ABI PRISM[™] 6700 Automated Nucleic Acid Workstation

User Guide



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1

Introduction

Overview

About This Chapter This chapter describes the organization of this User Guide. It also include information and a limited warranty statement for the ABI PRISM [™] 6700 Au Nucleic Acid Workstation.		Guide. It also includes safety ABI PRISM [™] 6700 Automated	
In This Chapter	This chapter contains the following topics:		
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6700 Workstation Manuals

6700	Wo	r	ks	ta	tio]
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n The manuals for the 6700 workstation are described below.

Manual Set

Title	Part Number	Description
ABI PRISM 6700 Automated Nucleic Acid Workstation Site Preparation and Safety Guide	4304419	This manual contains installation requirements, an installation checklist, and information about instrument and chemical safety.
ABI PRISM 6700 Automated Nucleic Acid Workstation User Guide	4304309	This manual contains a detailed description of instrument operation.
Database Administration Guide for the ABI PRISM 6700 Automated Nucleic Acid Workstation	4314342	This manual contains information about maintaining the 6700 database and managing database users. Note This manual is intended for the Database Administrator.

Purpose of This This User Guide describes the ABI PRISM 6700 Automated Nucleic Acid Workstation User Guide and contains procedures for operating, maintaining, and testing this instrument.

User Guide

Contents of This The table below describes the contents of this User Guide.

Chapter/Appendix	Topics
Chapter 2 System Description	This chapter describes the components of the 6700 workstation: the instrument, the computer hardware and accessories, and the software.
Chapter 3 Instrument Operation	This chapter describes how to log in to the 6700 workstation software, set up a run, set up the deckspace, and start the run.
Chapter 4 Protocol Creation	This chapter describes the 6700 workstation protocols and contains procedures for defining protocols.
Chapter 5 Maintenance	This chapter describes the maintenance schedules and procedures for the 6700 workstation.
Chapter 6 Function Tests and Instrument Calibration	This chapter describes function tests and instrument calibration and contains procedures for performing them.
Appendix A Instrument Decontamination	This appendix provides information for decontaminating the 6700 instrument.
Appendix B 6700 Workstation Materials	This appendix contains descriptions and part numbers of consumables and reagents designed for use on the 6700 workstation.
Appendix C Troubleshooting	This appendix contains troubleshooting information and a list of common error messages.
Appendix D References	This appendix contains a bibliography of references cited in this manual.
Appendix E Technical Support	This appendix provides information for contacting Applied Biosystems via telephone, fax, or Internet.

Limited Warranty Statement

Applera Corporation Limited Warranty Statement

Applera Corporation warrants to the customer that, for a period ending on the earlier of 1 year from the completion of installation or 15 months from the date of shipment to the customer (the "Warranty Period"), the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation purchased by the customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the functional test specifications set forth in the Installation Worksheet (the "Specifications").

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applera Corporation will repair or replace the Instrument so that it meets the Specifications, at Applera Corporation's expense. However, if the instrument becomes damaged or contaminated, or if the chemical performance of the Instrument otherwise deteriorates due to solvents and/or reagents other than those supplied or expressly recommended by Applera Corporation, Applera Corporation will return the Instrument to Specification at the customer's expense. After this service is performed, coverage of the parts repaired or replaced will be restored thereafter for the remainder of the original Warranty Period.

This Warranty does not extend to any Instrument or part which has been (a) the subject of an accident, misuse, or neglect, (b) modified or repaired by a party other than Applera Corporation, or (c) used in a manner not in accordance with the instructions contained in the Instrument User Guide. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User Guide. Those items are covered by their own warranties.

Applera Corporation's obligation under this Warranty is limited to repairs or replacements that Applera Corporation deems necessary to correct those failures of the Instrument to meet the Specifications of which Applera Corporation is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by Applera Corporation on site at the customer's location at Applera Corporation's sole expense.

No agent, employee, or representative of Applera Corporation has any authority to bind Applera Corporation to any affirmation, representation, or warranty concerning the Instrument that is not contained in Applera Corporation's printed product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of Applera Corporation will not be binding on Applera Corporation.

Applera Corporation shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the purchase or use of the Instrument. Applera Corporation makes no warranty whatsoever with regard to products or parts furnished by third parties.

This Warranty is limited to the original location and electrical power connection, unless the customer with written consent of Applera Corporation arranges for relocation of the instrument. This warranty is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS IN LIEU OF ANY OTHER EXPRESSED OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY

OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND IS IN LIEU OF ANY OTHER OBLIGATION ON THE PART OF Applera Corporation.

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.
	A WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.
	ADANCER 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 (<i>e.g.</i> , 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 (<i>e.g.</i> , 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.
	<u></u>

Chemical Waste Hazard 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 (<i>e.g.</i> , 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 (<i>e.g.</i> , fume hood). For additional safety guidelines, consult the MSDS.
	 After emptying the waste container, seal it with the cap provided.
	 Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.
Site Preparation and Safety Guide	A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.
About MSDSs	Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.
	Chemical manufacturers supply a current MSDS before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.
	We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.
	A 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 MSDSs	Then		
Over the Internet	 a. Go to our Web site a www.appliedbiosyste b. Click MSDSs 	t ems.com/techsupp	
	If you have	Then	
	The MSDS document number or the Docum on Demand index num	ent numbers in the appropriate field on this page.	
	The product part num	ber Select Click Here, then	
	Keyword(s)	enter the part number or keyword(s) in the field on this page.	
	c. You can open and download a PDF (using Adobe [®] Acrobat [®] Reader [™]) of the document by selecting it, or you can choose to have the document sent to you by fa or email.		
By automated telephone service	Use "To Obtain Documents on Demand" under "Technical Support."		
By telephone in the United States	Dial 1-800-327-3002 , then press 1 .		
By telephone from Canada	To order in	Dial 1-800-668-6913 and	
	English	Press 1, then 2, then 1 again	
	French	Press 2, then 2, then 1	
By telephone from any other country	See the specific region under "To Contact Technical Support by Telephone or Fax" under "Technical Support."		

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

Instrument Safety

Labels

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

- A signal word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
 - A message panel, which explains the hazard and any user action required.
 - A safety alert symbol, which indicates a potential personal safety hazard. See the *ABI PRISM 6700 Automated Nucleic Acid Workstation Site Preparation and Safety Guide* for an explanation of all the safety alert symbols provided in several languages.

About Waste Profiles	A waste profile was provided with this instrument and is contained in the <i>ABI PRISM</i> 6700 Automated Nucleic Acid Workstation Site Preparation and Safety Guide. Waste profiles list the percentage compositions of the reagents within the waste stream at installation and the waste stream during a typical user application, although this application may not be used in your laboratory. These profiles assist users in planning for instrument waste handling and disposal. Read the waste profiles and all applicable MSDSs before handling or disposing of waste.			
	IMPORTANT Waste profiles are not a substitute for MSDS information.			
About Waste Disposal	As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below.			
	 Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory. 			
	 Ensure the health and safety of all personnel in your laboratory. 			
	• Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, or national regulations.			
	Note Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.			
Before Operating the	Ensure that everyone involved with the operation of the instrument has:			
Instrument	 Beceived instruction in general safety practices for laboratories 			
	 Received instruction in specific safety practices for the instrument 			
	 Read and understood all related MSDSs 			
	ACAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.			
Safe and Efficient Computer Use	Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.			
	To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below.			
	ACAUTION MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD . These hazards are caused by the following potential risk factors which include, but are not limited to, repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.			
	 Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures. 			
	 The bulk of the person's weight should be supported by the buttocks, not the thighs. 			
	 Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs. 			

- Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
 - The proper height to position the forearms horizontally and upper arms vertically.
 - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
 - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
 - Positioning the screen to minimize reflections from ambient light sources.
 - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.
- When considering the user's distance from the screen, the following are useful guidelines:
 - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
 - For most people, the reading distance that is the most comfortable is approximately 20 inches.
 - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
 - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

2

System Description

Overview

About This Chapter	This chapter describes the features and functions of the ABI PRISM [™] 6700 Automated Nucleic Acid Workstation components.		
In This Chapter	This chapter contains the following topics:		
	Торіс	See Page	
	System Overview	2-2	
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	Computer Hardware and Accessories	2-6	
	About the Software	2-7	

System Overview

System Components The 6700 Automated Nucleic Acid Workstation is an automated, high-throughput system that consists of the following components.

- ♦ 6700 instrument
- Client computer for the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation software
- Server computer for the 6700 database



System Functions The 6700 workstation can perform the following nucleic acid preparation protocols:

Protocol	Function	
Lysis	Lyse cells with Applied Biosystems reagents	
DNA Precipitation	Precipitate DNA with Applied Biosystems reagents	
RNA Archive	Purify RNA from lysed cells	
DNA Archive	Purify DNA from precipitated DNA	
cDNA Archive	Synthesize cDNA from RNA	
Dilution Archive	Dilute nucleic acid samples	
Assay	Prepare plates for nucleic acid assays	

6700 Instrument Components

Instrument Diagram The picture below shows the 6700 instrument and its components.



Robotic Arm Functions

The robotic arm moves to different deckspace locations to transfer samples, standards, controls, reagents, and archive covers as specified in the protocols.

The table below describes the robotic arm components and functions.

Component	Function
Robotic arm tip	 Aspirate, deliver, and dispense reagents with disposable tips
assemblies (4)	 Pick up and transfer archive covers from the Archive Cover station to the archive plates
Diluters (4)	Control the volume and rate of liquid aspiration and dispensing

Deckspace Stations The deckspace is a 1.17 x 0.43-m (46 x 17-in.) plate divided into stations for holding samples, reagents, plastic consumables, and waste for automated protocols.

Station	Description	
Archive Cover station	Holds up to three archive covers	
Disposable Tips station	Holds:	
	 Four rear-position racks for 1000-mL or 200-mL tips 	
	 Four front-position racks for 200-mL tips 	
Dilutions/cDNA station	 Holds up to two archive plates 	
	♦ Can be heated to 50 °C	
	 Can be cooled by Peltier units to 4 °C 	
Heat-Seal station	Holds up to four optical heat-seal covers	
Output station	 Holds up to four output plates: 	
	 Four 96-well optical plates, OR 	
	 Three 96-well optical plates and one 384-well optical plate (for instruments with the 384-well upgrade) 	
	 Cooled by Peltier units (The Peltier units can be set to cool from 4 °C to 15 °C. The default is 4 °C.) 	
Standards, Master Mix/Cell	♦ Holds:	
Lysate Control station	 Up to twelve 2-mL microcentrifuge tubes for standards and controls 	
	 Up to eight 10-mL reagent tubes for master mixes or cell lysate controls 	
	♦ Cooled by Peltier units to 4 °C	
Reagent platform	Consists of the:	
	 Reagent reservoirs (holds up to eight) 	
	 Tip eject plate and tip eject bin 	
Vacuum station	♦ Holds:	
	 Splash guard in the waste position 	
	 Archive plate 	
	 Deep-well plate 	
	 Purification tray in the purification tray carriage 	
	 Moves the purification carriage over the waste position, the filtrate position, or the archive position 	
	Applies vacuum pressure to the purification tray	
Input station	 Holds 96-well cell culture plate (Falcon, Costar, or Nunc) in the Input 1 position 	
	 Holds deep-well plate in the Input 2 position 	
	 Input 1 position is cooled by Peltier units to 4 °C 	
System Flush station	Collects and recycles any system fluid dispensed when the diluters are primed or when the system fluid is flushed	

The table below describes the deckspace stations.

Enclosure Functions The 6700 instrument enclosure provides a controlled environment for performing protocols.

The table below describes the 6700 instrument enclosure components and functions.

Component	Description	Description		
Safety-interlocked door	Stops the instrument from opened by turning off por	Stops the instrument from operating when the door is opened by turning off power to the robotic arm		
Blower	Provides user protection	from aerosols		
HEPA filter	Filters airborne particles the instrument	Filters airborne particles and aerosols from the air leaving the instrument		
Prefilters	Filter airborne particles a the instrument	nd aerosols from the air entering		
Interior lighting	Consists of two 32-watt,	fluorescent bulbs		
LED lights	Indicate instrument statu	s as follows:		
	Color	Indication		
	Solid red	An instrument error occurred.		
	Flashing red (fast)	The instrument requires firmware download.		
	Flashing red (slow)There is an instrumentaccompanied withmalfunction. Call service.audible buzzermalfunction. Call service.			
	Flashing yellow	Data transfer is occurring.		
	Green	The instrument door is closed, the system is ready, and the HEPA fans are on low speed.		
	Green off	The instrument door is open and the HEPA fans are fully on.		

Computer Hardware and Accessories

	Computer Component	Function
	Server computer for the 6700 database (runs on the Windows NT [®] operating system)	 Stores the 6700 database, which contains protocols and run histories
		 Stores the user account information for the 6700 database
	Client computer for the 6700 software (runs on the Windows NT operating system)	 Used by the 6700 workstation database administrator, scientists, and operators to:
		 Access the 6700 database for protocols and run histories
		 Operate and maintain the 6700 instrument
		• Used by the 6700 workstation database administrator to:
-		 Manage the 6700 database user accounts
		 Troubleshoot the 6700 database
		 Maintain and back up the 6700 database
	Monitor, keyboard, and mouse	Accesses the server and client computers
	Switchbox	Switches the monitor, keyboard, and mouse between the server computer and the client computer
	Barcode reader	Scans the barcodes of plastic consumables and deckspace stations during run setup
	Keyboard wedge	Connects the barcode reader to the keyboard

Barcode Reader The barcode reader is a handheld model manufactured by PSC, Inc. It is connected to Description the keyboard by a keyboard wedge.



IMPORTANT Shut down both the server computer and client computer before disconnecting the barcode reader. If you try to disconnect the barcode reader while either computer is running, the systems will fail.

A 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.

About the Software

Software Overview	The ABI PRISM [™] 6700 Automated Nucleic Acid Workstation software provides:			
	Control of instrument functions			
	 Access to the 6700 database for protocols, run logs, sample information, detectors, dyes, and archive plates created 			
Software Tabs	There are three main tabs in the 6700 software for interacting with the instrument:			
	Protocol tab			
	Deckspace tab			
	Instrument tab			
Tab Commands	Each tab is designed for performing tasks related to specific stages of a 6700 workstation run.			

Tab	Run Stage	Possible Actions	
Protocol	Run setup	View stored protocols	
		Create new protocols	
		 Select protocols for a run 	
		 Enter archive sample names 	
		 Import archive sample names from a previous run 	
		 Import archive sample names from a file 	
		 Export archive sample names to a file 	
		 Select the input plate 	
		Define the input plate	
		 Select specific samples to start protocols 	
		 Select specific samples for the Assay protocol 	
		 Verify protocol setup 	
Deckspace	Deckspace	View the deckspace	
	preparation	 View the consumables and reagents required for the run 	
		 Load the deckspace with consumables and reagents 	
		 Enter barcodes 	
Instrument	Instrument run	 Cool Peltiers or stop Peltiers from cooling 	
		 Start, stop, or pause a run 	
		 Monitor the status of the instrument 	
		 Monitor the run log in real time 	
		 View and print protocol details and sample lists 	

Menu Bar Items The menu bar contains seven main menu items:

- ♦ File
- ♦ Edit
- View
- Setup
- Protocol
- Instrument
- ♦ Help

Menu Commands The table below describes the commands under each menu bar item

Description of the Menu Commands

Menu Items	Command	Function
Eile	View Run History	View stored runs.
View Run <u>H</u> istory Ctrl+H Import Samples From File Export Samples To File Ctrl+E	Import Samples From File	Import archive sample names from a tab-delimited text file.
Page Setup Print Ctrl+P	Export Samples To File	Export archive sample names to a tab-delimited text file.
Exit Ctrl+Q	Page Setup	These are standard commands. Refer to a Windows NT
	Print	platform manual for more information.
	Logout	Log out of the 6700 software.
	Exit	Log out and exit the 6700 software.
Edit	Undo	These are standard commands. Refer to a Windows NT
Undo Ctrl+Z	Cut	platform manual for more information.
<u>C</u> opy Ctrl+C	Сору	
Paste Ctrl+V	Paste	
Select <u>A</u> ll Ctrl+A	Clear	Clear all samples selected in the archive sample name list.
AutoFill Sample Names Preferences Ctrl+G	Select All	Select all samples in the archive sample name list.
	AutoFill Sample Names	Fill archive sample names according to a preset formula.
	Preferences	Set preferences for viewing protocols.
View	Protocol	View the Protocol tab.
Protocol Ctrl+1	Deckspace	View the Deckspace tab.
Deckspace Ctrl+2 Instrument Ctrl+3	Instrument	View the Instrument tab.
Setup	Protocol Browser	View protocols in the 6700 database.
Protocol Browser Ctrl+B	Detectors	View detectors used in master mixes for the Assay protocol.
Detectors Ctrl+D Dyes Ctrl+Y	Dyes	View dyes used in detectors.
Sample <u>T</u> ypes Ctrl+S	Sample Types	View sample types used in the Assay protocol.

Description of the Menu Commands (continued)

Menu Items	Command	Function	
Instrument	Start/Stop Run	Start or stop a run from the Instrument tab.	
Start/Stop Run Ctrl+R	Pause/Resume	Pause or resume a run from the Instrument tab.	
Utility + Tests +	Utility	Initialize Seal Plates System Flush Calibration Download Firmware Edit Calibration Values Set Instrument Serial Number Set Pressure Sensor Single Command Touchoff Adjust	
	♦ Initialize	Initialize the robotic arm, diluters, automatic Heat-Seal station, automatic Vacuum station, and safety-interlocked door.	
	 Seal Plates 	Command the heat sealer to seal the output plates independent of a protocol run.	
	 System Flush 	Purge the system fluid lines and/or prime and purge the diluters.	
	Calibration	Calibrate the robotic arm to the deckspace.	
		Note This function requires an Administrator login.	
	 Download Firmware 	Download the firmware for the instrument and the robotic arm.	
		Note This function requires an Administrator login.	
	 Edit Calibration Values 	These commands are for service use only.	
	 Set Instrument Serial Number 		
	 Set Pressure Sensor 		
	 Single Command 		
	◆ Touchoff Adjust		
	Tests	Fluid Delivery Function Tests	
	♦ Fluid Delivery	Test accuracy and precision of fluid delivery.	
	◆ Function Tests	Troubleshoot instrument performance.	

3

Instrument Operation

Overview

About This Chapter	This chapter contains procedures for operating the ABI PRISM [™] 6700 Automated Nucleic Acid Workstation.		
In This Chapter	This chapter contains the following topics:		
	Торіс	See Page	
	Instrument Run Overview	3-2	
	Software Login	3-5	
	Protocol Setup	3-10	
	Deckspace Setup	3-20	
	Instrument Run	3-30	
	After the Instrument Run	3-34	

Instrument Run Overview

Run Description	A run refers to a user-defined combination of various protocol types (as described on page 3-3). Once the parameters are set by the user, the run is automated by the 6700 workstation.			
Order of Operations	s The table below shows the order of operations required for a 6700 workstation run.			
	6700 Workstation Run: Order of Operations			
	Stage	Description		
	1	The user logs in to the ABI PRISM [™] 6700 Automated Nucleic Acid Workstation software from the client computer.		
	2	If needed, the user creates new protocol(s).		
	3	The user completes tasks on the Protocol tab:		
		a. Selects the protocol(s)		
		b. Names the archive samples		
		c. Selects the input plate type		
		d. Selects samples for the Assay protocol (optional)		
	4	The user completes tasks on the Deckspace tab:		
		a. Scans deckspace barcodes with the barcode reader		
		b. Scans consumable barcodes		
	c. Places required consumables and reagents on the deckspace			
	5	The user completes tasks on the Instrument tab:		
a. Cools the deckspace Peltier units		a. Cools the deckspace Peltier units		
		b. Starts the run		
	6	The 6700 instrument performs the specified protocol(s).		
	7	After the 6700 instrument completes the run, the user retrieves the archive(s) and/or output plate(s), stores the plates until needed, and clears the deckspace.		

About Input Plates The 6700 instrument requires an input plate to start a run. The table below describes the contents of each input plate type.

Description of the Input Plate Types

Input Plate Type	Description
Raw	Cells suspended in buffer
Lysed	Lysed cells
Deep-well	 Filtrate collected by the 6700 instrument during an RNA Archive protocol or DNA Archive protocol or Precipitated DNA
RNA archive	 Purified RNA or Dilution archive of an RNA Archive protocol

Description of the Input Plate Types (continued)

Input Plate Type	Description	
DNA archive	Purified DNA	
	or	
	 Dilution archive of a DNA Archive protocol 	
cDNA archive	Synthesized cDNA	
	or	
	Dilution archive of a cDNA Archive protocol	

About the Protocols The 6700 workstation can perform five basic protocols, which can be customized depending on your application. The five basic protocols are described briefly below. See Chapter 4, "Protocol Creation," for more detailed descriptions of the 6700 workstation protocols.

6700 Protocol	Purpose	See Page
Lysis/DNA Precipitation	 To lyse cells with Applied Biosystems reagents 	4-11, 4-15
Lysis/DNA Precipitation	• To precipitate DNA with Applied	
6700 Standard Lysis 💌	Biosystems reagents	
P View ♥ New		
RNA/DNA Archive	 To purify RNA from lysed cells 	4-19, 4-31
RNA/DNA Archive	 To purify DNA from precipitated DNA 	
6700 RNA Archive		
P View ♥ New		
cDNA Archive	To synthesize cDNA from RNA	4-43
🔲 cDNA Archive		
6700 Standard cDNA 🔽		
_		
Dilution Archive	To dilute RNA, DNA, or cDNA	4-47
Dilution Archive		
1:20/200 Dilution		
P View ♥ New		
Assay	To prepare output plates for assays	4-53
Assay		
6700 Install Assay 🔽		
_		

Protocol Flow The figure below shows the protocols possible on the 6700 workstation, the flow of these protocols, the input and output plates, and the location of the input and output plates on the deckspace.

IMPORTANT Assay protocols that specify sample dilutions other than "Neat" cannot follow a Dilution Archive protocol.



Configurations for the Output Station

The Output station can be configured as follows:

Platform	Configuration	
96-well	Four 96-well optical plates	
384-well	 Three 96-well optical plates One 384-well optical plate 	
	Note 96-well and 384-well optical plates cannot be run simultaneously.	

Software Login

Establishing an Account

The ABI PRISM[™] 6700 Automated Nucleic Acid Workstation software supports an
 electronic signature system to log system use and to log the creation, modification, and deletion of protocols.

Before you can use the 6700 workstation, you must establish a database account with the 6700 workstation database administrator. The database administrator will provide you with a user name and password and will assign a user level.

User Levels The table below lists the assigned privileges for each user level.

User Level	Privileges	
Operator	 Log in to the 6700 workstation 	
	 View protocols, detectors, dyes, and sample types 	
	 View run history, import samples from file, and export samples to file 	
	 Set up and begin 6700 workstation runs 	
	 Perform instrument utility functions: 	
	 Initialize the instrument 	
	 Seal plates 	
	 Flush the system 	
	Perform instrument tests:	
	 Function tests 	
Scientist	The operator privileges listed above, plus:	
	 Create protocols, detectors, dyes, and sample types 	
	 Duplicate or delete protocols created by the user (identified by the user name and password combination) 	
	 Edit or delete detectors, dyes, and sample types created by the user (identified by the user name and password combination) 	
Administrator	The scientist privileges listed above, plus:	
	 Add, delete, or edit user accounts 	
	 Perform database administration utilities 	
	Calibrate the instrument	
	Download firmware	

$Logging\ In$ $\ \ To\ log\ in\ to\ the\ 6700\ workstation:$

Step	Action				
1	Launch the 6700 software:				
	a. Go to the client computer's Start menu				
	b. Scroll to Programs .				
	c. Select ABI 6700 Instrument Application				
	A 6700 Log-In dialog box appears.				
2	Enter the correct User Name and Password combination.				
	IMPORTANT Entries are case-sensitive.				
	10 6700 Log-in				
	Please log-in to the 6700 System;				
	User Name: User				
	Password: Setup	1			
	Quit OK				
3	Click OK .				
	If login was	Then			
	successful	the Protocol tab of the 6700 software appears.			
	unsuccessful the first time	a Login Error dialog box appears.			
		Click OK and repeat step 2.			
	unsuccessful the second time	a Login Error dialog box appears.			
		a. Click OK .			
		b. Make sure the server computer is on and the Windows NT[®] platform is running			
		c Beneat step 1 and step 2			
		d. If login is still unsuccessful contact			
		your on-site 6700 workstation database administrator to confirm your account information.			
Setting Preferences To set preferences:

Step	Action						
1	From the Edit menu, select Preferences						
	A Preferences dialog box opens.						
	Preferences X						
	General						
D Show ToolTips							
	Protocols						
	Only Show Current User's Protocols in Browser						
	Only Show Current User's Protocols in Protocol View						
	96-Well Output Plate Setup Files						
	C SDS 1.x Setup File format						
	- Alart Sounda						
	✓ Play sound when the instrument stops with an error.						
	Sound Name : Temple 🔽 Try Sound						
	Play sound every 10 seconds.						
2	Set the following General preferences:						
	 Activate Error Checks for ABI Consumables (recommended) 						
	 Check this box if you want the 6700 workstation to display error messages if the concumption are incorrect or if the concumption are in the wrong place. 						
	MOODTANT It is strongly recommended that you shack this hay. If the have a set						
	PORTANT It is strongly recommended that you check this box. If the box is not elected, it may cause the run to abort.						
	Show ToolTips						
	Check the box if you want hints to appear as you move the cursor to different areas of each tab.						
	 Uncheck the box if you do not want hints to appear. 						
3	Set the following Protocols preferences:						
	Only Show Current User's Protocols in Browser						
	 Check the box if you want to see only protocols that you created in the Protocol Browser. 						
	 Uncheck the box if you want to see all protocols in the database in the Protocol Browser. 						
	Only Show Current User's Protocols in Protocol View						
	 Check the box if you want to see only protocols that you created in the protocol pop-up menus on the Protocol tab. 						
	 Uncheck the box if you want to see all protocols in the database in the protocol pop-up menus on the Protocol tab. 						

To set preferences: (continued)

Step	Action					
4	Set the following 96-Well Output Plate Setup Files preferences:					
	♦ SDS 1.x Setup File format					
	 Check this box if you are analyzing output plates only on the ABI PRISM[®] 7700 Sequence Detection System (7700 SDS). The list of consumables for the 7700 SDS is small and is incompatible with the ABI PRISM[®] 7900HT Sequence Detection System (7900HT SDS). 					
	 Uncheck this box if you will be analyzing output plates with both the 7700 SDS and 7900HT SDS. 					
	SDS 2.0 Setup File format					
	 Check this box if you are analyzing output plates only on the 7900HT SDS 					
	 Uncheck this box if you will be analyzing output plates with both the 7700 SDS and 7900HT SDS. 					
5	Set the following Alert Sounds preferences:					
	 Play sound when the instrument stops with an error. 					
	 Check this box if you want to hear a sound when the instrument stops with an error. Choose a Sound Name and frequency as desired. 					
	 Uncheck this box if you do not want to hear any sound. 					

 $\label{eq:constraint} Adding \ Users \quad \mbox{Only the database administrator and service engineers can add users.}$

To add users:

Action							
Go to the computer's Start menu, scroll to Programs , and select ABI User Account Manager .							
A Connect to User Account Database dialog box appears.							
Log into the user account database with a database administrator or service engineer account.							
Click Connect.							
A User Account Manager window appears.							
Click New.							
An Add User dialog box appears.							
Enter the Login Name, Password, Confirm Password, and Full Name.							
Note Spaces are not allowed in the login name or the password. The login name and password must begin with a letter of the alphabet.							
Select the type of user account to set up.							
Click Add.							
The new user information appears in the User Account Manager window.							

Deleting Users Only the database administrator and service engineers can delete users.

To delete users:

Step	Action						
1	Go to the computer's Start menu, scroll to Programs , and select ABI User Account Manager .						
	A Connect to User Account Database dialog box appears.						
2	Log into the user account database with a database administrator or service engineer account.						
3	Click Connect. A User Account Manager window appears.						
4	Select the user account to be deleted. IMPORTANT Do not delete the pebio account.						
5	Click Delete . A dialog box appears, requesting whether or not you wish to delete the selected account.						
6	Click OK to delete the user account.						

Protocol Setup

Protocol Setup Overview Protocol setup occurs via the Protocol tab of the 6700 software. The process involves the following stages:

Stage				
Selecting Protocols				
Viewing Protocols				
Creating Protocols				
Entering Archive Sample Names				
Selecting an Input Plate Type				
Selecting Samples for the Assay Protocol				

Protocol Tab View Accessing the Protocol Tab

To access the Protocol tab:

Step	Action				
1	Launch the 6700 software.				
	The Protocol tab is automatically displayed, as shown below.				

The figure below shows the different areas of the Protocol tab.



Selecting Protocols You can select up to four protocols for one run. The number of protocols required varies according to the input plate and the goal of the run.

