Analyze button on the toolbar become available. The status bar indicates that data must be reanalyzed.
Click the **Analyze** button.	The data is reanalyzed and the results are displayed in the Results tab.

Note: The analysis settings are different for each assay. Refer to the specific assay descriptions of the selections for analysis settings on pages 3-28, 3-41, 3-51 and 7-11.

Results Tab

About the Results Tab

In the main 7000 plate document, click the Results tab to open the tabbed pages that are available from that view. The Results tab has the results of the run where you can view them and change the parameters to run the plate document again, or reanalyze the data.



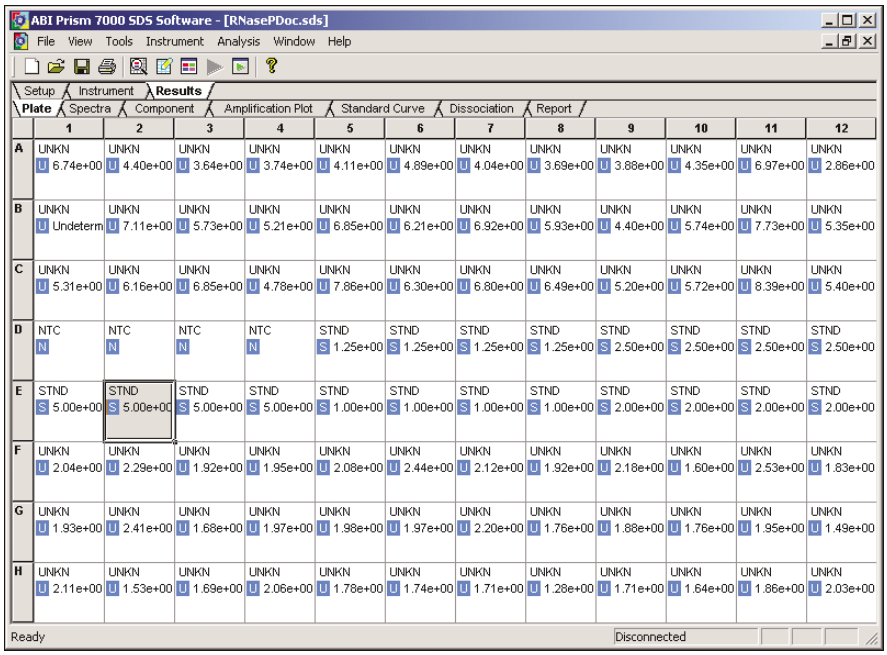
The Results tab has seven secondary tabs described below.

Results Tab

Tabs	Description
Plate	A plate document similar to the setup plate that shows the results data of each well. Each well displays up to five lines of data. a. Line 1 is the sample name. b. Lines 2 through 5 are the colored boxes representing the detectors. Numbers next to the boxes represent the data and results of the analysis.
Spectra	Shows the spectra of one to all the selected wells that represent the fluorescence of the wells. • The Cycles slider allows you to see the spectra for each cycle by dragging it with the pointer. • The Cycle # text box shows the cycle of the slider. Double-clicking the legend on the left opens the Graph Settings dialog box to reset the Y and X axes or allow autoscaling.
Component	Shows the complete spectral contribution of each dye in a selected well over the duration of the PCR run. Only the first selected well is shown at a time. Double-clicking the legend opens the Graph Settings dialog box. The X-axis of the graph is relative fluorescence and the Y-axis is time. The data plotted in this graph includes all the dyes.
Amplification Plot	This shows both real-time and post-run amplification of specific samples. It displays all selected samples in the plate matrix.
Standard Curve	This displays the standard curve of the standards. The X-axis is the starting copy number and the Y-axis is the threshold cycle (C_T).
Dissociation	Displays the melting (T_m) curves associated with a dissociation plate document.
Report	Displays a tabular report of data for the run.

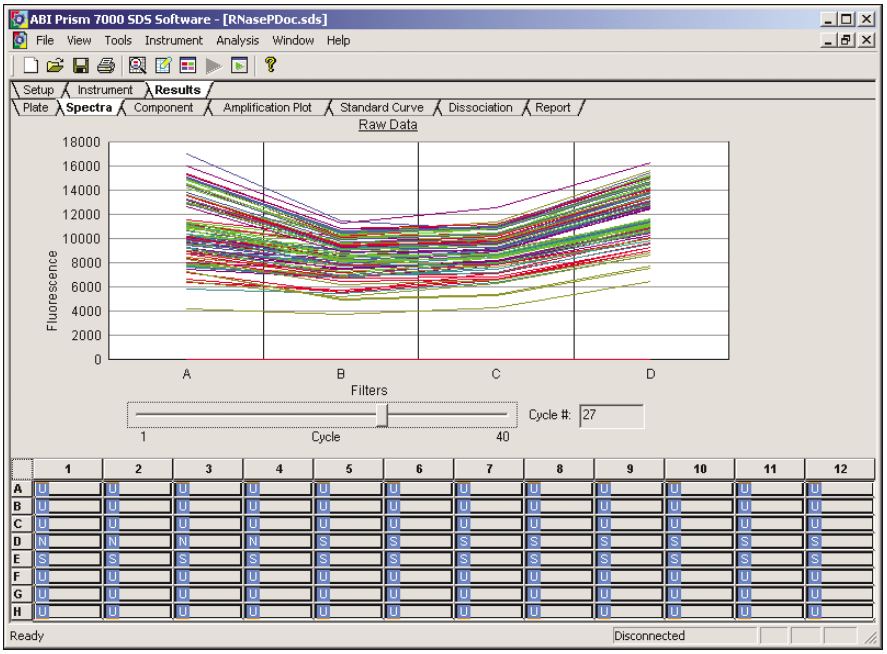
Plate Tab

The Plate tab is described in detail in “Plate Documents” on page 3-11. The figure below is an example of the Results Plate view.



Spectra Tab

The Spectra tab shows raw data (historical average fluorescence) for selected wells. It allows you to view data in an X – Y graph for all selected sample wells. X is a representation of the four filter positions, shown as A, B, C, D on the raw data graph. Y is the raw fluorescence intensity (0 to ~40,000) of the wells.

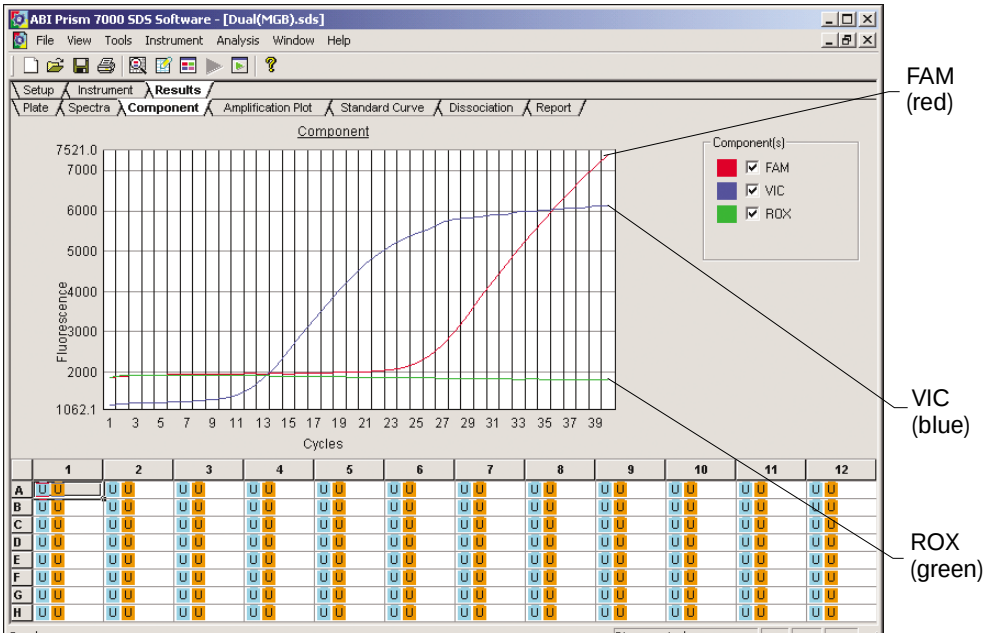


Spectra Tab

Element	Description
Cycles slider bar	Sets the Cycle Number for the data displayed in the graph. The current cycle number appears in the field to the right of the cycle slider bar.
Plate matrix	The section at the bottom of the window is a representation of the plate document that allows you to select the samples to view on the raw data graph. This view only displays the detector labels. When a sample is selected, a data point for that sample is added to the raw data graph. The legend is also updated.

Component Tab

The Component tab displays the complete spectral contribution of each dye in a selected well over the duration of the PCR run. The data shown is post-run data only. The graph of relative fluorescence shows how fluorescence signal changes over time. The Component graph displays only the components of the first selected well if more than one well is selected.



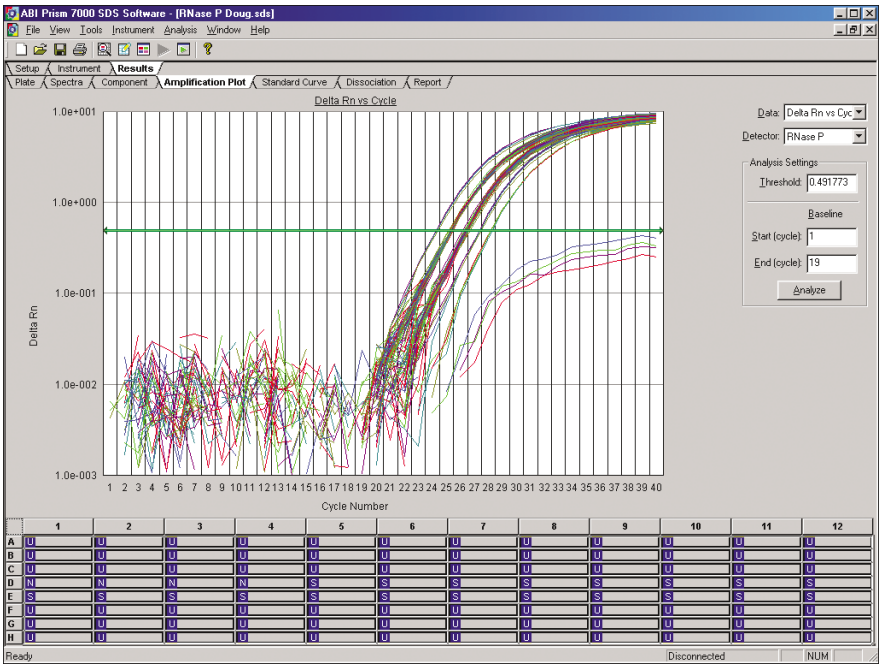
Elements of the Component Tab

Element	Description
Component graph	The Y-axis of the graph is relative fluorescence and the X-axis is cycle number. The data plotted in this graph includes all the dyes.
Components color legend	The Components legend to the right of the graph identifies the dyes in the multicomponent plot by color. In the figure above, A1 is selected with red as the FAM™ component, blue as the VIC™ component, and green as the ROX™ component.
Plate matrix	The section at the bottom of the window is a representation of the plate document that allows you to select the samples to view on the Component graph. This view only displays the detector labels. When a sample is selected, data for that sample is added to the graph. The legend is also updated. The components are displayed for only one well at a time.

Amplification Plot Tab

The Amplification Plot pane is provided to view both real-time and post-run amplification of specific samples. The Amplification Plot displays all samples selected in the wells. The specific features for this view include:

- Sample data (R_n, ΔR_n) for single and multiple samples
- Sample data for specific detectors
- Sample data for specific experiments
- Data in 2D (X is the cycle or well position; Y is C_T, R_n, ΔR_n)
- Data in real-time as well as post-run amplification



Description of the Amplification Plot

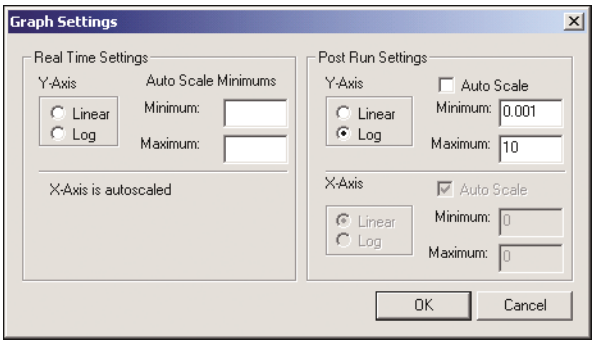
Element	Description
Amplification graph	The plot displays a visual graph of the sample amplification.
Graph Settings dialog box	Double-click the X-axis or Y-axis of the plot to display the Graph Settings dialog box. In the Graph Settings dialog box, choose: • Log view to scale the Y-axis in decimals, for example, 0, 0.1, 1.0, 10, 100. • Linear view to set the baseline.
Threshold bar	The Amplification Plot has a green threshold bar that can be dragged up and down to set the threshold for the data. Dragging the bar changes the bar to red until data is analyzed again. Click the Analyze button to reanalyze the data after dragging the bar.

Description of the Amplification Plot *(continued)*

Element	Description
Data menu	The Data menu sets the type of data to display in the plot: • **Rn vs Cycle** – The X-axis of the plot is the cycle, and the Y-axis of the plot is either R_n, or Log R_n. • **Delta Rn vs Cycle** – It shows the normalized reporter signal minus the baseline signal. Y-axis is ΔR_n, X-axis is cycle number. • **Ct vs Well** – The X-axis shows the well number (1 through 96) and the Y-axis is C_T.
Detector menu	Sets the graph to view the selected wells and updates the graph to show the data from the Detector Manager setup. You can view the wells according to their setup on the plate document or display all of them.
Analysis Settings	Contains settings for threshold and baseline start and end. Editing these fields enables the Analyze menu item and button (in this pane and on the toolbar). • **Threshold** sets the threshold to calculate the C_T values. • **Baseline Start (cycle):** Sets the cycle number for the start of the baseline calculation. To choose a number to start a new calculation for a baseline, the number must be less than the Baseline End value. • **Baseline End (cycle):** Sets the cycle number for the end of the baseline calculation. The number must be greater than the Baseline Start value. The Analyze button analyzes the data the same as the Analyze menu item and the Analyze button on the toolbar.
Plate matrix	This section is the same as on the Spectra and Component panes, a representation of the 96 wells.

Graph Settings Dialog Box

To open the Graph Settings dialog box, double-click the legend on either the Y-axis or the X-axis of the amplification plot graph.



Descriptions of the Graph Settings Dialog Box

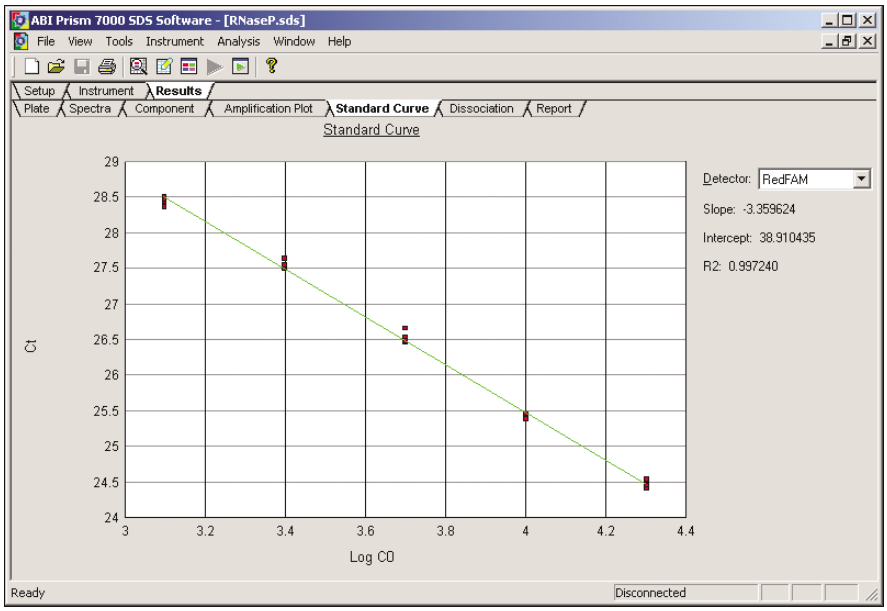
The Graph Settings dialog box allows you to set the scale of the Y-axis and the X-axis.

Graph Settings

Element	Description
Real Time Settings	Sets the graph scaling during real-time data collection. Auto Scale is the default. To manually change the default settings, you have to change them before you start the run. • **Linear** and **Log** radio buttons – Sets the Y-axis of the graph to log or linear. • **Auto Scale Minimums** fields – Sets the minimum values for the maximum R_n and minimum R_n on the Y-axis. These settings mean that the graph will never be autoscaled below these values. However, when the data exceeds these values, the graph will be autoscaled. Default value for Minimum is 0.
Post Run Settings	Sets the graph scaling for post-run data. • **Linear** and **Log** buttons – Sets the Y-axis of the graph to log or linear. • **Auto Scale** check box. Sets the graph to be autoscaled based on the data. Autoscaling sets the scale a bit above the maximum data point and a bit below the minimum data point. When Auto Scale is turned on, the Maximum R_n and Minimum R_n fields are disabled. • **Maximum** and **Minimum** R_n fields. Allows you to manually set the maximum and minimum values for the Y-axis scale. These fields are enabled when the Auto Scale check box is not checked.
OK button	Accepts the changes you have made, closes the dialog box, and changes the scale of the graph.
Cancel button	Discards the changes you have made and closes the dialog box.

Standard Curve Tab

The Standard Curve tab is available from the Results tab.



The Standard Curve pane displays the standard curve for samples designated as standards. The X-axis is the starting copy number, and the Y-axis is the threshold cycle (C_T). Standards appear on the curve as squares.

Elements of the Standard Curve

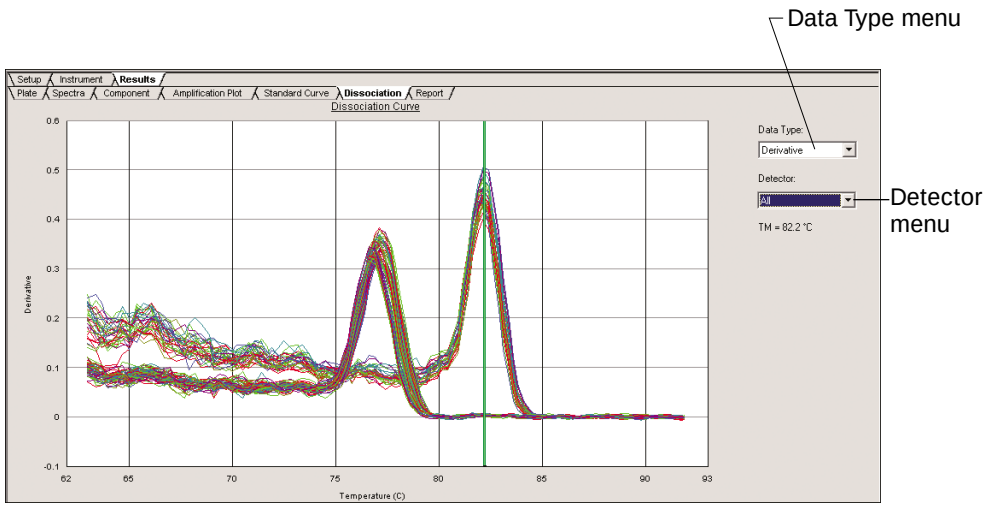
Element	Description
Standard Curve graph	The X-axis of the graph is the starting copy number. The Y-axis is C_T. Standards are plotted as data points, with a curve line extrapolated from the data points.
Detector menu	Sets the detector to view and update the graph to show the data from that detector. You can view the detectors that were added to the plate document, or you can choose All.
Standard Curve pane	The Standard Curve pane displays statistical data associated with the standard curve. Included in the box are statistics for: • Slope • Intercept • R2

Allelic Discrimination Tab

The Allelic Discrimination tab is only available from the Results tab when the plate is set up for this type of plate read. For a description of the Allelic Discrimination tab, refer to “Allelic Discrimination Assay” on page 3-44.

Dissociation Tab

The Dissociation tab displays the melting (T_m) curves associated with a dissociation assay. The data is only shown when using SYBR® Green and when Dissociation Protocol has been checked on the Instrument tab.



Elements of the Dissociation Tab

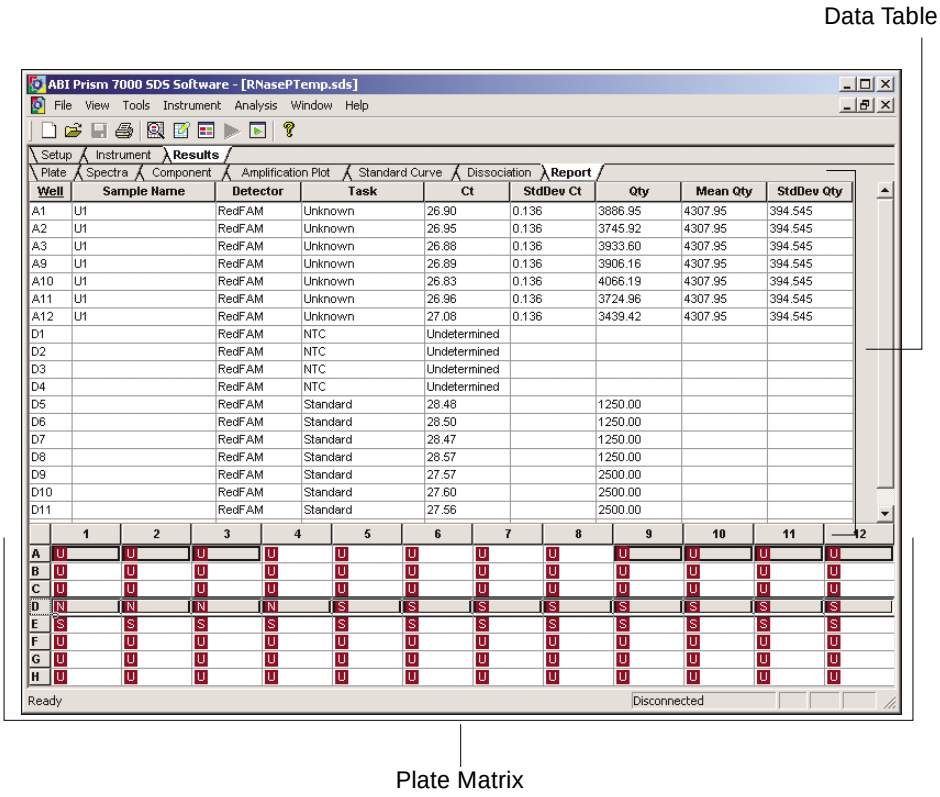
These selections are only available for data using SYBR Green and when the Dissociation check box on the Instrument pane is active.

Dissociation Tab

Element	Description
Dissociation graph	The X-axis is temperature. The Y-axis is the raw fluorescence or derivative of raw fluorescence.
Data Type menu	Sets the data being displayed in the curve. The menu contains Derivative and Fluorescence.
Detector menu	Shows the detectors and updates the graph to show the data from that detector. The menu contains the detectors that were added to the document, or shows all of them.
Plate matrix (not shown above)	Represents the wells in the plate document.

Report Tab

The Report tab displays a tabular report of data. The data columns associated with the report are determined by the assay you are running. Refer to the assay descriptions for details on what columns are displayed. The data displayed in the report depends on the wells you select in the plate matrix.

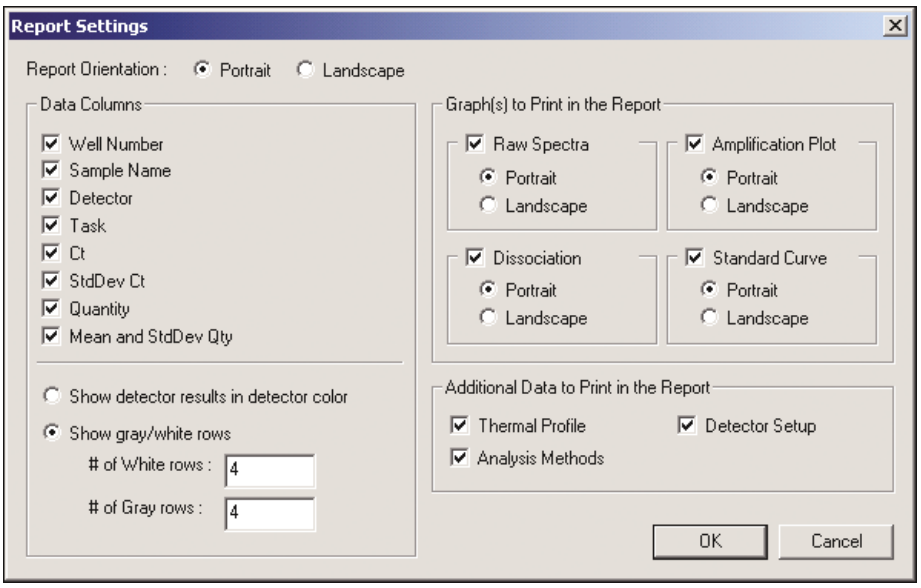


Elements of the Report Tab

Element	Description
Data table	Columns displayed are dependent on the assay you are running.
Plate matrix	Represents the wells in the plate document.

Report Settings Dialog Box

The Report Settings dialog box formats the display of the report and how the report will be printed. The following screen capture is a typical window for an absolute quantification assay. There are other data columns shown for an allelic discrimination assay.



Report Settings

Element	Description
Report Orientation	Specifies whether to print the report in portrait or landscape format.
Data Columns	This table contains the data columns associated with the document's assay. You can set columns to hide or show data. Print orientation of the column data is set in Page Setup. Other controls: • **Don't include column data** indicates not to include column data on the printed report. When this is checked, the printed report will not contain column data. • **Show results in detector color** shows results in the plate detector color. When this is checked, it displays the text of the detector results in the Report table in the detector color assigned to the wells. • **Show gray/white rows** displays a gray or white background for each row when checked. Frequency of the gray and white background is set by the number of white rows field and number of gray rows field. The range is 1 to 9.
Graph(s) to Print in the Report	Specifies which graphs to include in the printed report: Raw Spectra, Amplification Plot, Dissociation, or Standard Curve. Also, sets whether the graphs will be printed in portrait or landscape orientation in the report.

Report Settings *(continued)*

Element	Description
Additional Data to Print in the Report	Specifies other data to include in the printed report. • **Comments** – Includes comments you have typed during setup. • **Thermal Profile** – Includes a table containing the thermal profile on the printed report. • **Analysis Methods** – Includes the analysis methods used. • **Detector Setup** – Includes the list of detectors added to the document on the printed report.
OK	Accepts the changes you have made and closes the dialog box.
Cancel	Discards the changes you have made and closes the dialog box.

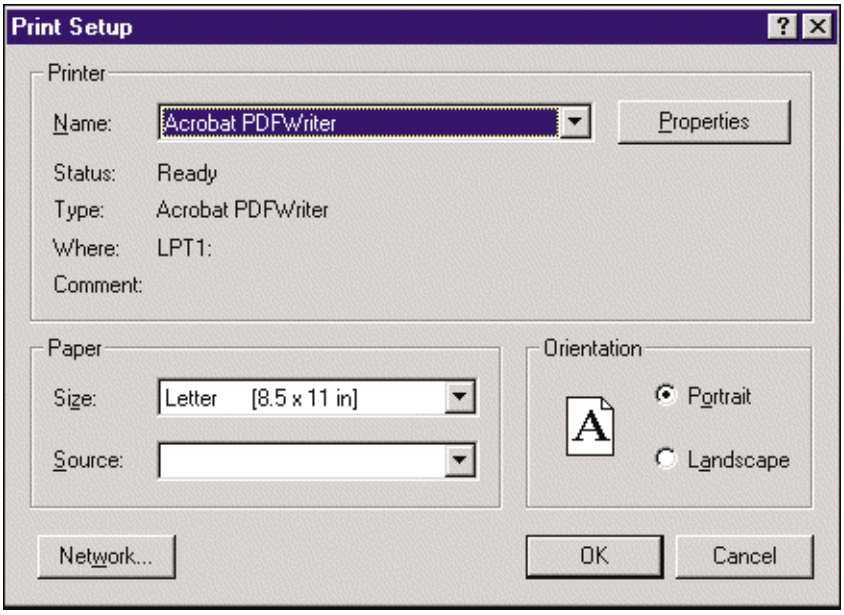
Printing

About Printing

The software prints individual windows as well as entire reports. The Print command prints the contents of the window to the printer in a WYSIWYG format, that is, one that prints the same way as you see it on the screen. Note that the Report tab has some special settings for printing, set in the Report Settings dialog box.

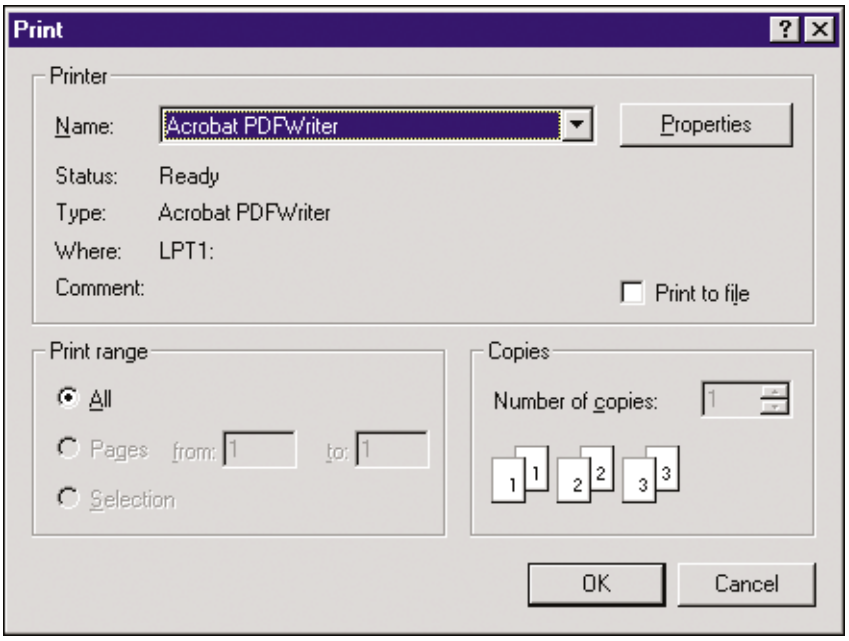
Page Setup

The Page Setup dialog box configures the printer and paper settings.



Print Dialog Box

The Print dialog box is the standard window as shown below.

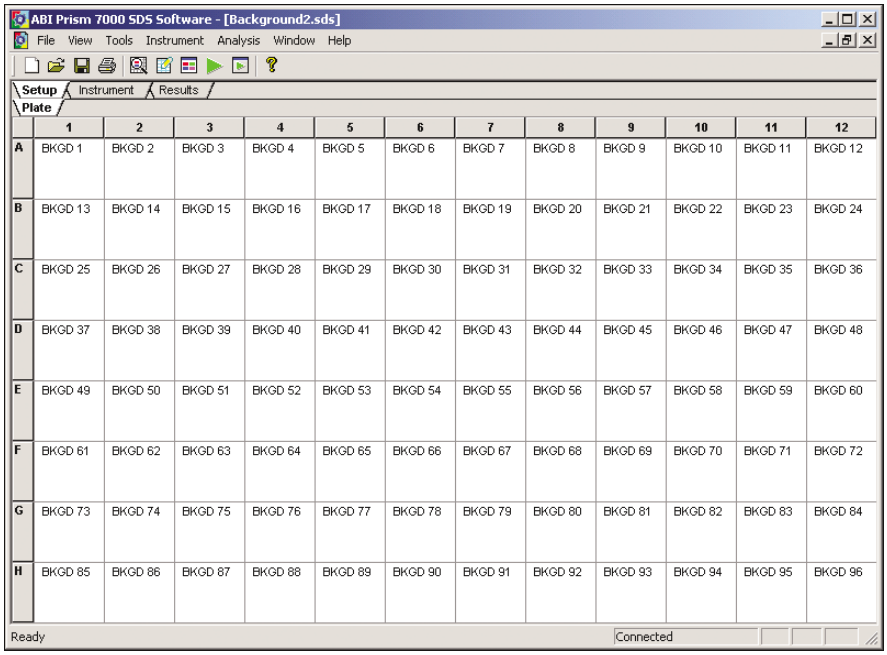


Background Assay

About the Background Assay

The background assay calibrates the background component. The background component, along with the spectra component [see “Pure Spectra Assay (Pure Dyes)” on page 3-38], helps the software determine the contribution of each fluorescent dye to the raw spectra.

This assay is a plate read. Thermal cycler conditions are fixed at 60 °C for a specified number of minutes.



	1	2	3	4	5	6	7	8	9	10	11	12
A	BKGD 1	BKGD 2	BKGD 3	BKGD 4	BKGD 5	BKGD 6	BKGD 7	BKGD 8	BKGD 9	BKGD 10	BKGD 11	BKGD 12
B	BKGD 13	BKGD 14	BKGD 15	BKGD 16	BKGD 17	BKGD 18	BKGD 19	BKGD 20	BKGD 21	BKGD 22	BKGD 23	BKGD 24
C	BKGD 25	BKGD 26	BKGD 27	BKGD 28	BKGD 29	BKGD 30	BKGD 31	BKGD 32	BKGD 33	BKGD 34	BKGD 35	BKGD 36
D	BKGD 37	BKGD 38	BKGD 39	BKGD 40	BKGD 41	BKGD 42	BKGD 43	BKGD 44	BKGD 45	BKGD 46	BKGD 47	BKGD 48
E	BKGD 49	BKGD 50	BKGD 51	BKGD 52	BKGD 53	BKGD 54	BKGD 55	BKGD 56	BKGD 57	BKGD 58	BKGD 59	BKGD 60
F	BKGD 61	BKGD 62	BKGD 63	BKGD 64	BKGD 65	BKGD 66	BKGD 67	BKGD 68	BKGD 69	BKGD 70	BKGD 71	BKGD 72
G	BKGD 73	BKGD 74	BKGD 75	BKGD 76	BKGD 77	BKGD 78	BKGD 79	BKGD 80	BKGD 81	BKGD 82	BKGD 83	BKGD 84
H	BKGD 85	BKGD 86	BKGD 87	BKGD 88	BKGD 89	BKGD 90	BKGD 91	BKGD 92	BKGD 93	BKGD 94	BKGD 95	BKGD 96

Elements of the Plate Document

The configurations for the background assay are listed in the table below.

Plate Document

Element	Description
Detectors	The background assay does not require probes, therefore, the Well Inspector is disabled.
Tabs and views	• Setup tab • Instrument tab: – The Thermal Profile has only one Stage and one Step. – The temperature field is enabled. – The following are disabled: time field, buttons for adding cycles, holds, and steps, Dissociation Protocol check box, and 9600 Emulation check box. • Results tab: Plate, Spectra, and Report.
Analysis menu	After viewing the raw background data in the Spectra tab, selecting Extract Background analyzes and extracts the background component. Executing this command automatically saves the background spectra information into the calibration folder.
Sample Setup	No sample setup is required.
Results tabs	• Plate tab – shows the background fluorescence intensity values of all wells. • Spectra tab – Shows the fluorescence of the selected wells. • Report tab – Shows results data for Well, Sample Name, and Intensity.

Pure Spectra Assay (Pure Dyes)

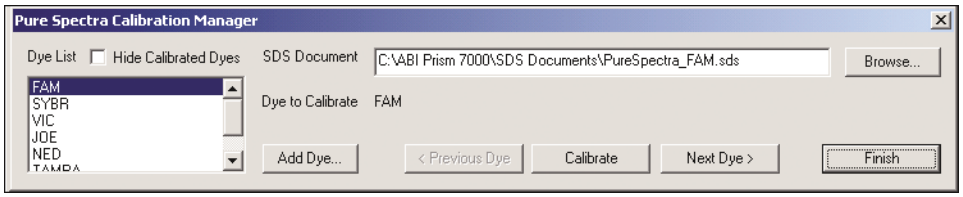
Overview

The Pure Spectra assay calibrates the pure spectra components. The pure spectra components, along with the background component, help the software determine the contribution of each fluorescent dye to the raw spectra.

Pure Spectra Calibration Manager

A Pure Spectra Calibration Manager provides a way to quickly calibrate the dyes on the 7000 system. The software will prompt you to load the appropriate dye plate in the 7000 system before running the calibration for each dye.

When you open a new plate document by selecting Pure Spectra from the Assay menu, a Pure Spectra Calibration Manager dialog box opens as shown below.



Pure Spectra Calibration Manager

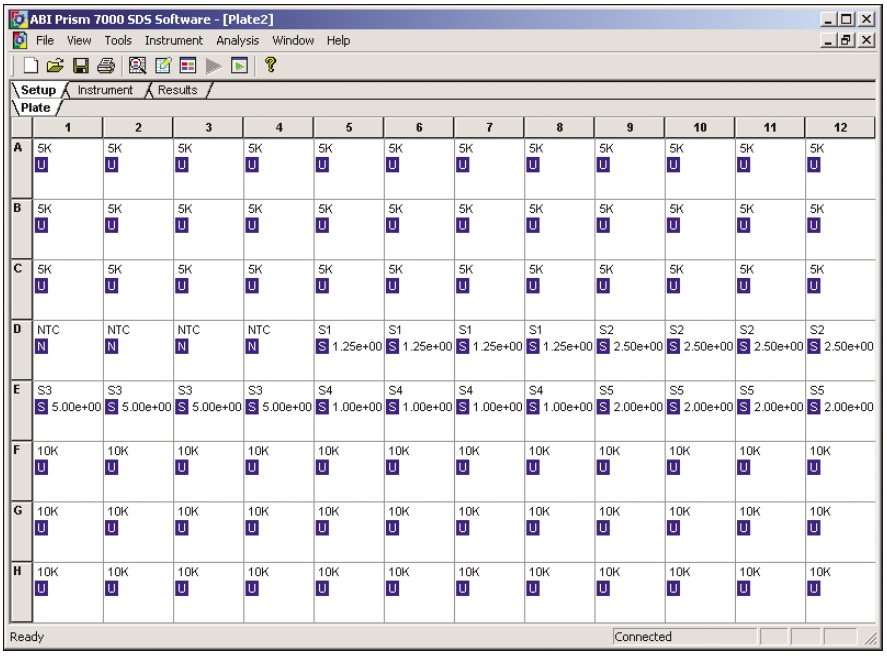
Item	Description
Dye List	List of dyes available to calibrate.
Hide Calibrated Dyes	Check box to show remaining dyes that have not yet been calibrated.
SDS Document field	Calibration directory listings.
Browse button	Button to look for a directory.
Dye to Calibrate	Shows the highlighted dye in the Dye List that will be calibrated.
Add Dye button	Opens the Dye Manager to select and add a new dye to the list to calibrate.
< Previous Dye button	Highlights the previous dye in the Dye List.
Calibrate button	Calibrates the highlighted dye in a few minutes. A message appears asking if you want to disconnect the active document. If you do not want to close the document, the dye will not be calibrated.
Next Dye > button	Highlights the next dye on the Dye List.
Finish button	Finishes the calibration.

Absolute Quantification Assay

About Absolute Quantification

The Absolute Quantification assay quantitates unknown samples by interpolating their quantity from a standard curve.

During data collection, the software will use estimated Delta R_n to display this real-time assay. This involves a fixed baseline offset subtraction where 1 to 3 points are used to compute an average that is subtracted from every point. On the amplification plot, a menu enables you to select R_n or Delta R_n, where Delta R_n, during real-time collection, is the estimated Delta R_n. At the end of the run, analyzing the data will use the true baseline correction. This plate runs in real-time mode only. The following plate document shows a sample setup for an absolute quantification run.



The configurations for the Absolute Quantification assay are listed in the table below.

Elements of Absolute Quantification

Element	Description
Detectors	The Well Inspector panel is available.
Tabs and views	• **Setup** • **Instrument** (Start and Stop buttons) • **Results** tabs: Plate, Spectra, Component, Amplification Plot, Standard Curve, Dissociation, and Report
Sample Setup	Requires detectors for each sample position. To build a standard curve, the task of some detectors needs to be Standard. Detector tasks include Standard, Unknown, and NTC.
Results Report Data columns	The data columns available for results are Well, Sample Name, Detector, Task, Ct, StdDev Ct, Qty, Mean Qty, and StdDev Qty.

Analysis Menu

The Analysis menu for absolute quantification has more selections available than for the background assay.

