ABI PRISM®

310 Genetic Analyzer

User's Manual

Applied Biosystems

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

NOTICE TO PURCHASER:

This instrument, Serial No. _____, is Authorized for use in DNA sequencing and fragment analysis. This authorization is included in the purchase
price of this instrument and corresponds to the up-front fee component of a license under process claims of U.S. patents and under all process claims for DNA sequence and fragment analysis of U.S. patents now or hereafter owned or licensable by Applied Biosystems for which an Authorization is required, and under corresponding process claims in foreign counterparts of the foregoing for which an Authorization is required. The running royalty component of licenses may be purchased from Applied Biosystems or obtained by using Authorized reagents purchased from Authorized suppliers in accordance with the label rights accompanying such reagents. Purchase of this instrument does not itself convey to the purchaser a complete license or right to perform the above processes. This instrument is also licensed under U.S. patents and apparatus and system claims in foreign counterparts thereof. No rights are granted expressly, by implication or by estoppel under composition claims or under other process or system claims owned or licensable by Applied Biosystems. For more information regarding licenses, please contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404.

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To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

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, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
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In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844**, then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS™, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
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Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858,** then press **14**	**1-508-383-7855**
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PNA Custom and Synthesis	**1-800-899-5858**, then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

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Region	Telephone Dial...	Fax Dial...
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Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**

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Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 22358 2838**	**886 2 2358 2839**
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Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
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Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
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Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
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South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
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 331400**	**31 (0)180 331409**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

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We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the **Troubleshooting** heading, click **Support Request Forms,** then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click **Ask Us RIGHT NOW**. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

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by phone for fax delivery	a. From the U.S. or Canada, call **1-800-487-6809,** or from outside the U.S. and Canada, call **1-858-712-0317**. b. Follow the voice instructions to order the documents you want. **Note** There is a limit of five documents per request.
through the Internet for fax or e-mail delivery	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Under **Resource Libraries**, click the type of document you want. c. Enter or select the requested information in the displayed form, then click **Search**. d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click **Deliver Selected Documents Now** (or click the PDF icon for the document to download it immediately). e. Fill in the information form (if you have not previously done so), then click **Deliver Selected Documents Now** to submit your order. **Note** There is a limit of five documents per request for fax delivery but no limit on the number of documents you can order for e-mail delivery.

Safety Information

For information on instrument, and chemical safety related to the operation of the 310 Genetic Analyzer, refer to the *Site Preparation and Safety Guide (P/N 903558)*.

How the ABI PRISM 310 Genetic Analyzer Works 1

Introduction

In This Chapter Topics in this chapter include the following:

ABI PRISM 310 Genetic Analyzer

Introduction The ABI PRISM® 310 Genetic Analyzer is a system composed of instrument hardware, a Macintosh® computer, several types of software, and consumables.

This overview will help you identify parts of the ABI PRISM® 310 Genetic Analyzer and understand how it works.

What the 310 Genetic Analyzer Does

DNA Sequencing

About DNA Sequencing

DNA sequencing experiments determine the order of the bases in a DNA sample.

Fluorescently labeled dyes are attached to ACGT extension products in DNA sequencing reactions. Dye labels are incorporated using either 5´-dye labeled primers or 3´-dye labeled dideoxynucleotide terminators. Polymerases such as AmpliTaq® FS are used for primer extension.

The sequencing reaction sample tubes are placed in a tray in the instrument's autosampler. The autosampler successively brings each sample into contact with the cathode electrode and one end of a glass capillary filled with polymer. An anode electrode at the other end of the capillary is immersed in buffer.

A portion of the sample enters the capillary as current flows from the cathode to the anode. This is called electrokinetic injection. The end of the capillary near the cathode is then placed in buffer. Current is applied again to continue electrophoresis.

When the nucleotides reach a detector window in the capillary coating, a laser excites the fluorescent dye labels. Emitted fluorescence from the dyes is collected by a CCD camera. The software interprets the result, calling the bases from the fluorescence intensity at each data point.

Required Software Files

A number of software files are required by the instrument to perform this process:

Software File	Purpose
Sample Sheet	The Sample Sheet associates sample information (name and type of analysis) with a sample tube position.
Injection List	The Injection List specifies the order for running samples, how many injections are made from each sample, and the module and running conditions for each injection.
Mobility Files	The mobility files, also called Dye Set/Primer Files, adjust the data for the mobility of the sequencing primer in the polymer.
Modules	The 310 Genetic Analyzer executes the steps in a run module to process a sample. Run time, temperature and voltage, prerun time, injection time and voltage are some of the run parameters controlled by the run module.
Matrix Files	Matrix files contain information that corrects for "spectral overlap". Fluorescent dye labels are sold in sets of four. The emission spectra of the dyes overlap slightly. Although an effort is made to select dyes for each set that overlap minimally, spectral overlap still occurs to some extent.
Preference Files	Preferences record the default for a number of different selections in the software.

Data Collection Software

The ABI PRISM® 310 Data Collection software collects raw data and allows real-time run monitoring in four software windows that show:

- Sample raw data
- Run status
- Electrophoresis record
- Instrument log file

ABI PRISM® DNA Sequencing Analysis Software analyzes the raw data and calls the bases.

GeneScan Software

About the GeneScan® Analysis Software

GeneScan® Analysis Software analyzes raw data to quantify the DNA fragments and determine the size of the fragments by comparing them to fragments contained in a size standard.

Each sample is labeled with one fluorescent dye. A dye-labeled internal size standard is also placed in the tube. Three samples and a size standard can be electrophoresed simultaneously, as dyes come in sets of four colors.

The sample tubes are placed in a tray in the instrument's autosampler. The autosampler successively brings each sample into contact with the cathode electrode and one end of a glass capillary filled with polymer. An anode electrode at the other end of the capillary is immersed in buffer.

A portion of the sample enters the capillary as current flows from the cathode to the anode. This is called electrokinetic injection. The end of the capillary near the cathode is then placed in buffer. Current is applied again to continue electrophoresis.

When the DNA fragments reach a detector window in the capillary coating, a laser excites the fluorescent dye labels. Emitted fluorescence from the dyes is collected by a CCD camera. The software interprets the result, calculating the size or quantity of the fragments from the fluorescence intensity at each data point.

Software Files Required

A number of software files are required by the instrument to perform these functions:

Software files:

Software File	Purpose
Sample Sheet	The Sample Sheet associates sample information (name and type of analysis) with a sample tube position.
Injection List	The Injection List specifies the order for running samples, how many injections are made from each sample, and the module and running conditions for each injection.
Modules	The ABI PRISM® 310 Genetic Analyzer executes the steps in a run module to process a sample. Run time, temperature and voltage, prerun time, injection time and voltage are some of the run parameters controlled by the run module.
Matrix Files	Matrix files contain information that corrects for "spectral overlap". Fluorescent dye labels are sold in sets of four. The emission spectra of the dyes overlap slightly. Although an effort is made to select dyes for each set that overlap minimally, spectral overlap still occurs to some extent.

Software files: *(continued)*

Software File	Purpose
Size Standards Files	The Size Standard file holds the results of a run performed with fragments of known length. The file can be used to analyze other samples run with the same size standard under the same conditions to determine the size of fragments of unknown length.
Analysis Parameters	The Analysis Parameters file holds the default start and stop point for data analysis, the default peak height and width threshold, and the default size-calling method.
Preference Files	Preferences record the default for a number of different selections in the software.

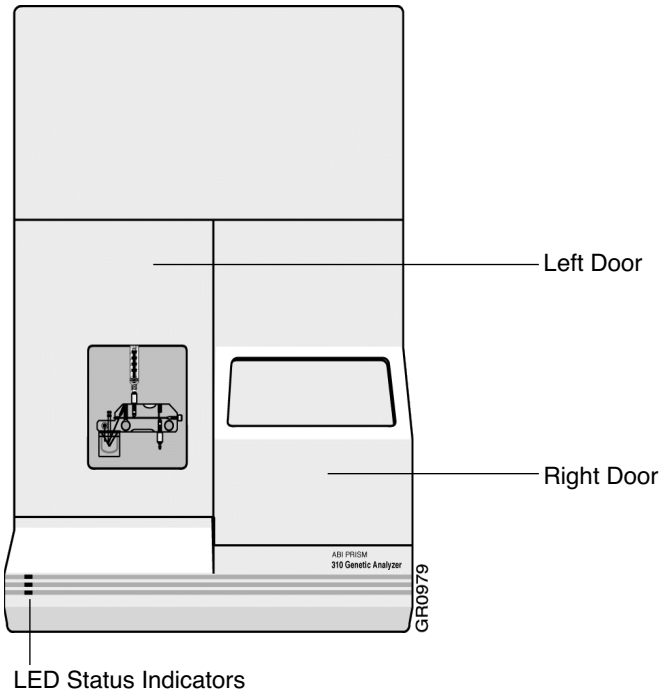
Data Collection Software

The Data Collection software collects raw data and allows real-time run monitoring in four software windows that show:

- Raw data
- Run status
- Electrophoresis record
- Instrument log file

Instrument Hardware: Front

Front of Instrument



Led Status Indicators

The LED Status Indicators are red, amber, and green lights. The pattern of lights indicates the instrument's state.

Instrument Status	LED Pattern
Ready For Use	Steady green
Running	Blinking green
End of Run	Steady green with blinking amber
Paused, Door Open, or Awaiting Completion of Self-test	Amber blinking
Failure (check the error log in the 310 Collection software for details)	Red

Doors

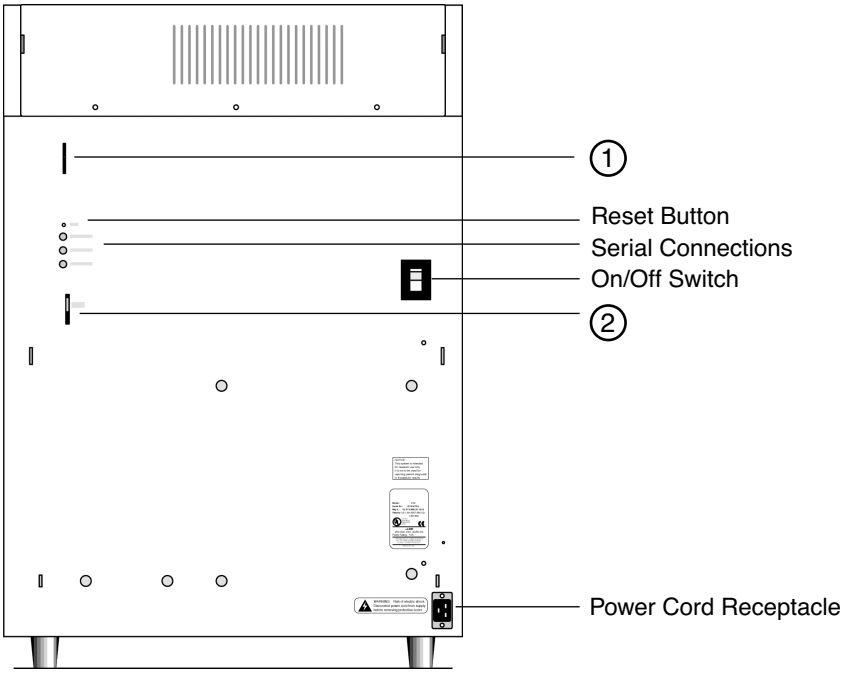
The doors protect you from exposure to the instrument's laser and electrophoresis power supply.

The doors must be closed to begin a run. The run interrupts if they are opened.

Instrument Hardware: Back

Back of Instrument

For a description of the call outs, see “Service LEDs” below.



On/Off Switch

This switch turns power to the instrument on and off.

Service LEDs

The following table lists the Service LEDs in the above figure. The LEDs are red lights. The pattern of lights indicates the instrument’s state.

Call Out	LED	Purpose
1	Init	Indicates board is functioning properly.
	Spare	Not used.
	Tray	Indicates 48- or 96-well tray. ♦ On = 48-well tray ♦ Off = 96-well tray
	X, Y, and Z limit	Lit when autosampler is at the furthest extent of its travel. Thus, when the electrode is at the front left of the autosampler, all lights should be on.
2	Firmware status	Lights 2 through 9 indicate the status of the firmware. ♦ Flashing lights indicate the firmware is loaded. ♦ All lights off mean the board has a problem. ♦ Four lights on and four off means you need to reload the firmware.
	XLX	When flashing, indicates the logic board is functioning properly.

Communications Reset Button

This button resets instrument–computer communications. Insert a pen or a similar thin object to push the recessed button and activate the reset.

For more information, see “How to Reset The Genetic Analyzer” on page 433.

Serial Connections

Port	Purpose
Control/Data	Connects the instrument to the Macintosh modem port.
Diagnostics	Connects the instrument to a diagnostics device or the Macintosh printer port.
Aux/Serial	Auxiliary serial connection.

Power Cord Receptacle

A power cord delivers power to the instrument from a wall receptacle and is rated for 110 or 230 VAC.

For information about the power, refer to the ABI PRISM® 310 *Genetic Analyzer Site Preparation and Safety Guide*.

Instrument Hardware: Behind the Doors

Overview

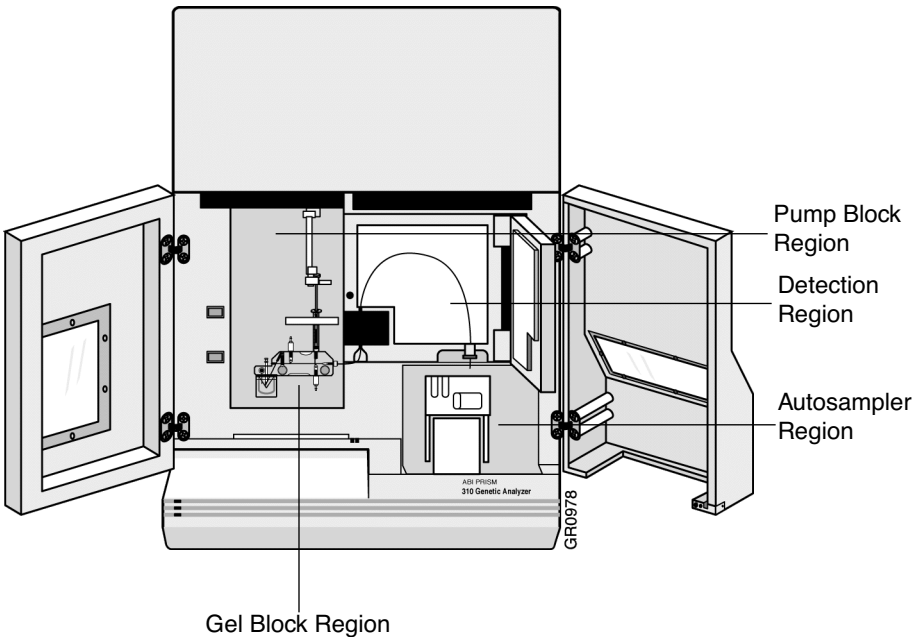
Hardware located inside the ABI PRISM 310 is responsible for:

- Automated sample handling
- Electrophoresis
- Fluorescence detection

Some of this hardware is located directly behind the doors. Other parts, such as power supplies, electronics, detection optics and the laser are only accessible by a service technician.

Instrument with Door Open

The following is a diagram of the instrument with the doors open.



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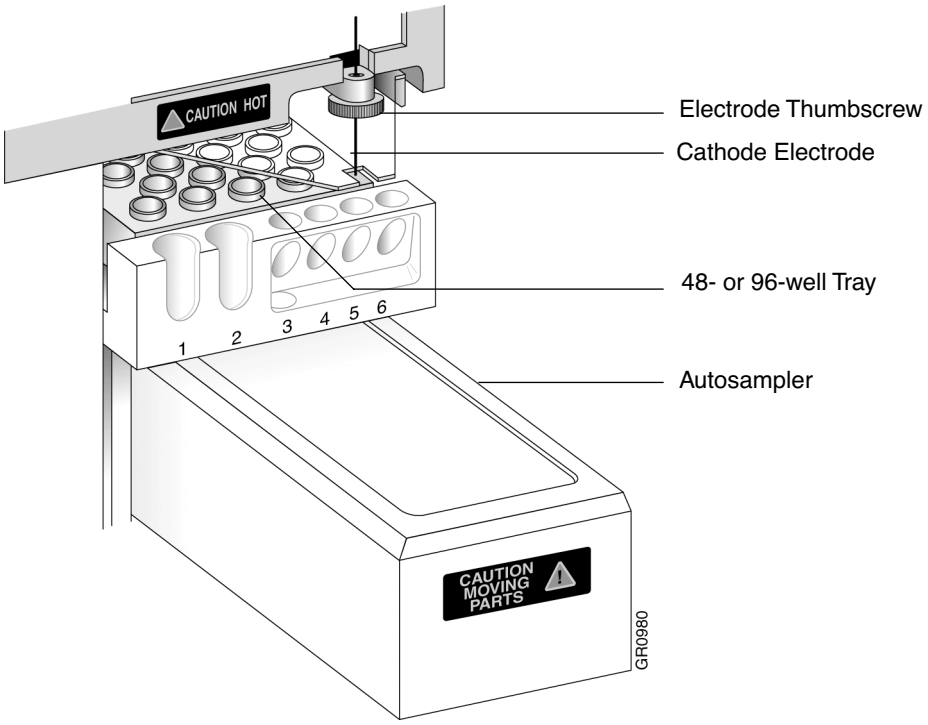
Autosampler Region Diagram

The following is a diagram of the autosampler region.



Autosampler Region Diagram Described

The following table lists the parts to the above diagram. The autosampler moves buffers and samples to the capillary.

Part	Purpose
Autosampler	Holds the sample tray and consumable tubes: ♦ Two 4-mL buffer vials ♦ One 1.5-mL Eppendorf tube ♦ Up to three 0.5-mL tubes The autosampler moves the tray and tubes so the capillary can be inserted into them.
Cathode Electrode	Provides a negative pole for electrical current for electrophoresis.
48-well Tray	Holds a maximum of 48 0.5-mL tubes in a 6 x 8 format.
96-well Tray	Holds a maximum of 96 0.2-mL tubes in an 8 x 12 format.
Electrode Thumbscrew	Holds the electrode. It also has a hole through which you thread the capillary.

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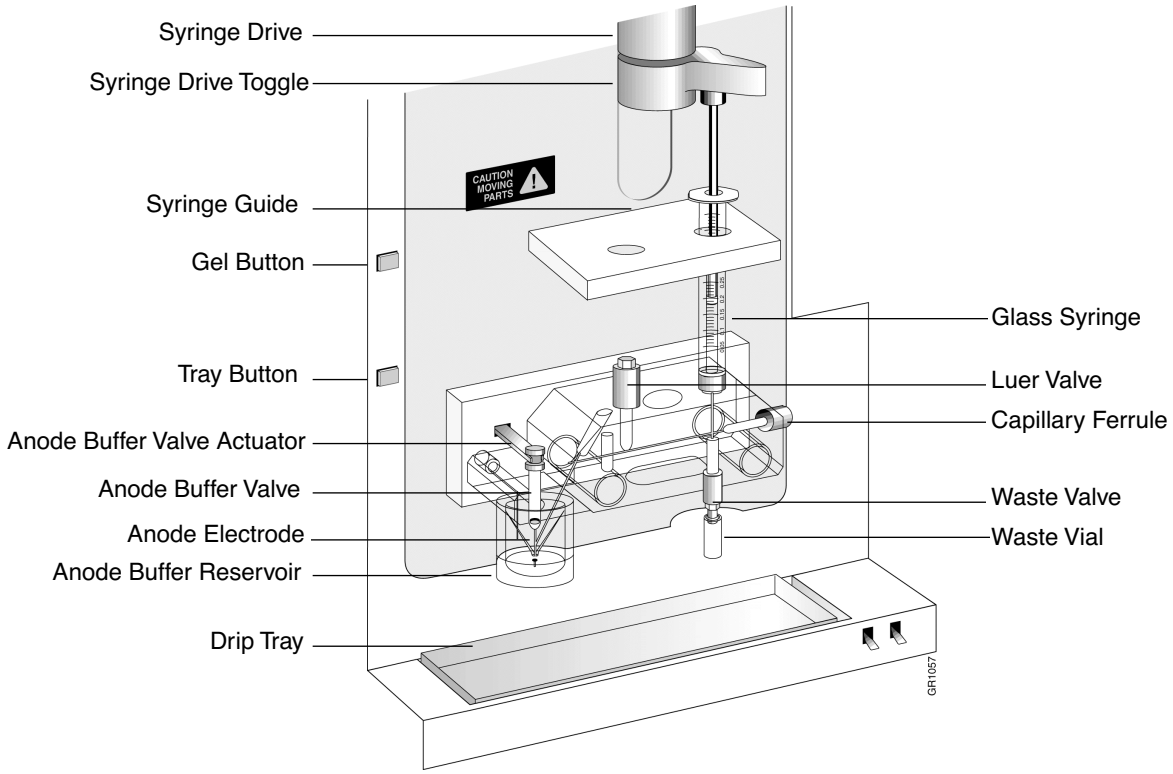
Gel Block Region Diagram

The following is a diagram of the Gel Block region.



Gel Block Region Described

The following table lists the parts to the above diagram. The pump block controls the flow of polymer and samples through the capillary.

Gel Block Region described:

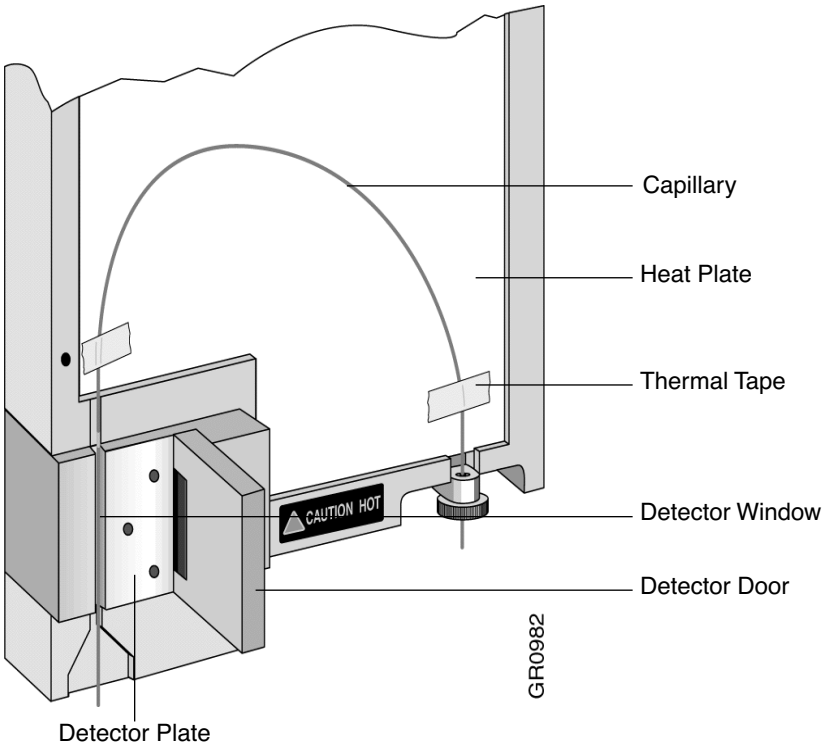
Part	Purpose
Gel Pump Button	Launches the Gel Pump Fill module to pump polymer from the plastic syringe into the 250-µL glass syringe through the pump block (DNA Sequencing Polymer).
Tray Button	Moves the autosampler in and out so you can put a sample tray on the autosampler or a sample into the sample tray.
Syringe Drive	Provides positive pressure to the syringe.
Syringe Drive Toggle	Allows you to switch the syringe drive to either the glass or plastic syringe.
Syringe Guide	Holds the plastic and glass syringes in position.
Luer Valve (plastic syringe)	Attaches the plastic syringe to the pump block.
Anode Electrode	Provides a positive electrical current for electrophoresis.
Anode Buffer Valve	Closes access to the buffer when filling the capillary. Opens access to the buffer for electrophoresis.
Anode Buffer Reservoir	Contains the buffer required for electrophoresis.
Glass Syringe	Stores the polymer between runs and generates the necessary force to fill the capillary with polymer.

Gel Block Region described: *(continued)*

Part	Purpose
Waste Valve	Valve for waste flow when priming and cleaning the pump block channels.
Waste Vial (optional)	Collects waste put through the waste valve.
Capillary Ferrule	Attaches the capillary to the pump block.
Anode Buffer Valve Actuator	Opens and closes the buffer valve.
Drip Tray	Catches any liquids that drip from the pump block.

Detection Region Diagram

The following is a diagram of the Detection Region.



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Detection Region Described

The following table lists the parts to the above diagram. The detection region collects data from the samples as they migrate past the capillary window.

Detection Region Described:

Part	Purpose
Charge-Coupled Device (CCD)[a]	The laser excites the dyes in the sample and their fluorescence is detected by the CCD camera.
Laser Detector Window and Door	The capillary window is placed over the laser detector window. The door holds the capillary in place and protects you from exposure to the laser.
Heat Plate	The heat plate heats the capillary during electrophoresis
Capillary	A capillary is a glass tube filled with polymer that carries the sample past the laser to the pump block.
Heater Door[b]	The heater door insulates the capillary during electrophoresis and protects you from burns.
Thermal Tape	Thermal tape holds the capillary in place against the heat plate.

a. The CCD is accessible to service technicians only. It is not shown in the drawing above.

b. The Heater Door covers the Heat Plate. It is not shown in the drawing above.

About the Macintosh Computer

Overview

The Macintosh® computer collects and analyzes data from the ABI PRISM 310 Genetic Analyzer.

The ABI PRISM® 310 Data Collection Software collects what is called the “raw data”: fluorescence which is detected on the CCD array as the labeled fragments, separated in the capillary by electrophoresis and excited by the laser, pass the detection window.

The ABI PRISM® DNA Sequencing Analysis Software and the GeneScan® Analysis Software programs are used to analyze the raw data, converting it to DNA sequence or DNA fragment size/quantitation data.

Other programs are available from Applied Biosystems to align sequences, identify previously unsequenced regions, archive data, identify patterns of heredity, and other data manipulations. See your sales representative and visit Applied Biosystems on the Applied Biosystems web site at www.appliedbiosystems.com/techsupport

System Requirements

The ABI PRISM® 310 Genetic Analyzer is shipped with a Power Macintosh® computer. An optional color printer is also available.

Note Contact technical support for the latest specifications for the Macintosh computer and the operating system (see “Technical Support” on page xvii).

If you need to replace the computer or printer, the following table lists the minimum specifications:

Item	Specification
Macintosh® Model	Power Macintosh® 6100/66 or better.
Monitor	Color monitor 14-inch display or larger.
Disk Drive	Hard disk with a minimum of 250 MB storage, preferably IG.
Memory	Minimum 16 MB random access memory, preferably 64 MB.
Printer	Color PostScript printer.
Operating system software	Macintosh system software v 7.5.3 or higher.

RAM Requirements

To check your available RAM, choose About this Macintosh... from the Apple (🍎) menu. RAM requirements for software applications are:

Software	RAM Required
ABI PRISM® 310 Data Collection Software	4.7 MB
ABI PRISM® DNA Sequencing Analysis Software and Basecallers	6 MB
GeneScan® Analysis Software	6 MB

Principles of Operation

Sample Labeling

The samples are labeled using a dye set—four dyes that fluoresce at different wavelengths. The four dyes might label different bases in the DNA sequence or different fragments in an experiment that will be analyzed with GeneScan® Analysis Software.

Automated Sample Handling

Fluorescently labeled samples are placed in the autosampler.

The cathode electrode and one end of a polymer-filled capillary are located near the autosampler. The autosampler successively brings samples in the sample tray into contact with them. A short period of electrophoresis injects the sample into the capillary. The autosampler then moves the cathode buffer reservoir to the capillary and electrode to continue electrophoresis.

The cathode buffer reservoir, a waste vial, and a water reservoir (used to wash the cathode electrode and capillary tip between samples) are vials located on the autosampler.

Electrokinetic Sample Injection

The short period of electrophoresis conducted while the capillary and cathode are immersed in the sample is called electrokinetic injection.

A portion of the sample forms a tight band in the capillary during this injection.

Electrophoresis

Samples are electrophoretically separated as they travel through polymer in the capillary.

! WARNING ! HIGH VOLTAGE HAZARD. Exposure to high voltage can cause serious injury and death. Do not touch the electrodes or capillary when the electrophoresis power supply is on.

An interlock switch shuts off the electrophoresis power supply if the front doors of the instrument are open.

Since the capillary is fastened along most of its length to a heat plate, temperature is controlled during electrophoresis.

Sample Fluorescence

As the DNA fragments pass through the window of the capillary, an argon-ion laser excites the attached dye labels and they fluoresce.

! WARNING ! LASER HAZARD. Exposure to direct or reflected laser light at 10 mW for 0.1 seconds can burn the retina and leave a permanent blind spot. Never look directly into the laser beam or allow a reflection of the beam to enter your eyes.

The laser is located behind the detector door and the front door of the instrument. An interlock switch protects you from the laser when the front door of the instrument is open.

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Detection

A series of lenses direct and focus the fluorescent light into a spectrograph.

A diffraction grating in the spectrograph disperses the light by wavelength and focuses the resulting light spectrum onto a CCD array.

Virtual Filters, Matrix Files and Dye Sets

The ABI PRISM 310 Data Collection software defines certain areas on the CCD array for the collection of the fluorescent emissions from the dye labels in the dye set. These areas are called virtual filters. There can be any number of virtual filters, since the filter is simply a software-designated site on the CCD array. Virtual filters are grouped into sets and referred to by a letter, such as Virtual Filter Set A, B, C, etc.

Fluorescent dye labels come in sets of four. There are several different dye sets, for use in different types of experiments. The fluorescence from each dye set must be collected using the correct virtual filter set.

You select the virtual filter set that corresponds to the experiment's dye set by choosing a module file when setting up the run. The virtual filter set's letter is in the module file name. For example, data is collected from BigDye™ Terminator DNA sequencing samples using Virtual Filter E. To run BigDye™ Terminator samples, choose a module file such as Seq POP6 (1.0-mL) E.

Some portion of a dye's emission profile may fall on a virtual filter other than the one intended to collect its emission maximum. The dyes in each dye set are selected to have widely spaced emission maximums to minimize overlap of the emission profiles on the CCD array. However, overlap still occurs to some extent.

The "crosstalk" between colors on the CCD array, known as spectral overlap, is corrected by a matrix file in the software. The matrix file is generated from a separate matrix run, and contains information about how much of the collected light falling on a filter is due to the intended light emission and how much is contaminating light.

When setting up the run, you select a matrix file to interpret the data and correct for spectral overlap.

One matrix file can be used for all runs sharing the same conditions. If run conditions are changed, a new matrix file must be generated using the new run conditions. Changes in run conditions affect dye fluorescence and the amount of spectral overlap.

Compatibility of Dyes and Virtual Filters

5-FAM and 6-FAM cannot be used in the same sample because there is no virtual filter that distinguishes one from the other.

HEX and JOE cannot be used in the same sample because there is no virtual filter that distinguishes one from the other.

TAMRA and NED cannot be used in the same sample because there is no virtual filter that distinguishes one from the other.

continued on next page

Color Display of Data

The Data Collection software creates a real-time display of the light intensities collected on the virtual filters. The wavelengths of the dyes in the set are color-coded from shortest to longest: blue, green, yellow, and red.The colors on the real-time display do not represent actual wavelengths. They represent the color-coded *relative* wavelengths of the four dyes in each dye set.

Each of the sequencing chemistries is associated with one of the dye sets. Each chemistry associates the four bases with dyes of different relative wavelengths. Therefore, the color associated with each base in the Data Collection software changes with the chemistry used to label it.

In DNA sequencing applications, Virtual Filter A uses the four colors to represent wavelengths within the dye set used with primer sequencing and the dye set used with terminator sequencing. Virtual Filter B uses the four colors to represent wavelengths within the dye set used for primer sequencing with T7 primers, an application that is no longer common. Virtual Filter E represents wavelengths within the dye set used for sequencing with dichlororhodamine-labeled terminators or BigDye™-labeled primers.

Virtual Filters C, D, and F are used only with the GeneScan® Analysis Software.

Color Guide for Sequencing Raw Data

The following are color guides for sequencing raw data displays.

Virtual Filter A				
	Taq Primer		Taq Terminator	
Color	Base	Dye	Base	Dye
Blue	C	5-FAM	G	R110
Green	A	JOE	A	R6G
Yellow	G	TAMRA	T	TAMRA
Red	T	ROX	C	ROX

Virtual Filter E						
	dRhodamine Terminator		BigDye™ Terminator		BigDye™ Primer	
Color	Base	Dye	Base	Dye	Base	Dye
Blue	G	dR110	G	dR110	C	dR110
Green	A	dR6G	A	dR6G	A	dR6G
Yellow	C	dTAMRA	T	dTAMRA	G	dTAMRA
Red	T	dROX	C	dROX	T	dROX

The ABI PRISM® Sequencing Data Analysis program converts the information collected by the Data Collection program to a color code in which blue represents C, green represents A, yellow represents G, and red represents T. When G is printed, black replaces yellow, because black is easier to read.

Color Guide for GeneScan Applications

In GeneScan® applications, the dyes corresponding to the colors for virtual filters are:

Color guide for GeneScan® Analysis Software raw data displays.

Corresponding Dye				
Color	**Virtual Filter A (Fluorescent dNTPs)**	**Virtual Filter C**	**Virtual Filter D**	**Virtual Filter F**
Blue	R110	6-FAM	6-FAM	5-FAM
Green	R6G	TET	HEX	JOE
Yellow	TAMRA	HEX	NED	NED
Red	ROX	TAMRA	ROX	ROX

Software for Data Collection and Analysis

Overview

The ABI PRISM 310 Genetic Analyzer comes with software programs that perform:

- Raw data collection
- Data analysis

These programs run on theMacintosh® computer.

Software Used with the Genetic Analyzer

Software Application	Purpose
ABI PRISM® 310 Data Collection Software	Controls, monitors, and collects data from the 310.
ABI PRISM® DNA Sequencing Analysis Software	Analyzes raw sequencing data and calls bases.
GeneScan® Analysis Software	Sizes and quantifies DNA fragments.

Data Collection Software

ABI PRISM® 310 Data Collection Software collects the raw data from either DNA sequencing or GeneScan® Analysis Software runs. It contains information about the dyes' emission (fluorescence) spectra and processes the raw data as base sequence, fragment size, or relative concentration for use by one of the analysis software applications.

The program is discussed thoroughly in Chapter 5, "Using Data Collection Software."

Data Analysis Software

Two software programs are available to analyze the raw data:

- ABI PRISM® DNA Sequencing Analysis Software
- GeneScan® Analysis Software

DNA Sequencing Analysis

The DNA Sequencing Analysis software analyzes the raw sequencing data collected by the Data Collection software. This program comes bundled with the Data Utility program. The Data Utility program enables you to make and copy matrices to correct for the spectral overlap of the fluorescent dye labels.

GeneScan Analysis

The GeneScan® Analysis Software enables you to use ABI PRISM® 310 Genetic Analyzer automated fluorescence detection to size and quantify DNA fragments. The program automates the sizing of DNA fragments. It also provides flexibility to interactively confirm and fine-tune the data analysis, and allows you to display the results of an experiment in several different ways.

Each analysis program is described in detail in separate manuals: the *ABI PRISM DNA Sequencing Analysis Software User's Manual* and the *ABI PRISM GeneScan Analysis Software User's Manual*.

Other Software Provided with Your Computer

Overview

If other software programs have been provided on the hard disk of your computer, send in the registration cards to receive future updates from their manufacturers.

Do *not* install other programs on the Macintosh® computer yourself unless directed to do so by a Applied Biosystems representative. Other programs may have features that interfere with correct operation of the ABI PRISM® 310 Genetic Analyzer software.

The Macintosh® operating system is described in manuals provided by Apple Computer, Inc. and shipped with your computer.

Consumables

Overview

There are specific consumables for use by the ABI PRISM® DNA Sequencing Analysis Software and the GeneScan® Analysis Software, as well as shared consumables.

Part numbers for many consumables are noted in this chapter. Refer to these part numbers when ordering from Applied Biosystems.

More information about Applied Biosystems kits and consumables is available from your sales representative or on the Applied Biosystems pages of the Applied Biosystems web site, at www.appliedbiosystems.com/techsupport

Shared Consumables

About Capillaries

A capillary is a glass tube with a small internal diameter. Filled with polymer, it carries the sample past the laser and detector to the pump block. The capillary has an opaque polyimide coating except in the window area. The laser and detector read samples during electrophoresis through the window in the coating. Capillaries are fragile, especially at the detection window.

Removing Capillaries from the Packaging

The capillaries are shipped in a curved plastic tube. Remove the capillaries by grasping one capillary between your thumb and forefinger, and then pulling and turning the capillary simultaneously.

Capillary Life

Capillaries can be used for up to a minimum of 100 runs.

Capillary Types, Labels and Part Numbers

The following table lists the capillary types, labels, and part numbers.

Capillary Type	Label	Polymer	Length (cm)	Length to Detector (cm)	Coated/ Uncoated	Internal Diameter (μ)	Part Number
47 cm x 50-μm i.d.	green	POP-6	47	36	internally uncoated	50	402839
61 cm x 50-μm i.d.	pink	POP-6	61	50	internally uncoated	50	402840
Sequencing	silver	DNA Sequencing Polymer	47	36	internally coated	75	401821
47 cm x 50-μm i.d.	green	POP-4 GeneScan Polymer	47	36	internally uncoated	50	402839

About Septa Strips for the 96-Well Tray

Septa strips seal 0.2-mL tubes in the 96-well tray. They require septa clips.

CAUTION **The septa strip will melt at high temperatures. Do not autoclave or reuse the septa strips.**

CAUTION **Do not close the lid when denaturing samples using the GeneAmp® 9600 or 9700. The septa strip may adhere to the lid.**

The retainer clip interferes with the insertion guides in the GeneAmp® 9600 and 9700 Thermal Cycler. Therefore, you cannot place the retainer clip on the sample tray while you are using the 9600 or the 9700 to heat denature your samples.

You can process fewer by leaving the unused wells in the sample tray empty. The reaction tube eight-strip and septa strip can be cut to accommodate the number of samples you want to process.

continued on next page

Using Septa Strips

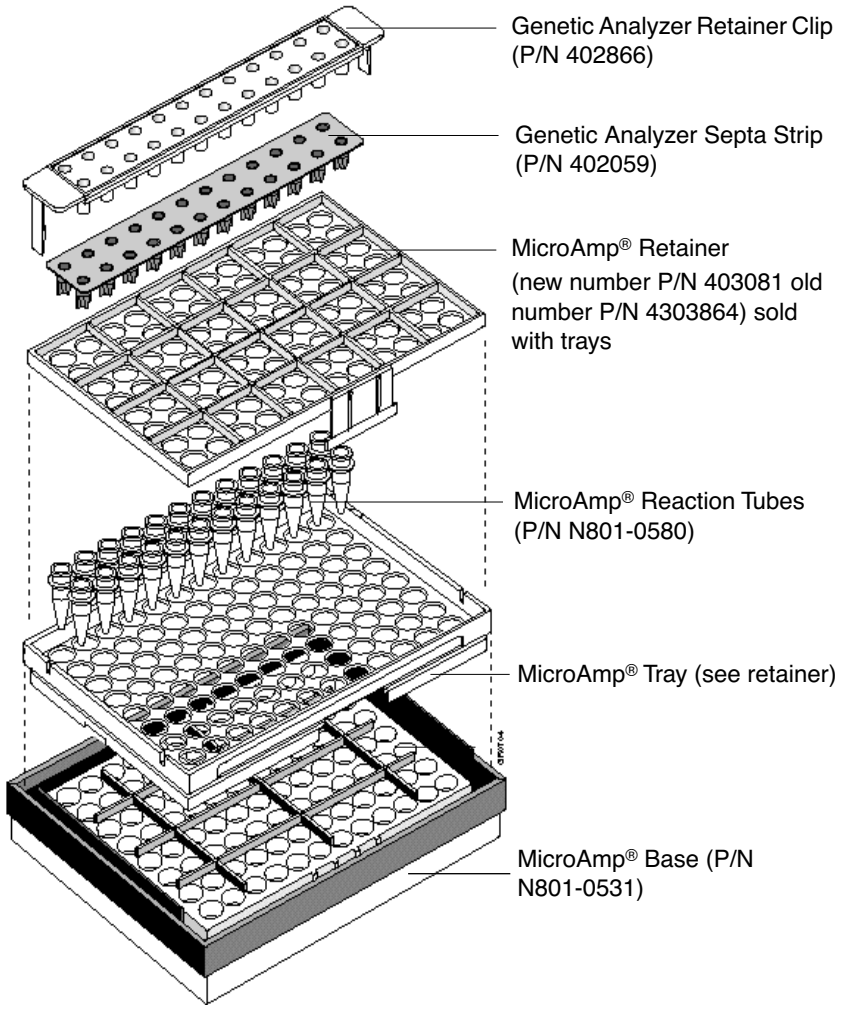
Using the Right Trays with the Thermocycler

Note At the time of printing of this manual, the 96 sample trays were in the process of being changed, so the use of two types of trays is described below. The old beige tray and retainer is P/N 4303864, and the new red one is P/N 403081.

For this thermocycler...	Use this Tray/Retainer...
2400 and 9600	P/N 4303864
9700	P/N 43081 **Note** This tray/retainer is not compatible with the 9700 unless used with an adaptor (P/N 4305051).

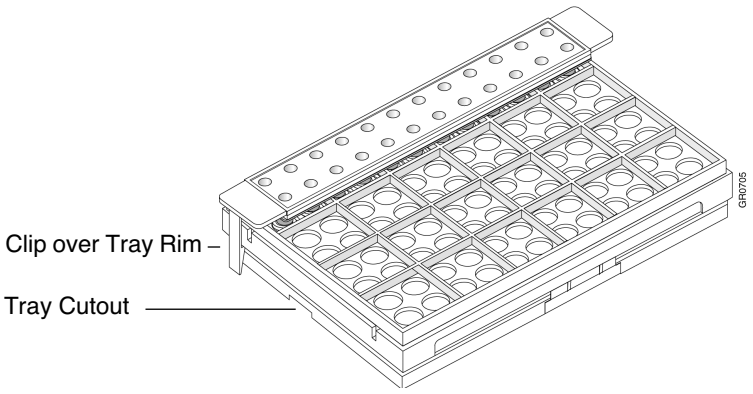
Preparing the Sample Tray

Step	Action
1	Place the MicroAmp® Base on a flat surface (do not put the base in the instrument).
2	Place the MicroAmp® Tray onto the base so that the well numbered A1 is located at the upper left corner of the tray. The cutout in the outside rim of the tray will then be located on the left side of the tray. This orients the tray for proper fit into the sample block.



Step	Action
3	Place the MicroAmp® Reaction Tubes in the tray. **Note** The wells in the tray are labeled left to right from 1 through 12 and A through H from back to front.
4	Place the MicroAmp® Retainer over the sample tray and tubes. The retainer and tray are keyed so that the retainer can fit only one way.
5	Pipette the solution into the sample tubes.
6	Sit the septa strip on top of the sample tubes, with each of the 24 septum placed into the opening of a sample tube. Gently and evenly press the septa strip down until the strip is seated securely against the tubes.
7	Place the retainer clip over the 24 openings on the septa strip and press down until the clip clicks into place over the rim on each side of the sample tray.

Inserting the Tray into the ABI PRISM® 310 Genetic Analyzer

Step	Action
1	Remove the tray assembly from the base for insertion into the ABI PRISM® 310 Genetic Analyzer. 
2	With the tray cutout pointed toward the instrument, take the following actions: ♦ Place the tray assembly on the autosampler platform. ♦ Press it flat against the rectangular, raised guide until the tray is flush with the platform. **Note** The newer red tray requires an adaptor (P/N 4305051) for use on the ABI PRISM® 310. A sensor in the platform recognizes which tray is present (the 96-well tray or the 48-well tray) so that the tube positions are correctly identified during the run.

continued on next page

Other Shared Consumables

These are the consumables for both GeneScan® Analysis Software and ABI PRISM® DNA Sequencing Analysis Software experiments:

Consumable	Purpose	Part Number
48-well tray	Holds a maximum of 48 0.5-mL tubes in a 6 x 8 format.	005572
4-mL glass buffer vial with cap adapter	Holds buffer and water on the autosampler.	401955
0.2-mL tubes	Holds samples on the 96-well tray.	N801-0580
0.5-mL tubes	Holds samples on the 48-well tray, as well as for consumables on the autosampler.	401957
1.5-mL Eppendorf	Holds water on the autosampler and acts as the waste vial.	—
Septa for 0.5-mL tubes	Prevents evaporation of samples and consumables. The capillary and electrode can pass through a slit in the septum.	401956
Septa strips for 0.2-mL tubes	Prevents evaporation of samples and consumables. The capillary and electrode can pass through a slit in the septum.	402059
Septa clip for 96-well tray	Secures septa strips on 0.2-mL tubes.	402866
Cathode electrode	Provides the negative pole for electrophoresis.	005914
Capillary cutters	Shortens the length of the capillary.	401958
Thermal tape	Hold the capillary in position on the heat plate.	310021
1.0-mL glass syringe	Delivers the polymer to the capillary.	604418
Syringe o-rings	O-rings for the glass syringe and ferrule. Two o-rings are required. See “How to Inspect the Syringe” on page 418.	221102
Syringe ferrule	Ferrule inside the glass syringe.	005401
Capillary fitting	Holds the capillary in the pump block.	005404
Waste vial	Collects waste from the waste valve on the pump block.	603796
Waste valve	Attaches the waste vial to the pump block, and controls the flow from the waste vial.	604076
Plastic syringe luer valve	Attaches the plastic syringe to the pump block, and controls the flow from the plastic syringe.	604075
Anode buffer jar	Holds anode buffer.	005402
MicroAmp Tray and Retainer	Holds 0.2-mL tubes for the 96-well tray.	Old: 4303864 New: 403081
MicroAmp Base	Holds 0.2-mL tubes for the 96-well tray.	N801-0531
96 well tray adaptor	Allows thermocycler trays to be used on the ABI PRISM® 310.	4305051

Sequencing Consumables

Sequencing Polymer

Two polymers are available for DNA sequencing:

- Performance Optimized Polymer, 6% (POP-6™)
- DNA Sequencing Polymer for specific applications

Sequencing Application Kits

Applied Biosystems sells Ready Reaction and Core Kits for the various sequencing chemistries. Please contact your sales representative or see the Applied Biosystems pages on the World Wide Web at www.appliedbiosystems.com/techsupport

Kit	Purpose
Ready Reaction	Ready Reaction kits contain all necessary reagents in stable premixes.
Core Kit	Core Kits separately package each reagent.

Sequencing Consumables Part Numbers

Use these part numbers to order sequencing consumables:

Consumable	Purpose	Part Number
Performance Optimized Polymer, 6 (POP-6), with 2 vials of TSR	Polymer for electrophoresing DNA fragments in the capillary.	402844
Performance Optimized Polymer, 6 (POP-6), with 8 vials of TSR	Polymer for electrophoresing DNA fragments in the capillary.	403076
Performance Optimized Polymer, 6% without TSR (POP-6)	Polymer for electrophoresing DNA fragments in the capillary.	402837
10X Genetic Analyzer Buffer with EDTA (for use with POP-6)	Running buffer for electrophoresis.	402824
DNA Sequencing Polymer with TSR[a] (DSP)	Polymer for electrophoresing DNA fragments in the capillary.	402091
10X Genetic Analyzer Buffer (for use with DSP)	Running buffer for electrophoresis.	401884

GeneScan Consumables

GeneScan Polymer

Two polymers are available for GeneScan®:

- Performance Optimized Polymer, 4% (POP-4).
- GeneScan Polymer for SSCP and native runs.

GeneScan Application Kits

A number of kits are available for specialized applications of GeneScan® Analysis Software with the ABI PRISM® 310. Please consult your sales representative or see the Applied Biosystems pages on the World Wide Web at www.appliedbiosystems.com/techsupport

GeneScan Size Standard Kits

Applied Biosystems offers several size standard kits for a variety of applications.

Kit	For Fragments Between…	Part Number
GeneScan® 350 TAMRA	35 bp to 350 bp	401736
GeneScan® 350 ROX	35 bp to 350 bp	401735
GeneScan® 400 HD ROX	50 bp to 400 bp	402985
GeneScan® 500 TAMRA	35 bp to 500 bp	401733
GeneScan® 500 ROX	35 bp to 500 bp	401734
GeneScan® 1000 ROX	100 bp to 900 bp	401098
GeneScan® 2500 ROX	100 bp to 2500 bp	401100
GeneScan® 2500 TAMRA	100 bp to 2500 bp	401545

GeneScan Consumables Part Numbers

Use these part numbers to order GeneScan consumables:

Consumable	Purpose	Part Number
Performance Optimized Polymer, 4% (POP-4)	Polymer for electrophoresing DNA under denaturing conditions.	402838
10X Genetic Analyzer Buffer with EDTA (for use with POP-4)	Running buffer for electrophoresis.	402824
GeneScan Polymer	Polymer for electrophoresing DNA under non-denaturing conditions.	401885

Running Sequencing Experiments 2

Introduction

In This Chapter Topics in this chapter include the following:

About Running Sequencing Experiments

About this Chapter

This chapter summarizes the steps for a DNA sequencing experiment, and, as an example, explains in detail the steps for cycle sequencing a single-stranded (M13) template using BigDye™ terminators and Performance Optimized Polymer, 6%.

This chapter explains the steps for making a matrix file from:

- Four separate matrix standards (one for each color)
- A single four-color sample

To quickly start a sequencing run, see the Quick Reference Card at the front of the manual.

For more detail about specific procedures, see Chapter 4, “Procedures for Operation.”

About Cycle Sequencing

Although the reaction mixes and temperature cycling are similar, it is important to understand the difference between PCR and cycle sequencing.

PCR uses two primers and a double-stranded template to exponentially amplify a sequence of interest. Template is denatured, primers are allowed to anneal to the template, and the primers are extended. The purpose is to amplify the unknown sequence for further experimentation, such as sequencing.

Cycle sequencing can use one or two primers and a single-stranded or double-stranded template. The purpose is to determine the base sequence of a DNA template.