To select protocols:



Viewing Protocols You can view protocols to confirm conditions.

To view protocols:

Step	Action					
1	In the Select Protocols section of the Protocol tab, click the View button underneath the protocol you wish to view.					
	The protocol conditions appear.					
	Note The windows displayed when viewing protocol conditions are identical to those displayed when creating new protocols (<i>i.e.</i> , changing protocol conditions). However, you cannot make any changes to the protocols using this procedure. If you want to make changes, see Chapter 4, "Protocol Creation."					
2	Click OK or Close to close the window and return to the Protocol tab.					

Creating Protocols Note Protocol creation requires the Scientist or Administrator login.

To create protocols:

Step	Action
1	In the Select Protocols section of the Protocol tab, click the New button underneath the protocol you wish to create.
2	For details on creating and defining protocols, see Chapter 4, "Protocol Creation."

Entering Archive Guidelines

Sample Names Follow the guidelines below when entering archive sample names.

- Enter names in one of three ways:
 - Use the Sample Name field (see below).
 - Use the AutoFill Sample Names menu command (see page 3-14).
 - Import archive sample names from a previous run (see page 3-16).
- Use names that are unique for the run.
- ◆ Create names that contain ≤94 characters.
- Enter names for all samples to be prepared from the input plate.

Using the Sample Name Field

To name the samples using the Sample Name field:

	Step	Action					
	1	In the Enter Archive Sample Names section of the Protocol tab, highlight the rows corresponding to the sample wells.					
Note To select all 96 samples, right-click the Name button and choose Se from the drop-down menu.							
		Enter Samp	Archive S	iample Names		— Bight-click the Name button to	
		Well	Pos	Name		view the drep down many	
		A1	1 03.	Name	Clear	view the drop-down menu	
		A2	2		Clear Non-Assay Samples		
		A3	3		Select All		
		A4	4		Select Active Samples		
		A5	5		AutoFill Sample Names		
		A6	6				
		A7	7				
		A8	8				
		A9	9				
		A10 A11	11				
		A17	12				
		B1	13				
		B2	14				
		B3	15				
		B4	16				
		B5	17				
		B6	18				
		B7	19				
		88	20				
		B9	21				
		810	22				
L							

Step	Actio	n				
2 Place the cursor in the Sample Name field and enter a name.				Sample Name field and enter a name.		
	The software assigns the Sample Name to the sample.					
	10/oll	Doe	Namo			
	oven G3	PUS. 75	Installation 75			
	G4	76	Installation 76			
	G5	77	Installation 77			
	G6	78	Installation 78			
	G7	79	Installation 79			
	G8	80	Installation 80			
	G9	81	Installation 81			
	G10	82	Installation 82			
	G11	83	Installation 83			
	G12	84	Installation 84			
	H1	85	Installation 85			
	H2	86	Installation 86			
	H3	87	Installation 87			
	H4	88	Installation 88			
	H5	89	Installation 89			
	Hb	90	Installation 90			
	HI/	91	Installation 91			
		92	Installation 92			
		9.5 Q.4	Installation 93			
	H11	94	Installation 95			
	H12	96	Installation 96			
	Note	lf v	you selected m	nore than one row in step 1, the software assigns the		
		11 J		ample with a number		
	Sample Name to each sample with a number.					
3	Repeat steps 1 and 2 until you name all the samples.					

To name the samples using the Sample Name field: (continued)

Using the AutoFill Menu Command

To name the samples using the AutoFill menu command:

Step	Action						
1	In the Enter Archive Sample Names section of the Protocol tab, highlight the rows corresponding to the sample wells.						
	Note To select all 96 samples, right-click the Name button and choose Select All from the drop-down menu.						
	Enter Archive Sample Names						
	Sample Name: Bight-click the Name button to						
	Well Pos. Name	Clear Clear Non Assay Samples	view the drop-down menu				
	A2 2 A3 3 A4 4	Select All Select Active Samples					
	A5 5 A6 6	AutoFill Sample Names					
	A7 7 A8 8	_					
	A10 10 A11 11						
	A12 12 B1 13 D2 14	_					
	B2 14 B3 15 B4 16						
	B5 17 B6 18 B7 19						
	B7 13 B8 20 B9 21						
	Biol 22						
2	Right-click the Name button and select AutoFill Sample Names from the drop-down menu.						
	Enter Archive Sample Names						
	Sample Name:						
	Well Pos. Name A1 1	Clear Clear Non-Assay Samples					
	A2 2 A3 3 A4 4	Select All Select Active Samples					
	A5 5 A6 6	AutoFill Sample Names					
	A7 7 A8 8 A9 9						
	A10 10 A11 11						
	A12 12 B1 13 B2 14						
	B3 15 B4 16						
	B5 17 B6 18 B7 19						
	B8 20 B9 21						
	The AutoFill Samples dialog box appears.						

Step	Action							
3	Place the cursor in the Sample Name field and enter a name, using the Sample Name Codes shown below.							
	Sample Name Code	Meaning						
	%n	Increment						
	%d	Today's Date (year-month-day)						
	AutoFill Samples	AutoFill Samples X						
	AutoFill these sample names into the selected sample table rows: Sample Name: Sample %n, %d Sample Name Codes: %d = Today's Date, %n = Increment							
	Increment: Start With 1 Increment By 1							
	AutoFill Preview: Sample 01, 2000-03-20 Sample 02, 2000-03-20 Sample 04, 2000-03-20 Sample 06, 2000-03-20 Sample 06, 2000-03-20 Sample 07, 2000-03-20 Sample 08, 2000-03-20							
	Cancel							
4	If you choose to name the same	e is sample %n, %d.						
4	Name Code) select the Increment parameters:							
	a. Enter the number for the first sample in the Start With field.							
	b. Enter the increment value in	the Increment By field.						
5	Preview the sample names in the AutoFill Preview pane.							

To name the samples using the AutoFill menu command: (continued)

To name the samples using the AutoFill menu command: (continued)	To name the sample	s using the	AutoFill menu	command:	(continued)
--	--------------------	-------------	---------------	----------	-------------

Step	Actio	n			
6	Click	OK.			
	The s	oftw	are names the sa	mp	ples using the formula in the Sample Name field.
	Enter . Samp	Archiv le Nar	re Sample Names me:		
	Well	Pos.	Name	ſ	-
	A1	1	Sample 01, 2000-03-20		
	A2	2	Sample 02, 2000-03-20		1
	A3	3	Sample 03, 2000-03-20		
	A4	4	Sample 04, 2000-03-20		
	A5	5	Sample 05, 2000-03-20		
	A6	6	Sample 06, 2000-03-20	_	1
	A7	7	Sample 07, 2000-03-20		
	A8	8	Sample 08, 2000-03-20		
	A9	9	Sample 09, 2000-03-20		
	A10	10	Sample 10, 2000-03-20		
	A11	11	Sample 11, 2000-03-20		
	A12	12	Sample 12, 2000-03-20		
	B1	13	Sample 13, 2000-03-20		
	B2	14	Sample 14, 2000-03-20	_	
	B3	15	Sample 15, 2000-03-20	_	
	B4	16	Sample 16, 2000-03-20	-	
	B5	17	Sample 17, 2000-03-20	-	
	B6	18	Sample 18, 2000-03-20		
	87	19	Sample 19, 2000-03-20		
	B8	20	Sample 20, 2000-03-20	_	
	B9	21	Sample 21, 2000-03-20		
	B10	22	Sample 22, 2000-03-20	-	1

Importing Archive Sample Names

If your input plate type is an archive plate from a previous run, you can import the archive sample names from the 6700 database.

To import archive sample names from the 6700 database:

Step	Action
1	In the Select Input Plate Type section of the Protocol tab, select one of the following plates from the Input Plate Type pop-up menu:
	RNA Archive
	◆ cDNA Archive
	DNA Archive
	Note If your input plate type is a dilution archive plate, select either RNA Archive , cDNA Archive , or DNA Archive .
	Select Input Plate Type
	Input Plate Type: Lysed
	Archive Plate Consumable ID: Raw Lysed
	Select Samples for Assay Prot
	1 2 3 4 5 6 DNA Archive the input plate type
	The Archive Plate Consumable ID field becomes active.

To import archive sample names from the 6700 database: (continued)

Step	Action
2	Using the barcode reader, scan the Archive Plate Consumable ID barcode on the archive plate.
	AWARNING 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.
	a. The software enters the barcode, then searches the 6700 database for the barcode and the corresponding archive sample names.
	Searching Database
	b. The software imports the Sample Names and enters the Consumable ID on the Deckspace tab.
	Note You cannot edit archive sample names imported from the database.

Selecting an Input Plate Type

Note These are general procedures for selecting an input plate type. If you are importing archive sample names, select an input plate type per step 1 of "Importing Archive Sample Names" on page 3-16.

To select an input plate type:

Step	Action	
1	In the Select Input Plate Type section of the Input Plate Type pop-up menu.	e Protocol tab, select a plate from the
	Note See "About Input Plates" on page	3-2 for descriptions of input plate types.
	Select Input Plate Type Input Plate Type: Lysed	
	If the first protocol in the run is	Then select
	Lysis	Raw
	DNA Precipitation	Deep-Well Filtrate Plate
	RNA Archive	Lysed
	DNA Archive (using a deep-well plate)	Deep-Well Filtrate Plate
	cDNA Archive	RNA Archive
	Dilution Archive	RNA Archive, DNA Archive, or cDNA Archive
	Assay	RNA Archive, DNA Archive, or cDNA Archive

 Selecting Samples for the Assay Protocol
 If you selected an Assay protocol, select the samples to transfer to output plates.

 To select samples for the Assay protocol:
 To select samples for the Assay protocol:

 Step
 Action

 1
 In the Select Samples for Assay Protocol section of the Protocol tab, click the

in ai	e hi	sponding Wells in ghlighted.	the	Enter Archive Sample Names section of the Pro
Enter	Archiv	e Sample Names		Select Input Plate Type
Samp	le Nar	ne:		Input Plate Type: Lysed
Well	Pos.	Name		Archive Plate Consumable ID:
A1	1	Sample 01, 2000-03-20		·
A2	2	Sample 02, 2000-03-20		Select Samples for Assay Protocol
A3	3	Sample 03, 2000-03-20		
A4	4	Sample 04, 2000-03-20		1 2 3 4 5 6 7 8 9 10 11 12
A5	5	Sample 05, 2000-03-20		
A6	6	Sample 06, 2000-03-20		
A7	7	Sample 07, 2000-03-20		
A8	8	Sample 08, 2000-03-20		¢ 25 26 27 28 29 30 31 32 33 34 35 36 Sele
A9	9	Sample 09, 2000-03-20		D 37 38 69 40 41 42 43 44 45 46 47 48
A10	10	Sample 10, 2000-03-20		
A11	11	Sample 11, 2000-03-20		E (49 (50 (51 (52 (53 (54 (55 (56 (57 (58 (59 (60)
A12	12	Sample 12, 2000-03-20		F 61 62 63 64 65 66 67 68 69 70 71 72 Pro
B1	13	Sample 13, 2000-03-20		A F3 F7
B2	14	Sample 14, 2000-03-20		
B 3	15	Sample 15, 2000-03-20		н 85 86 87 88 89 90 91 92 93 94 95 96
B4	16	Sample 16, 2000-03-20		J
B5	17	Sample 17, 2000-03-20		Proving Annoy Protocol Output
B6	18	Sample 18, 2000-03-20		
B7	19	Sample 19, 2000-03-20		·
B8	20	Sample 20, 2000-03-20		Import Sample Names From File
B9	21	Sample 21, 2000-03-20		· · · · · · · · · · · · · · · · · · ·
D1.0	22	Sample 22, 2000-03-20	-	

To select samples for the Assay protocol: (continued)

Step	Action	
2	Click the Preview Assay Protocol samples in the output plates.	Output button to preview the arrangement of
	A Preview Assay Protocol Output	dialog box appears.
	Note The dialog box indicates h plates, but the number of samples limits, <i>e.g.</i> , the number of tips the	ow many samples can be placed on the output the system can handle may be less due to other deckspace can hold.
	If you are using a	Then the dialog box shows
	96-well instrument	Output tabs 1, 2, 3, and 4.
		Output 1 Output 2 Output 3 Output 4 Image: Stress of the stress o
	384-well upgrade and running a 384-well optical plate	Output tab 1 only.
	384-well upgrade and running 96-well optical plate(s)	Output tabs 2, 3, and 4.
3	Click the Output tab(s) to view the	arrangement of samples in each output plate.
	Note The well color indicates w Master Mixes" on page 4-71 for m	hich master mix is present (see "Specifying the nore information).
4	Click Done.	

Deckspace Setup

Deckspace Setup Overview Deckspace setup occurs through the Deckspace tab of the 6700 software. The process involves the following stages:

Stage	See Page
Cooling the Deckspace	3-21
Selecting Plate Cover Options	3-22
Viewing Deckspace Information	3-22
Using the Barcode Reader and Deckspace Tab	3-23
Placing Required Consumables, Reagents, or Placeholders	3-26
Verifying the Deckspace	3-29

Deckspace Tab View Accessing the Deckspace Tab

To access the Deckspace tab:

Step	Action
1	Launch the 6700 software.
	The Protocol tab is automatically displayed.
2	Click the Deckspace tab.
	The Deckspace tab is displayed, as shown below.

The figure below shows the different areas of the Deckspace tab.



Output plate temperature setpoint

Deckspace Colors Deckspace tab station colors indicate the requirements of the deckspace position.

If the station color is	Then the position
Yellow	requires a consumable (<i>e.g.</i> , plate or tip rack) or reagent to perform the run.
Blue	requires a placeholder (<i>e.g.</i> , an empty tube or 96-well optical plate).
	IMPORTANT Placeholders are required to minimize condensation formation on the deckspace.
Green	is marked in the software as loaded with the appropriate consumable or reagent.
Gray	will not be used in the run.

Cooling the IMPORTANT Begin cooling the deckspace Peltier units before setting up the deckspace to **Deckspace** prevent degradation of the input, standards, controls, reagents, and master mixes.

To cool the deckspace:

Step	Action			
1	Go to the Deckspace tab.			
2	In the Peltier Temp field, select the temperature setpoint for the output plates. The range is 4 °C to 15 °C. The default is 4 °C.			
	Pettier Temp			
3	Go to the Instrument tab.			
4	Click the Cool Peltiers button.			
	Cool Peltiers			
	The 6700 instrument begins to cool the following stations:			
	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.			

Selecting PlateSelect plate cover options for all archive plates and for all assay output plates. The
6700 workstation covers and seals plates after completing the protocols.

To select plate cover options:

Step	Action
1	Go to the Deckspace tab.
2	For the 6700 instrument to place archive covers on all of the archive plates and dilution archive plates after performing the run, check Use Covers for 'Archive' and 'Dilution Archive' Plates .
	✓ Use Covers for 'Archive' and 'Dilution Archive' Plates
	An appropriate number of Cover locations turns yellow.
	Note The instrument always places archive covers on cDNA archive plates.
3	For the 6700 instrument to seal all of the assay output plates with optical heat-seal covers immediately after performing the Assay protocol, check Seal Output Plates .
	Seal Output Plates
	The Heat Seals location turns yellow.
	IMPORTANT Make sure to put enough optical heat-seal covers in the Heat-Seal station for your run, however, never add more than six. If you add more than six optical heat-seal covers, the instrument may fail to pick them up and seal the output plates.

Viewing Deckspace Information	To view deckspace information:		
	Step	Action	
	1	View the Consumables and Reagents Required for the run in the bottom-left panel.	
		Note See Appendix B, "6700 Workstation Materials," for descriptions and part numbers of consumables and reagents.	
	2	To print the list, click the Print Consumables List button.	
	3	Gather the consumables and reagents required for the run before setting up the	

deckspace.

Loading the Guidelines

Deckspace

Follow the guidelines below when loading the deckspace.

- Load the deckspace in one of two ways:
 - Use the barcode reader and the Deckspace tab (see below).
 - Use the Setup Deckspace window (see page 3-25).
- Enter unique barcodes or consumable IDs for archive plates and output plates.
- Load placeholder items to minimize condensation on Peltier-cooled deckspace stations.
- To reset the deckspace, click the Reset Deckspace button.

Using the Barcode Reader and Deckspace Tab

To load the deckspace using the barcode reader and the Deckspace tab:

Step	Action		
1	Empty the bin (see "Emptying the Tip Eject Bin" on page 5-4).		
2	In the Deckspace tab:		
	a. Click the TIP BIN button to activate the tip eject bin.		
	b. Using the mouse, check the On Deckspace check box.		
	ABI Prism 6700 Cur File Edit View Setup F Protocol Deckspace Tip Eject Bin Description: EMPTY the Tip Eject Bin On Deckspace Check the On Deckspace check box.		

Step Action 3 Using the barcode reader, scan a barcode on the deckspace. NWARNING 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 in the Archive section of the Deckspace tab. • The Consumable ID field becomes active. The software checks the On Deckspace check box. Archive **Description:** Archive Plate Deck ID: &A1S Consumable ID: On Deckspace 4 Then... If the required input... scan the input barcode. has a barcode The software updates the Consumable ID field with the barcode. does not have a barcode either: ♦ Leave the Consumable ID field empty. or Enter notes in the Consumable ID. field 5 Place the required input in the station. A CAUTION CHEMICAL HAZARD. RNA Purification Lysis Solution may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. A 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. A 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.

To load the deckspace using the barcode reader and the Deckspace tab: (continued)

To load the deckspace using the barcode reader and the Deckspace tab: (continued)

Step	Action		
6	Repeat step 1 through step 5 until you place all of the following items on the deckspace:		
	Plates		
	Purification tray		
	 Tips 		
	Reagent reservoirs		
	Master mix		
	Standards and controls		
	Optical heat-seal covers		
	Archive covers		
	Splash guard		

Using the Deckspace Setup Window

To load the deckspace using the Deckspace Setup window:

Step	Action		
1	Go to the Deckspace tab and click the Set Up Deckspace button.		
	The Deckspace Setup window appears.		
2	Using the barcode reader, perform the tasks listed on each tab:		
	♦ Plates		
	♦ Tips		
	♦ Reagents		
	♦ Master mix		
	♦ Standards		
	♦ Misc.		
	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.		
3	Click Done.		
	The Deckspace Setup dialog box closes and the Deckspace tab becomes active.		

Placing Required Consumables, Reagents, or Placeholders Most of the steps in this procedure require use of the barcode reader. Please note the warning below.

Iders A 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 place the required items on the deckspace:

Step	Action
1	 Set up the plates: a. Using the barcode reader, scan a plate location barcode on the deckspace. b. Scan the barcode of the plate. c. Place the plate in its correct location on the deckspace. d. Repeat steps a through c until you place all plates on the deckspace. IMPORTANT Load empty placeholder plates to minimize condensation on the
	deckspace.
2	 a. Using the barcode reader, scan the purification tray carriage barcode. b. Scan the barcode of the purification tray. IMPORTANT Make sure you load the appropriate purification tray for the nucleic acid you are purifying.
	c. Firmly place the purification tray in the purification tray carriage.
	d. Move the four pins to lock the purification tray in place.
	Image: Constraint of the state of

Step	Action		
3	Set up the disposable conductive pipette tips:		
	a. Using the barcode reader, scan a tip rack barcode on the deckspace.		
	b. Place the required tips on the deckspace.		
	c. Repeat steps a and b until you place all tips on the deckspace.		
	Note Disposable tip positions 1–4 only accommodate 200- μ L tips. Positions 5–8 accommodate 200- or 1000- μ L tips. Load the tip size indicated by the Deckspace tab in the 6700 software.		

To place the required items on the deckspace: (continued)

To place the required items on the deckspace: (continued)

Step	Action
4	Set up the reagent reservoirs:
	a. Using the barcode reader, scan a reagent reservoir barcode on the deckspace.
	b. Fill a reagent reservoir with the specified amount of reagent.
	c. Repeat steps a and b until you place all reagents on the deckspace.
	A CAUTION CHEMICAL HAZARD. RNA Purification Lysis Solution may
	cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	ACAUTION 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.
	A 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.
5	Set up the master mixes:
	a. Using the barcode reader, scan a master mix barcode on the deckspace.
	b. Place master mixes or an empty tube into the specified deckspace position.
	 Repeat steps a and b until you place all required master mix tubes on the deckspace.
	IMPORTANT Load empty placeholder tubes to minimize condensation on the deckspace.
6	Set up specified standards, controls, and empty placeholder tubes:
	 Place all specified standards, controls, and empty placeholder tubes in position on the deckspace.
	 b. Click each standard/control/placeholder and use the mouse to check each item as On Deckspace.
	c. Repeat step c until all standards positions are checked as On Deckspace .
	IMPORTANT Load empty placeholder tubes to minimize condensation on the deckspace.
7	Set up the optical heat-seal covers:
	 Using the barcode reader, scan the optical heat-seal covers barcode on the deckspace.
	b. Place the optical heat-seal covers in position.
	CAUTION Place optical heat-seal covers with the dull side facing downward and the shiny side facing upward. Incorrect placement will cause irreparable damage to the heat sealer.
	IMPORTANT Make sure to put enough optical heat-seal covers in the Heat-Seal station for your run, however, never add more than six. If you add more than six optical heat-seal covers, the instrument may fail to pick them up and seal the output plates.

To place the required items on the deckspace: (continued)

Step	Action			
8	Set up the archive covers:			
	a. Using the barcode reader, scan an archive cover barcode on the deckspace.			
	b. Place an archive cover in position on the deckspace.			
	 Repeat steps a and b until you place all required archive covers on the deckspace. 			
9	Set up the splash guard:			
	a. Scan the waste position barcode on the deckspace.			
	b. Place a splash guard in the waste position.			
	IMPORTANT The splash guard is a blue plate with bottomless wells. Do not load a 96-well optical plate in the waste position or waste will collect on the deckspace.			

Verifying the Deckspace	To verify the deckspace:		
Deckspace	Step	Action	
	1	Verify that all active stations on the Deckspace tab are green.	
		Note This indicates that all required deckspace stations are marked as on the 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 6700 instrument to quit the run.	

Instrument Run

Instrument Run	The instrument run occurs via the Instrument tab of the 6700 software. The process
Overview	involves the following stages:

Stage	
Starting a Run	3-31
Pausing a Run (optional)	
Completing a Run	

Instrument Tab Accessing the Instrument Tab

View _

To access the Instrumen	t tab:
-------------------------	--------

Step	Action
1	Launch the 6700 software.
	The Protocol tab is automatically displayed.
2	Click the Instrument tab.
	The Instrument tab is displayed, as shown below.

The figure below shows the different areas of the Instrument tab.

otocol Deckspace Instrument	
Cool Petitiers Instrument Status Archive and Lysis Plates: - Start Assay Plates: - Master Mix, Standards, Controls, Dilution Plates: -	Current Task: Idle Status: Idle Time Remaining: 0 Hr 0 Min 0 Sec
Consumable Status ***** Consumable Description - Tip Eject Bin Input Vacuum -	

Instrument Runs

Guidelines for Follow the guidelines below during an instrument run.

- ٠ Do not use the barcode reader during the run.
- Do not disconnect the barcode reader from the client computer or server • computer during the run or while either computer is turned on. If you try to disconnect the barcode reader while either computer is running, the systems will fail.
- If you pause the run, wait for the robotic arm to stop moving before opening the instrument door.

IMPORTANT Opening the instrument door before the robotic arm stops moving shuts down power to the robotic arm and quits the run.

Starting a Run To start a run:

Step	Action	
1	Close the instrument door.	
2	Go to the Instrument tab of the 6700 software.	
3	Click the Start button.	
	The software verifies that all required items are marked as On Deckspace . If you forgot to scan a station on the 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.	
	Please make sure that the door to the instrument is closed before proceeding! OK	
5	Enter a name for the run in the Name Run dialog box that appears.	
	Name Run Please enter a name for the Run: Installation Cancel	
	Note The run name must contain fewer than 25 characters.	
	Click OK .	
	If you have not already clicked the Cool Peltiers button, the Peltier units begin to cool.	
	Note If the run includes a cDNA Archive protocol, the instrument stops cooling the Dilutions/cDNA station at this time.	
6	Click the Run Log tab to monitor the process.	

Pausing a Run To pause a run:

Action
While the instrument is running, click the Pause button on the Instrument tab.
The robotic arm continues to move until the protocol reaches an appropriate time to pause. A message appears on the computer indicating that the robotic arm has reached a safe position and that you may now open the door.
Wait for the above message to appear, then open the instrument door.
CAUTION Opening the instrument door while the robotic arm is moving shuts off power to the robotic arm and causes the 6700 workstation to quit the run. If you open the instrument door before the robotic arm stops moving, you must restart the 6700 instrument and 6700 software before continuing. Failure to restart may damage the robotic arm.
Resume the run:
a. Close the instrument door.
b. In the 6700 software, click OK to close the error message.
c. Click the Resume button on the Instrument tab.
-

Completing a Run Cooled Stations

After a run is completed, the instrument maintains the following stations at the temperatures indicated below.

Station	Temperature
Input station	4 °C
Standards, Master Mix/Cell Lysate Control station	
Dilutions/cDNA station	
Output station	4 °C to 15 °C
	Note To select the temperature setpoint for the output plates, see "Cooling the Deckspace" on page 3-21.

Completing a Run

To complete a run:

Step	Action
1	Verify that the run is completed by checking the Instrument Status section of the Instrument tab.
2	Open the door of the 6700 instrument.

To complete a run: (continued)

Step	Action			
3	Clear the deckspace:			
	a. Remove the archive plate(s) and/or output plate(s) from the deckspace.			
	b. Store the plates until needed.			
	Output Plate	Deckspace Location	Recommended Storage Temperature	
	Lysed cells Input station 2 to 8 °C			
	RNA/DNA archiveVacuum station-15 to -25 °C			
	Deep-well Vacuum station N/A ^a			
	cDNA archive ^b	Dilutions/cDNA station	–15 to –25 °C	
	Dilution archive	Dilutions/cDNA station	–15 to –25 °C	
	Output plate	Output station	2 to 8 °C	
	a. Manually reposition the deep-well plate to obtain DNA output.			
	b. The archive cover may contain condensation. Briefly centrifuge the plate with the cover of to collect contents at the bottom of the wells before storing the plate or using it in another ru			
4	Click the Turn Peltiers Off button.			
	Note This is a toggle button: Turn Peltiers Off and Cool Peltiers.			
	ACAUTION If you leave the Peltier units on, condensation will collect on the deckspace. If this occurs, the temperature sensors may malfunction and report inaccurate deckspace temperatures.			

After the Instrument Run

Run Overvi

After the Instrument After the instrument run you can perform the following tasks:

Task	See Page
Using Output Plate Setup Files with the 7700 SDS	3-34
Using Output Plate Setup Files with the 7900HT SDS	3-35
About the Run History	3-35

About Output Plate After the run, the 6700 database exports information about each output plate in an Setup Files output plate setup file.

File Attribute	Output Plate Setup File Info	Output Plate Setup File Information	
Location	D:\pebio\6700\Output Plate S	D:\pebio\6700\Output Plate Setup Files	
Name	If you	Then the file is named with the	
	scanned consumable IDs	consumable ID.	
	did not scan consumable IDs	year-month-day and the output plate number.	

Setup Files with the 7700 SDS

Using Output Plate IMPORTANT Only 96-well optical plates can be used with the ABI PRISM® 7700 Sequence Detection System (7700 SDS). If you are using 384-well optical plates, see "Using Output Plate Setup Files with the 7900HT SDS" on page 3-35.

To use output plate setup files with the 7700 SDS:

Step	Action	
1	Locate the output plate setup files on the client computer's 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.	
2	Use a floppy disk to transfer the file to the 7700 SDS.	
3	Launch the 7700 SDS software on the 7700 SDS computer.	
	Close the untitled window that appears.	
4	Create a new file with the settings appropriate for your assay:	
	a. From the File menu, select New Plate	
	b. Choose appropriate options in the New Plate dialog box.	
	c. Click OK .	
	A new window appears in the setup view.	
5	From the File menu, scroll to Import and select Import Setup File.	
6	Locate the appropriate output plate setup file and click Open .	
	The 7700 SDS software imports the plate setup information.	

Using Output Plate Setup Files with the 7900HT SDS

To use (output plate setup files with the 7900HT	SDS:	
Step	Action		
1	Locate the output plate setup files on the client computer's hard drive:		
	a. Go to D:\pebio\6700\Output Plate Setup Files on the client computer.		
	b. Find the appropriate output plate setur	o file.	
	Note The setup file is named with the consumable ID or with the year-month-day and output plate number.		
2	Transfer the file to the ABI PRISM® 7900HT Sequence Detection System (7900HT SDS) computer.		
	If the 6700 database server is	Then transfer the file using	
	networked	the network.	
	not networked	a floppy disk.	
3	Launch the 7900HT SDS software on the 7900HT SDS computer.		
	Close the untitled window that appears.		
4	Create a new file with the settings appropriate for your assay:		
	a. From the File menu, select New Plateb. Choose appropriate options in the New Plate dialog box.		
	c. Click OK .		
	A new window appears in the setup view.		
5	From the File menu, scroll to Import and s	elect Import Setup File.	
6	Locate the appropriate output plate setup file and click Open .		

