ABI PRISM® Cell Lysate Control Kit

Protocol

Applied Biosystems

© Copyright 2001, Applied Biosystems. All rights reserved.

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

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Applied Biosystems be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

Notice to Purchaser:

The PCR process and 5´ nuclease process are covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd.

PLEASE REFER TO THE ABI PRISM™ 6700 or 6100 System USER GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

ABI PRISM, the ABI PRISM design, and Applied Biosystems are registered trademarks of Applera Corporation or its subsidiaries in the U.S. and certain other countries.

AB (Design), ABI, Applera, and VIC are trademarks of Applera Corporation or its subsidiaries in the U.S. and certain other countries.

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

AppleScript and Macintosh are registered trademarks of Apple Computer, Inc.

Microsoft is a registered trademark of Microsoft Corporation.

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

Printed in the USA, 7/2001
Part Number 4316607 Rev. C

Contents

Introduction and Safety 1

Introduction

Overview

The purpose of the ABI PRISM® Cell Lysate Control Kit is to qualify the performance of either of the following instruments:

- ABI PRISM™ 6700 Automated Nucleic Acid Workstation
- ABI PRISM™ 6100 Nucleic Acid PrepStation

The qualification process for these instruments in discussed in a separate chapter for each instrument.

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 material safety data sheet (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**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

To order documents by telephone:

In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	♦ To order in English, dial **1.800.668.6913** and press **1**, then **2**, then **1** ♦ To order in French, dial **1.800.668.6913** and press **2**, then **2**, then **1**
From any other country	See the specific region under "To Contact Technical Support by Telephone or Fax (Outside North America)" on page A-5.

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

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

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

6700 Workstation Qualification

2

Overview

Purpose of the Cell Lysate Control Kit

The purpose of the ABI PRISM® Cell Lysate Control Kit is to qualify the performance of the ABI PRISM™ 6700 Automated Nucleic Acid Workstation. This kit is designed to be used with Applied Biosystems reagents and plastic consumables and with the ABI PRISM® 7700 Sequence Detection System (7700 SDS).

About the Cell Lysate Control Kit

The Cell Lysate Control Kit contains the following from a single lot of human Raji cells:

- Two concentrations of lysed human Raji cells
  - High Cell Lysate Control (HCLC)
  - Low Cell Lysate Control (LCLC)
- Total RNA recovered from two concentrations of lysed human Raji cells and dissolved in Nucleic Acid Purification Elution Solution (P/N 4305983)
  - High Recovered RNA Standard (HRS)
  - Low Recovered RNA Standard (LRS)

About This Chapter

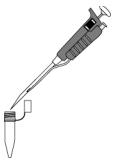
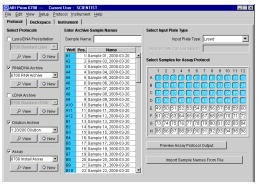
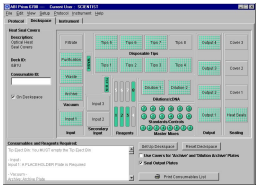
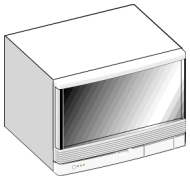
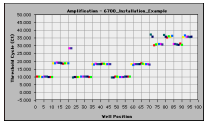
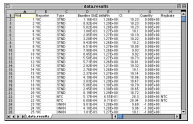
This chapter contains the following:

- An overview of the 6700 workstation qualification process
- A list of the equipment and materials required to qualify the 6700 workstation
- Procedures for setting up the 6700 workstation using the Cell Lysate Control Kit, Applied Biosystems reagents, and Applied Biosystems plastic consumables on the 6700 workstation
- Procedures for using the 7700 SDS to perform a real-time run and to analyze the 6700 workstation output[1]
- Procedures for analyzing the qualification

1. Verify the performance of the 7700 SDS with the TaqMan® RNase P Instrument Verification Plate (P/N 4310982) before analyzing the 6700 workstation output using the 7700 SDS.

6700 Workstation Qualification Overview

Process The qualification process involves the following stages:

Stage	Overview	Process
1		You prepare reagents and gather the materials required to perform the 6700 workstation qualification procedure.
2		Using the 6700 system software, you set up the 6700 workstation qualification protocols.
3		Using the barcode reader and 6700 system software, you set up the Cell Lysate Control Kit reagents, Applied Biosystems reagents, and Applied Biosystems plastic consumables on the 6700 workstation deckspace.
4		The 6700 workstation purifies total RNA from the two concentrations of Raji cell lysates (HCLC and LCLC).
5		The 6700 workstation dilutes the RNA from HCLC and LCLC samples 1:20 and 1:200.
6		The 6700 workstation prepares two output plates containing master mixes, standards, controls, and diluted total RNA samples.
7		You transfer one of the output plates from the 6700 workstation to the 7700 SDS for a real-time run.
8		Using the Sequence Detection Systems (SDS) software, you analyze the threshold cycles (C_T values).
9		You calculate the following to analyze the qualification process: ♦ % RNA recovery ♦ Precision of RNA recovery ♦ % DNA contamination

Materials and Equipment

Kit Contents

The Cell Lysate Control Kit (P/N 4315646) contains sufficient quantities to perform one 6700 workstation qualification procedure.

Item	Description[a]	Volume
High Cell Lysate Control (HCLC)	Raji cells diluted in lysis buffer[b] to a final concentration of 5×10^6 cells/mL	11 mL
Low Cell Lysate Control (LCLC)	Raji cells diluted in lysis buffer[b] to a final concentration of 5×10^4 cells/mL	11 mL
High Recovered RNA Standard (HRS)	Total RNA recovered from 1 mL of HCLC[c], and diluted 1:150	0.6 mL
Low Recovered RNA Standard (LRS)	Total RNA recovered from 1 mL of LCLC[c], and diluted 1:150	0.6 mL

a. All components in the packaged kit are produced from a single lot of human Raji cells.

b. Lysis buffer consists of a 1:1 mixture of Nucleic Acid Purification Lysis Solution (P/N 4305895) and calcium/magnesium-free phosphate-buffered saline solution.

c. The RNA is dissolved in 0.6 mL Nucleic Acid Purification Elution Solution (P/N 4305893).

Kit Storage and Stability

All components of the Cell Lysate Control kit should be stored at –15 to –25 °C.

Note If stored correctly, this kit is guaranteed for 1 year from the date of manufacture.

Equipment and Materials Required But Not Supplied

The following tables list the equipment and materials required in addition to the reagents supplied with the Cell Lysate Control Kit.

IMPORTANT Follow the instructions as indicated in this protocol to qualify the 6700 workstation. If you modify this protocol in any way, including changing the amounts used and substituting reagents or other materials, Applied Biosystems cannot guarantee the validity of your 6700 workstation qualification process.

Equipment Required

Equipment	Source
ABI PRISM 6700 Automated Nucleic Acid Workstation	See your Applied Biosystems sales representative
ABI PRISM 7700 Sequence Detection System	
Centrifuge with 96-well adapter	Major laboratory supplier (MLS)
Microcentrifuge	MLS
Pipettors	MLS
Vortexer	MLS

Applied Biosystems Materials Required

Applied Biosystems Materials	Amount Needed	P/N
6700 Splash Guards	1 guard	4311758
96-Well Optical Reaction Plate with Barcode	8 plates	4306737
Archive Covers	3 covers	4306286
Conductive Pipette Tips, 1000-µL	1 rack	4306377
Conductive Pipette Tips, 200-µL	6 racks	4306375
Microcentrifuge Tubes and Caps, 2-mL	10 tubes	4305936
Nucleic Acid Purification Elution Solution	57.1 mL	4305893
Optical Cover Compression Pad	1 pad	4312639
Optical Heat Seal Covers	2 covers	4307726
Reagent Reservoirs, 120-mL **Note** This product comes with a sheet of barcode labels for Applied Biosystems nucleic acid purification reagents.	4 reservoirs	4304831
Reagent Tubes with Caps, 10-mL	6 tubes	4305932
RNA Purification Wash Solution 1	44 mL	4305891

Applied Biosystems Materials Required *(continued)*

Applied Biosystems Materials	Amount Needed	P/N
RNA Purification Wash Solution 2	117 mL	4305890
TaqMan® One-Step RT-PCR Master Mix Reagents Kit ♦ 2X Master Mix without UNG Contains AmpliTaq Gold® DNA Polymerase, dNTPs with dUTP, Passive Reference, and optimized buffer components ♦ 40X Multiscribe and RNase Inhibitor Mix	3 kits	4309169
TaqMan® Ribosomal RNA Control Reagents (VIC™ Dye) ♦ Human Control RNA ♦ rRNA Probe (VIC™) ♦ rRNA Forward Primer ♦ rRNA Reverse Primer	1 kit	4308329
TaqMan® RNase P Instrument Verification Plate	2 plates	4310982
Total RNA Purification Tray	1 tray	4305673

Reagent Preparation

About Reagent Preparation

In this section, you prepare the following:

- 18S RT+ master mix
- 18S RT– master mix
- Cell lysate controls

Preparing 1.25X 18S RT+ Master Mix

Prepare 18S RT+ master mix for measuring RNA in samples prepared by the 6700 workstation. Use components from the TaqMan One-Step RT-PCR Master Mix Reagents Kit and the TaqMan Ribosomal RNA Control Reagents (VIC Dye).

To prepare 18S RT+ master mix:

Step	Action
1	Pipette the components into a 10-mL reagent tube. **Component** / **Volume** 2X Master Mix without UNG — 4.0 mL rRNA Forward Primer — 80 µL rRNA Reverse Primer — 80 µL rRNA VIC Probe — 10 µL 40X Multiscribe and RNase inhibitor mix — 200 µL Deionized water — 2.03 mL **Total** — 6.4 mL
2	Place the 18S RT+ master mix on ice until you load the deckspace.

Preparing 1.25X 18S RT– Master Mix

Prepare 18S RT– master mix for detecting DNA contamination in samples prepared by the 6700 workstation. Use components from the TaqMan One-Step RT-PCR Master Mix Reagents Kit and the TaqMan Ribosomal RNA Control Reagents (VIC Dye).

To prepare 18S RT– master mix:

Step	Action
1	Pipette the components into a 10-mL reagent tube. **Component** / **Volume** 2X Master Mix without UNG — 1.5 mL rRNA Forward Primer — 30 µL rRNA Reverse Primer — 30 µL rRNA VIC Probe — 3.75 µL Deionized water — 836 µL **Total** — 2.4 mL
2	Place the 18S RT– master mix on ice until you load the deckspace.

Preparing Cell Lysate Controls

Prepare the cell lysate controls as described below for accurate results.

⚠ WARNING CHEMICAL HAZARD. Raji cell lysate controls cause irritation to the eyes, skin, and respiratory tract. This material is harmful if swallowed, inhaled, or absorbed through the skin. It may cause damage to the nervous system and bone marrow. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare cell lysate controls:

Step	Action
1	Allow the cell lysate controls to thaw at room temperature or on ice. **IMPORTANT** Do not use heat to thaw cell lysate controls.

To prepare cell lysate controls: *(continued)*

Step	Action
2	Vortex cell lysate controls thoroughly to ensure that the controls are completely resuspended. ♦ Use the highest speed setting. ♦ Place the tubes at an angle while vortexing. ♦ You should observe liquid from the bottom of the tube mixing with liquid at the top of the tube. **IMPORTANT** Complete and thorough vortexing is essential for accurate results.
3	Pipette the bubbles into a 2-mL microcentrifuge tube, and briefly centrifuge the tube to recover liquid.
4	Pipette the recovered liquid from the bubbles back into the correct cell lysate tube.
5	Cap and invert the tubes to mix.
6	Place the tubes on ice until you load the tubes on the deckspace.

Protocol Setup

Overview

In this section you use the 6700 workstation client computer to set up the 6700 system software protocols for qualifying the 6700 workstation.

This involves the following procedures:

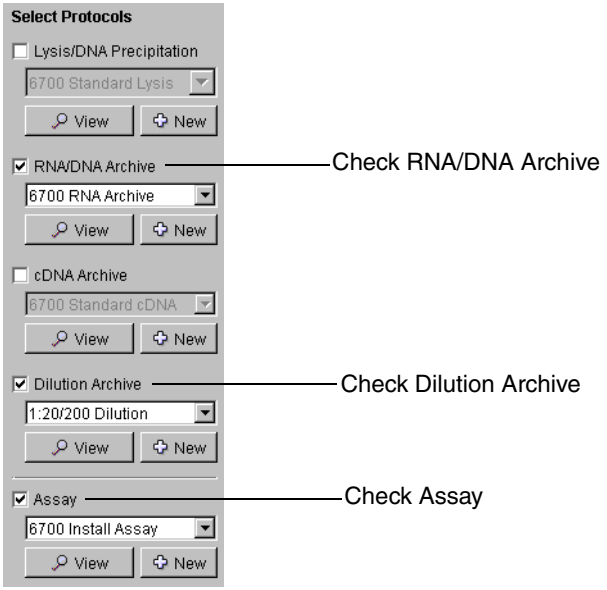
Logging In

To log in to the 6700 system:

Step	Action
1	Using the 6700 system client computer, go to the **Start** menu, scroll to **Programs**, and select **ABI 6700 Instrument Application** to launch the 6700 system software. A **6700 Log-In** dialog box appears.
2	Log in. a. Enter the correct user name and password combination. b. Click **OK**.

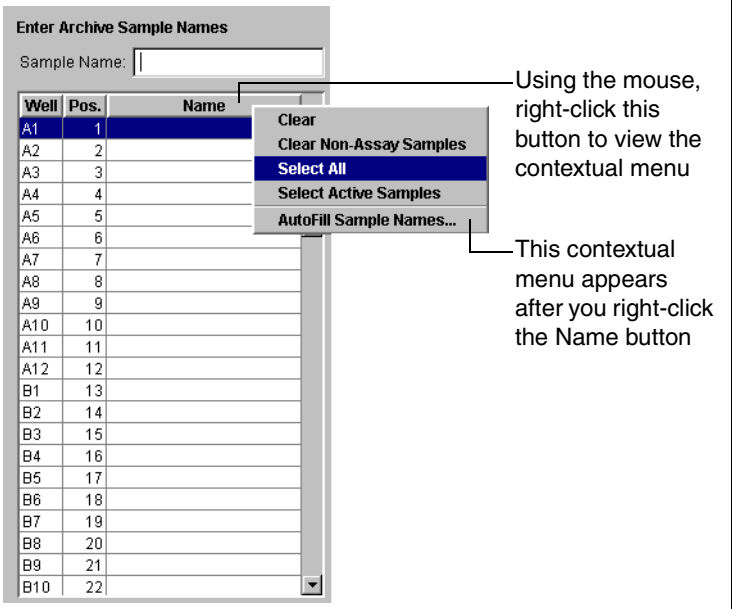
Selecting Protocols

To select protocols:

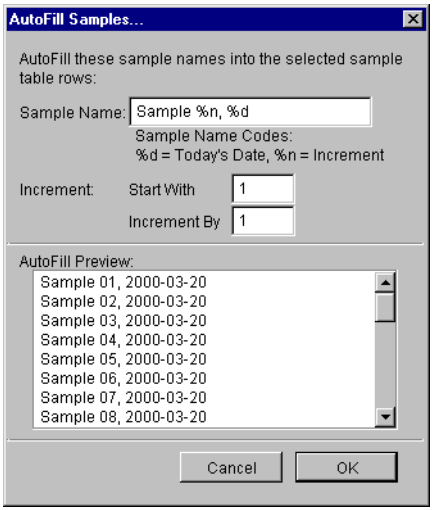
Step	Action
1	From the **Protocol** tab, check the box next to protocols required for the run. 
2	Select the following predefined protocols from the pop-up menu: **Protocol Type** / **Predefined Protocol Name** RNA/DNA Archive / 6700 RNA Archive Dilution Archive / 1:20/200 Dilution Assay / 6700 Install Assay

Naming Samples

To name samples:

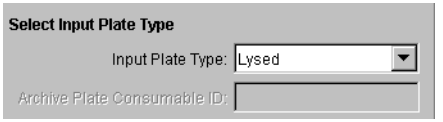
Step	Action
1	Right-click the **Name** button, and choose **Select All** from the contextual menu to select 96 samples.  The software highlights all 96 samples.

To name samples: *(continued)*

Step	Action
2	Right-click the **Name** button, and select **AutoFill Sample Names** from the contextual menu. A dialog box appears. 
3	Click **OK**. The software names the samples using the formula in the **Sample Name** field.

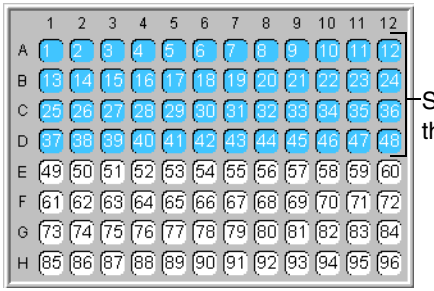
Selecting Input Plate Type

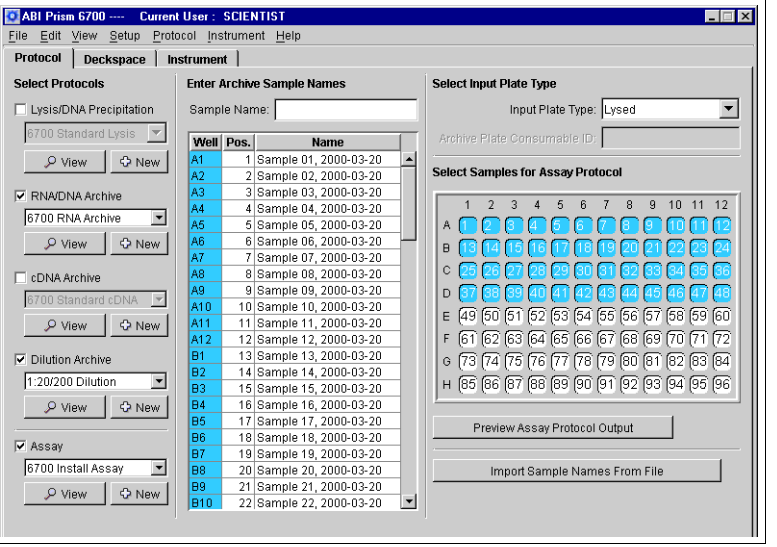
To select input plate type:

Step	Action
1	Select **Lysed** from the **Input Plate Type** pop-up menu. 

Selecting Samples for the Assay Protocol

To select samples for the Assay protocol:

Step	Action
1	Select wells 1–48 for the Assay protocol in the **Select Samples for Assay Protocol** section. **Note** Click and drag, or hold down the Ctrl key while selecting samples.  The software highlights the corresponding **Well** column in the **Archive Sample Names** section of the **Protocol** tab.
2	Verify that the screen looks like the figure shown below.



Verifying Protocol Setup

To verify protocol setup:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Click the Deckspace tab.

The software determines whether the protocols are set up properly.</td></tr>
<tr><td>2</td><td>
<table>
<tr><th>If the protocols…</th><th>Then…</th></tr>
<tr><td>are set up properly,</td><td>the Deckspace tab becomes active.</td></tr>
<tr><td>are not set up properly,</td><td>a Protocol Verification Errors dialog box appears.

Click the Print button to print the errors, and click Close to close the box.

