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ABI PRISM® 377 DNA Sequencer

For Sequencing and GeneScan® Analysis Software Applications

User's Manual

Applied Biosystems

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About This Instrument 1

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

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Getting Started Quickly

To Get Started Quickly

If you are not familiar with the ABI PRISM® 377 DNA Sequencer, follow the guidelines listed under "What to Do If You Are a New User" on page 1-3.

If you are familiar with the instrument and its operation, proceed as follows.

- Before using the instrument, read the safety information located on page 1-4, and in the *ABI PRISM® 377 Site Preparation and Safety Guide* (P/N 903393).
- If you are preparing your own gels, proceed to:
  - Appendix A, "Gel Recipes," for recommendations and instructions on preparing gel solutions
  - Chapter 2, "Pouring Gels," for instructions on how to pour the gel
- If you have a gel, proceed to Chapter 3, "Instrument Operation," for step-by-step instructions on instrument set up and operation.

Note Chapters 2 and 3, and Appendix A are bound separately for your convenience. They can be removed from the binder and used separately as quick reference guides for preparing gels and using the instrument.

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What to Do If You Are a New User

Safety Information

Before using the instrument, read the safety information located on page 1-4, and in the *ABI PRISM 377 Site Preparation and Safety Guide* (P/N 903393).

The Macintosh Computer

This manual is written with the assumption that you know how to operate a Macintosh® computer. If you are not familiar with this computer, refer to the Macintosh documentation shipped with this system for specific operating information.

What to Read

We recommend reading:

- "ABI PRISM 377 DNA Sequencer—System Components" on page 1-11
  - Provides an overview of system hardware and software
- "Theory of Operation" on page 1-13
  - Describes electrophoresis and how data is collected
- "Using the Instrument" on page 1-14
  - Provides an overview of the steps involved in using the instrument
  - Introduces terminology specific to system operation
- Chapter 2, "Pouring Gels" and Appendix A, "Gel Recipes" if you are preparing your own gels. Includes:
  - Detailed instructions for two methods of pouring gels
  - Recommendations and instructions for preparing a variety of gels that can be used on this instrument
  - Storage recommendations for reagents and stock solutions
  - Factors that affect gel quality and read lengths for sequencing
  - Supplier information
- Chapter 3, "Using the Instrument"
  - Provides detailed instructions for setting up and operating the instrument for both GeneScan® analysis software and sequencing analysis software applications

Note Chapters 2 and 3, and Appendix A are bound separately for your convenience. They can be removed from the binder and used separately as quick reference guides for pouring gels and using the instrument.

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Safety Information

Site Preparation and Safety Guide

The *ABI PRISM 377 Site Preparation and Safety Guide* (P/N 903393) was sent to you prior to instrument installation. This guide includes important safety information that should be read by all users before they operate the instrument. Topics covered in the guide include:

- An explanation of the safety labels affixed to the instrument
- Laser safety, including hazards and safe-operation requirements
- Chemical safety, including a waste profile and Material Data Safety Sheets for the chemicals commonly used with the instrument

We strongly recommend this guide be kept readily available for reference at all times.

Safety Warnings in this Manual

Safety warnings appear throughout this manual at relevant locations. These warnings address chemical, high voltage, and laser safety. They appear in the format shown below.

! WARNING ! ELECTRICAL SHOCK HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

! WARNING ! LASER HAZARD. Exposure to direct or reflected laser light at 40 mW for 0.1 seconds can burn the retina and leave permanent blind spots. Never look directly into the laser beam or allow a reflection of the beam to enter your eyes.

Ergonomic Hazard

! WARNING ! Ergonomic Hazard. Performing loading activities may increase risk of developing the following cumulative trauma disorders (repetitive motion or repetitive strain injuries) which include but are not limited to: tendinitis, tenosynovitis, epicondylitis, strains, and/or sprains. To reduce the risk of experiencing these types of disorders, the following recommendations have been developed to decrease awkward posture; repetitive motion; excessive force; static muscle loading; and soft tissue contact.

- **Use an automated multi-channel pipette loader**
- **Locate the instrument on a variable or predetermined-height worktable or lab bench**
- **Use a stable stool or stepladder**
- **Install adequate artificial lighting in the appropriate area to facilitate loading**
- **Ensure adequate front access to instrument while performing loading activities**

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Special Text Usage

User Attention Words

The text of this manual includes four user attention words designed to draw your attention to safety issues or issues relative to proper instrument operation. Each represents a certain level of attention or action, as described below.

Note Calls attention to information.

IMPORTANT Indicates information that is necessary for proper instrument operation or for effective chemistry.

CAUTION **Indicates that damage to the instrument could result if you do not comply with this information.**

! WARNING ! **Indicates that physical injury to the user or other persons could result if these precautions are not implemented.**

User Bulletins

What are User Bulletins

User Bulletins are the mechanism we use to inform our customers of technical information, products improvements, and new products that are related to the operation of the ABI PRISM 377 DNA Sequencer and related laboratory techniques. Examples of topics covered in user bulletins include:

- New versions of ABI PRISM® 377 Data Collection Software
- Improved and new accessories for the instrument
- New gel formulations and protocols

User bulletins related to the use of this instrument will be mailed to you. We recommend storing the bulletins in this manual. A tab labeled “User Bulletins” has been included for this purpose.

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Product Area	E-mail address
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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

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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**

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ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
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Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**
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Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
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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**
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Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

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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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ABI PRISM 377 DNA Sequencer—System Components

Hardware

The ABI PRISM 377 DNA Sequencer consists of two main components:

- The ABI PRISM 377 DNA Sequencer (the instrument)
- A Macintosh computer

A printer is available as optional equipment. The components are connected by an asynchronous RS-232C serial data line.

Software

The ABI PRISM 377 DNA Sequencing System is shipped with the following software:

- ABI PRISM Data Collection Software
- Data analysis software—one or both of the following:
  - ABI PRISM® GeneScan® Analysis Software
  - ABI PRISM® DNA Sequencing Analysis Software

  The type of data analysis software shipped with the instrument is determined when the system is ordered.
- Symantec AntiVirus for Macintosh (SAM™)
- The Norton Utilities™

The software is installed onto the computer during system installation.

IMPORTANT Complete and return any registration cards included with the software to ensure you receive notification of future updates.

Models Available

Four models of the ABI PRISM 377 DNA Sequencer are available. The hardware for each model is identical. The data collection software and firmware are different.

ABI PRISM 377 DNA Sequencer

The standard ABI PRISM 377 instrument is designed to accommodate three sizes of gel plates—12 and 36 cm for GeneScan applications, and 36 and 48 cm for sequencing applications. Combs available are 18, 24, 32, 36, and 48-well shark's tooth, and 24, 32, and 36-well square tooth. Data is collected from 194 channels during each scan.

ABI PRISM 377-18 DNA Sequencer

The ABI PRISM 377-18 DNA Sequencer is the lowest cost, lowest-throughput version of the instrument. It can run up to 18 lanes on a single gel.

ABI PRISM 377 DNA Sequencer with XL Upgrade

The ABI PRISM 377 DNA Sequencer with XL Upgrade increases the number of samples that can be analyzed simultaneously. Increased throughput is made possible by modifying the instrument to collect data from 388 channels instead of 194 during each scan.

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The XL Upgrade can accommodate the following combs: 48- and 64-well shark' s tooth combs for sequencing applications; 48-, 50-, 64-, and 66-well for GeneScan applications. You can still use 36-well or other lower lane density combs if desired. Refer to the *ABI PRISM® 377 DNA Sequencer XL Upgrade User's Manual* (P/N 904412) for more information.

ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade

The ABI PRISM 377 DNA Sequencer with 96-lane Upgrade increases the number of samples that can be run on each gel. Increased throughput is made possible by re-engineering the instrument to collect data from 480 channels instead of 388 for the ABI PRISM 377 DNA Sequencer with XL Upgrade or 194 for the ABI PRISM 377 DNA Sequencer. In addition, the scan region has been increased from 6 inches to 7.25 inches.

The 96-lane upgrade includes a 100-well shark's tooth comb, and a new front glass plate. The new front glass plate has a bevel where samples are loaded. The bevel increases the thickness of the gel from 0.2 mm to 0.4 mm in this region. Lower lane density combs can still be used, but only with the original front glass plates provided with the standard ABI PRISM 377 instrument.

Refer to the *ABI PRISM® 377 DNA Sequencer 96-Lane Upgrade User's Manual* (P/N 4305423) for more information.

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Theory of Operation

How the Instrument Works

The ABI PRISM 377 DNA Sequencer is an automated instrument designed for analyzing fluorescently-labeled DNA fragments by *gel electrophoresis.* Gel electrophoresis is the movement of charged molecules through a gel solution (such as polyacrylamide) in an electrical field. It is used to separate DNA fragments by size.

The instrument functions the same way for both sequencing and GeneScan applications. The main differences between these applications is in the preparatory chemistries used, and the type of analysis performed on the resulting data.

To use the instrument, DNA fragments labeled with up to four different fluorescent dyes are combined and loaded into one lane on a vertical slab gel made of polymerized acrylamide or acrylamide derivatives. You can load up to 96 lanes on one gel depending on the model instrument you have. The instrument is designed to accommodate different size gel plates so that the distance the sample migrates before detection can be varied. This feature permits run times and sample resolution to be optimized according to the type of analysis, either base calling (DNA sequencing), molecular sizing in base pairs, or molecular quantitation.

Once the samples are loaded, voltage is applied, causing the fragments to electrophorese through the gel and separate according to size. At the lower portion of the gel above the lower buffer chamber, the fragments pass through an area called the “read region” (see page 1-15), where a laser beam continuously scans across the gel.

The laser excites the fluorescent dyes attached to the fragments, and they emit light at a specific wavelength for each dye. The light is collected in 194, 388, or 480 channels (the 377, 377XL, or 377-96 respectively) during each scan. It is then separated according to wavelength by a spectrograph onto a cooled, charge coupled device (CCD) camera, so all four types of fluorescent emissions can be detected with one pass of the laser.

The data collection software collects the light intensities from the CCD at particular wavelength bands (software-selectable virtual filters) and stores them on the Macintosh as digital signals for processing. The collected data before it is analyzed is often referred to as *raw data.* The file in which the raw data is stored is referred to as a *gel file.* The raw data of the gel is a matrix of information from the number of channels across the gel (194, 388, or 480) in four colors, multiplied by the number of scans collected. The gel file contains an image of the data for display and the raw scan data. Gel files range in size from 20 MB to 40MB.

At the end of data collection, the analysis software (ABI PRISM DNA Sequencing Analysis or ABI PRISM GeneScan Analysis software) is used to manually or automatically process, analyze, and translate the collected data into either base sequence, fragment sizing information, or relative concentrations.

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Using the Instrument

Overview

The process of setting up the instrument, loading samples onto the gel, and electrophoresing the samples is commonly referred to as performing a *run.* An overview of the steps involved in performing a typical run are listed in the table below. Step-by-step instructions are listed in Chapter 3, “Instrument Operation.”

Step	Action
1	Set up the instrument.
2	Perform a *plate check.*
3	Configure the data collection software.
4	Perform a *prerun* and load the samples.
5	Start the *run*.

The term run is also used when referring to a *run module* (described below and in Chapter 9), and to step 5 of the process listed above. A description of the plate check, prerun, and run are provided on pages 1-15 through 1-17. Configuring the software is described below.

The Data Collection Software

ABI PRISM Data Collection Software is the interface between the instrument and the computer. It performs the following functions:

- Controls the instrument by sending it commands that are contained in files called *modules.*
- Collects raw data from the instrument and stores it in a gel file (Figure 1-1 on page 1-17; described on page 1-13)
- Transfers data automatically to either the sequencing or GeneScan analysis software for automatic data analysis at the end of a run (software must be configured for this to occur)

Modules contain the various settings (electrophoresis voltage, gel temperature, etc.) required for instrument operation. Two sets of modules are included:

- Standard modules—provide gel temperature control from 10°C above ambient to a maximum of 60°C
- Chiller modules—used when an external cold water bath is attached to the instrument, and temperatures below 10°C above ambient are required

There are three types of standard and chiller modules: plate check, prerun, and run. Prerun and run modules are specific for either sequencing or GeneScan applications.

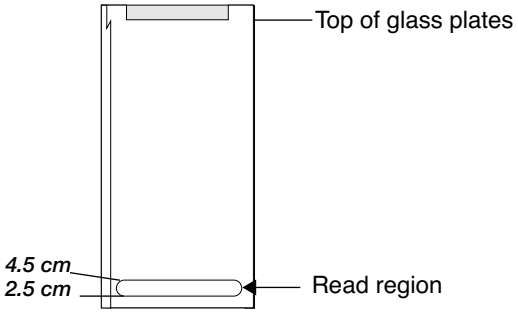
Configuring the software for a run means setting up a sample sheet and a run sheet. There are two types of sample and run sheets: one for sequencing and one for GeneScan applications. A sample sheet is a file that contains information used for sample identification, sample tracking, and data analysis.

A run sheet is a file that contains the sample identification, tracking, and data analysis information from the sample sheet; the gel comb configuration and other pertinent information used to direct instrument operation during a run (i.e. a plate check, prerun, and run module), and to analyze data automatically after the run. The data collection software is described in detail in Chapter 9, “Data Collection Software.”

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The Plate Check

The purpose of the plate check is to ensure that the glass plates and gel are clean and free of fluorescent contaminants before the samples are loaded. During a plate check, the *read region* of the plates is scanned without electrophoresis. The read region is the area of the glass scanned by the laser. It is approximately 2.5 to 4.5 cm from the bottom of the glass. It is 6 inches wide for standard and XL instruments, 7 inches wide for 96-lane instruments, and 3 inches wide for the ABI PRISM 377-18 instrument.

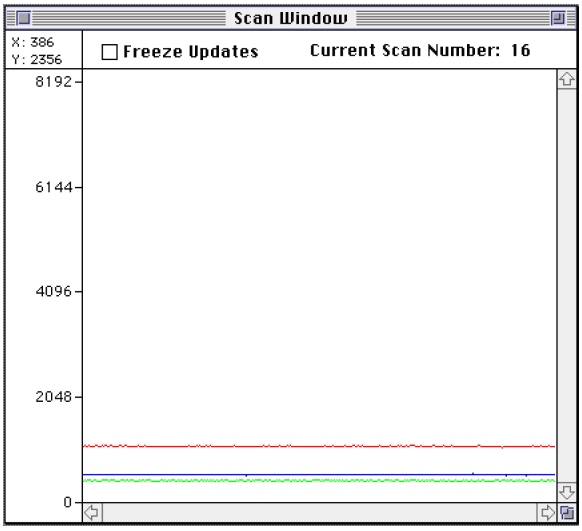


The number of channels in the read region are:

- 194 for the standard ABI PRISM 377 and 377-18 instruments (numbered 0 to 193)
- 388 for ABI PRISM 377 XL instruments (numbered 0 to 387)
- 480 for ABI PRISM 377 96-lane instrument (numbered 0 to 479)

The type of comb used determines the number of channels a lane will include. For example, one lane of a 36-well comb is equivalent to approximately 5 channels.

To perform a plate check, you open a new run sheet, select a plate check module, and click the Plate Check button on the run sheet. A *Scan window* opens automatically, and you observe the window for approximately 2 minutes.



Relatively flat scan lines as shown in the figure above indicate the plates are clean and there are no contaminants in the read region of the gel. If peaks appear, either the plates are dirty or the gel is contaminated. The plates can be cleaned and the plate check repeated. If peaks still appear, the gel is probably contaminated.

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If the gel is contaminated, you can:

- Load a new gel.
- Determine which lanes are safe to load with samples, and which lanes should be left empty. This is referred to as *skipping lanes.*
- Terminate the plate check, fill the buffer chambers, start the prerun, and allow the gel to electrophorese for a few minutes while watching the Scan window. Sometimes contaminants will migrate out of the read region.

Instructions for performing a plate check and determining which lanes to skip are listed in Chapter 3, “Instrument Operation.” The scan window and the run sheet are described in more detail in Chapter 3, “Instrument Operation,” and in Chapter 9, “Data Collection Software.”

The PreRun

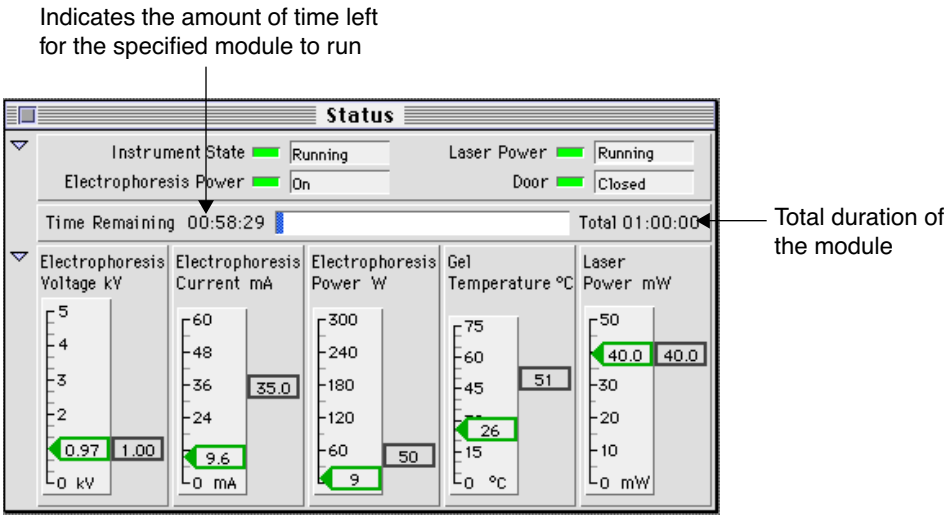
A prerun is typically performed after the plate check, and prior to starting the run. The purpose of the prerun is to:

- Equilibrate the gel temperature before samples are loaded
- Allow the operator to ensure the system is working properly

During a prerun the pump, heating system, and scanner are activated. Prerunning the gel is particularly important when performing a high speed (2400 scans/hour) run. The gel must be at *run temperature* (the temperature at which the samples will be electrophoresed) when the samples are loaded to ensure appropriate denaturation conditions exist. Prerunning also removes mobile ions from the gel, and prevents extrusion (gel oozing from between the plates) from occurring when the run begins and a higher voltage is applied.

To perform a prerun, the gel and all other necessary parts must be installed inside the instrument, and a prerun module must be selected on the run sheet. You start the prerun by clicking the PreRun button on the run sheet.

During the prerun, you open the *Status window* (shown below) to monitor instrument conditions including the gel temperature. Run temperature is typically reached after 15–25 minutes, even though the default duration of the prerun module may be longer.



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Once the gel reaches run temperature, the prerun is typically paused—not cancelled—and the samples are loaded. When paused, the pump and heater remain on, and the gel temperature designated by the module is maintained. Once the samples are loaded, the prerun is cancelled and the run is started.

Instructions for performing a prerun are listed in Chapter 3, “Instrument Operation.” The status window and the run sheet are described in more detail in Chapter 3, “Instrument Operation,” and in Chapter 9, “Data Collection Software.”

The Run

The run is typically performed after the plate check and prerun. During the run, samples are electrophoresed and data is collected.

To perform a run, the run sheet must be completed (i.e. the run module, sample sheet, number of lanes, well-to-read distance, etc. must be specified.) You start the run by cancelling the prerun, and then clicking the Run button on the run sheet.

Once the Run button is clicked, electrophoresis and data collection begin. The raw data is stored in a gel file along with sample information from the run sheet (imported from the sample sheet).

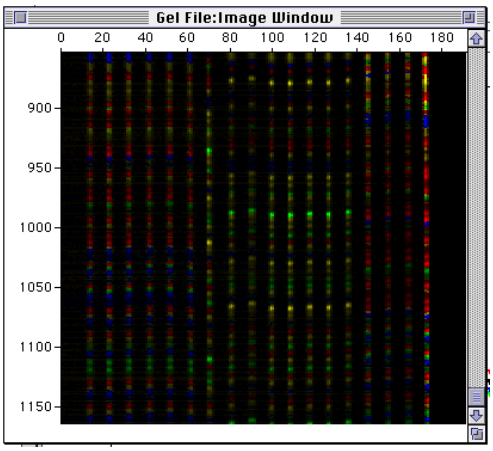


Figure 1-1 Gel file

Four different windows are available for viewing the data being collected and instrument status in real-time. The Scan and Gel windows are for viewing data. The Status window displays the current status of the instrument. The Electrophoresis History window displays the actual values for the electrophoresis power supply and gel temperature throughout the course of a run.

When the run is finished, data is analyzed either automatically or manually. Automatic data analysis must be specified on the run sheet prior to starting the run.

For more detailed information and instructions, see Chapter 3, “Instrument Operation,” and Chapter 9, “Data Collection Software.”

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Data Analysis

Overview

Once the run is finished, the collected data is transferred automatically (if specified on the run sheet) or manually to either the ABI PRISM DNA Sequencing Analysis Software or the ABI PRISM GeneScan Analysis Software for analysis. Electropherograms can be printed on a color printer. You can display the raw and analyzed data on the computer screen, or edit and print it at any time. Both types of analysis software are briefly described below.

ABI PRISM GeneScan Analysis Software

GeneScan Analysis Software automatically analyzes data collected from each sample. This software allows you to:

- Create custom analysis parameters, or use the default analysis settings.
- Size and quantitate PCR and other DNA fragments.
- Automatically identify all DNA bands, compare the mobility of each band to that of an internal lane standard, and size the bands based on the sizing curve of the internal lane standards.
- Interactively confirm and fine-tune data analysis.
- Display the results of an experiment as an electropherogram, reconstructed gel image, tabular data, or a combination of electropherograms and tabular data.
- Adjust the dye intensity of each sample individually or collectively.
- Customize colors to more easily view large quantities of data.
- Organize analyzed data in a variety of ways to fit the needs of your individual projects. For example, every project file can contain sample data from one or more runs.

Refer to the *ABI PRISM GeneScan® Analysis Software User's Manual* for more detailed information.

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ABI PRISM DNA Sequencing Analysis Software

Sequencing Analysis Software analyzes the raw data collected from each sample. This software:

- Tracks gel files
- Extracts sample information from gel files
- Performs multicomponent analysis
- Applies mobility corrections
- Normalizes base spacing
- Baselines data
- Determines analysis starting points
- Calls bases
- Includes the following two utilities:
  - GelDoc—removes tracking data from a gel file, repairs file resources, and removes and restores the image from a gel file
  - DataUtility—makes or copies matrices for instrument files

You can reanalyze and edit the sequence data. Also, data files are in formats that you can use with commercially available or user-generated programs on the Macintosh or on other compatible computers.

Refer to the *ABI PRISM DNA Sequencing Analysis Software User's Manual* for specific information about the Sequencing Analysis software.

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Additional ABI PRISM Software

Genotyper Fragment Analysis Software

Genotyper® software analyzes the data generated by GeneScan Analysis Software. It enables you to analyze and interpret nucleic acid fragment size and quantitation data by converting it to user-defined results specific to your genotyping studies. For example, Genotyper can convert GeneScan fragment data to called alleles.

Genotyper results can be transferred to databases for storage, spreadsheets for statistical analysis, or linkage analysis software.

GenoPedigree

GenoPedigree™ software is an interactive pedigree diagram editor that reads and writes its own documents containing pedigree, layout, and style information. GenoPedigree can also generate files for linkage analysis.

GenBase

GenBase™ software is a database application that stores data for genotypes, pedigrees, markers, traits (diseases), and other relevant information. You can import data from or export data to GenBase from Genotyper and GenoPedigree.

Primer Express

Primer Express™ software allows you to design, analyze, and order oligonucleotides. The process is simple: import or drag sequences into the application, then select primers using the Automatic Find mode, default settings, or custom parameters settings. The software performs the appropriate calculations, and automatically updates all relevant views.

You can select target amplicon sequences, automatically translate DNA sequences to amino acids, exclude DNA sequences from an amplicon, label exon-intron junctions, and annotate important sequence sites.

The software has specific, ready-to-use templates for many PCR and sequencing applications:

- TaqMan® probe and primer design
- Standard, nested, allele-specific, multiplex, and RT PCR
- Sequencing
- Cycle sequencing
- Batch processing of multiple DNA documents
- Primer test

Two choices are available for viewing DNA sequences: text or map format. The Map page shows a graphical representation of the sequence and allows viewing of all primers found in relation to template DNA. All sequence annotations are shown, including restriction endonuclease sites, exon-intron junction sites, and amino acid translations.

Primer information (primer pairs when applicable) is displayed in a primer table page. The table displays all relevant information according to your preference. You decide how the information display is sorted.

Primer Express software is fully compatible with our AutoAssembler and Sequence Navigator software.

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The GeneAssist Sequence Analysis System

GeneAssist® is a multi-user system used to edit, assemble, and analyze DNA and protein sequence data. The GeneAssist programs are designed to run on the Macintosh platform either independently or interactively with a UNIX server. They provide a robust set of tools for sequence analysis, and allow you to access and store data in a common file format.

GeneAssist has three Macintosh programs:

- Factura™ cleans up sequence data for analysis and alignment. It identifies specified vector and ambiguity ranges, restriction sites, and a specified confidence range. It also identifies multiple base positions with codes described by the International Union of Biochemists (IUB codes), based on a user-defined threshold. The program processes sequences in batches, speeding the process considerably.
- AutoAssembler™ allows you to quickly and efficiently assemble small pieces of DNA into larger segments of DNA, using ABI Prism 377 data as well as other types of data. It provides powerful tools for editing the sequences, including the ability to display constantly-spaced electropherograms that are synchronized with the assembled sequences. You can build a consensus from the assembled sequences and export it for use with other programs.
- GeneAssist Analysis allows you to analyze data from ABI Prism 377 sequence files, files processed in AutoAssembler, text files, or a database. You can use this program to rapidly search biological databases for specific sequences or motifs, and to interactively work with the resulting sequence lists. In addition to sequence lists the program can create dot plots, alignments, and restriction maps of sequence data.

AutoAssembler Sequence Assembly Software

AutoAssembler™ simplifies sequence assembly by automating each step of the process from vector deactivation, sequence “cleanup”, and assembly, to easy resolution of sequence ambiguities. It is designed for use on small to medium size sequencing projects.

AutoAssembler automatically examines all possible relationships between sequences in both orientations. Dynamic programming is used to determine the optimum sequence order, resulting in a precise consensus sequence.

Multiple projects are efficiently managed and easily updated. Convenient sequence and project reports monitor sequence quality and project progress. In addition, data integrity is always maintained because AutoAssembler allows you to go back and review all stages of your project at any time. No data is ever lost, and original sequence information is always preserved. Deactivated vector and ambiguity sequence regions, as well as edited base calls, can be viewed or reactivated as original primary base sequences. Moreover, unlike other software programs, this information is preserved within the original sequencer data files, making data management easier and more reliable.

Results can be graphically viewed and quickly interchanged as project layouts; bar and sequence alignments; and aligned, multiple, four-color electropherograms, allowing you to analyze your data from several different perspectives. The statistics view helps you quickly identify ambiguous sequence regions. Electropherograms, features tables, sequence annotations, and edited and primary sequence data are accessible and easily edited directly from within any part of the program and for any sequence.

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AutoAssembler can be upgraded to client-server based INHERIT® AutoAssembler for large scale sequencing projects. With INHERIT, any number of Macintosh clients are networked with a SUN Microsystems® server and FDF® Data Search System to provide shared-use efficiency and faster performance. The user-interface for INHERIT AutoAssembler and AutoAssembler are identical.

Sequence Navigator

Sequence Navigator® is a two-part software package designed for automated DNA and protein sequence comparisons. It accurately aligns sequences to a standard or a population, and identifies sequence variants. To accomplish this, Sequence Navigator integrates with data collected on the ABI PRISM 377 DNA Sequencer, as well as text files from other sources.

Sequence Navigator comes standard with two powerful software modules: Factura and Sequence Navigator. Factura automatically identifies critical patterns as it sorts through and deactivates needless data. Factura's automated batch processing for feature identification saves you time by recognizing features such as the vector, primer, and heterozygote/IUB base positions.

Once sequence files have been processed in Factura and a batch work sheet created, the work sheet is imported to Sequence Navigator. With Sequence Navigator you can perform basic sequence editing, use pair-wise alignments to assemble contigs, select multiple alignment to identify sequence variants, and display the electropherograms collected by the ABI PRISM 377 DNA Sequencer.

ABI PRISM BioLIMS Software System

ABI PRISM BioLIMS® software was designed by Applied Biosystems in collaboration with Molecular Informatics, a key player in the development of the Genome Sequence Database—the world's most widely used relational database for genetic sequence information. It is the first commercially available system for automated genetic information management and an excellent foundation on which to build your current and future bioinformatics capabilities.

Using BioLIMS software, you can automate DNA sequencing from data acquisition to assembled contig. Data flows automatically from your ABI PRISM 377 DNA Sequencer into the ABI PRISM BioLIMS relational database, and on to downstream analysis packages.

To simplify project tracking and automation, all sequences are stored by project name. Using UNIX and AppleScript® to control the process, sequence information flows seamlessly from your instruments to the database and into the analysis packages.

ABI PRISM GeneScan Analysis Software (BioLIMS version 2.0 and up only), ABI PRISM Sequencing Analysis Software, Factura, and AutoAssembler programs all read and write to the database. Just specify the sequences you want to analyze and the applications you want to use.

The ABI PRISM BioLIMS software system simplifies data retrieval, analysis, and reporting. It maintains data integrity by eliminating multiple copies of the same data, and it helps improve reporting efficiency by allowing you to quickly organize and locate critical information.

However you choose to configure and implement this software system, the integrity of your data is ensured. One copy of the original sequence data is preserved at all times, so the original data is never lost or written-over, not matter how often the data is analyzed and edited. For further protection, data backup and archiving utilities are built-in.

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The ABI PRISM BioLIMS system streamlines data retrieval and reporting by allowing you to locate specific sets of information. Database queries can be performed using various data file attributes, including project name, sequence name, instrument, date, and operator. Your query results can be saved and printed for documentation and reference.

The power of this centralized database gives local and remote users immediate access to their data. Whether you're in a different lab or working in your home, you can use ABI PRISM BioLIMS query tools to quickly retrieve the information you need.

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ABI PRISM® 377 DNA Sequencer

Pouring Gels

Quick Start Guide

Applied Biosystems

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Pouring Gels 2

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

Topics	See page
Selecting a Gel Formulation	2-2
Importance of Using High Quality Gels	2-3
About the Gel Pouring Methods in This Chapter	2-4
Before Using New Glass Plates	2-5
Cleaning Glass Plates, Spacers, and Combs	2-6
Method 1—Part 1—Mounting Glass Plates into the Gel Cassette	2-10
Method 1—Part 2—Attaching the Gel Injection Device	2-14
Method 1—Part 3—Pouring the Gel Using Mounted Plates	2-18
Method 2—Pouring the Gel Using Unmounted Plates	2-20

Related Information in Appendix A—Gel Recipes

Topics in Appendix A The following related topics and information are located in Appendix A, “Gel Recipes”:

Topics
Gel recipes and recommendations for ABI PRISM® DNA Sequencing and GeneScan® analysis software applications
How to store reagents and stock solutions
Factors that affect sequencing read lengths
Factors that affect gel quality including: ♦ Purity of reagents ♦ Rate of polymerization ♦ Air bubbles ♦ Age of the gel

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Selecting a Gel Formulation

Gel Recipes and Protocols

Protocols for preparing the following gel solutions are located in Appendix A, "Gel Recipes."

- 19:1 polyacrylamide (any percent)
- 29:1 polyacrylamide (4.25% and 4.5%)
- Long Ranger® (4.75% and 5.0%)
- PAGE-PLUS (4.8% and 5.25%)
- Mutation detection (MDE) gel for single-strand conformation polymorphism (SSCP)

For Sequencing Runs

A variety of gels can be used for sequencing runs on the ABI PRISM® 377 DNA Sequencer. Because conditions vary from lab-to-lab, one gel formulation may work better than another. We recommend testing each formulation, and selecting the one that performs best.

19:1 polyacrylamide gels are frequently used for sequencing applications. The gel formulations that typically provide the longest read lengths are as follows:

Plate Size and Run Speed	Gel Formulations	Expected Read Length in Bases
♦ 36-cm well-to-read (WTR) plates ♦ 1200 scans/hr	♦ 4.5% 29:1 polyacrylamide ♦ 5.0% Long Ranger (concentrate or Singel™ gel forms) ♦ 4.8% PAGE-PLUS	650–800
♦ 36-cm WTR plates ♦ 2400 scans/hr	♦ 4.5% 29:1 polyacrylamide	550–700
♦ 48-cm WTR plates ♦ 1200 scans/hr	♦ 4.25% 29:1 polyacrylamide ♦ 4.75% Long Ranger (concentrate or Singel™ gel forms) ♦ 5.25% PAGE-PLUS	750–900

For GeneScan Runs

Gels used for GeneScan runs on this instrument are:

- 5.0% Long Ranger
- 4.25% 19:1 polyacrylamide
- Mutation detection (MDE) gel for single-strand conformation polymorphism (SSCP)

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Importance of Using High Quality Gels

Why a High Quality Gel is Important

One of the most critical variables that determines the success or failure of both sequencing and GeneScan runs is the gel. The use of consistently prepared, high quality gels helps ensure the best experimental results, and minimizes the time spent troubleshooting gel-related problems. Poor quality gels often cause problems that are mistaken for instrument problems.

For sequencing runs, the quality of the gel directly effects the number of bases that can be called.

For GeneScan runs, the quality of the gel effects the mobility of DNA fragments from run-to-run, reproducibility of sizing, signal strength, and resolution.

Factors that affect gel quality include:

- Purity and freshness of reagents
- Rate of polymerization
- Presence of air bubbles
- Age of the gel

A discussion of these factors is located in Appendix A, “Gel Recipes.”

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About the Gel Pouring Methods in This Chapter

Methods Presented in This Chapter

Many methods and devices are available for pouring gels. The two methods presented in this chapter are:

- Method 1—Using Mounted Plates and the Gel Injection Device
- Method 2—Using Unmounted Plates

Method 1 Using Mounted Plates and the Gel Injection Device

In this method, the glass plates are mounted in the gel cassette. The gel injection device is attached to the plates, and the gel solution is injected between the plates using a syringe. Instructions for this method are present in three parts:

- "Method 1—Part 1—Mounting Glass Plates into the Gel Cassette" on page 2-10
- "Method 1—Part 2—Attaching the Gel Injection Device" on page 2-14
- "Method 1—Part 3—Pouring the Gel Using Mounted Plates" on page 2-18

The advantages of using this method are:

- The gel cassette:
  - Eliminates the need for binder clips
  - Ensures the proper amount of pressure is applied to the plates at the correct locations along the edges of the plates
- Gel preparation is more consistent

Method 2 Using Unmounted Plates

In this method, the glass plates are clamped together with binder clips, and the gel solution is manually injected between the plates. Instructions for this method are listed under "Method 2—Pouring the Gel Using Unmounted Plates" on page 2-20.

Summary of the Gel Preparation Procedure

A summary of the steps involved in preparing a gel are as follows:

Step	Action
1	Clean the glass plates, spacers, and comb.
2	Mount the plates and spacers in the gel cassette and attach the gel injection device (method 1 only). or Assemble the plates and spacers using binder clips (method 2 only).
3	Prepare the gel solution.
4	Add the polymerizing reagents to the gel solution.
5	Pour the gel.
6	Insert the comb.
7	Wrap bottom of plates with plastic wrap.
8	Allow the gel to polymerize.

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Before Using New Glass Plates

The Gasket Mark

The first time the front glass plate and the gasket of the upper buffer chamber make contact, an invisible, permanent, hydrophobic area is created on the surface of the glass. This hydrophobic area is referred to as the *gasket mark*. If subsequent gels are poured using the same plates with the gasket mark on the inside, the gel solution will not flow readily across this area of the plate. Therefore, always keep the same side of each plate facing out.

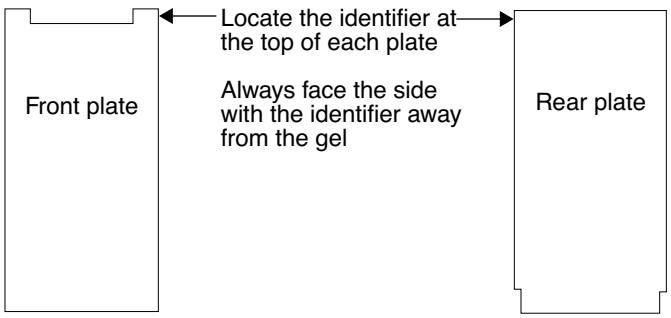
Gasket marks can be removed by cleaning the plates with an alcoholic KOH wash. See “Removing the Gasket Mark” on page 2-9.

Identifying the Front and Back of a Plate

The sides of glass plates can be identified several different ways. Applied Biosystems glass plates are scored with the part number and well-to-read distance. You can orient plates so these identifiers always face out. In addition you can:

- Number the front and back plates as a set, and always use the same two plates together. The benefit of this method is that gel-related problems can more easily be tracked back to a particular set of plates. Use an engraving pen to mark the glass near the top of the outside of each plate.
- Make a small scratch with an engraving pen, or place a small piece of waterproof tape near the top of the outside of each plate.

IMPORTANT Do not use a ballpoint pen or other liquid marker to identify plates. Mark plates near the top as shown below to ensure that the identifier is not near the read region (defined in Chapter 3) or seal area of the upper buffer chamber.



Refer to Chapter 3, “Instrument Operation,” for more information on the read region and upper buffer chamber.

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Cleaning Glass Plates, Spacers, and Combs

Why Clean Plates are Important

Clean plates, spacers, and combs are critical for successful gel preparation and a successful run. Plates that are cleaned thoroughly and consistently will also help avoid the temporary loss of signal that can occur sporadically on this instrument.

Our research indicates this loss of signal is due to contaminant molecules (surfactants, fatty acids, long chain polymers) attached to the surface of the plates. It manifests itself as a band of little or no signal across the entire width of the gel image. It usually occurs between 140 to 200 base pairs, and typically lasts the equivalent of 20 to 40 base pairs. Following this band, signal strength usually returns to normal.

Glass plates can be cleaned manually or in a dishwasher. The use of a laboratory dishwasher with a hot (195°F/90°C) deionized water rinse cycle has been found to effectively remove suspect contaminants, thereby eliminating any temporary loss of signal.

Gasket marks can be removed by cleaning the plates with an alcoholic KOH wash. See “The Gasket Mark” on page 2-5, and “Removing the Gasket Mark” on page 2-9.

Using a Dishwasher

We strongly recommend cleaning glass plates in a laboratory dishwasher with a hot (195°F/90°C) deionized water rinse cycle. Using a dishwasher helps ensure plates are cleaned effectively and consistently every time, and will also eliminate the sporadic, temporary loss of signal that can occur on this instrument (described above.) Deionized water is required for the rinse cycle only. Dishwasher recommendations are listed on page 2-8.

When using a dishwasher, we recommend you:

- Connect the dishwasher to a high-grade, deionized water source.
- Clean plates as soon as possible once the gel is removed.
- Rinse residual gel from plates before loading in the dishwasher.
- Initially use the longest deionized water rinse option on the dishwasher, followed by a drying cycle. After some experimentation, you may be able to reduce the rinse time.
- Do not use a detergent.
- Avoid excessive handling of dry plates with ungloved hands.

Cleaning by Hand

Detergents

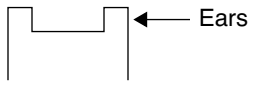
- Alconox—most major laboratory suppliers
- Multiterge—VWR Scientific Products, P/N 34171-010

! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are poisons, neurotoxins, irritants, carcinogens, and possible teratogens. Acrylamide and bisacrylamide sublime (the solids release toxic vapor) and are harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.

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Procedure

If a dishwasher is not available, follow this procedure to manually clean glass plates.

Step	Action	
1	Put on a pair of gloves. If necessary, wash gloves to remove talc powder. **IMPORTANT** Always wear gloves when handling glass plates to avoid skin contact with the gel, and to avoid contaminating clean glass plates.	
2	**If the plates …**	**Then …**
	contain a gel	a. Gently pry the plates apart starting at the bottom of the plates. Do not pry plates apart at the ears. Be careful not to chip the plates.  b. Remove the comb and spacers. c. Remove the gel using one of these methods: – Lay a large Kimwipe on the gel and roll it up. – Wash the gel off the plate with water. d. Properly dispose of the gel immediately.
	do not contain a gel	proceed to step 4.
3	Wash the glass plates with a very small amount of detergent (approximately 0.10 gram) such as Alconox or Multiterge that is non-fluorescent and will not leave a residue. **IMPORTANT** Do not soak plates in detergent. Use only a very small amount of detergent. Detergent residue can cause red or other fluorescent fronts to appear on the gel image.	
4	Thoroughly rinse the plates with hot water.	
5	Rinse the plates again with hot deionized water. **IMPORTANT** This step is critical for the complete removal of contaminants such as surfactants, fatty acids, and long chain polymers. These contaminants can cause the temporary loss of signal during instrument operation. Because the optimum water temperature is 195°F/90°C, we recommend cleaning plates in a dishwasher to avoid injury.	
6	Stand the plates up and allow them to air dry. Lean plates so the side that will contact the gel is angled downward. This will help keep dust and other airborne particles from settling on the inside surface of the plate. **Note** If there is not enough time to let the plates air dry, you can dry them gently with lint-free towels such as Kimwipes. Do not use compressed air to blow plates dry. Never use an organic solvent to speed up the drying process. Compressed air propellants and organic solvents may leave fluorescent residues.	

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Dishwasher Recommendations

The following dishwashers and plate rack are recommended for cleaning glass plates.

Item	P/N	Supplier(s)
Lancer 1600 Dishwasher with facility for drying	Lancer 1600 UP	Lancer USA Inc. 705 West Highway 434 Longwood, Florida 32750 Telephone: 407-332-1855 Lancer UK Ltd 1 Pembroke Ave Waterbeach, Cambridge CB5 9QR Telephone: 44-01223-861665 Fax: 44-01223-861990
Sequencing plate rack with a 50 plate capacity for Lancer 1600 dishwasher	SPR 16	
Labconco Undercounter SteamScrubber Washer/Dryer	15-352-801	Fisher Scientific U.S. Headquarters 585 Alpha Drive Pittsburgh, Pennsylvania 15238 Customer Service: 1-800-766-7000 Fax: 1-800-926-1166 Internet: http://www.fishersci.com

Cleaning Spacers and Combs

! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are poisons, neurotoxins, irritants, carcinogens, and possible teratogens. Acrylamide and bisacrylamide sublime (the solids release toxic vapor) and are harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.

Two methods can be used to clean spacers and combs.

Method 1

Clean the spacers and combs with hot water. Then rinse them with hot deionized water, and allow them to air dry.

Method 2

If the spacers and combs are very dirty, clean them with a very small amount of Alconox. Then rinse them with hot water, followed by a hot deionized water rinse. Allow them to air dry.

IMPORTANT Do not soak combs in detergent or even water for a long period of time such as overnight. Soaking will break down the glue, and the comb will eventually come apart.

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Removing the Gasket Mark

An alcoholic KOH wash can be used to remove buffer chamber gasket marks from the plates.

Preparation of all solutions should be carried out in a hood using safety glasses, gloves, and other appropriate protective clothing.