About the Run The 6700 database stores the history of all runs. The run history includes the following information:

The 7900HT SDS software imports the plate setup information.

- Date of the run
- Run name
- User name
- Protocol tab information
- Deckspace tab information
- Instrument tab information

Viewing the Run	To view the run his
History	

Step	Action				
1	In the 6700 softwar	re, go to the File me	nu.		
2	Select View Run History.				
	A View Dun History window oppose				
	A view Rull history	window appears.			
	🔯 View Run History			x	
	File				
	Date	Run Name	Run By	T	
	2000-Feb-15, 11:59 AM	test_021500	scientist		
	2000-Feb-11, 06:33 PM	Installation Test	scientist		
	2000-Feb-03, 05:23 PM	Lysis	pat	_	
	2000-Feb-03, 05:15 PM	DilutionArchive020300	pat		
	2000-Jan-28, 03:25 PM	Standards Test 012800	pat		
	2000-Jan-27, 01:16 PM	cnt0127001100	Administrator	_	
	 ₽ View		Done		
3	Locate the run you	want to view and se	Done Plect it by clicking it.		
3	Locate the run you Note Click Date,	want to view and se Run Name, or Run E	Done elect it by clicking it. By in the header row	to sort the runs.	
3	Locate the run you Note Click Date, Click the View butto	want to view and se Run Name, or Run E on.	Done elect it by clicking it. By in the header row	to sort the runs.	
3	P View Locate the run you Note Click Date, Click the View butto The Run History file appears.	want to view and se Run Name , or Run E on. e with the Protocol t	Done elect it by clicking it. By in the header row ab, Deckspace tab, a	to sort the runs.	
3 4 5	P view Locate the run you Note Click Date, Click the View butto The Run History file appears. Close the Run History	want to view and se Run Name , or Run E on. e with the Protocol t ory file when finishe	Done elect it by clicking it. By in the header row ab, Deckspace tab, a d.	to sort the runs.	

4

Protocol Creation

Overview

About This Chapter

This chapter describes the types of protocols that the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation can perform, how these protocols flow, the protocol conditions, and the procedures for creating and defining each type of protocol.

In This Chapter

Торіс	See Page
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Using the Protocol Browser	4-6
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Protocol Overview

About Designing Runs	The 6700 workstation automates cell lysis, nucleic acid purification, dilution, and assay setup. Use this section to familiarize yourself with the following:				
	 Descriptions of protocols that the 6700 workstation can perform How protocols flow on the 6700 workstation to generate the desired output 				
About the Protocols	The 6700 workstation can perform five basic protocols, which can be customized depending on your application.				
	6700 Protocol	Purpose	See Page		
	Lysis/DNA Precipitation	 To lyse cells with Applied Biosystems reagents 	4-11, 4-15		
	Lysis/DNA Precipitation 6700 Standard Lysis View View	 To precipitate DNA with Applied Biosystems reagents 			
	RNA/DNA Archive	◆ To purify RNA	4-19, 4-31		
	RNA/DNA Archive 6700 RNA Archive P View New	◆ To purify DNA			
	CDNA Archive CDNA Archive 6700 Standard cDNA ▼ P View O New	To synthesize cDNA from RNA	4-43		
	Dilution Archive Dilution Archive 1:20/200 Dilution View New	To dilute RNA, DNA, or cDNA	4-47		
	Assay Assay 6700 Install Assay View O New	To prepare output plates for assays	4-53		

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Protocol Flow The figure below shows the protocols possible on the 6700 workstation, the flow of these protocols, the input and output plates, and the location of input and output plates on the deckspace.

IMPORTANT Assay protocols that specify sample dilutions cannot follow Dilution Archive protocols.



Configurations for the Output Station

The Output station can be configured as follows:

Platform	Configuration		
96-well	 Four 96-well optical plates 		
384-well	Three 96-well optical plates		
	One 384-well optical plate		
	Note 96-well and 384-well optical plates cannot be run simultaneously.		

Protocol Flow for
RNA OutputThe flow chart below shows the protocols that the 6700 instrument performs to
prepare plates containing RNA, the flow of these protocols, the input and output
plates, and the location of input and output plates on the deckspace.

IMPORTANT Assay protocols that specify sample dilutions cannot follow Dilution Archive protocols.



Protocol Flow for
DNA OutputThe flow chart below shows the protocols that the 6700 instrument performs to
prepare plates containing DNA, the flow of these protocols, the input and output
plates, and the location of input and output plates on the deckspace.

IMPORTANT Assay protocols that specify sample dilutions cannot follow Dilution Archive protocols.



Protocol Flow for cDNA Output

The flow chart below shows the protocols that the 6700 instrument performs to prepare plates containing cDNA, the flow of these protocols, the input and output plates, and the location of input and output plates on the deckspace.

IMPORTANT Assay protocols that specify sample dilutions cannot follow Dilution Archive protocols.



Using the Protocol Browser

About the Protocol The Protocol Browser contains all 6700 workstation protocols created or used in an Browser instrument run.

Accessing the Protocol Browser Window

To access the Protocol Browser window:

Step	Action					
1	In the ABI PRISM [™] 6700 Automated Nucleic Acid Workstation software, go to the Setup menu.					
2	Select Protocol Brows The Protocol Browser	er. window opens.				
	Protocol Browser			_ 🗆 X]	
	File Protocol Tabs					
	Lysis/DNA Precipitation RNA	DNA Archive cDNA Ar	chive Dilution Archive /	Assay		
	Name	Created By	Date	In Use		
	DNA Precipitation	Administrator	2000-Jan-27, 01:30 PM			
	Lysis	scientist	2000-Jan-26, 04.14 PM 2000-Feb-11, 06:52 PM	- -		
	Q New _P View	C Duplicate		X Delete		
				Done		

When to Use the Use the Protocol Browser to perform the following:

- - Duplicate and edit protocols
 - Delete protocols
 - Change protocol use settings
Creating Protocols To create protocols with the Protocol Browser:

Step	Action		
1	In the Protocol Browser window, click the tab of the type of protocol you want to create:		
	Lysis/DNA Precipitation		
	RNA/DNA Archive		
	♦ cDNA Archive		
	Dilution Archive		
	◆ Assay		
2	Click the New button.		
	A New Protocol dialog box appears.		
3	Complete the dialog box as described in this chapter.		
	If you want to create	Then see page	
	a Lysis protocol	4-11	
	a DNA Precipitation protocol	4-15	
	an RNA Archive protocol	4-19	
	a DNA Archive protocol	4-31	
	a cDNA Archive protocol	4-43	
	a Dilution Archive protocol	4-47	
	an Assay protocol	4-53	
4	Click OK when you are finished ent	ering protocol conditions	Э.

Editing Protocols

Duplicating and To duplicate and edit a protocol:

Step	Action		
1	In the Protocol Browser window, click the tab of the type of protocol you want to duplicate:		
	Lysis/DNA Precipitation		
	RNA/DNA Archive		
	cDNA Archive		
	Dilution Archive		
	♦ Assay		
2	Select the protocol you want to duplicate by clicking it once.		
	The selected protocol is highlighted.		
	Note Default preference settings allow you to see all protocols in the database. To see only protocols that you created, change your preference settings ("Setting Preferences" on page 3-7).		
3	Click the Duplicate button.		
	A protocol dialog box appears with the settings of the protocol you selected.		

To duplicate and edit a protocol: (continued)

Step	Action		
4	Enter a Protocol Name.		
	Note The protocol name must be:		
	 A unique combination of letters, numbers, and spaces 		
	 No more than 32 characters long 		
5	To edit the protocol, change the settings.		
6	Click OK to add the duplicated or edited protocol and to return to the Protocol		
	Browser.		

Deleting Protocols To delete a protocol, it must fulfill the following criteria:

- You created the protocol.
- The protocol was never used in an instrument run.

To delete a protocol:

Step	Action			
1	In the Protocol Browser window, click the tab of the type of protocol you want to delete:			
	 Lysis/DNA Precipitation 			
	RNA/DNA Archive			
	cDNA Archive			
	Dilution Archive			
	♦ Assay			
2	Select the protocol you want to delete by clicking it once.			
	The selected protocol is highlighted.			
	Note The 6700 database requires protocol information for all protocols used in instrument runs to keep the run history intact. See the next section, "Changing Protocol Use Settings," to remove a protocol from use if you do not want it to appear in the protocol pop-up menu on the Protocol tab.			
3	Click the Delete button.			
	The software asks whether you really want to delete the protocol.			
4	Click OK to delete the protocol and to return to the Protocol Browser.			

Use Settings

Changing Protocol Change the use settings to perform the following:

- ٠ Remove a protocol from use if you do not want it to appear in the protocol pop-up menu on the Protocol tab.
- Add a protocol for use if you want to use the protocol in an instrument run.

To change use settings:

Step	Action		
1	In the Protocol Browser window, click the tab of the type of protocol for which you want to change In Use settings:		
	 Lysis/DNA Precipitation 		
	RNA/DNA Archive		
	◆ cDNA Archive		
	Dilution Archive		
	♦ Assay		
2	Double-click the protocol you want to s	et.	
	A View Protocol dialog box appears.		
3	If you want to	Then	
	remove the protocol from use	uncheck the In Use box.	
	add the protocol for use	check the In Use box.	
4	Click OK to change the setting and to r	eturn to the Protocol Browser.	

Section: Lysis Protocols

In This Section	The following topics are covered in this section:	
	Торіс	See Page
	Lysis Protocol Overview	4-11
	Lysis Protocol Creation	4-12

Lysis Protocol Overview

Description	During a Lysis protocol, the 6700 workstation lyses cells with Applied Biosystems
	reagents.

Lysis Process The table below describes how the 6700 workstation performs a Lysis protocol.

Lysis Process

Stage	Description	
1	The 6700 instrument adds lysis buffer to the samples in the input plate.	
2	The 6700 instrument mixes the samples and lysis buffer.	
3	The instrument incubates the plate containing the samples and lysis buffer at 4 °C.	
	Note Incubation is optional.	

Output Applications Lysis output can be used for RNA Archive protocols.

Lysis Protocol Creation

Lysis Conditions When you create a new Lysis protocol, you define the conditions displayed in the New Lysis/DNA Precipitation Protocol dialog box, as shown below.

Protocol Name: Lysis ✓ In Use Kind of Plate: Falcon 96-Well Plate ▼ Specify the conditions for creating the Lysis Plate Initial Sample Volume: 125 µL Lysis Buffer Volume: 125 µL Number of Mixes: 4
Г In Use Kind of Plate: Falcon 96-Well Plate Specify the conditions for creating the Lysis Plate Initial Sample Volume: 125 µL Lysis Buffer Volume: 125 µL Number of Mixes: 4
Kind of Plate: Falcon 96-Well Plate Specify the conditions for creating the Lysis Plate Initial Sample Volume: 125 µL Lysis Buffer Volume: 125 µL Number of Mixes: 4
Specify the conditions for creating the Lysis Plate Initial Sample Volume: 125 µL Lysis Buffer Volume: 125 µL Number of Mixes: 4
Initial Sample Volume: 125 µL Lysis Buffer Volume: 125 µL Number of Mixes: 4
Lysis Buffer Volume: 125 µL Number of Mixes: 4
Number of Mixes: 4
Incubation Time: 0 Minutes
DNA Solution 2 Volume µL
Number of Mixes:
Incubation Time: Minutes
Cancel OK

These conditions are described in the table below.

Lysis Protocol Conditions

Condition	Description	Accepted Values
Protocol Name	A unique name for a specific Lysis protocol	 A unique combination of letters, numbers, and spaces
		 Up to 32 characters
Kind of Plate	The kind of input plate to use	♦ Input 1:
		 Falcon 96-well plate
		 Costar 3596 plate
		 – Nunc 168055 plate
		♦ Input 2 or 3:
		 Deep-well plate
Initial Sample Volume	The volume of raw cells in the input plate	10 to 200 μL
		Note The sum of the Initial Sample Volume and Lysis Buffer Volume must be ≤300 μL.
Lysis Buffer Volume	The volume of lysis buffer to add to raw cells	10 to 200 μL
		Note The sum of the Initial Sample Volume and Lysis Buffer Volume must be ≤300 μL.
Number of Mixes	The number of times to mix the raw cells and lysis buffer by pipetting up and down	0 to 9 times
Incubation Time	The length of time to incubate the samples with lysis buffer at 4 °C	0 to 99 minutes

Creating Lysis	To creat	te Lysis protocols:
Protocols	Step	Action
	1	Go to the Protocol tab of the 6700 software.
	1 2	Go to the Protocol tab of the 6700 software. In the Protocol section, click the New button under the Lysis/DNA Precipitation protocol.
		Incubation Time: Minutes
	3	Enter a Protocol Name.
	_ -	

Defining Lysis To define lysis conditions:

Step	Action
1	Select the plate that will be used from the Kind of Plate pop-up menu.
2	Enter a value from 10 to 200 (μ L) for Initial Sample Volume .
	Note The sum of the Initial Sample Volume and Lysis Buffer Volume must be \leq 300 μ L.
3	Enter a value from 10 to 200 (μL) for Lysis Buffer Volume.
	Note The sum of the Initial Sample Volume and Lysis Buffer Volume must be \leq 300 μ L.
4	Enter a value from 0 to 9 for Number of Mixes.
5	Enter a value from 0 to 99 (minutes) for Incubation Time.
6	Click OK to save this protocol and to return to the Protocol tab.

Section: DNA Precipitation Protocols

In This Section The following topics are covered in this section:

Торіс					
DNA Precipitation Protocol Overview	4-15				
DNA Precipitation Protocol Creation					

DNA Precipitation Protocol Overview

Description	 During a DNA Precipitation protocol, the 6700 workstation adds up to two solutions to filtrate in a deep-well plate to precipitate DNA. The table below describes how the 6700 workstation performs a DNA Precipitation protocol. 						
DNA Precipitation Process							
	DNA Precipitation Process						
	Stage	Description					
	1 The 6700 instrument adds solution 1 to the filtrate plate.						
	2	The 6700 instrument mixes filtrate and solution 1.					
	3	The instrument incubates the plate containing the filtrate-solution 1 mixture at 4 °C.					
		Note Incubation is optional.					
	4	The 6700 instrument adds solution 2 to the filtrate-solution 1 mixture.					
	5	The 6700 instrument mixes the filtrate-solution 1-solution 2 mixture.					
	6	The instrument incubates the plate containing filtrate, solution 1, and solution 2 at 4 $^{\circ}\text{C}.$					
		Note Incubation is optional.					

Output Applications DNA precipitation output can be used for DNA Archive protocols.

DNA Precipitation Protocol Creation

DNA Precipitation
ConditionsWhen you create a new DNA Precipitation protocol, you define the conditions
displayed in the New Lysis/DNA Precipitation Protocol dialog box, as shown below.

New Lysis/DNA Precipitation Protocol
D. J. J. J. Blau DNA Brosinitation
Protocol Name: New DNA Precipitation
In Use
Kind of Plate: Deep-Well Plate
Specify the DNA Precipitation Protocol
لبر Starting Volume in Plate: 200
DNA Solution 1 Volume: 150 للس
Number of Mixes: 0
Incubation Time: 0 Minutes
DNA Solution 2 Volume 350 山
Number of Mixes: 3
Incubation Time: 0 Minutes
Cancel OK

These conditions are described in the table below.

DNA Precipitation Protocol Condition

Condition	Description	Accepted Values		
Protocol Name	A unique name for a specific DNA Precipitation protocol	 A unique combination of letters, numbers, and spaces 		
		Up to 32 characters		
Kind of Plate	The kind of input plate to use	Deep-well plate		
Starting Volume in Plate	The starting volume in the deep-well plate	≥10 μL Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be ≤850 μL.		
DNA Solution 1 Volume	The volume of solution 1 to add to the deep-well plate	≥10 μL Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be ≤850 μL.		
Number of Mixes	The number of times to mix the filtrate and solution 1 by pipetting up and down	0 to 9 times		
Incubation Time	The length of time to incubate the filtrate-solution 1 mixture at 4 °C	0 to 99 minutes		
DNA Solution 2 Volume	The volume of solution 2 to add to the filtrate-solution 1 mixture.	≥0 μL Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be ≤850 μL.		
Number of Mixes	The number of times to mix the filtrate-solution 1-solution 2 mixture by pipetting up and down	0 to 9 times		

DNA Precipitation Protocol Conditions (continued)

E.

Condition	Description	Accepted Values
Incubation Time	The length of time to incubate the filtrate-solution 1-solution 2 mixture at 4 °C	0 to 99 minutes

Creating DNA	To creat	e DNA Precipitation protocols:			
Protocols	Step	Action			
110000015	1	Go to the Protocol tab of the 6700 software.			
	2	In the Protocol section, click the New button under the Lysis/DNA Precipitation protocol.			
		I he New Lysis/DNA Precipitation Protocol dialog box appears.			
		DNA-Bolution 2 Volume - µL Number of Mixes: - Incubation Time: - Minutes Cancel OK			
	3	Enter a Protocol Name.			

Defining DNA	Defining DNA To define DNA precipitation conditions:						
Conditions	Step	Action					
Conditions	1	Select Deep-Well Plate from the Kind of Plate pop-up menu.					
		The dialog box changes to show conditions for defining a DNA Precipitation protocol.					
		DNA Solution 1 Volume: 150 μL Number of Mixes: 0 Incubation Time: 0 Minutes Incubation Time: 0 Minutes Incubation Time: O Minutes					
	2	Enter a value $\geq 10 \ (\mu L)$ for Starting Volume in Plate.					
		Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be \leq 850 µL.					
	3	Enter a value $\ge 10 \ (\mu L)$ for DNA Solution 1 Volume. Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be $\le 850 \ \mu L$.					
	4	Enter a value from 0 to 9 for Number of Mixes.					
	5	Enter a value from 0 to 99 (minutes) for Incubation Time.					
	6	Enter a value $\ge 0 \ \mu L$ for DNA Solution 2 Volume .					
		Note The sum of the Starting Volume in Plate, DNA Solution 1 Volume, and DNA Solution 2 Volume must be \leq 850 µL.					
	7	Enter a value from 0 to 9 for Number of Mixes.					
	8	Enter a value from 0 to 99 (minutes) for Incubation Time.					
	9	Click OK to save this protocol and to return to the Protocol tab.					

Section: RNA Archive Protocols

In This Section The following topics are covered in this section:

Торіс	See Page
RNA Archive Protocol Overview	4-19
RNA Archive Protocol Creation	4-21

RNA Archive Protocol Overview

Description	During an RNA Archive protocol, the 6700 workstation purifies RNA from cells lysed
	with Applied Biosystems reagents.

RNA Archive The table below describes how the 6700 workstation performs an RNA Archive process protocol.

RNA Archive Process

Stage	Description						
1	The 6700 instrument transfers cell lysate to a purification tray, which contains a filter in each well.						
2	The 6700 instrument applies vacuum pressure to the purification tray:						
	a. The filter captures the RNA.						
	b. DNA and other cellular debris flow through the filter into a filtrate plate or waste.						
	Note You can save the filtrate from this step for use in another run to purify DNA.						
3	The 6700 instrument washes the filter-bound RNA.						
4	The 6700 instrument elutes the RNA:						
	a. The instrument adds elution solution to release the RNA from the filter.						
	b. The instrument applies vacuum pressure to the purification tray.						
	c. The purified RNA elutes into the RNA archive plate at the vacuum station.						
5	If specified, the 6700 instrument adds a final addition fluid:						
	a. The instrument adds a final addition fluid, as specified by the user.						
	b. The instrument applies vacuum pressure to the purification tray.						
	c. The purified RNA elutes into the RNA archive plate at the vacuum station.						
6	If specified, the 6700 instrument covers the RNA archive plate with an archive cover.						

Output Applications RNA archive output can be used for:

- cDNA Archive protocols
- Dilution Archive protocols
- Assay protocols
- Northern blots, cDNA cloning, and transcript imaging
- ◆ Long-term storage at -80 °C

RNA Archive Protocol Creation

RNA Archive When you create a new RNA Archive protocol, you define the conditions displayed in Conditions the New RNA/DNA Archive Protocol dialog box, as shown below.

New RNA/DNA Archive Protocol									
Protocol Name: New RNA/DNA Archive Protocol 🔽 In Use									
Conditions for Transferring Samples to the Purification Tray									
Add Soln. Transfer Mix Starting									
Ealcon	Lysis/DNA Precipitation Input (µL) (µL) (#) (µL) Falcon 96.Well Plate First Transfer None 200 3 250								
1	Cocond Transfer 0 0 0								
Second transfer:									
Filtratio	n Condition	s			Create D	eep-Well I	Filtrate F	late	
Incuba	ation Time:	0 (m	nin.)		Vacuu	ım Time:	120	(sec.)	
	Vacuum Pressure: 20 💌 %								
Wash C	onditions		Volume	Temp	Incubatio	n Vacuum	Repea	at Vacuum	
Step	A	dd	(µL)	(°C)	(min)	(sec)	(count) (%)	
1. 🗹	Wash Sol	ution 1	400		0	120	1	20 💌	
2. 🗹	Wash Soli	ution 2	500		0	120	1	20 💌	
3. 🔽	Wash Soli	ution 2	300		0	120	2	20 💌	
4. 🗖			300		0	120	1	20 💌	
5. 🗖			300		0	120	1	20 💌	
6. 🗖			300		0	120	1	20 🔽	
7. 🗖			300		0	120	1	20 💌	
Pre-Elution Vacuum 300 9						90 💌			
	Elution So	lution	150	-	0	120	1	20 💌	
◄	Final Addi	tion Fluid	50		0	120	1	20 💌	
	Cancel OK								

These conditions are described in the table below.

RNA Archive Protocol Conditions

Conditions	Description	Accepted Values		
Protocol Name	A unique name for a specific RNA Archive protocol	 A unique combination of letters, numbers, and spaces 		
		 Up to 32 characters 		
Transfer Conditions				
Lysis/DNA Precipitation Input	The kind of input plate to use	Falcon 96-well plate		
		Costar 3596 plate		
		 Nunc 168055 plate 		
		Deep-well plate		

RNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values
First Transfer:	Transfer (μL)	The volume to transfer from the input plate to the purification tray	$\begin{tabular}{ c c c c } \hline & Accepted \\ \hline \mbox{Input Plate} & Values \\ \hline \mbox{Falcon 96-well} & 5 to 250 \ \mu L \\ \hline \mbox{plate} & 5 to 250 \ \mu L \\ \hline \mbox{Oostar 3596} & \\ \hline \mbox{plate} & \\ \hline \mbox{Costar 3596} & \\ \hline \mbox{plate} & \\ \hline \mbox{Deep-well plate} & 5 to 700 \ \mu L \\ \hline \mbox{IMPORTANT} & You must leave \\ \hline \mbox{behind $\geq 50 \ \mu L$ in the lysis input plate.} \\ \hline \mbox{To transfer more cells, enter Second} \\ \hline \mbox{Transfer conditions.} \\ \hline \end{tabular}$
	Mix (#)	The number of times to mix the lysed cells by pipetting up and down before aspirating	0 to 9 times
Second Transfer: Note Specify Second Transfer conditions to	Add Soln. (μL)	The volume of solution to add to the input plate before transferring to the purification tray Note Adding solution to the input plate permits the robotic arm to access more of the sample.	≤ First Transfer: Transfer volume
maximize transfer of lysed cells to the purification	Transfer (μL)	The volume to transfer from the input plate to the purification tray.	\leq Second Transfer: Add Soln. volume IMPORTANT You must leave behind \geq 50 µL in the lysis input plate.
tray.	Mix (#)	The number of times to mix the lysed cells by pipetting up and down before aspirating	0 to 9 times
High Viscosity Sample		Whether or not to decrease the rate of aspirating and dispensing samples by the robotic arm tips	 Checked Unchecked Note Check the box when the sample is viscous, <i>e.g.</i>, when using crushed tissue samples.
		Filtration Conditions	
Create Deep-Well Filtrate Plate		Whether or not to collect the first filtrate for a subsequent protocol	CheckedUnchecked
Incubation Time (min.)		The length of time to capture samples on the purification tray before applying vacuum pressure	0 to 99 minutes
Vacuum Time (se	ec.)	The length of time to apply vacuum pressure	0 to 999 seconds
Vacuum Pressure %		The level of vacuum pressure to apply during transfer of samples to the purification tray	10 to 100%, in 10% increments

RNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values	
		Wash Conditions		
Step		The number of wash steps to include	1 to 7 contiguous steps	
		Note At least one wash step is required to pre-wet the purification tray before elution.		
Add		For each wash step, the name of the wash solution to add over the purification tray	Up to 32 characters	
Volume (µL)		For each wash step, the volume of wash solution to add over the purification tray	40 to 650 μL	
Incubation (min)		For each wash step, the length of time to wash the samples on the purification tray before applying vacuum pressure	0 to 99 minutes	
Vacuum (sec)		For each wash step, the length of time to apply vacuum pressure	0 to 999 seconds	
Repeat (count)		For each wash step, the number of times to repeat the step	1 to 9	
Vacuum (%)		For each wash step, the amount of vacuum pressure to apply	10 to 100%, in 10% increments	
		Pre-Elution Vacuum Conditions		
Pre-Elution Vacuum (sec) Vacuum		The length of time to apply vacuum pressure before elution	1 to 999 seconds	
Vacuum (%)		The amount of vacuum pressure to apply before elution	10 to 100%, in 10% increments	
		Elution Conditions		
Elution Volume (μL) Solution		The volume of elution solution to add over the purification tray	40 to 200 μL Note The sum of the Elution Solution and Final Addition Fluid must be ≤200 μL.	
Incubation (min)		The length of time to incubate the elution solution and samples on the purification tray before applying vacuum pressure	0 to 99 minutes	
	Vacuum (sec)	The length of time to apply vacuum pressure during elution	1 to 999 seconds	
	Vacuum (%)	The amount of vacuum pressure to apply to the purification tray during elution	10 to 100%, in 10% increments	

RNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values
Final Addition Volume (μL) Fluid Note Note This is an optional		The volume of final addition fluid to add over the purification tray	5 to 200 μL Note The sum of the Elution Solution and Final Addition Fluid must be ≤200 μL.
step.	Incubation (min)	The length of time to incubate the final addition fluid and samples on the purification tray before applying vacuum pressure	0 to 99 minutes
	Vacuum (sec)	The length of time to apply vacuum pressure	1 to 999 seconds
	Vacuum (%)	The amount of vacuum pressure to apply to the purification tray	10 to 100%, in 10% increments

Creating RNA Archive Protocols	To creat	te RNA Archive protocols:					
	Step	Action					
	1	Go to the Protocol tab of the 6700 software.					
	2	In the Protocol section, click the New button under the RNA/DNA Archive protocol RNA/DNA Archive 6700 RNA Archive View Click this button The New RNA/DNA Archive Protocol dialog box appears.					
		New RNA/DNA Archive Protocol					
		Protocol Name: New RNA/DNA Archive Protocol					
		Conditions for Transferring Samples to the Purification Tray					
		Add Soln. Transfer Mix Starting Lysis/DNA Precipitation Input (µL) (µL) (µL)					
		Falcon 96-Well Plate First Transfer: None 200 3 250 Transfer conditions					
		Second Transfer: 0 0 0					
		Filtration Conditions Create Deep-Well Filtrate Plate					
		Incubation Time: 0 (min.) Vacuum Time: 120 (sec.) Filtration conditions Vacuum Pressure: 20 💌 %					
		Wash Conditions Step Add Temp. (μL) Incubation Vacuum (mini) Repeat Vacuum (count) Name 1. ■ Wash Solution 1 400 0 120 1 20 ■ 2. ■ Wash Solution 2 500 0 120 1 20 ■ 3. ■ Wash Solution 2 300 0 120 1 20 ■ 4. ■ 300 0 120 1 20 ■ ■ 5. ■ 300 0 120 1 20 ■ ■ 6. ■ 300 0 120 1 20 ■ ■ 7. ■ 300 0 120 1 20 ■ ■ Pre-Elution Vacuum 300 0 120 1 20 ■ ■ Pre-Elution Solution 150 0 120 1 20 ■ ■ Final Addition Fluid 50 0 120 1 20 ■ ■ Final Addition Fluid 50 0 120 1 20 ■ ■					
	3	Enter a Protocol Name.					