In cycle sequencing, the temperature cycles from a denaturation temperature of about 95 °C to an annealing temperature of about 56 °C. The primers sit down on the denatured template. The temperature is raised to 72 °C, and Taq polymerase extends the primer, incorporating ddNTPs that stop the extension reactions to randomly generate fragments that differ in length by one base. This temperature cycling can continue without the addition of enzyme because AmpliTaq® DNA Polymerase, FS tolerates high temperatures.

In primer sequencing, the primers are fluorescently labeled; in terminator sequencing, the ddNTPs are fluorescently labeled.

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About DNA Sequencing

There are currently five DNA sequencing chemistries:

Chemistry	Protocol Part Number
Dye-labeled terminator	402078
Dye-labeled primer	402113
Dichlororhodamine (dRhodamine) dye-labeled terminator	403041
BigDye™-labeled primer	403057
BigDye™-labeled terminators	4303237

Dye primer labeling matches a specific dye-labeled primer to each of the four bases in four different dideoxynucleotide extension reactions. The reaction products are pooled and electrophoresed.

Dye terminator labeling covalently attaches a specific fluorescent dye to each dideoxy nucleotide base. Extension product termination and labeling occur simultaneously for all bases in one tube.

All methods use AmpliTaq DNA Polymerase, FS. The required polymer and reagents used vary. Consult the protocol that comes with the Ready Reaction kit.

Description of Chemistry Table Levels

There are four levels of recommendations in the chemistry tables.

Level	Description
Recommended	This chemistry is the best choice for most customers most of the time.
Satisfactory	This chemistry will work. Customers will get good results but there is a better choice of chemistry of the given application or platform.
Not Recommended	This chemistry might work. Some customers will get good results but others will not. In most cases the results will be suboptimal in terms of readlength and or accuracy
Not Possible	This chemistry will not work and is not sold/specified for the given application or platform.

continued on next page

310 Chemistry Options Table

The following table describe the chemistry options for DNA sequencing applications.

310 Chemistry Options					
DNA Sequencing Application					
	BigDye™ Terminators	**dRhodamine Terminators**	**BigDye™ Primers**	**Standard Primers**	**Standard Terminators**
de novo Sequencing High Throughput	Recommended	Satisfactory	Recommended	Satisfactory	Recommended
de novo Sequencing Mid-Low Throughput	Recommended	Satisfactory	Satisfactory	Satisfactory	Recommended
Comparative Sequencing (Germline Mutations 50:50)	Recommended	Satisfactory	Recommended	Recommended	Not Recommended
Comparative Sequencing (Somatic Mutations 30:70)	Recommended	Not Recommended	Recommended	Recommended	Not Recommended
Comparative Sequencing (Somatic Mutations 10:90)	Not Recommended	Not Recommended	Recommended	Recommended	Not Recommended
DNA Sequence Context					
G-C rich > 65%	Recommended	Satisfactory	Recommended	Recommended	Recommended
A-T rich > 65%	Recommended	Recommended	Recommended	Recommended	Recommended
G-T rich (regions)	Not Recommended	Satisfactory	Recommended	Recommended	Recommended
Homopolymer A or T > 25 bp	Not Recommended	Recommended	Recommended	Recommended	Recommended
GA Motifs	Recommended	Satisfactory	Recommended	Recommended	Recommended
Template					
Plasmid (<15 bp)	Recommended	Recommended	Recommended	Recommended	Recommended
M13	Recommended	Recommended	Recommended	Recommended	Recommended
BAC, Cosmid, Lambda, XL PCR	Recommended	Satisfactory	Satisfactory	Not Recommended	Not Recommended
Bacterial genomic DNA	Recommended	Not Recommended	Not Recommended	Not Recommended	Not Recommended
PCR Amplicon	Recommended	Recommended	Recommended	Recommended	Recommended
PCR Amplicon (Heterozyous 50:50)	Recommended	Satisfactory	Recommended	Recommended	Not Recommended
PCR Amplicon (Heterozyous 30:70)	Recommended	Not Recommended	Recommended	Recommended	Not Recommended
PCR Amplicon (Heterozyous 10:90)	Not Recommended	Not Recommended	Recommended	Recommended	Not Recommended

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About Cycle Sequencing Using Terminators

This chapter uses BigDye™ terminator cycle sequencing chemistry as an example for DNA sequencing on the ABI PRISM® 310 Genetic Analyzer. This method for performing enzymatic extension reactions is quick, convenient and commonly used.

The benefits of this method include the following:

- Single-tube reactions.
- Less hands-on time required than with dye-labeled primer chemistry.
- Same protocol for both single- and double-stranded templates.
- Less starting template needed than with non-cycling protocols.
- Easier sequencing of large constructs compared to non-cycling protocols.
- More reproducible results.

Summary of Procedures

To perform a sequencing experiment on your samples using the ABI PRISM® 310 Genetic Analyzer, do the following:

- Prepare the ABI PRISM® 310 by:
  - Preparing the reagents.
  - Cleaning the pump block.
  - Installing the capillary.
  - Cleaning and installing the syringe.
  - Cleaning the electrode.
  - Recalibrating the autosampler if:
    - The electrode is removed, replaced or cleaned
    - The capillary is replaced
  - Priming the pump block.
  - Loading buffers.
  - Preheating the instrument.
- Prepare matrix standards (if necessary).
- Prepare the samples.
- Start the run by:
  - Selecting or creating a Sample Sheet.
  - Filling out an Injection List.

continued on next page

Materials Required

The following table lists what materials are required to work through the example provided in this chapter.

! WARNING ! CHEMICAL HAZARD. Before handling the chemical reagents needed for BigDye™ terminator cycle sequencing, read the safety warnings on the reagent bottles and in the manufacturers' Material Safety Data Sheets (MSDS). Always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) when handling chemicals. Dispose of waste in accordance with all local, state, and federal health and environmental regulations and laws.

Materials required for this example.

Item	Source
ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq FS	Applied Biosystems: ♦ P/N 4303149 for 100 reactions ♦ P/N 4303150 for 1000 reactions ♦ P/N 4303151 for 5000 reactions
ABI PRISM® dRhodamine Matrix Standards Kit	Applied Biosystems P/N 403047
Deionized formamide	Major laboratory suppliers (MLS)
Deionized water	MLS
25 mM EDTA (ethylenediaminetetraacetate) with 50 mg/mL blue dextran, pH 8.0	Applied Biosystems P/N 402055
Ethanol (EtOH), non-denatured, 95% or 70% Magnesium chloride ($MgCl_2$), 0.5 M or 2 mM	MLS MLS
Mineral oil, for the DNA Thermal Cycler (TC1) and the DNA Thermal Cycler 480	Applied Biosystems P/N 0186-2302
Shrimp alkaline phosphatase (SAP), 1 U/μL, and 10X SAP buffer (200 mM Tris-HCl, 100 mM $MgCl_2$, pH 8.0)	Amersham Life Science P/N E 70092 Z
Sodium acetate (NaOAc), 3 M, pH 4.6	Applied Biosystems P/N 400320
ABI PRISM Plasmid Miniprep Kit ♦ 100 purifications ♦ 500 purifications	Applied Biosystems: ♦ P/N 402790 ♦ P/N 402791
Adhesive-backed aluminum foil tape	3M (Scotch Tape P/N 425-3)
Centricon-100 Micro-Concentrator columns	Applied Biosystems P/N N930-2119
Microcentrifuge, variable speed, capable of reaching 14,000 × *g*	MLS
Spin column, Centri-Sep, 1-mL in North America	Princeton Separations P/N CS-901
Spin column, Centri-Sep, 1–mL outside North America ♦ 32 columns ♦ 100 columns	Applied Biosystems ♦ P/N 401763 ♦ P/N 401762
Table top centrifuge, with 96-tube tray adaptor	MLS
Vacuum centrifuge	Savant Speedvac (P/N DNA100) or equivalent
Vortexer	MLS

Materials required for this example. *(continued)*

Item	Source
Disposable, non-powdered, chemical-resistant gloves	MLS
A small-volume, calibrated pipette and tips (the Gilson Pipetman is recommended)	Rainin Instruments P/N P10
ABI PRISM® 310 10X Genetic Analyzer Buffer with EDTA	Applied Biosystems P/N 402824
One 1.0-mL glass syringe	Applied Biosystems P/N 604418
Performance Optimized Polymer 6 with TSR	Applied Biosystems P/N 402844
Two 4.0-mL Genetic Analyzer Buffer Vials	Applied Biosystems P/N 401955
Two septa for the 4.0-mL buffer vials	Applied Biosystems P/N 401956
For rapid sequencing: ABI PRISM® 310 Genetic Analyzer Capillary, 47-cm, 50-μm i.d., labeled with a green mark[a]	Applied Biosystems P/N 402839
For long-read sequencing: ABI PRISM® 310 Genetic Analyzer Capillary, 61-cm, 50-μm i.d., labeled with a pink mark	Applied Biosystems P/N 402840
ABI PRISM® DNA Sequencing Analysis Software User's Manual	Applied Biosystems P/N 904436
One of the following thermal cyclers from Applied Biosystems: ♦ GeneAmp® PCR System 9600 ♦ GeneAmp® PCR System 9700 ♦ GeneAmp® PCR System 2400 ♦ DNA Thermal Cycler 480 **Note If the GeneAmp® PCR System 2400 or DNA Thermal Cycler 480 will be used to denature your samples, you will need the appropriate materials (*i.e.,* tubes, tray, etc.) for these thermal cyclers. You will also be required to transfer the samples to either the 96- or 48-well sample tray to load the samples on the ABI PRISM® 310.**	

a. Do not use sequencing capillaries labeled with a silver mark (P/N 401821) with POP-6™ polymer. Resolution will be unsatisfactory if these capillaries are used with POP-6 polymer.

You will also need a 48-well or a 96-well sample tray and accessories.

Note If you are using a 9700 Thermocycler, and you do not have the 96 sample tray adaptor (P/N 4305051), then you will have to transfer the samples from the tray compatible with the 9700 after denaturation to the ABI PRISM® 310 96 sample tray before placing on the autosampler.

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96-Well Sample Tray Accessories

The following table lists the 96-well sample tray and accessories.

Part	Quantity	Old Part Number	New Part Number
310 Genetic Analyzer Tray/Retainer Set	1	4303864	403081
0.2-mL MicroAmp Reaction Tubes	1 box	N801-0580	N801-0580
MicroAmp® Base	1	N801-0531	N801-0531
MicroAmp® Full Plate Cover	1	N801-0550	N801-0550
Genetic Analyzer Septa Strips	1	402059	402059
310 Genetic Analyzer Retainer Clips	1	402866	402866
310 Genetic Analyzer 96 Well Sample Tray Adaptor	1	NA/	4305051

48-Well Sample Tray and Accessories

The following table lists the 48-well sample tray and accessories.

Part	Quantity	Part Number
48-well Sample Tray	1	005572
0.5-mL Samples Tubes	1 box	401957
Septa for 0.5-mL Sample Tubes	1 box	401956
1.5-mL Eppendorf tube	1	—

Software Required

- ABI PRISM® 310 Firmware, v 1.0.2 or higher
- ABI PRISM® 310 Data Collection Software, v 1.0.2 or higher
- ABI-CE1 Basecaller file
- DT POP-6 {BD Set-any primer} terminator mobility file
- POP-6 module file
  - For rapid sequencing: ABI PRISM® 310 module SEQ POP6 RAPID (1.0-mL) E
  - For long read sequencing: ABI PRISM® 310 module SEQ POP6 (1.0-mL) E
- ABI PRISM® DNA Sequencing Analysis Software, v 2.1.1 or higher

How to Switch Between DNA Sequencing and the GeneScan Analysis Software

Switching Between Applications

If the ABI PRISM® 310 was set up for experiments to be analyzed with the GeneScan® Analysis Software, then before following the instructions in this chapter to prepare an experiment that will be analyzed with the ABI PRISM® DNA Sequencing Analysis Software, it will be necessary to do the following.

Take this action	See page
Remove the 2.5-mL glass syringe (if present) or clean out the 1.0-mL syringe.	4-18
Remove the capillary and store it.	4-15
Clean the pump block.	4-8

How to Set Up the Reagents

Preparing Reagents

! WARNING ! CHEMICAL HAZARD. Urea is a potential mutagen. Dangers cited in toxicity studies show reproductive and tumorigenic effects. Urea can cause irritation to the skin, eyes, and respiratory tract. Avoid inhalation and contact with skin, eyes and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

CHEMICAL HAZARD. Some chemicals used with this instrument are potentially hazardous. Warnings are prominently displayed on the bottle labels of all hazardous chemicals. Material Safety Data Sheets (MSDSs) are provided by the manufacturer and contain information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. Please familiarize yourself with the information contained in these documents before attempting to operate the instrument or using hazardous reagents. Additional copies of the MSDSs for Applied Biosystems chemicals are available from Applied Biosystems at no cost.

For experiments that require DNA Sequencing Polymer (DSP), see “Setting Up the Reagents for Runs with DSP” on page 2-75 for instructions.

Prepare the polymer and buffer:

Step	Action
1	Equilibrate polymer to room temperature, then mix thoroughly by inversion.
2	Allow the polymer to sit for at least 5 minutes after mixing.
3	Dilute 5-mL of 10X Genetic Analyzer Buffer with EDTA to a 1X concentration (50-mL) with filter-sterilized, deionized water and bring to room temperature.

Storing the Buffer

Store electrophoresis buffer at 2–8 °C. The 1X buffer can be stored for 2 weeks.

When to Replace Reagents

Use the following table.

Reagent	Replace
Cathode and Anode buffers	Every week or every 200 runs.
Polymer	Every week.
Water in the water vial and waste vial	Before every set of runs.

Preparing the 310 Genetic Analyzer for a DNA Sequencing Run With POP-6

About These Procedures

To work through the example in this chapter using BigDye™ Terminators and POP-6 polymer, or for any experiment requiring POP-6 polymer, set up the instrument as detailed below.

For experiments that require DNA Sequencing Polymer (DSP), see “Preparing the Syringes for a DNA Sequencing Run with DNA Sequencing Polymer (DSP)” on page 2-66 for additional instructions.

These procedures can also be found in Chapter 4, “Procedures for Operation.” Some are explained there with more detail and with illustrations. If you have a question about a procedure, see Chapter 4.

Cleaning the Pump Block

IMPORTANT Do not expose the pump block to any organic solvents.

To clean the pump block:

Step	Action
1	Remove the pump block from the instrument.
2	Open all valves and ports on the pump block.
3	Hold the pump block under warm running water to thoroughly rinse it.
4	Force warm water through the channels with the 5-cc plastic syringe provided in the Basic Installation Kit. Direct the flow of water through each channel in turn by sealing channel openings with plugs provided in the Basic Installation Kit. Rinse each channel five times. **Note** If a 30-cc syringe is available, rinse each channel with it once. This can be more convenient than rinsing five times with a 5-cc syringe.
5	Rinse the valves with warm water. Soak valves that are coated with dried polymer.
6	Visually inspect the channels for dried polymer, which looks like white residue. Wash the channels until the polymer is gone.
7	Rinse the block and its channels with distilled, deionized water.
8	Remove residual water from the pump block and fittings to ensure that the running polymer is not diluted. Force air through the channels, using the plastic syringe or canned compressed air, until the channels are dry.
9	Replace the pump block and buffer reservoir.

continued on next page

Cleaning and Preparing the Syringe for Use

Use the 1.0-mL syringe with Performance Optimized Polymers.

CAUTION **The teflon plunger is very susceptible to wear caused by friction. Therefore the pulling up and pushing down of the plunger within the barrel of the syringe should be minimized.**

To clean the syringe:

Step	Action
1	Rinse the syringe with distilled water. Remove excess water, but do not dry completely. **IMPORTANT** Excessive wear occurs to the Teflon fitting of the plunger if it is completely dry.
2	Allow the polymer to equilibrate to room temperature before loading it into the syringe. **IMPORTANT** Precipitate present when the bottle is removed from cold storage should go back into solution at room temperature. Do not use the polymer until the precipitate dissolves.
3	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.
4	Inspect the syringe for the ferrule and two o-rings.
5	Draw a small amount of polymer solution (0.20-mL maximum) into the syringe. Pull plunger up to the 0.60-mL marker after the polymer solution is added. **! WARNING ! Gloves and eye protection should be worn when handling polymer.**
6	Invert the syringe gently five or six times to coat the walls with polymer and discard this polymer solution. This ensures that the running polymer is not diluted when added to the syringe.
7	For new, never-before-used syringes, perform this entire procedure twice to prevent air bubbles from sticking to the syringe walls.

continued on next page

Loading Polymer

To load polymer:

Step	Action
1	Allow the polymer to equilibrate to room temperature before loading it into the syringe. **IMPORTANT** Precipitate present when the bottle is removed from cold storage should go back into solution at room temperature. Do not use the polymer until the precipitate dissolves.
2	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.
3	Fill the syringe manually with a maximum of 0.5-mL of polymer. **Note** Do not use polymer that has been on the instrument for more than one week. Do not return unused polymer to the original bottle.
4	Remove all air bubbles by inverting syringe and pushing air bubbles out. **IMPORTANT** To avoid loss of polymer, any bubbles near the plunger head must be removed.
5	Rinse the outside of the syringe with distilled water to remove any polymer on the outside of the syringe. Dry the outside of the syringe with a lint-free paper.
6	Move the syringe drive toggle to the left to attach the syringe to the pump block.
7	Place the syringe through the right-hand port of the plastic syringe guide plate, and screw the syringe into the pump block finger-tight.
8	Manually close and tighten the waste valve below the syringe and the luer valve to the left of the syringe.

Setting Syringe Max Travel

Set the Syringe Max Travel once for each size of syringe. Record the value for each syringe (the calibration sticker on the door is a good place for this record) and input it in the Manual Control window when you install the syringe again.

To set the Syringe Max Travel:

Step	Action
1	Determine which version of Collection software is installed on your Macintosh® computer by clicking the Apple menu and selecting “About Collection Software”.
2	Use the following table to determine the next step: **If you have...** / **Then...** v. 1.04 or later Collection software / go to step 3. v. 1.0.2 Collection software / a. Open the Window menu. b. Under Preferences, select Sequence Injection List Defaults or GeneScan Injection List Defaults. c. Type 139115 in the Operator entry window and click OK. d. Re-launch the Collection software.
3	Open the Manual Control window, select Syringe Home from the Function pop-up menu, and click Execute.

To set the Syringe Max Travel: *(continued)*

Step	Action
4	Select Syringe Max Travel in the Function pop-up menu. **Note** You will enter the displayed value in the next step.
5	Select Syringe DOWN and enter the current Syringe Max Travel value and click Execute.
6	After the syringe drive reaches the syringe plunger and stops moving, select Status from the Window menu.
7	Under the Injection pop-up window, read the number in the Gel Pump At window. Subtract 15 from that number
8	Select Syringe Max Travel in the Function pop-up menu of Manual Control and type the number you just calculated in step 7 and click Execute.
9	Record this value on the Calibration sticker on the left side door. **Note** If you install a syringe of a different size, you must input the max travel value for that syringe size.
10	Select Syringe Up in the Function pop-up menu of Manual Control, enter 250 in the Value window, and click Execute.

Filling the Pump Block

To fill the pump block channels with polymer:

Step	Action
1	Open the Manual Control window. Select Buffer Valve Close in the Function pop-up menu to close the pin valve at the anode buffer reservoir on the pump block.
2	Manually open the waste valve below the syringe.
3	Press the syringe plunger until a drop of polymer forms on the bottom of the waste valve. This removes the air bubbles at this valve site, and uses about 0.1-mL of polymer.
4	Manually close the waste valve.
5	Open the Manual Control menu and select Buffer Valve Open in the Function pop-up menu to open the pin valve at the anode buffer reservoir on the pump block.
6	Press the syringe plunger until polymer fills the polymer channel in the block. This removes all of the air bubbles from the polymer channels, and should use about 0.1-mL of polymer. **IMPORTANT** There should be no air bubbles in the pump block channels.
7	Select Buffer Valve Close in the Function pop-up menu.
8	Move the syringe drive toggle to the right to position it over the syringe plunger.
9	Select Syringe Down in the Function pop-up menu.
10	Select 50-step intervals and click Execute until the toggle makes contact with the syringe plunger.

continued on next page

Cleaning the Electrode

To clean the electrode:

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software.
2	Press the Tray button on the 310 Genetic Analyzer to lower the autosampler and present the tray.
3	Wipe the electrode with lint-free paper that has been dampened with distilled, deionized water.
4	Dry the electrode with fresh lint-free paper.
5	Press the Tray button to return the autosampler to its original position and immerse the capillary in buffer.
6	Recalibrate the autosampler after cleaning, trimming or replacing the electrode.

Installing the Capillary

Connecting the Capillary to the Pump Block

Step	Action
1	Remove the new capillary from the storage tube. **Note** When removing capillaries, rotate and pull on the capillary simultaneously. Be careful not to bend the capillary at the capillary window.
2	Clean the capillary window.
3	Open the door covering the heat plate.
4	Remove the plastic capillary fitting on the right side of the pump block.
5	Partially screw the capillary fitting back into the pump block. **IMPORTANT** Do not tighten the fitting at this point in the procedure, or the opening on its tip will be crushed. The capillary must be properly inserted through the fitting before you tighten it.
6	Thread one end of the capillary through the capillary fitting.
7	Adjust the end of the capillary so that it is positioned directly below the opening to the glass syringe. The end of the capillary must protrude well beyond the opening at the tip of the capillary fitting. To avoid crushing the opening, be certain that you see the capillary in the pump block channel before proceeding to the next step.
8	Tighten the capillary fitting finger-tight to secure the capillary. **IMPORTANT** The capillary will twist as the fitting is tightened. Leave the other end of the capillary free to twist, or the capillary will break.

Positioning the Capillary in the Detector

Step	Action
1	Open the laser detector door, and position the capillary in the vertical track of the detector. Align the colored labelling mark on the capillary with the top edge of the detector plate and laser detector door.
2	Tape the capillary to the heat plate with thermal tape to secure the position of the capillary labelling mark relative to the detector plate.
3	Close the laser detector door to secure the position of the capillary window.

Positioning the Capillary Near the Electrode

Step	Action
1	Thread the capillary through the capillary hole in the electrode thumbscrew until it protrudes past the tip of the electrode by about 0.5 mm.
2	Tape the capillary to the heat plate just above the electrode thumbscrew and just above the detector door using thermal tape. This secures the position of the capillary tip relative to the electrode.
3	Close the door over the heat plate.
4	With the heat plate door closed, check that the capillary has not moved relative to the electrode.

Resetting the Injection Counter

Step	Action
1	Open the Instrument window and choose Change Capillary.
2	Click OK in the Reset window to set the injection counter to zero.

Calibrating the Autosampler

Calibrate the autosampler as follows:

Step	Action
1	Choose Autosampler Calibration from the Instrument menu of the Collection software. The Autosampler Calibration window appears.
2	Click Start and follow the directions that appear on the screen.
3	Move the autosampler using the arrow keys in the Autosampler Calibration window or the arrow keys on the Macintosh® computer keyboard. **Note** Hold down the arrow keys to move the autosampler with larger steps. This is often useful for z calibration.
4	Align the calibration dot on the front of the tray platform with the capillary. Center the end of the capillary on the x,y-calibration point. Almost touch the z-calibration point with the end of the capillary.
5	Click Set to save the calibration value.
6	Repeat for the rear calibration point and click Set.

continued on next page

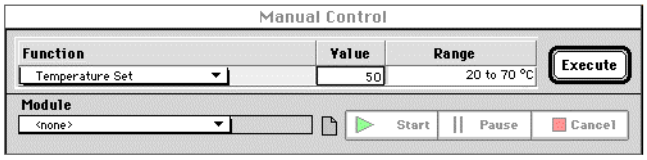
Loading Buffers

Load the buffer as follows:

Step	Action
1	Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer Buffer with EDTA, and install it on the pump block.
2	Label one of the buffer vials as Buffer, and fill it to the line with 1X Genetic Analyzer buffer with EDTA.
3	Cap the vial, insert the septum, and place it in position one on the autosampler.
4	Label the other glass buffer vial as H_2O, and fill it to the line with filter-sterilized, deionized water.
5	Cap the vial, insert the septum, and place it in position two on the autosampler.
6	If the 1.5-mL Eppendorf tube has a lid attached, cut the lid off. Completely fill the tube with filter-sterilized, deionized water, and place it in position three on the autosampler.

Preheating the Heat Plate

This is optional. Preheating the heat plate can take up to 20 minutes. You can denature the samples while the instrument preheats.

Step	Action
1	Open the Window pull-down menu, and choose Manual Control. The Manual Control window is displayed. 
2	Choose Temperature Set from the Function menu, and set the temperature to 50 °C in the Value window.
3	Click Execute. The instrument will preheat to 50 °C. While the instrument is preheating, you can denature your samples. The instrument doors must be closed. The safety interlock will prevent operation when the doors are open.

DNA Template Preparation

Overview

The DNA purification method used can affect the quality of the template preparation. Some recommendations for purifying DNA templates are given below.

Prepare adequate template to check purity (see "Determining DNA Quality" on page 2-21), to quantitate the DNA accurately (see "Quantitation" on page 2-22), and to perform the sequencing reactions. The recommended quantities for sequencing reactions are shown in Table 2-1 on page 2-22.

Refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for more information on DNA template preparation and specific protocols.

Single-stranded DNA Templates

You can use the following methods to prepare single-stranded templates such as M13:

- QIAGEN (http://www.qiagen.com) QIAprep Spin M13 Kit (P/N 27704, 50 reactions)
- Thermomax procedure
- PEG precipitation followed by phenol extraction

Plasmid DNA Templates

The optimal procedure for preparing a particular plasmid depends on the particular bacterial strain and the yield of each construct. Good sequencing data has been obtained from the following methods:

- ABI PRISM Plasmid Miniprep Kit (P/N 402790 or 402791)
- Cesium chloride (CsCl) banding
- Modified alkaline lysis/PEG precipitation method
- PureGene DNA Isolation Kit (Gentra Systems, Inc., P/N D-5500A)
- QIAGEN Plasmid Kits (http://www.qiagen.com):
  - Mini (P/N 12123, 25 reactions; 12125, 100 reactions)
  - Midi (P/N 12143, 25 reactions; 12144, 50 reactions; 12145, 100 reactions)
  - Maxi (P/N 12162, 10 reactions; 12163, 25 reactions; 12165, 100 reactions)

BAC DNA Templates

With larger DNA targets such as bacterial artificial chromosomes (BACs), the quality of DNA template is crucial to the success of the sequencing reaction. Three methods have given good sequencing results:

- Alkaline lysis,[1] with extra phenol extraction and isopropanol precipitation if very clean DNA is desired\
- Cesium chloride (CsCl) banding
- Modified AutoGen prep[2]

1. Marra, M., Weinstock, L.A., and Mardis, E.R. 1996. End sequence determination from large insert cloning using energy transfer fluorescent primers. *Genomic Methods* 6: 1118–1122.
2. "Bacterial Artificial Chromosome DNA Preparation" in *ISS AutoGen 740 Operator's Guide*, version 3.0.

PCR Templates

This section provides information regarding PCR template preparation, but it is not meant to be a detailed guide to PCR amplification. General information on PCR amplification can be found in the *Guide to PCR Enzymes* (Stock No. 700905) and in the product inserts included with GeneAmp® PCR reagents. For PCR amplification, use GeneAmp PCR Instrument Systems and GeneAmp PCR Core Reagents.

Cycle sequencing has been found to provide the most reproducible results when sequencing PCR templates. Although PCR fragments can be difficult to denature with traditional sequencing methods, cycle sequencing provides several chances to denature and extend the template, which ensures adequate signal in the sequencing reaction.

For more detailed information about PCR sequencing, refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) and *Comparative PCR Sequencing, A Guide to Sequencing-Based Mutation Detection* (Stock No. 770901-001).

Purifying PCR Products

- Commercially available products

  We recommend Centricon-100 columns (P/N N930-2119). These columns contain an ultrafiltration membrane that separates primers and dNTPs from larger PCR products. However, they may not work as well for short PCR products (<125 bases).

  QIAquick PCR Purification Kits (QIAGEN: P/N 28104, 50 reactions; 28106, 250 reactions) also work well for PCR products ranging from 100 bp–10 kbp.

- Shrimp alkaline phosphatase and exonuclease I treatment

  The removal of PCR primers is more critical for dye terminator sequencing, as both PCR primers can act as sequencing primers, creating two sets of extension products.

  An alternative to purification is treatment of PCR products with shrimp alkaline phosphatase (SAP) and exonuclease I (Exo I) before sequencing. The SAP/Exo I procedure degrades nucleotides and single-stranded DNA (primers) remaining after PCR (Werle *et al.*, 1994). This procedure is particularly useful in cases where limiting concentrations of primers and nucleotides cannot be used in PCR.

DNA Template Quality

Using Control DNA

Always use a control DNA template when preparing your sequencing reactions. This can help determine whether failed reactions are the result of poor template quality or sequencing reaction failure.

We recommend M13mp18 as a single-stranded control and pGEM-3Zf(+) as a double-stranded control. All Applied Biosystems DNA sequencing kits provide pGEM control DNA and a –21 M13 primer.

Sequencing Standards

The Long Read Cycle Sequencing Standards provide an additional control to help in troubleshooting electrophoresis runs. There are four standards available:

- Dye Primer Cycle Sequencing Standard (P/N 401920)
- Dye Terminator Cycle Sequencing Standard (P/N 402830)
- dRhodamine Terminator Cycle Sequencing Standard (P/N 4303120)
- BigDye Terminator Cycle Sequencing Standard (P/N 4304154)

Poor Template Preparation

Inadequate template preparation is the most common cause of sequencing problems. Always follow recommended procedures to prepare templates.

The following are characteristics of badly prepared templates:

- Noisy data or peaks under peaks
- Failed reactions
- Weak signal

Contamination

Potential contaminants include:

- proteins
- RNA or chromosomal DNA
- residual salts
- residual organic chemicals, *e.g.*, phenol, chloroform, and ethanol

Determining DNA Quality

The following methods can be used to examine DNA quality:

- Agarose gel electophoresis

  Purified DNA should run as a single band on an agarose gel.

- Spectrophotometry

  The A_{260}/A_{280} ratio should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals.

Agarose gels reveal the presence of contaminating DNAs and RNAs, but not proteins. Spectrophotometry can indicate the presence of protein contamination, but not DNA and RNA contamination. These methods should be used together to get the most information about your DNA template before sequencing.

Note Neither of these methods shows the presence of contaminating salts that can cause noisy data. If you suspect that your DNA is contaminated with salt, remove the salt before sequencing (see "Purifying PCR Products" on page 2-19).

Cleaning Up Dirty Templates

A "dirty" template preparation sometimes can be cleaned up with one of the following methods:

- Purify the DNA by ultrafiltration. Use Centricon-100 Micro-Concentrator columns (see "Purifying PCR Products" on page 2-19).
- Extract the DNA twice with 1 volume of chloroform or chloroform:isoamyl alcohol in a 24:1 (vol:vol) ratio.
- Precipitate the aqueous layer with PEG to remove all traces of chloroform. Add 0.16 volumes of 5M NaCl and 1 total volume of 13% PEG. Incubate on ice for 20 minutes, then centrifuge at 4 °C for 20 minutes. Rinse the pellet with 70% ethanol.

DNA Template Quantity

Quantitation

DNA template quantitation is critical for successful sequencing reactions. The most common way to determine DNA quantity is to measure the absorbance (optical density or O.D.) of a sample at 260 nm in a spectrophotometer.

One O.D. unit is the amount of a substance dissolved in 1.0 mL that gives an absorbance reading of 1.00 in a spectrophotometer with a 1-cm path length. The wavelength is assumed to be 260 nm unless stated otherwise. A_{260} values can be converted into ng/μL using Beer´s Law:

Absorbance (260 nm) = sum of extinction coefficient contributions × cuvette pathlength × oligonucleotide concentration

The following formulas, which are derived from Beer's Law, convert A_{260} readings into ng/μL concentrations:

- One A_{260} unit of single-stranded DNA contains 33 ng/μL.
- One A_{260} unit of double-stranded DNA contains 50 ng/μL.

Amount to Use

The amount of DNA template used in a sequencing reaction can affect the quality of the data. Too much template makes data appear top heavy with strong peaks at the beginning of the run that fade rapidly. Too little template or primer reduces the signal strength and peak height. In the worst case, the noise level increases so that bases cannot be called. Table 2-1 shows the recommended quantities for each method.

Table 2-1 Recommended DNA Template Quantity for Each Chemistry

	Cycle Sequencing Chemistry				
Template	**Dye Primer**	**Dye Terminator**	**dRhodamine Terminator**	**BigDye Primer**	**BigDye Terminator**
PCR product:					
100–200 bp	2–5 ng	1–3 ng	1–3 ng	2–5 ng	1–3 ng
200–500 bp	5–10 ng	3–10 ng	3–10 ng	5–10 ng	3–10 ng
500–1000 bp	10–20 ng	5–20 ng	5–20 ng	10–20 ng	5–20 ng
1000–2000 bp	20–50 ng	10–40 ng	10–40 ng	20–50 ng	10–40 ng
>2000 bp	50–150 ng	40–100 ng	40–100 ng	50–150 ng	40–100 ng
single-stranded	150–300 ng	100–250 ng	50–100 ng	200–400 ng	50–100 ng
double-stranded	300–600 ng	200–500 ng	200–500 ng	200–800 ng	200–500 ng
cosmid, BAC	0.5–1.0 μg	0.5–2.0 μg	not recommended	300–600 ng	300–600 ng
genomic DNA	not recommended				2–3 μg

Primer Design and Quantitation

Overview

The choice of primer sequence, method of primer synthesis, and approach to primer purification can have a significant effect on the quality of the sequencing data obtained in dye terminator cycle sequencing reactions with this kit. Dye primer cycle sequencing kits include dye-labeled primers that are already optimized and quantitated.

Some of the recommendations given here are based on information that is general knowledge, while others are based on practical experience gained by Applied Biosystems scientists.

Primer Design

The following recommendations are provided to help optimize primer selection:

- The primer should be as pure as possible, preferably HPLC grade.
- Primers should be at least 18 bases long to ensure good hybridization.
- Avoid runs of an identical nucleotide, especially runs of four or more Gs.
- Keep the G-C content in the range 30–80%.
- For cycle sequencing, primers with melting temperatures (T_m) above 45 °C produce better results than primers with lower T_m using our recommended thermal cycling parameters.
- For primers with a G-C content less than 50%, it may be necessary to extend the primer sequence beyond 18 bases to keep the T_m>45 °C.
- Use of primers longer than 18 bases also minimizes the chance of having a secondary hybridization site on the target DNA.
- Avoid primers that can hybridize to form dimers.
- Avoid palindromes because they can form secondary structures.
- Several computer programs for primer selection are available. They can be useful in identifying potential secondary structure problems and determining if a secondary hybridization site exists on the target DNA.

Quantitation

The following formula, which is derived from Beer's Law, converts A_{260} readings into pmol/μL concentrations:

$$C\ (\text{pmol}/\mu\text{L or } \mu\text{M}) = (A_{260} \times 100)/(1.54n_A + 0.75n_C + 1.17n_G + 0.92n_T),$$

where n_x = number of residues of base x in the oligonucleotide

Primer Problems and Possible Causes

Table 2-2 Primer Problems and Possible Causes

Problems	Possible Causes
Poor priming resulting in weak signal	Melting temperature is too low due to low GC content and/or short primer length
	Secondary structure of the primer, particularly at the 3' end
	Secondary structure of the template in the region of hybridization
Adequate signal strength with noisy data	Secondary hybridization site, which results in many extra peaks
	Impure primer. You may see a shadow sequence of N-1

Preparing Sequencing Reactions

Reagent Age and Reaction Storage

The freshest reagents are likely to perform the best. The following methods are recommended for guaranteeing reagent freshness.

- Store reagents at –15 to –25 °C when not in use, and thaw completely at room temperature or in an ice bath (do not heat) before use.

Note Do not use a frost-free freezer. The automatic cycling of the temperature for defrosting can damage reagents.

- Avoid excess (more than ten) freeze-thaw cycles. Aliquot reagents in smaller amounts if necessary.
- Shield reagents and sequencing reactions from light. Fluorescent dyes are susceptible to bleaching.
- If you would like to store sequencing reactions for future use, purify and dry them. Store at –15 to –25 °C in a non-frost-free freezer.

Reaction Tubes

The type of tube required depends on the type of thermal cycler used. For the DNA Thermal Cycler (TC1) and DNA Thermal Cycler 480, use 0.5-mL GeneAmp Thin-Walled PCR tubes. For the GeneAmp PCR Systems 9700, 9600, and 2400, use 0.2-mL MicroAmp® PCR tubes. If using the CATALYST 800 or ABI PRISM® 877 instrument, refer to your instrument user's manual for reaction setup.

Thermal Cyclers

The type and performance of the thermal cycler used to prepare sequencing reactions can affect the quality of the reactions. Ensure that the thermal cycler is calibrated regularly by the manufacturer and that ramping rates are 1 °C/second.

BigDye Terminators

The procedures given here are for the ABI PRISM® BigDye Terminator Cycle Sequencing Ready Reaction Kits. Refer to the ***Automated DNA Sequencing Chemistry Guide*** (P/N 4305080) for information about other chemistries.

The flexibility of the BigDye terminators allows three options for cycle sequencing:

Reaction Type	Template	Cycle
1X	♦ PCR product ♦ plasmid ♦ M13	standard
0.5X	♦ PCR product ♦ plasmid ♦ M13	standard
High-sensitivity (2X)	♦ BACs, PACs, YACs, cosmids ♦ extra long PCR products ♦ genomic DNA	modified

1X Reactions

Step	Action	
1	For each reaction, add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	8.0 µL
	Template	–
	single-stranded DNA	50–100 ng
	double-stranded DNA	200–500 ng
	PCR product DNA	2–100 ng (depending on size)
	Primer	3.2 pmol
	Deionized water	q.s.
	Total Volume	20 µL
2	Mix well and spin briefly.	
3	**If using the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:**	
	Overlay the reaction mixture with 40 µL of light mineral oil.	

0.5X Reactions

Dilute 5X Sequencing Buffer (400 mM Tris-HCl, 10 mM $MgCl_2$, pH 9.0—P/N 4305605, 600 reactions; 4305603, 5400 reactions) with an equal volume of deionized water to 2.5X for use in this procedure.

Step	Action	
1	For each reaction, add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	4.0 µL
	2.5X Sequencing Buffer	4.0 µL
	Template	–
	single-stranded DNA	50–100 ng
	double-stranded DNA	200–500 ng
	PCR product DNA	2–100 ng (depending on size)
	Primer	3.2 pmol
	Deionized water	q.s.
	Total Volume	20 µL
2	Mix well and spin briefly.	
3	**If using the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:**	
	Overlay the reaction mixture with 40 µL of light mineral oil.	

High-sensitivity (2X) Reactions for BACs, PACs, YACs, Cosmids, and Extra Long PCR Fragments

The cycle sequencing procedure is on page 2-29.

Step	Action	
1	For each reaction, add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix[a] (***keep or delete note?***)	16 µL
	DNA Template	400–600 ng
	Primer	5–10 pmol[b]
	Deionized water	q.s.
	Total Volume	40 µL
2	Mix well and spin briefly.	

a. Some laboratories have found that increasing the magnesium ion concentration gives better results.

b. Some laboratories use larger quantities of primer.

High-sensitivity (2X) Reactions for Genomic DNA

The cycle sequencing procedure is on page 2-29.

Step	Action	
1	For each reaction, add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	16 µL
	DNA Template[a]	2–3 µg (genomic DNA)
	Primer	6–13 pmol
	ThermoFidelase (optional)[b]	1.0 µL
	Deionized water	q.s.
	Total Volume	40 µL
2	Mix well and spin briefly.	

a. Shearing the DNA by passing it seven times through a 21-gauge, 1-inch long needle can improve signals.

b. Fidelity Systems (http://www.fidelitysystems.com): P/N A016 (100 µL), A056 (500 µL), A106 (1 mL)

Cycle Sequencing

Overview

These protocols have been optimized for all Applied Biosystems thermal cyclers, including the DNA Thermal Cycler (TC1), the DNA Thermal Cycler 480, the CATALYST 800 Molecular Biology LabStation, the ABI PRISM® 877 Integrated Thermal Cycler, and the GeneAmp PCR Systems 9700, 9600, and 2400. The protocols contained in this section should work for all seven instruments.

If you use a thermal cycler not manufactured by Applied Biosystems, you may need to optimize thermal cycling conditions. Ramping time is very important. If the thermal ramping time is too fast (>1°/sec), poor (noisy) data may result.

BigDye Terminators

These protocols are used for the BigDye terminator chemistry. These conditions work for a variety of templates and primers. However, if necessary, these parameters can be changed to suit particular situations, including the following:

- For short PCR products, you can use reduced numbers of cycles (*e.g.*, 20 cycles for a 300-bp fragment).
- If the T_m of a primer is >60 °C, the annealing step can be eliminated.
- If the T_m of a primer is <50 °C, increase the annealing time to 30 seconds or decrease the annealing temperature to 48 °C.
- For templates with high GC content (>70%), heat the tubes at 98 °C for 5 minutes before cycling to help denature the template.

GeneAmp 9700, 9600, or 2400

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 20 µL.
2	Repeat the following for 25 cycles: ♦ Rapid thermal ramp to 96 °C ♦ 96 °C for 10 sec. ♦ Rapid thermal ramp to 50 °C ♦ 50 °C for 5 sec. ♦ Rapid thermal ramp to 60 °C ♦ 60 °C for 4 min.
3	Rapid thermal ramp to 4 °C and hold until ready to purify.
4	Spin down the contents of the tubes in a microcentrifuge.

DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 20 µL.
2	Repeat the following for 25 cycles: ♦ Rapid thermal ramp to 96 °C ♦ 96 °C for 30 sec. ♦ Rapid thermal ramp to 50 °C ♦ 50 °C for 15 sec. ♦ Rapid thermal ramp to 60 °C ♦ 60 °C for 4 min.
3	Rapid thermal ramp to 4 °C and hold until ready to purify.
4	Spin down the contents of the tubes in a microcentrifuge.

BACs, PACs, YACs, Cosmids, and Extra Long PCR Fragments on the GeneAmp 9700 or 9600

Note This protocol is for use only with the BigDye terminator kits.

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 40 µL.
2	Heat the tubes at 95 °C for 5 minutes.
3	Repeat the following for 30 cycles:[a] ♦ Rapid thermal ramp to 95 °C ♦ 95 °C for 30 sec. ♦ Rapid thermal ramp to 50–55 °C (depending on template) ♦ 50–55 °C for 10 sec. ♦ Rapid thermal ramp to 60 °C ♦ 60 °C for 4 min.
4	Rapid thermal ramp to 4 °C and hold until ready to purify.
5	Spin down the contents of the tubes in a microcentrifuge.

a. Some laboratories have found that increasing the number of cycles gives better results.

Genomic DNA on the GeneAmp 9700 or 9600

Note This protocol is for use only with the BigDye terminator kits.

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 40 µL.
2	Heat the tubes at 95 °C for 5 minutes.
3	Repeat the following for 45 cycles: ♦ Rapid thermal ramp to 95 °C ♦ 95 °C for 30 sec. ♦ Rapid thermal ramp to 55 °C (depending on template) ♦ 55 °C for 20 sec. ♦ Rapid thermal ramp to 60 °C ♦ 60 °C for 4 min.
4	Rapid thermal ramp to 4 °C and hold until ready to purify.
5	Spin down the contents of the tubes in a microcentrifuge.

Cycle Sequencing on the CATALYST 800

Templates that have been prepared as described in this chapter should be suitable for use on the CATALYST 800 Molecular Biology LabStation using LabStation 3.0 protocols. Follow the protocols in the Turbo Appendix of the ***CATALYST 800 Molecular Biology LabStation User's Manual*** to set up your reactions.

Terminator Sequencing has two options:

- Using a reaction premix containing the sequencing primer or premixing template with primer in the sample tube
- Combining reaction cocktail (lacking primers), water, and primer from one tube and template from another tube

  This eliminates the requirement for premixing samples and primers.

Cycle Sequencing on the ABI PRISM® 877 ITC

Predefined temperature profiles are provided for the following on the ABI PRISM® 877 Integrated Thermal Cycler:

- Terminator Sequencing uses a reaction premix containing the sequencing primer, or else requires premixing template with primer in the sample tube.
- Terminator Automix Sequencing combines reaction cocktail (lacking primers), water, primer from one tube, and template from another tube. This eliminates the requirement for premixing of samples and primers.

The profile is chosen on the Chemistry page of the Sequencing Notebook and can be edited to make custom profiles. Refer to Chapter 4, "Using the ABI PRISM® 877 Software," in the ABI PRISM® 877 ***Integrated Thermal Cycler User's Manual***.

Preparing Extension Products for Electrophoresis

Overview

Unincorporated dye terminators must be removed before the samples can be analyzed by electrophoresis. Excess dye terminators in sequencing reactions obscure bases in the early part of the sequence and can interfere with basecalling.

- Precipitation methods are cheaper and faster, but they remove less of the unincorporated dye-labeled terminators that can obscure data at the beginning of the sequence.
- The spin column procedure removes more terminators, but is more costly than precipitation methods.

Spin Column Purification

We recommend Centri-Sep™ spin columns from Princeton Separations (P/N CS-901).

Tips for optimizing spin column purification:

- Use one column for each sample.
- Do not process more columns than you can handle conveniently at one time.
- Hydrate the spin columns for at least 2 hours.
- Load the sample in the center of the column bed. Make sure that the sample does not touch the sides of the column. If samples are not properly loaded, peaks from unincorporated dye terminators can result.
- Spin the column at 325–730 × *g* for best results. Use the following formula to calculate the best speed for your centrifuge:

  $g = 11.18 \times r \times (\text{rpm}/1000)^2$

  where:

  g = relative centrifugal force

  rpm = revolutions per minute

  r = radius of the rotor in cm
- Do not spin for more than 2 minutes.
- Perform the entire procedure without interruption to ensure optimal results. Do not allow the column to dry out.

To perform spin column purification:

Step	Action
1	Gently tap the column to cause the gel material to settle to the bottom of the column.
2	Remove the upper end cap and add 0.8 mL of deionized water.
3	Replace the upper end cap and vortex or invert the column a few times to mix the water and gel material.
4	Allow the gel to hydrate at room temperature for 30 minutes (at least 2 hours for BigDye terminators). **Note** Hydrated columns can be stored for a few days at 2–6 °C. Longer storage in water is not recommended. Allow columns that have been stored at 2–6 °C to warm to room temperature before use.
5	Remove any air bubbles by inverting or tapping the column and allowing the gel to settle.

To perform spin column purification: *(continued)*

Step	Action
6	Remove the upper end cap first, then remove the bottom cap. Allow the column to drain completely by gravity. **Note** If flow does not begin immediately, apply gentle pressure to the column with a pipette bulb.
7	Insert the column into the wash tube provided.
8	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes to remove the interstitial fluid.
9	Remove the column from the wash tube and insert it into a sample collection tube (e.g., a 1.5-mL microcentrifuge tube).
10	Remove the extension reaction mixture from its tube and load it carefully onto the center of the gel material. **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 2 of the EtOH/NaOAc procedure on page 2-37.
11	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes. **Note** If using a centrifuge with a fixed-angle rotor, place the column in the same orientation as it was in for the first spin. This is important because the surface of the gel will be at an angle in the column after the first spin.
12	Discard the column. The sample is in the sample collection tube.
13	Dry the sample in a vacuum centrifuge for 10–15 minutes, or until dry. Do not over-dry. (***check time w/R&D or tech support***)

Isopropanol Precipitation

Precipitation in 96-Well MicroAmp Trays

Reagents and equipment required:

- Variable speed table-top centrifuge with microtiter plate tray, capable of reaching at least 1400 × *g*
- Strip caps or adhesive-backed aluminum foil tape (3M Scotch Tape 425-3)[3]
- 75% Isopropanol (2-propanol) or 100% isopropanol (anhydrous) at room temperature

Note This procedure does not use salt.

To precipitate extension products in MicroAmp Trays:

Step	Action
1	Remove the MicroAmp Tray from the thermal cycler. Remove the caps from each tube.
2	Add one of the following: ♦ 80 µL of 75% isopropanol or ♦ 20 µL of deionized water and 60 µL of 100% isopropanol The final isopropanol concentration should be 60 ± 5%.
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 425-3 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the tray a few times to mix.
5	Leave the tray at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tray in a table-top centrifuge with tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: ♦ 1400–2000 × *g*: 45 minutes ♦ 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp Tray can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the tray onto a paper towel folded to the size of the tray.
8	Place the inverted tray with the towel into the table-top centrifuge and spin at 700 × *g* for 1 minute.

3. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
9	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

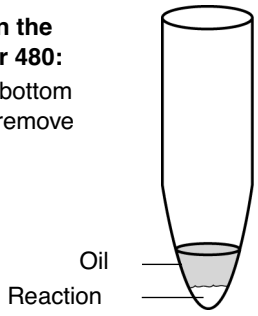
Precipitation in Microcentrifuge Tubes

Reagents and equipment required for this method:

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- 75% Isopropanol (2-propanol) or 100% isopropanol (anhydrous) at room temperature

Note This procedure does not use salt.

To precipitate extension products in microcentrifuge tubes:

Step	Action
1	Pipet the entire contents of each extension reaction into a 1.5-mL microcentrifuge tube. **To remove reactions run on the TC1 or DNA Thermal Cycler 480:** Place the pipette tip into the bottom of the reaction and carefully remove the reaction from the oil.  **IMPORTANT** Transfer as little oil as possible.
2	Add one of the following: ♦ 80 µL of 75% isopropanol or ♦ 20 µL of deionized water and 60 µL of 100% isopropanol The final isopropanol concentration should be 60 ± 5%.
3	Close the tubes and vortex briefly.
4	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
5	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.

To precipitate extension products in microcentrifuge tubes: *(continued)*

Step	Action
6	Carefully aspirate the supernatants with a separate pipette for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
7	Add 250 µL of 75% isopropanol to the tubes and vortex them briefly.
8	Place the tubes in the microcentrifuge in the same orientation as in step 5 and spin for 5 minutes at maximum speed.
9	Aspirate the supernatants carefully as in step 6.
10	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)

Ethanol Precipitation

With ethanol precipitation, traces of unincorporated terminators may be seen at the beginning of the sequence data (up to base 40), but this is usually minimal. Some loss in the recovery of the smallest fragments may also be observed.