Step	Action
1	Add 30–35 g of potassium hydroxide (KOH) or sodium hydroxide (NaOH) pellets to a plastic bottle. ! WARNING ! **Potassium hydroxide is hygroscopic and caustic. It can cause severe burns and blindness if it comes in contact with the skin or eyes. Always work in a fume hood. Obtain a copy of the from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
2	Add 200 mL of absolute ethanol to the bottle. ! WARNING ! **CHEMICAL HAZARD. Ethanol is a flammable chemical and is irritating to the skin, eyes, respiratory system. It can cause nerve and liver damage, CNS depression, nausea, vomiting, and headache. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
3	Mix the solution well. It will take at least 15 minutes for most of the pellets to dissolve. **Note** This recipe is for a saturated solution, so some pellets will remain. Store the solution with the bottle capped tightly. During storage, the color of the solution will turn dark red-brown. The solution can still be used, and is good for 1 year.
4	Place some uncolored absorbent towels or other covering in the hood to catch spills.
5	Place the gel plates on the towels with the inside surfaces facing up. **Note** The plates should be nearly level so that the cleaning solution does not run off onto the bench. Only the inside (gel side) surface of the plates need be cleaned, though the outside surfaces can be cleaned similarly.
6	Pour approximately 15 mL of the cleaning solution onto the area of the plate where the gasket mark is.
7	Allow the solution to remain on the plates for 10 minutes. CAUTION **Longer times can harm the plates.**
8	Repeat steps 2 and 3.
9	Rinse thoroughly with deionized water.
10	Clean plates as usual.

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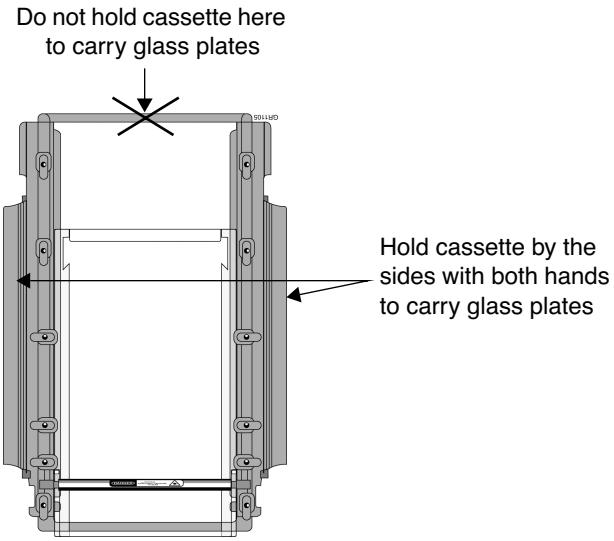
Method 1—Part 1—Mounting Glass Plates into the Gel Cassette

Using Unmounted Plates

Gels can be prepared using mounted or unmounted plates. The following procedure is for using mounted plates. Refer to “Method 2—Pouring the Gel Using Unmounted Plates” on page 2-20 if you wish to prepare the gel using unmounted plates.

Carrying a Cassette with Plates

When carrying plates mounted in the gel cassette, always hold the cassette by the sides with both hands. Do not carry the cassette with plates by holding the top bar only. The weight of the plates may cause the bar to break, resulting in broken glass plates.



Materials Required

- Comb, sharks- or square-tooth
- Gel cassette
- Glass plates, front and rear, one set
- Kimwipes
- Spacers, two 0.2-mm
- Water, distilled and deionized

Before Mounting the Plates

Before mounting the glass plates into the gel cassette, verify that:

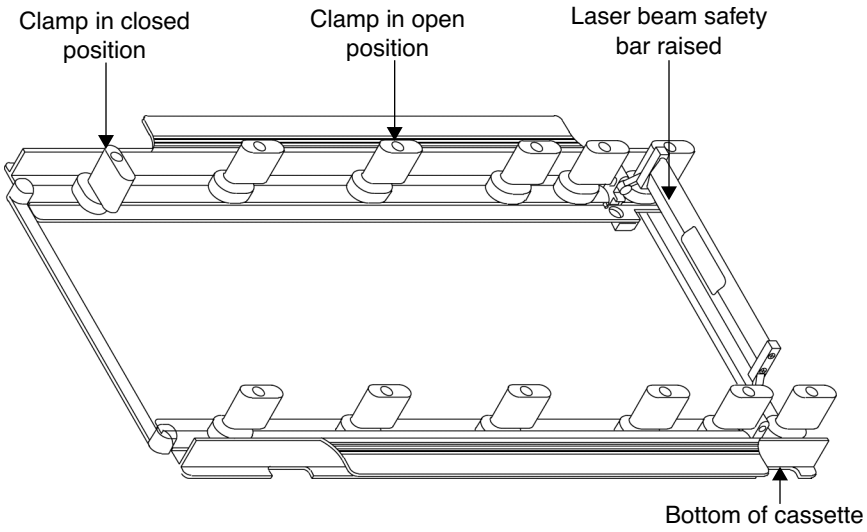
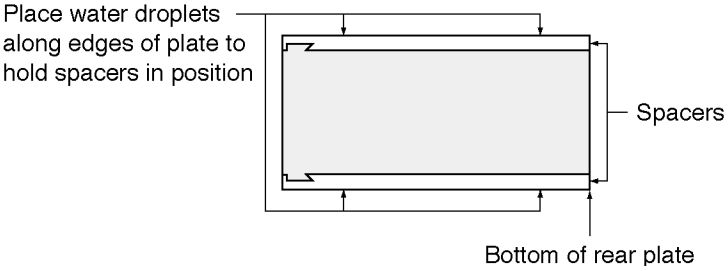
- A clean, level working area such as a bench top is available for mounting the plates and pouring the gel.
- The plates, spacers, and comb are clean and dry (cleaning procedure on page 2-6).
- The spacers are the same length as the glass plates. If the spacers are too long, the gel injection device may leak. Trim them to the proper size if necessary.

If the plates have never been used before, read “Before Using New Glass Plates” on page 2-5.

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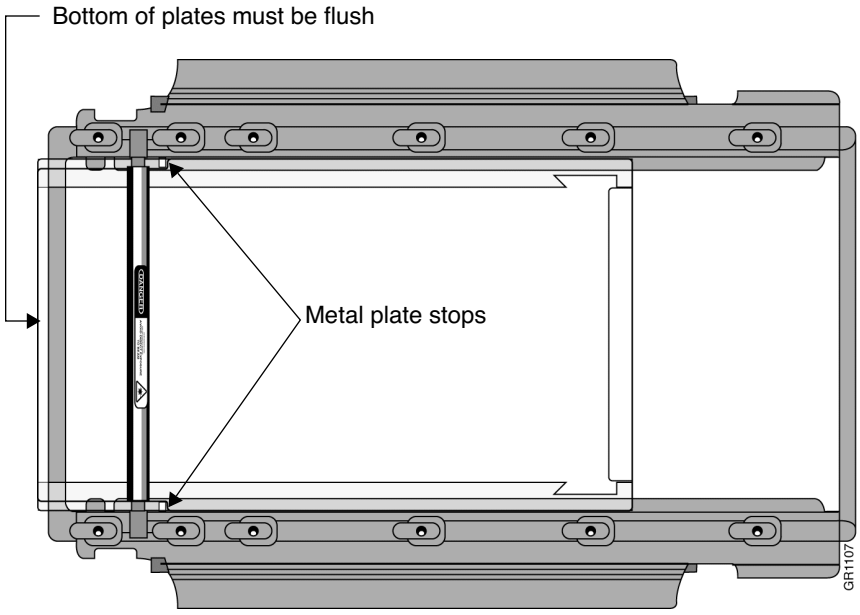
Procedure

To mount glass plates in the gel cassette:

Step	Action
1	Place the cassette on a level, clean surface.
2	Raise the laser beam safety bar, and turn all the clamps to the open position. 
3	Check the inside surface of both plates for water droplets, dust, lint, or anything else that might fluoresce or scatter light. Clean the plates with a damp Kimwipe if necessary.
4	Place the rear plate in the cassette with the notched end oriented toward the bottom of the cassette. **IMPORTANT To avoid difficulty pouring the gel, always load the front and rear plates with the same side of the glass facing out. Refer to "Before Using New Glass Plates" on page 2-5 for more information.**
5	Place the spacers on the rear plate as shown below (notched side facing the middle of the plate). Two to three droplets of water can be applied to the edge of the glass where the spacers will rest to keep them from moving. **IMPORTANT Align the outside edge of each spacer with the outside edge of the plate. Spacers must cover the notched areas of the plate.** 

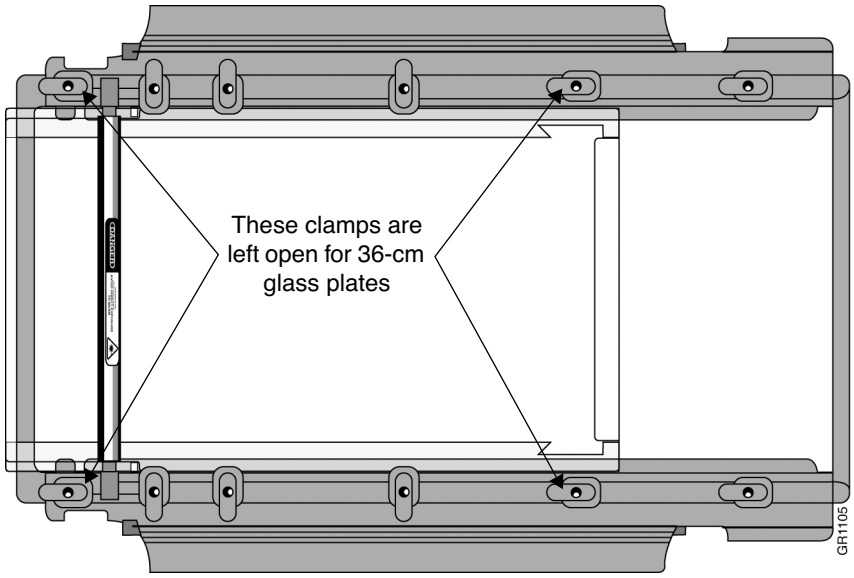
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To mount glass plates in the gel cassette: *(continued)*

Step	Action
6	Place the front plate on top of the rear plate and spacers so that the: ♦ Gasket mark faces up (hydrophobic area, described on page 2-5) ♦ Bottom of the plates are flush ♦ Large notch in top of plate is oriented toward the top of the cassette (see illustration in next step) **IMPORTANT** To avoid difficulty pouring the gel, always load the front and rear plates with the same side of the glass facing out. Refer to “Before Using New Glass Plates” on page 2-5 for more information.
7	Keeping both plates together, push the plates to the bottom of the cassette until the notches of the rear plates are seated firmly against the metal plate stops in the cassette. Push the plates from the top to ensure firm contact. 
8	Run the tip of your finger along the bottom of the plates to make sure they are flush. **IMPORTANT** The bottom edges of the plates must be flush with each other. If the plates are even slightly misaligned, the gel injection device will leak.  Bottoms of plates correctly aligned Misalignment like this causes the gel injection device to leak

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To mount glass plates in the gel cassette: *(continued)*

Step	Action
9	Press on the center of the front plate with the fingertips of one hand to hold the plates in position. At the same time, lock the plates in place by turning all the cassette clamps to the closed position *except the clamps that secure the top and bottom of the plates* (shown below). 
10	Run the tip of your finger along the bottom of the plates again to make sure they are still flush. If the plates shifted, unlock the clamps and adjust the plates. Repeat this procedure until the plates are locked in position against the plate stops, and the bottoms of the plates are flush.

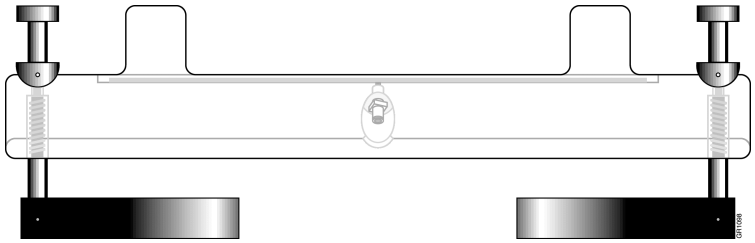
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Method 1—Part 2—Attaching the Gel Injection Device

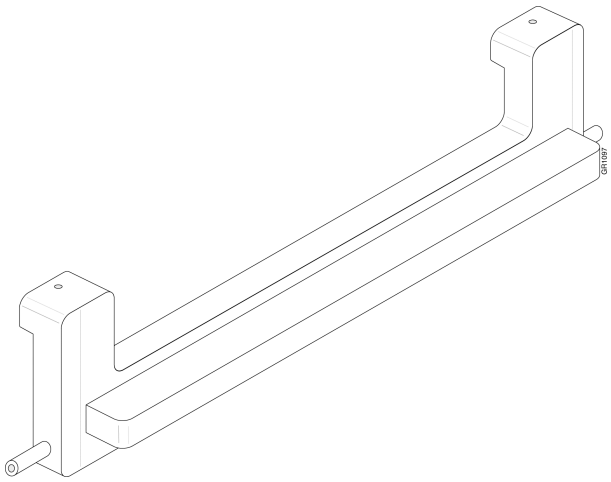
Materials Required

The materials required for this procedure are as follows:

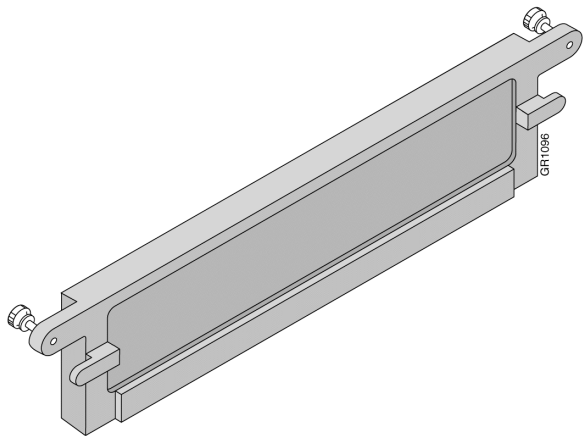
- Gel injection device—three pieces as illustrated below
  - Gel injection fixture, clear
  - Two braces that hold the comb in place (one clear and one black)
- Level, raised support (for example, two empty pipet tip boxes)
- Absorbent paper



Gel injection fixture



Clear brace



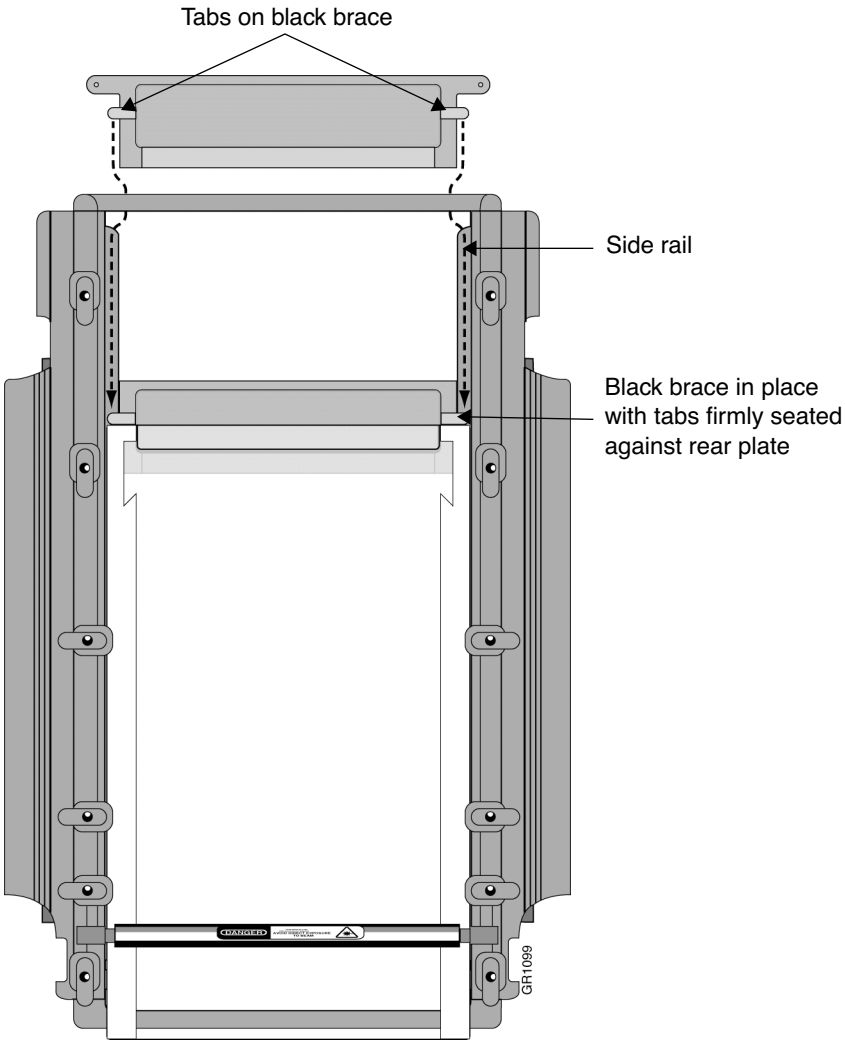
Black brace

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Attaching the Gel Injection Device

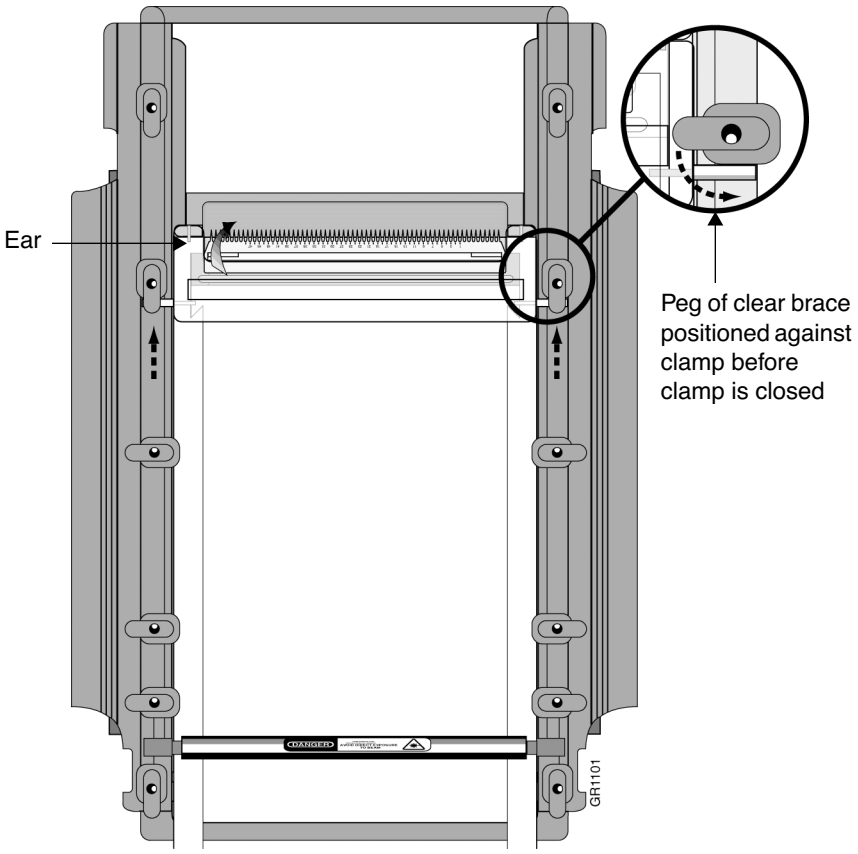
If you are pouring gels using unmounted plates, proceed to “Method 2—Pouring the Gel Using Unmounted Plates” on page 2-20.

To attach the gel injection device:

Step	Action
1	Place the gel cassette with plates on a level, raised support. The support(s) should be under the cassette—not under the glass plates.
2	Attach the black brace as follows: a. Hold the brace with the tabs pointing up. Move the tabs up through the gap between the side rails and top bar of the cassette as shown below, so the tabs rest on the rails. b. Slide the brace along the rails and under the glass. c. Firmly push the brace under the plates until the tabs press against the rear plate. 

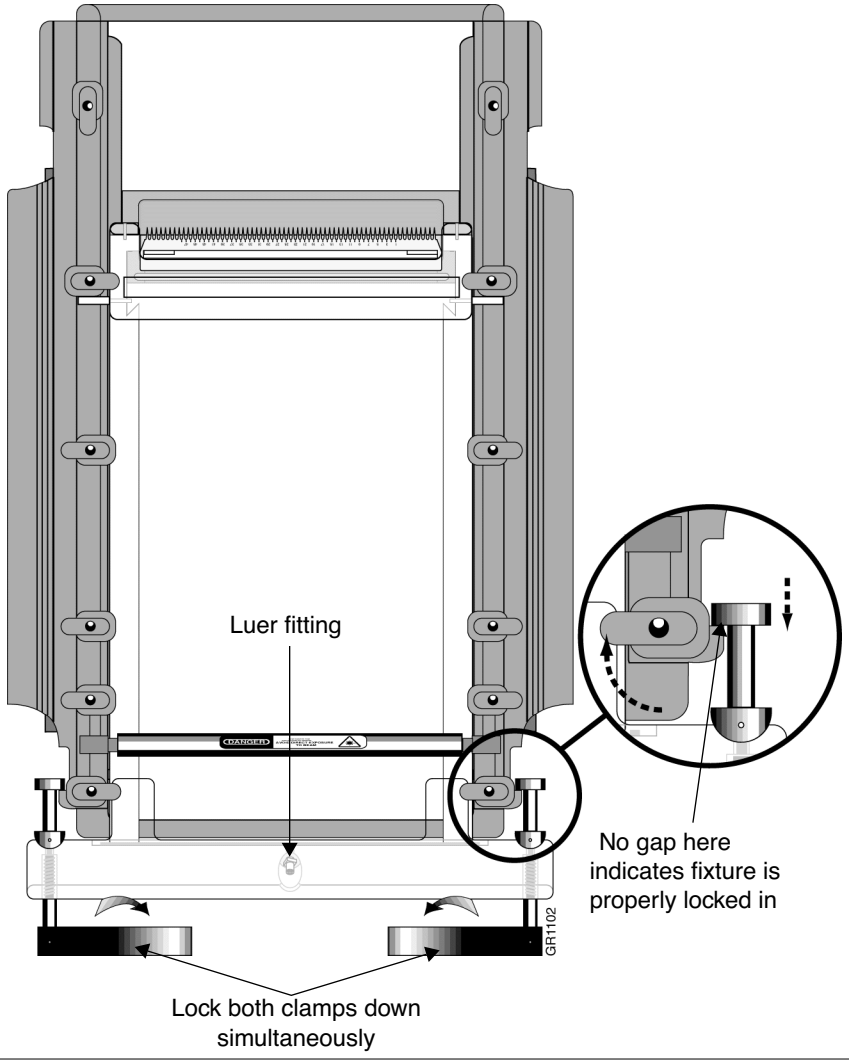
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To attach the gel injection device: *(continued)*

Step	Action
3	Attach the clear brace as follows: a. Slide the pegs of the brace up against the cassette clamps at the top of the plates. b. Fit the ears of the brace over the ears of the front glass plate. **IMPORTANT** The brace must cover the ears of the front plate to prevent leakage and hold the comb in place. 
4	Insert the flat edge of the comb between the plates as far as it will go.
5	Lock the clear brace in position by turning the appropriate two clamps.
6	Test the tightness of the comb between the plates by carefully pulling on the tips of the teeth. If either side or both sides of the comb can be pulled out from between the plates, tighten the screws on the underside of the black brace. **Note** Testing the fit of the comb between the plates at this point in the procedure is more efficient than waiting until you are ready to pour the gel. You may not have enough time to make the appropriate adjustments if you perform this test after the polymerizing reagents are added to the gel solution.
7	**IMPORTANT** Keep the braces locked in place throughout the remainder of this procedure. Check the luer fitting in the gel injection fixture. Finger-tighten the fitting if it is loose.
8	If the fixture is still wet from previous use, briefly blow air through the luer fitting to expel any trapped moisture. An empty syringe or compressed air can be used.

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To attach the gel injection device: *(continued)*

Step	Action
9	Attach the gel injection fixture as follows: a. Hold the fixture underneath the plates at a 45° angle. b. Rotate the fixture up and onto the plates as shown below. c. Close the cassette clamps to lock the fixture in place. d. Simultaneously lock down both clamps on the fixture. e. Verify that the tension springs lock the fixture firmly against the notches on the outside of the gel cassette (see exploded view below) Verify that the edges of the plates are flush against the rubber gasket inside the fixture. 
10	Place absorbent paper on the table at each end of the plates to catch any gel solution that spills during injection. **IMPORTANT** Do not raise the cassette by placing objects such as blocks or pipet tip boxes under the plates. This can cause the gel to be thinner in middle.

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Method 1—Part 3—Pouring the Gel Using Mounted Plates

Preparing the Gel Solution

Prepare the gel solution now. See “Selecting a Gel Formulation” on page 2-2 for guidelines. Gel recipes are listed in Appendix A, “Gel Recipes.”

IMPORTANT The polymerizing reagents—ammonium persulfate and TEMED—are the catalysts that initiate polymerization. Once these reagents are added to the gel solution, you must work quickly to pour the gel. Polymerization can occur within 15 minutes.

Using Mounted Plates and the Gel Injection Device

At this point in the gel preparation procedure, the glass plates, spacers, and comb should be mounted in the gel cassette. The gel injection device should be attached to the plates. The entire assembly should be resting on a level, raised support.

! WARNING ! CHEMICAL HAZARD. Long Ranger gel solution contains acrylamide. Acrylamide is a neurotoxin. Avoid skin contact with Long Ranger gel solution because acrylamide can be absorbed through the skin. 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. Acrylamide and bisacrylamide are poisons, neurotoxins, irritants, carcinogens, and possible teratogens. Acrylamide and bisacrylamide sublime (the solids release toxic vapor) and are harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.

To inject the gel solution:

Step	Action
1	Remove the clear brace and comb.
2	Draw at least 35 mL of gel solution into a 60-cc syringe, filling it slowly to avoid introducing air bubbles. **IMPORTANT** Draw enough solution into the syringe to inject the entire gel without having to stop and draw more solution.
3	Check the tip of the syringe. The gel solution should be at the tip, and no air bubbles should be present. Adjust the solution and remove air bubbles if necessary.
4	Screw the tip of the syringe into the luer fitting. **IMPORTANT** Do not overtighten. Overtightening will strip the threads.

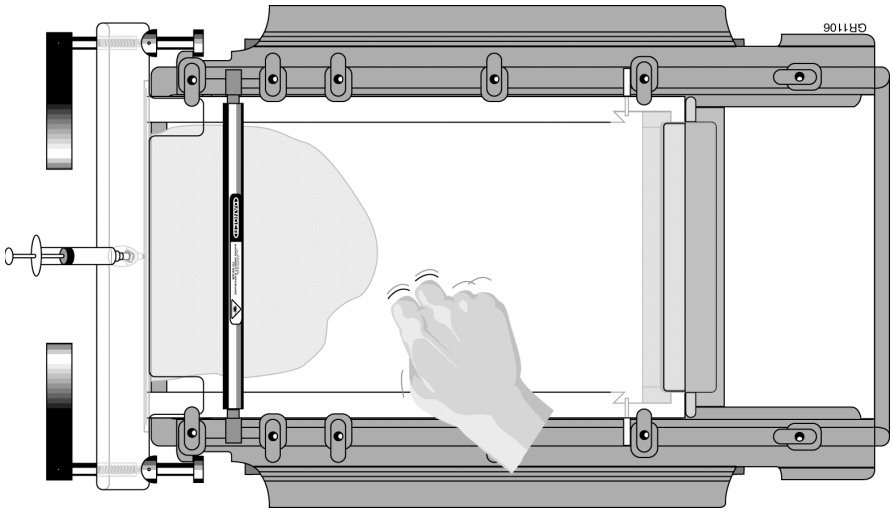
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To inject the gel solution: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>5</td><td colspan="2">Slowly and steadily inject the solution between the plates. At the same time, constantly tap the top plate directly in front of the gel path to eliminate air bubble formation. Injection takes approximately 60 seconds. Stop when the solution fills the space between the plates. Allow a small amount to pool in the well of the black brace.

CAUTION Tapping the plate with a tool such as a rubber mallet or reflex hammer can break the plates.

Note If you need to refill the syringe, be careful not to introduce air through the fitting. Keep excess solution in a beaker to verify polymerization.

</td></tr>
<tr><td>6</td><td colspan="2">Unscrew the syringe. Do not remove the luer fitting.</td></tr>
<tr><td rowspan="3">7</td><td>If using a …</td><td>Then …</td></tr>
<tr><td>shark’s-tooth comb</td><td>a. insert the straight edge of the comb to form one large well.
b. Verify no air bubbles are trapped where the gel and comb meet.</td></tr>
<tr><td>square-tooth comb</td><td>a. insert the teeth of the comb.
b. Verify no air bubbles are trapped where the gel and comb meet.</td></tr>
<tr><td>8</td><td colspan="2">Reinstall the clear brace and lock it in place.</td></tr>
<tr><td>9</td><td colspan="2">Remove the gel injection fixture.</td></tr>
<tr><td>10</td><td colspan="2">Close the bottom cassette clamps to secure the bottom of the plates.</td></tr>
<tr><td>11</td><td colspan="2">Flush the gel injection fixture immediately with water to clean it. Clean the syringe in a similar manner if it is to be reused.

Note Allow the luer fitting to dry completely before using it again.</td></tr>
<tr><td>12</td><td colspan="2">Cover the bottom of the plates with plastic wrap or damp paper towel to prevent the bottom of the gel from drying out.</td></tr>
<tr><td>13</td><td colspan="2">Allow the gel to polymerize the amount of time recommended by the gel solution protocol.</td></tr>
</table>

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Method 2—Pouring the Gel Using Unmounted Plates

Materials Required

- Binder clips
  - Eight medium size
  - Three large size
- Comb
- Kimwipes
- Plastic wrap
- Raised, level platform (e.g. two empty pipet tip boxes)
- Glass plates, front and rear, one set
- Spacers, two 0.2 mm
- Syringe, 60-cc

Preparing the Gel Solution

Prepare the gel solution now. See “Selecting a Gel Formulation” on page 2-2 for guidelines. Gel recipes are listed in Appendix A, “Gel Recipes.”

IMPORTANT The polymerizing reagents—ammonium persulfate and TEMED—are the catalysts that initiate polymerization. Once these reagents are added to the gel solution, you must work quickly to pour the gel. Polymerization can occur within 15 minutes. If desired, you can prepare the solution up to the point of adding the polymerizing reagents, then 1) prepare the work area and glass plates, 2) add the polymerizing reagents to the gel solution, and 3) pour the gel.

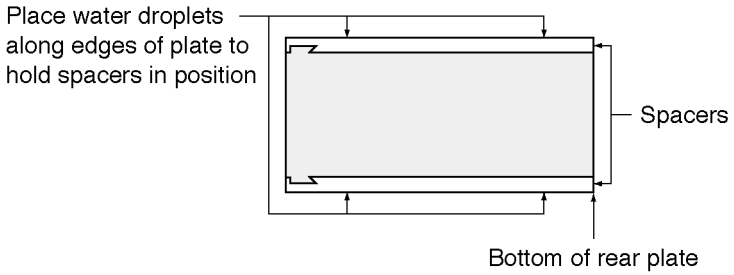
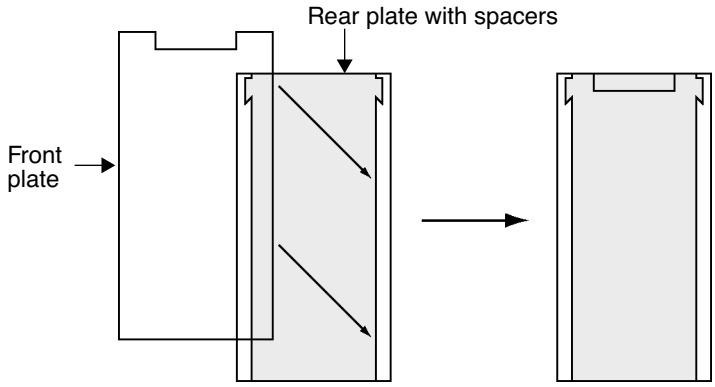
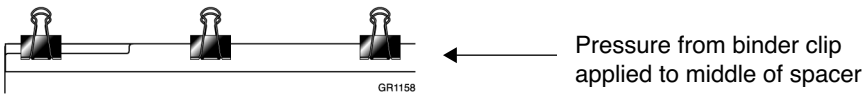
Preparing the Work Area and Glass Plates

To prepare the work area and glass plates:

Step	Action
1	Prepare a raised platform that is stable and level. For example, two empty pipet tip boxes work well.
2	Check the inside surface of both plates for water droplets, dust, lint, or anything else that might fluoresce or scatter light. Clean the plates with a damp Kimwipe if necessary.
3	Place the rear plate on the platform.
4	Place absorbent paper towels under the plate to catch spills or overflow.
5	Check the spacers and comb. If necessary, wipe them off with a damp Kimwipe.

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To prepare the work area and glass plates: *(continued)*

Step	Action
6	Place the spacers on the rear plate as shown below (notched side facing the middle of the plate). Two to three droplets of water can be applied to the edge of the glass where the spacers will rest to keep them from moving. **IMPORTANT** Align the outside edge of each spacer with the outside edge of the plate. Spacers must cover the notched areas of the plate. 
7	Place the front plate on top of the rear plate with the gasket mark facing up (hydrophobic area, described on page 2-5). Align the bottom of the plates so they are flush. 
8	Attach four medium binder clips along the length of each side of the plates. Evenly space the clips, and clamp them so pressure is applied to the middle of the spacers. **IMPORTANT** To avoid uneven gel thickness, do not position binder clips any further onto the plates than the middle of the spacers. 
9	Slightly raise the end of the glass plates with the large notch in the front plate. This will help the solution flow more easily to the bottom of the plates.

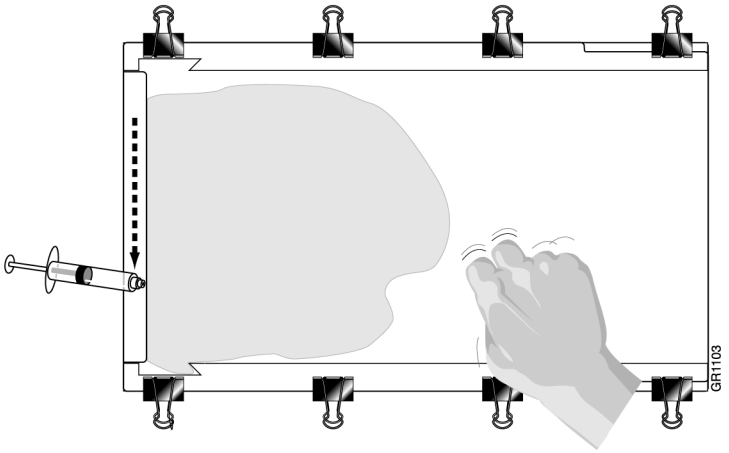
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Injecting the Gel Solution

! WARNING ! CHEMICAL HAZARD. Long Ranger gel solution contains acrylamide. Acrylamide is a neurotoxin. Avoid skin contact with Long Ranger gel solution because acrylamide can be absorbed through the skin. 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. Acrylamide and bisacrylamide are poisons, neurotoxins, irritants, carcinogens, and possible teratogens. Acrylamide and bisacrylamide sublime (the solids release toxic vapor) and are harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.

To inject the gel solution:

Step	Action
1	Draw at least 35 mL of gel solution into a 60-cc syringe, filling it slowly to avoid introducing air bubbles. **IMPORTANT** Draw enough solution into the syringe the first time to inject the entire gel without having to stop and draw more solution.
2	Check the tip of the syringe. The gel solution should be at the tip, and no air bubbles should be present. Adjust the solution and remove air bubbles if necessary.
3	Starting at one end of the large notch in the front plate, slowly and steadily inject gel solution between the plates across the entire width of the notch. 
4	Simultaneously: ♦ Continue injecting the gel solution while moving the syringe back and forth along the width of the notch ♦ Constantly tap the top plate directly in front of the gel path to eliminate air bubble formation Injection takes approximately 60 seconds. Stop when the solution completely fills the space between the plates. **Note** If you need to refill the syringe, be careful not to introduce any air bubbles. Keep excess solution in the beaker to verify polymerization.

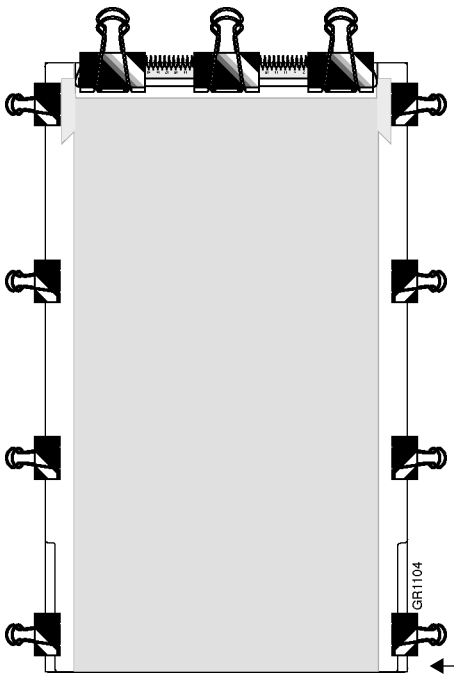
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To inject the gel solution: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td rowspan="3">5</td><td>If using a …</td><td>Then …</td></tr>
<tr><td>shark’s-tooth comb</td><td>insert the straight edge of a shark’s-tooth comb to form one large well.</td></tr>
<tr><td>square-tooth comb</td><td>insert the teeth of a square-tooth comb to form the wells.</td></tr>
<tr><td>6</td><td colspan="2">Verify no air bubbles are trapped where the gel and comb meet.</td></tr>
<tr><td>7</td><td colspan="2">Attach three large binder clips to the top of the plates directly over the comb. Evenly space the clips. If using a shark’s-tooth comb, be careful not to damage any teeth while clamping the plates.

IMPORTANT Do not attach binder clips to the bottom of the gel. This can result in an uneven gel thickness.

IMPORTANT To prevent well leakage, the 96-lane plates and casting combs require 10–12 pounds clamping pressure.


</td></tr>
<tr><td>8</td><td colspan="2">Cover the bottom of the plates with plastic wrap or damp paper towel to keep the bottom of the gel from drying out.</td></tr>
<tr><td>9</td><td colspan="2">Allow the gel to polymerize the amount of time recommended by the gel solution protocol.</td></tr>
</table>

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ABI PRISM® 377 DNA Sequencer

Instrument Operation

Quick Start Guide

Applied Biosystems

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Instrument Operation 3

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

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Preparing the Formamide/Blue Dextran Loading Solution	3-3
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Summary of Procedures for Performing a Run

To Perform a Run

To perform a run (electrophorese samples on the instrument) you will:

1. Prepare the buffer and loading solutions before you start or as needed.
2. Clean the outside of the gel plates and mount them in the gel cassette.
3. Install the lower buffer chamber and gel cassette.
4. Perform a plate check.
5. Install the upper buffer chamber, and fill both chambers with buffer solution.
6. Install the front heat-transfer plate.
7. Set up the software by completing a sample sheet and a run sheet.
8. Perform a prerun and load the samples.
9. Start the run.

Materials Required but not Supplied

Available from Applied Biosystems

- 25 mM EDTA with 50 mg/mL blue dextran, pH 8.0 (P/N 402055)
- Matrix standard samples (may not be required; refer to your protocol and Chapter 6, “Making Matrix Files for GeneScan,” or Chapter 7, “Making Instrument Files for Sequencing,” as appropriate for more information)
- Sequencing standard (for sequencing runs only; may not be required; refer to your protocol for the recommended sequencing standard)
- Size standard (required for GeneScan® analysis software runs only; refer to your protocol for the recommended size standard)
- Thermal cycler (we recommend the GeneAmp® PCR System 9700 or 9600)
- Thermal cycler accessories, MicroAmp® tray/retainer sets, reaction tubes, and caps

Available from Major Laboratory Suppliers

- Formamide, deionized (see Appendix A for deionization procedure)
- Gel, freshly prepared (recipes in Appendix A)
- Gloves, disposable, powder-free
- Kimwipes®
- Microcentrifuge or centrifuge adapted for spinning microtiter plates
- Pipet and tips, small volume, calibrated
  - Gilson Pipetman (Rainin Instruments, P/N P10 or P20)
  - Flat pipet tips (Rainin Instruments, P/N GT-1514)

Note Pipet tips listed above fit both the P10 and P20. However, they eject off the P10 only.

- Syringe, 60-cc, with a 16- to 18-G needle
- Tris Borate EDTA (TBE) stock solution, 10X, pH 8.3
- Water, sterile, deionized

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Preparing the 1X Tris Borate EDTA (TBE) Buffer Solution

Materials Required

- Graduated cylinder, large
- 10X Tris Borate EDTA (TBE) stock solution, pH 8.3 at ambient temperature (Procedure for preparing 10X TBE in Appendix A.)
- Water, deionized

IMPORTANT If the pH of the 10X TBE stock solution is not 8.3 (± 0.2), discard and use fresh solution. Do not attempt to adjust the pH.

Preparing the 1X TBE Buffer Solution

! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

Prepare this solution before you start or as needed based on the procedures in this chapter. To prepare the 1X TBE buffer solution:

Step	Action
1	Pour 120 mL of 10X TBE stock solution into the graduated cylinder.
2	Dilute with deionized water to a total volume of 1200 mL, and mix well.

Preparing the Formamide/Blue Dextran Loading Solution

Materials Required

- Formamide, deionized, pH > 7.0 (Deionization procedure in Appendix A.)
- 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL)

Storage Recommendations

We recommend storing the materials required separately.

- Formamide—store at –20°C
  - Freeze/thaw formamide stored in Eppendorf tubes a maximum of 5–10 times before discarding
- EDTA with blue dextran—store at room temperature

Preparing the Loading Solution

! 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.

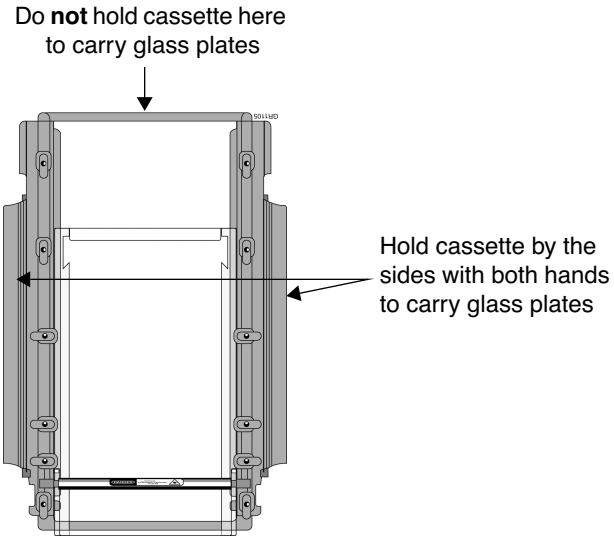
Prepare this solution before you start or as needed based on the procedures in this chapter. Prepare loading solution fresh daily by mixing the following ingredients in a 5:1 ratio (formamide : EDTA with blue dextran). The amount you will need to prepare will vary depending on the number of lanes used.

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Cleaning the Gel Plates Before Loading the Gel

How to Carry the Cassette with Plates

IMPORTANT When carrying a cassette with plates, always hold the cassette by the sides with both hands. Do not carry the cassette by the top bar. The weight of the plates may cause the bar to break, resulting in broken glass plates.



Cleaning the Plates Before Using the Gel

Follow this procedure to clean the outside surface of the glass plates before loading the gel on the instrument.

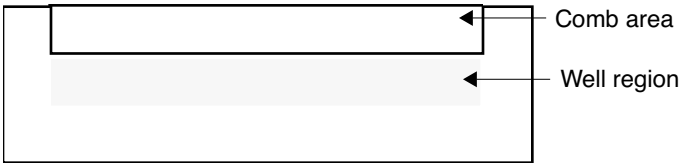
IMPORTANT Wear gloves throughout this procedure for your protection, and to avoid transferring fluorescent contaminants from your hands to the glass plates.

To clean the glass plates:

Step	Action	
1	**If the plates are …**	**Then …**
	very dirty	a. remove the plates from the cassette to clean them (if applicable). CAUTION **Always remove the plates from the cassette before rinsing in a sink with tap water. Arcing can occur inside the instrument if the cassette and plates are installed with even a small amount of moisture on them. Arcing is a luminous, low voltage, high current electrical discharge that can severely damage the instrument.** b. Put the plates in a sink and rinse them with cool tap water, making sure the read region (see page 3-47) is thoroughly cleaned. c. Dry the plates with Kimwipes.
cont.		