Resolve the errors before proceeding.</td></tr>
</table>
</td></tr>
</table>

Deckspace Setup

Purpose In this section, you use the 6700 system software and the barcode reader to set up the Cell Lysate Control Kit reagents, Applied Biosystems reagents, and Applied Biosystems plastic consumables on the 6700 workstation deckspace.

Deckspace setup involves the following procedures:

Cooling the Deckspace

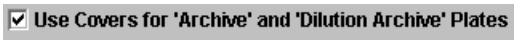
Begin cooling the deckspace Peltier units before setting up the deckspace to prevent degradation of your input, standards, controls, and master mixes.

To cool the deckspace:

Step	Action
1	Go to the **Instrument** tab.
2	Click the **Cool Peltiers** button.  The 6700 instrument begins to cool the following stations to 4 °C: ♦ Input Station ♦ Standards, Master Mix/Cell Lysate Control Station ♦ Dilutions/cDNA Station ♦ Output Station **Note** It takes up to 20 minutes to cool the stations to 4 °C.

Selecting Plate Cover Options

To select plate cover options:

Step	Action
1	Click the **Deckspace** tab.
2	Check **Use Covers for Archive and Dilution Archive Plates** for the robotic arm to automatically place archive covers on the Archive plates and Dilution archive plates after the run.  Three of the **Cover** stations turn yellow.
3	Check **Seal Output Plates** to seal the Output plates with optical heat seal covers for the heat sealer to automatically place optical heat seal covers on output plates at the end of the run.  The **Heat Seals** location turns yellow.

Consumables and Reagents Required

Gather the consumables and reagents required before setting up the deckspace.

Consumables and Reagents	Amount Needed
18S RT+ Master Mix	6.4 mL
18S RT– Master Mix	2.4 mL
6700 Splash Guard	1 guard
96-Well Optical Reaction Plate with Barcode	5 plates
96-Well Optical Reaction Plate with Barcode (placeholders)[a]	3 plates
Archive Covers	3 covers
Conductive Pipette Tips, 1000-µL	1 rack
Conductive Pipette Tips, 200-µL	6 racks
High Cell Lysate Control	11 mL
High Recovered RNA Standard	0.6 mL
Low Cell Lysate Control	11 mL
Low Recovered RNA Standard	0.6 mL
Microcentrifuge Tubes and Caps, 2-mL	1 tube
Microcentrifuge Tubes and Caps, 2-mL (placeholders)[a]	9 tubes
Nucleic Acid Purification Elution Solution	57.1 mL
Reagent Reservoirs, 120-mL	4 reservoirs
Reagent Tubes with Caps, 10-mL (placeholders)[a]	4 tubes
RNA Purification Wash Solution 1	44 mL
RNA Purification Wash Solution 2	63 mL + 54 mL
Total RNA Purification Tray	1 tray

a. Placeholders are required to minimize condensation fomation on Peltier-cooled deckspace stations.

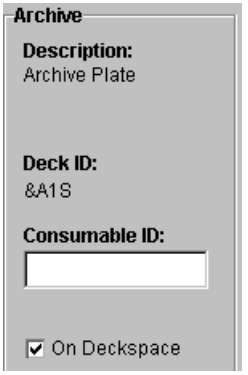
Loading the Deckspace

Follow the guidelines below when loading the deckspace:

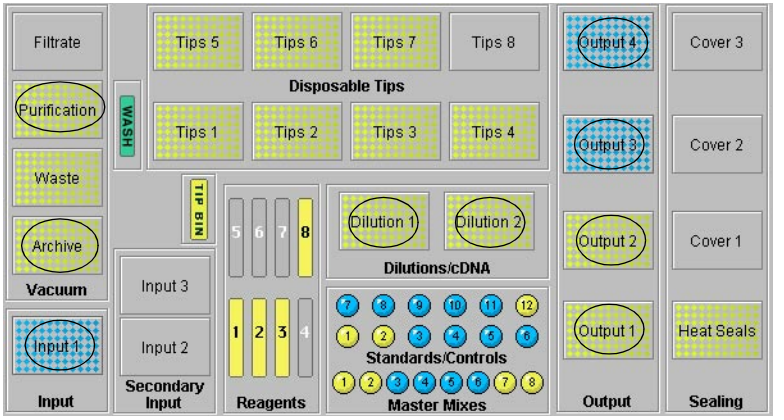
- Load the deckspace using the barcode reader and the Deckspace tab.
- Enter unique barcodes or Consumable IDs for archive plates and output plates.
- Load placeholder items to minimize condensation formation on Peltier-cooled deckspace stations.
- To reset the deckspace, click the Reset Deckspace button.

Loading Plates

To load plates:

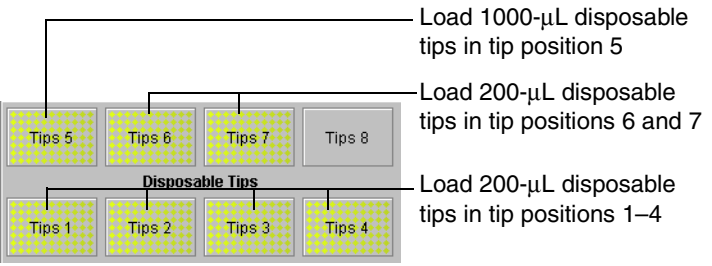
Step	Action
1	Using the barcode reader, scan a plate location on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam. ♦ Details about the deckspace location appear to the left of the deckspace diagram. ♦ The software checks the **On Deckspace** check box, and the **Consumable ID** box becomes active. 
2	Scan the barcode of the plate. **Note** You need to perform this step only if the plate is not a placeholder plate.

To load plates: *(continued)*

Step	Action
3	Place the plate on the deckspace. **Deckspace Location** / **Plate Required** Input 1 — Placeholder 96-well plate Archive — 96-well barcoded microplate Purification — Total RNA purification tray Dilution 1 — 96-well barcoded microplate Dilution 2 — 96-well barcoded microplate Output 1 — 96-well barcoded microplate Output 2 — 96-well barcoded microplate Output 3 — Placeholder 96-well plate Output 4 — Placeholder 96-well plate 
4	Repeat step 1 through step 3 until you place all required plates on the deckspace. **IMPORTANT** Loading placeholder plates is required to minimize condensation formation on Peltier-cooled deckspace stations.

Loading Tips

To load tips:

Step	Action
1	Using the barcode reader, scan a tip location on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam. ♦ Details about the deckspace location appear to the left of the deckspace diagram. ♦ The software checks the **On Deckspace** check box, and the **Consumable ID** box becomes active.
2	Scan the barcode of the tip rack (optional).
3	Place the tip rack on the deckspace. 
4	Repeat step 1 through step 3 until you place all required tips on the deckspace.

Loading Reagents

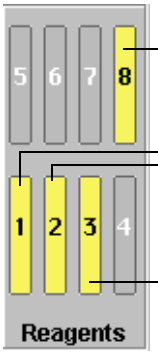
To load reagents:

Step	Action
1	Place 44 mL of RNA Purification Wash Solution 1 in a reagent reservoir, and place a Wash Solution 1 barcode label on the reagent reservoir. **⚠ CAUTION CHEMICAL HAZARD**. **RNA Purification Wash Solution 1** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Note** Barcode labels for the reagent reservoirs are packaged with the reagent reservoirs.

To load reagents: *(continued)*

Step	Action
2	Place 54 mL of RNA Purification Wash Solution 2 in a reagent reservoir, and place a Wash Solution 2 barcode label on the reagent reservoir. **⚠ WARNING CHEMICAL HAZARD. RNA Purification Wash Solution 2** is a flammable liquid and vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Place 63 mL of RNA Purification Wash Solution 2 in a reagent reservoir, and place a Wash Solution 2 barcode label on the reagent reservoir. **⚠ WARNING CHEMICAL HAZARD. RNA Purification Wash Solution 2** is a flammable liquid and vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Place 57 mL of Nucleic Acid Purification Elution Solution in a reagent reservoir, and place an Elution Solution barcode label on the reagent reservoir.
5	Using the barcode reader, scan a reagent reservoir location on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam. ♦ Details about the deckspace location appear to the left of the deckspace diagram. ♦ The software checks the **On Deckspace** check box, and the **Consumable ID** box becomes active.
6	Scan the barcode of the reagent reservoir.

To load reagents: *(continued)*

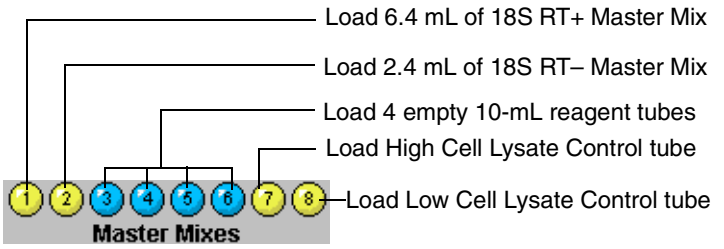
Step	Action
7	Place the reagent reservoir on the deckspace.  Load 57 mL of Nucleic Acid Purification Elution Solution in reagent position 8 Load 44 mL of RNA Purification Wash Solution 1 in reagent position 1 Load 54 mL of RNA Purification Wash Solution 2 in reagent position 2 Load 63 mL of RNA Purification Wash Solution 2 in reagent position 3 **⚠ CAUTION CHEMICAL HAZARD**. **RNA Purification Wash Solution 1** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ WARNING CHEMICAL HAZARD**. **RNA Purification Wash Solution 2** is a flammable liquid and vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
8	Repeat step 5 through step 7 until you place all required reagents on the deckspace.

Loading Master Mixes

To load master mixes:

Step	Action
1	Using the barcode reader, scan a master mix location on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam. ♦ Details about the deckspace location appear to the left of the deckspace diagram. ♦ The software checks the **On Deckspace** check box, and the **Consumable ID** box becomes active.

To load master mixes:

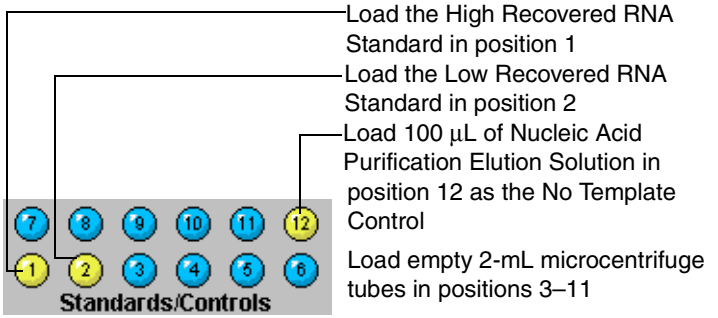
Step	Action
2	Place the appropriate master mix on the deckspace.  **⚠ WARNING CHEMICAL HAZARD. Raji cell lysate controls** cause irritation to the eyes, skin, and respiratory tract. This material is harmful if swallowed, inhaled, or absorbed through the skin. It may cause damage to the nervous system and bone marrow. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Repeat step 1 and step 2 until you scan all master mix barcodes and place all required master mix tubes on the deckspace.

Loading Standards

To load standards:

Step	Action
1	Using the barcode reader, scan the standards location on the deckspace. Standard position 1 becomes active. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

To load standards: *(continued)*

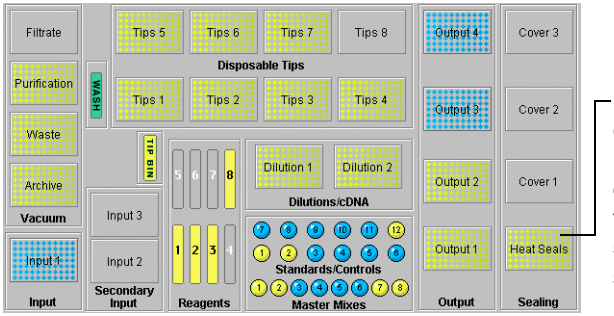
Step	Action
2	Load the standards, control, and placeholder tubes on the deckspace. 
3	Mark the standards, control, and placeholder tubes as **On Deckspace**. a. Using the mouse, click a standard position on the **Deckspace** tab. b. Using the mouse, click the **On Deckspace** check box. Repeat steps 3a and 3b until all standards and controls are marked as **On Deckspace**.

Loading the Covers

To load the covers:

Step	Action
1	Using the barcode reader, scan the optical heat seal covers barcode on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

To load the covers: *(continued)*

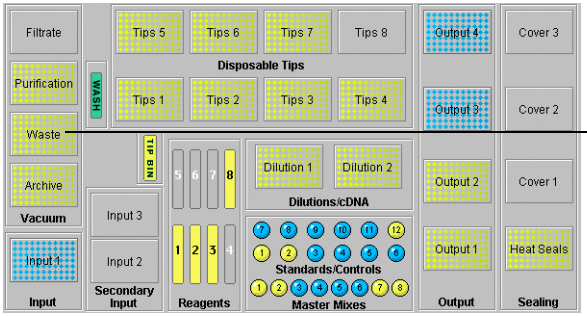
Step	Action
2	Place no more than four optical heat seal covers in position. **IMPORTANT** Place the optical heat seal covers with the dull side facing downward and the shiny side facing upward. 
3	Using the barcode reader, scan a Cover barcode on the archive cover shelf.
4	Place an archive cover on the archive cover shelf.
5	Repeat step 3 and step 4 until you place all archive covers.

Loading the Splash Guard

To load the splash guard:

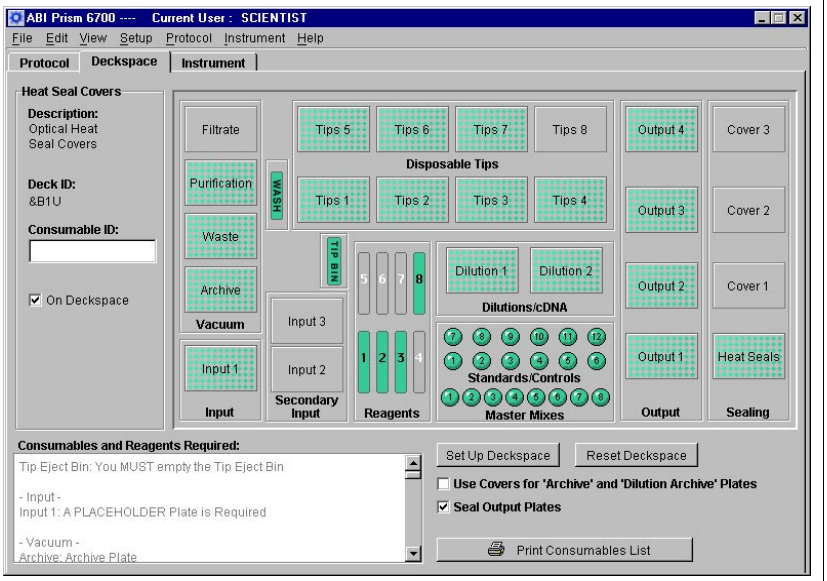
Step	Action
1	Using the barcode reader, scan the waste position barcode on the deckspace. **⚠ WARNING LASER HAZARD**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

To load the splash guard: *(continued)*

Step	Action
2	Load the splash guard in the waste position. **IMPORTANT** The splash guard is a blue plate with bottomless wells. Do not load a 96-well plate in the waste position. If you do, waste will collect on the deckspace. 

Verifying the Deckspace

To verify the deckspace:

Step	Action
1	Verify that all active stations on the **Deckspace** tab are green. **Note** This indicates that all required materials were marked as **On Deckspace**. 
2	Verify that all required items are placed in the appropriate deckspace position.
3	Verify that the volumes for reagents, master mixes, standards, and controls are correct. **IMPORTANT** Missing items or insufficient amounts may cause the instrument to pause during the qualification run. This will require manual intervention to restart and complete the qualification run.

6700 Instrument Run

Purpose

In this section you start the 6700 instrument run, and the 6700 workstation purifies the RNA, dilutes the RNA, and prepares output plates.

This involves the following procedures:

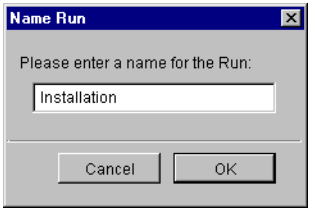
Procedure	See Page
Starting the Run	2-29
Finishing the Run	2-30

Starting the Run

To start the run:

Step	Action
1	Close the instrument door.
2	Go to the **Instrument** tab of the software.
3	Click the **Start** button. The software verifies that all locations are marked as **On Deckspace**. If you did not mark all locations as **On Deckspace**, an error message will appear, asking you to return to the **Deckspace** tab.
4	Make sure the instrument door is completely closed before proceeding. 
5	Click **OK**.

To start the run: *(continued)*

Step	Action
6	Enter a name for the run in the **Name Run** dialog box that appears.  **Note** The run name must contain fewer than 23 characters. If you have not already clicked the **Cool Peltiers** button, the Peltiers begin to cool.
7	Click **OK**.
8	View the **Run Log** pane to monitor the process.

Finishing the Run

To finish the run:

Step	Action
1	Verify that the run is completed by checking the status section of the **Instrument** tab.
2	Open the door of the 6700 instrument.
3	Clear the deckspace. a. Remove the archive plates and output plates from the deckspace. b. Store the archive plates and output plates until needed. **Plate** / **Deckspace Location** / **Recommended Storage Temperature** RNA archive / Vacuum station / –15 to –25 °C Dilution archive plates / Dilution/cDNA Archive station / –15 to –25 °C Output plates / Output station / 2 to 8 °C
4	Click the **Turn Peltiers Off** button. **IMPORTANT** If you leave the Peltiers on, water will collect on the deckspace and flood it. If this occurs, the temperature sensors may malfunction and fail to report deckspace temperatures accurately.

Output Plate Setup Files Description

After the run, the 6700 database exports information about each output plate in an output plate setup file.

<table>
<tr><th>File Attribute</th><th>Output Plate Setup File Information</th></tr>
<tr><td>Location</td><td>6700_client\D:\pebio\6700\Output Plate Setup Files</td></tr>
<tr><td>Name</td><td>
<table>
<tr><th>If you…</th><th>Then the file is named with the…</th></tr>
<tr><td>scanned Consumable IDs</td><td>Consumable ID.</td></tr>
<tr><td>did not scan Consumable IDs</td><td>year-month-day and the output plate number.</td></tr>
</table>
</td></tr>
</table>

Real-Time PCR Run

Purpose

In this section, you select one Assay output plate from the 6700 workstation qualification run for a real-time run on the ABI PRISM 7700 Sequence Detection System (7700 SDS).

IMPORTANT Verify the performance of the 7700 SDS with the TaqMan® RNase P Instrument Verification Plate (P/N 4310982) before analyzing the 6700 workstation output using the 7700 SDS.

A real-time run involves the following procedures:

Procedure	See Page
Setting Up Real-Time Run Files	2-32
Programming Thermal Cycler Conditions	2-37
Loading the Reaction Plate	2-38

Setting Up Real-Time Run Files

Set up a real-time run file with sample types, reporters, dyes, and sample location. You can do this two ways:

- Importing sample type setup files created by the 6700 system software
- Setting up the run file manually using 7700 SDS software

Use the table below to determine which procedure to follow.

If the 6700 database server is…	Then set up the real-time run files by following the procedure in…
networked	"Importing Setup Files" on page 2-33.
not networked and the 7700 SDS computer has a floppy drive	"Importing Setup Files" on page 2-33.
not networked and the 7700 SDS computer does not have a floppy drive	"Performing Sample Type Setup" on page 2-34.