Precipitation in 96-Well MicroAmp Trays

Reagents and equipment required:

- Variable speed table-top centrifuge with microtiter plate tray, capable of reaching at least 1400 × *g*
- Strip caps or adhesive-backed aluminum foil tape (3M Scotch Tape 425-3)[4]
- 95% Ethanol (ACS reagent grade), non-denatured

Note This procedure does not use salt.

To precipitate extension products in MicroAmp Trays:

Step	Action
1	Remove the MicroAmp Tray from the thermal cycler. Remove the caps from each tube.
2	Add the following: ♦ 16 µL of deionized water ♦ 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%.
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 425-3 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the tray a few times to mix.

4. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
5	Leave the tray at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tray in a table-top centrifuge with tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: ♦ 1400–2000 × *g*: 45 minutes ♦ 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp Tray can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the tray onto a paper towel folded to the size of the tray.
8	Place the inverted tray with the towel into the table-top centrifuge and spin at 700 × *g* for 1 minute.
9	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Precipitation in Microcentrifuge Tubes

Reagents and equipment required for this method:

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- 95% Ethanol (ACS reagent grade)

Note This procedure does not use salt.

To precipitate extension products in microcentrifuge tubes:

Step	Action
1	Pipet the entire contents of each extension reaction into a 1.5-mL microcentrifuge tube. **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 1 on page 2-34.
2	Add the following: ♦ 16 µL of deionized water ♦ 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%.
3	Close the tubes and vortex briefly.

To precipitate extension products in microcentrifuge tubes: *(continued)*

Step	Action
4	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
5	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
6	Carefully aspirate the supernatants with a separate pipette for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
7	Add 250 µL of 70% ethanol to the tubes and vortex them briefly.
8	Place the tubes in the microcentrifuge in the same orientation as in step 5 and spin for 10 minutes at maximum speed.
9	Aspirate the supernatants carefully as in step 6.
10	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)

Ethanol/Sodium Acetate Precipitation

Precipitation in 96-Well MicroAmp Trays

Reagents and equipment required:

- Variable speed table-top centrifuge with microtiter plate tray, capable of reaching at least 1400 × *g*
- Strip caps or adhesive-backed aluminum foil tape (3M Scotch Tape 425-3)[5]
- Sodium acetate (NaOAc), 3 M, pH 4.6 (P/N 400320)
- 95% Ethanol (ACS reagent grade)

To precipitate extension products in MicroAmp Trays:

Step	Action
1	Remove the MicroAmp Tray from the thermal cycler. Remove the caps from each tube.
2	Add the following: ♦ 2.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 ♦ 50 µL of 95% ethanol (EtOH) The final ethanol concentration should be 65%.

5. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

To precipitate extension products in MicroAmp Trays: *(continued)*

Step	Action
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 425-3 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the tray a few times to mix.
5	Leave the tray at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tray in a table-top centrifuge with tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: ♦ 1400–2000 × *g*: 45 minutes ♦ 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp Tray can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the tray onto a paper towel folded to the size of the tray.
8	Place the inverted tray with the towel into the table-top centrifuge and spin at 700 × *g* for 1 minute.
9	Add 150 µL of 70% ethanol to each pellet.
10	Cap or seal the tubes, then invert the tray a few times to mix.
11	Spin the tray for 10 minutes at maximum speed.
12	Repeat steps 7 and 8.
13	Remove the tray and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Precipitation in Microcentrifuge Tubes

- 1.5-mL microcentrifuge tubes
- Benchtop microcentrifuge, capable of reaching at least 14000 × *g*
- Vacuum centrifuge
- Sodium acetate (NaOAc), 3 M, pH 4.6 (P/N 400320)
- 95% Ethanol (ACS reagent grade)

Step	Action
1	For each sequencing reaction, prepare a 1.5-mL microcentrifuge tube containing the following: ♦ 2.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 ♦ 50 µL of 95% ethanol (EtOH) **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 1 on page 2-34.

Step	Action
2	Pipet the entire contents of each extension reaction into a tube of sodium acetate/ethanol mixture. Mix thoroughly.
3	Vortex the tubes and leave at room temperature for 15 minutes to precipitate the extension products. Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
4	Spin the tubes in a microcentrifuge for 20 minutes at maximum speed.
5	Carefully aspirate the supernatant with a pipette and discard. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
6	Rinse the pellet with 250 µL of 70% ethanol.
7	Vortex briefly.
8	Spin for 5 minutes in a microcentrifuge at maximum speed. Again, carefully aspirate or decant the supernatant and discard.
9	Dry the pellet in a vacuum centrifuge for 10–15 minutes, or until dry. Do not over-dry. (Alternatively, place the tubes with the lids open in a heat block or thermal cycler at 90 °C for 1 minute.)

Denaturing and Loading Samples

About TSR and Samples in TSR

The protocol uses a sample preparation reagent designated the Template Suppression Reagent (TSR).

Store TSR at 2–8 °C.

At room temperature, samples in TSR are stable for a maximum of 48 hours.

Although not recommended on a routine basis, you can keep samples prepared in TSR frozen for several weeks before running on the ABI PRISM 310 Genetic Analyzer with no detectable loss in resolution or base calling.

Preparing the TSR-Sample Mix

Prepare the TSR-sample mix as follows:

Step	Action
1	Add 25-µL of TSR to each sample pellet.
2	Vortex thoroughly and heat for 2 minutes at 95 °C.
3	If you are using the 96-well tray assembly, you can denature samples directly in the tray. See "Using Septa Strips" on page 1-21.
4	Chill samples on ice, vortex thoroughly, then spin briefly in a microcentrifuge.
5	Hold samples on ice or keep frozen until ready to load on the instrument.
6	Transfer the samples to 0.5- or 0.2-mL sample tubes and cover with a tube septum. **Note** You must use tube septa to prevent evaporation of samples, especially if samples are put in the autosampler more than 6 hours before analysis.

Minimum Sample Volume

The minimum sample volume per tube is 10-µL.

IMPORTANT If using 10-µL sample volumes, it is extremely important that the autosampler tray be calibrated (see "How to Calibrate the Autosampler" on page 4-27).

You can use a single sample for more than one analysis. Either inject out of one sample tube repeatedly, or split the sample into several tubes.

continued on next page

Preparing a Portion of a Reaction Mixture for Analysis

Occasionally, you may want to prepare only a portion of a sequencing reaction mixture for analysis on the 310 Genetic Analyzer and reserve the rest of the sample for analysis later or elsewhere.

To prepare a portion of a sequencing reaction mixture:

Step	Action
1	Add 6-μL of TSR to the dried DNA sequencing reaction.
2	Vortex to dissolve the extension products.
3	Heat the sample for 1 minute at 95 °C to ensure denaturation.
4	Transfer 2-μL of the sample to 10-μL of TSR in a sample tube.
5	Cover the tube with a septum and vortex well.
6	Heat the TSR mixture for 2 minutes at 95 °C and place it on ice until ready to place in the instrument.

Loading the Samples

Move the samples into the autosampler as follows:

Step	Action
1	If necessary, transfer the denatured samples to a 48- or 96-well tray. **IMPORTANT** The tube arrangement and order of the samples in the tray and on the Sample Sheet must be the same. Make note of the tube arrangement you use, so that you can prepare the Sample Sheet correctly.
2	Seal each tube with a septum, and place the tray into the autosampler.

About Run Modules, Dye Set/Primer, and Matrix Files

Overview

DNA sequencing on the 310 Genetic Analyzer requires you to have these types of files:

- Module
- Basecaller
- Dye set/Primer (also known as mobility)
- Matrix

You select the particular files appropriate to your experiment on the Sample Sheet and Injection List.

The module, Basecaller, and Dye Set/Primer files are supplied with the Data Collection Software. Updated files can be obtained from the Applied Biosystems site on the World Wide Web (www.appliedbiosystems.com/techsupport), from Applied Biosystems Technical Support, or from your local field applications specialist (call your local sales office for more information). For new applications, a disk containing the files is often included with the reagent kit.

Matrix files are all created using ABI PRISM® DNA Sequencing Analysis Software. See the ***ABI PRISM DNA Sequencing Analysis Software User's Manual***.

Run Modules

Run modules are software files. The 310 Genetic Analyzer executes the steps in a run module to process a sample. Run time, temperature and voltage, prerun time, injection time and voltage are some of the run parameters controlled by the run module.

Run modules are selected through the Injection List. The reference table on page 2-43 will help you select the appropriate run module.

If a custom module is necessary, see "Editing Modules Using Manual Control" on page 5-17.

Dye Set/Primer Files

Also known as mobility files, these contain information that adjusts the sample data collected during electrophoresis for:

- The dye set
- Mobility of the primers or terminators

Dye Set/Primer files are selected through the Sample Sheet. The reference table below will help you select the appropriate run module.

Dye Set/Primer files cannot be edited.

continued on next page

Reference Table for Run Modules and Dye Set/Primer Files

The following table lists the Run modules and the Dye/Set Primer files.

Chemistry	Syringe	Polymer	Run Module	Mobility File	Base Caller	Capillary Size	Capillary Mark
Terminator	250-µL	DSP	Seq Run (250 uL) A	DP5%CEHV{A Set– any primer}	CE-1	47 cm x 75 µ	silver
Terminator, long-read sequencing	1.0-mL	POP-6	Seq POP6 (1.0\-mL) A	DT POP 6	CE-2	61 cm x 50 µ	pink
Terminator, RAPID sequencing	1.0-mL	POP-6	Seq POP6 (1.0 -mL) RAPID A	DT POP 6	CE-2	47 cm x 50 µ	green
Primer	250-µL	DSP	Seq Run (250 uL) A	DP5%CEHV{-21M13 } or DP5%CEHV{M13rev }	CE-1	47cm x 75 µ	silver
Dichlororhodamine (dRhodamine) Terminator	250-µL	DSP	Seq Run (250 uL) E	DP5%CEHV{dR Set– any primer}	CE-1	47cm x 75 µ	silver
dRhodamine Terminator, long-read sequencing	1.0-mL	POP-6	Seq POP6 (1.0-mL) E	DT POP6 {dR Set– any primer}	CE-1	61 cm x 50 µ	pink
dRhodamine Terminator, RAPID sequencing	1.0-mL	POP-6	Seq POP6 (1.0-mL) RAPID[a] E	DT POP6 {dR Set– any primer}	CE-1	47 cm x 50 µ	green
BigDye™ Primer[b], long-read sequencing	1.0-mL	POP-6	Seq POP6 (1.0 -mL) E	DP POP6 {BD Set-21M13} or DP POP6 {BD Set-M13rev}	CE-1	61 cm x 50 µ	pink
BigDye™ Primer, RAPID sequencing	1.0-mL	POP-6	Seq POP6 (1.0–mL) RAPID E	DP POP6 {BD Set-21M13} or DP POP6 {BD Set-M13rev}	CE-1	47 cm x 50 µ	green
BigDye™ Terminator[c], long-read sequencing	1.0-mL	POP-6	Seq POP6 (1.0–mL) E	DT POP6 {BD Set-any primer}	CE-1	61 cm x 50 µ	pink
BigDye™ Terminator, RAPID sequencing	1.0-mL	POP-6	Seq POP6 (1.0–mL) RAPID E	DT POP6 {BD Set-any primer}	CE-1	47 cm x 50 µ	green

a. Rapid sequencing

b. There are no BigDye™ Primer mobility files for the DNA Sequencing Polymer on the ABI PRISM® 310 instrument.

c. There are no BigDye™ Terminator mobility files for the DNA Sequencing Polymer on the ABI PRISM® 310 instrument.

continued on next page

Matrix Files

Matrix files contain information that corrects for “spectral overlap”. Spectral overlap occurs when part of one dye’s emission spectrum falls onto a portion of the detection hardware that is collecting the fluorescent peak of another dye. Dye sets are created to minimize spectral overlap, but it occurs to some extent.

When choosing matrix files in the Sample Sheet, match the matrix file to the samples by dye set and sequencing chemistry. For example, BigDye™ terminator samples run with a module file for Virtual Filter E should be analyzed with a matrix file that was also run with Virtual Filter E.

The dye set used to create the matrix file must be the same as the dye set used to run the sample. If you analyze data with a matrix file that does not reflect the conditions of your run, the run will appear to fail. Re-analyze with the correct matrix file if you have made this error.

Preparing the Matrix Standards

Preparing Matrix Standard Samples

You must create a matrix file the first time you use any sequencing method (*i.e.*, dye terminators, BigDye™ terminators, dye primers, etc.).

Preparing matrix standards is optional. The matrix file also can be made directly from a sample or a Sample Standard.

For each of the four matrix standards:

Step	Action
1	Mix in a sample vial: a. 12-µL of TSR b. 1-µL of matrix standard
2	Label each vial with the base and dye.
3	Gently vortex the mixture for 3–5 seconds.
4	Store the mix at 2–6 °C until ready to use.

About dRhodamine Matrix Standards

The dRhodamine matrix standards are used for BigDye™ primer and BigDye™ terminator matrix files as well as dRhodamine matrix files. The dRhodamine matrix standards are:

Tube Label	Color of Raw Data	Base
dR110 Matrix Standard	blue	G
dR6G Matrix Standard	green	A
dTAMRA Matrix Standard	black	C
dROX Matrix Standard	red	T

Matrix standards are stable for six months at 2–6 °C. Avoid freeze-thaw cycles.

The dRhodamine matrix standards are provided in a ready-to-use format and are premixed with a blue dye for use on slab gels. The blue dye is not necessary nor is it detrimental to use with the 310 Genetic Analyzer.

The matrix is consistent with current base-calling conventions, *i.e.*, C is blue, A is green, G is black, and T is red in analyzed files. See “Color Display of Data” on page 1-16 for more information about color display in raw and analyzed data.

Denaturing the Matrix Standard Samples

You can denature the matrix standard samples in the thermal cycler with your samples or follow this procedure:

Step	Action
1	Heat the sample for 2 minutes at 95 °C.
2	Chill the sample on ice.
3	Hold on ice until ready to load in the autosampler.

Preparing the Sample Standard

About Sample Standards

A Sample Standard verifies operation of the instrument's hardware, just as the control template verifies the correct preparation of the sample template. Running a Sample Standard is optional.

Applied Biosystems sells sequencing standards for dye terminator and dye primer sequencing. Contact your sales representative for more information.

Preparing a TSR-Sample Standard Mix

To prepare a TSR-Sample Standard mix:

Step	Action
1	Add 25-µL of TSR to a tube containing a dried DNA sequencing Sample Standard.
2	Mix thoroughly on a vortex mixer and heat for 2 min at 95 °C.
3	Chill on ice, vortex thoroughly, then spin briefly in a microfuge.
4	Hold on ice or keep frozen until ready to load on the instrument.
5	Just before loading in the autosampler, transfer the samples to small sample tubes. Cover with a tube septum. **IMPORTANT** You must use tube septa to prevent evaporation of samples—especially samples that sit in the autosampler for more than 6 hours before being analyzed.

Preparing the Sample Sheet and Injection List

Purpose

The Sample Sheet associates sample information (name and type of analysis) with a sample tube position in the autosampler.

The Injection List specifies the order for running samples, how many injections are made from each sample, and the module and running conditions for each injection.

Preparing the Sample Sheet

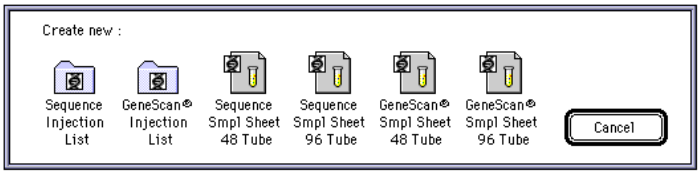
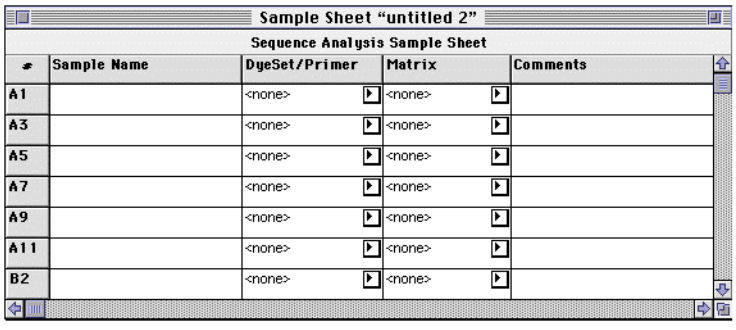
The first time you use any group of samples, you must create a Sample Sheet.

Note This chapter takes BigDye™ terminator sequencing as a detailed example, and the illustrations in the procedure below are from a BigDye™ terminator sequencing Sample Sheet. If you use rhodamine or dichlororhodamine dye terminator sequencing, or rhodamine or BigDye™ primer sequencing, choose mobility files and matrix files appropriately. See the Quick Reference Card (P/N 904579 rev A) at the front of this manual or “About Run Modules, Dye Set/Primer, and Matrix Files” on page 2-42, for help when filling out the Sample Sheet and Injection List.

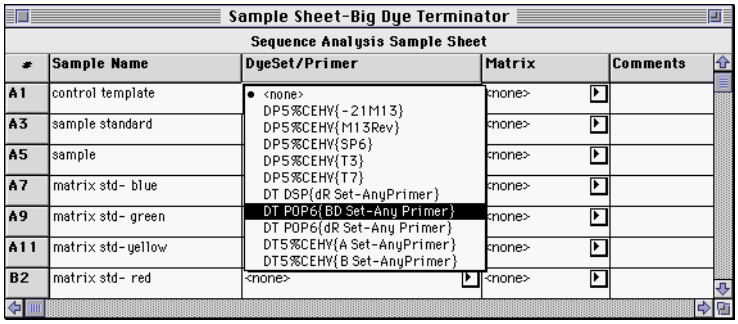
The Sample Sheet will be saved in the Sample Sheet folder on the Macintosh® computer’s hard drive, and if you need to sequence the same group of samples again, you can simply select the previously created Sample Sheet. If there is already a Sample Sheet prepared, go to“Using a Previously Created Sample Sheet” on page 2-48.

Creating a New Sample Sheet

To create a new Sample Sheet:

Step	Action
1	In the ABI PRISM® 310 Data Collection Software, select New from the File menu.
2	Click Sequence Smpl Sheet 48 or 96 Tube (match the type of tray used in the autosampler). 
3	The Sample Sheet window appears.  **Figure 2-1** Sample Sheet window for a 48-well sample tray

To create a new Sample Sheet: *(continued)*

Step	Action
4	The number in the first column corresponds to the location of the sample in the autosampler tray. Put Sample Names in the second column.
5	Select Dye Set/Primer and Matrix files through the pop-up menus. See “About Run Modules, Dye Set/Primer, and Matrix Files” on page 2-42 if you need help selecting the correct file. **Note** The first time you run sequencing reactions under new conditions, any matrix file can be used for the run. After the run, you must make a matrix file for the new conditions.  **Figure 2-2** Dye Set/Primer pop-up menu
6	Enter any additional comments that you want to link to the samples.
7	Open the File pull-down menu, and select Save As. Name the Sample Sheet, and press Return to save it in the Sample Sheets folder.

Using a Previously Created Sample Sheet

If you are running the same group of samples for a second or third time, you can use the original Sample Sheet. Simply select the previously created Sample Sheet when filling out the Injection List.

If you want to modify an existing Sample Sheet:

Step	Action
1	Open the Sample Sheet. It is stored in the Sample Sheet folder on the Macintosh hard drive.
2	Make changes and save the Sample Sheet. Give it a new name if you want to keep the original. Choosing Save without renaming the Sample Sheet will overwrite the original.
3	Select the modified Sample Sheet when filling out the Injection List.

continued on next page

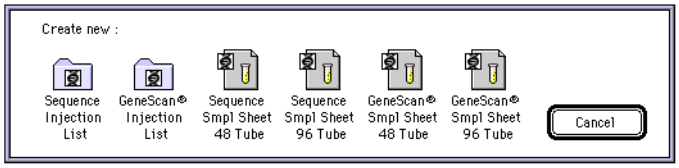
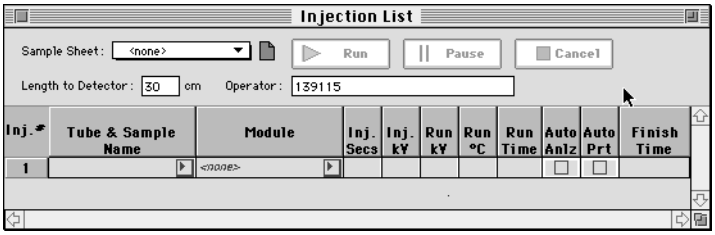
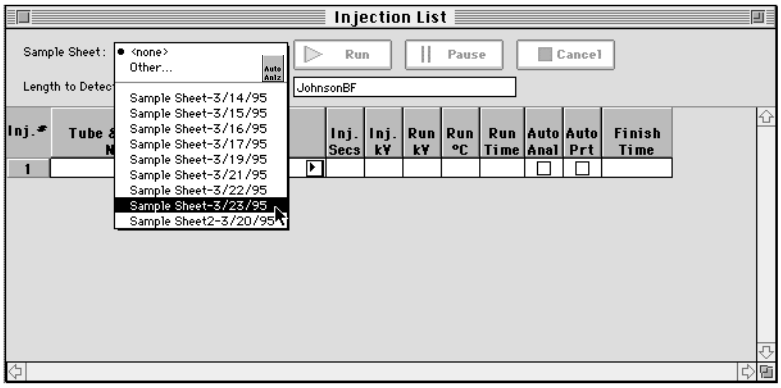
Preparing an Injection List

Fill out an Injection List to start a run.

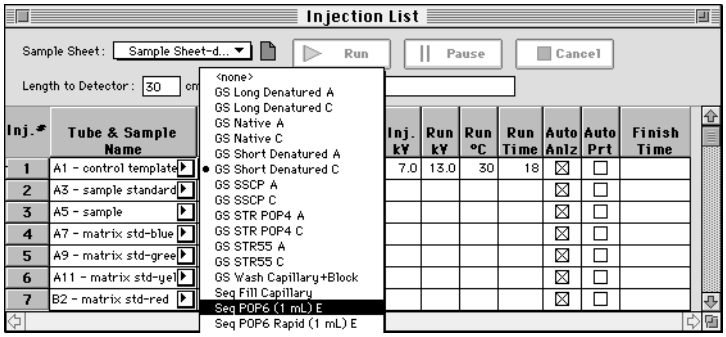
Note This chapter takes BigDye™ terminator sequencing as a detailed example, and the illustrations in the procedure below are from a BigDye™ terminator sequencing Injection List. If you use rhodamine or dichlororhodamine dye terminator sequencing, or rhodamine or BigDye™ primer sequencing, choose run modules appropriately. See the Quick Reference Card at the front of this manual or “About Run Modules, Dye Set/Primer, and Matrix Files” on page 2-42, for help when filling out the Sample Sheet and Injection List.

Creating an Injection List

To create an Injection List:

Step	Action
1	In the ABI PRISM® 310 Data Collection Software, select New from the File menu. The following box of icons is displayed. 
2	Select Sequence Injection List. 
3	Click the arrow in the Sample Sheet field to display a pop-up menu of Sample Sheets stored in the Sample Sheet folder and select a Sample Sheet. 
4	The Injection List is automatically filled in with information from the selected Sample Sheet.

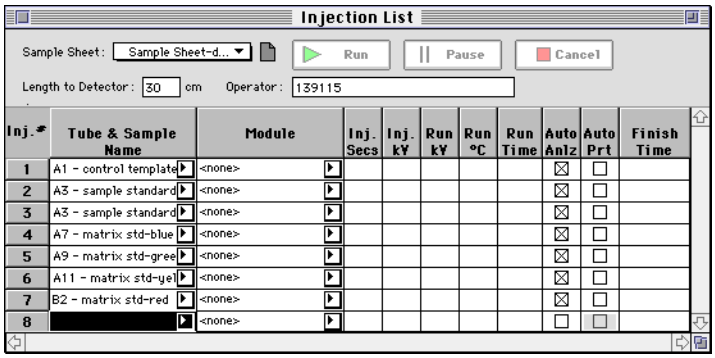
To create an Injection List: *(continued)*

Step	Action
5	Select a module from the Module pop-up menu. See “About Run Modules, Dye Set/Primer, and Matrix Files” on page 2-42 if you need help selecting the correct file. 
6	You can turn on automatic analysis and automatic printing by checking the boxes.

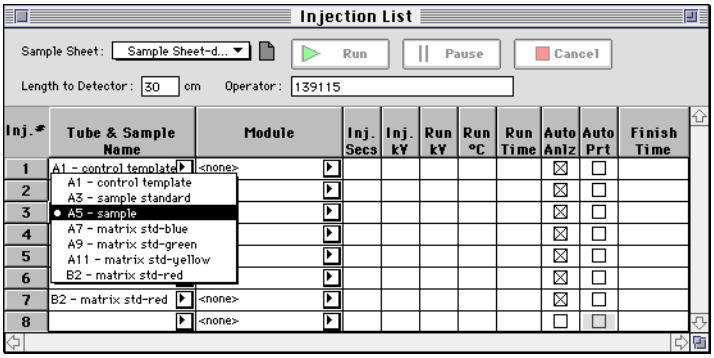
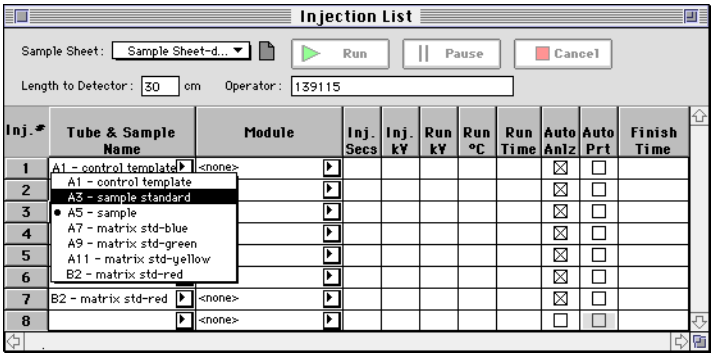
Making Multiple Injections from One Sample Tube

The default setting for the Injection List assumes that each sample will be injected once. If you prefer multiple injections, modify the Sample Name column so that the sample appears multiple times.

To make multiple injections from 1 sample tube:

Step	Action
1	In the example used here, the original Sample Sheet has been modified to inject the sample standard twice. 
2	For the injection you are modifying, click the arrow next to the Tube & Sample Name.

To make multiple injections from 1 sample tube: *(continued)*

Step	Action
3	When a pop-up menu of the original sample list appears, select the sample you want to inject. Figure 2-3 and Figure 2-4 show the sequence for editing the injection. Instead of injecting a sample in position A5 of the autosampler as the third injection, sample standard in position A3 will be the third injection.  **Figure 2-3** Select Sample  **Figure 2-4** Change Sample for Third Injection

Modifying an Injection List

Adding Rows

The Injection List has the same number of rows as the Sample Sheet. To add rows, highlight a row, press Command-I, and a new row will be inserted above the highlighted row.

Changing Run Parameters

As you select a module file for each injection, the run parameters are filled in according to the values in the module. You can edit any parameter by clicking in the field and typing in the new value. Click another field or press Enter to save the change.

continued on next page

Changing the Order of the Samples in a Run

The default setting for the Injection List assumes that each sample will be injected in the order listed in the Sample Sheet. If you prefer a different injection order, modify the Sample Name column.

Testing the Capillary Window

Run a test to ensure that the capillary window is clean and positioned correctly before running your samples:

Step	Action
1	Insert a line into the Injection List before the first sample. See “Modifying an Injection List” on page 2-51 for instructions, if necessary.
2	Set the module to Test CCD 4-Color.
3	Change the collection time from 5 minutes to 1 minute.
4	When the module runs, check the raw data for excessive noise. The ideal baseline falls at or below about 2068. Use the following table to help determine whether the capillary is positioned correctly.

If...	Then...
the baseline is too high	remove the capillary and clean the capillary window with a lab wipe and dampened ethanol.
there is a single blue line at 8000 or at the bottom of the window	the capillary window is not positioned in the detector.

Starting the Run

Click the Run button in the Injection List to start the run.

Note If you did not preheat the instrument as suggested under “Preheating the Heat Plate” on page 2-17, it can take up to 25 minutes for the instrument to heat to 50 °C.

Monitoring the Run

During the run, you can monitor the run and your samples four ways. From the Window menu, select one of the following:

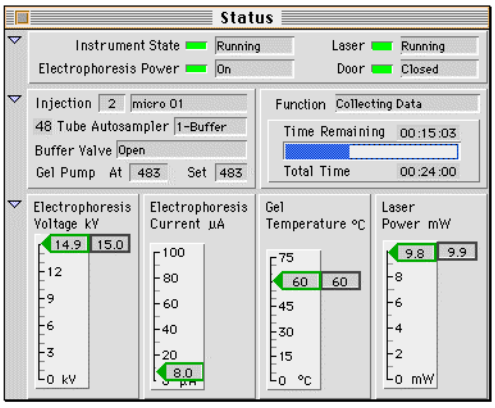


Figure 2-5 Status

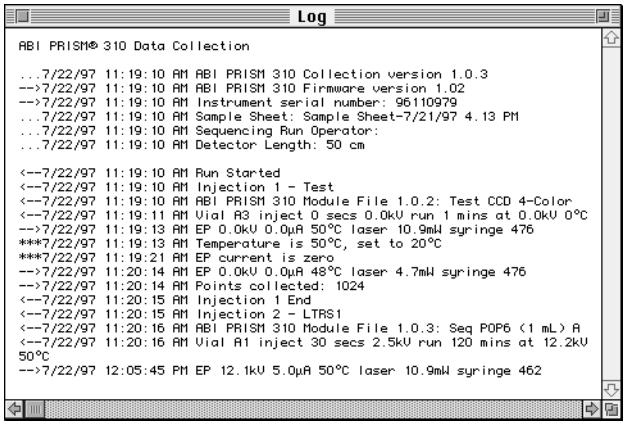


Figure 2-6 Log

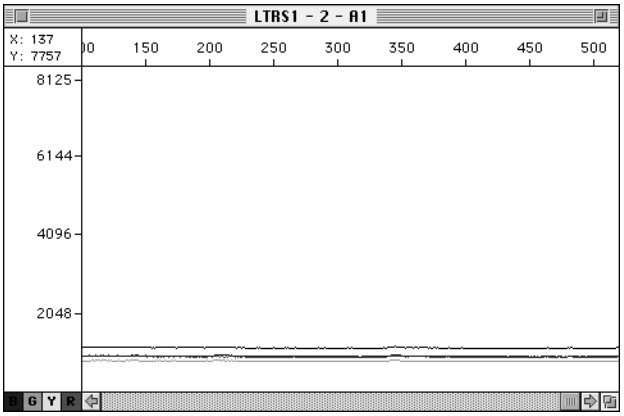


Figure 2-7 Raw Data

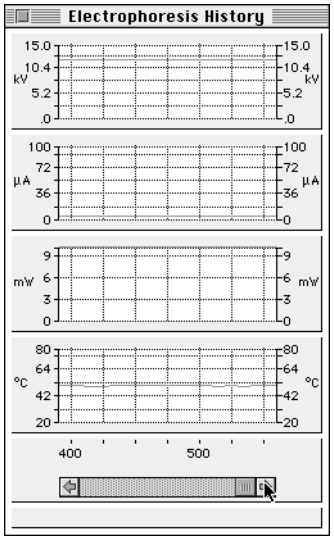


Figure 2-8 Electrophoresis History

Creating the DNA Sequencing Matrix File from the Matrix Standards

Overview

The matrix file contains the information necessary for software to correct the overlap of the dyes emission spectra on the virtual filter. Once a matrix file has been created, it can be used for subsequent runs performed:

- With the same kit
- On the same instrument
- Using the same:
  - Run modules
  - Set of dyes
  - Polymer

After running the matrix standards, use their sample files to generate a matrix file using ABI PRISM® DNA Sequencing Analysis Software.

Verifying the Raw Data for the Matrix Standards Run

Raw data of the matrix standards run can be viewed using the Data Utility program that accompanies the ABI PRISM® DNA Sequencing Analysis Software.

Step	Action
1	Open the Data Utility program.
2	Under File, choose Open.
3	Search for the Run Folder containing the four sample files.
4	Click on the first sample. The corresponding windows open are Raw data, Analyzed, EPT, and Information. **Note** The Analyzed window in Data Utilities and ABI PRISM® DNA Sequencing Analysis Software are not identical.
5	Check the current for the run. Typically, the range is 4–8 μA.
6	Check the location of the peaks. Peaks typically first appear about 1000 scans into the run. Verify that the baseline is stable.
7	Check the peak height. Peak height should be greater than 200.

Generating a Matrix File from Matrix Standards

About Matrix Files

The matrix is made using ABI PRISM® DNA Sequencing Analysis Software. For more information on the steps provided here, see the ***ABI PRISM DNA Sequencing Analysis Software User's Manual***.

When you create matrix files, you will be asked for the name of the file containing data for C, data for G, etc.

Put the correct data file for each matrix standard into the correct "box" in the Data Utility application. The example used here is for BigDye™ terminators, which use Virtual Filter E. The dyes for Virtual Filter E are entered in the Dye Primer Matrix boxes. If you make matrices for other filter sets, you will need to enter the appropriate dyes.

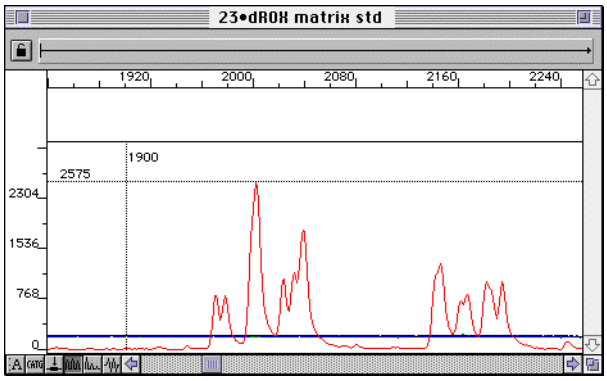
Filter Dyes

Box	Dye Primer Matrix	Taq Terminator Matrix	T7 Terminator Matrix
C…	dR110	dROX	dR6G
A…	dR6G	dR6G	dTAMRA
G…	dTAMRA	dR110	dROX
T…	dROX	dTAMRA	dR110

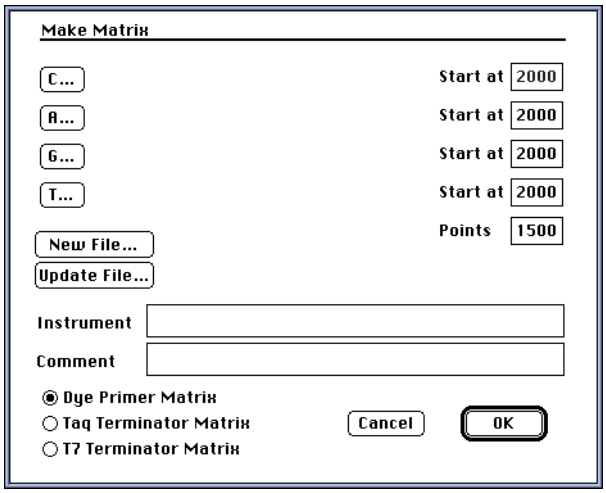
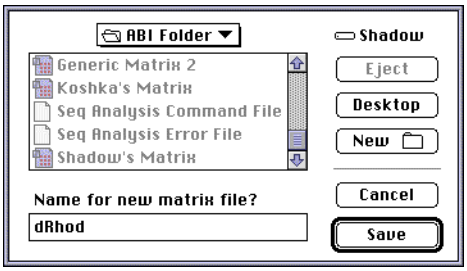
IMPORTANT You need to make all three matrices, even if you are only using one chemistry. T7 algorithms are used for analysis of Taq reactions, so T7 cannot be left blank.

Making a Dye Primer Matrix

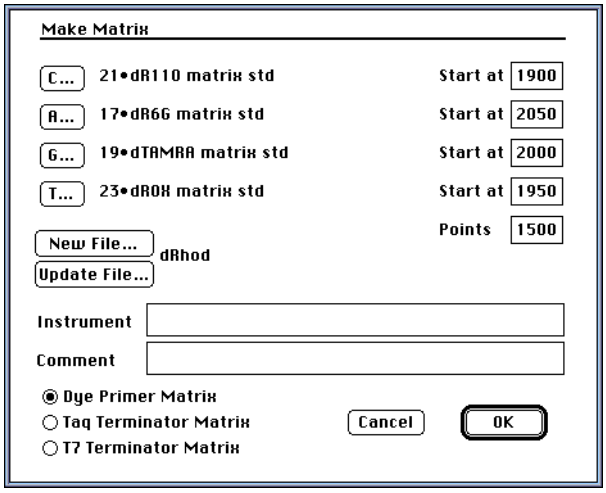
To make a dye primer matrix:

Step	Action
1	Set the analysis start point and the number of data points to analyze. a. In the SABI PRISM® DNA Sequencing Analysis Software, examine the raw data for one of the matrix standard samples as shown below. b. Select a starting point where there are no peaks and the baseline is flat. c. Select a number of data points to analyze such that no peaks in the range are off-scale, *i.e.*, above 4000 relative fluorescence units (RFU) and where the baseline at the end of the range is flat. A typical number of data points is 1500. 
2	Repeat step 1 for each matrix standard sample. Record the results for later use. **IMPORTANT** The number of data points analyzed is the same for each matrix standard. Choose starting points for each sample such that all peaks are less than 4000 RFU and where both the starting and ending points have flat baselines and no peaks.
3	Launch the Data Utility software.

To make a dye primer matrix: *(continued)*

Step	Action
4	From the Utilities menu, choose Make Matrix… The Make Matrix dialog box appears as shown below. Verify that the Dye Primer Matrix button at the lower left is selected. 
5	Click on the box for each nucleotide base and enter the data file that corresponds to the matrix standard.
6	Enter the analysis start point for each matrix standard sample as determined in step 1 on page 2-56.
7	Click New File… A dialog window appears as shown below. Name the file dRhod and save it in the ABI folder within the System folder. 

To make a dye primer matrix: *(continued)*

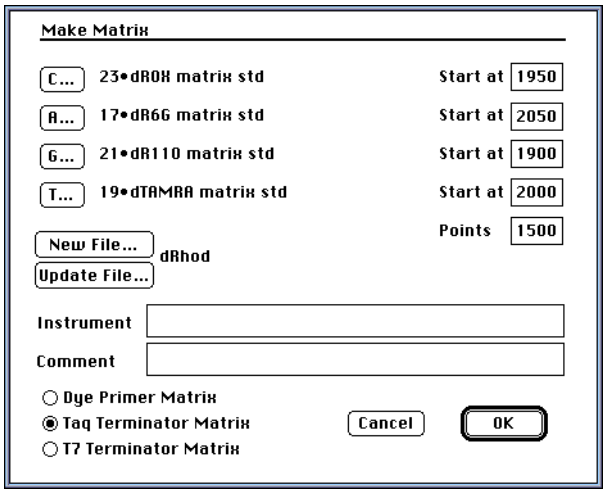
Step	Action
8	The Make Matrix dialog box should look like that shown below. **Note** The numbers in the Start at… and Points… boxes below are typical values. Your numbers may vary. 
9	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” b. Click OK.
10	If the computer is unable to make a matrix, examine the raw data again in the Sequencing Analysis software. If many peaks are off-scale, dilute the matrix standards and rerun them.

Making the Taq Terminator Matrix

To make the Taq Terminator matrix:

Step	Action
1	In the Data Utility application, choose Make Matrix… from the Utilities menu. The Make Matrix dialog box appears.
2	In the Make Matrix dialog box, click the Taq Terminator Matrix button at the lower left.
3	Click on the box for each nucleotide base and enter the data file that corresponds to the matrix standard.
4	Enter the same numbers for each matrix standard sample in the Start at… and Points… boxes as for the Dye Primer Matrix.
5	Click Update File… A dialog window appears.

To make the Taq Terminator matrix: *(continued)*

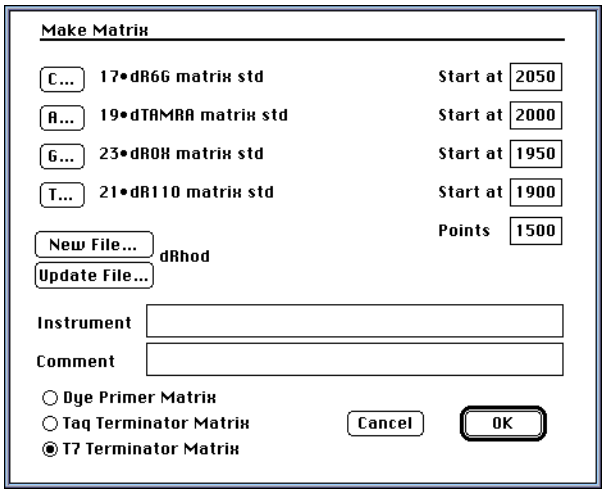
Step	Action
6	Choose dRhod from the ABI folder within the System folder and click Save. The Make Matrix dialog box should look like that shown below. 
7	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” b. Click OK.

Making the T7 Terminator Matrix

To make the T7 Terminator matrix:

Step	Action
1	In the Data Utility application, choose Make Matrix… from the Utilities menu. The Make Matrix dialog box appears.
2	In the Make Matrix dialog box, click the T7 Terminator Matrix button at the lower left.
3	Click on the box for each nucleotide base and enter the data file that corresponds to the matrix standard.
4	Enter the same numbers for each matrix standard sample in the Start at… and Points… boxes as for the Dye Primer Matrix and Taq Terminator Matrix.
5	Click Update File… A dialog window appears.

To make the T7 Terminator matrix: *(continued)*

Step	Action
6	Choose dRhod from the ABI folder within the System folder and click Save. The Make Matrix dialog box should look like that shown below. 
7	a. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message "Make matrix successfully completed." b. Click OK.

Checking Matrix Quality

What to Review in the Matrix

Check the quality of the matrix by reviewing the:

- Raw data
- Values in the Copy Matrix window
- Analyzed data of the matrix run

To check the raw data, examine the electropherogram of the raw data.

The matrix standards should display the following colors:,

Matrix Standard	Color in Raw Data
dR110	blue
dR6G	green
dTAMRA	black
dROX	red

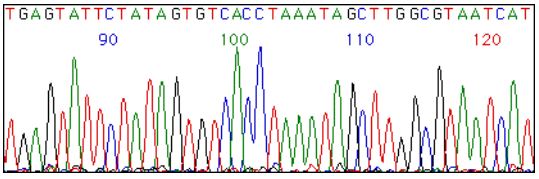
Checking the Copy Matrix Window

To check the Copy Matrix window:

Step	Action
1	From the Utilities menu in ABI PRISM® DNA Sequencing Analysis Software, choose Copy Matrix…
2	Under Source, select Instrument file and choose the matrix file from the ABI folder within the System folder.
3	Make sure that all three boxes have numbers in them. The numbers on the diagonal (Blue against Blue, Green against Green, etc.) must all be 1.00. The numbers off the diagonal are less than 1.00. An exception is Virtual Filter C, where Green under Blue is sometimes more than 1.00. This is acceptable. If the file is not correct, then repeat the matrix-making procedure starting with “Making a Dye Primer Matrix” on page 2-56. **Note** The corresponding numbers in the boxes for all three matrices will be the same.
4	Click Cancel.
5	Restart the Sequencing Analysis software and use the matrix file, also known as the Instrument file, to analyze your sequencing data.

Checking Matrix Quality

To check matrix quality:

Step	Action	
1	Open the Sample Manager window in the ABI PRISM® DNA Sequencing Analysis Software.	
2	Click the Add Files button.	
3	Select the sample files for each standard in the directory dialog box and choose Done.	
4	The P and F checkboxes should not be checked. Bring the Instrument File column of the Sample Manager window into view by scrolling with the horizontal scrollbar.	
5	Click Start.	
6	To check the electropherograms, double-click on each file in the Electropherogram view.	
7	**If…**	**Then…**
	each peak is one color with the other colors flat under it: 	the matrix is good.
	The other colors are not flat under the peaks	the matrix is poor.

To check matrix quality:

Step	Action	
8	**If the…**	**Then…**
	matrix is good	Save the matrix file to the ABI folder.
	matrix is poor	Re-analyze the matrix run. If this does not improve the matrix data, run new matrix standards.

Creating a DNA Sequencing Matrix from a Sample

Overview

It is not necessary to use matrix standards to generate a sequencing matrix. Any sample that yields good raw data can be used to create a matrix file.

The Sample Standard is used to make a matrix in the procedures below. The method can be used with other types of samples.

Making Copies of the Sample File for the Sample Standard

Make three copies of the sample file for the Sample Standard run:

Step	Action
1	Click on the sample folder for the Sample Standard run.
2	Choose Duplicate from the File menu. A duplicate file is created and named “File name copy”.
3	Repeat the above steps twice more to create “File name copy-2” “File name copy-3”.

Verifying Raw Data for the Sample Standard Matrix Run

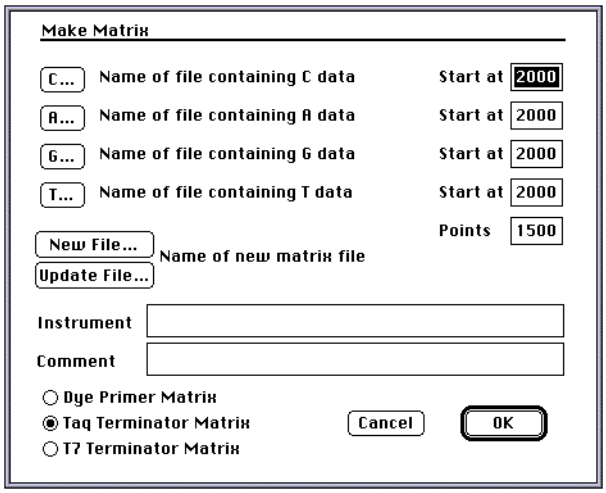
Raw data of the Sample Standard run can be viewed using the Data Utility program that accompanies the ABI PRISM® DNA Sequencing Analysis Software.

Step	Action
1	Open the Data Utilities program.
2	Under File, choose Open.
3	Search for the Run Folder containing the four sample files (one original and 3 duplicates).
4	Click on the first sample. The corresponding windows open are Raw data, Analyzed, EPT, and Information. **Note** The Analyzed window in Data Utilities and ABI PRISM® DNA Sequencing Analysis Software are not identical.
5	Verify that the baseline is stable, and peak heights are on scale in the raw data.
6	Choose a scan point (e.g., 2000) that occurs after the first peaks, though still in the beginning of the run.

continued on next page

Generating the Matrix File Using a Sample

Create the matrix file by following this procedure:

Step	Action
1	Under Data Utilities, select Make Matrix.
2	A dialog box appears.  a. Click the C button, then choose the “File Name” sample file (the original sample file for the Sample Standard run) in the Run Folder. b. Click open. c. Do the same for the A (File Name copy”), G (File Name copy-2”), and T (File Name copy-3”) buttons.
3	Enter the starting point for each file. The Start At point should be after the first peaks, though still in the beginning of the run. Modify the default setting of 2000 if necessary. Modify the Points value if necessary. This is the number of points after the start point to be analyzed. Good data points include a single color with three clean peaks that do not have overlapping dyes. Set the Points value so that the end point falls after these three peaks.
4	Click the New File button and name the new matrix file. Choose a name that reflects the chemistry and run conditions.
5	Locate and open the ABI Folder in the System Folder and save the matrix file.
6	Enter the last three numbers of the instrument serial number. Comment: seq matrix created.
7	Click the button for the appropriate type of sequencing chemistry and click OK. The message “Make matrix successfully completed” appears.

Note If you get an error message, and the software will not make a matrix, you may have designated the wrong files, or used the terminator standards for a primer matrix, etc. It is possible also that the signal is too weak to make a matrix, but this happens rarely. If you don't get adequate signal to make a matrix, you will have to rerun the standard.

continued on next page

Checking Matrix Quality

Check the quality of the matrix by:

- Reviewing the values in the Matrix Values window
- Reviewing the analyzed data of the matrix run

Review the matrix values in the Matrix Values window as follows:

Step	Action
1	Under Utilities, open the Copy Matrix dialog box.
2	Choose Instrument File.
3	From the ABI Folder, choose the matrix file that was just created.
4	Click Open to view the matrix file values. The numbers on the diagonal (Blue against Blue, Green against Green, etc.) must all be 1.00. The numbers off the diagonal are less than 1.00.
5	Click Cancel to close the matrix file values without altering them.

Review the analyzed matrix data as follows:

Step	Action	
1	Open the matrix standard sample file in the Data Utility program.	
2	Examine the electropherogram in the Analyzed window.	
3	**If…**	**Then the matrix is…**
	each peak is one color with the other colors flat under it	good.
	the other colors are not flat under the peaks	poor.
4	**If the…**	**Then…**
	matrix is good	save the matrix file to the ABI folder.
	matrix is poor	re-analyze the matrix run. If this does not improve the matrix data, run new matrix standards.

Analyzing The Data

Overview

ABI PRISM® DNA Sequencing Analysis Software automatically analyzes the samples when the run finishes if the automatic analyze checkbox on the Sample Sheet is checked.

See the ***ABI PRISM DNA Sequencing Analysis software User's Manual*** for more information.

Preparing the Syringes for a DNA Sequencing Run with DNA Sequencing Polymer (DSP)

Overview

To set up a run with DNA Sequencing Polymer (DSP), rather than POP-6 polymer, you will need to install both the 5-mL plastic syringe containing the DNA Sequencing Polymer and a 250-µL glass syringe. Then the DSP must be transferred from the plastic syringe to the glass syringe before beginning the run.

If your experiment requires DNA Sequencing Polymer, follow the procedures below to set up the syringes.

Cleaning and Preparing the Syringe for Use

DNA Sequencing Polymer requires a 250-µL syringe. Prepare it as follows:

Step	Action
1	Rinse the glass syringe with distilled water. Remove excess water but do not dry completely. **IMPORTANT** Excessive wear occurs to the Teflon fitting of the plunger if it is completely dry.
2	Inspect the syringe for the ferrule and two o-rings.
3	Install the syringe.