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To clean the glass plates: *(continued)*

Step	Action	
1 cont.	not very dirty	a. you can leave them in the cassette to clean them (if applicable). b. Using deionized water and Kimwipes thoroughly clean the read region (see page 3-47). c. Dry the plates with Kimwipes.
	IMPORTANT Be sure to clean the outside of the back plate. Gel solution can sometimes leak out the back while pouring the gel, and polymerizes as drops on the glass. If not removed, the back plate will not make full contact with the rear heat-transfer plate, and instrument sensitivity will be greatly reduced. Over time, gel solution can also damage the rear heat-transfer plate.	
2	For square-tooth combs only: Place the overlay strip on the clean class. Align the well outlines on the overlay strip with the teeth of the comb.	
3	Carefully remove the comb from between the plates, and clean it with deionized water and Kimwipes. Allow the comb to air dry.	
4	Clean the comb area with deionized water and Kimwipes. Remove residual acrylamide from the well region.  Very carefully use the teeth of the comb to remove residual acrylamide in the well region formed between the plates.	
5	Visually examine the plates for dust, lint, water spots, and fingerprints. Clean again if necessary.	
6	**If the plates are …**	**Then …**
	mounted in the gel cassette, and you are using a square-tooth comb	proceed to “Installing the Gel Cassette and Lower Buffer Chamber” on page 3-8.
	mounted in the gel cassette, and you are using a shark’s-tooth comb	proceed to step 5 on page 3-6.
	not in the gel cassette	proceed to “Loading the Gel Into the Cassette” on page 3-6.

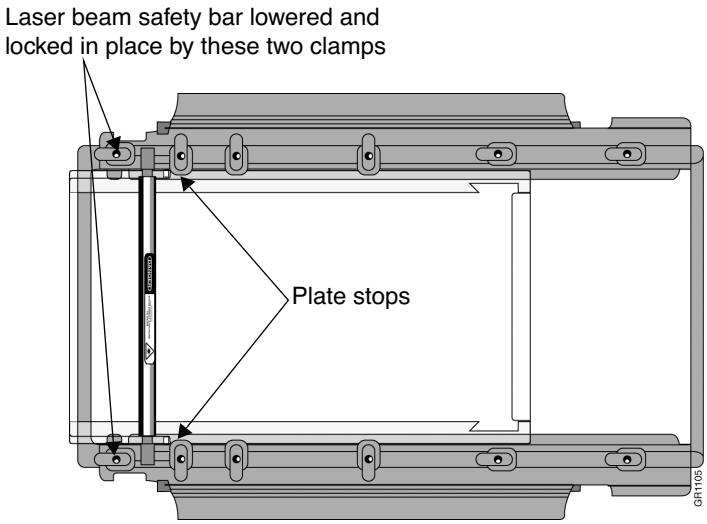
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Loading the Gel Into the Cassette

Procedure

IMPORTANT When carrying a cassette with plates, always hold the cassette by the sides with both hands. Do not carry the cassette by the top bar. The weight of the plates may cause the bar to break, resulting in broken glass plates. See the illustration on page 3-4.

To load the gel into the cassette:

Step	Action	
1	Place the cassette on a clean, level surface.	
2	Orient the plates in the cassette as shown below so the front plate is on top. Then push the plates to the bottom of the cassette until the notches in the rear plate are seated firmly against the metal plate stops in the cassette. Push the plates from the top to ensure firm contact.	
3	Press on the center of the front plate with the fingertips of one hand to hold plates in place. At the same time, lock the plates into position by turning all the cassette clamps to the closed position except for the bottom pair of clamps.	
4	Lower the laser beam safety bar, and turn the bottom cassette clamps to lock the bar into position.  	
5	**If using a …**	**Then …**
	shark's-tooth comb	a. make sure the comb is clean and dry. b. Proceed to the next step.
	square-tooth comb	proceed to "Installing the Gel Cassette and Lower Buffer Chamber" on page 3-8.
6	**If this is …**	**Then …**
	not a 96-lane upgrade instrument	proceed to the next step.
	a 96-lane upgrade instrument	using a syringe, add 1X TBE buffer to the loading area. Adding TBE makes comb insertion and removal much easier.

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To load the gel into the cassette: *(continued)*

Step	Action
7	Insert the teeth of the shark's-tooth comb between the plates as follows: a. Hold the comb at both ends. b. Slowly and carefully insert the teeth of the comb between the plates without bending any of the teeth or introducing air bubbles. c. Continue sliding the comb between the plates until the tips of all the teeth penetrate the gel approximately 1–2 mm. If the top of the gel is not completely flat in the sample loading region, you may have to insert some of the teeth further than 1–2 mm so that all the teeth penetrate the gel surface. **IMPORTANT** If any of the teeth penetrate the gel too much, do not pull them out. Leave them in the gel. Removing teeth that have penetrated the gel causes samples to leak into adjacent wells.
8	Proceed to "Installing the Gel Cassette and Lower Buffer Chamber" on page 3-8.

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Installing the Gel Cassette and Lower Buffer Chamber

The Electrophoresis Chamber

The following illustration shows the various components of the electrophoresis chamber when it is empty.

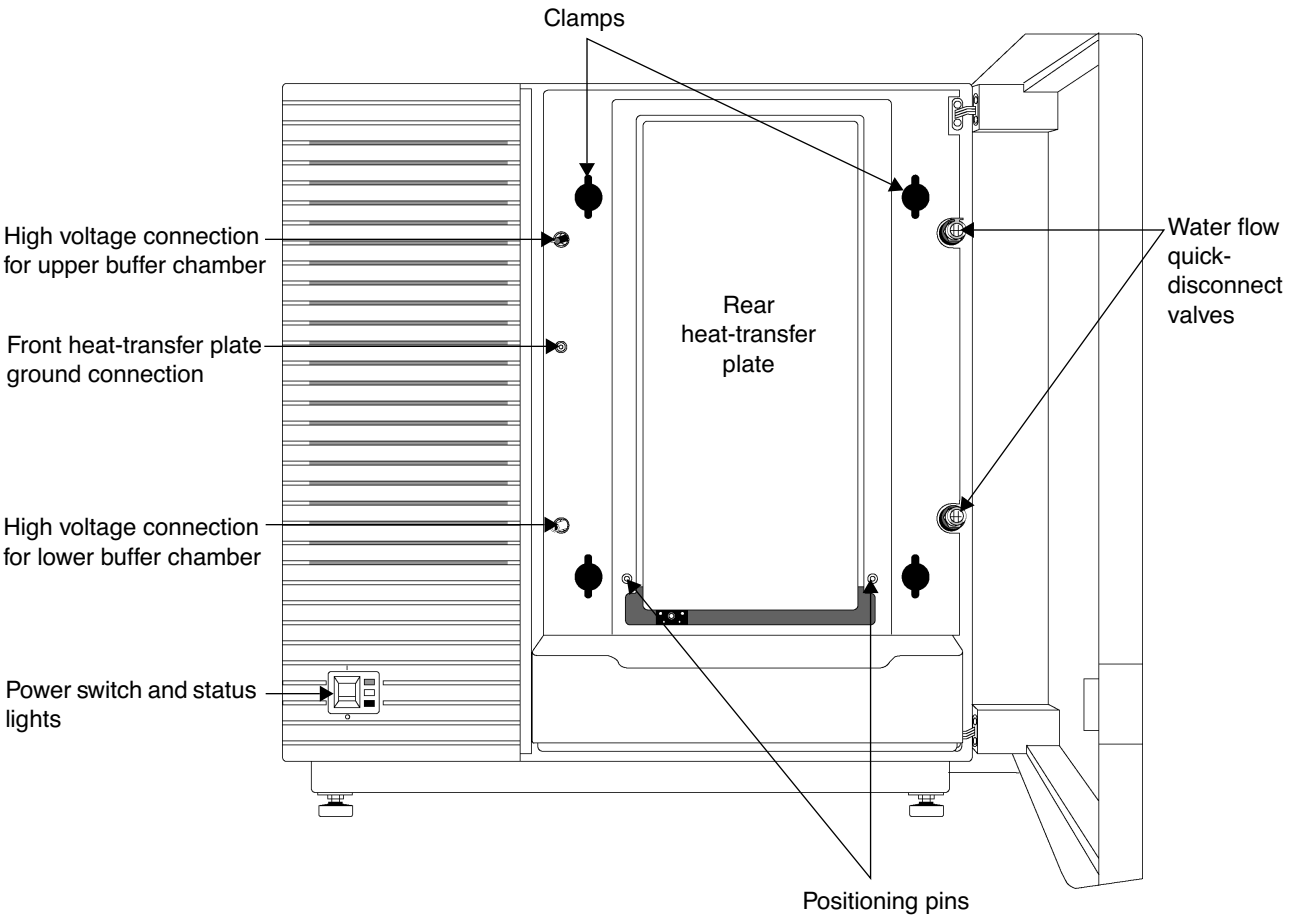


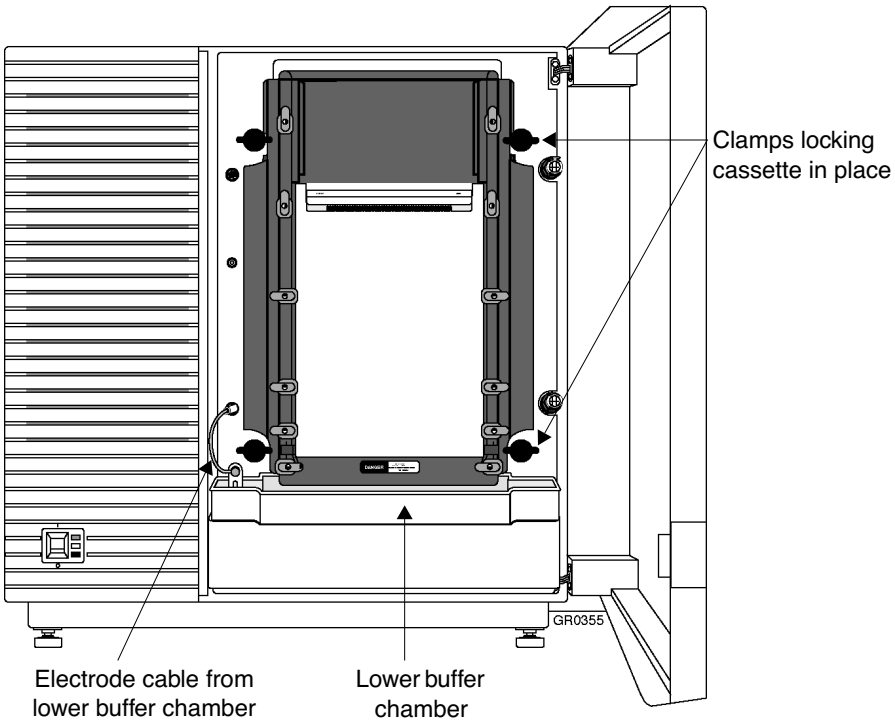
Figure 3-1 Electrophoresis chamber

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Installation Procedure

! WARNING ! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result if you defeat the safety interlocks located in the door of the instrument. Close the panel door before operating the instrument.

To install the gel cassette and lower buffer chamber:

Step	Action
1	Open the right front panel of the ABI PRISM® 377 instrument.
2	Inspect the front and rear heat-transfer plates, and the two positioning pins inside the electrophoresis chamber (Figure 3-1 on page 3-8) for residue. If dirty, clean with a Kimwipe. **CAUTION** **TBE crystals and dried acrylamide can accumulate on the positioning pins and heat-transfer plates. The presence of these residues can result in arcing. Arcing is a luminous, low voltage, high current electrical discharge that can severely damage the instrument.** **Note** TBE crystals and dried acrylamide can also cause misalignment of plates, resulting in a colored haze over the gel image.
3	Place the lower buffer chamber on the bottom shelf of the electrophoresis chamber, and connect the electrode cable to the lower high voltage connection.
4	Fit the cassette up against the rear heat-transfer plate between the four clamps in the chamber as shown below. Turn the clamps to lock the cassette in place. **IMPORTANT** Do not touch the read region of the plates (see page 3-47). 

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To install the gel cassette and lower buffer chamber: *(continued)*

Step	Action
5	Carefully check cassette installation. The positioning pins in the chamber (Figure 3-1 on page 3-8) must be touching the spacers through the two holes on the back of the cassette. The pins should be visible through the glass. Repeat the installation if necessary. **IMPORTANT** Proper installation is critical to ensure good results. The rear heat-transfer plate is spring loaded, and is designed to apply even pressure to the glass plates. If the cassette is not properly installed against the positioning pins, instrument sensitivity will be greatly reduced, and experimental results will be poor.
6	Close the front panel of the instrument, and proceed to “Performing a Plate Check” on page 3-11.

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Performing a Plate Check

Plate Check Procedure

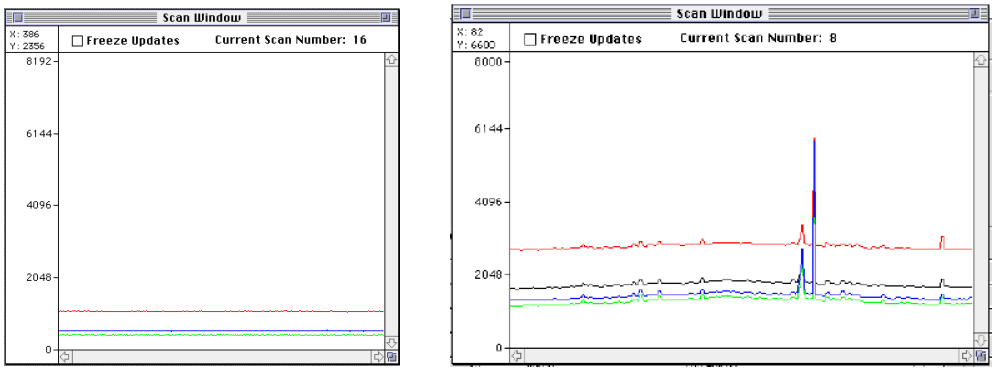
See “Using the Instrument” in Chapter 1 for a description of the plate check and its purpose.

! WARNING ! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result if you defeat the safety interlocks located in the door of the instrument. Close the panel door before operating the instrument.

To check the plates:

Step	Action	
1	Open the Special menu and choose Restart to restart the computer. We recommend restarting the computer once a day or before each run to reduce memory fragmentation, and to quit any applications that might be running in the background.	
2	If not launched automatically, launch the ABI PRISM® Data Collection Software (data collection software) by double-clicking the icon.	
3	Open a new run sheet (also referred to as the “run window”) as follows: a. Open the File menu and select New. b. Select Sequence Run or GeneScan Run as appropriate. c. Open the Plate Check Module pop-up menu, and select a plate check module.	
4	Click Plate Check on the run sheet. The Log and Scan windows are displayed. The plates are scanned without electrophoresis.	
5	Observe the Scan window. After approximately 45 seconds, four lines are displayed. The typical pattern from top to bottom is red, black, blue, and green. The blue and green lines can be transposed, or displayed on top of each other. This is acceptable.	
6	Watch the scan lines for approximately 30 seconds.	
7	**If …**	**Then …**
	the scan lines are relatively flat (Figure 3-2 on page 3-12)	the plates are clean. a. Click Cancel and terminate the Plate Check. b. Leave the run window open. c. Proceed to “Installing the Upper Buffer Chamber” on page 3-15.
	peaks appear in the scan window (Figure 3-2 on page 3-12)	the plates are dirty. Proceed to “Reclean the Plates” on page 3-13.
	the green line is higher than the red and black lines	the plates or gel are contaminated with a large amount of fluorescent material. Proceed to “Reclean the Plates” on page 3-13.

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Clean plates

Dirty plates

Figure 3-2 Scan windows during the plate check showing clean and dirty plates. The typical order of colors for the scan lines from top to bottom is red, black, blue, and green

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Reclean the Plates

Peaks in the scan lines during a Plate Check indicate contaminants on the glass in the read region, or contaminating fluorescence in the gel. Clean the plates again as described below, and repeat the plate check.

To clean the plates and repeat the plate check:

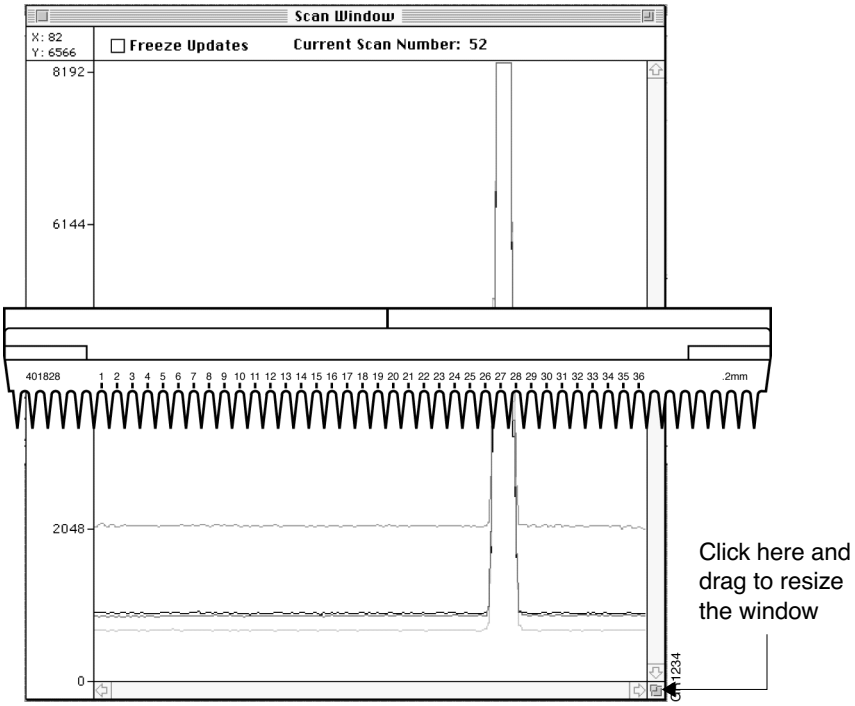
Step	Action	
1	Click Pause to pause the plate check.	
2	Open the right front panel of the instrument, and remove the gel cassette. **! WARNING ! LASER HAZARD. Exposure to direct or reflected laser light at 40 mW for 0.1 seconds can burn the retina and leave permanent blind spots. Never look directly into the laser beam or allow a reflection of the beam to enter your eyes.**	
3	Carefully clean both sides of the glass with deionized water and Kimwipes, particularly the read region.	
4	Remount the cassette in the electrophoresis chamber.	
5	Close the front panel of the instrument.	
6	Click Resume to check the plates again.	
7	**If …**	**Then …**
	the plates are clean	a. Click Cancel and terminate the plate check. b. Leave the run window open. c. Proceed to “Installing the Upper Buffer Chamber” on page 3-15.
	peaks are still present	the gel is probably contaminated. Choose one of the following: ♦ Determine which lanes are contaminated, and leave those lanes empty when loading samples. See “Skipping Lanes” on page 3-14 to determine which lanes are contaminated. ♦ Cancel the plate check, fill the buffer chambers, install the front heat-transfer plate, and start the prerun. Prerun the gel approximately three minutes and watch the Scan window. Contaminants may migrate out of the read region and peaks will disappear. All lanes can then be used. (See pages 3-15, 3-16, 3-18, and 3-35.) ♦ Click Cancel, terminate the plate check, and install a new gel.

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Skipping Lanes

Determining Which Lanes to Skip

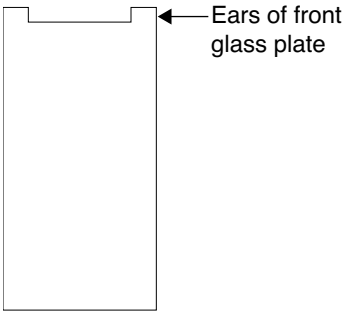
The Scan window displays data as channel numbers. Follow this procedure to determine the lane number(s) that correspond to the channel number(s) containing fluorescent contaminants. Skip these lanes when loading samples.

Step	Action	
1	While the Scan window is open and the plate check is running, hold a spare, clean comb up against the scan window. The comb must be of the exact configuration as the one used to prepare the gel.	
2	Resize the Scan window to the exact size of the **numbered lanes only** on the comb. 	
3	The lanes in which the peaks appear are the lanes to skip when loading samples. In the example shown above, sample would not be loaded in lanes 26, 27, and 28. If the peaks are close to the edge of a lane, we recommend skipping an extra lane.	
4	Click Cancel and terminate the plate check.	
5	**If a sample sheet …**	**Then …**
	has not been completed	proceed to “Installing the Upper Buffer Chamber” on page 3-15.
	has already been completed	reopen the sample sheet and make the necessary changes to reflect the empty lanes.
6	If the sample sheet has already been imported to the run sheet: a. Open the sample sheet pop-up menu and select <none>. b. Open the sample sheet pop-up menu and reselect the sample sheet. (See “Select the Sample Sheet” on page 3-29.)	
7	Proceed to “Installing the Upper Buffer Chamber” on page 3-15.	

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Installing the Upper Buffer Chamber

Procedure To install the upper buffer chamber:

Step	Action
1	Open the front panel of the instrument.
2	Open the two top clamps holding the gel in the cassette.
3	Rest the two tabs on the back of the chamber on the ears at the top of the front glass plate. 
4	Close the clamps to lock the chamber in place.
5	Connect the electrophoresis cable to the upper high voltage connection. (See Figure 3-1 on page 3-8.)
6	Proceed to “Filling the Buffer Chambers” on page 3-16.

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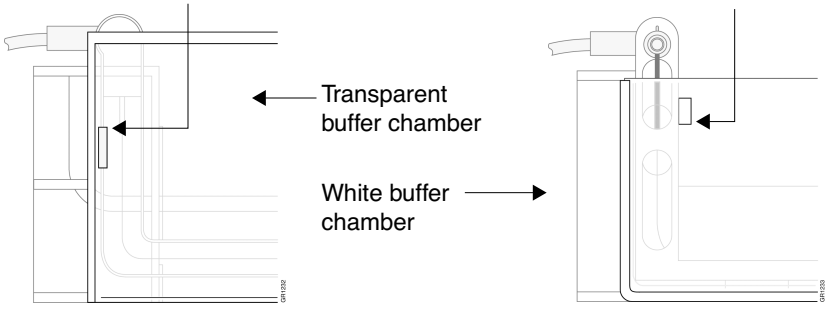
Filling the Buffer Chambers

To Fill the Buffer Chambers

IMPORTANT The concentration of the buffer in both chambers must match the concentration of the buffer in the gel. Otherwise, samples will run slower or faster than expected, or you may get a “No EP Current Detected” error message. The gel recipes listed in Appendix A use 1X Tris Borate EDTA buffer (TBE).

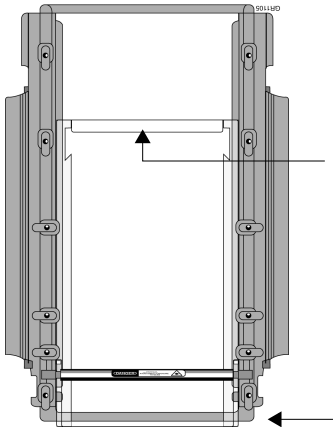
! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

To fill the buffer chambers:

Step	Action
1	If not already prepared, prepare the 1X Tris Borate EDTA (TBE) buffer solution by following the instructions listed on page 3-3.
2	Slowly and carefully fill the upper buffer chamber with approximately 600 mL of 1X TBE buffer. Do not allow buffer to splash onto the plates. Fill levels for the two styles of upper buffer chambers for this instrument are shown below. Filling the buffer chamber to the recommended level helps reduce the amount of buffer that evaporates during a run. Fill to top of the two level marks located on each side of the front of the buffer chamber Fill so buffer just touches the bottom of tab the lid rests on (tab is located on the rear of the buffer chamber) 
3	Wait approximately 30 seconds, and then check for leaks as follows: a. Look for fluid dripping down the front of the plates. This indicates the buffer chamber gasket may be worn and should be replaced. b. Look for fluid inside the lower buffer chamber. This indicates that buffer is leaking over the ears of the plates and down the sides of the cassette.

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To fill the buffer chambers:

Step	Action
4	If leakage occurs, try one or more of the following: ♦ Check the buffer level. If the chamber is overfilled, remove the excess buffer. ♦ Plug the suspected areas with melted agarose. If buffer is leaking over the ears of the plates, apply a bead or ribbon of agarose to seal this area. ♦ Carefully empty the buffer chamber and clean the gasket. Also clean the front of the glass plate where the gasket makes contact. If leakage continues, replace the gasket. Follow the procedure “Replacing the Upper Buffer Chamber Gasket” in Chapter 8. **IMPORTANT** If the buffer level drops below the notch in the front plate (in the upper buffer chamber) or below the bottom of both plates (in the lower buffer chamber), electrical contact is lost and the instrument shuts down automatically to prevent arcing (a luminous, low voltage, high current electrical discharge that can severely damage the instrument.)  Instrument shuts down automatically if buffer level drops below the notch at the top of the front plate, or below the bottom of the plates during a run
5	Carefully fill the lower buffer chamber with 1X TBE buffer to the top edge of the overflow dam. Do not overfill. **Note** Be careful not to splash buffer up onto the back of the rear plate. Buffer on the plate will dry during the run, resulting in a green haze on the gel image.
6	Proceed to “Installing the Front Heat-Transfer Plate” on page 3-18.

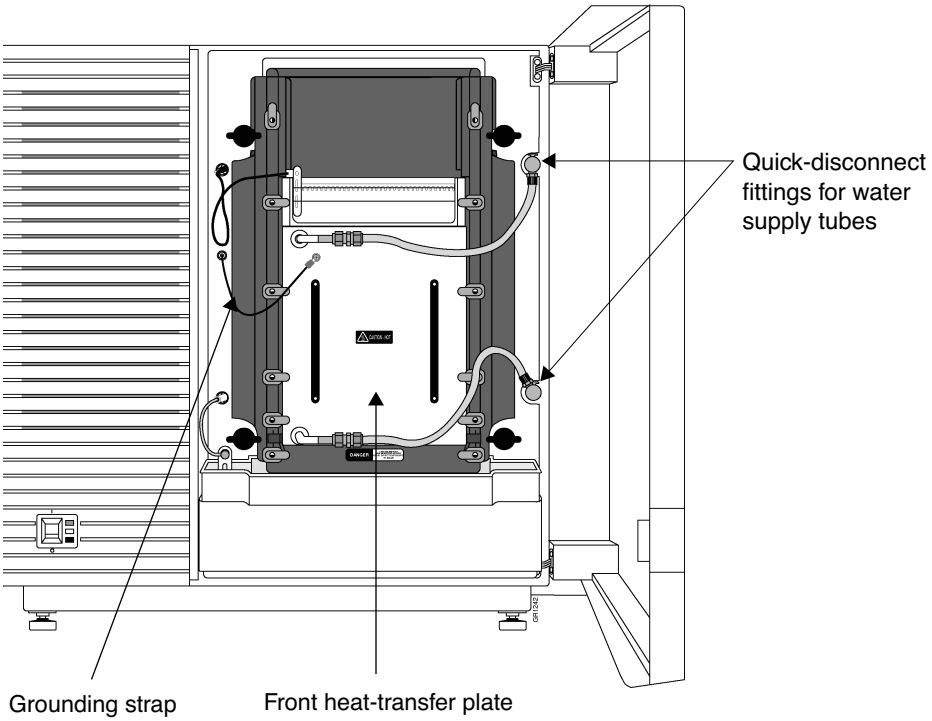
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Installing the Front Heat-Transfer Plate

Install the Front Heat-Transfer Plate

To install the front heat-transfer plate:

Step	Action	
1	**If this is a …**	**Then …**
	48-cm run	use of the front heat-transfer plate is optional, and is not necessary for good data. Proceed to step 2 and install the plate, or proceed to “Setting Up the Software for a Run” on page 3-19.
	36-cm run	Proceed to step 2.
2	Open the six gel cassette clamps between the upper buffer chamber and the laser beam safety bar.	
3	Make sure the laser beam safety bar is down and locked into position.	
4	Carefully position the front heat-transfer plate up against the glass plates so it rests on the laser beam safety stop. **CAUTION The front heat-transfer plate is heavy, and can be damaged if dropped or bumped against a hard surface. Exposed metal on a damaged plate will cause arcing. Arcing is a luminous, low voltage, high current electrical discharge that can severely damage the instrument. Handle the plate with care.**	
5	Close the cassette clamps to lock the plate in position.	
6	Attach the grounding strap to the heat plate ground connection.	
7	Connect the water supply tubes to the quick-disconnect fittings.	
8	Proceed to “Setting Up the Software for a Run” on page 3-19.	



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Setting Up the Software for a Run

Overview

This chapter does not include a detailed description of the data collection software. See Chapter 9, “Data Collection Software,” for more information.

Before starting a run, you must set up a sample sheet and a run sheet specific to your application (sequencing or GeneScan). The location of instructions for preparing sample and run sheets are listed in the following table.

Application	Type of Sheet	Location of Instructions
Sequencing	Sample sheet	page 3-19
	Run sheet	page 3-28
GeneScan	Sample Sheet	page 3-24
	Run sheet	page 3-28

Preparing a Sequencing Sample Sheet

IMPORTANT Do not mix sequence and GeneScan analysis samples on the same sample sheet or in the same run.

Save Time by Setting Sample Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up sample sheets by *setting sequencing sample sheet default preferences*. Setting sample sheet preferences means changing the default value of certain fields on the sample sheet template to the values used most often. The following fields on sequencing sample sheets can be set as preferences:

- DyeSet/Primer
- Instrument File

Once these preferences have been set, the preferred values appear automatically on each new sequence sample sheet. Preferences can be changed as often as necessary either by setting new preference values, or by opening the pop-up menus and manually selecting new values. Instructions for setting sequencing sample sheet default preferences are located in Chapter 5, “Setting Preferences.”

Project Names and BioLIMS

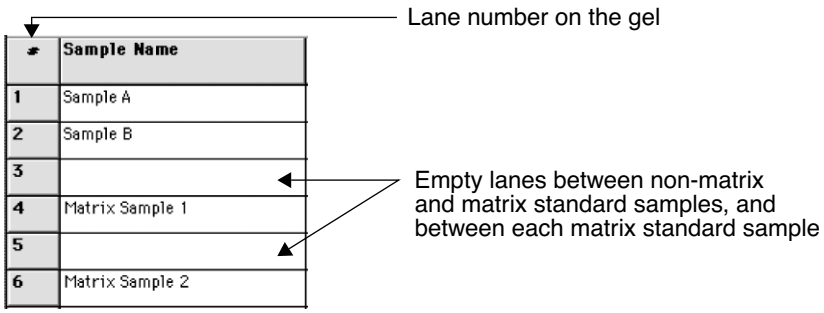
BioLIMS® and Setting Preferences

The Project Name field on the sample sheet is used to identify data transferred to BioLIMS. If this field is left empty, the data transferred to BioLIMS is identified by the gel file name only. Project names are defined prior to setting up sample sheets by opening the Windows menu, and selecting Project Info under Preferences. For more information and instructions, see Chapter 5, “Setting Preferences.”

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Open a New Sample Sheet and Enter Sample Names

To open a new sequencing sample sheet and enter sample names:

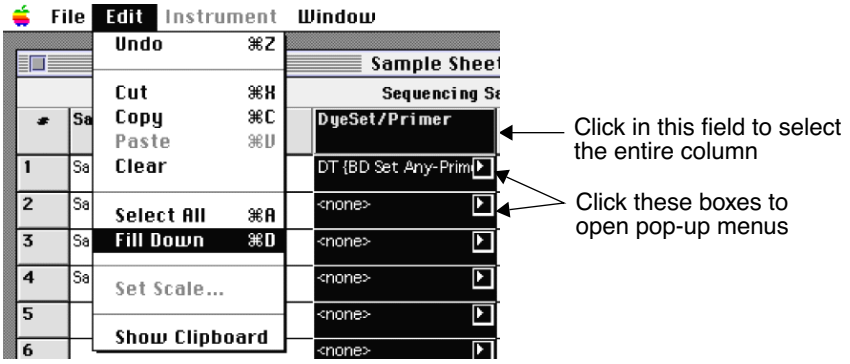
Step	Action
1	Open the File menu and select New (leave the run window open).
2	Click the icon named Sequence Sample.
3	Enter sample names in the Sample Name column by clicking in the Sample Name field, and typing the sample name. Enter names in the exact order the samples will be loaded onto the gel. The numbers to the left of the Sample Name column represent the gel lane numbers. Leave fields blank that correspond to empty lanes. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters.* Do not use colons, slashes, or symbols in sample names. **Note** More text can be entered than is visible. Text automatically shifts as the information is entered. Use the keyboard arrow keys to scroll through long entries. To import sample names from tab-delimited text files, follow the instructions listed in Chapter 9, “Data Collection Software.”
4	If matrix standard samples are being run, enter a name for each matrix sample. To help ensure a robust matrix is produced, we strongly recommend you follow these guidelines: ♦ Leave at least one empty lane between non-matrix standard samples and matrix standard samples. ♦ Leave one empty lane between each matrix standard sample. 

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Select a DyeSet/Primer File

Different dye set/primer files can be used for the same run as long as the virtual filter set is the same for all samples. Dye set/primer file names for dRhodamine Terminators are similar to those for BigDye™ Terminators, and can easily be mistaken for one another. If the wrong file is selected, base spacing in the data will not be noticeably affected. C and T bases will be miscalled. If you are not sure which file to select, refer to the chemistry kit protocol, and to “DyeSet/Primer Files” in Chapter 9.

To select a dye set/primer file:

Step	Action
1	Open the DyeSet/Primer pop-up menu and select the appropriate file for the first sample. You must select a file if you want the data to be analyzed automatically at the end of the run. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the dye set/primer files. To correct this, you must set the Settings Folder Preference to the ABI Folder. See “Setting Folder Location Preferences” on page 3-51.
2	If the file is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate DyeSet/Primer file for each sample individually. 

Select an Instrument File

The instrument file must be the same for all the samples.

To select the instrument file:

Step	Action
1	Open the Instrument File pop-up menu for the first sample, and select the appropriate file. You must select a file if you want the data to be analyzed automatically at the end of the run. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the instrument files. To correct this, you must set the Settings Folder Preference to the ABI Folder. See “Setting Folder Location Preferences” on page 3-51.
2	Click in the column heading to select the entire column, open the Edit menu, and select Fill Down to enter the same instrument file for the remaining samples.

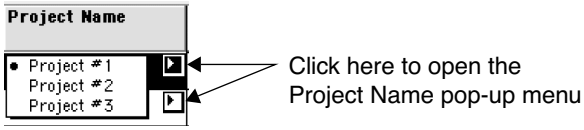
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Select Project Names and Enter Comments

The Project Name field is for BioLIMS users only. Otherwise, the field can be left blank. If a project name is not identified, the data transferred to BioLIMS is identified by the gel file name only.

Project names must be defined prior to setting up the sample sheet as Project Info Preferences. Refer to “Project Information Preferences” in Chapter 5 for instructions and more information.

To select project names and enter comments:

Step	Action
1	Open the Project Name pop-up menu for each sample, and select the appropriate project name. 
2	If the Project Name is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate Project Name for each sample individually.
3	To enter comments, click in the Comments field, and type the information.

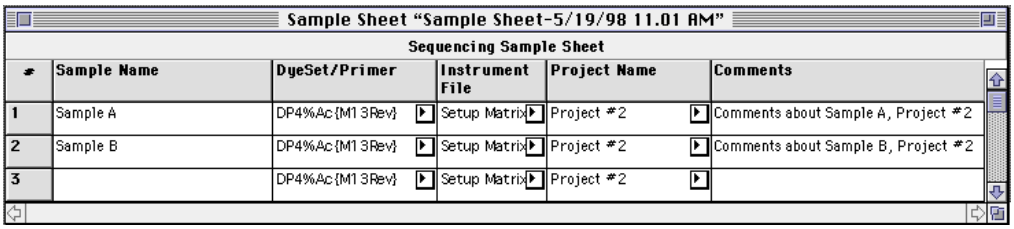
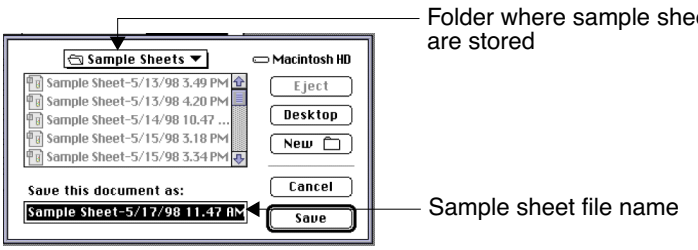


Figure 3-3 Example of a completed sequencing sample sheet

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Save and Close the Sample Sheet

To save and close the sample sheet:

Step	Action
1	Do one of the following: ♦ Open the File menu and select Close. ♦ Click the box in the upper left-hand corner of the window, and then click Save.
2	A dialog box showing the default sample sheet file name, and the location where the sample sheet will be stored is displayed. a. Change the file name now if desired. b. Click Save. **IMPORTANT** Although it is an option, we do not recommend changing the storage location of the sample sheet. If the location is changed, software will not be able to locate the sample sheet when you set up the run sheet.  **Note** You can also save the sample sheet by opening the File menu and selecting Save, Save As, or Save A Copy In.
3	Proceed to “Preparing a Run Sheet” on page 3-28 to finish setting up the software.

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Preparing a GeneScan Sample Sheet

IMPORTANT Do not mix GeneScan and sequence analysis samples on the same sample sheet or in the same run.

Save Time by Setting Sample Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up sample sheets by *setting GeneScan sample sheet default preferences*. Setting sample sheet preferences means changing the default value of certain fields on the sample sheet template to the values used most often. The following field can be set as a preference:

- Std (size standard dye color)

Once you have set this preference, the preferred value appears automatically on each new GeneScan sample sheet. This preference can be changed as often as necessary either by setting a new preference value, or by manually selecting a different value on the sample sheet. Instructions for setting GeneScan sample sheet default preferences are located in Chapter 5, “Setting Preferences.”

Open a New Sample Sheet and Enter Sample Names

To open a new GeneScan sample sheet and enter sample names:

Step	Action
1	Open the File menu and select New (leave the run window open).
2	Click the icon named GeneScan Sample.
3	Enter sample names in the Sample Name column by clicking in the appropriate Sample Name field, and typing the sample name. Enter names in the exact order the samples will be loaded onto the gel. The numbers to the left of the Sample Name column represent the gel lane numbers. Leave fields blank that correspond to empty lanes. See Figure 3-4 on page 3-25. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters*. Do not use colons, slashes, or symbols in sample names. **Note** More text can be entered than is visible. Text automatically shifts as the information is entered. Use the keyboard arrow keys to scroll through long entries. To import sample names from tab-delimited text files, follow the instructions listed under “Importing and Exporting Sample Sheet Information” in Chapter 9.
4	If matrix standard samples are being run, enter a name for each matrix sample. To help ensure a robust matrix is produced, we strongly recommend you follow these guidelines (see Figure 3-4 on page 3-25): ♦ Leave at least one empty lane between non-matrix standard samples and matrix standard samples. ♦ Leave one empty lane between each matrix standard sample. **IMPORTANT** Matrices are dye set, instrument, and run condition dependent. As such, matrices must be remade when any of these conditions change. For more information, refer to Chapter 6, “Making Matrix Files for GeneScan.”

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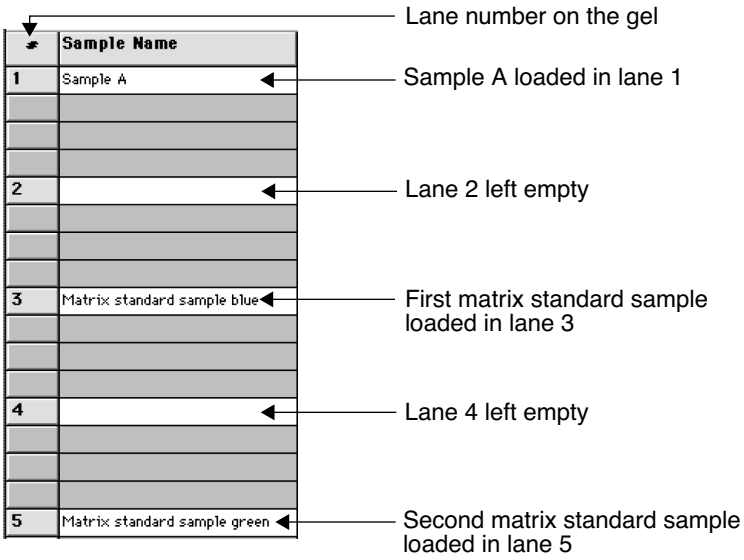


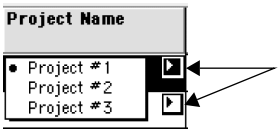
Figure 3-4 Sample name column on a GeneScan sample sheet

Select Project Names

The Project Name field is for BioLIMS version 2.0 and up users only. Otherwise, the field can be left blank. If a project name is not identified, the data transferred to BioLIMS is identified by the gel file name only.

Project names must be defined prior to setting up the sample sheet as Project Info Preferences. Refer to “Project Information Preferences” in Chapter 5 for instructions and more information.

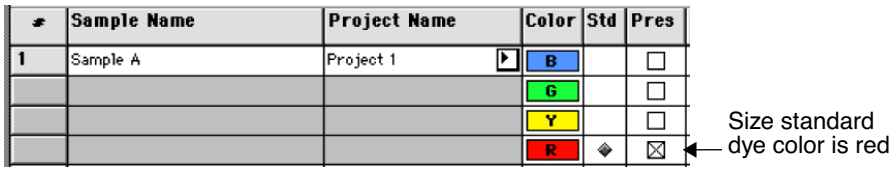
To select project names:

Step	Action
1	Open the Project Name pop-up menu for each sample, and select the project name. 
2	If the Project Name is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate Project Name for each sample individually.

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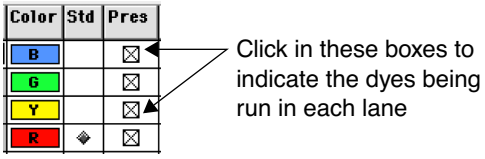
Specify the Size Standard Dye Color

To specify the size standard dye color:

Step	Action
1	For all non-matrix standard samples, designate the color of the size standard by clicking in the box to the right of the appropriate letter in the Color column (B = blue; G = green; Y = yellow; R = red). When selected, a diamond is displayed in the Std column. This must be specified for data to be analyzed automatically. 
2	Leave the Std column blank for matrix standard samples. If necessary, remove diamonds for matrix standard samples by clicking on the diamond.

Specify the Dyes Run in Each Lane

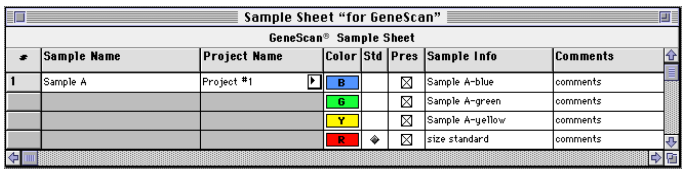
To specify the dye colors run together in each lane:

Step	Action
1	Click in the appropriate boxes in the Pres (present) column to select the dye colors that will be run in each lane for each sample. Codes for the colors are displayed in the Color column (B = blue; G = green; Y = yellow; R = red). Dyes must be specified for data to be analyzed automatically. 
2	To deselect a color, click in the appropriate box to remove the X.

Enter Sample Info and Comments

The information in the Sample Info and Comments fields is imported into ABI PRISM Genotyper® DNA Fragment Analysis Software, and is used for sample identification and sorting. If these fields are left blank, only the words “sample file” will appear when sample information is displayed in Genotyper.

To enter Sample Info and Comments:

Step	Action
1	To enter Sample Info, click in the Sample Info field and type the information. 
2	To enter comments, click in the Comments field and type the information.