Analysis Menu

Item	Description
Analyze	Starts an analysis of the data.
Analysis Settings	Opens the Analysis Settings dialog box to reset the threshold or baseline cycles and to reanalyze the data.
Display	Displays the values in the wells when you select the Results tab. • **Quantity** – Displays the quantity values for the detectors. • **Delta Rn** – Displays the Delta R_n value for the detector. • **Ct** – Displays the C_T values for the detectors

File Menu

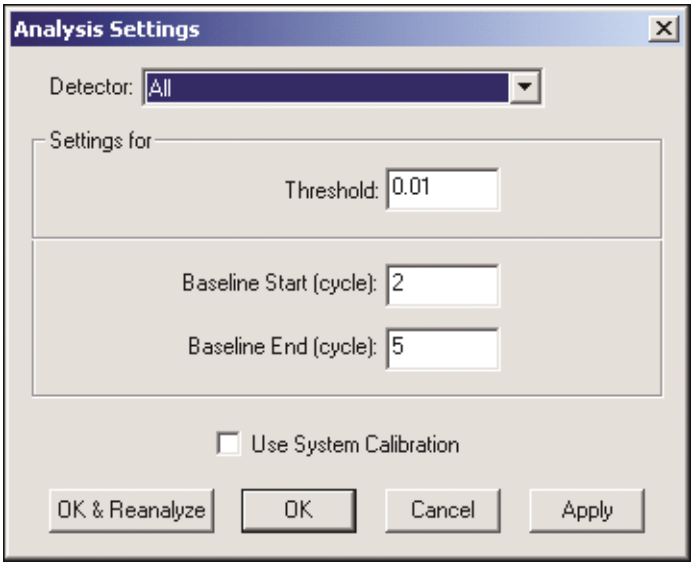
The File menu contains the export selections shown below.

File Menu

Item	Description
Export	• **Sample Setup** – Exports the setup of the plate document. • **Calibration Data** – Exports the data from the calibration file. The remaining selections allow you to export the data shown in the wells when you select the Results tab. • **Spectra** – Exports the spectra of the run. • **Component** – Exports the components. • **Delta Rn** – Exports the Delta R_n value for the detector. • **Ct** – Exports the C_T values. • **Dissociation** – Exports the dissociation values. • **Results** – Exports all the results of the run.

Analysis Settings Dialog Box

The Analysis Settings dialog box contains the editable default system analysis settings used to create new documents.



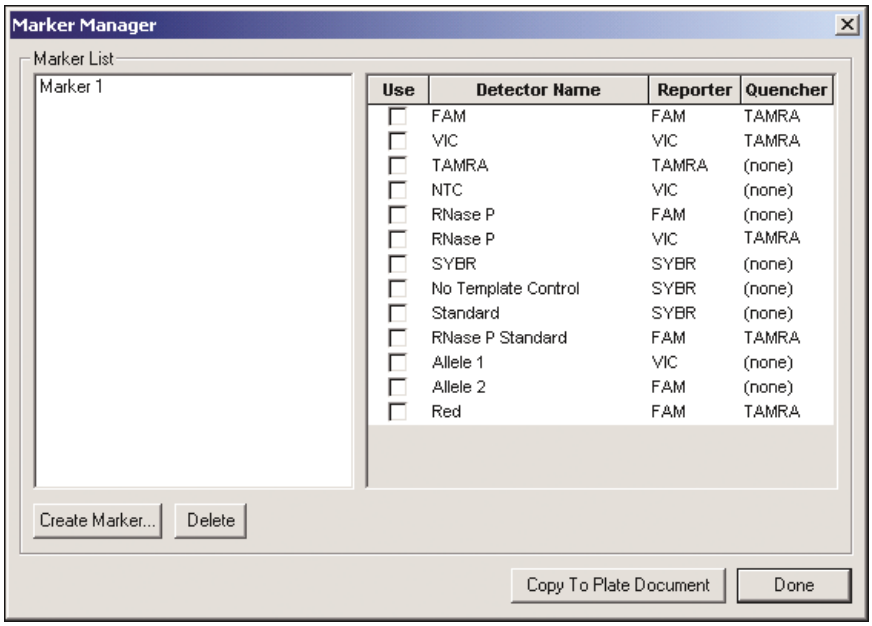
Elements of the Analysis Settings Dialog Box

Element	Description
Detector	Menu to select all detectors for the analysis or specific detectors in the plate document.
Threshold field	The Threshold field contains the manual threshold value. This is the value of the threshold bar.
Baseline settings	Baseline has two settings: • **Baseline Start (cycle)** sets the cycle number for the start of the baseline calculation. It must be less than the Baseline End value. • **Baseline End (cycle)** sets the cycle number for the end of the baseline calculation. It must be greater than the Baseline Start value.
Use System Calibration check box	Allows you to reanalyze a document using the current system calibration files.
OK & Reanalyze button	Accepts the changes and reanalyzes the data.
OK button	Accepts the changes you have made and closes the dialog box.
Cancel button	Discards the changes you have made and closes the dialog box.
Apply button	Applies the changes to the settings.

Marker Manager

About the Marker Manager

A marker is a named combination of two detectors. A marker is used to define two alleles (a target) when performing allelic discrimination assays. The Marker Manager is an editor that enables you to create, edit, and delete markers associated with the installed application and to add created markers to a document.



Marker Manager Definitions

The Marker Manager contains the list of marker elements for allelic discrimination.

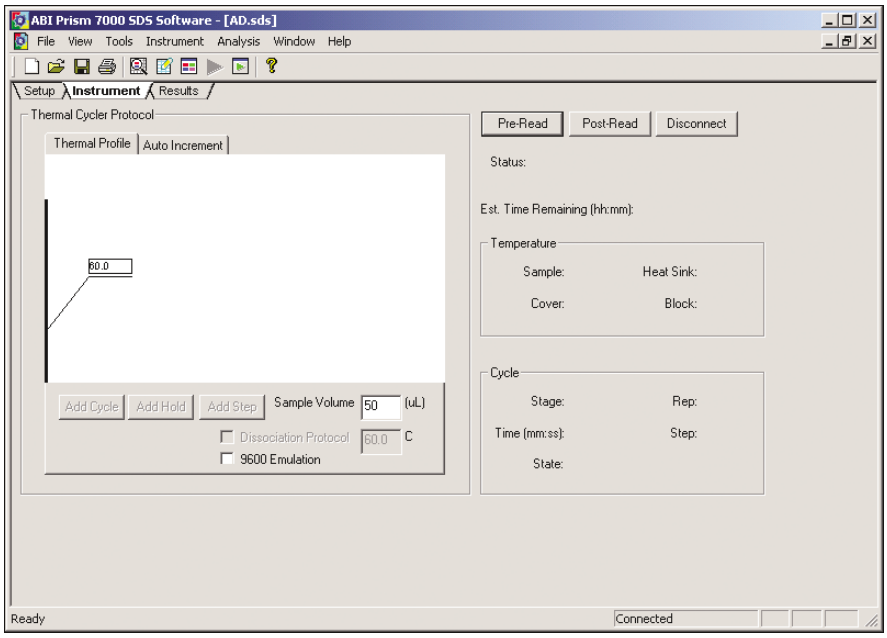
Marker Manager

Element	Description
Marker List	Contains a list of the markers that can be associated with the open document. Select a marker name to display which detectors in the detector list are associated with the marker. A marker is a combination of two detectors.
Detector list	Contains the list of detectors that can be associated with the open document. • **Use** – Check box that associates the detector with the selected marker. Only two detectors may be selected for a marker. • **Detector Name** – Name of the detector. • **Reporter** – Name of the detector reporter. • **Quencher** – Name of the detector quencher.
Create Marker	Opens the Create Marker dialog box. Enter a name for the new marker in the Name field. You must enter a unique name for each marker.
Delete	Deletes the selected marker. This action will remove the marker from all wells where it is defined. You cannot undo this operation.
Copy To Plate Document	Copies the marker with its checked detectors to the Well Inspector, and to the plate document.
Done	Accepts the selections and closes the Marker Manager.

Allelic Discrimination Assay

Allelic Discrimination Plate Document

The Allelic Discrimination tab is a secondary tab under the Results tab. It is only available when it has been set up as an allelic discrimination plate document to perform a pre-read, and then a post-read of a plate after it has been thermal cycled. The plate may be thermal cycled either using an absolute quantification assay on the 7000 system or offline. Allelic discrimination is a plate-read assay.



Elements of Allelic Discrimination

Configurations for the allelic discrimination assay are listed in the table below.

Allelic Discrimination

Element	Description
Detectors	The Well Inspector is enabled.
Detector tasks	The detector tasks are one of the following: • Unknown – Unknown sample. • NTC – No Template Control.
Tabs	• Setup tab • Instrument tab – The thermal cycler shows only one Stage with one Step. – Temperature field is enabled. – The buttons are Pre-Read, Post-Read, and Disconnect. – Buttons for adding or removing cycles, holds, and steps, fields for setting time and step, the Dissociation Protocol check box and field, and 9600 Emulation check box are all disabled. • Results tab – The Results tab has the following tabs: Plate, Spectra, Allelic Discrimination, and Report.
Results data columns	The data columns shown in the Report window are Well, Sample Name, Marker, Allele X Delta Rn, Allele Y Delta Rn, and Call.

Results Tabs

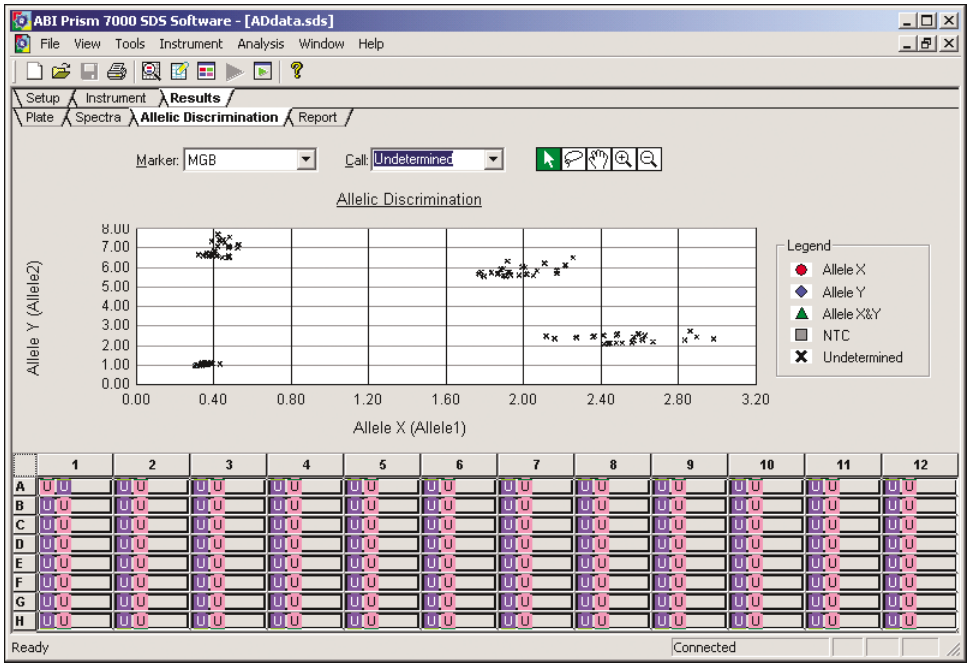
Secondary Tabs Under the Results Tab

Tab	Description
Plate tab	Representation of the plate document
Spectra tab	• Spectra of the pre-read or post-read of the allelic discrimination • Representation of the plate document
Allelic Discrimination tab	Graph and plate representation of allelic discrimination results. See “Elements of Allelic Discrimination Tab” on page 3-46.

Allelic Discrimination Tab

The Allelic Discrimination tab allows you to view a pre-read and a post-read analysis of a run. It is available from the Results tab when the plate document has been set up for allelic discrimination and a pre-read or post-read has been run. You can collect data from a pre-read and a post-read run, but you can only view post-read data. The pre-read is optional.

The following figure is an example of a post-read allelic discrimination run showing the calls before they have been associated with the alleles shown on the legend.



Elements of Allelic Discrimination Tab

Item	Description
Marker menu	Sets the marker to display in the graph. The menu contains the marker list.
Call menu	Menu that displays the list of valid calls.
Arrow tool	Enables you to select individual data points in the graph or drag a rectangle around multiple data points to select them. Use the Control key and click to select multiple data points individually.
Lasso tool	Identifies the markers when you draw a circle around them.
Hand tool	The hand tool changes to a hand when you click and hold it on the plot. When it is selected, use it to move the scatter plot around in the pane.
Zoom In tool	Zooms in on the data. There are two ways to zoom: • Click data points on the scatter plot to zoom in by two times magnification. • Click and drag a rectangle on the scatter plot to define a region to zoom.

Elements of Allelic Discrimination Tab *(continued)*

Item	Description
Zoom Out tool 	Zooms the data out. There are two ways to zoom out: • Click on the scatter plot to zoom out two times the magnification. • Double-click the Zoom Out icon to zoom out to full view.
Allelic Discrimination graph	The scatter plot region displays a scatter plot of dye components.
Graph Settings	Double-clicking the Y-axis or X-axis legends opens the Graph Settings dialog box for the currently displayed graph.
Legend	Displays the symbol and color data for the data points related to the various allele calls.
Plate matrix	Represents the wells in the plate document.

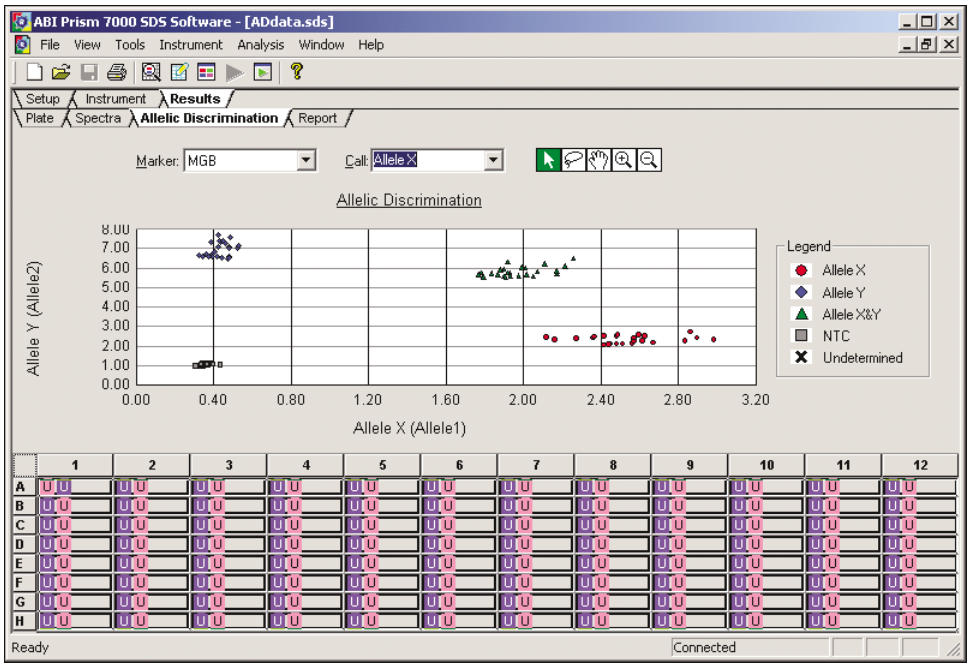
Meanings of Calls

The allelic discrimination assay enables you to classify results. The possible calls in this analysis are shown in the table below.

Possible Calls

Name	Meaning
Allele X	Sample contains the gene for Allele X.
Allele Y	Sample contains the gene for Allele Y.
Allele X & Y	Sample contains both Allele X and Allele Y genes.
NTC	No template control.
Undetermined	Sample that can not be associated with one population or another (no growth in X or Y).

The following figure is an example of a post-read allelic discrimination run showing the calls that have been associated with the alleles shown on the legend.



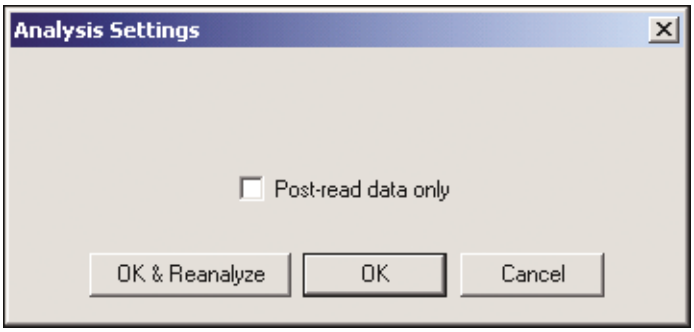
To open the Analysis menu, select it on the menu bar.

Analysis Menu

Item	Description
Analyze	Analyzes the data.
Analysis Settings	Opens the Analysis Settings dialog box.
Display	Menu containing the data elements that can be displayed in the Results/Plate tab. • Delta Rn – displays the R_n value for the marker. • AD Calls – displays the call for the marker (combination of two detectors).

Analysis Settings Dialog Box

To open the Analysis Settings dialog box, select it from the Analysis menu.



Elements of Analysis Settings

The analysis settings associated with the Allelic Discrimination assay are described below.

Analysis Settings

Element	Description
Post-read data only check box	Uses post-read data only in the analysis when this box is checked.
OK & Reanalyze button	Accepts the pending changes, closes the dialog box, and reanalyzes the data based on the new settings. This is available when analysis parameters are changed. It has the same function as the Analyze button on the toolbar and the menu item.
OK	Accepts the changes you made and closes the dialog box.
Cancel	Discards the changes you made and closes the dialog box.

Plus/Minus Assay

Overview

The Plus/Minus plate type reports R_n and ΔR_n results for unknown samples, and compares those results to a given threshold. If a sample is above the baseline, the sample is labeled “Plus.” (Threshold is determined by X standard deviations above the NTC. This is set in the Analysis Settings dialog box and should be expressed as a confidence value – particularly settings related to T-values.) If a sample is at or below the threshold, the sample is labeled “Minus.”

Plus/Minus Components

These are the configurations for the Plus/Minus assay.

Plus/Minus

Element	Description
Detectors	The Well Inspector panel is available.
Tabs and Views	• Setup • Instrument • Results tabs: Plate, Spectra, and Report
Sample Setup	Requires detectors for each sample position. Detector tasks include Unknown, NTC (No Template Control), IPC (Internal Positive Control), and IPC+.
Results Data Columns	The columns that show data for each well are Well (location), Sample Name, Task, Detector, Call (+/-/?), Delta Rn, Mean Delta Rn, and Std Dev Delta Rn.

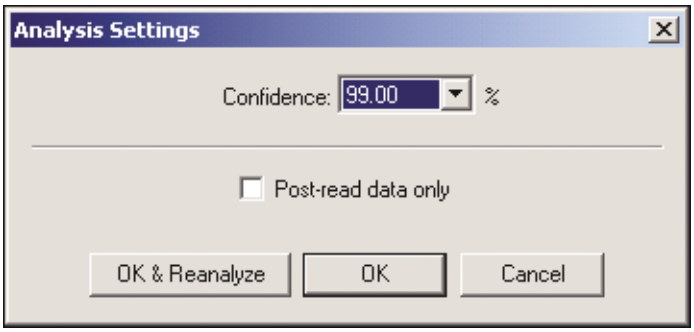
Analysis Menu

Analysis Menu

Item	Description	Status
Analyze Ctrl+G	Starts an analysis of the data.	Available when a document is open and analysis is required.
Analysis Settings… Ctrl+H	Opens the Analysis Settings dialog box.	Available when a plate document is open.
Display	Hierarchical menu containing the data elements that can be displayed in the Results/Plate tab. • Delta Rn – displays the ΔR_n value for the detector. • Call – displays the call, either +, -, or ? (undetermined).	Available when the Results/Report tab is active.

Analysis Settings Dialog Box

The Analysis Settings dialog box contains the analysis parameters associated with an assay.



Analysis Settings Components

Analysis Settings

Item	Description
Confidence menu	Select the confidence level for the calls in a +/– pre-read or post-read run.
Post-read data only	When this box is checked, the data in a plus/minus assay from a pre-read run is not used in the analysis if a pre-read has been done.
OK & Reanalyze	After making changes, they are accepted and the data is reanalyzed.
OK	Accepts the changes in the dialog box.
Cancel	Cancels any changes made.

Online Help

About Online Help

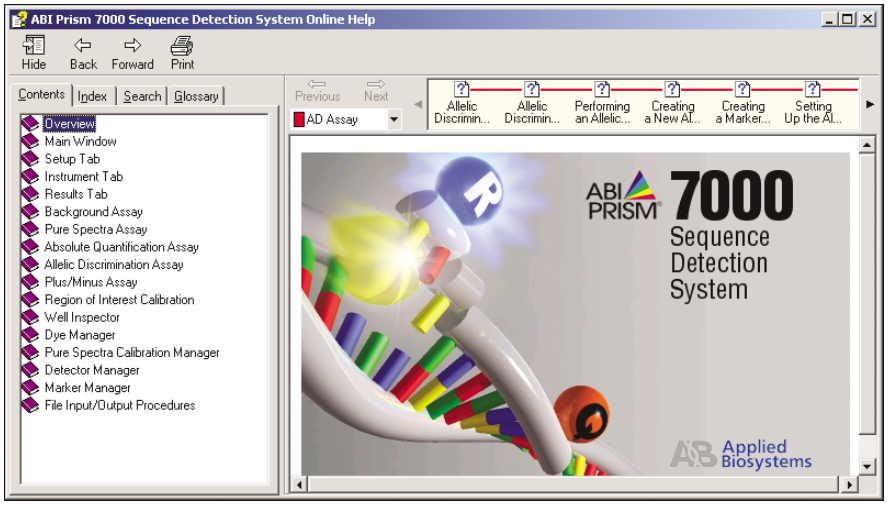
Online help is provided as a guide for the 7000 system accessed from the question mark on the toolbar. The online help feature has four tabs: Contents, Index, Search, and Glossary. Within the help pages there are underlined headings and buttons. Click the underlined headings to go to the page with that heading.

HTML Help

The HTML Help file provides online Help for the 7000 software. You can access this file at any time from the Window menu by choosing **Help ▸ Contents and Index**.

Help Topics

All Help topics are accessed by selecting the Contents, Index, Search, and Glossary tabs in the Navigation pane. You can Hide/Show the Navigation pane at any time or resize the Help window to manage space on the desktop.



Toolbar Icons

The table below describes the actions of the online help toolbar.

Online Help Toolbar

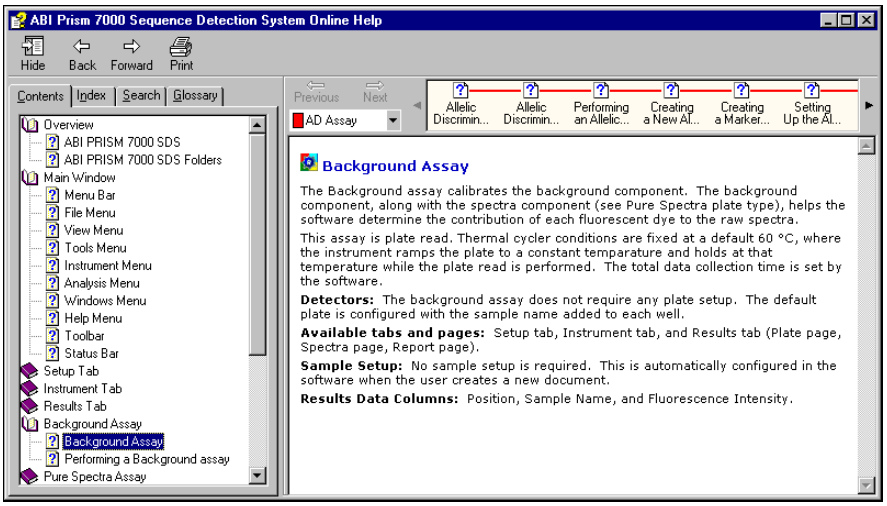
Toolbar Button	Action	Toolbar Icon
Hide	This button hides the Contents pane of the window. To open it again, click the Show button.	
Show	Shows the Contents pane when it is hidden.	
Back arrow	Goes back to the previous page.	
Forward arrow	Goes forward to the next page.	
Print	Opens the print dialog box where you can set it to print the selected topic or the selected heading and all subtopics. It then opens the printer connection.	

Online Help Toolbar *(continued)*

Toolbar Button	Action	Toolbar Icon
Previous arrow	Arrow in the topic browse sequence that goes back to the previous topic.	Previous
Next arrow	Arrow in the topic browse sequence that goes to the next topic.	Next

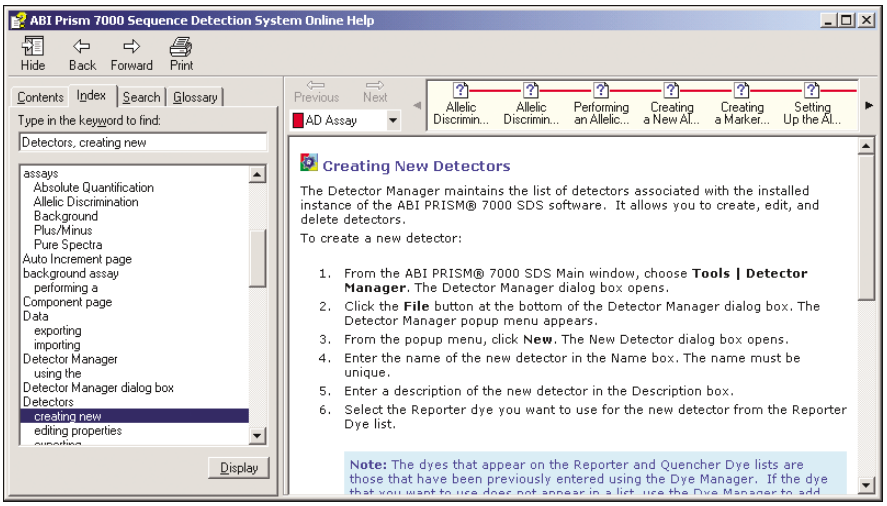
Contents Tab

The selections on the Contents tab have instructions on how to use the features in the 7000 software. The Contents tab organizes all of the Help topics into books that have other books or pages linked to topics. Double-click the book icon to display other books or pages. Click the topic to open a page and display the linked topic.



Index Tab

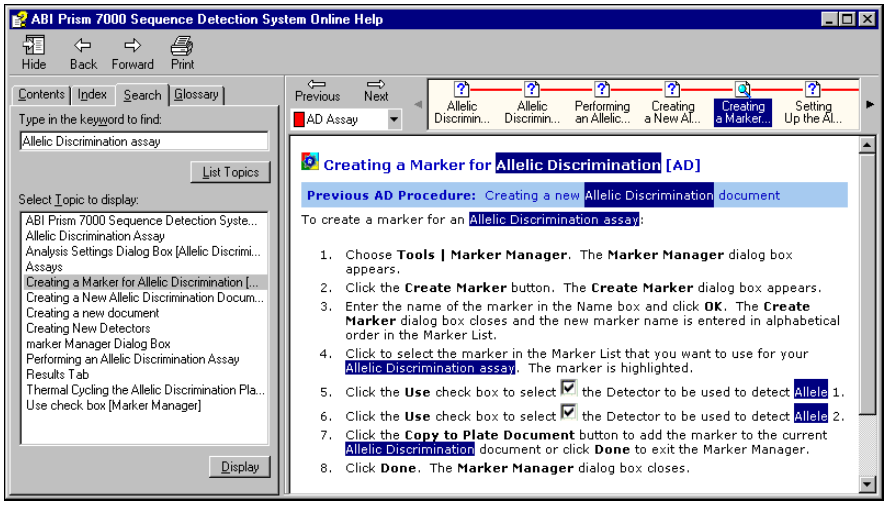
The Index tab provides an alphabetical list of words or phrases that link to corresponding topics.



The Index tab shows all the help subjects on one page. You can double-click each label to access the help information, or click a subject and select Display. In the text field above the list, type a one-word subject you are searching for and press Enter to show the available subject.

Search Tab

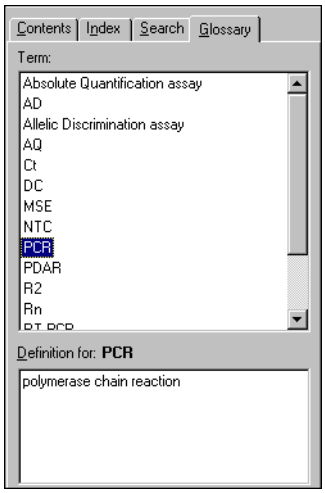
The Search tab allows you to search all topics for a specific word or phrase. All topics that contain this word or phrase are then listed for review, selection, and display.



You can type any word in the text field and press Enter or select the List Topics button to view the topics for help. Then highlight one of the subjects in the list and click Display or press Enter to go to the help page on the subject.

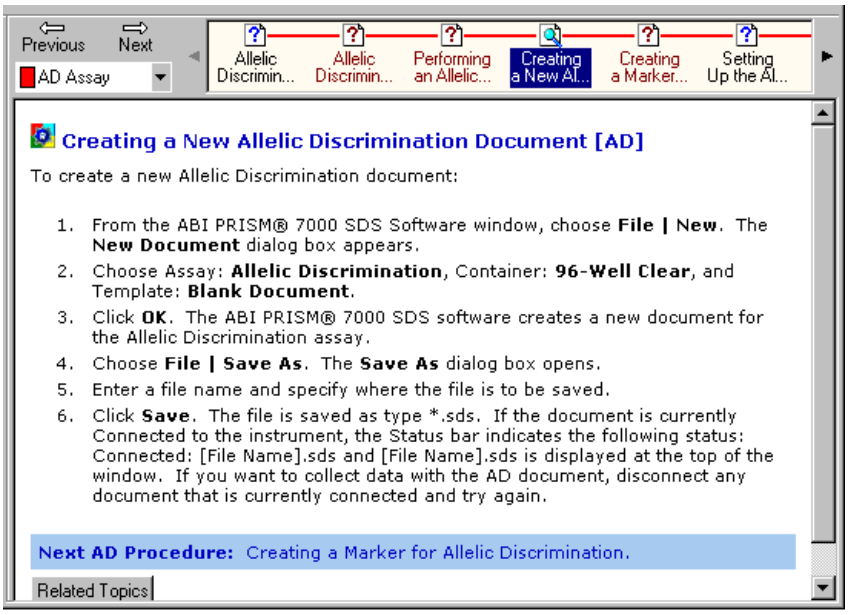
Glossary Tab

The Glossary tab provides an alphabetical list of terms and abbreviations that are used in many of the online Help topics. The definition is displayed in the bottom text box when you select the term.



Topic Browse Sequences

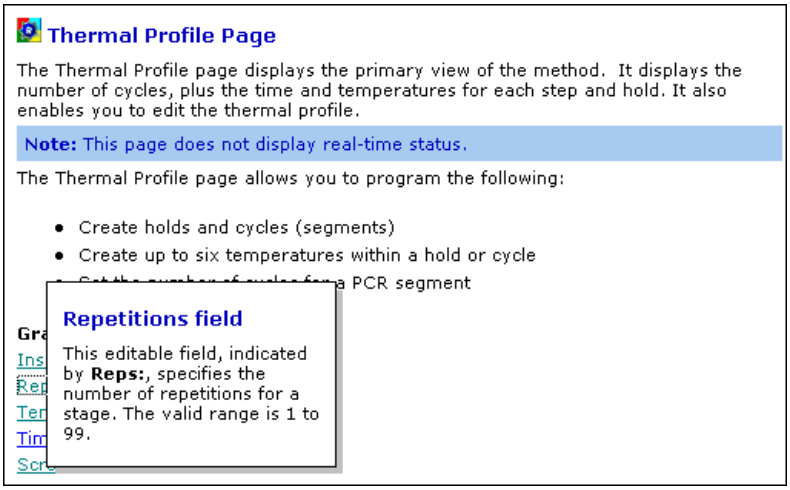
Topic browse sequences are provided for Allelic Discrimination (AD), Absolute Quantification (AQ), and Plus/Minus (PM) on the top right of the Help window.



Information Topics

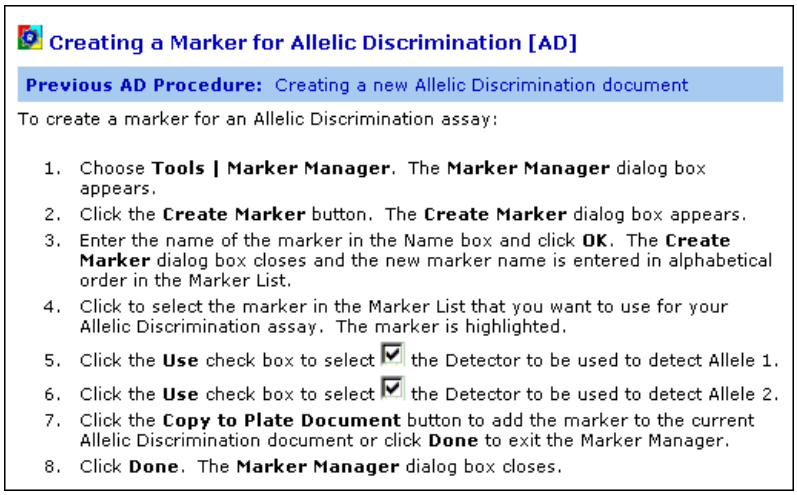
The Information topic describes how a page or dialog box works. All options and settings are described.

Popups are used within topics to reduce the space required and to minimize scrolling.



Procedure Topics

The Procedure topic gives the step-by-step sequence of events required to accomplish a specific task. The title of a procedure topic starts with a gerund, for example, Creating or Using.



Creating a Marker for Allelic Discrimination [AD]

Previous AD Procedure: Creating a new Allelic Discrimination document

To create a marker for an Allelic Discrimination assay:

1. Choose **Tools | Marker Manager**. The **Marker Manager** dialog box appears.
2. Click the **Create Marker** button. The **Create Marker** dialog box appears.
3. Enter the name of the marker in the Name box and click **OK**. The **Create Marker** dialog box closes and the new marker name is entered in alphabetical order in the Marker List.
4. Click to select the marker in the Marker List that you want to use for your Allelic Discrimination assay. The marker is highlighted.
5. Click the **Use** check box to select the Detector to be used to detect Allele 1.
6. Click the **Use** check box to select the Detector to be used to detect Allele 2.
7. Click the **Copy to Plate Document** button to add the marker to the current Allelic Discrimination document or click **Done** to exit the Marker Manager.
8. Click **Done**. The **Marker Manager** dialog box closes.

Basic Operation 4

Introduction

About This Chapter

This chapter provides basic procedures to help you become familiar with the ABI PRISM® 7000 Sequence Detection System software.

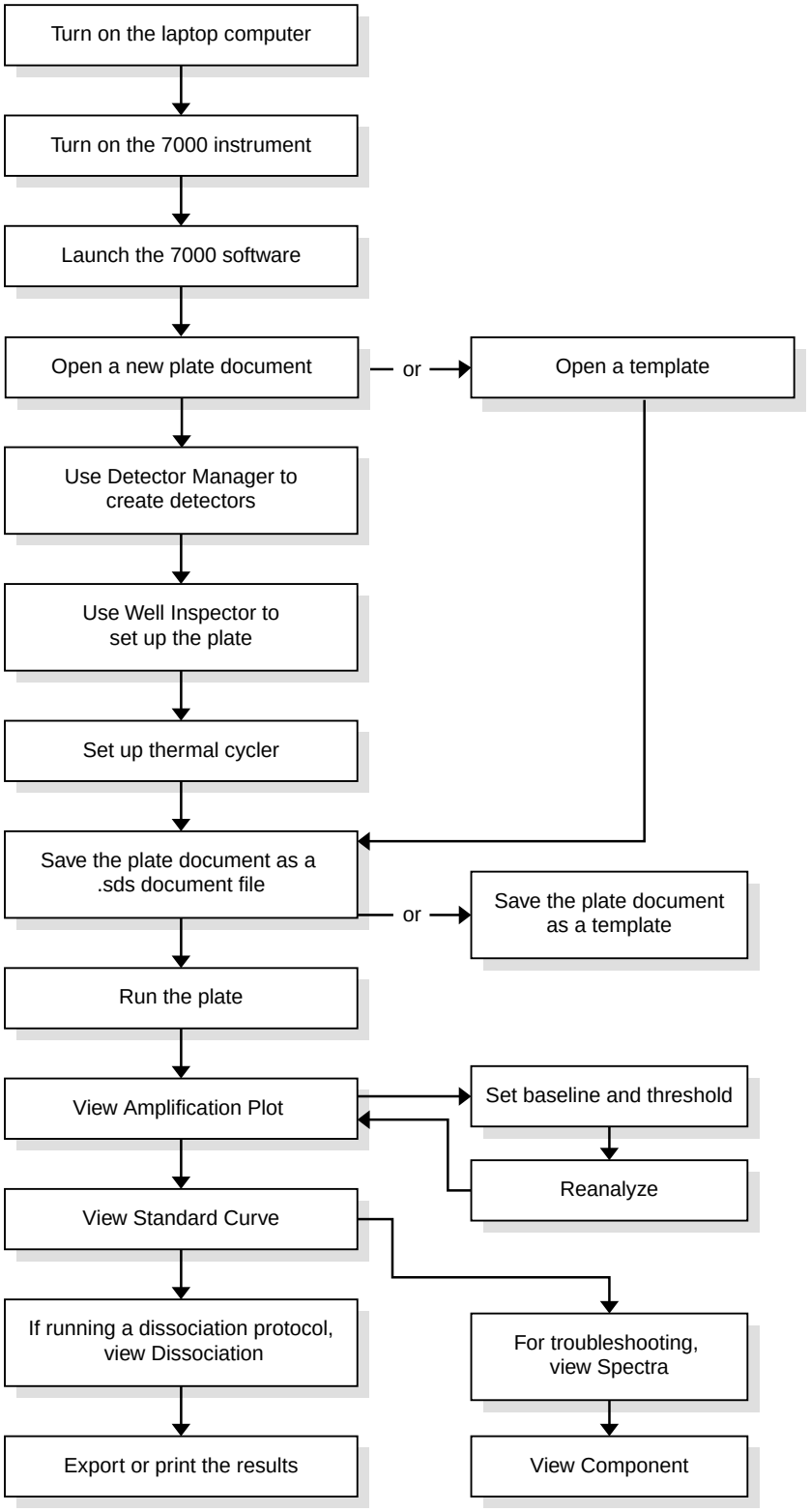
In This Chapter

This chapter contains the following topics:

Procedure Flowchart

Operation and Analysis Flowchart

The following figure illustrates the flow for setting up, running, and analyzing runs.



System Overview

About the 7000 System

The ABI PRISM 7000 Sequence Detection System is a second-generation sequence detection instrument capable of quantitative and qualitative detection with fluorescent-based PCR chemistries. The instrument is capable of quantitative detection using real-time analysis, and qualitative detection using end-point and dissociation curve analysis. The 7000 system analyzes assays arrayed in a 96-well format. It is designed for use with Applied Biosystems chemistries and assay development guidelines.

About Plate Documents

Overview

A plate document is an electronic file that represents the 96-well reaction plate in the thermal cycler. The plate document contains information on the sample types, primers and probes used, and sample locations on the 96-well reaction plate.

You can open a plate document in one of three ways:

- Create a new plate document
- Open a plate document template
- Open an existing plate document

What You Will Do

In this chapter you will learn:

- The purpose of a plate document
- How to set up a plate document
- A basic RNase P run
- Results of the run
- How to analyze the results

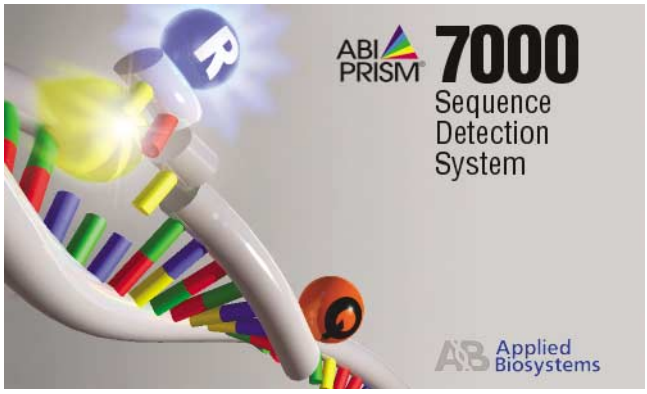
Creating a Plate Document

Creating a New Plate Document

To create a new plate document:

1. Double-click the **ABI Prism 7000 SDS Software** shortcut located on the desktop.

The splash screen like the one shown below will open, followed by a blank ABI Prism 7000 SDS Software window.



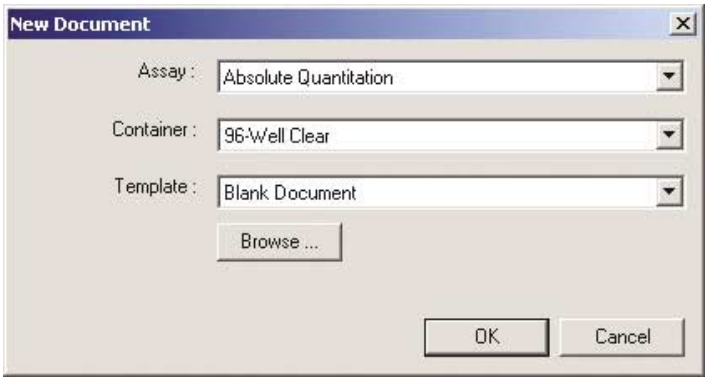
The plate document will show status bar messages. In the lower left, it will show Ready and in the lower right it will show Disconnected.

2. Select **File** ▸ **New** to open a new document.

To create a new plate document: *(continued)*

3. In the New Document window, make the appropriate selections for an absolute quantification plate document from the Assay, Container, and Template menus, as shown below and click **OK**.