Methods of Filling the Glass Syringe

The DNA Sequencing Polymer is packaged in a 5-mL plastic syringe. The polymer must be transferred to the 250-µL glass syringe.

There are two methods for transferring DNA Sequencing Polymer from the plastic syringe to the glass syringe:

- Using the ABI PRISM® 310 Data Collection Software, which will prompt you to perform some manual steps
- Manually

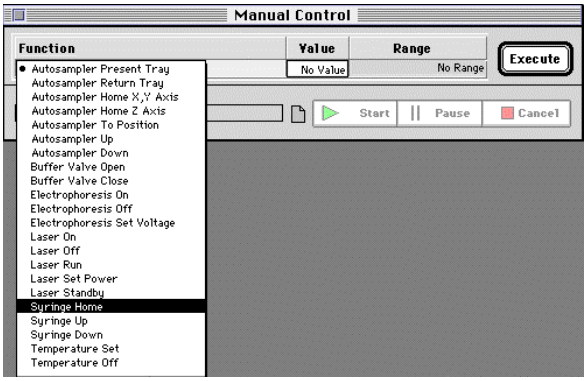
Filling the Glass Syringe Manually

Installing the Plastic Syringe

Step	Action
1	Remove DNA Sequencing Polymer from the refrigerator and equilibrate to room temperature. This takes about 1 hour.
2	Remove the cap from the room temperature DNA Sequencing Polymer.
3	Put the plastic syringe through the syringe guide. It goes in the plastic luer fitting in the hole to your left as you face the 310 Genetic Analyzer.
4	Partially screw it onto the luer fitting. Do not tighten it yet.

Homing the Glass Syringe

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software.
2	Open the Manual Control window.

Step	Action
3	Select Syringe Home from the Function pop-up menu, and click Execute. 
4	Install the glass syringe.

Priming the Pump Block

Always clean the pump block before filling it with fresh polymer.

Step	Action
1	Select Buffer Valve Open from the Function pop-up menu in Manual Control, then click Execute.
2	Turn the plastic syringe to tighten its connection to the luer fitting, and open the valve below the luer fitting 3/4 turn.
3	Push the plunger of the plastic syringe to fill the channel to the buffer reservoir with polymer solution. Stop when the channel is free of air bubbles.
4	Select Buffer Valve Close from the Function pop-up menu, then click Execute.
5	Open the valve to the waste vial.
6	Push the plunger of the plastic syringe until the channel to waste is filled.
7	Close the valve to the waste vial.

Priming the Glass Syringe

If the glass syringe is new, it may be difficult to prime manually. The plunger of the glass syringe must be lubricated with water to minimize wear, and this will help the plunger move when priming the syringe.

IMPORTANT Do not try to prime the glass syringe by pulling its plunger to draw sequencing polymer through the pump block. This creates air bubbles.

Step	Action
1	Push the plunger of the plastic syringe until 50-µL of sequencing polymer enters the glass syringe. If the glass syringe plunger resists moving, gently lift it while pressing the plastic syringe plunger.
2	Open the valve to the waste vial, then push the plunger of the glass syringe. **Note** You may need to exert a small amount of pressure on the plastic syringe to prevent polymer from moving up the channel toward the plastic syringe.
3	Repeat steps 1 and 2 until the glass syringe is free of air bubbles.

Filling the Glass Syringe

Fill the glass syringe with only enough polymer to perform the planned sequencing analyses. Two hundred microliters is adequate for 40–50 runs.

Step	Action
1	Push the plunger of the plastic syringe to fill the glass syringe.
2	Close the valve under the luer fitting to the plastic syringe.

Setting Up the Syringe Drive

Step	Action
1	Move the syringe drive toggle to the right.
2	Select Syringe Down from the Function menu.
3	Enter the value 230 steps for a syringe filled with 200-µL of polymer (use a larger value for smaller volumes). Click Execute to move the syringe drive toggle close to the end of the plunger.
4	Enter smaller step values (for example, 20 steps) and click Execute until the syringe drive toggle is 1–2 mm from the top of the glass syringe. **Note** If the syringe drive travels too far without encountering resistance, the instrument assumes that the cause is a polymer leak. An alert message about the leak will be posted to the Macintosh screen. Put the syringe drive as close as possible to the syringe plunger to avoid false warnings about leaks.

Replacing the Plastic Syringe

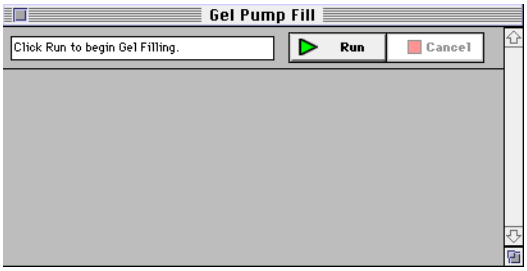
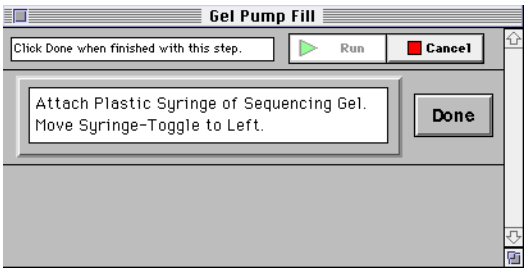
Step	Action
1	Remove the plastic syringe containing polymer, cap it, and store it in the refrigerator.
2	Fill the 5-mL plastic syringe from the Basic Installation Kit with water.
3	Put the plastic syringe filled with water through the syringe guide. It goes in the hole to your left as you face the 310 Genetic Analyzer.
4	Screw it onto the luer fitting.

Filling the Glass Syringe Using the 310 Data Collection Software

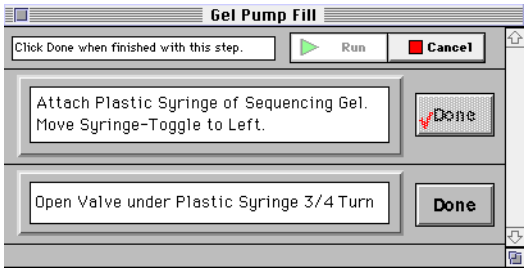
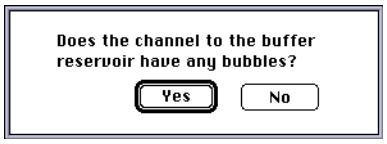
Installing the Plastic Syringe

Step	Action
1	Remove DNA Sequencing Polymer from the refrigerator and equilibrate to room temperature. This takes about one hour.
2	Remove the cap from the room-temperature DNA Sequencing Polymer.
3	Put the plastic syringe through the syringe guide. It goes in the hole to your left as you face the 310 Genetic Analyzer.
4	Partially screw it onto the luer fitting. Do not tighten it yet.

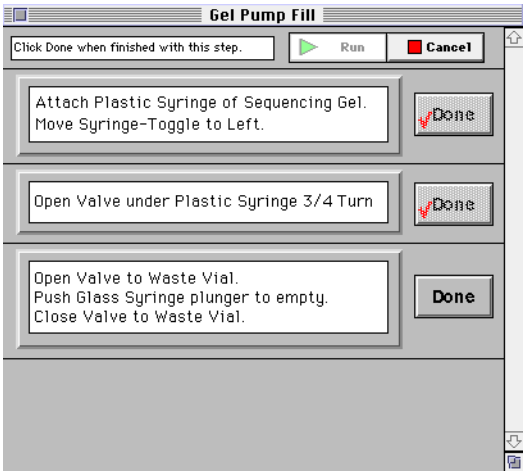
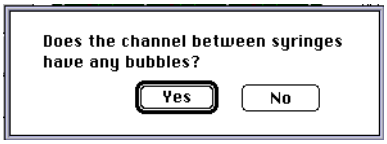
How to Use the Gel Pump Fill Window

Step	Action
1	Open the 310 Data Collection software.
2	Open the Instrument menu and choose Gel Pump Fill. The Gel Pump Fill window appears. Click Run. The syringe homes and the Buffer Valve opens. 
3	When the Gel Pump Fill window directs you to attach the plastic syringe, loosen the valve under the luer fitting, and tighten the plastic syringe on the luer fitting Do not click Done yet. 
4	Manually push the syringe plunger to fill the loosened valve and luer fitting with polymer.
5	Close the valve under the luer fitting.
6	Move the syringe drive toggle to the left.
7	Click Done in the Gel Pump Fill window. The buffer reservoir valve closes and the syringe drive moves down. **Note** Pressing the Gel Pump button is equivalent to clicking Done in the Gel Pump Fill window. Press and hold the button until the computer responds.

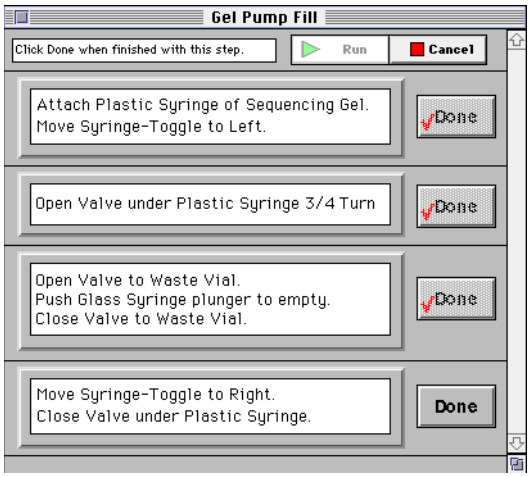
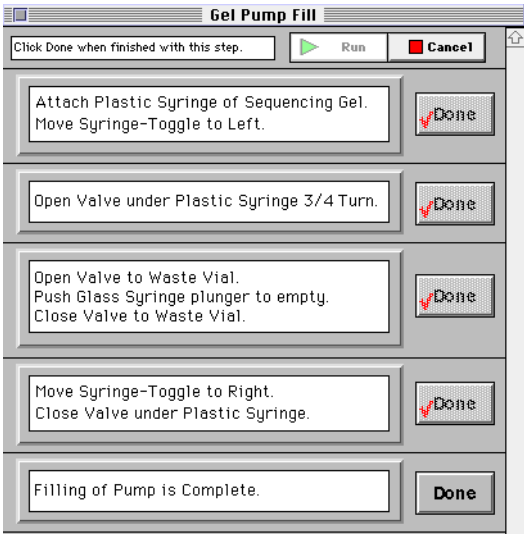
Priming the Pump Block Using Gel Pump Fill

Step	Action	
1	Open the valve under the plastic syringe luer fitting 3/4 turn, then click Done in the Gel Pump Fill window (see Figure 2-9). The instrument pumps sequencing polymer into the block, filling the passage between the plastic syringe and the buffer reservoir.  **Figure 2-9** Gel Pump Fill window–Open Valve	
2	When this message appears, check the pump block channel. 	
3	**If there is…**	**Then click…**
	air in the pump block channel	Yes.
	no air in the pump block channel	No.
4	When No is selected, polymer is pumped to the glass syringe.	

Priming the Glass Syringe Using Gel Pump Fill

Step	Action	
1	Open the valve to the waste vial, push the polymer and air out of the glass syringe, close the valve to the waste vial, and click Done in the Gel Pump Fill window (see Figure 2-10). The instrument pumps sequencing polymer into the block, filling the passage between the plastic and glass syringe.  **Figure 2-10** Gel Pump Fill window–Prime the Glass Syringe	
2	When this message appears, check the pump block channel.  	
3	**If there is…**	**Then click…**
	air in the pump block channel	Yes.
	no air in the pump block channel	No.
4	When No is selected, about 200-µL of polymer is pumped into the glass syringe.	

Setting Up the Syringe Drive Using Gel Pump Fill

Step	Action
1	Move the syringe drive toggle to the right, close the valve under the plastic syringe, and click Done. The syringe drive moves to the top of the plunger. 
2	Make a final inspection for air bubbles, and click Done. 

Replacing the Plastic Syringe

The polymer in the pump block passageways must not dry out. Install a plastic syringe filled with water to prevent dried polymer from plugging the channel leading to the plastic syringe luer fitting.

Step	Action
1	Remove the plastic syringe containing polymer, cap it, and store it in the refrigerator.
2	Fill the 5-mL plastic syringe from the Basic Installation Kit with water.
3	Put the plastic syringe filled with through the syringe guide. It goes in the hole to your left as you face the 310 Genetic Analyzer.
4	Screw it onto the luer fitting.

How to Fill the Capillary with DNA Sequencing Polymer (DSP)

Filling the Capillary

The Seq Fill Capillary module is used to fill the capillary with polymer. When you start the module, the instrument pumps polymer to the capillary for 5 seconds, pauses for 10 seconds, and then fills the capillary for 10 minutes.

If you note anomalies early in the pumping operation, pause or cancel the run during the programmed 10-second delay.

Initially, the glass syringe plunger should rapidly move 1–2 mm before stopping. If the plunger moves rapidly for a greater distance, there may be a leak.

During the 10-minute fill, pumping automatically stops if the instrument detects movement of the plunger corresponding to 25-μL or more of polymer. Check for leaks if the fill stops early.

To fill the capillary:

Step	Action
1	Open the 310 Data Collection software and the Manual Control window.
2	Press the Tray button. The autosampler tray moves forward.
3	Open the instrument doors, and place a small tube containing 0.5 mL water in tube position 6 in the front of the autosampler.
4	Press the Tray button again to move the autosampler back to its original position.
5	Select Seq Fill Capillary from the Module pop-up menu on the Manual Control screen. 
6	Note the position of the end of the glass syringe plunger and write it down.
7	Click Start.
8	After the module has run, again note the position of the plunger, and subtract it from the initial value. The pump should consume 4—7-μL of polymer per 10-minute fill. Check for leaks if more than 7-μL of polymer was consumed.

How to Store the DNA Sequencing Polymer (DSP)

Storing DSP

Remove, cap and refrigerate the plastic syringe of sequencing polymer at 5–10 °C to extend the life of the polymer.

The sequencing polymer contains urea and will slowly increase in conductivity as the urea decomposes. Polymer left at room temperature is good for 1 week. Each sequencing analysis uses 4–5-μL of polymer, so fill the glass syringe appropriately.

Setting Up the Reagents for Runs with DSP

Preparing Reagents

! WARNING ! CHEMICAL HAZARD. Urea is a potential mutagen. Dangers cited in toxicity studies show reproductive and tumorigenic effects. Urea can cause irritation to the skin, eyes, and respiratory tract. Avoid inhalation and contact with skin, eyes and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

CHEMICAL HAZARD. Some chemicals used with this instrument are potentially hazardous. Warnings are prominently displayed on the bottle labels of all hazardous chemicals. Material Safety Data Sheets (MSDSs) are provided by the manufacturer and contain information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. Please familiarize yourself with the information contained in these documents before attempting to operate the instrument or using hazardous reagents. Additional copies of the MSDSs for Applied Biosystems chemicals are available from Applied Biosystems at no cost.

Prepare the polymer and buffer:

Step	Action
1	Equilibrate DSP polymer to room temperature.
2	Dilute 5-mL of 10X Genetic Analyzer Buffer (without EDTA) to a 1X concentration (50-mL) with filter-sterilized, deionized water and bring to room temperature.

Storing the Buffer

Store electrophoresis buffer at 2–8 °C.

When to Replace Reagents

Use the following table.

Reagent	Replace
Cathode and Anode buffers	Every week or every 200 runs.
Polymer	Every week.
Water in the water vial and waste vial	Before every set of runs.

GeneScan Analysis Software Experiments 3

Overview

About This Chapter

This chapter summarizes the steps for an experiment that is analyzed with GeneScan® Analysis Software and, as an example, explains in detail the steps for using the ABI PRISM® Fluorescent Genotyping Demonstration Kits A and B.

To quickly start a GeneScan® Analysis Software run, see the Quick Reference Card at the front of the manual.

For more detail about specific procedures, see Chapter 4, “Procedures for Operation.”

In This Chapter

Topics in this chapter include the following:

About GeneScan Analysis

Type of Experiments

There are many types of experiments that are analyzed using the 310 Genetic Analyzer and the GeneScan® Analysis Software. They all attempt to determine the size or relative quantity of DNA fragments. Applied Biosystems sells a number of kits for applications that are analyzed with the GeneScan® Analysis Software. Contact your sales representative for information.

Applications	Experiments
Microsatellite Analysis	♦ Fluorescent genotyping for genetic linkage studies ♦ Paternity identification ♦ Forensic identification of samples ♦ Determination of loss of heterozygosity ♦ Microsatellite instability ♦ Trisomy analysis
Amplified Fragment Length Polymorphism (AFLP™) Analysis	♦ Gene mapping using AFLP
Gene Expression Profiling	♦ Differential display ♦ Quantitative expression of gene products ♦ RNase protection assays
Mutation Detection	♦ Single strand conformation polymorphisms (SSCP) ♦ Heteroduplex mobility assays (HMA) ♦ Mismatch cleavage ♦ Oligonucleotide ligation assays (OLA) ♦ Allele-specific PCR

About Using the Fluorescent Demonstration Kits

The Fluorescent Genotyping Demonstration Kits are typically used to show that the 310 Genetic Analyzer is properly sizing fluorescently labeled PCR fragments. Use the kits to become familiar with sizing DNA fragments on the 310 Genetic Analyzer or to troubleshoot the process.

For Kit A, you amplify the PCR products; Kit B provides pre-amplified products.

continued on next page

Summary of Procedures

To perform a GeneScan® Analysis Software experiment with pooled DNA using the ABI PRISM® 310 Genetic Analyzer, you will need to:

- Prepare the 310 Genetic Analyzerr by:
  - Preparing the reagents
  - Cleaning the pump block
  - Installing the capillary
  - Cleaning and installing the syringe
  - Cleaning the electrode
  - Recalibrating the autosampler if:
    - The electrode is removed, replaced or cleaned
    - The capillary is replaced
  - Priming the pump block
  - Loading buffers
  - Preheating the instrument
- Prepare matrix standards (if necessary)
- Amplify and denature (if necessary) the samples
- Load the samples
- Start the run by:
  - Selecting or creating a Sample Sheet
  - Filling out an Injection List

Materials Required

To work through the example provided in this chapter, you will need:

! WARNING ! CHEMICAL HAZARD. Before handling the chemical reagents needed for the Fluorescent Genotyping Demonstration Kits, read the safety warnings on the reagent bottles and in the manufacturers' Material Safety Data Sheets (MSDS). Always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) when handling chemicals. Dispose of waste in accordance with all local, state, and federal health and environmental regulations and laws.

Item	Source
Fluorescent Genotyping Demonstration Kits A or B	Applied Biosystems P/N 402246 or P/N 402247
Fluorescent Amidite Matrix Standards	Applied Biosystems P/N 401546
Deionized formamide	Major laboratory suppliers (MLS)
Filter-sterilized, deionized water	MLS
Disposable, non-powdered, chemical-resistant gloves	MLS
GeneScan–350 [TAMRA] Size Standard (required only if using Kit B)	Applied Biosystems P/N 401736
A small-volume, calibrated pipette and tips. We recommend the Gilson Pipetman	Rainin Instruments P/N P10
ABI PRISM® 310 10X Genetic Analyzer Buffer with EDTA	Applied Biosystems P/N 402824

Item	Source
One 1.0-mL glass syringe (or one 2.5-mL glass syringe)	Applied Biosystems P/N 604418
Performance Optimized Polymer, 4% (POP-4™)	Applied Biosystems P/N 402838
Two 4.0-mL Genetic Analyzer Buffer Vials with cap adapters	Applied Biosystems P/N 401955
Two septa for the 4.0-mL buffer vials	Applied Biosystems P/N 401956
ABI PRISM® 310 Genetic Analyzer Capillary, 47-cm, 50-μ i.d., labeled with a green mark[a]	Applied Biosystems P/N 402839
ABI PRISM GeneScan Chemistry Guide and GeneScan Chemistry Guide Update	Applied Biosystems P/N 903560 and P/N 904580
Microcentrifuge or centrifuge adapted for spinning microtiter plates (96-well tray)	MLS
One of the following thermal cyclers from Applied Biosystems: ♦ GeneAmp® PCR System 9700 ♦ GeneAmp® PCR System 9600 ♦ GeneAmp® PCR System 2400 ♦ DNA Thermal Cycler 480 **Note** If the GeneAmp® PCR System 2400 or DNA Thermal Cycler 480 will be used to denature your samples, you will need the appropriate materials (***i.e.,*** tubes, tray, etc.) for these thermal cyclers. You will also be required to transfer the samples to either the 96-or 48-well sample tray to load the samples on the ABI PRISM® 310.	

a. Do not use GeneScan capillaries labeled with a yellow mark (P/N 401823) with POP-4™ polymer. Resolution will be unsatisfactory if these capillaries are used with POP-4 polymer.

You will also need one sample tray and related accessories as listed in Table 3-1 or Table 3-2 on page 3-5:

Table 3-1 96-well sample tray and accessories

Part	Quantity	Part Number
MicroAmp® Tray/Retainer Set	1	403081
0.2-mL MicroAmp® Reaction Tubes	2—10*	N801-0580
MicroAmp® Base	1	N801-0531
MicroAmp® Full Plate Cover	1	N801-0550
Genetic Analyzer Septa Strips	1	P/N 402059
Genetic Analyzer Retainer Clips	1	P/N 402866

* Two tubes for Kit A; four tubes for Kit B; four tubes for matrix standard samples

Table 3-2 48-well sample tray and accessories

Part	Quantity	Part Number
48-well Sample Tray (4.0-mL)	1	401955
0.5-mL Samples Tubes	2—10*	401957
* Two tubes for Kit A; four tubes for Kit B; four tubes for matrix standard samples		
Septa for 0.5-mL Sample Tubes	2—10*	401956
1.5-mL Eppendorf tube	1	—

Materials Required for PCR

With the GeneAmp® 9700, 9600 or 2400

- One MicroAmp® tray and retainer, 96-well or 24-well
- 14 MicroAmp reaction tubes and caps
- One MicroAmp base, 96-well, or 24-well
- Microcentrifuge adapted for spinning microtiter plates

With the 877

- Six 1.5-mL capless tubes (Sarstedt P/N 72.607)
- Two 96-well MicroAmp Tray/Retainer Sets filled with tubes
- Two microcentrifuge tubes
- *ABI PRISM 877 Integrated Thermal Cycler User's Manual* (P/N 904414)
- Bleach, 30 mL sodium hypochlorite solution diluted to 2% in available chlorine (we recommend JT Baker sodium hypochlorite solution which may be 5–7% in available chlorine, P/N 9416-03)
- TrIS, 30-mL (0.01% Tween20 and 50 mM Tris [pH 8.0])
- TE buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA)

With the GeneAmp 480

- 14 GeneAmp® Thin-Walled Reaction Tubes with flat caps, 0.5-mL (P/N N801-0737)
- Mineral oil, molecular biology grade

Software Required

- ABI PRISM® 310 Firmware, version 1.0.2 or higher
- ABI PRISM® 310 Module GS STR POP4 (1.0-mL) C
- ABI PRISM® 310 Collection Software, v 1.0.2 or higher
- ABI PRISM® GeneScan® Analysis Software, v 2.0.2 or higher

How to Switch Between DNA Sequencing and GeneScan

Switching Between Applications

If the ABI PRISM® 310 was set up for experiments to be analyzed with the ABI PRISM® DNA Sequencing Analysis Software, then before following the instructions in this chapter to prepare an experiment that will be analyzed with the GeneScan® Analysis Software, it will be necessary to do the following.

Take this action	See page
Remove the 2.5-mL glass syringe (if present) or clean out the 1.0-mL syringe.	4-18
Remove the capillary and store it.	4-15
Clean the pump block.	4-8

How to Set Up the Reagents

Preparing Reagents

! WARNING ! CHEMICAL HAZARD. Urea is a potential mutagen. Dangers cited in toxicity studies show reproductive and tumorigenic effects. Urea can cause irritation to the skin, eyes, and respiratory tract. Avoid inhalation and contact with skin, eyes and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

CHEMICAL HAZARD. Some chemicals used with this instrument are potentially hazardous. Warnings are prominently displayed on the bottle labels of all hazardous chemicals. Material Safety Data Sheets (MSDSs) are provided by the manufacturer and contain information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. Please familiarize yourself with the information contained in these documents before attempting to operate the instrument or using hazardous reagents. Additional copies of the MSDSs for Applied Biosystems chemicals are available from Applied Biosystems at no cost.

Preparing the Polymer and Buffer

Step	Action
1	Equilibrate polymer to room temperature, then mix thoroughly by inversion.
2	Allow the polymer to sit for at least 5 minutes after mixing.
3	Dilute 5-mL of 10X Genetic Analyzer Buffer with EDTA to a 1X concentration (50-mL) with filter-sterilized, deionized water and bring to room temperature.

Storing the Buffer

Store electrophoresis buffer at 2–8°C. The 1X buffer can be stored for 2 weeks.

When to Replace Reagents

Use the following table.

Reagent	Replace
Cathode and Anode buffers	Every week or every 200 runs.
Polymer	Every week.
Water in the water vial and waste vial	Before every set of runs.

Preparing the 310 Genetic Analyzer for a GeneScan Run With POP-4

About These Procedures

To work through the example in this chapter using a Fluorescent Genotyping Demonstration Kit and POP-4 polymer, or for any experiment requiring POP-4 polymer, set up your instrument as detailed below.

These procedures can also be found in Chapter 4, “Procedures for Operation.” Some are explained there with more detail and with illustrations. If you have a question about a procedure, see Chapter 4.

Cleaning the Pump Block

IMPORTANT Do not expose the pump block to any organic solvents.

Step	Action
1	Remove the pump block from the instrument.
2	Open all valves and ports on the pump block.
3	Hold the pump block under warm running water to thoroughly rinse it.
4	Force warm water through the channels with the 5-cc plastic syringe provided in the Basic Installation Kit. Direct the flow of water through each channel in turn by sealing channel openings with plugs provided in the Basic Installation Kit. Rinse each channel five times. **Note** If a 30-cc syringe is available, rinse each channel with it once. This can be more convenient than rinsing five times with a 5-cc syringe.
5	Rinse the valves with warm water. Soak valves that are coated with dried polymer.
6	Visually inspect the channels for dried polymer, which looks like white residue. Wash the channels until the polymer is gone.
7	Rinse the block and its channels with distilled, deionized water.
8	Remove residual water from the pump block and fittings to ensure that the running polymer is not diluted. Force air through the channels, using the plastic syringe or canned compressed air, until the channels are dry.
9	Replace the pump block and buffer reservoir.

Cleaning and Preparing the Syringe for Use

Use the 1.0-mL syringe with Performance Optimized Polymers.

Cleaning the Syringe

To clean the syringe:

Step	Action
1	Rinse the syringe with distilled water. Remove excess water, but do not dry completely. **IMPORTANT** Excessive wear occurs to the Teflon fitting of the plunger if it is completely dry.
2	Allow the polymer to equilibrate to room temperature before loading it into the syringe. **IMPORTANT** Precipitate present when the bottle is removed from cold storage should go back into solution at room temperature. Do not use the polymer until the precipitate dissolves.
3	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.

To clean the syringe: *(continued)*

Step	Action
4	Draw a small amount of polymer solution (0.20-mL maximum) into the syringe. Pull plunger up to the 0.60-mL marker after the polymer solution is added. **! WARNING ! Gloves and eye protection should be worn when handling polymer.**
5	Invert the syringe gently five or six times to coat the walls with polymer and discard this polymer solution. This ensures that the running polymer is not diluted when added to the syringe.
6	For new, never-before-used syringes, perform this entire procedure twice to prevent air bubbles from sticking to the syringe walls.

Loading Polymer

Step	Action
1	Allow the polymer to equilibrate to room temperature before loading it into the syringe.
2	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.
3	Fill the syringe manually with a maximum of 0.5-mL of polymer. **Note** Do not use polymer that has been on the instrument for more than one week. Do not return unused polymer to the original bottle.
4	Remove all air bubbles by inverting syringe and pushing air bubbles out. **IMPORTANT** To avoid loss of polymer, any bubbles near the plunger head must be removed.
5	Rinse the outside of the syringe with distilled water to remove any polymer on the outside of the syringe. Dry the outside of the syringe with a lint-free paper.
6	Move the syringe drive toggle to the left to attach the syringe to the pump block.
7	Place the syringe through the right-hand port of the plastic syringe guide plate, and screw the syringe into the pump block finger-tight.
8	Manually close and tighten the waste valve below the syringe and the luer valve to the left of the syringe.

Setting Syringe Max Travel

Set the Syringe Max Travel once for each syringe. Record the value for each syringe (the calibration sticker on the door is a good place for this record) and input it in the Manual Control window when you install the syringe again.

To set Syringe Max Travel:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Determine which version of Collection software is installed on your Macintosh® computer by clicking the Apple menu and selecting “About Collection Software.”</td></tr>
<tr><td>2</td><td>Use the following table to determine the next step:
<table>
<tr><th>If you have...</th><th>Then...</th></tr>
<tr><td>v. 1.04 or later Collection software</td><td>go to step 3.</td></tr>
<tr><td>v. 1.0.2 Collection software</td><td>a. Open the Window menu.
b. Under Preferences, select Sequence Injection List Defaults or GeneScan Injection List Defaults.
c. Type 139115 in the Operator entry window and click OK.
d. Re-launch the Collection software.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Open the Manual Control window, select Syringe Home from the Function pop-up menu, and click Execute.</td></tr>
<tr><td>4</td><td>Select Syringe Max Travel in the Function pop-up menu. Note the displayed value, as you will enter it in the next step.</td></tr>
<tr><td>5</td><td>Select Syringe DOWN and enter the current Syringe Max Travel value. Click Execute.</td></tr>
<tr><td>6</td><td>After the syringe drive reaches the syringe plunger and stops moving, select Status from the Window menu.</td></tr>
<tr><td>7</td><td>Under the Injection pop-up window, read the number in the Gel Pump At window.
Subtract 15 from that number</td></tr>
<tr><td>8</td><td>Select Syringe Max Travel in the Function pop-up menu of Manual Control, type the number you just calculated in step 7, and click Execute.</td></tr>
<tr><td>9</td><td>Record this value on the Calibration sticker on the left side door.
Note If you install a syringe of a different size, you must input the max travel value for that syringe size.</td></tr>
<tr><td>10</td><td>Select Syringe Up in the Function pop-up menu of Manual Control, enter 250 in the Value window, and click Execute.</td></tr>
</table>

continued on next page

Filling the Pump Block

To fill the pump block channels with polymer:

Step	Action
1	Open the Manual Control window. Select Buffer Valve Close in the Function pop-up menu to close the pin valve at the anode buffer reservoir on the pump block.
2	Manually open the waste valve below the syringe.
3	Press the syringe plunger until a drop of polymer forms on the bottom of the waste valve. This removes the air bubbles at this valve site, and uses about 0.1-mL of polymer.
4	Manually close the waste valve.
5	Open the Manual Control menu and select Buffer Valve Open in the Function pop-up menu to open the pin valve at the anode buffer reservoir on the pump block.
6	Press the syringe plunger until polymer fills the polymer channel in the block. This removes all of the air bubbles from the polymer channels, and should use about 0.1-mL of polymer. **IMPORTANT** There should be no air bubbles in the pump block channels.
7	Select Buffer Valve Close in the Function pop-up menu.
8	Move the syringe drive toggle to the right to position it over the syringe plunger.
9	Select Syringe Down in the Function pop-up menu.
10	Select 50 step intervals and click Execute until the toggle makes contact with the syringe plunger.

Cleaning the Electrode

To clean the electrode:

Step	Action
1	Open the 310 Data Collection software.
2	Press the Tray button on the 310 Genetic Analyzer to lower the autosampler and present the tray.
3	Wipe the electrode with lint-free paper that has been dampened with distilled, deionized water
4	Dry the electrode with fresh lint-free paper.
5	Press the Tray button to return the autosampler to its original position and immerse the capillary in buffer.
6	Recalibrate the autosampler after cleaning, trimming or replacing the electrode.

continued on next page

Installing the Capillary

Connecting the Capillary to the Pump Block

Step	Action
1	Remove the new capillary from the storage tube.
2	Clean the capillary window.
3	Open the door covering the heat plate.
4	Partially unscrew the capillary fitting on the right side of the pump block.
5	Partially screw the capillary fitting back into the pump block. **IMPORTANT** Do not tighten the fitting at this point in the procedure, or the opening on its tip will be crushed. The capillary must be properly inserted through the fitting before you tighten it.
6	Thread one end of the capillary through the capillary fitting.
7	Adjust the end of the capillary so that it is positioned directly below the opening to the glass syringe. The end of the capillary must protrude well beyond the opening at the tip of the capillary fitting. To avoid crushing the opening, be certain that you see the capillary in the pump block channel before proceeding to the next step.
8	Tighten the capillary fitting finger-tight to secure the capillary. **IMPORTANT** The capillary will twist as the fitting is tightened. Leave the other end of the capillary free to twist, or the capillary will break.

Positioning the Capillary in the Detector

Step	Action
1	Open the laser detector door, and position the capillary in the vertical track of the detector. Align the colored labelling mark on the capillary with the top edge of the detector plate and laser detector door.
2	Tape the capillary to the heat plate with thermal tape to secure the position of the capillary labelling mark relative to the detector plate.
3	Close the laser detector door to secure the position of the capillary window.

Positioning the Capillary Near the Electrode

Step	Action
1	Thread the capillary through the capillary hole in the electrode thumbscrew until it protrudes past the tip of the electrode by about 0.5 mm (maximum).
2	Tape the capillary to the heat plate with thermal tape to secure the position of the capillary tip relative to the electrode. Tape the capillary just above the electrode thumbscrew and just above the detector door.
3	Close the door over the heat plate.
4	With the heat plate door closed, check that the capillary has not moved relative to the electrode.

Resetting the Injection Counter

Step	Action
1	Open the Instrument window and choose Change Capillary.
2	Click OK in the Reset window to set the injection counter to zero.

Calibrating the Autosampler

Calibrate the autosampler as follows:

Step	Action
1	Choose Autosampler Calibration from the Instrument menu of the Collection software. The Autosampler Calibration window appears.
2	Click Start and follow the directions that appear on the screen.
3	Move the autosampler using the arrow keys in the Autosampler Calibration window or the arrow keys on the Macintosh® computer keyboard. **Note** Hold down the arrow keys to move the autosampler with larger steps. This is often useful for z calibration.
4	Align the calibration dot on the front of the tray platform with the capillary. Center the end of the capillary on the x, y-calibration point. Almost touch the z-calibration point with the end of the capillary.
5	Click Set to save the calibration value.
6	Repeat for the rear calibration point and click Set.

Loading Buffers

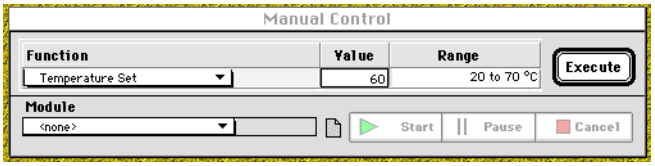
Load the buffer as follows:

Step	Action
1	Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer Buffer with EDTA, and install it on the pump block.
2	Label one of the buffer vials as *Buffer*, and fill it to the line with 1X Genetic Analyzer with EDTA buffer.
3	Cap the vial, insert the septum, and place it in position one on the Genetic Analyzer autosampler.
4	Label the other glass buffer vial as H_2O, and fill it to the line with filter-sterilized, deionized water.
5	Cap the vial, insert the septum, and place it in position two on the Genetic Analyzer autosampler.
6	If the 1.5-mL Eppendorf tube has a lid attached, cut the lid off. Completely fill the tube with filter-sterilized, deionized water, and place it in position three on the Genetic Analyzer autosampler.

continued on next page

Preheating the Heat Plate

This is optional. Preheating the heat plate can take up to 30 minutes. You can prepare the samples while the instrument preheats.

Step	Action
1	Open the Window pull-down menu, and choose Manual Control. The Manual Control window is displayed. 
2	Choose Temperature Set from the Function menu, and set the temperature to 60 °C in the Value window.
3	Click Execute. The instrument will preheat to 60 °C. While the instrument is preheating, you can prepare your samples. The instrument doors must be closed. The safety interlock will prevent operation when the doors are open.

Performing PCR

Overview

Fluorescent Genotyping Demonstration Kit A, used in this chapter as an example, requires a procedure typical of most experiments that will be analyzed with the GeneScan® Analysis Software: performing a PCR amplification of the samples.

Optimized PCR protocols for Kit A on Applied Biosystems thermal cyclers, including the GeneAmp® PCR Systems 9700, 9600, and 2400, ABI PRISM® 877 Integrated Thermal Cycler, and the DNA Thermal Cycler 480 are described in this section. If you use a thermal cycler not manufactured by Applied Biosystems, you may need to optimize thermal cycling conditions.

You must optimize thermal cycling conditions for other types of samples.

Performing PCR with the GeneAmp PCR System 9700, 9600, or 2400

! WARNING ! Always follow proper laboratory procedures when working with the chemicals in the kit. Wear protective eyewear, gloves and a laboratory coat at all times.

Preparing the Reaction Tray

You will need the MicroAmp® base, reaction tray, retainer and reaction tubes for this procedure.

Step	Action
1	If the MicroAmp® tray is fully assembled, disassemble it now.
2	Place the base on a flat surface.
3	Place the reaction tray in the base, so that the well numbered A1 is located in the upper left corner of the tray.
4	Place 14 reaction tubes in the tray. We recommend using a 2 x (6 + 1) array such as the one shown in Figure 3-1, which illustrates this array in a 96-well MicroAmp reaction tray. Each row will contain a single DNA sample; each column will contain a single primer pair. The tube placed at the end of each row will be used later to pool the PCR products.
5	Secure the retainer onto the tray and base.

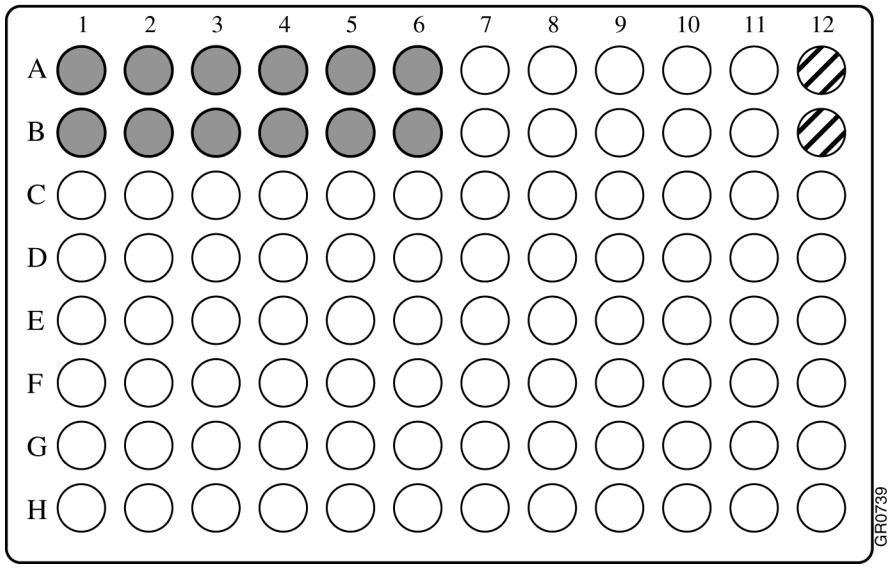


Figure 3-1 Recommended tube configuration for a 96-well tray

Loading the Reaction Tray

IMPORTANT To prevent cross-contamination of samples, change the pipette tip each time you load a different solution.

Refer to Figure 3-2 as you load the reaction tray:

Step	Action
1	Thaw all components of Kit A on ice.
2	Pipette 9-µL Reagent Mix into the first six tubes in each row. Remember, the tube at the end of each row remains empty.
3	Referring to Figure 3-2 for the next three steps, add 3-µL of Control DNA 1347-02 to the first six tubes in Row A.
4	Add 3-µL of Control DNA 1347-10 to the first six tubes in Row B.
5	Take the following action: Add 3-µL of each Primer Mix as follows: ♦ D12S83-[FAM] to both tubes in column 1 ♦ D7S517-[FAM] to both tubes in column 2 ♦ D2S391-[TET] to both tubes in column 3 ♦ D13S171-[TET] to both tubes in column 4 ♦ D1S220-[HEX] to both tubes in column 5 ♦ D3S1266-[HEX] to both tubes in column 6
6	Cap all the tubes, making sure they are tightly sealed.
7	Spin or shake down the tray to collect solutions at the bottom of each tube.

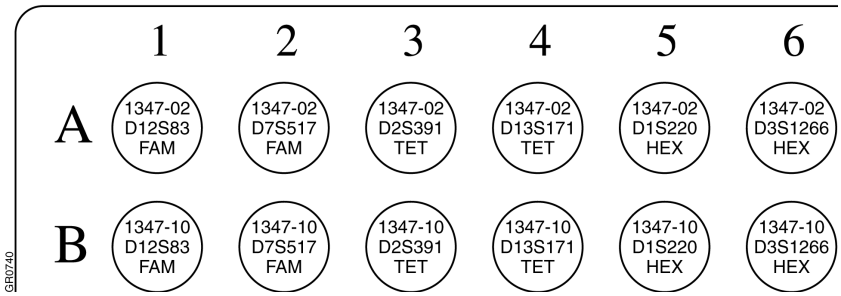


Figure 3-2 Control DNA and primer mix loading diagram for Kit A

Performing PCR

To program the thermal cycler and start PCR:

Step	Action
1	Program the thermal cycler using the conditions listed in Table 3-3.
2	Place the loaded tray into the thermal cycler. Make sure the tray is properly oriented.
3	Close and tighten the cover.
4	Start PCR.
5	When PCR is finished, proceed to page 3-22, “About Pooling PCR Products”.

Table 3-3 PCR conditions for GeneAmp® PCR System 9700, 9600, or 2400

Repetitions of Each Cycle	Cycle Conditions
1	95 °C for 12 minutes
10	Melt at 94 °C for 15 seconds Anneal at 55 °C for 15 seconds Extend at 72 °C for 30 seconds
20	Melt at 89 °C for 15 seconds Anneal at 55 °C for 15 seconds Extend at 72 °C for 30 seconds
1	Final Extension at 72 °C for 30 minutes
HOLD	4 °C (forever)

continued on next page

Performing PCR with the DNA Thermal Cycler 480 by GeneAmp

! WARNING ! Always follow proper laboratory procedures when working with the chemicals in the kit. Wear protective eyewear, gloves and a laboratory coat at all times.

Preparing the GeneAmp Reaction Tubes

To prepare the GeneAmp® reaction tubes:

Step	Action
1	Label 12 of the 14 reaction tubes with the control DNA and Primer Mix each will contain. Use Figure 3-3 on page 3-17 as a guide. The remaining two tubes will be used later to pool the PCR products.
2	Place the tubes on ice or in a tube rack in a 6 x 2 array.

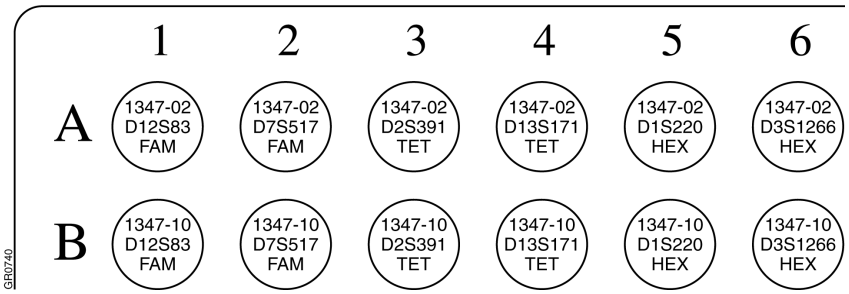


Figure 3-3 Control DNA and primer mix loading diagram for Kit A

Loading the GeneAmp Reaction Tubes

Note To prevent cross-contamination of samples, change the pipette tip each time you load a different solution.

To load the GeneAmp® Reaction Tubes:

Step	Action
1	Thaw all the components of Kit A on ice.
2	Pipette 9-µL Reagent Mix into each tube.
3	Add 3-µL of Control DNA 1347-02 to the six tubes marked for this DNA.
4	Add 3-µL of Control DNA 1347-10 to the remaining six tubes.
5	Add 3-µL of the appropriate Primer Mix to each tube as shown in Figure 3-3.
6	Add two drops of mineral oil to each tube.
7	Cap all the tubes, making sure they are tightly sealed.
8	Spin or shake down the tubes to collect solutions at the bottom of the tubes.

Performing PCR

To program the thermal cycler and start PCR:

Step	Action
1	Program the thermal cycler using the conditions listed in Table 3-4 on page 3-18.
2	Load the tubes into the thermal cycler.
3	Close the cover.
4	Start PCR.
5	When PCR is finished, proceed to page 3-22, “About Pooling PCR Products”.

Table 3-4 PCR conditions for the DNA Thermal Cycler 480

Repetitions of Each Cycle	Cycle Conditions
1	95 °C for 12 minutes
10	Melt at 94 °C for 45 seconds Anneal at 55 °C for 1 minute Extend at 72 °C for 1 minute
20	Melt at 89 °C for 1 minute Anneal at 55 °C for 1 minute Extend at 72 °C for 1 minute
1	Final Extension at 72 °C for 30 minutes
HOLD	4 °C (forever)

Performing PCR with the ABI PRISM 877 Integrated Thermal Cycler

The ABI PRISM® 877 Integrated Thermal Cycler performs PCR and pools the reaction products in a single run. The software supplied with the ABI PRISM® 877 includes two notebooks designed as tutorials to be used with Kit A of the Fluorescent Genotyping Demonstration Kit. These notebooks are located in a folder called ***Tutorial Files***, and are:

- ***PCR demo***—designed for a 64-sample run
- ***32-sample PCR demo***—designed for a 32-sample run

Instructions for performing PCR using Kit A are listed below, and are also located in Chapter 2, “Getting Started”, of the *ABI PRISM **877 Integrated Thermal Cycler User’s Manual***.

Preparing the ABI PRISM 877 Integrated Thermal Cycler

! WARNING ! Always follow proper laboratory procedures when working with this kit. Wear protective eyewear, gloves and a laboratory coat at all times.

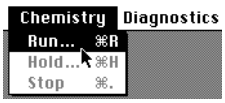
To prepare the thermal cycler:

Step	Action
1	After turning on the power to the instrument and Macintosh® computer, inspect the syringes for leaks.
2	Tighten the syringes into the Luer fittings, and adjust the set screws to finger tightness.
3	Thoroughly rinse and refill the small buffer (Tris) and bleach bottles on the work surface.
4	Thoroughly rinse and refill the external water and ethanol bottles.

Step	Action
5	Empty and clean the 1.5-mL microtube in position AC4–1.
6	Inspect the plastic tubing routed into the diluent bottles. If necessary, tighten the tubing where it enters each bottle, and adjust the tubing so the ends are well below the surface of the diluents.
7	Check the fluid level in the external waste bottle. Empty the bottle if necessary to ensure adequate space is available for the waste from the next run.

Opening and Printing the Load Map

To open and print the Load Map:

Step	Action
1	Open the ABI PRISM 877 folder, and double click the ABI PRISM 877 icon to launch the instrument control software. 
2	Open the folder labeled “Tutorial Files”.
3	Take the following action: **To run PCR on...** / **Then...** 64 samples / open the file called “PCR demo” to display the PCR demo notebook (Figure 3-4 on page 3-20). 32 samples / open the file called “32-sample PCR demo”.
4	Open the Chemistry pull-down menu and choose Run to display the Load Map (Figure 3-5 on page 3-20).  The following windows are displayed automatically before the Load Map: ♦ Checking ♦ Verify ♦ Save File As (displayed only if the Notebook has not already been saved) ♦ Build
5	If desired, click Print Details to print the Load Map.

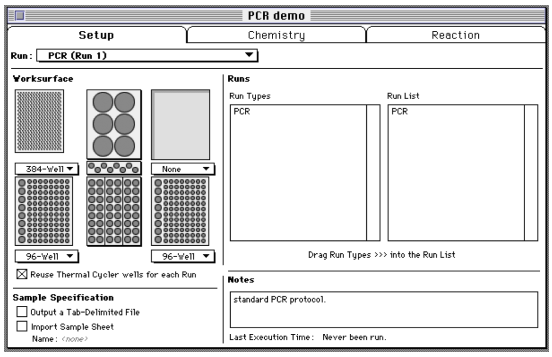


Figure 3-4 PCR Demo tutorial notebook

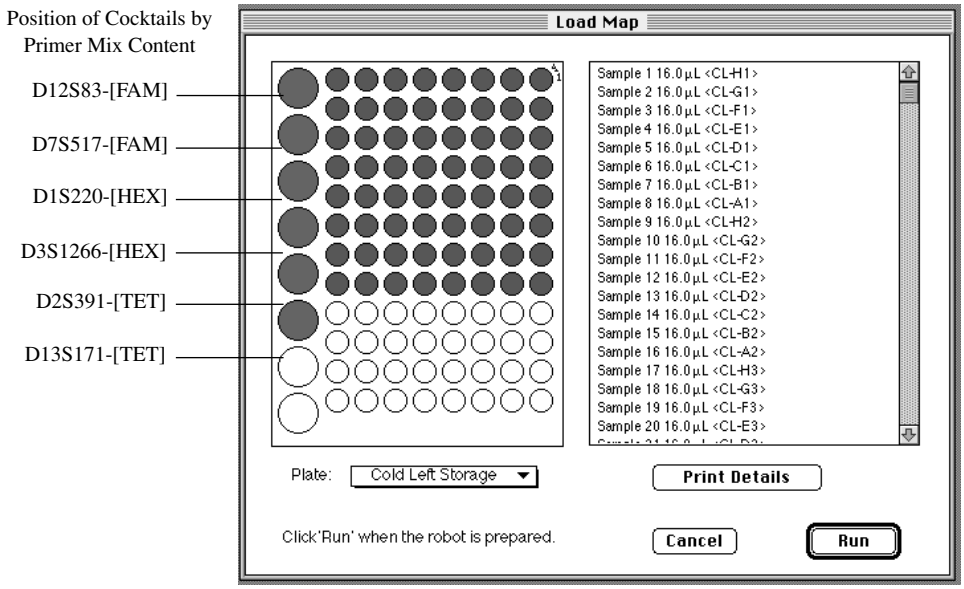


Figure 3-5 Load Map for the PCR Demo tutorial

Preparing the Cocktails and Control DNA

Instructions for preparing the cocktails and control DNA are also included in Chapter 3, “Preparing Chemistries”, of the *ABI PRISM 877 Integrated Thermal Cycler User’s Manual*.