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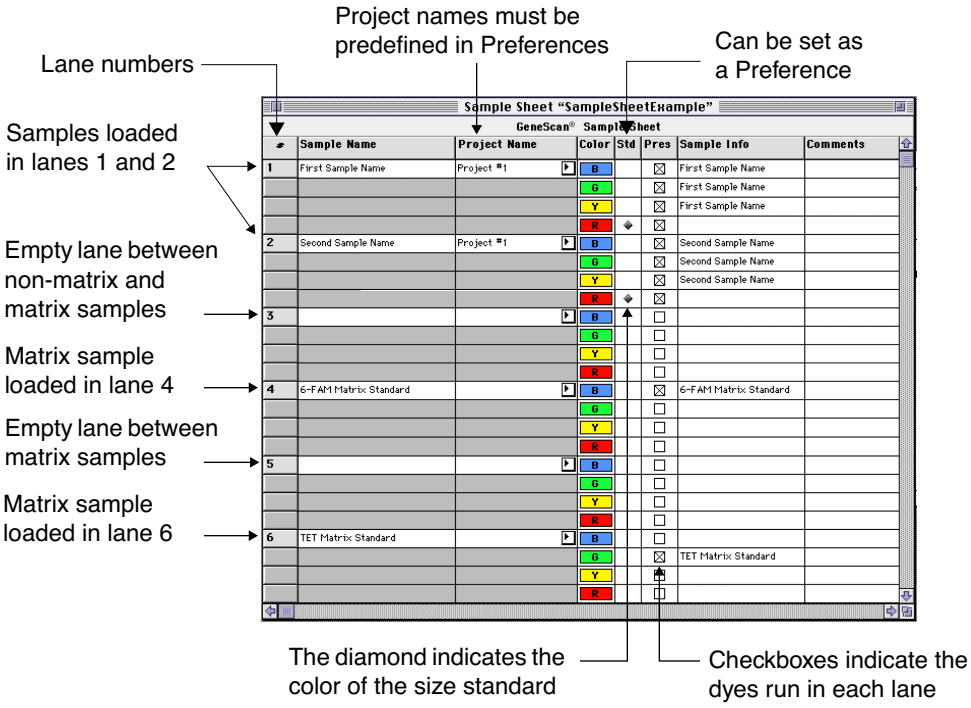
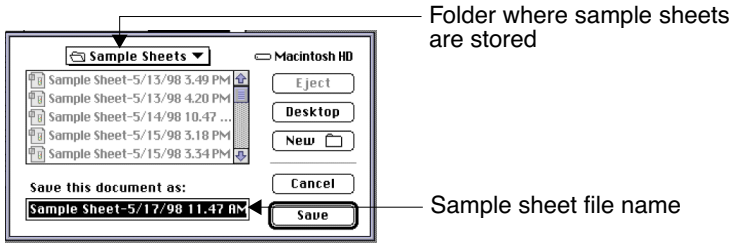


Figure 3-5 Example of a completed GeneScan sample sheet

Save and Close the Sample Sheet

To save and close the sample sheet:

Step	Action
1	Do one of the following: a. Open the File menu and select Close. b. Click the box in the upper left-hand corner of the window, and then click Save.
2	A dialog box showing the default sample sheet file name, and the location where the sample sheet will be stored is displayed. a. Change the file name now if desired. b. Click Save. **IMPORTANT** Although it is an option, we do not recommend changing the storage location of the sample sheet. If the location is changed, software will not be able to locate the sample sheet when you set up the run sheet. 
3	Proceed to "Preparing a Run Sheet" on page 3-28 to finish setting up the software.

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Preparing a Run Sheet

Save Time by Setting Run Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up run sheets by *setting sequencing or GeneScan run sheet default preferences*. Setting run sheet preferences means changing the default value of certain fields on the run sheet template to the values used most often. The following fields can be set as preferences:

- Operator
- Lanes
- Well-to-read distance
- Prerun and run modules
- Auto print
- Analysis parameters (GeneScan run sheets only)
- Gel's matrix file (GeneScan run sheets only)
- Size standard (GeneScan run sheets only)

Once these preferences are set, the preferred values appear automatically on each new run sheet. Preferences can be changed as often as necessary either by setting new preference values, or by manually selecting new values on the run sheet. Instructions for setting run sheet preferences are located in Chapter 5, "Setting Preferences."

Open a New Run Sheet

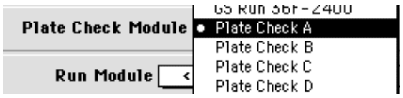
To open a new run sheet:

Step	Action	
1	**If the run window used for the plate check is**	**Then …**
2	still open	click on that window to make it the active window.
3	not open	open a new run sheet as follows: a. Open the File menu and select New. b. Click either the Sequence Run or GeneScan Run icon as appropriate. A new Run folder is created automatically in the Runs folder inside the ABI PRISM 377 folder. **Note** We recommend using the same run sheet for the plate check, prerun, and run. This will limit the number of run folders created per run to one.

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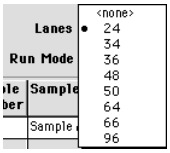
Select the Plate Check, PreRun, and Run Modules

To select the plate check, prerun, and run modules:

Step	Action
1	Open the Plate Check Module pop-up menu, and select a plate check module.  **IMPORTANT** If the pop-up menus list <none> only, software cannot find the folder that contains the modules. See “Setting Folder Location Preferences” on page 3-51.
2	Open the PreRun Module pop-up menu, and select a prerun module.
3	Open the Run Module pop-up menu, and select a run module.

Select the Number of Lanes

To select the number of lanes:

Step	Action
1	Open the Lanes pop-up menu and select the appropriate value.  For GeneScan applications: Full Scan–24, 34, 36 lanes XL Scan–48, 50, 64, 66 lanes 96 Lane Scan–96 lanes For Sequencing applications: Full Scan–24, 32, 36 lanes XL Scan–48, 64 lanes 96 Lane Scan–96 lanes **Note** If this is an XL or 96-lane upgrade instrument, the appropriate value for the Run Mode field is selected automatically when this parameter is set. The run sheet for the standard ABI PRISM 377 instrument does not have a Run Mode field.

Select the Sample Sheet

IMPORTANT Select the number of lanes before selecting the sample sheet. If the default number of lanes is less than the number of samples, the sample information on the run sheet will be truncated. For example, if you entered 36 samples on the sample sheet, but the default number of lanes on the run sheet is 24, only the information for the first 24 samples will be imported to the run sheet if the sample sheet is selected before the number of lanes is changed to 36.

To select a sample sheet:

Step	Action
1	Open the sample sheet pop-up menu, and select the sample sheet prepared for this run. Once selected, the information on the sample sheet is imported to the run sheet. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the sample sheets. To correct this, see “Setting Folder Location Preferences” on page 3-51.

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Step	Action
2	The information imported from the sample sheet cannot be changed on the run sheet. If changes are made to the sample sheet after it has been selected on the run sheet, you must reimport the sample sheet data. To make changes to the sample sheet after selecting it on the run sheet: a. Open the sample sheet by clicking the icon next to the sample sheet pop-up menu on the run sheet.  b. Make changes to the sample sheet. c. Close and save the sample sheet. d. Open the sample sheet pop-up menu on the run sheet, and select <none>. e. Open the sample sheet pop-up menu on the run sheet, and reselect the sample sheet. **Note** You cannot make changes to the sample sheet while a module is running.

Select the Instrument or Gel's Matrix File

For GeneScan runs in particular, matrices are dye set, instrument, and run condition dependent. As such, matrices must be remade when any of these conditions change. For more information, refer to Chapter 6, "Making Matrix Files for GeneScan," and the *ABI PRISM GeneScan® Reference Guide,* P/N 4303188.

To select an instrument or gel matrix file:

If this is a …	Then …
sequencing run	open the Instrument File pop-up menu, and select the appropriate file. If one does not yet exist, leave the field set to <none>. A file must be selected for automatic data analysis.
GeneScan run	open the Gel's Matrix File pop-up menu, and select the appropriate file. If one does not yet exist, leave the field set to <none>. A file must be selected for automatic data analysis.
IMPORTANT If the pop-up menu lists <none> only, software cannot find the folder that contains the instrument/matrix files. To correct this, you must set the Settings Folder Preference to the ABI Folder. See "Setting Folder Location Preferences" on page 3-51.	

Select the Run Mode, Well-to-Read Distance, and Operator

To select the run mode, well-to-read distance, and operator:

Step	Action	
1	**If this is …**	**Then …**
	a standard instrument	proceed to the next step.
	an XL or 96-lane upgrade instrument	verify that the correct mode is displayed in the Run Mode field. 
2	Open the Well-to-Read distance pop-up menu and select the appropriate value.	
3	Click in the Operator field and type your name.	

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Enter the Collection Time

To enter the collection time:

<table>
<tr><th>Step</th><th colspan="4">Action</th></tr>
<tr><td>1</td><td colspan="4">Enter the duration of the run in the Collect time field. Use the run time recommended in your protocol. The following are suggested run times only.</td></tr>
<tr><td></td><td rowspan="2">Well-to-Read Length in cm</td><td rowspan="2">Type of Run (Scans per Hour/Application)</td><td colspan="2">Suggested Collection Time in Hours</td></tr>
<tr><td></td><td>19:1 Acrylamide Gels</td><td>Long Ranger™ (LR), PAGE-PLUS (PP), 29:1 Acrylamide Gels</td></tr>
<tr><td></td><td>12</td><td>1200/GS</td><td>1.0</td><td>1.0</td></tr>
<tr><td></td><td>12</td><td>2400/GS</td><td>1.0</td><td>1.0</td></tr>
<tr><td></td><td>36</td><td>1200/GS & Seq</td><td>7.0</td><td>9.0</td></tr>
<tr><td></td><td>36</td><td>2400/GS
2400/Seq</td><td>2.0
3.5</td><td>4.0
4.0</td></tr>
<tr><td></td><td>48</td><td>1200/Seq</td><td>10.0</td><td>11.0 (29:1 & LR)
12.0 (PP)</td></tr>
<tr><td></td><td colspan="4">GS = GeneScan application; Seq = Sequencing Application

Note The number of scans that can be collected is limited by the software.</td></tr>
<tr><td>2</td><td colspan="2">If ...</td><td colspan="2">Then ...</td></tr>
<tr><td></td><td colspan="2">you do not want the data to be analyzed automatically</td><td colspan="2">a. deselect the boxes in the Auto Analyze column by clicking in each box to remove the X.
b. Proceed to “Starting the Run or Closing the Run Sheet” on page 3-34.</td></tr>
<tr><td></td><td colspan="2">this is a sequencing run and you want the data to be analyzed automatically</td><td colspan="2">proceed to “Auto Analysis for Sequencing Runs” on page 3-32.</td></tr>
<tr><td></td><td colspan="2">this is a GeneScan run and you want the data to be analyzed automatically</td><td colspan="2">proceed to “Auto Analysis for GeneScan Runs” on page 3-33.</td></tr>
</table>

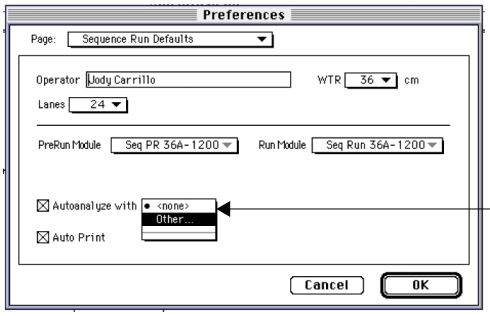
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Auto Analysis for Sequencing Runs

See “About Automatic Data Analysis” in Chapter 9 for more information.

IMPORTANT Do not automatically analyze matrix standard samples.

To set up the run sheet for automatic data analysis:

Step	Action
1	Verify that the data analysis software is selected in Preferences. To do this: a. Open the Window menu, and select Preferences. b. Select Sequence Run Defaults … c. Look at the “Autoanalyze with …” field. 
2	**If the analysis software is …** / **Then …** selected: click OK to return to the run sheet. not selected: a. Open the “Autoanalyze with” pop-up menu, and select Other. b. In the dialog box, locate the ABI PRISM® DNA Sequencing Analysis Software and click Open. c. Close the current run sheet, and create a new run sheet. **Note** Changing the preference has no affect on run sheets created prior to the change.
3	On the run sheet, verify that the Auto Analyze boxes are selected for each non-matrix standard sample. If the boxes are not selected: a. Open the sample sheet by clicking the icon next to the sample sheet pop-up menu on the run sheet.  b. Select a dye set/primer file for each sample you want analyzed automatically. c. Close and save the sample sheet. d. Open the sample sheet pop-up menu, and select <none>. e. Open the sample sheet pop-up menu, and reselect the sample sheet to update the information.
4	If matrix standard samples are being run, deselect Auto Analyze for all matrix standard samples.
5	Select Auto Print for analyzed data to be printed automatically.
6	Proceed to “Starting the Run or Closing the Run Sheet” on page 3-34.

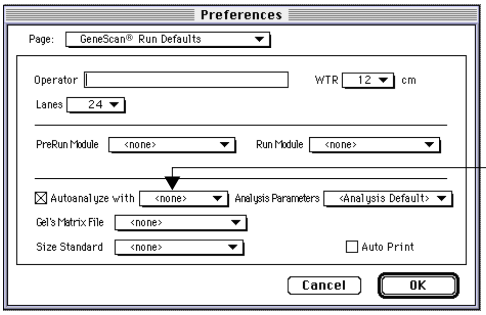
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Auto Analysis for GeneScan Runs

See “About Automatic Data Analysis” in Chapter 9 for more information.

IMPORTANT Do not analyze matrix standard samples.

To set up the run sheet for automatic data analysis:

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>1</td><td colspan="2">Verify that the data analysis software is selected in Preferences. To do this:
a. Open the Window menu, and select Preferences.
b. Select GeneScan Run Defaults …
c. Look at the “Autoanalyze with …” field.

</td></tr>
<tr><td rowspan="3">2</td><td>If the analysis software is …</td><td>Then …</td></tr>
<tr><td>selected</td><td>click OK to return to the run sheet.</td></tr>
<tr><td>not selected</td><td>a. Open the “Autoanalyze with” pop-up menu, and select Other.
b. In the dialog box, locate the ABI PRISM GeneScan® Analysis Software and click Open.
c. Close the current run sheet, and create a new run sheet.

Note Changing the preference has no affect on run sheets created prior to the change.</td></tr>
<tr><td>3</td><td colspan="2">Verify that the Auto Analyze boxes are selected for each non-matrix standard sample (Figure 3-5 on page 3-27). Selecting Auto Analyze allows you to select analysis parameters, a size standard, and the auto print option.</td></tr>
<tr><td>4</td><td colspan="2">If matrix standard samples are being, run, deselect Auto Analyze for all matrix standard samples.</td></tr>
<tr><td>5</td><td colspan="2">If not already selected, or if you wish to change the preference settings, set the remaining fields as follows:
a. Open the Analysis Parameters pop-up menu, and select an analysis parameters file. Analysis parameters must be set up in the analysis software program prior to completing the run sheet. Refer to the ABI PRISM GeneScan Analysis Software User’s Manual for instructions.
b. Open the Size Standard pop-up menu, and select the appropriate size standard file.
c. Select the Auto Print boxes for analyzed data to be printed automatically.

Note If the pop-up menus list <none> only, software cannot find the folders that contain the analysis parameters and size standard files. To correct this, see “Setting Folder Location Preferences” on page 3-51.</td></tr>
<tr><td>6</td><td colspan="2">Proceed to “Starting the Run or Closing the Run Sheet” on page 3-34.</td></tr>
</table>

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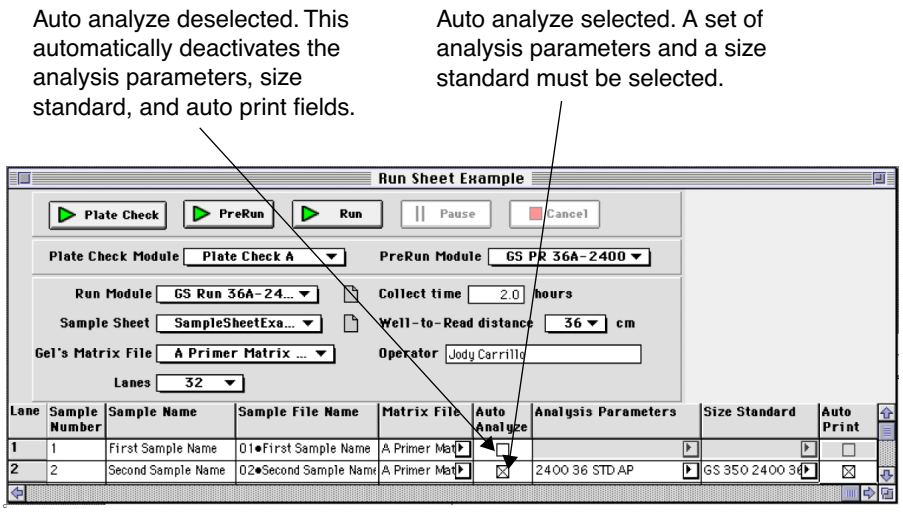
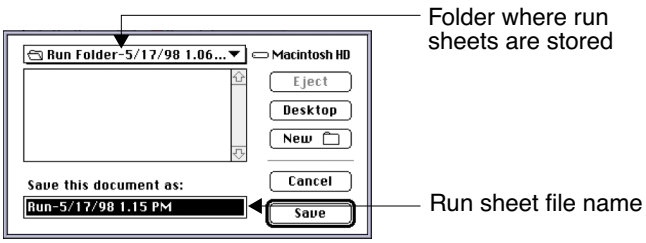


Figure 3-6 Auto analyze selected and deselected on a GeneScan run sheet

Starting the Run or Closing the Run Sheet

To start the run or close the run sheet:

If …	Then …
you are ready to proceed with instrument operation	a. open the File menu and choose Save. A dialog box showing the default run sheet name, and the location where the file will be saved is displayed. Change the file name and storage location now if desired.  b. Click Save. c. Proceed to “Performing a PreRun and Loading the Samples” on page 3-35.
you wish to close the run sheet	a. open the File menu and choose Close. b. Click Save. A dialog box showing the default run sheet name, and the location where the file will be saved is displayed (shown in previous step). Change the file name and storage location now if desired. c. Click Save.

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Performing a PreRun and Loading the Samples

Prepare the Loading Solution

If not already prepared, prepare the loading solution now. Instructions are listed under “Preparing the Formamide/Blue Dextran Loading Solution” on page 3-3.

Flush the Wells

! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

Before starting the prerun, it is important to remove all the bubbles from the wells in the gel by flushing the wells with buffer.

To flush the wells:

Step	Action
1	Fill the syringe (needle attached) with 1X TBE buffer from the upper buffer chamber.
2	For shark’s-tooth combs only: Starting at one end of the comb, slowly inject the buffer across the well area to force out the air bubbles.
3	Repeat this procedure until all air bubbles are gone. **Note** Bubbles present during electrophoresis result in an uneven electrical field and poor electrical contact. Bubbles will also electrophorese into the gel and alter the loading surface.
4	If desired, install the lid onto the upper chamber now. However, sample loading may be easier with the lid off.

Start the PreRun

See “Using the Instrument” in Chapter 1 for a description of the prerun and its purpose.

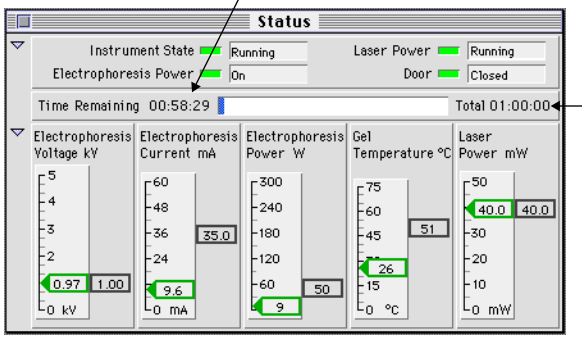
IMPORTANT To avoid gel extrusion, the electrophoresis voltage during a prerun should never exceed 1 kV. Prerunning the gel is particularly important when performing a high speed (2400 scans/hour) run. The gel must be at run temperature when the samples are loaded to ensure appropriate denaturation conditions.

To prerun the gel:

Step	Action
1	If not already specified in the Run window, open the PreRun pop-up menu and select the appropriate PreRun module.
2	Optional–recommended when using a square-tooth comb and if you are a new user. Follow these steps to stain the surface of the gel. Staining will help you visualize the wells and make sample loading easier. a. Load approximately 25–50 µL of formamide/blue dextran loading solution into a pipet. b. Starting at one end of the wells, slowly drag and release the formamide/blue dextran loading solution across the tops of the wells. **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.**
3	Close the front panel of the instrument.

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To prerun the gel: *(continued)*

Step	Action
4	Click PreRun. The Scan window is displayed.
5	Open the Window menu and select Status to display the Status window. Time remaining for the module being executed  Total amount of time the module will run ♦ Green arrow indicates actual reading from instrument. ♦ Gray box indicates value set by the module. ♦ Electrophoresis voltage, gel temperature, and laser power are setpoints. ♦ Electrophoresis current and power are limits.
6	Prerun the gel only until run temperature (gel temperature in the Status window) is reached (approximately 15–25 minutes). The default duration of the prerun module may be longer, so watch the “Time Remaining” in the Status window (shown above). **Note** Prerunning the gel longer than it takes to reach run temperature can cause it to swell up around the comb. If this occurs, the samples may not run straight in the gel, and resolution will be adversely affected. The surface of the gel where the samples are loaded should be as level as possible.
7	If contaminants are present in the gel, and you have elected to skip contaminated lanes when loading samples: a. Click on the Scan window to activate it. b. Watch the scan lines during the PreRun. Sometimes contaminants migrate out of the gel during the PreRun, and all the lanes can then be used.
8	Once the gel has reached run temperature, click Pause to temporarily halt the prerun. **IMPORTANT** Pause the prerun—do not cancel it. When paused, the pump and heater remain on, thereby maintaining the gel temperature.

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Denature the Samples

To denature the samples:

Step	Action
1	Finish preparing the samples and bring them up to the final volume as directed in the protocol you are using.
2	Load the samples and any other reagents as directed by the protocol into reaction tubes.
3	Cap each tube and vortex for 3–5 seconds.
4	Spin down the contents of the tubes, and load them onto a thermal cycler or heat block.
5	Denature the samples as directed by the protocol.
6	Remove the samples from the thermal cycler or heat block, and immediately place them on ice until ready to load.

Load the Samples

! WARNING ! Ergonomic Hazard. Performing loading activities may increase risk of developing the following cumulative trauma disorders (repetitive motion or repetitive strain injuries) which include but are not limited to: tendinitis, tenosynovitis, epicondylitis, strains, and/or sprains. To reduce the risk of experiencing these types of disorders, the following recommendations have been developed to decrease awkward posture; repetitive motion; excessive force; static muscle loading; and soft tissue contact.

- **Use an automated multi-channel pipette loader**
- **Locate the instrument on a variable or predetermined-height worktable or lab bench**
- **Use a stable stool or stepladder**
- **Install adequate artificial lighting in the appropriate area to facilitate loading**
- **Ensure adequate front access to instrument while performing loading activities**

Remember to load the samples in the same lanes as specified on the run sheet. The information on the run sheet at the start of the run is the information used to identify each sample in the Gel file. Changes made to sample and run sheets after starting the run are not implemented.

To load the samples:

Step	Action	
1	**If this is …**	**Then …**
	a 96-lane instrument	proceed to “Starting and Monitoring the Run” on page 3-40. Sample loading instructions are part of this procedure.
	not a 96-lane instrument	If not already paused, click Pause in the Run window to pause the PreRun. Pausing stops electrophoresis, but maintains the temperature of the gel while loading samples.

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To load the samples: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>2</td><td colspan="2">Carefully flush all of the wells with 1X TBE buffer to remove urea.

! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.</td></tr>
<tr><td>3</td><td colspan="2">Optional: Load formamide/blue dextran loading solution in the well to the left of the first sample lane, and in the well to the right of the last sample lane (the comb creates additional wells on either side of the numbered wells). This helps focus the bands in the first and last lanes.

! 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.</td></tr>
<tr><td rowspan="4">4</td><td>If using a …</td><td>Then …</td></tr>
<tr><td>square-tooth comb</td><td>♦ load one sample per lane into each consecutive well.
♦ Refer to your protocol for the load volume, or to “Suggested Load Volumes” on page 3-39.
♦ Change the pipet tip for each sample.</td></tr>
<tr><td>shark’s-tooth comb</td><td>select Load Method 1 or 2 below.</td></tr>
<tr><td>Load Method 1—
≤ 50% of lanes used
♦ Load one sample into every other well.
♦ Refer to your protocol for the load volume or to “Suggested Load Volumes” on page 3-39.
♦ Change the pipet tip for each sample.</td><td>Load Method 2—≥ 50% of lanes used
Refer to your protocol for the load volume, or to “Suggested Load Volumes” on page 3-39. Change the pipet tip for each sample.
a. Load one sample into each odd-numbered well.
b. Close the front panel of the instrument.
c. Click Resume and allow the samples to electrophoresis into the gel for two minutes.
d. Click Pause.
e. Open the front panel of the instrument.
f. Carefully flush all of the wells with 1X TBE buffer to remove any residual formamide from the previously loaded wells.
g. Load one sample into each even-numbered well.</td></tr>
<tr><td colspan="2">IMPORTANT Whenever possible, load a sample in lane one (unless contaminants are present and you are skipping that lane). It is better to leave empty lanes at the right end of the gel. Also, avoid skipping more than one lane between samples. The tracker may not work properly if more than one lane is left empty between samples.

IMPORTANT For shark’s-tooth comb users—loading samples in every other lane is necessary for good tracking. Although the formamide in the loading solution helps narrow the lane width, loading samples in adjacent lanes can blur the definition between lanes. Loading samples in every other lane creates discrete spaces between samples to properly identify the lanes.</td></tr>
<tr><td>5</td><td colspan="2">Close the front panel of the instrument, and proceed to “Starting and Monitoring the Run” on page 3-40.</td></tr>
</table>

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Suggested Load Volumes

Total resuspension volume depends on the application and chemistry. Refer to your protocol or the following guides as appropriate:

- *ABI PRISM GeneScan® Reference Guide,* P/N 4303188
- *ABI PRISM DNA Sequencing Chemistry Guide,* P/N 4305080

For GeneScan Applications

Number of Wells	Load Volume (µL)
24 and 36	1.5
50	1.0–1.5
66	0.5–1.0
96	0.5—1.0

For Sequencing Applications

Number of Wells	Load Volume (µL)
18	0.75—2.0
36	0.75—2.0
48	0.5—2.0
64	0.5—1.5
96	0.5—1.0

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Starting and Monitoring the Run

See Chapter 3, “Instrument Operation,” for a description of the run and its purpose.

To Start the Run

To start the run:

Step	Action
1	Click Cancel on the Run sheet, and then click Terminate to cancel the PreRun.
2	Wait five seconds, and then click Run. The data collected throughout the run is stored in a Gel file. A dialog box displaying the default gel file name and storage location appears. You can change the name and storage location of the gel file now if desired. Do not use colons, slashes, or symbols in gel file names.
3	Click Save. The Scan window is displayed. **Note** If a dialog box appears indicating there is not enough room on the hard disk to save the files created by a run, delete files (particularly Gel files) to create enough space. First, back up important files onto floppy disks or another storage device, then delete files off the hard disk and click Run again.
4	Verify electrophoresis has resumed by viewing the Status window.
5	Verify scanning has resumed by checking the following in the Scan window: ♦ All four scan lines should be above zero. ♦ Current scan number should increment. If either electrophoresis or scanning does not resume, cancel the run and restart it.
6	If this is a 96-lane instrument, load your samples now as follows: a. Click Pause to pause the run. b. Open the front panel of the instrument. c. Carefully flush all of the wells with 1X TBE buffer to remove urea. **! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.** d. Load samples in the odd numbered lanes. e. Click Resume and allow the samples to electrophorese for one minute. f. Click Pause. g. Carefully flush all of the wells with 1X TBE buffer to remove any residual formamide from the previously loaded wells. h. Load the remaining samples. i. Close the front panel of the instrument. j. Click Resume to continue the run.
7	To cancel a run in progress and analyze the data collected up to that point, click Cancel, and then click Stop & Analyze. The gel file is closed, sent to the analysis software, and then analyzed. Data collection software automatically quits, and the run is terminated. **Note** To use the Stop & Analyze feature, the run sheet must be configured for automatic data analysis before the run is started. The run cannot be resumed after selecting Stop & Analyze.

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The amount of time it takes for peaks to appear varies, and is dependent upon the well-to-read distance, type of gel, and run speed. General guidelines are listed below.

Well-to-Read Distance (cm)	Run Speed (scans/hour)	Approximate Amount of Time Before Peaks are Detected	
		Sequencing	GeneScan
12	1200/2400	N/A	10–20 minutes
36	1200	40 minutes	20–25 minutes
36	2400	20 minutes	20–25 minutes
48	1200	1 hour	N/A

Monitoring the Run

Four types of windows are available for viewing data and instrument status.

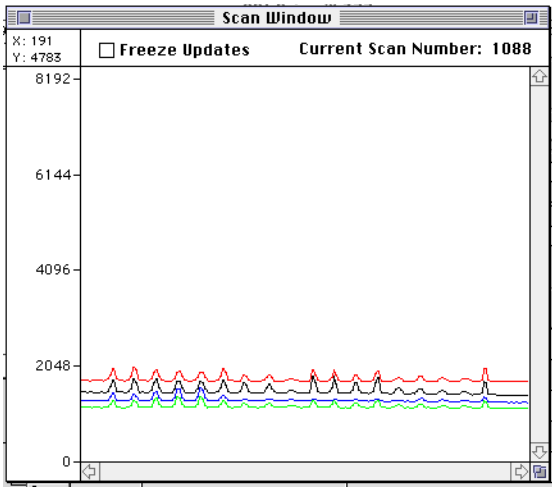
- Data windows are:
  - Scan
  - Gel
- Instrument windows are:
  - Status
  - Electrophoresis History

Refer to Chapter 9, “Data Collection Software,” for more information on these windows.

Note To use as little RAM as possible, we recommend leaving a minimum number of windows open (for example, only the Status and Scan windows). The Gel window requires the largest amount of RAM to display.

Scan Window

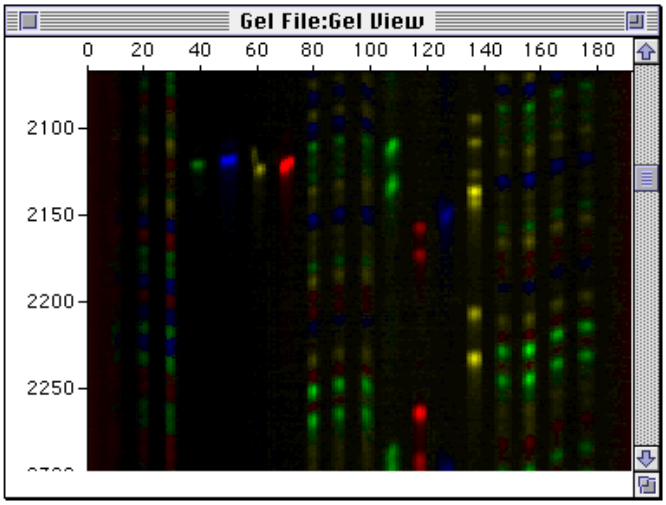
The Scan window displays the intensity of the fluorescent emissions being collected as samples pass through the read region of the gel and the dyes are excited by the laser. The emissions are displayed as peaks. Each dye/virtual filter is represented by a different colored line. The display is continuously updated during instrument operation. The current scan number is displayed in the upper right hand corner of the window.



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Gel Window

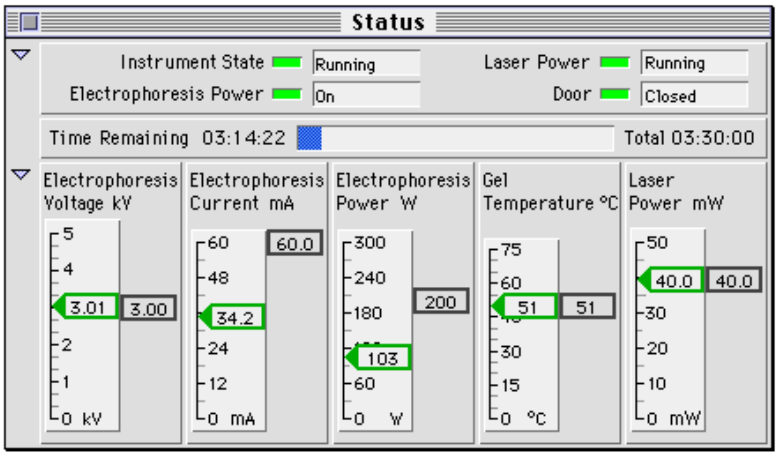
Like the Scan window, data in the Gel window also represents the fluorescent emissions collected from the samples as they pass through the read region of the gel. The first fragments scanned by the laser appear at the top of the window, and move down as new data is collected.



Status Window

The Status window displays the current status of the instrument (green boxes). This information is updated approximately every three seconds. Open the Status window by choosing Status from the Window menu.

The electrophoresis voltage, gel temperature, and laser power are setpoints specified by the module currently being executed. Electrophoresis current and electrophoresis power are limits. The instrument shuts down automatically if limits are exceeded. Setpoints and limits are shown in the grey boxes.

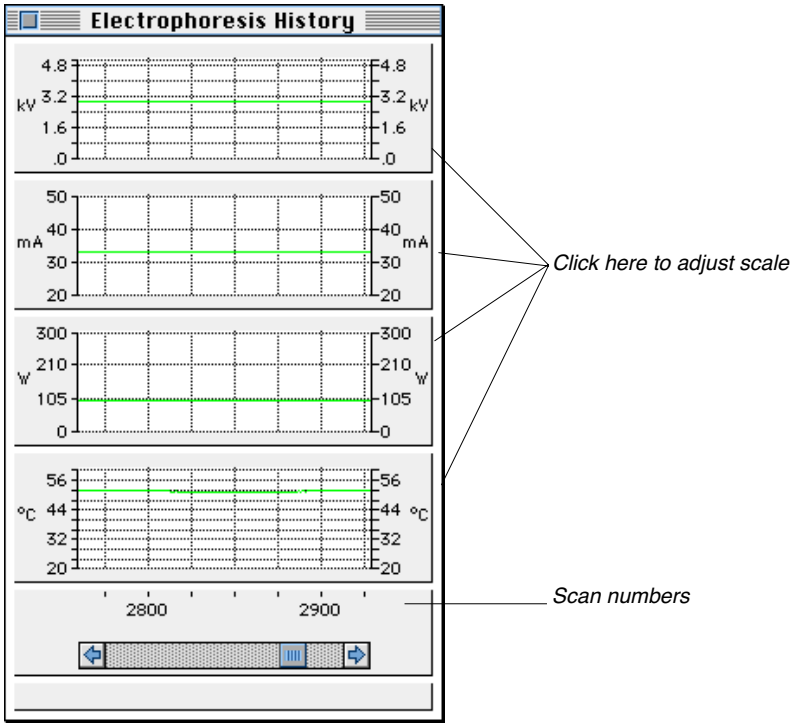


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Electrophoresis History Window

The Electrophoresis History window displays the set and actual values for the electrophoresis power supply and gel temperature throughout the course of a run. The scale for each panel is adjustable.

The information in the Electrophoresis History window is also stored in the Gel file. To display the electrophoresis history window, open the Window menu and choose Electrophoresis History.



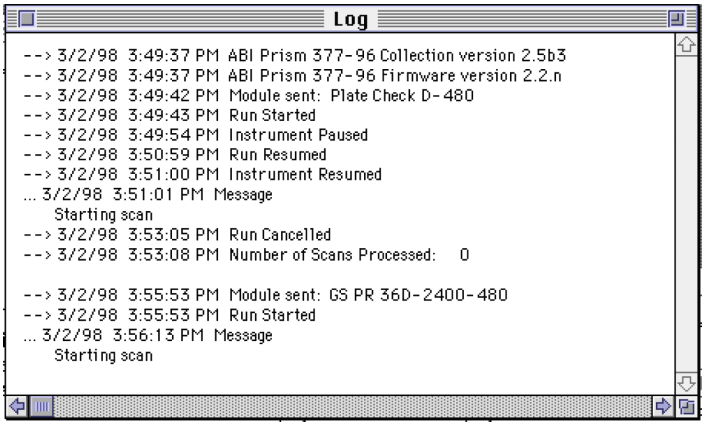
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Viewing the Log File

A Log file is created for each run when the run is started. Log files contain a comprehensive record of all error and status messages generated by data collection software during a run including:

- Start and stop times of the run
- Instrument and Macintosh® errors

Log files are stored in the Run folder. To view the Log file, open the Windows menu and select Log. Refer to Chapter 9, “Data Collection Software,” for more information on this window.



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Cleaning Up After the Run

Cleaning Procedure

To clean up after a run:

Step	Action
1	Optional. Turn the instrument off.
2	Open the front panel of the instrument.
3	Disconnect the upper and lower buffer chamber electrode cables.
4	If the front heat-transfer plate was used, disconnect the grounding strap and water supply tubes.
5	Remove the front heat-transfer plate by releasing the six cassette clamps that hold the plate in place and lifting it away from the instrument. **IMPORTANT** Always remove the front heat-transfer plate from the gel cassette before removing the cassette from the instrument. The front heat-transfer plate is heavy, and removing them together can damage the cassette.
6	Siphon the buffer from the upper and lower buffer chambers into an appropriate waste container. **! WARNING ! Tris-borate-EDTA (TBE) buffer can be harmful if inhaled, ingested, or absorbed through the skin. It is irritating to the eyes, skin, and mucous membranes. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
7	Open the four clamps that hold the cassette in place, and remove the cassette from the instrument.
8	Holding the cassette over a sink, carefully open the cassette clamps holding the upper buffer chamber to the cassette, and remove the buffer chamber.
9	Remove the gel plates from the cassette.
10	Clean the cassette with damp Kimwipes and allow it to air dry it.
11	Remove the lower buffer chamber and discard any remaining buffer.
12	Clean up any liquid left in the electrophoresis chamber.
13	Clean the front and rear heat-transfer plates and the positioning pins with damp Kimwipes. Place the front heat-transfer plate on a non-scratch surface. Allow all the parts to air dry.
14	Rinse the buffer chambers with deionized water, and allow them to air dry. **IMPORTANT** To avoid damaging the electrode inside the buffer chambers, rinse the buffer chambers only. Do not scrub or clean them with a sponge.
15	Follow the procedures listed in Chapter 2, Gel Preparation, to: ♦ Remove the gel from between the glass plates. ♦ Clean the glass plates, spacers, and comb.

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Analyzing the Data

For Sequencing Runs

Refer to the *ABI PRISM® DNA Sequencing Analysis Software User's Manual* for instructions on how to analyze data from a sequencing run.

For GeneScan Runs

Refer to the *ABI PRISM GeneScan® Analysis Software User's Manual* for instructions on how to analyze data from a GeneScan run.

Archiving and Printing Data from Runs

Refer to Chapter 9, "Data Collection Software," for information on archiving and printing data.

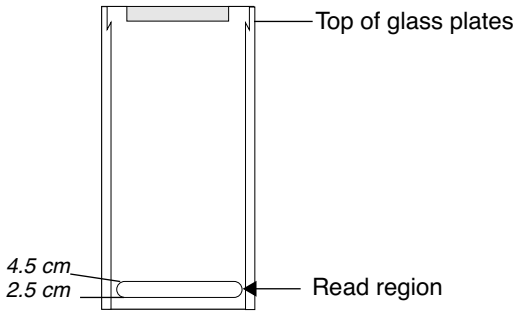
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The Read Region

What is the Read Region

The read region is the area of glass scanned by the laser. It is approximately 2.5 to 4.5 cm from the bottom of the glass, and is:

- 6 inches wide for standard and XL instruments
- 7.25 inches wide for 96-lane instruments
- 3 inches wide for ABI PRISM 377-18 instruments



The number of channels in the read region are:

- 194 for the standard ABI PRISM 377 and 377-18 instruments (numbered 0 to 193)
- 388 for ABI PRISM 377 XL instruments (numbered 0 to 387)
- 480 for ABI PRISM 377 96-lane instrument (numbered 0 to 479)

The type of comb used determines the number of channels per lane. For example, one lane of a 36-well comb is equivalent to approximately 5 channels.

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Manually Controlling the ABI PRISM 377 Instrument

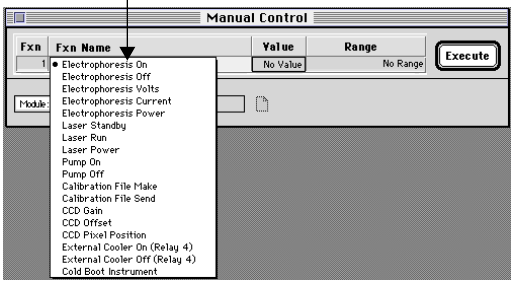
Procedure

In manual control mode, the instrument settings can be changed in real time. The instrument must be idle before you can control it manually, because commands sent via standard operation mode (from a run sheet) override commands entered manually.

Functions specified from the Manual Control function pop-up menu are executed when the Execute button is clicked. Functions continue running until:

- The function is cancelled
- A new function is selected

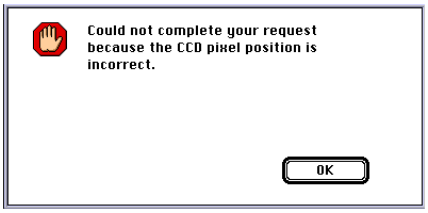
To manually operate the instrument:

Step	Action
1	Open the Window menu and choose Manual Control.
2	Open the Function pop-up menu (Fxn Name), and select a function. Function pop-up menu 
3	If appropriate, enter a number in the Value field. Guidelines are shown in the Range box, and in the following table. Per the guidelines shown in the Range field, enter a value between 0 and 60. 
4	Click Execute. If necessary, click Cancel to terminate a function.

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Manual Control Functions

The following tables describe the functions that can be performed from the Manual Control window.

Function Name	Description
Electrophoresis On	Starts electrophoresis.
Electrophoresis Off	Stops electrophoresis.
Electrophoresis Volts	Sets electrophoresis volts to a value between 0.00 and 5.00 kV.
Electrophoresis Current	Sets electrophoresis current to a value between 0.00 and 60.0 mA.
Electrophoresis Power	Sets electrophoresis power to a value between 0 and 300 W.
Laser Standby	Sets the laser to minimum power.
Laser Run	Turns the laser on.
Laser Power	Sets laser power to a value between 0.00 and 40.0 mW (in 10ths of mW).
Pump On	Turns the temperature control system on, and sets the temperature to a value between 20 and 60°C.
Pump Off	Turns the temperature control system off.
CCD Pixel Position	The charge coupled device (CCD) pixel position value is a reference point for alignment of the CCD camera with the laser beam. The instrument is shipped with the correct CCD pixel position value in memory. When a run is started, data collection software checks for a value greater than zero. If not found, the error message shown below is displayed.  If this occurs, the value must be reentered. See “CCD Pixel Position Value” in Chapter 4, “Troubleshooting.” For convenience, the value can be stored in a calibration file. The value can be sent to the instrument using the Calibration File Send command. See “Using Calibration File Make and Send” in Chapter 4 for more information.
Calibration File Make	Used to create a file called ABI 377 Calibration that contains the CCD pixel position value and instrument serial number. The file is stored in the Preferences folder inside the System folder. See “Using Calibration File Make and Send” in Chapter 4 for more information.
Calibration File Send	Used to send the CCD pixel position value and instrument serial number to the instrument. The serial number is transferred into all Sample files created by the data analysis software, and can be viewed in the annotative view of the sample file. This is useful for instrument identification, particularly if more than one instrument is being operated. See “Using Calibration File Make and Send” in Chapter 4 for more information.
CCD Gain	Typically does not require adjustment. When running dRhodamine terminators on an ABI PRISM 377 with XL Upgrade, we recommend increasing the gain to 4. The default setting is 2.

