ABI PRISM® 377 DNA Sequencer

96-Lane Upgrade

User's Manual

Applied Biosystems

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

Contents

1 Introduction

Overview

About This Manual

This manual describes the enhancements to the ABI PRISM® 377 DNA Sequencer included in the 96-lane upgrade.

Be sure to place this manual in your *ABI PRISM 377 DNA Sequencer User's Manual*.

Technical Support Contacts

For technical support contact information refer to the *ABI PRISM 377 DNA Sequencer User's Manual*.

In This Chapter

The following topics are covered in this chapter.

Upgrade Overview

Product Overview

The ABI PRISM® 377 DNA Sequencer 96-lane Upgrade Kit enhances the capabilities of the 377 DNA sequencer to support up to 96 lanes for both Sequencing and GeneScan applications.

Key Features

The following list provides an overview of the key features of the 96-lane upgrade.

- Increased scan window by 20%, allowing additional lanes to be added without losing sensitivity or increasing scan time
- Increased number of data collection to 480 channels, allowing data collection of three channels plus two-channel separation per lane
- Improved Neural Net Tracker, decreasing labor to process gels
- Increased comb thickness in loading area to 0.4 mm while using 0.2-mm gel for electrophoresis, causing no change in run time
- Improved comb durability and geometry, allowing easier loading of volumes up to 1.5 µL
- Added position-based CCD integration with time scaling, allowing collection while accelerating the stage, which minimizes noise
- Upgraded instruments still run 36-, 48-, and 64-lane gels

Hardware Required

The following hardware is required to upgrade to 96-lane capability.

- ABI PRISM 377-36 or 377XL DNA Sequencer
- Macintosh® computer with the following specifications
  - Power PC processor
  - 32 MB RAM (RAM modules supplied if required)
  - Mac OS 8 (supplied in kit)

Important Upgrade Notes

Matrices

To ensure data quality, we strongly recommend rerunning matrices at installation and semiannually for applications where matrices may be critical for optimal signal-to-noise ratio (e.g., heterozygote detection and any GeneScan application).

Combs

The 96-lane run mode only supports the use of a shark's-tooth comb.

Clamps

The 96-lane plates and casting combs require 10–12 lbs. clamping pressure. To prevent well leakage, only use clamps that meet this requirement. Use our stainless steel "bulldog" clips (P/N 4305386) or measure other clamps with a force gauge.

Mac OS 8 Extension Conflicts

There are known conflicts with some of the Mac OS 8 extensions. It is important to turn off these extensions before beginning any 96-lane run.

From the Extensions Manager window turn off the following extensions:

- Open Tpt Modem
- Open Tpt Remote Access
- Open Tpt Serial Arbitrator

Computer Notes

Can Run on Any Macintosh Computer

96 lanes can be run on any Macintosh computer supplied with the 377 instrument.

Processor Speed

The processor speed does not impact Collection, but it does impact the speed of analysis.

Hard Drive Disk Space

The hard drive must have enough disk space to hold a 70 MB gel file.

A CD Drive Is Required to Load Analysis Software

Analysis will work on any Macintosh computer. However, the 7100 Macintosh computers supplied with the 377 instrument were not shipped with a CD drive, which is required to load the Analysis software.

Kit Configurations

Kit Configurations Table The following table lists the components and quantity of components included in ABI PRISM® 377 DNA Sequencer 96-lane upgrade kits.

Component (Quantity)	Part No. (P/N)	Kit Contents					
		377-96-66/80B	377-96-90B	377-96-90C	377-96-120C	377-96-C	377-96-XL
Kit, stepped plates, 36-cm, pair of spacers[a]	4305693	2	2	2	2	2	2
Disk, 377 Collection s/w v. 2.5	4305535	1	1	1	1	1	1
Manual, user's 96-lane upgrade	4305423	1	1	1	1	1	1
Clamps, glass, 2-in.	4305386	3	3	3	3	3	3
Comb, 100-well, shark's-tooth cast, 0.4-mm, 1.8-mm center	4305385	2	2	2	2	2	2
Kit, EEPROM, 96-lanes	—	1	1	1	1	1	1
PCA, tested 16 MHz, 377XL	—	1	1	—	—	—	—
Upgrade 8 MB Power Mac RAM SIMM	—	2	—	—	—	—	—
Upgrade 8 MB Power Mac RAM DIMM	—	—	2	2	1	—	—
Mac OS 8	—	1	1	1	1	1	1
Seq Anal 3.2[b]	—	1	1	1	1	1	1
GeneScan 3.0[b]	—	1	1	1	1	1	1

a. Spacers are 48 cm and need to be cut to size before use.

b. As licensed.

Note The 96-lane upgrade also includes hardware modifications that will be made by the service engineer at installation.

2 *Gels*

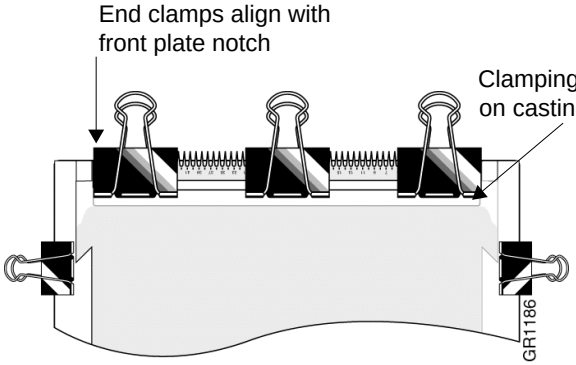
Overview

In This Chapter The following topics are covered in this chapter.