Importing Setup Files

Import setup files created by the 6700 workstation client computer if you can transfer the output plate setup files via the network or floppy disks.

To import setup files:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Locate the output plate setup files on the 6700 workstation client computer hard drive.
a. Go to D:\pebio\6700\Output Plate Setup Files on the client computer.
b. Find the appropriate output plate setup file.

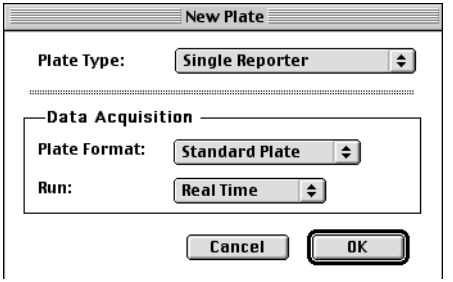
Note The setup file is named with the Consumable ID or with the year, month, day and output plate number.</td></tr>
<tr><td>2</td><td>Transfer the file to the 7700 SDS computer.

<table><tr><th>If the 6700 database server is…</th><th>Then…</th></tr><tr><td>networked</td><td>transfer the file using the network.</td></tr><tr><td>not networked and the 7700 SDS computer has a floppy drive</td><td>transfer the file using a floppy disk.</td></tr><tr><td>not networked and the 7700 SDS computer does not have a floppy drive</td><td>go to the next section, “Performing Sample Type Setup,” and follow the procedures for manually setting up the run file.</td></tr></table></td></tr>
<tr><td>3</td><td>Launch SDS software on the computer connected to the 7700 SDS.

Close the untitled window that appears.</td></tr>
<tr><td>4</td><td>From the File menu, select New Plate...

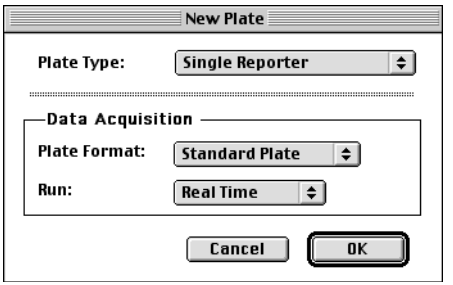
A New Plate dialog box appears.</td></tr>
</table>

To import setup files: *(continued)*

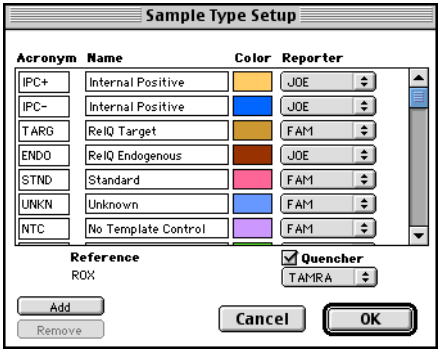
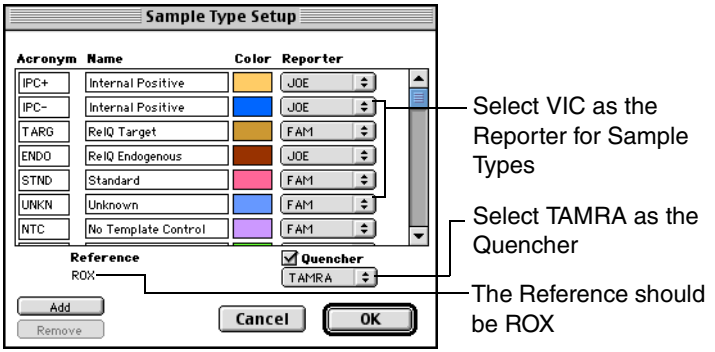
Step	Action
5	Complete the **New Plate** dialog box as shown below: 
6	Click **OK**. An untitled window appears in the setup view.
7	Go to the **File** menu, scroll to **Import**, and select **Import Setup File**.
8	Locate the output plate setup file, and click **Open**. The SDS software imports plate setup information.
9	Go to “Programming Thermal Cycler Conditions” on page 2-37.

Performing Sample Type Setup

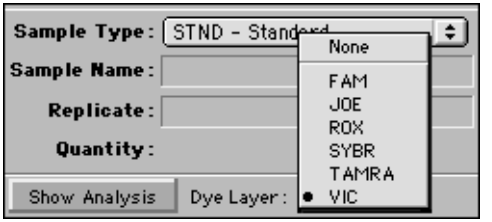
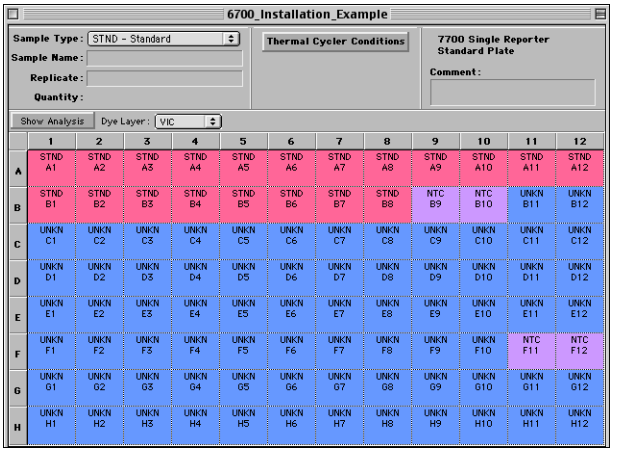
To perform sample type setup:

Step	Action
1	Launch SDS software on the computer connected to the 7700 SDS. Close the untitled window that appears.
2	From the **File** menu, select **New Plate...** A **New Plate** dialog box appears.
3	Complete the **New Plate** dialog box as shown below: 

To perform sample type setup: *(continued)*

Step	Action
4	Click **OK**. An untitled window appears in the setup view.
5	From the **Sample Type** pop-up menu, select **Sample Type Setup...** The **Sample Type Setup** dialog box appears. 
6	Complete the **Sample Type Setup** dialog box. a. Set the following sample types with the reporter dye **VIC**: ♦ NTC ♦ UNKN ♦ STND b. Verify that **ROX** is shown as the **Reference** dye. c. Verify that **TAMRA** is selected as the **Quencher** dye.  d. Click **OK**. The dialog box closes and the untitled window becomes active.

To perform sample type setup: *(continued)*

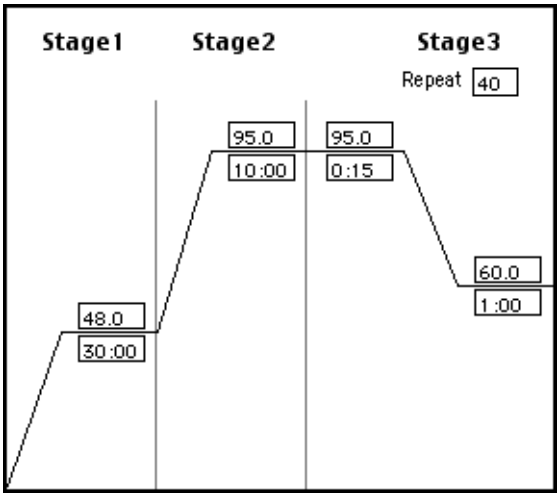
Step	Action
7	Select **VIC** from the **Dye Layer** pop-up menu. 
8	Label **STND-Standard** wells. a. Select wells A1–A12 and B1–B8. b. From the **Sample Type** pop-up menu, select **STND-Standard**.
9	Label **NTC-No Template Control** wells. a. Select wells B9, B10, F11, and F12. **Note** Hold down the Control key to select multiple wells. b. From the **Sample Type** pop-up menu, select **NTC-No Template Control**.
10	Label **UNKN-Unknown** wells. a. Select wells B11, B12, C1–C12, D1–D12, E1–E12, F1–F10, G1–G12, and H1–H12. b. From the **Sample Type** pop-up menu, select **UNKN-Unknown**. The samples are set up as shown in the figure below. 
11	Save the changes to the run file.

To perform sample type setup: *(continued)*

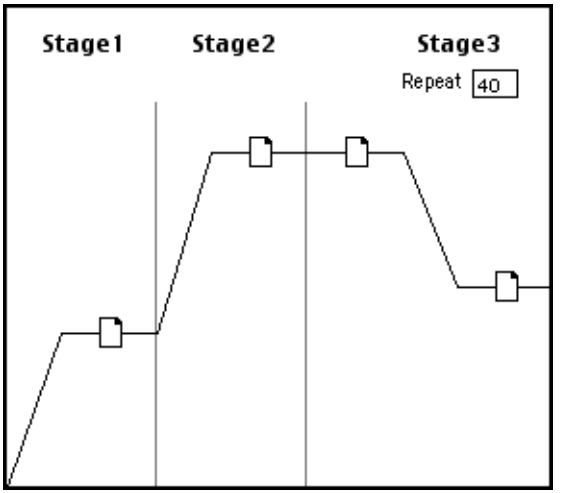
Step	Action
12	Go to “Programming Thermal Cycler Conditions” on page 2-37.

Programming Thermal Cycler Conditions

To program thermal cycler conditions:

Step	Action
1	Program thermal cycling settings. a. Click the **Thermal Cycler Conditions** button. A **Thermal Cycler Conditions** dialog box appears. b. Program the **Thermal Cycler Conditions** as shown below.  c. Set the **Reaction Volume** to **50 µL**.

To program thermal cycler conditions: *(continued)*

Step	Action
2	Program data collection settings. a. Click the **Show Data Collection** button in the dialog box. Document icons indicate when the instrument will collect data. b. Set the document icons as shown in the figure below.  **Note** Click a line segment to add or remove document icons.
3	Click **OK**. The dialog box closes, and the plate read window becomes active.

Loading the Reaction Plate

To load the reaction plate:

Step	Action
1	Place an optical cover compression pad on top of one of the two output plates from the 6700 workstation run.
2	Load the output plate into the 7700 SDS sample block. **IMPORTANT** Start the 7700 SDS run immediately to prevent degradation of the reaction components.

To load the reaction plate: *(continued)*

Step	Action
3	Secure the heated cover over the output plate. a. Pull the heated cover forward. b. Turn the knob clockwise to lower and secure the heated cover over the output plate **⚠ CAUTION** When securing the heated cover in place, be careful not to overtighten the knob. Damage to the instrument could occur.
4	Start the 7700 SDS run. a. Click the **Show Analysis** button. The analysis view appears. b. Make sure that the temperature of the heated cover (**Cov Temp**) is > 100 °C. c. Click the **Run** button. d. Wait for the instrument to beep and the shutters to click before leaving.

Amplification Plot Analysis

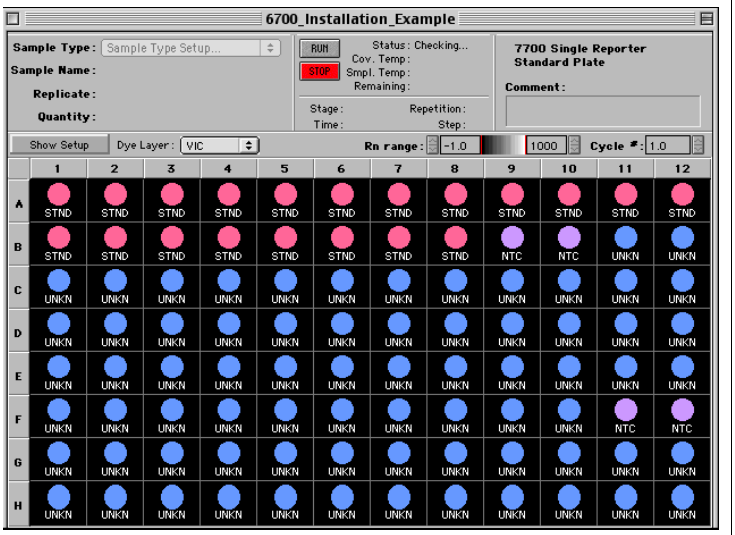
Purpose

In this section, you use Sequence Detection Systems (SDS) software v. 1.6 or later to analyze the amplification plot and export the data.

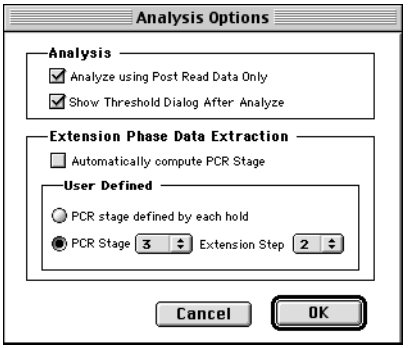
This involves the following procedures:

Setting Analysis Options

To set analysis options:

Step	Action
1	Save the real-time run file.
2	Click the **Show Analysis** button. The analysis view appears. 
3	From the **Analysis** menu, select **Options.** An **Analysis Options** dialog box appears.

To set analysis options: *(continued)*

Step	Action
4	Set the **Analysis Options** dialog box as shown below. 
5	Click **OK**.

Baseline and Threshold Guidelines

In analyzing threshold cycles (see the next page), you set the baseline and threshold values for analysis. Follow the guidelines below to ensure accurate analysis of the data.

Setting the Baseline

- Set the baseline to start at a cycle when background fluorescence is detected by the 7700 SDS.

  Baselines usually start between 2 and 3.

- Set the baseline to stop at a cycle before fluorescence signals from amplification are generated and detected.

  Baselines usually stop between 6 and 8.

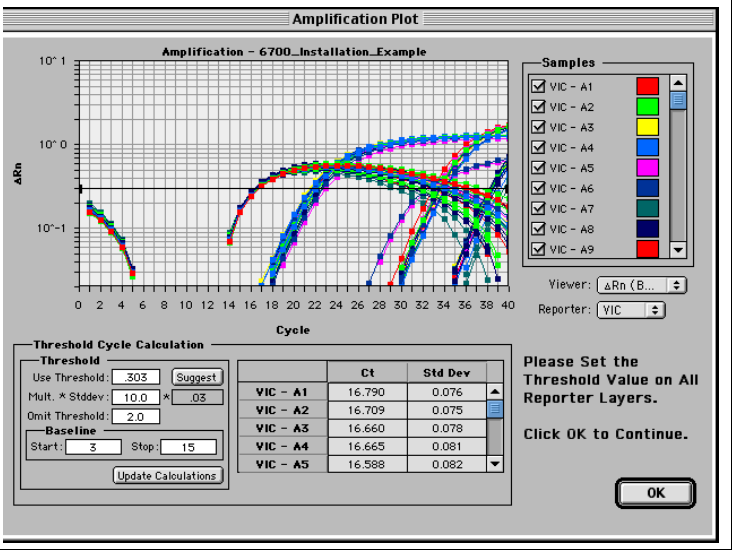
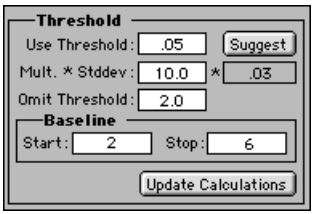
Setting the Threshold

- Set the threshold value to 0.05.

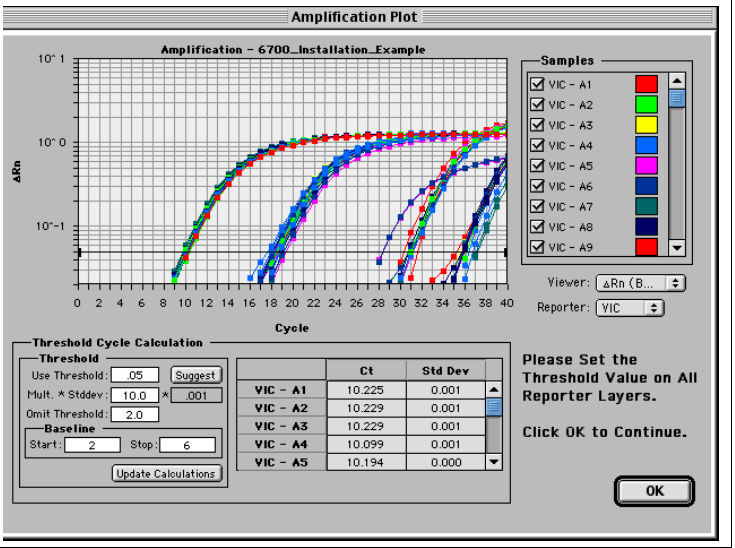
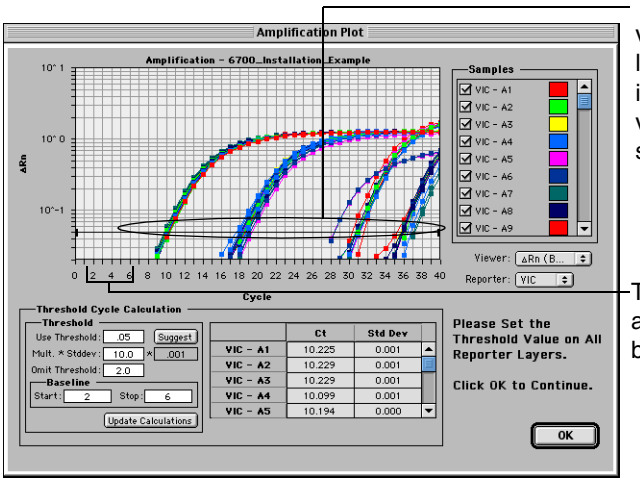
  At this value, fluorescence signals generated from RT+ samples should be increasing logarithmically. Adjustment of the threshold value from 0.05 may be necessary for optimal results.

Analyzing Threshold Cycles

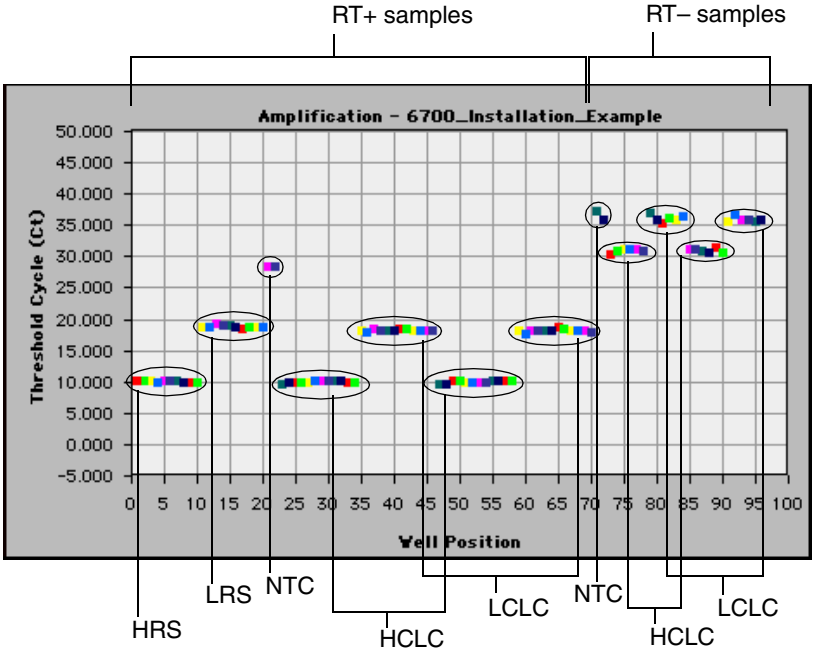
To analyze threshold cycles:

Step	Action
1	Select all wells.
2	From the **Analysis** menu, select **Analyze**. The software analyzes the data, and an **Amplification Plot** window appears. 
3	Set the **Threshold Cycle Calculation** values. a. Set the **Threshold** value to **0.05**. b. Set the **Baseline** to **Start** at **2–3**. c. Set the **Baseline** to **Stop** at **6–8**. 