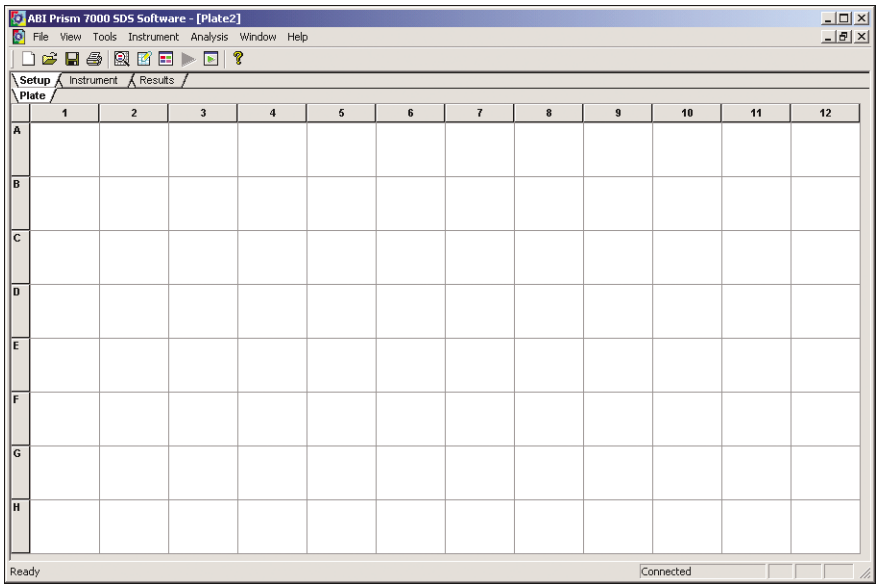
Menu	Selection
Assay	Absolute Quantitation
Container	96-Well Clear
Template	Blank Document



Instrument Messages

Each time you launch the software and create a new document, the software will initialize the instrument. You should see a message indicating that the instrument is being initialized. This message will last for a few seconds and then the instrument status will show Connected. Under normal conditions you should be able to hear the instrument initializing during this process. If the instrument fails to initialize, an error will be reported and the status will be Disconnected.

When the instrument is ready, the status bar will show Ready in the lower left and Connected in the lower right of the window, and a new plate document window will open as shown below.



Note: When you move the pointer over the icons on the toolbar, the status bar shows the action of the icon under the pointer.

Setting Up the Plate Document

Overview

The plate document is a representation of the 96-well reaction plate. A plate document must be set up before starting a PCR run. The setup process requires the following:

- Creating detectors
- Adding detectors to the plate document using the Detector Manager
- Adding detectors to the wells using the Well Inspector
- Selecting sample types
- Modifying the sample types
- Setting up the standards, if applicable

Setting Up the Dye Manager

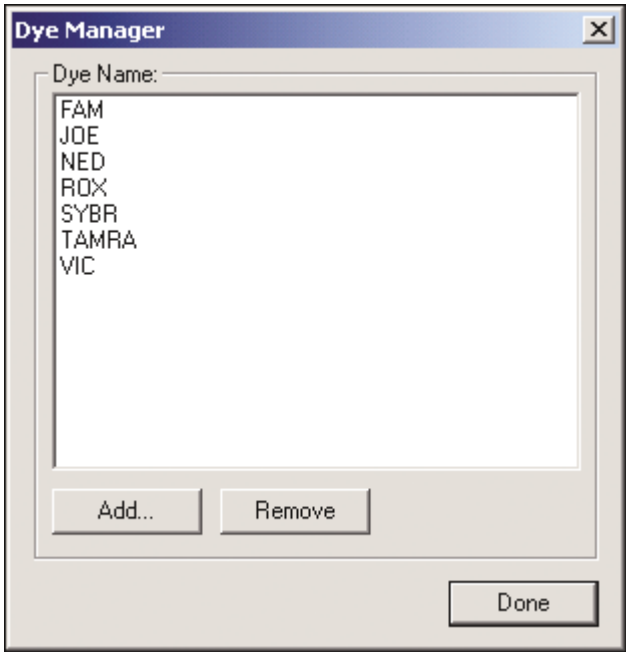
The Dye Manager maintains the list of dyes associated with the installed application. It also manages dyes that are created and deleted. The Dye Manager system dyes cannot be removed.

Note: The Dye Manager will be configured with the seven dyes shown in the figure in step 1 below. You will not need to set it up unless you have custom dyes that you want to use in your plate document.

When adding new dyes, the Dye Manager must be set up before setting up the plate document. To do this, make sure the Setup/Plate tabs are selected.

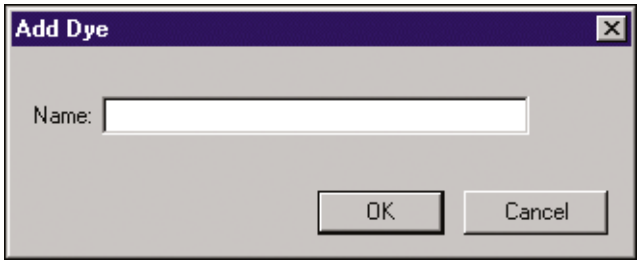
To set up dyes for the Dye Manager:

1. Select **Tools ▸ Dye Manager** to name the dyes and make them accessible to the plate document if they are not already in the Dye Manager.



To set up dyes for the Dye Manager: *(continued)*

2. Click the **Add** button and type a new name for one of the dyes you are using in the Add Dye dialog box, for example, **Custom**.



3. Click **OK**. The name will appear in the Dye Name field in the Dye Manager.

4. Continue naming and adding dyes until you have named all the ones you wish to add, then click **Done**.

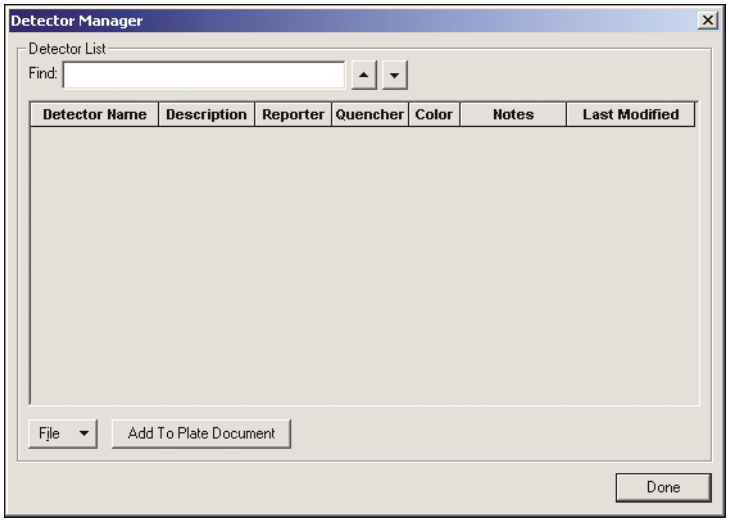
   Note: New dyes added to the Dye Manager must be calibrated and analyzed before they can be used in a document. For information on calibrating pure dyes, refer to "Pure Spectra Assay (Pure Dyes)" on page 8-17.

Using the Detector Manager

Detectors are the labels that are in the plate setup associated with the samples of the plate. In the Detector Manager, you can create detectors using dyes that have been calibrated and add them to the plate document.

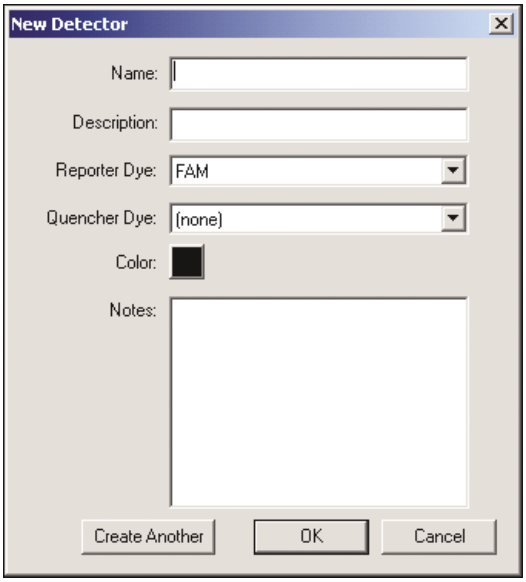
Using the Detector Manager:

1. Select **Tools** ▸ **Detector Manager**. The Detector Manager is where you will add detectors to the plate document.



Using the Detector Manager: *(continued)*

2. In the bottom left of the Detector Manager window, select **File** ▸ **New** to create new detectors.



3. Click in the New Detector window **Name** field and type a name for the new detector. For example, you can use the name of a unique gene.

4. In the **Description** field, type a specific description of the detector.

   Note: This field is optional.

5. From the **Reporter Dye** menu, select one of the dyes available in the Dye Manager, for example, **FAM**.

6. From the **Quencher Dye** menu, select one of the available dyes (for example, **TAMRA** or **none**).

7. Next to the **Color** label, click the black color square, which will open the color picker.

8. Select a color for the detector, and click **OK**.

9. This is optional:

   In the **Notes** field, type any notes appropriate for the detector.

10. To continue setting up the rest of the detectors, click **Create Another** and follow steps 3 though 9.

11. When you have finished setting up all the detectors, click **OK** in the New Detector window.

12. Click **Done** in the Detector Manager window.

Options for Detectors

Duplicating Detectors

This procedure and all of the following ones must be done from the Detector Manager.

To duplicate detectors in the Detector Manager:

1. Select an existing detector from the list, then click **File ▸ Duplicate**.
2. Give the detector a unique name.
3. Click **Done**.

Importing Detectors

To import detectors from another file into the Detector Manager:

1. Click **File ▸ Import**.
2. Select the detector to import.
3. Click **Import**.

   The Import dialog box closes and the selected detectors are added to the Detector Manager.

   Dyes will be added automatically to the Dye Manager if they do not exist.

Exporting Detectors

To export detectors from the Detector Manager to a flat file:

1. Select one or more detectors, then click **File ▸ Export**.

   The Export dialog box opens and the Detector name appears in the File Name field.
2. Click **Export** and specify a directory.

   The Detector is exported as a flat file to the directory that you specified.

Editing Detector Properties

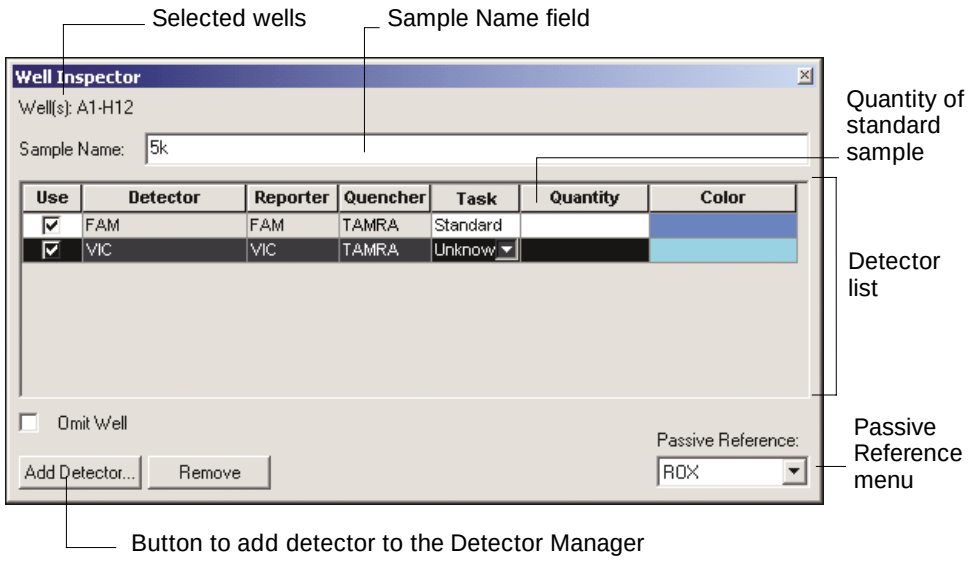
To edit detector properties in the Detector Manager:

1. Double-click a detector or select a detector and click **File ▸ Properties**.

   The Detector Properties dialog box opens.
2. Edit the appropriate properties and click **OK**.
3. The dialog box closes and the properties are updated and displayed in the Detector Manager.

Adding Detectors to the Well Inspector

After setting up the dyes and the detectors, you need to add the detectors to the wells of the plate. You can open the Well Inspector from the View menu, or click the icon located on the toolbar.



Note: When you highlight one of the detectors, the color will change temporarily to a color complementary to the one you selected. This will not change the color on this window or on the analysis windows.

To add detectors:

1. On the toolbar, click the **Well Inspector** icon .
2. In the **Well Inspector**, click the **Add Detector** button to open the Detector Manager.

   Note: If the Detector Manager window is covering the Well Inspector, you may want to move it to the side so you can see both.
3. In the **Detector Manager**, highlight the detector you want to use for the first sample, and click the **Add to Plate Document** button. This adds the detectors to the plate document and the Well Inspector, but you will not see them in the Plate Setup yet.
4. Highlight each remaining detector in the Detector Manager that you want for this plate document and click the **Add to Plate Document** button, then click **Done**.

Using the Well Inspector

When the Well Inspector is set up, you are ready to set up the plate document.

To set up the plate document:

1. Click and drag in the **Plate/Setup** to highlight all the wells (or all the wells in use).

   Note: You can highlight the wells with the Well Inspector open. They will highlight under the window.

2. In the **Well Inspector**, select the **Use** check box for detectors in those wells.

   Note: If the check mark appears gray, it is not checked. Clicking the Use column indicates which detectors to use for this sample.

3. Highlight the group of wells that are unknown samples, click the **Task** column and change it to **Unknown**.

   Note: The Passive Reference may be changed for the plate. The default Passive Reference setting is ROX.

4. Select one replicate of unknown samples and assign a unique name in the **Sample Name** field.

   Note: Unique names are used to identify replicates and report their average and standard deviation in the Report window.

5. Highlight the group of wells that are standards.

6. Click the **Task** column, and change it to **Standard**.

   Note: The task can be changed at any time during the setup.

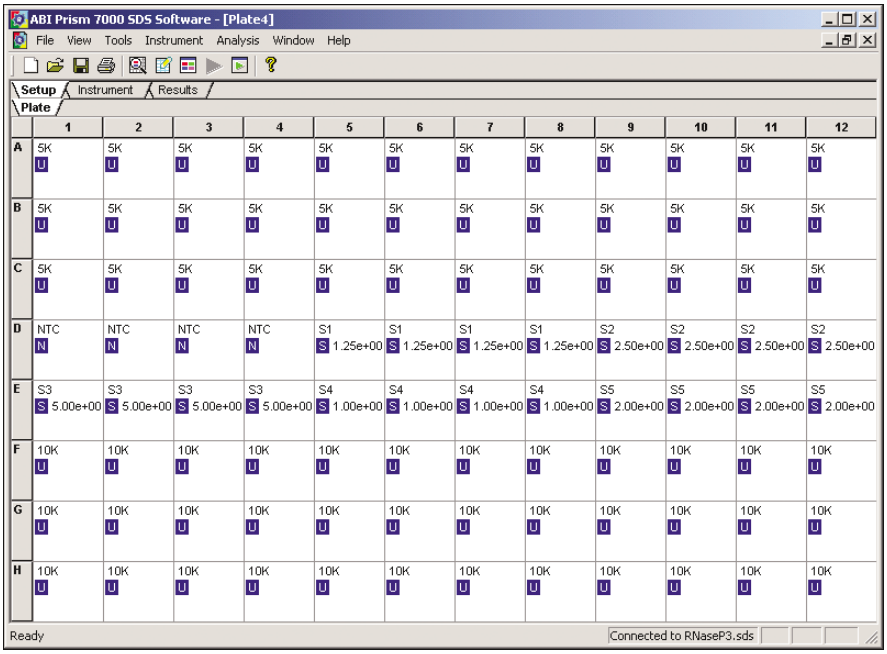
7. Select replicate standards, one group at a time, click the **Quantity** field and enter the numerical quantity (for example, 1250, 5000) for that standard group.

To set up the plate document: *(continued)*

8. Highlight negative control samples, click the **Task** column and select **NTC**.

   Note: For multiplex assays (multiple detectors per well), you will have to repeat steps 1 through 8 for each detector.

   The following figure is a sample of an RNase P setup of a plate document.



9. If at any time you want to change detectors in wells, highlight the wells, click the **Use** check box to uncheck the detector and remove it from the wells.

10. To apply a new detector to the highlighted wells, click the **Use** check box for the new detector.

11. If you want to delete a detector completely from the Well Inspector, highlight the detector and click **Remove**.

    This removes it from the plate document and the Well Inspector, but not from the Detector Manager. It can be added into the Well Inspector again from the Detector Manager.

12. When you have finished setting up the plate document, click the **X** in the upper-right corner in the Well Inspector dialog box to close it.

Any of the setups in the plate document can be changed by selecting them, then opening the Well Inspector, unchecking the Use column, and setting up and using a different detector for as many wells as you want in the new setup.

Saving the Plate Document as a Template

Once the plate document has been set up, it can be saved as a template so that it can be used for future runs. If you do not want to save this file as a template, go to "Saving the Plate Document for the Run," below.

To save the plate document as a template:

1. Select **File ▸ Save as**.

2. To save the file as a template, configure the **Save as** dialog box as follows:

   a. Click the **File name** field, and type a name for the file.

   b. Select **SDS Templates (*.sdt)** from the Save as type menu.

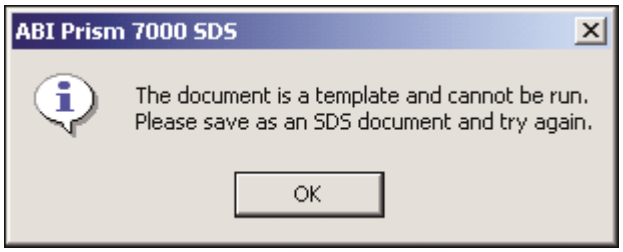
   Before you save the plate document it will not have an extension. After it is saved, it will have the .sdt designation.

3. Click **Save**.

   The software saves the plate document template as a **.sdt** file.

   This document can be used later as a template, and will be listed in the templates files when you open a new file.

4. To use the template, open the **.sdt** file from the **File** menu and save it as a new plate document (**.sds**) before starting a run. If you do not save the document, there will be a message to do so.



Saving the Plate Document for the Run

Once the plate document has been set up or saved as a template (.sdt), it needs to be saved as a .sds document before it can be run.

To save the plate document:

1. Select **File ▸ Save as**.

2. Configure the **Save as** dialog box as follows:

   a. Click the **File name** field, and type a name for the file.

   b. Select **SDS Document (*.sds)** from the Save as type menu.

   Before you save the plate document it will not have an extension. After it is saved, it will have the .sds designation.

3. Click **Save**.

   The software saves the plate document as a .sds file.

   This is the document you will be using to run the plate document in the next procedure.

Run Phases

An experiment performed completely on the 7000 system goes through three phases: Setup, Run, and Analysis, which are described below:

Run Phases

Phase	Name	Description
1	Setup	Define the experiment setup and thermal cycler conditions.
2	Run	Run the 7000 system with fluorescent signal changes as the polymerase chain reaction (PCR) progresses.
3	Analysis	• View the PCR amplification plots • View the standard curves • Calculate starting template amounts • Print and export data

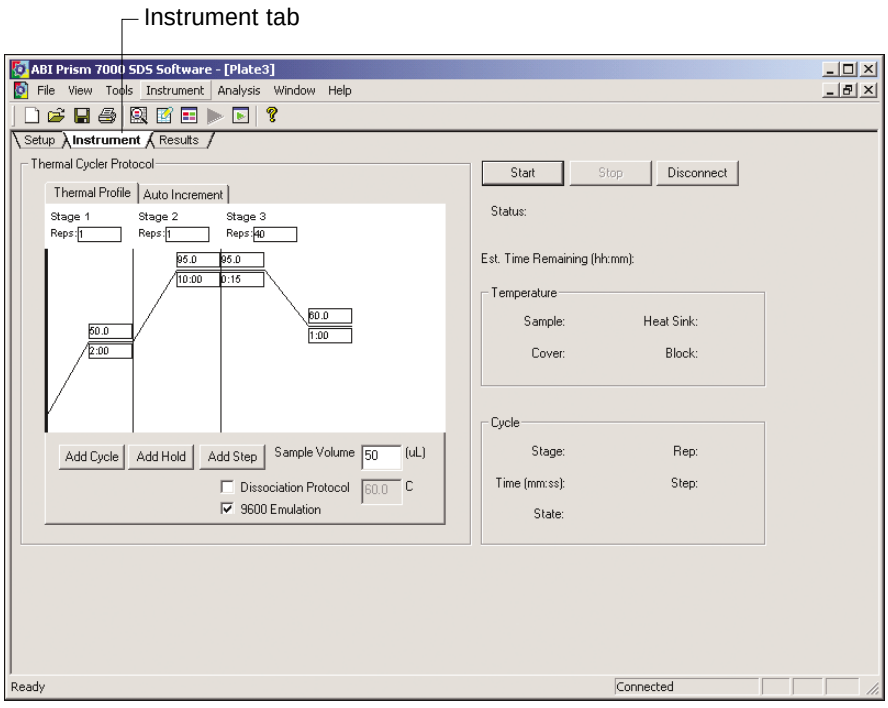
Running the Plate Document

The plate document has been set up. Now you are ready to check the thermal cycler settings and run the PCR.

To check thermal cycler settings and run the plate document:

1. In the plate document, click the **Instrument** tab.

   You can add cycles, holds, or steps and edit the time and temperature fields to fit the run. The following figure is set up with the conditions to run an RNase P plate.



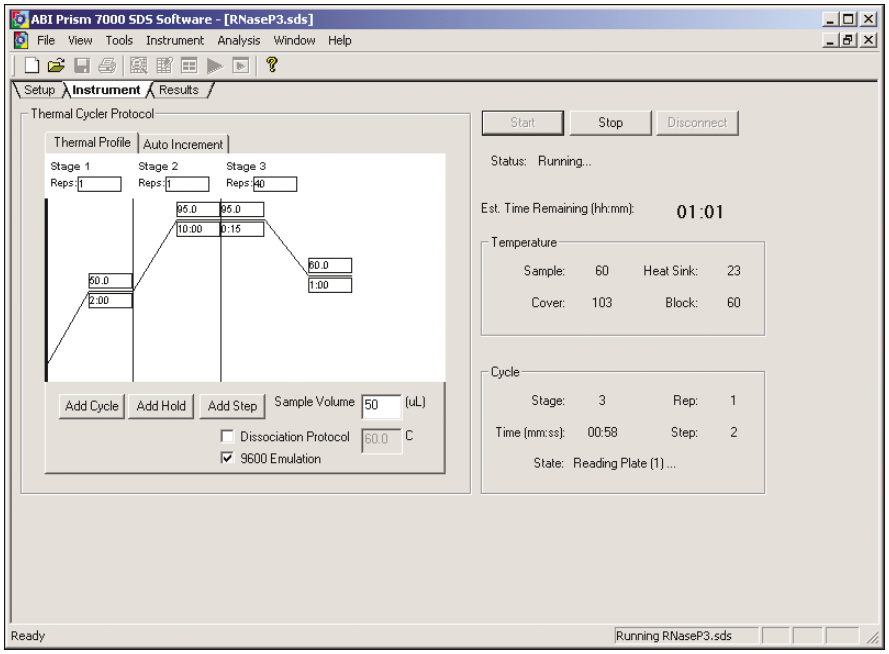
To check thermal cycler settings and run the plate document: *(continued)*

2. To change the conditions for the thermal cycler, click in the text boxes in the Thermal Profile pane, and type a new number of repetitions, new temperature, or time. You can add cycles, holds, or steps by clicking the pane, then clicking one of the Add buttons. Refer to "Thermal Cycler Protocol Buttons" on page 3-20 for these procedures.

3. Save the plate document.

4. Click **Start**.

   Status messages appear next to Status under the Start button as shown in the figure below, then the run starts, showing the time, temperature, and cycle of the run.



   The run time will vary depending on the experiment you are running. When it has finished, the time remaining is 00:00 and Status shows Idle.

Running the RNase P Installation Plate

RNase P Template

An RNase P template is included in the software and is available for running an RNase P installation plate. For this procedure, you will need the TaqMan® RNase P 96-Well Instrument Verification Plate (P/N 4310982). For more information on the Detector Manager, refer to “Detector Manager” on page 3-15.

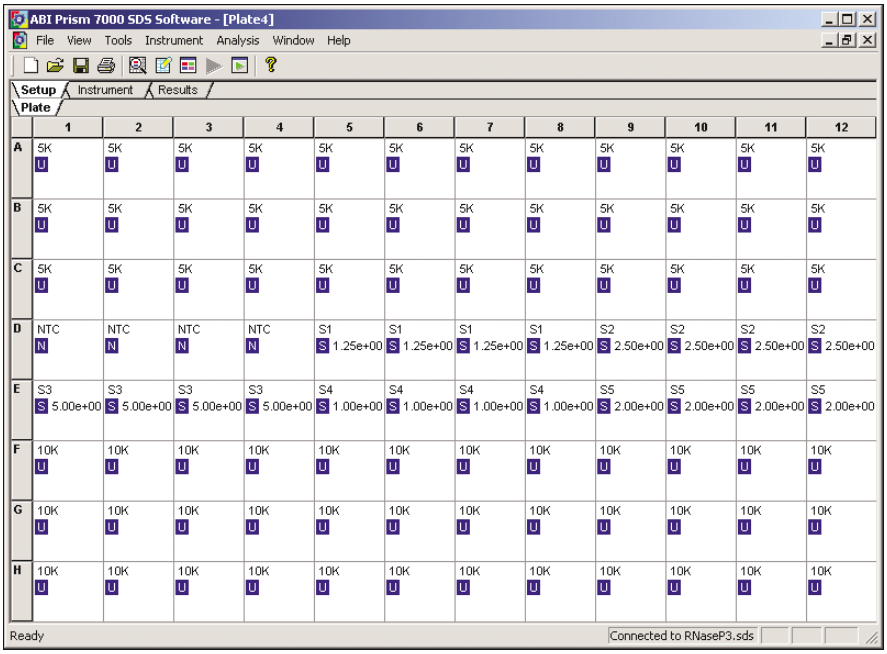
To open the RNase P template:

1. Select the **New Document** icon ( ) located on the toolbar.

2. From the New Document dialog box, make the following selections:

Menu	Selection
Assay	Absolute Quantitation
Container	96-Well Clear
Template	AQ RNase P Install.sdt

3. An RNase P plate document will open like the one shown below.



Since this template is already set up, save the document as a **.sds** file.

a. Select the **Save Document** icon ( ).

b. Type a name in the **File name** field.

c. Select **SDS Document** in the Save as type menu.

To open the RNase P template: *(continued)*

4. Select the **Instrument** tab ▸ **Start** button.

   During the run, you can view the real-time data in the Amplification Plot.

5. When the run has finished, you can view the results and reanalyze the data.

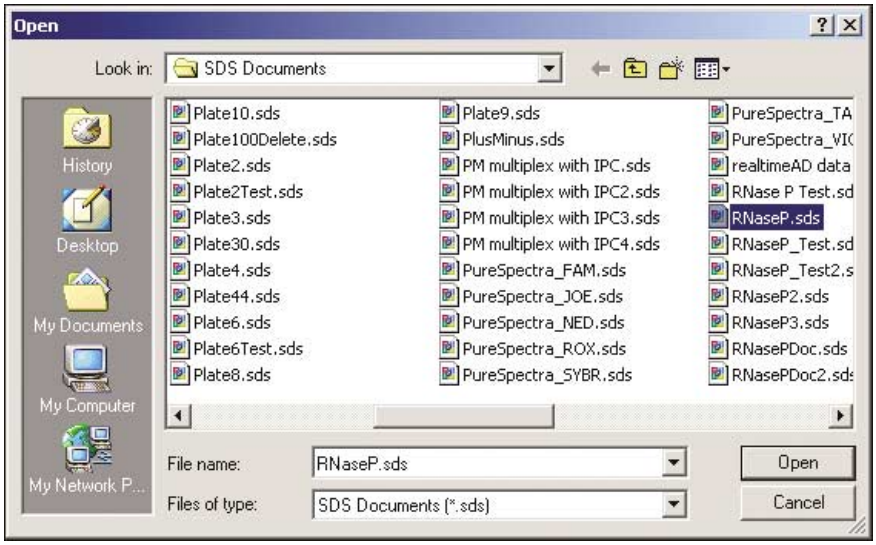
Opening an Existing Plate Document

How to Open a Plate Document

Once you have created a plate document, you can open it to review the data.

To open an existing plate document:

1. Select **File** ▸ **Open** to navigate to the file you want to open.



2. Double-click the desired plate icon ( ) in the directory.

Reviewing the Results of the Run

Results Tab

There are seven tabbed pages that you can use to view the results once the run has finished. The following descriptions are presented in the order that you may want to view the results. Refer to “Creating a New Plate Document” on page 4-4 for information on the Plate tab. Refer to “Viewing Data From the Dissociation Tab” on page 4-30 and “Report Tab” on page 4-36 for information on the Dissociation tab and the Report tab.

- Plate
- Spectra
- Component
- Amplification Plot
- Standard Curve
- Dissociation
- Report

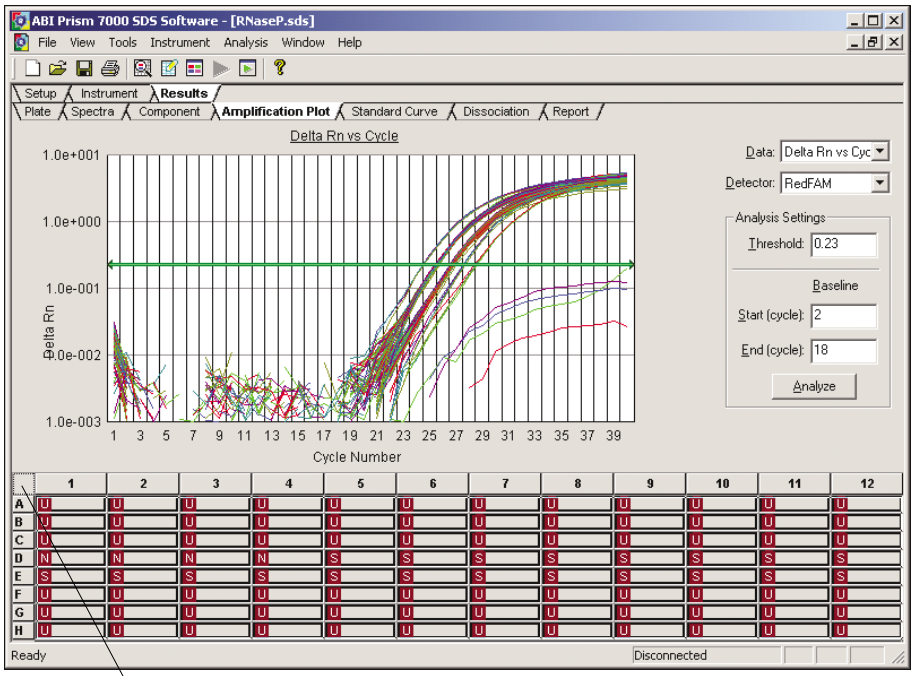
Amplification Plot Tab

When you open the Amplification Plot window while the run is in progress, you can see the plot update while the data is being collected so you may want to view it first. The green threshold indicates that the run has been analyzed. The red threshold indicates that it needs to be analyzed.

Viewing Results

To view the results of the run:

1. Select the **Results** tab ▸ **Amplification Plot** tab.



To view the results of the run: *(continued)*

2. Select the wells of interest to view amplification.

3. To view all wells, select the square above A in the plate representation, as shown in step 1, to see the amplification of all 96 wells.

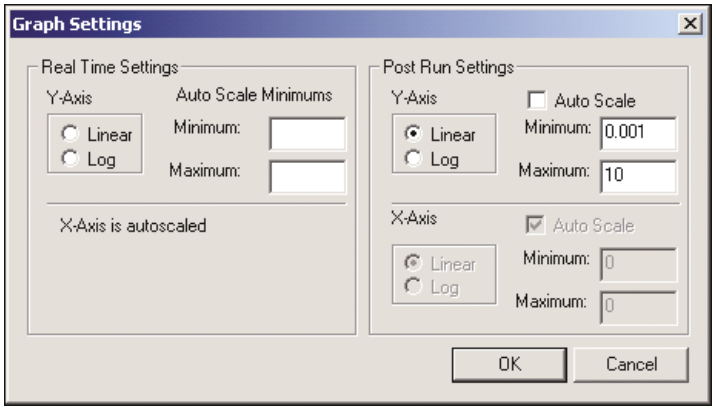
Setting the Baseline

In the Amplification Plot pane, check all the results that appear when you select the items on the Data menu.

To reset the baseline:

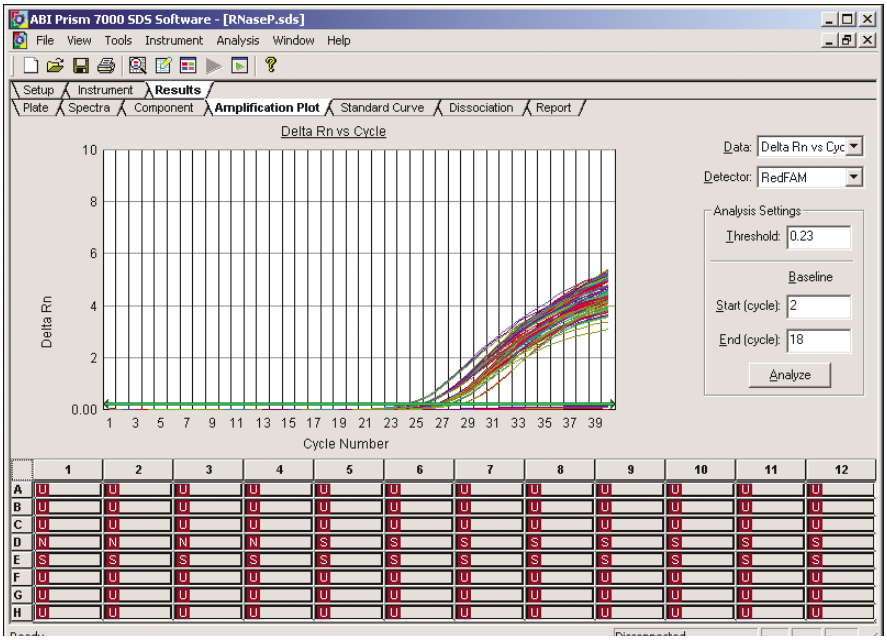
1. Select **Data** menu ▸ **Delta Rn vs Cycle**.

2. Double-click the numbers on the left side of the graph to open the **Graph Settings** dialog box. The Log button under Y-Axis will be selected as the default. Change it to **Linear**. The linear view has the best resolution to set the baseline.



To reset the baseline: *(continued)*

3. Click **OK**. The figure below is an example of a linear view of the amplification plot.



4. In the **Amplification Plot Baseline** fields, choose baseline start and end cycles that correspond to the initial cycles that show no amplification.

   a. Select the narrowest linear part of the baseline.

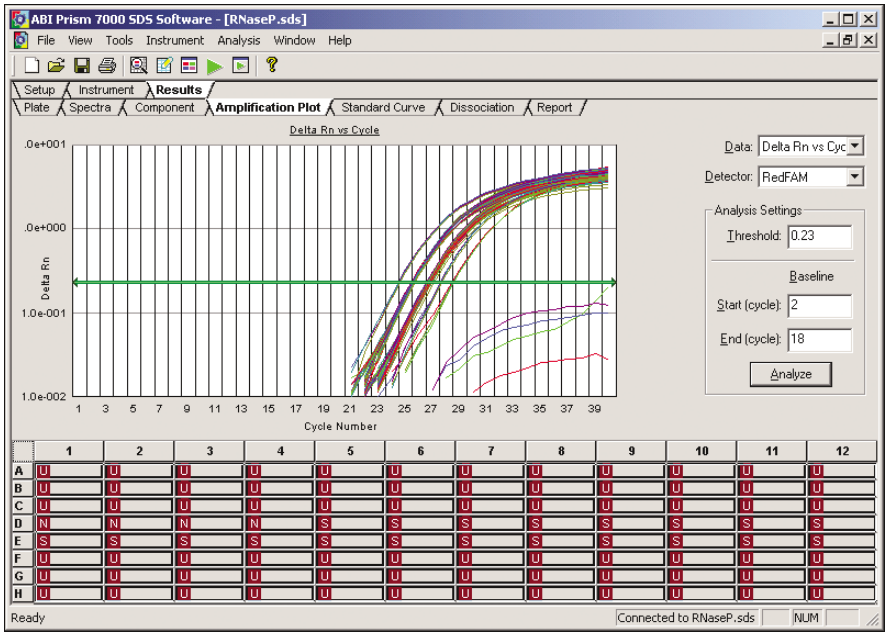
   b. Place the end cycle approximately 5 cycles before amplification starts.

5. Zoom in on the plot by adjusting the graph settings, so that you can see where the baseline is closest to being linear.

6. View the results. The baseline correction sets each curve at the origin. Threshold cycles (C_Ts), correspond to where the threshold line intersects each amplification trace.

To reset the baseline: *(continued)*

7. After resetting the baseline, click the **Analyze** button. The amplification plot below shows the analyzed data after the baseline has been corrected.



Resetting the Threshold

After resetting the threshold to the best place on the plot above the noise, the run must be analyzed. Both the baseline and the threshold are reset before being analyzed. On your computer screen, the red threshold bar indicates that it has not been analyzed since it was moved.

To reset the threshold of the amplification plot:

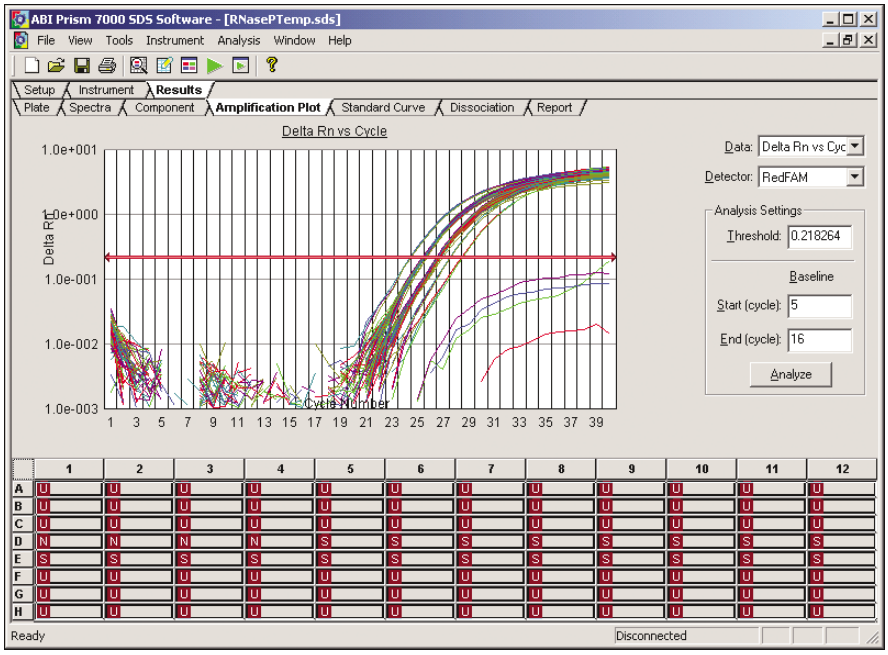
1. Select all the wells and look at the plot to see where the noise occurs and where the linear part of the curve occurs.

2. To set the threshold, move the green threshold bar up on the plot by dragging it with the pointer into the linear part of the curve.

To reset the threshold of the amplification plot: *(continued)*

3. Move it to a position above the noise to the linear part of the growth curve, at its narrowest point, as shown in the figure below.

   Once you start moving the threshold, it turns red to indicate that the new threshold value has not been applied to the data.



4. After selecting a new threshold, click the green **Analyze** button to recalculate the C_Ts. When the threshold bar turns green, the data has been analyzed.

Viewing Results

R_n, or normalized reporter, is calculated by dividing the reporter signal by the passive reference signal. During PCR, R_n increases as target nucleic acid is amplified until the reaction approaches a plateau.

ΔR_n represents the normalized reporter signal minus the baseline signal established by setting the baseline in the first few cycles of PCR. Like R_n, ΔR_n increases during PCR as target is amplified, until the reaction approaches a plateau.

Note: C_T, represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal can first be detected. Assuming that PCR is 100% efficient, a relationship exists between threshold cycle and template concentration so that the threshold cycle decreases by one cycle as concentration of template doubles. The software generates a standard curve of C_T vs. Log (starting copy number) for all standards, and then determines the starting copy number of unknowns by interpolation.