To prepare the cocktails and control DNA:

Step	Action
1	Thaw all components of Kit A on ice.
2	Prepare six cocktails, one primer mix plus reagent mix per cocktail, as shown in Table 3-5 below. Prepare each cocktail in a 1.5-mL capless tube.
3	Place the tubes with cocktails into the large tube holders in Cold Left Storage in the order shown on the Load Map in Figure 3-5 on page 3-20.
4	Transfer 175-µL of control DNA 1347-02 to a clean microcentrifuge tube.

To prepare the cocktails and control DNA: *(continued)*

Step	Action
5	Dilute the DNA by adding 350-µL of filter-sterilized, deionized water or TE buffer. Vortex briefly.
6	Transfer 175-µL of control DNA 1347-10 to a clean tube, and dilute the DNA by adding 350-µL of filter-sterilized, deionized water or TE Buffer. Vortex briefly.
7	Pipette 16-µL of diluted control DNA 1347-02 into each of the first 32 tubes in the MicroAmp tray in Cold Left Storage (rows one through four; tubes A through H).
8	Pipette 16-µL of diluted control DNA 1347-10 into each of the remaining 32 tubes in Cold Left Storage (rows five through eight; tubes A through H).
9	Spin or shake down the tray to collect solutions at the bottom of each tube.
10	Place the MicroAmp® tray with samples in Cold Left Storage. Place the MicroAmp® tray with empty tubes in Cold Right Storage.

Executing the PCR Run

To load the thermal cycler and start the run:

Step	Action
1	Double check your instrument set up, and the placement of samples and reagents on work surface.
2	Check all liquids. Liquids should be at the bottom of the sample and reagent tubes, and there should be no bubbles in the tubes. Centrifuge reagents and samples if necessary.
3	Check that the aluminum cold storage blocks and microtiter trays are properly seated.
4	Close the lids on both cold storage blocks. The lids should lie flat.
5	Close the instrument door, and click Run on the Load Map to start the PCR run.
6	Watch for reagent delivery to begin. This should occur approximately 15 minutes after the run starts. The run will take approximately five hours.

Removing the Pooled PCR Products and Cleaning Up

To remove the pooled PCR products and clean up:

1	Open Cold Right Storage and remove the pooled PCR products. The following step is optional: To pause the robot during the Purge cycle to remove the pooled PCR products: a. Select HOLD from the Chemistry menu, or click the PAUSE/RESUME button on the Robot. b. When the green light by the PAUSE/RESUME button goes out, open the lid and remove the pooled PCR products from Right Cold Storage. c. Click the PAUSE/RESUME button, or choose RESUME from the Chemistry menu to complete the purge. **IMPORTANT** Resume the purge. Do **not** select Stop; otherwise, the instrument will not be clean for the next run.
2	Check the residual volumes in the sample and reagent tubes, and the product volumes in the product tubes. **Note** Check any unusual volumes for later investigation.
3	If the Log File contains an error message, note the following: ♦ The exact wording of the message. ♦ The statements immediately before and after the error message.
4	To reduce condensation, keep the cold storage lids and instrument door closed after you are finished.

About Pooling PCR Products

After performing PCR on a thermal cycler (other than the ABI PRISM® 877 Integrated Thermal Cycler), the PCR products must be combined (pooled).

The ratio for pooling the products depends upon the relative intensity of the dye labels and the efficiency of PCR amplification of each product.

Dye	Relative Intensity
FAM	High
NED or HEX	Medium
TET	Low

When using Virtual Filter C, a ratio of 1:2:1 is the general rule for pooling products labeled with FAM, HEX, and TET. For Virtual Filter D, use a ratio of 1:2:2 for FAM, HEX, and NED. Refer to the protocol in the application kit for the specific pooling ratio. For some applications, it may be necessary to optimize the pooling ratio in your laboratory.

With Kit A, you will have two pools (one for each DNA sample).

continued on next page

Pooling PCR Products from Kit A

How to Pool PCR Products from Kit A Produced on the GeneAmp PCR System 9700, 9600, or 2400

The following procedure describes how to pool PCR products From Kit A produced on the GeneAmp® PCR System 9700, 9600, or 2400.

Pool as follows:

Step	Action
1	Using the volumes listed in Table 3-5, transfer the appropriate volume of each reaction product in Row A to the empty reaction tube at the end of Row A.
2	Using the same table, transfer the appropriate volume of each reaction product in Row B to the empty reaction tube at the end of Row B.
3	Cap the reaction tubes.
4	Invert the tray to mix the samples.
5	Place the reaction tray in the MicroAmp base.
6	Spin down the tray to collect solutions at the bottom of each tube.
7	Store the pooled samples and remaining reaction products at –15 °C to –25 °C until you are ready to use them again.

Table 3-5 Kit A PCR Products Pooling Ratios: GeneAmp® 9700, 9600, or 2400

Tube Position and Primer Mix	Volume	Dilution Ratio
A1 or B1 with D12S83-[FAM]	7.5-µL	1:6.67
A2 or B2 with D7S517-[FAM]	7.5-µL	1:6.67
A3 or B3 with D2S391-[TET]	5.0-µL	1:10
A4 or B4 with D13S171-[TET]	10.0-µL	1:5
A5 or B5 with D1S220-[HEX]	10.0-µL	1:5
A6 or B6 with D3S1266-[HEX]	10.0-µL	1:5
	Total volume = 50.0-µL	

How To Pool PCR Products From Kit A Produced on the DNA Thermal Cycler 480

Pool as follows:

Step	Action
1	Pool each of the six reaction products for control DNA 1347-02 by pipetting the volumes listed below in Table 3-6 to a clean, empty GeneAmp® reaction tube.
2	Repeat this procedure for control DNA 1347-10.
3	Cap the tubes, and invert to mix the samples.
4	Spin down the tubes to collect solutions at the bottom of each tube.
5	Store the pooled samples and remaining reaction products at –15 °C to –25 °C until you are ready to use them again.

Table 3-6 Kit A PCR Products Pooling Ratios: DNA Thermal Cycler 480

Primer Mix	Volume	Dilution Ratio
D12S83-[FAM]	7.5-µL	1:6.67
D7S517-[FAM]	7.5-µL	1:6.67
D2S391-[TET]	5.0-µL	1:10
D13S171-[TET]	10.0-µL	1:5
D1S220-[HEX]	10.0-µL	1:5
D3S1266-[HEX]	10.0-µL	1:5
Total volume = 50.0-µL		

About Run Modules, Analysis Parameters, Size Standard and Matrix Files

Overview

Runs on the 310 Genetic Analyzer that will be analyzed with GeneScan® Analysis Software require you to have these types of files:

- Module
- Size standard
- Analysis parameters
- Matrix

You select the particular files appropriate to your experiment on the Sample Sheet and Injection List.

Updated

The module files are supplied with the Data Collection Software. Updated files can be obtained from the:

Applied Biosystems site on the World Wide Web (www.appliedbiosystems.com/techsupport),

Applied Biosystems Technical Support, or from your local field applications specialist (call your local sales office for more information).

For new applications, a disk containing the files is often included with the reagent kit.

Size Standard, Analysis Parameters, and matrix files are all created using GeneScan® Analysis Software. See the *ABI PRISM GeneScan Analysis Software User's Manual*.

Run Modules

Run modules are software files. The 310 Genetic Analyzer executes the steps in a run module to process a sample. Run time, temperature and voltage, prerun time, injection time and voltage are some of the run parameters controlled by the run module.

Run modules are selected through the Injection List. Select the run module based on the polymer, glass syringe size, dye set, and application you are using.

If a custom module is necessary, see "Editing Modules Using Manual Control" on page 5-17.

continued on next page

Run Module Reference Table

Use the following table as a guide when selecting a run module:

Chemistry	Syringe	Polymer	Run Module	Capillary Size	Capillary Mark
AFLP Plant Mapping Kits I and II	1.0-mL	POP-4	GS STR POP4 (1.0mL) A	47 cm x 50μ i.d.	green
Linkage Mapping Set version 1	1.0-mL	POP-4	GS STR POP4 (1.0mL) C	47 cm x 50μ i.d.	green
StockMarks Kits	1.0-mL	POP-4	GS STR POP4 (1.0mL) C	47 cm x 50μ i.d.	green
Linkage Mapping Set version 1	1.0-mL	POP-4	GS STR POP4 (1.0mL) C	47 cm x 50μ i.d.	green
StockMarks Kits	1.0-mL	POP-4	GS STR POP4 (1.0mL) C	47 cm x 50μ i.d.	green
Linkage Mapping Set version 2	1.0-mL	POP-4	GS STR POP4 (1.0mL) D	47 cm x 50μ i.d.	green
Custom Primers for fragment analysis	1.0-mL	POP-4	GS STR POP4 (1.0mL) D	47 cm x 50μ i.d.	green
AFLP Microbial Identification Kit	1.0-mL	POP-4	GS STR POP4 (1.0mL) F	47 cm x 50μ i.d.	green
Human Identification Profiler Kits	1.0-mL	POP-4	GS STR POP4 (1.0mL) F	47 cm x 50μ i.d.	green

Size Standard Files

About Size Standard Files

The Size Standard file holds the results of a run performed with fragments of known length. The file can be used to analyze other runs performed under the same conditions to determine the size of fragments of unknown length.

Applied Biosystems offers several size standard kits. Contact your sales representative for more information.

Generating New Size Standard Files

To generate new Size Standard files, set up the instrument and run the mixture of known-length fragments as a single sample. Create a new Size Standard file using GeneScan® Analysis Software (see the *GeneScan Analysis Software User's Manual*).

Analysis Parameters Files

This file holds the default start and stop point for data analysis, the default peak height threshold, and the default size calling method.

New Analysis Parameters files are created using GeneScan® Analysis Software (see the *GeneScan Analysis Software User's Manual*).

continued on next page

Matrix Files

Matrix files contain information that corrects for “spectral overlap”. Spectral overlap occurs when part of one dye’s emission spectrum falls onto a portion of the detection hardware that is collecting the fluorescent peak of another dye. Dye sets are created to minimize spectral overlap, but it occurs to some extent.

When choosing matrix files in the Sample Sheet, match the matrix file to the samples by dye set and chemistry. For example, samples run with a module file for Virtual Filter D should be analyzed with a matrix file that was also run with Virtual Filter D.

The dye set used to create the matrix file must be the same as the dye set used to run the sample. If you analyze data with a matrix file that does not reflect the conditions of your run, the run will appear to fail. Re-analyze with the correct matrix file if you have made this error.

Denaturing and Loading the Samples

About Formamide and Samples in Formamide

! 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.

The protocol uses formamide as a sample preparation reagent. Fresh formamide must be deionized and aliquotted into smaller volumes for storage. Each aliquot should be adequate for about one week's work.

Store aliquots of formamide at -20°C for up to three months. Formamide stored at 4°C is good for about one week.

At room temperature, samples in formamide are stable for a maximum of 48 hours.

Although not recommended on a routine basis, you can keep samples prepared in formamide frozen for no more than three days before running on the ABI PRISM® 310 Genetic Analyzer with no detectable loss in resolution.

Deionizing Formamide

To deionize formamide:

Step	Action	
1	Mix 50-mL of formamide and 5 g of ion-exchange resin (AG501 X8 from BioRad is recommended).	
2	Stir the mixture at room temperature for 30 minutes.	
3	Check the pH. It must be 7.0–9.0.	
4	**If the pH…**	**Then…**
	is in the proper range	filter the mixture through a 2-micron filter.
	is not in the proper range	a. Decant the formamide into a beaker with 5g of resin. b. Stir at room temperature for 30 minutes. c. Check the pH; it must be 7.0–9.0.
5	Make 500-μL aliquots and store them at minus 20°C for up to 3 months.	

continued on next page

Preparing the Formamide-Size Standard Mix

To ensure reproducibility of results for all samples, prepare the formamide-size standard mix using the 12:1 ratio of reagents stated in the procedure below.

IMPORTANT The formamide-size standard mix for the ABI PRISM® 310 differs from the mix prepared for the ABI® 373 and ABI PRISM 377, and do not use on other instruments.

! 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.

Prepare the formamide-size standard mix as follows:

Step	Action
1	Mix in a sample vial: a. 0.5-µL GeneScan–350 [TAMRA] Size Standard (for example) b. 12.0-µL deionized formamide
2	Label the vial.
3	Gently vortex the mixture for 3–5 seconds.
4	Spin down the mixture.
5	Store the mix at 2–6 °C until ready to use.

Preparing Matrix Standard Samples

About Matrix Standards

You must run matrix standards and create a matrix file the first time you use a new chemistry or change the run conditions.

Do not prepare matrix standards more than two hours in advance.

To ensure reproducibility of results for all samples, prepare the matrix standard mix using the 12:1 ratio of reagents stated in the procedure below.

IMPORTANT Do **not** add size standard mix to the matrix standard samples.

IMPORTANT The matrix standard mix for the ABI PRISM® 310 differs from the mix prepared for the ABI® 373 and ABI PRISM 377, and should not be used on other instruments.

When using the Fluorescent Genotyping Demonstration Kit, prepare Fluorescent Amidite Matrix Standards (P/N 401546). For fluorescent dNTPs, prepare Fluorescent dNTP Matrix Standards (P/N 402792). Remember that the matrix standards run must always match the sample run chemistry and conditions.

Preparing Matrix Standard Samples

For each matrix standard:

Step	Action
1	Mix in a sample vial: a. 1.0-µL of matrix standard b. 12.0-µL of de-ionized formamide
2	Label each vial according to the dye.
3	Gently vortex the mixture for 3–5 seconds.
4	Spin down the mixture.
5	Store at 2–6 °C until ready to use.

Denaturing the Matrix Standard Samples

You can denature the matrix standard samples in the thermal cycler with your samples (See "Denaturing the Samples" below), or follow this procedure:

Step	Action
1	Heat the sample for five minutes at 95°C.
2	Chill the sample on ice.
3	Hold on ice until ready to load in the autosampler.

Denaturing the Samples

IMPORTANT To prevent PCR carry-over contamination, we recommend working with amplified PCR products in an area separate from where reaction trays are loaded prior to PCR.

To denature your samples:

Step	Action
1	If the GeneAmp® PCR System 9600 or 9700 will be used to denature your samples, determine the order in which you will place the samples in the 96-well tray. Suggested configurations are shown in Figure 3-8 on page 3-32 and Figure 3-9 on page 3-33. **Note** The 96-well tray adaptor (P/N 4305051) is required to use the same tray on the ABI PRISM® 310. If the GeneAmp® PCR System 2400 or DNA Thermal Cycler 480 will be used to denature your samples, you will be required to transfer the samples to either a 96 or 48-well sample tray to load the samples onto the ABI PRISM® 310.
2	Load the pooled PCR products. For example: ♦ For Kit A, pipette 1.0-µL of each pooled PCR product into a MicroAmp or 0.5-mL reaction tube, one pooled product per tube. ♦ For Kit B, pipette 2.0-µL of each pooled PCR product into a MicroAmp or 0.5-mL reaction tube, one pooled product per tube.
3	Add 12-µL formamide-size standard mix to each tube. **IMPORTANT** Do **not** add size standard mix to matrix standard samples.
4	Place matrix samples in the 96-well tray.

To denature your samples: *(continued)*

Step	Action
5	Cap each tube, or seal the tubes with a MicroAmp full plate cover (available for the GeneAmp® PCR System 9700, 9600, and 2400 only). **IMPORTANT** Do not seal the tubes with the septa because the septa will melt inside the thermal cycler.
6	Spin down the contents of each tube.
7	Place the tubes into the thermal cycler.
8	Denature the samples as follows: a. 95 °C for 5 minutes b. Hold at 2–6 °C **Note** You can denature matrix standard samples using these conditions even though they differ from the conditions listed on the matrix standard samples product insert. Product performance will not be affected.
9	Remove your samples from the thermal cycler, and cool them in an ice water bath before loading them onto the Genetic Analyzer.

Loading the Samples

Load the samples into the autosampler as follows:

Step	Action
1	If necessary, transfer the denatured samples to a 48-or 96-well tray. Suggested tube arrangements for the example using the 48-well tray are shown in Figure 3-6 and Figure 3-7 on page 3-32. Suggested tube arrangements for the example using the 96-well tray are on pages 3-32 and 3-33. **IMPORTANT** The tube arrangement and order of the samples in the tray and on the Sample Sheet must be the same. Make note of the tube arrangement you use, so that you can prepare the Sample Sheet correctly.
2	Seal each tube with a septum, and place the tray into the autosampler.

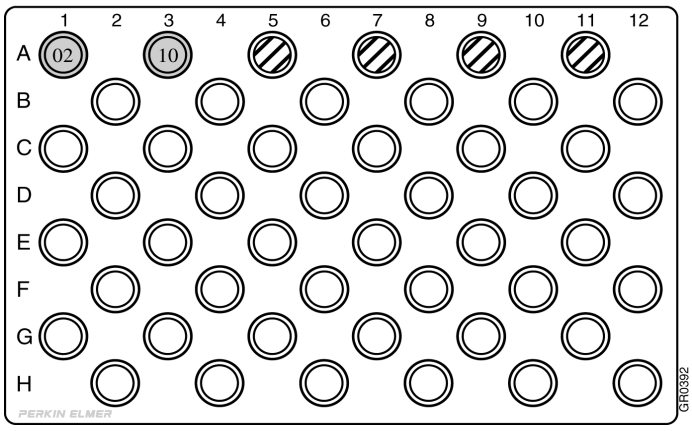


Figure 3-6 Kit A pooled PCR products in positions A1 and A3. Matrix standard samples in positions A5, A7, A9 and A11.

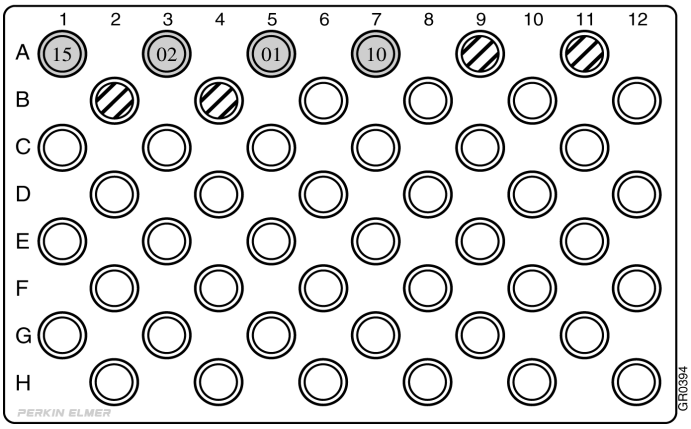


Figure 3-7 Kit B pooled PCR products in positions A1, A3, A5 and A7. Matrix standard samples in positions A9, A11, B2 and B4.

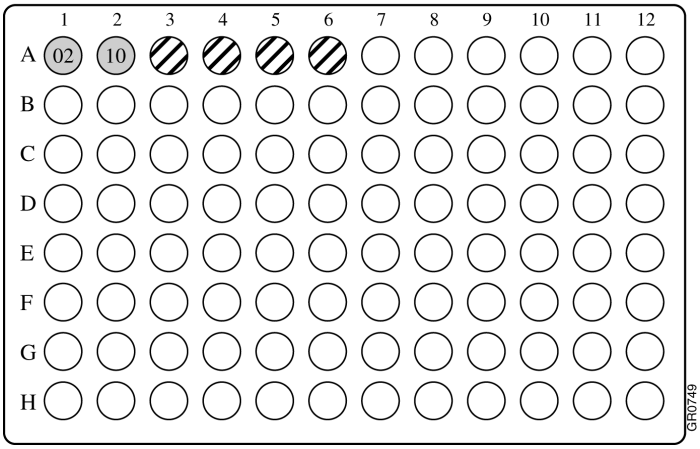


Figure 3-8 Kit A pooled PCR products in positions A1 and A2. Matrix standard samples in positions A3 through A6.

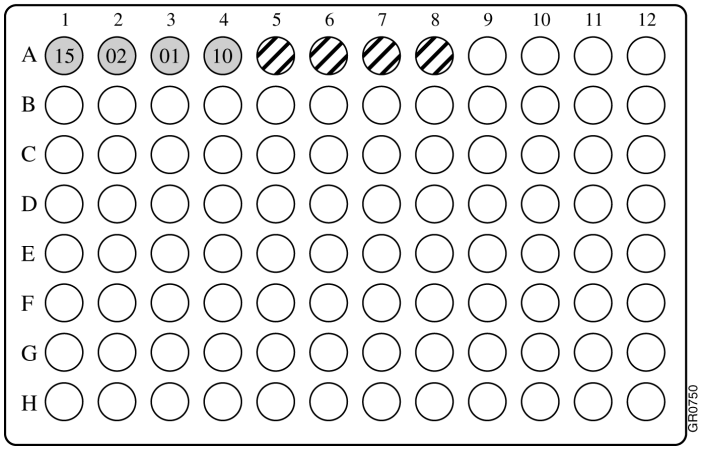


Figure 3-9 Kit B pooled PCR products in positions A1 through A4. Matrix standard samples in positions A5 through A8.

Minimum Sample Volume

The minimum sample volume per tube is 10-μL. If you choose to use 10-μL samples, routine autosampler calibration is necessary (see "How to Calibrate the Autosampler" on page 4-27).

You can use a single sample for more than one analysis. Either inject out of one sample tube repeatedly, or split the sample into several tubes.

About Quantitating and Diluting the PCR Mixture

Unlike DNA sequencing samples, samples requiring GeneScan® Analysis Software are not typically quantitated on a spectrophotometer prior to loading on the 310 Genetic Analyzer.

The 12:1 ratio of formamide-size standard:PCR sample mix is usually an adequate dilution. If the peaks go off-scale at this dilution, further dilute the mixture.

How to dilute the PCR mixture

Step	Action
1	Transfer 1-μL of the 12:1 formamide-size standard:PCR sample mix to 9-μL of formamide in a sample tube.
2	Cover the tube with a septum and vortex well.
3	Heat the mixture for 2 minutes at 95°C and place it on ice until ready to place in the instrument.

How to Prepare the Sample Sheet and Injection List

Purpose

The Sample Sheet associates sample information (name and type of analysis) with a sample tube position in the autosampler.

The Injection List specifies the order for running samples, how many injections are made from each sample, and the module and running conditions for each injection.

Preparing the Sample Sheet

About Preparing the Sample Sheet

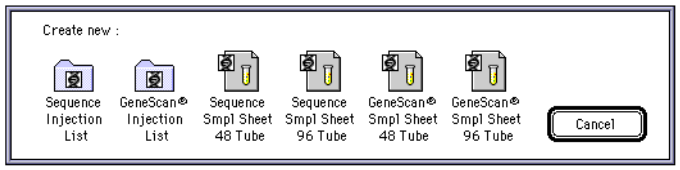
The first time you use Kit A or B of the Fluorescent Genotyping Demonstration Kit, or any group of samples, you must create a Sample Sheet.

Note This chapter takes the Fluorescent Genotyping Demonstration Kit as a detailed example, and the illustrations for the procedure below are from a Fluorescent Genotyping Demonstration Kit Sample Sheet. When you fill out Sample Sheets for other types of experiments to be analyzed with the GeneScan® Analysis Software, choose the colors present and color of the standard that reflect the set up of your samples.

Save the Sample Sheet in the Sample Sheet folder on the Macintosh® computer's hard drive. If you use the same type of kit or group of samples again, select the previously created Sample Sheet. If there is already a Sample Sheet for the kit in the Sample Sheet folder, see "Preparing an Injection List" on page 3-37.

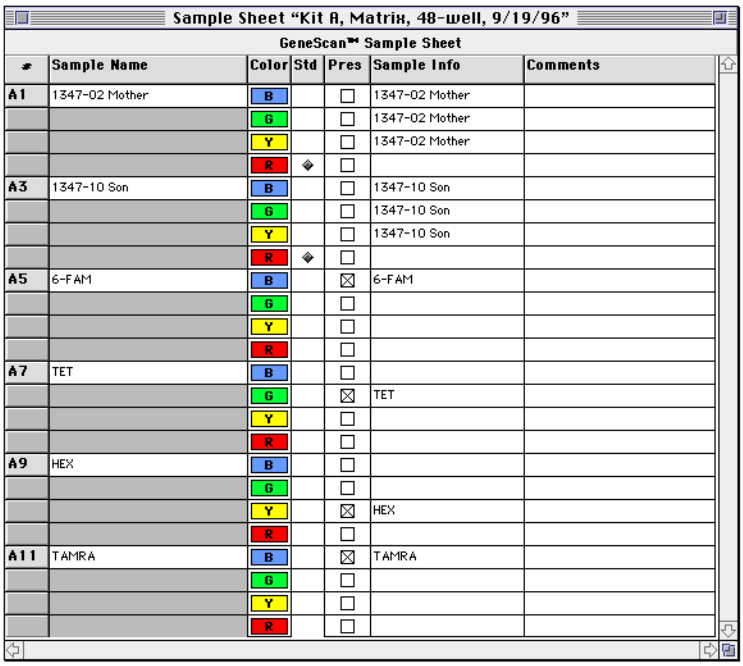
Creating a New Sample Sheet

To prepare a new Sample Sheet:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>In the ABI PRISM® 310 Data Collection Software, select New from the File menu.</td></tr>
<tr><td>2</td><td>Click GeneScan Smpl Sheet 48 or 96 Tube as appropriate.
 
</td></tr>
<tr><td>3</td><td>Refer to these examples to see how a Sample Sheet is filled out:
<table>
<tr><th>If you are using...</th><th>Then...</th></tr>
<tr><td>Kit A</td><td>♦ Prepare the Sample Sheet as shown in Figure 3-10 on page 3-35.
♦ Include the matrix standard samples if you need to create a matrix file.</td></tr>
<tr><td>Kit B</td><td>♦ Prepare the Sample Sheet as shown in Figure 3-11 on page 3-36.
♦ Include the matrix standard samples if you need to create a matrix file.</td></tr>
</table></td></tr>
<tr><td>4</td><td>Check the Pres column when a sample is present for that color.</td></tr>
</table>

To prepare a new Sample Sheet: *(continued)*

Step	Action
5	Put a diamond in the Std column next to the color for the standard. **IMPORTANT** Do not specify a color in the standard (Std) column for the matrix samples. **Note** Remember, the tube arrangement in the tray and the order of the samples on the Sample Sheet must be the same.
6	Open the File pull-down menu, and select Save As. Name the Sample Sheet, and press Return to save it in the Sample Sheets folder.

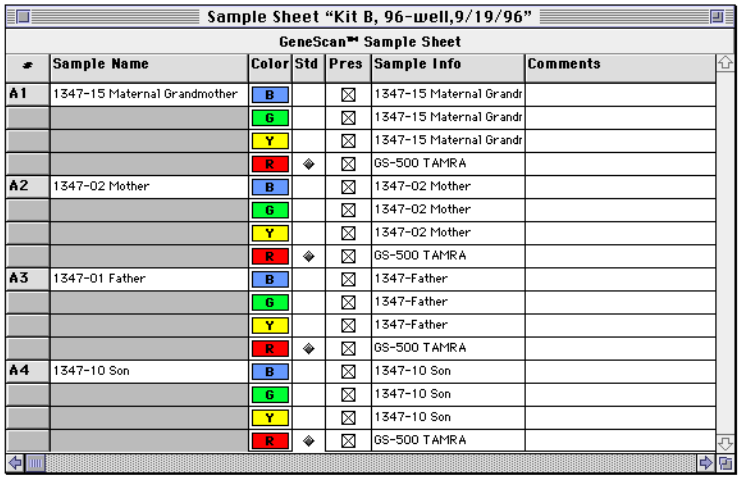


Sample Sheet "Kit A, Matrix, 48-well, 9/19/96"

GeneScan™ Sample Sheet

*	Sample Name	Color	Std	Pres	Sample Info	Comments
A1	1347-02 Mother	B		☐	1347-02 Mother	
		G		☐	1347-02 Mother	
		Y		☐	1347-02 Mother	
		R	◆	☐		
A3	1347-10 Son	B		☐	1347-10 Son	
		G		☐	1347-10 Son	
		Y		☐	1347-10 Son	
		R	◆	☐		
A5	6-FAM	B		☒	6-FAM	
		G		☐		
		Y		☐		
		R		☐		
A7	TET	B		☐		
		G		☒	TET	
		Y		☐		
		R		☐		
A9	HEX	B		☐		
		G		☐		
		Y		☒	HEX	
		R		☐		
A11	TAMRA	B		☒	TAMRA	
		G		☐		
		Y		☐		
		R		☐		

Figure 3-10 Sample Sheet for Kit A with matrix standard samples when using a 48-well tray. Note the size standard is specified for Kit A samples only, not for the matrix standard samples.



Sample Sheet "Kit B, 96-well,9/19/96"

GeneScan™ Sample Sheet

#	Sample Name	Color	Std	Pres	Sample Info	Comments
A1	1347-15 Maternal Grandmother	B		☒	1347-15 Maternal Grandr	
		G		☒	1347-15 Maternal Grandr	
		Y		☒	1347-15 Maternal Grandr	
		R	◆	☒	GS-500 TAMRA	
A2	1347-02 Mother	B		☒	1347-02 Mother	
		G		☒	1347-02 Mother	
		Y		☒	1347-02 Mother	
		R	◆	☒	GS-500 TAMRA	
A3	1347-01 Father	B		☒	1347-Father	
		G		☒	1347-Father	
		Y		☒	1347-Father	
		R	◆	☒	GS-500 TAMRA	
A4	1347-10 Son	B		☒	1347-10 Son	
		G		☒	1347-10 Son	
		Y		☒	1347-10 Son	
		R	◆	☒	GS-500 TAMRA	

Figure 3-11 Sample Sheet for Kit B without matrix standard samples when using a 96-well tray

Using a Previously Created Sample Sheet

If you are running the same group of samples for a second or third time, you can use the original Sample Sheet. Select the previously created Sample Sheet when filling out the Injection List.

To use a previously created Sample Sheet:

Step	Action
1	Open the Sample Sheet. It is stored in the Sample Sheet folder on the Macintosh® hard drive.
2	Make changes and save the Sample Sheet. You can take the following action:
3	Select the modified Sample Sheet when filling out the Injection List.

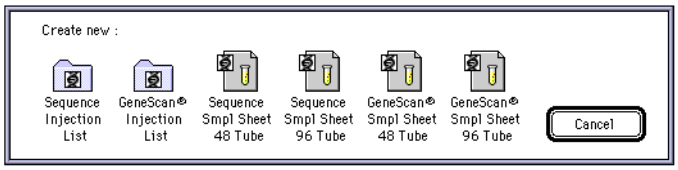
If you want to...	Then...
keep the original Sample Sheet	enter a new name.
overwrite the original	choose Save without renaming the Sample Sheet.

continued on next page

Preparing an Injection List

Note This chapter takes the Fluorescent Genotyping Demonstration Kit as a detailed example, and the illustrations for the procedure below are from a Fluorescent Genotyping Demonstration Kit Injection List. When you fill out Injection Lists for other types of experiments to be analyzed with the GeneScan® Analysis Software, choose the module and matrix files appropriately.

To prepare an Injection List:

Step	Action
1	In the ABI PRISM® 310 Data Collection Software, select New from the File menu. The following box of icons is displayed. 
2	Select GeneScan Injection List.
3	Complete the Injection List as follows. Sample Injection Lists for Kits A and B are shown in Figure 3-12 and Figure 3-13 on page 3-37. a. Open the Sample Sheet pop-up menu, and select the Sample Sheet prepared for the kit you are using. b. Enter your name as the Operator. c. Open the Module pop-up menu for each sample, and select GS STR POP4 (2.5-mL) C, or GS STR POP4 (1.0-mL) C if using the 1.0-mL glass syringe. d. Leave the remaining parameters at their default settings.

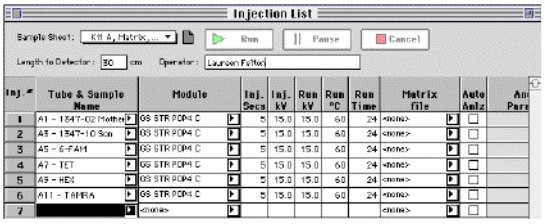


Figure 3-12 Injection List for Kit A with matrix standard samples when using a 48-well tray

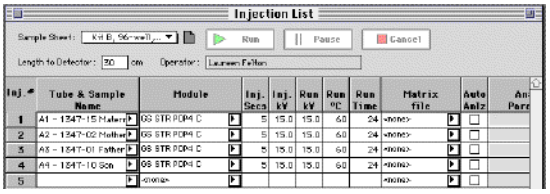


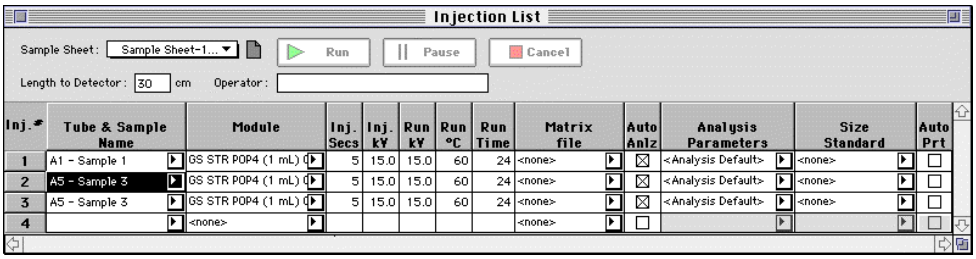
Figure 3-13 Injection List for Kit B without matrix standard samples when using a 96-well MicroAmp tray

continued on next page

Making Multiple Injections from One Sample Tube

The default setting for the Injection List assumes that each sample will be injected once. If you prefer multiple injections, modify the Sample Name column so that the sample appears multiple times.

Step	Action
1	In the example used here, the original Sample Sheet has been modified to inject Sample 3 twice.



Step	Action
2	For the injection you are modifying, click the arrow next to the Tube & Sample Name.
3	When a pop-up menu of the original sample list appears, select the sample you want to inject. Figure 3-14 and Figure 3-15 show the sequence for editing the injection. Instead of injecting a sample in position A3 of the autosampler as the second injection, Sample 3 in position A5 will be the second injection.

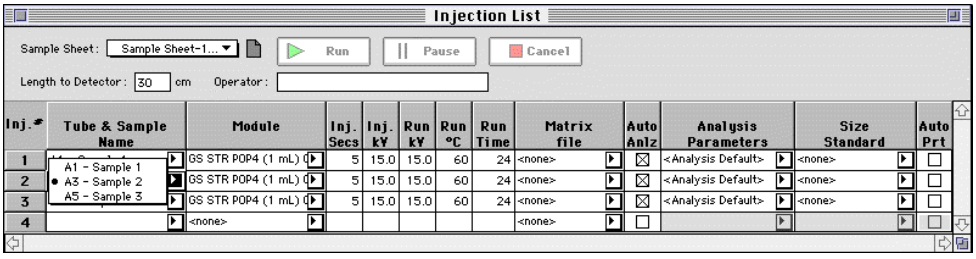


Figure 3-14 Select Sample

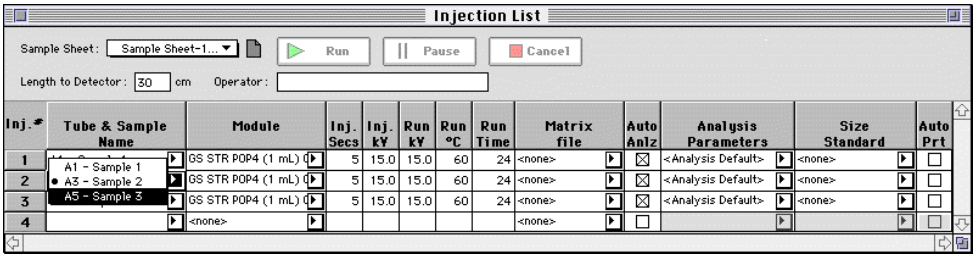


Figure 3-15 Change Sample for Third Injection

continued on next page

Modifying an Injection List

Adding Rows

The Injection List has the same number of rows as the Sample Sheet. To add rows, highlight a row, press Command-I, and a new row will be inserted above the highlighted row.

Changing Run Parameters

As you select a module file for each injection, the run parameters are filled in according to the values in the module. You can edit any parameter by clicking in the field and typing in the new value. Click another field or press Enter to save the change.

Changing the Order of the Samples in a Run

The default setting for the Injection List assumes that each sample will be injected in the order listed in the Sample Sheet. If you prefer a different injection order, modify the Sample Name column.

Testing the Capillary Window

Run a test to ensure that the capillary window is clean and positioned correctly before running your samples:

Step	Action
1	Insert a line into the Injection List before the first sample. See “Modifying an Injection List” on page 3-39 for instructions, if necessary.
2	Set the module to Test CCD 4-Color.
3	Change the collection time from 5 minutes to 1 minute.
4	When the module runs, check the raw data for excessive noise. The ideal baseline falls at or below about 2068. Use the following table to help determine whether the capillary is positioned correctly.

If...	Then...
the baseline is too high	remove the capillary and clean the capillary window with a lab wipe and dampened ethanol.
there is a single blue line at 8000 or at the bottom of the window	the capillary window is not positioned in the detector.

Starting the Run

Click the Run button in the Injection List to start the run.

Note If you did not preheat the instrument as suggested under “Preheating the Heat Plate” on page 3-13, it can take up to 30 minutes for the instrument to heat to 60 °C and begin electrophoresis

Monitoring the Run

During the run, you can monitor the run and your samples four ways. From the Window menu, select one of the following:

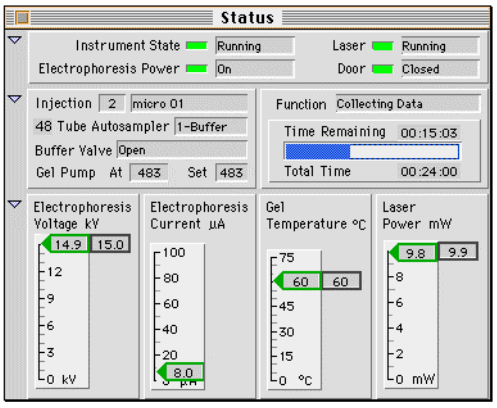


Figure 3-16 Status

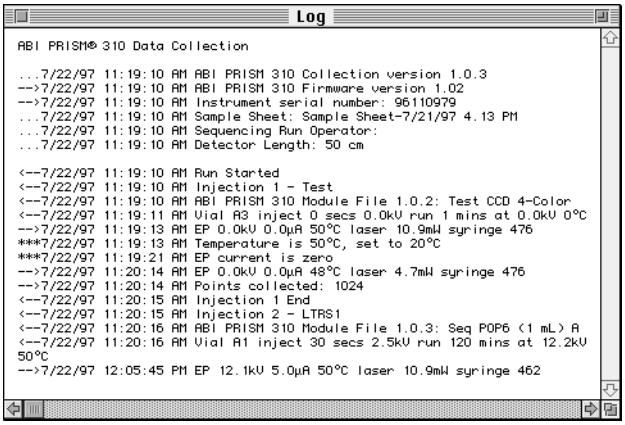


Figure 3-17 Log

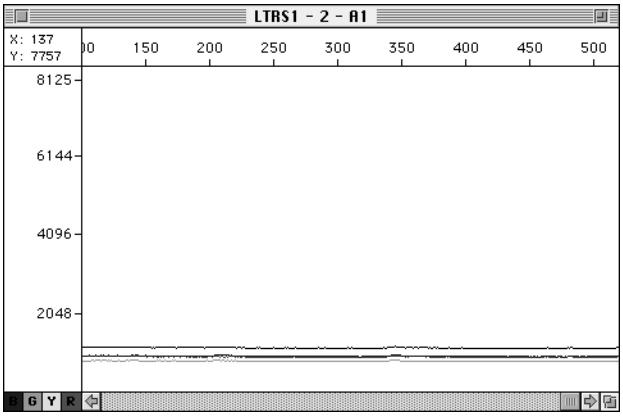


Figure 3-18 Raw Data

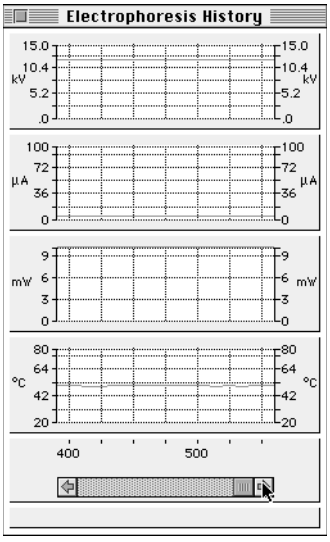


Figure 3-19 Electrophoresis History

How to Create the GeneScan Matrix File

Overview

The matrix file contains the information necessary for software to correct the overlap of the dyes emission spectra on the virtual filter. Once a matrix file has been created, it can be used for subsequent runs performed:

- With the same kit or chemistry
- On the same instrument
- Using the same:
  - Run modules
  - Set of dyes
  - Polymer

After running the matrix standards, use their sample files to generate a matrix file using GeneScan® Analysis Software.

Verifying the Raw Data

Before creating the matrix file, verify that the raw data from the standards is good.

Viewing the Raw Data in the GeneScan® Analysis Software

Step	Action
1	Create a new project: a. Choose New from the File menu. b. Select the Project icon. An untitled Analysis Control window opens. c. Choose Add Sample Files from the Project menu. d. Find and open the Run Folder for the matrix standards run. e. Select the four Sample files representing the blue, green, yellow, and red dye-labeled “runs,” and then click Add. f. Click Done after the Sample files are transferred.
2	In the Analysis Control window, select the four matrix standard Sample files by clicking on the first Sample file, holding down the mouse button, and releasing on the last Sample file.
3	Choose Raw Data from the Project menu. Electropherograms displaying raw data from the four matrix standard Sample files appear.

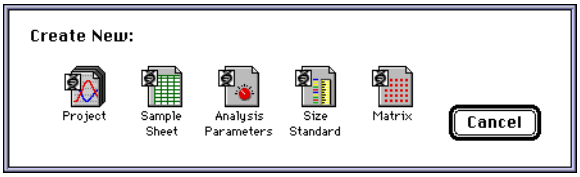
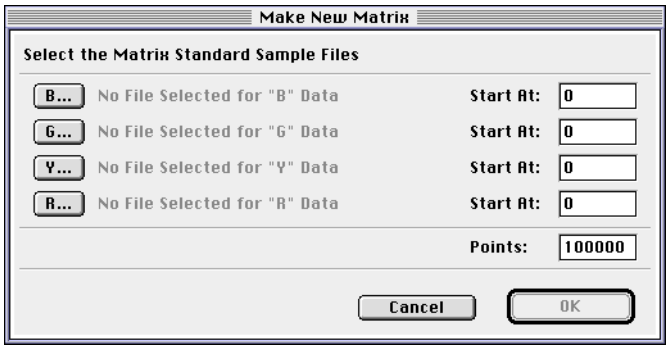
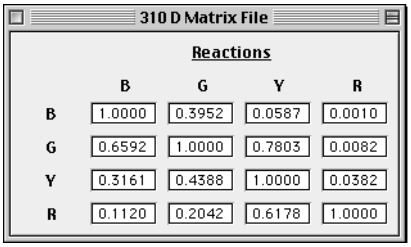
Verifying the Raw Data

Step	Action
1	Verify data peaks are present in all four samples. Peak data should be on-scale and the dye of interest should have a value of at least 200.
2	Check for any data anomalies, such as an unstable baseline. Rerun samples that have an unstable baseline.
3	Select a starting point for the matrix data. The starting point for matrix data should be slightly beyond the point where the primer peak falls back to the baseline.

continued on next page

Generating The Matrix File

To generate the matrix files:

Step	Action
1	Choose New from the File menu. 
2	Click the Matrix icon. This opens the Make New Matrix dialog box. 
3	Click the B, G, Y, and R buttons to choose the standard sample files. Choose the sample file representing blue dye for B, green dye for G, etc.
4	Enter the starting point for each file. The Start At point should be after the primer peak. If necessary: ♦ Modify the default setting of 2000. ♦ Modify the Points value. This is the number of points after the start point to be analyzed.
5	Click OK. A successful matrix opens an untitled Matrix Values window with a 4x4 matrix of numerical values. 
6	Use the Save As command to name and save the matrix file. Choose a name that reflects the chemistry and run conditions.

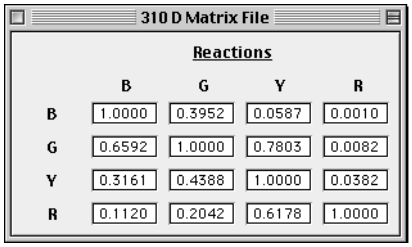
continued on next page

Checking Matrix Quality

Check the quality of the matrix by reviewing the:

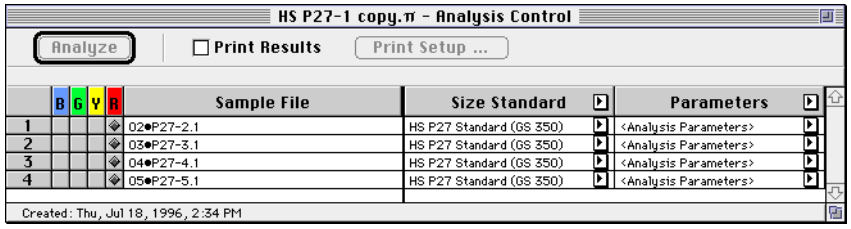
- Values in the Matrix Values window.
- Analyzed data of the matrix run.

Reviewing the Matrix Values in the Matrix Values Window

Step	Action
1	View the Matrix Values window. 
2	The numbers on the diagonal (Blue against Blue, Green against Green, etc.) must all be 1.00. The numbers off the diagonal are less than 1.00. In Virtual Filter C, Green under Blue is sometimes slightly above 1.00. This is acceptable.

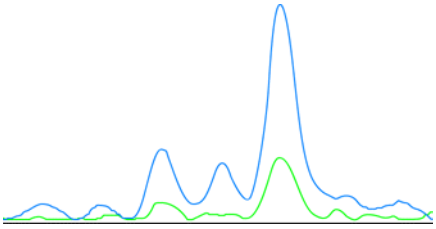
Checking the Matrix Quality

To change matrix quality:

Step	Action
1	From the Project containing your matrix standard Sample files, open the Analysis Control window. In the Analysis Control window, select the colors for each sample. 
2	Select the four matrix standard Sample files.
3	Choose Assign New Matrix in the Project menu and select the matrix file.
4	Select numbers 1, 2, 3, and 4 on the left side of the window to highlight the colors for each row.
5	Use the Set Analysis Parameters dialog box in the Settings menu to set the Analysis Range and click OK to return to the Analysis Control window. A typical setting is 2000.
6	Click Analyze.

To change matrix quality: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>7</td><td colspan="2">Choose Results from the Windows menu and check each electropherogram by taking the following action:

<table><tr><th>Clicking</th><th>In the</th></tr><tr><td>4</td><td># of Panels pop-up menu.</td></tr><tr><td>1</td><td>Dye Samples.</td></tr><tr><td>#1</td><td>on the Sample Files side of the Results window.</td></tr></table></td></tr>
<tr><td rowspan="3">8</td><td>If…</td><td>Then the matrix is…</td></tr>
<tr><td>each peak is one color with the other colors flat under it</td><td>good.</td></tr>
<tr><td>the other colors are not flat under the peaks:
  </td><td>poor.</td></tr>
<tr><td rowspan="3">9</td><td>If the matrix is…</td><td>Then…</td></tr>
<tr><td>good</td><td>save the matrix file to the ABI folder.</td></tr>
<tr><td>poor</td><td>re-analyze the matrix run.

If this does not improve the matrix data, run new matrix standards.</td></tr>
</table>

Analyzing The Data

Automatically Analyze Data

GeneScan® Analysis Software automatically analyzes the samples when the run finishes if the automatic analyze checkbox on the Sample Sheet is checked.

See the *ABI PRISM GeneScan Analysis Software User's Manual* for more information.

Procedures for Operation 4

Introduction

In This Chapter Topics in this chapter include the following:

How to Turn the Genetic Analyzer On and Off

Turning On the Instrument Without a Capillary Installed

Follow these steps anytime you turn on the 310 Genetic Analyzer without a capillary installed:

Step	Action
1	Turn the 310 Genetic Analyzer on.
2	Check the LEDs on the front. The green light should be illuminated.
3	Turn on the Macintosh® computer.
4	Open the Manual Control window, choose Syringe Home and click Execute.
5	In the Manual Control window, do the following: ♦ Choose Autosampler Home X,Y and click Execute. ♦ Choose Autosampler Home Z and click Execute.

Turning On the Instrument With a Capillary Installed

Follow these steps anytime you turn on the 310 Genetic Analyzer with a capillary installed:

Step	Action
1	The capillary is inserted in a buffer vial on the autosampler when it is stored on the instrument. Remove the capillary from the buffer tube.
2	Thread the capillary through the electrode thumbscrew. It should extend 0.5 mm beyond the electrode. See “Positioning the Capillary Near the Electrode” on page 4-14.
3	Turn on the 310 Genetic Analyzer.
4	Check the LEDs on the front. The green light should be illuminated.
5	Turn on the Macintosh® computer.
6	Open the Manual Control window, choose Syringe Home, and click Execute.
7	In the Manual Control window, do the following: ♦ Choose Autosampler Home X,Y and click Execute. ♦ Choose Autosampler Home Z and click Execute.
8	Calibrate the autosampler. See “Calibrating the Autosampler” on page 4-28.