To analyze threshold cycles: *(continued)*

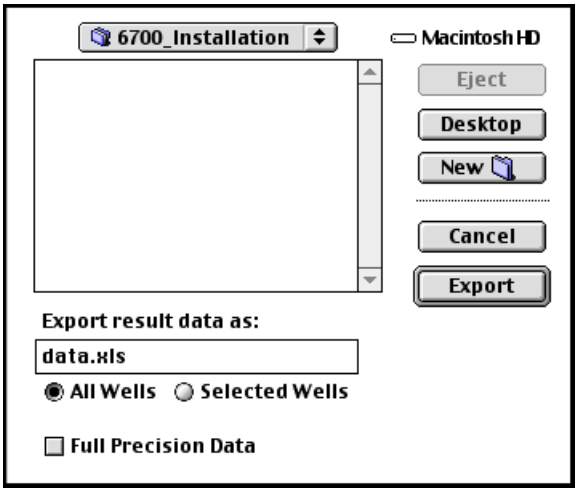
Step	Action
4	Click **Update Calculations**. The software updates the **Amplification Plot**. 
5	Verify that the baseline and threshold are set properly. a. The cycles set for the baseline should show no amplification. b. The threshold value should indicate logarithmic growth of ΔR_n values. 

To analyze threshold cycles: *(continued)*

Step	Action
6	From the **Viewer** pop-up menu, select **Ct vs Well position**. The view of the amplification plot changes. 
7	Save the file.

Exporting Data

To export data:

Step	Action
1	From the **File** menu, scroll to **Export**, and select **Results.** A file dialog box appears. 
2	Enter the file name for the exported data, select a location for the data file, and click **Export**. The data are exported into a data file in the selected location.

Threshold Cycle Analysis

Purpose

In this section you manipulate the exported data using Microsoft® Excel to calculate C_T averages and standard deviations for the different sample groups. See “Example Qualification Data and Calculations” on page 2-57 for an example of a qualification analysis.

Threshold cycle analysis involves the following procedures:

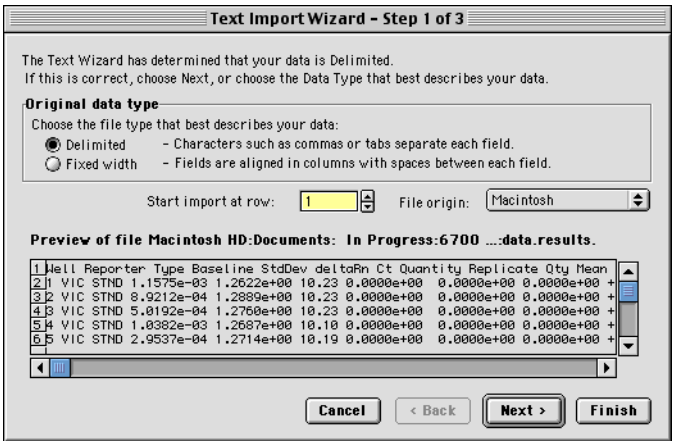
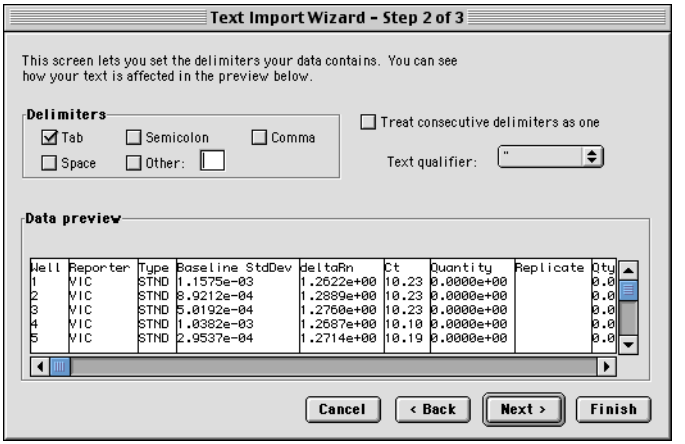
Procedure	See Page
Preparing the Data File	2-46
Calculating Average CT Values and Standard Deviations	2-49
Eliminating Outliers From Analysis	2-52
Eliminating Dropouts From Analysis	2-52

Preparing the Data File

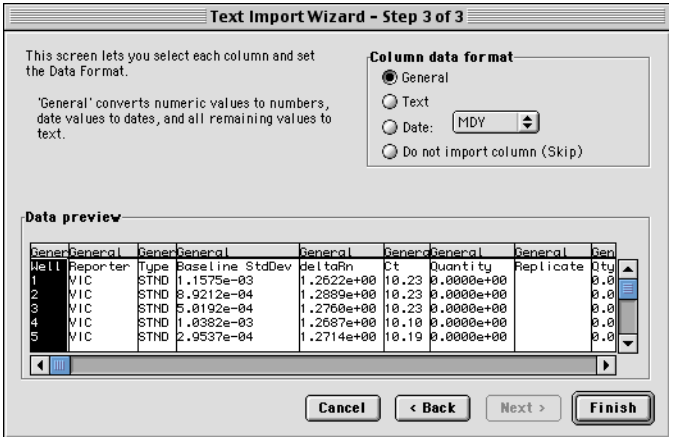
To prepare the data file:

Step	Action
1	Using Microsoft Excel, open the **data.xls** file you saved in “Exporting Data” on page 2-45. A **Text Import Wizard** dialog box appears.

To prepare the data file: *(continued)*

Step	Action
2	Complete Step 1 of the **Text Import Wizard** dialog box. a. Select **Delimited** as the **Original data type**. b. Start at row **1**. c. Select the appropriate **File Origin**. d. Click **Next**. 
3	Complete Step 2 of the **Text Import Wizard** dialog box. a. Check the **Tab** box. b. Click **Next**. 

To prepare the data file: *(continued)*

Step	Action
4	Complete Step 3 of the **Text Import Wizard** dialog box. a. Select **General** as the **Column data format**. b. Click **Finish**. The data file opens as a spreadsheet. 
5	Delete the following columns: ♦ Baseline StdDev ♦ deltaRn ♦ Quantity ♦ Replicate ♦ Qty Mean ♦ Qty StdDev ♦ IPC ♦ Sample Name
6	Insert five columns: ♦ Avg C_T (2x) ♦ Avg C_T (2x) sorted ♦ Avg C_T ♦ STDEV ♦ Specs

To prepare the data file: *(continued)*

Step	Action
7	Add cell borders to distinguish between the sample types. a. Select row **11**. b. Click the Borders button.  The software applies a border to the bottom of row **11**. c. Repeat steps 7a and 7b for the following rows. <table><tr><td>21</td><td>59</td><td>85</td></tr><tr><td>23</td><td>71</td><td>91</td></tr><tr><td>34</td><td>73</td><td>97</td></tr><tr><td>47</td><td>79</td><td></td></tr></table>

Calculating Average C_T Values and Standard Deviations

To calculate average C_T values:

Step	Action
1	Calculate the average C_T value for each set of duplicates (HCLC RT+ and LCLC RT+ samples) in the **Avg C_T (2x)** column. a. In cell E24, enter the formula **=AVERAGE(D24:D25)**, and press Return. The software calculates the average for samples 23 and 24, a set of duplicates. b. Select cells E24 and E25, and from the **Edit** menu, select **Copy**. c. Select cells E26 through E71, and from the **Edit** menu, select **Paste**. The software calculates the average for the remaining sets of duplicates.
2	Paste the average C_T values for HCLC RT+ samples into column F. a. Hold down the Command key, and select the average C_T values in column E for HCLC RT+ samples. b. From the **Edit** menu, select **Copy**. c. Place the cursor in cell F24. d. From the **Edit** menu, select **Paste**.

To calculate average C_T values: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Paste the average C_T values for LCLC RT+ samples into column F.
a. Hold down the Command key, and select the average C_T values in column E for LCLC RT+ samples.
b. From the Edit menu, select Copy.
c. Place the cursor in cell F36.
d. From the Edit menu, select Paste.</td></tr>
<tr><td>4</td><td>Sort the C_T values for the sets of duplicates for the HCLC RT+ and for the LCLC RT+ samples in the Avg C_T (2x) sorted column.
a. Select samples F24:F35, and from the Data menu select Sort.
b. Select samples F36:F47, and from the Data menu select Sort.</td></tr>
<tr><td>5</td><td>Calculate the average C_T value for each sample group in the Avg C_T column.
<table><tr><th>In cell…</th><th>Enter the formula…</th><th>Sample Group</th></tr><tr><td>G2</td><td>=AVERAGE(D2:D11)</td><td>HRS RT+</td></tr><tr><td>G12</td><td>=AVERAGE(D12:D21)</td><td>LRS RT+</td></tr><tr><td>G22</td><td>=AVERAGE(D22:D23)</td><td>NTC RT+</td></tr><tr><td>G24</td><td>=AVERAGE(D24:D35, D48:D59)</td><td>HCLC RT+</td></tr><tr><td>G36</td><td>=AVERAGE(D36:D47, D60:D71)</td><td>LCLC RT+</td></tr><tr><td>G72</td><td>=AVERAGE(D72:D73)</td><td>NTC RT–</td></tr><tr><td>G74</td><td>=AVERAGE(D74:D79, D86:D91)</td><td>HCLC RT–</td></tr><tr><td>G80</td><td>=AVERAGE(D80:D85, D92:D97)</td><td>LCLC RT–</td></tr></table></td></tr>
</table>

To calculate average C_T values: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>Calculate the standard deviation for C_T values for each sample group in the STDEV column (column I).
<table>
<tr><th>In cell…</th><th>Enter the formula…</th><th>Sample Group</th></tr>
<tr><td>H2</td><td>=STDEV(D2:D11)</td><td>HRS RT+</td></tr>
<tr><td>H12</td><td>=STDEV(D12:D21)</td><td>LRS RT+</td></tr>
<tr><td>H22</td><td>=STDEV(D22:D23)</td><td>NTC RT+</td></tr>
<tr><td>H24</td><td>=STDEV(F24:F29)</td><td>HCLC RT+ below avg</td></tr>
<tr><td>H25</td><td>=STDEV(F30:F35)</td><td>HCLC RT+ above avg</td></tr>
<tr><td>H36</td><td>=STDEV(F36:F41)</td><td>LCLC RT+ below avg</td></tr>
<tr><td>H37</td><td>=STDEV(F42:F47)</td><td>LCLC RT+ above avg</td></tr>
<tr><td>H72</td><td>=STDEV(D72:D73)</td><td>NTC RT–</td></tr>
<tr><td>H74</td><td>=STDEV(D74:D79, D86:D91)</td><td>HCLC RT–</td></tr>
<tr><td>H80</td><td>=STDEV(D80:D85, D92:D97)</td><td>LCLC RT–</td></tr>
</table></td></tr>
<tr><td>7</td><td>Eliminate samples following the guidelines in the following sections, “Eliminating Outliers From Analysis” and “Eliminating Dropouts From Analysis,” before proceeding with the specification calculations.</td></tr>
</table>

Eliminating Outliers From Analysis

An outlier exhibits a C_T value that differs by greater than three standard deviations from the average C_T value for the sample group.

Eliminate from analysis up to four outliers from each output plate:

- Eliminate an RT+ sample from analysis if the C_T value of the RT+ sample differs by greater than three standard deviations from the average C_T value for the sample group and the C_T value of the sample's replicate is within three standard deviations of the average C_T value for the sample group.

Note If the C_T values of a sample and its replicate both differ by greater than three standard deviations of the average C_T value for the sample group, then the sample set meets the definition of a dropout (See the next section, "Eliminating Dropouts From Analysis.").

- Eliminate an RT– sample from analysis if the C_T value of the RT– sample differs by greater than three standard deviations from the average C_T value for the sample group.
- Eliminate a standard from analysis if the C_T value of the standard differs by greater than three standard deviations from the average C_T value for the sample group

Eliminating Dropouts From Analysis

A dropout consists of a set of duplicate samples that both exhibit C_T values that differ by greater than three standard deviations from the average C_T value for the sample group.

Eliminate up to two dropouts from each output plate from analysis.

Qualification Analysis

Purpose

In this section you use the C_T values to measure the quality of the RNA prepared by the 6700 workstation using three performance parameters.

Performance	Specification
% RNA Recovery	The 6700 workstation can recover ≥50% of the RNA content of the cell lysate control.
Precision of RNA Recovery	The 6700 workstation can recover RNA with a precision of <30% coefficient of variation (CV).
% DNA Contamination	The 6700 workstation can recover RNA containing <0.5% of DNA by weight.

Calculating % RNA Recovery

For the RNA recovery to meet or exceed 50% of the RNA content of the lysate, the following equations must be true.

C_T(HCLC RT+) – C_T(HRS RT+) < 1.0

C_T(LCLC RT+) – C_T(LRS RT+) < 1.0

To calculate % RNA recovery:

Step	Action
1	Calculate ΔC_T values for HCLC RT+ samples compared to standard HRS RT+ samples. a. In cell I2, enter the formula **=G24 – G2**. b. Verify that the difference is less than 1.0.
2	Calculate ΔC_T values for LCLC RT+ samples compared to standard LRS RT+ samples. a. In cell I12, enter the formula **=G36 – G12**. b. Verify that the difference is less than 1.0.

Calculating Precision of RNA Recovery

For the precision of RNA recovery to be less than 30% CV, the following equations must be true for the HCLC RT+ population and for the LCLC RT+ population.

For the standard deviation of C_T values below the average C_T:

$[10^{(\text{Std. Dev. } C_T/3.33)} - 1] \times 100 < 30\%$

For the standard deviation of C_T values above the average C_T:

$[10^{(-\text{Std. Dev. } C_T/3.33)} - 1] \times 100 < 30\%$

To calculate precision of RNA recovery:

Step	Action
1	Calculate the % CV for the standard deviation of HCLC RT+ C_T values below the average. a. In cell I24, enter the formula **=(POWER(10, A/ 3.33)–1)*100** Where A is the value from cell H24, the standard deviation for HCLC RT+ C_T values below the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.
2	Calculate the % CV for the standard deviation of HCLC RT+ C_T values above the average. a. In cell K25, enter the formula **=(POWER(10, – B/ 3.33)–1)*100** Where B is the value from cell H25, the standard deviation for HCLC RT+ C_T values above the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.

To calculate precision of RNA recovery: *(continued)*

Step	Action
3	Calculate the % CV for the standard deviation of LCLC RT+ C_T values below the average. a. In cell I36, enter the formula **=(POWER(10, C/ 3.33)–1)*100** Where C is the value from cell H36, the standard deviation for LCLC RT+ C_T values below the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.
4	Calculate the % CV for the standard deviation of LCLC RT+ C_T values above the average. a. In cell K37 for LCLC RT+ samples, enter the formula **=(POWER(10, – D/ 3.33)–1)*100** Where D is the value from cell H37, the standard deviation for LCLC RT+ C_T values above the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.

Calculating % DNA Contamination

For DNA contamination in the RNA samples to be less than 0.5% by weight, the following equation must be true:

C_T(HCLC RT–) – C_T(HCLC RT+) ≥ 16

To calculate % DNA contamination:

Step	Action
1	In cell I74, enter the formula **=G74–G24**.
2	Press Return to calculate.
3	Verify that the value is greater than or equal to 16.

6700 Qualification Requirement

Qualification of the 6700 instrument using the Cell Lysate Control kit requires that the purified RNA meets Applied Biosystems specifications for % RNA recovery, precision of RNA recovery, and % DNA contamination.

If one or more of the specifications is not met, please do one of the following:

- Contact technical support (see “Technical Support” on page A-1) for assistance with using the kit or analyzing the data.
- Contact your Applied Biosystems service representative for service of your 6700 workstation.

Example Qualification Data and Calculations

Purpose

This appendix contains data and calculations from an example 6700 workstation qualification run.

Example % RNA Recovery

The figure below shows example data and calculations for determining % RNA recovery.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
2	1	VIC	STND	10.53			10.44	0.12409674	-0.0491667
3	2	VIC	STND	10.6					
4	3	VIC	STND	10.58					
5	4	VIC	STND	10.43					
6	5	VIC	STND	10.49					
7	6	VIC	STND	10.48					
8	7	VIC	STND	10.44					
9	8	VIC	STND	10.35					
10	9	VIC	STND	10.23					
11	10	VIC	STND	10.27					
12	11	VIC	STND	19			18.866	0.28975852	-0.5780833
13	12	VIC	STND	18.76					
14	13	VIC	STND	19.43					
15	14	VIC	STND	19.02					
16	15	VIC	STND	19.16					
17	16	VIC	STND	18.74					
18	17	VIC	STND	18.47					
19	18	VIC	STND	18.79					
20	19	VIC	STND	18.75					
21	20	VIC	STND	18.54					
22	21	VIC	NTC	28.36			28.29	0.09899495	
23	22	VIC	NTC	28.22					

ΔC_T values for HCLC RT+ samples compared to HRS RT+ samples is less than 1.0

ΔC_T values for LCLC RT+ samples compared to LRS RT+ samples is less than 1.0

Example Precision of RNA Recovery

The next figure shows example data and calculations for determining precision of RNA recovery.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
24	23	VIC	UNKN	10.12	10.19	10.18	10.3908	0.08489209	6.0457139
25	24	VIC	UNKN	10.26		10.19		0.08769265	-5.8834778
26	25	VIC	UNKN	10.37	10.375	10.315			
27	26	VIC	UNKN	10.38		10.34			
28	27	VIC	UNKN	10.4	10.4	10.35			
29	28	VIC	UNKN	10.4		10.375			
30	29	VIC	UNKN	10.49	10.43	10.4			
31	30	VIC	UNKN	10.37		10.43			
32	31	VIC	UNKN	10.49	10.43	10.43			
33	32	VIC	UNKN	10.37		10.495			
34	33	VIC	UNKN	10.32	10.315	10.56			
35	34	VIC	UNKN	10.31		10.625			
36	35	VIC	UNKN	18.16	18.14	18.085	18.2879	0.06658954	4.71209745
37	36	VIC	UNKN	18.12		18.085		0.12320714	-8.1665587
38	37	VIC	UNKN	18.46	18.31	18.14			
39	38	VIC	UNKN	18.16		18.205			
40	39	VIC	UNKN	18.26	18.23	18.22			
41	40	VIC	UNKN	18.2		18.23			
42	41	VIC	UNKN	18.55	18.505	18.31			
43	42	VIC	UNKN	18.46		18.31			
44	43	VIC	UNKN	18.4	18.365	18.365			
45	44	VIC	UNKN	18.33		18.38			
46	45	VIC	UNKN	18.21	18.085	18.505			
47	46	VIC	UNKN	17.96		18.62			
48	47	VIC	UNKN	10.17	10.18				
49	48	VIC	UNKN	10.19					
50	49	VIC	UNKN	10.51	10.495				
51	50	VIC	UNKN	10.48					
52	51	VIC	UNKN	10.31	10.34				
53	52	VIC	UNKN	10.37					
54	53	VIC	UNKN	10.3	10.35				
55	54	VIC	UNKN	10.4					
56	55	VIC	UNKN	10.58	10.56				
57	56	VIC	UNKN	10.54					
58	57	VIC	UNKN	10.59	10.625				
59	58	VIC	UNKN	10.66					
60	59	VIC	UNKN	18.09	18.085				
61	60	VIC	UNKN	18.08					
62	61	VIC	UNKN	18.39	18.38				
63	62	VIC	UNKN	18.37					
64	63	VIC	UNKN	18.28	18.31				
65	64	VIC	UNKN	18.34					
66	65	VIC	UNKN	18.7	18.62				
67	66	VIC	UNKN	18.54					
68	67	VIC	UNKN	18.2	18.22				
69	68	VIC	UNKN	18.24					
70	69	VIC	UNKN	18.22	18.205				
71	70	VIC	UNKN	18.19					

Example % DNA Contamination

The figure below shows example data and calculations for determining % DNA contamination.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
72	71	VIC	NTC	36.91			36.53	0.53740115	
73	72	VIC	NTC	36.15					
74	73	VIC	UNKN	30.11			30.895	0.42530203	20.5041667
75	74	VIC	UNKN	30.5					
76	75	VIC	UNKN	31.01					
77	76	VIC	UNKN	31.71					
78	77	VIC	UNKN	31.17					
79	78	VIC	UNKN	31.01					
80	79	VIC	UNKN	36.7			35.8867	0.41220766	
81	80	VIC	UNKN	35.66					
82	81	VIC	UNKN	35.62					
83	82	VIC	UNKN	35.82					
84	83	VIC	UNKN	35.68					
85	84	VIC	UNKN	36.19					
86	85	VIC	UNKN	30.69					
87	86	VIC	UNKN	31.17					
88	87	VIC	UNKN	30.6					
89	88	VIC	UNKN	30.52					
90	89	VIC	UNKN	31.13					
91	90	VIC	UNKN	31.12					
92	91	VIC	UNKN	35.59					
93	92	VIC	UNKN	36.59					
94	93	VIC	UNKN	36.1					
95	94	VIC	UNKN	35.61					
96	95	VIC	UNKN	35.56					
97	96	VIC	UNKN	35.52					

Table of Formulas

The table below describes the formula or contents for each cell.