To view the results:

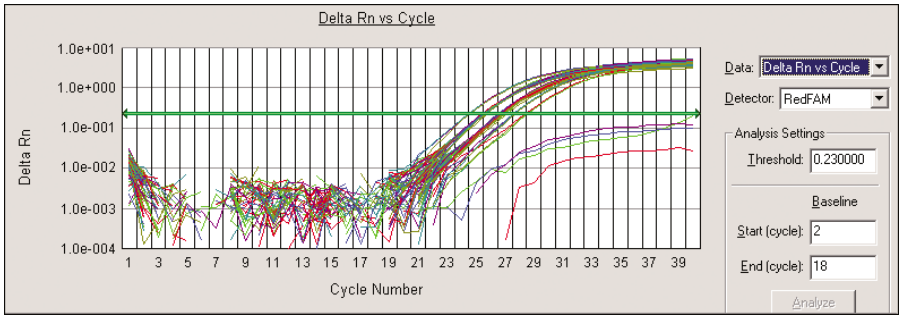
1. Select **Data ▸ Delta Rn vs Cycle** and view the results. The baseline correction sets each curve at the origin. C_T reads where the amplification curve goes across the threshold.
2. Select all the wells.
3. Select **Data ▸ Ct vs Well Position** to change the plot and review that data.
4. Select **Data ▸ Rn vs Cycle** and review the data.

   Samples of these three views are shown in step 5 on page 4-25.

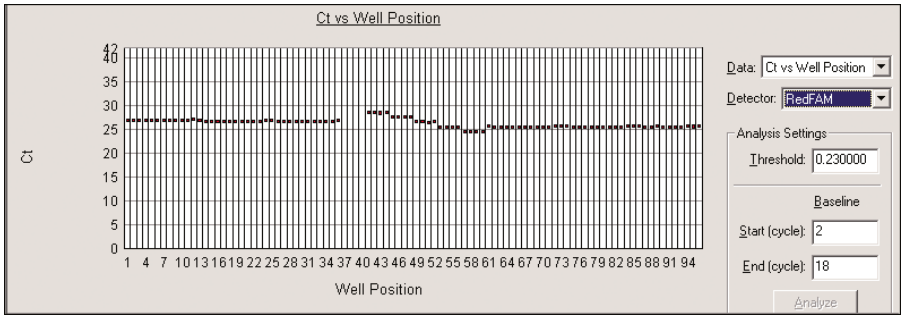
To view the results: *(continued)*

5. From the **Amplification Plot** tab, select a detector used in the setup and view the results in the window. Change between **Linear** and **Log** view in the graph settings to look at data in each of the three plot formats.

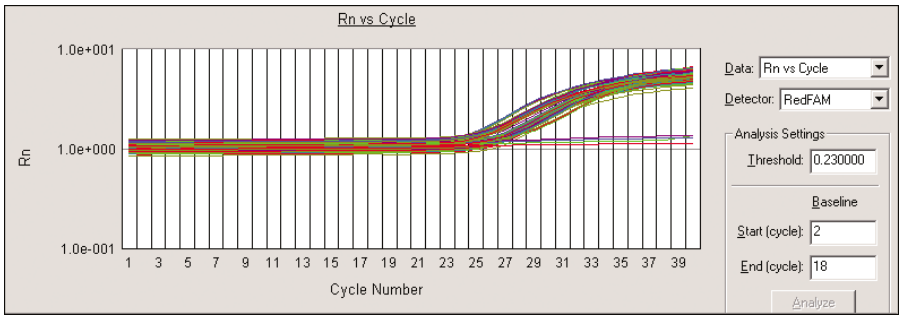
   Delta Rn vs Cycle



   Ct vs Well Position



   Rn vs Cycle



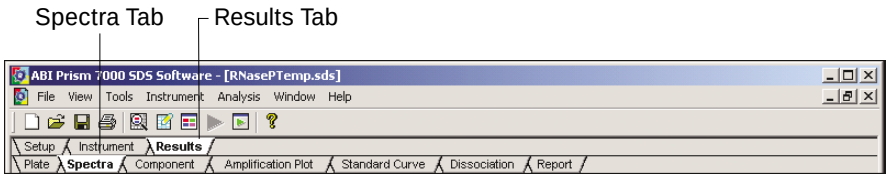
6. When the threshold is set, analyze the data by clicking the green **Analyze** button on the toolbar and check the results.

7. Once you are satisfied with the results, save the plate document.

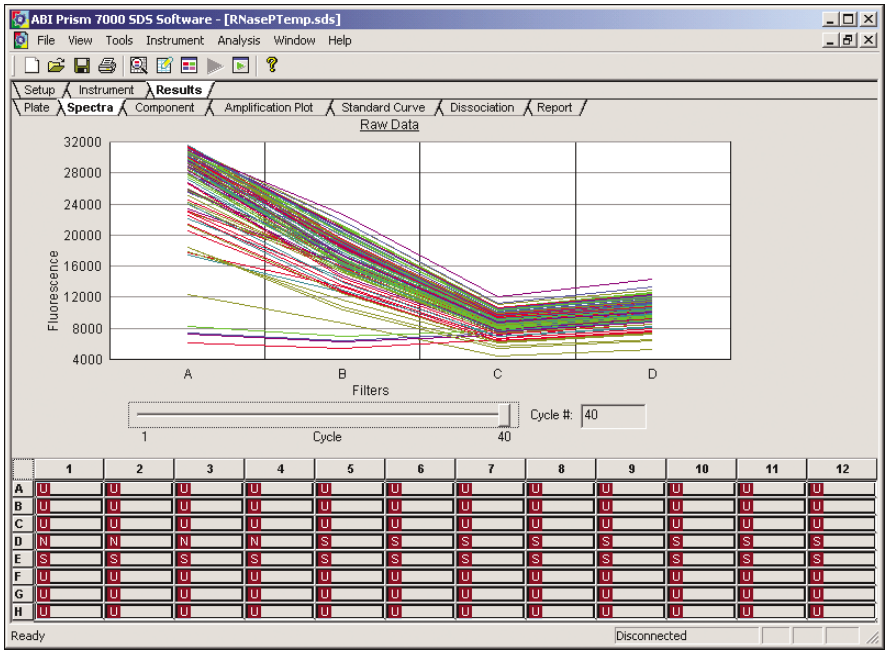
Spectra Tab

To view the spectra of the run:

1. Select **Spectra** to see the fluorescence of all 96 wells.

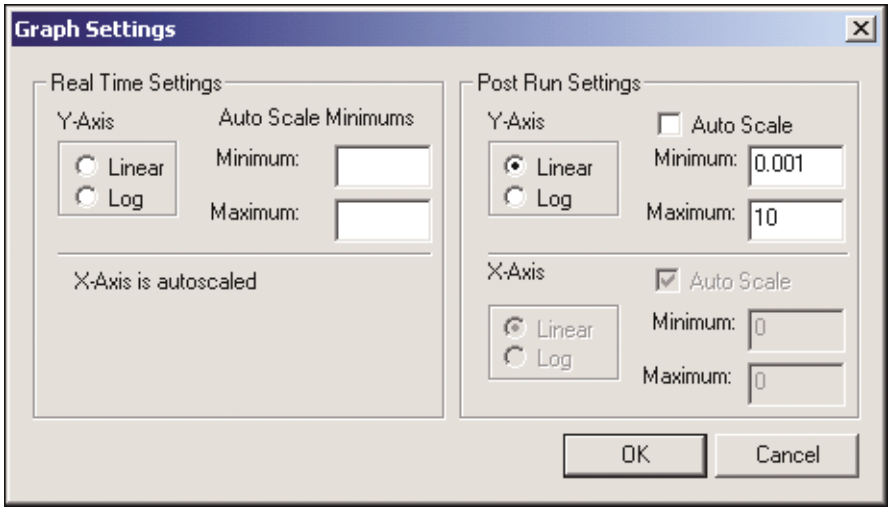


2. In the bottom of the window, click the top left corner of the columns to select all wells in the run, or select a row or column of wells.



To view the spectra of the run: *(continued)*

3. To change the scale of the spectral graph, double-click the numbers on the left column of the scale. This opens the Graph Settings dialog box.



4. Unclick **Auto Scale**, then change the scale in the **Maximum** and **Minimum** fields until the graph appears the way you want it to appear.

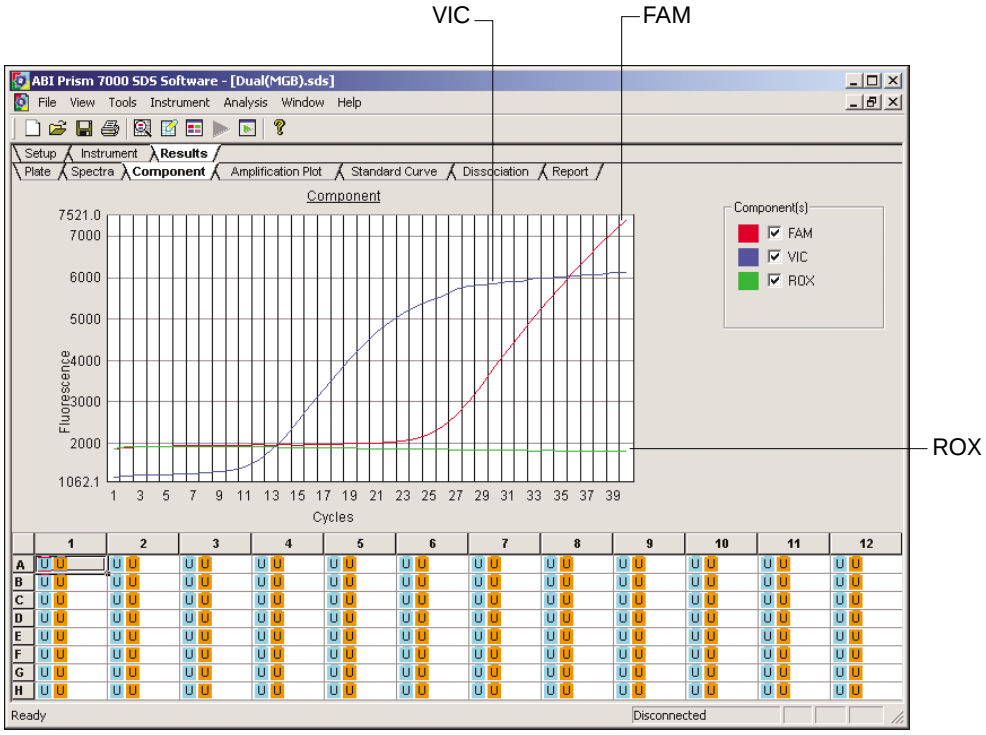
Component Tab

The Component tab displays the component dye signals that contribute to the composite signal for a selected well.

During dye componenting, a multicomponenting algorithm distinguishes the contribution of each individual dye spectra from the spectra data collected during PCR. The multicomponenting algorithm uses the pure spectra component files defined during Pure Dye Spectra calibration and applies a matrix calculation to determine the contribution of each dye.

Note: The Component window is only available after the Pure Dyes have been calibrated and the run data has been analyzed.

The Component window is a representation of the dyes in the run. In this window, VIC™ (blue line) and FAM™ (red line) are the reporter dyes for the selected well, A-1. ROX™ (green line) is the Passive Reference.



Viewing the Components of the Run

The Component view displays the complete spectral contribution of each dye in the well over the duration of the PCR run. Show and hide the dye components by clicking the check boxes under the Component(s) legend.

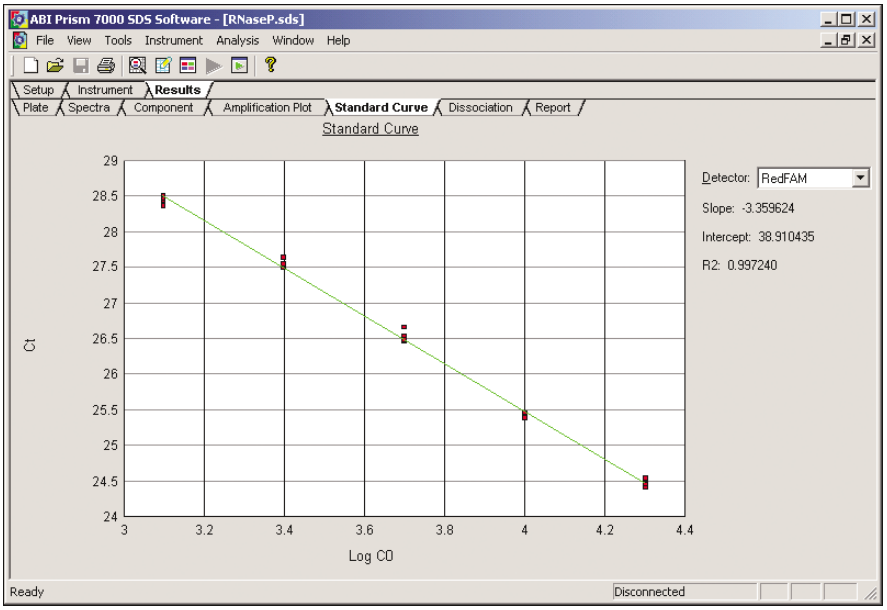
To view the components:

1. Select the **Results** tab ▸ **Component** tab.
2. Select one well at a time from the bottom of the window to see the components of the well.

When you look at the components of the run, it shows only one well at a time or the first selected well if more than one is selected. The Y-axis in the graph is fluorescence and the X-axis is cycle number.

Standard Curve

The Standard Curve view, as shown in the following figure, displays the results of the standard curve for the selected detector. The X-axis is the log of the starting copy number or quantity and the Y-axis is the C_T. Standards appear on the curve as squares, and unknowns appear as X.



Note: Standards with the highest starting copy number will have the lowest C_Ts.

Viewing Data From the Dissociation Tab

Overview

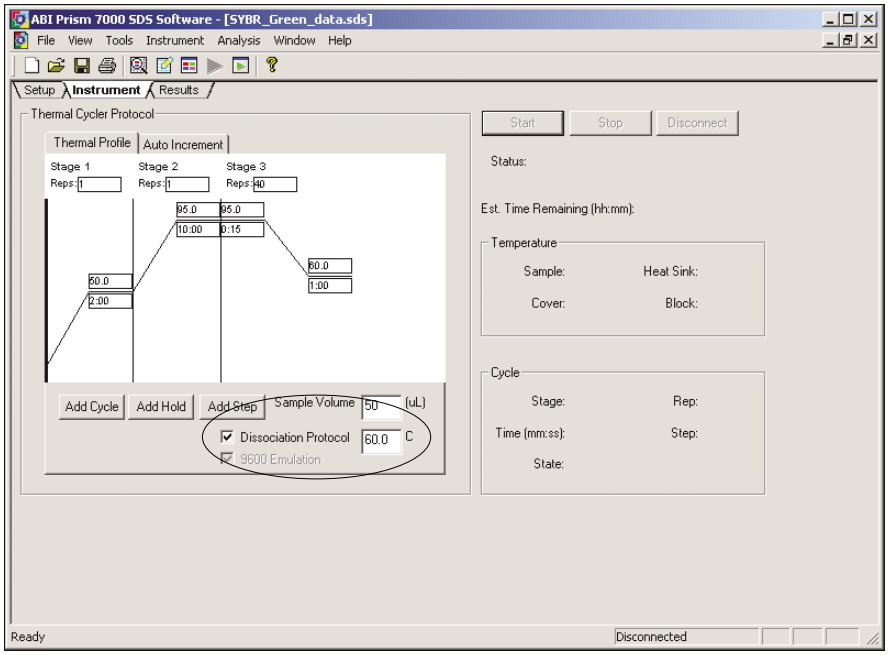
The Dissociation window displays dissociation data from the amplicons of quantitative PCR runs. If you are using SYBR® Green and want to determine if there is contamination and/or the dissociation temperature, this option will be available.

IMPORTANT: The accuracy and precision for the T_ms determined from this data are constrained by the specifications for the thermal cycler, which controls the thermal profiles for these reactions.

Capturing Dissociation Data

To automatically capture dissociation data at the end of a PCR run:

1. Click the **Instrument** tab.
2. In the **Thermal Cycle Protocol** pane, click the **Dissociation Protocol** check box.



3. Enter an appropriate starting temperature in the temperature field.

   Note: There is a 35 °C window in which dissociation data can be acquired.

   A dissociation curve will be generated after the last cycle of the PCR reaction.

4. Save the plate document with a **.sds** extension.
5. Click **Start**.

Capturing Dissociation Data on a Previously Run Plate

You can also generate a dissociation curve for a previously run PCR reaction plate that has been stored at 2 to 8 °C.

To do this:

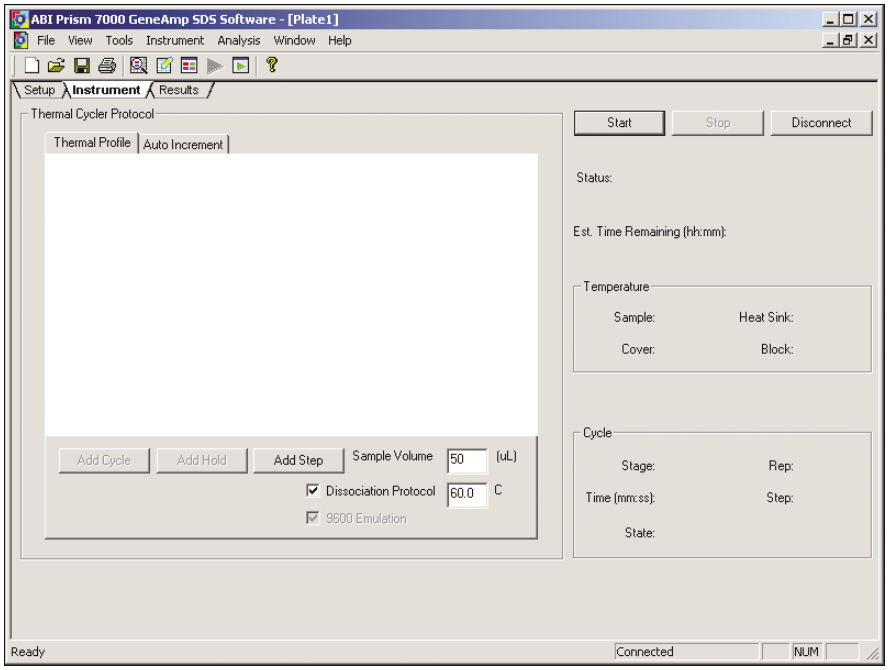
1. Delete all default stages from either the **Thermal Profile** tab or the Auto Increment tab:

   a. Hold the **Shift** key down and click in each of the stages.

   b. Press the **Delete** key.

2. Click the **Dissociation Protocol** check box.

   The Instrument view will look like the figure below.



3. Save the plate document with a **.sds** extension.

4. Click **Start**.

Viewing Data in the Dissociation Window

When running SYBR Green 1 Dye chemistry, you should verify that a single specific PCR product has been amplified to ensure that quantification results are valid. You do this with the Dissociation graph. The X-axis is temperature and the Y-axis is either fluorescence or the derivative of the fluorescence, as set by the Data Type menu. This selection enables you to select the detector to display.

Verifying Amplification

To verify amplification:

1. To determine the product purity, select all the wells using the SYBR Green 1 Dye chemistry. This provides an overview of whether a single or multiple PCR products have been generated.

2. If required, you can use the green bar on the left to obtain the specific melting temperature of a PCR product. Click and drag the bar to view the melting temperatures.

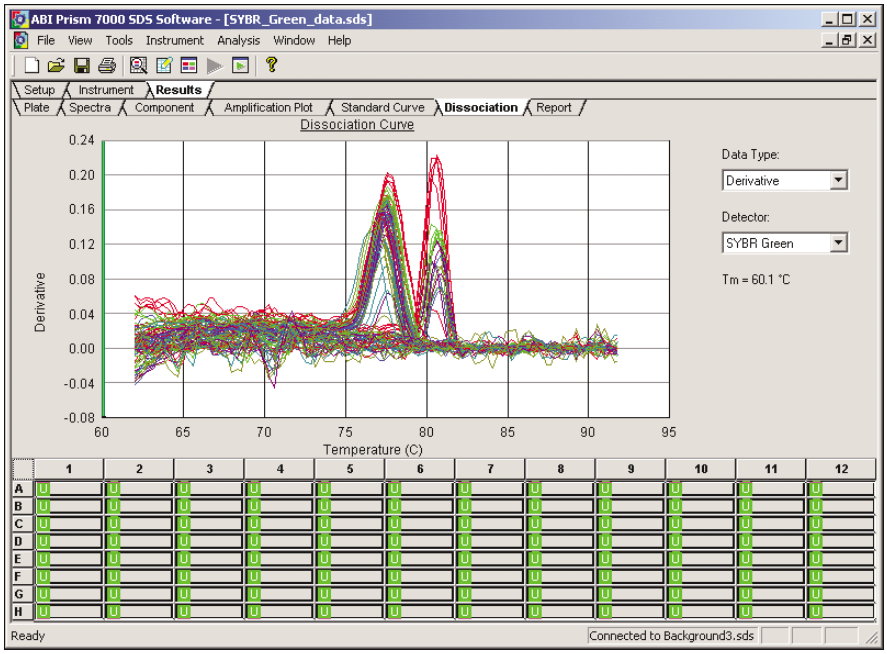
3. In the Derivative Data Type plot, wells showing a single strong peak represent wells in which a single PCR product has been amplified. Wells showing two or more peaks contain multiple PCR products. If multiple PCR products are present, you need to identify the wells. For each set of replicates, select the samples and view the data.

Even when multiple PCR products are present, the amplification plots may look normal. If multiple PCR products are produced with the SYBR Green 1 Dye chemistry, you may need to reoptimize the assay. Refer to the *SYBR Green PCR Master Mix and RT-PCR Protocol* (P/N 4310251) for details.

Dissociation Graph

The dissociation graph displays dissociation data from the amplicons of quantitative PCR runs. The data captured is the change in fluorescence as a function of temperature from a dye or probe interacting with double-stranded DNA. This is particularly useful as a quality control tool to identify primer-dimers and other products different from the specific amplicon products in a quantitative PCR reaction measured with SYBR Green dye.

Dissociation curves are useful for the detection of nonspecific products in an amplification reaction.



This analysis may also be run for the primer concentration optimization matrix defined for the optimization of SYBR Green reactions. The amplification curves from this matrix are analyzed to obtain the forward and reverse primer concentrations that yield the widest separation between the C_Ts for samples containing template and NTC samples.

Dissociation data can be viewed in two ways:

- Derivative – Derivative data is the negative of the rate of change in fluorescence as a function of temperature.

Note: By taking the derivative of the dissociation curve, the inflection point, or T_m point, is enhanced.

$$\left(\frac{dF}{dT}\right)$$

- Raw – Raw data is a graphical view of fluorescence versus temperature.

T_m Value Display

There are two definitions for the T_m value. The chemical definition is the temperature at which 50% of the DNA amplicons are in a double-stranded configuration. The mathematical definition is the maximum value for the first derivative curve within a specific peak.

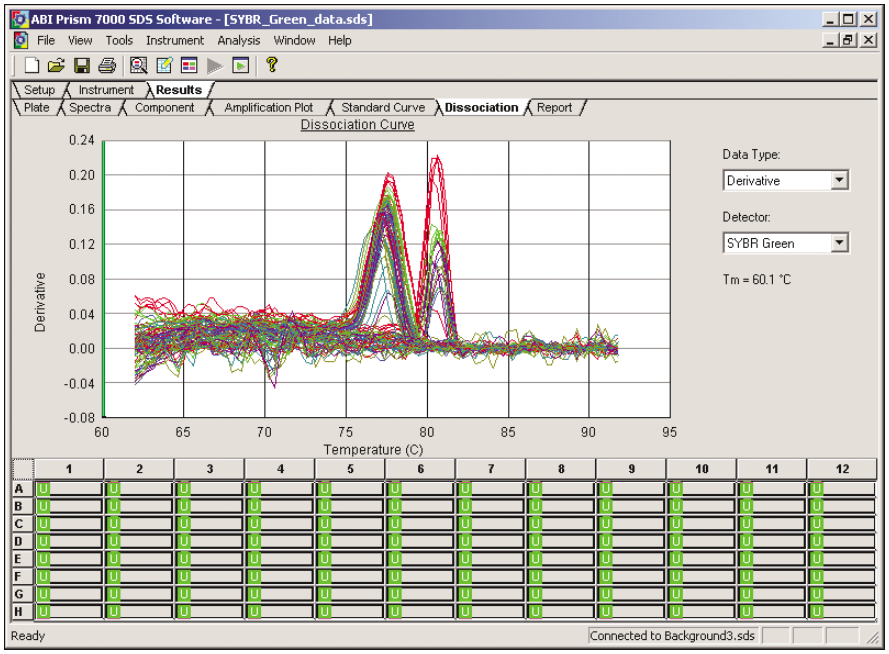
Viewing Data

To view data in the Dissociation window:

1. Click **Results** ▸ **Dissociation**.

2. In the plate matrix, select one or more wells.

   A Dissociation Curve graph is shown in the upper pane for the selected wells.



3. Click the **Data Type** menu and select the type of dissociation data you want to view:

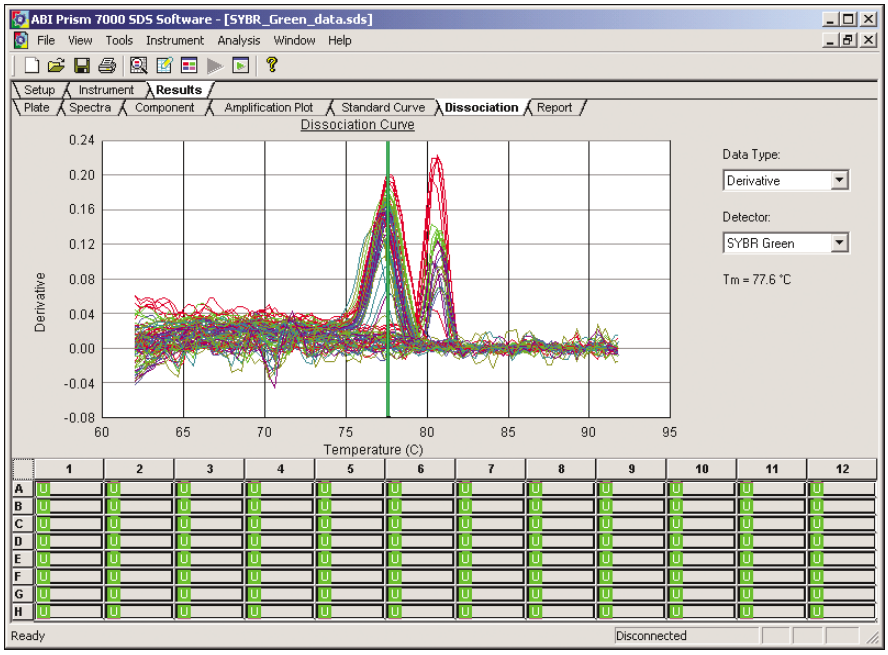
   - Derivative
   - Raw

4. Inspect the plot for one single peak.

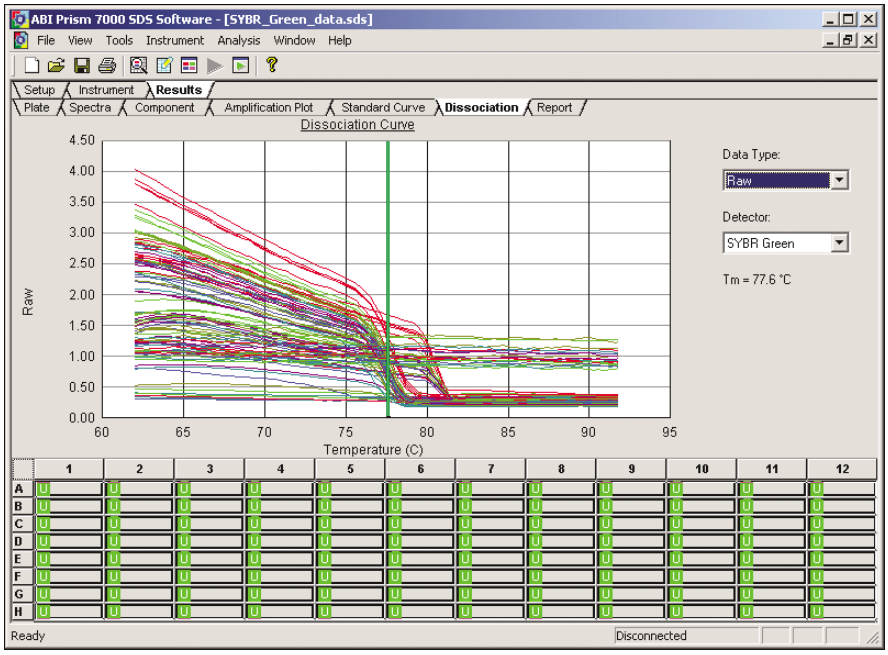
To view data in the Dissociation window: *(continued)*

5. Drag the green bar on the left of the first derivative dissociation graph to the peak for which the T_m is being measured.

   If you selected Derivative in step 3 on page 4-34, the graph will look similar to the one below.



   If you selected Raw in step 3 on page 4-34, the graph will look similar to the one below.



Report Tab

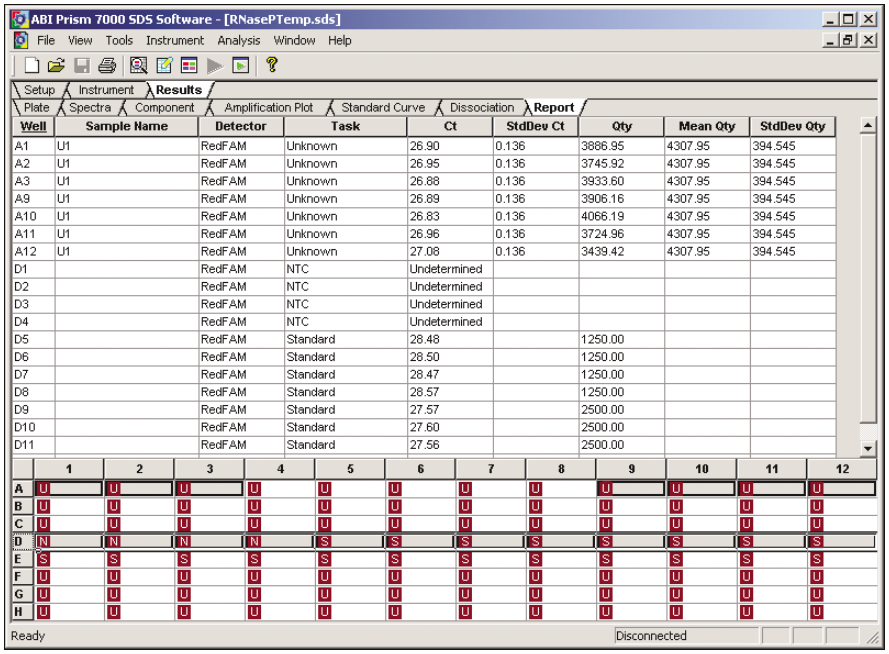
About the Report Tab

The Report window displays the analyzed quantitative data in a table. You can customize the data you want to appear in the table in the Report Settings dialog box. Use the plate matrix at the bottom of the window to select the wells you want to view in the data table.

Viewing Data in the Report Window

To view data in the Report window:

1. Click the **Report** tab.



2. In the lower pane, select one or more wells.

   Quantification data are displayed in the upper pane for the selected wells. Note the following:

   - For standard wells, the Quantity column displays the starting quantity entered for the DNA template.
   - For other well categories, the quantity is calculated using the C_T value for the well and the standard curve.
   - To calculate the quantity for unknown wells, the standards must be associated with those unknowns (that is, same detectors).

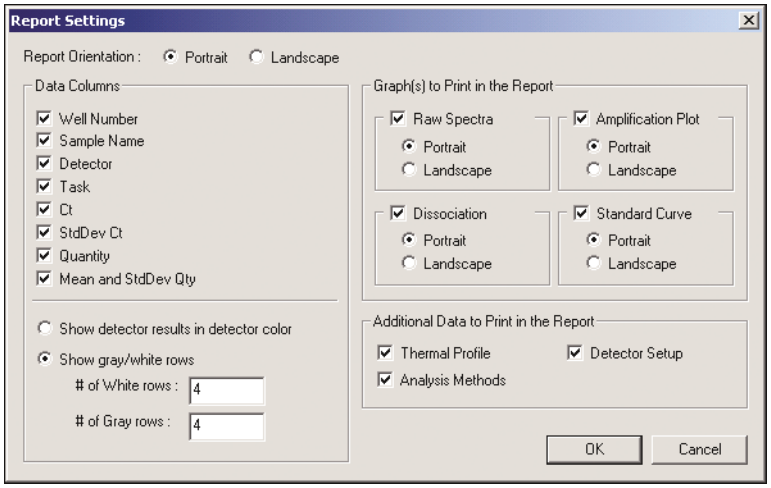
3. If desired, customize your report. See “Customizing the Report Window” on page 4-37.

Customizing the Report Window

You can customize the printed report using the Report Settings dialog box and export the data to another application to analyze it.

To customize the Report window:

1. Select **Tools ▸ Report Settings** to open the dialog box shown below.



2. Select the options you want from the columns that appear in the Report Settings dialog box.

3. Click **OK** to close the Report Settings dialog box. The Report window changes accordingly.

4. Select **File ▸ Export ▸ Results**.

5. Type a file name and click **OK** to export and analyze the data in a comma-separated (*.csv) format in an application that reads this format.

Printing Results and Exporting Data 5

Introduction

About This Chapter

This chapter provides procedures to print results of the runs and to export data to another file.

In This Chapter

This chapter contains the following topics:

Printing the Analysis Results

Overview

Once you have completed a run and analyzed the data, you can print the analysis results. Selecting Print from the Report tab will print all the windows in the document. Selecting Print from one of the other tabs will print only that window.

Printing Analysis Results

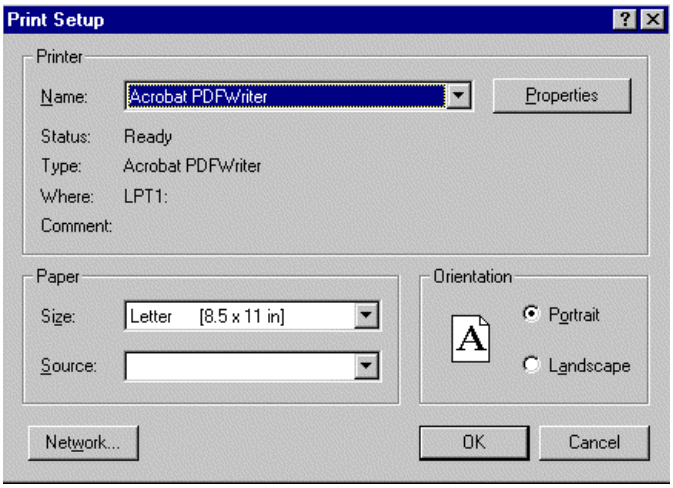
Note: You must have a printer installed on your computer to use the print function. Make sure you have the correct printer drivers installed for your printer, then select the printer. If necessary, contact the manufacturer of the printer for this information.

To print the analysis results of a sample file:

1. Click the **Results** tab in the plate document.

2. Select one of the tabs from Results to print one of the following:
   - Plate
   - Spectra
   - Component
   - Amplification plot
   - Standard Curve
   - Dissociation
   - Report

3. Select **File ▸ Print Setup** to open the following dialog box.

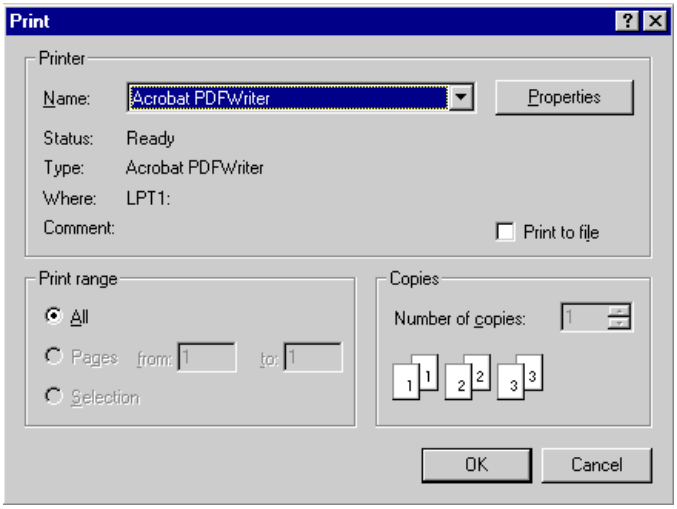


4. Check the settings and change the Orientation to **Landscape** if it is for one of the graphs or the plate document and click **OK**.

5. Select **File ▸ Print Preview** to see how the pages will be printed.

To print the analysis results of a sample file: *(continued)*

6. Select **File ▸ Print** to open the Print dialog box.



7. Choose the options you want for the printout and click **OK** to print.

If You Get Unexpected Results

If you get unexpected printing results, reselect the printer you are using.

To prevent unexpected printing results:

1. The first time you print after changing the printer configuration, select **File ▸ Page Setup**, and click **OK**.

2. Select **File ▸ Print** (Ctrl+P) to open the Print dialog box.

Exporting Data

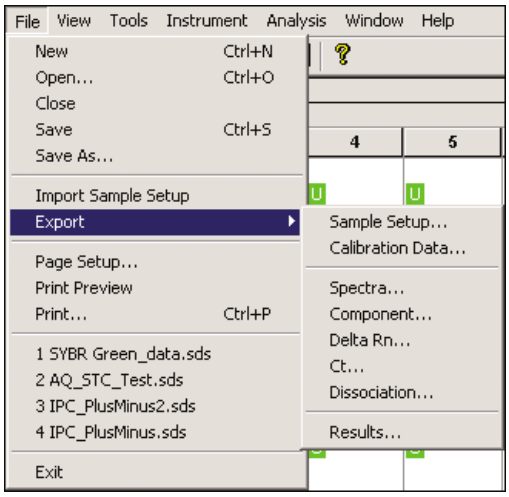
Overview

You can export analyzed data in a comma-separated (*.csv) format. You can open these exported files with any application that reads comma-separated text.

Exporting Data

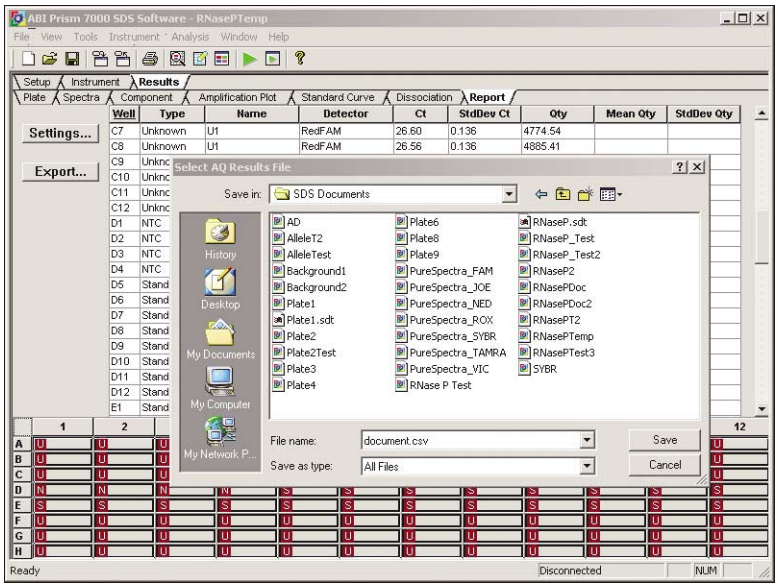
To export data in comma-separated format:

1. Select **File ▸ Export** in the plate document as shown in the following figure.



2. Choose the type of data to export (for example, **Sample Setup**).

3. Choose an appropriate directory.



4. Type a file name and select **Save**.

   Note: You can open the exported file with any application that reads comma-separated text.

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 that can 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 5-6

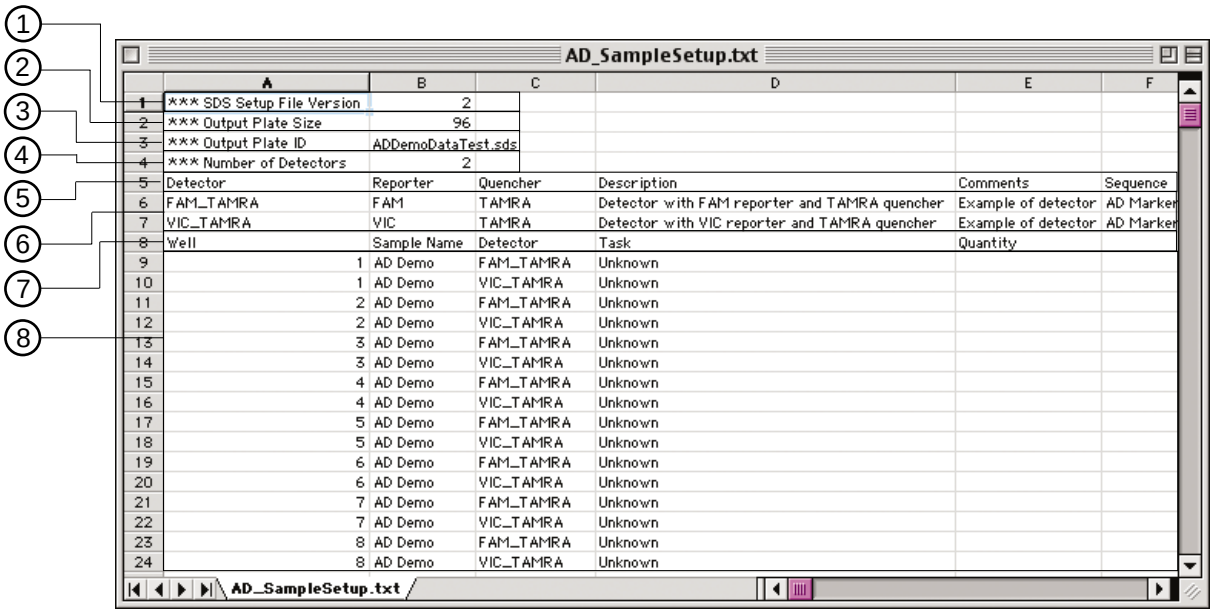
Note: The blank setup table file can be created using a secondary application (such as Microsoft® Excel or a text editor) so long as it is saved in tab-delimited format and is configured according to the file structure explained on page 5-6.