Preparing Gels

Pouring Gels

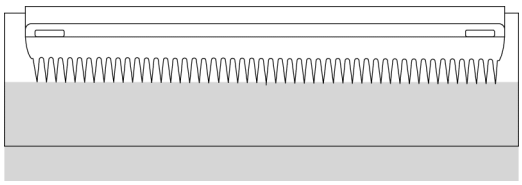
To pour gels for use on the ABI PRISM 377 96-lane DNA sequencer:

Step	Action
1	Cast the gels as instructed in the *ABI PRISM 377 DNA Sequencer User's Manual.* Use 0.2-mm spacers (P/N 401837) and a 0.4-mm 96-lane casting comb (P/N 4305385) with the new stepped front plate (P/N 4305384). **! WARNING ! CHEMICAL HAZARD. Acrylamide and Bis-Acrylamide are both poisons, neurotoxins, irritant, carcinogens, and possible teratogens. Acrylamide and Bis-Acrylamide sublimes (the solid releases toxic vapor) and is harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear personal protection (i.e., lab coat, safety glasses, and chemical resistant gloves) and use in a well ventilated area. Thoroughly clean surfaces subject to contamination (i.e., binder clips, combs, and glass plates).**
2	Clamp the gels as shown below. Use three stainless steel "bulldog" binder clips (P/N 4305386) on the top. **IMPORTANT** To prevent well leakage, the 96-lane plates and casting combs require 10–12 lbs. clamping pressure.  **IMPORTANT** Be careful not to damage the teeth of the comb when attaching the clamps.

Preparing a Gel

To prepare a gel for a run:

Step	Action
1	Let the gel polymerize for at least two hours.
2	Remove the clamps from the gel. Leave the casting comb in place until you are ready to insert the comb.
3	Rinse the plate thoroughly with dH_2O and let dry. **IMPORTANT** Carefully clean the read region of the gel.
4	Slide out the casting comb without bending it. **IMPORTANT** Do not pry the casting comb.
5	Using a razor blade, scrape off all excess acrylamide from the glass in the loading area.
6	Using a squirt bottle, rinse the loading area with dH_2O and dry with at lint-free tissue.
7	Using a syringe, add 1x TBE in to the loading area. **Note** Adding TBE eases the insertion of the comb. **IMPORTANT** Be very careful not to introduce bubbles. They are very difficult to remove once the comb has been inserted.
8	If necessary, clean the shark's tooth comb with dH_2O and a lint-free tissue.

Step	Action
9	Carefully insert the comb into the gel. a. Carefully align the center registration line on the comb with the registration mark on the back plate. b. Slide the comb between the plates. **IMPORTANT** To avoid bending or breaking the teeth of the comb, ensure all teeth enter the space between the plates at the same time. Do not force the comb into the gel because the teeth will bend, causing leaking. c. Continue to slide the comb down until the tips of the teeth just touch or slightly depress the surface of the gel.  d. Teeth should just barely indent the surface of the gel. If the surface of the gel is not completely flat in the loading region, insert some of the teeth below the surface of the gel (up to 0.5 mm) so that all of the teeth touch the gel surface. e. If a tooth has penetrated the gel surface do not attempt to withdraw the comb. This will cause sample to leak into adjacent wells.
10	Place the gel and the cassette in the 377 instrument. **Note** For instructions on setting up the 377 instrument for a run refer to the *ABI PRISM 377 DNA Sequencer User's Manual*.

Setting Run Conditions

Selecting a Run Mode

Use the following table to select the type of comb to use based on the number of lanes you are running.

Note The correct run mode is automatically chosen when the number of lanes is selected.

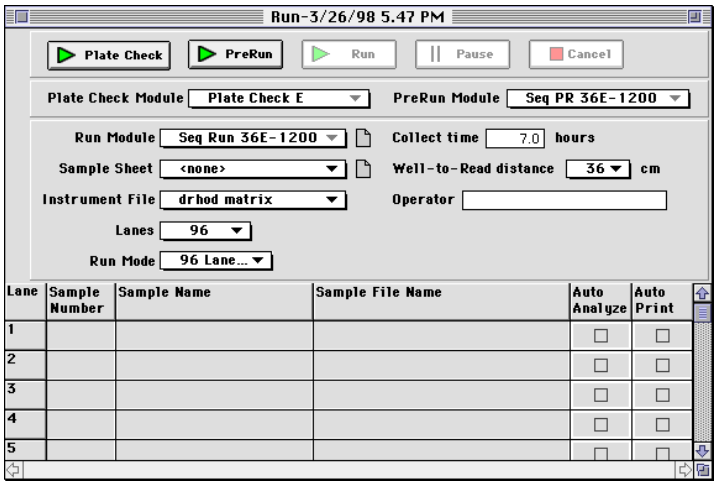
No. of Lanes	Comb	Run Mode
24	Shark's-tooth	Full Scan
24	Square-tooth	Full Scan
32	Shark's-tooth	Full Scan
34	Square-tooth	Full Scan
36	Shark's-tooth	Full Scan
36	Square-tooth	Full Scan
48	Shark's-tooth	XL Scan
50	Square-tooth	XL Scan
64	Shark's-tooth	XL Scan
66	Square-tooth	XL Scan
96	Shark's-tooth	96 Scan

Note It is possible to run a gel of any number lanes in 96 Scan mode. There will be the same number of data collection points per lane, but there will be an area of blank space to the left and right of the samples due to extra scan width. However, the Neural Net Tracker has been trained using gels run according to the default parameters. (For example: 48-lane gels run in XL mode and 36 lane gels run in Full Scan mode.) Any deviation from the default is likely to confuse the tracker resulting in mistracked lanes.

Setting Run Conditions

To set gel run conditions:

Step	Action
1	Open the 377-96 Collection software.
2	Prepare a sample sheet as described in the *ABI PRISM 377 DNA Sequencer User's Manual*. **IMPORTANT** Preparing a sample sheet prior to the run is required for optimal tracker operation.

Step	Action
3	Select a new GeneScan or Sequencing run. The Run window is displayed. 
4	Within the Run window perform the following: a. Select 96 from the Lanes pulldown menu. **Note** The correct run mode is then automatically selected. b. Select the plate check Pre-run and Run modules that corresponds to your desired filter set from the appropriate pulldown menus. c. Select the proper instrument file (matrix) for your run. **IMPORTANT** The tracker will not function unless the matrix file was selected before starting the run. d. Select the proper sample sheet.
5	Perform the plate check, prerun, and run procedures as instructed in the *ABI PRISM 377 DNA Sequencer User's Manual*.

Loading Gels

Loader Options

The following loaders can be used to load a 96-lane gel.