Cell	Formula/Contents
E24	=AVERAGE(D24:D25)
E26:E71	pasted from E24:E25
F24:F35	pasted from E24:E34, E48:E58 and sorted in ascending order
F36:F47	pasted from E36:E46, E60:E70 and sorted in ascending order
G2	=AVERAGE(D2:D11)
G12	=AVERAGE(D12:D21)
G22	=AVERAGE(D22:D23)

Cell	Formula/Contents
G24	=AVERAGE(D24:D35, D48:D59)
G36	=AVERAGE(D36:D47, D60:D71)
G72	=AVERAGE(D72:D73)
G74	=AVERAGE(D74:D79, D86:D91)
G80	=AVERAGE(D80:D85, D92:D97)
H2	=STDEV(D2:D11)
H12	=STDEV(D12:D21)
H22	=STDEV(D22:D23)
H24	=STDEV(F24:F29)
H25	=STDEV(F30:F35)
H36	=STDEV(F36:F41)
H37	=STDEV(F42:F47)
H72	=STDEV(D72:D73)
H74	=STDEV(D74:D79, D86:D91)
H80	=STDEV(D80:D85, D92:D97)
I2	=G24–G2
I12	=G36–G12
I24	=(POWER(10,A/3.33)–1)*100 **Note** A is the value from cell H24.
I25	=(POWER(10,–B/3.33)–1)*100 **Note** B is the value from cell H25.
I36	=(POWER(10,C/3.33)–1)*100 **Note** C is the value from cell H36.
I37	=(POWER(10,–D/3.33)–1)*100 **Note** D is the value from cell H37.
I74	=G74–G24

6100 PrepStation Qualification

3

Overview

Purpose of the Cell Lysate Control Kit

The purpose of the ABI PRISM® Cell Lysate Control Kit is to qualify the performance of the ABI PRISM™ 6100 Nucleic Acid PrepStation. This kit is designed to be used with Applied Biosystems reagents and plastic consumables and with either the ABI PRISM® 7700 Sequence Detection System (7700 SDS) or a spectrophotometer.

About the Cell Lysate Control Kit

The Cell Lysate Control Kit contains the following from a single lot of human Raji cells:

- Two concentrations of lysed human Raji cells
  - High Cell Lysate Control (HCLC)
  - Low Cell Lysate Control (LCLC)
- Total RNA recovered from two concentrations of lysed human Raji cells and dissolved in Nucleic Acid Purification Elution Solution (P/N 4305983)
  - High Recovered RNA Standard (HRS)
  - Low Recovered RNA Standard (LRS)

About This Chapter

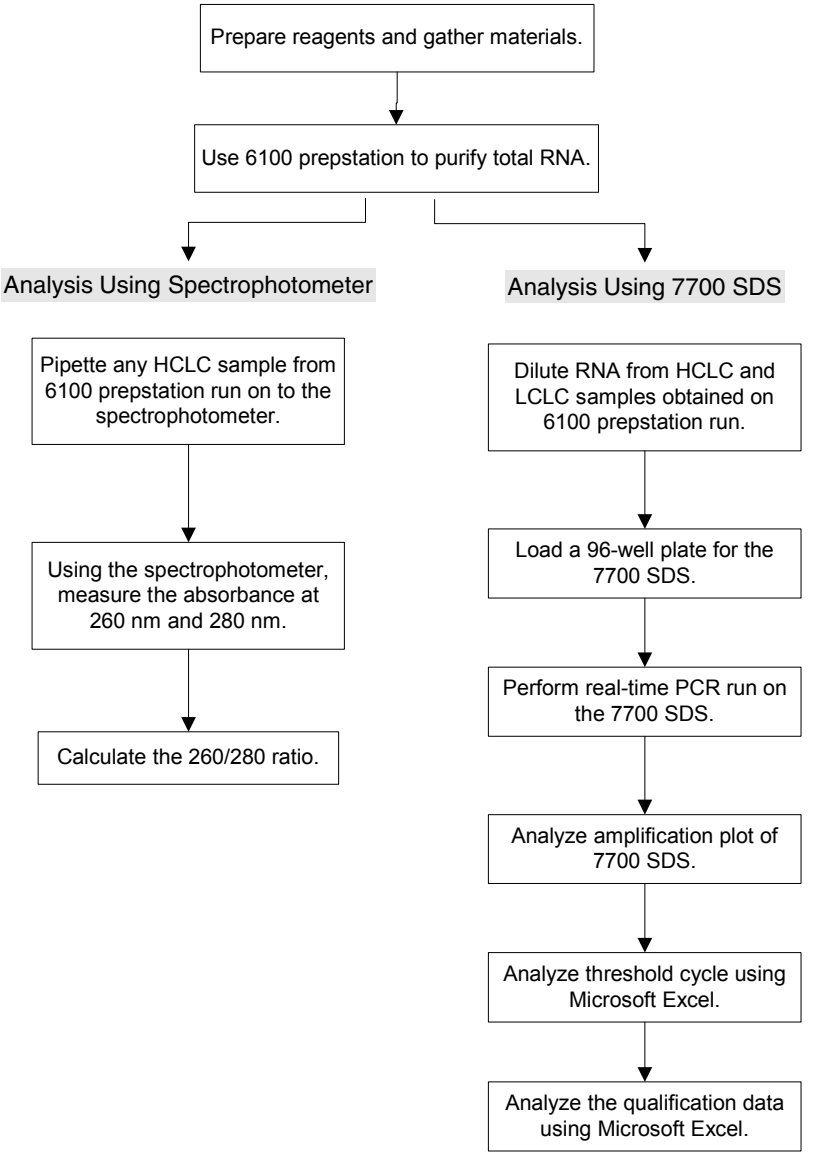
This chapter contains the following:

- An overview of the 6100 prepstation qualification process
- A list of the equipment and materials required to qualify the 6100 prepstation
- Procedures for using the 6100 prepstation with the Cell Lysate Control Kit, Applied Biosystems reagents, and Applied Biosystems plastic consumables
- If using a spectrophotometer for analysis:
  - Procedures for verifying the purity of the total RNA by measuring the absorbance at 260 nm and 280 nm for the total RNA from the High Cell Lysate Control
- If using the 7700 SDS for PCR and analysis:
  - Procedures for using the 7700 SDS to perform a real-time run and to analyze the 6100 prepstation output[1]
  - Procedures for analyzing the qualification

1. Verify the performance of the 7700 SDS with the TaqMan® RNase P Instrument Verification Plate (P/N 4310982) before analyzing the 6100 prepstation output using the 7700 SDS.

6100 Instrument Qualification Overview

Process The qualification process involves the following stages:



Materials and Equipment

Kit Contents

The Cell Lysate Control Kit (P/N 4315646) contains sufficient quantities to perform one 6100 prepstation qualification procedure.

Item	Description[a]	Volume
High Cell Lysate Control (HCLC)	Raji cells diluted in lysis buffer[b] to a final concentration of 5×10^6 cells/mL	11 mL
Low Cell Lysate Control (LCLC)	Raji cells diluted in lysis buffer[b] to a final concentration of 5×10^4 cells/mL	11 mL
High Recovered RNA Standard (HRS)	Total RNA recovered from 1 mL of HCLC[c], and diluted 1:150	0.6 mL
Low Recovered RNA Standard (LRS)	Total RNA recovered from 1 mL of LCLC[c], and diluted 1:150	0.6 mL

a. All components in the packaged kit are produced from a single lot of human Raji cells.

b. Lysis buffer consists of a 1:1 mixture of Nucleic Acid Purification Lysis Solution (P/N 4305895) and calcium/magnesium-free phosphate-buffered saline solution.

c. The RNA is dissolved in 0.6 mL Nucleic Acid Purification Elution Solution (P/N 4305893).

Kit Storage and Stability

All components of the Cell Lysate Control kit should be stored at –15 to –25 °C.

Note If stored correctly, this kit is guaranteed for 1 year from the date of manufacture.

Equipment and Materials Required But Not Supplied

The following tables list the equipment and materials required in addition to the reagents supplied with the Cell Lysate Control Kit.

IMPORTANT Follow the instructions as indicated in this protocol to qualify the 6100 prepstation. If you modify this protocol in any way, including changing the amounts used and substituting reagents or other materials, Applied Biosystems cannot guarantee the validity of your 6100 prepstation qualification process.

Equipment Required

Equipment	Source
ABI PRISM 6100 Nucleic Acid PrepStation	See your Applied Biosystems sales representative
ABI PRISM 7700 Sequence Detection System or Spectrophotometer	
Centrifuge with 96-well adapter	Major laboratory supplier (MLS)
Microcentrifuge	MLS
Pipettors	MLS
Vortexer	MLS

Applied Biosystems Materials Required

Applied Biosystems Materials	Amount Needed	P/N
For 6100 prepstation		
Microcentrifuge Tubes and Caps, 2-mL	10 tubes	4305936
6100 Splash Guard	1 guard	4311758
96-Well Optical Reaction Plate with Barcode	1 plate	4306737
Total RNA Purification Tray	1 tray	4305673
RNA Purification Wash Solution 1	44 mL	4305891
RNA Purification Wash Solution 2	117 mL	4305890
Nucleic Acid Purification Elution Solution	57.1 mL	4305893

Applied Biosystems Materials Required *(continued)*

Applied Biosystems Materials	Amount Needed	P/N
For 7700 SDS		
Reagent Tubes with Caps, 10-mL	6 tubes	4305932
96-Well Optical Reaction Plate with Barcode	3 plates	4306737
Optical Adhesive Cover	1 cover	4311971
Optical Cover Compression Pad	1 pad	4312639
TaqMan® One-Step RT-PCR Master Mix Reagents Kit ♦ 2X Master Mix without UNG Contains AmpliTaq Gold® DNA Polymerase, dNTPs with dUTP, Passive Reference, and optimized buffer components ♦ 40X Multiscribe and RNase Inhibitor Mix	3 kits	4309169
TaqMan® Ribosomal RNA Control Reagents (VIC™ Dye) ♦ Human Control RNA ♦ rRNA Probe (VIC™) ♦ rRNA Forward Primer ♦ rRNA Reverse Primer	1 kit	4308329
TaqMan® RNase P Instrument Verification Plate	2 plates	4310982

Reagent Preparation

About Reagent Preparation

In this section, you prepare the following:

- Cell lysate controls
- 18S RT+ master mix (for 7700 SDS only)
- 18S RT– master mix (for 7700 SDS only)

Preparing Cell Lysate Controls

Prepare the cell lysate controls as described below for accurate results.

⚠ WARNING **CHEMICAL HAZARD**. **Raji cell lysate controls** cause irritation to the eyes, skin, and respiratory tract. This material is harmful if swallowed, inhaled, or absorbed through the skin. It may cause damage to the nervous system and bone marrow. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare cell lysate controls:

Step	Action
1	Allow the cell lysate controls to thaw at room temperature or on ice. **IMPORTANT** Do not use heat to thaw cell lysate controls.
2	Vortex cell lysate controls thoroughly to ensure that the controls are completely resuspended. ♦ Use the highest speed setting. ♦ Place the tubes at an angle while vortexing. ♦ You should observe liquid from the bottom of the tube mixing with liquid at the top of the tube. **IMPORTANT** Complete and thorough vortexing is essential for accurate results.
3	Pipette the bubbles into a 2-mL microcentrifuge tube, and briefly centrifuge the tube to recover liquid.
4	Pipette the recovered liquid from the bubbles back into the correct cell lysate tube.
5	Cap and invert the tubes to mix.
6	Place the tubes on ice until you load the purification tray.

Preparing 1.25X 18S RT+ Master Mix

If using the 7700 SDS for analysis, perform the following procedure. Prepare 18S RT+ master mix for measuring RNA in samples prepared by the 6100 prepstation. Use components from the TaqMan One-Step RT-PCR Master Mix Reagents Kit and the TaqMan Ribosomal RNA Control Reagents (VIC Dye).

To prepare 18S RT+ master mix:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Pipette the components into a 10-mL reagent tube.

<table>
<tr><th>Component</th><th>Volume</th></tr>
<tr><td>2X Master Mix without UNG</td><td>4.0 mL</td></tr>
<tr><td>rRNA Forward Primer</td><td>80 µL</td></tr>
<tr><td>rRNA Reverse Primer</td><td>80 µL</td></tr>
<tr><td>rRNA VIC Probe</td><td>10 µL</td></tr>
<tr><td>40X Multiscribe and RNase inhibitor mix</td><td>200 µL</td></tr>
<tr><td>Deionized water</td><td>2.03 mL</td></tr>
<tr><td>Total</td><td>6.4 mL</td></tr>
</table></td></tr>
<tr><td>2</td><td>Place the 18S RT+ master mix on ice until you load the 96-well plate for the 7700 SDS.</td></tr>
</table>

Preparing 1.25X 18S RT– Master Mix

If using the 7700 SDS for analysis, perform the following procedure. Prepare 18S RT– master mix for detecting DNA contamination in samples prepared by the 6100 prepstation. Use components from the TaqMan One-Step RT-PCR Master Mix Reagents Kit and the TaqMan Ribosomal RNA Control Reagents (VIC Dye).

To prepare 18S RT– master mix:

Step	Action
1	Pipette the components into a 10-mL reagent tube. **Component** / **Volume** 2X Master Mix without UNG — 1.5 mL rRNA Forward Primer — 30 µL rRNA Reverse Primer — 30 µL rRNA VIC Probe — 3.75 µL Deionized water — 836 µL **Total** — 2.4 mL
2	Place the 18S RT– master mix on ice until you load the 96-well plate for the 7700 SDS.

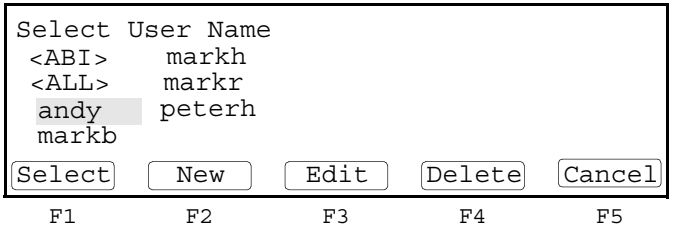
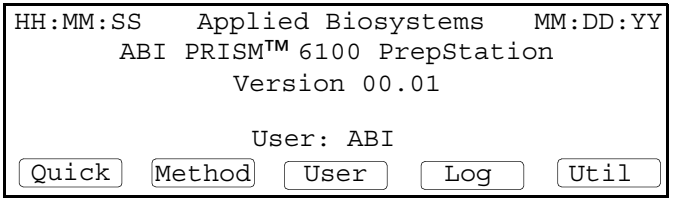
6100 PrepStation Run

Overview

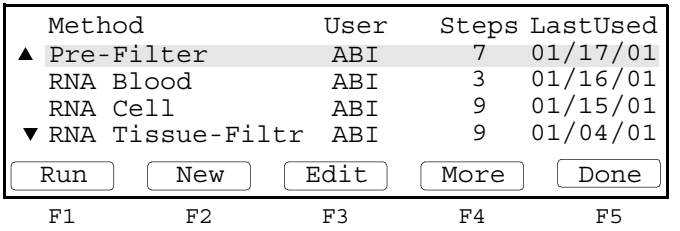
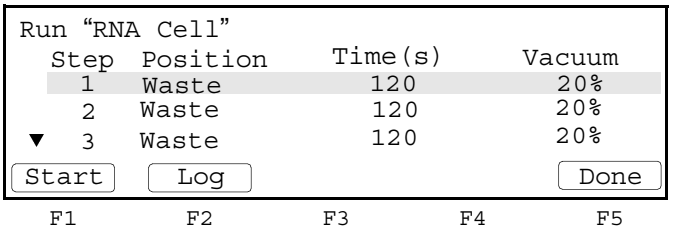
Performing the 6100 prepstation run consists of the following procedures, which must be done in order:

Accessing Predefined Method

To access the RNA Cell predefined method:

Step	Action
1	From the main menu, press **F3** (User). The Select User Name screen appears. 
2	Use the arrow keys to highlight user ABI.
3	Press **F1** (Select). The main menu appears showing ABI as the user name. 

To access the RNA Cell predefined method: *(continued)*

Step	Action
4	Press **F2** (Method). The Method Select 1 screen appears. 
5	Use the up and down arrow keys to highlight the RNA Cell method.
6	Press **F1** (Run). The Method View screen appears. 

Loading Disposables

To load disposables on the 6100 prepstation:

Step	Action
1	Place a 96-well archive plate on top of the adapter in the collection compartment.
2	Place a splash guard in the waste compartment.
3	Place a total RNA purification tray in the carriage. Turn the two knobs to secure the tray in place.
4	Move the carriage to the waste position. Push the carriage handle down until the carriage locks into position (seals).