Setup Table File Format

Example Setup Table Files

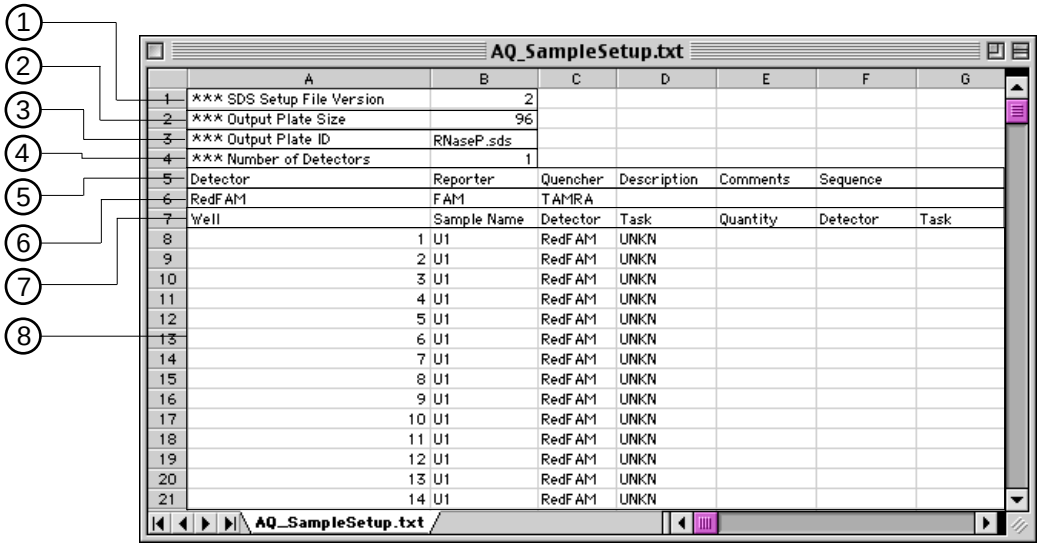
To guarantee a successful importation of setup table data into a plate document, the imported setup table file must be configured in the correct format for the assay type. The following figures illustrate the orientation of information in tab-delimited setup table files as viewed in a Microsoft Excel spreadsheet document. The numbered elements of the setup table files are explained on page 5-7.

Example Setup Table File from an Allelic Discrimination Run



	A	B	C	D	E	F
1	*** SDS Setup File Version	2				
2	*** Output Plate Size	96				
3	*** Output Plate ID	ADDemoDataTest.sds				
4	*** Number of Detectors	2				
5	Detector	Reporter	Quencher	Description	Comments	Sequence
6	FAM_TAMRA	FAM	TAMRA	Detector with FAM reporter and TAMRA quencher	Example of detector	AD Marker
7	VIC_TAMRA	VIC	TAMRA	Detector with VIC reporter and TAMRA quencher	Example of detector	AD Marker
8	Well	Sample Name	Detector	Task	Quantity	
9	1	AD Demo	FAM_TAMRA	Unknown		
10	1	AD Demo	VIC_TAMRA	Unknown		
11	2	AD Demo	FAM_TAMRA	Unknown		
12	2	AD Demo	VIC_TAMRA	Unknown		
13	3	AD Demo	FAM_TAMRA	Unknown		
14	3	AD Demo	VIC_TAMRA	Unknown		
15	4	AD Demo	FAM_TAMRA	Unknown		
16	4	AD Demo	VIC_TAMRA	Unknown		
17	5	AD Demo	FAM_TAMRA	Unknown		
18	5	AD Demo	VIC_TAMRA	Unknown		
19	6	AD Demo	FAM_TAMRA	Unknown		
20	6	AD Demo	VIC_TAMRA	Unknown		
21	7	AD Demo	FAM_TAMRA	Unknown		
22	7	AD Demo	VIC_TAMRA	Unknown		
23	8	AD Demo	FAM_TAMRA	Unknown		
24	8	AD Demo	VIC_TAMRA	Unknown		

Example Setup Table File from an Absolute Quantification Run



	A	B	C	D	E	F	G
1	*** SDS Setup File Version	2					
2	*** Output Plate Size	96					
3	*** Output Plate ID	RNaseP.sds					
4	*** Number of Detectors	1					
5	Detector	Reporter	Quencher	Description	Comments	Sequence	
6	RedFAM	FAM	TAMRA				
7	Well	Sample Name	Detector	Task	Quantity	Detector	Task
8	1	U1	RedFAM	UNKN			
9	2	U1	RedFAM	UNKN			
10	3	U1	RedFAM	UNKN			
11	4	U1	RedFAM	UNKN			
12	5	U1	RedFAM	UNKN			
13	6	U1	RedFAM	UNKN			
14	7	U1	RedFAM	UNKN			
15	8	U1	RedFAM	UNKN			
16	9	U1	RedFAM	UNKN			
17	10	U1	RedFAM	UNKN			
18	11	U1	RedFAM	UNKN			
19	12	U1	RedFAM	UNKN			
20	13	U1	RedFAM	UNKN			
21	14	U1	RedFAM	UNKN			

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.

Setup Table Format

Format/Symbol	Definition
courier	Text appearing in bold courier font must be applied to a setup table file exactly as it 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 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 successful importing of the setup table file into a plate document, the file must contain all of the sections in the following table in the order in which 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 Reporter Quencher 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 ...Detector Task Quantity</td></tr>
<tr><td rowspan="2">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, 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>

Analyzing Assays 6

Introduction

About This Chapter

This chapter describes the polymerase chain reaction (PCR) assays that you can perform using the ABI PRISM® 7000 SDS Software and how to analyze them.

In This Chapter

This chapter contains the following topics:

Target Quantification

Chemistries for Absolute Quantification

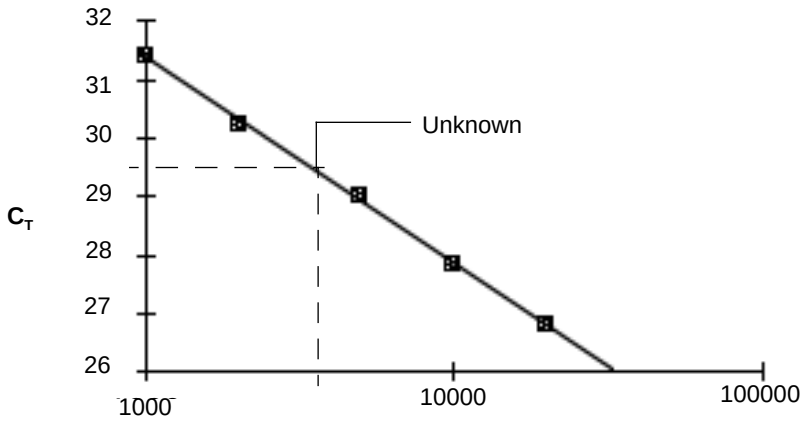
Two chemistries are available for use on the ABI PRISM® 7000 Sequence Detection System. The fluorogenic 5´ nuclease assay, or assay using TaqMan® probes, uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR. The fluorogenic probe design patented by Applied Biosystems that incorporates the reporter dye on the 5´ end and the quencher on the 3´ end has greatly simplified the design and synthesis of effective TaqMan probes. Coupled with latest assay design guidelines by Applied Biosystems, this has resulted in success rates approaching 100 percent for quantitative assays using TaqMan probes.

The second assay chemistry available uses SYBR® Green 1 dye, a highly specific double-stranded DNA binding dye, which also allows the detection of product accumulation during PCR. The most important difference between the two chemistries is that SYBR Green 1 assay chemistry will detect all double-stranded DNA, including non-specific reaction products. A well optimized reaction is, therefore, essential for accurate quantitative results. The advantage of SYBR Green 1 assay chemistry is that no probe is required, thus reducing assay setup and running costs.

Employing the 5´ Nuclease Assay

Absolute quantification on the 7000 system is accomplished through the use of the polymerase chain reaction and the fluorogenic 5´ nuclease assay. Unknown samples and standards diluted over several orders of magnitude are loaded onto an ABI PRISM® Optical Reaction Plate containing master mix and assay using TaqMan probe reagents targeting a specific nucleic acid sequence. The reaction plate is loaded and run on a 7000 system which has been configured for real-time analysis. During the run, the instrument records the fluorescence emission resulting from the cleavage of TaqMan probes in the presence of the target sequence. After the run, the software processes the raw fluorescence data to produce threshold cycle (C_T) values for each sample. The software 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).

The figure below illustrates a standard curve generated from a standard RNase P Installation Plate.



SYBR Green

The SYBR® Green PCR Master Mix is a convenient premix of all the components, except primers, necessary to perform real-time PCR using SYBR Green 1 Dye. It is designed for use with the 7000 system. Direct detection of PCR product is monitored by measuring the increase in fluorescence caused by the binding of SYBR Green dye to double-stranded (ds) DNA.

One-Step or Two-Step RT-PCR can also be performed using the SYBR Green RT-PCR Reagents Kit.

In RNA quantification assays, the SYBR Green PCR Master Mix is used in the second step of a two-step reverse-transcription polymerase chain reaction (RT-PCR) protocol. In a One-Step RT-PCR protocol, MultiScribe™ Reverse Transcriptase and RNase Inhibitor are added to the SYBR Green PCR Master Mix.

For the best quantification results, use the following:

- Primer Express™ software for primer design
- Applied Biosystems reagents
- Applied Biosystems universal thermal cycling conditions

Absolute Quantification on the 7000 Instrument

The 7000 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.

Absolute Standard Curve

It is possible to use the 7000 system data to obtain absolute quantification, but it requires that the absolute quantities of the standard 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.

The following critical points must be considered for the proper use of absolute standard curves:

- It is important that the standard DNA or RNA 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.
- Accurate pipetting is required 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. This concentrated DNA or RNA must be diluted 10^6–10^{12} -fold to be at a concentration similar to the target in biological samples.
- The stability of the diluted standards must be considered, 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 report on the steps they used in developing an absolute RNA standard for viral quantification.
- Generally, it is not possible to use DNA as a standard for absolute quantification of RNA because there is no control for the efficiency of the reverse transcription step.

Absolute Quantification Chemistry Kits

Refer to Appendix C, "Consumables and Reagents," for kits that are available from Applied Biosystems to design and run real-time quantification chemistries.

Terms Defined

The following definitions are assumed in this description.

Absolute Quantification Terms

Controls/Terms	Definitions
Standard	A sample of known concentration used to construct a standard curve.
Reference	Endogenous and exogenous controls are examples of active references. Active reference means the signal is generated as the result of PCR amplification. The active reference has its own set of primers and probe. Whether or not an active reference is used, it is important to use a passive reference containing the ROX™ dye.
Endogenous Control	An RNA or DNA that is present in each experimental sample as isolated.
Exogenous Control	A characterized RNA or DNA spiked into each sample at a known concentration. An exogenous active reference is usually an *in vitro* construct that can be used as an internal positive control (IPC) to distinguish true target negatives from PCR inhibition.
Calibrator	A sample used as the basis for comparative results.

Relative Standard Curve

It is easy to prepare standard curves for relative quantification because quantity is expressed relative to some basis sample, such as the calibrator. For all experimental samples, target quantity is determined from the standard curve and divided by the target quantity of the calibrator. Thus, the calibrator becomes the 1× sample, and all other quantities are expressed as an *n*-fold difference relative to the calibrator. As an example, in a study of drug effects on expression, the untreated control would be an appropriate calibrator.

Because the sample quantity is divided by the calibrator quantity, the unit from the standard curve drops out. Thus, all that is required of the standards is that their relative dilutions be known. For relative quantification, this means any stock RNA or DNA containing the appropriate target can be used to prepare standards.

The critical points listed below must be considered for the proper use of relative standard curves:

- It is important that stock RNA or DNA be accurately diluted, however the units used to express this dilution are irrelevant. If two-fold dilutions of a total RNA preparation from a control cell line are used to construct a standard curve, the units could be the dilution values 1, 0.5, 0.25, 0.125, and so on. By using the same stock RNA or DNA to prepare standard curves for multiple plates, the relative quantities determined can be compared across the plates.
- It is possible to use a DNA standard curve for relative quantification of RNA. Doing this requires the assumption that the reverse transcription efficiency of the target is the same in all samples, however the exact value of this efficiency need not be known.
- For quantification normalized to an endogenous control, standard curves are prepared for both the target and the endogenous reference. For each experimental sample, the amount of target and endogenous reference is determined from the appropriate standard curve. Then, the target amount is divided by the endogenous reference amount to obtain a normalized target value.

Again, one of the experimental samples is the calibrator, or 1× sample. Each of the normalized target values is divided by the calibrator normalized target value to generate the relative expression levels.

Allelic Discrimination Assay

Allelic Discrimination on the 7000 Instrument

The 7000 system supports allelic discrimination chemistries available from Applied Biosystems. 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 7000 system is possible by using the fluorogenic 5´ nuclease assay. Initially, a plate is prepared by aliquoting to each well: PCR master mix, primers, and fluorogenic probes constructed for the two possible allele sequences. After adding unknown samples to the plate, it is run on a thermal cycler.

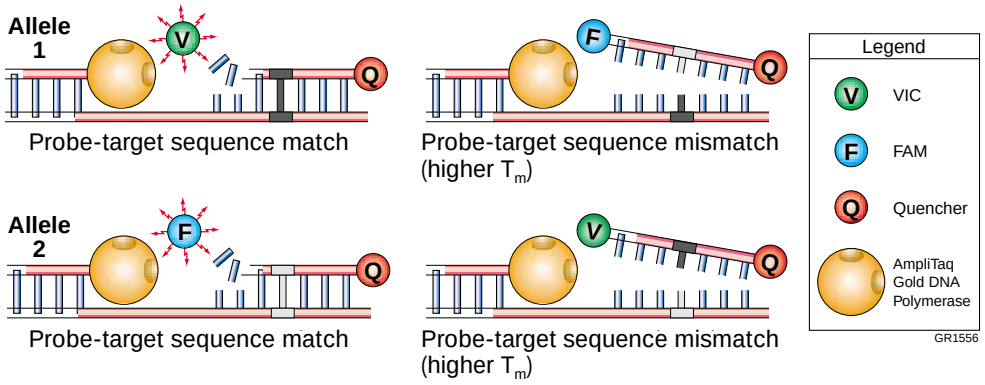
During the PCR, the fluorogenic probes anneal specifically to complementary sequences between the forward and reverse primer sites on the template DNA. Then during extension, AmpliTaq Gold® DNA polymerase cleaves the probes hybridized to the matching allele sequence(s) present in each sample. The cleavage of each matched probe separates the reporter dye from the quencher dye, which results in increased fluorescence by the reporter. The 7000 system reads the fluorescence generated during the PCR amplification. By quantifying and comparing the fluorescent signals using the software, it is possible to determine the allelic content of each sample on the plate.

Mismatches Between Probe and Target Sequences

Determining Variants

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 rather than cleave to it, releasing the reporter dye. By running 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).



Fluorescence and Allelic Content

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

Fluorescence Signals and Allelic Content

A substantial increase in…	Indicates…
VIC™ fluorescence only	homozygosity for Allele X (1).
FAM™ fluorescence only	homozygosity for Allele Y (2).
both fluorescent signals	heterozygosity.

Algorithmic Manipulation of Raw Allelic Discrimination Data

The software automatically analyzes raw data upon completion of each allelic discrimination run. The term raw data refers to the spectral data between 500 nm to 660 nm collected by the 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.

Transforming Raw Data into Dye Components

The first mathematical transformation involves the conversion of the raw data, expressed in terms of fluorescent signal versus wavelength, using the extracted pure dye standards, to pure dye components.

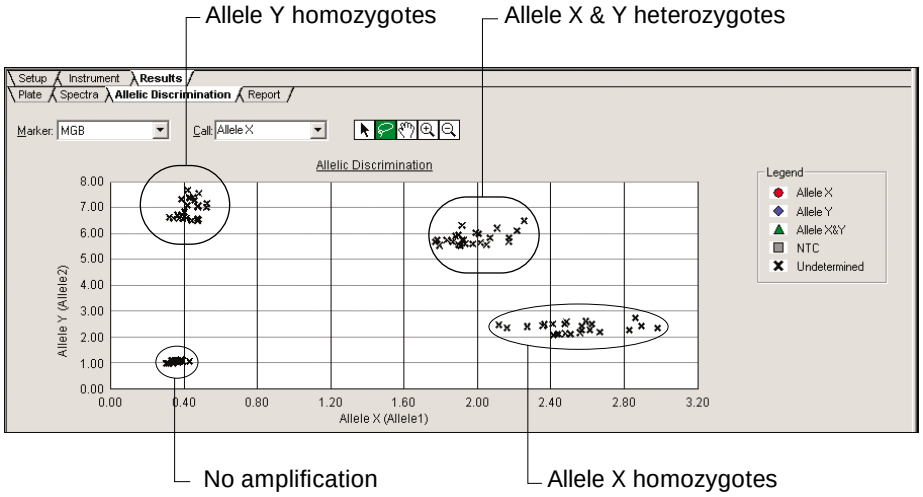
Dye Components

After the dye components have been identified, the software determines the contribution of each dye in the raw data using a mathematical algorithm.

Cluster Variations

The software graphs the results of the allelic discrimination run on a scatter plot of Allele X (allele 1) R_n versus Allele Y (allele 2) R_n. The software represents each well of the 96-well plate as an X on the graph. The clustering of cross marks 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 variation in clustering due to differences in the extent of PCR amplification.



Genotypic Segregation of Data Point Clusters

The figure above 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.

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)

Allelic Discrimination Chemistry Kit

The Pre-Developed TaqMan® Assays and Reagents for Allelic Discrimination kit (P/N 4312561) is available from Applied Biosystems to design and run allelic discrimination chemistries. For a list of other PDAR kits, refer to Appendix C, “Consumables and Reagents.”

Creating Markers for Allelic Discrimination

Overview

The allelic discrimination procedure consists of three steps:

- Setting up a plate document for a pre-read
- Running an absolute quantification plate document
- Running a post-read of the allelic discrimination plate document

Creating the Detectors

Before creating markers for allelic discrimination, you must create detectors and set up the plate document. You will use the same plate for all the procedures in this section.

To create the detectors:

1. Open a new document by selecting **File** ▸ **New** then select:

   Assay: **Allelic Discrimination**

   Container: **96-Well Clear**

   Template: **Blank Document**

2. Click **OK**.

3. Select **Tools** ▸ **Detector Manager**.

4. Create detectors for **Allele 1** and **Allele 2** in the New Detector window.

5. When you have created the detectors, click **OK**.

6. In the Detector Manager, click **Done**.

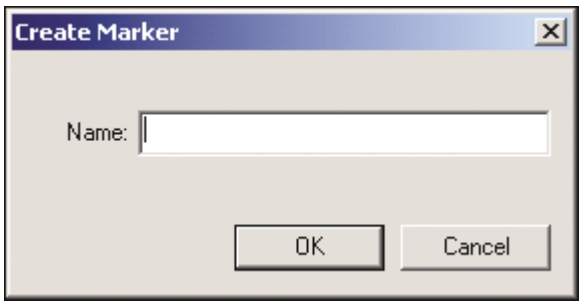
Creating Markers

After creating the detectors, create and add the markers for the allelic discrimination.

To create markers in the Marker Manager:

1. Select **Tools** ▸ **Marker Manager**.

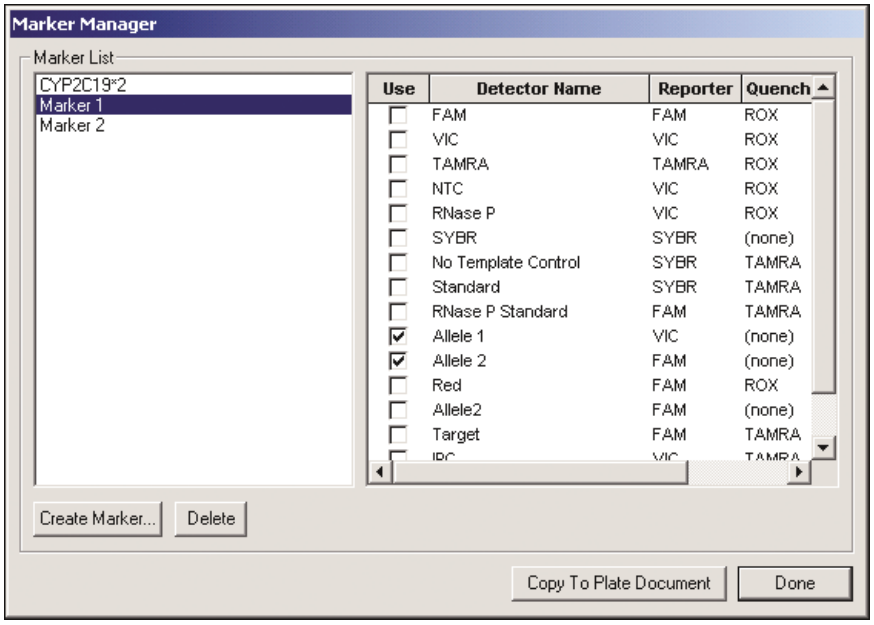
2. Click **Create Marker** in the Marker Manager window and name the marker you want to use, for example, **MGB**, and click **OK**.



3. Select the marker you named from the Marker List on the left side of the window.

To create markers in the Marker Manager: *(continued)*

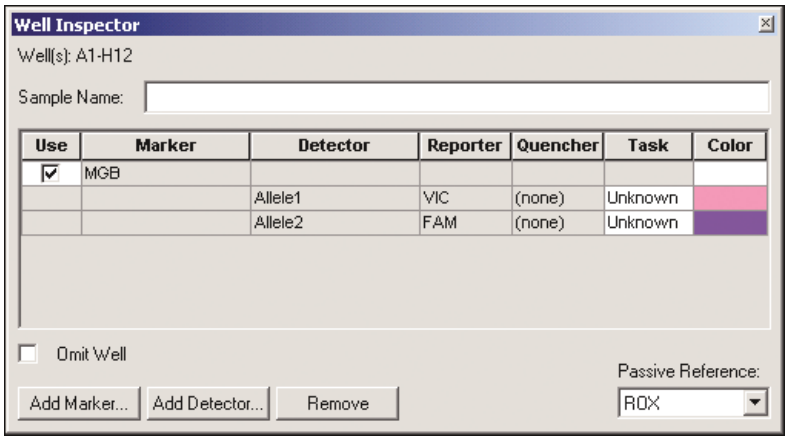
4. On the right side of the Marker Manager window, click the **Use** check box for **Allele 1** and **Allele 2**.



5. Click the **Copy to Plate Document** button, then click **Done**.

6. Select **View** ▸ **Well Inspector**.

7. Highlight the wells and click the **Use** box for **MGB** to add it to the whole plate document.



8. Save the document as a **.sds** plate document and keep it open.

Running a Pre-Read Document

If you want to do a pre-read of a plate document, you must run it before running the plate through the thermal cycler. If you do not want to do a pre-read, go to the next procedure, "Running the PCR" on page 6-11.

To run the pre-read of the plate:

1. With the allelic discrimination plate document still open, click the **Instrument** tab and select the **Pre-Read** button. The pre-read will take about one minute to complete.
2. Select the **Spectra** tab to make sure there was signal generated.
3. Save the file again and close it.

Running the PCR

To perform a post-read allelic discrimination assay, the plate must be thermal cycled. Thermal cycle the plate according to normal recommendations for the assay. For more information, refer to the *Primer Express Version 1.5* and *TaqMan MGB Probes for Allelic Discrimination User Bulletin* (P/N 4317594). Thermal cycling can be done on the ABI PRISM 7000 Sequence Detection System or another instrument. After thermal cycling has finished, perform the post-read on the plate.

To cycle the plate using the 7000 system, use the absolute quantification assay:

1. With the same plate in the system, open a new plate document by selecting **File ▸ New** then select:

   Assay: **Absolute Quantitation**

   Container: **96-Well Clear**

   Template: **Blank Document**
2. Set up the plate document with the same detectors you used in the allelic discrimination plate document you created earlier in "Creating Markers for Allelic Discrimination" on page 6-9.
3. Run the plate document and save the data.
4. Continue to the next procedure to run a post-read on the new plate document.

Running a Post-Read

To run a post-read of the plate document, you must load the same plate into the instrument that has been thermal cycled. Thermal cycling can be done elsewhere and then transferred to the 7000 system for data collection and analysis. Then open the pre-read allelic discrimination plate document.

IMPORTANT: The same plate must be in the system or reloaded to continue this procedure.

To run the post-read of the plate:

1. Open the pre-read allelic discrimination plate document.
2. Select the **Instrument Tab**.

   The thermal cycler is set up for one cycle for a post-read run.
3. Click the **Post-Read** button to run it for allelic discrimination.

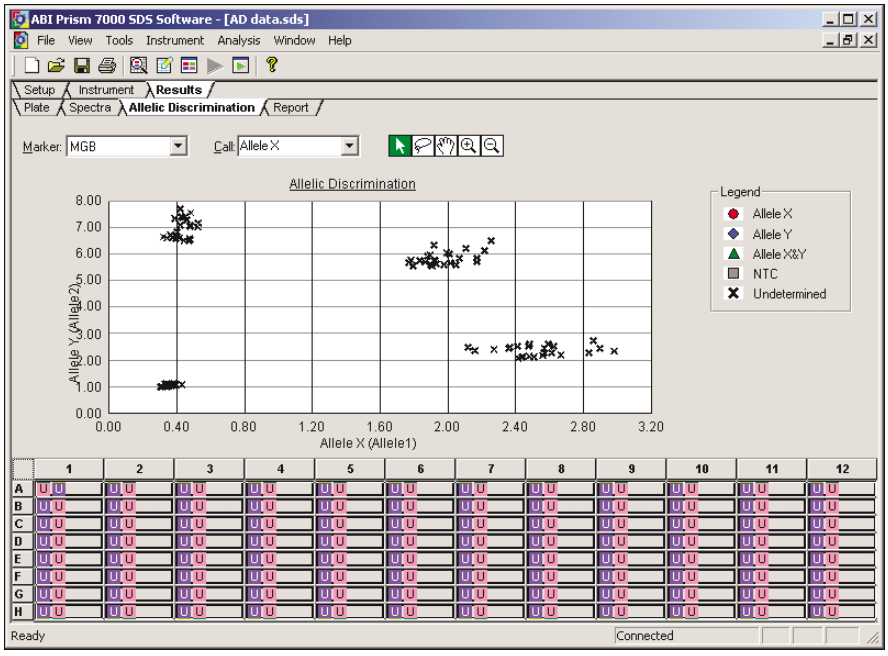
   The results will show the pre-read data subtracted from the post-read data for the run.

Using the Arrow Tool and Lasso Tool to Mark the Alleles

To check the results:

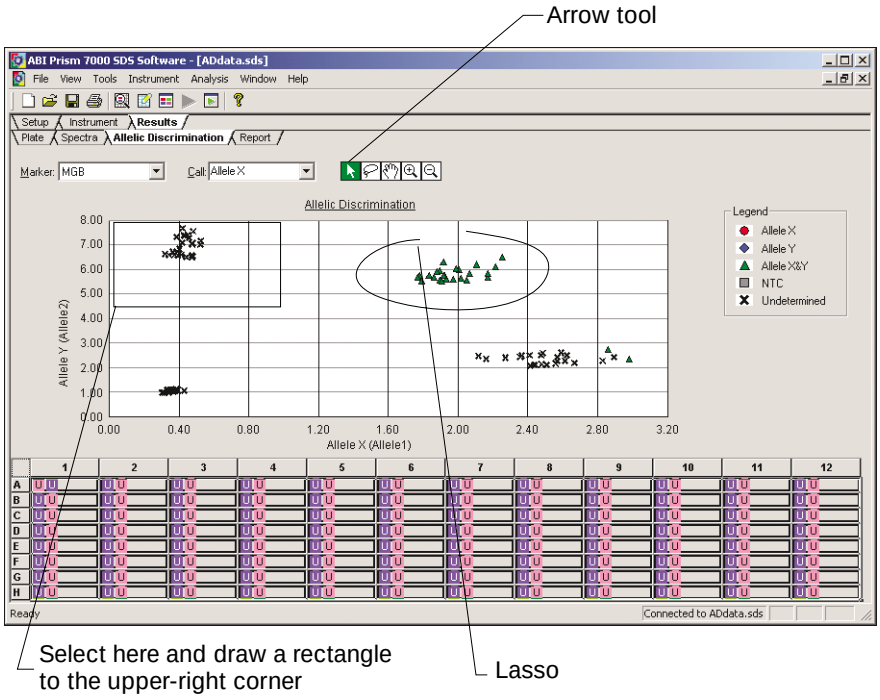
1. Select the **Results** tab ▸ **Allelic Discrimination**.
2. Highlight all the wells in the matrix at the bottom of the window.

   Each highlighted well will be represented as an X on the Allelic Discrimination graph before calls have been assigned to them.



To check the results: *(continued)*

3. Identify the alleles by using the arrow tool or the lasso tool and the selections on the Call menu. In the graph, select the samples with Allele Y (Allele 2) Rn by clicking the arrow tool and dragging a rectangle around the population.



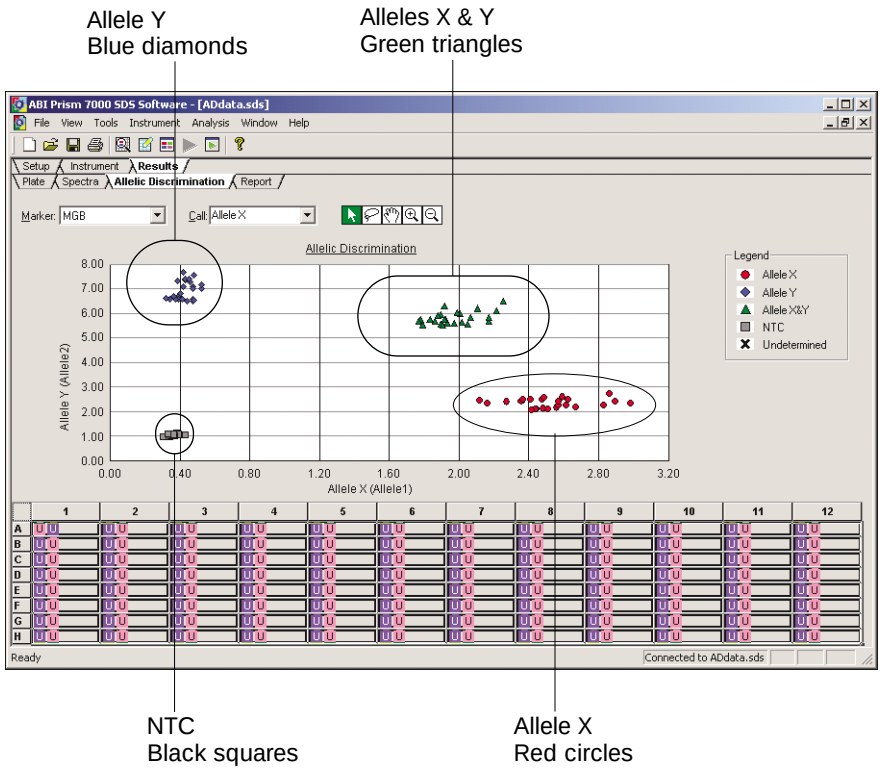
4. From the Call menu, select **Allele Y**. All the samples within the rectangle will change in the scatter plot to blue diamonds.

5. Select the lasso tool and draw around the **X** marks that you have identified as **Allele X & Y**. You do not need to complete the circle. All of the wells with Allele X & Y markers will be identified by the green triangle shown on the Legend on the right of the graph.

To check the results: *(continued)*

6. Select **Call** menu ▸ **Allele X & Y**. These are represented by green triangles.

7. Follow the same procedure for the remaining markers for alleles. When you have completed this, the graph will show all of the wells identified as shown in the Legend.



Dissociation Curve Analysis

Dissociation Curve Analysis on the 7000 Instrument

The 7000 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 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 7000 instrument is made possible through the use of the fluorogenic SYBR Green 1 double-stranded DNA binding dye chemistry. The process begins by loading an ABI PRISM Optical Reaction Plate with PCR samples and the SYBR Green 1 Dye. The plate is then loaded into a 7000 instrument that has been programmed to perform a 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 7000 instrument to monitor the hybridization activity of nucleic acids. During the run, the instrument records the decrease in SYBR Green fluorescence resulting from the dissociation of dsDNA.

Mathematical Transformations

After the run, the 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.

Transforming Raw Data into Dye Components

The first mathematical transformation involves the conversion of the raw data, expressed in terms of fluorescent signal versus wavelength, using the extracted pure dye standards, to pure dye components.

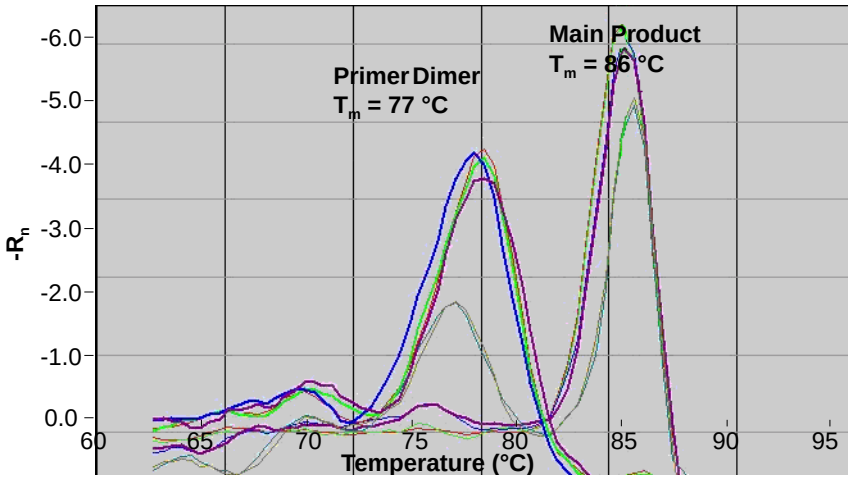
Dye Components

After the dye components have been identified, the software determines the contribution of each dye in the data using the component algorithm.

The software plots the negative of the resulting derivative data on a 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 dissociation curve 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 86 °C, while the primer-dimer product has a characteristically lower T_m of 77 °C.

Designing Dissociation Curve Analysis Experiments

For a detailed explanation of the SYBR Green 1 double-stranded DNA binding dye chemistry, we recommend the following protocols:

- SYBR® Green PCR and RT-PCR Reagents Protocol (P/N 4304965)
- SYBR® Green PCR Master Mix Protocol (P/N 4310251)

Chemistry Kits for Dissociation Curve Analysis

The following Applied Biosystems kits are available for dissociation curve analysis:

Kit	Part Number
SYBR Green RT-PCR Reagents Kit	4310179
SYBR Green PCR Core Reagents Kit	4304886
SYBR Green PCR Master Mix	4309155

Plus/Minus Assays 7

Introduction

About This Chapter

This chapter covers the description of plus/minus assays, their applications, and analysis.

In This Chapter

This chapter contains the following topics:

Overview

Plus/Minus Scoring on the 7000 Instrument

The ABI PRISM® 7000 Sequence Detection System supports plus/minus assays. Plus/minus scoring is the process by which the 7000 system detects the presence of a specific nucleic acid sequence in a prepared sample. Plus/minus assays can be accomplished with or without the use of an internal positive control (IPC); however an IPC ensures that a failed polymerase chain reaction (PCR) is not mistaken for a negative test result. This assay is not real-time, it is an end-point application.

The plus/minus plate type reports normalized reporter signal (R_n), post-read only, or change in normalized reporter signal (ΔR_n), post-read minus pre-read, results for unknown (UNKN) samples.

Employing the 5´ Nuclease Assay for Plus/Minus Scoring

Plus/minus scoring on the 7000 system is made possible by using the fluorogenic 5´ nuclease assay. Plus/minus experiments begin by aliquoting the following to each well of a plate: PCR master mix, primers, and fluorogenic probe constructed for the target nucleic acid sequence. Test samples are then added to the plate and loaded into a thermal cycler for thermal cycling.

During the PCR, the fluorogenic probes anneal specifically to the complementary target sequence between the forward and reverse primer sites on the template DNA. Then during extension, AmpliTaq Gold® DNA polymerase cleaves the hybridized probes in each sample containing the target. 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 7000 system, which reads the fluorescence generated during the PCR amplification. The fluorescent signals, measured by the software, can determine the presence or absence of the target nucleic acid in each sample on the plate.

For plates not using an IPC, the calls for the unknown samples are based on a threshold determined by the no template control (NTC). This threshold determines the minimum fluorescent signal that must be achieved to assign a (+) call to the sample. The confidence value assigned to this call can be determined by selecting a value in the Analysis Settings dialog box.

Using a TaqMan Exogenous IPC Kit for Plus/Minus Scoring

The incorporation of an IPC brings added value to plus/minus experiments by providing a means for validating negative calls made by the SDS software. An IPC assay is a second TaqMan® probe and primer set added to the plate that targets a low-copy, constitutive nucleic acid. If a well does not exhibit amplification, the software uses the positive signal from the IPC assay to confirm that the well failed to amplify because of a lack of template, rather than a pipetting error.

Note: IPC target sequences must be both constituent, so that it provides a consistent positive standard for comparison in all test samples, and low-copy, to minimize the competition between the target and IPC reactions.

The configuration of samples on a plus/minus plate is slightly different when using an IPC. For plates containing an IPC, primers, probe, and template for the IPC assay are pipetted with the target assay to all unknown wells on the plus/minus plate. In addition to the NTC and unknown samples, a positive control for the IPC is also arrayed on the plate. This group of wells (IPC+) contains IPC template, IPC primers and probe, target primers and probe, but no target template.

Plate-read detection collects fluorescent data after PCR is completed. Pre-PCR plate read is not necessary, but it is recommended. If the pre-read data is collected, it will be subtracted from the post-read fluorescence for each well.

Mathematical Transformation of Plus/Minus Data

The SDS software automatically analyzes raw data upon completion of each plus/minus run. 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 spectra changes in the unknown samples and the control reactions (NTC and IPC+).

Transforming Raw Data into Dye Components

The first mathematical transformation involves the conversion of the raw data, expressed in terms of fluorescent signal versus wavelength, using the extracted pure dye standards, to pure dye components.

Multicomponenting

After identifying the dye components, the software determines the contribution of each dye in the raw data using the multicomponent algorithm.

Calling Unknowns Using an Internal Positive Control

First, the software compares the normalized reporter signals (R_n or ΔR_n) generated by unknown samples to the IPC+ threshold. It assigns a positive call to any sample yielding an R_n or ΔR_n value greater than the IPC+ threshold. If negative calls are made, the software compares the R_n or ΔR_n of the IPC for the negative well to the NTC threshold. If the IPC yields an R_n or ΔR_n value greater than the NTC threshold indicating that IPC amplification occurred, the software assigns a negative call (–) to the well. If the IPC yields an R_n or ΔR_n value less than the NTC threshold, the software assigns an undetermined call (?) to the well.

Possible Plus/Minus Calls

If the Detectable Target Template call is...	And the Detectable IPC call is...	Then the Resulting Call is...
+	+, –[a]	+
–	+	–
–	–	No Amplification (?)

[a] In the presence of a strong target signal for the target assay, a negative assignment and/or signal can be obtained in the IPC. This results from the limiting primer concentrations used in the IPC assay.

Note: Because the data from the IPC+ wells contains spectral overlap from the IPC probe signal, the IPC+ threshold minimizes the possibility of a false positive call due to spectral crosstalk.

Chemistry Kits

The following kit is available from Applied Biosystems for designing and running plus/minus scoring chemistries.

Kit	Part Number
TaqMan® Exogenous Internal Positive Control Reagents with TaqMan Universal PCR Master Mix	4308320
TaqMan® Universal PCR Master Mix Kit	4304437

Getting Started

Using the Online Help

The SDS software features an online help system that can guide you through the procedures for setting up, running, and analyzing a plus/minus experiment. To get help at any time during the procedure, click the Help menu, Contents and Index, located on the menu bar.