R

Protocol Creation 4-25

 Step	Action		
1	Select the plate that will be used from the Lysis/DNA Precipitation Input pop-up menu.		
	Conditions for Transferring Samples to the Pu	ification Tray	
	A Lysis/DNA Precipitation Input	ddSoln. Transfer Mix Starting	
	Falcon 96-Well Plate First Transfer:	None 200 3 250	
	Second Transfer:	0 0 0	
	Γ	High Viscosity Sample	
2	Enter the First Transfer conditions:		
	a. In the Transfer (μL) field, enter the amo tray.	ount of lysed cells to add to the purification	
	Input Plate	Accepted Value (µL)	
	Falcon 96-well plate	5 to 250	
	Costar 3596 plate		
	Nunc 168055 plate		
	Deep-well plate	5 to 700	
	IMPORTANT You must leave behind ≥5 cells, enter the Second Transfer condition	$0 \ \mu L$ in the input plate. To transfer more s using step 3.	
	b. In the Mix (#) field, enter a value from 0 aspirating samples.	to 9 for the number of times to mix befo	
3	To transfer more lysed cells, enter the Se	cond Transfer conditions:	
	a. In the Add Soln. (μ L) field, enter a volume \leq First Transfer: Transfer volume for the amount of solution to add to the input plate.		
	b. In the Transfer (μ L) field, enter a volume \leq Second Transfer: Add Soln. volume for the amount of lysed cells-solution mixture to add to the purification tray.		
	IMPORTANT You must leave behind \geq 50 µL in the input plate.		
	c. In the Mix (#) field, enter a value from 0 aspirating samples.	to 9 for the number of times to mix befor	
4	To decrease the rate of aspiration for high	n-viscosity samples (e.g., crushed tissue	

-

Defining Filtration To define filtration conditions:						
Conditions	Step Action 1 To save the filtrate, check the Create Deep-Well Filtrate Plate box.					
		Note When this box is checked, the 6700 instrument saves the filtrate that flow through the purification tray from sample transfer(s).				
		Filtration Conditions	Create Deep-Well Filtrate Plate			
		Incubation Time: 0 (min.)	Vacuum Time: 120 (sec.)			
			Vacuum Pressure: 20 💌 %			
	2	In the Incubation Time (min.) field, enter a volume to capture samples on the purificat pressure.	value from 0 to 99 (minutes) for the length ion tray before applying vacuum			
	3	B Enter a value from 0 to 999 (seconds) in the Vacuum Time (sec.) field.				
	4	Select the Vacuum Pressure % from the pop-up menu. Select the vacuum based on sample viscosity:				
		If sample viscosity is	Then select			
		low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%)			
		high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%)			

Defining Wash С

Conditions	

Го	define	wash	conditions:
	acinic	waon	001101101101

Step	Action							
1	Check up to 7 boxes in the Step column for each wash step to perform. Note At least one wash step is required to pre-wet the purification tray before elution.							
	Wash Conditions	Volume	Temp.	Incubation	Vacuum	Repeat	Vacuum	
	Step A	dd (للبر) olution 1 400	(°C)	(min)	(sec)	(count)	(%)	
	2. 🔽 Wash S	olution 2 500			120	1	20 -	
	3. 🔽 🛛 Wash S	olution 2 300		0	120	2	20 -	
	4.	300		0	120	1	20 💌	
	5. 🗖	300		0	120	1	20 💌	
	6. 🔲	300		0	120	1	20 💌	
	7. 🗖	300		0	120	1	20 💌	
	Pre-Eluti	on Vacuum			300		90 🔻	
	Elution S	olution 150	-	0	120	1	20 💌	
2	In the Add field for each wash step, enter the name of the wash solution to add.							
3	In the Volume (μ L the volume of wa	.) field for each was sh solution to add	ash step d over th	o, enter a ne purifica	volume tion tray	from 40 /.) to 650 (μL) for

To define wash conditions: (continued)

Step	Action				
4	In the Incubation (min) field for each wash step, enter a value from 0 to 99 (minutes) for the length of time to wash the samples on the purification tray before applying vacuum pressure.				
5	In the Vacuum (sec) field for each wash sto for the length of time to apply vacuum pre	ep, enter a value from 0 to 999 (seconds) ssure.			
6	In the Repeat (count) field for each wash step, enter a value from 1 to 9 for the number of times to repeat the wash step.				
7	Select the vacuum pressure for each wash step from the Vacuum (%) pop-up menu:				
	If sample viscosity is Then select				
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).			
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).			

Defining Pre-Elution To define pre-elution vacuum conditions:

Vacuum Conditions

Step	Action				
1	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure before performing elution.				
2	Select the vacuum pressure from the Vacuum (%) pop-up menu: If sample viscosity is Then select				
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).			
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).			

Defining Elution To define elution conditions: Conditions

Step	Action		
1	In the Volume (μ L) field, enter a volume from 40 to 200 (μ L) for the volume of elution solution to add over the purification tray.		
	Note The sum of the Elution Solution ar	nd Final Addition Fluid must be ≤200 μL.	
2	In the Incubation (min) field, enter a value from 0 to 99 (minutes) for the length of time to incubate the elution solution and samples on the purification tray before applying vacuum pressure.		
3	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure during elution.		
4	Select the vacuum pressure from the Vacuum (%) pop-up menu:		
	If sample viscosity is	Then select	
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).	
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).	

To define elution conditions: (continued)

Step	Action
5	Click OK to save this protocol and to return to the Protocol tab.

Defining Final Addition Fluid Conditions

Defining Final Note This is an optional step.

To define final addition fluid conditions:

Step	Action				
1	If you would like to add a final addition fluid, check the Final Addition Fluid checkbox and, if desired, type the name of the fluid in the text field (replacing Final Addition Fluid).				
	Note This is often a second elution step, in which more elution solution is added. However, you may add a different fluid per your specific chemistry requirements.				
2	In the Volume (μ L) field, enter a volume from 5 to 200 (μ L) for the volume of elution solution to add over the purification tray.				
3	In the Incubation (min) field, enter a value from 0 to 99 (minutes) for the length of time to incubate the final addition fluid and samples on the purification tray before applying vacuum pressure.				
4	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure.				
5	Select the vacuum pressure from the Vacuum (%) pop-up menu:				
	If sample viscosity is	Then select			
	lowa lower vacuum pressure(similar to the consistency of water)(e.g., from 20% to 50%).				
	higha higher vacuum pressure(similar to the consistency of glycerol)(e.g., from 50% to 90%).				
6	Click OK to save this protocol and to retur	n to the Protocol tab.			

Section: DNA Archive Protocols

In This Section The following topics are in this section:

Торіс	See Page
DNA Archive Protocol Overview	4-31
DNA Archive Protocol Creation	4-33

DNA Archive Protocol Overview

Description	During a DNA Archive protocol, the 6700 workstation purifies DNA from DNA
	precipitate.

DNA Archive The table below describes how the 6700 workstation performs a DNA Archive Process protocol.

DNA Archive Process

Stage	Description				
1	The 6700 instrument transfers DNA precipitate to a purification tray, which contains a filter in each well.				
2	The 6700 instrument applies vacuum pressure to the purification tray:				
	a. The filter captures the DNA.				
	b. Cellular debris flows through the filter.				
	Note You can save the filtrate from this step for use in a subsequent protocol.				
3	The 6700 instrument washes the filter-bound DNA.				
4	The 6700 instrument elutes the DNA:				
	a. The instrument adds elution solution to release the DNA from the filter.				
	b. The instrument applies vacuum pressure to the purification tray.				
	c. The purified DNA elutes into a DNA archive plate at the Archive Cover station.				
5	If specified, the 6700 instrument adds a final addition fluid:				
	a. The instrument adds a final addition fluid, as specified by the user.				
	b. The instrument applies vacuum pressure to the purification tray.				
	c. The purified RNA elutes into the RNA archive plate at the vacuum station.				
6	If specified, the 6700 instrument covers the DNA archive plate with an archive cover.				

Output Applications DNA archive output can be used for:

- Dilution Archive protocols
- Assay protocols
- Southern blots, cloning, and sequencing
- Long-term storage at -80 °C

DNA Archive Protocol Creation

DNA Archive
ConditionsWhen you create a new DNA Archive protocol, you define the conditions displayed in
the New RNA/DNA Archive Protocol dialog box, as shown below.

New RNA/DNA Archive Protocol							
Proto	col Name: New RN	IA/DNA Arch	nive Proto	ocol	I	🗸 In Us	e
Conditio	ons for Transferring S	Samples to	the Puri	fication Tra	ay		
			Ad	d Soln. Ti	ransfer	Mix	Starting
Lysis/D	NA Precipitation Input			(止) · · · · · ·	(<u>)</u>	(#)	(山)
Deep-v	vell Plate 🗾	First Frai	nster:	None	600	3	650
	8	Second Trai	nsfer:	0	0	0	
				High Visc	osity Sam	nple	
Filtratio	n Conditions			Create De	eep-Well F	iltrate P	late
Incub	ation Time: 0 (min.)		Vacuu	m Time: 🛛	120	(sec.)
				Vacuum Pi	ressure:	20	▼ %
Wash C	Conditions	Volumo	Tomo	Incubation		Donoo	+ \/oouum
Step	Add	volume (山)	(°C)	(min)	(sec)	(count) (%)
1. 🗹	Wash Solution 1	400		0	120	1	20 💌
2. 🗹	Wash Solution 2	500		0	120	1	20 💌
3. 🔽	Wash Solution 2	300		0	120	2	20 💌
4. 🗖		300		0	120	1	20 💌
5. 🗖		300		0	120	1	20 💌
6. 🗖		300		0	120	1	20 💌
7. 🗖		300		0	120	1	20 💌
	Pre-Elution Vacuum				300		90 💌
	Elution Solution	150	-	0	120	1	20 💌
~	Final Addition Fluid	50		0	120	1	20 💌
				С	ancel		Ж

These conditions are described in the table below.

DNA Archive Protocol Conditions

Conditions	Description	Accepted Values	
Protocol Name	A unique name for a specific DNA Archive protocol	 A unique combination of letters, numbers, and spaces 	
		 Up to 32 characters 	
Transfer Conditions			
Lysis/DNA Precipitation Input	The kind of input plate to use	 Falcon 96-well plate 	
		 Costar 3596 plate 	
		 Nunc 168055 plate 	
		Deep-well plate	

DNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values		
First Transfer:	Transfer (μL)	The volume to transfer from the input plate to the purification tray	Input Plate	Accepted Values	
			♦ Falcon	5 to 250 μL	
			 Costar 		
			♦ Nunc		
			Deep-well plate	5 to 700 μL	
			IMPORTANT You me behind ≥50 μL in the ly To transfer more cells, Transfer conditions.	ust leave ysis input plate. , enter Second	
	Mix (#)	The number of times to mix the lysed cells by pipetting up and down before aspirating	0 to 9 times		
Second Transfer: Note Specify	Add Soln. (μL)	The volume of solution to add to the input plate before transferring to the purification tray	≤ First Transfer: Transf	ier volume	
Second Transfer conditions to		Note Addition of solution to the input plate permits the robotic arm to access more of the sample.			
maximize transfer of lysed cells to the purification	Transfer (μL)	The volume to transfer from the input plate to the purification tray.	\leq Second Transfer: Ad IMPORTANT You minimize behind \geq 50 µL in the light	d Soln. volume ust leave ysis input plate	
tray.	Mix (#)	The number of times to mix the lysed cells by pipetting up and down before aspirating	0 to 9 times		
High Viscosity Sample		Whether or not to decrease the rate of aspirating and dispensing samples by the robotic arm tips	 Checked Unchecked Note Check the box sample is viscous, <i>e.g</i> crushed tissue sample chromosomal DNA. 	when the ., when using es or	
		Filtration Conditions	1		
Create Deep-Wel	I Filtrate Plate	Whether or not to collect the first filtrate for	 Checked 		
			Unchecked		
Incubation Time (min.)		The length of time to capture samples on the purification tray before applying vacuum pressure	0 to 99 minutes		
Vacuum Time (sec.)		The length of time to apply vacuum pressure	0 to 999 seconds		
Vacuum Pressure %		The level of vacuum pressure to apply during transfer of samples to the purification tray	10 to 100%, in 10% increments		

DNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values			
	Wash Conditions					
Step		The number of wash steps to include	1 to 7 contiguous steps			
		Note At least one wash step is required to pre-wet the purification tray before elution.				
Add		For each wash step, the name of the Wash Solution to add over the purification tray	Up to 32 characters			
Volume (µL)		For each wash step, the volume of Wash Solution to add over the purification tray	40 to 650 μL			
Incubation (min)		For each wash step, the length of time to wash the samples on the purification tray before applying vacuum pressure	0 to 99 minutes			
Vacuum (sec)		For each wash step, the length of time to apply vacuum pressure	0 to 999 seconds			
Repeat (count)		For each wash step, the number of times to repeat the step	1 to 9			
Vacuum (%)		For each wash step, the amount of vacuum pressure to apply	10 to 100%, in 10% increments			
		Pre-Elution Vacuum Conditions				
Pre-Elution Vacuum (sec) Vacuum		The length of time to apply vacuum pressure before elution	1 to 999 seconds			
	Vacuum (%)	The amount of vacuum pressure to apply before elution	10 to 100%, in 10% increments			
		Elution Conditions				
Elution Solution Incubation (min) Vacuum (sec)		The volume of elution solution to add over the purification tray	40 to 200 μL Note The sum of the Elution Solution and Final Addition Fluid must be ≤200 μL.			
		The length of time to incubate the elution solution and samples on the purification tray before applying vacuum pressure	0 to 99 minutes			
		The length of time to apply vacuum pressure during elution	1 to 999 seconds			
	Vacuum (%)	The amount of vacuum pressure to apply to the purification tray during elution	10 to 100%, in 10% increments			

DNA Archive Protocol Conditions (continued)

Conditions		Description	Accepted Values
Final Addition Fluid Note This is an optional	Volume (μL)	The volume of final addition fluid to add over the purification tray	5 to 200 μL Note The sum of the Elution Solution and Final Addition Fluid must be ≤200 μL.
step.	Incubation (min)	The length of time to incubate the final addition fluid and samples on the purification tray before applying vacuum pressure	0 to 99 minutes
	Vacuum (sec)	The length of time to apply vacuum pressure	1 to 999 seconds
	Vacuum (%)	The amount of vacuum pressure to apply to the purification tray	10 to 100%, in 10% increments

Creating DNA	e DNA Archive protocols:	
Archive Protocols	Step	Action
	1	Go to the Protocol tab of the 6700 software.
	2	In the Protocol section, click the New button under the RNA/DNA Archive protocol.
		New RNA/DNA Archive Protocol
		Protocol Name: New RNA/DNA Archive Protocol 🔽 In Use
		Conditions for Transferring Samples to the Purification Tray Add Soln, Transfer Mix Starting
		Lysis/DNA Precipitation Input (µL) (µL) (#) (µL) Deen-Well Plate First Transfer Noos 500 2 550
		Second Transfer: 0 0 0 Transfer conditions
		Incubation Time: 0 (min.) Vacuum Time: 120 (sec.) Filtration conditions
		Wash Conditions Volume Temp. Incubation Vacuum Repeat Vacuum Step Add (µL) 0 120 1 20 1 1 Wash Solution 1 400 0 120 1 20 1 2. Wash Solution 2 500 0 120 1 20 1 3. Wash Solution 2 300 0 120 1 20 1 4. 300 0 120 1 20 1 20 1 6. 300 0 120 1 20 1 20 1 7. 300 0 120 1 20 1 20 1 7. 300 0 120 1 20 1 Pre-elution vacuum cond. Cond. Pre-Elution Vacuum 300 90 1 20 Elution conditions Final Addition Fluid 50 0 120 1 20 Final addition conditions
	3	Enter a Protocol Name.

0 0 0 - 0 - 0 - 0	Step	Action			
	1	Select the plate that will be used from the menu.	Lysis/DNA Precipitation Input pop-up		
		Conditions for Transferring Samples to the Pur	ification Tray dd Soln Transfer Miv Starting		
		Lysis/DNA Precipitation Input	(д.) (д.) (#) (д.) None 200 3 250		
		Second Transfer:	U U U U		
	2				
		 a. In the Transfer (μL) field, enter the amo tray. 	ount of lysed cells to add to the purification		
		Input Plate	Accepted Values (µL)		
		Falcon 96-well plate	5 to 250		
		Costar 3596 plate			
		Nunc 168055 plate			
		Deep-well plate	5 to 700		
		IMPORTANT You must leave behind ≥5 cells, enter the Second Transfer condition	0 μ L in the input plate. To transfer more s using step 3.		
		b. In the Mix (#) field, enter a value from 0 aspirating samples.	to 9 for the number of times to mix before		
	3	To transfer more lysed cells, enter the Second Transfer conditions:			
		 a. In the Add Soln. (μL) field, enter a volume ≤ First Transfer: Transfer volume for the amount of solution to add to the input plate. 			
		b. In the Transfer (μL) field, enter a volum the amount of lysed cells-solution mixt	e ≤ Second Transfer: Add Soln. volume fo ure to add to the purification tray.		
		IMPORTANT You must leave behind $\geq 50 \ \mu$ L in the input plate.			
		c. In the Mix (#) field, enter a value from 0 aspirating samples.	to 9 for the number of times to mix before		
	4	To decrease the rate of aspiration for high samples or chromosomal DNA), check the	n-viscosity samples (<i>e.g.</i> , crushed tissue		

Defining Filtration	To defin	e filtration conditions:			
Conditions	Step	Action			
	1	To save the filtrate, check the Create Deer	-Well Filtrate Plate box.		
		Note When this box is checked, the 670 through the purification tray from sample the purification tray fro	00 instrument saves the filtrate that flows transfer(s).		
		Filtration Conditions	Create Deep-Well Filtrate Plate		
		Incubation Time: 0 (min.)	Vacuum Time: 120 (sec.)		
			Vacuum Pressure: 20 💌 %		
	2	In the Incubation Time (min.) field, enter a of time to capture samples on the purifica pressure.	value from 0 to 99 (minutes) for the length tion tray before applying vacuum		
	3	Enter a value from 0 to 999 (seconds) in the Vacuum Time (sec.) field.			
	4	Select the Vacuum Pressure % from the pop-up menu. Select the vacuum pressure based on sample viscosity:			
		If sample viscosity is	Then select		
		low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).		
		high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).		

Defining Wash To

С	ond	lit	io	ns
\sim	one	***		110

0	define	wash	conditions:

Step	Action				
1	Check up to 7 boxes in the Step column for each wash step to perform.				
	Note At least one wash step is required to pre-wet the purification tray before elution.				
	Wash Conditions Volume Temp. Incubation Vacuum Repeat Vacuum Step Add (山) (°C) (min) (sec) (count) (%)				
	1. Wash Solution 1 400 0 120 1 20 💌				
	2, 🔽 Wash Solution 2 500 0 120 1 20 💌				
	3. 🗹 Wash Solution 2 300 0 120 2 20 💌				
	4. 300 0 120 1 20 💌				
	5. 300 0 120 1 20 🗸				
	6. 300 0 120 1 20 •				
	7.				
	Pre-Elution Vacuum 300 90 💌				
	Elution Solution 150 - 0 120 1 20 🗸				
2	In the Add field for each wash step, enter the name of the wash solution to add.				
3	In the Volume (μ L) field for each wash step, enter a volume from 40 to 650 (μ L) for the volume of wash solution to add over the purification tray.				

To define wash conditions: (continued)

Step	Action		
4	In the Incubation (min) field for each wash step, enter a value from 0 to 99 (minutes) for the length of time to wash the samples on the purification tray before applying vacuum pressure.		
5	In the Vacuum (sec) field for each wash step, enter a value from 0 to 999 (seconds) for the length of time to apply vacuum pressure.		
6	In the Repeat (count) field for each wash step, enter a value from 1 to 9 for the number of times to repeat the wash step.		
7	Select the vacuum pressure for each wash step from the Vacuum (%) pop-up menu:		
	If sample viscosity is Then select		
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).	
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).	

Defining Pre-Elution To define pre-elution vacuum conditions:

Vacuum Conditions

Step	Action		
1	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure before performing elution.		
2	Select the vacuum pressure from the Vacuum (%) pop-up menu: If sample viscosity is		
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).	
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).	

Defining Elution To define elution conditions: Conditions

Step	Action		
1	In the Volume (μ L) field, enter a volume from 40 to 200 (μ L) for the volume of elution solution to add over the purification tray.		
	Note The sum of the Elution Solution and Final Addition Fluid must be $\leq 200 \ \mu$ L.		
2	In the Incubation (min) field, enter a value from 0 to 99 (minutes) for the length of time to incubate the elution solution and samples on the purification tray before applying vacuum pressure.		
3	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure during elution.		
4	Select the vacuum pressure from the Vacuum (%) pop-up menu:		
	If sample viscosity is Then select		
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).	
	high (similar to the consistency of glycerol)	a higher vacuum pressure (<i>e.g.</i> , from 50% to 90%).	

To define elution conditions: (continued)

Step	Action
5	Click OK to save this protocol and to return to the Protocol tab.

Defining Final Addition Fluid Conditions

Defining Final Note This is an optional step.

To define final addition fluid conditions:

Step	Action		
1	If you would like to add a final addition fluid, check the Final Addition Fluid checkbox and, if desired, type the name of the fluid in the text field (replacing Final Addition Fluid).		
	Note This is often a second elution step, in which more elution solution is added. However, you may add a different fluid per your specific chemistry requirements.		
2	In the Volume (μ L) field, enter a volume from 5 to 200 (μ L) for the volume of elution solution to add over the purification tray.		
3	In the Incubation (min) field, enter a value from 0 to 99 (minutes) for the length of time to incubate the final addition fluid and samples on the purification tray before applying vacuum pressure.		
4	In the Vacuum (sec) field, enter a value from 1 to 999 (seconds) for the length of time to apply vacuum pressure.		
5	Select the vacuum pressure from the Vacuum (%) pop-up menu:		
	If sample viscosity is Then select		
	low (similar to the consistency of water)	a lower vacuum pressure (<i>e.g.</i> , from 20% to 50%).	
	higha higher vacuum pressure(similar to the consistency of glycerol)(e.g., from 50% to 90%).		
6	Click OK to save this protocol and to return to the Protocol tab.		
Section: cDNA Archive Protocols

In This Section This section covers the following topics:

Торіс	See Page
cDNA Archive Protocol Overview	4-43
cDNA Archive Protocol Creation	4-44

cDNA Archive Protocol Overview

Description During a cDNA Archive protocol, the 6700 workstation transfers RNA and reverse transcription (RT) master mix to a cDNA archive plate and heats the plate to reverse transcribe cDNA from RNA.

cDNA Archive The table below describes how the 6700 workstation performs a cDNA Archive Process protocol.

cDNA Archive Process

Stage	Description	
1	The 6700 instrument transfers RNA from the input plate to the cDNA archive plate.	
2	The 6700 instrument adds RT master mix to the cDNA archive plate.	
3	The 6700 instrument's robotic arm places an archive cover on the cDNA archive plate.	
4	The 6700 instrument heats the cDNA archive plate to perform RT.	
5	After completing the cDNA Archive protocol, the instrument cools the Dilutions/cDNA station to 4 °C.	

Output Applications cDNA archive output can be used for:

- Dilution Archive protocols
- Assay protocols
- cDNA cloning
- ♦ Long-term storage at –80 °C

cDNA Archive Protocol Creation

cDNA Archive Conditions When you create a new cDNA Archive protocol, you define the conditions displayed in the New cDNA Archive Protocol dialog box, as shown below.

New cDNA Archive Protocol	×		
Protocol Name: New cDNA Archive Protocol In Use: 🗹	j		
Specify the conditions for transferring samples:			
RT Master Mix Add Volume: 50 µL Number of Mixes: 2			
Specify the temperature and time conditions:			
(°C) (min)			
Step 1: 25 10			
Step 2: 37 120			
Cancel OK			

These conditions are described in the table below.

cDNA Archive Protocol Conditions

Condition		Description	Accepted Values
Protocol Name		A unique name for a specific cDNA Archive protocol	 A unique combination of numbers, letters, and spaces
			 Up to 32 characters
Sample Transfer Volume		The volume of sample to transfer to the cDNA archive plate	5 to 145 μL Note The sum of the Sample Transfer Volume and RT Master Mix Add Volume must be ≤150 μL.
RT Master Mix Add Volume		The volume of RT master mix to transfer to the cDNA archive plate	5 to 145 μL Note The sum of the Sample Transfer Volume and RT Master Mix Add Volume must be ≤150 μL.
Number of Mixes		The number of times to mix sample and master mix by pipetting up and down	0 to 9 times
Step 1:	Temp (°C)	The temperature of the cDNA archive plate for Step 1	4 to 50 °C
	Duration (min)	The length of time to incubate the cDNA archive plate for Step 1	1 to 180 minutes
Step 2:	Temp (°C)	The temperature of the cDNA archive plate for Step 2	4 to 50 °C
	Duration (min)	The length of time to incubate the cDNA archive plate for Step 2	0 to 180 minutes

Creating cDNA	To creat	te cDNA Archive protocols:		
Archive Frotocols	Step	Action		
	1	Go to the Protocol tab of the 6700 software.		
	2	In the Protocol section, click the New button under the cDNA Archive protocol.		
		Click this button The New cDNA Archive Protocol dialog box appears. New cDNA Archive Protocol dialog box appears. New cDNA Archive Protocol Protocol Name: New cDNA Archive Protocol In Use: ♥ Specify the conditions for transferring samples: Sample Transfer Volume: 50 µL Number of Mixes: 2 Specify the temperature and time conditions: Temp Duration (°C) (°C) Step 1: 25 10 Step 2: 37 120 Cancel OK		
	3	Enter a Protocol Name.		

Defining cDNA Archive Conditions 1

 $Defining \ cDNA \quad \mbox{To define cDNA archive conditions:}$

Step	Action		
1	In the Sample Transfer Volume field, enter a value from 5 to 145 (μ L) for the volume of sample to transfer to the cDNA archive plate.		
	Note The sum of the Sample Transfer Volume and RT Master Mix Add Volume must be $\leq 150 \ \mu L$.		
2	In the RT Master Mix Add Volume field, enter a value from 5 to 145 (μ L) for the volume of RT master mix to add to the cDNA archive plate.		
	Note The sum of the Sample Transfer Volume and RT Master Mix Add Volume must be $\leq 150 \ \mu$ L.		
3	In the Number of Mixes field, enter a value from 0 to 9 for the number of times to mix sample and master mix by pipetting up and down.		
4	Specify temperature and duration conditions for Step 1:		
	a. In the Temp (° C) field, enter a value from 4 to 50 (°C) for the temperature of the cDNA archive plate.		
	b. In the Duration (min) field, enter a value from 1 to 180 (minutes) for the length of time to incubate the cDNA archive plate for Step 1.		

To define cDNA archive conditions: (continued)

Step	Action		
5	Specify temperature and duration conditions for Step 2:		
	a. In the Temp (° C) field, enter a value from 4 to 50 (°C) for the temperature of the cDNA archive plate.		
	b. In the Duration (min) field, enter a value from 0 to 180 (minutes) for the length of time to incubate the cDNA archive plate for Step 2.		
6	Click OK to save this protocol and to return to the Protocol tab.		

Section: Dilution Archive Protocols

In This Section This section covers the following topics:

Торіс	See Page
Dilution Archive Protocol Overview	4-47
Dilution Archive Protocol Creation	4-48

Dilution Archive Protocol Overview

Description	During a Dilution Archive protocol, the 6700 workstation performs up to two serial
_	dilutions of an RNA archive, DNA archive, or cDNA archive plate.

Dilution Archive The table below describes how the 6700 workstation performs a Dilution Archive Process protocol.

Dilution Archive Process

Stage	Description
1	The 6700 instrument adds diluent (dilution solution) to dilution archive plate 1.
2	The 6700 instrument transfers the amount of RNA, DNA, or cDNA to be diluted to dilution archive plate 1.
3	The 6700 instrument mixes the sample and diluent in dilution archive plate 1.
4	If a serial dilution is specified in the Dilution Archive protocol, the 6700 instrument adds diluent (dilution solution) to dilution archive plate 2.
5	If specified, the 6700 instrument transfers diluted sample from dilution archive plate 1 into dilution archive plate 2.
6	The 6700 instrument mixes the sample and diluent in dilution archive plate 2.
7	If specified, the 6700 instrument covers the dilution archive plates with archive covers.

Output Applications Dilution archive output can be used for:

- RNA Archive protocol output applications (see page 4-20) •
- DNA Archive protocol output applications (see page 4-32)
- cDNA Archive protocol output applications (see page 4-43)

Dilution Archive Protocol Creation

Dilution Archive
ConditionsWhen you create a new Dilution Archive protocol, you define the conditions displayed
in the New Dilution Archive Protocol dialog box, as shown below.

New Dilution Archive Protocol			
Protocol Name: New Dilution Archive Protocol			
In Use: 💌			
Specify the conditions for creating Dilution Archive Plate 1			
Dilute the Archive Sample at 1:2 💌			
for a total volume of 📃 200 🖃 🔟			
and mix 1 time(s).			
Specify the conditions for creating Dilution Archive Plate 2			
Dilute the Archive Sample at			
for a total volume of			
and mix 1 time(c)			
and mix interest.			
Cancel OK			

These conditions are described in the table below.