Loading Purification Tray

To load the purification tray:

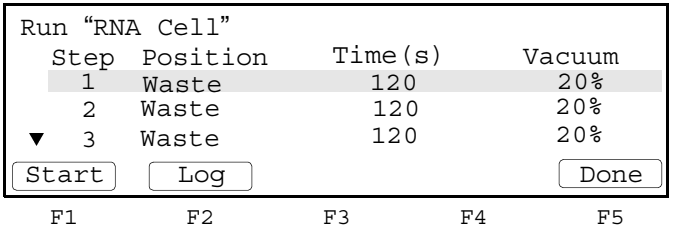
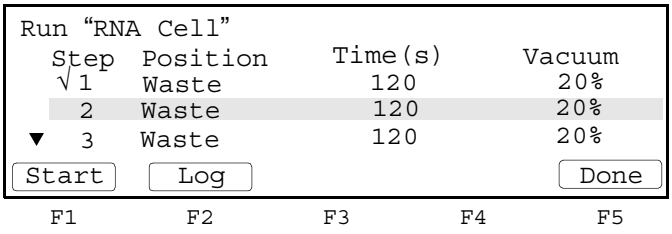
Step	Action
1	Pre-wet the purification tray by pipetting 40 µL of RNA purification wash solution 1 over each well of the purification tray. **⚠ CAUTION CHEMICAL HAZARD. RNA purification wash solution 1** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Pipette 200 µL of High Cell Lysate Control (HCLC) or Low Cell Lysate Control (LCLC) into each well according to the plate map shown page 3-13. **⚠ WARNING CHEMICAL HAZARD. Raji cell lysate controls** cause irritation to the eyes, skin, and respiratory tract. This material is harmful if swallowed, inhaled, or absorbed through the skin. It may cause damage to the nervous system and bone marrow. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Ensure that the highlighter is at step 1 of the RNA Cell method, then press **F1** (Start). 

Plate Map for 6100 prepstation

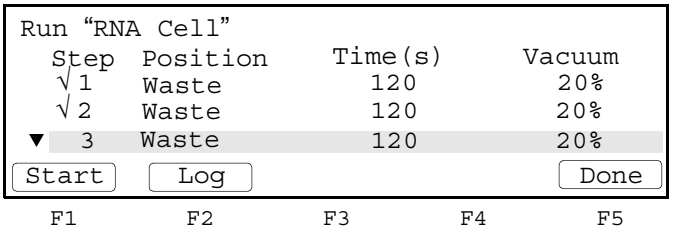
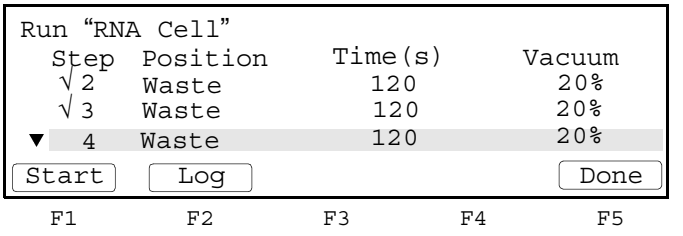
	1	2	3	4	5	6	7	8	9	10	11	12
A	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
B	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
C	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
D	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
E	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
F	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
G	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC
H	HCLC	HCLC	HCLC	HCLC	HCLC	HCLC	LCLC	LCLC	LCLC	LCLC	LCLC	LCLC

Running the RNA Cell Method

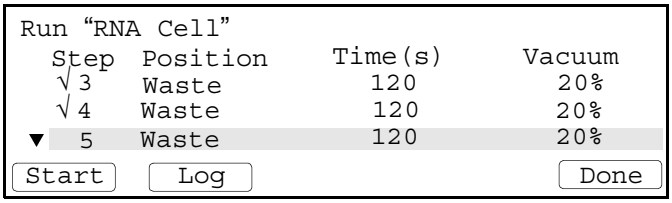
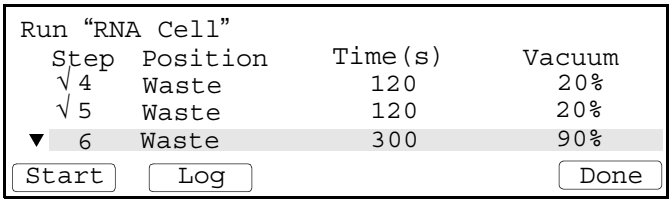
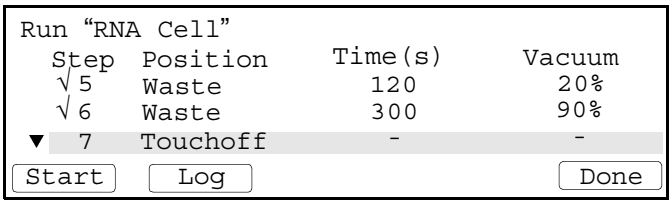
To run the rest of the RNA Cell method:

Step	Action
1	Perform the first wash. a. Pipette 500 µL of RNA purification wash solution 1 over each sample in the purification tray. **⚠ CAUTION CHEMICAL HAZARD. RNA purification wash solution 1** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Ensure that the highlighter is at step 2 of the RNA Cell method, then press **F1** (Start). 

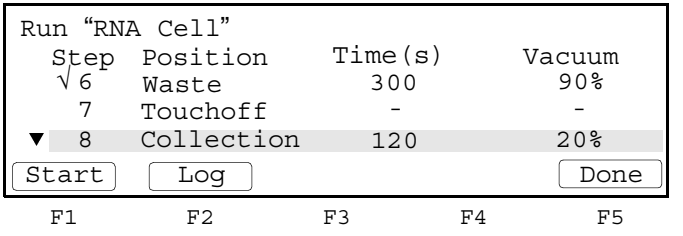
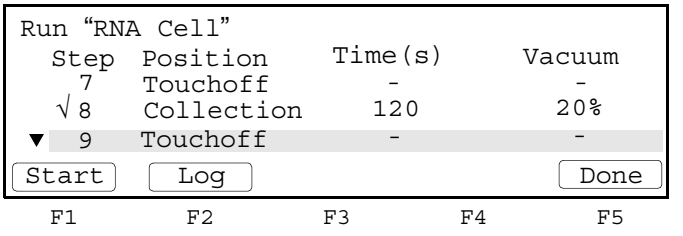
To run the rest of the RNA Cell method: *(continued)*

Step	Action
2	Perform the second wash. a. Pipette 400 µL of RNA purification wash solution 2 over each sample in the purification tray. **⚠ CAUTION CHEMICAL HAZARD. RNA purification wash solution 2** is a flammable liquid and vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Ensure that the highlighter is at step 3 of the RNA Cell method, then press **F1** (Start). 
3	Perform the third wash. a. Pipette 300 µL of RNA purification wash solution 2 over each sample in the purification tray. **⚠ CAUTION CHEMICAL HAZARD. RNA purification wash solution 2** is a flammable liquid nad vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Ensure that the highlighter is at step 4 of the RNA Cell method, then press **F1** (Start). 

To run the rest of the RNA Cell method: *(continued)*

Step	Action
4	Perform the fourth wash. a. Pipette 300 µL of RNA purification wash solution 2 over each sample in the purification tray. **⚠ CAUTION CHEMICAL HAZARD. RNA purification wash solution 2** is a flammable liquid and vapor. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Ensure that the highlighter is at step 5 of the RNA Cell method, then press **F1** (Start). 
5	Ensure that the highlighter is at step 6 of the RNA Cell method, then press **F1** (Start). 
6	Ensure that the highlighter is at step 7 of the RNA Cell method, then perform touchoff. 

To run the rest of the RNA Cell method: *(continued)*

Step	Action
7	Move the carriage to the collection position and push the handle down until the carriage locks.
8	Perform an elution. a. Pipette 150 µL of nucleic acid purification elution solution over each sample in the purification tray. b. Move the highlighter to step 8 of the RNA Cell method, then press **F1** (Start). 
9	Ensure that the highlighter is at step 9 of the RNA Cell method, then perform touchoff. 
10	Clear the instrument. a. Remove the purification tray, archive plate, and splash guard from the instrument. b. Save the archive plate for the rest of the qualification procedure. If necessary, store it at –15 to –25 °C until needed.
11	Continue with one of the following: ♦ "Analysis Using a UV Spectrophotometer" on page 3-17 ♦ "Analysis Using the 7700 SDS" on page 3-17

Analysis Using a UV Spectrophotometer

Introduction You can use a spectrophotometer to estimate the purity of the nucleic acid from the run on the 6100 prepstation. You take UV absorbance readings at wavelengths of 260 nm and 280 nm. If the ratio of the two readings (260/280) is > 1.8, the nucleic acid is considered pure.

Note It is important that the absorbance be measured within the quantitative range of the spectrophotometer you are using. Excessive dilution of the RNA sample will seriously affect the $A_{260/280}$ ratio measured by your spectrophotometer. Generally, the apparent $A_{260/280}$ ratio of your sample will be decreased by over-dilution.

Procedure To perform analysis using a spectrophotometer:

Step	Action
1	Pipette any HCLC sample (from the left side of the 96-well archive plate) from the run on the 6100 prepstation to a cuvette on a spectrophotometer.
2	Using the spectrophotometer, measure the absorbance at 260 nm and 280 nm.
3	Calculate the $A_{260/280}$ ratio. If the $A_{260/280}$ ratio is > 1.8, the 6100 prepstation passes the qualification test.

Analysis Using the 7700 SDS

Overview Analysis on the 7700 SDS consists of the following procedures, which must be done in order:

Procedure	See Page
Sample Preparation	3-18
Real-Time PCR Run	3-21
Amplification Plot Analysis	3-27
Threshold Cycle Analysis	3-33
Qualification Analysis	3-40

Sample Preparation

Overview

This section describes how to prepare samples from the 6100 prepstation run for analysis on the 7700 SDS. Preparation includes the following procedures:

Diluting RNA

To dilute RNA:

Step	Action
1	Assemble the materials you will need: ♦ 96-Well output plate (archive plate) from the run on the 6100 prepstation ♦ Two 96-well plates ♦ Nucleic Acid Purification Elution Solution
2	Perform a 1:20 dilution in a new 96-well plate. a. Pipette 190 µL of elution solution into each of the following wells: A1–A12 and B1–B12. b. Pipette 10 µL of purified RNA from each well of the output plate from the 6100 prepstation to each of the corresponding wells (A1 to A1, B1 to B1, etc.) to which you added elution solution (A1–A12 and B1–B12). Pipette up and down to mix thoroughly.
3	Perform another dilution in a second new 96-well plate. In this step you perform a 1:10 dilution of the samples prepared in step 2, giving an overall dilution factor of the 1:200. a. Pipette 180 µL of elution solution into each of the following wells: A1–A12 and B1–B12. b. Pipette 20 µL of the 1:20 dilution from step 2 above from each well of the 96-well plate into each of the corresponding wells (A1 to A1, B1 to B1, etc.) on this plate (A1–A12 and B1–B12). Pipette up and down to mix thoroughly.

Loading the Assay Plate

To load the assay plate:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Assemble the materials you will need:
♦ New 96-well plate
♦ Last 96-well plate from “Diluting RNA” on page 3-18
♦ 18S RT+ master mix from “Preparing 1.25X 18S RT+ Master Mix” on page 3-8
♦ 18S RT– master mix from “Preparing 1.25X 18S RT– Master Mix” on page 3-9
♦ Nucleic Acid Purification Elution Solution
♦ High Recovered RNA Standard (HRS) from the kit
♦ Low Recovered RNA Standard (LRS) from the kit</td></tr>
<tr><td>2</td><td>Load master mix.
a. Pipette 40 µL of 18S RT+ master mix into each of the following wells: A1–F10 (70 wells total).
b. Pipette 40 µL of 18S RT– master mix into each of the remaining wells: F11–H12 (26 wells total).</td></tr>
<tr><td>3</td><td>Load 10 µL of “sample” into each well, according to the plate map shown on the following page. Pipette up and down to mix thoroughly.
<table><tr><th>“Sample”</th><th>Description</th></tr><tr><td>HRS</td><td>High Recovered RNA Standard</td></tr><tr><td>LRS</td><td>Low Recovered RNA Standard</td></tr><tr><td>A1–B12</td><td>From designated wells of 96-well plate of dilution</td></tr><tr><td>NTC</td><td>Elution solution</td></tr></table></td></tr>
<tr><td>4</td><td>Cover the plate with an optical adhesive cover.</td></tr>
</table>

Assay Plate Map for 7700 SDS

	1	2	3	4	5	6	7	8	9	10	11	12
A	HRS	HRS	HRS	HRS	HRS	HRS	HRS	HRS	HRS	HRS	LRS	LRS
B	LRS	LRS	LRS	LRS	LRS	LRS	LRS	LRS	NTC	NTC	A1	A1
C	A2	A2	A3	A3	A4	A4	A5	A5	A6	A6	A7	A7
D	A8	A8	A9	A9	A10	A10	A11	A11	A12	A12	B1	B1
E	B2	B2	B3	B3	B4	B4	B5	B5	B6	B6	B7	B7
F	B8	B8	B9	B9	B10	B10	B11	B11	B12	B12	NTC	NTC
G	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
H	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12

Real-Time PCR Run

Purpose

In this section, you use the assay plate you prepared in "Loading the Assay Plate" on page 3-19 for a real-time run on the ABI PRISM 7700 Sequence Detection System (7700 SDS).

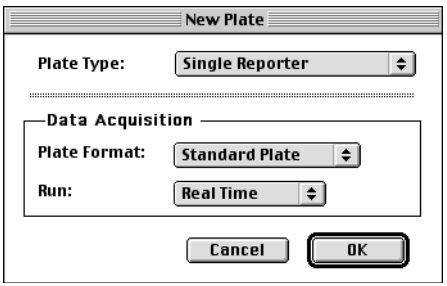
IMPORTANT Verify the performance of the 7700 SDS with the TaqMan® RNase P Instrument Verification Plate (P/N 4310982) before analyzing the 6100 prepstation output using the 7700 SDS.

A real-time run involves the following procedures:

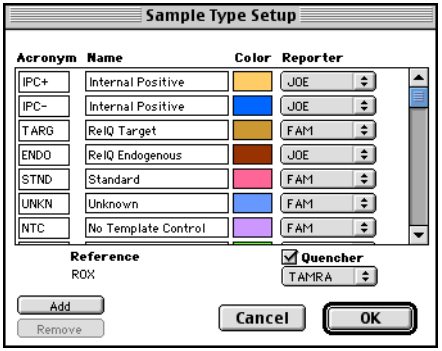
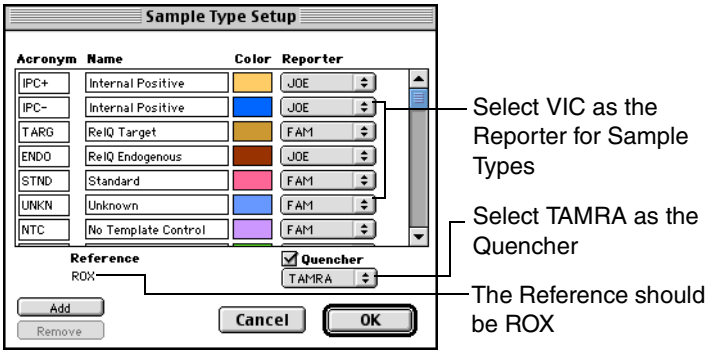
Procedure	See
Performing Sample Type Setup	3-21
Programming Thermal Cycler Conditions	3-24
Loading the Reaction Plate	3-25

Performing Sample Type Setup

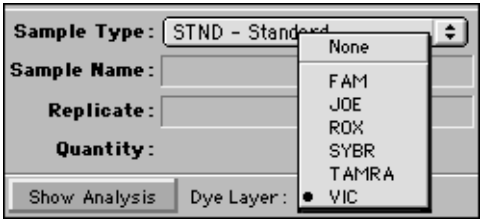
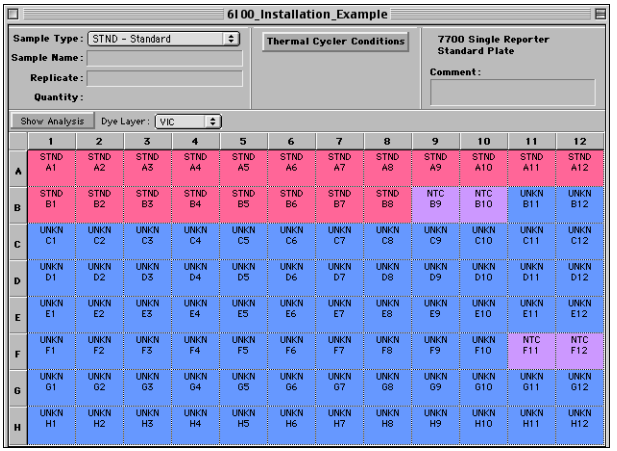
To perform sample type setup:

Step	Action
1	Launch SDS software on the computer connected to the 7700 SDS. Close the untitled window that appears.
2	From the **File** menu, select **New Plate...** A **New Plate** dialog box appears.
3	Complete the **New Plate** dialog box as shown below: 
4	Click **OK**. An untitled window appears in the setup view.

To perform sample type setup: *(continued)*

Step	Action
5	From the **Sample Type** pop-up menu, select **Sample Type Setup...** The **Sample Type Setup** dialog box appears. 
6	Complete the **Sample Type Setup** dialog box. a. Set the following sample types with the reporter dye **VIC**: ♦ NTC ♦ UNKN ♦ STND b. Verify that **ROX** is shown as the **Reference** dye. c. Verify that **TAMRA** is selected as the **Quencher** dye.  d. Click **OK**. The dialog box closes and the untitled window becomes active.

To perform sample type setup: *(continued)*

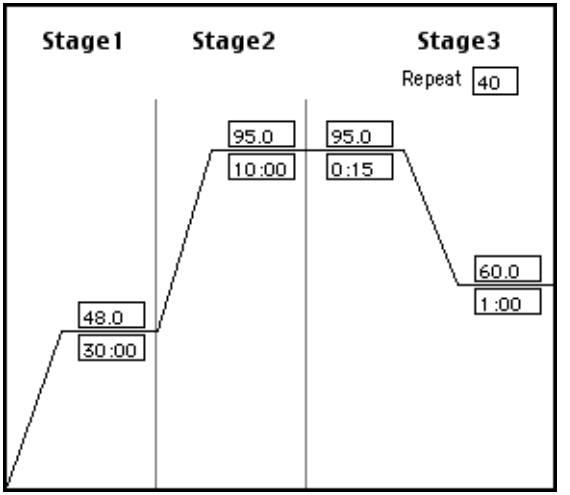
Step	Action
7	Select **VIC** from the **Dye Layer** pop-up menu. 
8	Label **STND-Standard** wells. a. Select wells A1–A12 and B1–B8. b. From the **Sample Type** pop-up menu, select **STND-Standard**.
9	Label **NTC-No Template Control** wells. a. Select wells B9, B10, F11, and F12. **Note** Hold down the Control key to select multiple wells. b. From the **Sample Type** pop-up menu, select **NTC-No Template Control**.
10	Label **UNKN-Unknown** wells. a. Select wells B11, B12, C1–C12, D1–D12, E1–E12, F1–F10, G1–G12, and H1–H12. b. From the **Sample Type** pop-up menu, select **UNKN-Unknown**. The samples are set up as shown in the figure below. 
11	Save the changes to the run file.

To perform sample type setup: *(continued)*

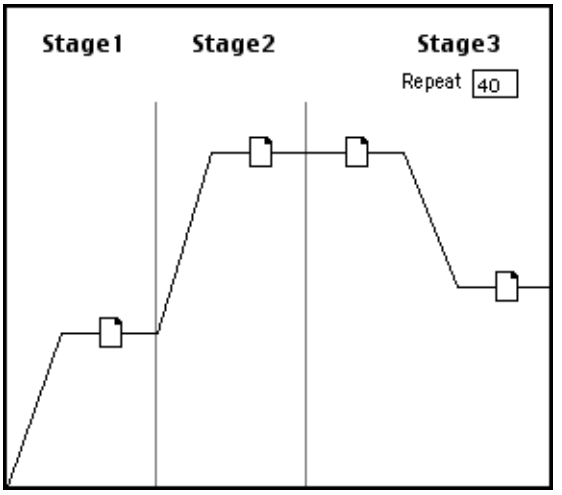
Step	Action
12	Go to “Programming Thermal Cycler Conditions” on page 3-24.