Electrodes Overview

Anode and Cathode

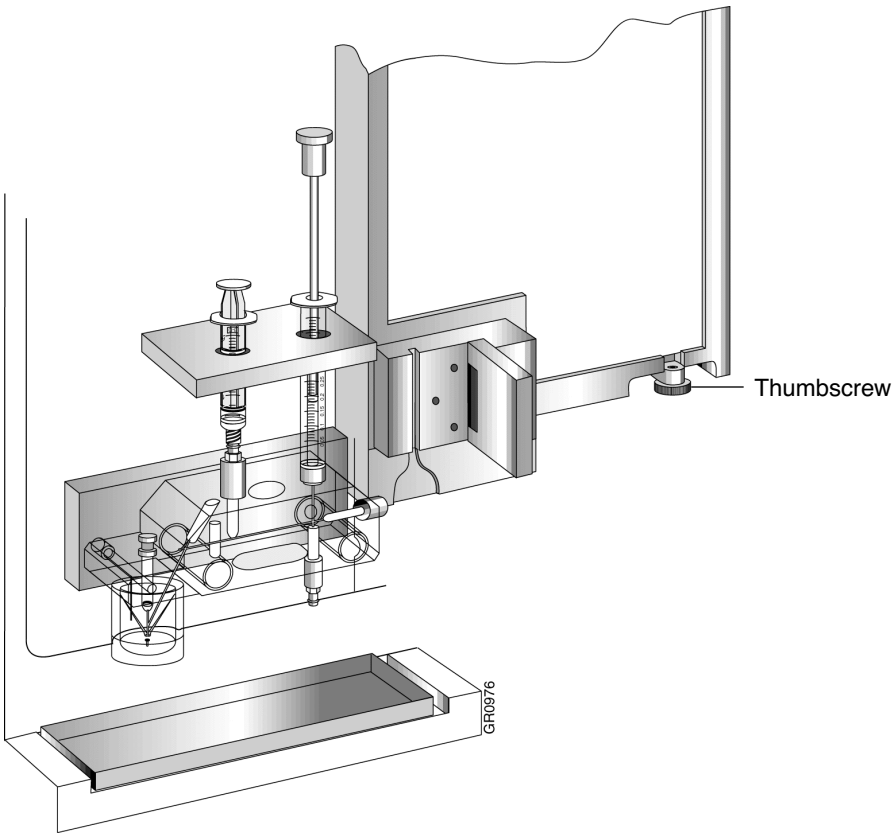
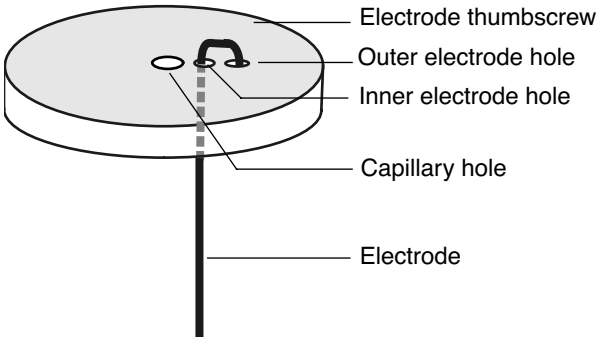
Both electrodes are platinum.

Electrode	Description
Anode	This electrode is on the left as you face the instrument, and is always immersed in the electrophoresis buffer.
Cathode **Note** Always recalibrate the autosampler if the cathode electrode is removed, replaced, or cleaned.	This electrode is a U-shaped wire located above the autosampler. It is placed in the sample during electrophoresis. You can remove and replace the cathode electrode.

How to Prepare the Cathode Electrode

Installing the Electrode

To Install the electrode:

Step	Action
1	Remove the capillary if it is on the instrument.
2	Unscrew and remove the cathode electrode thumbscrew. 
3	There are three holes in the electrode thumbscrew: the capillary hole, an inner electrode hole, and an outer electrode hole (See Figure 4-1). Put the long arm of the cathode electrode in the inner electrode hole.  **Figure 4-1** Electrode inserted in the electrode thumbscrew (top view)

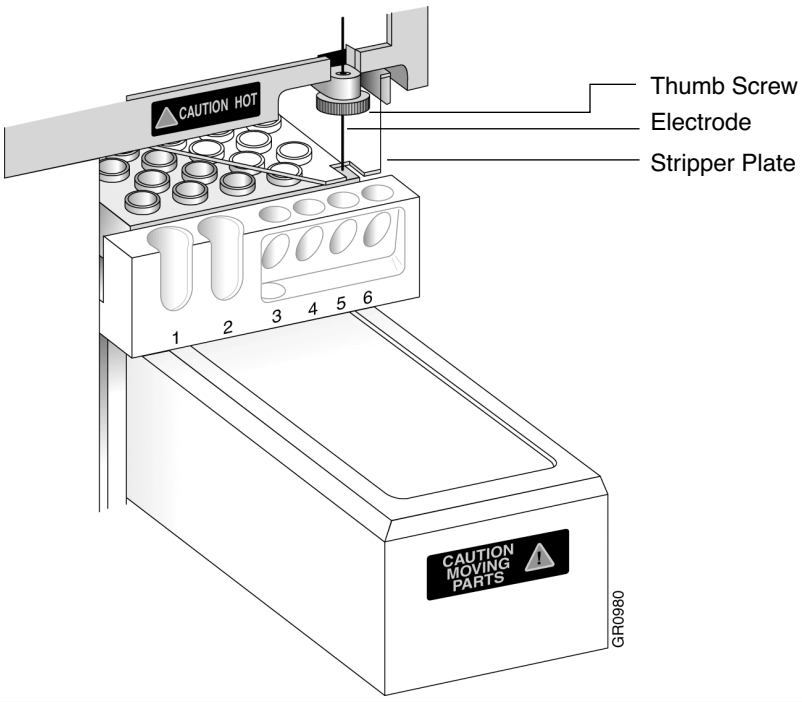
To Install the electrode: *(continued)*

Step	Action
4	Put the short arm of the electrode in the outer hole.
5	Push the electrode down until it is flush with the top of the electrode thumbscrew.
6	Screw the electrode thumbscrew back onto the instrument.
7	Recalibrate the autosampler after cleaning, trimming or replacing the electrode.

Trimming the Electrode

It may be necessary to trim a new electrode so that the end is flush with the stripper plate.

To trim the electrode:

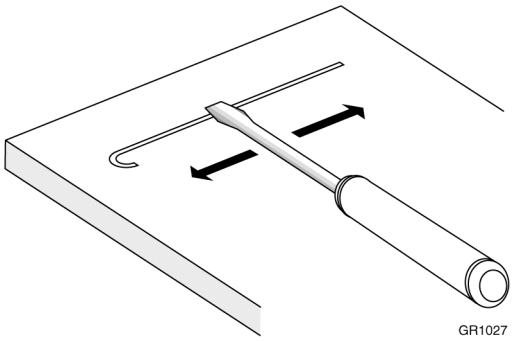
Step	Action
1	From the Manual Control window, select the Function pop-up menu and choose Home Z-Axis.
2	Use the wire cutter (P/N T-6157) provided in the instrument packing kit. Hold the cutters with the flat cutting face toward the top of the instrument. **Note** The wire cutter provided in the instrument packing kit is a flush-cutting wire cutter. Do not use other types of wire cutter.
3	Cut the electrode flush with the lower surface of the stripper plate. Do not flex the stripper plate upwards while cutting. Do not cut the electrode too short. 
4	Recalibrate the autosampler after cleaning, trimming or replacing the electrode.

continued on next page

Straightening the Electrode

Note Recalibrate the autosampler after installing an electrode.

To straighten the electrode, if the cathode electrode is bent:

Step	Action
1	Remove the electrode from the electrode thumbscrew.
2	Lay it on a flat surface.
3	Rub a flatblade screwdriver back and forth along the length of the electrode to straighten it.  GR1027

When to Clean the Electrode

Clean the electrode if it has been touched, if it is new, when autosampler buffer solutions are replaced, or every 48 hours. Crystals formed on the electrode can fall into the sample and clog the capillary.

Since the capillary is removed from buffer while the electrode is cleaned, complete the procedure quickly, so that the capillary does not dry out.

Cleaning the Electrode

To clean the electrode:

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software.
2	Press the Tray button on the 310 Genetic Analyzer to lower the autosampler and present the tray.
3	Wipe the electrode with lint-free paper that has been dampened with distilled, deionized water
4	Dry the electrode with fresh lint-free paper.
5	Press the Tray button to return the autosampler to its original position and immerse the capillary in buffer.
6	Recalibrate the autosampler after cleaning, trimming or replacing the electrode.

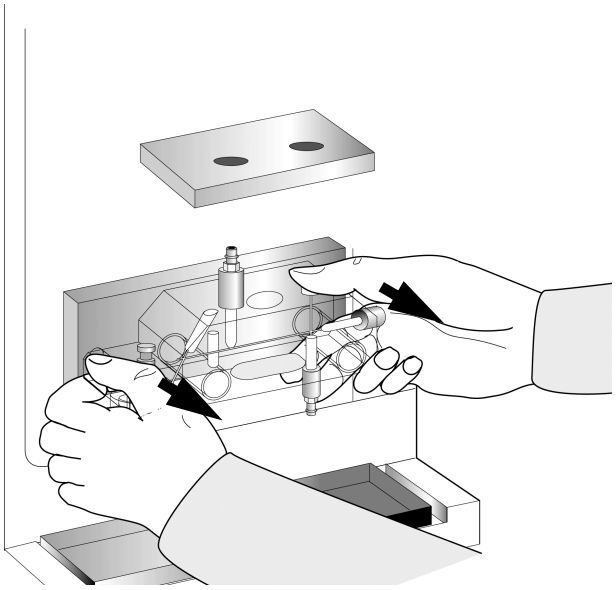
How to Remove the Pump Block

Removing the Pump Block

The pump block rides on two steel shafts. It slides freely after a spring plunger in the block moves past an indentation on one of the steel shafts.

For a diagram of the inside of the instrument, see “Instrument Hardware: Behind the Doors” on page 1-8.

To remove the pump block:

Step	Action
1	Disconnect the glass syringe plunger from the syringe drive.
2	Open the Manual Control window and move the syringe drive up.
3	Unscrew the glass syringe and plastic syringe.
4	Remove the capillary.
5	Remove the buffer reservoir by gently twisting and pulling it straight down.
6	Remove the pump block by pulling it straight toward you. 

How to Clean the Pump Block

When to Clean the Pump Block

Clean the pump block when:

- Installing the syringe.
- Removing the capillary.
- Changing between the ABI PRISM® DNA Sequencing Analysis Software and the GeneScan® Analysis Software.
- Shutting down the instrument.

IMPORTANT The pump block can be irreversibly damaged if polymer dries in the channels.

It is especially important to clean the pump block if a syringe containing polymer was installed on the block for more than 1 week. At room temperature, sufficient urea decomposition occurs in 1 week to cause transient current increases during electrophoresis.

Cleaning the Pump Block

IMPORTANT Do not expose the pump block to any organic solvents.

To clean the pump block:

Step	Action
1	Remove the pump block from the instrument.
2	Open all valves and ports on the pump block.
3	Hold the pump block under warm running water to thoroughly rinse it.
4	Take the following actions: ♦ Force warm water through the channels with the 5-cc plastic syringe provided in the Basic Installation Kit. ♦ Direct the flow of water through each channel in turn by sealing channel openings with plugs provided in the Basic Installation Kit. ♦ Rinse each channel five times. **Note** If a 30-cc syringe is available, rinse each channel with it once. This can be more convenient than rinsing five times with a 5-cc syringe.
5	Rinse the valves with warm water. Soak valves that are coated with dried polymer.
6	Visually inspect the channels for dried polymer, which looks like white residue. Wash the channels until the polymer is gone.
7	Rinse the block and its channels with distilled, deionized water.
8	Take the following actions: ♦ Remove residual water from the pump block and fittings to ensure that the running polymer is not diluted. ♦ Force air through the channels, using the plastic syringe or canned compressed air, until the channels are dry.
9	Replace the pump block and buffer reservoir.

How to Replace the Pump Block

Replacing the Pump Block

To replace the pump block:

Step	Action
1	Verify that the gold electrode socket on the back of the block is dry.
2	Align the steel shafts with the two large holes of the block.
3	Before sliding the pump block all the way to the back of the shafts, align the anode buffer reservoir valve with the activator arm.
4	Slide the U-shaped end of the activator arm into the collar at the top of the valve's plunger.
5	Lift the plunger about 3 mm, if necessary, and slide the pump block until flush against the instrument.

How to Fill the Pump Block

Filling the Pump Block

To fill the pump block channels with polymer:

Step	Action
1	Open the Manual Control window and select Buffer Valve Close in the Function pop-up menu. This closes the pin valve at the anode buffer reservoir on the pump block.
2	Manually open the waste valve below the syringe.
3	Press the syringe plunger until a drop of polymer forms on the bottom of the waste valve. This removes the air bubbles at this valve site, and uses about 0.1-mL of polymer.
4	Manually close the waste valve.
5	Open the Manual Control menu and select Buffer Valve Open in the Function pop-up menu This opens the pin valve at the anode buffer reservoir on the pump block.
6	Press the syringe plunger until polymer fills the polymer channel in the block. This removes all of the air bubbles from the polymer channels, and should use about 0.1-mL of polymer. **IMPORTANT** There should be no air bubbles in the pump block channels.
7	Select Buffer Valve Close in the Function pop-up menu. **Note** If using the 250-μL syringe, you may have to refill the syringe now.
8	Move the syringe drive toggle to the right to position it over the syringe plunger.
9	Select Syringe Down in the Function pop-up menu.
10	Select 50-step intervals and click Execute until the toggle makes contact with the syringe plunger.

Capillary Overview

About the Capillary

Do not expose the ends of a polymer-filled capillary to air for more than 30 minutes. This allows the polymer to dry and adversely affects capillary performance. Make sure the autosampler is moved back and the capillary immersed in buffer as soon as samples are loaded.

Be careful when handling the capillary. The capillary window for the detector is very fragile.

If maintained properly, one capillary can be used for at least 100 electrophoretic separations.

Why Cut the Capillary

For some applications, you may want to reduce the length of the 61cm or 47 cm capillary to reduce the run time. A capillary cutter (P/N 401958) is available for this purpose. Capillary cutters are small, flat squares of a hard ceramic material.

You may want to practice this technique on an old capillary before cutting new capillaries.

Cutting the Capillary

To cut a capillary:

Step	Action
1	Scratch the capillary with the capillary cutter using one firm, even stroke.
2	Break the capillary at the scratch mark on the glass.
3	Examine the capillary. The cut end must be flat, without jagged edges.

Cleaning the Capillary Window

Clean the capillary window with a lint-free laboratory wipe moistened with ethanol.

How to Install the Capillary on an Empty Pump Block

What to Do First

The capillary can break at the detector window if twisted. Always connect the capillary to the pump block first, because the capillary will turn slightly as you connect it there. If the other end is not free to move, the capillary will twist and break.

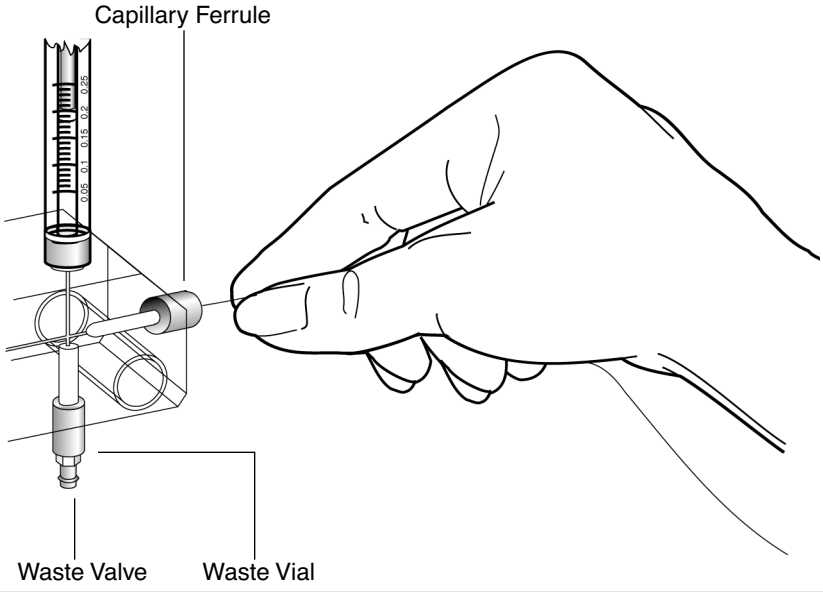
The pump block should be clean.

Removing Capillaries from the Packaging

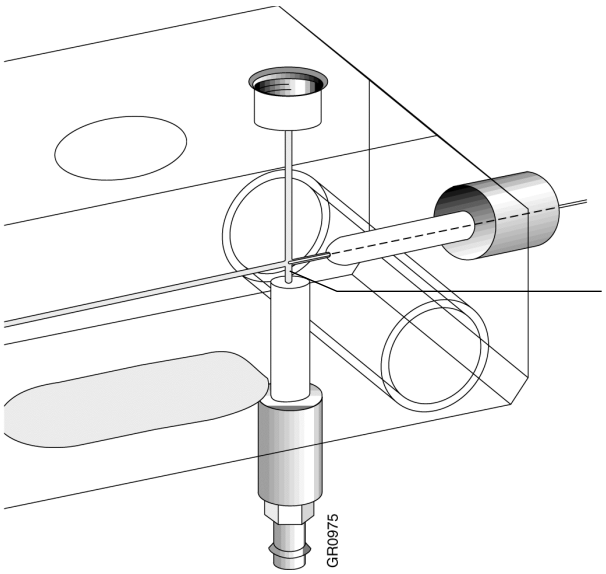
The capillaries are shipped in a curved plastic tube. Remove the capillaries by grasping one capillary between your thumb and forefinger, and then pulling and turning the capillary simultaneously.

Connecting the Capillary to the Pump Block

To connect the capillary to the pump block:

Step	Action
1	Clean the capillary window with an ethanol-dampened lab wipe.
2	Open the door covering the heat plate.
3	Remove the plastic capillary fitting on the right side of the pump block.
4	Partially screw the capillary fitting back into the pump block. **IMPORTANT** Do not tighten the fitting at this point in the procedure, or the opening at the tip will be crushed. The capillary must be properly inserted through the opening before you tighten the fitting.
5	Thread one end of the capillary through the capillary ferrule. 

To connect the capillary to the pump block: *(continued)*

Step	Action
6	Adjust the end of the capillary so that it is positioned directly below the opening to the glass syringe. It is also acceptable to position the end of the capillary between the intersection of the channels and the capillary fitting. **IMPORTANT** The end of the capillary must protrude well beyond the opening at the tip of the capillary fitting. To avoid crushing the opening, be certain that you see the capillary in the pump block channel before proceeding to the next step. 
7	Tighten the capillary fitting finger-tight to secure the capillary. CAUTION **Overtightening can cause cracks in the gel block which will result in polymer leakage.** **IMPORTANT** The capillary will twist as the fitting is tightened. Leave the other end of the capillary free to twist, or the capillary will break.

Positioning the Capillary in the Detector

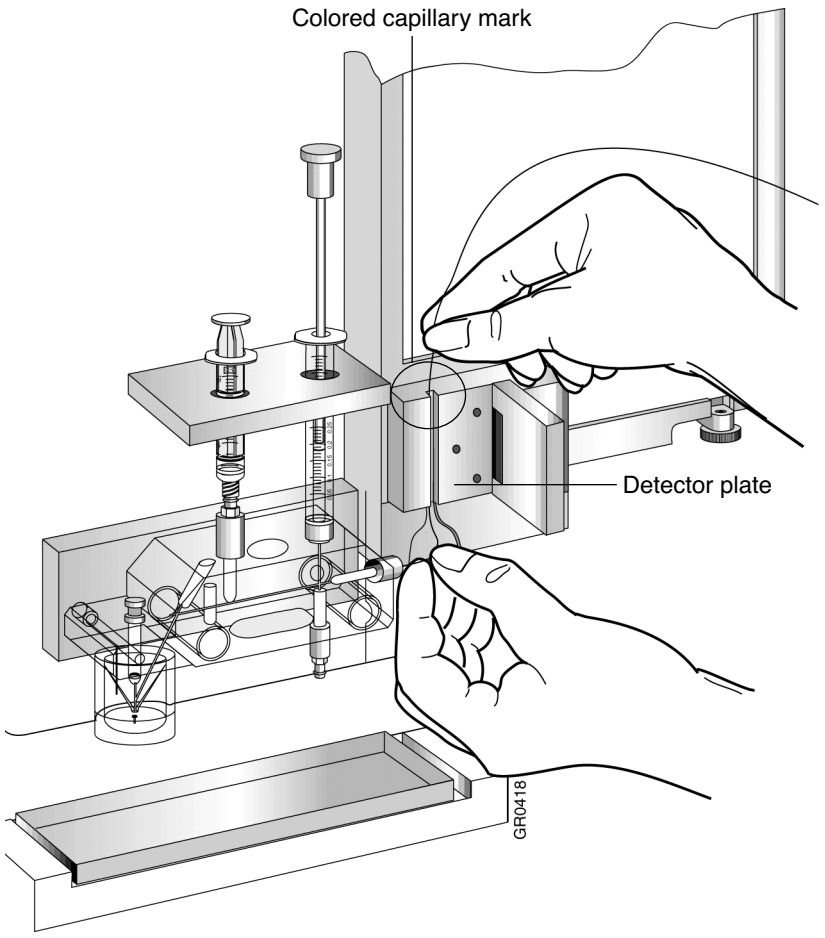
The central portion of the capillary window should rest over the laser detector window without putting strain or tension on the capillary. The window is fragile; be careful not to break it when you fit the capillary into its track in the detector.

Align the colored labelling mark on the capillary with the top edge of the detector plate and laser detector door. This will position the capillary window over the laser detector opening.

continued on next page

Procedure for Positioning the Capillary in the Detector

To position the capillary:

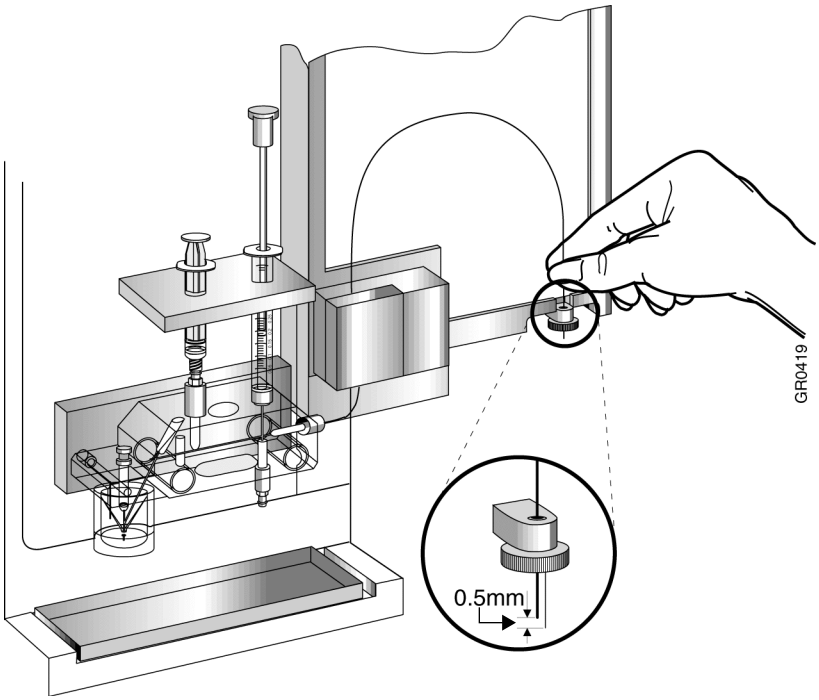
Step	Action
1	Open the laser detector door, and position the capillary in the vertical track of the detector. 
2	Tape the capillary to the heat plate with thermal tape to secure the position of the capillary labelling mark (colored capillary mark) relative to the detector plate. For a diagram showing how the capillary is taped to the heat plate, see “Detection Region Diagram” on page 1-11.
3	Close the laser detector door to secure the position of the capillary window.

continued on next page

Positioning the Capillary Near the Electrode

The capillary and cathode electrode should be as close to each other as possible. The capillary should protrude beyond the electrode a maximum of 0.5 mm.

To position the electrode end of the capillary:

Step	Action
1	Thread the capillary through the capillary hole in the electrode thumbscrew until it protrudes past the tip of the electrode by about 0.5 mm. 
2	Tape the capillary to the heat plate with thermal tape to secure the position of the capillary tip relative to the electrode. Tape the capillary just above the electrode thumbscrew and just above the detector door. **IMPORTANT** To avoid damaging the 61 cm sequencing capillary, verify that the entire capillary is secured to the heat plate before closing the heat plate door.
3	Close the door over the heat plate.
4	With the heat plate door closed, check that the capillary has not moved relative to the electrode.

Resetting the Injection Counter

Reset the injection counter as follows:

Step	Action
1	Open the Instrument window and choose Change Capillary.
2	Click OK in the Reset window to set the injection counter to zero.

continued on next page

After the Capillary is Installed

Calibrate the autosampler after installing a capillary (see “How to Calibrate the Autosampler” on page 4-27).

Do not turn the capillary fitting with the detector door closed. Torque on the capillary can break it. Release the capillary from the detector if the capillary fitting needs adjustment.

How to Remove the Capillary for Storage

Removing the Capillary for Short-term Storage

Use this procedure if the capillary will be re-installed within one week.

Step	Action
1	Run the “Seq Fill Capillary” module in Manual Control to remove any sample still in the capillary
2	Leave the polymer in the capillary.
3	Remove the capillary by following the installation steps in reverse order.
4	Clean the pump block.

Removing the Capillary for Long-term Storage

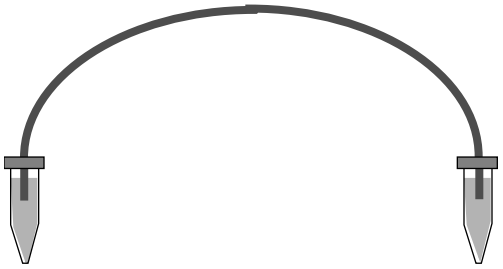
To remove the capillary when it will not be re-installed within one week:

Step	Action
1	Flush the capillary free of polymer as follows: a. Rinse the pump block. b. Fill the glass syringe with 200-µL of water. c. Close all valves. d. Move the syringe drive to position 250. e. Run the Seq Fill Capillary module from the Manual Control window to force the polymer out of the capillary. It requires about 6 minutes to force the polymer from the capillary. **Note** When the capillary is completely flushed of polymer, the water flowing through the capillary may trigger the leak detector. If this happens, click OK in the Pause Alert window, resume the run from the menu, and immediately cancel the run.
2	Remove the capillary by following the installation steps in reverse order.
3	Clean the pump block.

How to Store the Capillary

Storing the Capillary Off the Instrument

To store the capillary off the instrument:

Step	Action
1	Label on the capillary with the number of runs performed. A capillary can be used for up to 100 runs. Keep track of the number of runs performed on each capillary.
2	Maintain the capillary ends in two sample tubes filled with sterile deionized water and closed with septa. 
3	Water evaporates very slowly from the tubes. Check them every week and add water if necessary.
4	Always clean the pump block after removing the capillary.

Storing the Capillary On the Instrument

IMPORTANT Leave the power off when storing the capillary with this method. The autosampler moves up when the power is turned on. Position the capillary end 0.5-mm below the end of the electrode and securely tape the capillary to the heat plate in this position before turning on the power after storing the capillary with this method. The autosampler will break the capillary if the instrument is turned on before moving the capillary up.

To store the capillary on the instrument:

Step	Action
1	Run the “Seq Fill Capillary” module in Manual Control to remove any sample still in the capillary.
2	Turn off the instrument. The autosampler drops when the power is turned off.
3	Remove the thermal tape holding the capillary to the heat plate.
4	Lower the end of the capillary into the buffer vial in the autosampler.
5	Tape the capillary in this position and close all the doors.

After Re-installing a Stored Capillary

Run a known standard to verify the condition of the capillary.

Recalibrate the autosampler after installing a capillary.

Syringe Overview

Types There are two types of syringes used with the 310 Genetic Analyzer:

- Glass
- Plastic disposable

Purpose The following table lists the purpose of the glass and plastic syringes.

Syringe	Purpose
Glass	♦ Polymer reservoir during the run. ♦ Contains adequate polymer to complete the run. ♦ Remains installed on the instrument throughout the run. ♦ Used for the ABI PRISM® DNA Sequencing Analysis Software and the GeneScan® Analysis Software.
Plastic	♦ Packaging for DNA Sequencing Polymer (DSP). ♦ Transfers polymer to the glass syringe before a run begins. ♦ Removed before starting the run. ♦ Used for DNA sequencing applications. ♦ Used for cleaning the pump block.

Syringe Sizes

Glass Syringes Future applications will be developed for the 1.0-mL syringe only. There will be no new modules for the 250-µL or 2.5-mL syringe in software releases after ABI PRISM® 310 Data Collection Software v.1.0.2.

Glass syringe sizes and intended applications are:

Size	Application
250-µL	DNA sequencing or the GeneScan® Analysis Software with DNA Sequencing Polymer
1.0-mL	DNA sequencing with POP-6, GeneScan® Analysis Software with POP-4®, and the GeneScan® Analysis Software with the GeneScan Polymer

Plastic Syringes Plastic syringes are 5-mL disposable syringes. DNA Sequencing Polymer is packaged in 5-mL syringes. An empty plastic syringe is provided in the Basic Installation Kit.

How to Remove the Syringe

Removing the Syringe

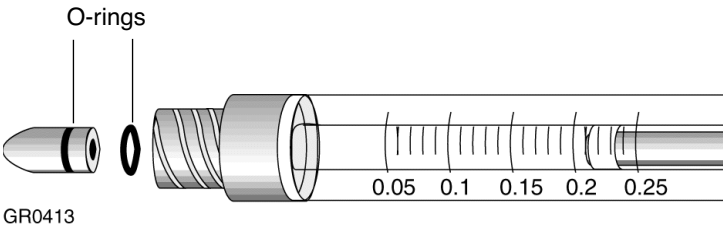
To remove the syringe:

Step	Action
1	Start the ABI PRISM® 310 Data Collection Software 310 Data Collection software by double-clicking the icon, if the program is not currently open.
2	Open the Manual Control window and select Home Syringe from the Function pop-up menu.
3	Click the Execute button.
4	Unscrew the syringe from the pump block.

How to Inspect the Syringe

Inspecting the Syringe

To inspect the syringe:

Step	Action
1	Inspect the glass syringe for two O-rings (P/N 221102), one behind the ferrule and one around the ferrule.  GR0413
2	Verify that the ferrule is firmly seated in the end of the syringe.

How to Clean the Syringe

When to Clean the Syringe

Thoroughly clean the syringe whenever it is removed from the pump block. Verify that the syringe is clean before use.

Cleaning and Preparing the Syringe for Use

CAUTION **The teflon plunger is very susceptible to wear caused by friction. Therefore the pulling up and pushing down of the plunger within the barrel of the syringe should be minimized.**

To clean and the prepare the syringe:

Step	Action
1	Rinse the syringe with distilled water. Remove excess water, but do not dry completely. **IMPORTANT** Excessive wear occurs to the Teflon fitting of the plunger if it is completely dry.
2	Allow the polymer to equilibrate to room temperature before loading it into the syringe. **IMPORTANT** Precipitate present when the bottle is removed from cold storage should go back into solution at room temperature. Do not use the polymer until the precipitate dissolves.
3	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.
4	Draw a small amount of polymer solution (0.20-mL maximum) into the syringe. Pull plunger up to the 0.60-mL marker after the polymer solution is added. ! WARNING ! **Gloves and eye protection should be worn when handling polymer.**
5	Invert the syringe gently five or six times to coat the walls with polymer and discard this polymer solution. This ensures that the running polymer is not diluted when added to the syringe.
6	For new, never-before-used syringes, perform this entire procedure twice to prevent air bubbles from sticking to the syringe walls.

How to Install the Syringe

Installing the Syringe

To install the glass syringe:

Step	Action
1	Install the prepared syringe on the pump block with the plunger positioned at the bottom of its travel.
2	Move the syringe drive toggle to the right, so it is positioned over the syringe plunger.

Syringe Max Travel

Determine the Syringe Max Travel for each size of glass syringe that you use. Record the values on the Calibration sticker on the left side door.

When you install a syringe, input the correct Syringe Max Travel value in the Function pop-up menu of Manual Control.

Setting Syringe Max Travel

The syringe must be empty when setting Syringe Max Travel.

To set Syringe Max Travel:

Step	Action	
1	Determine which version of ABI PRISM® 310 Data Collection Software is installed on your Macintosh® computer. With the Collection software open, select “About Collection Software” from the Apple menu.	
2	**If you have…**	**Then…**
	v. 1.0.4 or later Collection software…	skip to step 3.
	v. 1.0.2 Collection software…	a. Open the Window menu. b. Under Preferences, select Sequence Injection List Defaults or GeneScan Injection List Defaults. c. Type “139115” in the Operator entry window and click OK. d. Quit the Collection software and then re-launch it.
3	Open the Manual Control window, select Syringe Home from the Function pop-up menu, and click Execute.	
4	Install a clean, empty glass syringe with the syringe plunger at the bottom of its travel.	
5	Select Syringe Max Travel in the Function pop-up menu. Note the displayed value, as you will enter it in the next step.	
6	Select Syringe DOWN and enter the current Syringe Max Travel value. Click Execute.	
7	After the syringe drive reaches the syringe plunger and stops moving, select Status from the Window menu. **Note** If the plunger is not completely depressed, then choose Manual Control from the Window menu and Syringe Down from the Function pop-up menu. Use steps of 10.	

To set Syringe Max Travel: *(continued)*

Step	Action
8	Under the Injection pop-up window, read the number in the Gel Pump At window. Subtract 15 from that number.
9	Select Syringe Max Travel in the Function pop-up menu of Manual Control and type the number you just calculated in step 8. Click Execute.
10	Record this value on the Calibration sticker on the left side door. **Note** If you install a syringe of a different size, you must input the max travel value for that syringe size.
11	Select Syringe Up in the Function pop-up menu of Manual Control and enter 250 in the Value window. Click Execute.

How to Home the Syringe

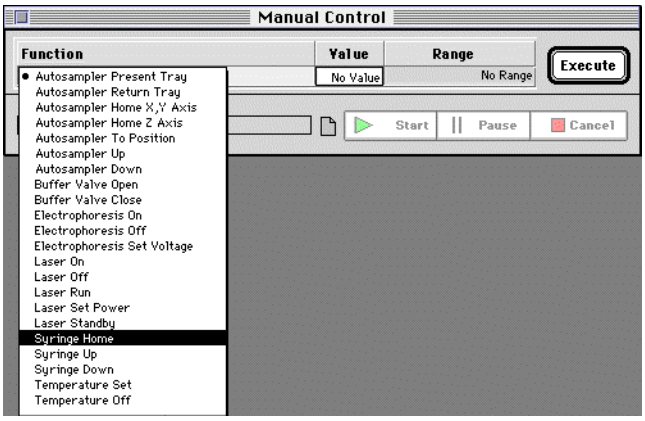
When to Home the Syringe

Home the syringe after:

- Turning on the main power switch
- Resetting the ABI PRISM 310 Genetic Analyzer

Homing the Syringe

To home the syringe:

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software.
2	Open the Manual Control window.
3	Select Syringe Home from the Function pop-up menu, and click Execute. 

How to Load Polymer into the Syringe

Before Loading Polymer

Make sure the syringe has been properly cleaned.

Loading Polymer

! WARNING ! CHEMICAL HAZARD. Urea is a potential mutagen. Dangers cited in toxicity studies show reproductive and tumorigenic effects. Urea can cause irritation to the skin, eyes, and respiratory tract. Avoid inhalation and contact with skin, eyes and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

! WARNING ! CHEMICAL HAZARD. Some chemicals used with this instrument are potentially hazardous. Warnings are prominently displayed on the bottle labels of all hazardous chemicals. Material Safety Data Sheets (MSDSs) are provided by the manufacturer and contain information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. Please familiarize yourself with the information contained in these documents before attempting to operate the instrument or using hazardous reagents. Additional copies of the MSDSs for Applied Biosystems chemicals are available from Applied Biosystems at no cost.

To load polymer into the syringe:

Step	Action
1	Allow the polymer to equilibrate to room temperature before loading it into the syringe. **IMPORTANT** Precipitate present when the bottle is removed from cold storage should go back into solution at room temperature. Do not use the polymer until the precipitate dissolves.
2	Mix the polymer thoroughly by inversion before use. Let it settle for at least 5 minutes before use.
3	Fill the syringe manually with a maximum of 0.5-mL of polymer. **Note** Do not use polymer that has been on the instrument for more than one week. Do not return unused polymer to the original bottle.
4	Remove all air bubbles by inverting syringe and pushing air bubbles out. **IMPORTANT** To avoid loss of polymer, any bubbles near the plunger head must be removed.
5	Take the following actions: a. Rinse the outside of the syringe with distilled water to remove any polymer on the outside of the syringe. b. Dry the outside of the syringe with a lint-free paper.
6	Move the syringe drive toggle to the left to attach the syringe to the pump block.
7	Place the syringe through the right-hand port of the plastic syringe guide plate, and screw the syringe into the pump block finger-tight.
8	Manually close and tighten the waste valve below the syringe and the luer valve to the left of the syringe.

How to Fill the Buffer Reservoirs

Filling the Buffer Reservoirs

To fill the buffer reservoirs:

Step	Action
1	Dilute 5-mL of 10X Genetic Analyzer EDTA Buffer to 1X concentration (50-mL total volume) with distilled, deionized water. **Note** The 10X Genetic Analyzer buffer with EDTA can be diluted in a smaller volume. Diluted buffer can be stored at 4 °C and used within two weeks. Change diluted buffer on the instrument every week or 200 injections, whichever comes first.
2	Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer EDTA Buffer and place the reservoir on the pump block.
3	Fill a 4-mL glass buffer vial to the fill line with 1X Genetic Analyzer EDTA Buffer.
4	Insert the plastic cap adapter with attached septum into the glass vial and place the buffer vial into position 1 on the autosampler. This serves as the cathode buffer. **IMPORTANT** Change the septum on the buffer vial every two days to avoid build-up of dried polymer.
5	Fill a second 4-mL glass buffer vial to the fill line with distilled water, insert the plastic vial lid with attached septum, and place the vial into position 2 on the autosampler.
6	Clip the lid off a 1.5-mL Eppendorf tube, fill with distilled water, and place it into position 3 on the autosampler. **Note** Do not use a screw-cap tube. The screw-cap tubes are too high to clear the electrode and capillary.

When to Change the Buffer

Replace the buffer every week or every 200 runs.

Making Buffer for a Single Run

You can prepare fresh electrophoretic buffer for a single run by mixing 1.3-mL of 10X Genetic Analyzer Buffer with EDTA (P/N 402824) with 11.7-mL deionized water. You can also prepare a stock 1X solution of analysis buffer (for example, 5-mL 10X Genetic Analysis Buffer with EDTA and 45-mL deionized water). Store the stock buffer at 2–8°C.

Autosampler Overview

When to Clean the Autosampler

Remove dried buffer from the autosampler with a water-dampened lab wipe once a week.

! WARNING ! Salt accumulation on the autosampler can cause an arc during electrophoresis.

Tray Types

One of two trays can be attached to the tray platform. A sensor in the platform automatically detects the type of tray.

Number of Tube Positions	Size of sample tubes (mL)
48	0.5
96	0.2

Installing the Tray

Press the tray flat against the rectangular, raised guide until the tray is flush with the platform.

Sample Numbering in the 48-well Tray

Figure 4-2 shows the numbering of the tube positions in the 48-well tray:

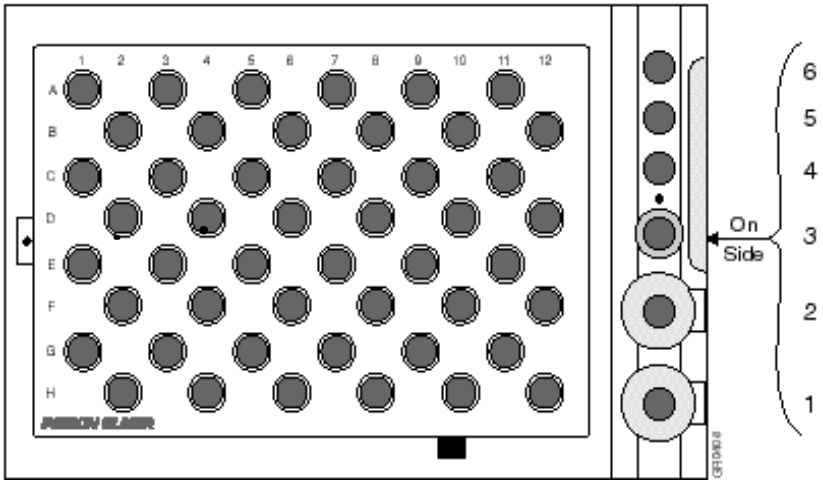


Figure 4-2 Autosampler tray numbering (48 tube positions)

Note Because of the staggered arrangement of sample tubes in the 48-tube sample tray, the numbering of the tube positions across the first row is A1, A3, A5, etc. The numbering of the tube positions across the second row is B2, B4, B6, etc.

continued on next page

Sample Numbering in the 96-well Tray

Figure 4-3 shows the numbering of the tube positions in the 96-well tray:

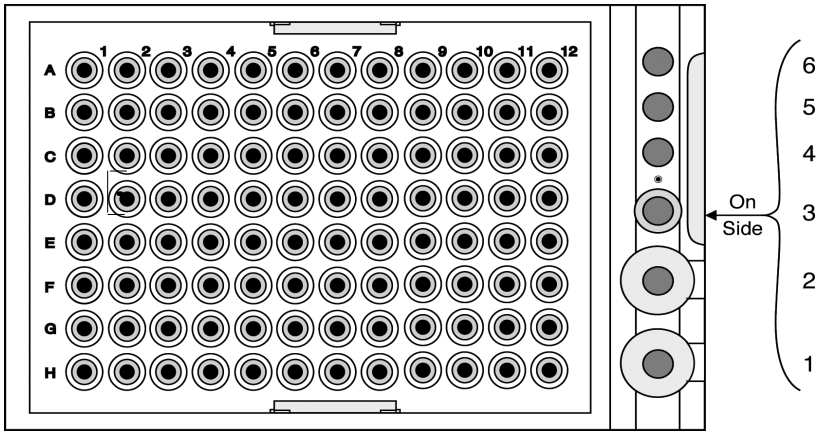


Figure 4-3 Autosampler tray numbering (96 tube positions)

How to Home the Autosampler

When to Home the Autosampler

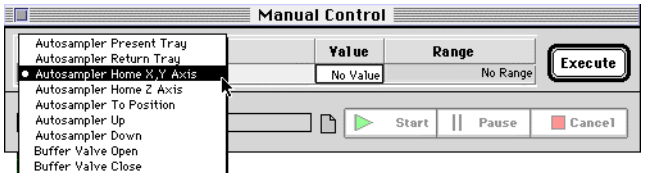
Home the autosampler after:

- Turning on the main power switch
- Resetting the ABI PRISM 310 Genetic Analyzer

Homing the Autosampler

IMPORTANT Always home the X, Y positions before the Z position. Homing the Z position first can bend the electrode.

To home the autosampler:

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software and choose Manual Control from the Window menu.
2	Select Autosampler Home X, Y Axis from the Function pop-up menu, and click execute. 
3	Select Autosampler Home Z Axis, and click execute. 

How to Calibrate the Autosampler

When to Calibrate the Autosampler

The autosampler calibration settings are maintained in the permanent memory of the instrument.

Recalibrate:

- After changing the electrode
- After changing the capillary
- If the capillary and the septa caps collide
- After a reset that clears memory

IMPORTANT The sample tray must be removed before calibrating the autosampler. If the sample tray is not removed, the electrode will be bent.

Calibration Markers

The autosampler tray platform is shown in Figure 4-4. Use the black or silver dots on the front and rear of the platform to calibrate the autosampler.

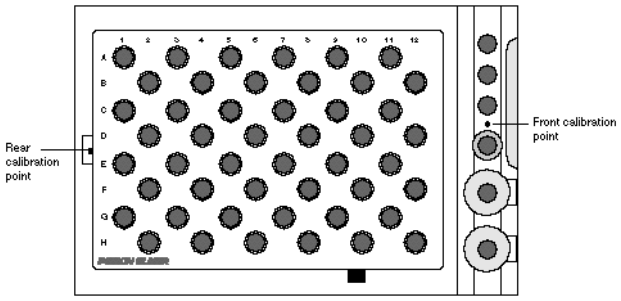


Figure 4-4 Autosampler Calibration Marks

Autosampler Calibration Keyboard Equivalents

These are the keyboard equivalents for the autosampler calibration:

Move to the	Key
Rear	Up Arrow
Front	Down Arrow
Right	Right Arrow
Left	Left Arrow
Up	Page Up
Down	Page Down
Half Steps	Use Shift Key with the above keys

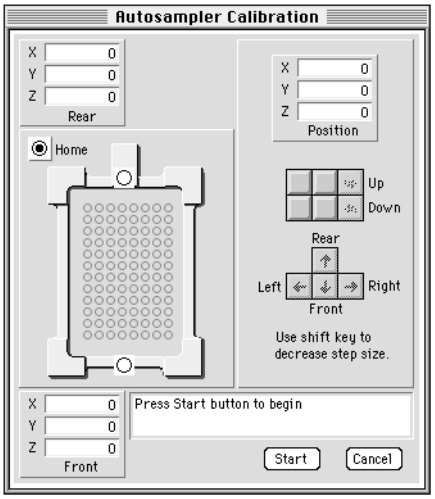
IMPORTANT By holding the keys down (rather than tapping them), the autosampler will move in larger increments. Use caution when holding down the keys or the autosampler will travel farther than intended, causing damage to the electrode and the Teflon block of the autosampler.

continued on next page

Calibrating the Autosampler

IMPORTANT The sample tray must be removed before calibrating the autosampler. If the sample tray is not removed, the electrode will be bent.

To calibrate the autosampler:

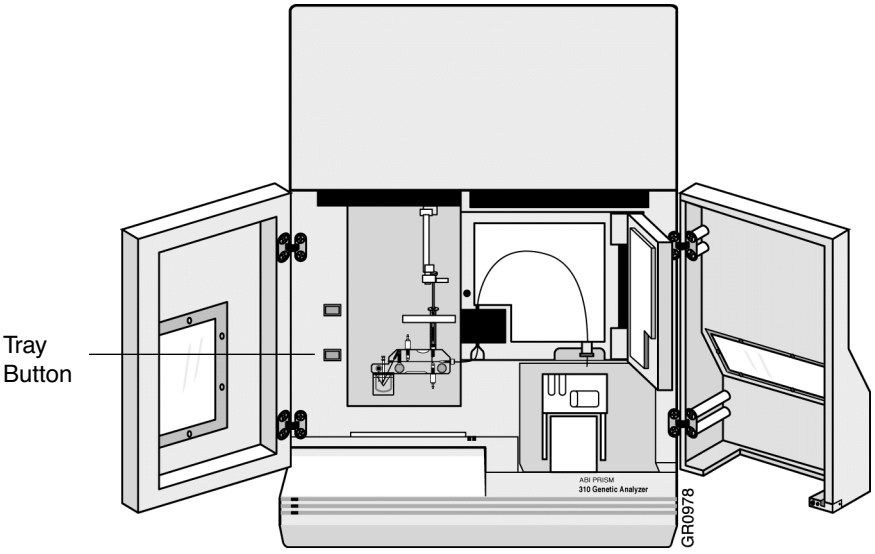
Step	Action
1	Remove the sample tray and Eppendorf tube from the autosampler.
2	Choose Autosampler Calibration from the Instrument menu of the Collection software. The Autosampler Calibration window appears.  **Figure 4-5** Autosampler Calibration window
3	Click Start and follow the directions that appear on the screen.
4	Move the autosampler using the arrow keys in the Autosampler Calibration window or the arrow keys on the Macintosh® computer keyboard.
5	Align the calibration dot on the front (Front dot in Figure 4-3 on page?4-25) of the tray platform with the capillary. Center the capillary on the calibration point for x,y calibration. Touch the capillary to the calibration point for z calibration.
6	Click Set to save the calibration value.
7	Repeat for the rear calibration point and click Set. Follow the instructions on the screen to complete the calibration.

How to Load Samples and Buffers on the Autosampler

Loading the Autosampler

The following steps describe how to load samples, buffers, and wash solutions on the autosampler.

To load the autosampler:

Step	Action
1	To access the autosampler on an idle instrument, press the Tray button (see Figure 4-6). The autosampler moves forward, allowing access to all sample and buffer positions.  **Figure 4-6** Location of Tray button to access the autosampler **Note** On some early ABI PRISM® 310 instruments, the Tray button is located on the front panel where the figure shows status indicators.

To load the autosampler: *(continued)*

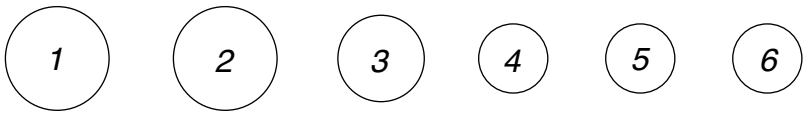
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Load solutions on the autosampler in the numbered positions at the right side of the autosampler, as shown below.
<table>
<tr><th>Position</th><th>Volume</th><th>Vial</th><th>Solution</th></tr>
<tr><td>1</td><td>4.0-mL</td><td>buffer vial</td><td>Electrophoresis buffer</td></tr>
<tr><td>2</td><td>4.0-mL</td><td>buffer vial</td><td>Deionized water</td></tr>
<tr><td>3</td><td>1.5-mL</td><td>Eppendorf tube</td><td>Deionized water</td></tr>
<tr><td>4,5, 6</td><td colspan="3">Not Currently Used</td></tr>
</table>


Figure 4-7 Positions for solutions on the autosampler

Note The 1.5-mL tube in position 3 should not be a screw-cap tube. The screw-cap tube is too high to clear the electrode and capillary. Use a 1.5-mL Eppendorf tube with the lid clipped off for position 3.

! WARNING ! CHEMICAL HAZARD. Some chemicals used with this instrument are potentially hazardous. Warnings are prominently displayed on the bottle labels of all hazardous chemicals. Material Safety Data Sheets (MSDSs) are provided by the manufacturer and contain information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. The MSDSs can be kept in the Safety Supplement provided with the chemistry guide. Please familiarize yourself with the information contained in these documents before attempting to operate the instrument or using hazardous reagents. Additional copies of the MSDSs for Applied Biosystems chemicals are available from Applied Biosystemsat no cost.</td></tr>
<tr><td>3</td><td>Attach the anode buffer reservoir containing the buffer solution to the pump block.</td></tr>
<tr><td>4</td><td>Load samples in the autosampler tray.</td></tr>
<tr><td>5</td><td>Press the Tray button to bring the autosampler platform forward and install the tray on the platform.</td></tr>
<tr><td>6</td><td>Press the Tray button to move the autosampler back.</td></tr>
</table>

How to Shut Down the Genetic Analyzer

What is a Short-Term Shutdown

A short-term shutdown is typically for no more than two days and used to conserve power.