Maximizing Throughput

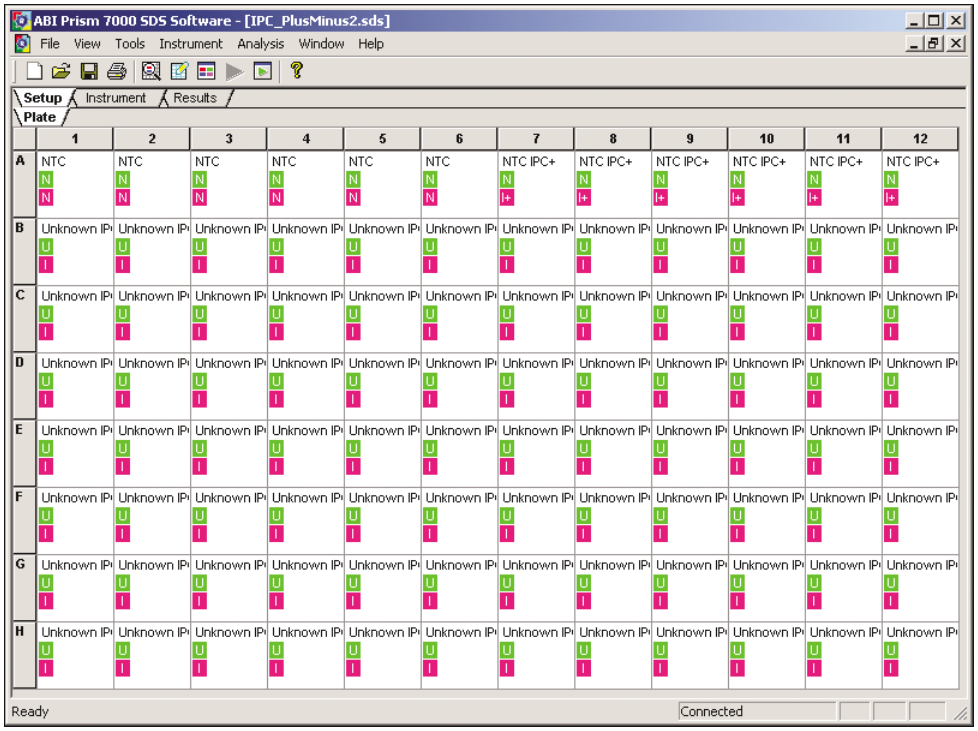
The throughput of the ABI PRISM® 7000 Sequence Detection System can be increased by dividing the workload between the 7000 system and several thermal cyclers. Because plus/minus experiments are end-point runs, the 7000 system does not collect data during thermal cycling. Consequently, the thermal cycling of plus/minus scoring plates can be done elsewhere and then transferred to the 7000 system afterward for data collection and analysis.

IMPORTANT: The SDS software is configured to maximize the throughput of the 7000 system. Consequently, it does not provide the option to thermal cycle plus/minus plate documents. If you would like to perform both the thermal cycling and the plate read using the 7000 system, then you must run the plate first as a real-time plate document and then as a plus/minus scoring plate document.

Examples

Each well of the plate contains 1X TaqMan® PCR Master Mix, and the target and IPC primers and probe sets (FAM™-labeled probe detectors for the target sequence and VIC™-labeled probe detectors for the IPC sequence).

The following figure illustrates the arrangement of the controls and unknown samples, IPCs, and NTCs on the plate. Wells A1–A6 = NTC (no IPC template and no target template), wells A7–A12 = IPC+ samples (IPC template, but no target template), and rows B–H = unknown samples.



Performing End-Point Detection

Setting Up the Software

To perform end-point analysis, follow the procedure below.

To set up the software:

1. Launch the 7000 SDS software from the desktop shortcut.
2. Select **File ▸ New**.
3. In the New Document dialog box, select **Plus/Minus** from the **Assay** menu and leave the rest of the choices as they appear.
4. Click **OK**.
5. Define the detectors for the target as shown in “Target Detector” on page 7-9.
6. Define the detectors for the IPC as shown in “IPC Detector” on page 7-9.

Setting Up the Plate Document for Use With IPC

Overview

Sample plate setups for the target detector and the IPC detector are shown below. There is only one setup shown here although there are other ways of setting up the plate for experiments you may use.

Setting Up Detectors

Detectors need to be set up for both the target and the IPC samples.

To set up detectors:

1. Select **View ▸ Well Inspector**.
2. In the Well Inspector, select the **Add Detector** button. This opens the Detector Manager window.
3. In the Detector Manager, click **File ▸ New**, which opens the New Detector window.
4. Set up the first detector as shown below:

   Name: **Target**

   Reporter Dye: **FAM**

   Quencher Dye: **TAMRA**

   Color: choose whatever color you want and click **OK**.
5. Click **Create Another**.
6. Set up the second detector as shown below:

   Name: **IPC**

   Reporter Dye: **VIC**

   Quencher Dye: **TAMRA**

   Color: choose whatever color you want and click **OK**.
7. Click **OK** in the New Detector dialog box.
8. In the Detector Manager, highlight each of the new detectors, **Target** and **IPC**, and click the **Add To Plate Document** button.
9. Click **Done**.

Adding Detectors to the Plate Document

The Well Inspector should still be open for this procedure.

To add the new detectors to the plate document:

1. Highlight six wells on the plate document (for example, **A1** through **A6**) for the NTC samples.

2. a. In the Well Inspector, type a name for the sample in the **Sample Name** field, if necessary (for this example, we use **NTC**).
   b. Highlight the **Target** detector.
   c. Click **Task** ▸ **NTC**.
   d. Click **Use**.

   An **N** will appear in the six selected wells.

3. a. Highlight the **IPC** detector.
   b. Click **Task** ▸ **NTC**.
   c. Click **Use**.

   A second **N** will appear in the six wells.

4. Highlight 6 different wells on the plate document for the second target (for example, **A7** through **A12**).

5. a. In the Well Inspector, type a name for the sample in the **Sample Name** field, if necessary (for this example, we use **NTC IPC+**).
   b. Highlight the **Target** detector.
   c. Click **Task** ▸ **NTC**.
   d. Click **Use**.

   An **N** will appear in the six wells.

6. a. Highlight the **IPC** detector:
   b. Click **Task** ▸ **IPC+**.
   c. Click **Use**.

   An **I+** will appear in the six wells.

7. Highlight all the remaining wells, rows **B** through **H**.

8. a. In the Well Inspector, type a name for the sample in the **Sample Name** field, if necessary (for this example, we use **Unknown IPC**).
   b. Highlight the **Target** detector.
   c. Click **Task** ▸ **Unknown**.
   d. Click **Use**.

   A **U** will appear in the highlighted wells.

To add the new detectors to the plate document: *(continued)*

9. a. Highlight the **IPC** detector.
   b. Click **Task** ▸ **IPC**.
   c. Click **Use**.
   An **I** will appear in the highlighted wells.

10. Close the Well Inspector and save the plate document.

11. Select **Instrument** tab ▸ **Pre-Read**.
    Note: A pre-read is optional, but is recommended.

Target Detector

In this example, the target detector is used in the following manner:

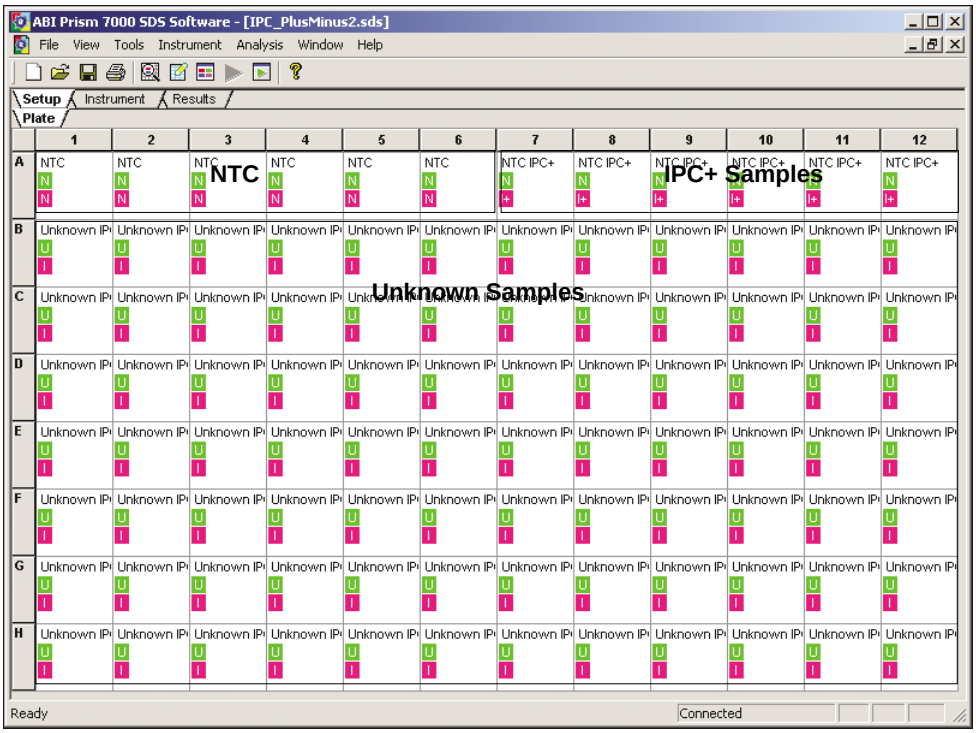
- 12 No Target Template Control (NTC) wells
- 84 Unknown (U) wells

IMPORTANT: Six replicates of each control (NTC and IPC+) must be run to make +/– calls at a 99.7% confidence level. These are required to accurately define the +/– thresholds.

IPC Detector

In this example, the IPC detector is used in the following manner:

- 6 No Target Template Control (NTC) wells
- 6 Internal Positive Controls (IPC+) sample wells
- 84 Internal Positive Controls (IPC) sample wells



Note: Plus/minus plate documents can be configured with detectors for multiple targets. The procedures in this chapter are written for a single target.

Pre-Reading the Plate Document

It is not necessary to perform a pre-read on the plate document, but it is recommended. If pre-read data is collected, it will be subtracted from the post-read fluorescence for each well. This procedure records the background fluorescence of each well of the plus/minus plate prior to thermal cycling.

Starting the Run

Starting a Plate Read

To start a plus/minus plate read:

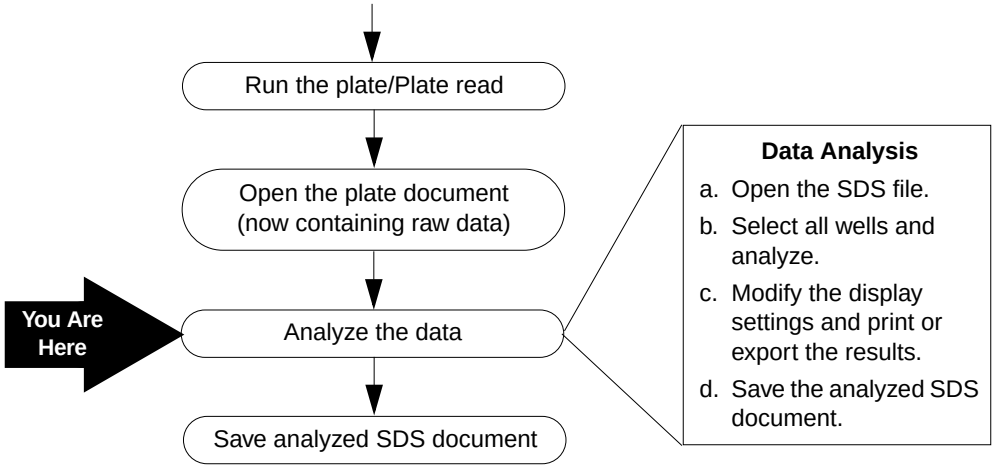
1. Select **Instrument** tab ▸ **Thermal Profile**.
2. Click the **Pre-Read** button if you are doing a pre-read or click the **Post-Read** button if you are doing a post-read to start the run.

Note: If you would like to perform both the thermal cycling and the plate read using the 7000 system, then you must run the plate first as an absolute quantitation document and then as a plus/minus plate document.

Analyzing the Run Data

Where You Are in the Procedure

The following flowchart illustrates your current position in the overall procedure.



Immediately following the analysis of the plus/minus scoring run, you can view the results in both the Results/Plate tab and the Results/Report tab. The results of the analysis can be printed or exported in a tab-delimited format for reference.

Configuring the Analysis Settings

To configure the analysis settings for the plus/minus run:

1.	Select **Analysis** menu ▸ **Analysis Settings**.
2.	In the **Confidence** menu, select the confidence level you want the software to use. The software uses the confidence level to determine the threshold cutoff. The higher the confidence level setting, the higher the cutoff will be.
3.	Click **OK**. The software configures the analysis with new settings.

Analyzing Data for End-Point Runs

To analyze the run:

1.	Select all wells.
2.	In the toolbar, click the **Analyze** icon ( ) to analyze the run data. The results will be displayed in the Results tab.
3.	Select **Results**/**Plate**, or **Results**/**Report** and highlight the wells to view the calls.

Note: If you wish to view the post-read data only, click the **Post-read data only** check box in the Analysis Settings dialog box and reanalyze the data.

Viewing Results

Interpretation of Results

A positive result (+) indicates that the target is present in the sample based on the controls used. On the other hand, a negative result (–) could mean that the sample contains no target or, for some reason, PCR failed in that well. Referring to the results with the IPC probe can distinguish between these two possibilities. A positive signal with the IPC probe shows that PCR amplification can occur in that well and, thus, the lack of target signal means the sample is a true negative. If amplification of the IPC is not detected (?), then PCR was inhibited in that well and it is not possible to determine whether target is present in the sample or not.

Viewing the Results/Report

The software displays the results of the plus/minus run in the Results/Report table of the plate document. The figure below shows an example of the results table containing the data from the run (see “Examples” on page 7-4). Column titles may change to Rn, Mean Rn, and Std Dev Rn if post-read data only is analyzed.

Setup | Instrument | **Results**
Plate | Spectra | **Report**

Well	Sample Name	Detector	Task	Call	Delta Rn	Mean Delta Rn	Std Dev Delta Rn
A11	NTC IPC+	Target	NTC		-0.319	-0.2943	0.0205
		IPC	IPC+		1.320	1.2521	0.1123
A12	NTC IPC+	Target	NTC		-0.303	-0.2943	0.0205
		IPC	IPC+		1.440	1.2521	0.1123
B1	Unknown IPC	Target	Unknown	(+)	1.146	3.4407	2.4817
		IPC	IPC		4.068	2.1643	1.0128
B2	Unknown IPC	Target	Unknown	(+)	1.073	3.4407	2.4817
		IPC	IPC		3.820	2.1643	1.0128
B3	Unknown IPC	Target	Unknown	(+)	1.081	3.4407	2.4817
		IPC	IPC		3.597	2.1643	1.0128
B4	Unknown IPC	Target	Unknown	(+)	1.072	3.4407	2.4817
		IPC	IPC		3.445	2.1643	1.0128
B5	Unknown IPC	Target	Unknown	(+)	1.064	3.4407	2.4817
		IPC	IPC		3.317	2.1643	1.0128
B6	Unknown IPC	Target	Unknown	(+)	1.046	3.4407	2.4817
		IPC	IPC		3.140	2.1643	1.0128
B7	Unknown IPC	Target	Unknown	(+)	1.056	3.4407	2.4817
		IPC	IPC		3.175	2.1643	1.0128

Results/Report Descriptions

Column	Displays
Well	Location of the highlighted well.
Sample Name	The sample name applied to the well.
Detector	Representation of a TaqMan probe or amplicon(s) being detected in a well.
Task	The task (Unknown, NTC, IPC, or IPC+) assigned to the detector.
Call	The call (+, –, or ?) assigned to the target detector.
Delta Rn	The normalized reporter signal generated in the well during the PCR.
Mean Delta Rn	The average value of the Delta R_n data of the samples of the same name.
Std Dev Delta Rn	The standard deviation of the Delta R_n data of the samples of the same name.

After the Analysis

Post-Analysis Options

After analyzing and scrutinizing the run data, you have the following options:

Post-Analysis Options

Action	Description
Reconfigure the appearance of the Results view	The SDS software can alter the appearance of elements in the plus/minus Results views.
Print the results	You can print individual windows, where the software prints an exact copy of what appears on the screen, or you can generate and print an experimental report for the active plate document.
Export the results	The SDS software can export raw or analyzed data in text format for all wells on a plate document. Exported files are compatible with any application that reads text files.

System Maintenance 8

Introduction

About This Chapter

This chapter provides procedures to calibrate the ABI PRISM® 7000 Sequence Detection System to operate properly. It also covers maintenance procedures that you should follow.

In This Chapter

This chapter contains the following topics:

Calibrating the ROIs

Overview

Before obtaining any results from your 7000 system, the regions of interest (ROIs) must be calibrated. When your instrument is installed, the service engineer will perform this task before calibrating the pure dyes or running the installation plate. In the rare event in which the computer or software application loses these values, referred to as a mask for each filter, you should follow the procedures below to calibrate new ROIs.

Materials Needed

The FAM™ Pure Dye Plate located in the ABI PRISM® Spectral Calibration Kit (P/N 4328895) may be used to calibrate the ROIs for the 7000 system.

Note: The Pure Dye plate is recommended, if it is available. The calibration plate is included in the packing kit and can be used if the Pure Dye Plate is not available.

Loading the Calibration Tray

To load the plate or tray to calibrate the ROIs:

1. Open the instrument sample door by lifting up and pushing back, then lock the door in the open position.

   IMPORTANT: Do not push the top of the door until you have lifted the handle to open the door. This will cause the door to break or damage the mechanism.

2. Place the plate in the sample block.

3. Push the door of the instrument to unlock it until it moves out, then pull the handle down to close it in position.

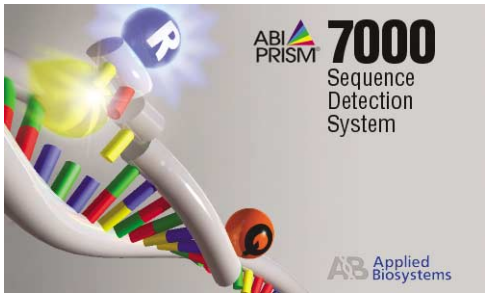
Preparing to Calibrate ROIs

IMPORTANT: When you are doing calibration runs, do not open or manipulate any documents. Wait for the calibrations to finish before opening or setting up documents so that the data is not compromised. It is safe to open documents during a normal run but not during calibration runs.

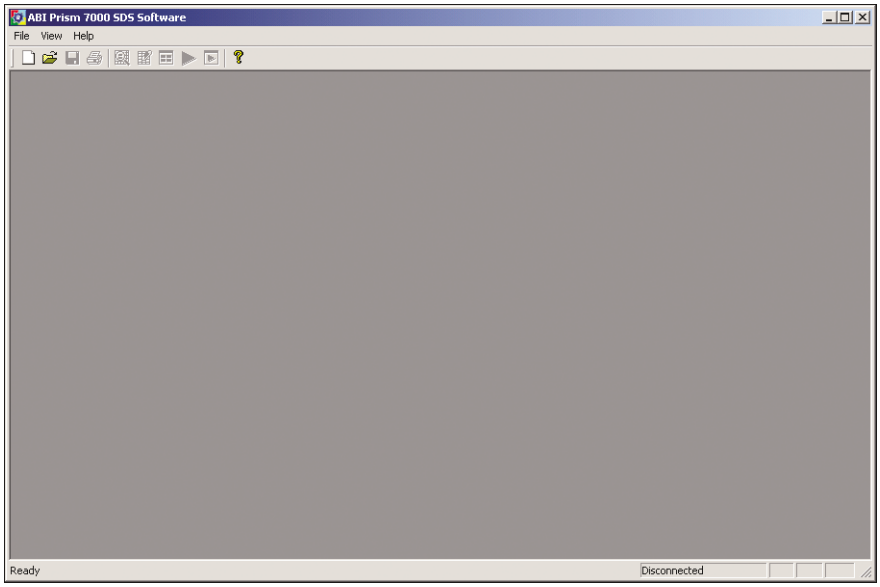
To prepare the instrument for calibration:

1. Double-click the **ABI Prism 7000 SDS Software** shortcut located on the desktop.

The splash screen will open and then there will be an initializing message.

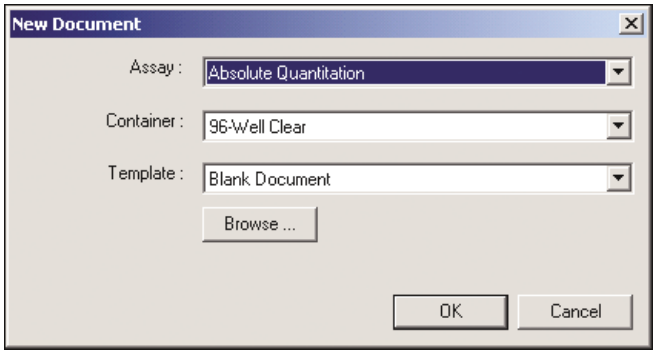


A blank 7000 SDS software window will open like the one shown below.



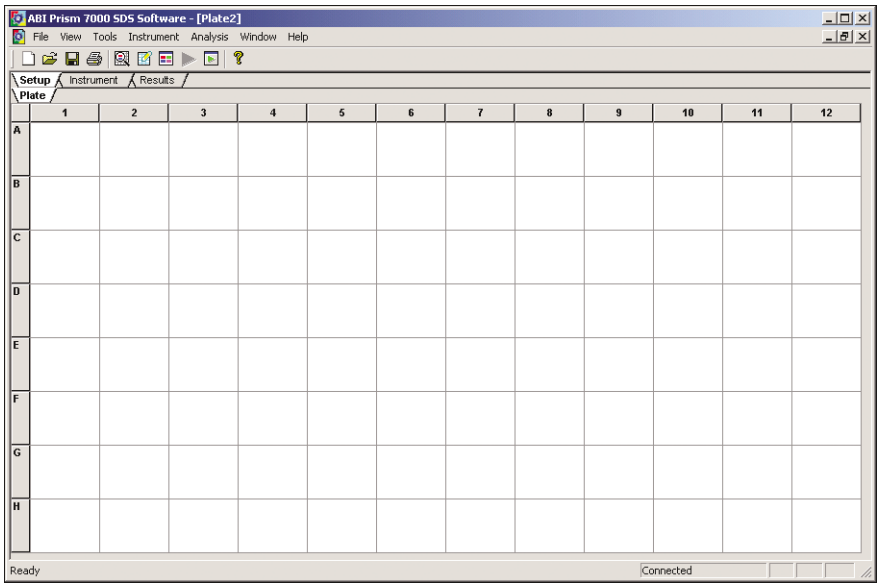
To prepare the instrument for calibration: *(continued)*

2. Select **File** ▸ **New** to open the New Document dialog box as shown below.



3. Click **OK** to accept the default parameters, with **Absolute Quantitation** highlighted as the assay.

   A plate document opens like the one shown in the following figure.



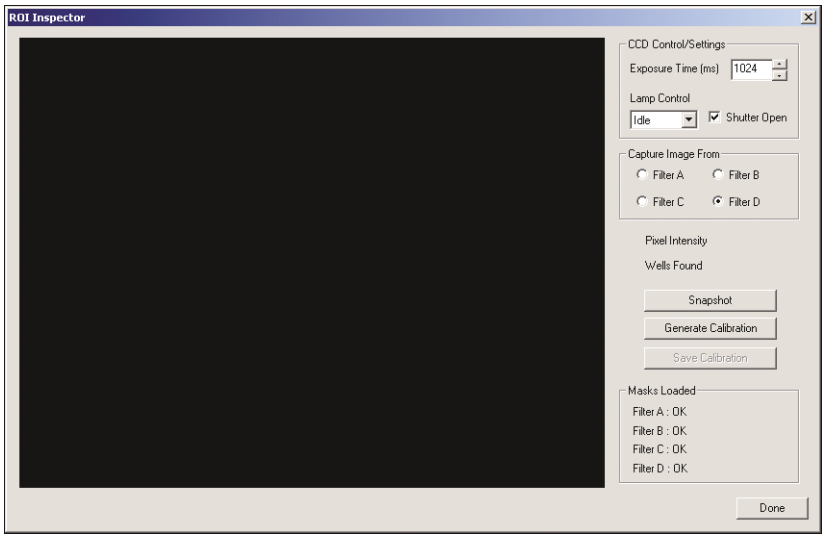
   Note: The instrument message on the status bar changes from Disconnected to Connected.

Checking for Saturation

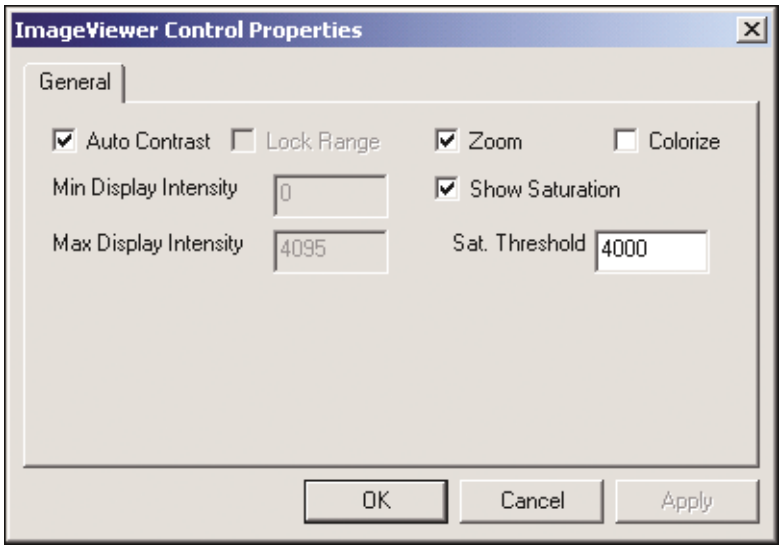
To check for saturation in the image:

1. Select **Instrument** menu ▸ **Calibrate**.

2. When the ROI Inspector window opens, place the pointer in the black area of the window, right-click the mouse, and select **Display Properties** from the menu.



3. The Image Viewer Control Properties window will open as shown below. In the Sat. Threshold text box, enter **4000**. Make sure **Show Saturation** is checked and click **OK**.



4. In the ROI Inspector window, make the following selections:

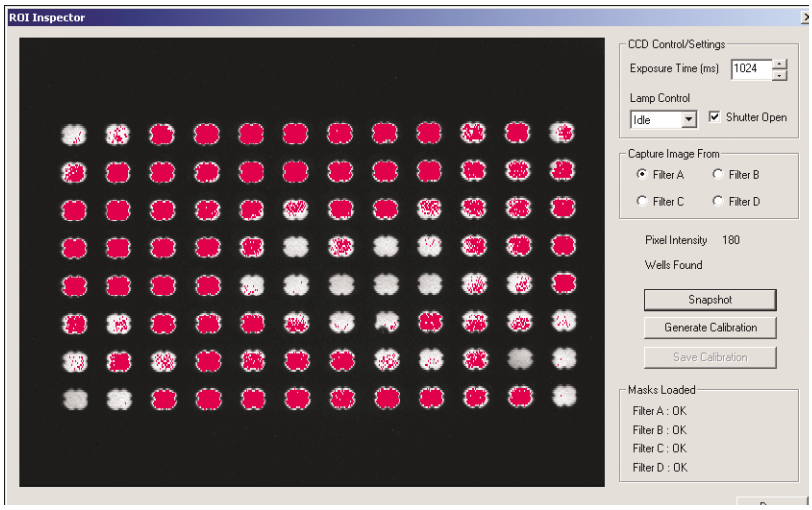
   a. Under Capture Image From, click **Filter A**.

   b. Under Exposure Settings, change the Exposure Time to **256** ms.

   c. Click **Snapshot**.

To check for saturation in the image: *(continued)*

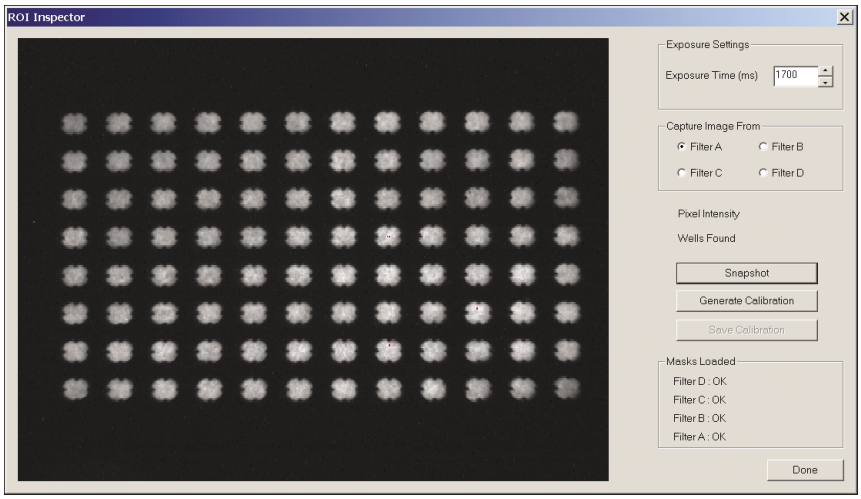
5. Check the image for saturation. The wells in the image must be as bright as possible without oversaturating. Oversaturation is indicated by red pixels appearing inside any of the wells. The image below shows oversaturation.



6. If there is no indication of saturation in the image captured at 256 ms, increase the Exposure Time and click **Snapshot** again. Increase the Exposure Time until saturation is in the image, then move back to the previous time (ms).

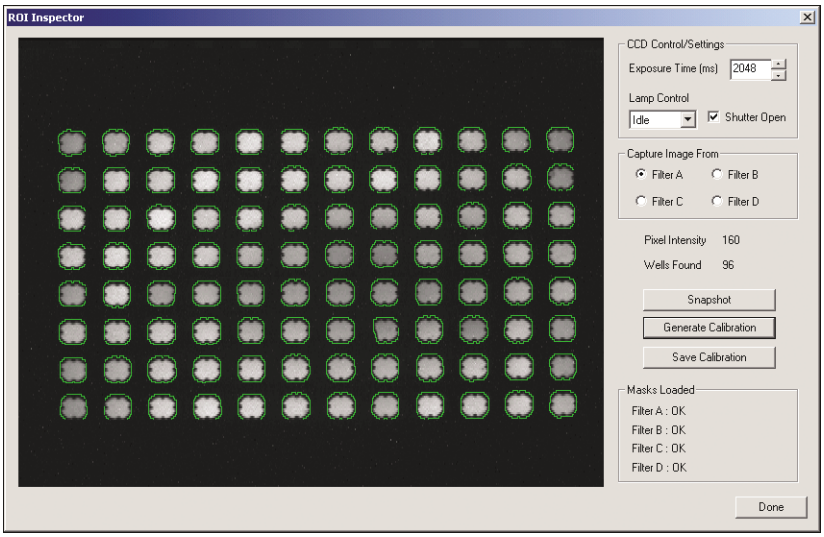
   Note: You can magnify the well areas to look more closely for the slightest indication of saturation. To do this, place the pointer over the well areas in the image. The pointer will change to a Z+. Click the mouse several times to increase the size of the image. To zoom back out, hold the **Shift** key (the pointer changes to a Z-), and click the mouse.

7. Once the proper exposure time has been selected and the wells in the image appear as bright as possible without any saturation present, click the **Generate Calibration** button.



To check for saturation in the image: *(continued)*

8. If all the ROIs in the image are bright enough, green circles will appear around each well area as shown in the image below. If the ROIs cannot be calibrated, repeat steps 5 through 7.

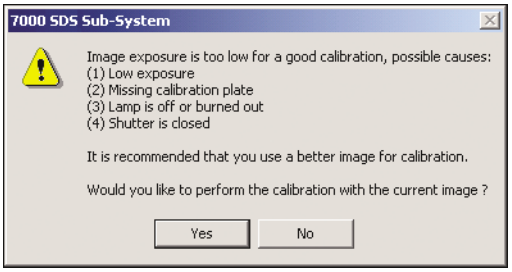


9. Click **Save Calibration**.

   This will save the newly generated ROI calibration for Filter A. **OK** should appear next to Filter A in the Masks Loaded area of the ROI Inspector window.

10. Repeat steps 2 through 9 for the other three filters, then select **Done** in the ROI Inspector when you are finished.

11. If the ROIs in the image are not bright enough or are too bright (oversaturated), there will be an error message indicating this. Select a more appropriate exposure time and regenerate the calibration until the ROIs are calibrated properly.



12. Remember to select **Save Calibration** before moving on to the next filter or selecting **Done** to complete the procedure.

Performing a Background Run

Overview

The background assay calibrates the background component. The background component, along with the spectra component, helps the software determine the contribution of each fluorescent dye to the raw spectra.

This assay is in real time. Thermal cycler conditions are fixed at 60 °C for 10 minutes.

About the Background Component

Fluorescent signals collected by the 7000 system include signal inherent in the system, or a "background" component. To accurately measure gains in fluorescence produced by the PCR, the background component must be measured. Excessive background signal can interfere with the sensitivity of the sequence detection software and its ability to determine threshold cycles (C_Ts).

When to Perform

Because the background signal can change with instrument age, Applied Biosystems recommends regenerating the background component calibration once a month or as often as necessary depending on how much you use the instrument.

⚠ CAUTION Do not set up plate documents or perform other processes on the system during the 10-minute background run as it may compromise the background data file.

Purpose of Background Runs

The SDS software uses the data in a calibration file to compensate for the background component during every run. The software generates the calibration data as the result of a background run in which the instrument measures the level of ambient signal in a MicroAmp® plate containing TaqMan® buffer.

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

Materials Needed

The following materials will be necessary to run a background plate:

- 96-well background tray provided in the Spectral Dye Kit, or
- 96-well tray with 50 µL of deionized water or PCR buffer pipetted in each well
- Talc-free gloves

Preparing a Background Plate

Note: Always wear talc-free gloves when handling the background plate.

To prepare a plate for a background run:

1. Immediately before using the background tray, remove it from the refrigerator and remove the foil packaging.
2. Centrifuge the plate briefly to force all liquid to the bottom of the wells and eliminate any air bubbles from the mixture.
3. Place the prepared plate into the plate holder in the instrument and close the door.

Creating a Plate Document

About Plate Documents

A plate document is an electronic file that represents the 96-well reaction plate. The plate document contains information on the sample types, primers, and probes, and sample locations on the 96-well reaction plate.

Creating a New Background Plate Document

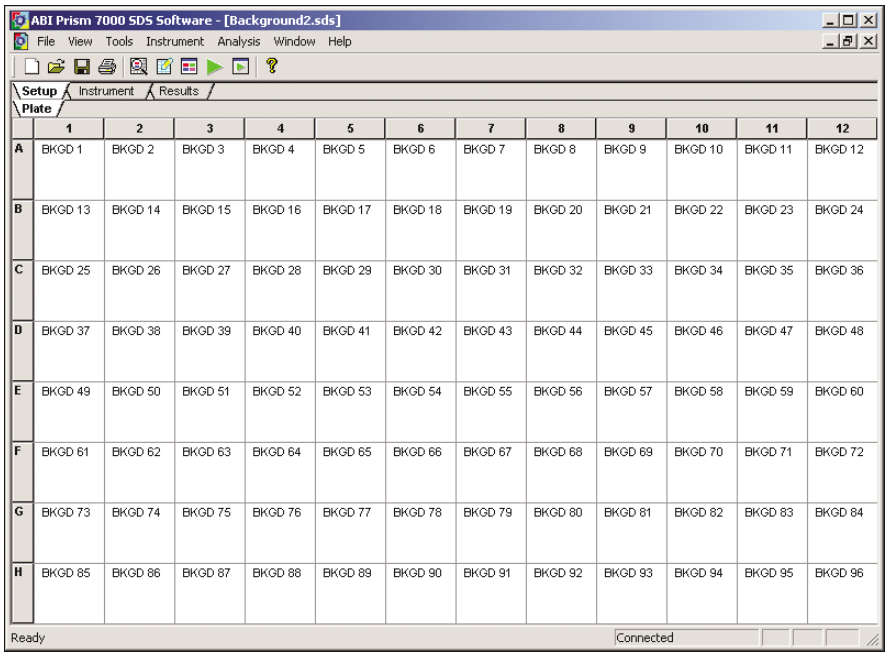
To create a new background plate document:

1. Select **File** ▸ **New** to open a new document.

2. In the New Document window, make the selections shown below from the **Assay**, **Container**, and **Template** menus, and click **OK**.

Menu	Select
Assay	Background
Container	96-Well Clear
Template	Blank Document

The background plate document opens with labels in each of the wells with the attributes for a background run.



The status bar at the bottom of the window shows Ready in the left corner and Connected in the right corner as shown in the figure above to indicate that the instrument is connected to the background document.

Saving the Background Plate Document

You must save the background plate document before the background run will start.

To save the background plate document:

1. Select **File ▸ Save as**.

2. Configure the Save as dialog box as follows:

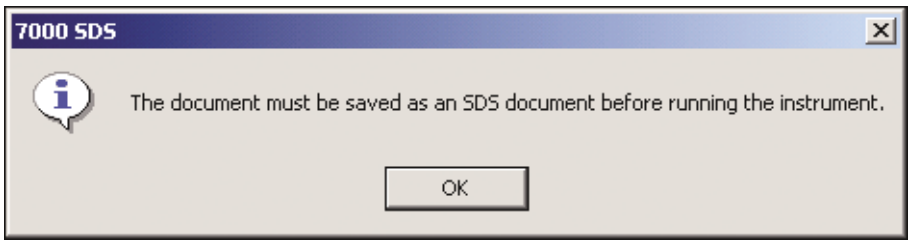
   a. Type **Background** in the **File name** field.

   b. Select **Save as type ▸ SDS Documents (*.sds)**.

3. Click **Save**.

   The software saves the plate document as Background.sds.

   Note: If you do not save the background plate document before starting the run, you will receive a message that you must save it before the run can start.



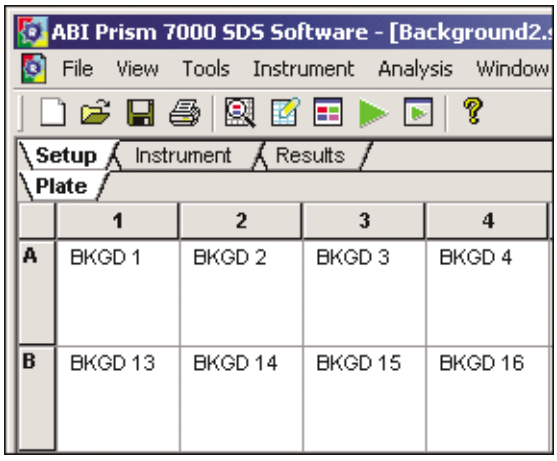
Running the Plate Document

The background run may take 10 minutes to run. Do not set up new plate documents, open existing files, or perform other processes on the system during the background run as this may compromise the background data file.

To run the plate document:

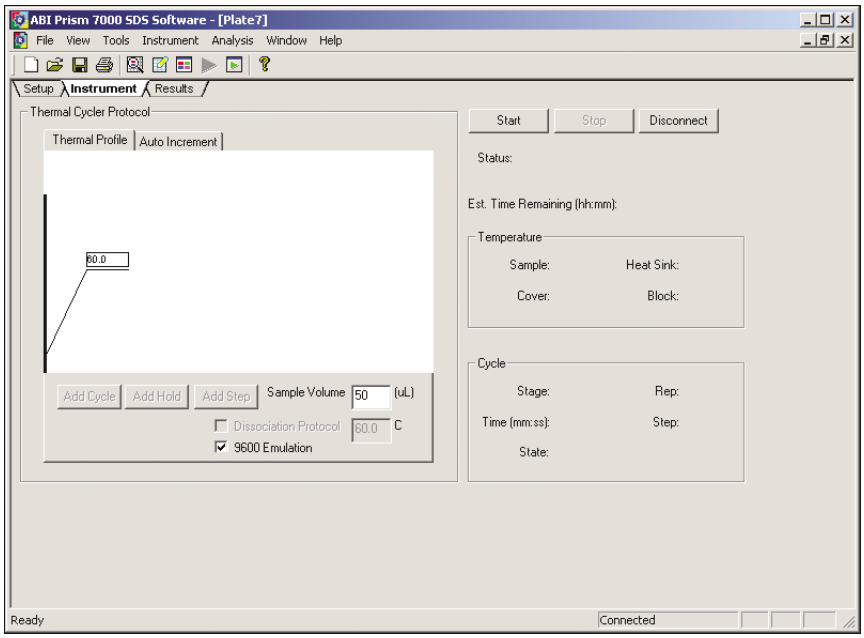
1. In the background plate document, click the **Instrument** tab.

   Note: The tabs under the icons in the window allow you to move around the Setup Plate, Instrument, and Results views.