Programming Thermal Cycler Conditions

To program thermal cycler conditions:

Step	Action
1	Program thermal cycling settings. a. Click the **Thermal Cycler Conditions** button. A **Thermal Cycler Conditions** dialog box appears. b. Program the **Thermal Cycler Conditions** as shown below.  c. Set the **Reaction Volume** to **50 µL**.

To program thermal cycler conditions: *(continued)*

Step	Action
2	Program data collection settings. a. Click the **Show Data Collection** button in the dialog box. Document icons indicate when the instrument will collect data. b. Set the document icons as shown in the figure below.  **Note** Click a line segment to add or remove document icons.
3	Click **OK**. The dialog box closes, and the plate read window becomes active.

Loading the Reaction Plate

To load the reaction plate:

Step	Action
1	Place an optical cover compression pad on top of the assay plate you prepared in “Loading the Assay Plate” on page 3-19.
2	Load the assay plate into the 7700 SDS sample block. **IMPORTANT** Start the 7700 SDS run immediately to prevent degradation of the reaction components.

To load the reaction plate: *(continued)*

Step	Action
3	Secure the heated cover over the output plate. a. Pull the heated cover forward. b. Turn the knob clockwise to lower and secure the heated cover over the output plate **⚠ CAUTION** When securing the heated cover in place, be careful not to overtighten the knob. Damage to the instrument could occur.
4	Start the 7700 SDS run. a. Click the **Show Analysis** button. The analysis view appears. b. Make sure that the temperature of the heated cover (**Cov Temp**) is > 100 °C. c. Click the **Run** button. d. Wait for the instrument to beep and the shutters to click before leaving.

Amplification Plot Analysis

Purpose

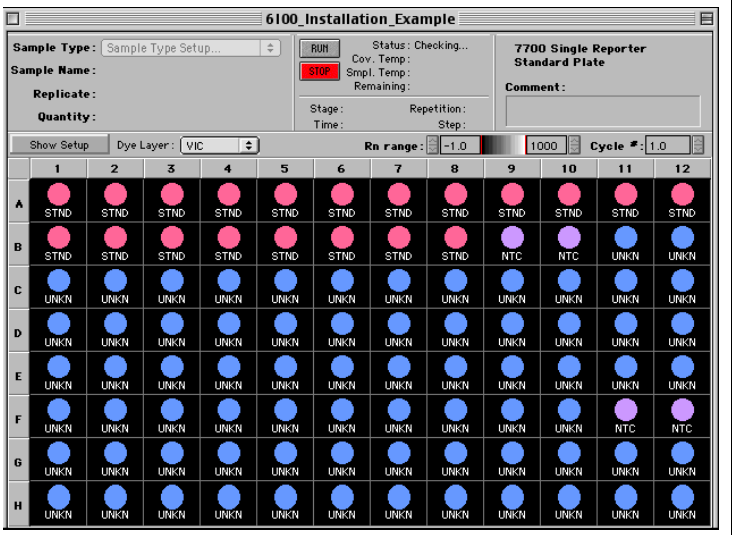
In this section, you use Sequence Detection Systems (SDS) software v. 1.6 or later to analyze the amplification plot and export the data.

This involves the following procedures:

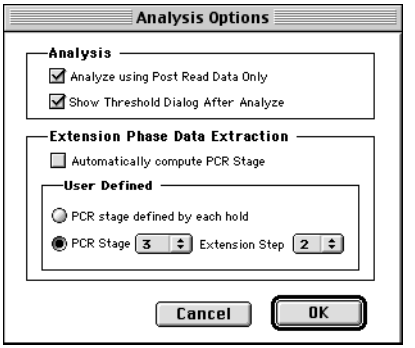
Procedure	See Page
Setting Analysis Options	3-27
Analyzing Threshold Cycles	3-29
Exporting Data	3-32

Setting Analysis Options

To set analysis options:

Step	Action
1	Save the real-time run file.
2	Click the **Show Analysis** button. The analysis view appears. 
3	From the **Analysis** menu, select **Options.** An **Analysis Options** dialog box appears.

To set analysis options: *(continued)*

Step	Action
4	Set the **Analysis Options** dialog box as shown below. 
5	Click **OK**.

Baseline and Threshold Guidelines

In analyzing threshold cycles (see the next page), you set the baseline and threshold values for analysis. Follow the guidelines below to ensure accurate analysis of the data.

Setting the Baseline

- Set the baseline to start at a cycle when background fluorescence is detected by the 7700 SDS.

  Baselines usually start between 2 and 3.

- Set the baseline to stop at a cycle before fluorescence signals from amplification are generated and detected.

  Baselines usually stop between 6 and 8.

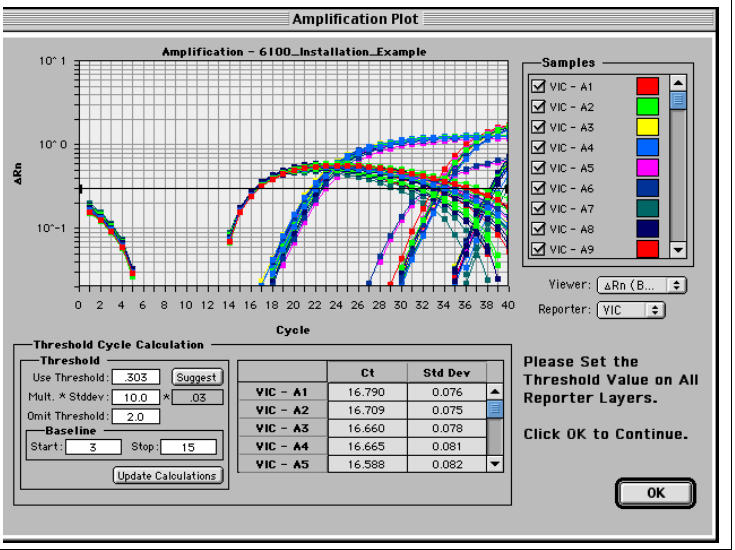
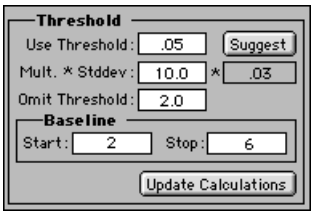
Setting the Threshold

- Set the threshold value to 0.05.

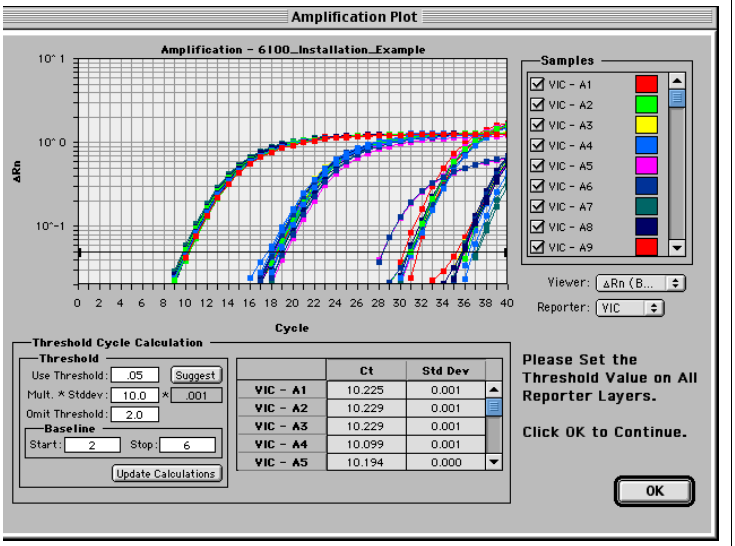
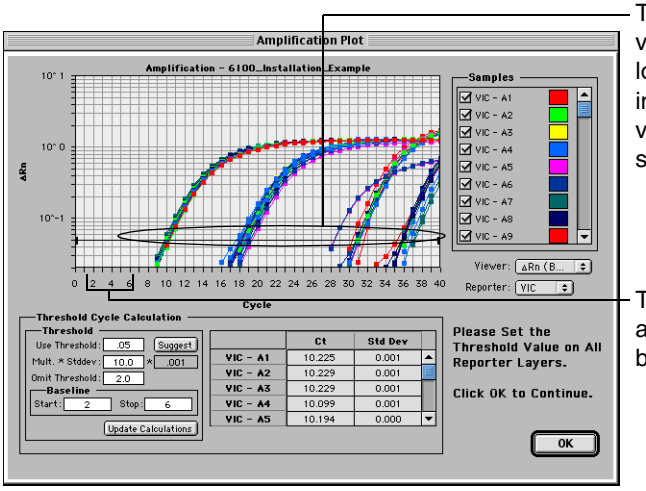
  At this value, fluorescence signals generated from RT+ samples should be increasing logarithmically. Adjustment of the threshold value from 0.05 may be necessary for optimal results.

Analyzing Threshold Cycles

To analyze threshold cycles:

Step	Action
1	Select all wells.
2	From the **Analysis** menu, select **Analyze**. The software analyzes the data, and an **Amplification Plot** window appears. 
3	Set the **Threshold Cycle Calculation** values. a. Set the **Threshold** value to **0.05**. b. Set the **Baseline** to **Start** at **2–3**. c. Set the **Baseline** to **Stop** at **6–8**. 

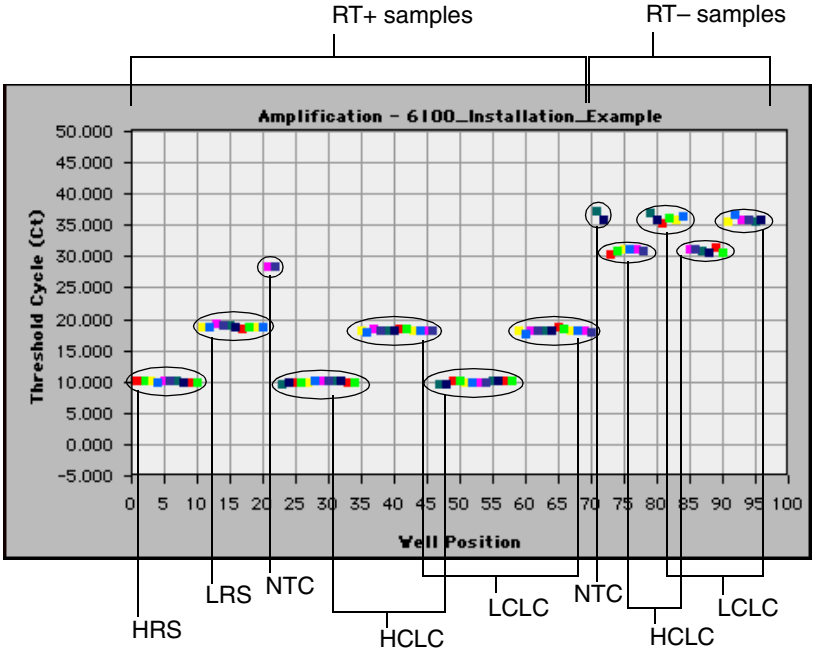
To analyze threshold cycles: *(continued)*

Step	Action
4	Click **Update Calculations**. The software updates the **Amplification Plot**. 
5	Verify that the baseline and threshold are set properly. a. The cycles set for the baseline should show no amplification. b. The threshold value should indicate logarithmic growth of ΔR_n values. 

The threshold value indicates a logarithmic increase in ΔR_n values for all samples

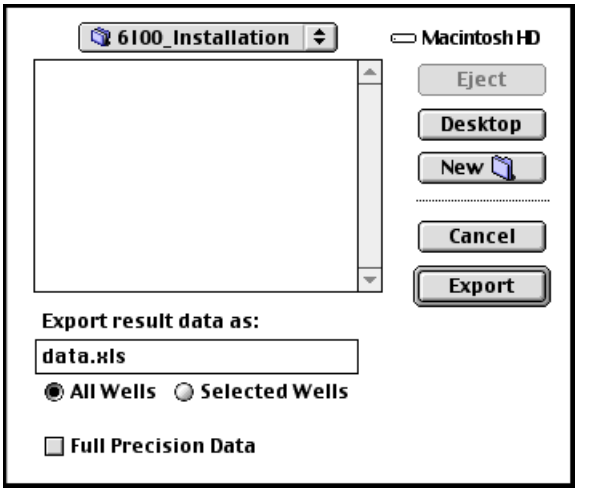
There is no amplification in baseline cycles

To analyze threshold cycles: *(continued)*

Step	Action
6	From the **Viewer** pop-up menu, select **Ct vs Well position**. The view of the amplification plot changes. 
7	Save the file.

Exporting Data

To export data:

Step	Action
1	From the **File** menu, scroll to **Export**, and select **Results.** A file dialog box appears. 
2	Enter the file name for the exported data, select a location for the data file, and click **Export**. The data are exported into a data file in the selected location.

Threshold Cycle Analysis

Purpose

In this section you manipulate the exported data using Microsoft® Excel to calculate C_T averages and standard deviations for the different sample groups. See “Example Qualification Data and Calculations” on page 3-44 for an example of a qualification analysis.

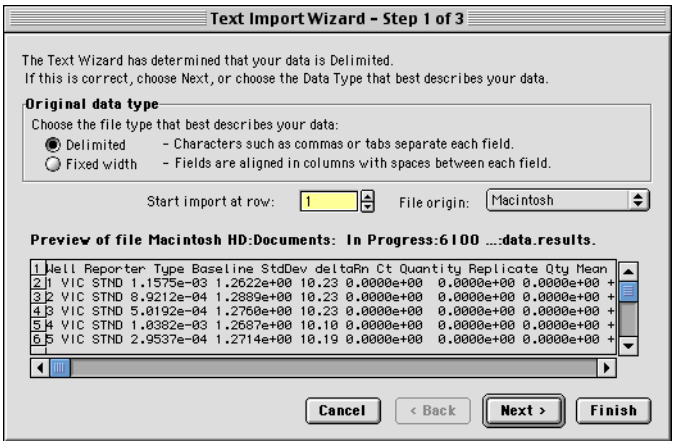
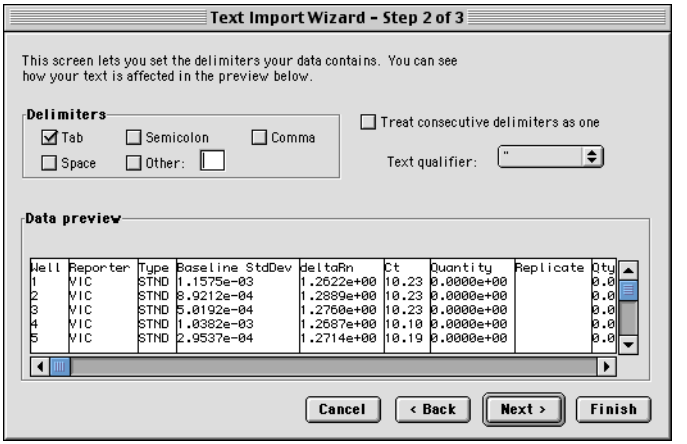
Threshold cycle analysis involves the following procedures:

Preparing the Data File

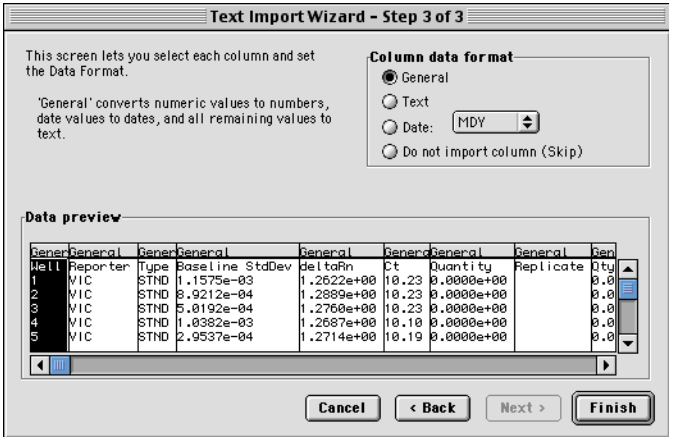
To prepare the data file:

Step	Action
1	Using Microsoft Excel, open the **data.xls** file you saved in “Exporting Data” on page 3-32. A **Text Import Wizard** dialog box appears.

To prepare the data file: *(continued)*

Step	Action
2	Complete Step 1 of the **Text Import Wizard** dialog box. a. Select **Delimited** as the **Original data type**. b. Start at row **1**. c. Select the appropriate **File Origin**. d. Click **Next**. 
3	Complete Step 2 of the **Text Import Wizard** dialog box. a. Check the **Tab** box. b. Click **Next**. 

To prepare the data file: *(continued)*

Step	Action
4	Complete Step 3 of the **Text Import Wizard** dialog box. a. Select **General** as the **Column data format**. b. Click **Finish**. The data file opens as a spreadsheet. 
5	Delete the following columns: ♦ Baseline StdDev ♦ deltaRn ♦ Quantity ♦ Replicate ♦ Qty Mean ♦ Qty StdDev ♦ IPC ♦ Sample Name
6	Insert five columns: ♦ Avg C_T (2x) ♦ Avg C_T (2x) sorted ♦ Avg C_T ♦ STDEV ♦ Specs

To prepare the data file: *(continued)*

Step	Action
7	Add cell borders to distinguish between the sample types. a. Select row **11**. b. Click the Borders button.  The software applies a border to the bottom of row **11**. c. Repeat steps 7a and 7b for the following rows. <table><tr><td>21</td><td>59</td><td>85</td></tr><tr><td>23</td><td>71</td><td>91</td></tr><tr><td>34</td><td>73</td><td>97</td></tr><tr><td>47</td><td>79</td><td></td></tr></table>

Calculating Average C_T Values and Standard Deviations

To calculate average C_T values:

Step	Action
1	Calculate the average C_T value for each set of duplicates (HCLC RT+ and LCLC RT+ samples) in the **Avg C_T (2x)** column. a. In cell E24, enter the formula **=AVERAGE(D24:D25)**, and press Return. The software calculates the average for samples 23 and 24, a set of duplicates. b. Select cells E24 and E25, and from the **Edit** menu, select **Copy**. c. Select cells E26 through E71, and from the **Edit** menu, select **Paste**. The software calculates the average for the remaining sets of duplicates.
2	Paste the average C_T values for HCLC RT+ samples into column F. a. Hold down the Command key, and select the average C_T values in column E for HCLC RT+ samples. b. From the **Edit** menu, select **Copy**. c. Place the cursor in cell F24. d. From the **Edit** menu, select **Paste**.

To calculate average C_T values: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Paste the average C_T values for LCLC RT+ samples into column F.
a. Hold down the Command key, and select the average C_T values in column E for LCLC RT+ samples.
b. From the Edit menu, select Copy.
c. Place the cursor in cell F36.
d. From the Edit menu, select Paste.</td></tr>
<tr><td>4</td><td>Sort the C_T values for the sets of duplicates for the HCLC RT+ and for the LCLC RT+ samples in the Avg C_T (2x) sorted column.
a. Select samples F24:F35, and from the Data menu select Sort.
b. Select samples F36:F47, and from the Data menu select Sort.</td></tr>
<tr><td>5</td><td>Calculate the average C_T value for each sample group in the Avg C_T column.