Dilution Archive Protocol Conditions

Condition	Description	Accepted Values
Protocol Name	A unique name for a specific Dilution Archive protocol	 A unique combination of letters, numbers, and spaces
		 Up to 32 characters
	Dilution Archive Plate 1	
Dilute the Archive Sample at	The ratio of input archive sample volume to total diluted volume	1:2 to 1:20
(dilution factor)		
for a total volume of	The total volume of diluted material to prepare	Values in the pop-up menu vary according to the dilution factor for plate 1
		 Minimum value is 20 μL (1:2 dilution)
		 Maximum value is 200 μL
and mix	The number of times to mix the diluent and sample by pipetting up and down	1 to 9
	Dilution Archive Plate 2	
Create Dilution Archive	Indicates whether or not to perform a second dilution	♦ Checked
Plate 2		Unchecked
Dilute the Archive Sample at	The ratio of input archive sample volume to total diluted volume	Values in the pop-up menu vary according to the dilution factor for plate 1
(dilution factor)	Note This dilution factor indicates the final dilution factor of the input archive sample in dilution archive plate 2.	

Dilution Archive Protocol Conditions (continued)

Condition	Description	Accepted Values
for a total volume of	The total volume of diluted material to prepare	 Values in the pop-up menu vary according to the dilution factor for plate 2 Minimum value is 20 μL Maximum value is 200 μL
and mix	The number of times to mix the diluent and sample by pipetting up and down	1 to 9

Creating Dilution To create Dilution Archive protocols:		te Dilution Archive protocols:		
Archive Protocols	Step	Action		
	1	Go to the Protocol tab of the 6700 software.		
	2	In the Protocol section, click the New button under the Dilution Archive protocol.		
		New Dilution Archive Protocol Protocol Name: New Dilution Archive Protocol In Use: ✓ Specify the conditions for creating Dilution Archive Plate 1 Dilute the Archive Sample at 1:2 ▼ for a total volume of 200 ▼ µL and mix 1		
		Specify the conditions for creating Dilution Archive Plate 2		
		Create Dilution Archive Plate 2 Dilute the Archive Sample at for a total volume of and mix 1 time(s).		
	3	Cancel OK Enter a Protocol Name.		

Defining Dilution	To define dilution archive plate 1 conditions:			
Conditions	Step	Action		
Conditions	1	Select a dilution factor from the Dilute the	Archive Sample at pop-up menu.	
		Specify the conditions for creating Dilution Are Dilute the Archive Sample at 1:2 for a total volume of 200 and mix 1 time	c hive Plate 1 µ⊥ a(s).	
	2	Select the volume of diluted material to pr pop-up menu.	repare from the for a total volume of	
	3	In the and mix field, enter a value from 1 t (<i>e.g.</i> , dilution solution) and sample by pipe	to 9 for the number of times to mix diluent etting up and down.	
	4	If this is	Then	
		the only dilution you require	click OK to save this protocol and to return to the Protocol tab.	
		not the only dilution you require	continue with "Defining Dilution Archive Plate 2 Conditions" below.	

Defining Dilution	To define dilution archive plate 2 conditions:			
Conditions	Step	Action		
	1	Check the Create Dilution Archive Plate 2 check box.		
		Specify the conditions for creating Dilution Archive Plate 2		
		Dilute the Archive Sample at 1:40		
		for a total volume of 200 ▼ µL		
		and mix 1 time(s).		
	2	Select a dilution factor from the Dilute the Archive Sample at pop-up menu.		
		Note This dilution factor indicates the final dilution factor of the input archive sample in dilution archive plate 2.		

To define dilution archive plate 2 conditions: (continued)

Step	Action		
3	Select the volume of diluted material to prepare from the for a total volume of pop-up menu.		
	Note The software calculates the final volume that will replate 1 and updates the dialog box with this value.	main in dilution archive	
	New Dilution Archive Protocol		
	Protocol Name: New Dilution Archive Protocol		
	In Use: 🗹		
	Specify the conditions for creating Dilution Archive Plate 1		
	Dilute the Archive Sample at 1:2 🔽 for a total volume of 200 💌 山	— The input archive sample is diluted 1:2 in dilution archive	
	and mix interess. NOTE: The final well volume for Dilution Archive Plate 1 will be reduced to 190 للم after creating Dilution Archive Plate 2.	plate 1	
	Specify the conditions for creating Dilution Archive Plate 2		
	Create Dilution Archive Plate 2		
	Dilute the Archive Sample at 1:40 🔽 for a total volume of 200 💌 الله and mix 1 time(s).	The input archive sample is diluted 1:40 in dilution archive plate 2	
	Cancel OK		
4	In the and mix field, enter a value from 1 to 9 for the number (<i>e.g.</i> , dilution solution) and sample by pipetting up and dow	er of times to mix diluent n.	
5	Click OK to save this protocol and to return to the Protocol	tab.	

Section: Assay Protocols

In This Section This section covers the following topics:

Торіс	See Page	
Assay Protocol Overview		
Assay Protocol Setup		
Assay Protocol Creation Overview		
Assay Protocol Creation for 96-Well Output		
Assay Protocol Creation for 384-Well Output		

Assay Protocol Overview

Description	During an Assay protocol, the 6700 workstation prepares assay output plates: up to four 96-well optical plates or one 384-well optical plate. These output plates contain master mixes, standards, controls, and samples from an RNA archive, DNA archive, cDNA archive, or dilution archive plate.		
Assay Protocol Process	The table below describes how the 6700 workstation performs an Assay protocol. Assay Protocol Process		
	Description		
1 The 6700 instrument transfers master		The 6700 instrument transfers master mix to the output plates.	
	2 If specified, the 6700 instrument dilutes the samples into dilution archive p		
	3 The 6700 instrument transfers standards, controls, and samples to the output plates.		
	4 If specified, the heat sealer places optical heat-seal covers on the output plates.		
Output Applications	 Assay output can be used for a variety of applications: Standard Curve 		
	Comparative Quantification		

- Allelic Discrimination ٠
- Plus/Minus
- Custom ٠

Assay Protocol Setup

Overview Before you create an Assay protocol, you need to set up the following in the 6700 database:

- Dyes
- Detectors
- Sample Types

 $Setting \ Up \ Dyes \quad Set \ up \ the \ dyes \ in \ the \ 6700 \ database \ before \ creating \ an \ Assay \ protocol.$

To set up dyes:

ur assay
ctors ing a inue.
w the ee ting"
nove See o

Creating a New Dye

To create a new dye:

Step	Action		
1	In the Dye Setup dialog box, click the New button.		
	An Add Dye dialog box appears.		
	Add Dye Name: Description: In Use: Cancel		
2	Enter the Name of the dye and a Description.		
3	Make sure that the In Use check box is checked.		
4	Click OK to return to the Dye Setup dialog box, then click Done.		

Changing the Dye's In Use Setting

To change the In Use setting:

Step	Action		
1	In the Dye Setup dialog box, double-click the dye you want to reset.		
	A View Dye dialog box appears.		
2	Change the In Use check box by clicking it.		
	Note You can change settings only for dyes you created.		
3	Click OK to return to the Dye Setup dialog box, then click Done .		

Deleting a Dye

To delete a dye:

Step	Action		
1	In the Dye Setup dialog box, select the dye by clicking it once.		
	The dye is highlighted.		
2	Click the Delete button.		
	Note To delete a dye, it must fulfill the following criteria:		
	 You created the dye. 		
	 The dye is not used in any detectors in the 6700 database. 		
	Note Remove the dye from use if you do not want it to appear in the Reporter or Quencher pop-up menus in the Add Detector dialog box. See "Creating a New Detector" on page 4-57 for more information.		

Setting Up Detectors Set up detectors in the 6700 database before creating an Assay protocol.

To set up detectors:

Step	Action			
1	Determine the detectors in the master mixes that your assay uses.			
2	Go to the Setup menu of the 6700 software.			
3	Select Detectors . The Detector Setup dialog box appears.			
	Detector Setup Name Description Task Reporter 188 Ribosomal RNA Comparative Qua VIC	Quench Color In Use Created By TAMRA Image: Color prebio pebio		
4	☑ New ♀ View ★ Delete View the list of detectors by Name.	Done		
	If the detector is	Then		
	not listed and you want to use the detector when creating Assay protocols	create a new detector. See "Creating a New Detector" on page 4-57 to continue.		
	listed but not marked in the In Use column and you want to use the detector when creating Assay protocols	change the In Use setting. See "Changing the Detector's In Use Setting" on page 4-58 to continue.		
	listed and marked in the In Use column and you want to remove the detector from use in new Assay protocols,	 change the In Use setting. See "Changing the Detector's In Use Setting" on page 4-58 to continue. 		
		 Delete the detector. See "Deleting a Detector" on page 4-58 to continue. 		

Creating a New Detector

To create a new detector:

Action				
In the Detector Setup dialog box, click the New button.				
The Add Detector dialog box appears.				
Add Detector				
Reporter: JOE				
Color:				
In Use: 🔽 Notes:				
Cancel				
Enter the Name and Description.				
Select the type of task this detector performs from the Task pop-up menu.				
Select the reporter dye from the Reporter pop-up menu.				
Note If the reporter dye is not available, see "Setting Up Dyes" on page 4-54 to create a reporter dye or to mark an appropriate dye as In Use .				
Select the quencher dye from the Quencher pop-up menu.				
Note If the quencher dye is not available, see "Setting Up Dyes" on page 4-54 to create a quencher dye or to mark an appropriate dye as In Use .				
Set the color for the detector:				
a. Double-click the Color box.				
I ne Select Detector Color dialog box appears.				
Swatches use and				
Swatches [HSB] KGB]				
Preview Sample Text Sample Text				
Sample Text Sample Text				
OK Cancel Reset				
b. Set the colors using the Swatches , HSB , or RGB tabs. c. Click OK .				

To create a new detector: (continued)

Step	Action
7	Make sure that the In Use check box is checked.
8	Click OK to return to the Detector Setup dialog box, then click Done.

Changing the Detector's In Use Setting

To change the In Use setting:

Step	Action
1	In the Detector Setup dialog box, double-click the detector that you want to reset.
	An Edit Detector dialog box appears.
2	Change the In Use check box by clicking it.
	Note You can change settings only for detectors you created.
3	Click OK to return to the Detector Setup dialog box, then click Done .

Deleting a Detector

To delete a detector:

Step	Action					
1	In the Detector Setup dialog box, select the detector by clicking it once.					
2	Click the Delete button.					
	Note To delete a detector, it must fulfill the following criteria:					
	You created the detector.					
	 The detector is not used in any Assay protocols. 					
	Note Remove the detector from use if you do not want it to appear in the Detector pop-up menus when specifying master mixes for Assay protocols.					

Setting Up Sample
TypesSet up sample types in the 6700 database before creating a new Assay protocol.
To set up sample types:

Step	Action								
1	Determine the sample types for standards and controls that your assay uses.								
2	Go to the Setup menu of the 6700 software.								
3	Select Sample Types								
Ū									
	The Sample Type Setup dialog box appears.								
	Sample Type Setup								
		1	[10 (10					
	Name	Description	Color	Created B	By				
	BOFFER	Builer Blark		peblo					
	IPC-	Internal Positive II		pebio					
	NAC	No Amplification Control		pebio					
	NPC	No Probe Control		pebio					
	NTC	No Template Control		pebio					
	STND	Standard		pebio					
	UNKN	Unknown		pebio					
	Q New	X Delete		Done					
4	View the	list of sample types	by Na	ime.					
	If the sa	ample type is			Then				
	not liste	ed and you want to i	use the	e	create a new sample type. See				
	sample type when creating Assay protocols			"Creating a New Sample Type" on page 4-60 to continue.					
	listed a	nd you want to rem	ove the	<u>_</u>	delete the sample type. See "Deleting				
	sample type from use in new Assay protocols.								

Creating a New Sample Type

To create a new sample type:

1	In the Sample Type Setup dialog box, click the New button to add a new sample type to the 6700 database.						
	The Add Sample Type dialog box appears.						
	Add SampleType						
	Name:						
	Description:						
	Color:						
	Cancel						
2	Enter a Name and a Description.						
3	Set the color for this sample type:						
	a. Double-click the Color box.						
	The Select Sample Type Color dialog box appears.						
	b. Set the colors using the Swatches, HSB, or RGB tabs.						
	c. Click OK .						
4	Click OK to return to the Sample Type Setup dialog box, then click Done .						

Deleting a Sample Type

To delete a sample type:

1	In the Sample Type Setup dialog box, select a sample type by clicking it once.					
2	Click the Delete button.					
	Note To delete a sample type, it must fulfill the following criteria:					
	 You created the sample type. 					
	• The sample type is not used in any Assay protocols in the 6700 database.					

Assay Protocol Creation Overview

Assay Protocol Defining the Assay protocol occurs through the Assay Protocol wizard.

Wizard

Assay Protocol Wizard Procedures

The Assay Protocol wizard takes you through the following procedures:

Торіс	See Page
Assay Protocol Creation for 96-Well Output	4-70
Specifying the Master Mixes	4-71
Specifying Master Mix Detectors	4-72
Specifying Replicates for Master Mixes	4-73
Specifying Dilutions	4-74
Specifying Standards for Master Mixes	4-75
Specifying Standard Quantities	4-76
Specifying Controls for Master Mixes	4-77
Completing the Protocol	4-78
Assay Protocol Creation for 384-Well Output	4-79
Specifying the Master Mixes	4-80
Specifying Master Mix Detectors	4-81
Specifying Replicates for Master Mixes	4-82
Specifying Dilutions	4-83
Specifying Standards for Master Mixes	4-84
Specifying Standard Quantities	4-85
Specifying Controls for Master Mixes	4-86
Completing the Protocol	4-87

Assay Protocol Wizard Bar

The bar below is present on every Assay Protocol wizard dialog box when you create a new Assay protocol.

. Votes	X Cancel	🗢 Back	_ Next ⇔	O Finish
Click to add notes to accompany the protocol	Click to cancel Assay protocol creation at any time	 Click to go back to the previous dialog box	Click to proceed to the next dialog box	 Click to finish defining the Assay protocol

Assay Conditions When you create a new Assay protocol, you define the conditions displayed on Pages 1–8 of the New Assay Protocol wizard. Each page is shown below.

Note The screen captures of New Assay Protocol Pages 1–9 were taken from a 96-well instrument. If you have a 384-well upgrade, your pages may differ slightly in appearance.

New Assay Protocol Page 1	X
	Please name the new Assay Protocol. Protocol Name: New Assay Protocol I In Use Experiment type: Standard Curve Number of wells per Output Plate: 96 💌
🔍 Notes 🗶 Car	ncel 🗢 Back Next 🔿 🍦 Finish

New Assay Protocol Page 1

Page 1 Conditions

Condition	Description	Accepted Values
Protocol Name	A unique name for a specific Assay protocol	 A unique combination of letters, numbers, and spaces
		 Up to 32 characters
Experiment type	The type of assay to perform with the output	Standard Curve
	plates	 Comparative Quantification
	Note The Experiment type specifies the controls available for the Assay protocol.	 Allelic Discrimination
		♦ Plus/Minus
		◆ Custom
Number of wells per	Determines the kind of output plate being used	♦ 96 wells
Output Plate	in the assay	 384 wells, for customers with the 384-well upgrade

New Assay Protocol Page 2: Master Mixes

New Assay Protocol Page 2							×
~	Specify Master Mixes you will use in the assay:						
1.	5.		Name	Color	Mix Vol.	Sample Vol.	Total Vol.
		<u> </u>	Mastermix #1		40	10	50
2.	6.	☑ 2	Mastermix #2		40	10	50
		🗖 3	Mastermix#3		40	10	50
3		4	Mastermix #4		40	10	50
			Mastermix #5		40	10	50
			Mastermix #6		40	10	50
4. 🔾 🔠	8.	□ 7	Mastermix #7		40	10	50
Master	Mixes	<u>∏</u> 8	Mastermix #8		40	10	50
Note: All volumes are in microliters (للله)							
Notes		🗙 Cancel	🗢 Back 🛛 🕅 Ne	xt⇔		🖗 Finish	

Page 2 Conditions

Condition	Description	Accepted Values	
Check boxes 1 through 8	The number of master mixes to use in the assay	For 96-well output:	
		If you sort outputThen select upplates byto	
		Master mix four master mixes.	
		Master mix and eight master sample mixes.	
		 For 384-well output, select up to eight master mixes 	
Name	A unique name for a specific master mix	 A unique combination of letters, numbers, and spaces 	
		 Up to 32 characters 	
Color	The color to identify the master mix in the Assay protocol output preview	Swatches, HSB, or RGB	
Mix Vol. The volume of master mix to transfer to the		96-Well 384-Well	
		5 to 195 μL 5 to 15 μL	
		$\begin{array}{c c c c c c c c c c c c c c c c c c c $	
Sample Vol. The volume of sample to transfer to the sample to transfer to the states		96-Well 384-Well	
plates	5 to 195 μL 5 to 15 μL		
		Note Mix Vol. Note Mix Vol.	
		plus Sample Vol.plus Sample Vol.must be $\leq 200 \ \mu$ L.must be $\leq 20 \ \mu$ L.	

New Assay Protocol Page 3: Detectors

New Assay Protocol Page 3		×
		Specify Detectors in the Master Mix: Mastermix #1
1. 🔛 📃	5.	Detectors for 'Mastermix #1'
		Name [Probe] [F-Primer] [R-Primer]
2.	6.	
3.		
4.	8.	
Maste	er Mixes	
		O New X Delete
V Notes	X Cance	el 🗢 Back Next 🔿 🍈 Finish

Page 3 Conditions

Condition	Description	Accepted Values	
Name	The name of the detector(s) in the master mix	 Detectors set up in the database 	
		 ♦ Up to 12 	
[Probe]	Probe concentration	0.00001 to 9999999	
[F-Primer]	Forward primer concentration	0.00001 to 9999999	
[R-Primer]	Reverse primer concentration	0.00001 to 9999999	

New Assay Protocol Page 4: Master Mix Samples

New Assay Protocol Page 4	×
Output 1 Output 2 Output 3 Output 4	Specify samples for MasterMix: Mastermix #1
1 2 3 4 5 6 7 8 9 10 11 12 A t t 2 2 9 9 4 4 6 6 6 6	Make 2 replicate(s) of each Sample.
B [7 [7 [8 [8 [9 [9 [10 [10 [11 [11 [12 [12 C [13 [13 [14 [14 [15 [15 [16 [16 [16 [17 [17 [18 [18	
D 19 19 20 20 21 21 22 22 23 23 24 24 E 25 25 26 28 27 27 28 28 29 29 30 30	
F 31 91 92 92 93 33 94 94 95 95 96 96	
H 43 43 44 44 45 45 46 46 46 47 47 48 48	
This protocol can handle 48 samples.	
Sort Assay Plates by: Master Mix	
🔍 Notes 🗶 Can	cel 🗢 Back Next 🔿 🖉 Finish

Page 4 Conditions

Condition	Description	Accepted Values
Make replicate(s) of each Sample	The number of sample replicates to set up	1 to 6
Sort Assay Plates by	How to arrange the samples on the output plates	Master mix
	Note This is not applicable to a 384-well optical plate.	 Master mix and sample

New Assay Protocol Page 5: Sample Dilutions

New Assay Protocol Page 5	×	
Output 1 Output 2 Output 3 Output 4 1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 2 5 6 7 8 9 10 11 12 A 1 1 2 2 3 4 5 6 6 6 B 7 7 8 8 9 10 10 11 11 12 12 C 13 14 14 15 15 16 16 17 17 18 18 D 19 19 20 20 21 21 22 22 23 24 24 E 26 26 26 27 27 28 28 29 30 30	Specify dilutions for the samples: Initial Dilution: Neat Dilution Factor: Neat 1:2 1:4 Select which Dill 1:5 Dilutions: 1 1:20 4. 1:8	
F 91 91 92 92 93 93 94 94 95 96 96 G 97 97 98 98 99 40 40 41 41 42 42 H 43 43 44 46 46 46 46 47 47 48 48 This protocol can handle 48 samples. 48 48 48 48 48 48	2. 1:2 5. 1:16 3. 1:4 6. 1:32	
Sort Assay Plates by: Master Mix		

Page 5 Conditions

Condition	Description	Accepted Values
Initial Dilution	The dilution factor for the first dilution	♦ Neat
		◆ 1:2
		♦ 1:4
		◆ 1:5
		◆ 1:10
		◆ 1:20
Dilution Factor	The dilution factor for the second dilution	Values in the pop-up menu vary according to the initial dilution factor
Dilutions	Serial dilutions to use	Values that appear vary according to the initial and second dilution factors
		Note You must check at least one box.

New Assay Protocol Page 6: Standards

New Assay Protocol Page 6		×
Output 1 Output 2 Output 3 Output 4	Specify standards setup for each Master Mix:	Mastermix #1 💌
1 2 3 4 5 6 7 8 9 10 11 12 A (st	Make 2 replicate(s)	of each Standard.
B (3 (3 (3 (3 (4 (4 (4 (6 (6 (6 (6 (6	Deck Position Description	Deck Position Description
	I Standard 1	7 Standard 7
E (12 (12 (12 (12 (13 (13 (13 (13 (14 (14 (14	2 Standard 2	B Standard 8
F 15 15 16 16 16 16 16 16 17 17 17 17	4 Standard 4	10 Standard 10
G [18 [18 [18 [19 [19 [19 [19 [19 [10 [20 [20 [20 [20]	5 Standard 5	11 Standard 11
This protocol can handle 23 samples.	🗖 6 Standard 6	12 Standard 12
Sort Assay Plates by: Master Mix	Note: You will set quantitie next screen.	s for the standards on the
🔇 Notes 🗶 Canc	el 🗢 Back Ne	xt 🔿 🕼 Finish

Page 6 Conditions

Condition	Description	Accepted Values
Make replicate(s) of each Standard	The number of replicates of standards to set up	1 to 6
Deck Positions	The location of the standard	1 to 12

New Assay Protocol Page 7: Standard Quantities

New Assay Protocol Page 7		×
Output 1 Output 2 Output 3 Output 4	Specify standard quantities for each probe in MasterMix:	Mastermix #1
1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 2 3 4 4 6 6 6 6	Detector:	
B 7 7 8 8 9 9 10 10 11 11 12 12 C 13 13 14 14 15 15 16 16 16 17 17 18 18	Deck Quantity Position (nM)	Deck Quantity Position (nM)
D 19 19 20 20 21 21 22 22 23 23 24 24	1. 0.0	7. 0.0
E 25 25 26 26 27 27 28 28 29 29 30 30	2. 0.0	8. 0.0
F 31 31 32 32 33 33 34 34 35 35 36 36	3. 0.0	9. 0.0
G 37 37 38 38 39 39 40 40 41 41 42 42	4. 0.0	10. 0.0
H 43 43 44 44 46 45 46 46 47 47 48 48	5. 0.0	11. 0.0
This protocol can handle 48 samples.	6. 0.0	12. 0.0
Sort Assay Plates by: Master Mix		
😲 Notes 🗶 Cano	el 🗢 Back Next	⇒ Finish

Page 7 Conditions

Condition	Description	Accepted Values
Detector	The name of a detector in the master mix	Values in the pop-up menu vary according to the detectors specified for the master mix
Quantity (nM)	The standard template quantity	0.00001 to 9999999

New Assay Protocol Page 8: Controls

New Assay Protocol Page 8	×
Output 1 Output 2 Output 3 Output 4	Specify Controls for Master Mix: Mastermix #1 💌
1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 2 3 4 4 6 6 6 6	Controls
	Deck Replicate Deck Replicate Position # Control 1. 2 V NTC V 7. 2 NTC V
E 25 25 26 26 27 27 27 28 28 29 20 30 30	
6 37 38 38 39 39 40 41 41 42 42 H <td< td=""><td>4. 2 V NTC V 10. 2 V NTC V</td></td<>	4. 2 V NTC V 10. 2 V NTC V
This protocol can handle 48 samples.	5. 2 NTC 11. 2 NTC 6. 2 NTC 12. 2 NTC
Sort Assay Plates by: Master Mix	
🔍 Notes 🗶 Cano	el 🗢 Back Next 🔿 🖉 Finish

Page 8 Conditions

Condition	Description		Accepted Values
Deck Position	The location of the control		1 to 12
Replicate #	The number of replicates of	f each control to set up	1 to 6
Control	The sample type of the control		Values in the pop-up menu vary according to the experiment type
	Experiment Type	Controls Available	
	Standard Curve	NTC, NPC, NAC	
	Comparative Quantification	NTC, NPC, NAC	
	Allelic Discrimination	AL1, AL2, NTC	
	Plus/Minus	IPC+, IPC-, Buffer, NTC, NAC	
	Custom	All sample types available	

Assay Protocol Creation for 96-Well Output

Protocol for 96-Well Output

Creating an Assay Note To create an assay protocol for 384-well output, see "Assay Protocol Creation for 384-Well Output" on page 4-79.

To create an Assay protocol for 96-well output:

Step	Action		
1	Go to the Protocol tab of the 6700 software.		
2	In the Protocol section, click the New button under the Assay protocol.		
	Notes Cancel Hack Next - Finish		
3	Enter a Protocol Name.		
4	Select an experiment type from the Experiment type pop-up menu.		
5	Select 96 from the Number of wells per Output Plate pop-up menu.		
	Note Assay protocols with 384-well output and 96-well output cannot be run simultaneously.		
6	Click Next.		

Specifying the	To spec	ify the master mixes:			
whaster whites	Step	Action			
	1	In the New Assay Protocol Page 2 dialog box, check the box for each master mix. Note The number of master mixes you can use varies according to how you sort the output plates in "Specifying Sorting" on page 4-73.			
		If you sort output plates by	Then you can use up to		
		Master mix	four master mixes.		
		Master mix and sample	eight master mixes.		
		New Assay Protocol Page 2			
		1.	Specify Master Mixes you will use in the assay: Mix Sample Tota Name Color Vol. Vol. Vol. Vol. Vol. Vol. Vol. 00		
		2.	✓ 2. Mastermix #2 40 10 50 □ 3. Mastermix #3 40 10 50		
		3.	4. Mastermix #4 40 10 50 5. Mastermix #5 40 10 50		
		4.	0. Mastermix #6 40 10 50 7. Mastermix #7 40 10 50		
		Master Mixes	🗖 8. Mastermix #8 📃 40 10 50		
			Note: All volumes are in microliters (الله)		
		Car X Car	ncel Cristing Contraction Contraction		
	2	Enter the Name of each master mix.			
	3	Specify the color for each master mix:			
		a. Click the Color box.			
		A Select Master Mix Color dialog box a	ppears.		
		b. Choose a color using the Swatches, H	SB, or RGB tabs.		
		d Click OK to change the color			
		present in Assay protocol output previews Mixes" on page 4-73).	s here to indicate the master mixes s (see "Specifying Replicates for Master		
4 In the Mix Vol. column, enter a value from 5 to 195 (μL) for the volume of master mix to transfer to the output plates.		ι 5 to 195 (μL) for the volume of each s.			
		Note The sum of the Mix Vol. and Samp	ble Vol. must be ≤200 μL.		
5 In the Sample Vol. column, enter a value from 5 to 195 (μL) for the v to transfer to the output plates.			rom 5 to 195 (μ L) for the volume of sample		
	IMPORTANT The sum of the Mix Vol. and Sample Vol. must be \leq 200 µL.				
	The software calculates and updates the Total Vol. column (Mix Vol.+ Sample Vol.).				
	6 Click Next.				

Specifying Master	To spec	ify master mix detectors:		
WIX Detectors	Step	Action		
	1	In the New Assay Protocol Page 3 dialog box, select a master mix from the Specify Detectors in the Master Mix pop-up menu.		
		New Assay Protocol Page 3		
		5. Detectors for 'Mastermix #1' Name [Probe] [F-Primer]		
		2.		
		3.		
		4.		
		Master Mixes		
		♥ Notes ★ Cancel ← Back ● Finish		
	2	Specify detectors:		
		a. To add a detector, click New .		
		A detector with [Probe], [F-Primer], and [R-Primer] values appears.		
		b. To select a detector, click the detector name to access the detector pop-up menu.		
		c. To change [Probe], [F-Primer], and [R-Primer] values, double-click the numbers.		
		Note If the detector is not available in the pop-up menu, see "Setting Up Detectors" on page 4-56 to verify detector setup.		
	3	Repeat steps 1 and 2 until you specify all detectors in all master mixes.		
	4	Click Next.		