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Function Name	Description
CCD Offset	Used to move baselines and signals up and down the scale in the Scan window and in the recorded data. CCD Offset can be useful if baselines look unusually high or low. Be aware that baselines normally drop over the course of a run. Therefore, the lowest baseline should be set high enough so that it does not drop below zero by the end of the run. We recommend setting the lowest baseline at the beginning of the run between 300 and 500 points.
External Cooler On (Relay 4)	Used to manually control power to an external water bath. This function gives you control of the internal system pump from the rear of the instrument at relay 4. Manual control is not required if the recommended external water bath and chiller modules are used. Refer to Appendix B, “Subambient Temperature Operation,” for further information regarding external cold water baths. Refer to “Modules” on page 9-42 to see a list of all the modules available including chiller modules.
External Cooler Off (Relay 4)	External Cooler On and Off can be used to manually control power to an external water bath.
Cold Boot Instrument	Downloads the firmware image to the instrument. Use this command if the computer and instrument are communicating, but the instrument is not responding appropriately. This symptom indicates the firmware image on the instrument is corrupt. See “Performing a Cold Boot” in Chapter 4 for more information.

Function range of values:

Function	Range of Values
Electrophoresis Volts*	0.00 to 5.00 kV
Electrophoresis Current*	0.00 to 60.0 mA
Electrophoresis Power*	0 to 300 W
Laser Power	0.00 to 40.0 mW (in 10ths of mW)
Pump On	20 to 60°C
CCD Gain†	1, 2, 4, or 8 CCD Output
CCD Offset	0 to 4096 CCD Output
CCD Pixel Position‡	1 to 512 pixels
* These parameters can be controlled manually to make them limiting. However, you should be thoroughly familiar with the principles of electrophoresis before doing so.	
† This parameter amplifies the signal, and should not require adjustment.	
‡ Use this only if instrument memory has been reset, and the value has been lost.	

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Setting Folder Location Preferences

Overview

The folder locations for the following types of files and folders must be designated to perform a run, and to handle the data generated during a run:

- Sample sheets
- Module files
- Run folders
- Firmware image file
- Settings
- GeneScan analysis parameters (for GeneScan users only)
- GeneScan size standards (for GeneScan users only)

Software must know where to put and locate these files to operate the instrument. These folders are typically located on the hard disk of the Macintosh attached to the instrument. The folder names and locations listed below are the ones specified during system installation. The settings can be changed at any time. However, if changes are made, the new folder location and/or name must be specified in the Preferences Folder Locations dialog box (page 3-52). Refer to Chapter 5, “Setting Preferences,” and Chapter 9, “Software,” for more information.

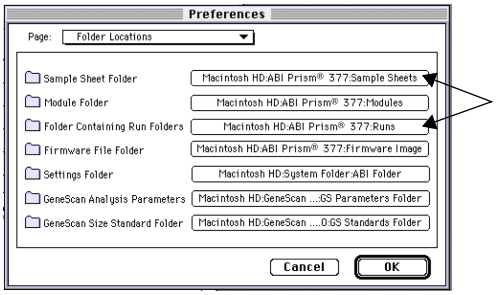
Folder	Location and Folder Name*	What the Folder Contains
Sample Sheet Folder	Macintosh HD: ABI Prism 377: Sample Sheets	Contains all the sample sheets. When setting up a run sheet, the selections displayed in the pop-up menu for sample sheets are the files stored in this folder.
Module Folder	Macintosh HD: ABI Prism 377: Modules	Contains the Plate Check, PreRun, and Run modules. When setting up a run sheet, the selections displayed in the Plate Check, PreRun, and Run Module pop-up menus are the files stored in this folder.
Chiller Modules Folder	Macintosh HD: ABI Prism 377: Chiller Modules	Contains the Plate Check, PreRun, and Run modules. When setting up a run sheet, the selections displayed in the Plate Check, PreRun, and Run Module pop-up menus are the files stored in this folder.
Folder Containing Run Folders	Macintosh HD: ABI Prism 377: Runs	Contains the Run folders. A new Run folder is created for each run. At the end of analysis, each new Run folder will contain a gel file, log file, sample files, and a run file.
Firmware File Folder	Macintosh HD: ABI Prism 377: Firmware Image	Contains the firmware image file.
* **IMPORTANT** Locations and folder names listed are those at the time of system installation. We strongly recommend not changing folder names and locations. ‡ = Displayed in Folder Locations dialog box for GeneScan users only.		

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Folder	Location and Folder Name*	What the Folder Contains
Settings Folder	Macintosh HD: System folder: ABI Folder	Typically set as the ABI Folder. The choices in other Preferences dialog boxes and in Run windows use the information contained in this folder for their pop-up menus. **IMPORTANT** To automatically analyze data after a run using the sequencing analysis software, the Settings folder must be designated as the ABI folder.
GeneScan Analysis Parameters‡	Macintosh HD: GeneScan Analysis x.x: GS Parameters Folder	Contains the parameters used to analyze GeneScan run data, and is located in the GeneScan Analysis software folder.
GeneScan Size Standard Folder‡	Macintosh HD: GeneScan Analysis x.x: GS Standards Folder	Contains the size standard definitions used to analyze GeneScan run data, and is located in the GeneScan Analysis software folder.
*** IMPORTANT** Locations and folder names listed are those at the time of system installation. We strongly recommend not changing folder names and locations. ‡ = Displayed in Folder Locations dialog box for GeneScan users only.		

Setting Folder Location Preferences

To set the Folder Location preferences:

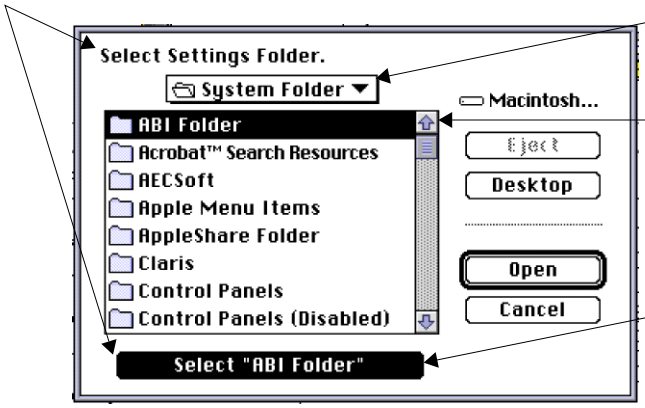
Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Window menu, select Preferences, and then select Folder Locations.
	is open	open the Page pop-up menu and select Folder Locations.
2	Click the box that corresponds to the folder location you wish to set.  	

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<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>3</td><td colspan="2">Using the pop-up menu in the dialog box that is displayed, locate and select the folder the appropriate folder.

IMPORTANT To automatically analyze data with ABI PRISM DNA Sequencing Analysis Software on the same computer used for data collection, the Settings Folder must be designated as the ABI Folder (shown below).

The text above the menu indicates the folder location preference currently being set, and corresponds to the “select” button at the bottom of the box.


</td></tr>
<tr><td>4</td><td colspan="2">Click the Select box underneath the list of folders. Do not click Open.</td></tr>
<tr><td rowspan="3">5</td><td>If …</td><td>Then …</td></tr>
<tr><td>you are finished</td><td>click OK to save your changes, or Cancel to quit without making any changes.</td></tr>
<tr><td>you wish to change more preferences</td><td>open the Page pop-up menu, and select another Preference.</td></tr>
</table>

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4 Troubleshooting

Chapter Contents

In this Chapter

This chapter contains information you can use to troubleshoot and solve many of the problems that can occur when using this system. Quick reference tables are listed on pages 4-3 through 4-19. The remaining portion of the chapter contains procedures referenced in the tables, and provides more information on the data collection software and firmware.

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For More Troubleshooting Information

References For more troubleshooting information, refer to these manuals as appropriate for your application:

- *GeneScan® Reference Guide : ABI™ 373 and ABI PRISM® 377 DNA Sequencers* (P/N 4303188)
- *Automated DNA Sequencing Chemistry Guide: ABI™ 373 and ABI PRISM® 377 DNA Sequencers,* P/N 4305080

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Gel and Gel Image Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
Misshapen wells **Note** If only a few wells are misshapen, you can still load the gel using only those lanes with flat, well-formed wells	Suction when comb removed	When removing the comb: 1. Lay gel flat. 2. Pour 1X TBE over comb. 3. Remove comb slowly.
	Wet comb or well-former	Ensure that comb is clean and dry before inserting into gel. **Note** To ease insertion of comb, you can "wet" the comb with acrylamide (or the appropriate gel polymer).
	Air bubbles trapped when comb inserted	Ensure that no air is trapped by comb.
Swirls in gel (Schlieren pattern)	Excessive TEMED or APS	Prepare new solutions following protocol
	Temperature too high	Polymerize at 20–23°C
	Gel polymerized too quickly	Do not heat gel solution to dissolve urea
	Insufficient reagent mixing	Mix reagents gently but thoroughly
Polymerization too slow (gels should polymerize within 15–20 minutes)	Excessive dissolved oxygen	♦ Keep vacuum filter strength and time constant ♦ Stir and pour solutions gently ♦ Filter/pour gels at 20–23°C
	♦ Not enough TEMED or APS ♦ Degraded TEMED or APS	♦ Prepare new solutions following the protocol using fresh high-quality reagents ♦ Store APS at room temperature in a desiccator ♦ Store TEMED in tightly sealed container at room temperature
	Temperature too low during casting	Polymerize at 20–23°C
	Did not use deionized water	Use only distilled or deionized water for making all solutions
Gel solution leaks out when injecting gel	♦ Bottom of plates not flush ♦ Gel pouring fixtures not mounted correctly ♦ Plates not positioned properly in the cassette	Review the gel pouring procedures in Chapter 2, "Pouring Gels."
Gel extrudes from between plates into upper buffer chamber during electrophoresis	Buildup of charge on the surface of the glass plate. See "Gel Extrusion" on page 4-29 for more information.	Clean the plates with an alcoholic KOH wash (page 4-30) or a 3 M HCl wash (page 4-31).
Large migrating region showing no fluorescence	Plates not rinsed free of Alconox	Rinse plates thoroughly with distilled, deionized water.
Lane-wide region(s) of fluorescence	Contaminated loading buffer or sample	Remake solutions co-loaded with sample.

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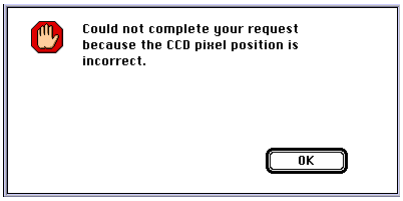
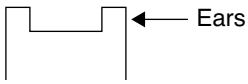
Observation	Possible Causes	Recommended Actions
Severely bowed gel image	Clamping bottom of gel plates	Do not clamp the bottom of the gel. **Note** Use clamps of equal tension along the side of the gel. The clamps must be placed over the spacers.
	Gel extruded between plates into upper buffer reservoir. See "Gel Extrusion" on page 4-29 for more information.	Clean the plates with an alcoholic KOH wash (page 4-30) or a 3 M HCl wash (page 4-31).
Red or green smearing on gel	Gel dried out before running	♦ Before storing a poured gel, wrap the gel ends with damp Kimwipes and plastic wrap. ♦ Use gel within 2–6 hours after pouring
Appearance of donut-shaped band (with black central hole)	Too much sample loaded	Load less sample or remake sample with a higher dilution.
Gel-wide region of migrating fluorescence (can be of any color)	Contamination of buffer with fluorescent species (*e.g.*, laboratory pen used to label lanes, gel plates, or comb)	Do not mark gel plates or comb.
	Lower buffer chamber not clean (*i.e.*, contaminated with fluorescent species)	Clean and replenish buffer in lower buffer chamber.
Tilted or deformed bands	Small bubble in lane	Cast gel as described in protocol. Make sure plates are lint-free before pouring gel.
	Gel not clamped properly	Use clamps of equal tension along the side of the gel. The clamps must be placed over the spacers only. **IMPORTANT** Do not clamp the bottom of the gel.
	Dirty plates	Clean plates using one of these methods: ♦ Soak the plates overnight in a 5% solution of Multiterge detergent (VWR Scientific, P/N 34171-010) ♦ Use a 3 M HCl wash (page 4-31) ♦ Use an alcoholic KOH wash (page 4-30)
Lanes appear as smears	Impure or degraded TEMED or APS	Use fresh reagents.
	Samples are overloaded	Follow loading procedure in your protocol or in Chapter 3.
	Electrophoresis failure due to buffer leak	Make sure that the plates are clamped correctly, and that the upper buffer chamber gasket makes a proper seal. Do not spill buffer behind the upper buffer chamber. Wicking can occur.
Gel image contains vertical red streaks ("red rain") near end of run (top of gel image)	Gel destruction in read region	♦ Wrap bottom of gel to prevent drying. ♦ Run at a lower temperature or voltage.

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Observation	Possible Causes	Recommended Actions
Gel image contains green/blue streaks throughout run	Fluorescent contaminant in gel	Vacuum filter gel solution. Cast gel in dust-free environment.
	Urea crystals present in gel	Use room temperature reagents. Pour at 20–23°C. **IMPORTANT** Do not refrigerate.
	Particles on outer surface of plates in read region	Wipe read region with damp, lint-free Kimwipe.
Blue or green streaks (“curtain”) at top of gel image	Buffer leak	Make sure that the plates are clamped correctly, and that the upper buffer chamber gasket makes a proper seal. Do not spill buffer behind the upper buffer chamber. Wicking can occur.
Blue or green curtain obscuring entire gel image	Warped gel plate	Use gel plates from Applied Biosystems.
Green streak through entire gel lane	Protein in template	Clean up the template before performing sequencing reactions.
Greenish-yellow haze	Poor gel plate alignment	Remove the gel plates and realign them correctly.
	Fluorescent contaminant in gel	Use fresh reagents.
		Do not write on gel plates with marking pens.
	Residual detergent on plates	Rinse plates thoroughly with hot deionized water.

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Instrument Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
A dialog box appears indicating there is not enough room on the hard disk to start a plate check, prerun, or run.	Hard disk is too full.	Delete files (particularly gel files) to create enough space. If the files are important, back them up first. Delete files by dragging them to the trash and emptying the trash. See Chapter 8 for more information.
The following message is displayed: 	CCD pixel position value erased from instrument memory.	Re-enter the CCD pixel position value. Follow the procedure “Location of the CCD Pixel Position Value” on page 4-26.
The buffer level in upper buffer chamber is going down, but no external leaks are visible.	Buffer is wicking over ears of the plates and leaking down spacers 	Follow this procedure: a. Siphon the buffer out of the upper buffer chamber. b. Dry the corners of the notched area at the top of the front plate. c. Apply a small amount of molten agarose to the corners of the notches. d. Refill the upper buffer chamber.
Buffer splashes and crystallizes around the read region.	Buffer poured into lower buffer chamber too quickly	Fill the chamber to the top edge of the overflow dam. Do not overfill.
No communication between instrument and computer.	♦ Instrument memory cleared due to a power outage. ♦ Corrupted firmware on instrument. ♦ Cable is loose.	Perform a single reset. See “Locating the Firmware Image File” on page 4-24. If this does not solve the problem, perform a total reset. See “Performing a Total Reset” on page 4-22.
Flat scan lines or no scan lines.	♦ Instrument memory cleared due to a power outage. ♦ Corrupted firmware on instrument.	Perform a single reset. See “Locating the Firmware Image File” on page 4-24.
Error message: EP Voltage Deviation Exceeds Tolerance	The EP voltage deviated outside the tolerance range. Instrument operation is paused.	Call service.
Error message: No EP Current Detected	Concentration of TBE buffer in buffer chambers differs from the concentration in the gel. All should be 1X TBE.	TBE buffer concentration must be the same in the gel and both buffer chambers. Remake the gel and buffer chamber solutions so the TBE buffer concentration is the same in each.

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Observation	Possible Causes	Recommended Actions
Error message: Warning: Possible Plate P43/J43 Thermistor Open/Short Circuit Warning: Plate Out. Thermistor P43/J43 Open/Short Circuit Warning: Possible Plate P44/J44 Thermistor Open/Short Circuit Warning: Plate In. Thermistor P44/J44 Open/Short Circuit Warning: Possible Heater Thermistor Open/Short Circuit	♦ Possible open or short circuit exists with the thermistor/cable connected to J43 or J44. ♦ Temperature of the plate in an instrument with 100k ohm thermistors is 21.9°C or less. ♦ One of two temperature sensors (thermistors) on the rear heat-transfer plate may be bad.	The instrument will function normally with one sensor. Schedule a service call to have the thermistors checked. Click OK, and continue operating the instrument. This message may appear when you launch data collection software and start a plate check, prerun, or run.
Error message: Flow Detected with Pump Off – External Cooling in Use!	♦ The wrong module is being used for a run where an external cooling device is attached. ♦ Internal coolant system valve is stuck in the on or open position.	If an external cooling device is in use, check the modules selected on the run sheet. Use Chiller modules. If no external cooling system is in use, try to start the run as follows, and place a service call. ♦ Click OK in the error message box and try to start the run. ♦ Open the Manual Control window and try to turn the pump on manually.
Error message: Err: Coolant Flow Failure!	Pump turned on and off three times to see if coolant flow was detected.	Open the Manual Control window and try to turn the pump on manually. If the problem persists, call service.
Error message: No Flow Detected! Attempted Pump Restart	Coolant pump was turned on, but no coolant flow was detected by the flow switch.	Check the water reservoir. Refill the bottle if necessary. Procedure listed in Chapter 8, “System Maintenance.”
Error message: Scanner Did Not Find Its Home Position	Scanner did not find home position prior to collecting data for a plate check, prerun, or run.	Press the Reset button on the back of the instrument once. Then click Resume in the run window.
Error message: A Valid XL Lane Firmware Image is Required!	Non-XL data collection software tried to establish communication with an ABI PRISM® 377 instrument with XL upgrade.	Install and use ABI PRISM® 377 XL Upgrade Data Collection Software.
Error message: A Valid 96 Lane Firmware Image is Required!	Non-96 data collection software tried to establish communication with an ABI PRISM® 377 instrument with the 96-lane upgrade.	Install and use ABI PRISM® 377 96-Lane Upgrade Data Collection Software.
Error message: Heat plate temperature exceeds 70°C even when status window indicates less than 70°C.	Clog in coolant system.	Flush the coolant system to remove the clog, and refill the water reservoir with deionized water and 5.0% antifreeze. See “Removing Coolant System Clogs” on page 4-32.

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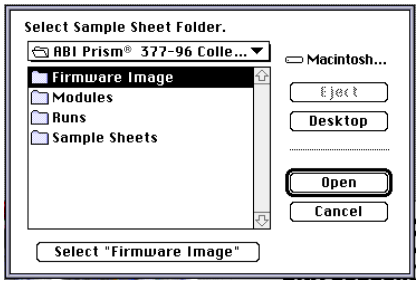
Observation	Possible Causes	Recommended Actions
Error message: The A2D Converter is Not Functioning	Converter not functioning. No data being transmitted from instrument to computer.	Turn the instrument power switch off and then on again. If the message appears again, perform a plate check to see if a signal is displayed. If no signal is displayed, call service. If a signal is displayed, and the reoccurrence of this error is not severe, you should be able to run samples and collect data. If the problems persists, call service.
Flow switch sticks and plugs up.	Clog in coolant system.	Flush the coolant system to remove the clog, and refill the water reservoir with deionized water and 5.0% antifreeze. See “Removing Coolant System Clogs” on page 4-32.
High resistance to flow through heat plates.	Clog in coolant system.	Flush the coolant system to remove the clog, and refill the water reservoir with deionized water and 5.0% antifreeze. See “Removing Coolant System Clogs” on page 4-32.
Quick-disconnect fittings leak.	Buildup behind the seals.	Flush the coolant system to remove the clog, and refill the water reservoir with deionized water and 5.0% antifreeze. See “Removing Coolant System Clogs” on page 4-32.
Pump shuts down during run (warning displayed), or Pump shuts down during run (warning displayed) and poor resolution due to gel running too cold.	Clog in coolant system.	Flush the coolant system to remove the clog, and refill the water reservoir with deionized water and 5.0% antifreeze. See “Removing Coolant System Clogs” on page 4-32.
Rear panel LEDs are stuck in one pattern, e.g. all on or all off.	♦ Instrument memory cleared due to a power outage. ♦ Corrupted firmware on instrument.	Perform a total reset or cold boot the instrument. See: ♦ “Performing a Total Reset” on page 4-22 ♦ “Performing a Cold Boot” on page 4-23.
Computer cannot load a new firmware image onto the instrument, or A new firmware image appears to have been downloaded, however, ♦ The instrument still does not operate ♦ The rear panel LEDs remain stuck in one pattern.	♦ Instrument memory cleared due to a power outage. ♦ Corrupted firmware on instrument.	Perform a total reset or cold boot the instrument. See: ♦ “Performing a Total Reset” on page 4-22 ♦ “Performing a Cold Boot” on page 4-23.

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Observation	Possible Causes	Recommended Actions
No current/electrophoresis	Incorrect TBE buffer formulation	Use correct buffer concentrations: ♦ 10X TBE in the gel (for a final concentration of 1X) ♦ 1X TBE running buffer
	Instrument set up incorrectly	Verify that: ♦ The electrode leads are secure and plugged in. ♦ The bottom and top of the gel are immersed in running buffer.
	Broken instrument parts	Inspect the electrophoresis cables, electrodes, and platinum wire in both buffer chambers. If anything is visibly broken, replace the electrophoresis cable and electrode assembly. Procedure in Chapter 8, “System Maintenance.”
	Corrupted firmware on instrument.	Resend firmware by performing a total reset. See “Performing a Total Reset” on page 4-22.
Front heat-transfer plate sticks to the gel plates.	Upper buffer chamber leak.	Follow this procedure: a. Empty and remove the upper buffer chamber. b. Open the plate clamps. c. Slide the front heat transfer plate up along the gel plate until it is released. d. Clean the front heat-transfer plate e. Check the upper buffer chamber for leaks and repair or replace as appropriate. **IMPORTANT** Always remove the front heat transfer plate from the gel cassette before removing the cassette from the instrument. The front heat transfer plate is heavy. Removing them together can damage the cassette.

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Data Collection Software Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
The following dialog box is displayed when data collection software is launched. 	♦ Folder names have been changed. ♦ Preferences file corrupt.	Reset the Folder Locations Preference. Instructions are listed in Chapter 9 on page 9-39, and in Chapter 5, “Setting Preferences.”
Message indicating there is not enough room to store data from run.	Not enough room on the hard disk.	Delete files (particularly gel files) from the hard disk. Gel files are large and are stored in the corresponding Run folder created for each run.
Data not analyzed automatically	Sample Sheet not completed or completed incorrectly	Complete Sample Sheet as described.
	GeneScan® Analysis Software Run Defaults set incorrectly in data collection program (in the Preferences under the Window menu)	Make sure that the Autoanalyze box is checked and that the GeneScan Run Default appears with the correct path name.
	Insufficient free RAM	Restart the computer before collecting data. **Note** You should always restart the computer before collecting data.
	Conflicting extensions	Choose Extensions Manager from the Control Panels. Turn off any extensions that were not part of the original installation and restart computer.
Error messages displayed during data collection.	See “About Troubleshooting Software” on page 4-20 for more information on possible causes.	Error messages displayed during data collection are also logged in the Log file. The Log lists both computer errors and instrument firmware errors.
Computer cannot load a new firmware image onto the instrument, or A new firmware image appears to have been downloaded, however, ♦ The instrument still does not operate ♦ The rear panel LEDs remain stuck in one pattern.	♦ Instrument memory cleared due to a power outage. ♦ Corrupted firmware on instrument.	Perform a total reset or cold boot the instrument. See: ♦ “Performing a Total Reset” on page 4-22 ♦ “Performing a Cold Boot” on page 4-23.

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Signal Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
Loss of signal (50–75%)	Plates and gel cassette not installed properly against positioning pins.	Repeat the run after reviewing the instructions in Chapter 2 for installing the plates and gel cassette.
Temporary loss of signal, typically between 140 to 200 bp lasting about 20 to 40 bp	Contaminants on glass plates See "Temporary Loss of Signal" on page 4-29 for more information.	Try the following: ♦ Wash glass plates in laboratory dishwasher with hot deionized water rinse cycle (195°F/90°C) ♦ Soak plates overnight in a 5.0% solution Multiterge See Chapter 2 for more information on cleaning glass plates.
Inconsistent signal from lane to lane	Some samples not thoroughly mixed with formamide/size standard mixture	Mix samples into formamide/size standard mixture by pipetting up and down several times.
Signal too low (peak heights lower than usual)	Insufficient sample added to some or all lanes	♦ Check your protocol. ♦ Examine the efficiency of the PCR. ♦ Check pipette calibration. Refer to the GeneScan or Sequencing guides listed on page 4-2 for more information.
	Cassette not flush with back heat transfer plate and alignment pins	Place cassette flush against back heat transfer plate. **IMPORTANT** The spacers must touch the alignment pins. You should be able to see the alignment pins pressing against the spacers.
	Difficulty in loading sample because wells not flushed	Flush the wells prior to loading the gel.
	Bad formamide	Use freshly deionized formamide. (Procedure in Appendix A.) **Note** Formamide pH should be between 7 and 9.
	Insufficient [F]dNTPs added to PCR reaction	Reamplify using more [F]dNTPs or examine the efficiency of the PCR.
	Optics/detector misaligned	Call service representative.

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Observation	Possible Causes	Recommended Actions
Signal too high (streaks appearing, or peaks heights greater than 4000 RFU)	Too much sample added to some or all lanes	Load less sample.
		Reamplify using less [F]dNTPs.
	Unincorporated [F]dNTPs	Purify the PCR product.
	Unincorporated dye terminators	Follow the protocols for excess dye terminator removal carefully. See the user bulletin: *Precipitation Methods to Remove Residual Dye Terminators from Sequencing Reactions* (P/N 4304655). Obtain from our website at: www.appliedbiosystems.com/techsupport
High/noisy baseline	Matrix made incorrectly resulting in too much correction (also indicated by troughs under peaks)	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 gel formulation, buffer, and run conditions as samples.
	Contamination of buffer with fluorescent species (for example, from laboratory pen used to label lanes gel plates or comb)	Do not label gel plates or comb.
	Lower buffer chamber not clean (*i.e.*, contaminated with fluorescent species)	Clean and replenish buffer in lower buffer chamber.
Signal gets progressively weaker for successive gels run over time	Degraded primers	Store primers in the dark at –15 to –25°C when not in use. Avoid unnecessary freeze-thaw cycles. Keep primers on ice during run setup.
	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 signal strength of the same samples prepared and run using fresh reagents.

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Observation	Possible Causes	Recommended Actions
Faint or no signal from sample DNA and from positive control	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 10–15 minutes. ♦ Use the recommended buffer. **Note** Both buffer pH and buffer composition affect enzyme activation. **Note** At temperatures >95°C, the enzyme is susceptible to irreversible denaturation. If you suspect insufficient activation, increase the incubation time, not the incubation temperature.
	Too little sample DNA added to reaction. **Note** This is especially critical in human identification experiments because sample quality is often poor.	Quantitate DNA and use the amount recommended in the protocol. **Note** For accurate quantitation of human DNA samples, use the QuantiBlot® Human DNA Quantitation Kit (P/N N808-0114).
	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 10–15 minutes. ♦ Use the recommended buffer. **Note** Both buffer pH and buffer composition affect enzyme activation. **Note** At temperatures >95°C, the enzyme is susceptible to irreversible denaturation. If you suspect insufficient activation, increase the incubation time, not the incubation temperature.
	Too little sample DNA added to reaction. **Note** This is especially critical in human identification experiments because sample quality is often poor.	Quantitate DNA and use the amount recommended in the protocol. **Note** For accurate quantitation of human DNA samples, use the QuantiBlot Human DNA Quantitation Kit (P/N N808-0114).
	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 or increase time at the denaturation plateau.)

(continued)

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Observation	Possible Causes	Recommended Actions
Faint or no signal from sample DNA and from positive control *(continued from previous page)*	PCR Master Mix not well mixed before aliquoting	Vortex PCR Master Mix thoroughly.
	Primer concentration too low	Use the recommended primer concentration.
	Primers degraded	Use new primers. **Note** Preincubation at 95°C for 5–10 minutes should inactivate proteases or nucleases. **Note** To prevent primer degradation during storage, store primers at –15 to –25°C, either lyophilized or in TE. Avoid excessive (more than 3–4) freeze-thaw cycles.
	Too little free Mg^{2+} in reaction	Check that you added sufficient total Mg^{2+} given concentration of the dNTPs and EDTA. **Note** [Free Mg^{2+}] = [Total Mg^{2+}] – [Total dNTP] – 2[EDTA]
	Incorrect pH	Verify buffer pH and buffer concentration.
	Wrong PCR tube	Use: ♦ GeneAmp® Thin-Walled Reaction Tubes for DNA Thermal Cycler 480 ♦ MicroAmp® Reaction Tubes with Caps for the GeneAmp PCR Systems 9600 and 2400
	MicroAmp Base used with tray/retainer set and tubes in GeneAmp® PCR System 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 during amplification (DNA Thermal Cycler 480)	Push reaction tubes firmly into contact with block after first cycle. Repeat amplification.
	GeneAmp PCR System 9600 heated cover misaligned	Align the heated cover so that white stripes align after twisting the top portion clockwise.
	Poor thermal cycler performance	Check instrument calibration.

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Observation	Possible Causes	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. (Use 8–16 µg BSA for every 50 µL PCR reaction volume.)
	Sample DNA is degraded	Evaluate the quality and concentration of the DNA sample by: ♦ Using the QuantiBlot Human DNA Quantitation Kit (for human DNA) ♦ Running an agarose yield gel If DNA is degraded or inaccurately quantitated, reamplify with an increased amount of DNA.
	Insufficient sample DNA added because of inaccurate quantitation	Evaluate the quality and concentration of the DNA sample by: ♦ Using the QuantiBlot Human DNA Quantitation Kit (for human DNA) ♦ Running an agarose yield gel If DNA is degraded or inaccurately quantitated, 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. Refer to the *GeneScan Reference Guide* for more information.
	T_m of primers is lower than expected	Decrease the annealing temperature by 2°C increments.

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Amplification Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
Poor yield for multiplex PCR	Non-optimal thermal cycling parameters	Between the denaturation and annealing stages, add a 2 minute down-ramp time to thermal cycling profile. For multiplex PCR, a short down-ramp time is not necessarily optimal.
	Competition from mispriming and other competing side reactions	Use AmpliTaq Gold DNA Polymerase. Refer to the Refer to the *GeneScan Reference Guide* for more information on designing custom primers, multiplexing PCR, and Hot Start PCR.
	Problems with primer choice, concentration, or degradation	Refer to the troubleshooting guide in the *GeneScan Reference Guide*.
Inconsistent yields with control DNA	Combined reagents not spun to bottom of PCR 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)	Put tubes in block immediately after combining reagents.
	Combined reagents not thoroughly mixed	Vortex all primers, reagents, and reaction mixes (minus enzyme) thoroughly to ensure uniform concentration.
	Primers not uniformly suspended before adding to reaction mixture. (Primers can aggregate and settle to the bottom of the tube.)	
	Pipetting errors.	Follow all these precautionary measures: ♦ Calibrate pipettes ♦ Attach tips firmly ♦ Check pipetting technique ♦ Minimize pipetting small volumes (for example, make master mixes) You may also want to consider using a 2-µL or other high-precision pipette
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.
Poor yield for multiplex PCR	Non-optimal thermal cycling parameters	Between the denaturation and annealing stages, add a 2 minute down-ramp time to thermal cycling profile. For multiplex PCR, a short down-ramp time is not necessarily optimal.

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Resolution Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
Fuzzy or smeared bands in electropherogram	Dirty gel plates	Clean plates with Alconox cleaner and a soft cloth, then rinse thoroughly with distilled, deionized water.
	Excess urea in wells before loading	Flush wells immediately before loading.
	Incorrect TBE buffer formulation	Remake buffer, carefully following protocol.
	Worn or damaged spacers causing variable gel thickness	Replace damaged spacers.
Poor resolution throughout size range	Poor quality or old reagents	Remake 10X TBE and gel solution stock using fresh reagents from a reliable source. **IMPORTANT** Urea must be ultrapure.
	Formamide degraded to formic acid and formate ions (denaturing applications only)	Use freshly deionized formamide. (Deionization procedure in Appendix A.) **Note** Formamide pH should be between 7 and 9.
	Small bubble between load and read region	Cast gel as described in protocol. **Note** Make sure plates are lint-free before pouring gel.
	Incomplete strand separation due to insufficient heat denaturation	Make sure the samples are heated at 95 °C for 5 minutes prior to loading in gel.
	Misshapen wells **Note** If only a few wells are misshapen, you can still load the gel using only those lanes with flat, well-formed wells.	To prevent suction when removing the comb: a. Lay gel flat. b. Pour 1X TBE over comb. c. Remove comb slowly. Ensure that no air is trapped by comb during insertion.
	Wrong TBE buffer formulation	Remake buffer, carefully following protocol.
Catastrophic loss of resolution (bands tilted and poorly resolved)	Gel extruding from between plates. See “Gel Extrusion” on page 4-29 for more information.	Clean the plates with an alcoholic KOH wash (page 4-30) or a 3 M HCl wash (page 4-31).

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Observation	Possible Causes	Recommended Actions
Poor resolution	Poor quality reagents, especially acrylamide	Use fresh reagents from a reliable source
	Small bubble(s) between load and read region	♦ Clean plates thoroughly ♦ Cast gel carefully. Remove bubbles by tapping plates while pouring. See Chapter 2 for more information.
	Well shape not flat	♦ Check for air bubbles trapped by comb ♦ Remove comb carefully ♦ Only load in flat wells ♦ Do not push shark's-tooth combs too far into the gel
	Old buffer	♦ Make 1X fresh daily ♦ Do not use 10X TBE which has precipitated
	Old gel	♦ Use gels within 2–6 hours of casting **IMPORTANT** Do not refrigerate.
	Gel not allowed to polymerize long enough	♦ Allow gel to polymerize the full length of time recommended by the protocol
	Gel stored under/in bright light	♦ Do not store gel under or in bright light
	Variation in spacers	Use spacers and comb sets that are of equal thickness.
	Temperature of room, gel solution, or glass too warm or cool during polymerization	20–23°C is optimal.

For GeneScan applications only, see also “Optimizing Electrophoresis Conditions for GeneScan Applications” on page 4-34.

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Sample Mobility Troubleshooting Guide

Observation	Possible Causes	Recommended Actions
Inconsistent mobilities from gel to gel	Total % polymer wrong	♦ Follow protocol carefully
	Wrong concentration of Bis	♦ Follow protocol carefully
	Wrong buffer concentration	♦ Follow protocol carefully ♦ Do not use 10X TBE which has precipitated
	Poor quality reagents or old urea	♦ Remake the 10X TBE and acrylamide stock solution using highest grade reagents. ♦ Use ultra pure urea
	Dissolved O_2 concentration	♦ Keep vacuum strength/time constant ♦ Stir/pour gel solutions gently ♦ Filter/pour gels at 20–23°C
	Variation in spacers	♦ Use spacers and comb sets of equal thickness
	Temperature of room, gel solution or glass varies	♦ 20–23°C is optimal
Slow mobility	Total polymer concentration too high	Check reagents. Prepare new solutions using fresh reagents.
	Bisacrylamide concentration too high	
	Buffer concentration too low	
	Old gel	Pour a fresh gel and use within 2 to 6 hours of casting **IMPORTANT** Do not refrigerate.
Mobility too quick	Total polymer concentration too low	Check reagents. Prepare new solutions using fresh reagents. **Note** Do not use TBE buffer if it has precipitate in it.
	Bisacrylamide concentration too low	
	Buffer concentration too high	

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About Troubleshooting Software

Log File Error Messages

Macintosh® computer operating errors are recorded in Log files. A Log file is created for each run, and is stored in the corresponding Run folder. The log lists error messages displayed during data collection including computer and instrument firmware errors.

Software-related errors are usually due to problems with:

- Memory

  For example, the computer might not have enough memory to handle a certain function, or the data collection program might not have been allocated enough memory when launched.

- File searching

  For example, data collection software may not be able to locate a file it needs to open or save. Or, a file might be corrupted.

- Hard Disk

  Any hard disk problem will result in an error message.

Check these areas if the Log file lists Macintosh errors. Run Norton Utilities™ Disk Doctor to check the hard disk, and correct recurring problems.

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About Troubleshooting Firmware

About the Firmware

The firmware required to run the instrument is stored in memory on the instrument. A copy of the firmware is also included as part of the data collection software, and is stored as a file in the ABI PRISM 377 folder.

The firmware is downloaded from the computer to the instrument during system installation. This occurs automatically when the instrument is turned on and data collection software is launched for the first time. If the instrument is turned off between runs, a battery backup keeps the firmware stored in instrument memory.

New versions of data collection software received after system installation can also include new firmware. When installed on the computer and launched for the first time, the software will ask if the firmware on the instrument should be upgraded.

A power outage can sometimes clear the instrument memory, or the firmware can become corrupt. Below is a list of symptoms that can indicate corrupt firmware on the instrument, and recommended corrective actions.

Symptoms of Corrupt Firmware

If any of the following symptoms occur, perform a single reset (see page 4-22):

- Flat scan lines or no scan lines when they should be present in the Scan window
- No response to commands from the computer

If any of the following symptoms occur, perform a total reset or cold boot as appropriate (see pages 4-22 and 4-23):

- No response to commands from the computer even after performing a single reset
- Rear panel LEDs are stuck in one pattern, *e.g.* all on or all off
- The computer cannot load a new image onto the instrument
- The computer appears to have downloaded a new firmware image onto the instrument, however,
  - the instrument still does not run
  - the rear panel LEDs remain stuck in one pattern

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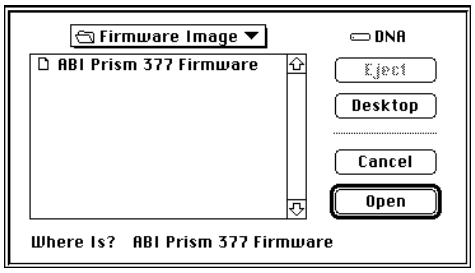
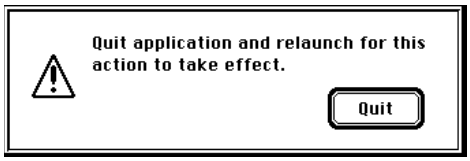
Performing a Single Reset

Procedure Using the eraser end of a pencil or similar object, press the red reset button on the back of the instrument once. If communication is not restored, perform a total reset as described below.

Performing a Total Reset

What is a Total Reset When a total reset is performed, the firmware currently installed on the instrument is erased. Data collection software must be restarted so that a new copy of the firmware is loaded automatically onto the instrument

Procedure To perform a total reset:

Step	Action
1	Using the eraser end of a pencil or similar object, press the red reset button on the back of the instrument twice in rapid succession.
2	Quit the data collection software program.
3	Relaunch the data collection software program. The firmware is automatically downloaded to the instrument. This will take 60–90 seconds.  **Note** If the following dialog box is displayed, see “Locating the Firmware Image File” on page 4-24. 
4	In response to this prompt, quit and then relaunch the data collection software program again. 

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Step	Action
5	Check the CCD pixel position value as follows: a. Open the Window menu and select Manual Control. b. Open the Fxn Name menu and select CCD Pixel Position Value. The value is displayed in the Value box. If it matches the value on the instrument, it is ok. If it does not match, correct the value. See “CCD Pixel Position Value” on page 4-25 for more information.

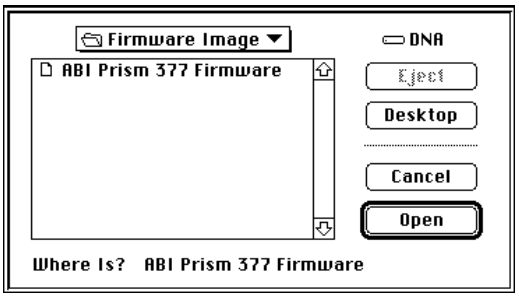
Performing a Cold Boot

Overview

If the computer and instrument are communicating, but the instrument does not respond appropriately, the firmware image may be corrupted. Download a new copy of the firmware using Cold Boot Instrument.

Procedure

To cold boot the instrument:

Step	Action
1	Select Manual Control from the Window menu in the data collection software program.
2	Open the Fxn Name pop-up menu.
3	Select Cold Boot Instrument, and click Execute. The current firmware image is erased from memory, and the status lights on the front of the instrument change from green (ready) to flashing yellow.
4	Open the File menu, and select Quit.
5	Launch the data collection software program. The firmware is automatically downloaded to the instrument. **Note** If the following dialog box is displayed, see “Locating the Firmware Image File” on page 4-24. 

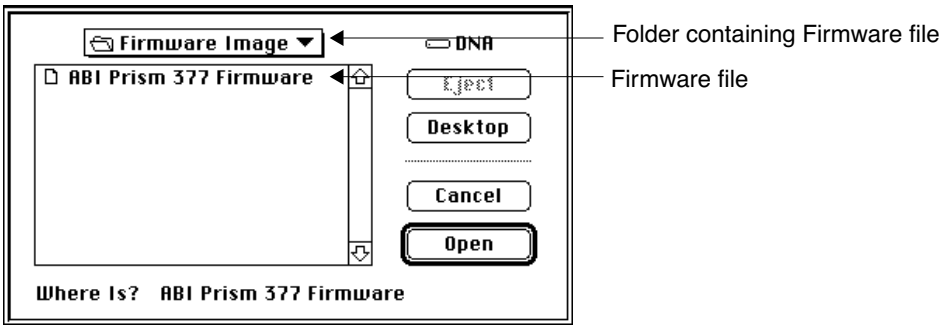
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Locating the Firmware Image File

Location of the Firmware File

The firmware required to run the instrument is stored in memory on the instrument. A copy of the firmware is also included as part of the data collection software, and is stored in the ABI PRISM 377 folder.

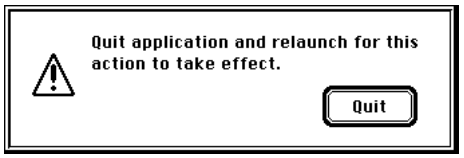
The location of the firmware on the computer must be specified as one of several Folder Location Preferences. Folder Location Preferences are set during system installation. If files or folders are renamed or moved after installation, data collection software may not be able to locate the firmware image file when necessary. If this occurs, a dialog box asking “Where Is? ABI Prism 377 Firmware” is displayed.



Follow the procedure listed below to locate and download the firmware. Refer to Chapter 5, “Setting Preferences,” for more information.

Procedure

To locate and download the firmware:

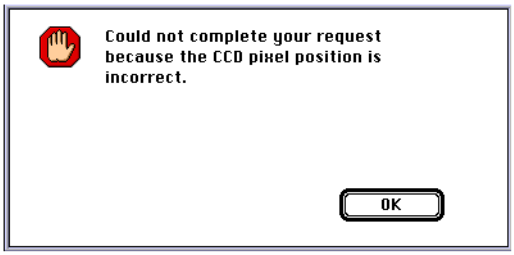
Step	Action
1	Open the pop-up menu in the dialog box, and locate the folder containing the firmware image file. Unless changed after system installation, the filename is ABI Prism 377 Firmware. It is located in a folder named Firmware Image inside the ABI PRISM 377 folder.
2	Click Open. Software automatically downloads the file to the instrument. 
3	Once the file is downloaded, the following alert box is displayed. Click Quit. 
4	Double-click the ABI Prism 377 Collection icon to launch the data collection software.

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CCD Pixel Position Value

Overview

The charge coupled device (CCD) pixel position value is a reference point for the alignment of the CCD camera with the laser beam. The instrument is shipped with the correct CCD pixel position value stored in its memory. When you start a run, the Data Collection software checks for a value greater than zero. If not found, the following error message is displayed.



Follow the procedures “Location of the CCD Pixel Position Value” and “Checking and Entering the CCD Pixel Position Value” on page 4-26 to locate and reenter the value.