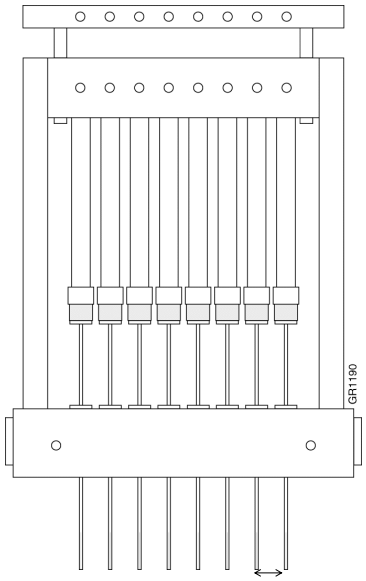
- Fixed-pitch, 10.8-mm loader and a plate rack holding micro-amp tubes spaced 10.8 mm apart
- P-10 microliter pipet with a flat loading tip
- Single-barrel syringe with 0.2-mm or 0.3-mm needles
- Two-pitch, eight-channel loader

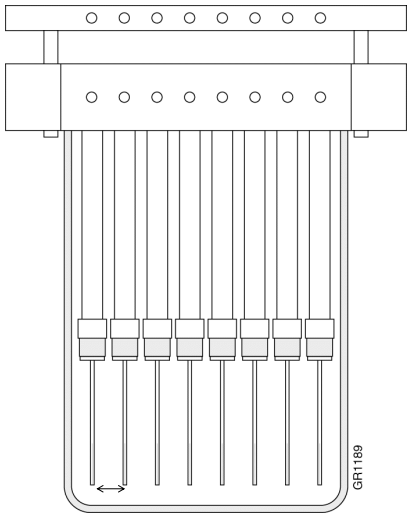
Note For reasons of loading speed and accuracy, Applied Biosystems highly recommends using a two-pitched, eight-channel loader to load 96-lane gels.

Two-Pitch, Eight-Channel Loader

The following schematics depict generic two-pitched, eight-channel loaders in their closed position.

Note For specific vendor information, see “Two-Pitch, Eight-Channel Loader Suppliers” on page B-1.





Distance between needles:

- 9 mm in closed position
- 10.8 mm in open position

Suggested Sequencing Load Volumes

The following table lists the suggested load volumes and sample resuspension volumes for sequencing.

No. of Wells	Resuspension Vol. (µL)[a]	Loading Vol. (µL)
24/36	6–9	1.5
48	2–4	1.0–1.5
64	2–4	1.0–1.5
96	2–4	1.0–1.5[b]

a. 5:1 Deionized formamide to 50 mg blue dextran/mL in 25 mM EDTA

b. Loading 1.5 µL requires a syringe with a 0.2-mm tip to facilitate loading at the bottom of the well.

! WARNING ! CHEMICAL HAZARD. Formamide is a known teratogen. It can cause birth defects. Wash thoroughly after handling formamide. Wear appropriate protective eyewear, clothing, and gloves. Obtain a copy of the MSDS from the manufacturer. Wash thoroughly after handling formamide.

Suggested GeneScan Load Volumes

The following table lists the suggested load volumes for GeneScan.

No. of Wells	Loading Vol. (µL)
24/36	1.5
50	1.0–1.5
66	0.5–1.0
96	1.0–1.5[a]

a. Loading 1.5 µL requires a syringe with a 0.2-mm tip to facilitate loading at the bottom of the well.

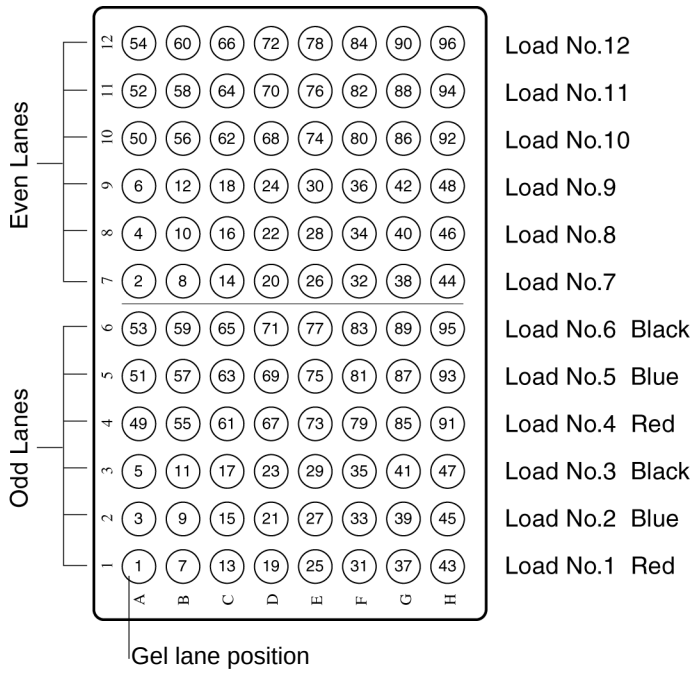
Note For more details, refer to the *GeneScan Reference Guide* and the *LMS v. 2 User's Manual.*

Suggested Load Mapping

The following schematic depicts a microtiter plate showing the suggested load mapping for an eight-channel loader.

Notes:

- Loading in staggered format with a two-pitch, eight-channel loader takes 12 loading steps (Load No. 1–12).
- The number in each well represents the respective gel lane position.
- Odd-lane loading positions are color coded on the comb.



Suggested Loading Sequence

Follow the loading procedure to load the odd lanes first, electrophorese for 2 min, then load the even lanes.

IMPORTANT If any wells leak, flush the contaminated lanes then follow the table below.

If you are running...	Then...
Sequencing	Electrophorese immediately, then after each three loads.
GeneScan	Leaking wells are not tolerated in GeneScan applications. If a well leaks, it is best to run another gel. At the very least, do not use the wells around the leaking lane.

Loading Procedure

To load the gel:

Step	Action
1	Press PAUSE during the prerun.
2	Flush the wells with 1x TBE using a syringe. **Note** Use care when flushing the wells: Too much pressure could tear the wells, and touching the teeth with the syringe could damage the comb and displace the teeth, which could cause leakage.
3	Using the two-pitch, eight-channel loader, draw 2 µL of sample into the needles.
4	Clear any air gaps in the needles by dispensing 0.5 µL of sample, or by dispensing until sample is visible in the tips of the syringe.
5	Using the comb markers as a guide, align the needles into their respective lanes.
6	Very slowly dispense up to 1.5 µL of samples into the wells. Load the odd lanes first. **Note** For longer reads, load the samples close to the gel surface rather than from the top of the well. To accomplish this, the two-position loading syringe must have needles with 0.2-mm or 0.25-mm outer diameters. With 0.3-mm outer diameter needles, the samples must be gravity loaded.
7	After each loading, rinse the needles with warm dH_2O and blot dry with a lint-free tissue to remove residual salt and prevent clogging.
8	Continue to load until all odd lanes have been loaded.