Specifying	To specify replicates for master mixes:					
Replicates for Master Mixes	Step	Step Action				
Master Mixes	1	In the New Assay Protocol Page 4 dialog b samples for Master Mix pop-up menu. New Assay Protocol Page 4 Output 1 Output 2 Output 3 Output 4 1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 2 9 4 4 6 6 6 6 B 7 7 8 8 9 9 10 10 11 11 12 12 C 13 13 14 14 15 15 16 16 17 17 18 18 D 19 19 20 20 21 21 22 22 23 23 24 24 E 25 25 26 26 26 27 27 28 28 29 29 30 30 F 31 31 22 32 33 34 34 36 36 36 36 G 37 37 38 38 39 39 40 40 41 41 42 42 H 43 43 44 44 6 45 46 46 47 47 48 48	ox, select a master mix fr Specify samples for MasterMix: Make 2 replicate(s) of eact	om the Specify Mastermix #1 💌		
		This protocol can handle 48 samples. Sort Assay Plates by: Master Mix Image: Canadian Content of C	cel	Finish		
	2	For the master mix specified, select the nu replicate(s) of each Sample pop-up menu. Note A message is displayed below the samples can be run with the current protoc changes to the protocol (<i>e.g.</i> , change the dilutions for output, etc.).	mber of replicates to make output graphic indicating col. The number updates number of replicates, the	e from the Make how many when you make number of		
	3	Repeat steps 1 and 2 until you specify the	number of replicates for a	all master mixes.		

Specifying Sorting To specify sorting of output plates:

Step	Action				
1	Choose a sorting method from the Sort Assay Plates by pop-up menu.				
	If you sort output plates by Then you can use up to				
	Master mix	four master mixes.			
	Master mix and sample	eight master mixes.			
2	Click Next.				

Specifying Dilutions IMPORTANT Assay protocols that specify dilutions cannot follow Dilution Archive protocols in an instrument run.

To specify dilutions:

Step	Action		
1	In the New Assay Protocol Page 5 dialog box, select the first dilution conditions from the Initial Dilution pop-up menu.		
	New Assay Protocol Page 5		
	Output 1 Output 2 Output 3 Output 4 Specify dilutions for the samples:		
2	1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 3 4 4 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 8 9 10 11 12 12 12 12 12 12 12 12 12 12 12 14 5 6 12 12 12 12 14 5 6 12 12 14 5 16 16 17 17 18 8 8 8 110 111 15 are to use: 1110 14 13 1120 111 110 111 110 111		
Note The dilutions available for output plates vary according to the Dilutic you select.			
	lew Assay Protocol Page 5		
	Output 1 Output 2 Output 3 Output 4 Specify dilutions for the samples:		
	1 2 3 4 5 6 7 8 9 10 11 12		
	B 7 7 8 8 9 9 10 10 11 11 12 12 Dilution Factor: 1:2		
	C [13 [14 [14 [16 [15 [16 [17 [17 [18 [18]		
	D 19 19 20 20 21 21 22 22 23 23 24 24 24 Select Which Din 1:5 E 25 25 26 26 27 27 28 28 29 29 30 30 10 Dilutions: 1 1:10 4. □ 1:8		
	F 31 31 32 32 33 33 34 34 36 36 36 36 36 2.		
	G [37 [37 [38 [38 [39 [39 [40 [40 [41 [41 [42 [42] 3. ■ 1:4 6. ■ 1:32 H [43 [43 [44 [44 [45]46 [46 [47 [47 [47 [48]48]		
	This protocol can handle 48 samples.		
	Sort Assay Plates by: Master Mix		
	♥ Notes ★ Cancel ← Back Next ← ● Finish		

To specify dilutions: (continued)

-			
Step	Action		
3	Check the Dilutions check boxes to select the dilutions to use for output plates.		
	The software adjusts the amount of color in each well according to the serial dilution present in the well.		
	Note You must check at least one box.		
	Output 1 Output 2 Output 3 Output 4		
	1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 1 1 2 2 2 3 3 5 5		
	H 22 22 22 23 23 23 23 24 24 24 24		
	This protocol can handle 24 samples.		
4	Click Next.		

Specifying Standards for Master Mixes

 $\label{eq:specifying} \quad \mbox{To specify standards for master mixes:}$

Step	Action		
1	In the New Assay Protocol Page 6 dialog be standards setup for each Master Mix pop-up	ox, select a master mix from the Specify p menu.	
	New Assay Protocol Page 6	د	
	Output 1 Output 2 Output 3 Output 4 1 2 3 4 5 6 7 8 9 10 11 12	Specify standards setup Mastermix #1 💌	
	A (S1 (S1 (S2 (S2 (1 (1 (+ (+ (2 (2 (2 (2)	Make 2 💌 replicate(s) of each Standard.	
	B 3 3 3 3 4 4 4 5 6 5 5 C 6 6 6 6 7 7 7 7 8 8 8 8	Deck Deck Position Description Position Description	
		2 Standard 2 8 Standard 8	
	E 12 12 12 12 12 13 13 13 13 14 14 14 14	3 Standard 3 9 Standard 9	
	G 18 18 18 18 19 19 19 19 20 20 20 20	□ 4 Standard 4 □ 10 Standard 10	
	H (21 (21 (21 (21 (22 (22 (22 (22 (22 (23 (23 (23 (23 (23	□ 5 Standard 5 □ 11 Standard 11	
	This protocol can handle 23 samples.	□ 6 Standard 6 □ 12 Standard 12	
	Sort Assay Plates by: Master Mix	Note: You will set quantities for the standards on the next screen.	
	Canc	el 🗢 Back Next 🔿 🕼 Finish	
2	Select the number of replicates to make from pop-up menu.	om the Make replicate(s) of each Standard	
3	Check the Deck Position check boxes to de the deckspace.	esignate the location of the standards on	
4	Repeat steps 1 through 3 until you specify	standards for all master mixes.	
5	Click Next.		

Specifying	Standard
(Duantities

To specify standard quantities:

Quantities

Step	Action				
1	In the New Assay Protocol Page 7 dialog box, select a master mix from the Specify standard quantities for each probe in Master Mix pop-up menu.				
	New Assay Protocol Page 7 Output 1 Output 2 Output 3 Output 4 1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 3 4 5 6 7 8 9 10 11 12 A 1 1 2 2 3 4 4 5 6 7 7 8 8 0 7 0 0 8 0 7 0 0 8 0 9 0 0 0 10 0 0 11 0 0 11 0				
2	Select a detector from the Detector population menu				
-	 Note Detectors available in the pop-up menu vary according to the detectors indicated in the New Assay Protocol Page 3 dialog box (see "Specifying Master Mix Detectors" on page 4-72). Quantity fields become active for deckspace positions that contain the selected master mix, the selected detector, and standards. 				
3	In the Quantity fields, enter the standard template quantity for each standard.				
	Note If you are going to use the Output Setup Plate file in the ABI PRISM [®] 7900HT Sequence Detection System, you must type in the starting copy number (1 to 99,000) in the Quantity field.				
4	Repeat steps 1 through 3 until you specify standard template quantities for each detector in all master mixes.				
5	Click Next.				

Specifying Controls To for Master Mixes

o specify controls for master m	ixes:
---------------------------------	-------

Step	Action				
1	In the New Assay Protocol Page 8 dialog box, select a master mix from the Specify				
	New Assay Protocol Page 8				
	Output 1 Output 2 Output 3 Output	t 4]	Specify Controls for Master M	lix: Mastermix #1 💌	
			Controls		
	C [13 [13 [14 [14 [15 [15 [16 [16 [17	17 18 18	Position # Control	Position # Control	
		23 24 24			
	E [26 [26 [26 [26 [27 [27 [28 [28 [29 F]31]31]32]32]33]33]34]34]35	29 (30 (30 35 (36 (36	3. 2 V NTC V	9. 2 VIC	
	G 37 37 38 38 39 39 40 40 41	41 42 42	🗆 4. 2 💌 NTC 💌	🗆 10. 2 💌 NTC 💽	
	H 43 43 44 44 45 45 46 48 48 47	47 (48 (48	5. 2 💌 NTC 💌	🗆 11. 2 💌 NTC 💌	
	This protocol can handle 48 samples.		6. 2 💌 NTC 💌	12. 2 V NTC	
	Sort Assay Plates by: Master Mix	V			
	Notes	🗶 Canc	el 🖉 🗢 Back 🛛 🕅	t ➡ 🖉 Finish	
2	Check the Deck Position check	boxes to de	esignate the location	of the controls on	
	the deckspace.				
3	For each control, select the nul pop-up menu.	mber of repl	icates to set up from	the Replicate #	
4	For each control, specify the sample type from the Control pop-up menu.				
	Note Sample types available in the pop-up menu vary according to the Experiment type selected in "Creating an Assay Protocol for 96-Well Output" on page 4-70.				
	Experiment Type	Controls	Available		
	Standard Curve	NTC, NPC	C, NAC		
	Comparative Quantification	NTC, NPC	C, NAC		
	Allelic Discrimination	AL1, AL2,	NTC		
	Plus/Minus	IPC+, IPC NAC	-, Buffer, NTC,		
	Custom	All sample	e types availableª		
	a. See "Setting Up Sample Types" on page 4-59 for more information about sample types.				
5	Repeat steps 1 through 4 until	you specify	controls for all maste	er mixes.	
6	Click Next.				

Ste	Action			
1	In the New Assay Protocol Page 9 dialog box, view the output plates by clicking the different Output tabs.			
	New Assay Protocol Page 9			
	Output 1 Output 2 Output 3 Output 4 The Assav Protocol setup is going to finish			
	1 2 3 4 4 6 6 6 7 8 9 10 11 12 Click the Finish button to save the protocol. A 1 1 2 3 4 4 6 6 6 6			
	B 7 7 8 8 9 9 10 10 11 11 12 12			
	C 13 13 14 14 16 16 18 17 17 18 18			
	D [19 [19 [20 [20 [21 [21 [22 [22 [23 [23 [23 [24 [24 F [25 [25 [26 [26 [27 [27 [28 [28 [29 [29 [30 [30]			
	F 31 31 32 32 33 33 34 34 36 36 36 36			
	G 37 37 38 38 39 39 40 40 41 41 42 42			
	H 43 43 44 44 45 45 46 46 47 47 48 48			
	This protocol can handle 48 samples.			
	Sort Assay Plates by: Master Mix			
	♥ Notes ★ Cancel ► Back Next → ● Finish			
	the Master Mixes" on page 4-71 for more information).			
Assay Protocol Creation for 384-Well Output

Creating an Assay Protocol for 384-Well Output

Note To create a protocol for 96-well output, see "Assay Protocol Creation for 96-Well Output" on page 4-70.

Action Step Go to the Protocol tab of the 6700 software. 1 2 In the Protocol section, click the New button under the Assay protocol. 🖂 Assay 6700 Install Assay • Click this button 🔎 View 🗘 New -The New Assay Protocol Page 1 dialog box appears. New Assay Protocol Page 1 Please name the new Assay Protocol. Protocol Name: New Assay Protocol 🔽 In Use Experiment type: Standard Curve Number of wells per Output Plate: 384 💌

💢 Cancel

Choose 384 from the Number of wells per Output Plate pop-up menu.

Note Assay protocols with 384-well output and 96-well output cannot be run

🗢 Back

Next ⇔

To create an Assay protocol for 384-well output:

🔍 Notes

simultaneously. Click **Next**.

Enter a Protocol Name.

Select the Experiment type from the pop-up menu.

3

4

5

6

•

🕂 Finish

Specifying the	To spec	ify the master mixes:
whaster whites	Step	Action
	1	In the New Assay Protocol Page 2 dialog box, check the box for each master mix. You may use up to eight master mixes.
		New Assay Protocol Page 2
		Specify the Master Mixes that you will use in the assay:
		1 5 Mix Sample Total Name Color Vol. Vol. Vol. Vol.
		□ 1. Mastermix #1 □ 10 10 20
		2. 6. 7 Masterrix #2 10 10 20
		3. [Mastermix #3] 10 10 20
		3. 7. 6. Mastermix #5 10 10 20
		6. Mastermix #6 10 10 20
		4. 8. 7. Mastermix #7 10 10 20
		Master Mixes
		Note: All volumes are in microliters (µL)
		🔨 Notes 🗶 Cancel 🗢 Back Next 🔿 🗣 Finish
	2	Enter the Name of each master mix.
	3	Specify the color for each master mix.
		a. Click the Color box.
		A Select Master Mix Color dialog box appears.
		c. Preview the color in the Preview page
		d. Click OK to change the color.
		Note The 6700 software uses the colors here to indicate the master mixes present in Assay protocol output previews (see "Specifying Replicates for Master Mixes" on page 4-82)
	4	In the Mix Vol. column, enter a value from 5 to 15 (μ L) for the volume of each master
		mix to transfer to the output plates.
		Note The sum of the Mix Vol. and Sample Vol. must be $\leq 20 \ \mu$ L.
	5	In the Sample Vol. column, enter a value from 5 to 15 (μ L) for the volume of sample to transfer to the output plates.
		Note The sum of the Mix Vol. and Sample Vol. must be $\leq 20 \ \mu$ L.
		The software calculates and updates the Total Vol. column (Mix Vol.+ Sample Vol.).
	6	Click Next.

Specifying Master	To specify master mix detectors:			
MIX Detectors	Step	Action		
	1	In the New Assay Protocol Page 3 dialog box, select a master mix from the Specify Detectors in the Master Mix pop-up menu.		
		New Assay Protocol Page 3		
		Specify Detectors in the Master Mix: Mastermix #1		
		1. Detectors for 'Mastermix #1'		
		3.		
		4.		
		Master Mixes		
		O New X Delete		
		♥ Notes ♥ Cancel ♥ Back Next ♥ ● Finish		
	2	Specify detectors:		
		a. To add a detector, click New.		
		A detector with [Probe], [F-Primer], and [R-Primer] values appears.		
		 b. To select a detector, click the detector name to access the detector pop-up menu. 		
		c. To change [Probe], [F-Primer], and [R-Primer] values, double-click the numbers.		
		Note If the detector is not available in the pop-up menu, see "Setting Up Detectors" on page 4-56 to verify detector setup.		
	3	Repeat steps 1 and 2 until you specify all detectors in all master mixes.		
	4	Click Next.		

Step	Action					
1	In the New Assay Protocol Page 4 dialog box, note the display:					
	If you are using the 384-well upgrade and using	Then the dialog box shows				
	a 384-well optical plate	Output tab 1 only.				
		New Assay Protocol Page 4				
		Output 1 1 2 3 4 5 6 7 8 9 1011 12 1314 1516 17 18 19 2021 22 23 24 4 000000000000000000000000000000000000				
		tab 1 by clicking the Zoom In/Zoom Out toggle button. To see the wells in more detail, click Zoom In , and scroll if necessary.				
	a 96-well optical plate(s)	Output tabs 2, 3, and 4.				
		For these procedures, see "Assay Protocol Creation for 96-Well Output" on page 4-70.				
2	Select a master mix from the Spe	cify samples for each Master Mix pop-up menu.				
3	For the master mix specified, sele replicate(s) of each Sample pop-u	ect the number of replicates to make from the Ma p menu.				
	Note A message is displayed b samples can be run with the curre changes to the protocol (<i>e.g.</i> , cha dilutions for output, etc.).	elow the output graphic indicating how many ent protocol. The number updates when you mal ange the number of replicates, the number of				
4	Repeat steps 1 and 2 until you sp	ecify the number of replicates for all master mixe				

Specifying Dilutions IMPORTANT Assay protocols that specify dilutions cannot follow Dilution Archive protocols in an instrument run.

To specify dilutions:

Step	Action
1	In the New Assay Protocol Page 5 dialog box, select the first dilution conditions from the Initial Dilution pop-up menu.
	New Assav Protocol Page 5
	Output 1 Specify dilutions for the samples:
	1 2 3 4 5 6 7 8 9 1011 12 13 14 15 16 17 18 19 2021 2223 24 8 000000000000000000000000000000000000
	Votes Cancel ← Back Next ⇔ @ Finish
	Note The dilutions available for output plates vary according to the Dilution Factor you select.
	New Assay Protocol Page 5
	Output 1 Specify dilutions for the samples: 1 2 3 4 5 6 7 8 9 1011 12 1314 15 16 17 18 19 2021 2223 24 Initial Dilution: Neat 8 CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	L CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	Sort Assay Plates by: Single Plate
	♥ Notes ♥ Cancel ♥ Back Next ♥ ● Finish

To specify dilutions: (continued)

Step	Action
3	Check the Dilutions check boxes to select the dilutions to use for output plates.
	The software adjusts the amount of color in each well according to the serial dilution present in the well.
	Note You must check at least one box.
	New Assay Protocol Page 6
	Output 1 1 2 3 4 5 6 7 8 9 1011 12 13 14 15 16 17 18 19 2021 2223 24 A CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
4	Click Next.

Specifying Standards for Master Mixes

To specify standards for master mixes:



.	
step	Action
1	In the New Assay Protocol Page 7 dialog box, select a master mix from the Specify standard quantities for each Master Mix pop-up menu. Note The dialog box below shows an enlarged ("zoomed") view of the Output 1 tab. You can change your view of the Output 1 tab by clicking the Zoom In/Zoom Out toggle button.
	New Assay Protocol Page 7
	Output 1 Image: Comparison of the standard quantities Mastermix #1 1 2 3 4 5 6 7 8 9 10 11 12 12 2 2 1 1 1 12 2 2 1 1 1 12 2 2 1 Detector: 18S Image: Comparison of the standard quantities Image: Comparison of the standard quantities <t< th=""></t<>
	C 12 12 13 13 13 14 <td< th=""></td<>
-	
2	 Select a detector from the Detector pop-up menu. Note Detectors available in the pop-up menu vary according to the detectors indicated in the New Assay Protocol Page 3 dialog box (see "Specifying Master Mix Detectors" on page 4-81). Quantity fields become active for deckspace positions that contain the selected
2	master mix, the selected detector, and standards.
5	Note If you are going to use the Output Setup Plate file in the ABI PRISM [®] 7900HT Sequence Detection System, you must type in the starting copy number (1 to 99,000) in the Quantity field.
4	Repeat steps 1 through 3 until you specify standard template quantities for each detector in all master mixes.
5	Click Next.
	1 2 3 4 5

Specifying Controls	To spec	ify controls for master mixes:		
IUI WIASTEI WIIXES	Step	Action		
	1	In the New Assay Protocol Page 8 dialog box, select a master mix from the Specify Controls for Master Mix pop-up menu.		
		Note The dialog box below sl tab. You can change your view toggle button.	nows an enlarged ("zoomed") of the Output 1 tab by clicking	view of the Output 1 the Zoom In/Zoom Out
		New Assay Protocol Page 8		×
		Output 1	Specify Controls for each N	Master Mix: Mastermix #1
		1 2 3 4 5 6 7 8 9 1 A (5) (52) (1) (1) (1) (1) (2) (2) B 6 6 6 7 7 7 8 (2) C 12 12 12 13 13 13 14 11 D 18 18 18 10 10 10 10 20 2 E 24 24 24 25 25 26 2	D 11 12 2 2 4 4 4 4 14 4 14 14 2 2 2 4 5 8 8 4 14 14 2 2 2 2 2 2 2 2 2 1 2 2 1 1 2 1 1 2 2 1 1 2 1 1 2 2 1 1 2 1	Controls Deck Replicate Position # Control T. 2 V NTC V R. 8. 2 V NTC V V 9. 2 V NTC V V 10. 2 V NTC V
		$H \left[42^{2} \left[42^{2} \left[42^{2} \left[42^{2} \left[43^{2} \left[43^{2} \right] \left[43^{2} \left[43^{2} \left[43^{2} \left[43^{2} \right] \left[43^{2} \right] \left[43^{2} \left[43^{2} \left[43^{2} \right] \left[43^{2} \left[43^{2} \left[43^{2} \left[43^{2} \right] \left[43^{2} \left[$		
		This protocol can handle 47 samples.	Coom Out 6. 2 V NTC	▼ 12. 2 ▼ NTC ▼
		Sort Assay Plates by: Single Plate	Y	
		V Notes	🗶 Cancel 🤄 Back 📗	lext 🔿 🔮 Finish
	2	Check the Deck Position check the deckspace.	boxes to designate the location	on of the controls on
	3	For each control, select the nur pop-up menu.	nber of replicates to set up fro	om the Replicate #
	4	For each control, specify the sa	mple type from the Control po	op-up menu.
		Note Sample types available Experiment type selected in "Cr page 4-79.	in the pop-up menu vary accore ating an Assay Protocol for a	ording to the 384-Well Output" on
		Experiment Type	Controls Available	
		Standard Curve	NTC, NPC, NAC	
		Comparative Quantification	NTC, NPC, NAC	_
		Allelic Discrimination	AL1, AL2, NTC	_
		Plus/Minus	IPC+, IPC-, Butter, NTC, NAC	
		Custom	All sample types available ^a	
		a. See "Setting Up Sample Types" about sample types.	on page 4-59 for more information	n
	5	Repeat steps 1 through 4 until	ou specify controls for all ma	ster mixes.
	6	Click Next.		

-

11010001	Step	Action
	1	In the New Assay Protocol Page 8 dialog box, view the output plate.
		New Assay Protocol Page 9
		Output 1 The Assay Protocol setup is going to finish!
		1 2 3 4 5 6 7 8 9 1011 12 13 14 15 16 17 18 19 2021 2223 24 Click the Finish button to save the protocol. A C
		This protocol can handle 96 samples. Zoom In
		Sort Assay Plates by: Single Plate
		♥ Notes ♥ Cancel Next ⇒ ♥ Finish
		Note The colors of the wells indicate which master mix is used (see "Specifying the Master Mixes" on page 4-80 for more information).
	2	Click Finish to save this protocol and to return to the Protocol tab

Maintenance

Overview

About This Chapter	out This Chapter This chapter contains information about schedules and procedures for maintainin ABI PRISM [™] 6700 Automated Nucleic Acid Workstation.		
In This Chapter	This chapter contains the following topics:		
	Торіс	See Page	
	Instrument Maintenance Overview	5-2	
	Maintenance Procedures	5-4	

Instrument Maintenance Overview

Maintenance Recommendation	IMPORTANT Preventive maintenance of the 6700 workstation is required to ensure instrument reliability and accuracy.
Maintenance Schedules	 Maintenance of the 6700 workstation should occur as follows: Daily maintenance Perform at the end of each day or after 8 hours of operation. Weekly maintenance Perform at the end of each week or after 40 hours of operation. Periodic maintenance Perform after approximately 6 months of operation.
Maintenance and Chemical Waste	 IMPORTANT Some of the maintenance procedures require that you handle chemical waste. Please read and follow the chemical waste hazard warning below. AWARNING 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 and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (<i>e.g.</i>, safety glasses, gloves, or protective clothing). After emptying the waste container, seal it with the cap provided. Dispose of the contents of the waste tray and waste container in accordance with good laboratory practices and local, state/provincial, or national environmental
Maintenance and Biologically Hazardous Material	and health regulations. If you expose the 6700 instrument enclosure to potentially biologically hazardous material (<i>e.g.</i> , blood or plasma), you need to contact a certified professional to decontaminate the 6700 instrument enclosure with formaldehyde vapor. IMPORTANT These decontamination procedures must be performed by a certified professional before an Applied Biosystems service engineer can service the instrument. See Appendix A, "Instrument Decontamination," for more information.

Checklist

Daily Maintenance IMPORTANT Performing daily maintenance will improve the 6700 workstation's performance and reliability.

To perform daily maintenance:

Step	Action	See Page
Before Every Run:		
1	Check the waste container:	5-5
	a. Empty and clean the container if it is more than 50% full.	
	IMPORTANT If the waste container overfills, liquid waste will flow into the inline filter. This makes it impossible to pull any vacuum and requires inline filter replacement.	
	b. Verify that the lid of the waste container is tightened.	
	IMPORTANT If the lid of the waste container is loose, the instrument may not be able to apply sufficient vacuum pressure during RNA/DNA Archive protocols.	
2	Flush system lines for 60 seconds.	5-6
3	Tighten the robotic arm tips with the white Teflon tip tightener.	5-8
	CAUTION Never touch the gold robotic arm tips with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.	
4	Finger-tighten diluter syringes.	5-9
After Ev	/ery Run:	•
5	Empty the tip eject bin. Replace the disposable tip eject bin liner (P/N 4316565) if needed.	5-4
6	Clean the deckspace with an appropriate cleaning agent.	5-9
7	Check the level of system fluid. If the container is less than 25% full, add system fluid until the container is 75% full.	5-8
	IMPORTANT Do not overfill the system fluid container. Overfilling causes bubbles to form in the fluid lines.	

Weekly Maintenance To perform weekly maintenance:

Checklist

See Step Action Page 1 Perform the tasks listed in the "Daily Maintenance Checklist." 5-3 2 Check the diluter valves for leaks. 5-9 3 Check the system fluid lines. 5-7 4 Check the fluid lines for microbial growth. 5-7 Note If microbial growth is present, perform the procedure in "Replacing System Fluid" on page 5-8.

Annual Maintenance Applied Biosystems service representatives perform annual maintenance of the 6700 workstation.

Maintenance Procedures

Emptying the Tip To empty the tip eject bin:

Eject Bin

Step	Action
1	Remove any reagent reservoirs from the reagent reservoir platform.
2	Loosen the captive screw on the reagent reservoir platform.
3	Carefully detach the reagent reservoir platform by moving it slightly forward, then lifting upward.
4	Remove the tip eject bin liner and dispose of the liner and the pipette tips. WARNING Always follow the safety precautions regarding waste in the waste profile. Dispose of the waste in accordance with all local, state, and federal health and environmental regulations and laws.
5	Place a new disposable tip eject bin liner (P/N 4316565) in the tip eject bin.
6	Replace the reagent reservoir platform on the deckspace.
7	Finger-tighten the captive screw on the platform until it fits snugly.
	Note It is not necessary to use a screwdriver to tighten the captive screw.

Emptying and Cleaning the Waste Container

Empty and clean the waste container if it is more than 50% full.

IMPORTANT If the waste container overfills, liquid waste will flow into the inline filter. This makes it impossible to pull any vacuum and requires inline filter replacement.

To empty the waste container:

Step	Action				
1	Remove any plates from the vacuum station.				
2	Pour 50 mL of a germicidal detergent into the waste container to inactivate any potentially infectious biohazardous chemicals. For a germicidal detergent we recommend:				
	Envirocide disinfectant ^a				
	OR				
	 Process Vesphene IIst[™] Environmental Disinfectant^b 				
	Prepare each according to package instructions.				
	WARNING CHEMICAL HAZARD. Envirocide disinfectant may cause eye and skin irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.				
	A DANGER CHEMICAL HAZARD. Process Vesphene llst Environmental Disinfectant is corrosive. Exposure may cause eye and skin damage (burns). It is harmful if swallowed. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.				
	WARNING BIOHAZARD. Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in <i>Biosafety in Microbiological and</i> <i>Biomedical Laboratories</i> (stock no. 017-040-00547-4) and in Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site http://www.cdc.gov.				
3	Close the 6700 instrument door.				
4	In the 6700 software, set the instrument to pull a vacuum into the waste container:				
	a. From the Instrument Menu, scroll to Tests and select Function Tests.				
	A Function Tests window appears.				
	b. Click the Purification tab.				
	Purification tests appear.				
	c. Check the box next to Perform 'Vacuum' Test .				
	d. From the Vacuum Location pop-up menu, select Waste.				
	e. From the Carriage Location pop-up menu, select Filtrate.				
	f. From the vacuum intensity pop-up menu, select 50%.				
-	y. Enter so in the time/secs box.				
5	Once the vacuum is completed, exit the 6700 software.				
6	Using the power button on the front of the instrument, turn off the instrument.				
	The LED lights and the interior lights turn off.				

To empty the waste container: (continued)

Step	Action
7	Disconnect the waste fluid lines from the waste container using the quick-disconnectors.
	Note The quick-disconnectors self-seal to limit the amount of fluid that spills from them.
8	Wipe off any drops from the quick-disconnectors with lint-free tissues and the germicidal detergent (Envirocide disinfectant or Process Vesphene IIst Environmental Disinfectant).
	A WARNING CHEMICAL HAZARD. Envirocide disinfectant may cause eye and skin irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	A DANGER CHEMICAL HAZARD. Process Vesphene list Environmental Disinfectant is corrosive. Exposure may cause eye and skin damage (burns). It is harmful if swallowed. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
9	Empty the waste container in an appropriate waste disposal receptacle.
	WARNING Always follow the safety precautions regarding waste in the waste profile. Dispose of the waste in accordance with all local, state, and federal health and environmental regulations and laws.
10	Reconnect the waste container to the waste fluid lines.
11	IMPORTANT Make sure:
	 All quick-disconnectors are fully seated
	 The waste cap is fully seated and its vent plug is in place

a. Envirocide disinfectant is available from Viro Research (P/N 30128).

b. Process Vesphene IIst Environmental Disinfectant is available from Steris Corporation at telephone number 1-800-JIT-4-USE (1-800-548-4873) or through their Web site at http://www.steris.com.

Flushing the System To flush the system:

Step	Action		
1	Firmly tighten all tubing connections.		
2	From the Instrument Menu, scroll to Utility and select System Flush.		
	The System Flush dialog box appears.		
	Flush System		
	Purge Lines Purge Lines Exit		
3	Click Purge Diluters.		
	The instrument initializes, then purges the diluter lines.		