Calibration File Make and Send

Two functions—Calibration File Make and Calibration File Send—allow you to create a file that contains the CCD pixel position value and instrument serial number. This file is called ABI 377 Calibrations, and is stored in the Preferences folder inside the System folder. The benefits to using these functions are as follows.

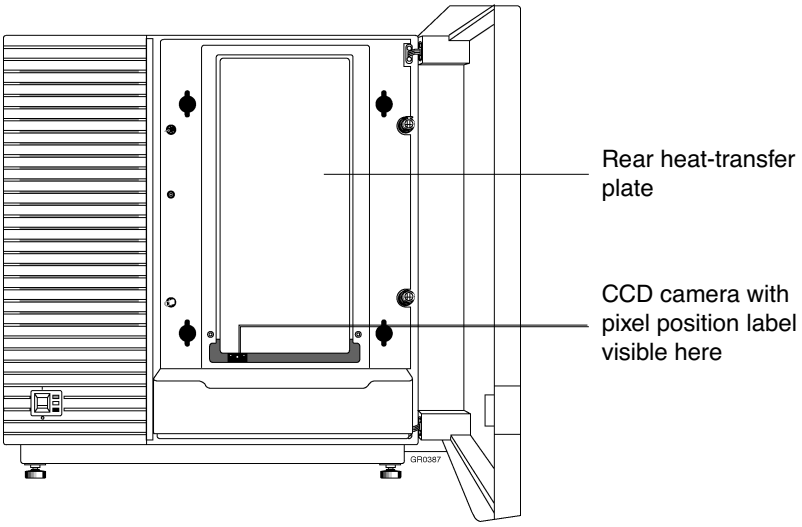
- Simply use Calibration File Send to reenter the CCD pixel position value, rather than manually locating and reentering the value.
- Once Calibration File Send is executed, the instrument serial number is added to all sample files, and is visible when sample files are opened in the annotative view. This is useful for tracking and troubleshooting, particularly when more than one instrument is in operation.

See “Using Calibration File Make and Send” on page 4-27 for more information.

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Location of the CCD Pixel Position Value

The correct CCD pixel position value is printed on a white label affixed to the CCD camera. The label is visible from the front of the instrument through the opening below the rear heat-transfer plate.



Locating the CCD Pixel Position Value

To locate the CCD pixel position value:

Step	Action
1	Have a flashlight available.
2	Open the front door of the instrument.
3	Shine the flashlight through the opening below the rear heat-transfer plate, and locate the white label on the CCD camera.
4	Record the value from the white label.
5	If you cannot find the label, call Applied Biosystems technical support (telephone numbers listed in Chapter 1.)

Checking and Entering the CCD Pixel Position Value

To enter the CCD pixel position value

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu in the data collection software.
3	Open the Fxn Name pop-up menu, and select the CCD Pixel Position function. The current pixel position value is displayed. If it is the same as the value on the white label, do not complete the remaining steps. If the value is different, continue.
4	Select the text box, and type the correct CCD pixel position value.
5	Click Execute.
6	If the CCD Pixel Position error occurs again, call Applied Biosystems technical support (telephone numbers listed in Chapter 1.)

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Using Calibration File Make and Send

Function Description

Two functions—Calibration File Make and Calibration File Send—allow you to create a file that contains the CCD pixel position value and instrument serial number. This file is called ABI 377 Calibrations, and is stored in the Preferences folder inside the System folder. The benefits to using these functions are as follows.

- Simply use Calibration File Send to reenter the CCD pixel position value, rather than manually locating and reentering the value as described under “CCD Pixel Position Value” on page 4-25.
- Once Calibration File Send is executed, the instrument serial number is added to all sample files, and is visible when sample files are opened in the annotative view. This is useful for tracking and troubleshooting, particularly when more than one instrument is in operation.

Using Calibration File Make

IMPORTANT The correct CCD pixel position value must be entered in instrument memory before using Calibration File Make. See “Location of the CCD Pixel Position Value” and “Checking and Entering the CCD Pixel Position Value” on page 4-26.

Creating the ABI 377 Calibrations File

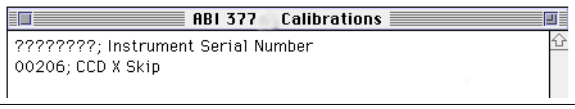
To create an ABI 377 Calibrations file:

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu.
3	Open the Fxn Name pop-up menu.
4	Select Calibration File Make.
5	Click Execute, and select Save from the File menu.

Adding the Serial Number to the ABI 377 Calibrations File

Add the instrument serial number to the ABI 377 Calibrations File.

To enter the instrument serial number in the calibrations file:

Step	Action
1	Make a note of the serial number from the back of the instrument.
2	Open the System folder, then the Preferences folder, and then the ABI 377 Calibration file.
3	Select the series of question marks displayed, and then type the instrument serial number in their place. 
4	Open the File menu and select Save.
5	Close the ABI 377 Calibrations file.
6	Quit the SimpleText program.

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Using Calibration File Send

To send the ABI 377 Calibrations file information to the instrument:

Step	Action
1	Turn power on to the instrument.
2	Open the Window menu and select Manual Control.
3	Open the Fxn Name pop-up menu and select Calibration File Send.
4	Click Execute. The CCD pixel position value and instrument serial number are sent to the instrument.

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Temporary Loss of Signal

Description This problem manifests itself as a band of little or no signal across the entire width of the gel image. It usually occurs between 150 and 250 bases. Temporary loss of signal has been traced to contaminants on the gel plates. These contaminants include surfactants, fatty acids, and long chain polymers.

Solution Rinsing glass plates in a dishwasher with hot deionized water (90°C) has been found in most cases to remove the contaminants that cause temporary loss of signal.

In a few cases where a dishwasher did not work well, soaking the plates overnight in a 5% solution of Multiterge detergent (VWR Scientific, P/N 34171-010) eliminated the temporary loss of signal.

Gel Extrusion

Description When voltage is applied on the ABI PRISM® 377 DNA Sequencer, the polyacrylamide gel sometimes moves from between the glass gel plates toward the cathode (upper electrode) and into the upper buffer chamber. Up to about five centimeters of gel in a folded sheet can be deposited in the chamber.

This “gel extrusion” usually begins at the start of a run or even during the prerun. It is believed to be caused by a buildup of charge on the surface of the glass plate such that the gel is not bound to the plate after pouring. As the voltage is applied, the gel migrates toward the upper electrode.

The gel image can show a variety of anomalous effects, including catastrophic loss of resolution, lane splitting, extreme band tilt, and band distortion.

Solution Almost all known cases of gel extrusion have been resolved by alcoholic KOH washing or acid washing. See “Performing an Alcoholic KOH Wash” on page 4-30 and “Performing a 3 M HCl Wash” on page 4-31.

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Performing an Alcoholic KOH Wash

Procedure The following procedures are not meant to be used for regular gel plate maintenance, but for decontamination. For regular plate cleaning, we recommend using a dishwasher with a hot, deionized water rinse.

! WARNING ! Preparation of all solutions should be carried out in a hood using safety glasses, gloves, and other appropriate protective clothing.

To perform an alcoholic KOH wash:

Step	Action
1	Add 30–35 g of potassium hydroxide (KOH) or sodium hydroxide (NaOH) pellets to a plastic bottle. **! WARNING ! Potassium hydroxide is hygroscopic and caustic. It can cause severe burns and blindness if it comes in contact with the skin or eyes. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
2	Add 200 mL of absolute ethanol to the bottle. **! WARNING ! CHEMICAL HAZARD. Ethanol is a flammable chemical and is irritating to the skin, eyes, respiratory system. It can cause nerve and liver damage, CNS depression, nausea, vomiting, and headache. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
3	Mix the solution well. It will take at least 15 minutes for most of the pellets to dissolve. **Note** This recipe is for a saturated solution, so some pellets will remain. Store the solution with the bottle capped tightly. During storage, the color of the solution will turn dark red-brown. The solution can still be used, and is good for 1 year.
4	Place some uncolored absorbent towels or other covering in the hood to catch spills.
5	Place the gel plates on the towels with the inside surfaces facing up. **Note** The plates should be nearly level so that the cleaning solution does not run off onto the bench. Only the inside (gel side) surface of the plates need be cleaned, though the outside surfaces can be cleaned similarly.
6	Pour approximately 15 mL of the cleaning solution onto the center of each plate to be cleaned. Spread the solution over the surface of plate.
7	Allow the solution to remain on the plates for 5 minutes. **CAUTION Longer times can harm the plates.**
8	Rinse the plates thoroughly with distilled, deionized water. Allow plates to dry. **Note** Avoid other cleaning procedures or solutions that may reintroduce contaminants to the plates.

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Performing a 3 M HCl Wash

Procedure To perform a 3 M HCl wash:

Step	Action
1	Place some uncolored absorbent towels or other covering in the hood to catch spills.
2	Pour 10 mL of concentrated HCl (12 N, 37%) carefully into 30 mL of water and mix thoroughly. ! WARNING ! **Hydrochloric acid (HCl) is a very corrosive liquid. Always work in a fume hood to avoid inhalation. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
3	Place the plates on the towels with the inside surfaces facing up. **Note** The plates should be nearly level so that the cleaning solution does not run off onto the bench. Only the inside (gel side) surface of the plates need be cleaned, though the outside surfaces can be cleaned similarly.
4	Pour approximately 15 mL of the cleaning solution in the center of each plate to be cleaned. Spread the solution over the surface of plate.
5	Allow the solution to remain on the plates for 5 minutes. **Note** Longer times will not harm the plates but are unnecessary.
6	Rinse the plates thoroughly with distilled, deionized water. Allow plates to dry. **Note** Avoid other cleaning procedures or solutions that can reintroduce contaminants to the plates.

Removing Gasket Marks from Glass Plates

Procedure An alcoholic KOH wash can also be used to remove buffer chamber gasket marks from the plates.

Step	Action
1	Perform steps 1–5 above.
2	Pour approximately 15 mL of the cleaning solution onto the area of the plate where the gasket mark is.
3	Allow the solution to remain on the plates for 10 minutes. CAUTION **Longer times can harm the plates.**
4	Repeat steps 2 and 3.
5	Rinse thoroughly with deionized water.
6	Clean plates as usual.

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Removing Coolant System Clogs

Clog Indicators

If tap water is used in the water reservoir, calcium buildups can occur, particularly in regions with hard water. Calcium buildup can cause the following problems:

- Flow switch sticks and plugs up
- High resistance to flow through heat plates
- Quick-disconnect fittings leak due to buildup behind the seals
- Warning displayed that the pump has shut down during a run
- Poor resolution due to a gel running too cold (accompanied by a warning that the pump has shut down)
- Error message that the heat plate temperature exceeds 70°C even though the status window indicates less than 70°C

Note The error message, "Heat plate temperature exceeds 70°C," is triggered by either the plate sensors or heater sensor. If the flow switch sticks in the flowing position due to calcium deposits, the pump shuts down, but the heater continues to operate in an attempt to raise the temperature of the front heat-transfer plate. When the temperature of the heater exceeds 70°C, the error message is displayed.

Recommendations

- Flush the system with a mild acid solution to remove calcium deposits. The cleaning solution and procedure are listed below.
- Refill the water reservoir with a solution of deionized water and 5.0% antifreeze. Refer to "Refilling the Water Reservoir" in Chapter 8, "System Maintenance," for more information.

Cleaning Solutions

Calcium deposits can usually be removed by flushing the system with a mild acid solution. Commercially available de-scaling solutions for removing deposits in coffee pots or hot water systems are recommended. Follow all precautions on the label when working with these solutions. If necessary, use a 3.0% by volume nitric acid solution.

! WARNING ! Do not use nitric acid with a higher concentration. Full strength nitric acid is 15.99N and should be diluted for use in this procedure.

! WARNING ! CHEMICAL HAZARD. Nitric acid (fuming) is an extremely corrosive oxidant. 3% nitric acid should be prepared by trained personnel only. Do not inhale vapor. Work in a well-ventilated area in a fume hood, and wear respiratory protection suitable for acid vapor. When diluting nitric acid, ALWAYS ADD ACID TO WATER or to aqueous solutions. Obtain a copy of the MSDS from the manufacturer. Wear full protective clothing, including eye and face coverings, rubber gloves, and rubber apron.

! WARNING ! HAZARDOUS WASTE/EXPLOSION HAZARD. Nitric acid waste must be emptied into the AQUEOUS waste bottle. DO NOT mix nitric acid waste with ORGANIC waste products, such as phenol. The mixture is potentially highly explosive. Always work in a fume hood. Wear appropriate eyewear, clothing, and gloves when handling TEMED solutions. Dispose of the contents of the waste tray and waste bottle in accordance with all applicable local, state, and federal health and environmental regulations.

! WARNING ! ELECTRICAL SHOCK HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

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Procedure To flush the system with a cleaning solution:

Step	Action
1	Power on the instrument.
2	Open the panel on the right side of the instrument to access the water reservoir.
3	Place paper towels under the bottle.
4	Unscrew the water bottle and properly dispose of the fluid.
5	Fill the bottle with 800 mL of cleaning solution and reattach the bottle to the instrument.
6	Open the Collection software, if it is not already open.
7	Turn the pump on. a. Open the Window menu and select Manual Control. b. Open the Fxn Name pop-up menu and select Pump On. c. Enter 51 in the Value box and click Execute.
8	Allow the solution to circulate for 15 to 30 minutes.
9	Turn the pump off. a. Open the Fxn Name menu and select Pump Off. b. Click Execute.

To flush the cleaning solution out of the instrument:

Step	Action
1	Open the panel on the right side of the instrument to access the water reservoir.
2	Unscrew the water bottle and properly dispose of the cleaning solution.
3	Fill the bottle with 800 mL of deionized water and reattach the bottle to the instrument.
4	Turn the pump on. a. Open the Window menu and select Manual Control. b. Open the Fxn Name pop-up menu and select Pump On. c. Enter 51 in the Value box and click Execute.
5	Allow the water to circulate for 1-2 minutes.
6	Turn the pump off. a. Open the Fxn Name menu and select Pump Off. b. Click Execute.
7	Repeat steps 1-6 three more times to ensure the cleaning solution has been removed.

To fill the water bottle:

Step	Action
1	Unscrew the water bottle and properly dispose of the solution.
2	To prevent calcium and other mineral deposits from reoccurring, refill the system with a solution of deionized water and 5.0% antifreeze. Refer to “Refilling the Water Reservoir” in Chapter 8, “System Maintenance,” for more information.

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Optimizing Electrophoresis Conditions for GeneScan Applications

Introduction

Optimizing electrophoresis conditions (run time, run voltage, and run temperature) for GeneScan applications can greatly improve data quality, run-to-run precision, and/or throughput. When selecting values for these parameters, consider the following factors:

- Range of fragment lengths
- Required degree of resolution
- Type of genetic analysis you will be performing

  For example, does the application require native or denaturing conditions?

The preset electrophoresis parameters in the application modules are set to ensure the following:

- Detection of all fragments in the typical size range permitted by the application

  For example, microsatellite loci are rarely over 400 base pairs in length.
- Acceptable run times
- Acceptable resolution

Definition of Resolution

The resolution, R_s, of two peaks in an electropherogram is defined as follows:

$$R_s = \frac{|P_1 - P_2|}{0.5 \times (W_1 + W_2)}$$

where the P_i are the peak positions measured below the peak apex and the W_i are the peak widths measured at half peak maximum.

Modifying Run Time

Determining Required Run Time

To determine the minimal acceptable run time for a given run voltage, you will need to perform a trial run. To ensure that you collect sufficient data to perform analysis, set the electrophoresis run time approximately 10% higher than the migration time of the largest fragment of interest.

In general, the migration time of the 400-bp fragment in the GeneScan-400HD Internal Lane Size Standard is 2 hours on a 36-cm well-to-read 5% Long Ranger gel.

Note The “largest fragment of interest” will most probably be a size-standard peak that is needed for sizing the largest sample fragments of interest. The set of size-standard peaks that GeneScan uses to generate the sizing curve can vary with the size-calling method. In general, be sure to include the two size-standard peaks immediately larger than the largest sample fragment of interest.

Decreasing Run Time

For faster run times, you can increase the electrophoresis voltage, but this can decrease the resolution.

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Modifying Electrophoresis Temperature

Perform native applications and non-denaturing applications, such as SSCP, at lower temperatures (27–42°C).

Protocols for most denaturing applications specify a 51°C run temperature.

For More Information

For information on setting electrophoresis parameters, refer to “Modifying and Creating Modules” on page 9-45 in Chapter 9.

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5 Setting Preferences

Chapter Contents

In This Chapter The following topics are discussed in this chapter:

Topic	See page
What Are Preferences	5-1
Where are Preferences Stored	5-2
Viewing and Modifying Preferences	5-3
If the Preferences File Becomes Corrupt	5-4
Folder Location Preferences	5-5
Default File Name Preferences	5-8
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GeneScan Run Sheet Preferences	5-13
General Settings	5-15
Dye Indicator Preferences	5-17
Project Information Preferences	5-20

What Are Preferences

About Preferences Preferences are default settings selected by the user in the data collection software. Certain preferences, such as the folder locations, must be set to operate the instrument, save and retrieve files, and process the data generated by runs. Other preferences are designed to save you time and customize the system. For example, you can reduce the time spent setting up sample and run sheets by changing certain sample and run sheet defaults to reflect the type of run performed most often. Most preferences have factory-set default values. All preferences (including the factory-set defaults) can be changed at any time.

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List of Preferences

The following is a brief description of each preference.

Preference	Description	See page
Folder Locations	Location of the folders on the hard disk in which the following are stored: sample sheets, standard and chiller modules, Run folders and files, the firmware image, general settings, matrix/instrument files, GeneScan® software analysis parameters, and GeneScan size standard files.	5-5
Default File Names	Naming convention used by data collection software to automatically name the new files and folders created for each run.	5-8
Sequencing Sample Sheet Defaults	User-designated parameters on sequencing sample sheets.	5-10
GeneScan Sample Sheet Defaults	User-designated parameters on GeneScan sample sheets.	5-12
Sequencing Run Sheet Defaults	User-designated parameters on sequencing run sheets.	5-11
GeneScan Run Sheet Defaults	User-designated parameters on GeneScan run sheets.	5-13
General Settings	Instrument name, global serial number, plate check module, communication port, and minimum number of scans.	5-15
Dye Indicators	Colors used to display data on the computer screen and to print data.	5-17
Project Information	For BioLIMS® software users only. Project information required to properly identify data transferred to BioLIMS.	5-20

Where are Preferences Stored

Location

Preferences are stored in a file that has one of the following names:

- ABI 377 (for standard ABI PRISM® 377 instruments)
- ABI 377XL (for ABI PRISM 377 instruments with the XL upgrade)
- ABI 377-96 (ABI PRISM 377 instruments with the 96-lane upgrade)
- ABI 377-18 (ABI PRISM 377-18 instruments)

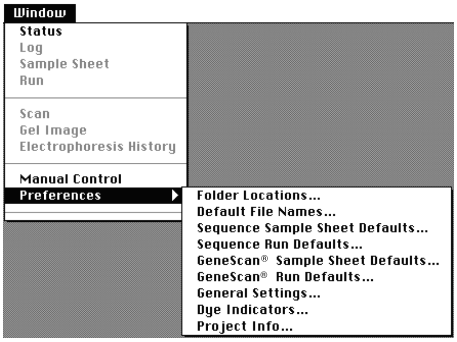
The preferences file is located inside the Preferences folder which is located inside the System Folder on the hard disk.

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Viewing and Modifying Preferences

How To The information in the preferences file cannot be viewed or modified by double-clicking on the file icon.

To view and/or modify preferences:

Step	Action
1	Launch the data collection software.
2	Open the Window menu, and select Preferences to open the preferences menu. 
3	Select a preference. A dialog box is displayed in which you can view and modify the current settings for that particular preference. The preferences file is modified whenever you click OK in a preferences dialog box.

Detailed instructions for modifying each preference are located in this chapter.

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If the Preferences File Becomes Corrupt

Indicators

Occasionally, the preferences file becomes corrupted. When this occurs, you will either be unable to launch the data collection software, or you will experience problems with the application.

Note If you launch the data collection software and a dialog box asking you to set folder locations preferences is displayed, you need only to reset the folder locations preferences to continue. See “Folder Location Preferences” on page 5-5.

To Create a New Preferences File

To create a new preferences file:

Step	Action
1	If open, quit the data collection software program. **Note** Data collection software must not running while you perform this procedure.
2	Open the System Folder.
3	Open the Preferences folder.
4	Click on and drag the existing preferences file to the trash, and empty the trash. Depending on the type of ABI PRISM 377 instrument you have, the preferences file will be named ABI 377, ABI 377XL, ABI 377-96, or ABI 377-18.
5	Launch the data collection software.
6	Set the folder locations preferences. See “Folder Location Preferences” on page 5-5 for more detailed instructions.
7	Reset the remaining preferences that you use. Detailed instructions are listed by preference in this chapter.

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Folder Location Preferences

Setting Folder Location Preferences

The folder locations for the following types of files and folders must be designated to perform a run, and to handle the data generated during a run:

- Sample sheets
- Module files
- Run folders
- Firmware image file
- Settings
- GeneScan analysis parameters (for GeneScan users only)
- GeneScan size standards (for GeneScan users only)

Software must know where to put and/or locate these files to operate the instrument and automate various tasks. For example, the selections displayed in the various pop-up menus on sample and run sheets are located in these folders.

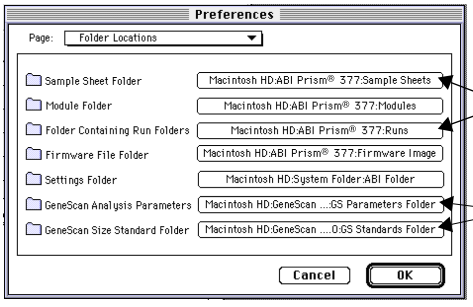
Typically these folders are located on the local hard disk. The folder names and locations listed below are the ones specified during system installation. The settings can be changed at any time. If changes are made, the new folder location and/or name must be specified in the Preferences Folder Locations dialog box (page 5-6).

Folder	Location and Folder Name*	Description
Sample Sheet Folder	Macintosh HD: ABI Prism 377: Sample Sheets	Contains all the sample sheets. When setting up a run sheet, the selections displayed on the pop-up menu for sample sheets are the files stored in this folder.
Module Folder	Macintosh HD: ABI Prism 377: Modules	Contains the Plate Check, PreRun, and Run modules. When setting up a run sheet, the selections displayed on the Plate Check, PreRun, and Run Module pop-up menus are the files stored in this folder.
Chiller Modules Folder	Macintosh HD: ABI Prism 377: Chiller Modules	Contains chiller Plate Check, PreRun, and Run modules. When setting up a run sheet, the selections displayed on the Plate Check, PreRun, and Run Module pop-up menus are the files stored in this folder.
Folder Containing Run Folders	Macintosh HD: ABI Prism 377: Runs	Contains the Run folders. A new Run folder is created for each run. At the end of data analysis, each new Run folder contains a gel file, log file, run sheet, and sample files.
Firmware File Folder	Macintosh HD: ABI Prism 377: Firmware Image	Contains the firmware image file.
*** IMPORTANT** Locations and folder names listed are those at the time of system installation. We strongly recommend not changing folder names and locations. ‡ = Displayed in Folder Locations dialog box for GeneScan users only.		

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Folder	Location and Folder Name*	Description
Settings Folder	Macintosh HD: System folder: ABI Folder	Typically set as the ABI Folder. The choices in other Preferences dialog boxes and in Run windows use the information contained in this folder for their pop-up menus (*e.g.* dye/set primer files, instrument/matrix files.) **IMPORTANT** To automatically analyze data after a run using the sequencing analysis software, the Settings folder must be designated as the ABI folder.
GeneScan Analysis Parameters‡	Macintosh HD: GeneScan Analysis x.x: GS Parameters Folder	Contains the parameters used to analyze GeneScan run data, and is located in the GeneScan Analysis software folder.
GeneScan Size Standard Folder‡	Macintosh HD: GeneScan Analysis x.x: GS Standards Folder	Contains the size standard definitions used to analyze GeneScan run data, and is located in the GeneScan Analysis software folder.
*** IMPORTANT** Locations and folder names listed are those at the time of system installation. We strongly recommend not changing folder names and locations. ‡ = Displayed in Folder Locations dialog box for GeneScan users only.		

To set the Folder Location preferences:

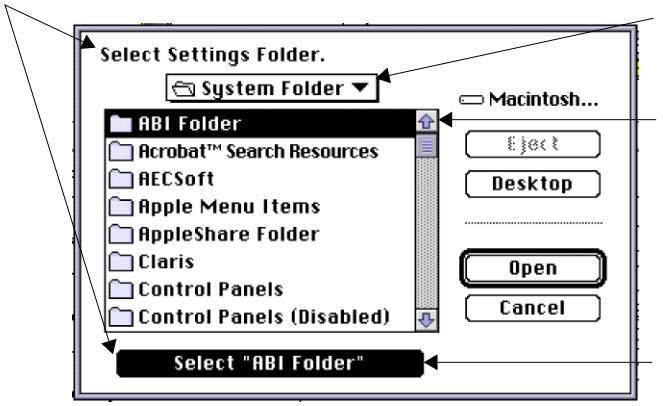
Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Window menu, select Preferences, and then select Folder Locations.
	is open	open the Page pop-up menu and select Folder Locations.
2	Click the box that corresponds to the folder location you wish to set.  	

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To set the Folder Location preferences: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td>3</td><td colspan="2">Using the pop-up menu in the dialog box that is displayed, locate and select the appropriate folder by clicking the folder once only.

IMPORTANT To automatically analyze data with ABI PRISM® DNA Sequencing Analysis Software on the same computer used for data collection, the Settings Folder must be designated as the ABI Folder (shown below).

1. The text above the menu indicates the folder location preference currently being set, and corresponds to the “select” button at the bottom of the box.


2. Use this pop-up menu to locate the desired folder
3. Click the folder once only to highlight it
4. Click here to set the preference</td></tr>
<tr><td>4</td><td colspan="2">Click the Select box underneath the list of folders (step 4 in the illustration above.) Do not click Open.</td></tr>
<tr><td rowspan="3">5</td><td>If …</td><td>Then …</td></tr>
<tr><td>you are finished</td><td>click OK to save your changes, or Cancel to quit without making any changes.</td></tr>
<tr><td>you wish to change more preferences</td><td>open the Page pop-up menu, and select another Preference.</td></tr>
</table>

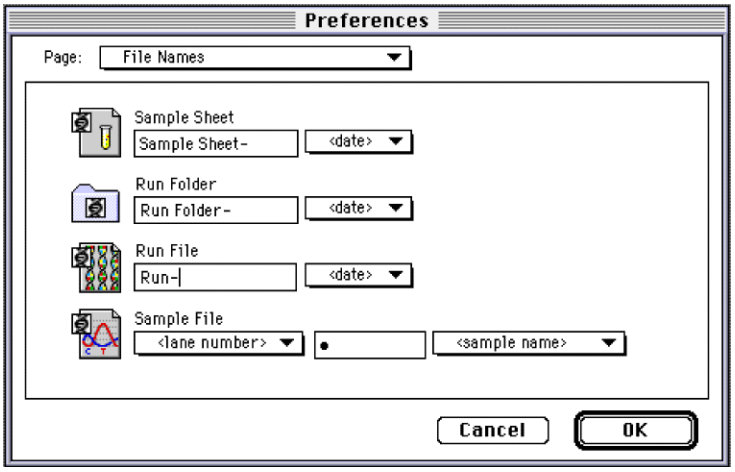
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Default File Name Preferences

Default File Name Preferences

Data collection software automatically names the new files and folders created for each run. These files and folders consist of:

- Sample sheets
- Run folders
- Run files
- Gel files
- Sample files (created after a run from the gel file)



Sample Sheet, Run Folder, and Run Sheet Names

The default naming convention for sample sheets, run folders, and run sheets is shown in the File Names Preferences window above. For example, a sample sheet created at 3:39 PM on June 30, 1998 would have a default name of: Sample Sheet–1/30/98 3.39 PM. The date and time are added as a suffix.

Folder and file names can be any text string or nothing at all. You can also choose not to display the date and time.

Sample File Names

By default, Sample File names have a prefix and a suffix. Choices for the prefix are lane number or sample name. Choices for the suffix are:

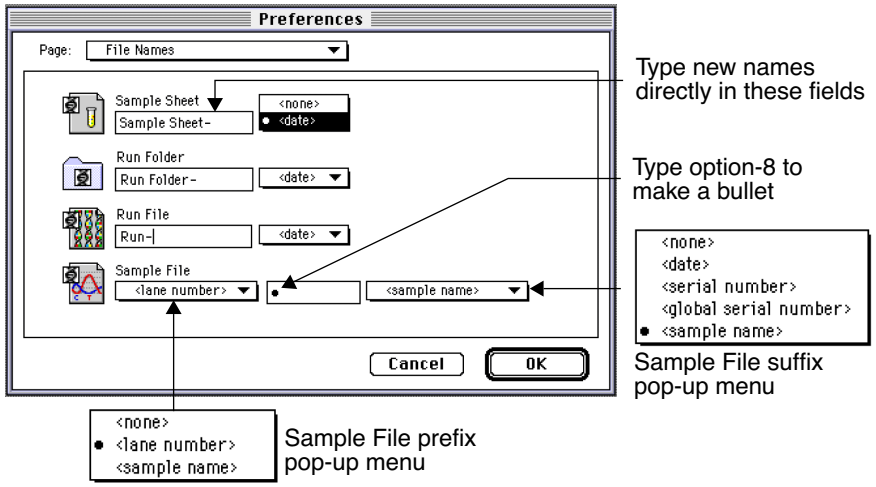
- Nothing <none>
- The current date and time <date>
- A serial number reset to zero at the start of each run <serial number>
- A serial number that increments across runs <global serial number>
- The sample name from the file's sample sheet <sample name>

IMPORTANT Limit the number of characters in sample file names to **27 or less**. Do not use colons, slashes, or symbols in sample names.

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Changing Folder and File Name Preferences

To change folder and file name preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select File Names.
	is open	open the Page pop-up menu and select File Names.
2	For sample sheets, run folders, and run sheets: a. Enter the file or folder name desired in the appropriate field. b. Open the associated pop-up menu to specify the date and time (<date>), or nothing (<none>) as a suffix. 	
3	For Sample Files: a. To change the prefix, open the prefix pop-up menu and make a new selection. b. To change the suffix, open the suffix pop-up menu and make a new selection. **IMPORTANT** Limit the number of characters in sample file names to 27 or less. Do not use colons, slashes, or symbols in sample names. **Note** The global serial number can be reset by changing the value in the General Settings Preferences window. Refer to page 5-15 for more information.	
4	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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Sequencing Sample Sheet Preferences

Setting Sequencing Sample Sheet Preferences

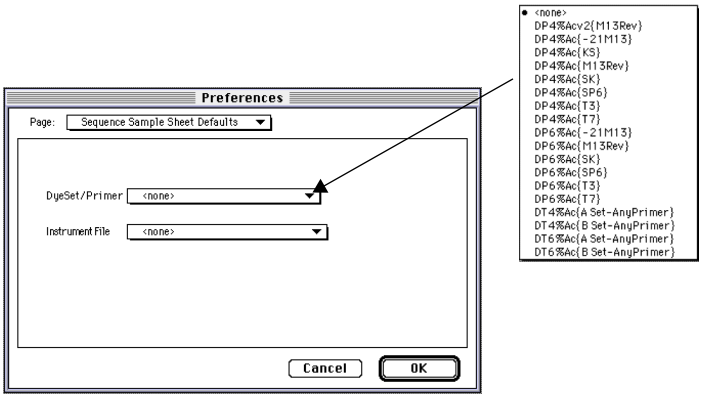
Two parameters can be set as preferences on Sequencing sample sheets:

- DyeSet/Primer
- Instrument File

The factory-default setting for both these parameters is none. As such, the DyeSet/Primer and Instrument File must be manually selected each time a new sample sheet is created.

To save time preparing sample sheets, set the defaults for these parameters to reflect the type of run most commonly performed.

To change sequencing sample sheet preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select Sequence Sample Sheet Defaults.
	is open	open the Page pop-up menu and select Sequence Sample Sheet Defaults.
2	To change the default settings: a. Open the DyeSet/Primer or Instrument File pop-up menu. b. Select the desired file from the pop-up menu.  DyeSet/Primer pop-up menu	
3	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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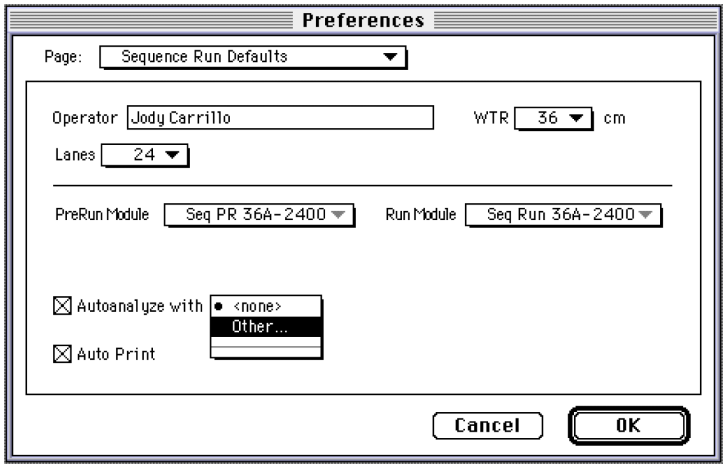
Sequencing Run Sheet Preferences

Setting Sequencing Run Sheet Preferences

The following parameters can be set as preferences for sequencing run files:

- Operator
- Well-to-read (WTR) distance in centimeters
- Lanes
- PreRun module
- Run module
- Autoanalyze
- Auto Print

To change sequencing run file preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select Sequence Run Defaults.
	is open	open the Page pop-up menu and select Sequence Run Defaults.
2	To enter an operator's name, type the name directly into the Operator field. 	
3	To change the default setting for the WTR distance, Lanes, PreRun Module, and Run Modules, open the pop-up menu for each parameter, and select the desired default.	
4	To specify that data be automatically analyzed by the sequencing analysis software: a. Select the box labeled "Autoanalyze with". b. Open the autoanalyze pop-up menu, select Other … c. Locate and select (double-click or OPEN) the appropriate analysis application (ABI PRISM DNA Sequencing Analysis Software).	
5	To specify that data be printed automatically at the end of each run, select the check box labeled "Auto Print".	

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To change sequencing run file preferences: *(continued)*

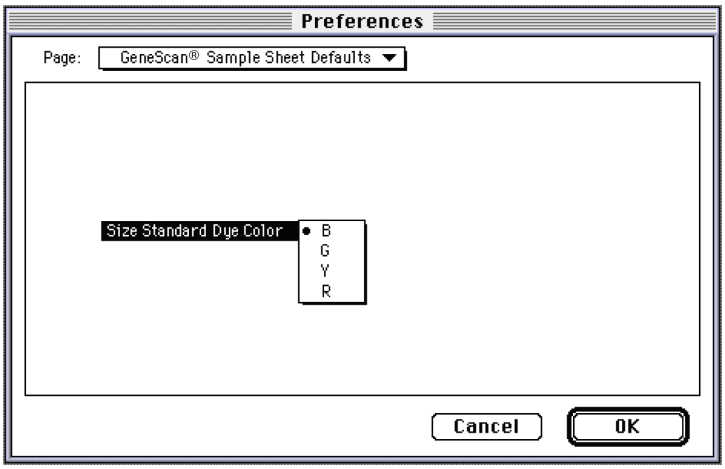
Step	Action	
6	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

GeneScan Sample Sheet Preferences

Setting GeneScan Sample Sheet Preferences

For GeneScan sample sheets, one parameter can be set as a preference—the size standard dye color.

To change GeneScan sample sheet preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select GeneScan Sample Sheet Defaults.
	is open	open the Page pop-up menu and select GeneScan Sample Sheet Defaults.
2	To change the size standard dye color, open the size standard pop-up menu and select the appropriate color (B=blue; G=green; Y=yellow; R=red). 	
3	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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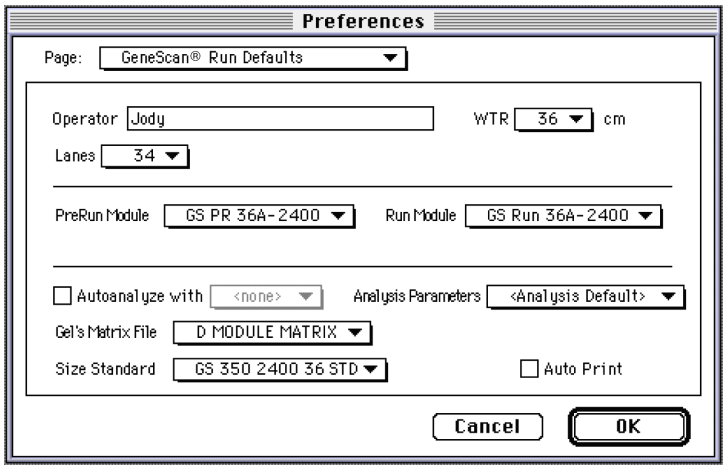
GeneScan Run Sheet Preferences

Setting GeneScan Run Sheet Preferences

The following parameters can be set as preferences for GeneScan run sheets:

- Operator
- Well-to-read (WTR) distance in centimeters
- Lanes
- PreRun module
- Run module
- Autoanalyze
- Analysis Parameters
- Gel's Matrix File
- Size Standard
- Auto Print

To change GeneScan run sheet preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select GeneScan Run Defaults.
	is open	open the Page pop-up menu and select GeneScan Run Defaults.
2	To enter an operator's name, type the name directly into the Operator field. 	
3	To change the default setting for the WTR distance, Lanes, PreRun Module, Run Module, Gel's Matrix File, and Size Standard, open the pop-up menu for each parameter, and select the desired default.	

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To change GeneScan run sheet preferences: *(continued)*

Step	Action	
4	To specify that data be automatically analyzed by GeneScan analysis software: a. Select the box labeled “Autoanalyze with”. b. Open the autoanalyze pop-up menu, select Other …, and then select the appropriate analysis application (ABI PRISM GeneScan® Analysis Software). c. Open the pop-up menu for Gel’s Matrix File, and select the appropriate file. d. Open the pop-up menu for Size Standard, and select the appropriate file. e. Open the pop-up menu for Analysis parameters, and select the appropriate file. **Note** Refer to the *ABI PRISM GeneScan® Analysis Software User’s Manual* for more information on matrix files, analysis parameters, and size standard files.	
5	To specify that data be printed automatically at the end of each run, select the check box labeled “Auto Print”.	
6	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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General Settings

About General Setting Preferences

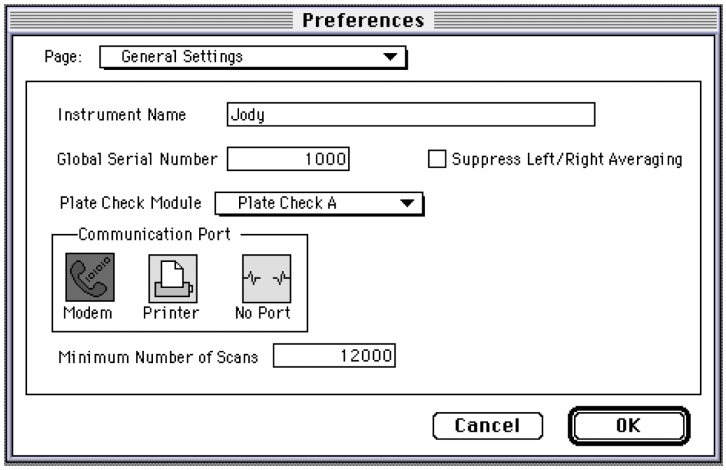
The General Settings window allows you to specify the following:

Setting	Description
Instrument Name	Used to track which instrument a gel is run on. The name is recorded in the Gel and Log files for each run.
Global Serial Number	Increments with each run. The number can be automatically added as a suffix to all Sample File names. This feature is set via the File Names preference window (sample file name).
Suppress Left/Right Averaging	*Do not use this feature.* Current versions of the ABI PRISM 377 DNA sequencing and GeneScan analysis software cannot process unaveraged data. This feature may be added to future versions of software.
Plate Check Module	Used to designate a default module for plate checks.
Communication Port	The Macintosh® computer port (Modem or Printer) to which the instrument is attached, or No Port. The Macintosh computer has two standard connection ports: Modem and Printer. Typically, the instrument is attached to the Modem port, and a printer or network connection is attached to the Printer port. If, however, the instrument must be connected to the Printer port, you must specify this change in the General Settings preferences window. The No Port option allows you to use the data collection software when no instrument is attached to the computer, or the instrument is turned off.
Minimum Number of Scans	Determines the amount of hard disk space software sets aside for the gel file at the beginning of every run. The default factory setting is 12000. If necessary, this number can be increased. We strongly recommend you never decrease this number. If decreased, the Macintosh may not have enough hard disk space to store the additional data.

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To Change General Settings Preferences

To change general settings preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select General Settings.
	is open	open the Page pop-up menu and select General Settings.
2	Set the preferences as follows: a. To enter an instrument name, type the name in the Instrument Name field. b. To change the global serial number, select the current value in the box, and type in the new value (use positive integers only). c. Do not select Suppress Left/Right Averaging. See explanation on previous page. d. To select a plate check module, open the pop-up menu and choose the appropriate module. e. To change the communication port, click the appropriate port icon. f. To change the minimum number of scans, select the current value in the box, and type in a new value.  **Note** Data collection software collects data for the run time specified on the run sheet. We recommend you do not enter a value below the default minimum number of scans (12000). If the value is less, data collection software may not allocate enough hard disk space to collect all the data.	
3	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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Dye Indicator Preferences

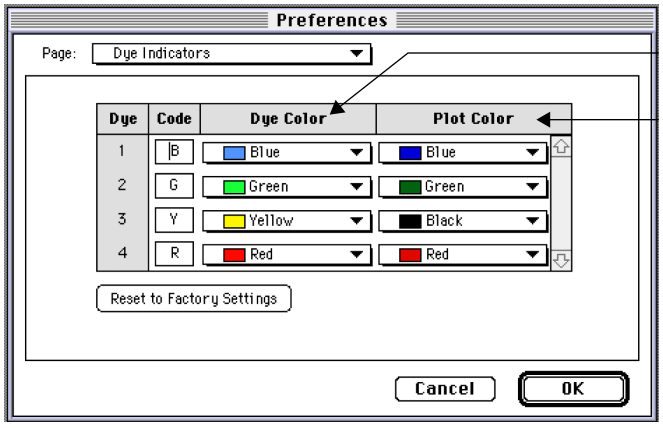
Setting Dye Indicator Preferences

Two categories of color definitions are available for the dyes used during a run:

- Dye Color—colors used to display data on the computer screen
- Plot Color—colors used to print data

Dye and plot colors can be changed at any time. This feature is useful if you are color blind, and the default factory settings are not appropriate for your needs.

To change Dye Indicator preferences:

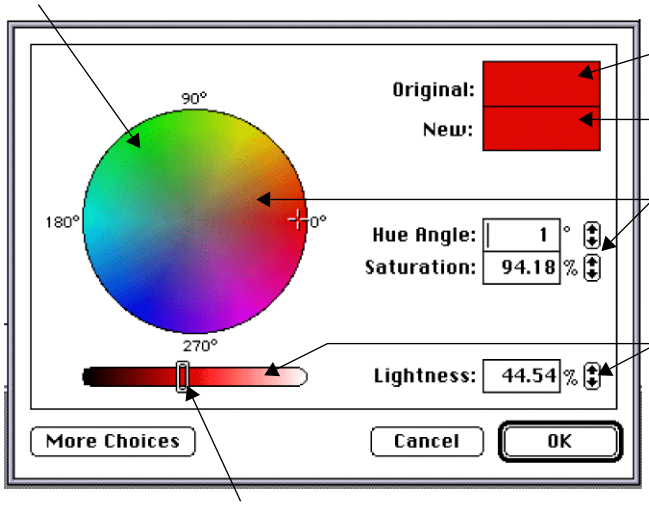
Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select Dye Indicators.
	is open	open the Page pop-up menu and select Dye Indicators.
2	To change the code used for a particular color, type a different character in the appropriate field in the Code column. 	
3	To change a color, open the appropriate pop-up menu and select a new color, or select Other.	