<table>
<tr><th>In cell…</th><th>Enter the formula…</th><th>Sample Group</th></tr>
<tr><td>G2</td><td>=AVERAGE(D2:D11)</td><td>HRS RT+</td></tr>
<tr><td>G12</td><td>=AVERAGE(D12:D21)</td><td>LRS RT+</td></tr>
<tr><td>G22</td><td>=AVERAGE(D22:D23)</td><td>NTC RT+</td></tr>
<tr><td>G24</td><td>=AVERAGE(D24:D35, D48:D59)</td><td>HCLC RT+</td></tr>
<tr><td>G36</td><td>=AVERAGE(D36:D47, D60:D71)</td><td>LCLC RT+</td></tr>
<tr><td>G72</td><td>=AVERAGE(D72:D73)</td><td>NTC RT–</td></tr>
<tr><td>G74</td><td>=AVERAGE(D74:D79, D86:D91)</td><td>HCLC RT–</td></tr>
<tr><td>G80</td><td>=AVERAGE(D80:D85, D92:D97)</td><td>LCLC RT–</td></tr>
</table>
</td></tr>
</table>

To calculate average C_T values: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>Calculate the standard deviation for C_T values for each sample group in the STDEV column (column I).
<table>
<tr><th>In cell…</th><th>Enter the formula…</th><th>Sample Group</th></tr>
<tr><td>H2</td><td>=STDEV(D2:D11)</td><td>HRS RT+</td></tr>
<tr><td>H12</td><td>=STDEV(D12:D21)</td><td>LRS RT+</td></tr>
<tr><td>H22</td><td>=STDEV(D22:D23)</td><td>NTC RT+</td></tr>
<tr><td>H24</td><td>=STDEV(F24:F29)</td><td>HCLC RT+ below avg</td></tr>
<tr><td>H25</td><td>=STDEV(F30:F35)</td><td>HCLC RT+ above avg</td></tr>
<tr><td>H36</td><td>=STDEV(F36:F41)</td><td>LCLC RT+ below avg</td></tr>
<tr><td>H37</td><td>=STDEV(F42:F47)</td><td>LCLC RT+ above avg</td></tr>
<tr><td>H72</td><td>=STDEV(D72:D73)</td><td>NTC RT–</td></tr>
<tr><td>H74</td><td>=STDEV(D74:D79, D86:D91)</td><td>HCLC RT–</td></tr>
<tr><td>H80</td><td>=STDEV(D80:D85, D92:D97)</td><td>LCLC RT–</td></tr>
</table></td></tr>
<tr><td>7</td><td>Eliminate samples following the guidelines in the following sections, “Eliminating Outliers From Analysis” and “Eliminating Dropouts From Analysis,” before proceeding with the specification calculations.</td></tr>
</table>

Eliminating Outliers From Analysis

An outlier exhibits a C_T value that differs by greater than three standard deviations from the average C_T value for the sample group.

Eliminate from analysis up to four outliers from each output plate:

- Eliminate an RT+ sample from analysis if the C_T value of the RT+ sample differs by greater than three standard deviations from the average C_T value for the sample group and the C_T value of the sample's replicate is within three standard deviations of the average C_T value for the sample group.

Note If the C_T values of a sample and its replicate both differ by greater than three standard deviations of the average C_T value for the sample group, then the sample set meets the definition of a dropout (See the next section, "Eliminating Dropouts From Analysis.").

- Eliminate an RT– sample from analysis if the C_T value of the RT– sample differs by greater than three standard deviations from the average C_T value for the sample group.
- Eliminate a standard from analysis if the C_T value of the standard differs by greater than three standard deviations from the average C_T value for the sample group

Eliminating Dropouts From Analysis

A dropout consists of a set of duplicate samples that both exhibit C_T values that differ by greater than three standard deviations from the average C_T value for the sample group.

Eliminate up to two dropouts from each output plate from analysis.

Qualification Analysis

Purpose

In this section you use the C_T values to measure the quality of the RNA prepared by the 6100 prepstation using three performance parameters.

Performance	Specification
% RNA Recovery	The 6100 prepstation can recover ≥50% of the RNA content of the cell lysate control.
Precision of RNA Recovery	The 6100 prepstation can recover RNA with a precision of <30% coefficient of variation (CV).
% DNA Contamination	The 6100 prepstation can recover RNA containing <0.5% of DNA by weight.

Calculating % RNA Recovery

For the RNA recovery to meet or exceed 50% of the RNA content of the lysate, the following equations must be true.

C_T(HCLC RT+) – C_T(HRS RT+) < 1.0

C_T(LCLC RT+) – C_T(LRS RT+) < 1.0

To calculate % RNA recovery:

Step	Action
1	Calculate ΔC_T values for HCLC RT+ samples compared to standard HRS RT+ samples. a. In cell I2, enter the formula **=G24 – G2**. b. Verify that the difference is less than 1.0.
2	Calculate ΔC_T values for LCLC RT+ samples compared to standard LRS RT+ samples. a. In cell I12, enter the formula **=G36 – G12**. b. Verify that the difference is less than 1.0.

Calculating Precision of RNA Recovery

For the precision of RNA recovery to be less than 30% CV, the following equations must be true for the HCLC RT+ population and for the LCLC RT+ population.

For the standard deviation of C_T values below the average C_T:

$[10^{(\text{Std. Dev. } C_T/3.33)} - 1] \times 100 < 30\%$

For the standard deviation of C_T values above the average C_T:

$[10^{(-\text{Std. Dev. } C_T/3.33)} - 1] \times 100 < 30\%$

To calculate precision of RNA recovery:

Step	Action
1	Calculate the % CV for the standard deviation of HCLC RT+ C_T values below the average. a. In cell I24, enter the formula **=(POWER(10, A/ 3.33)–1)*100** Where A is the value from cell H24, the standard deviation for HCLC RT+ C_T values below the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.
2	Calculate the % CV for the standard deviation of HCLC RT+ C_T values above the average. a. In cell K25, enter the formula **=(POWER(10, – B/ 3.33)–1)*100** Where B is the value from cell H25, the standard deviation for HCLC RT+ C_T values above the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.

To calculate precision of RNA recovery: *(continued)*

Step	Action
3	Calculate the % CV for the standard deviation of LCLC RT+ C_T values below the average. a. In cell I36, enter the formula **=(POWER(10, C/ 3.33)–1)*100** Where C is the value from cell H36, the standard deviation for LCLC RT+ C_T values below the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.
4	Calculate the % CV for the standard deviation of LCLC RT+ C_T values above the average. a. In cell K37 for LCLC RT+ samples, enter the formula **=(POWER(10, – D/ 3.33)–1)*100** Where D is the value from cell H37, the standard deviation for LCLC RT+ C_T values above the average b. Press Return to calculate. The % CV should be less than 30% for the installation to pass performance verification.

Calculating % DNA Contamination

For DNA contamination in the RNA samples to be less than 0.5% by weight, the following equation must be true:

C_T(HCLC RT–) – C_T(HCLC RT+) ≥ 16

To calculate % DNA contamination:

Step	Action
1	In cell I74, enter the formula **=G74–G24**.
2	Press Return to calculate.
3	Verify that the value is greater than or equal to 16.

6100 Qualification Requirement

Qualification of the 6100 prepstation using the Cell Lysate Control kit requires that the purified RNA meets Applied Biosystems specifications for % RNA recovery, precision of RNA recovery, and % DNA contamination.

If one or more of the specifications is not met, contact technical support (see “Technical Support” on page A-1) for assistance with using the kit or analyzing the data.

Example Qualification Data and Calculations

Purpose

This appendix contains data and calculations from an example 6100 prepstation qualification run.

Example % RNA Recovery

The figure below shows example data and calculations for determining % RNA recovery.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
2	1	VIC	STND	10.53			10.44	0.12409674	-0.0491667
3	2	VIC	STND	10.6					
4	3	VIC	STND	10.58					
5	4	VIC	STND	10.43					
6	5	VIC	STND	10.49					
7	6	VIC	STND	10.48					
8	7	VIC	STND	10.44					
9	8	VIC	STND	10.35					
10	9	VIC	STND	10.23					
11	10	VIC	STND	10.27					
12	11	VIC	STND	19			18.866	0.28975852	-0.5780833
13	12	VIC	STND	18.76					
14	13	VIC	STND	19.43					
15	14	VIC	STND	19.02					
16	15	VIC	STND	19.16					
17	16	VIC	STND	18.74					
18	17	VIC	STND	18.47					
19	18	VIC	STND	18.79					
20	19	VIC	STND	18.75					
21	20	VIC	STND	18.54					
22	21	VIC	NTC	28.36			28.29	0.09899495	
23	22	VIC	NTC	28.22					

ΔC_T values for HCLC RT+ samples compared to HRS RT+ samples is less than 1.0

ΔC_T values for LCLC RT+ samples compared to LRS RT+ samples is less than 1.0

Example Precision of RNA Recovery

The next figure shows example data and calculations for determining precision of RNA recovery.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
24	23	VIC	UNKN	10.12	10.19	10.18	10.3908	0.08489209	6.0457139
25	24	VIC	UNKN	10.26		10.19		0.08769265	-5.8834778
26	25	VIC	UNKN	10.37	10.375	10.315			
27	26	VIC	UNKN	10.38		10.34			
28	27	VIC	UNKN	10.4	10.4	10.35			
29	28	VIC	UNKN	10.4		10.375			
30	29	VIC	UNKN	10.49	10.43	10.4			
31	30	VIC	UNKN	10.37		10.43			
32	31	VIC	UNKN	10.49	10.43	10.43			
33	32	VIC	UNKN	10.37		10.495			
34	33	VIC	UNKN	10.32	10.315	10.56			
35	34	VIC	UNKN	10.31		10.625			
36	35	VIC	UNKN	18.16	18.14	18.085	18.2879	0.06658954	4.71209745
37	36	VIC	UNKN	18.12		18.085		0.12320714	-8.1665587
38	37	VIC	UNKN	18.46	18.31	18.14			
39	38	VIC	UNKN	18.16		18.205			
40	39	VIC	UNKN	18.26	18.23	18.22			
41	40	VIC	UNKN	18.2		18.23			
42	41	VIC	UNKN	18.55	18.505	18.31			
43	42	VIC	UNKN	18.46		18.31			
44	43	VIC	UNKN	18.4	18.365	18.365			
45	44	VIC	UNKN	18.33		18.38			
46	45	VIC	UNKN	18.21	18.085	18.505			
47	46	VIC	UNKN	17.96		18.62			
48	47	VIC	UNKN	10.17	10.18				
49	48	VIC	UNKN	10.19					
50	49	VIC	UNKN	10.51	10.495				
51	50	VIC	UNKN	10.48					
52	51	VIC	UNKN	10.31	10.34				
53	52	VIC	UNKN	10.37					
54	53	VIC	UNKN	10.3	10.35				
55	54	VIC	UNKN	10.4					
56	55	VIC	UNKN	10.58	10.56				
57	56	VIC	UNKN	10.54					
58	57	VIC	UNKN	10.59	10.625				
59	58	VIC	UNKN	10.66					
60	59	VIC	UNKN	18.09	18.085				
61	60	VIC	UNKN	18.08					
62	61	VIC	UNKN	18.39	18.38				
63	62	VIC	UNKN	18.37					
64	63	VIC	UNKN	18.28	18.31				
65	64	VIC	UNKN	18.34					
66	65	VIC	UNKN	18.7	18.62				
67	66	VIC	UNKN	18.54					
68	67	VIC	UNKN	18.2	18.22				
69	68	VIC	UNKN	18.24					
70	69	VIC	UNKN	18.22	18.205				
71	70	VIC	UNKN	18.19					

Example % DNA Contamination

The figure below shows example data and calculations for determining % DNA contamination.

	A	B	C	D	E	F	G	H	I
1	Well	Reporter	Type	Ct	Avg Ct (2x)	Avg Ct (2x) sorted	Avg Ct	STDEV	Specs
72	71	VIC	NTC	36.91			36.53	0.53740115	
73	72	VIC	NTC	36.15					
74	73	VIC	UNKN	30.11			30.895	0.42530203	20.5041667
75	74	VIC	UNKN	30.5					
76	75	VIC	UNKN	31.01					
77	76	VIC	UNKN	31.71					
78	77	VIC	UNKN	31.17					
79	78	VIC	UNKN	31.01					
80	79	VIC	UNKN	36.7			35.8867	0.41220766	
81	80	VIC	UNKN	35.66					
82	81	VIC	UNKN	35.62					
83	82	VIC	UNKN	35.82					
84	83	VIC	UNKN	35.68					
85	84	VIC	UNKN	36.19					
86	85	VIC	UNKN	30.69					
87	86	VIC	UNKN	31.17					
88	87	VIC	UNKN	30.6					
89	88	VIC	UNKN	30.52					
90	89	VIC	UNKN	31.13					
91	90	VIC	UNKN	31.12					
92	91	VIC	UNKN	35.59					
93	92	VIC	UNKN	36.59					
94	93	VIC	UNKN	36.1					
95	94	VIC	UNKN	35.61					
96	95	VIC	UNKN	35.56					
97	96	VIC	UNKN	35.52					

Table of Formulas

The table below describes the formula or contents for each cell.

Cell	Formula/Contents
E24	=AVERAGE(D24:D25)
E26:E71	pasted from E24:E25
F24:F35	pasted from E24:E34, E48:E58 and sorted in ascending order
F36:F47	pasted from E36:E46, E60:E70 and sorted in ascending order
G2	=AVERAGE(D2:D11)
G12	=AVERAGE(D12:D21)
G22	=AVERAGE(D22:D23)

Cell	Formula/Contents
G24	=AVERAGE(D24:D35, D48:D59)
G36	=AVERAGE(D36:D47, D60:D71)
G72	=AVERAGE(D72:D73)
G74	=AVERAGE(D74:D79, D86:D91)
G80	=AVERAGE(D80:D85, D92:D97)
H2	=STDEV(D2:D11)
H12	=STDEV(D12:D21)
H22	=STDEV(D22:D23)
H24	=STDEV(F24:F29)
H25	=STDEV(F30:F35)
H36	=STDEV(F36:F41)
H37	=STDEV(F42:F47)
H72	=STDEV(D72:D73)
H74	=STDEV(D74:D79, D86:D91)
H80	=STDEV(D80:D85, D92:D97)
I2	=G24–G2
I12	=G36–G12
I24	=(POWER(10,A/3.33)–1)*100 **Note** A is the value from cell H24.
I25	=(POWER(10,–B/3.33)–1)*100 **Note** B is the value from cell H25.
I36	=(POWER(10,C/3.33)–1)*100 **Note** C is the value from cell H36.
I37	=(POWER(10,–D/3.33)–1)*100 **Note** D is the value from cell H37.
I74	=G74–G24

Technical Support

A

Contacting Technical Support

You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems web site. (Please see the section "To Obtain Technical Documents" following the telephone information below.)

To Contact Technical Support by E-Mail

You can contact Applied Biosystems Technical Support by e-mail for help in the following product areas:

Product/Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems (Real-Time PCR) and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com

Product/Product Area	E-mail address
♦ Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products) ♦ Expedite™ 8900 Nucleic Acid Synthesis Systems ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ PNA Custom and Synthesis ♦ Pioneer™ Peptide Synthesizers ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent ♦ FMAT™ 8100 HTS Systems ♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

To Contact Technical Support by Telephone or Fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

Note To schedule a service call for other support needs, or in case of an emergency, dial **1.800.831.6844**, then press **1**.

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1**.**800**.**831**.**6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1**.**800**.**831**.**6844**, press **2**, then press **1**[a]	1.650.638.5981

Product/Product Area	Telephone	Fax
Fluorescent DNA Sequencing	**1.800.831.6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1.800.831.6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1.800.831.6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1.800.831.6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43x Systems)	**1.800.831.6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1.800.831.6844**, press **3**, then press **2**[a]	1.650.638.5981
Sequence Detection Systems (Real-Time PCR) and PCR	**1.800.762.4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI Prism, 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1.800.831.6844**, then press **5**[a]	1.240.453.4613

Product/Product Area	Telephone	Fax
♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent	**1.800.899.5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1.800.899.5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ 8900 Nucleic Acid Synthesis Systems	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
Pioneer™ Peptide Synthesizers	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
♦ FMAT™ 8100 HTS Systems ♦ CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 AM to 5:00 PM Pacific time.

b. 8:00 AM to 6:00 PM Eastern time.

c. 9:00 AM to 5:00 PM Eastern time.

To Contact Technical Support by Telephone or Fax (Outside North America)

To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608** or **86 800 8100497**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
India (New Delhi)	**91 11 653 3743/3744**	**91 11 653 3138**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 79588268**	**60 3 79549043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 532 4484**	**32 (0)2 582 1886**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0)6150 101 0**	**49 (0)6150 101 101**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Portugal (Lisboa)	**351.(0)22.605.33.14**	**351.(0)22.605.33.15**
Spain (Tres Cantos)	**34.(0)91.806.1210**	**34.(0)91.806.12.06**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 392400**	**31 (0)180 392409** or **31 (0)180 392499**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**

Region	Telephone	Fax
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	**27 11 478 0411**	**27 11 478 0349**
Africa, French speaking (Paris, France)	**33 1 69 59 85 11**	**33 1 69 59 85 00**
India (New Delhi)	**91 11 653 3743** **91 11 653 3744**	**91 11 653 3138**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 22 866 40 10**	**48 22 866 40 20**
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia)	**44 1925 282481**	**44 1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Caribbean countries, Mexico, and Central America	**52 55 35 3610**	**52 55 66 2308**
Brazil	**0 800 704 9004** or **55 11 5070 9654**	**55 11 5070 9694/95**
Argentina	**800 666 0096**	**55 11 5070 9694/95**
Chile	**1230 020 9102**	**55 11 5070 9694/95**
Uruguay	**0004 055 654**	**55 11 5070 9694/95**

To Reach Technical Support Through the Applied Biosystems Web Site

At the Applied Biosystems web site, you can search through frequently asked questions (FAQs) or a solution database, or you can submit a question directly to Technical Support.

Search FAQs

To search for FAQs:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Click you geographic region for the product area of interest.
4	Follow the instructions under the **Frequently Asked Questions** section (1) to display a list of FAQs for your area of interest.

Search the Solution Database

To search for solutions to problems using the Solution Database:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Follow the instructions under the **Search the Solution Database** section (2) to find a solution to your problem.

Submit a Question

To submit a question directly to Technical Support:

1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	In the **Personal Assistance – E-Mail Support** section (3), click **Ask Us RIGHT NOW**.
4	In the displayed form, enter the requested information and your question, then click **Ask Us RIGHT NOW**. Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

To Obtain Technical Documents

You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering Documents by Telephone

To order documents by telephone:

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

Obtaining Documents Through the Web Site

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

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Documents on Demand**.
3	In the search form, enter and select search criteria, then click **Search** at the bottom of the page.
4	In the results screen, do any of the following: ♦ Click the pdf icon to view a PDF version of the document. ♦ Right-click the pdf icon, then select **Save Target As** to download a copy of the PDF file. ♦ Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you. ♦ Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you. **Note** There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.

To Obtain Customer Training Information

To obtain Applied Biosystems training information:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Training**.

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

Worldwide Sales Offices
Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For international office locations, please call our local office or refer to our web site at www.appliedbiosystems.com or the Technical Support appendix in this document.

www.appliedbiosystems.com

Applied Biosystems

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Printed in the USA, 07/2001
Part Number 4316607 Rev. C

an Applera business