To flush the system: (continued)

Step	Action	
4	Using the Flush System dialog box, enter 60 (seconds) in the Purge Lines Pump Time field.	
	Flush System	
	Purge Lines Purge Lines Pump Time: 60 Seconds	
	Exit	
5	Click Purge Lines.	
	The instrument initializes, then purges the lines for the specified time.	
6	Inspect the fluid lines:	
	a. Verify that liquid is present in the lines.	
	b. Verify that no bubbles are present in the lines.	
	c. Verify that no leaks are present.	
	d. Verify that liquid does not drip from the lines after a few minutes.	

Fluid Line

Checking the To check the fluid lir

0	C	nec	K	the	fluid	lines:	

Step	Action
1	Firmly tighten all tubing connections.
2	Check the system fluid lines for microbial growth.
	If any growth is present:
	 Replace the system fluid with fresh Model 6700 System Fluid (P/N 4308456). See "Replacing System Fluid" on page 5-8.
	A CAUTION Model 6700 System Fluid. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	b. Flush the system. See "Flushing the System" on page 5-6.
3	Check the fluid lines for air bubbles.
	If the fluid lines contain air bubbles, flush the system. See "Flushing the System" on page 5-6.
4	Check the tubing connections for leaks.
	IMPORTANT If any leaks are present, contact an Applied Biosystems service representative for assistance.

Replacing System	To replace the system fluid:				
Fluid	Step	Action			
	1	Using the power button on the front of the instrument, turn off the instrument.			
		The LED lights and the interior lights turn off.			
	2	Disconnect the system fluid lines from the system fluid container using the quick-disconnectors.			
		Note The quick-disconnectors self-seal to limit the amount of fluid that spills from them.			
	3	Wipe off any drops from the quick-disconnectors with lint-free tissues.			
	4 Empty the system fluid in an appropriate waste disposal receptacle.	Empty the system fluid in an appropriate waste disposal receptacle.			
		CAUTION Model 6700 System Fluid. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.			
	5	Pour fresh Model 6700 System Fluid (P/N 4308456) into the system fluid container until the container is 75% full.			
		IMPORTANT Do not overfill the system fluid container. Overfilling causes bubbles to form in the fluid lines.			
		IMPORTANT Model 6700 System Fluid. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.			
	6	Reconnect the system fluid container to the system fluid lines.			

Maintaining the Ta Tips

		0
Robotic	Arm	Tip

Fo maintain the	robotic a	arm tips:
-----------------	-----------	-----------

Step	Action
1	Put on appropriate gloves.
	CAUTION Never touch the robotic arm tips with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.
2	Check the gold coating of the tips for scratches or bending.
	IMPORTANT If a tip adapter is scratched or bent, call an Applied Biosystems service representative to replace it.
3	Clean the cones and tips with isopropanol.
	WARNING CHEMICAL HAZARD. Isopropanol is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Tighten loose cones with the white Teflon tip tightener.

Diluter Syringes

 $Maintaining \ the \ \ \, To \ maintain \ the \ \, diluter \ \, syringes:$

Step	Action			
1	Finger-tighten the diluter syringes toward the back of the instrument enclosure.			
	Note Turn the diluter syringes toward the right to tighten.			
	CAUTION Do not overtighten the diluter syringes. Overtightening will damage the three-way valve and the diluter syringes.			
2	Check the diluter syringes for leaks.			
	IMPORTANT If the diluter syringes are leaking, contact an Applied Biosystems service representative to replace the Teflon seals.			

 $Cleaning \ the \quad \mbox{To clean the deckspace:}$

Decks	pace
-------	------

Step	Action
1	Wear appropriate protective clothing, eyewear, and gloves.
2	Apply a germicidal detergent to the deckspace with a squirt bottle, cloth, sponge, or brush. Thoroughly wet the surfaces to be cleaned. For a germicidal detergent we recommend:
	Envirocide disinfectant ^a
	OR
	 ♦ Process Vesphene IIst[™] Environmental Disinfectant^b
	Prepare each according to package instructions.
	A WARNING CHEMICAL HAZARD. Envirocide disinfectant may cause eye and skin irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	A DANGER CHEMICAL HAZARD. Process Vesphene llst Environmental Disinfectant is corrosive. Exposure may cause eye and skin damage (burns). It is harmful if swallowed. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	IMPORTANT Do not use bleach. Bleach will damage the aluminum surface.
	IMPORTANT Do not use ethanol or isopropanol in any concentration as a surface disinfectant. Alcohols coagulate proteins and may not work quickly as germicides. Furthermore, due to rapid evaporation, alcohols do not contact open surfaces for adequate time periods. Never use 100% alcohol because it may preserve some microorganisms.
3	Allow the germicidal solution to contact the deckspace surface ≥ 10 minutes.
4	If necessary, rinse with deionized water.
5	Wipe the surfaces dry.

a. Envirocide disinfectant is available from Viro Research (P/N 30128).

b. Process Vesphene IIst Environmental Disinfectant is available from Steris Corporation at telephone number 1-800-JIT-4-USE (1-800-548-4873) or through their Web site at http://www.steris.com.

Cleaning the Splash If your protocols use tissue or blood, you may need to clean the splash guard holder. Guard Holder To clean the splash guard holder:

Step	Action
1	Wear appropriate protective clothing, eyewear, and gloves.
2	Prepare a germicidal detergent such as:
	Envirocide disinfectant ^a
	OR
	 Process Vesphene IIst Environmental Disinfectant^b
	Prepare each according to package instructions.
	A WARNING CHEMICAL HAZARD. Envirocide disinfectant may cause eye and skin irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	A DANGER CHEMICAL HAZARD. Process Vesphene llst Environmental Disinfectant is corrosive. Exposure may cause eye and skin damage (burns). It is harmful if swallowed. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	A WARNING BIOHAZARD . Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in <i>Biosafety in Microbiological and Biomedical Laboratories</i> (stock no. 017-040-00547-4) and in Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site http://www.cdc.gov.
3	Using a 3/32 hex wrench (Allen key), loosen the two screws securing the splash guard holder on either side of the waste position.
4	Remove the splash guard holder and place it in a tray deep enough for soaking it.
5	Pour enough germicidal detergent into the tray to completely cover the splash guard holder.
6	Allow the splash guard holder to soak in the germicidal detergent \geq 10 minutes.
7	Remove the splash guard holder from the germicidal detergent.
8	Rinse with water.
9	Wipe the splash guard holder dry with a lint-free tissue.
10	Return the splash guard holder to the instrument and tighten the screws to secure it in place.

a. Envirocide disinfectant is available from Viro Research (P/N 30128).

b. Process Vesphene IIst Environmental Disinfectant is available from Steris Corporation at telephone number 1-800-JIT-4-USE (1-800-548-4873) or through their Web site at http://www.steris.com.

Function Tests and Instrument Calibration



Overview

About This Chapter In This Chapter	This chapter provides procedures for initializing, testing, and calibrating the ABI PRISM [™] 6700 Automated Nucleic Acid Workstation.	
-	Торіс	See Page
	6700 Instrument Initialization	6-2
	Instrument Function Tests Overview	6-3
	Instrument Function Tests	6-5
	Instrument Calibration	6-15

6700 Instrument Initialization

Initialization Operation of the 6700 instrument occurs through the ABI PRISM 6700 Automated Nucleic Acid Workstation software. The proper function of the 6700 software requires access to the 6700 database. If access to the 6700 database is blocked for any reason, the 6700 software will not work.

 Reporting Firmware Versions and Calibration Values
 Note
 Reporting firmware versions and calibration values requires the Administrator login.

 Calibration Values
 To report firmware versions and calibration values:
 To report firmware versions and calibration values:

 Step
 Action
 1
 From the Instrument menu, scroll to Tests and select Function Tests.

 A Function Tests window appears.
 2
 Select the Mise Tests tab

2	Select the Misc Tests tab.
	The Misc Tests appear.
3	Check the following check boxes:
	Report Firmware Version
	Report Calibration Values
4	Click Start.
	a. The instrument initializes.
	 b. The firmware version and instrument calibration values appear in the Test Log box.
	c. The software unchecks the check boxes.
5	Click Print to print the Test Log .
6	Verify firmware versions.
7	Click Exit.

Downloading Note Downloading firmware requires the Administrator login.

Firmware To download firmware:

	-	
Step	Action	
1	Close the instrument door.	
2	From the Instrument menu, scroll to Utility and select Download Firmware.	
3	Click Choose File.	
	A Choose Firmware Download File dialog box appears.	
4	Locate the primary controller firmware:	
	a. Look in the D drive on the client computer.	
	b. Find the pebio folder, open the 6700 folder, and open the firmware folder.	
	c. Select the file HtspXXXX.abs to download the primary controller firmware.	
	Note Do not select the GeniXXXX.hex file, which is the robotic arm firmware.	
	d. Click Open .	
5	Click Start.	
	Note Downloading firmware takes approximately 2 minutes.	

To download firmware: (continued)

Step	Action
6	After the firmware is downloaded, click Exit.

Instrument Function Tests Overview

Function TestsInstrument function tests are performed through the 6700 software, which requires
access to the 6700 database. If access to the 6700 database is blocked for any
reason, the 6700 software will not launch.

Function Tests The Function Tests are separated into seven groups, as described in the table below. **Description**

Function Test Descriptions

Function Test	Test	Test Objective	See Page
Arm Move	Arm Move	The robotic arm can locate a designated deckspace location	6-5
		 The robotic arm can move to a second designated deckspace location 	
	Random Arm Move	The robotic arm can move randomly in the X-Y direction well above the deckspace.	6-6
Disposable Tips	Get Disp. Tips	The robotic arm can locate the tip racks and the tip eject bin	6-6
		 The pipette assemblies can sense tips, pick up tips, and eject tips 	
	Sense Disp. Tips	 The robotic arm can locate the tip racks 	6-7
		 The pipette assemblies can sense tips 	
Purification	Move Vacuum Station	The purification carriage can move properly	6-8
		 The purification carriage can locate the different vacuum station positions 	
		 The carriage can perform touchoff 	
	Vacuum	The vacuum functions	6-9
		 The vacuum can maintain intensity for a specified amount of time 	
Liquid Detect	Liquid Level Detect	 The robotic arm can locate the tip racks and a reagent reservoir 	6-10
		 The pipette assemblies can sense tips, pick up tips, and eject tips 	
		 The pipette assemblies can sense liquid 	
Diluters	Diluters	 The diluters are online and functional 	6-11
	 Diluter 1 	 Encoders on the diluters are functioning 	
	Diluter 2		
	Diluter 3		
	Diluter 4		

Function Test Descriptions (continued)

Function Test Group	Test	Test Objective	See Page
Misc	Report Firmware Version	The instrument components possess the most recent firmware versions	6-2
	Report Calibration Values	The instrument calibration values match the calibration values printed out at installation	6-2
	Test Peltiers	The Peltier units cool certain deckspace stations	6-11
	Cool Peltiers to	The Peltier units cool certain deckspace stations to 4 °C	6-12
	4 °C	 The temperature sensors report accurate deckspace temperatures 	
	Test Vacuum Pumps	Both the small and large vacuum pumps function	6-13
	Test Valves	All valves are receiving electrical input	6-13
		All valves can open and close	
Archive Cover	 Test archive cover 1 	The robotic arm can locate the archive station and transfer an archive cover to a specified archive plate	6-14
	 Test archive cover 2 		
	Test archive cover 3		

Instrument Function Tests

Instructions

Performing Function You can access the instrument function tests through the 6700 software. Follow the Tests: General general instructions below to perform function tests. Read the following sections for parameter descriptions and specific instructions for each test.

To perform function tests:

Step	Action
1	From the Instrument menu, scroll to Tests and select Function Tests.
	A Function Tests window appears.
2	Click a tab to view the tests.
3	Check the box next to the tests you wish to perform.
4	Set the test parameters as described in the following sections.
5	Verify that boxes are checked only next to the tests that you wish to perform.
6	Click Start to perform the selected tests.
	The instrument initializes, performs all checked tests, then unchecks the function test boxes.
7	Print the Test Log box by clicking the Print button.
8	Click Exit to exit the Function Tests.

Performing the Arm Procedure **Move Test**

To perform the Arm Move test:

	-
Step	Action
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.
	A Function Tests window appears.
2	Click the Arm Move tab.
3	Check the Perform 'Arm Move' Test box.
4	From the First Destination pop-up menu, select the first destination for the arm to move to.
5	From the Second Destination pop-up menu, select the second destination for the arm to move to.
6	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.
7	Click Start.

Process

The instrument moves the arm to the First Destination, lowers the tips to the deckspace, moves the arm to the Second Destination, and lowers the tips to the deckspace.

If Failure Occurs

If the arm does not move to the specified destinations, repeat "Calibrating the Deckspace" on page 6-15.

Performing the Procedure Random Arm Move Test To perform the Random Arm Move test:

•	
Step	Action
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.
	A Function Tests window appears.
2	Click the Arm Move tab.
3	Check the Perform 'Random Arm Move' Test box.
4	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.
5	Click Start.

Process

The robotic arm moves randomly in the X-Y axis while it stays at a constant height well above the deckspace. During this random movement, the arm looks for any X and Y positions where the arm binds.

If Failure Occurs

If the robotic arm does not locate positions properly:

- Repeat "Calibrating the Deckspace" on page 6-15.
- Repeat this test.

If failure still occurs after performing the above actions, contact an Applied Biosystems service representative to service the robotic arm.

Performing the Procedure Get Disp. Tips Test

To perform the Get Disp. Tips test:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Disposable Tips tab.		
3	Check the Perform 'Get Disp. Tips' Test box.		
4	Place a full 200- μ L disposable tip rack in the designated tip rack position (1–8).		
	IMPORTANT You must always use a full 200- μ L disposable tip rack in the tip rack position.		
5	From the Use Tip Rack Position pop-up menu, select a tip rack that contains disposable tips.		
6	Enter a number from 1 to 768 in the Repeat field for the number of times to repeat the test.		
	Note The number of times to repeat the test is limited by the number of available tips. One full tray is equal to 24 repeats.		
7	Check the tips to test in the Tips To Use box.		
8	Click Start.		

Process

The robotic arm moves to the designated tip rack and uses the designated tips to pick up the disposable tips. The robotic arm proceeds to eject the tips into the tip eject bin.

If Failure Occurs

If the robotic arm does not get the tips properly, take the following actions:

- Make sure the robotic arm tips are tight.
- Check the white cable that runs from the tips to the robotic arm for crimps or cuts.
- Make sure the tip eject bin is not full and is secured properly.
- Repeat "Calibrating the Deckspace" on page 6-15.
- Repeat this test.

If failure still occurs after performing the above actions, contact an Applied Biosystems service representative to service the robotic arm.

Performing the Sense Disp. Tips Test

he Procedure

To perform the Sense Disp. Tips test:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Disposable Tips tab.		
3	Check the Perform 'Sense Disp. Tips' Test box.		
4	Place a full 200- μ L disposable tip rack in the designated tip rack position (1–8).		
	IMPORTANT You must always use a full 200- μ L disposable tip rack in the tip rack position.		
5	From the Use Tip Rack Position pop-up menu, select a tip rack that contains disposable tips.		
6	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.		
7	Check the tips to test in the Tips To Use section of the tab.		
8	Click Start.		

Process

The robotic arm moves to the designated tip rack, then lowers the designated tip to sense whether or not tips are present.

If Failure Occurs

If the robotic arm fails to sense the presence of tips, take the following actions:

- Make sure the robotic arm tips are tight.
- Check the white cable that runs from the tips to the robotic arm for crimps or cuts.
- Repeat "Calibrating the Deckspace" on page 6-15.
- Repeat this test.

Performing the Procedure

Move Vacuum **Station Test**

To perform the Move Vacuum Station test:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Purification tab.		
3	Check the Perform 'Move Vacuum Station' Test box.		
4	From the First Destination pop-up menu, select the first location for the carriage to locate.		
5	From the Second Destination pop-up menu, select the second location for the carriage to locate.		
6	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.		
7	Check the Vacuum box for the vacuum pump to pull a vacuum. IMPORTANT If you check the Vacuum box, insert a purification tray in the Second Destination position, or the vacuum will fail.		
8	Check the Touch Off box for the carriage to perform touchoff.		
9	Click Start.		

Process

The Vacuum station moves from the First Destination to the Second Destination, then repeats this movement if specified. If selected, the vacuum pump is activated and the Vacuum station performs touchoff.

If Failure Occurs

If the Vacuum station does not move properly, take the following actions:

- ٠ Make sure that there are no objects on the deckspace blocking the path of the purification carriage (e.g., stray tips or an archive plate, splash guard, or deep-well plate not fully seated in position).
- Repeat this test after removing any obstructions.

If the vacuum station still fails to move properly, contact an Applied Biosystems service representative.

Performing the Procedure

Vacuum Test

To perform the Vacuum test:

Step	Action	
1	Place an empty purification tray in the purification carriage and close the instrument door.	
2	Go to the Instrument menu, scroll to Tests, and select Function Tests.	
	A Function Tests window appears.	
3	Click the Purification tab.	
4	Check the Perform 'Vacuum' Test box.	
5	From the Vacuum Location pop-up menu, select the location for the vacuum.	
6	From the Carriage Location pop-up menu, select the location for the carriage.	
	Note If you select a carriage location that is different from the vacuum location, you must place a block on the vacuum location for the vacuum pump to pull a vacuum.	
7	Select a value from the Vacuum Intensity pop-up menu.	
8	Enter a value from 1 to 999 (seconds) in the Time/secs field for the length of time to pull a vacuum.	
9	Click Start.	

Process

The vacuum and the carriage move to the designated locations. The vacuum pump attempts to pull a vacuum at the designated vacuum location for the time specified.

If Failure Occurs

- If the vacuum station does not pull sufficient vacuum, take the following actions:
 - Check pumps, valves, and tubing, as described in "Testing Vacuum Pumps" and "Testing Valves" on page 6-13.
 - Check the tightness of the waste container and the vent plug.
 - Check the waste/vacuum lines for pinches.
 - Check the inline filter on the waste/vacuum line for liquid. If the waste was not emptied in a timely manner, the liquid may have flowed into the inline filter. When the inline filter becomes wet, it blocks the filter and prevents the 6700 instrument from pulling a vacuum. Replace the waste/vacuum line (P/N 4326865) if it has become wet.
 - Make sure the vacuum carriage can lower and form an intact seal to the deckspace. Remove any objects preventing intact sealing of the vacuum carriage to the deckspace.
 - Repeat this test.
- If the vacuum pumps do not turn on, take the following actions:
 - Check pumps, valves, and electrical connections (as described in "Testing Vacuum Pumps" and "Testing Valves" on page 6-13).
 - Repeat this test.

If the test fails after performing all of the above actions, test all vacuum positions individually to isolate the problem and contact an Applied Biosystems service representative.

Performing the	Procedure To perform the Liquid Level Detect test:		
Liquid Level Detect Test			
	Step	Action	
	1	Go to the Instrument menu, scroll to Tests, and select Function Test.	
		A Function Tests window appears.	
	2	Click the Liquid Detect tab.	
	3	Check the Perform 'Liquid Level Detect' Test box.	
	4	Select a reagent reservoir to test:	
		a. Place non-deionized water in a reagent reservoir.	
		b. Place the reagent reservoir in any reagent reservoir position.	
		c. From the Use Reagent pop-up menu, select the reagent reservoir location that contains water.	

	contains water.	
5	Select tips to test:	
	a. Place a full 200- μ L disposable tip rack in the designated tip rack position (1–8).	
	IMPORTANT You must always use a full 200- μ L disposable tip rack in the tip rack position.	
	 From the Use Tip Rack Position pop-up menu, select the tip rack that contains disposable tips. 	
6	Enter a number from 1 to 768 in the Repeat field for the number of times to repeat the test.	
	Note The number of times to repeat the test is limited by the number of available tips.	
7	Check the tips to test in the Tips To Use section of the tab.	
8	Click Start.	

Process

The robotic arm moves to the designated tip rack, uses the designated tips to pick up the disposable tips, moves to the designated reagent reservoir, lowers the arm until the tips sense liquid, and proceeds to eject the disposable tips into the tip eject bin.

If Failure Occurs

If the tips do not detect liquid properly, take the following actions:

- Verify that the tips are tight and disposable tips are all positioned properly.
- Check the white cable that runs from the tips to the robotic arm for crimps or cuts.
- Repeat this test.

If the test fails after performing all of the above actions, test each robotic arm tip individually to isolate the problem and contact an Applied Biosystems service representative.

Performing the Procedure

Diluters Test

Та		م ما ا		40.04.
10	periorm	the	Diluters	test:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Diluters tab.		
3	Check the Perform 'Diluters' Test box.		
4	Check the box next to the diluters you want to test.		
5	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.		
6	Click Start.		

Process

The selected diluters initialize, check encoder counts, and check motor function.

If Failure Occurs

If the selected diluters fail to initialize or fail to function, take the following actions:

- Make sure that the diluters are fully seated. Push them toward the back of the instrument.
- Restart the 6700 instrument.
- Restart the 6700 software.
- Repeat this test.

If the test fails after performing the above actions, test each diluter individually to isolate the problem and contact an Applied Biosystems service representative.

Testing Peltiers Procedure

To test Peltiers:

Step	Action
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.
	A Function Tests window appears.
2	Click the Misc tab.
3	Check the Test Peltiers box.
4	Noting the time, click Start.

Process

The Peltier units cool, then the Test Log reports the deckspace temperatures.

If Failure Occurs

If the Peltier units fail to cool, take the following actions:

 Using appropriate personal protective equipment, open the instrument door and touch the Peltier-cooled deckspace stations to see if they are cool. Temperature sensors may malfunction and report inaccurate deckspace temperatures when condensation forms on the deckspace. In this case, allow time for the temperature sensors to dry before retesting.

- Restart the 6700 instrument.
- Restart the 6700 software.
- Repeat this test.

If the test fails after performing all of the above actions, contact an Applied Biosystems service representative.

Cooling Peltiers to Procedure

4 °C To cool Peltiers to 4 °C:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Misc tab.		
3	Check the Cool Peltiers to 4 °C box.		
4	Place temperature probes in wells within the following deckspace locations:		
	Input station		
	 Vacuum station: filtrate and archive positions 		
	Dilutions/cDNA station		
	 Standards, Master Mix/Cell Lysate Control station 		
	Output station		
5	Click Start.		

Process

The Peltiers cool to 4 °C, then the Test Log reports the deckspace temperatures.

If Failure Occurs

If the Peltier units fail to cool to 4 °C, take the following actions:

- Using appropriate personal protective equipment, open the instrument door and touch the Peltier-cooled deckspace stations to see if they are cool. Temperature sensors may malfunction and report inaccurate deckspace temperatures when condensation forms on the deckspace. In this case, allow time for the temperature sensors to dry before retesting.
- Restart the 6700 instrument.
- Restart the 6700 software.
- Repeat this test.

If the test fails after performing all of the above actions, contact an Applied Biosystems service representative.

Testing Vacuum Perform this test if the System Flush test is not flushing the system fluid or if the vacuum pressure is not sufficient to complete a run.

Procedure

To test vacuum pumps:

Step	Action
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.
	A Function Tests window appears.
2	Click the Misc tab.
3	Check the Test Vacuum Pumps box.
4	Click Start.

Process

The small vacuum pump turns on and then off. Then the large vacuum pump turns on and then off. Listen for both pumps to turn on and then off.

If Failure Occurs

Note which pump fails to turn on and off, then contact an Applied Biosystems service representative.

Testing Valves Perform this test if the vacuum carriage does not move up/down or if the vacuum pressure is not sufficient to complete a run.

Procedure

To test valves:

Step	Action	
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.	
	A Function Tests window appears.	
2	Click the Misc tab.	
3	Check the Test Valves box.	
4	Enter a number from 1 to 999 in the Repeat field for the number of times to repeat the test.	
5	Click Start.	

Process

All liquid and pressure valves sequentially turn on and then off. Listen for the valves to click on and off. Note any gaps in the sequence.

If Failure Occurs

Note any gaps in the sequence, then contact an Applied Biosystems service representative.

Performing the Procedure Archive Cover Test To perform the Archive Cover test:

Step	Action		
1	Go to the Instrument menu, scroll to Tests, and select Function Tests.		
	A Function Tests window appears.		
2	Click the Archive Cover tab.		
3	Check the Perform 'Archive Cover' Test box.		
4	Check the boxes next to the archive covers to test.		
5	For each archive cover test, select the Cover Destination from the pop-up menu.		
6	Place plastic consumables on the deckspace:		
	a. Place archive covers in the appropriate positions on the Archive Cover station.		
	b. Place archive plates in the appropriate positions on the deckspace.		
	c. Close the instrument door.		
7	Click Start.		

Process

The robotic arm transfers the archive covers to the designated cover destination and seals the archive plates.

If Failure Occurs

If the robotic arm fails to transfer the archive covers properly, take the following actions:

- Repeat "Calibrating the Deckspace" on page 6-15.
- Make sure the archive covers are placed on the Archive Cover station properly.
- Make sure tips 1 and 4 (*i.e.*, the tips closest and farthest from you) are tightened properly.
- Repeat this test.

If the test fails after performing all of the above actions, then:

- Check for a damaged archive cover.
- Check the Archive Cover station for bending or damage.
- Check tips 1 and 4 (*i.e.,* the tips closest and farthest from you) for bending or damage.
- Note any damage, then contact an Applied Biosystems service representative.

Instrument Calibration

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Calibration	All calibrations are performed through the 6700 software. The proper function of the			
Requirement	6700 software requires access to the 6700 database. If access to the 6700 database is blocked for any reason, the 6700 software will not work			
Calibrating the	To calib	rate the deckspace:		
Deckspace	Step	Action		
	1	From the Instrument menu of the 6700 software, scroll to Litility and select		
		Calibration.		
		The Instrument Calibration dialog box appears.		
Instrument Colibration		Instrument Calibration		
		Status		
2 Make sure that the deckspace is clear: a. Remove the reagent reservoir platform.		Make sure that the deckspace is clear:		
		a. Remove the reagent reservoir platform.		
b. Remove all consumables from the deckspace.		b. Remove all consumables from the deckspace.		
	3	Click the Calibrate button.		
 This initializes the instrument. The instrument proceeds by lowering all four tips to the deck surface next to the 		 This initializes the instrument. The instrument proceeds with calibrating Deck Z by lowering all four tips to the deck surface next to the Input station. 		
 After the robotic arm completes I calibrating Deck X-Y. 		 After the robotic arm completes Deck Z calibration, the instrument proceeds with calibrating Deck X-Y. 		
 The robotic arm moves Tip 1 to the right of the calibration location. (The calibration location is deckspace). The Move Arm dialog box appears. 		 The robotic arm moves Tip 1 to the right of the Input 1 position, over the calibration location. (The calibration location is a square hole cut in the deckspace). 		
		 The Move Arm dialog box appears. 		
		Move Arm X Move tip over hole		
		Back Icft Forward Forward During		

To calibrate the deckspace: (continued)

Step	Action				
4	Using the mouse, click the arrows on the screen to move the arm until Tip 1 is centered above the calibration location.				
	 The robotic arm moves upward, slightly toward the back of the instrument, and back downward. 				
	 The arm lowers Tip 1 to the deck surface and "walks" it toward the front of the instrument until Tip 1 falls into the calibration location. The robotic arm moves upward, slightly to the right, and back downward. 				
	 The arm lowers Tip 1 to the deck surface and "walks" it toward the left un falls into the calibration location. 				
5	After the instrument calibrates the location, click Done .				
	The robotic arm moves to the next calibration location.				
6	Repeat steps 4 through 5 until the robotic arm calibrates all deckspace locations.				
	Note Deckspace calibration requires about 40 minutes to complete.				
	If this is a	The calibration positions are			
	96-well instrument	Output 1			
		 Behind the Output 4 position 			
		 At the edge of all three archive cover shelves (elevated above the deckspace) 			
		 At the eight disposable tip racks 			
		• At four points below the disposable tip racks			
	384-well upgrade	 Output 1 at two positions: back left and back right corners 			
		 Behind the Output 4 position 			
		Center of well A1			
		 At the edge of all three archive cover shelves (elevated above the deckspace) 			
		 At the eight disposable tip racks 			
		• At four points below the disposable tip racks			
7	Click Exit.				
Instrument Decontamination



Overview

About This Appendix	 If you expose the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation to potentia biologically hazardous material (<i>e.g.</i>, blood or plasma), you need to contact a certif professional to decontaminate the 6700 instrument enclosure with formaldehyde vapor. This appendix contains the recommended decontamination procedures for instrument enclosure. 		
	IMPORTANT These decontamination procedures must be performed by a certified professional before an Applied Biosystems service representative can service the instrumentation of the service representative can service the instrumentation of the service representative can service the service the service representative can service the servi		
	IMPORTANT This appendix does not provide any decontamination procedures for the instrument enclosure if it contains residual hazardous, nonmicrobiological materials. Remediation of such hazards may require a case-by-case review by a qualified safety professional.		
In This Appendix This appendix contains the following topics:			
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	Decontamination Requirements	A-2	
	Formaldehyde Vapor Decontamination Overview	A-3	
	Formaldehyde Vapor Decontamination Procedures A-5		

Decontamination Requirements

When to
Decontaminate the
InstrumentThe instrument should be decontaminated if the enclosure has been used with
potentially biologically hazardous materials (*e.g.*, blood or plasma) and before any of
the following events:

- · Repair or replacement of potentially contaminated components
- Relocation
- Decommissioning

 Who Can
 IMPORTANT
 Execution of the procedures in this appendix should be performed only by adequately trained individuals.