Step	Action
9	Electrophorese for 2 min.
10	Repeat steps 2–7 to load the even lanes.
11	End the prerun and begin the run.

Software and Firmware 3

Overview

In This Chapter The following topics are covered in this chapter.

Software

GeneScan Analysis

The current GeneScan Analysis software is v. 3.0 with GSGelTracker.

Sequence Analysis

The current Sequence Analysis software is v. 3.2 with SAGelTracker.

Collection Software

The current Collection software for 96 lanes is v. 2.5.

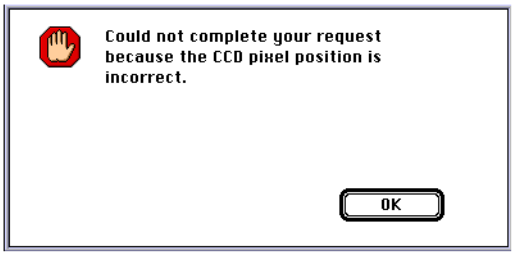
Preference File

Define default values in the new software following the procedures described under “Setting Preferences” in the *ABI PRISM 377 DNA Sequencer User’s Manual*.

The default values you define for Sequencing or GeneScan run modules and sample sheets are maintained in the Preference file.

CCD Pixel Position

The instrument is shipped with the correct CCD pixel position value in memory. When a run is started, the software checks for a value greater than zero. If the value is lost from memory, an error message is displayed at the beginning of the run as shown below.



The CCD pixel position value may become corrupted as the result of a power surge or power failure. If this occurs, you must enter the correct value before starting the run. For details, refer to the *ABI PRISM 377 DNA Sequencer User’s Manual*.

Module Files

There are many choices available among the module files provided in the ABI PRISM 377-96 upgrade. Because you may not use all of the module files, move those you do not intend to use from the Modules folder into the Unused Modules folder.

377-96 and Chiller Module Files[a]

377-96 Modules	Chiller Modules
GS PR 12A-1200	GS PR 12A-1200 CHILLER
GS PR 12A-2400	GS PR 12A-2400 CHILLER
GS PR 12C-1200	GS PR 12C-1200 CHILLER
GS PR 12C-2400	GS PR 12C-2400 CHILLER
GS PR 12D-1200	GS PR 12D-1200 CHILLER
GS PR 12D-2400	GS PR 12D-2400 CHILLER
GS PR 12F-1200	GS PR 12F-1200 CHILLER
GS PR 12F-2400	GS PR 12F-2400 CHILLER
GS PR 36A-1200	GS PR 36A-1200 CHILLER
GS PR 36A-2400	GS PR 36A-2400 CHILLER
GS PR 36C-1200	GS PR 36C-1200 CHILLER
GS PR 36C-2400	GS PR 36C-2400 CHILLER
GS PR 36D-1200	GS PR 36D-1200 CHILLER
GS PR 36D-2400	GS PR 36D-2400 CHILLER
GS PR 36E-1200	GS PR 36E-1200 CHILLER
GS PR 36E-2400	GS PR 36E-2400 CHILLER
GS PR 36F-1200	GS PR 36F-1200 CHILLER
GS PR 36F-2400	GS PR 36F-2400 CHILLER
GS Run 12A-1200	GS Run 12A-1200 CHILLER
GS Run 12A-2400	GS Run 12A-2400 CHILLER
GS Run 12C-1200	GS Run 12C-1200 CHILLER
GS Run 12C-2400	GS Run 12C-2400 CHILLER
GS Run 12D-1200	GS Run 12D-1200 CHILLER
GS Run 12D-2400	GS Run 12D-2400 CHILLER
GS Run 12F-1200	GS Run 12F-1200 CHILLER
GS Run 12F-2400	GS Run 12F-2400 CHILLER
GS Run 36A-1200	GS Run 36A-1200 CHILLER
GS Run 36A-2400	GS Run 36A-2400 CHILLER

377-96 and Chiller Module Files[a] *(continued)*

377-96 Modules	Chiller Modules
GS Run 36C-1200	GS Run 36C-1200 CHILLER
GS Run 36C-2400	GS Run 36C-2400 CHILLER
GS Run 36D-1200	
GS Run 36D-2400	GS Run 36D-2400 CHILLER
GS Run 36F-1200	GS Run 36F-1200 CHILLER
GS Run 36F-2400	GS Run 36F-2400 CHILLER
Plate Check A	Plate Check A CHILLER
Plate Check C	Plate Check C CHILLER
Plate Check D	Plate Check D CHILLER
Plate Check E	Plate Check E CHILLER
Plate Check F	Plate Check F CHILLER
Seq PR 36A-1200	Seq PR 36A-1200 CHILLER
Seq PR 36A-2400	Seq PR 36A-2400 CHILLER
	GS Run 36D-1200 CHILLER
Seq Run 36A-1200	Seq Run 36A-1200 CHILLER
Seq Run 36A-2400	Seq Run 36A-2400 CHILLER
Seq Run 36E-1200	Seq Run 36E-1200 CHILLER
Seq Run 36E-2400	Seq Run 36E-2400 CHILLER
Seq Run 48A-1200	Seq Run 48A-1200 CHILLER
	Seq Run 48B-1200 CHILLER
Seq Run 48E-1200	Seq Run 48E-1200 CHILLER
	GS Run 60W D CHILLER
	GS Run 2140V A CHILLER
	GS Run 2140V C CHILLER
	GS Run 2140V D CHILLER
	GS Run 60W A CHILLER
	GS Run 60W C CHILLER

a. PR = Prerun; Seq = Sequencing; GS = GeneScan

Firmware

Collection Channels Increased

The 96-Lane Scan mode uses 480 collection channels per scan, up from 388 in an XL Scan mode. This provides a 5x oversampling for analysis.