The key to a short-term shutdown is keeping the capillary in solution. This prevents polymer from drying in the capillary.

Performing a Short-Term Shutdown

To perform a short-term shutdown:

Step	Action
1	Shut down the Macintosh® computer.
2	Turn off the 310 Genetic Analyzer. The LED status lights will turn off.
3	Store the ends of the capillary in solution. You can store the capillary on or off the Genetic Analyzer. For more information, see: ♦ "Storing the Capillary Off the Instrument" on page 4-16 or, ♦ "Storing the Capillary On the Instrument" on page 4-16.
4	Close the instrument doors.

About a Long-Term Shutdown

A long-term shutdown is appropriate when you want to turn off the 310 Genetic Analyzer for more then two days.

You may need to refer to the following procedures:

Procedure	See page
Removing the Capillary for Long-term Storage	4-15
Removing the Pump Block	4-7
How to Remove the Syringe	4-18
How to Clean the Pump Block	4-8
Cleaning and Preparing the Syringe for Use	4-19
Executing Functions	5-15
Starting a Run Module	5-15

continued on next page

Performing a Long-Term Shutdown

To perform a long-term shutdown:

Step	Action
1	Remove the capillary, syringe, and pump block.
2	Wash the syringe and pump block.
3	Re-install the pump block and the capillary.
4	Fill the syringe with distilled and deionized water and attach it to the pump block.
5	Flush the capillary by running the Run Seq Fill Capillary module or the Syringe Down function in Manual Control. The polymer in the capillary will be flushed to the waste tube on the autosampler.
6	Press the Tray button to present the autosampler.
7	Shut down the Macintosh® computer.
8	Turn off the 310 Genetic Analyzer.
9	Remove the capillary and store it.
10	Take the following actions: a. Remove all chemistry and samples from the autosampler. b. Wipe the autosampler, trays, drip tray, and electrode with lab wipes dampened with water.

How to Reset The Genetic Analyzer

About Firmware

Firmware is software stored and executed in the 310 Genetic Analyzer's battery backed-up memory. It communicates with the software on the Macintosh® computer, enabling the system to function together.

Types of Resets

There are three types of reset:

Type of reset	Result
Soft	Re-starts the firmware.
Cold	Deletes the firmware and reloads it.
Clear Memory	Deletes the firmware and calibration data and reloads the firmware. You must re-enter the calibration data manually.

When to Reset

You should reset the 310 Genetic Analyzer when you experience the following situations:

Reset the...	If...
310 Genetic Analyzer	♦ It is behaving unpredictably. ♦ It does not respond to software commands.
Macintosh® computer and the 310 Genetic Analyzer	They are not communicating correctly **Note** If No Port is selected in the General Settings in Preferences, the Macintosh® and 310 Genetic Analyzer cannot communicate. Check this setting and select Modem before resetting. 

Troubleshooting with Resets

Perform the various types of resets in this order to resolve a problem with the Genetic Analyzer:

- Soft reset
- Cold boot
- Clear Memory

Call Technical Support if the problem persists after a Clear Memory reset.

continued on next page

Performing a Soft Reset

To do a soft reset:

Step	Action
1	Push the reset button on the back of the 310 Genetic Analyzer with a pointed object, such as a pen.
2	Quit the ABI PRISM® 310 Data Collection Software.
3	Re-launch the Data Collection program.
4	Open the Manual Control window, choose Syringe Home, and click Execute.
5	In the Manual Control window, choose: ♦ Autosampler Home X,Y, and click Execute. ♦ Autosampler Home Z and click Execute.

Performing a Cold Boot

Performing a Cold Boot through Manual Control

Step	Action
1	Open the Manual Control Window.
2	Select Cold Boot Instrument from the pop up menu.
3	Click Execute.
4	Open the Manual Control window, choose Syringe Home, and click Execute.
5	In the Manual Control window, choose: ♦ Autosampler Home X,Y and click Execute. ♦ Autosampler Home Z and click Execute.

Performing a Cold Boot if Manual Control is not Working

Step	Action
1	Quit the ABI PRISM® 310 Data Collection Software.
2	Shut down the Macintosh® computer.
3	Turn off the 310 Genetic Analyzer.
4	Hold the Tray button down for ten seconds while you turn on the Genetic Analyzer. This deletes the firmware. The three lit LED status lights indicate the absence of firmware.
5	Restart the Macintosh® computer and launch the Data Collection software. A dialog box appears as the firmware reloads.
6	Open the Manual Control window, choose Syringe Home, and click Execute.
7	In the Manual Control window, choose: ♦ Autosampler Home X,Y and click Execute. ♦ Autosampler Home Z and click Execute.

continued on next page

Performing a Clear Memory Reset

Recording the Calibration Data

Step	Action
1	Open Manual Control and record the values for: ♦ CCD Pixel Position X ♦ CCD Pixel Position Y ♦ Syringe Pump Force ♦ Syringe Max Travel **IMPORTANT** Since a Clear Memory reset will delete the calibration data, it is imperative that you record the current calibrated values. If you forget to record this data before a Clear Memory reset, a service engineer will need to reestablish the calibration values. You can find calibration data on the calibration sticker, through Manual Control, or through LabView software. In this procedure, you will record the values manually and as a Calibration File.
2	In Manual Control, select Calibration File Make.
3	Quit the ABI PRISM® 310 Data Collection Software and shut down the Macintosh® computer.
4	Turn off the 310 Genetic Analyzer.

Resetting the 310 Genetic Analyzer

Step	Action
1	Hold the Tray and Gel buttons down for ten seconds while you turn on the Genetic Analyzer. This will delete the firmware and clear the RAM. The three lit LED status lights indicates the absence of firmware.
2	Restart the Macintosh® computer and launch the ABI PRISM® 310 Data Collection Software. A dialog box will appear as the firmware reloads.
3	Open the Manual Control window and manually re-enter the calibration values for: ♦ CCD Pixel Position X (horizontal) ♦ CCD Pixel Position Y (vertical) ♦ Syringe Pump Force ♦ Syringe Max Travel or choose Calibration File Send.
4	Choose Syringe Home and click Execute.
5	In the Manual Control window, choose: ♦ Autosampler Home X,Y and click Execute. ♦ Autosampler Home Z and click Execute.
6	Calibrate the autosampler. See “Calibrating the Autosampler” on page 4-28.
7	Wait 10 minutes after a Clear Memory reset to allow the temperature circuitry to calibrate before starting a run.

Recovering from a Power Failure

Overview

The 310 Genetic Analyzer and the Macintosh® computer can automatically recover from a power failure and continue a run. The Macintosh® computer must be properly configured for automatic recovery.

IMPORTANT Under power down or power fail situations, the autosampler drops, exposing an open end of the capillary to air. Check the EPT file during the next electrophoresis to be sure the capillary is carrying current. Polymer may have dried in the capillary and created a blockage that requires cleaning or replacement.

How the Genetic Analyzer and Macintosh Recover from a Power Failure

When the 310 Genetic Analyzer starts a run, the ABI PRISM® 310 Data Collection Software automatically creates a recovery file in the ABI PRISM® 310 folder. Data about the status of the run is continuously recorded in this recovery file.

When a power failure occurs, the run stops and the autosampler drops. The capillary is exposed to air.

When the power comes back on, the Macintosh® computer and the 310 Genetic Analyzer restart. The Data Collection software will review the recovery file and resume the run.

About the Recovery File

The recovery file is a temporary file that is created at the start of every run and deleted when the run ends. It contains information necessary to recover from a power failure.

Do not throw away the recovery file, unless the recovery file is not deleted at the end of a run, and you are certain that a run is not in progress or waiting to be resumed.

After a Power Failure

Check the current in the Status window when the run resumes. If it is below 4 microamps, then its likely the polymer in the capillary dried out. This happens if the capillary is exposed to air for longer than 30 minutes.

Try clearing and refilling the capillary if the current is too low. However, in most cases, you will have to replace the capillary.

How to Configure Your Macintosh for Power Failure Recovery

You must configure the Macintosh® computer so that it can automatically recover from a power failure.

Step	Action
1	Open the General Controls control panel. The shut down warning must be off.
2	Open the Energy Saver control panel. Automatic restart must be on. The sleep timer must be set to never.
3	An alias of the ABI PRISM® 310 Data Collection Software must be in the Start-up Items folder.

Using Data Collection Software 5

Introduction

In This Chapter Topics in this chapter include the following:

About ABI PRISM 310 Data Collection Software

Overview

The ABI PRISM® 310 Data Collection Software provides instructions to firmware running on the instrument and displays instrument status and raw data in real time. As the instrument records sample fluorescence on the detection system hardware, the Data Collection software running on the Macintosh® computer collects the data, interprets it, and stores it to the computer's hard drive.

Four Key Tools in the Software

To successfully run the ABI PRISM® 310 Genetic Analyzer, you need to know how to operate four key tools in the Data Collection software:

- Manual Control
- Sample Sheets
- Injection Lists
- Raw Data Display

Data Collection Allows

The following table lists the functions you can do with the ABI PRISM® 310 Data Collection Software.

monitor	♦ Instrument Status. ♦ Electrophoresis History. ♦ Instrument Log.
automatically	♦ Start the ABI PRISM® DNA Sequencing Analysis Software or the GeneScan® Analysis Software. ♦ Direct Analysis programs to print the data.

How to Find Files

When files are stored on a Macintosh® disk or hard drive, the user organizes them by saving each one in a folder with related files. The organization of the disk can be taken a step further by gathering related folders together inside yet another folder.

If...	Then...
you want the location of a file that is not open	a. Select Find from the Macintosh® File menu. b. Use the pop-up menus to search the hard drive, attached servers, floppy disks, or the desktop. Search by name, date created, or other attributes of the file that you remember.
a file is open	the name of the file and its location are displayed at the top of the window. The name of the hard disk is displayed first. Folders and sub-folders are listed, separated by colons (:). For example, if the top of the window reads "Hard Disk:ABI Prism 310®:Sample Sheets:Sample Sheet A", a file named Sample Sheet A is located inside a folder named Samples Sheets, which is inside a folder named ABI Prism 310®, which is on the Macintosh drive named Hard Disk.

continued on next page

About Sample Sheets

The Sample Sheet is a form that you fill out in the ABI PRISM® 310 Data Collection Software to name each sample, associate it with a position in the autosampler, and make some choices about the sample's processing.

There are four kinds of Sample Sheets:

- Sequencing 48 sample tubes
- Sequencing 96 sample tubes
- GeneScan® 48 sample tubes
- GeneScan® 96 sample tubes

From the Sequencing Sample Sheets, you select a:

- Mobility file (Dye Set/Primer file)
- Matrix file

About Injection Lists

The Injection List is a form that you fill out in the Data Collection software to direct the processing of your samples.

The Injection List automatically pulls sample names and associated tube positions from a Sample Sheet and lists them as sequential injections to the capillary.

Other information pulled from the Sample Sheet or created by the Injection List is displayed according to settings in the Injection List preference files.

Run parameters for each injection and the order of the injections can be changed by editing the Injection List.

You begin the run by clicking the Run button in the Injection List.

Two Kinds of Injection Lists

There are two kinds of Injection Lists:

From this Injection List...	You select a...
Sequencing	♦ Sample Sheet ♦ Module file
GeneScan	♦ Sample Sheet ♦ Module file ♦ Matrix file ♦ Analysis Settings file ♦ Size Standard file

continued on next page

About Run Folders

The data from each sample is stored in a sample file.

Sample files from the same run are stored in a Run folder.

Data Collection automatically creates a Run folder to hold the sample files when you fill out a new Injection List.

About Preference Files

Preference files set:

- Where the software looks for files
- How files are named
- Default settings for Sample Sheets and Injection Lists
- Macintosh®/310 Genetic Analyzer communications port
- Starting number for automatic file numbering
- How dyes are represented on screen and paper

How to Install and Configure Data Collection Software

Installing Data Collection Software

The Installer disk is shipped with the instrument. The Field Service Engineer installs 310 Data Collection on the Power Macintosh® when setting up the 310 Genetic Analyzerr in your laboratory. Keep the Installer disk as a back up copy.

Reinstalling the Data Collection Software

To reinstall your ABI PRISM® 310 Genetic Analyzer:

Step	Action
1	Insert the Installer disk into the Macintosh® computer.
2	Double click the Installer icon.
3	Click Continue on the splash screen.
4	Click Install on the dialog box.
5	Follow the prompts.

Installing Run Modules and Mobility Files

From time to time, you may need to add files for new applications that have been released since the software was written. User Bulletins may inform you of a new application requiring updated run module and mobility or dye set/primer files. If so, the files are available on disks from local representatives or through the Applied Biosystems web site at www.appliedbiosystems.com/techsupport.

To install these run modules and mobility files:

Step	Action
1	Copy the run modules into the Module folder within the Collection software folder.
2	Copy the mobility files into the ABI folder within the System folder.
3	Relaunch the Collection and/or Analysis software if either was open while the files were installed.

continued on next page

Setting Macintosh General Controls

About the General Controls

The Macintosh's General Controls allows you to:

- Set a default preference for the folder to which a file will be saved
- Disable the improper shut down warning dialog box that appears when a Macintosh restarts after a power failure

Note Disable the shut down warning, as it interferes with the Data Collection software's ability to resume after a power failure.

Setting the Macintosh General Controls

Step	Action
1	Choose Control Panels from the Apple Menu.
2	Open General Controls.
3	In the Documents box, select "Folder that is set by the application."
4	In the Shut Down Warning box, uncheck the "Warn me if the computer was shut down improperly" checkbox.

About Virtual Memory

Turn Virtual Memory off. The software programs for the 310 Genetic Analyzerr are not tested for operation with Virtual Memory on.

About Extension Manager

Extension Manager is a Macintosh® utility that allows you to turn off start-up programs. Some start-up programs (also known as inits) and control panel programs can conflict with the ABI PRISM® 310 Genetic Analyzer Software.

If you believe a program to be the source of a conflict, turn it off using Extension Manager and restart the computer. Refer to the documentation shipped with the Macintosh for more instructions on Extension Manager.

Setting PrintMonitor Preferences

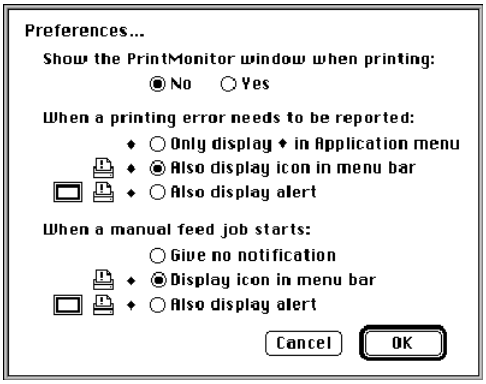
About the PrintMonitor

PrintMonitor is a Macintosh® program used to control all printing functions.

If you reinstall the Macintosh operating system, you will need to set the Preferences for the PrintMonitor program. PrintMonitor's Preferences must be set so that printing problems do not give alerts. Printing problem alerts open a dialog box on the computer screen and suspend all data collection until the problem is solved and the dialog box is closed.

Set Preferences as described here so the Macintosh® operating system can indicate a printing problem without suspending data collection. You can solve the problem when discovered, and the run in progress is not interrupted. The data is spooled to the printer and prints after the problem is solved.

Setting the PrintMonitor's Preferences

Step	Action
1	Find the PrintMonitor program in the Extensions folder inside the System folder.
2	Double-click on PrintMonitor.
3	Select Preferences under the File menu.
4	Set the Preferences as shown: 

The setting Display icon in menu bar allows the run to continue.

PrintMonitor is further described in the documentation shipped with your computer.

Setting Background Printing

Set Background Printing to On in the Chooser (in the Apple menu).

ABI PRISM 310 Data Collection Preference Files

Overview

The Preferences dialog boxes are under the Window menu. Through these dialog boxes you select defaults–the files and locations that the Data Collection software will automatically use unless directed to do otherwise.

The preference files are:

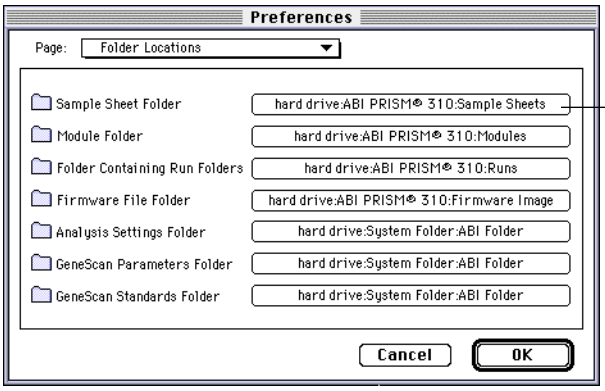
- Folder Locations
- Default File Names
- Sequence Sample Sheet Defaults
- GeneScan Sample Sheet Defaults
- Sequence Injection List Defaults
- GeneScan Injection List Defaults
- General Settings
- Dye Indicators

Folder Location Preferences

The Folder Location preferences file point the Injection List where to find the:

- Sample Sheet folder
- Module folder
- Folder Containing Run folders
- Analysis Settings folder (this folder contains mobility files, matrix files, and analysis settings files)
- GeneScan Parameters folder
- GeneScan Size Standard folder

The name in the button identifies the path to a folder. The files in that folder will appear in the pop-up menu on the Injection List.

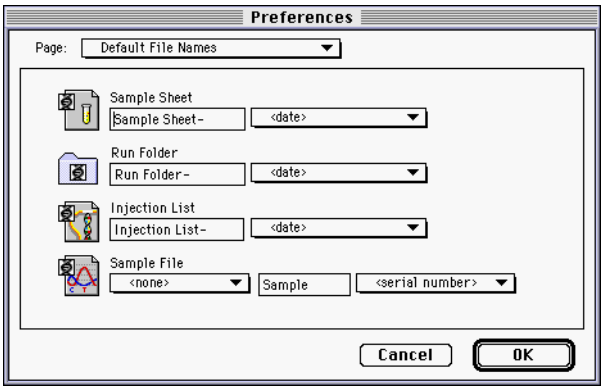


Identifies the path to a folder

continued on next page

File Names Preferences

The Data Collection software automatically creates and names certain files. Your preferences for automatic file naming can be entered in this part of the Data Collection software.

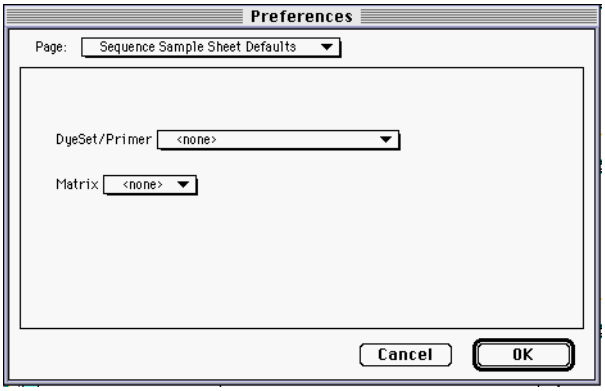


Choose none, today's date, or global serial number as a file name suffix from the pop-up menus.

The names you enter into the fields can only have alphanumeric characters. Do not use non-alphanumerics such as # * , (.) ! : . Such characters can disrupt the automatic creation of files.

Sample Sheets Preferences

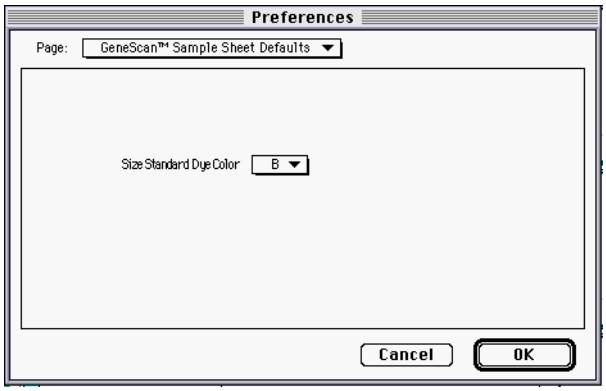
When you create a new Sample Sheet, a portion of the form is automatically filled in for you. The information comes from a preference file, which you can modify.



Sequencing Analysis Software

For Sequencing Sample Sheets, set these Sample Sheet preferences:

Preference	Description
Dye Set/Primer	Default Dye Set/Primer file that will automatically appear on a newly-created Sample Sheet.
Matrix	Default Matrix file that will automatically appear on a newly-created Sample Sheet.



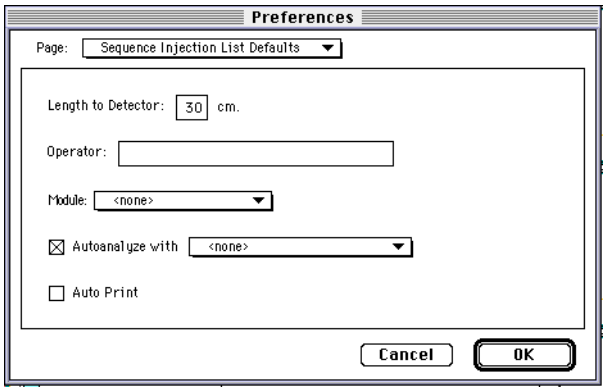
GeneScan Analysis Software

For GeneScan Sample Sheets, set the Size Standard Dye Color. This is the default dye color that will automatically be selected as the color for the size standard on a newly-created Sample Sheet.

Injection List Preferences

When you create a new Injection List and choose a Sample Sheet, the software automatically fills out the Injection List from the Sample Sheet. You can set how you want the software to fill out the Injection List through the Injection List Preferences.

The Page pop up menu lets you go to another preferences page.



Sequencing Analysis Software

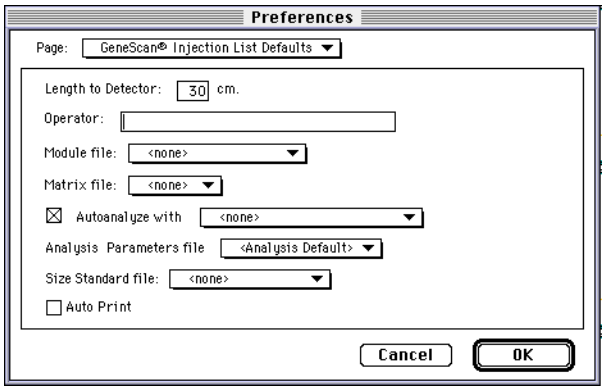
For Sequencing Injection Lists, set the following preferences:

Sequencing Injection List preferences.

Preference	Description
Length To Detector	The distance between the end of the capillary that is in the sample and the capillary window. Enter the length that will automatically appear on a newly-created Injection List. The length you enter does not affect the configuration of the instrument. It's for your record-keeping purposes only.
Operator.	Enter the name of the operator that will automatically appear on a newly-created Injection List.

Sequencing Injection List preferences. *(continued)*

Preference	Description
Module File.	A module file contains the instructions that tell the 310 Genetic Analyzer how to run a sample. It includes such things as injection time, voltage, and heat plate temperature. Choose here the default module file that will automatically appear on a newly-created Injection List.
Autoanalyze	Automatically analyzes your samples using the analysis program you specify in the pop-up menu. Check the box to have Autoanalysis automatically enabled on a newly-created Injection List. From the pop-up menu, choose the software program you want to use to analyze your data.
Autoprint.	Automatically prints your analyzed data. Check the box to have autoprint automatically enabled on a newly-created Injection List.



GeneScan Analysis Software

For GeneScan Injection Lists, set the following preferences:

GeneScan Injection List.

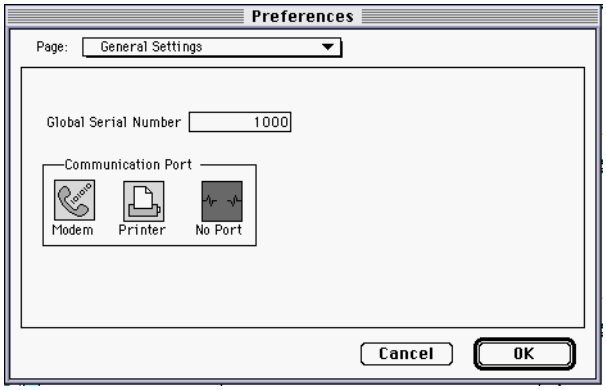
Preference	Description
Length To Detector	The distance between the end of the capillary that is in the sample and the capillary window. Enter the length that will automatically appear on a newly-created Injection List. The length you enter does not affect the configuration of the instrument. It's for your record-keeping purposes only.
Operator	Enter the name of the operator that will automatically appear on a newly-created Injection List.

GeneScan Injection List. *(continued)*

Preference	Description
Module File	A module file contains the instructions that tell the 310 Genetic Analyzer how to run a sample. It includes such things as injection time, voltage, and heat plate temperature. Choose here the default module file that will automatically appear on a newly-created Injection List
Matrix File.	A matrix file contains an algorithm that adjusts the data for spectral overlap of the fluorescent peaks in a dye set. Choose here the default matrix file that will automatically appear on a newly-created Injection List.
Autoanalyze	Automatically analyzes your samples using the analysis program you specify in the pop-up menu. Check the box to have Autoanalysis automatically enabled on a newly-created Injection List. From the pop-up menu, choose the software program you want to use to analyze your data.
Analysis Parameters File	Holds the default start and stop point for data analysis, the default peak height threshold, and the default size calling method.
Size Standard File	Holds the results of a run performed with fragments of known length. The file can be used to analyze other runs performed under the same conditions to determine the size of fragments of unknown length.
Autoprint	Automatically prints your analyzed data. Check the box to have autoprint automatically enabled on a newly-created Injection List.

General Settings

General Settings sets preferences for communication between the Macintosh® computer and the 310 Genetic Analyzer and sets up automated file numbering.



The following are the General Settings preferences.

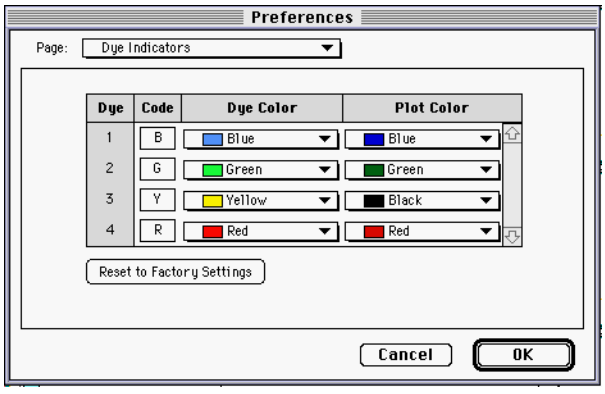
Preference	Description
Page	Use pop-up menu to view other preferences pages.
Global Serial Number	Files can be automatically numbered. This is the starting point for automated numbering here. Set which files to include in automatic numbering through the File Names preferences page.
Communication Port	Tell the Data Collection software which communications port (modem or printer) on the Macintosh® is connected to the 310 Genetic Analyzer.

If you are using...	Then...
for normal use	Set to Modem
the printer port for diagnostics or communication	Go to the Chooser (in the Apple menu) and turn AppleTalk off.
the Data Collection software when the Macintosh® is not connected to a 310 Genetic Analyzer	Choose No Port When No Port is selected, the Macintosh® will not communicate with the 310 Genetic Analyzer or generate alert messages about failed communication.

Dye Indicators Preferences

The Dye Indicators dialog box allows you to change:

- Dye Code
- Dye Color
- Plot Color



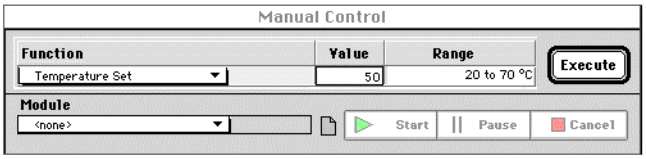
The following are the Dye Indicator preferences.

Preference	Description
Dye Code	A single letter, usually related to the dye's representational color or its name. To change the dye code, type a new letter in the code box.
Dye Color	The color displayed on the computer screen to represent fluorescence from that dye. To change the color used to represent a dye, choose a new color from the pop-up menu.
Plot Color	Color displayed on the printed electropherogram to represent fluorescence from that dye. \ To change the color used to represent a dye, choose a new color from the pop-up menu.
Reset To Factory Settings	Click to set all the codes and colors back to their factory settings.

Using Manual Control

Overview

Manual Control is in the Window menu.



Manual Control allows you to manually operate functions and some run modules.

Functions are specific mechanical tasks, such as moving the autosampler, moving the syringe, and turning the laser on and off.

Modules are files that tell the 310 Genetic Analyzer how to run a sample. Module files execute a series of functions.

Executing Functions

To execute a function:

Step	Action
1	Choose the function from the Manual Control pop-up menu.
2	Some functions require a value (such as temperature in °C or voltage in kV) to work. If the function you choose requires a value, enter it in the Value field.
3	Click the Execute button to start the function.

Starting a Run Module

To start a module:

Step	Action
1	Choose the module file you want to run from the pop-up menu. **Note** The pop-up menu will display the contents of the modules folder selected as the default in Folder Location preferences. To use a modules file in a folder other than the default folder, choose Other from the pop up menu and locate the file.
2	Click the Start button to start the module.
3	To pause or cancel a running module, click the Pause or Cancel buttons.

How to Edit Modules

Editing Modules

You can customize module files to optimize your runs. It is possible to modify the module files supplied with the software, but we recommend that you make copies and modify them.

To edit a module:

Step	Action
1	Locate the module file in a folder on the Macintosh® hard drive. Select it by clicking once.
2	In the Macintosh® File menu, choose Duplicate to create a copy of the module file.
3	Give the copy a descriptive file name.
4	Double click the copy. This opens a Settings dialog box.
5	Edit the values you wish to change.
6	Click the Save as Default button. This will save your new settings. **Note** The Save as Default button is enabled only for unlocked files. The original module files are locked, but copies are not locked. The Use Settings button is enabled only when an Injection List is open.

Editable Parameters

The parameters that can be edited differ from module to module. You may be able to edit:

- Injection time
- Injection voltage
- Run voltage
- Run temperature
- Run time
- Syringe pump time
- Pre-injection electrophoresis time
- Prerun time

Except syringe pump time, pre-injection electrophoresis, and prerun time, the parameters above can be altered for individual samples through the Injection List without permanently modifying the module. See “About Editing the Injection List” on page 5-23.

The Settings dialog box cannot be printed. The Log file contains the Injection List parameters used with the sample and it can be printed.

continued on next page

Editing Modules Using Manual Control

Through the Manual Control window, you can customize module files to optimize your runs. It is possible to modify the module files supplied with the software, but we recommend that you make copies and modify them.

To edit a module:

Step	Action
1	Locate the module file in a folder on the Macintosh hard drive. Select it by clicking once.
2	In the Macintosh File menu, choose Duplicate to create a copy of the module file.
3	Give the copy a descriptive file name.
4	Open the Manual Control window.
5	Choose the copy from the pop-up list in Manual Control.
6	Click the page icon. This opens a Settings dialog box.
7	Edit the values you wish to change.
8	Click the Save as Default button. This will save your new settings.

Editable Parameters

The parameters that can be edited differ from module to module. You may be able to edit:

- Injection time
- Injection voltage
- Run voltage
- Run Temperature
- Run time
- Syringe pump time
- Pre-injection electrophoresis
- Prerun time

Except syringe pump time, pre-injection electrophoresis, and prerun time, the parameters above can be altered for individual samples through the Injection List without permanently modifying the module. See “About Editing the Injection List” on page 5-23.

continued on next page

About Module Parameters

The following parameters can be changed only by editing a module.

Parameter	Description
Syringe Pump Time	This is the time used to fill the capillary with polymer. You may wish to experiment with slightly shorter times to maximize throughput.
Pre-injection Electrophoresis	Pre-injection electrophoresis is a prerun of the refilled capillary for ion equilibration, before samples are electrokinetically injected.
Prerun Time	After electrokinetic injection, the sample is electrophoresed prior to data collection at the running voltage. This improves resolution of peaks and stability of electrical current during the run. You you may wish to experiment with increased prerun times to improve peak resolution and current stability for some samples.

How to Fill Out a Sample Sheet

Sequencing Sample Sheets

Drag the column markers at the top of columns to change their width.

To fill out a sequencing Sample Sheet:

Step	Action
1	Choose New from the File menu.
2	In the dialog box, click the appropriate icon for either a 48- or 96-well sample tray Sample Sheet. A Sample Sheet opens.
3	Type the name of your sample in the Sample column.
4	Choose the Dye Set/Primer and a Matrix file for your sample. Enter comments for the sample in the Comments field.
5	Repeat this process for all the samples.
6	When finished, save the Sample Sheet. We recommend that you save it to the default Sample Sheet folder.

Dye Set/Primer

The Dye Set/Primer file contains mobility correction data for the sample. Note that the Dye Set/Primer file choice must be consistent with the sequencing reaction chemistry, polymer, and filter set.

Matrix

The Matrix file contains data that corrects for spectral overlap between the fluorescent dyes.

GeneScan Sample Sheets

Drag the column markers at the top of columns to change their width.

To fill out a GeneScan Sample Sheet:

Step	Action
1	Choose New from the File menu.
2	In the dialog box, click the appropriate icon for either a 48- or 96-well sample tray Sample Sheet. A Sample Sheet opens.
3	Type the name of your sample in the Sample column.
4	Identify the size standard color by clicking in the Std column. A diamond appears next to the color used for the size standard.
5	Indicate the colors present in the sample by checking boxes in the Pres column.
6	Enter sample information into the Sample Info column, and any comments in the Comments column. **Note** The Genotyper™ software requires that you fill out the Sample Information column. See the *Genotyper DNA Fragment Analysis Software User's Manual*.
7	Repeat this process for all the samples.
8	When finished, save the Sample Sheet. We recommend that you save it to the default Sample Sheet folder.

Color

These are the colors of your dyes. You set them in the Dye Indicators preferences.

Std

A marker appears in this column to indicate that a particular dye in your sample is a standard. Click in the box to turn it on or off.

Pres

This checkbox indicates that a sample or standard is present for that dye.

If you do not choose to launch and run ABI PRISM® GeneScan® Analysis Software automatically, or if you re-analyze data later, you can make changes to this selection through the analysis software. Raw data is collected for all colors, but only the colors for which a sample is present are automatically analyzed by ABI PRISM® GeneScan® Analysis Software.

How to Fill Out an Injection List

Filling Out a Sequencing Injection List

To complete a sequencing Injection List:

Step	Action
1	Choose New from the File menu.
2	In the dialog box, click the Sequence Injection List icon. This will create the folder for the run and open the Sequence Injection List.
3	Choose the Sample Sheet for the experiment from the pop-up menu. This will automatically transfer the Sample Sheet information to the Injection List.
4	For each sample, choose a module file from the pop up menu in the Module column. The default set in the Preferences will automatically appear.
5	Check the AutoAnlz box to automatically launch Sequencing Analysis software.
6	Check the AutoPrt box to automatically print the data

There is a shortcut if several samples require the same module:

Step	Action
1	For the first sample in the Injection List that requires the module, use the pop up field to select it.
2	Select the module fields for all the subsequent samples that require that module.
3	Choose Fill Down from the Edit menu. All selected module fields will be filled in with the module.

Sequencing Injection List Fields

Drag the column markers at the top of columns to change their width.

Fields in the Injection List form.

Field	Description
Sample Sheet	Displays the selected Sample Sheet.
Page Icon	Opens the selected Sample Sheet.
Length to Detector	The distance between the end of the capillary that is in the sample and the capillary window. The length you enter will not affect the configuration of the instrument. It is for record-keeping purposes only.
Operator	The name you enter here will appear on printed data.
Tube and Sample Name	The sample's name and position in the autosampler.
Module	The file that contains the specific functions executed to process samples.
Inj Secs	The duration of the injection in seconds.
Inj kV	The voltage during the injection in kilovolts.
Run kV	The voltage during the run in kilovolts.
Run °C	The temperature of the heat plate during the run in degrees Celsius.
Run Time	The duration of the data collection in minutes. This is less than the total run time.
AutoAnlz	Determines if raw data is automatically analyzed by the Sequence Analysis software.

Fields in the Injection List form. *(continued)*

Field	Description
AutoPrt	Determines if analyzed data is automatically printed.
Finish Time	Displays the time raw data collection for the sample finished.

Drag the column markers at the top of columns to change their width.

Filling Out a GeneScan Injection List

Completing a GeneScan Injection List

Step	Action
1	Choose New from the File menu.
2	In the dialog box, click the GeneScan Injection List icon. This will create the folder for the run and open the GeneScan Injection List.
3	Choose the Sample Sheet for the experiment from the pop-up menu. This will automatically transfer the Sample Sheet information to the Injection List.
4	For each sample, choose: ♦ A module file from the pop up menu in the Module column ♦ A matrix file from the pop up menu in the Matrix file column ♦ An Analysis Parameters file from the pop up menu in the Analysis Parameters column ♦ A Size Standard file from the pop up menu in the Size Standard column These are filled out automatically if the Injection List Preferences have been set.
5	Check the AutoAnlz box to automatically launch the GeneScan® Analysis Software.
6	Check the AutoPrt box to automatically print the data

Using the Fill Down Function

There is a shortcut if several samples require the same files:

Step	Action
1	For the first sample in the Injection List that requires the file, use the pop up field to select it.
2	Select the field for all the subsequent samples that require that file.
3	Choose Fill Down from the Edit menu. All selected fields will be filled in with the file.

continued on next page

GeneScan Injection List Fields

The fields in the Injection List form are:

Fields in the GeneScan Injection form.

Field	Description
Sample Sheet	Displays the selected Sample Sheet.
Page icon	Opens the selected Sample Sheet.
Length to Detector	The distance between the end of the capillary that is in the sample and the capillary window. The length you enter in the Injection List will not affect the physical configuration of the instrument. It is for record-keeping purposes only.
Operator	The name you enter here will appear on printed data.
Tube and Sample Name	The sample's name and position in the autosampler.
Module	The file that contains the specific functions executed to process samples.
Inj Secs	The duration of the injection in seconds.
Inj kV	The voltage during the injection in kilovolts.
Run kV	The voltage during the run in kilovolts.
Run °C	The temperature of the heat plate during the run in degrees Celsius.
Run Time	The duration of the data collection in minutes. This is less than the total run time.
Matrix File	The matrix file to be used.
AutoAnlz	Determines if raw data is automatically analyzed by the GeneScan® Analysis Software.
Analysis Parameters	The parameters file to be used.
Size Standard	The size standard file to be used.
AutoPrt	Determines if analyzed data is automatically printed.
Finish Time	Displays the time raw data collection for the sample finished.

About Editing the Injection List

Change Run Parameters

After a module is selected in the Injection List, the following fields are automatically filled in with information from the module:

- Injection time
- Injection voltage
- Run voltage
- Run Temperature
- Run time

You can edit these parameters by selecting a field and typing in a new value. This changes the run conditions for one run. It does not permanently alter the module.

For information about editing a module, see "Editing Modules Using Manual Control" on page 5-17.

Change Order of Samples

The injections are sequentially numbered. In the Tube and Sample Name column, use the pop up menus to select a sample for each injection.

Remember that the samples in the tray must match the positions in the Injection List.

Lines in the Injection List can be cut from one location and pasted into another. Holding down the option key when selecting an injection number selects the whole line.

Add a Line to the Injection List

Choose Insert from the Edit menu after selecting an injection. The new line will add above the selected injection.

Do not add lines above an injection that is running.

Run a Sample More Than Once

The injections are sequentially numbered. Add a line to the Injection List. Use the pop up menus in the Tube and Sample Name column to select the sample for more than one injection or copy and paste the entire injection on the new line.

Change the Module for a Sample

Use the pop up menu in the Module column to select a module.

Change Sample Sheet Items from the Injection List

The following files on a Sample Sheet can be changed after viewing the Sample Sheet from the Injection List:

- Dye Set/Primer
- Matrix
- Analysis parameters
- Size standard

The color of standards and which colors are present in a sample can be changed on GeneScan Sample Sheets.

Changing Items on the Sample Sheet from the Injection List

Step	Action
1	Click the Page icon to view the Sample Sheet from the Injection List.
2	Change the Sample Sheet.
3	Save the Sample Sheet.
4	From the Injection List, select Sample Sheet <none>.
5	Re-select the Sample Sheet from the Injection List.

Note It is not possible to add samples to a Sample Sheet once it has been saved to a folder.

continued on next page

About Run Parameters

Electrokinetic Injection (Voltage and Time)

The electrokinetic injection time and voltage can be varied to regulate the amount of DNA brought into the capillary for analysis.

The objective is to inject into the capillary just enough signal for adequate peak height (good signal to noise) while minimizing the peak widths.

There are no specific requirements for the voltage and current during the electrokinetic injection. Injections at 200 volts/cm are not distinguishable from injections at 25 volts/cm; however, a lower voltage and current are preferred since timing of the injection is more accurate.

Typically, a voltage of 50 volts/cm is used for the electrokinetic injection. At this value injection times of 5–60 seconds are required to obtain adequate peak height, depending on the application. Excessive injection time can cause loss of resolution.

Electrophoresis Running Voltage: Sequencing

Resolution and base calling are better at lower field strengths. Increasing the field strength allows shorter run times, but reduces base calling accuracy.

For Sequencing Samples using...	The standard running voltage is...
DSP	160 volts/cm. For a 47-cm capillary, this translates to 7.5 kV. The current at this voltage is 7–10 μA. The time required for a 450 base fragment to reach the detector window is about 135 minutes with these run conditions.
POP-6 and long-read sequencing	is 200 volts/cm. For a 61-cm capillary, this translates to 12.2 kV. The current at this voltage is 4–6 μA. The time required for a 600 base fragment to reach the detector window is about 120 minutes with these run conditions.
POP-6 and RAPID sequencing,	s 320 volts/cm. For a 47-cm capillary, this translates to 15 kV. The current at this voltage is 5–8 μA. The time required for a 400 base fragment to reach the detector window is about 35 minutes with these run conditions.

Electrophoresis Running Voltage: GeneScan Analysis Software

For GeneScan samples using POP-4, the standard running voltage is 319 volts/cm. For a 47-cm capillary, this translates to 15 kV. The current at this voltage is 8–9 μA. The time required for a 400bp fragment to reach the detector window is about 25 minutes with these run conditions.

Resolution is better at lower field strengths. Increasing the field strength allows shorter run times, but reduces resolution.

Electrophoresis Temperature

The heat plate of the instrument is routinely set at 50 °C for Sequencing Analysis applications and 60 °C for denaturing GeneScan® Analysis Software applications.

The temperature limits of the heat plate are 60 °C down to 5 °C above room temperature.

Electrophoresis Running Time

You can change the running time for special requirements. For example, you can shorten the data collection time if you only need information about short extension products.

Typically, set the electrophoresis running time at an interval 10% higher than the average migration time of the maximum fragment size of interest to ensure sufficient data collection.

Starting the Run

To begin a run, click the Run button on the Injection List.

Displaying Raw Data

Overview

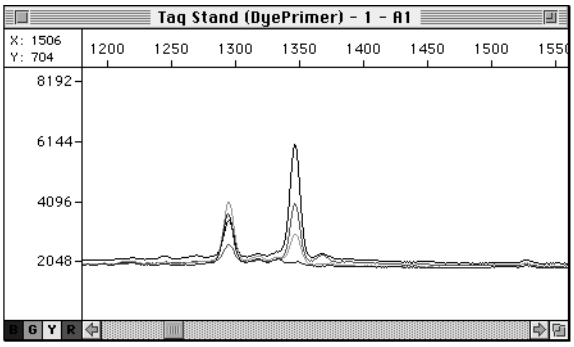
During a run you can monitor:

- Real-time raw data
- Instrument status
- Instrument errors

The Raw Data Window

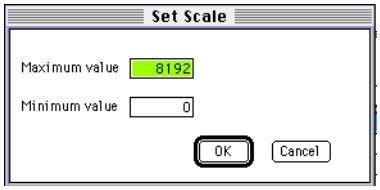
The Raw Data window shows data detected as it passes the capillary window, with a different colored line representing each filter. The computer simultaneously updates the Raw Data window with four lines every few seconds during instrument operation.

Choose Raw Data from the Window menu.



Resizing Raw Data

Double-click the left side of the Raw Data window to display the Set Scale dialog box where you can enter values to scale the Raw Data.



Why Raw Data and Analyzed Data Differ in Color

The colors in the real-time data display differ from the colors in the display of data after analysis.

The ABI PRISM® 310 Data Collection Software defines specific areas on the CCD camera from which to collect light intensities. These areas correspond to different wavelengths of light. The result is similar to using a physical filter to separate the light wavelengths. This is referred to as a “virtual” filter, since no physical filtering hardware is used.

On the real-time display (the Raw Data window), the data collection software displays these intensities, color-coded according to wavelength. Blue, green, yellow, and red (in that order) represent the wavelengths of the dye emissions within each dye set. Blue represents the shortest wavelength, and red represents the longest. The colors on the real-time displays therefore represent the relative wavelengths of the dyes being detected.

A virtual filter set represents the relative wavelengths of the dye set as blue, green, yellow, and red regardless of the absolute value of the wavelengths in nanometers. The colors do not represent actual wavelengths. They represent the *relative* wavelengths of the four dyes in each dye set.

Each of the chemistries is associated with a dye set. The relative wavelengths of the dye labels are not consistent across dye sets. Therefore the color associated with a base in the raw data depends on the chemistry used to label it.

The analysis software may re-assign the colors to standardize data collected with different labelling chemistries.

Data Display after Analysis: Sequencing

The analysis program converts the information collected by the Data Collection software, so that after analysis the color assigned to a base is consistent regardless of the sequencing chemistry used for labelling:

Color of Analyzed Data	Base
Blue	C
Green	A
Black	G
Red	T

Virtual Filters for DNA Sequencing

In sequencing applications, the dyes corresponding to the colors in the raw data are listed below.

	Virtual Filter A		Virtual Filter E
Color	*Dye* Primer	*Dye* Terminator	dRhodamine Terminators BigDye™ Primers, or BigDye™ Terminators
Blue	5-FAM	R110	dR110
Green	JOE	R6G	dR6G
Yellow	TAMRA	TAMRA	dTAMRA
Red	ROX	ROX	dROX

Virtual Filters for GeneScan

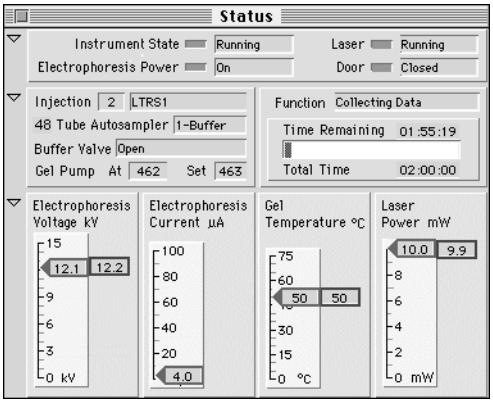
In GeneScan® Analysis Software applications, the dyes corresponding to the colors are listed below.

		Corresponding Dye		
Color	Virtual Filter A	Virtual Filter C	Virtual Filter D	Virtual Filter F
Blue	5-FAM or R110	6-FAM	6-FAM	5-FAM
Green	JOE or R6G	TET	HEX	JOE
Yellow	TAMRA	HEX	NED	NED
Red	ROX	TAMRA	ROX	ROX

How to Display Run Status and History

Observing Instrument Status

During a run, the current status of the instrument is displayed in the Status window. Choose Status from the Window menu.



The Status window displays:

- Time remaining in the current function.
- Actual electrophoresis voltage, heat plate temperature, and laser power.
- Set points for the electrophoresis voltage, heat plate temperature, and laser power.
- Actual electrophoresis current.

The Status window is updated about once per second.

The Log File

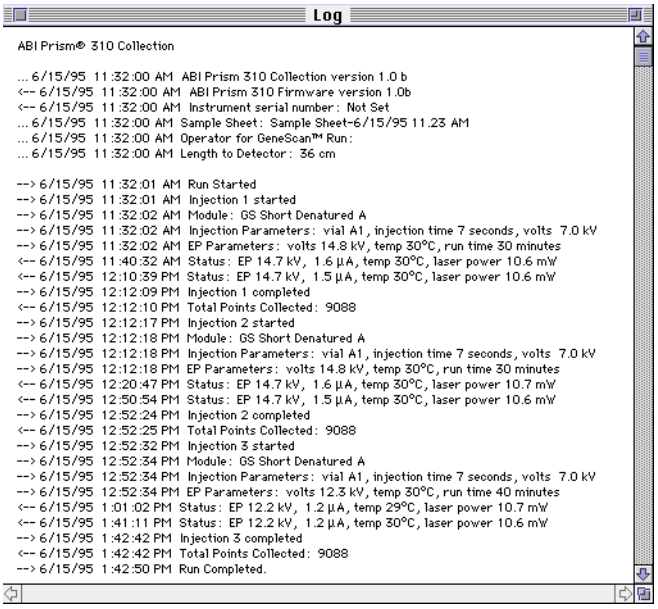
The Log file contains a comprehensive record of all error and status messages generated by the Data Collection software during a run. A Log file is created for each run and stored in the Run folder.

The Log File records:

- The injection parameters.
- Electrophoresis parameters.
- Instrument status at a few different times during data collection for each sample.
- The module.
- Total number of data points collected for each sample.

The Log File is useful for troubleshooting. If there is a problem with the data, compare the information recorded in the Log File against your expectations for the run. Was a parameter set incorrectly? Was the wrong module selected? Does a component of the instrument fail to reach the set parameters?

Choose Log from the Window menu to view the Log file. The Log file is a text file. Only 32K of the Log file can be viewed from Data Collection. If it is larger than 32K, open it in a word processing application to view the complete file.