 Instrument
 Individuals who perform this decentamination procedure must:

Individuals who perform this decontamination procedure must:

- Know safe handling practices for paraformaldehyde and ammonium bicarbonate
- Have successfully completed a respiratory fitness evaluation for the use of a full-face respirator by a licensed physician within the preceding calendar year
- Be currently listed as a Biohazard Cabinet Field Certifier accredited by the National Sanitation Foundation, International (NSF)

The list of current NSF-Accredited Biohazard Cabinet Certifiers is available at the NSF web site: http://www.nsf.org/.

Formaldehyde Vapor Decontamination Overview

Process Description The process of formaldehyde vapor decontamination involves the following stages:

Stage	Process	
1	All potentially biologically contaminated work surfaces are isolated and placed under negative pressure with respect to the environment local to the instrument enclosure.	
2	All potentially biologically contaminated surfaces in the enclosure are exposed to the following conditions for a minimum of 4 hours:	
	 Formaldehyde vapor at a concentration of 0.3 g per cubic foot (approximately 8000 ppm) 	
	 High relative humidity targeted at 70% 	
3	After the minimum exposure time, the formaldehyde vapor is neutralized using ammonium bicarbonate vapor and/or externally vented.	

Definitions Enclosure

The enclosure is the 6700 instrument cabinet that provides a controlled environment for automation of nucleic acid sample purification and preparation.



Decontamination

Decontamination is the reduction of bioburden on the potentially biologically contaminated surfaces of the enclosure to an acceptable level. This should not be confused with either surface cleaning or sterilization.

Equipment and F Supplies Required

Equipment and Formaldehyde vapor decontamination requires the following equipment and supplies:

Category	Description
Chemicals	 15 g of paraformaldehyde (ACS)
	 16 g of ammonium bicarbonate (ACS)
	♦ Water
Documents	MSDSs for paraformaldehyde and ammonium bicarbonate
	Warning signs
	Decontamination labels
Instruments	 Temperature and relative humidity meter
	 Formaldehyde vapor permissible exposure level (PEL) monitor wit a detection limit ≤ 0.1 ppm over 4 hours of exposure
	 ◆ Spontaneous formaldehyde vapor sensor with a detection limit ≤ 0.2 ppm
Miscellany	Pen, calculator, and hand tools
Process	 Two small evaporator pans
equipment	 Two electrical extension cords
	 Plastic sheeting and adhesive tape
	 Approximately 4 mm of ID tubing for connection to an anemomete
	Auxiliary fan
	 New inline carbon adsorber cell (carbon mass ≥ 1 kg) for the removal of formaldehyde vapor
	 Ducting, approximately 100 mm of ID tubing for connecting to the a filter and/or auxiliary fan
Safety equipment	Respirator with formaldehyde cartridges
	Rubber gloves
	 Safety glasses
	 Small dry chemical spill kit

Preliminary Setup
 Verify the function of the equipment.
 Verify the scope of work with Safety.
 Verify the enclosure is available for decontamination with the users.

Chemical Removal
Before beginning this procedure, remove all chemicals from the enclosure that may produce exceptionally toxic or dangerous compounds upon exposure to formaldehyde or ammonium vapors.

Formaldehyde Vapor Decontamination Procedures

Overview

Decontamination Formaldehyde vapor decontamination involves the following procedures:

Торіс	See Page
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Preparing the Enclosure	A-5
Sealing the Enclosure	A-6
Generating Formaldehyde Vapor	A-7
Exposing the Enclosure to Formaldehyde Vapor	A-8
Neutralizing the Enclosure	A-8
Ventilating the Enclosure	A-8
Re-establishing Pre-existing Conditions	A-8
Wiping Down the Decontaminated Enclosure	

Enclosure

Inspecting the To inspect the enclosure:

Step	Action
1	Wearing appropriate protective equipment, inspect the enclosure for damage that would indicate a breach of the biohazard containment zone within the enclosure.
2	Record any unexpected damage.
3	If you cannot perform this decontamination procedure safely, notify the users and Safety immediately.

Enclosure

Preparing the To prepare the enclosure:

Sten	Action		
1	Switch off any controls and place tape over the switches.		
2	Place the two small evaporator pans into the enclosure with the power controllers set to \leq 200 °C (392 °F). The unplugged power cords should be extended outside the enclosure.		
3	Label one evaporator pan power cord for paraformaldehyde and the other for ammonium bicarbonate.		
4	Measure and record the temperature and relative humidity (rH) within the enclosure.		
	If the temperature is < 15.6 °C (60 °F), then raise the ambient temperature to > 15.6 °C before proceeding.		
5	Determine the amount of water required, as follows:		
	If the relative humidity is Then		
	> 60%	do not add any additional water.	
	< 60%	calculate the amount of water to add using the following equation:	
		Number of grams of water (g) = 0.0055 x (Enclosure Volume (ft ³)) x (70-(ambient %rH)).	

To prepare the enclosure: (continued)

Step	Action
6	Measure the appropriate amount of water and add it to the paraformaldehyde evaporator pan.
	Record the actual amount of water used.
7	Place the paraformaldehyde evenly across the corresponding evaporator pan.
	A WARNING CHEMICAL HAZARD. Paraformaldehyde is a flammable solid. Exposure causes eye, skin, and respiratory tract irritation. Paraformaldehyde may cause allergic reactions and may be harmful if inhaled or swallowed. It may cause nervous system damage and is a cancer hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
8	Place the ammonium bicarbonate evenly across the corresponding evaporator pan.
	A WARNING CHEMICAL HAZARD. Ammonium bicarbonate may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Sealing the To seal the enclosure:

Enclosure

Ston	Action		
Siep			
1	Disconnect any exhaust system from the enclosure and cap the system ends.		
2	a. Connect the low pressure leg of the gauge to 4 mm of tubing.		
	b. Extend the tubing to lie within the enclosure.		
	c. Place the gauge outside the enclosure where it may be easily seen.		
3	a. Close the sash on the enclosure.		
	b. Cover the sash and all openings with plastic sheeting held airtight with adhesive tape.		
4	a. Cut a small hole in the plastic sheeting (or exhaust port).		
	b. Attach approximately 100 mm of plastic ducting with adhesive tape so that the only obvious gas exchange between the enclosure and the local environment occurs through the duct.		
5	Connect the duct to the inlet side of the adsorber cell.		
6	Connect a T-fitting to the outlet side of the adsorber cell.		
7	Connect a short length of duct to one of the available ports on the T-fitting and extend the duct to the inlet side of the auxiliary fan.		
8	Finish sealing the enclosure, giving special attention to the power cords and anemometer connections.		
9	Switch on the auxiliary fan.		
10	By controlling the area of the bypass port on the T-fitting and the speed of the auxiliary fan, adjust the differential pressure so that the enclosure is between $25 \text{ mm} (-0.01 \text{ in.})$ water column and $13 \text{ mm} (-0.005 \text{ in.})$ water column with respect to the adjoining area.		

Generating	rating To generate formaldehyde vapor:			
Formaldehyde	Step	Action		
, apor	1	Place decontamination warning signs with your pager number on the enclosure and by the doorway (primary and secondary containment).		
	2	a. Activate and affix a PEL monitor badge to the outer face of the enclosure.		
		b. Record the PEL monitor ID and start time.		
	3	Switch on the paraformaldehyde evaporator pan.		
		AWARNING CHEMICAL HAZARD. Paraformaldehyde is a flammable solid. Exposure causes eye, skin, and respiratory tract irritation. Paraformaldehyde may cause allergic reactions and may be harmful if inhaled or swallowed. It may cause nervous system damage and is a cancer hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.		
		WARNING CHEMICAL HAZARD. Formaldehyde is harmful if inhaled or swallowed. Exposure to formaldehyde vapors causes eye, skin, and respiratory tract irritation. Formaldehyde may cause allergic reactions and is a cancer hazard. Please obtain and read an MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.		
	4	If the concentration of formal dehyde vapor outside the enclosure increases to \geq 0.2 ppm at any time:		
		a. Disconnect the paraformaldehyde evaporator pan.		
		b. Switch the ammonium bicarbonate evaporator pan on for the same amount of time as the paraformaldehyde pan.		
		WARNING CHEMICAL HAZARD. Ammonium bicarbonate may cause eye, skin, and respiratory tract irritation. Please read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.		
		c. Proceed immediately to "Ventilating the Enclosure" on page A-8.		
	5	Observe the evaporator pan until the paraformaldehyde is completely evaporated, then turn off the pan. If the pan is not visible, allow 30 minutes for complete evaporation.		

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Exposing the	To expose the enclosure to formaldehyde vapor:		
Formaldehvde	Step	Action	
Vapor	1	After the paraformaldehyde has evaporated or 30 minutes after switching on the evaporator, record the time.	
	2	Allow the formaldehyde vapor to contact the surfaces within the enclosure for at least 4 hours.	
		A WARNING CHEMICAL HAZARD. Formaldehyde is harmful if inhaled or swallowed. Exposure to formaldehyde vapors causes eye, skin, and respiratory tract irritation. Formaldehyde may cause allergic reactions and is a cancer hazard. Please obtain and read an MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.	

Enclosure

Neutralizing the To neutralize the enclosure:

Step	Action
1	After the formaldehyde vapor has been allowed to contact the enclosure surfaces for at least 4 hours, switch on the ammonium bicarbonate evaporator pan.
	A WARNING CHEMICAL HAZARD. Ammonium bicarbonate 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	Observe the evaporator pan until the ammonium bicarbonate is completely evaporated.
	Turn off the pan. If the pan is not visible, allow 30 minutes for complete evaporation.
3	Allow 15 minutes for the two vapors to complex.

Ventilating the To ventilate the enclosure: Enclosure

Step	Action
1	After neutralization is complete, gradually increase the flow rate and negative pressure within the enclosure. Do this by carefully slitting the plastic covering until the negative pressure remains < 1.3 mm (0.05 in.) water column.
2	Allow the enclosure to vent at this rate for at least 1 hour.

Pre-existing

 $Re\text{-establishing} \quad \text{To re-establish pre-existing conditions:}$

Conditions

Step	Action
1	Record the stop time.
2	Cover and seal the PEL monitor badge.
3	Record the results from the PEL monitor immediately, or when they become available if an outside laboratory is used.
4	Wipe residue, if any, from the immediately accessible work surfaces.

Wiping Down the
Decontaminated
Enclosure

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To eliminate trace residues from the decontaminated enclosure:

Step	Action
1	Monitor and record the concentration of formaldehyde vapor in the enclosure and in the immediate vicinity.
2	Wearing appropriate protective equipment, spray all of the exposed surfaces of the enclosure with a weak ammonia solution at ambient temperature.
	ADANGER CHEMICAL HAZARD. Ammonium hydroxide solution (aqueous ammonia) causes burns to the eyes, skin, and digestive and respiratory tracts. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Allow a 3-minute contact time.
4	Dry the exposed surfaces thoroughly with lint-free tissues. Discard tissues as chemical waste.
	WARNING Always follow the safety precautions regarding waste in the waste profile. Dispose of the waste in accordance with all local, state, and federal health and environmental regulations and laws.
5	Spray all of the exposed surfaces again using 70% isopropanol (30% deionized water).
	A WARNING CHEMICAL HAZARD . Isopropanol is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. It may cause central nervous system effects such as drowsiness, dizziness, and headache, etc. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6	Allow a 10-minute contact time.
7	Dry the exposed surfaces thoroughly with lint-free tissues.
8	Monitor and record the concentration of formaldehyde vapor in the enclosure and in the immediate vicinity.

6700 Workstation **Materials**



Applied Biosystems Materials

6700 Workstation This appendix contains part numbers for the Applied Biosystems reagents and plastic Materials Overview consumables that are designed for preparing nucleic acids on the ABI PRISM™ 6700 Automated Nucleic Acid Workstation.

Reagents

Reagents	P/N
Model 6700 System Fluid	4308456
Nucleic Acid Purification Elution Solution	4305893
Nucleic Acid Purification Lysis Solution	4305895
RNA Purification Wash Solution 1	4305891
RNA Purification Wash Solution 2	4305890

Consumables

Plastic Consumable	Illustration	P/N
MicroAmp [®] 96-Well Optical Reaction Plate with Barcode		4306737
MicroAmp [®] 384-Well Optical Reaction Plate with Barcode		4309849
6700 Splash Guards	A DOLOGONO COLO	4311758
Archive Covers		4306286
Conductive Pipette Tips, 1000-µL		4306377
Conductive Pipette Tips, 200-µL		4306375

Plastic Consumable	Illustration	P/N
Deep-well plate		4308641
Disposable Tip Eject Bin Liner (box of five)		4316565
Microcentrifuge Tubes and Caps, 2-mL		4305936
Optical Heat Seal Covers		4307726
Optical Cover Compression Pads		4312639
Reagent Reservoirs, 120-mL	CHING STATE	4304831
Reagent Tubes with Caps, 10-mL		4305932
Total RNA Purification Trays	No. Lange	4305673
Waste/vacuum line		4326865

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Troubleshooting

Overview

About ThisThis appendix describes error messages and provides troubleshooting informatioAppendixthe ABI PRISM [™] 6700 Automated Nucleic Acid Workstation.		
In This Appendix	is Appendix This appendix contains the following topics:	
	Торіс	See Page
	Error Messages and Recoveries in 6700 Software v1.1	C-2

Error Messages and Recoveries in 6700 Software v1.1

Overview The ABI PRISM[™] 6700 Automated Nucleic Acid Workstation software may display the following error messages:

- Tip Not Fetched
- ILID Pulse
- Tip Not Ejected
- Unable to Reach Position/Drive
- Liquid Not Detected

The possible causes (states) for these error messages and the action(s) recommended to recover are discussed in the following sections.

Tip Not Fetched IMPORTANT Wear gloves for these procedures.

Error

CAUTION Never touch the robotic arm tips (gold) with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.

Possible Cause(s)	Recommended Action(s)	
Incorrect number of tip	a. Open the instrument door.	
racks added at the start	b. Add the correct number of tip racks.	
	 Remove any remaining disposable tips and raise all robotic arm tips to their maximum height. 	
	d. Close the door and click OK .	
Missing disposable tips	a. Open the instrument door.	
in rack	b. Replace the used rack with a new, full tip rack.	
	 Remove any remaining disposable tips and raise all robotic arm tips to their maximum height. 	
	d. Close the door and click OK .	
Rack mounted	a. Open the instrument door.	
incorrectly	b. Replace the used rack with a new, full tip rack. Make sure the rack is correctly seated.	
	 Remove any remaining disposable tips_and raise all robotic arm tips to their maximum height. 	
	d. Close the door and click OK .	
Loose robotic arm tips	a. Open the instrument door.	
	b. Replace the rack currently in use with a new, full tip rack.	
	c. Remove any remaining disposable tips from the robotic arm.	
	d. Tighten the robotic arm tips with the white Teflon tip tightener.	
	e. Raise all robotic arm tips to their maximum height.	
	f. Close the door and click OK .	

ILID Pulse Error The ILID pulse is the electronic signal that informs the 6700 instrument that a tip has been properly mounted.

IMPORTANT Wear gloves for these procedures.

A CAUTION Never touch the robotic arm tips (gold) with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.

Possible Cause(s)	Recommended Action(s)
A Tip Not Fetched error had	a. Open the instrument door.
already occurred. The user clicked OK without fixing the	 b. Check that the correct number of racks and tips are correctly mounted.
problem(s).	 Check that the tip eject bin is empty and secured to the deckspace.
	d. Remove any remaining disposable tips.
	 e. Tighten the robotic arm tips with the white Teflon tip tightener.
	f. Replace the rack currently in use with a new, full tip rack.
	g. Raise all robotic arm tips to their maximum height.
	h. Close the door and click OK .

Error

Tip Not Ejected IMPORTANT Wear gloves for these procedures.

A CAUTION Never touch the robotic arm tips (gold) with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.

Possible Cause(s)	Recommended Action(s)	
Tip incompletely ejected	a. Open the instrument door.	
from the robotic arm	b. Manually remove the tip.	
	 c. Check that the tip eject bin deckspace. 	is empty and secured to the
	d. Tighten the robotic arm tips tightener.	s with the white Teflon tip
	e. Raise all robotic arm tips to their maximum height.	
	f. Close the door and click OK .	
	Note If this error continues to occur or if all the tips did eject properly, the instrument may have a damaged tip mount switch. See "Damaged tip mount switch" below.	
Damaged tip mount switch	Test the tip mount switch by running the Get Disp. Tips function test (see page 6-6 for instructions).	
	IMPORTANT Test each indiv problems (12 ejections/tip).	vidual tip while watching for
	If the error message comes up even though all tips eject properly	If one or more tips continue to eject incompletely
	Call your Applied Biosystems service representative.	See "Loose reagent reservoir/tip eject holder unit" on page C-4.

Possible Cause(s)	Recommended Action(s)	
Loose reagent reservoir/tip eject plate	 Tighten the captive screw securing the front of the tip eject plate. 	
Note If this is loose, the whole reagent reservoir assembly lifts as the tip ejects.	b. Adjust the height of the setscrew at the back of the tip eject plate (the adjustment may have altered over time).The tip eject plate is secure if it no longer moves when the tips eject.	
	Note If you cannot adjust the screws or secure the tip eject plate, call your Applied Biosystems service representative.	

Unable to Reach This error displays when the robotic arm is unable to reach a position, or when the Position/Drive Error 6700 instrument cannot detect that the position has been reached.

Possible Cause(s)	Recommended Action(s)		
The robotic arm's torque sensor has been tripped. This sensor prevents damage to the arm by pausing when there is too much pressure on the arm as it moves between positions	 a. Open the instrument door. b. Close the door and click OK to resume the run. Note If this error continues to occur, call your Applied Biosystems service representative. 		
Damage to the tip mount switch caused the robotic	If the arm is	Then	
arm to increase its height beyond its maximum ^a	over the eject position and the tip has ejected correctly	a. open the instrument door.b. Close the door and click OK to resume the run.	
	over the eject position but the tip has not ejected correctly	 a. open the instrument door. b. Remove the disposable tips. c. Tighten the robotic arm tips. d. Raise all tips to their maximum height. e. Close the door and click OK to resume the run. 	
	Note If this error occurs aga the run and call your Applied representative.	ain at the next tip eject, abort Biosystems service	
Racks are set at incorrect height due to incorrect instrument calibration	 a. Abort the run. b. Recalibrate the 6700 instrument. See "Instrument Calibration" on page 6-15 for instructions. c. Restart the run. Note If this error continues to occur, run the Get Disp. Tips Test to check the tip mounting/ejection function. See page 6-6 for instructions. 		

Possible Cause(s)	Recommended Action(s)
Liquid in the primary input position is highly viscous	See "Liquid Not Detected Error" on page C-5.

a. If the tip mount switch is broken, the 6700 instrument will not be able to detect when the tips have ejected. The robotic arm will increase its height as it continues to try ejecting tips. Once beyond the maximum height, the torque sensor shuts down power to the arm.

Error

Liquid Not Detected IMPORTANT Wear gloves for these procedures.

ACAUTION Never touch the robotic arm tips (gold) with bare fingers. The oil from your skin will affect the functionality of the 6700 instrument.

Possible Cause(s)	Recommended Action(s)		
Insufficient liquid in a	a. Open the instrument door.		
position (<i>e.g.,</i> master mix)	b. Raise all tips to their maximum height.		
	c. Determine which position i	s low:	
		1	
	If you	Then	
	do not know which position is low	 close the door and click OK to resume the run. 	
		 Let the error happen again and note which position is low. 	
		 Open the instrument door. 	
		 Raise all tips to their maximum height. 	
		 Continue with step d. 	
	do know which position is low	continue with step d.	
	d. Add the appropriate liquid	to the position.	
	e. Close the door and click O	K to resume the run.	
Liquid in the primary input position is highly viscous	a. Retry the liquid detection. These errors can be generated intermittently.		
	b. If the error occurs multiple one of the following:	times, abort the run and do	
	 Dilute the solution 1:1 or hiviscosity). OR 	igher with 1X buffer (to reduce	
	 Set the number of mixes to or RNA/DNA Archive proto thoroughly mixed before pr the samples at least three pipette. OR 	o 0 (zero) in the Lysis protocol col. Ensure that the lysate is rocessing. To do this, hand-mix times with a multi-channel	
	Check the High Viscosity E protocol.	ox in the RNA/DNA Archive	

What Causes High Viscosity

For certain cell lines, adding total RNA lysis reagent to cell counts $>1x10^{6}$ cells per well results in a highly viscous lysate with the consistency of honey. The high viscosity is the result of the complete lysis of cells, with the release of genomic DNA (gDNA) into solution. The high efficiency of the lysis solution makes this phenomenon occur at cell counts and volumes of lysate not typically seen with other reagents.

In addition, cell lines with higher ploidy may show this phenomenon at a lower cell count, due to the higher concentration of gDNA. Tetraploid HeLa cells, for example, have twice as much gDNA per cell.

Cell Lines More Likely to Become Highly Viscous

This phenomenon has been noted for the following cell lines and concentrations:

- ♦ Human Raji cells at ≥1x10⁷ cells/well
- HeLa cells at approximately ≥5x10⁶ cells/well
- ◆ U937 cells at approximately ≥1x10⁶ cells/well

Impact of High Viscosity Liquids

The 6700 instrument uses the change in capacitance between air and liquid to sense when the tip reaches a liquid surface. Viscous liquid can leave adherent strings of material on the tips. The physical connection between the liquid on the tip and the liquid in the well prevents the instrument from sensing the capacitance change.

This happens more frequently if mixing is set to anything other than 0 (zero) in the Lysis protocol or RNA/DNA Archive protocol, since the strings of material are drawn up with the tip after the last mix step. If the change in capacitance cannot be sensed, the tip drives to the bottom of the well, triggering the Liquid Not Detected error or Unable to Reach Position error.

D

References

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, and National Institutes of Health. 1999. *Biosafety in Microbiological and Biomedical Laboratories, 4th ed.* Richmond, J.Y. and McKinney, R.W., eds. Washington, DC: U.S. Government Printing Office. For sale by the Superintendent of Documents, U.S. Government Printing Office. Stock no. 017-040-00547-4.

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Technical Support

Contacting	You can contact Applied Biosystems for tec	chnical support:		
Technical Support	• By e-mail			
 By telephone or fax Through the Applied Biosystems web site 				
To Contact Technical Support by E-Mail	To contact Applied Biosystems Technical S product areas:	upport by e-mail for help in the following		
	Product/Product Area	E-mail address		
	Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com		
	Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com		
	Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com		
	Biochromatography	tsupport@appliedbiosystems.com		
	PerSeptive DNA, PNA and Peptide Synthesis systems			
	FMAT™ 8100 HTS System			
	CytoFluor [®] 4000 Fluorescence Plate Reader			
	Mariner™ Mass Spectrometers			
	Voyager™ Mass Spectrometers			
	MassGenotyping Solution 1™ (MGS1) System			
	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 Technical 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
1.800.8	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
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 3ª	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM [®] 877 and Catalyst 800 instruments)	1.800.831.6844, press 2, then press 4ª	1.650.638.5981
ABI PRISM [®] 3100 Genetic Analyzer	1.800.831.6844, press 2, then press 6ª	1.650.638.5981
Peptide Synthesis (433 and 43x Systems)	1.800.831.6844, press 3, then press 1ª	1.650.638.5981
Protein Sequencing (Procise [®] Protein Sequencing Systems)	1.800.831.6844, press 3, then press 2ª	1.650.638.5981
PCR and Sequence Detection	1.800.762.4001, then press:	1.240.453.4613
	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	
Voyager™ MALDI-TOF Biospectrometry Workstations	1.800.899.5858, press 1, then press 3 ^b	1.508.383.7855
Mariner™ ESI-TOF Mass Spectrometry Workstations		
MassGenotyping Solution 1™ (MGS1) System		
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™ Nucleic Acid Synthesis Systems	1.800.899.5858, press 1, then press 5 ^b	1.508.383.7855

Product/Product Area	Telephone	Fax
Peptide Synthesis (Pioneer™ and 9050 Plus 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 System	1.800.899.5858,	1.508.383.7855
CytoFluor [®] 4000 Fluorescence Plate Reader	press 1, then press 6 ^b	

Chemiluminescence (Tropix)	1.800.542.2369 (U.S. only), or 1.781.271.0045 °	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	1.800.952.4716	1.508.383.7899

a. 5:30 A.M. to 5:00 P.M. Pacific time.

b. 8:00 A.M. to 6:00 P.M. Eastern time.

c. 9:00 A.M. to 5:00 P.M. 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
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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

Region	Telephone	Fax
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

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 3138
	91 11 653 3744	
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
Lat	in 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

To Reach Technical To contact Technical Support through the Applied Biosystems web site:

Step	Action
1	Go to http://www.appliedbiosystems.com
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3	Click Contact Support in the contents list at the left of the screen.
4	Click your geographic region for the product area of interest.
5	In the Personal Assistance form, enter the requested information and your question, then click Ask Us RIGHT NOW .

To contact Technical Support through the Applied Biosystems web site: (continued)

Step	Action
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4	In the results screen, do any of the following:			
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	 Right-click the pdf icon, then select Save Target As to download a copy of the PDF file. 			
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Information	Step	Action	
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Glossary

- archive plate Plate that contains nucleic acid purified or diluted by the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation.
- archive cover Plastic consumable that seals archive plates for long-term storage.
- Assay output plates Plate prepared by the 6700 workstation that contains Master Mixes, standards, controls, and samples from an RNA archive, DNA archive, cDNA archive, or Dilution archive plate. Assay output plates can be used for a variety of applications, including standard curve analysis, comparative quantification, allelic discrimination, plus/minus analysis, and custom analysis.
- Assay protocol 6700 workstation protocol to prepare up to four Assay output plates containing Master Mixes, standards, controls, and samples from an RNA archive, DNA archive, cDNA archive, or Dilution archive plate.
- Assay wizard 6700 system software tool for creating Assay protocols.
- cDNA archive plate Plate that contains cDNA prepared by the 6700 workstation.
- cDNA Archive protocol 6700 workstation protocol to reverse transcribe cDNA from RNA by mixing RNA and reverse transcription master mix together in a cDNA archive plate and then heating the plate for reverse transcription.
- Consumable ID Barcode label on Applied Biosystems consumables.
- deckspace A 1.17 x 0.43-m (46 x 17-in.) plate within the 6700 instrument that holds samples, reagents, plastic consumables, and waste for automated protocols.
- detector A nucleic acid probe in a master mix used for standard curve analysis, comparative quantification, allelic discrimination, plus/minus analysis, or custom analysis. Detectors contain a reporter dye and a quencher dye.
- diluters Control the volume and rate of liquid aspiration and dispensing by the robotic arm tips.

Dilution archive plate Plate that contains nucleic acid diluted by the 6700 workstation.

- **Dilution Archive protocol** 6700 workstation protocol to perform up to two serial dilutions of an RNA archive, DNA archive, or cDNA archive plate.
- DNA archive plate Plate that contains DNA purified by the 6700 workstation.
- DNA Archive protocol 6700 workstation protocol to purify DNA from DNA precipitate.
- DNA Precipitation protocol 6700 workstation protocol to precipitate DNA.
- dye Fluorescent marker on the detectors.
- elution The process of displacing nucleic acid from the purification tray filter by adding solvent (e.g., elution solution).
- filtrate Sample solution that flows through the purification tray filter.
- Lysis protocol 6700 workstation protocol to lyse cells by mixing cells with lysis solution.
- optical heat seal cover Covers designed to seal Assay output plates.
- Peltier units Used to cool the Input station; Standards, Master Mix/Cell Lysate Control station; Dilutions/cDNA station; and Output station to 4 °C.
- RNA archive plate Plate containing RNA purified by the 6700 workstation.
- RNA Archive protocol 6700 workstation protocol to purify RNA from lysed cells.
- RT master mix Solution that contains reverse transcriptase, nucleotides, and other components required to reverse transcribe cDNA from RNA.

- sample types Define the samples in an Assay protocol. Default sample types include buffer, internal positive control I (IPC+), internal positive control II (IPC-), no amplification control (NAC), no probe control (NPC), no template control (NTC), standard (STND), and unknown (UNKN).
- touchoff Movement of the purification tray carriage to release drops from the purification tray into filtrate, archive, and waste positions.
- viscosity The state of fluid cohesiveness and consistency. For example, water is a low-viscosity liquid and glycerol is a high-viscosity liquid.

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