Position-Based Integration Scheme

Due to the increased demand for positional accuracy of the detection optics, a new integration scheme is used. Previously, a given time was given to each channel, before reading the CCD camera and switching to the next channel. This release of firmware introduces position-based CCD integration, where predetermined stage positions determine when to switch.

Expanded Scan Region

The 96-Lane Scan mode uses a larger scan region to accommodate the increased comb size. The stage travels farther towards the edge on each side, and accelerates at a faster pace as it reenters the scan region. This offsets the increased number of collection channels, so that the integration time per channel remains approximately that of an XL scan. As a result, there is no loss of sensitivity for a 96-lane scan compared to an XL scan.

The instrument firmware automatically adjusts the size of the read region according to the selected scan mode. Users who wish to prevent the firmware from redefining the size of the scan window may be provided with special module files for this purpose.

Reloading the Firmware

If the instrument does not respond to commands or responds inappropriately, the firmware image may be corrupted. You can reset the firmware by performing a total reset (also known as a double reset).

Performing a Total Reset

A total reset erases the current firmware image from instrument memory. This is indicated by the instrument status lights changing from green (ready) to flashing yellow.

To reset the firmware image with a total reset:

Step	Action
1	Exit Collection.
2	Press the reset button on the back of the 377 instrument.
3	Immediately press the reset button again.

After a total reset, a new copy of the firmware will be downloaded to the instrument when you relaunch the ABI PRISM 377-96 Collection software.

4 Troubleshooting

Overview

In This Chapter

The following topics are covered in this chapter.

Troubleshooting References

For more information on troubleshooting, refer to the following manuals:

- *GeneScan Reference Guide*
- *ABI PRISM 377 DNA Sequencer User's Manual*

Troubleshooting

Gels

Problem	Possible Cause	Solution
Leaking wells	Loose combs	Sequencing: Electrophorese immediately, then after each three loads with the eight-channel loader. GeneScan: Leaking wells are not tolerated in GeneScan applications. If a well leaks, it is best to run another gel. At the very least, do not use the wells around the leaking lane.
	Bad clamps	Be sure to use three “bulldog” clamps (P/N 4305386) with 10–12 lbs. clamping pressure.
	Burrs or bent teeth on comb	Remove the burrs or replace the comb.
	Bent, kinked, or damaged spacers	Replace the spacers.
Error: “Your CCD offset is too high. I will reset it to zero.”	The CCD reading is below zero during calibration scan	Reset the CCD offset value: a. Open 377-96 Collection. b. In the Run window select the Run module. c. Double-click the small document icon next to the Run Module pulldown menu. d. Change the CCD offset value to zero. e. Click Save as Default.
Comb is difficult to insert	Using a different comb	Be sure to use same comb for loading that was used for casting.
	Clamps are too tight	♦ Insert comb slowly. Fix any misaligned teeth with a syringe before they touch the gel. ♦ Use looser clamps on future gels.

Thermistors

Problem	Possible Cause	Solution
Error: “Thermistor Failure”	One or more thermistors are bad	Schedule a service call to replace the thermistors. Continue to use the instrument as usual.
Error: “Temperature below thermistor limit.”	Ambient temperature is too low (< 21.9 °C) for 100k thermistor	♦ Turn on the pump to warm the coolant to above 21.9 °C. ♦ Scedule a service call service to replace the thermistors. ♦ Continue to use the instrument as usual.

Results

Problem	Possible Cause	Solution
Odd and even lanes overlap	Running too long between staggered loadings	Shorten the run time between loadings.
	Too much salt in the sample	♦ Resuspend samples in formamide only. ♦ Perform extra 70% ethanol rinse of samples if precipitated (may lead to slight loss in signal).
Signal showing up in neighboring lanes	Leaky lanes	Check clamps and comb fit.
	Signal intensity very high and signal is being detected in neighboring lanes due to closeness of spacing	♦ Move tracker lane position from center of band to the edge of the band away from the strong signal and extract as usual. ♦ Use one or two lane averaging to extract lanes. ♦ Load less volume.

Problem	Possible Cause	Solution
Signal too weak	Multiple	♦ Increase the CCD gain to four: a. Open 377-96 Collection. b. In the Run window select the Run module. c. Double-click the small document icon next to the Run Module pulldown menu. d. Change the CCD gain to four. ♦ Resuspend samples in less volume (concentrate).

377-96 Error Messages

Message	Possible Cause	Solution
A Valid 96 Lane Firmware Image is Required!	A non-96 collection software has tried to establish communications with a 377 instrument that has the 96-lane option installed.	Install the 96-lane collection software and firmware.
EP Voltage Deviation Exceeds Tolerance	The EP voltage deviated outside its tolerance range. The instrument operation is paused.	Call service.
Warning: Plate Out. Thermistor P43/J43 Open/Short Circuit Warning: Plate In. Thermistor P44/J44 Open/Short Circuit Warning: Possible Heater Thermistor Open/Short Circuit	Indicates one of the following: ♦ Possible open or short circuit exists with the thermistor/cable connected to J43 or J44. ♦ Temperature of the plate in an instrument with the 100k ohm thermistors is 21.9 °C or less.	One of the thermistors is not functioning properly. Schedule a service call, and continue to operate the instrument as usual. This message may appear when you launch data collection software and start a plate check, prerun, or run.

Message	Possible Cause	Solution
Flow Detected With Pump Off –External Cooling In Use!	Either: The wrong module is being used for a run where an external cooling device is attached, or The internal coolant system valve is stuck on or in the open position	If an external cooling device is in use: ♦ Check the modules selected on the run sheet. Use Chiller modules. If no external cooling system is in place: ♦ Try to start a run as follows: a. Click OK in the error message box and try to start the run. b. Open the Manual Control window and try to turn on the pump manually. ♦ Call service.
Err: Coolant Flow Failure!	Occurs after the pump was turned on and off three times to see if coolant flow was detected.	Open the Manual Control window and try to turn on the pump manually. If the problem persists call service.
No flow detected! Attempted Pump Restart	Indicates the coolant pump was turned on, but no coolant flow was detected by the flow switch.	Check the reservoir to see if there is liquid in the cooler.
Scanner Did Not Find Its Home Position	Indicates the scanner did not find its home position prior to collecting data for a plate check, prerun, or run.	Reset by pressing the Reset button once on the back of the 377. Click the Resume button in the Collection Run window.