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To change Dye Indicator preferences: *(continued)*

Step	Action	
4	**If …**	**Then …**
	Other is not selected	proceed to step 6.
	Other is selected	a. use the Hue Angle and Saturation scroll bars/fields, or the color wheel as described in the illustration to change the hue and saturation. b. Use the Lightness scroll bar/field, or the lightness bar as described in the illustration to make the color lighter or darker. As changes are made, the new color is displayed in the New box. c. When finished, choose one of the following: – Click OK to save the change and return to the Dye Indicators preference window – Click Cancel to cancel the change and return to the Dye Indicators preference window – Click More Choices, and proceed to step 5.

To use the wheel, move cursor onto wheel. Cursor turns to crosshair. Click and drag the



Color before changes are made

Color as you make changes

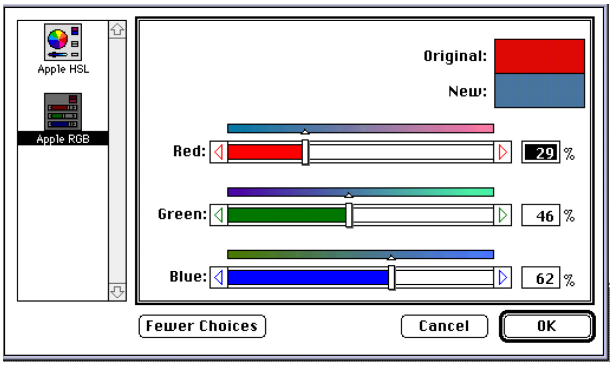
Use the wheel or these boxes to change hue and saturation

Use the bar or this box to change the lightness

Click and drag this bar to make changes

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To change Dye Indicator preferences: *(continued)*

<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td rowspan="5">5</td><td>If …</td><td>Then …</td></tr>
<tr><td>More Choices is not selected</td><td>proceed to step 6.</td></tr>
<tr><td>More Choices is selected</td><td>a. Click the Apple RGB icon to display the window shown below.
b. Use the bars or the % fields to create a new color.
c. When finished, choose one of the following:
– Click OK to save the change and return to the Dye Indicators preference window.
– Click Cancel to cancel the change and return to the Dye Indicators preference window.
– Click the Apple HSL icon to return to the previous window.</td></tr>
<tr><td colspan="2">
</td></tr>
<tr><td>6</td><td colspan="2">To revert back to the factory settings, click the Reset to Factory Settings button.</td></tr>
<tr><td rowspan="3">7</td><td>If …</td><td>Then …</td></tr>
<tr><td>you are finished</td><td>click OK to save your changes, or Cancel to quit without making any changes.</td></tr>
<tr><td>you wish to change more preferences</td><td>open the Page pop-up menu, and select another Preference.</td></tr>
</table>

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Project Information Preferences

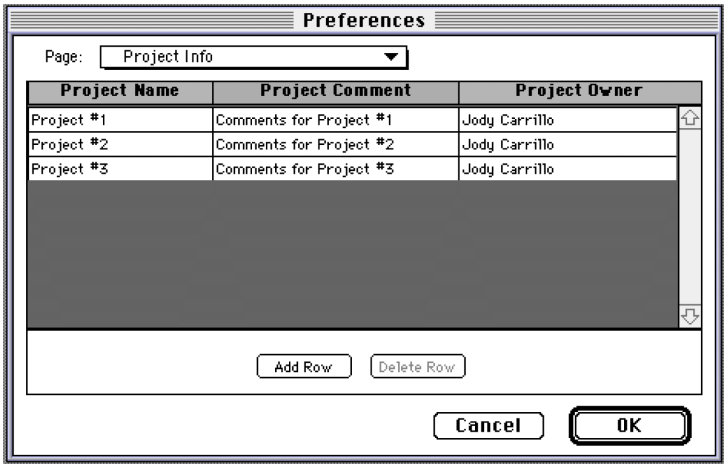
About Project Info Preferences

This preference is for BioLIMS users only (sequencing applications—BioLIMS version 1.0 and up; GeneScan applications—BioLIMS version 2.0 and up). The project information is used to identify the data transferred to BioLIMS, a nd must be defined prior to setting up sample sheets. If this information is not specified, data transferred to BioLIMS is identified by the gel file name only.

The Project Info preference allows you to enter a project name, information about the project in a comments field, and a person's name as the project owner. This information is transferred to the sample sheet when a project name is selected.

Setting Project Info Preferences

To set or change Project Info preferences:

Step	Action	
1	**If the Preferences dialog box …**	**Then …**
	is not open	open the Windows pull-down menu, select Preferences, and then select Project Info.
	is open	open the Page pop-up menu and select Project Info.
2	Click Add Row to add a row for each project to be defined. 	
3	To define the first project, select the first cell in the Project Name column, and enter a name.	
4	Press the tab or right arrow key to move to the Project Comment column, and enter comments or leave the field blank.	
5	Press the tab or right arrow key to move to the Project Owner column, and enter an owner's name or leave the field blank.	
6	Continue adding information in the same manner for the remaining projects.	
7	To delete a row, select the row and click Delete Row.	
8	**If …**	**Then …**
	you are finished	click OK to save your changes, or Cancel to quit without making any changes.
	you wish to change more preferences	open the Page pop-up menu, and select another Preference.

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Making Matrix Files for GeneScan 6

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

Topic	See page
Terminology	6-1
Purpose of the Matrix File for GeneScan Applications	6-2
Why Matrices Must Be Remade	6-2
Evaluating Matrix File Quality	6-3
Creating a Matrix File for GeneScan Applications	6-6
Checking the Quality of a New Matrix File	6-8

Terminology

Matrix vs. Instrument Files The terms matrix file and instrument file are sometimes used interchangeably. In this chapter, the term:

- "Matrix file" refers files that must be created to properly analyze data for GeneScan® analysis software applications.
- "Instrument file" refers to the files that must be created to properly analyze data for sequencing applications.

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Purpose of the Matrix File for GeneScan Applications

Purpose

While the most intense fluorescence emitted by an ABI PRISM® dye will fall within a small wavelength detection range, some fluorescence emission in the detection ranges of the other dyes will always occur. The multicomponent matrix compensates for this overlap by subtracting out, in each dye's detection range, the portion of the signal due to fluorescence from other dyes.

Why Matrices Must Be Remade

Factors Affecting Matrix Quality

When creating a matrix, each relevant dye matrix standard must be run separately to determine the proportional amount of fluorescence that is emitted in all four detection regions. Because the emission spectra of the dyes vary with the physical environment (such as the pH or polymer type and concentration), the matrix must be remade if run conditions change. Factors that affect matrix quality are:

- Aging reagents
- Buffer type and concentration
- Gel polymer type
- Denaturing vs. non-denaturing conditions
- Run temperature
- Change in instrument optics (CD camera or lenses)

Virtual Filter Set C

The emission maximum of 6-FAM, the recommended blue-displaying dye for this filter set, is very close to the laser wavelength of 514.5 nm. Thus, the window for collected blue light-intensity data is offset to longer wavelengths and does not contain the emission maximum of 6-FAM. It is also very close to the detection region for the green-displaying dye TET.

Matrix files made for Virtual Filter Set C are especially susceptible to minor changes in run conditions. If you are using Virtual Filter Set C for GeneScan applications, watch for evidence of matrix problems and remake the matrix as soon as problems appear.

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Evaluating Matrix File Quality

How to Recognize Matrix Problems

A poor or incorrect matrix results in too much or too little subtraction of dye spectral overlap during data analysis. Each causes a recognizable electropherogram anomaly:

- Bleedthrough peaks, also called “pull-ups” (caused by too little subtraction)
- Elevated interpeak baseline (caused by too much subtraction)

Bleedthrough Peaks (or Pull-ups)

Bleedthrough peaks are small peaks of one color lying directly under a large peak of another color even though there is no PCR product corresponding to the smaller peak. As shown Figure 6-1, the large-peak signal is “pulling-up” peaks in other colors.

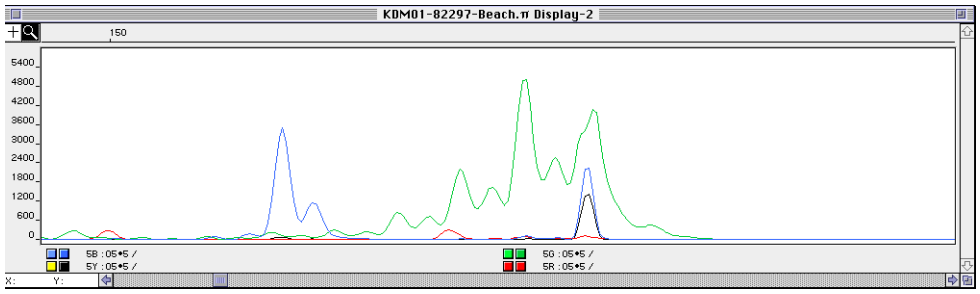


Figure 6-1 Characteristic appearance of bleedthrough peaks

Bleedthrough can occur for two reasons:

- The matrix was made with the wrong dyes or filter set.

  For a list of recommended dye/filter set combinations, refer to the *GeneScan Reference Guide: ABI™ 373 and ABI PRISM® 377 DNA Sequencers,* P/N 4303188.

- The signal from the large peak is off-scale because of sample overloading. In the example shown in Figure 6-1, the peak showing bleedthrough is actually off-scale (Figure 6-2).

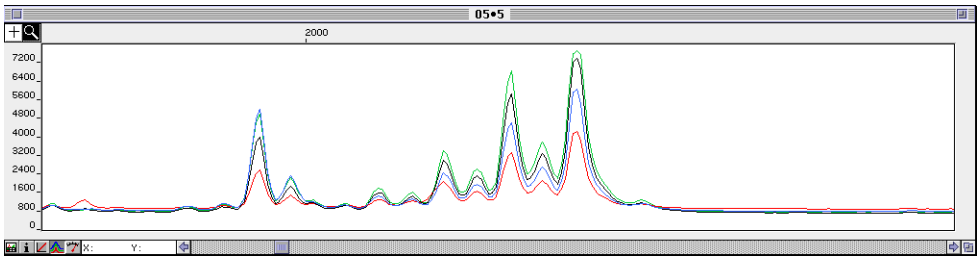


Figure 6-2 Raw data from the bleedthrough example shown in Figure 6-1

Keep peak heights between approximately 150 and 4000 RFU. If sample data is off-scale, do one of the following:

- Rerun the samples loading a smaller volume.
- Dilute the samples and rerun them.

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Elevated Interpeak Baseline

Figure 6-3 shows a typical example of an elevated interpeak baseline. In this example, the green baseline is elevated in the region between two large black peaks (representing the yellow dye signal) because too much green signal is subtracted from the yellow signal (see Figure 6-4 on page 6-5). The GeneScan software uses these low data points to calculate the baseline for the green signal. Therefore the original baseline is elevated.

Note If the baseline is sufficiently elevated, random fluctuations in the baseline can lie above the Peak Amplitude Threshold and might be falsely interpreted as product peaks.

If you suspect that an elevated interpeak baseline is caused by matrix problems, inspect the data before baselining. This can be done by reanalyzing the data with baselining deselected in the Analysis Parameters dialog box. As shown in Figure 6-4, low data points are apparent as troughs in one color beneath peaks in another.

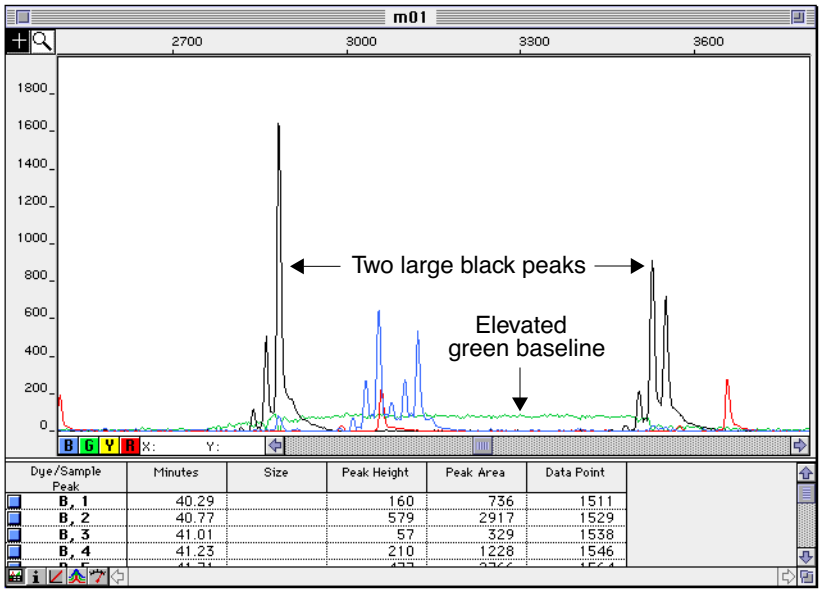


Figure 6-3 Characteristic appearance of an elevated baseline caused by a bad or incorrect matrix

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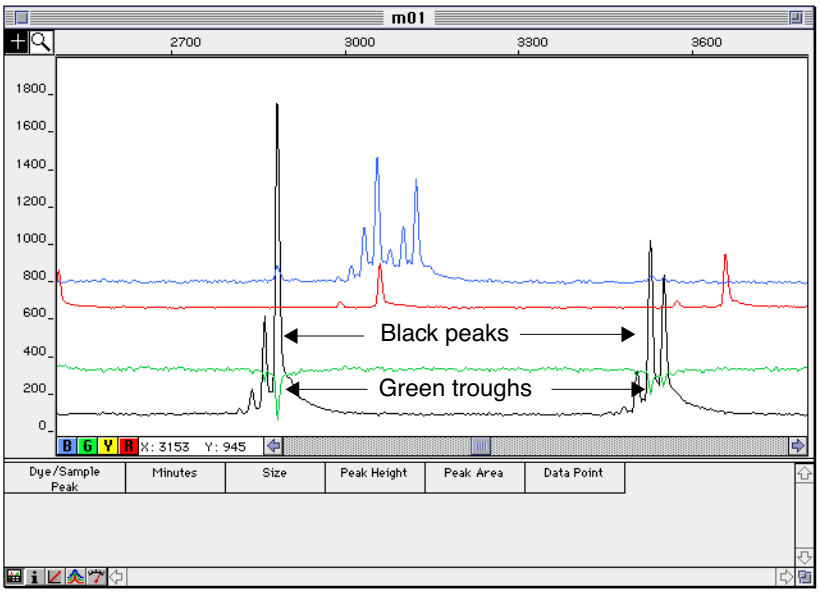


Figure 6-4 The data of Figure 6-3 before baselining

What To Do If You Have Matrix Problems

If problems related to bleedthrough peaks or to an elevated interpeak baseline appear with any regularity, you should remake the matrix. Apply the new matrix to the old sample data and reanalyze the data.

Instructions for creating matrix files are listed under “Creating a Matrix File for GeneScan Applications” on page 6-6.

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Creating a Matrix File for GeneScan Applications

Overview

The matrix file contains the information necessary for software to correct the spectral overlap of the dyes in the virtual filter sets. 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
  - gel parameters (gel polymer type, buffer, denaturing or non-denaturing conditions, etc.)

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

Verify the Raw Data

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

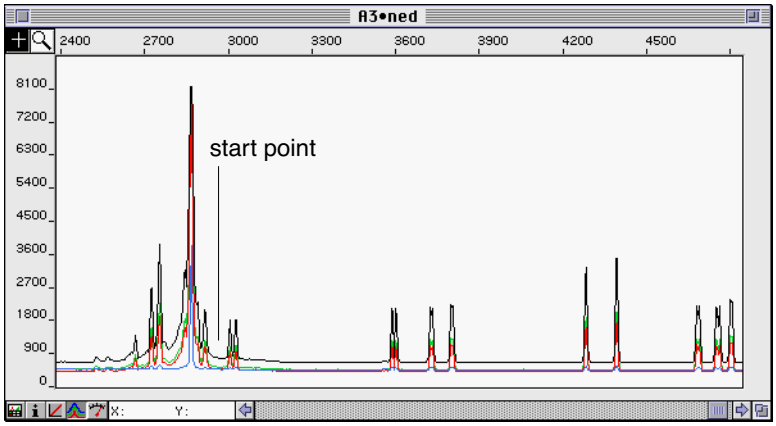
To view the raw data in GeneScan analysis software:

Step	Action
1	Create a new project if you did not select Autoanalyze in the GeneScan Run Defaults preferences in the data collection software: 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.

To verify the raw data:

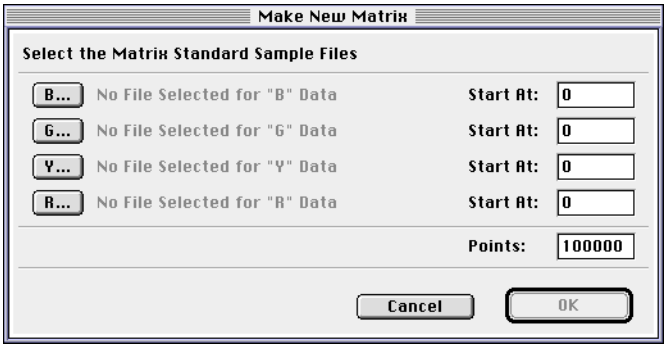
Step	Action
1	Verify data peaks are present in all four samples. Peak data should be on-scale and the dye of interest should have a value of at least 200.
2	Check for any data anomalies, such as an unstable baseline. Rerun samples that have an unstable baseline.

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Step	Action
3	Select a starting point for the matrix data as shown below. The starting point for matrix data should be slightly beyond the point where the primer peak falls back to the baseline (approximately 2950 scans in this example). 
4	Choose a stop point such that at least three matrix standard peaks will be within the range analyzed.

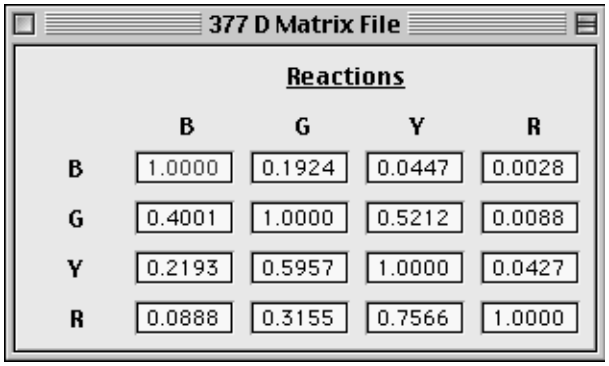
Generating the Matrix File

To generate the matrix file:

Step	Action
1	Choose New from the File menu.
2	Click the Matrix icon. The Make New Matrix dialog box is displayed. 
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. Define the Points value. This is the number of points after the start point to be analyzed.

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To generate the matrix file: *(continued)*

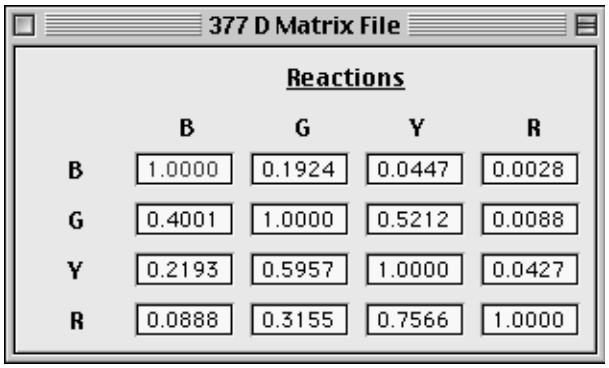
Step	Action
5	Click OK. A successful matrix opens an untitled Matrix Values window with a 4x4 matrix of numerical values. 
6	Use the Save As… command to name and save the matrix file. Choose a name that reflects the chemistry, the virtual filter set, and the run conditions.
7	Check the quality of the matrix file by following the procedure below “Checking the Quality of a New Matrix File.”

Checking the Quality of a New Matrix File

Procedure Check the quality of the matrix file by reviewing the:

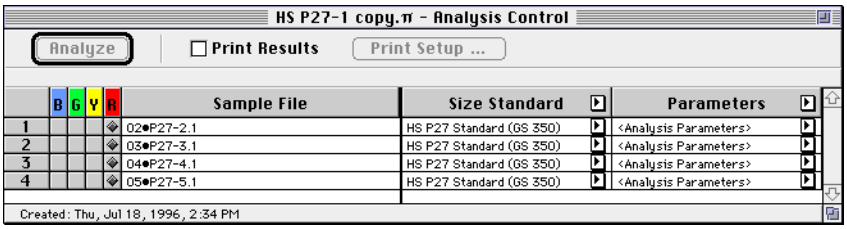
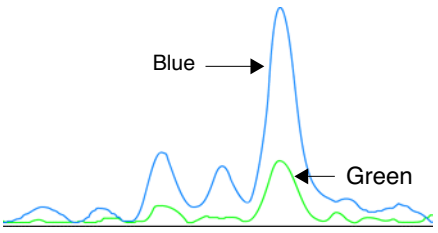
- Values in the Matrix Values window
- Analyzed data of the matrix run

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

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 should be less than 1.00. **Note** For virtual filter set C, Green under Blue (the second box from the top in the first column on the left) is sometimes slightly above 1.00. This is acceptable.

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Check matrix quality as follows:

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. 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.	
6	Click Analyze.	
7	Choose Results from the Windows menu and check each electropherogram by: a. Clicking 4 in the # of Panels menu b. Clicking 1 under Dye/Samples c. Clicking #1 on the Sample Files side of the Results window	
8	**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 (as shown below) 	the matrix is poor.
9	**If…**	**Then…**
	the matrix is good	Save the matrix file to the ABI folder.
	the matrix is poor	Redo the matrix by using different start and stop points. If this does not improve the matrix data, run new matrix standards.

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Making Instrument Files for Sequencing 7

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

Topic	See page
Terminology	7-1
Instrument File Overview	7-2
When to Make a New Instrument File	7-3
The Data Utility Program	7-3
Summary of Chemistries	7-4
Color Guide for Data Display Windows	7-4
Making an Instrument File for Virtual Filter Set A from Matrix Standards	7-6
Making an Instrument File for Virtual Filter Set E from Matrix Standards	7-10
Checking Instrument Files	7-16
Making an Instrument File from a Sample File	7-18
Storing and Backing Up the Instrument File	7-19
Adding or Replacing a Matrix in an Existing Instrument File	7-20
Correcting Errors in Matrix Creation	7-22

Terminology

Matrix vs. Instrument Files The terms matrix file and instrument file are sometimes used interchangeably. In this chapter, the term:

- "Matrix file" refers files that must be created to properly analyze data for GeneScan® analysis software applications. Refer to Chapter 6, "Making Matrix Files for GeneScan," for more information.
- "Instrument file" refers to the files that must be created to properly analyze data for sequencing applications.

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Instrument File Overview

Purpose

Although the dyes in a set fluoresce at different wavelengths, some overlap in the spectra still occurs. To correct for this overlap when analyzing data, a mathematical matrix is created for each dye set and is stored in a file called the instrument file.

The instrument file must contain a matrix for each chemistry run on the instrument. During data analysis the appropriate matrix is applied to remove any spectral overlap. During system installation, an instrument file is created specifically for your instrument. However, additional instrument files may be required. See “When to Make a New Instrument File” on page 7-3.

What Does the Instrument File Contain?

The instrument file normally contains:

- Three matrices
- A comment field
- An instrument name field

These can be seen in the Copy Matrix window in the DataUtility program. A copy of this instrument file is attached to every gel file and sample file when these files are first created. For this reason, each computer on which you use the Sequencing Analysis program must have an instrument file in the ABI folder (which is located in the System Folder).

IMPORTANT Due to slight variations in the filters of the ABI™ 373 instruments, and the CCD cameras of the ABI PRISM® 377 and ABI PRISM 310 instruments, the instrument file created for your ABI PRISM genetic analysis instrument is sub-optimal for other ABI PRISM genetic analysis instruments.
If you analyze sample files on a different computer from the one that was used to collect data, be sure to copy the correct instrument file(s) to the analysis computer.

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When to Make a New Instrument File

Guidelines

During system installation, an instrument file is created specifically for your instrument. If a valid instrument file exists in the ABI folder (inside the System Folder) on your Macintosh® computer, you need not create one. A new instrument file should be created if any of the optics in the instrument change either due to service or age. Some specific situations that require a new instrument file are:

- The CCD camera in the instrument is replaced.
- A run shows consistent and proportional pull-up peaks, indicating poor or incorrect spectral separation. (Pull-up peaks appear as smaller peaks of one color directly under larger peaks of another.)
- Fluorescence and spectral overlap are affected by the gel used for the run. You *may* need to make a new instrument file if the type of acrylamide or other gel reagents is changed.

If you need to replace a lost or damaged instrument file, and you do not have a backup copy, refer to the *Automated DNA Sequencing Chemistry Guide: ABI™ 373 and ABI PRISM® 377 DNA Sequencers,* P/N 4305080. This manual contains information on viewing and copying matrices. You may want to try restoring the instrument file by following the procedures in this manual before you recreate the entire instrument file.

The Data Utility Program

What it is Used For

The DataUtility program has two main functions for users: to make instrument files, and to copy matrices from one instrument file to another. The program is located in the Utilities folder within the Sequencing Analysis folder.

When creating instrument files, the correct data file for each matrix standard must be entered in the correct “box” in the Data Utility Make Matrix dialog box.

The Measure Noise function of the program is used by Applied Biosystems Service personnel and is not discussed here.

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Summary of Chemistries

The Sequencing Chemistries

Five cycle sequencing chemistries are currently available to prepare DNA samples for the ABI PRISM® 377 DNA Sequencer. Each chemistry requires the use of a specific virtual filter set as listed in the table below.

Chemistry	Virtual Filter Set
Fluorescein/Rhodamine Dye Primer	A
Rhodamine Dye Terminators	
dRhodamine Terminators	E
BigDye™ Terminators	
BigDye™ Primers	

Color Guide for Data Display Windows

The CCD Camera and Virtual Filter Sets

Data collection software collects the fluorescent signal from specific locations on a CCD camera inside the instrument. These locations correspond to different wavelengths of light. The result is the same as using a physical filter to separate the light wavelengths. This is referred to as a virtual filter, since no physical filtering hardware is used.

Real-Time Display Colors Vary

On the real-time displays (the Scan and Gel windows), the data collection program displays the light 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 wavelengths of the dyes being detected, rather than the bases being detected.

Colors Represent Relative Wavelengths

Different virtual filter sets use the same four colors to represent different wavelengths, so the colors do not represent actual wavelengths. They represent the *relative* wavelengths of the four dyes in each dye set. For example, Filter Set A uses the four colors to represent wavelengths within Dye Set 1 and Dye Set 2.

Each of the chemistries used for preparing DNA is associated with a dye set. Each dye set labels the four bases differently, so the relative wavelength, and therefore the color, associated with each base varies with the chemistry used to label it. Due to this, the four colors on the real-time displays represent different bases, depending on the chemistry used for labeling. The tables below describe the colors that represent each of the four bases on the real-time displays.

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Raw Data Color Guide

The following tables lists the raw data display colors and dyes for the gel image and raw data based on the virtual filter set used.

Raw Data Colors for Virtual Filter Set A

	Fluorescein/Rhodamine Dye Primers		Rhodamine Dye Terminators	
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

Raw Data Colors for Virtual Filter Set E

	dRhodamine Terminators		BigDye Primers		BigDye Terminators	
Color	**Base**	**Dye**	**Base**	**Dye**	**Base**	**Dye**
Blue	G	dR110	C	FAM-dR110	G	FAM-dR110
Green	A	dR6G	A	FAM-dR6G	A	FAM-dR6G
Yellow	C	dTAMRA	G	FAM-dTAMRA	T	FAM-dTAMRA
Red	T	dROX	T	FAM-dROX	C	FAM-dROX

Analyzed Data Color Guide

The Sequencing Analysis program converts the information collected by the data collection program, so that after analysis the colors representing each base are consistent regardless of the chemistry used. The colors on all displays of analyzed data, including printed electropherograms, are as follows:

Color Guide for All Analyzed Data

Base	Color
C	Blue
A	Green
G	Black[a]
T	Red

a. G is shown as yellow in AutoAssembler™ software.

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Making an Instrument File for Virtual Filter Set A from Matrix Standards

Summary of Procedure

The following is a brief outline of the steps involved in making an instrument file.

Step	Action
1	Run the appropriate matrix standards for your instrument, verify that lane tracking is correct, and verify that peaks exist in the raw data.
2	Backup the raw sample files for the standards.
3	Make a new instrument file using the DataUtility program. **Note** You can also add matrix data to an existing instrument file. See “Adding or Replacing a Matrix in an Existing Instrument File” on page 7-20.
4	Verify the accuracy of the new instrument file by using it to analyze each matrix standard file (the raw data). See “Making an Instrument File for Virtual Filter Set E from Matrix Standards” on page 7-10.
5	Store the new instrument file. See “Storing and Backing Up the Instrument File” on page 7-19.

What Matrix Standards are Required?

An instrument file can contain a Dye Primer matrix, a Taq Terminator matrix, and/or a T7 Sequences Terminator matrix.

The instrument file for virtual filter set A must contain a dye primer matrix, even if this chemistry will not be used. Otherwise, the data collection software will not function properly.

This instrument file can also contain the matrix information for the Rhodamine Dye Terminators. (Chemistries used with virtual filter sets A and E are listed on page 7-4.)

Running Matrix Standards to Obtain the Raw Data

Perform a run on the instrument using the matrix standard samples required to create the instrument file.

IMPORTANT Do not configure the run sheet to automatically analyze the matrix standard samples. Select <none> for the Instrument File on the sample sheet. If necessary, deselect the Auto Analyze boxes on the run sheet by clicking the box to remove the X.

Proceed to “Verifying the Raw Data” on page 7-7 to verify the raw data is satisfactory, and to generate sample files.

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Verifying the Raw Data

After you run the matrix standards, the next step is to verify that the run was successful and you have raw data for the matrix.

The following procedure requires you open gel files; track and extract lanes; and create and view sample files. Refer to the *ABI PRISM® DNA Sequencing Analysis Software User's Manual* for instructions on how to perform these operations.

To verify lane tracking and peaks in the raw data:

Step	Action
1	Launch the Sequencing Analysis Software program.
2	Open the gel file, and make sure auto-analysis is deselected.
3	Track the matrix standard sample lanes, and extract them into sample files. **IMPORTANT** Because the Tracker program only recognizes red data, you have to adjust the tracker lines by hand for the green, blue, and yellow standards. **Note** The raw matrix data must be contained in sample files. If you extracted the matrix data in BioLIMS® mode, export the data to sample files using the Sample2DB program. (For more information on exporting BioLIMS database records, see the *ABI Prism BioLIMS Sample2DB Software User's Manual,* (P/N 4304072).)
4	Open each sample file in a Sample window and verify that peaks are present.
5	Open the File menu and select Quit to quit the Sequencing Analysis program.

Make Backup Copies of Raw Data

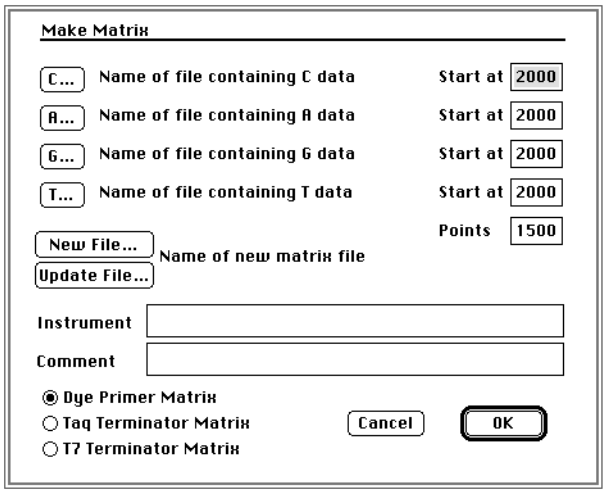
The sample files used to make an instrument file are altered by the DataUtility program when the instrument file is generated. Therefore, it is important to make backup copies of the standard sample files (the raw, unanalyzed data) before making the instrument file. If the new instrument file is no good, you can create another one using the backup copies of the sample files.

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Making the Instrument File

At this point in the procedure, you should have run the necessary matrix standard samples (“Running Matrix Standards to Obtain the Raw Data” on page 7-6), generated sample files and verified the raw data (“Verifying the Raw Data” on page 7-7), and made backup copies of the sample files.

To make an instrument file for virtual filter set A:

Step	Action
1	Open the DataUtility program. This program is located in the Utilities folder inside the Sequencing Analysis folder.
2	Open the Utilities menu and select Make Matrix. The Make Matrix dialog box appears. 
3	Specify the sample file to be used for each standard as follows: a. Click the C… button. b. In the directory dialog box that appears, select the file that contains the data from the C standard, then choose Open. c. Continue this selection process for the A, G, and T standards by clicking the A… button to select the A standard and so on. **Note** A separate matrix must be generated for each chemistry.

Box	Fluorescein/Rhodamine Dye Primer Matrix	Taq Terminator Matrix	T7 Terminator Matrix
C…	FAM	ROX	not used
A…	JOE	R6G	not used
G…	TAMRA	R110	not used
T…	ROX	TAMRA	not used

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To make an instrument file for virtual filter set A: *(continued)*

Step	Action
4	In each “Start at” text box, leave the default as is, or enter a different value. **Note** The defaults of 2000 for start point and 1500 for data points to be used for the matrix are almost always appropriate. Refer to the *ABI PRISM DNA Sequencing Analysis Software User's Manual* for information on how to determine values for the “Start at” and “Points” boxes.
5	In the Points text box, leave the default value as is or enter a different value. (See the Note in the preceeding step.)
6	Verify that the correct radio button for the type of chemistry being used is selected (Dye Primer, Taq Terminator, or T7 Terminator). If not, select the correct button.
7	Type information in the Instrument and Comment text boxes (e.g. the instrument serial number or name, and the date the matrix was created).
8	Click the New File… button, and enter a descriptive name for the file in the dialog box. Since instrument files are specific to instruments and chemistries use these to name the file, e.g. “<instrument name>-BigDye-InstFile”. **Note** Only alpha-numeric characters, the period (.), the dash (–), and the comma (,) are permissible characters for instrument file names.
9	Click Save. The dialog box closes, and the instrument file is saved in the ABI Folder in the System Folder.
10	Click OK to start the matrix calculation. The calculation takes about one minute. When the matrix is complete, the message “Make matrix successfully completed” is displayed. If an error message appears and the matrix is not made, see “Correcting Errors in Matrix Creation” on page 7-22.
11	Choose OK to close the Make Matrix dialog box, or wait about 20 seconds for the dialog box to disappear.
12	If you wish to add a matrix for another chemistry to the instrument file, repeat steps 2–11 with the following exceptions: a. For step 8, choose Update File… instead of New File… Select the instrument file to be modified. b. For step 9, click Open instead of Save.
13	Quit the DataUtility program.
14	Verify the instrument file by following the procedure “Checking Instrument Files” on page 7-16.

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Making an Instrument File for Virtual Filter Set E from Matrix Standards

Overview

When creating an instrument file for virtual filter set E, you must create three matrix files: dye primers, Taq terminator, and T7 terminator. All three matrix files must be made, even if you are only using one dRhodamine-based chemistry. Data collection software will not run with only a Taq or T7 terminator matrix in the file.

The T7 Terminator Matrix file is needed to analyze dRhodamine terminator and BigDye terminator sequencing data. It has a baselining algorithm associated with it that works well with these chemistries. The dRhodamine terminator and BigDye terminator dye set/primer files have tags in them that cause the Sequencing Analysis software to select this matrix file.

The correct placement of standards in the data utility application for making a Filter Set E instrument file is shown in the table below.

Table 7-1 Placement of Standards for Virtual Filter Set E

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

Perform the following procedures to make an instrument file for Filter Set E (dRhodamine-based chemistries):

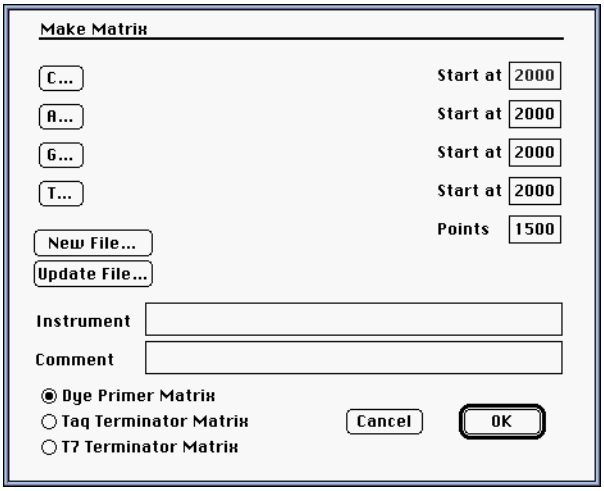
- “Making the Dye Primer Matrix” on page 7-11
- “Making the Taq Terminator Matrix” on page 7-14
- “Making the T7 Terminator Matrix” on page 7-15

When finished, check the instrument file by following the procedure “Making an Instrument File for Virtual Filter Set E from Matrix Standards” on page 7-10.

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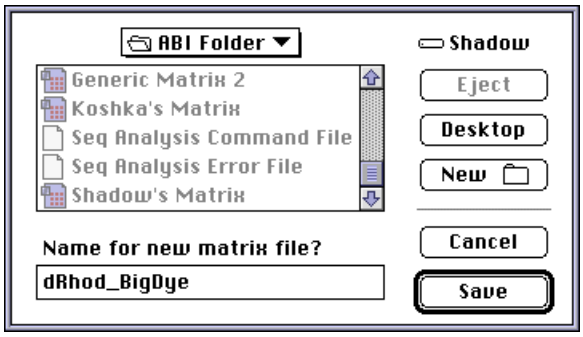
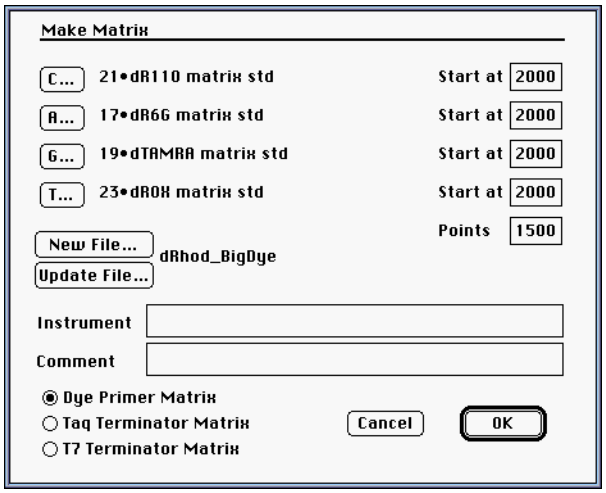
Making the Dye Primer Matrix

To make the Dye Primer Matrix:

Step	Action
1	Launch the Data Utility software (located in the Utilities folder within the Sequencing Analysis folder). The icon looks like this: 
2	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. 
3	Click on the box for each nucleotide base and select the sample file that corresponds to the correct matrix standard as shown in the table below. **Box** / **Dye Primer Matrix** C… — dR110 A… — dR6G G… — dTAMRA T… — dROX
4	For each matrix standard sample, start with the default value of 2000 for the start point. Start with the default value of 1500 for the number of data points to analyze. **Note** If the default values do not work, follow the instructions for using other values in steps 8 and 9 below.

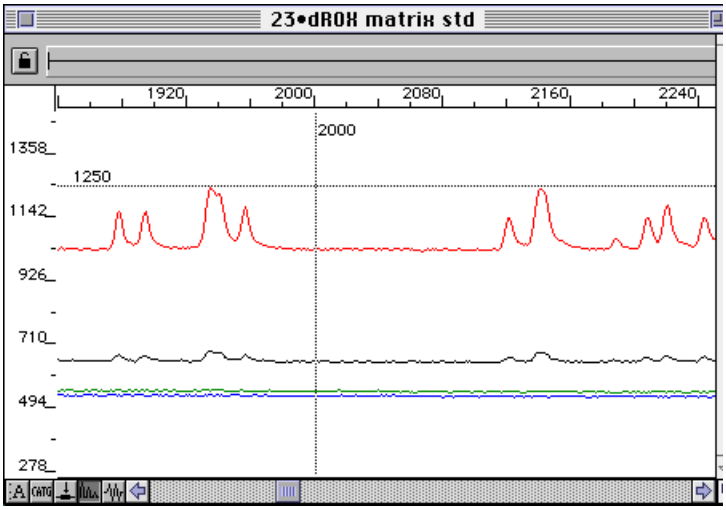
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To make the Dye Primer Matrix: *(continued)*

Step	Action
5	Click New File… A dialog window appears as shown below. Name the file dRhod_BigDye (or another appropriate name) and save it in the ABI folder within the System folder. 
6	The Make Matrix dialog box should look like that shown below.  a. Type information in the Instrument and Comment text boxes (e.g. the instrument serial number or name, and the date the matrix was created). b. Click OK. The computer makes the matrix. When finished, a dialog window appears with the message “Make matrix successfully completed.” c. Click OK.
7	If the computer is unable to make a matrix, examine the raw data again in the Sequencing Analysis software. If you used the default values, then select new start points as directed in steps 8 and 9. If many peaks are off-scale, dilute the matrix standards and rerun them.

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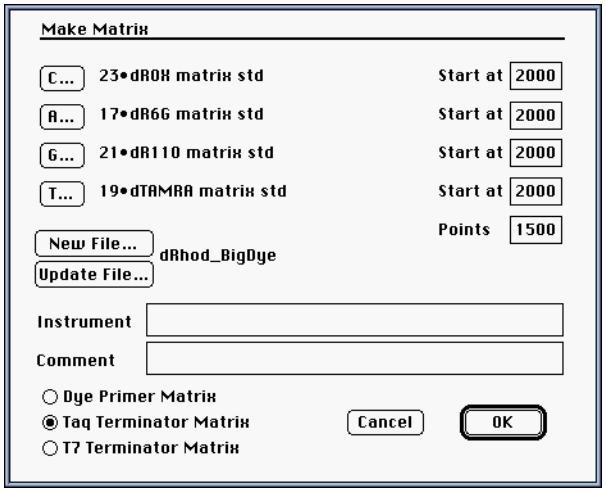
To make the Dye Primer Matrix: *(continued)*

Step	Action
8	If the matrix cannot be made with the default values, proceed with steps a, b, and c below. a. In the Sequencing Analysis software, open a matrix standard sample and examine the raw data. An example is 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 that the baseline at the end of the range is flat. A typical number of data points is 1500. 
9	Repeat step 8 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 that both the starting and ending points have flat baselines and no peaks.

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Making the Taq Terminator Matrix

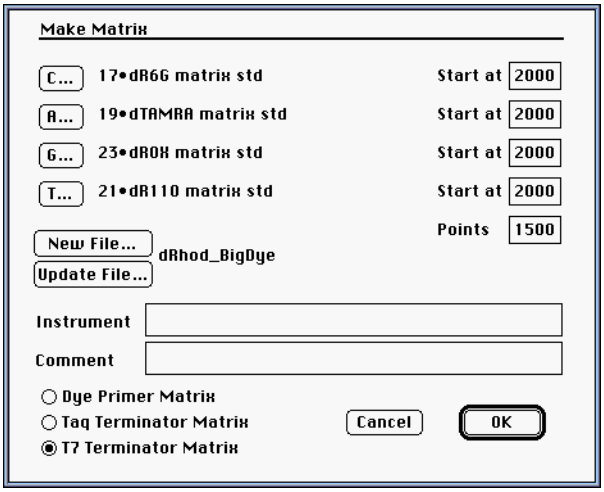
To make the Taq Terminator Matrix:

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 correct matrix standard as shown in the table below. **Box** / **Taq Terminator Matrix** C… / dROX A… / dR6G G… / dR110 T… / dTAMRA **IMPORTANT** The order of matrix standard data files is different from that in the Dye Primer Matrix (see Table 7-1 on page 7-10).
4	Enter the same numbers for each matrix standard sample in the Start at and Points boxes as were used for the Dye Primer Matrix.
5	Click Update File… A dialog window appears.
6	Choose dRhod_BigDye from the ABI folder within the System folder and click Open. The Make Matrix dialog box should look like that shown below. **Note** The numbers in the Start at and Points boxes are default values. Your numbers may vary. 
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.