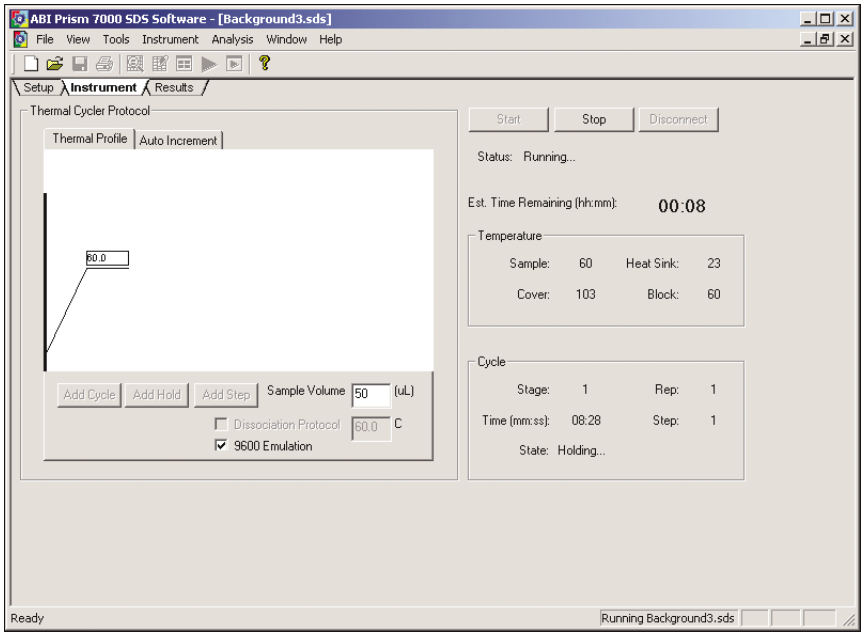
To run the plate document: *(continued)*

2. Click the **Start** button on the Instrument window.



Messages appear next to Status under the Start button as shown in the figure below, then the run starts, showing the time, temperature, and cycle of the run.

Note: The method for a background run is fixed in the 7000 SDS Software and consists of a single hold at 60 °C for 10 minutes. Because a background plate contains only TaqMan buffer, the plate document does not contain or require sample or detector labels.



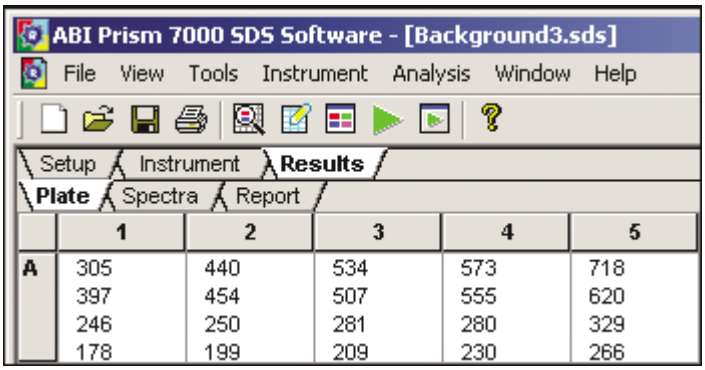
Checking the Results

When the run has finished, you can check to see that it was successful.

To check the background results:

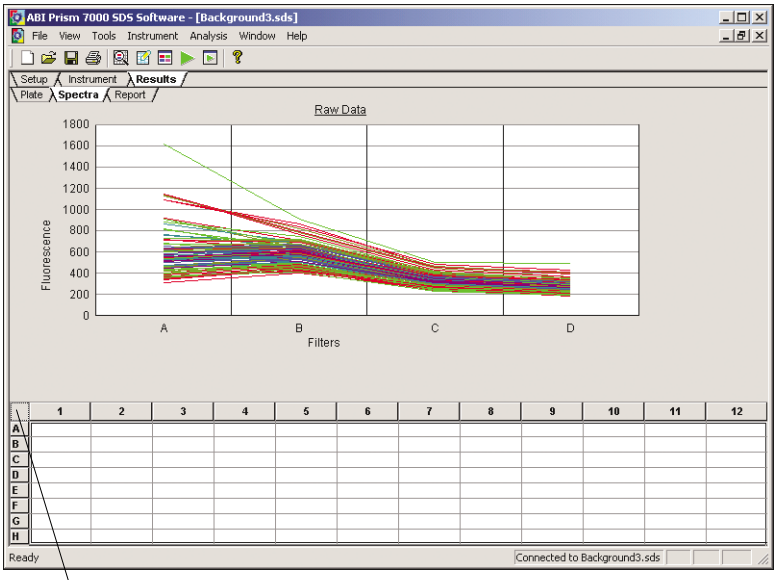
1. When the run has finished, the time remaining is 00:00 and the status is idle.

   Select the **Results** tab to view the raw background data.



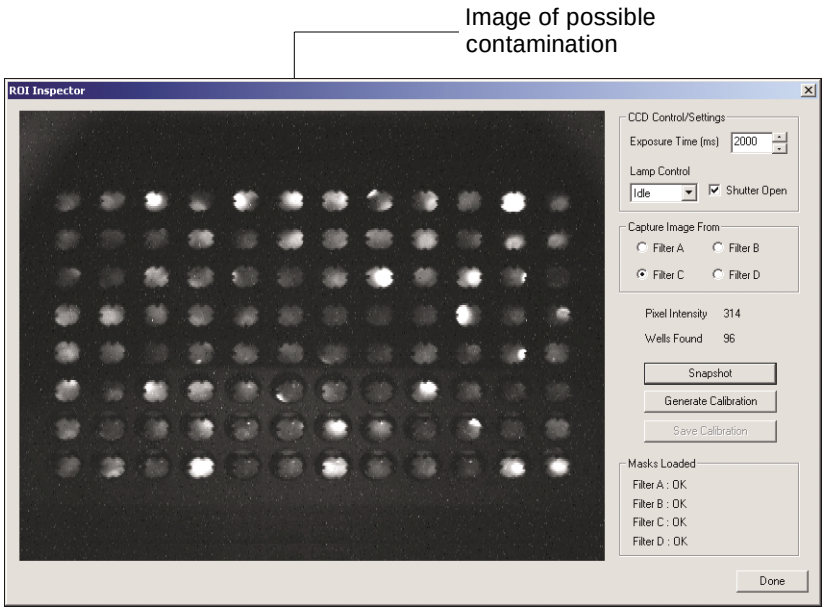
2. Select the **Spectra** tab.

3. Click the box above the A label to select all wells to see the background response of raw data of all 96 wells. Any outlier wells should be identified and cleaned.



To check the background results: *(continued)*

4. If one or more raw spectra do not conform, the run is unsuccessful. The image below is an example of possible contamination. The background plate or the sample block may be contaminated. Check the sample block for contamination as explained in “Cleaning the Sample Wells” on page 8-24.



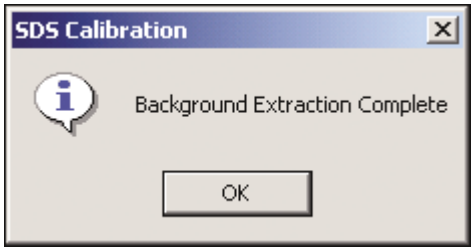
Extracting the Background

After checking the background spectra, you must extract the background.

To extract the background component from the run data:

1. Select **Analysis** menu ▸ **Extract Background**.

   When the analysis is finished, a message appears to notify you that the extraction is finished.

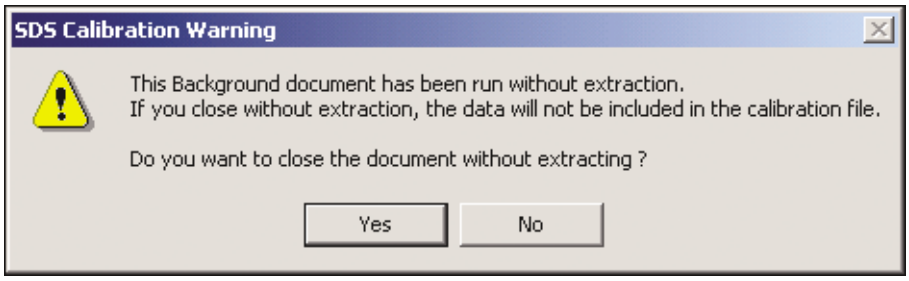


2. After extraction, the file will automatically be stored in the following location:

   C:\Program Files\ABI Prism 7000\Calibration

To extract the background component from the run data: *(continued)*

3. If you try to close the background plate document without extracting the data, you will receive a warning asking if you want to continue. Select **No** and run the extraction, unless you are going to replace the background plate document.



4. To check the numbers in the file at a later date, double-click the **Background.sds** file.

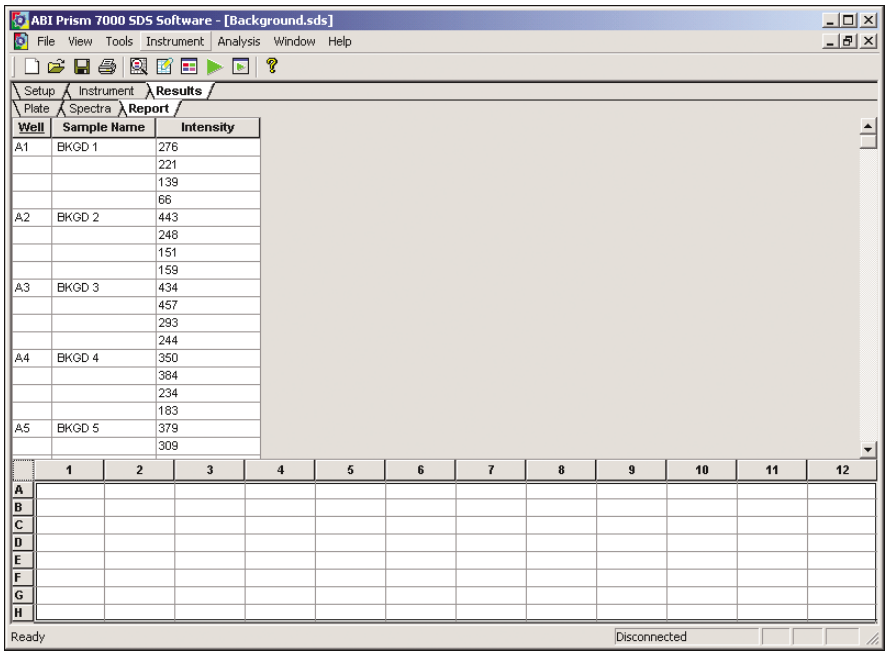
   Note: You will be able to open this file and the PureDyes.sds files later, however, you will not be able to open the calibration files.

Exporting the Background

If you want to export the background assay results, it can be done before or after extracting. Normally, you would not want to export the background results unless they did not pass the specification of fluorescent units. In that case, the results can be saved or exported without extracting them. Try to bring the background into specification by finding the source of contamination and eliminate it.

To export the background:

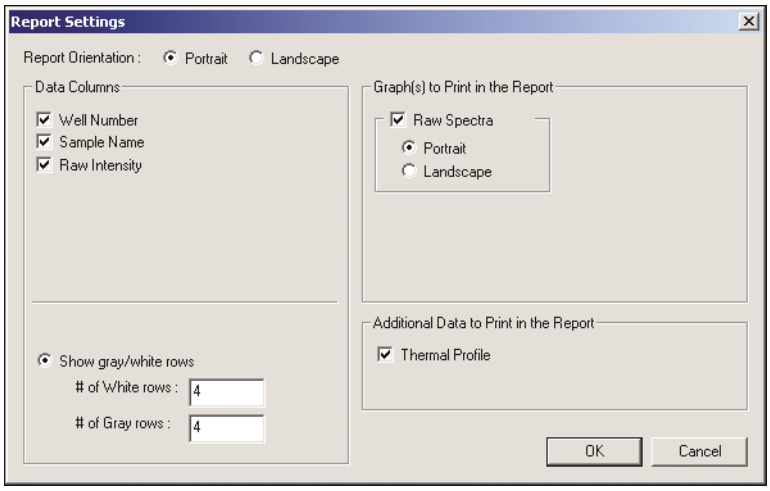
1. Click **Results** ▸ **Report**. Use the scrollbar on the right to scroll through the results.



To export the background: *(continued)*

2. Select all the wells, rows, or columns to see the report of each dye in each well.

3. Select **Tools ▸ Report Settings** to open the Report Settings window. Check the settings and click **OK**.



4. To export data, select **File ▸ Export ▸ Sample Setup**, name the file **Background.csv** and click **Save**.

5. When you have finished the calibration, close the plate document.

Pure Spectra Assay (Pure Dyes)

Overview

The Pure Spectra assay calibrates the pure spectra components. The pure spectra components, along with the background component, help the software determine the contribution of each fluorescent dye to the raw spectra.

Purpose of Pure Dye Runs

The 7000 SDS Software stores pure spectra information for the pure dye standards in the calibration file. Pure dye data is generated from the results of a pure dye run in which the software collects spectral data from a set of dye standards during a 3-minute hold at 60 °C.

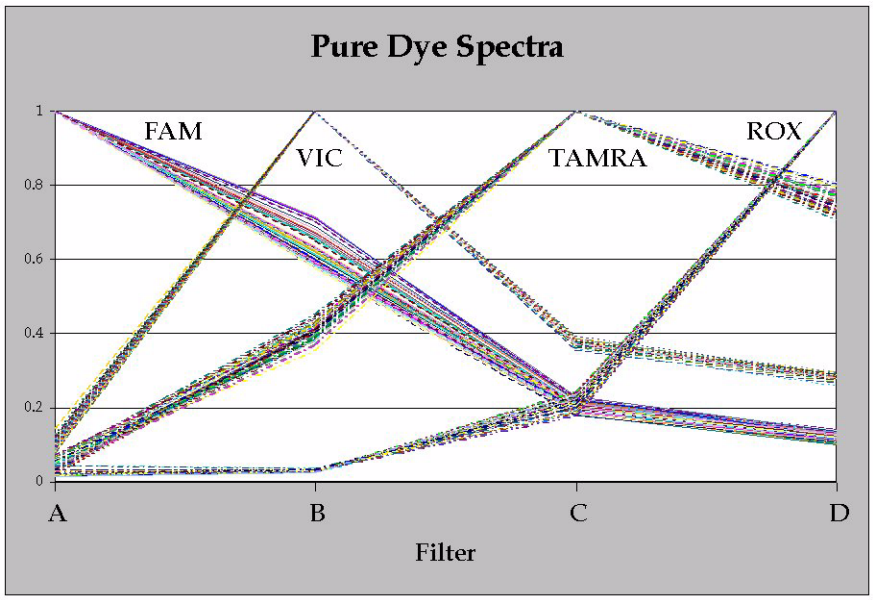
IMPORTANT: Always generate a background plate document before performing a Pure Dye spectra calibration.

When to Perform Spectral Calibration

IMPORTANT: Pure dyes are calibrated during installation of the 7000 system. 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 a year depending on instrument use.

Components of the Pure Dye Spectra

The 7000 system features real-time monitoring of fluorescent signals generated during PCR by FAM, NED™, ROX™, SYBR®, TAMRA™, and VIC™ dyes. The figure below compares the pure spectra for FAM, VIC, TAMRA, and ROX dyes.



After a run, the 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. The software accomplishes the separation by comparing the raw spectra with a set of pure dye standards contained in the calibration file. When a plate document is saved after data analysis, the software stores the pure spectra information with the rest of the collected fluorescent data for that experiment.

The pure spectra data used to analyze the data can be viewed by navigating to the C drive:

C:/Program Files/7000 SDS/Calibration

IMPORTANT: Do not modify, change, move, or delete any of these files. Any of these changes could make your software inoperable.

Materials Required

The Pure Dye run procedure requires the following materials:

- Dye calibration trays for each dye
- Centrifuge, with plate adapter

Preparing a Spectral Calibration Plate

To prepare the spectral calibration plate for the pure dye run:

1. Remove the plate from the freezer and allow it to thaw to room temperature.
2. Briefly centrifuge the plate.
3. Place the prepared plate into the sample block.

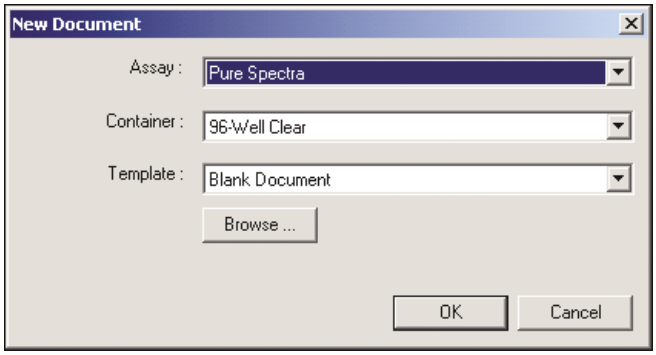
Creating a Pure Dye Plate Document

The information stored during calibration of the pure dyes is used to analyze data. Before performing any runs, you must calibrate the dyes.

To create a plate document for a pure dye run:

1. Double-click the **ABI Prism 7000 SDS Software** shortcut 

on the desktop to start the software, if it is not already open.
2. Click the **New** icon (□) on the toolbar.
3. In the New Document dialog box, select the following setups and click **OK**:



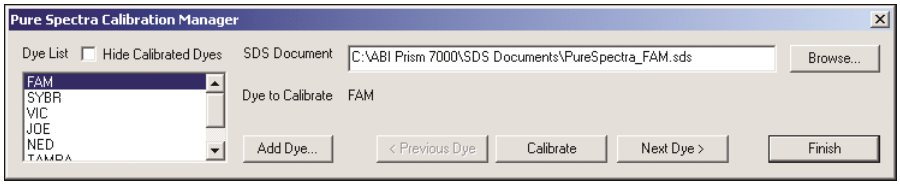
Note: The Pure Dye plate document contains fixed detector labels.

Calibrating the Pure Dyes

The Pure Spectra Calibration Manager software will lead you through the process with prompts to calibrate the dyes. Each dye must be calibrated using a separate dye and plate for each run.

To calibrate each dye:

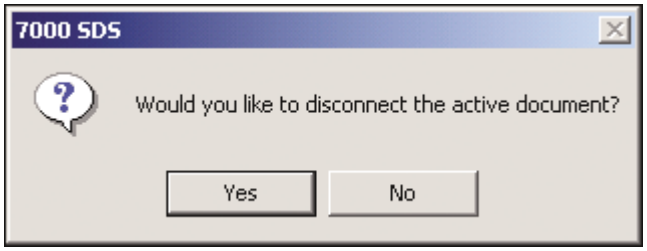
1. The Pure Spectra Calibration Manager shown below and a new blank plate document will open. The window shows the instrument as Ready on the left of the status bar and Connected on the right.



   Select the dye from the **Dye List** for the plate you will be calibrating.

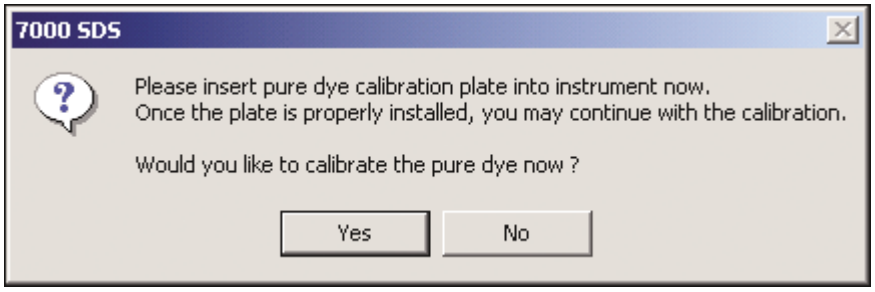
2. Select the **Calibrate** button.

3. Click **Yes** to the message asking if you want to disconnect the active document. The blank plate document will be disconnected so that the dye can be calibrated.



   The plate document now shows the dye name in the Plate Setup and Connected to the dye plate document in the status area.

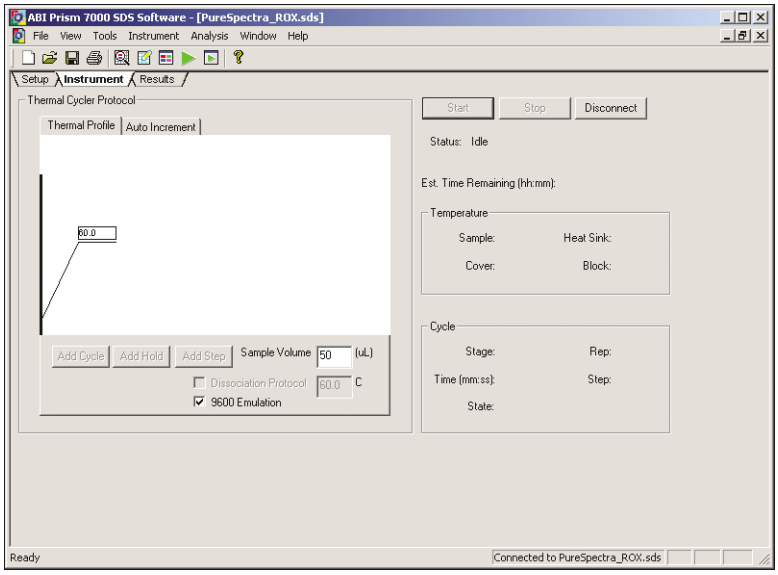
4. Follow the prompts onscreen by loading the dye plate you are going to calibrate and click **Yes** to calibrate the dye.



To calibrate each dye: *(continued)*

5. After selecting **Yes** to begin the calibration, select the **Instrument** tab to open the Instrument view.

   The calibration takes about three to five minutes. The following graphic shows the ROX dye after calibration.



   When calibration is complete, Status will show Idle.

6. Select the green **Analyze** button on the toolbar to extract the spectra.

   You will receive a message when it has completed the extraction. The software extracts the pure spectra and stores the data as a component of the calibration file.

7. To calibrate the next dye in the Dye List, click **Next Dye >** or select another dye from the Dye List.

8. Continue with the procedure by selecting **Calibrate**. Remember to extract each dye spectra after each calibration.

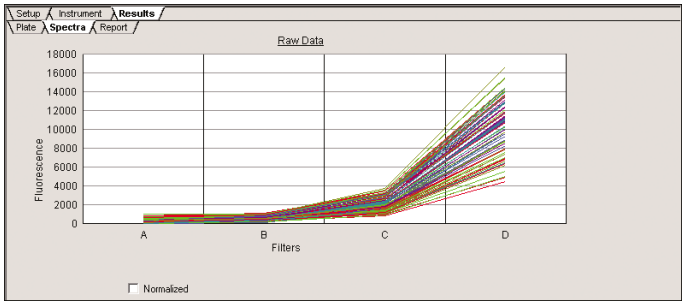
   Note: If you do not extract the pure spectra, you will receive a message asking if you want to close the document without extracting. The dye will still be on the Dye List if you click the Hide Calibration Dyes check box.

9. Follow this same procedure for the rest of the trays of pure dyes. After you have loaded each dye plate and calibrated the dyes you are using, click **Finish** in the Pure Spectra Calibration Manager.

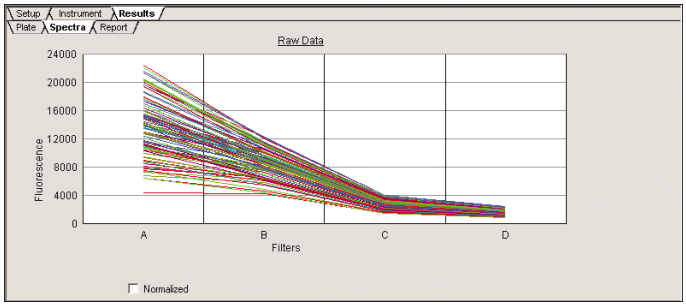
To calibrate each dye: *(continued)*

10. After all of the dyes have been calibrated, compare the results of each dye by clicking **Results ▸ Spectra**. Below are examples of pure spectral calibrations for ROX, FAM, VIC, and TAMRA dyes.

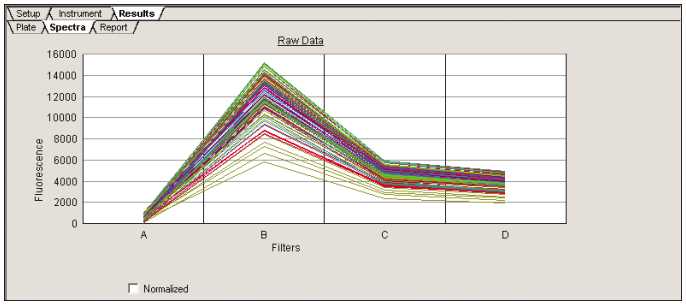
ROX spectra



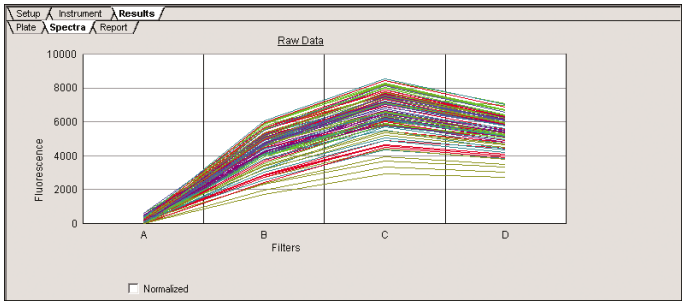
FAM spectra



VIC spectra



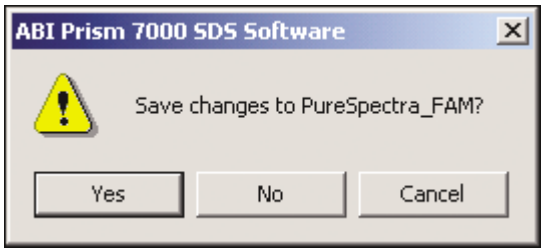
TAMRA spectra



To calibrate each dye: *(continued)*

11. When you select **Results ▸ Report**, it shows the fluorescence data of each well. This data and the spectral calibrations can be printed or exported.

12. When closing the Pure Spectra plate document, you will be asked if you want to save the changes. Select **No** to close the window.



13. If there is a detector in any of the wells that contains an uncalibrated dye, there will be an error message before the run is started.

To calibrate the dyes, refer to “Calibrating the Pure Dyes” on page 8-19.

Quick System Tests

Overview

You have limited access to the diagnostic procedures in the 7000 software. You may use the software for the following instrument tests and settings:

- Checking the thermal cycler for well contamination
- Verifying the shutter and lamp operation

Checking the Thermal Cycler for Well Contamination

To check the thermal cycler for well contamination:

1. Turn on the 7000 system.
2. Launch the software from the **ABI Prism 7000 SDS Software** shortcut located on the desktop.
3. Remove the reaction plate from the thermal cycler sample block.
4. Pull the carriage forward over the sample block and lock it down.
5. Use the FAM filter position, open the **ROI Inspector** and take an image at 2048 ms.
6. Observe the background fluorescence in the 96 wells. Note any wells that have significant fluorescence; fluorescence indicates contamination.

   Note: The view of the wells is a top-down view, with A1 in the upper left and H12 in the lower right.
7. To determine an acceptable background fluorescence level:
   a. Push back the 7000 instrument carriage.
   b. Put a new, clean, empty reaction plate without the cover into the sample block.
   c. Pull the carriage forward and pull the door down to close it.
   d. Take another image at 2048 ms.
8. If some of the wells still appear to have significant fluorescence, clean them per the instructions in “Cleaning the Sample Wells” on page 8-24.

   Recheck the wells after cleaning, following steps 1 through 6 above.

Maintenance of the Thermal Cycler

Overview

This section describes how to perform routine maintenance on the 7000 system thermal cycler without the aid of an Applied Biosystems Service Representative.

⚠ WARNING PHYSICAL INJURY HAZARD. Do not remove the instrument cover. There are no components inside the 7000 system that you can safely service yourself. If you suspect a problem, contact an Applied Biosystems Service Representative.

Cleaning the Sample Wells

The sample wells should be checked at least once a month or more frequently if needed. Clean only wells with high backgrounds. See "Checking the Thermal Cycler for Well Contamination" on page 8-23.

Materials Needed

To clean the sample wells, you will need:

- Cotton or nylon swabs and lint-free cloths
- 10% bleach solution
- 95% EtOH solution

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

To clean the sample wells:

1. Turn off the instrument and wait 15 minutes.
2. Remove the sample tray from the block and set it aside.
3. Put a small amount of 10% bleach solution into the sample block wells and use a swab to scrub inside the wells.

   ⚠ 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.
4. Absorb excess bleach with lint-free cloths.
5. Put a small amount of 95% EtOH solution into the sample block wells and use a swab to scrub inside the wells.

   ⚠ WARNING CHEMICAL HAZARD. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6. Absorb excess EtOH with lint-free cloths.
7. With the carriage **not** over the wells, turn on the thermal cycler and ramp it up to about 50 °C to evaporate any residual bleach or EtOH.

Replacing the Halogen Bulb

Overview

The bulb in the halogen lamp should be replaced after approximately 2000 hours of life. If you need more information on this procedure, contact your Field Service Engineer.

Materials Needed

The following materials will be necessary to replace the halogen bulb:

- Protective disposable gloves
- New halogen bulb
- Phillips screwdriver
- Sample plate to keep the optics assembly from moving

Replacing the Bulb

To replace the bulb in the halogen lamp:

1. Turn off the 7000 system. Allow it to cool for 15 minutes.

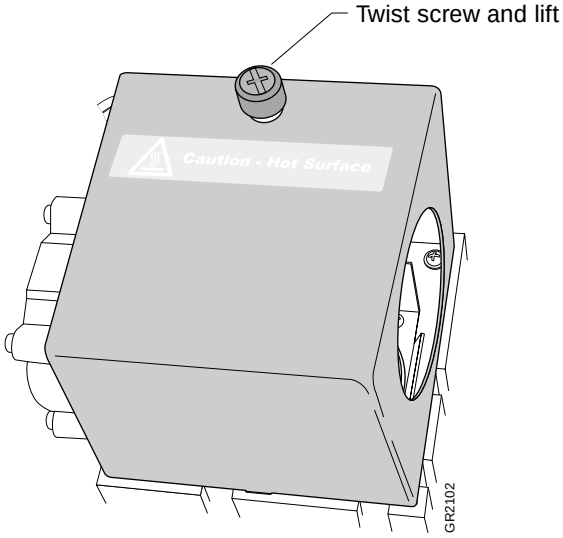
   ⚠ WARNING **PHYSICAL INJURY HAZARD**. The 7000 Sequence Detector and halogen lamp are hot! The lamp can become very hot while in use. Allow sufficient time for the lamp to cool, and put on protective gloves before handling it.

   IMPORTANT: You should always wear disposable gloves when handling the new replacement bulb to prevent burning and bulb contamination.

2. Load a sample plate into the instrument and close the door. This will hold the optics assembly securely in place while you change the lamp, otherwise the optics may move while you are replacing the lamp.

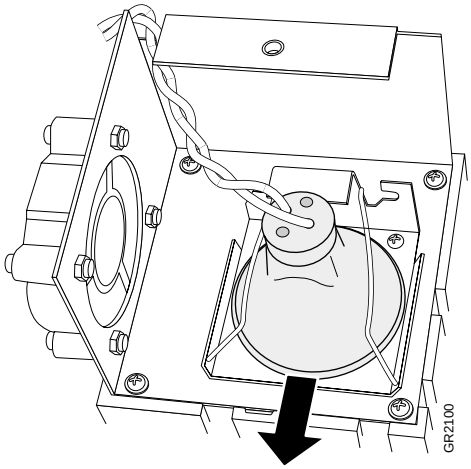
3. On the top of the instrument, lift open the lamp access door.

4. Loosen the screw holding the lamp cover in place and remove the cover from the assembly by sliding it forward.



To replace the bulb in the halogen lamp: *(continued)*

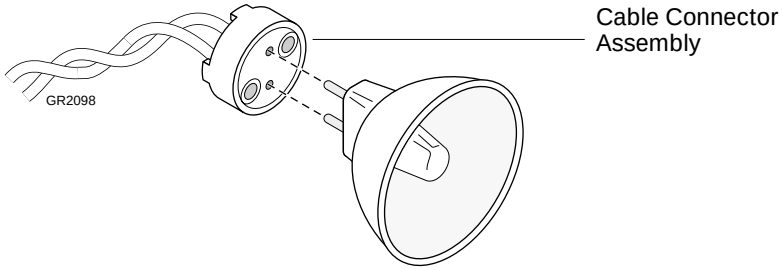
5. Remove the old bulb from the slotted mount by sliding it forward, and remove the cable by sliding it off the lamp connector.



⚠ CAUTION Handle the lamp with gloves only. Finger prints on the lamp will shorten lamp life. **Never** touch the bulb itself.

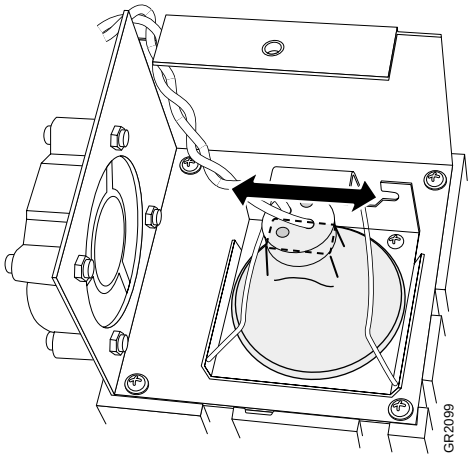
6. Attach the lamp connector to the back of the new bulb as shown in the following figure.

⚠ CAUTION Remove and replace the cable from the lamp away from the exposed filter in the optics compartment. Do not push the bulb into the cable connector assembly so far that it damages the lamp connection points or the ceramic. The connection needs to be made, but the points on the lamp do not need to be jammed into the holes.



To replace the bulb in the halogen lamp: *(continued)*

7. Align the long axis of the lamp parallel to the front of the instrument and slide the new bulb back into place, being careful of the cable. Refer to the following figure.

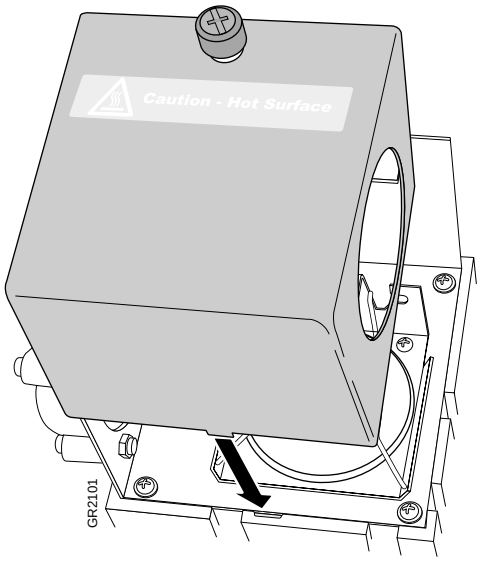


8. Make sure the cable is in the notch in the back as shown above before putting the cover on so that it does not get caught.

9. With the lamp access door open, turn on the instrument. Make sure the lamp turns on when the instrument is on.

   Note: If the new halogen bulb does not work, there may be a problem with the 7000 electronics fuse. Refer to the procedure, “Replacing the Fuses” on page 8-28.

10. Reattach the lamp cover and make sure the cover tab fits into the notch at the base of the lamp assembly. The arrow in the following graphic points to the notch.



11. Tighten the thumbscrew.

Replacing the Fuses

Overview

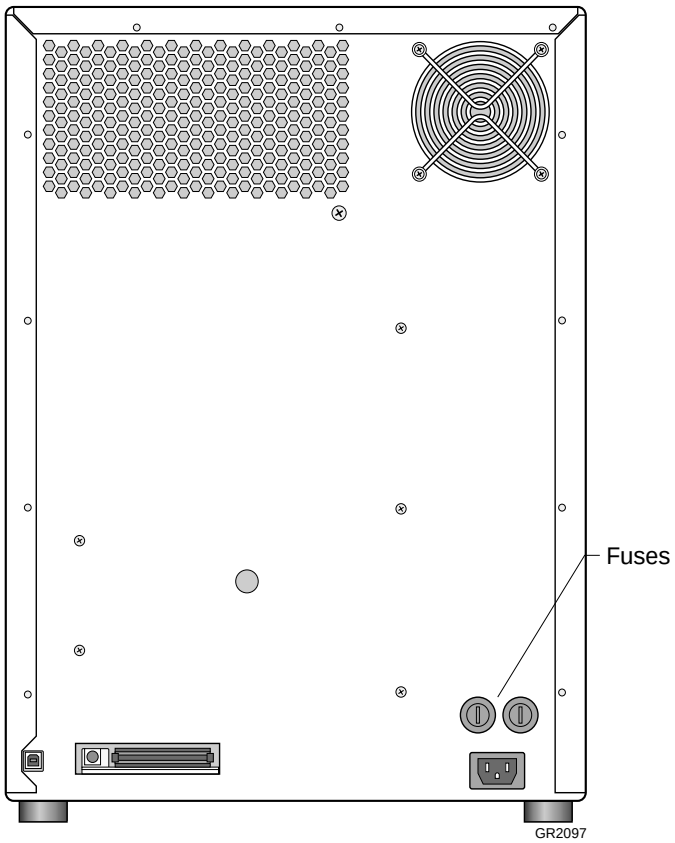
If the halogen lamp is not working after you have replaced the bulb, the fuses in the instrument may be blown.

⚠ CAUTION **FIRE HAZARD**. For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the same type and rating as those currently in the instrument.

To replace the fuses in the instrument:

1. Turn off the instrument and unplug it.

2. Remove the fuse holder and inspect each fuse.



Note: The fuse holders are next to the power cord.

3. If a fuse is blown, replace it with the appropriate fuse for the instrument.

   IMPORTANT: The fuses used to replace the blown ones **MUST** be 12.5A, 250V, 5 x 20 MM fuses.

   Note: The voltage and amperage ratings are on the fuse holder.

4. Put the fuse holder back into the instrument.

5. Plug in the instrument.

System Hardware Test

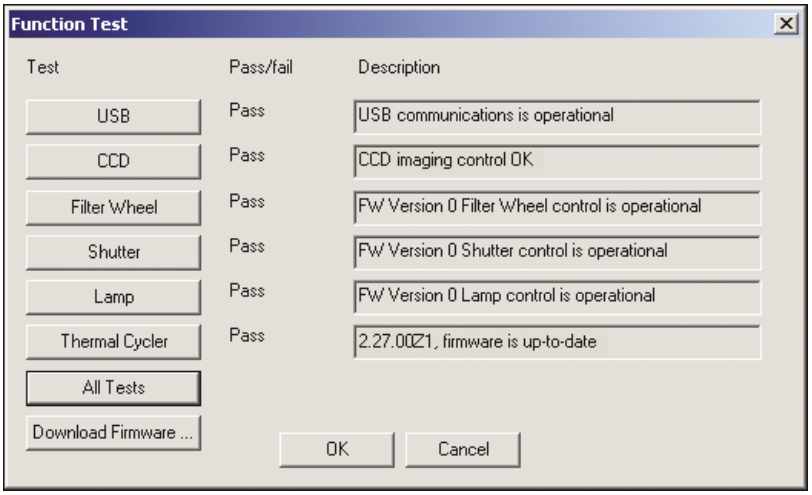
Checking the Hardware

There is a system hardware test available to make sure everything is operating properly.

To check the system hardware:

1. Select **Instrument** menu ▸ **Function-Test**.
2. To test an individual operation, select one of the buttons on the left side of the window to test it. There will be a Pass or Fail shown and a description of the test.
3. To test all the components of the system, click the **All Tests** button.
4. If all tests pass, click **OK** to close the window.
5. If all tests do not pass, contact your service representative.

The screen capture below is an example of a function test that has passed all the tests.



Theory of Operation A

Introduction

About This Appendix

This appendix provides the theory of operation for the ABI PRISM® 7000 Sequence Detection System.

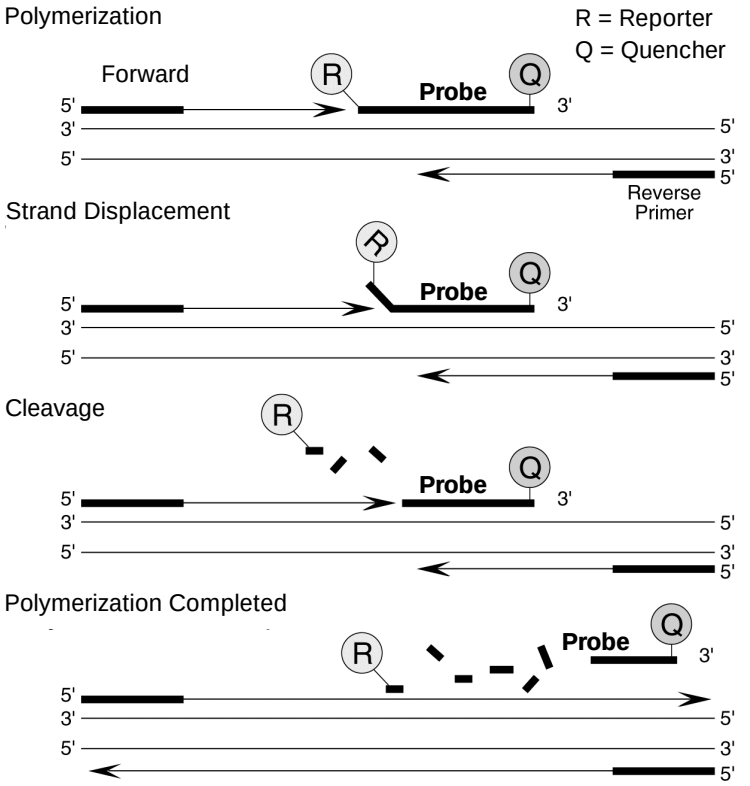
In This Appendix

This appendix contains the following topics:

Fluorescent-Based Chemistries

Basics of the 5´ Nuclease Assay

The polymerase chain reaction (PCR) 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´ to 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.