Glass Plates

Applied Biosystems does not support the use of third party plates or combs on the 377 instrument.

We have been manufacturing glass plates to exact tolerances for slab gel electrophoresis for over 10 years. Our plates are highly refined. Third party plates are not made to our proprietary process tolerances and may exhibit variances from the necessary dimensions.

Filter Set/Dye Combinations

A

Virtual Filter Set	Dyes		Chemistry
A	GeneScan: R110, R6G, TAMRA, ROX		[F} dNTP
	Sequencing:	JOE (A), 5-FAM (C), TAMRA (G), ROX (T)	Dye primer
		R6G (A), ROX (T), R110 (G), TAMRA (C)	Dye terminator
C	GeneScan: 6-FAM,TET, HEX, TAMRA		Linkage Mapping Set V. 1
	Sequencing: None		None
D	GeneScan: 6-FAM, HEX, NED, ROX		Linkage Mapping Set V. 2
	Sequencing: None		None
E	GeneScan: None		None
	Sequencing: dR6G, dTAMRA, dR110, dROX		♦ BigDye™ Terminator ♦ BigDye™ Primer ♦ dRhodamine terminator
F	GeneScan: 5-FAM, JOE, NED, ROX		♦ AmpFℓSTR Profiler™ PCR Amplification Kit ♦ AmpFℓSTR Profiler Plus™ PCR Amplification Kit ♦ Plus PCR Amplification Kit ♦ AFLP™ Plant Mapping Kit
	Sequencing: None		None

Two-Pitch, Eight-Channel Loader Suppliers

B

Supplier Information Tables

Introduction

For your convenience, the following tables provide information on suppliers of two-pitch, eight-channel loaders.

IMPORTANT Contact the companies listed for availability, pricing, and technical information regarding these products.

Suppliers Inside the U.S.

Supplier	Supplier Headquarters	Product	Part No.
Kloehn Company	10000 Banburry Cross Dr. Las Vegas, NV 89134 USA Voice: (702) 243-7727 Fax: (702) 243-6036 World Wide Web: http://www.kloehn.com **Outside U.S. offices are listed on the following page.**	Loader, 0.25-mm	18597
		Loader, 0.3-mm	18663
		Needle, 0.25-mm (8)	18597
		Needle, 0.3-mm (8)	18628

Supplier	Supplier Headquarters	Product	Part No.
World Precision Instruments, Inc.	Sarasota International Trade Center 175 Sarasota Center Blvd. Sarasota, FL 34240-9258 USA Voice: (941) 371-1003 Fax: (941) 377-5428 World Wide Web: http://www.wpiinc.com **Outside U.S. offices are listed on the following page**.	Loader	Gel Mate 96
		Needle, 0.25-mm (10)	67124

Note Hamilton Co. (702-858-3000) also supplies loaders that may work with this upgrade.

Suppliers Outside the U.S.

Supplier	Supplier Contact	Geographic Areas Served
Kloehn Europe	Bahnhofstrasse 12 Postfach 55 CH-7402 Bonaduz, Switzerland Voice: 41 81 630 2303 Fax: 41 81 641 3488 E-mail: kloehneurope@bluewin.ch	♦ Europe
World Precision Instruments, Inc. Australia	P.O. Box 1191 Glen Waverly, Victoria 3150 Australia Voice: 61 (0) 3 9887-6262 Fax: 61 (0) 3 9887-9585 E-mail: wpiau@c031.aone.net.au	♦ Australia ♦ Indonesia ♦ Malaysia ♦ New Guinea ♦ New Zealand

Supplier	Supplier Contact	Geographic Areas Served
World Precision Instruments, Inc. Germany	Liegnitzer Str. 15 D-10999 Berlin, Germany Voice: 49 (0) 30-6188845 Fax: 49 (0) 30-6188670 E-mail: wpi@wpi.sireco.de	♦ Austria ♦ Bulgaria ♦ Czechoslovakia ♦ Germany ♦ Greece ♦ Holland (Netherlands) ♦ Hungary ♦ Italy ♦ Poland ♦ Rumania ♦ Russia ♦ Switzerland ♦ Yugoslavia
World Precision Instruments, Inc. Japan	1-4-2-702 Naka-Meguro, Meguro Tokyo 153-0061, Japan Voice: 81 (0) 3-3760-5050 Fax: 81 (0) 3-3760-5055 E-mail: wpi@tkb.att.ne.jp	♦ Japan
World Precision Instruments, Inc. United Kingdom	Astonbury Farm Business Centre Aston, Stevenage Hertfordshire SG2 7EG England Voice: 44 (0) 1438-880025 Fax: 44 (0) 1438-880026 E-mail: wpi@piuk.demon. co.uk	♦ Belgium ♦ Denmark ♦ England ♦ Finland ♦ France ♦ Ireland ♦ Norway ♦ Portugal ♦ Scotland ♦ Spain ♦ Sweden

Supplier	Supplier Contact	Geographic Areas Served
World Precision Instruments, Inc. Other world-wide areas	Sarasota International Trade Center 175 Sarasota Center Blvd. Sarasota, FL 34240-9258 USA Voice: (941) 371-1003 Fax: (941) 377-5428 E-mail wpi@wpiinc.com	♦ Areas not listed above

Part Numbers

C

ABI PRISM 377 DNA Sequencer Parts

Plates and Spacers

P/N	Item
401878	48-cm Glass plates/spacers kit includes two sets of 48-cm well-to-read glass plates and gel spacers
401876	36-cm Glass plates/spacers kit: includes two sets of 36-cm well-to-read glass plates and gel spacers
401877	12-cm Glass plates/spacers kit: Includes two sets of 12-cm well-to-read glass plates and gel spacers
401835	48-cm Rear glass plate
401838	48-cm Front glass plate
401837	48-cm Gel spacers, 0.2-mm (2)
401839	36-cm Rear glass plate
401840	36-cm Front glass plate
4305384	36-cm Front stepped plates (2)
401833	12-cm Rear glass plate
401834	12-cm Front glass plate
4305384	36-cm Front stepped plates (2)