The information in the file is formatted as follows:

xxx	month/day/year	hour:minute:second	Description of event

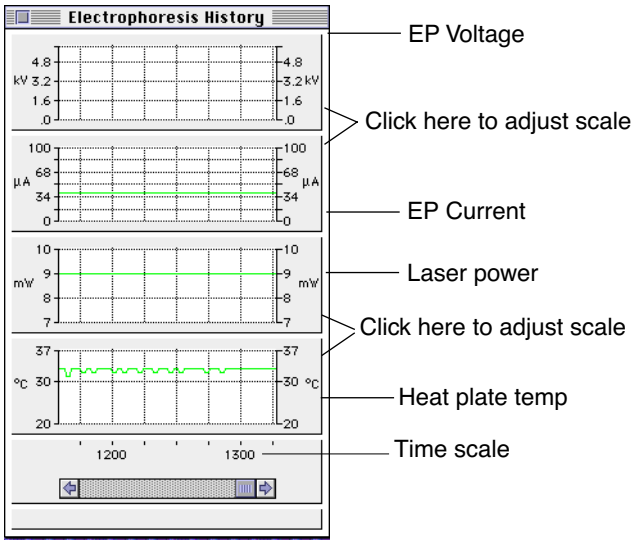
The entry in the first column, xxx, is variable. The possible entries are:

Entry	Meaning
…	System start, system stop or file created
-->	message sent to instrument
<--	message received from instrument
***	warning
###	Macintosh or instrument error

Electrophoresis History

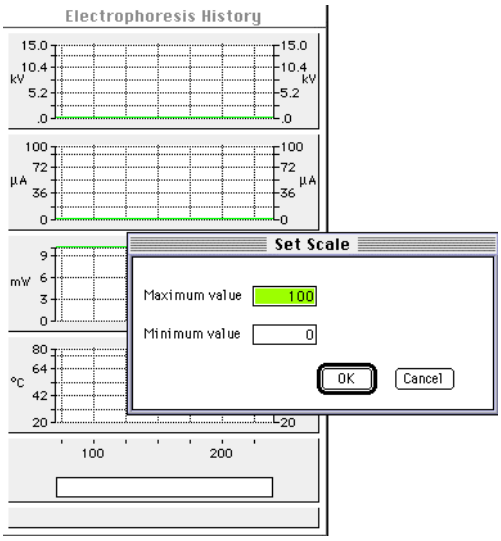
The Electrophoresis History window plots the actual values for the electrophoresis power supply, laser power, and heat plate temperature over the course of the run. The information in the Electrophoresis History Window is stored in each sample file.

Choose Electrophoresis History from the Window menu during a run to view the Electrophoresis History.



The amps, volts, laser mW, and temperature scales are adjustable. The pop-up windows for adjusting scale overlay the history window, and all four are identical.

Adjusting the Amp, Volts, Laser, and Temperature Scales.

Step	Action
1	Double-click one of the fields in the window, or click a field once and choose Scale from the Edit menu. The Set Scale dialog box appears. 
2	In the fields of the Set Scale dialog box, type the scale's maximum and minimum values.
3	Click OK.

How to Import and Export Files

Importing Files

You can import from tab-delimited text files into the grids of the Sample Sheet and Injection List windows.

Each row in the text file should contain the information for one row in the grid in the same order as the columns of the grid. Everything up to the first tab in the text file is imported into the first field in the grid.

Importing Tab-Delimited Text Files into Data Collection Software Windows

Step	Action
1	Open the window into which you wish to import information, and click the top left column of the grid.
2	Choose Import from the File menu.
3	In the dialog box that appears, choose the name of the file containing the text you wish to import.
4	Click OK.

Exporting Files

Use the Export command to save the contents of a window grid (for example, from the Sample Sheet or the Injection List) into a tab-delimited text format. This format is useful with most database, word processing, or spreadsheet programs.

To export information to tab-delimited text format:

Step	Action
1	Click the window containing the information you wish to export, to make it active. The window must be one of the windows containing a grid. It is the information in the grid that is exported.
2	Choose Export from the File menu.
3	Type a file name in the dialog box that appears.
4	Click OK.

Recovery Files

About Recovery Files

The 310 Genetic Analyzer is designed to resume operation after power failure. To resume the run, the instrument must have some record of the instrument status at the moment power failed.

At the start of a run, the Data Collection software creates a temporary file called the Recovery file. This file is updated continuously during the run. If the laboratory loses power, the instrument status is saved in the Recovery file. The 310 Genetic Analyzer searches for the Recovery file when power is restored and uses the information in it to resume the run at the correct step.

What to Do with Recovery Files

Do nothing with the Recovery file. The 310 Genetic Analyzerr will delete the Recovery file when the run ends. If the Recovery file has not been deleted, but you are certain that a run is neither in progress nor waiting to be resumed, put the Recovery file in the Trash.

About Matrix Files

Why You Need Matrix Files

A good matrix file is required to analyze raw data.

Each fluorescent dye contains components of other colors. A matrix file corrects for this spectral overlap in the filter set. This is an example of a matrix file:

Color	Blue	Green	Yellow	Red
Blue	1.0000	0.3952	0.0587	0.0010
Green	0.6592	1.0000	0.7803	0.0082
Yellow	0.3161	0.4388	1.0000	0.0382
Red	0.1120	0.2042	0.6178	1.0000

The amount of blue color in the blue dye, green color in the green dye, etc., is forced to 1.00 when the software creates a matrix.

The amount of green, yellow, and red in the blue dye is determined and recorded in the matrix file. The same process is repeated for the other dyes. The overlap will be subtracted from the data peaks when the matrix is applied, allowing the analysis software to “call the peaks, that is, identify the predominant dye in a fluorescent peak.

How Many Matrix Files?

Create a separate matrix file for each run condition that affects the collection of spectra. Some examples are:

- A new dye is used.
- The pH of polymers or buffers has changed.
- A different type of polymer is used.

Run the matrix standards under the sample run conditions and make a matrix file. Use that matrix file to analyze any and all samples run under those conditions.

6 Maintenance

Introduction

In This Chapter Topics in this chapter include the following:

General Care

Laboratory Temperature

Ensure that the ambient temperature is between 15 and 30 °C whenever the instrument is in operation, including nights and weekends.

Do not keep the instrument in direct sunlight or under heating or air conditioning vents.

Laboratory Humidity

Ensure that the humidity is below 80%. Avoid excessive condensation on the instrument.

Voltage Spikes

Do not plug the Macintosh® computer and 310 Genetic Analyzer into the same circuit used by other laboratory devices, especially centrifuges, refrigerators or freezers. Ensure that the circuit is grounded. Check with your laboratory's building facilities personnel if you have questions about the quality of power in your laboratory.

Put the Macintosh® computer and 310 Genetic Analyzer on a line conditioner or an uninterruptible power supply if voltage spikes and power outages are a persistent problem.

Cleaning

Clean the electrode, heat plate, and autosampler often to avoid the build up of conductive debris that causes arcing. Wiping with a water-dampened lab wipe is sufficient.

Clean the pump block as often as recommended. Polymer that dries in the pump block can be difficult or impossible to remove.

Protect the instrument from excessive dust.

Cables

Avoid twisting power cords around 310 Genetic Analyzer data communication cables. Power cords can affect the performance of computer cables.

The distance over which data communications are reliable depends upon both networking protocol and the type and quality of the cable. Connect the 310 Genetic Analyzerr and the Macintosh® computer that is collecting data with no more than six feet of cable. Use the cables provided with the instrument.

Caring for the Pump Block

When to Clean the Pump Block

Clean the pump block when:

- Installing the syringe.
- Removing the capillary.
- Changing between the ABI PRISM® DNA Sequencing Analysis Software and the GeneScan® Analysis Software applications
- Shutting down the instrument.
- The instrument has run for 4 days without any of the above occurring.

It is especially important to clean the pump block if a syringe containing polymer was installed on the block for more than a week. At room temperature, sufficient urea decomposition occurs in one week to cause transient current increases during electrophoresis.

IMPORTANT If polymer is allowed to dry in the pump block channels, the pump block may be ruined. It can be difficult or impossible to resolubilize dried polymer. Clean the pump block as often as recommended.

Do not clean the pump block with organic solvents or boiling water.

For more information, see “Cleaning the Pump Block” on page 4-8.

Caring for the Electrode

How to Avoid Bending the Electrode

Always recalibrate the autosampler if the cathode electrode is removed, replaced, or cleaned.

The electrode may bend if:

- A screw top tube is used in position 3 of the autosampler.
- The Z position of the autosampler is homed before the X, Y positions.
- The autosampler is not properly calibrated.
- Keys are held down when moving the autosampler with keyboard equivalents.
- The electrode is too long. The electrode should be flush with the translucent stripper plate.

Read “Autosampler Overview” on page 4-24, “How to Home the Autosampler” on page 4-26, and “How to Calibrate the Autosampler” on page 4-27 for instructions on operating the autosampler correctly.

Cleaning the Electrode

Clean the electrode if it has been touched, if it is new, when autosampler buffer solutions are replaced, or every 48 hours. Crystals formed on the electrode can fall into the sample and clog the capillary.

Since the capillary is removed from buffer while the electrode is cleaned, complete the procedure quickly, so that the capillary does not dry out.

Caring for the Autosampler

When to Calibrate the Autosampler

The autosampler calibration settings are maintained in the permanent memory of the instrument.

Recalibrate:

- After changing the electrode.
- After changing the capillary.
- If the capillary and the septa caps collide.
- After a reset that clears memory.
- When prompted by the instrument.

IMPORTANT The sample tray and Eppendorf tube must be removed before calibrating the autosampler. If the sample tray is not removed, the electrode will be bent.

An autosampler that is not calibrated can cause damage to the cathode electrode, capillary, and autosampler, and can result in poor data collection—or no data—due to improper capillary position in a sample tube.

When to Clean the Autosampler

Remove dried buffer from the autosampler with a water-dampened lab wipe at least once a week.

! WARNING ! Salt accumulation on the autosampler can cause arcing during electrophoresis.

Caring for the Glass Syringe

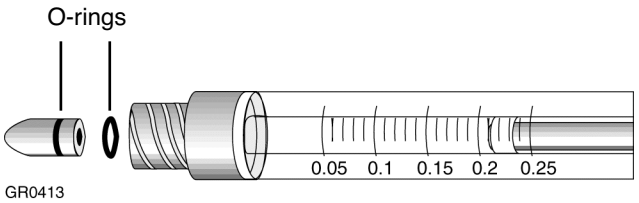
General Care

Syringe barrels and plungers are matched at the factory. Keep barrels and plungers as a set.

The glass syringes are fragile. Do not drop them.

About Syringe O-Rings

Inspect the two o-rings for degradation before each use. Replace them if they appear cracked, torn, flattened or otherwise worn.



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About Syringe Plungers

Always lubricate the plunger with water before inserting it into the barrel.

IMPORTANT Moving the plunger up and down in the barrel when the parts are dry will damage the syringe.

When to Clean the Syringe

Thoroughly clean the syringe whenever it is removed from the pump block.

Verify that the syringe is clean before use.

How to Store the Syringe

Syringe barrels and plungers are matched at the factory. Keep barrels and plungers as a set. Do not mix up barrels and plungers during storage.

Store the syringe away from direct sunlight. Sunlight degrades plastics.

Store the syringe dry. Lubricate the plunger with water before inserting it or moving it up and down in the barrel.

Loading Enough Polymer

Running Out of Polymer During a Run

If the instrument runs out of polymer during an injection, the injection is cancelled. An alert appears on the Macintosh® computer screen.

Check the Log file. Injections that last 1–2 minutes and Out of Polymer errors are recorded there when the syringe empties.

Starting a Run without Enough Polymer

The run will not start if the syringe encoder indicates that the syringe is located more than 500 steps from the Home position, because there is not enough polymer for the number of injections indicated in the injection list.

If the run will not start, remove the syringe and pump block and take the following actions:

Action	See page
Clean the pump block	4-8
Clean the syringe	4-19

How to Manually Test the Autosampler Calibration

Manually Test the Autosampler Calibration

The electrode and capillary must be immersed in the sample during electrokinetic injection. Minimum sample volume is 10-μL. Briefly microcentrifuging the sample to bring it to the bottom may be necessary.

If the electrode and capillary do not make contact with the sample, current problems and low signal problems can result.

To manually test that the autosampler calibration is allowing the capillary and electrode to make contact with your samples:

Step	Action
1	Put an empty tube in position A5 of the autosampler.
2	Open Manual Control from the Window menu of Data Collection software.
3	Use the Autosampler to Position function to send the autosampler to position A5.
4	Execute the Autosampler Up function.
5	Use the Autosampler Z Max Travel function to lower capillary and electrode into the tube.
6	Visually inspect the depth of the capillary and electrode in the tube.

How to Check for Polymer Leaks

Overview

Leaks can cause high rates of polymer consumption.

Severe leaks usually mean one or more of the manual valves are not closed.

Leaks may also occur at the ferrule sealing the capillary to the pump block, at the Teflon seal of the plunger in the glass syringe or at the valve to the buffer reservoir.

Check the base of the glass syringe for polymer leaks (visible as a white residue), especially at the interface between the glass barrel and the metal tip.

About the Leak Detect Error

If the instrument pauses with a Leak Detect error message, check for:

- Polymer leaks
- Bubbles in the glass syringe

Bubbles in the glass syringe will compress as the syringe tries to inject polymer into the pump block and capillary. The software assumes the syringe travel for compressing the bubble is for polymer delivery. More than 15 counts on the syringe drive encoder triggers a Leak Detect error. These counts might be the result of compressing a bubble in the syringe.

Avoid bubbles in the glass syringe when filling it with polymer.

Checking for Leaks

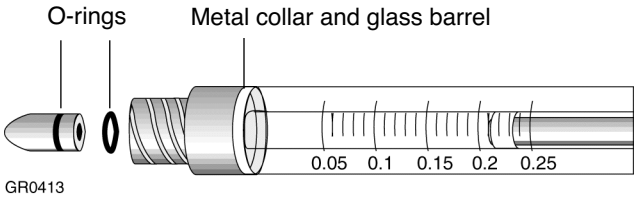
Run the Seq Fill Capillary module to check for leaks:

Step	Action
1	Open the ABI PRISM® 310 Data Collection Software and the Manual Control window.
2	Press the Tray button. The autosampler tray moves forward.
3	Place a small tube containing 0.5-mL of water in tube position 3 in the front of the autosampler.
4	Press the Tray button again to move the autosampler back to its original position.
5	Select Seq Fill Capillary from the Module pop-up menu on the Manual Control screen. 
6	Note the position of the end of the glass syringe plunger and write it down.
7	Click Start.
8	After the module has run, again note the position of the plunger, and subtract it from the initial value. The module should consume 4 - 7 µL of polymer per 10-minute fill. Check for leaks if more than 7-µL of polymer was consumed.

continued on next page

Correcting Leaks at the Syringe

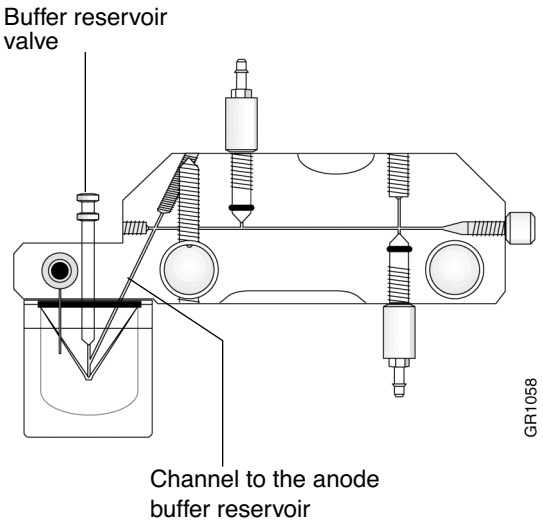
To correct leaks at the syringe:

Step	Action
1	Replace the syringe if polymer leakage is: ♦ Excessive past the plunger's seal. ♦ Between the metal collar and the glass barrel. Loss of only a few microliters of polymer out of a full syringe is normal and acceptable.
2	If polymer has leaked from the base of the glass syringe, check for the presence of two o-rings and a plastic ferrule. Replace the o-rings if they are worn. 

Correcting Leaks at the Valve to the Buffer Reservoir

To correct leaks at the valve to the buffer reservoir:

Step	Action
1	Remove the pump block and rinse it with warm water.
2	Flush the pump block channel to the anode buffer reservoir with warm water and take the following actions (see figure below): a. Attach a 5-mL plastic syringe filled with warm water to the pump block. b. Plug the other pump block openings with plugs provided in the Basic Installation Kit. c. Clean each pump block channel with distilled, deionized water, removing and replacing plugs to direct the water through each channel in turn. d. Clean the channel to the buffer reservoir thoroughly. e. Re-install the block on the instrument.

Step	Action
	
3	If leaks continue at the buffer reservoir valve, contact an ABD Service Representative.

If the Buffer Reservoir Fills Up

If the buffer reservoir is filling up, check for:

- Leaks at the valve to the buffer reservoir.
- Bubbles in the glass syringe.

Bubbles in the syringe compress and expand. During expansion, they can drive polymer into the buffer reservoir.

How to Avoid Capillary Clogs

Verifying That the Capillary Fills Correctly

Capillaries used with Performance Optimized Polymers are uncoated. A proper coat of polymer is especially important for these capillaries. Routinely check that capillaries fill properly.

To determine if the capillary is filling properly, check the Log File. The syringe drive encoder should move one or two counts per injection for the 1.0-mL syringe.

If there are...	This...
zero counts	Indicates that the capillary did not fill and may be clogged.
more than 15 counts	May indicate a polymer leak. For more information, see “Checking for Leaks” on page 6-7.

Avoiding Capillary Clogs

Clogged capillaries cause current problems during electrophoresis.

Cause	Prevention
Proteins	Purify your DNA templates. Run samples with TSR to avoid drawing protein into the capillary.
Large molecular weight DNA	Purify your DNA templates. Run samples with TSR to avoid drawing large molecular weight DNA into the capillary.
Bacteria	Use deionized, distilled water. Water is the most common source of bacterial contamination.
Crystallized buffer or polymer	Clean the electrode every two days. Check for crystals at the connection of the glass syringe and pump block. Bring polymer to room temperature before use. Do not expose the end of a filled capillary to air for more than 30 minutes.

Monitoring Communication Between the Macintosh Computer and the Instrument

About the Communications Diagnostics Window

The window shows information for all Send and Reply messages since the ABI PRISM® 310 Data Collection Software was last launched.

The window displays the:

- Number of messages
- Number of characters
- Duration of last message in milliseconds
- Number of retries
- Number of serial errors

Large numbers of retries indicate poor cable routing (see “Cables” on page 6-2), broken pins on connectors, or bad cables.

Serial retries indicate broken pins on connectors or bad cables.

Long duration for replies indicates a Macintosh® serial driver problem.

Take the following actions:

- Resetting the PRAM (see “Resetting the Macintosh PRAM” on page 6-13).
- Reloading the Mac OS.

Accessing the Communications Diagnostics Window

Accessing the Communications Diagnostics Window

To access the Communications Diagnostics window, the Status window must be active. Open or click on the Status window before trying to access the Communications Diagnostics window.

Opening the Communications Diagnostics window

Hold down the control and option keys simultaneously.

If there is no communication, the Communications Diagnostics window is not accessible.

How to Maintain and Care for Your Macintosh Computer

Overview

Computers require regular attention and maintenance to operate efficiently and consistently.

Because the ABI PRISM® 310 Genetic Analyzer software on the Macintosh® computer works with large files and accesses the hard disk often, it is especially important to follow the procedures described here to minimize errors during operation.

Hard Disk Maintenance

Follow these guidelines for optimal performance of the ABI PRISM® 310 Genetic Analyzer programs and the Macintosh® computer.

- Install only one System on a hard disk.
- Back up all programs and files regularly.
- Use a hard disk maintenance program (such as Norton Utilities) regularly to reorganize a fragmented disk.

  The Norton Utilities program is provided with the 310 Genetic Analyzer.
- Use discretion when adding software programs, especially Control Panels and extension files.

Each of these guidelines is discussed in more detail below.

Only One System File Required

The Macintosh® requires only one System file to operate. This System file is located in the System Folder on the hard disk and is essential for all operations.

You do not receive a System file with the ABI PRISM® 310 Genetic Analyzer programs, but other applications might furnish an additional System file. If you copy a program to your hard disk, avoid copying additional System files.

For more complete information about the System file and the System Folder, refer to the manuals that came with the computer.

Regularly Backing Up All Programs and Files

Although the hard disk is extremely reliable, it is still subject to the whims of fate and power. Always back up your computer's hard disk so you do not lose the data you have spent hours collecting and analyzing. Keep the back ups in a safe place.

When you use the ABI PRISM® 310 Data Collection Software and analysis programs regularly, a large number of data files may accumulate on your hard disk and consume available disk space. Make floppy copies of the data files you use infrequently, and remove the originals from your hard disk to reclaim storage space for future work.

Using a Hard Disk Maintenance Program Regularly

Whenever files are written to the hard disk, opened and rewritten, their physical location on the disk changes. This "fragments" the disk. If a significant amount of fragmentation occurs, the system runs slowly, and files may be lost. Protect your data by running a disk optimizer program at least once a month.

Use Discretion When Adding Software Programs

- Keep an anti-viral software application loaded on your hard disk and use it to inspect your hard disk either when you start up or shut down (at least once a day).

  Viruses can simply be annoying, but they can also, in the worst case, destroy all information stored on your hard disk. This could include the System software, ABI PRISM® 310 Genetic Analyzerr software, and data files.

- Use the Macintosh® computer only for ABI PRISM® 310 software. Prevent program conflicts before they occur. Do not load games or other software programs onto your hard disk.
- Do not use any Extensions or games (this includes custom start-up sounds and graphics) other than those that came on your original System Disk, or on the ABI PRISM® 310 software disks.

Eliminating Conflicts Between Inits

Use the Extension Manager control panel to select a group of extensions that starts up with conflicting inits. Refer to the documentation shipped with the Macintosh® computer for more instructions on Extension Manager. The best practice is to avoid using the Macintosh® computer for anything except Applied Biosystems software.

How to Turn Off All Extensions

You can turn off all Extensions by holding down the Shift key while you restart your computer. (You will need to restart it again to turn them back on.) Start the Macintosh® computer with all Extensions off if you suspect that programs are conflicting. If the problem disappears, an “init” conflict is the likely source.

Resetting the Macintosh PRAM

Restart the Macintosh while holding down these four keys:

- Apple (
- Option
- p
- r

When to Reset the Macintosh PRAM

Reset the PRAM if the Macintosh® computer:

- Crashes
- Gives Type 11 error messages
- Has problems communicating through the serial port
- Gives Coprocessor Not Found messages

Resetting the PRAM on a regular basis may help prevent errors. Consult the information provided with your computer.

continued on next page

Communication Port Settings

In General Settings (in the Preferences menu of Data Collection software), choose the modem port for optimum communication between the instrument and Macintosh® computer.

Turn AppleTalk off if the Macintosh® printer and instrument Diagnostic ports are connected.

EtherTalk will default to the printer port if the network is disconnected or down. If the Macintosh® computer is not connected to an Ethertalk network, do not select the Ethertalk protocol in the Network or AppleTalk control panels (in the Macintosh® Apple menu).

The Macintosh® computer locks up if AppleTalk or EtherTalk try to use the Macintosh® printer port when it is connected to the instrument Diagnostic port.

7 Troubleshooting

Introduction

In This Chapter Topics in this chapter include the following:

How to Troubleshoot Data

Troubleshooting Data

When troubleshooting 310 Genetic Analyzer problems:

Step	Action
1	Understand: ♦ The chemistry ♦ Labeling of the samples ♦ How the 310 Genetic Analyzerr collects data ♦ How the data analysis software programs work Review the experiment for errors in primer design, sample quantitation and purification, pipetting problems, software preference settings and other common mistakes.
2	Examine the data. Describe the problem as specifically as possible: ♦ Is it a problem with the sample peaks, the baseline, or the peaks of only one color? ♦ Does the problem exist in all parts of the run or does it affect only DNA fragments of a certain length? ♦ Is the problem visible in raw data? analyzed data? log files? Continue to ask these types of questions until you have described the problem as specifically and thoroughly as possible.
3	List possible causes of the problem. See the troubleshooting tables in this chapter for help.
4	For each “possible cause” you have listed, ask: Does other information support or contradict this as the cause of the problem?
5	If necessary, collect more information to narrow the list of possible causes.
6	Correct the problem and test the fix.

Check Our Web Address

Often a small amount of time invested in troubleshooting a problem yourself can save you inconvenience. Our site on the World Wide Web includes answers to frequently asked questions and allows downloads of software upgrades.

Of course, Applied Biosystems Field Application Specialists, Technical Support Specialists, Field Service Engineers and Sales Representatives are available to fully support the 310 Genetic Analyzer. Please contact the nearest Applied Biosystems office, visit us on the Web atwww.appliedbiosystems.com/techsupport, or call us toll-free in the U.S.

Problems with Automatic Data Analysis

Problems with automatic data analysis.

Observation	Possible Causes	Recommended Actions
Data was not automatically analyzed	Sample Sheet not completed or completed incorrectly	Review and correct Sample Sheet
	Injection List not completed or completed incorrectly	Complete Injection Lists as described
	Analysis preferences set incorrectly in 310 Data Collection software	Check the ABI PRISM® 310 Data Collection Software preferences: Select Autoanalyze with GeneScan® Analysis Software under the GeneScan® Injection List Defaults Select Autoanalyze with ABI PRISM® DNA Sequencing Analysis under the Sequencing Injection List Defaults
	Insufficient free RAM	Restart computer before collecting data **Note** Always restart the computer before collecting data.
	Conflicting extensions (inits)	Choose Extensions Manager from the Control Panels. Turn off any conflicting extensions and restart computer. See "How to Maintain and Care for Your Macintosh Computer" on page 6-12.

Problems with Current

Problems with current.

Observation	Possible Causes	Recommended Actions
No current	Too little or no buffer in anode buffer reservoir	Replenish buffer reservoir.
	Too little or no buffer in autosampler position 1	Replenish buffer in position 1 of autosampler.
	Electrode bent	a. Remove electrode. b. Straighten electrode. c. Replace electrode. d. Recalibrate autosampler.
	Capillary bent away from electrode	a. Tape capillary securely to heat plate to keep capillary from shifting position. b. Recalibrate autosampler.
	Unfilled capillary, bubbles in capillary and block	a. Examine the instrument for leaks. See “How to Check for Polymer Leaks” on page 6-7. b. Rerun module.
	Pump block is plugged with urea or crystallized buffer	Remove and clean block.
	Loose valve fittings or syringe	Tighten valve fittings and syringe.
	Anode buffer valve does not open	Open buffer valve: a. Push down on the valve with your finger. b. Release the valve. It should spring to the “open” position. **Note** If the valve is stuck, it should be cleaned. See “Correcting Leaks at the Valve to the Buffer Reservoir” on page 6-8 for directions.
	Plugged, broken, or nonconducting capillary	Replace the capillary.
	Poor quality water in buffer solutions	Remake buffer with fresh distilled, deionized water.
	Old, defective, or incorrectly made buffer or polymer	Replace buffer or polymer.
	Corrupted firmware	Resend firmware.

Problems with current. *(continued)*

Observation	Possible Causes	Recommended Actions
Low current	Small bubble in capillary blocking current flow	Rerun module.
	Plugged, broken, or nonconducting capillary	Replace the capillary.
	Poor quality water in buffer solutions	Remake buffer with fresh distilled, deionized water.
	Old, defective, or incorrectly made buffer or polymer	Replace buffer or polymer.
Fluctuating current	Too little buffer in anode buffer reservoir	Replenish buffer reservoir.
	Small bubble in capillary blocking current flow	Rerun module.
	Broken or cracked capillary	Replace the capillary.
	Arcing to conductive surface on the instrument	a. Clean the heat plate, electrode and autosampler. b. Ensure that the ambient temperature is between 15 and 30 °C and the humidity is below 80%. Check for excessive condensation on the instrument.
Current is normal at beginning of run and then decreases rapidly over the next several minutes	Loss of anode buffer capacity	Replace the buffer.
	Overloaded capillary	a. Use TSR or formamide. b. Decrease the electrokinetic injection time or voltage.
Current too high	Decomposition of urea in the polymer	a. Clean the syringe and pump block. b. Install new polymer.
	Incorrect buffer formulation (most likely too concentrated)	Replace buffer with appropriate 1X running buffer.
	Arcing to conductive surface on the instrument	Clean the heat plate, electrode and autosampler. Ensure that the ambient temperature is between 15 and 30 °C and the humidity is below 80%. Check for excessive condensation on the instrument.

Problems with Signal Strength and Quality

Problems with signal strength and quality.

Observation	Possible Causes	Recommended Actions
No signal	No sample added	a. Check pipet calibration. b. Examine the efficiency of the PCR and/or cycle sequencing reactions. c. Remake sample.
	Template not accurately quantitated (DNA sequencing)	Quantitate template. Refer to the sequencing kit protocol for appropriate DNA quantities.
	Sample not at bottom of tube	Spin sample tube in microcentrifuge.
	Air bubble at bottom of sample tube	Spin sample tube in microcentrifuge to remove air bubbles.
	Volume of sample too low	Sample volume must be at least 10-μL.
	Capillary misaligned with cathode	Align capillary and cathode. **Note** The capillary should be adjacent to, but not touching, the cathode. The capillary should protrude 0.5 mm past the cathode.
	Capillary bent out of sample tube	Align capillary and cathode. Recalibrate autosampler. Verify whether a bent capillary is the problem by watching: ♦ The movement of the autosampler tray during run operation. ♦ For injection current and EP current during data collection.
	Autosampler not calibrated correctly	Calibrate autosampler in X, Y, and Z directions. **IMPORTANT** The capillary should almost touch the Z calibration point.
	Sealed sample tube septum (that is, septum will not open to allow electrode into sample tube)	You can either: ♦ Replace septum or, ♦ Open the septum by pinching it.
	Septum not placed in the sample tube properly	Replace septum.
	Insufficient injection time	Increase the electrokinetic injection time or voltage.

Problems with signal strength and quality. *(continued)*

Observation	Possible Causes	Recommended Actions
Signal too low	Insufficient sample added	a. Check pipet calibration. b. Examine the efficiency of the PCR and/or cycle sequencing reactions c. Remake sample
	Dust or particulates have collected on the capillary window and or the internal optics	Clean window with ethanol and a Kimwipe. If no improvement, contact your service representative to clean the internal optics.
	Capillary not correctly aligned in the window	Align capillary in window. See "Procedure for Positioning the Capillary in the Detector" on page 4-13.
	Volume of sample too low	Sample volume must be 10 μL.
	Samples added to formamide that has degraded to formic acid and formate ions (leading to injection of insufficient sample)	Use freshly deionized formamide. See "Denaturing and Loading Samples" on page 2-40 or 3-28.
	Salts in sample (leading to injection of insufficient sample)	Remove ions by: ♦ Dialyzing sample ♦ Ethanol precipitation ♦ Spin column purification
	Sample not thoroughly mixed	Mix sample by pipetting up and down several times.
	Template not accurately quantitated (DNA sequencing)	Quantitate template. Refer to the sequencing kit protocol for appropriate DNA quantities.
	Insufficient [F]dNTPs added to PCR reaction (GeneScan®)	Reamplify using more [F]dNTPs or examine the efficiency of the PCR.
	Capillary and/or electrode not immersed in sample	Recalibrate the autosampler.
	Insufficient injection time	Increase the electrokinetic injection time or voltage.
	CCD camera not calibrated	Contact you r service representative.

Problems with signal strength and quality. *(continued)*

Observation	Possible Causes	Recommended Actions
Signal too high	Too much sample injected into capillary	Decrease injection time or injection voltage.
		Dilute sample.
		Reamplify using less [F]dNTPs (GeneScan®)
	Unincorporated [F]dNTPs (GeneScan® Analysis Software)	Purify the PCR product.
	Unincorporated ddNTPs (ABI PRISM® DNA Sequencing Analysis Software)	Use ethanol precipitation or spin column purification
Signal too high in first part of run (sequencing)	Excess DNA template depletes nucleotides in reaction mix, leading to excess of short fragments	Quantitate template. Refer to the sequencing kit protocol for appropriate DNA quantities.
Peak height decreases throughout run	Sample evaporation	Use septa.
High baseline	Dirty capillary window	Clean capillary window using 95% ethanol and lint free paper.
	Dirty syringe	Clean the syringe with warm water.
	Dirty pump block	Clean the pump block with warm water as described in Chapter 4.
	Capillary moved out of position in front of detector window	Position capillary in front of laser window.
	Precipitate in polymer	Allow polymer to equilibrate to room temperature before using.
	Old, defective, or incorrectly made buffer or polymer	Replace buffer and polymer.
	Dirty detector window	Clean the detector window carefully using lense paper.
	Defective capillary	Replace the capillary.
	Matrix made incorrectly resulting in *too much* correction (indicated by troughs under peaks) **Note** Matrix problems only show in analyzed data and not the raw data.	Remake matrix. Be sure to: ♦ Remove primer peak (or aberrant off-scale peaks) from scan range. ♦ Pick start and stop points on flat parts of the baseline when viewing raw data. ♦ Make matrix using same polymer, buffer, and run conditions as sample injections.

Problems with signal strength and quality. *(continued)*

Observation	Possible Causes	Recommended Actions
Noisy baseline	Old, defective, or incorrectly made buffer or polymer	Replace buffer or polymer.
	Weak or low signals and or an elevated baseline	See “Problems with Signal Strength and Quality” on page 7-6.
	A noisy baseline in raw data could be electrical noise	Contact your service representative.
	Dirty detector window	Clean the detector window.
	Plugged, broken, or nonconducting capillary	Replace the capillary.
	Salts in sample (leading to injection of insufficient sample)	Remove ions by: ♦ Dialyzing sample. ♦ Ethanol precipitation. ♦ Spin column purification.
	Capillary not refilling	a. Check for leaks. b. Check for bubbles in the syringe. c. Replace capillary if clogged. d. Increase capillary refill time.
Spikes in baseline	Precipitate in polymer	Allow polymer to equilibrate to room temperature before adding to capillary.
	Old polymer (POP 4™ or POP 6™)	Use fresh polymer.
Low baseline with no color separation	Laser problem	Contact your service representative.
	CCD problem	
	Cap misaligned	Ensure that the window is in the groove in the front of the laser.

Problems with Peak Number and Position

Problems with peak number and position.

Observation	Possible Causes	Recommended Actions
Extra peaks in additional colors displayed underneath each peak. **Note** This shows up clearly in analyzed data and may not be an obvious problem in the raw data. This is because it is common to have extra peaks in other colors displayed in the raw data.	Too much sample injected into capillary	Decrease injection time or injection voltage.
	Problem with sample preparation. See "Problems with Extra Peaks" on page 7-20.	Dilute sample.
	Too little sample injected into capillary (matrix overcorrects)	Increase injection time or injection voltage.
		Quantitate DNA in sample. Refer to protocol for correct quantity.
	Incorrect matrix chosen	Check matrix selection on Injection List. If correct, you may want to create a new matrix.
	Incorrect Run Module Filter set chosen	Verify module filter set with dyes being run with charts on page 2-43 and page 3-26.
	Wrong filter set used	
Many small extraneous peaks appearing next to a high-intensity peak	Background above Minimum Peak Height value.	Increase value for Minimum Peak Height. Reanalyze data.
	Sample DNA overloaded. (Extraneous peaks represent nonspecific DNA comigrating with main fragment peak.)	Load less sample and repeat electrophoresis.
	Too much sample injected into capillary	Decrease injection time or injection voltage.

Problems with peak number and position. *(continued)*

Observation	Possible Causes	Recommended Actions
Extra peaks when sample is known to contain DNA from a single source	Samples not fully denatured	Make sure the samples are heated at 95 °C for five minutes prior to loading onto autosampler.
	Unoptimized PCR	Check efficiency of the PCR. See *ABI PRISM® GeneScan Chemistry Guide for* detailed suggestions.
	Renaturation of denatured samples	Load samples immediately following denaturation, or store on ice until you are ready to load. **IMPORTANT** Do not store samples on ice for more than two hours before loading. **Note** Too much DNA also promotes renaturation, but before you add less DNA you will need to assess the signal strength and quality.
Extra peaks are 1-4 nt larger or smaller than expected peak (GeneScan®)	PCR artifact	Refer to Troubleshooting PCR Amplification on page 7-16.
Size-standard peaks not recognized when defining size standard (GeneScan®)	Height of a size-standard peak less than the Peak Amplitude Threshold for the size-standard color (in Analysis Parameters) **Note** 50 RFU is the default threshold	Rerun sample, adding the recommended amount of size standard.
		Lower the value for the size-standard color in the Peak Amplitude Threshold field.
	Peaks missing from size-standard definition.	Check GeneScan® Analysis Parameters to make sure the correct scan range is defined.
	Minimum Peak Half Width is set too high (in Analysis Parameters)	Lower the value for the Minimum Peak Half Width.

Problems with peak number and position. *(continued)*

Observation	Possible Causes	Recommended Actions
Peak positions off throughout size range (GeneScan®) **Note** Refer to the *ABI PRISM® GeneScan Chemistry Guide* for detailed information on factors that affect sizing.	Incorrect Sample Sheet	Check Sample Sheet selection in data collection program.
	Change in size-calling method.	Use consistent size-calling method.
	Incorrect internal size standard.	Use correct GeneScan® size standard.
	Incorrect polymer composition.	Check urea concentration and polymer composition against protocol.
	Incorrect electrophoresis temperature	Check the Injection List for temperature setting. If correct on Injection List, check the Log for a recording of the actual electrophoresis temperature.
	Incorrectly defined size standard	Define size-standard peak sizes separately for each incorrectly sized injection.
Runs get progressively slower (peaks come off at higher and higher scan numbers)	Capillary not refilling	a. Check for leaks. b. Check for bubbles in the syringe. c. Replace capillary if clogged. d. Increase capillary refill time.
	Syringe out of polymer	Fill syringe with fresh polymer.
	Ambient temperature in laboratory changing	Ensure that the ambient temperature is between 15 and 30 °C whenever the instrument is in operation, including nights and weekends. Do not keep the instrument in direct sunlight.
Runs get progressively faster (peaks come off at lower and lower scan numbers)	Water in syringe	When loading syringe: a. Prime syringe with small volume of polymer. b. Invert syringe to coat capillary walls. c. Discard polymer. d. Fill syringe with fresh running polymer.
	Ambient temperature in laboratory changing	Ensure that the ambient temperature is between 15 and 30 °C whenever the instrument is in operation, including nights and weekends. Do not keep the instrument in direct sunlight.

Problems with peak number and position. *(continued)*

Observation	Possible Causes	Recommended Actions
Peak spacing too high (sequencing)	Capillary clog	See "Avoiding Capillary Clogs" on page 6-10.
	Arcing to conductive surface on the instrument	a. Clean the heat plate, electrode and autosampler. b. Ensure that the ambient temperature is between 15 and 30 °C and the humidity is below 80%. c. Check for excessive condensation on the instrument.
Irregular peak spacing (sequencing)	Wrong mobility file	Use correct mobility file.
Some but not all loci visible on electropherogram (GeneScan®)	Sample DNA is degraded	Quantitate DNA and add more template. Repeat amplification.
		Wash the sample in an Amicon Centricon-100 column and repeat amplification. **Note** For fragments smaller than 130 bp the Amicon Centricon-30 column is preferable.
	Sample contains PCR inhibitor (for example, heme compounds, EDTA, or certain dyes)	a. Quantitate DNA and add minimum necessary volume of PCR product. b. Repeat amplification.

Problems with Peak Quality and Resolution

Problems with peak quality and resolution.

Observation	Possible Cause	Recommended Actions
Fuzzy/smeared peaks	Too much sample DNA	Decrease injection time and/or voltage.
		Dilute PCR sample before adding to formamide/size-standard mix.
Poor resolution	Poor capillary performance	Replace capillary.
	Capillary clog (especially peaks that start normally but abruptly become broad at some point in run)	See “Avoiding Capillary Clogs” on page 6-10.
	Old, defective, or incorrectly made buffer or polymer	Replace buffer or polymer.
	Injection time too long (broad peaks)	Decrease injection time.
	Incorrectly prepared or degraded sample	Prepare new sample.
	Incorrect buffer formulation	Check if buffer formulation matches protocol requirements.
	Incorrect polymer composition	Check if polymer composition matches protocol requirements.
	Electrophoresis voltage too high	Decrease electrophoresis voltage by as much as 4 kV. **Note** Increase electrophoresis time accordingly.
	Sample concentrated by evaporation leaving excess salt behind.	Do not concentrate sample by evaporation. Use an Amicon Centricon®-100 column if necessary.
	Incomplete strand separation due to insufficient heat denaturation	Make sure the samples are heated at 95 °C for 5 minutes prior to loading onto autosampler.
	Too much DNA in sample	Dilute sample before adding to formamide.
	Wrong capillary used for POP-4™ or POP-6™ runs	Verify that you are using the correct capillary.

Problems with peak quality and resolution. *(continued)*

Observation	Possible Cause	Recommended Actions
Poor resolution	Oil in sample (from DNA Thermal Cycler 480)	Carefully pipette PCR product without oil carryover.
		Remove oil by organic extraction.
	Poor quality water	Use freshly autoclaved, distilled, deionized water.
	Bacterial contamination in water vials or buffer	Change water and buffer vials regularly.
	Syringe empty or incorrect Syringe Max Travel value	Fill syringe if necessary and recalibrate Syringe Max Travel value.
	Capillary too short	Increase capillary length. **Note** Increase electrophoresis time accordingly.
	Ethanol contamination (sequencing)	Remove ethanol by evaporation.
Peaks “trail” or shoulder into next peak	Template not accurately quantitated (DNA sequencing)	Quantitate template. Refer to the sequencing kit protocol for appropriate DNA quantities.
False stop (large peak in all four colors in dye primer sequencing)	Secondary structure	a. Denature at 95° C for 5 minutes. b. Try dye terminator sequencing.
	Failure to separate transformed and non-transformed vectors.	Use proper techniques to isolate transformed vector.
	Primer-dimer formation during PCR amplification	Use different primers. For more information, see the following user manuals: ♦ *ABI PRISM® GeneScan Chemistry Guide* ♦ *ABI PRISM® DNA Sequencing Chemistry Guide*

Problems with Poor Amplification

Problems with poor amplification.

Observation	Possible Cause	Recommended Actions
Faint or no signal from sample DNA and from positive control	Insufficient injection or a mis-positioned capillary	Check that the capillary is 0.5-mm or less, and is aligned with electrode end. If you adjust the capillary, then recalibrate the autosampler.
	Insufficient enzyme in reactions	Use the recommended amount of enzyme.
	Incomplete activation of AmpliTaq Gold™ DNA Polymerase.	Repeat amplification, making sure to hold reactions initially at 95 °C for 11 minutes.
	Too little sample DNA added to reaction.	Quantitate DNA and use the amount recommended in the protocol.
	Incorrect or suboptimal thermal cycler parameters	Check protocol for correct thermal cycler parameters. If the correct parameters were used, they may need to be optimized for your specific application. For example, allow a linear increase in extension time with increasing cycle number, increase time at denaturation plateau, and so on.
	PCR Master Mix not well mixed before aliquoting	Vortex PCR Master Mix thoroughly.
	Primer concentration too low	Use the recommended primer concentration.

Problems with poor amplification. *(continued)*

Observation	Possible Cause	Recommended Actions
Faint or no signal from sample DNA and from positive control (continued)	Primers degraded	Use new primers. **Note** Preincubation at 95 °C for 5 - 10 minutes should inactivate proteases or nucleases.
	Too little free Mg^{2+} in reaction	Check that you added sufficient total Mg^{2+} given the total dNTP concentration. **Note** [Free Mg^{2+}] = [Total Mg^{2+}] - [Total dNTP]
	Incorrect pH	Verify buffer pH and buffer concentration.
	Wrong PCR tube	Use: ♦ Applied Biosystems GeneAmp® Thin-Walled Reaction Tubes for the DNA Thermal Cycler 480. ♦ MicroAmp® Reaction Tubes with Caps for the GeneAmp® PCR System (9700, 9600, and 2400).
	MicroAmp® Base used with tray/retainer set and tubes in GeneAmp® PCR System (9700, 9600, or 2400)	Remove MicroAmp® Base from tray/retainer set and repeat amplification.
	Verify GeneAmp® PCR System protocols and programmed parameters	Refer to the thermal cycler user's manual and check instrument calibration.
	Tubes not seated tightly in the thermal cycler (DNA Thermal Cycler 480)	a. Push reaction tubes firmly into contact with block after first cycle. b. Repeat amplification.
	GeneAmp® PCR System 9600 heated cover misaligned	Align 9600 heated cover so that white stripes align after twisting the top portion clockwise.

Problems with poor amplification. *(continued)*

Observation	Possible Cause	Recommended Actions
Good signal from positive control but faint or no signal from sample DNA	Sample contains PCR inhibitor (for example, heme compounds, EDTA, or certain dyes)	Quantitate DNA. Dilute if possible in order to add minimum necessary volume. Repeat amplification.
		Wash the sample in an Amicon Centricon-100 column and repeat amplification. **Note** For fragments smaller than 130 bp use the Amicon Centricon-30 column instead.
		Add bovine serum albumin (BSA) to the PCR reaction mixture.
	Sample DNA is degraded	If possible, evaluate the quality of DNA sample by ♦ Using the QuantiBlot Human DNA Quantitation kit. ♦ Running an agarose yield gel. If DNA is degraded, reamplify with an increased amount of DNA.
	Incorrect pH	Verify buffer pH and concentration. If correct, quantitate sample DNA. Too little or too much DNA can alter the pH.
	Primer choice not optimal (for example, primers may be annealing to sites of template secondary structure or may have internal secondary structure)	Use different primers. For more information, see the following: ♦ *ABI PRISM® GeneScan Chemistry Guide* ♦ *ABI PRISM® DNA Sequencing Chemistry Guide*
Poor yield for multiplex PCR	Incorrect thermal cycling parameters	Add a 2 minute down-ramp time to thermal cycling profile between the denaturation and annealing stages, **Note** For multiplex PCR, a short down-ramp time is not necessarily optimal.

Problems with poor amplification. *(continued)*

Observation	Possible Cause	Recommended Actions
Yield gets progressively poorer for successive PCR amplifications performed over time	Expired or mishandled reagents	Check expiration dates on all reagents. If not expired, verify that reagents are being stored and used according to manufacturer's instructions. Compare with PCR performance using fresh reagents.
Inconsistent yields with control DNA	Combined reagents not spun to bottom of sample tube	Place all reagents in apex of tube and spin briefly after combining.
	Combined reagents left at room temperature or on ice for extended periods of time (encouraging mispriming and other primer artifacts)	Keep reactions on ice and load immediately.
	Pipetting errors	Follow all these precautionary measures: ♦ Calibrate pipettes. ♦ Attach tips firmly. ♦ Check all phases of pipetting technique. ♦ Whenever possible minimize pipetting small volumes (for example, make master mixes). **Note** You may also want to consider using a 2-µL or other high-precision pipette

Problems with Extra Peaks

Problems with extra peaks.

Observation	Possible Causes	Recommended Actions
Extra peaks appear with no discernible pattern	Mixed sample	Verify quality and integrity of sample.
	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Nonspecific priming (that is, primer-template mismatch)	Check for good primer design.
		Add less template DNA. **Note** High DNA concentrations promote nonspecific annealing.
		Add less primer DNA **Note** High primer concentrations promote nonspecific annealing.
		If you are ***not*** using AmpliTaq Gold DNA Polymerase, consider performing Hot-Start™ Technique.
		Increase annealing temperature in 2 - 5 °C increments.
		Decrease annealing and/or extension times.
		Increase primer length.
		Perform a second amplification with nested primers.
		Perform Touchdown PCR.
	Primer-dimer and primer-oligomer artifacts	Check primers for 3' complementarity.
		Design longer primers.
		Reduce primer concentration.
		Reduce number of PCR cycles.
		Raise the annealing temperature in 2 - 5 °C increments.
		Increase amount of target DNA.
	Incomplete restriction (and/or ligation if performing AFLP)	Repeat restriction (and/or ligation).
	If performing AFLP, too much DNA in reaction so that insufficient adaptor is present	Use the recommended amount of template DNA.

Problems with extra peaks. *(continued)*

Observation	Possible Causes	Recommended Actions
Extra peaks with pattern	PCR run contaminated with Amplicons	a. Decontaminate equipment, tools, and working area. b. Check disposables and reagents as potential contaminated source.
Presence of split peaks differing in size by one base pair (GeneAmp®) (Extra peak of size n + 1)	Partial nontemplate addition of an extra nucleotide (usually adenosine) to the blunt end of the PCR product	Add the correct amount of Mg^{2+} to the reaction mix. **Note** High Mg^{2+} concentrations can lower the frequency of nontemplate nucleotide addition and vice versa. Increasing the extension time at 72 °C will increase the frequency of nontemplate nucleotide addition. For more suggestions refer to the *ABI PRISM® GeneScan Chemistry Guide*.

Applied Biosystems Limited Warranty

A

To What This Warranty Applies

Applied Biosystems warrants to the Customer that, for a period ending on the earlier of one year from completion of installation or fifteen (15) months from the date of shipment to the Customer (the “Warranty Period”), the ABI PRISM® 310 DNA Sequencer purchased by the Customer (the “Instrument”) will be free from defects in material and workmanship, and will perform in accordance with the performance specifications contained in the Instrument User’s manual that accompanies the instrument (the “Specifications”). During the Warranty Period, if the Instrument fails to perform in accordance with such specifications, Applied Biosystems will repair or replace the Instrument at no charge to the Customer, subject to the conditions below.

To What This Warranty Does Not Apply

This Warranty does not apply to the Instrument's valves, reagent lines, or performance, unless the Customer uses only reagents and solvents supplied by Applied Biosystems or expressly recommended by Applied Biosystems, or to damages caused by reagents or solvents not supplied by Applied Biosystems, even though recommended by Applied Biosystems. This Warranty does not extend to any Instrument or part thereof (i) that has been the subjected of misuse, neglect or accident, (ii) that has been modified or repaired by any party other than Applied Biosystems or (iii) that has been used in a manner not in accordance with the instructions contained in the Instrument User's Manual. This Warranty does not cover the customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual.

Applied Biosystems Obligation

Applied Biosystems obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct covered defects or failures of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty shall be performed by Applied Biosystems on-site at the Customer's location at Applied Biosystems expense.

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

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products or parts furnished by third parties; such products or parts will be subject to the warranties, if any, of their respective manufacturers.

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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

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