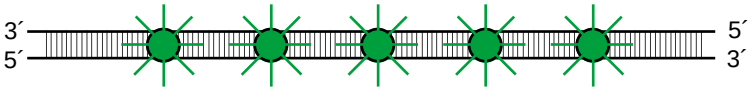
The 5´–3´ nucleolytic activity of the AmpliTaq Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3´ end of the probe is blocked 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.

SYBR Green 1 Dye Chemistry

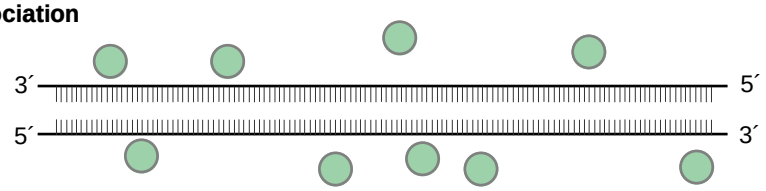
The SYBR® Green 1 Double-Stranded Binding Dye is used for the fluorescent detection of double-stranded DNA (dsDNA) generated during PCR. The SYBR Green 1 Dye binds non-specifically to dsDNA and generates an excitation-emission profile similar to that of the FAM™ reporter dye. When used in combination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

SYBR Green 1 During a Single Cycle of a PCR



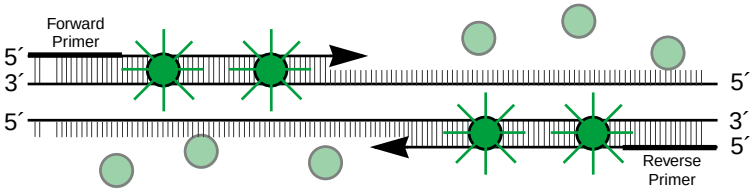
When added to the reaction, the SYBR Green 1 Dye binds non-specifically to the hybridized dsDNA and fluoresces.

Dissociation



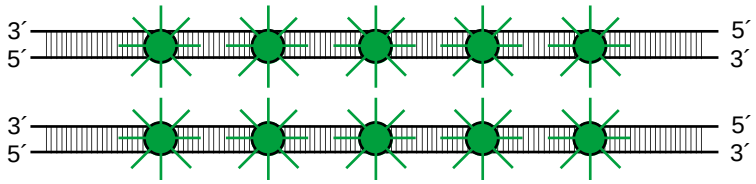
Denaturation complete, the SYBR Green 1 Dye dissociates from the strand, resulting in decreased fluorescence.

Polymerization



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

Polymerization Complete



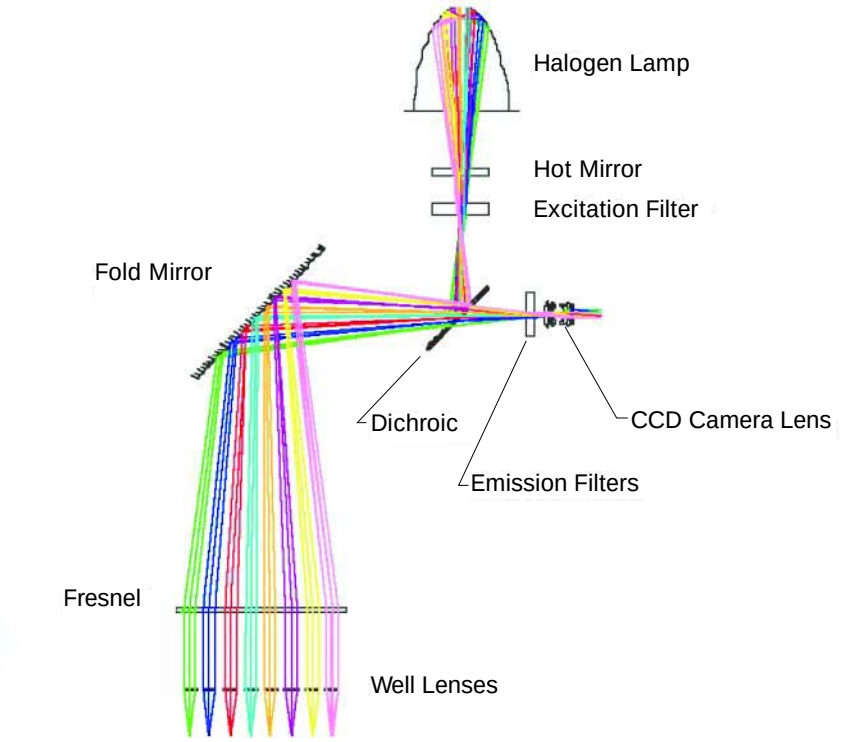
Polymerization is complete and SYBR Green 1 Dye is completely bound, resulting in a net increase in fluorescence.

Fluorescence Detection and Data Collection

Fluorescent Sequence Detection

During PCR, light from a tungsten-halogen lamp is focused simultaneously to each well on the microplate. The light passes through the opening of the sample well and the light 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 during the extension phase of the PCR reaction.

A system of lenses, filters, and a dichroic mirror focus the fluorescence emission into a charge-coupled device (CCD) camera. The filters separate the light (based on wavelength) into a predictably spaced pattern across the CCD camera. The 7000 software collects the fluorescent signals from the CCD camera and applies data analysis algorithms. Refer to the figure below.



Mathematical Transformations

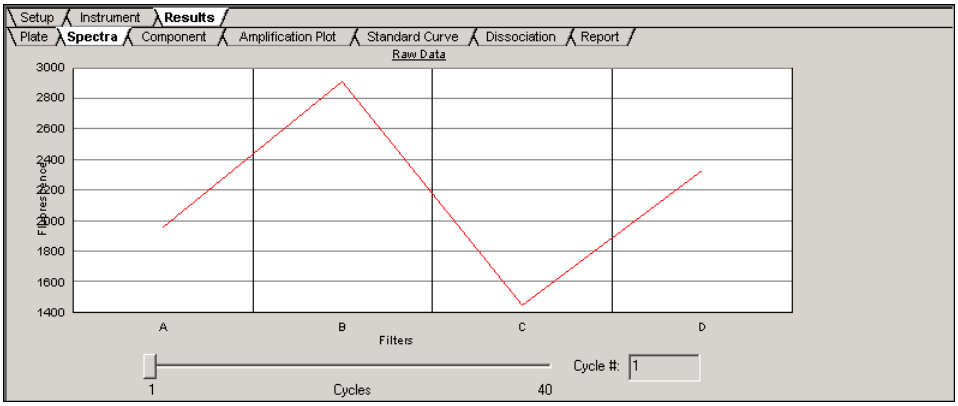
Overview

The 7000 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 and 660 nm collected by the 7000 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 runs by the 7000 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 7000 system. During the multicomponent transformation, the 7000 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 in the background calibration file. Next, the software employs the extracted pure dye standards 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 software multicomponenting algorithm applies matrix calculations to determine the contributions of each component dye spectra.



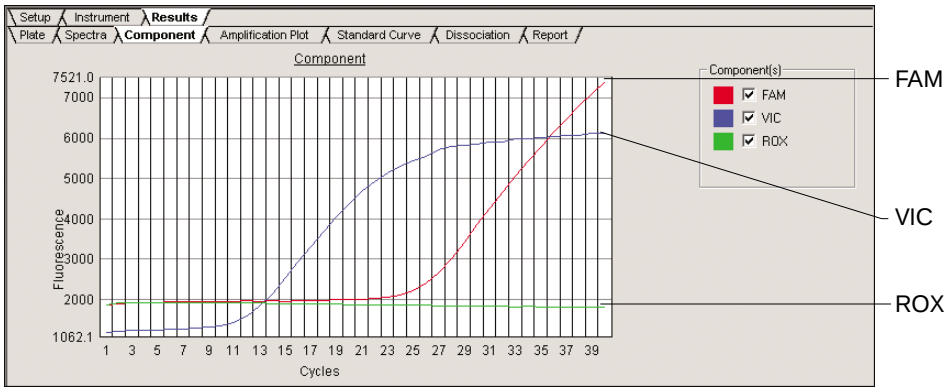
The 7000 software uses the pure dye spectra, generated as part of instrument calibration, to solve for coefficients a, b, and c in the following equation:

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

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.

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 7000 software displays, cycle-by-cycle, changes in normalized reporter signal (R_n). The 7000 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 normalizing is displayed as FAM R_n and VIC R_n.

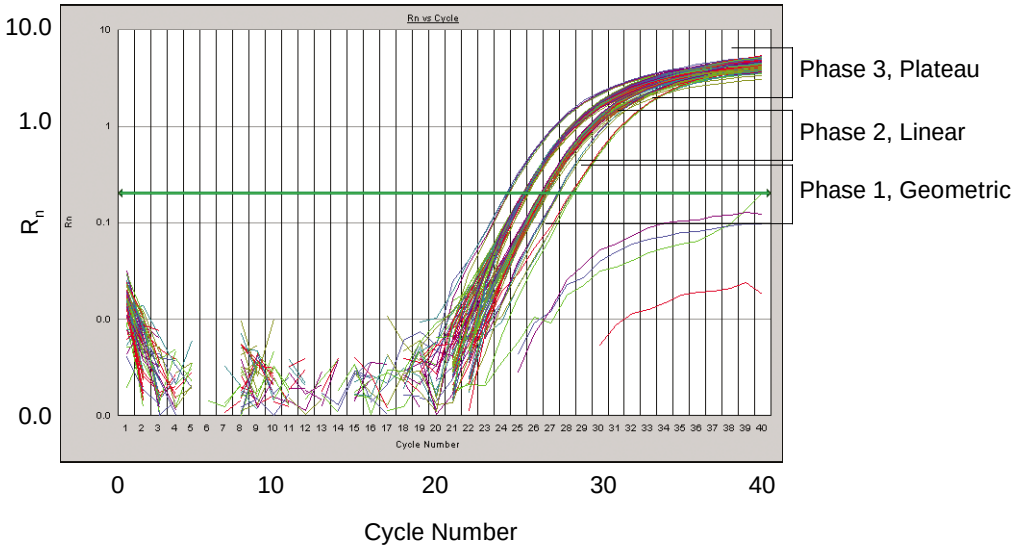
Real-Time Data Analysis

Overview

Analysis of real-time data requires several additional modifications to the run data to create meaningful relationships from the normalized reporter fluorescence.

Kinetic Analysis/ Quantitative PCR

The 7000 system 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 log 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 it 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 versus cycle number, the curve generated by the geometric phase should approximate a straight line with a slope. The 7000 system typically delivers sufficient sensitivity to detect at least three cycles in the geometric phase, assuming reasonably optimized PCR conditions.

Phase 2: Linear

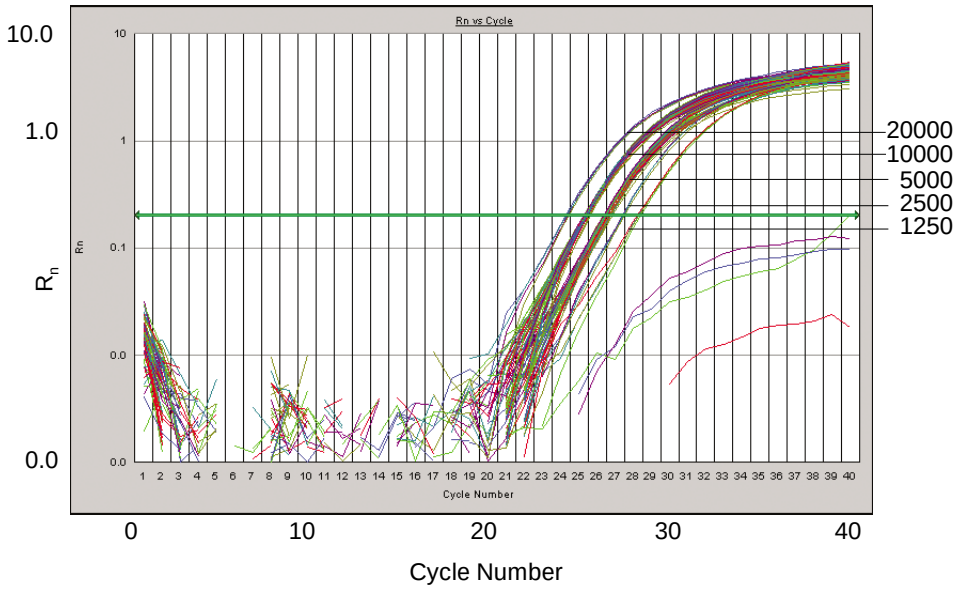
The linear phase is characterized by a leveling effect where the slope of the amplification curve decreases steadily. At this point, one or more components have fallen below a critical concentration and the amplification efficiency has begun to decrease. This phase is termed linear, because amplification 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 in 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, the ratio of template concentration to detectable signal is preserved in the exponential phase for all dilutions (see below). This relationship appears to change as the rate of amplification approaches a plateau.



Fluorescence Versus Amplified Product

When using TaqMan fluorogenic probes with the 7000 system, fluorescence emission increases in direct proportion to the amount of specific amplified product. As the figure on page A-7 demonstrates, the graph of normalized reporter (R_n) versus 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

About the Threshold Cycle

The 7000 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 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 7000 system and is dependent on two factors:

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

How the SDS Software Determines C_Ts

To determine the C_T for an amplification plot, the software uses data collected 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 using the baseline cycles’ R_n values to generate a baseline subtracted amplification plot (showing ΔR_n on the Y-axis). Then, an algorithm searches for the point on the amplification plot at which the ΔR_n value crosses the default threshold setting of 0.2. The fractional cycle at which this occurs is defined as the C_T.

Note: It may be necessary to adjust baseline and threshold settings to obtain accurate and precise data. For further information on resetting the baseline and threshold, see “Reviewing the Results of the Run” on page 4-19.

Significance of Threshold Cycles

Begin 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 ≥ 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:

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

Thus, 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.

Designing Assays Using TaqMan Probes

B

Introduction

About This Appendix

This appendix provides design tips for assays and guidelines for precision and the use of replicates.

In This Appendix

This appendix contains the following information:

Rapid Assay Development Guidelines

Processes

Processes for developing custom 5´ nuclease assays are listed in the table below.

Stage	Process	See Page
1	Identify Target Sequences	B-2
2	Design Probes and Primers	B-2
3	Order Reagents	B-3
4	Prepare Master Mix	B-4
5	Optimize Primer/Probe Concentrations	B-4
6	Run Your Custom Assay	B-4

Identify Target Sequences

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 B-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 B-5, for Quantitative PCR see page B-6.

Design Probes 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 B-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.

Design Guidelines

Application	Reporter Dye	
	First Probe	Second Probe
Allelic Discrimination	FAM™	VIC™
Plus/Minus Scoring	FAM	VIC

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 Gs 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 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, Passive Reference I, and optimized buffer components)

IMPORTANT: PCR master mix used with the 7000 system must contain a passive reference dye. The 7000 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
- TaqMan Spectral Calibration Reagents
- Centrifuge with 96-well plate adapter

- Deionized water or Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Disposable Gloves

Prepare Master Mix

If designing primers and TaqMan probes, 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 7000 system must contain a passive reference dye. The 7000 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, http://www.appliedbiosystems.com. See Appendix E, "Technical Support," for more information.

Optimize Primer/Probe Concentrations

Refer to the *TaqMan Universal PCR Master Mix Protocol* for specific information about preparing the master mix for use.

Run Your Custom Assay

Run your experiment.

Note: If conducting a quantitative PCR experiment (absolute), consider the use of replicate assays to enhance the precision of your data. See "Precision and the Use of Replicates" on page B-7 for more information.

Design Tips for Allelic Discrimination Assays

Discrimination by Multiple Probes

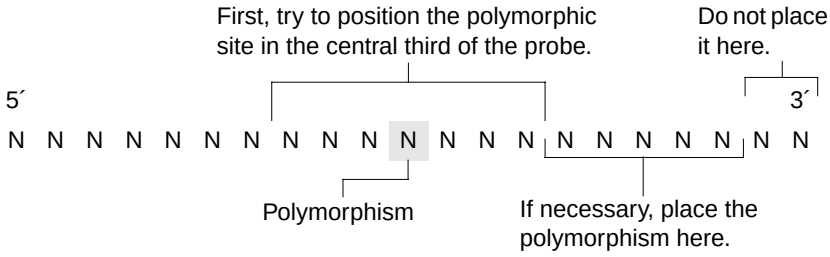
By using different reporter dyes, cleavage of multiple probes can be detected in a single PCR. One application of this multi-probe capability is to use allele-specific probes to distinguish genetic polymorphisms (Bloch, 1991; Lee , *et al.*, 1993). Probes that differ by as little as a single nucleotide 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:

Design Guidelines

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 "Rapid Assay Development Guidelines" on page B-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.

Note: 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.
- 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.

Precision and the Use of Replicates

Overview

Reproducibility, or precision, is important for all quantitative applications and can be measured through the use of replicates. For absolute quantification, poor precision increases the risk that the experimental values will differ significantly from the actual values.

Description of Replicates

Replicates are repetitions of an assay on a consumable (reaction plate). They fall into two categories: identical replicates and experimental replicates.

Identical Replicates

Identical replicates are amplifications performed in multiple wells using the same template preparation and the same PCR reagents. Identical replicates serve two functions:

- They can preserve data. If one amplification fails, other wells can potentially still provide data.
- They can be used to monitor the precision of the PCR amplification and detection steps.

Experimental Replicates

Experimental replicates are amplifications that share the same PCR reagents, while the template preparations come from similar, but not identical samples. They provide crucial information about the overall precision of the experiment. For example, if a researcher wishes to examine the effect of drug treatment on the relative level of a mouse mRNA, it is inappropriate to drug treat just one mouse. A number of mice must be treated similarly with the drug to determine the variation of response in the mouse population. A group of ten mice would represent ten experimental replicates.

Disadvantages

The disadvantages of replicates are that they result in additional cost, occupy more space in the thermal cycler block, and consume more sample. Each researcher should weigh these disadvantages against the need for precision information when deciding how many replicates to run in the assay.

Consumables and Reagents C

Introduction

About This Appendix

This appendix covers the consumables that are available to use with the ABI PRISM® 7000 Sequence Detection System.

In This Appendix

This appendix contains the following topics:

Consumables

US Part Numbers

Note: Part numbers listed are for US only and are subject to change. Contact your local Regional Sales Office for local part numbers, prices, and updates. See Appendix E, "Technical Support," for information on contacting Technical Support.

US Part Numbers

Part Number	Description	Quantity
	ABI PRISM Optical Adhesive Covers	
4313663	ABI PRISM® Optical Adhesive Cover Starter Kit Includes ABI PRISM® Optical Adhesive Covers (quantity 20), Applicator (quantity 1), ABI PRISM® Optical Cover Compression Pad (quantity 1).	20 Covers/Pkg
4311971	ABI PRISM Optical Adhesive Covers	100 Covers/Pkg
	96-Well Optical Reaction Plates	
4306737	ABI PRISM® 96-Well Optical Reaction Plate with Barcode (code 128)	20 Plates/Pkg
4326659	25-Pack, ABI PRISM 96-Well Optical Reaction Plate with Barcode (code 128) Includes 25X (4306737) ABI PRISM 96-Well Optical Reaction Plate with Barcode (code 128).	500 Plates/Pkg
4314320	ABI PRISM 96-Well Optical Reaction Plate with Barcode (code 128) and ABI PRISM Optical Adhesive Covers Includes 4311971 and 5X (4306737) ABI PRISM Optical 96-Well Reaction Plates.	100 Plates/Pkg 100 Covers
N801-0560	MicroAmp® Optical 96-Well Reaction Plate (no Barcode)	10 Plates/Pkg
403012	MicroAmp Optical 96-Well Reaction Plates and Optical Caps Includes 4306737 and 4323032.	20 Plates 2400 Caps/Pkg
	Miscellaneous	
4323032	ABI PRISM® Optical Caps, 8 Caps/Strip	300 Strips/Pkg 2400 Caps/Pkg
4312063	MicroAmp® Splash Free Support Base for 96-Well Reaction Plates	10 Bases/Pkg

Instrument Maintenance and Verification

The following sequence detection kits and reagents are used to perform routine maintenance and verify the function of the 7000 system. For more information about the use of the kits below, see Chapter 8, "System Maintenance."

Routine Maintenance

Part Number	Description	Quantity
Sequence Detection Systems Spectral Calibration Kits		
4328895	Sequence Detection Systems Spectral Calibration Kit	—
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 TaqMan primers and probe to detect and quantitate genomic copies of the human RNase P gene.	1 x 96-Well Plate

Sequence Detection Preformatted Assays

Applied Biosystems offers several preformatted assays for use on the 7000 system.

Preformatted Assays

Part Number	Description	Quantity
4307265	TaqMan® Cytokine Gene Expression Plate 1 with TaqMan® Universal PCR Master Mix Includes two MicroAmp Optical 96-Well Reaction Plates pre-loaded with TaqMan primers and probes for 12 human cytokine targets (replicates of 8) and the 18S Ribosomal RNA endogenous control (in all 96 wells). TaqMan primer and probe concentrations are optimized for multiplex PCR at 50 µls utilizing FAM™ and VIC™ dyes. Configuration includes TaqMan Universal PCR Master Mix and MicroAmp Optical Caps.	2 x 96-Well Plates
4307266	TaqMan Cytokine Gene Expression Plate 1 with TaqMan Universal PCR Master Mix and Control Total RNA Includes two MicroAmp Optical 96-Well Reaction Plates pre-loaded with TaqMan primers and probes for 12 human cytokine targets (replicates of 8) and the 18S Ribosomal RNA endogenous control (in all 96 wells). TaqMan primer and probe concentrations are optimized for multiplex PCR at 50 µLs utilizing FAM and VIC dyes. Configuration includes TaqMan Universal PCR Master Mix, MicroAmp Optical Caps, Control Total RNA (Human), and Protocol.	2 x 96-Well Plates
4306744	TaqMan Cytokine Gene Expression Plate 1 Protocol	—
4309920	TaqMan® Human Endogenous Control Plate with TaqMan Universal PCR Master Mix and Control Total RNA Includes two MicroAmp Optical 96-Well Reaction Plates pre-loaded with TaqMan primers and probes for 11 human endogenous control targets and an internal positive control (IPC) in replicates of 8. TaqMan primer and probe concentrations are optimized for 50 µL reactions and utilize VIC dye. Configuration includes TaqMan Universal PCR Master Mix, MicroAmp Optical Caps, Control Total RNA (Human), and Protocol.	2 x 96-Well Plates
4309921	TaqMan Human Endogenous Control Plate with TaqMan Universal PCR Master Mix Includes two MicroAmp Optical 96-Well Reaction Plates pre-loaded with TaqMan primers and probes for 11 human endogenous control targets and an internal positive control (IPC) in replicates of 8. TaqMan primer and probe concentrations are optimized for 50 µl reactions and utilize VIC dye. Configuration includes TaqMan Universal PCR Master Mix and MicroAmp Optical Caps.	2 x 96-Well Plates
4308134	TaqMan® Human Endogenous Control Plate Protocol	—

Reagents

Sequence Detection PCR Reagents Kits

Applied Biosystems offers sequence detection PCR reagents kits listed below for use on the 7000 system.

PCR Reagents Kits

Part Number	Description	Reactions
	TaqMan Universal PCR Master Mix Kits and Reagents	
4304437	TaqMan Universal PCR Master Mix Supplied at a 2X concentration. The mix is optimized for TaqMan reactions and contains AmpliTaq Gold® DNA Polymerase, AmpErase® UNG, dNTPs with dUTP, Passive Reference 1, and optimized buffer components.	200
4318157	TaqMan Universal PCR Master Mix, 2000 Reactions Includes 10 x 5 mL vials in one box.	2000
4305719	10-Pack, TaqMan Universal PCR Master Mix Includes: 10 x 4304437.	2000
4326717	50 mL, TaqMan Universal PCR Master Mix	2000
4324018	TaqMan Universal PCR Master Mix, No AmpErase UNG Supplied at a 2X concentration. The mix is optimized for TaqMan reactions and contains AmpliTaq Gold DNA Polymerase, dNTPs with dUTP, Passive Reference 1, and optimized buffer components.	200
4324020	10-Pack, TaqMan Universal PCR Master Mix, No AmpErase UNG Includes 10 x 5 mL vials in one box.	2000
4326614	50 mL, TaqMan Universal PCR Master Mix, No AmpErase UNG	2000
4304449	TaqMan Universal PCR Master Mix Protocol Instructions and optimization guidelines for the use of TaqMan Universal PCR Master Mixes.	—
	TaqMan PCR Core Reagents Kits	
N808-0228	TaqMan® PCR Core Reagents Kit Includes 250 Units AmpliTaq Gold DNA Polymerase, 100 Units AmpErase UNG, dUTP, dATP, dCTP, dGTP, 10X TaqMan Buffer A, 25 mM $MgCl_2$ Solution.	200

PCR Reagents Kits *(continued)*

Part Number	Description	Reactions
	TaqMan PCR Core Reagents Kits *(continued)*	
4304439	TaqMan® 1000 RXN PCR Core Reagents Includes 1250 Units AmpliTaq Gold DNA Polymerase, 500 Units AmpErase UNG, dUTP, dATP, dCTP, dGTP, 10X TaqMan® Buffer A, 25 mM $MgCl_2$ Solution.	1000
402930	10-Pack, TaqMan PCR Core Reagents Kit Includes: 10 x N808-0228.	2000
402823	TaqMan® PCR Reagent Kit Protocol	—
	SYBR Green Kits and Reagents	
4304886	SYBR® Green PCR Core Reagents Includes 250 Units AmpliTaq Gold DNA Polymerase, 100 Units AmpErase UNG, dNTP Mix with dUTP, 10X SYBR Green PCR Buffer, 25 mM $MgCl_2$ Solution.	200
4306736	10-Pack, SYBR Green PCR Core Reagents Includes: 10 x 4304886.	2000
4304965	SYBR® Green PCR Core Reagents Protocol	—
4309155	SYBR® Green PCR Master Mix Supplied at a 2X concentration. The mix is optimized for SYBR Green reactions and contains SYBR Green 1 dye, AmpliTaq Gold DNA Polymerase, dNTPs with dUTP, Passive Reference 1, and optimized buffer components.	200
4312704	10-Pack, SYBR Green PCR Master Mix Includes: 10 x 4309155.	2000
4310251	SYBR® Green PCR Master Mix Protocol Combined protocol for SYBR Green PCR Master Mix and SYBR Green RT-PCR Reagents.	—

RT-PCR Kits and Reagents

RT-PCR kits and reagents are listed below.

RT-PCR Kits and Reagents

Part Number	Description	Reactions
	TaqMan One-Step RT-PCR Master Mix Kits	
4309169	TaqMan® One-Step RT-PCR Master Mix Reagents Kit Contains two components. Vial 1: AmpliTaq Gold DNA Polymerase mix (2X) is optimized for TaqMan reactions and contains AmpliTaq Gold DNA Polymerase, dNTPs with dUTP, Passive Reference 1, and optimized buffer components. Vial 2: RT enzyme mix (40X) contains MultiScribe™ Reverse Transcriptase and RNase Inhibitor.	200
4313803	10-Pack, TaqMan One-Step RT-PCR Master Mix Reagents Kit Includes: 10 x 4309169.	2000
4310299	TaqMan® One-Step RT-PCR Master Mix Reagents Kit Protocol	—
	TaqMan Gold RT-PCR Reagents	
N808-0232	TaqMan® Gold RT-PCR Reagents without controls Includes TaqMan PCR Core Reagents Kit (N808-0228), TaqMan® Reverse Transcriptase Reagents (N808-0234).	200
4304133	10-Pack, TaqMan Gold RT-PCR Reagents without controls Includes: 10 x N808-0232.	2000
402876	TaqMan® Gold RT-PCR Protocol	—
	TaqMan Reverse Transcription Reagents	
N808-0234	TaqMan Reverse Transcription Reagents Includes MultiScribe Reverse Transcriptase, RNase Inhibitor, dNTP Mixture, Oligo $d(T)_{16}$, Random Hexamers, 10X RT Buffer, $MgCl_2$ Solution.	200
4304134	10-Pack, TaqMan Reverse Transcription Reagents Includes: 10 x N808-0234.	2000

RT-PCR Kits and Reagents *(continued)*

Part Number	Description	Reactions
	TaqMan EZ RT-PCR Core Reagents	
N808-0236	TaqMan® EZ RT-PCR Core Reagents Includes 1000 Units r*Tth* DNA Polymerase, 100 Units AmpErase UNG, dUTP, dATP, dCTP, dGTP, 5X TaqMan® EZ Buffer, 25 mM $Mn(OAc)_2$.	200
403028	10-Pack, TaqMan EZ RT-PCR Core Reagents Includes: 10 x N808-0236	2000
402877	TaqMan® EZ RT-PCR Kit Protocol	—
	SYBR Green RT-PCR Kits and Reagents	
4310179	SYBR® Green RT-PCR Reagents Includes SYBR Green PCR Master Mix (4309155), TaqMan Reverse Transcriptase Reagents (N808-0234).	200
4310251	SYBR Green PCR Master Mix Protocol Combined protocol for SYBR Green PCR Master Mix and SYBR Green RT-PCR Reagents.	—
	High Capacity cDNA Archive Kit	
4322171	High Capacity cDNA Archive Kit Includes Random Primers, Optimized RT Buffer, dNTPs and MultiScribe™ MULV/RNase Inhibitor mix for the conversion of up to 10 μg of total RNA in a single 100 μl reaction to single stranded cDNA.	200
4322169	High Capacity cDNA Archive Kit Protocol	—

Sequence Detection Control Kits and Reagents

Sequence detection control kits and reagents are listed below.

Sequence Detection Control

Part Number	Description	Reactions
	Sequence Detection Control Kits	
N808-0230	TaqMan® PCR Reagents Kit Includes TaqMan PCR Core Reagents Kit, 100 reaction B-actin control reagents, and protocol.	200
N808-0233	TaqMan Gold RT-PCR Reagents with controls Includes TaqMan PCR Core Reagent Kit, TaqMan Reverse Transcriptase Reagents, and TaqMan® GAPDH Control Reagents, and protocol.	200
N808-0235	TaqMan® EZ RT-PCR Kit Includes 1000 Units r*Tth* DNA Polymerase, 100 Units AmpErase UNG, dUTP, dATP, dCTP, dGTP, 5X TaqMan EZ Buffer, 25 mM $Mn(OAc)_2$, 100 GAPDH control reactions, and protocol.	200
	Sequence Detection Control Reagents	
4308329	TaqMan® Ribosomal RNA Control Reagents (VIC Dye) Includes Human Control RNA, rRNA Probe (VIC), rRNA Forward Primer, and rRNA Reverse Primer.	1000
4308310	TaqMan® Ribosomal RNA Control Reagents Protocol (VIC Dye)	—
4316844	TaqMan® RNase P Control Reagents (VIC Dye) Includes 20X primer and probe (VIC) mix and Human Genomic Control DNA (100 µLs at 10 ng/µL).	1000
4308313	TaqMan® Rodent GAPDH Control Reagents (VIC Dye) Includes Rodent Control RNA, rodent GAPDH Probe (VIC), rodent GAPDH Forward Primer, and rodent GAPDH Reverse Primer.	1000
4308318	TaqMan® Rodent GAPDH Control Reagents Protocol (VIC Dye)	—
402869	TaqMan® GAPDH Control Reagents (Human) Includes Human Control RNA, GAPDH Probe (JOE™), GAPDH Forward Primer, and GAPDH Reverse Primer.	100
401846	TaqMan® β-actin Detection Reagents	100
401970	TaqMan® DNA Template Reagents	—

Sequence Detection Control *(continued)*

Part Number	Description	Reactions
	Sequence Detection Control Reagents *(continued)*	
4308323	TaqMan® Exogenous Internal Positive Control Reagents (VIC Dye) Includes 10X exogenous IPC primer and probe (VIC) mix, 10X exogenous IPC blocking reagent, and 50X exogenous IPC target. Kit represents an IPC assay (optimized with TaqMan Universal PCR Master Mix) to be used with custom +/– assays.	200
4308321	5-Pack, TaqMan Exogenous IPC Reagents (VIC Dye) Includes: 5 x 4308323.	1000
4308320	TaqMan Exogenous Internal Positive Control Reagents (VIC Dye) with TaqMan Universal PCR Master Mix Includes: 4308323 and 4304437.	200
4308335	TaqMan® Exogenous Internal Positive Control Reagents Protocol (VIC Dye)	—
4316831	TaqMan® RNase P Detection Reagents Kit Includes 20X primer and probe (FAM) mix and Human Genomic Control DNA (100 µLs at 10 ng/µL).	100

Sequence Detection Reagent Components

Sequence detection reagent components are listed below.

Sequence Detection Reagent Components

Part Number	Description	Quantity
	Sequence Detection Reagent Components	
4304441	TaqMan® 1000 RXN Gold with Buffer A Pack Includes 1250 Units AmpliTaq Gold DNA Polymerase, 10X TaqMan Buffer A, 25 mM $MgCl_2$ Solution.	1000 Reactions
4305822	Sequence Detection Systems Spectral Calibration Kit	—
4311235	MultiScribe Reverse Transcriptase	5000
N808-0119	RNase Inhibitor	2000
N808-0260	dNTP Mixture (10 mM)	1mL
N808-0128	Oligo $d(T)_{16}$ (50 μM)	0.1mL
N808-0127	Random Hexamers (50 μM)	0.1mL
4307281	Control Total RNA (Human)	100 μls at 50 ng/μl
N808-0096	AmpErase Uracil N-glycosylase (UNG)	100 μls at 1 unit/μl
4312660	Control Total DNA (Human)	2 tubes each 100 μl at 10 ng/μl
402929	20% Glycerol Solution, Molecular Biology Grade	100 mL

Pre-Developed Assays and Reagents Using TaqMan Probes

For the latest information on PDARs using TaqMan probes covering gene expression quantification and allelic discrimination, visit the PDAR list using TaqMan probes on the Applied Biosystems web site at: www.appliedbiosystems.com/pdarlist.

Custom Oligonucleotide Synthesis

Order custom oligonucleotides online at the Applied Biosystems web site:
http://oligos.appliedbiosystems.com
or by e-mail:
OligosUS@appliedbiosystems.com

Custom Oligonucleotide Synthesis

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

Using Windows D

Introduction

About This Appendix

This appendix contains a brief overview of the common window operations you will be using in the software. If you need more information about the Microsoft® Windows® operating system, refer to the online help index located on your computer Windows Explorer directory or the user's manual that came with your Microsoft Windows software.

In This Appendix

This appendix contains the following topics:

Computer Vocabulary and Operations

Overview

To use the ABI PRISM® 7000 Sequence Detection System, you should be familiar with the following basic computer vocabulary and operations:

Computer Vocabulary Descriptions

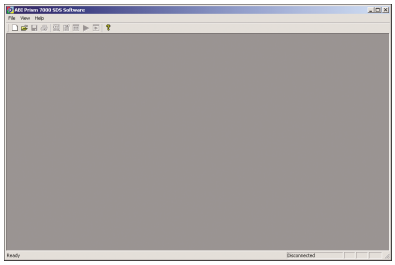
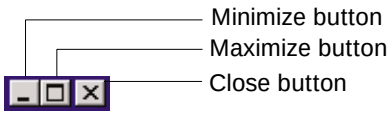
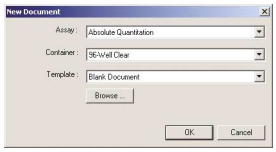
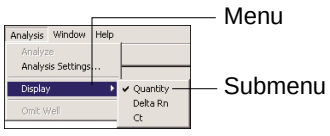
Vocabulary and Operations	Description
Using the mouse	Clicking, double-clicking, selecting, and dragging.
Choosing commands	Using drop-down and pop-up menus, dialog boxes, radio buttons, and check boxes.
Working with windows	Opening, closing, resizing, repositioning, scrolling, and understanding the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

Using a Windows Environment

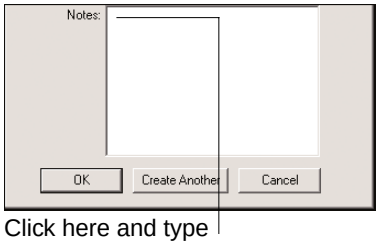
Windows Terms Used in This Manual

The following terms are used frequently in this manual to describe how to use the ABI PRISM® 7000 SDS Software.

Window Descriptions

Term	Description
Windows 	Display information and allow you to edit or enter additional information. If many windows are open, click one to make it active. When a window is active, you can click the top border, hold down the mouse button, and drag the window to another location on the screen. The Minimize button temporarily minimizes the window to a location on the taskbar at the bottom of the screen. Click the label to restore the window. The Maximize button enlarges the window to cover the whole screen. The Close button removes the window from the screen. When you are finished working with a window, click the Close button or click another window. 
Dialog boxes 	Appear when you must make a decision or enter information. All other actions on the monitor screen are suspended until you close the dialog box by clicking a button, such as **Cancel**, **OK**, **Open**, or **Done**.
Menus 	Provide access to various functions you can perform with the software. A heavy arrow (▶) after a menu item indicates that a submenu appears when you click that choice and hold down the mouse button.
Pop-up menus 	Display a heavy arrow (▼) and are in dialog boxes or windows. When you click a pop-up menu and hold down the mouse button, a submenu opens. These menus allow you to choose entries from item lists.

Window Descriptions *(continued)*

Term	Description
Text fields 	Rectangular areas in which you can enter information. Click in an entry field and type the information.
Buttons 	Rectangles with rounded corners that allow you to accept or cancel the contents of a dialog box or perform functions (such as taking a snapshot) in the dialog box. A button with a heavy outline is the default button that applies if you press the **Enter** key.
Check boxes 	Boxes that you click to select certain options in a dialog box. A check appears in a check box to indicate that the option is selected.

Technical Support

E

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 for specific products.

Limited Warranty

F

PE Corporation (NY) Limited Warranty Statement

Limited Warranty Statement

PE Corporation (NY), through its Applied Biosystems Group, ("PE/ABD") 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® 7000 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 specifications set forth in the ABI PRISM 7000 Sequence Detection System specifications sheet (the "Specifications").

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, PE/ABD will repair or replace the Instrument so that it meets the Specifications, at PE/ABD's expense. However, if the Instrument 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 PE/ABD, PE/ABD 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 PE/ABD, or (c) used in a manner not in accordance with the instructions contained in the Instrument User Guide. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User Guide. Those items are covered by their own warranties.

PE/ABD's obligation under this Warranty is limited to repairs or replacements that PE/ABD deems necessary to correct those failures of the Instrument to meet the Specifications of which PE/ABD is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by PE/ABD on site at the Customer's location at PE/ABD's sole expense.

No agent, employee, or representative of PE/ABD has any authority to bind PE/ABD to any affirmation, representation, or warranty concerning the Instrument that is not contained in PE/ABD's printed product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of PE/ABD will not be binding on PE/ABD.

PE/ABD 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.

PE/ABD 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 PE/ABD.

Glossary

analysis	Integration, calibration, and reporting of data.
auto increment value	Method setting that increases or decreases the value for any PCR setpoint parameter (time or temperature) a fixed amount every cycle.
background	An inherent spectral component of the signal generated by a fluorescent assay that is attributed to the ABI PRISM® 7000 Sequence Detection System. The background signal can interfere with the sensitivity of the software and its ability to determine C_Ts.
C_T	Threshold cycle, which represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal can first be detected. The software generates a standard curve of C_T versus starting copy number (LogN) for all standards and then determines the starting copy number of unknowns by interpolation.
ΔR_n	Represents the normalized reporter signal minus the baseline signal established in the first few cycles of PCR. Like R_n, ΔR_n increases during PCR as amplicon copy number increases until the reaction approaches a plateau.
detector	Virtual representation of a nucleic acid probe in a master mix used for standard curve analysis, comparative quantification, allelic discrimination, plus/minus analysis, or custom analysis. Examples of common detectors include SYBR® Green Dye and TaqMan® probes which are labeled at the 5´ end with a reporter dye and a quencher dye at the 3´ end. Each SDS detector has a designated task or function that describes the purpose of the detector when applied to a plate document. Tasks vary depending on the type of application for which they are used, such as standards for PCR runs.
hold	A setpoint that maintains a specified temperature for an extended period of time.
pure dye	Pure dye data is generated from the results of a pure dye run in which the software collects spectral data from a set of dye standards.
ramp	The interval between two setpoints during which the instrument heats or cools the samples.
ramp rate	Method setting that determines the time the instrument will take to ramp from one setpoint to another.
R_n	The normalized reporter signal, which represents the fluorescence signal of the reporter dye divided by the fluorescence signal of the passive reference dye. During PCR, R_n increases as amplicon copy number increases until the reaction approaches a plateau. When using the instrument in its plate-read mode, the fluorescence signal is read at a single point in time after the completion of PCR rather than continuously during the course of PCR.

task	A setting applied to the detectors within a well of a plate document that determines the way the software will use the data collected from the well during analysis.

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Index

Symbols

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

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