Cassette, Buffer Chambers, Heat Plate, and Clamps

P/N	Item
603627	Gel cassette
603947	Top and bottom gel pouring fixtures
401969	Top pouring fixture
604014	Bottom pouring fixture
603873	Upper buffer chamber
603875	Lower buffer chamber
603822	Upper buffer electrode assembly
603823	Lower buffer electrode assembly
4303201	Front 36-cm well-to-read heat plate
4305386	Clamps, glass, 2-in., “Bulldog”

ABI PRISM DNA Fragment Analysis Kits and Reagents

Internal-Lane Size Standards

GeneScan-350, 500, and 400HD contain enough material for 800 lanes. GeneScan-1000 and 2500 contain enough material for 400 lanes. GeneScan-500XL contains enough material for 1600 lanes. Loading buffer is included.

P/N	Item
401735	GeneScan-350 [ROX]
401736	GeneScan-350 [TAMRA]
402985	GeneScan-400HD [ROX]
401734	GeneScan-500 [ROX]
401733	GeneScan-500 [TAMRA]
403040	GeneScan-500XL [TAMRA]
403039	GeneScan-500XL [ROX]
401098	GeneScan-1000 [ROX]
401100	GeneScan-2500 [ROX]
401545	GeneScan-2500 [TAMRA]
401144	Loading buffer

Fluorescent dNTPs

For fluorescent labeling of DNA during PCR amplification:

P/N	Item	Quantity
401894	[F]dUTP Set: [R110], [R6G], and [TAMRA]	3, 3, and 12 nmol (3 x 30 µL)
401896	[R110]dUTP	6 nmol (2 x 30 µL)
401897	[R6G]dUTP	6 nmol (2 x 30 µL)
401895	[TAMRA]dUTP	24 nmol[a] (2 x 30 µL)
402793	[F]dCTP Set: [R110], [R6G], and [TAMRA]	3, 3, and 12 nmol (3 x 30 µL)
402795	[R110]dCTP	6 nmol (2 x 30 µL)
402796	[R6G]dCTP	6 nmol (2 x 30 µL)
402794	[TAMRA]dUTP	24 nmol[a] (2 x 30 µL)

a. [TAMRA]dNTP is supplied at a concentration four times higher than [R110]dNTP and [R6G]dNTP because it produces approximately four times less signal.

Fluorescent dNTP PCR Kits

Each kit listed below includes a GeneAmp® kit as specified (100 reactions) along with an [F]dNTP set that contains 30 µL each of [R110]dNTP (3 nmol), [R6G]dNTP (3 nmol), and [TAMRA]dNTP (12 nmol).

P/N	Kit
N808-0220	GeneAmp PCR Reagent Kit with AmpliTaq® DNA Polymerase with [F]dUTP Set
N808-0221	GeneAmp PCR Core Reagents with [F]dUTP Set
N808-0222	GeneAmp Thermostable r*Tth* Reverse Transcriptase RNA PCR Kit with [F]dUTP Set
N808-0223	GeneAmp PCR Reagent Kit with AmpliTaq DNA Polymerase with [F]dCTP Set
N808-0224	GeneAmp PCR Core Reagents with [F]dCTP Set
N808-0225	GeneAmp Thermostable r*Tth* Reverse Transcriptase RNA PCR Kit with [F]dCTP Set

Fluorescent Phosphoramidites

For direct 5' end labeling on an automated DNA synthesizer:

P/N	Item	Quantity
401527	[6-FAM] Phosphoramidite	85 mg
401533	[TET] Phosphoramidite	100 mg
401526	[HEX] Phosphoramidite	105 mg

Fluorescent NHS-Esters

For post-synthesis labeling of primers containing a 5' Aminolink 2:

P/N	Item	Quantity
400981	[TAMRA] NHS-Ester	5 mg/60 µL in DMSO
400980	[ROX] NHS-Ester	5 mg/60 µL in DMSO
400808	Aminolink 2	0.25 g

Matrix Standard Sets

P/N	Kit
401114	Dye Primer Matrix Standards Kit (Filter Set A) for NHS-ester labeling Contains one tube each of 5-FAM-, JOE-, TAMRA-, and ROX-labeled DNA
402792	[F]dNTP matrix standards Contains one tube each of R110-, R6G-, TAMRA-, and ROX-labeled DNA
401546	Fluorescent Amidite Matrix Standards Kit (Filter Set C) for fluorescent phosphoramidite labeling Contains one tube each of 6-FAM-, TET-, HEX-, TAMRA- and ROX-labeled DNA
402996	NED matrix standard Used in combination with the 5-FAM, JOE and ROX dyes in the Dye Primer Matrix Standards Kit or the 6-FAM, HEX, and ROX dyes in the Fluorescent Amidite Matrix Standards Kit

Fluorescent Genotyping Demonstration Kits A and B

P/N	Kit
402246	Kit A: PCR reagents Contains six fluorescent labeled PCR primer pairs labeled with [HEX], [TET] & [FAM], two control DNAs (CEPH 1347-02 and 1347-10), and a ready made mix of PCR reagents containing AmpliTaq Gold™ DNA Polymerase, GeneAmp PCR Buffer II, dNTPs, and magnesium chloride Also includes GeneScan-350 Internal Lane Size Standard and loading buffer
402247	Kit B: Amplified PCR products Contains four tubes of pooled (combined) PCR products. To generate the products each DNA sample (CEPH 1347-01, 1347-02, 1347-10, 1347-15) has been amplified with the same six fluorescent-labeled PCR primer pairs in kit A. All of the PCR products from one tube can be detected in one gel lane.

ABI PRISM Linkage Mapping Set Version 2

50-Rxn Kits	300-Rxn Kits	Panel	Chromosome
403089	403118	Complete Set	1–22, X
403090	403119	1	1
403091	403120	2	1
403092	403121	3	2
403093	403122	4	2
403094	403123	5	3,4
403095	403124	6	3,4
403096	403125	7	3,4
403097	403126	8	5,6
403998	403127	9	5,6
403099	403128	10	5,6
403100	403129	11	7,8
403101	403130	12	7,8
403102	403131	13	9,10,11
403103	403132	14	9,10,11
403104	403133	15	9,10,11
403105	403134	16	9,10,11
403106	403135	17	12,13
403107	403136	18	12,13
403108	403137	19	12,13
403109	403138	20	14
403110	403139	21	15,16
403111	403140	22	15,16
403112	403141	23	17,18
403113	403142	24	17,18
403114	403143	25	19,20,21,22
403115	403144	26	19,20,21,22
403116	403145	27	19,20,21,22
403117	403146	28	X

ABI PRISM Linkage Mapping Set Version 2 (*continued*)

P/N	Kit	Quantity
450096	Individual Primer Pairs from the ABI PRISM™ Linkage Mapping Set Version 2 Must be ordered through Applied Biosystems Custom Oligonucleotide Synthesis Service (specify locus name)	3000 pmol
403061	True Allele™ PCR Premix	18 mL, enough for 2000 rxns
403062	Control DNA CEPH 1347-02	180 µL, enough for 150 rxns

ABI PRISM DNA Sequencing Kits and Reagents

dRhodamine Terminator Cycle Sequencing Kits with AmpliTaq® DNA Polymerase, FS

P/N	Kit	Reactions
403044	Ready Reaction	100
403045	Ready Reaction	1000
4303143	Ready Reaction	5000

BigDye™ Primer Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase, FS

P/N	Primer	Reactions
403051	–21 M13	100
403049	–21 M13	5000
403052	M13 Reverse	100
403050	M13 Reverse	5000

BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq DNA Polymerase, FS

P/N	Kit	Reactions
4303573	Ready Reaction	24
4303149	Ready Reaction	100
4303150	Ready Reaction	1000
4303151	Ready Reaction	5000

Dye Primer Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase, FS

P/N	Primer	Reactions
402111	–21 M13	100
402109	M13 Reverse	100

Dye Primer Cycle Sequencing Core Kits with AmpliTaq DNA Polymerase, FS

Core Kit configurations contain all essential reagents packaged in separate tubes. Each kit sequences both single-stranded and double-stranded templates.

P/N	Primer	Reactions
402071	–21 M13	100
402072	M13 Reverse	100
402073	–21 M13/M13 Reverse	100
402125	Kit reagents only (primerless)	100
402126	T7	100
402127	T3	100
402128	SP6	100
402129	T7/SP6	100
402130	T3/T7	100
402799	SK	100
402798	KS	100
402797	SK/KS	100

Dye Terminator Cycle Sequencing Kits with AmpliTaq DNA Polymerase, FS

Dye Terminator Kits sequence single-stranded and double-stranded templates. Ready Reaction formulations contain all necessary reagents in one stable premix. The Core Kit configuration contains all essential reagents packaged in separate tubes.

P/N	Kit	Reactions
402123	Ready Reaction	24
402080	Ready Reaction	100
402119	Ready Reaction	1000
402124	Ready Reaction	5000
402118	Core Kit	100

Dye Primers

Kits include 20 pmol of FAM- and JOE-labeled primer, 40 pmol of TAMRA- and ROX-labeled primer, and a control template in quantity enough for 50 ss- or ds-DNA sequencing reactions.

P/N	Primers
401131	–21 M13 Dye Primers (4 x 50), 5´ TGT AAA ACG ACG GCC AGT 3´
401130	M13 Reverse Dye Primers (4 x 50), 5´ CAG GAA ACA GCT ATG ACC 3´
401127	T7 Dye Primers (4 x 50), 5´ TAA TAC GAC TCA CTA TAG GG 3´
401128	T3 Dye Primers (4 x 50), 5´ ATT AAC CCT CAC TAA AGG GA 3´
401129	SP6 Dye Primers (4 x 50), 5´ ATT TAG GTG ACA CTA TAG 3´
402787	SK Dye Primers (4 x 50), 5´ CGG CCG CTC TAG AAC TAG TGG ATC 3´
402786	KS Dye Primers (4 x 50), 5´ CCT CGA GGT CGA CGG TAT CG 3´
403013	PI (+) Dye Primers (4 x 50), 5´ CAG GAC ATT GGA TGC TGA GAA TTC G 3´
403014	PI (–) Dye Primers (4 x 50), 5´ CAG GAG CCG TCT ATC CTG CTT GC 3´

Matrix and Sequencing Standards

P/N	Standard
403047	dRhodamine Matrix Standards Kit
401114	Dye Primer Matrix Standards Kit
401071	Dye Terminator Matrix Standards Kit
401920	Dye Primer Cycle Sequencing Standard
402830	Dye Terminator Cycle Sequencing Standard
4303120	dRhodamine Terminator Cycle Sequencing Standard
4304154	BigDye Terminator Cycle Sequencing Standard

Application Kits

P/N	Kit
4303557	HLA-A Sequencing-Based Typing Starter Kit
4305026	HLA-DRB Sequencing-Based Typing Starter Kit
403085	MicroSeq 16S rRNA Gene Kit
4003015	Primer Island Transposition Kit

Reagent Kit Protocols

P/N	Protocol
402113	*ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kit Protocol*
402114	*ABI PRISM Dye Primer Cycle Sequencing Core Kit Protocol*
402078	*ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit Protocol*
402116	*ABI PRISM Dye Terminator Cycle Sequencing Core Kit Protocol*
403041	*ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit Protocol*
403057	*ABI PRISM BigDye Primer Cycle Sequencing Ready Reaction Kit Protocol*
4303237	*ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit Protocol*

User’s Manuals

903433	*ABI PRISM® 377 DNA Sequencer User’s Manual*
902376	*373 DNA Sequencing System User’s Manual*
904435	*GeneScan® Analysis Software User’s Manual*
902842	*GeneScan 672 Software User’s Manual*
4303188	*GeneScan Reference Guide*

Part Number Updates

Part numbers are subject to change. Consult the Applied Biosystems World Wide Web site (www.appliedbiosystems.com/techsupport) for updated information.

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

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

Technical Support
For technical support:
Toll Free: +1 800.831.6844 ext 23
Fax: +1 650.638.5891

www.appliedbiosystems.com

Applied Biosystems

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

Printed in the USA, 09/2000
Part Number 4305423B