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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 correct matrix standard as shown in the table below (note the order of the matrix standard files). **Box** / **T7 Terminator Matrix** C… / dR6G A… / dTAMRA G… / dROX T… / dR110
4	Enter the same numbers for each matrix standard sample in the Start at and Points boxes as were used in the Dye Primer Matrix and Taq Terminator Matrix.
5	Click Update File… A dialog window appears.
6	Choose dRhod_BigDye from the ABI folder within the System folder and click Open. The Make Matrix dialog box should look like that shown below. **Note** The numbers in the Start at and Points boxes are default values. Your numbers may vary. 
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.
8	Verify the instrument file by following the procedure “Checking Instrument Files” on page 7-16.

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Checking Instrument Files

Introduction

Instrument files can be verified two ways:

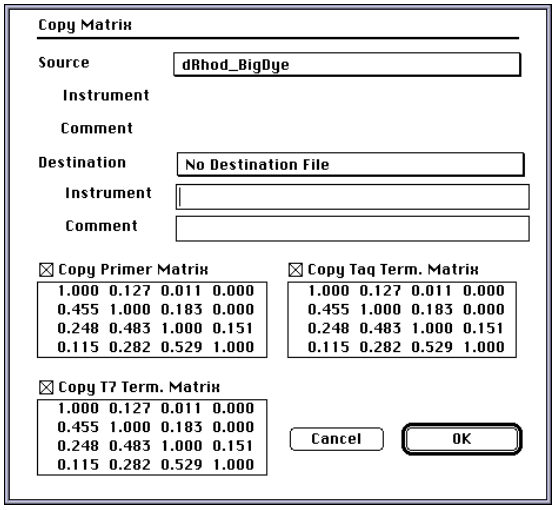
- Using the DataUtility program
- Using analyzed matrix standard samples

Using the DataUtility Program

This operation allows you to:

- Check the quality of the matrices in the instrument file
- Verify that the matrix is appropriate for the chemistry being used
- Determine if the matrix is responsible for poor data

To view the instrument file:

Step	Action
1	Open the DataUtility program.
2	From the Utilities menu, choose Copy Matrix…
3	Under Source, select Instrument file, and then select the instrument file name. The instrument file is displayed as shown below.  *For virtual filter set E,* all three matrix boxes will contain numeric values as shown here. The numeric values will be the same in each box. *For virtual filter set A,* numeric values will typically be displayed in the Primer and Taq Terminator matrix boxes only. The numeric values will not be the same in each box. **Note** The numbers shown here are not representative values, and will differ with each chemistry. If the numbers in the matrix appear misaligned, change the System Font from Charcoal to Chicago. (From the Finder, choose Options in the Appearance control panel.)
4	Make sure the numbers in all the boxes range from 0 to 1. The numbers on the diagonals from top left to bottom right should all be 1.
5	Click Cancel, and quit the DataUtility program.

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Using Matrix Standards

To verify the instrument file using matrix standards:

Step	Action
1	Open the Sequencing Analysis program.
2	Open the matrix standard files that were used to create the instrument file.
3	Use the electropherogram (analyzed) data view to confirm that the analyzed data looks good. In each file, you should see one color trace with obvious peaks, and all other color traces should be flat throughout the run. A pattern of pronounced peaks or dips in any of the other three colors indicate that something is wrong.
4	If all the data looks good, store and backup the new instrument file. See “Storing and Backing Up the Instrument File” on page 7-19**.** If the data does *not* look good, pick a different range of raw data points and remake the matrix. Be sure to use the backup copies of raw, unanalyzed data files. An analyzed file cannot be used to make an instrument file. Refer to the *ABI PRISM DNA Sequencing Analysis Software User’s Manual* for guidelines and a worksheet for selecting new start and data points.

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Making an Instrument File from a Sample File

Introduction

An instrument file can be made from matrix standards as explained above, or it can be made from a sample file. This procedure requires fewer steps than running matrix standards; however, the matrix made from a sample file may not be as good as one made from matrix standards. The quality of an instrument file made from a sample file depends on the quality of the sample file used.

The best samples to choose for making a matrix have approximately 25% each of A, C, G, and T. A good example of this is the pGEM DNA with the –21M13 primer that is included as a control in every Ready Reaction Sequencing Kit.

Making the Instrument File from a Sample File

To create an instrument file from a sample file:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Before making the matrix, verify that lane tracking is accurate. Adjust if necessary.</td></tr>
<tr><td>2</td><td>Duplicate the unanalyzed sample file four times. Use the Duplicate command from the File menu in the Finder. The four copies will have the following names:
♦ Sample name
♦ Sample name Copy 1
♦ Sample name Copy 2
♦ Sample name Copy 3
♦ Sample name Copy 4
These four sample file copies are used in the same way as the four matrix standard samples.</td></tr>
<tr><td rowspan="4">3</td><td>For Filter Set A instrument files:</td></tr>
<tr><td>Follow the procedure “Making an Instrument File for Virtual Filter Set A from Matrix Standards” on page 7-6. Whenever the procedure indicates a specific matrix standard to be used, follow the table below:
<table><tr><th>Matrix Standard</th><th>Standard File</th></tr><tr><td>C…</td><td>Sample name Copy 1</td></tr><tr><td>A…</td><td>Sample name Copy 2</td></tr><tr><td>G…</td><td>Sample name Copy 3</td></tr><tr><td>T…</td><td>Sample name Copy 4</td></tr></table></td></tr>
<tr><td>For Filter Set E instrument files:</td></tr>
<tr><td>Follow the procedure “Making an Instrument File for Virtual Filter Set E from Matrix Standards” on page 7-10. Whenever the protocol indicates a specific matrix standard to be used, follow the table below:
<table><tr><th>Matrix Standard</th><th>Standard File</th></tr><tr><td>dR110</td><td>Sample name Copy 1</td></tr><tr><td>dR6G</td><td>Sample name Copy 2</td></tr><tr><td>dTAMRA</td><td>Sample name Copy 3</td></tr><tr><td>dROX</td><td>Sample name Copy 4</td></tr></table></td></tr>
</table>

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To create an instrument file from a sample file: *(continued)*

Step	Action
4	Apply the newly made matrix back to the original sample file. There should be defined peaks and a flat baseline. If the baseline is not flat, or if there are dips or pull-up peaks, then the instrument file is wrong and should not be used.

Storing and Backing Up the Instrument File

Introduction

Valid instrument files must be placed in the ABI Folder in the System Folder. New instrument files should also be backed up. Obsolete instrument files should be deleted or archived.

Follow the steps in the table below after creating and verifying a new instrument file.

Storing and Backing Up Instrument Files

To properly store and backup instrument files:

Step	Action
1	Use the Finder to make sure the new instrument file is stored in the ABI Folder inside the System Folder. If you saved the file to a different location, drag it to the ABI folder now. To be used by the Sequencing Analysis program, the instrument file must be in the ABI folder.
2	Clean up the ABI folder by deleting any invalid instrument files.
3	Put a backup copy of the instrument file on a server or a disk (and put the disk in a safe location). It is a good idea to put the raw sample files for the standards in the same place.

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Adding or Replacing a Matrix in an Existing Instrument File

Introduction

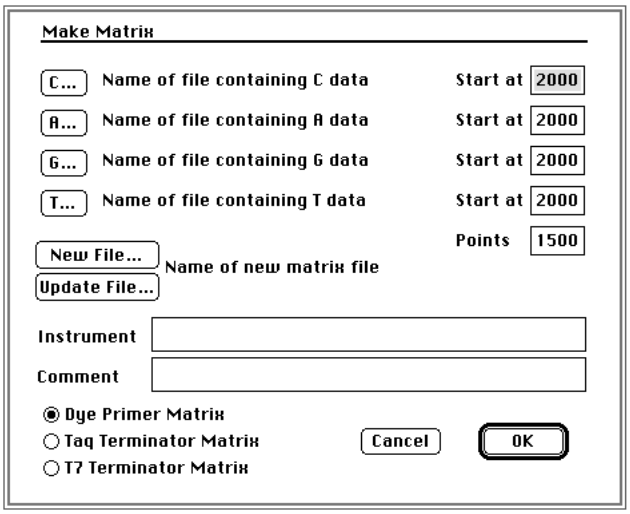
Use the procedure described below to:

- Add a matrix to an incomplete instrument file.
- Replace an existing matrix.
- Make an additional instrument file for testing purposes.

Note Be sure to make a backup copy of the original instrument file before you modify it.

Adding or Replacing a Matrix

To add or replace a matrix in an existing instrument file:

Step	Action
1	Open the DataUtility program.The program icon looks like this:  The program is located in the Utilities folder inside the Sequencing Analysis folder.
2	Choose Make Matrix from the Utilities menu. The Make Matrix dialog box appears.  **Note** The files you select for the four nucleotides are the sample files you named on the Sample Sheet when you electrophoresed the matrix standards.
3	Specify the sample file to be used for each standard. a. Click the C… button. In the directory dialog box that appears, select the file that contains the data from the C standard, then choose Open to close the dialog box. b. Repeat this selection process with for the A, G, and T standards.

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To add or replace a matrix in an existing instrument file: *(continued)*

Step	Action
4	In each “Start at” text box, leave the default as is, or enter a different value. **Note** The defaults of 2000 for start point and 1500 for data points to be used for the matrix are almost always appropriate. Refer to the *ABI PRISM DNA Sequencing Analysis Software User's Manual* for information on how to determine values for the “Start at” and “Points” boxes.
5	In the Points text box, leave the default value as is or enter a different value. (See the Note in the preceeding step.)
6	Click the Update File… button.
7	In the directory dialog box that appears: a. Select the name of the instrument file to which you want to add the new matrix. b. Click Open.
8	Click the appropriate button for the chemistry (Dye Primer, Taq Terminator, or T7 Terminator) at the bottom of the Make Matrix dialog box. **IMPORTANT** When you add a new matrix to an instrument file, it overwrites any existing matrix of the same type. Other matrices in the file are not affected.
9	Click OK to start the matrix calculation. The calculation takes about one minute.
10	When the message “Make matrix successfully completed” appears, click OK or wait approximately 20 seconds for the dialog box to disappear. If an error message appears and the matrix is not made, see “Correcting Errors in Matrix Creation” on page 7-22.
11	Verify the accuracy of the instrument file by following the appropriate procedure (“Making an Instrument File for Virtual Filter Set E from Matrix Standards” on page 7-10, or “Making an Instrument File from a Sample File” on page 7-18).
12	Store and backup the file as described under “Storing and Backing Up the Instrument File” on page 7-19.

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Correcting Errors in Matrix Creation

Common Problems

The two most common problems that can occur during matrix creation are:

- Signal too weak
- Incorrect files or chemistry selected, resulting in an error message stating the matrix was not made successfully

Signal Too Weak

If the signal size for any of the data is too small, an error message appears and the matrix is not made.

To correct for weak signal:

Step	Action
1	Open the Sequencing Analysis program.
2	Open sample file for the standard in the Sample window.
3	Open the Window menu and select Raw Data.
4	Find a data range with about 1500 points with reasonable signal strength. Write down the start and end points for that range. If the file does not contain enough good data, run a new set of matrix standards.
5	Repeat the Make Matrix process (or the Add/Replace Matrix process on page 7-20), using the new start point and data range numbers.

Incorrect Files or Chemistry

If any of the files selected are obviously incorrect, or if the wrong chemistry button was selected, an error message appears and the matrix is not made.

To correct file or chemistry selection:

Step	Action
1	Repeat the Make Matrix process selecting the correct chemistry button for the correct set of matrix sample files.
2	Use the gel file to verify that matrix sample files contain the dye that the file indicates. “Color Guide for Data Display Windows” on page 7-4 explains the correlation between the colors in the gel file and the base that each color represents. **IMPORTANT** The gel file in the data collection program shows unconverted raw data, so the colors displayed represent different bases depending on the chemistry.

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8 System Maintenance

Chapter Contents

In this Chapter

This chapter describes how to maintain the instrument and computer. The maintenance procedures in this chapter can be performed by users of the ABI PRISM® 377 DNA Sequencer. All other maintenance procedures should be performed by a trained Applied Biosystems service technician.

Topic	See Page
Instrument Maintenance Recommendations	8-2
Cleaning Instrument Accessories	8-3
Refilling the Water Reservoir	8-5
Replacing the Upper Buffer Chamber Gasket	8-6
Replacing Electrophoresis Cables and Electrodes	8-12
CCD Pixel Position Value	8-14
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Optimizing the Hard Disk	8-19
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Instrument Maintenance Recommendations

How to Maintain the Instrument

ELECTRICAL HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

Recommendation	For more information see …
After every run: ♦ Wipe the front and rear heat-transfer plates and positioning pins with damp, lint-free towels and allow to air dry. ♦ Remove any liquid present in the electrophoresis chamber. ♦ Wipe the gel cassette clean with a damp, lint-free towel and allow to air dry. ♦ Clean the comb and spacers with deionized water. Do not use an organic solvent. ♦ Rinse the buffer chambers with deionized water and allow to air dry.	♦ "Cleaning Instrument Accessories" on page 8-3 ♦ Chapter 2, "Pouring Gels" ♦ Chapter 3, "Instrument Operation"
Weekly: ♦ Check the water reservoir level, and refill as necessary.	"Refilling the Water Reservoir" on page 8-5

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Cleaning Instrument Accessories

How To

Thoroughly clean instrument accessories after each run as described below:

Accessory	Method of Cleaning
Glass plates	We strongly recommend cleaning glass plates in a laboratory dishwasher with a hot, deionized water rinse cycle. See “Cleaning Glass Plates” below for more information.
Heat transfer plates	Wipe with damp, lint-free towels and allow to air dry on a non-scratch surface.
Positioning pins in electrophoresis chamber	Wipe with damp, lint-free towels and allow to air dry. Gel or TBE crystals collect on them (white, cakey substance) and can cause arcing. CAUTION **Arcing is a luminous, low voltage, high current electrical discharge. Arcing can severely damage the instrument.**
Spacers and comb	Clean with deionized water and allow to air dry. Refer to Chapter 2, “Pouring Gels,” for more information. **Note** Do not clean spacers and combs with organic solvents. These tend to adhere and do not evaporate well. Residual solvent can prevent the gel from polymerizing right up against the spacer. Do not mark spacers or combs with liquid markers such as Sharpie® markers.
Gel cassette	Wipe with damp, lint-free towel and allow to air dry. Must be completely dry before the next run or arcing can occur. See the Caution statement above.

Cleaning Glass Plates

Clean plates, spacers, and combs are critical for successful gel preparation and a successful run. Plates that are cleaned thoroughly and consistently will also help avoid the temporary loss of signal that can occur sporadically on this instrument.

Our research indicates this loss of signal is due to contaminant molecules (surfactants, fatty acids, long chain polymers) attached to the surface of the plates. It manifests itself as a band of little or no signal across the entire width of the gel image. It usually occurs between 140 to 200 base pairs, and typically lasts the equivalent of 20 to 40 base pairs. Following this band, signal strength usually returns to normal.

Glass plates can be cleaned manually or in a dishwasher. The use of a laboratory dishwasher with a hot (195°F/90°C) deionized water rinse cycle has been found to effectively remove suspect contaminants, thereby eliminating any temporary loss of signal.

Using a Dishwasher

We strongly recommend cleaning glass plates in a laboratory dishwasher with a hot (195°F/90°C) deionized water rinse cycle. Using a dishwasher helps ensure plates are cleaned effectively and consistently every time, and will also eliminate the sporadic, temporary loss of signal that can occur on this instrument (described above.) Deionized water is required for the rinse cycle only. Dishwasher recommendations are listed on page 8-4.

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When using a dishwasher, we recommend you:

- Connect the dishwasher to a high-grade, deionized water source.
- Clean the plates as soon as possible once the gel is removed.
- Rinse residual gel from the plates before loading in a dishwasher.
- Initially use the longest deionized water rinse option on the dishwasher, followed by a drying cycle. After some experimentation, you may be able to reduce the rinse time.
- Do not use a detergent.
- Avoid excessive handling of dry plates with ungloved hands.

Recommended dishwashers are listed below.

If a dishwasher is not available, follow the procedure listed in Chapter 2, “Pouring Gels.”

Recommended Dishwashers

The following dishwashers have been found to work well. A customized plate rack may be required.

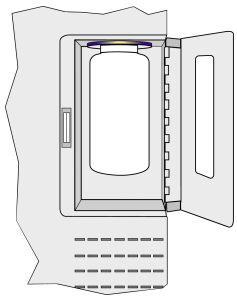
Item	Part Number	Supplier
Lancer 1600 dishwasher with facility for drying	Lancer 1600 UP	Lancer USA Inc. 705 West Highway 434 Longwood, Florida 32750 Telephone: 407-332-1855 Lancer UK Ltd. 1 Pembroke Avenue Waterbeach, Cambridge CB5 9QR Telephone: 44-01223-861665 Fax: 44-01223-861990
Sequencing plate rack (50 plate capacity) for Lancer dishwasher	SPR 16	Lancer USA Inc. as listed above
Labconco Undercounter SteamScrubber Washer/Dryer	15-352-801	Fisher Scientific U.S. Headquarters 585 Alpha Drive Pittsburgh, Pennsylvania 15238 Customer Service: 1-800-766-7000 Fax: 1-800-926-1166 Internet: http://www.fishersci.com

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Refilling the Water Reservoir

Procedure Check the water level in the reservoir once a week. Refill the reservoir when it is between one-third and one-half full.

To refill the water reservoir:

Step	Action
1	Turn the pump off if it is running. **IMPORTANT** Do not remove the water reservoir while a run is in progress.
2	Open the water reservoir compartment on the right side of the instrument by pulling gently on the door. 
3	Unscrew the water reservoir and remove it by pulling downward.
4	Place a paper towel under the tubes connecting the reservoir to the pump to catch any drips.
5	Fill the reservoir approximately 3/4 full (750–850 mL) with a solution of deionized water and 5.0% antifreeze (any brand).
6	Replace the reservoir, and insert the tubes that connect it to the pump before screwing the reservoir into place.
7	Screw the reservoir into place, and close the compartment door.

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Replacing the Upper Buffer Chamber Gasket

Two Styles of Upper Buffer Chambers

Your instrument will have one of two styles of upper buffer chambers. The earlier version (illustrated on page 8-7; P/N 604078) is white with a convex window, and is referred to in this section as the white upper buffer chamber. The version released in mid-1998 (illustrated on page 8-10; P/N 4304406) is transparent with a flat window, and is referred to in this section as the transparent upper buffer chamber. Separate instructions are provided for each chamber.

Parts and Tools Required

For the Transparent Upper Buffer Chamber

Parts and Tools	Kit Part Number	Supplier
ABI PRISM 377 Buffer Chamber Gasket Kit (contains 2 gaskets (P/N 430440), sealant (P/N 201480), and instructions)	7307172	Applied Biosystems
Latex gloves	N/A	Major laboratory supplier
Kimwipes® or similar lint-free wipes	N/A	Major laboratory supplier
Spatula (must fit into groove of buffer chamber to remove old sealant)	N/A	Major laboratory supplier

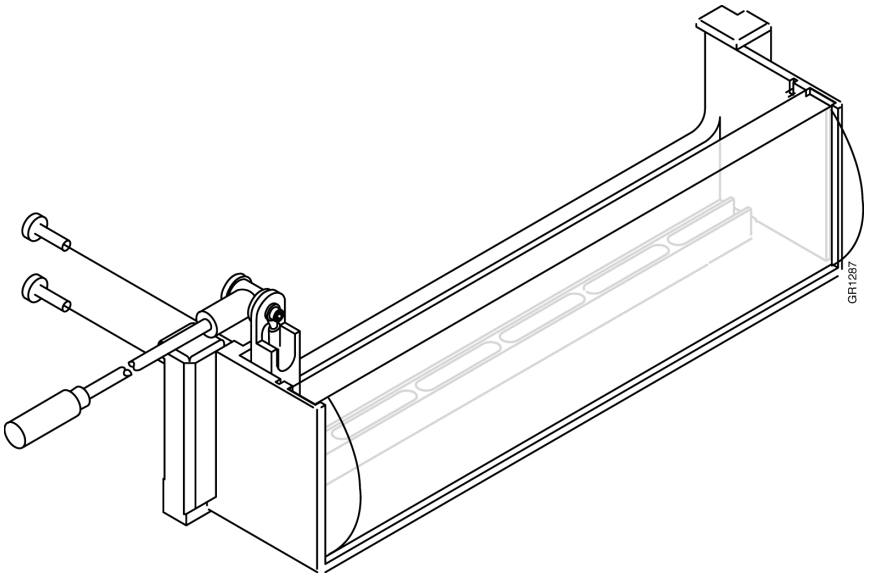
For the White Upper Buffer Chamber

Parts and Tools	Kit Part Number	Supplier
ABI PRISM 377 Buffer Chamber Gasket Kit (contains 5 gaskets, sealant, and instructions)	604524	Applied Biosystems
Latex gloves	N/A	Major laboratory supplier
Kimwipes or similar lint-free wipes	N/A	Major laboratory supplier
Scrap glass plate or flat rigid plastic plate at least 10” x 4”	N/A	Major laboratory supplier
Spatula (must fit into groove of buffer chamber to remove old sealant)	N/A	Major laboratory supplier

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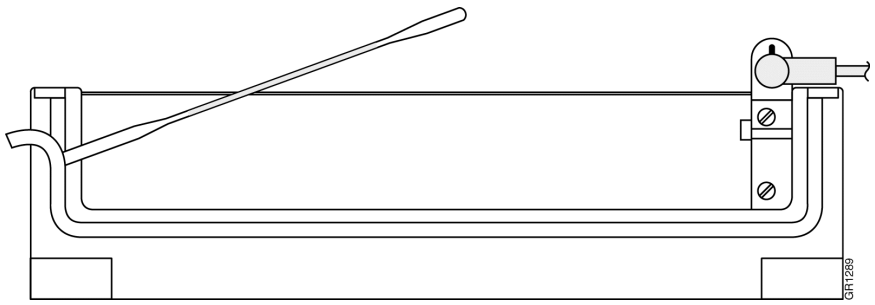
Procedure for White Upper Buffer Chamber

The following is an illustration of the white upper buffer chamber.



Removing the Old Gasket

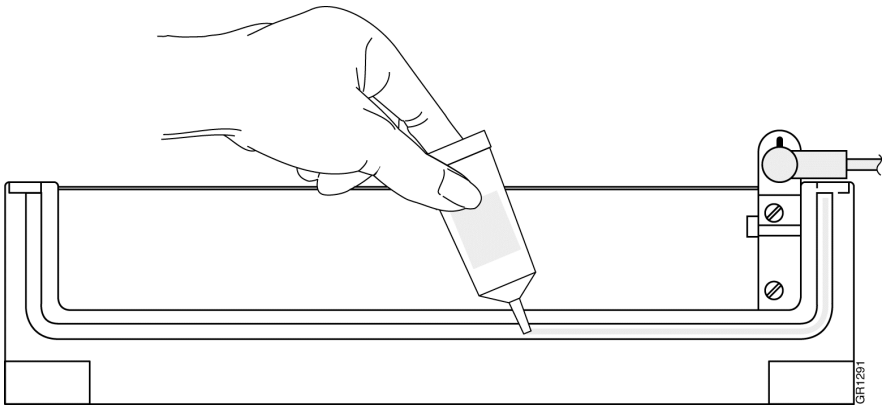
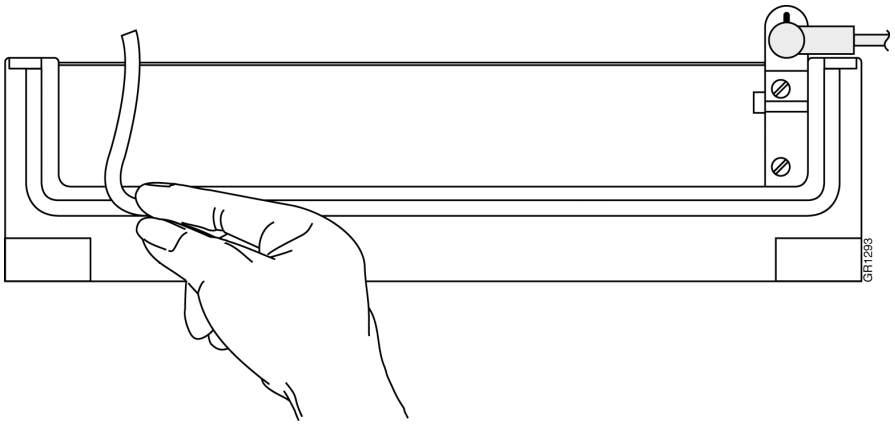
ELECTRICAL SHOCK HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

Step	Action
1	Disconnect the electrophoresis cable from the instrument and remove the buffer chamber.
2	Place the buffer chamber on a piece of clean lab paper so the lens is not scratched and does not touch the work surface.
3	Using the spatula, remove the old gasket and sealant from the groove of the buffer chamber. Be sure to remove all residual glue from the bottom of the groove. **IMPORTANT** Do not use solvents to remove residual glue. Solvents can damage the chamber. 

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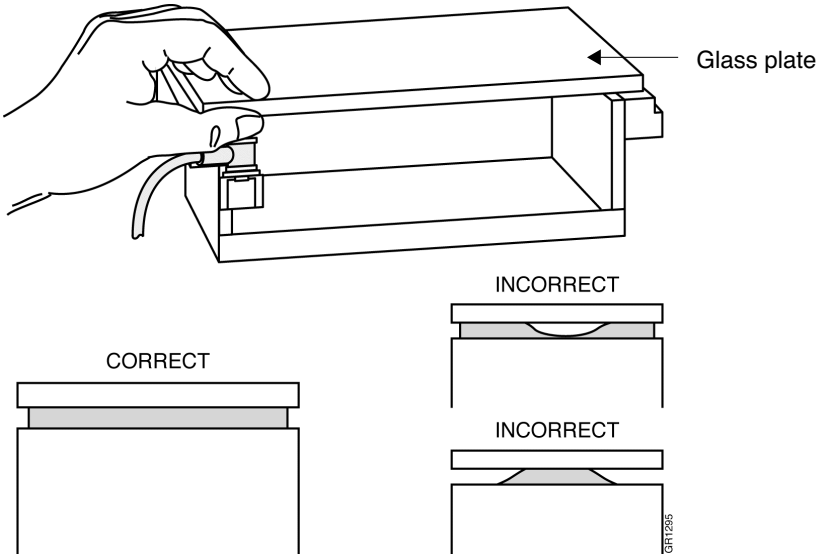
Installing the New Gasket

To install the new gasket:

Step	Action
1	Fit the new gasket into the groove to ensure a proper fit. The rounded surface faces out. The surface with the channel must contact the bottom of the groove in the chamber. When properly fitted, remove the gasket. ♦ Trim the gasket if too long. ♦ The gasket can be short within 1 mm of each end.
2	Wearing latex gloves, apply a 1/16" (1.5 mm) diameter bead of sealant into the center of the groove. Apply a consistent amount throughout the groove. 
3	Place the gasket in the groove.
4	Starting from one side of the chamber, use your index finger to gently press the gasket into the groove with a rolling motion. **IMPORTANT** Do not use excessive force to push the gasket into the groove. Excessive force or stretching the center portion of the gasket can distort the material and adversely affect sealing. 
5	Remove excess sealant with a Kimwipe. Be especially careful that no sealant is left on the surface of the gasket that will contact the glass plate.
6	Stretch the ends of the gasket to the ends of the groove if necessary, and hold the ends in place for 10 seconds. CAUTION **Never stretch the center portion of the gasket.**

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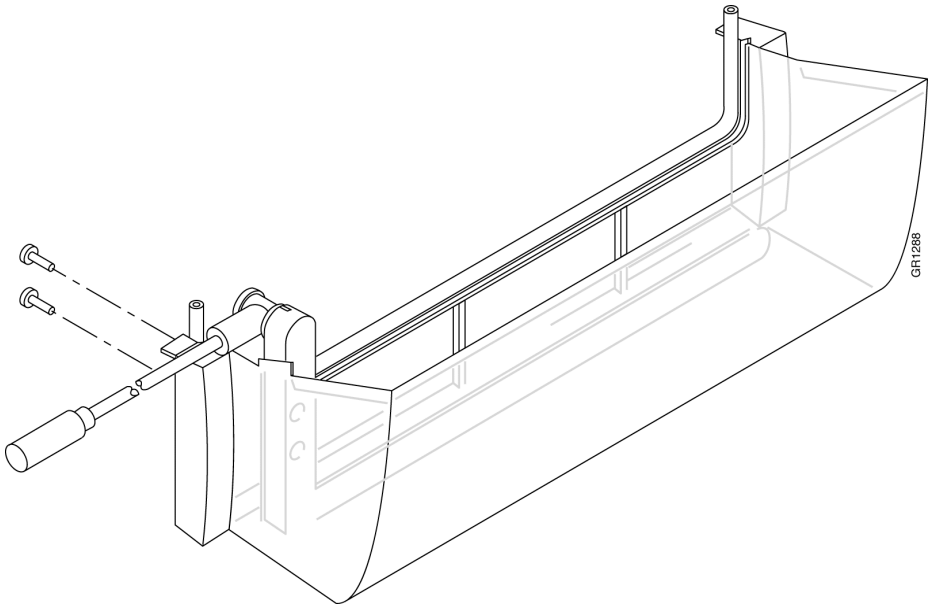
To install the new gasket: *(continued)*

Step	Action
7	Place the scrap glass or plastic plate on top of the gasket, making sure the plate sits flat. **IMPORTANT** Use scrap glass only. Do not use glass plates that are also used for gels. Residual sealant will ruin gel plates.
8	Press gently and evenly on the plate with your fingertips, and hold for 15 seconds.
9	Visually verify that there are no gaps between the plate and the gasket. If a gap exists, remove the plate and use your fingertips to flatten the gasket. Then return to step 7. If a gap still exists, remove the gasket, clean off the sealant, and return to step 1. Once there are no gaps, remove the glass plate and proceed to the next step. 
10	Carefully remove all excess sealant with a Kimwipe.
11	Allow the sealant to cure for a minimum of 2 hours to overnight before use.

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Procedure for Transparent Upper Buffer Chamber

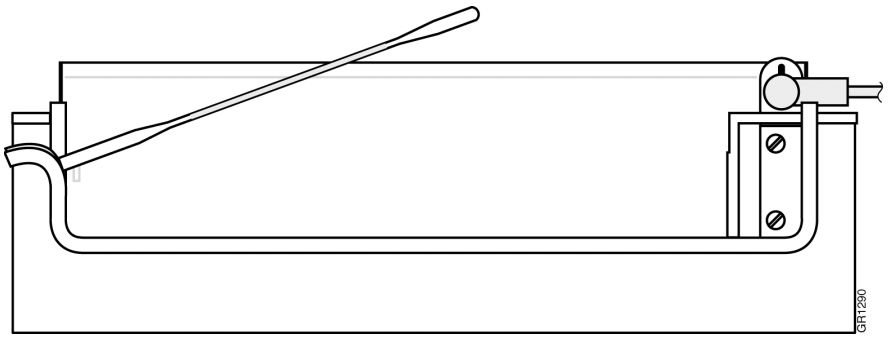
The following is an illustration of the transparent upper buffer chamber.



Removing the Old Gasket

ELECTRICAL SHOCK HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

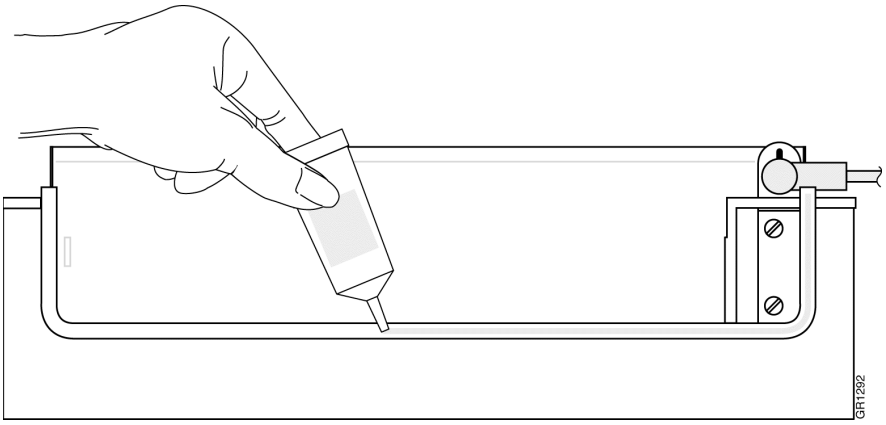
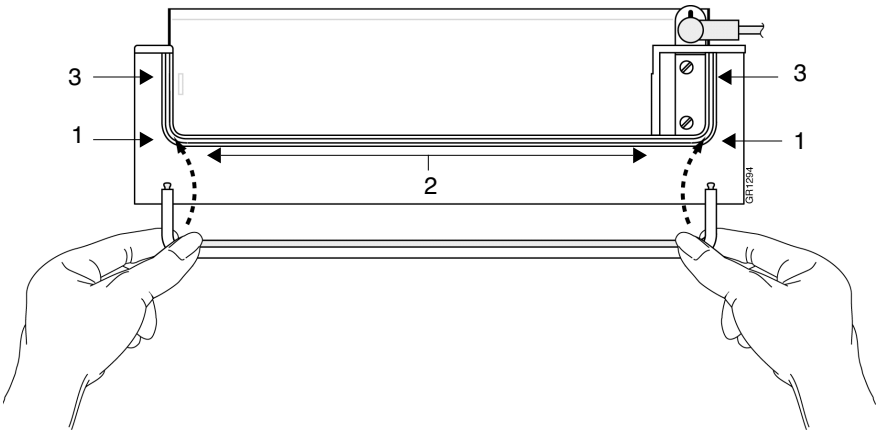
To remove the old gasket and sealant:

Step	Action
1	Disconnect the electrophoresis cable from the instrument and remove the buffer chamber.
2	Place the buffer chamber on a piece of clean lab paper so the lens is not scratched and does not touch the work surface.
3	Using the spatula, remove the old gasket and sealant from the groove of the buffer chamber. Be sure to remove all residual glue from the bottom of the groove. **IMPORTANT** Do not use solvents to remove residual glue. Solvents can damage the chamber. 

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Installing the New Gasket

To install the new gasket:

Step	Action
1	Place the buffer chamber on clean lab paper to avoid scratching it.
2	Wearing latex gloves, apply a 1/16” (1.5 mm) diameter bead of sealant into the center of the groove. Apply a consistent amount throughout the groove. 
3	Place the gasket in the groove as follows: a. Hold the curves of the gasket as shown below, and place the gasket into the groove. b. Gently press the gasket in place starting with both positions 1 (the two curves of the gasket), then position 2, and then each position 3. The gasket is longer than the groove. Do **not** cut off the ends of the gasket, otherwise the gasket will leak. It is a sealed gasket that must remain sealed to work effectively. 
4	Remove excess sealant with a Kimwipe. Be especially careful that no sealant is left on the surface of the gasket that will contact the glass plate.
5	Install the buffer chamber onto a cassette with glass plates for at least two hours before filling it with buffer. This will ensure good adhesion and allow the sealant to cure.

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Replacing Electrophoresis Cables and Electrodes

Overview

Electrophoresis cables and electrodes are built as assemblies to simplify their installation. Therefore, change the entire assembly when replacing a cable or electrode. Part numbers for the assemblies are as follows.

Buffer Chamber	Electrode Assembly Part Number
Upper	254304
Lower	254303

Procedure

ELECTRICAL SHOCK HAZARD. The ABI Prism 377 contains a high voltage power supply. Although the instrument has been designed with safety features in the door to disconnect the power supply when the door is open, please follow procedures as prescribed. As with any electrophoresis apparatus, be careful during instrument operation and when handling electrodes and liquids.

To replace an electrophoresis cable and electrode assembly:

Step	Action	
1	Disconnect the electrophoresis cable from the instrument and remove the buffer chamber.	
2	**To remove the cable/electrode assembly from the …**	**Follow these steps …**
	upper buffer chamber	a. Remove the two plastic screws on the back left end of the upper buffer chamber (Figure 8-1 or Figure 8-2 on page 8-13). b. Remove the assembly from the buffer chamber.
	lower buffer chamber	The assembly in the lower buffer chamber is held under a hook at the right end of the chamber. a. Remove the flathead plastic screw. b. Lift the electrode assembly from the left side. c. Pull it from beneath the hook at the right end of the buffer chamber.
3	Insert the new assembly into the buffer chamber. If replacing the assembly in the lower buffer chamber, be sure the assembly is properly situated under the hook at the right end of the chamber.	
4	Reinstall the screw(s).	

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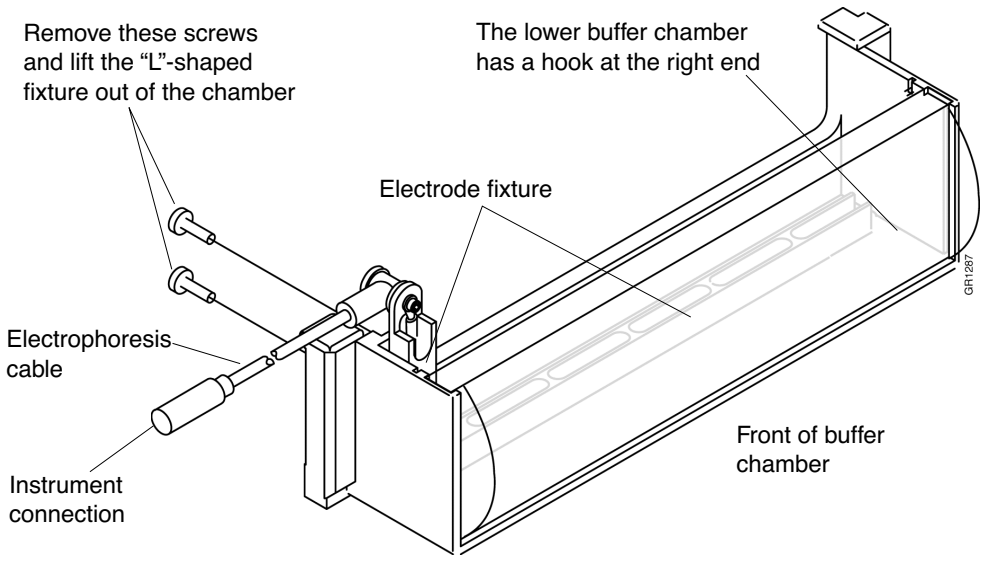


Figure 8-1 Cable and electrode assembly in the white upper buffer chamber (P/N 604078)

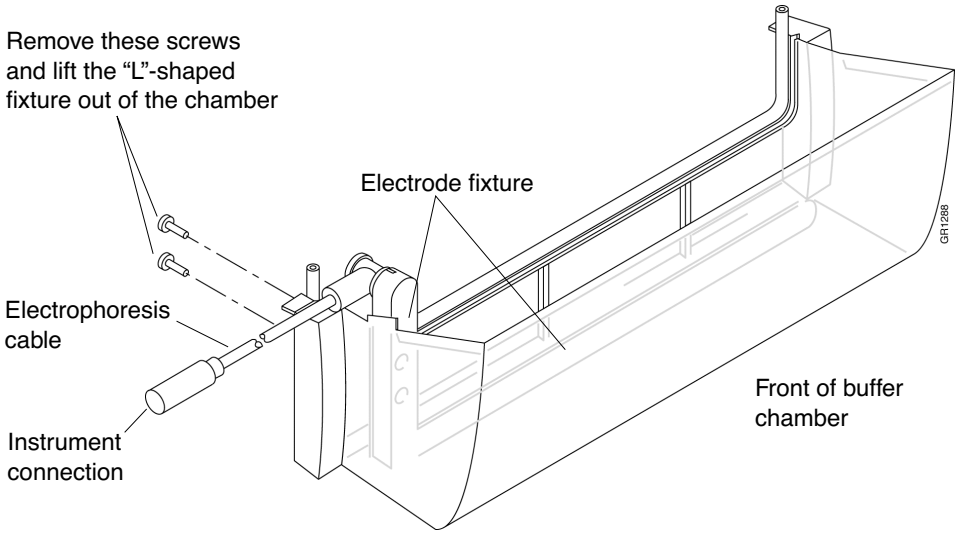


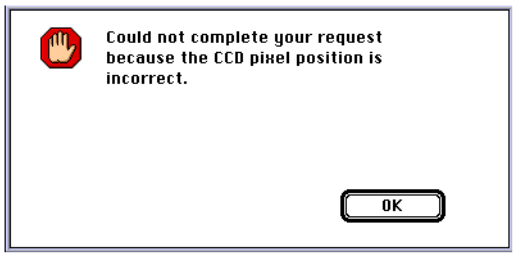
Figure 8-2 Cable and electrode assembly in the transparent upper buffer chamber (P/N 4304406)

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CCD Pixel Position Value

Overview

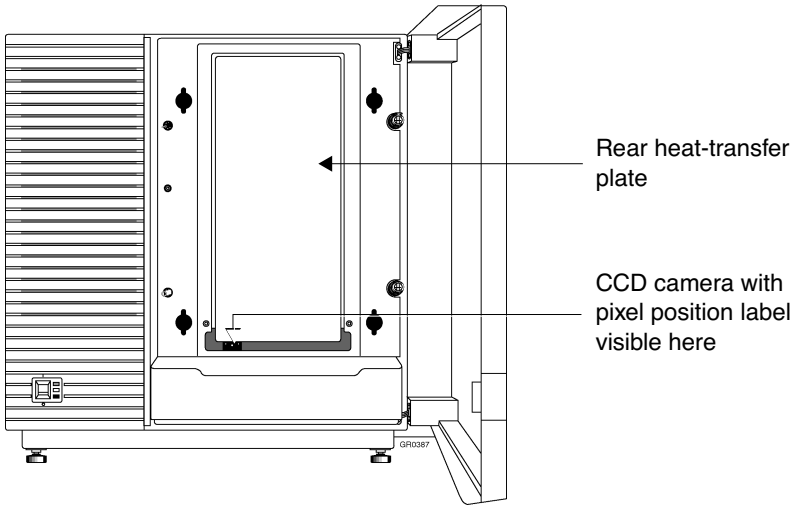
The charge coupled device (CCD) pixel position value is a reference point for alignment of the CCD camera with the laser beam. The instrument is shipped with the correct CCD pixel position value in memory. When you start a run, data collection software checks for a value greater than zero. If not found, the following error message is displayed.



Perform the following procedures to locate and reenter the correct CCD pixel position value before using the instrument.

Locating and Entering the CCD Pixel Position Value

The correct CCD pixel position value is printed on a white label affixed to the CCD camera. The label is visible from the front of the instrument through the opening below the rear heat-transfer plate.



To locate the CCD pixel position value:

Step	Action
1	Have a flashlight available.
2	Open the front door of the instrument.
3	Shine the flashlight through the opening below the rear heat-transfer plate, and locate the white label on the CCD camera.
4	Record the value on the white label.
5	If you cannot find the label, call technical support (telephone numbers in Chapter 1.)

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To enter the correct CCD pixel position value:

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu in data collection software.
3	Open the Fxn Name pop-up menu, and select the CCD Pixel Position function. The current pixel position value is displayed. If it is the same as the value on the white label, do not complete the remaining steps. If the value is different, continue.
4	Select the text box, and type the correct CCD pixel position value.
5	Click Execute.
6	If the CCD Pixel Position error occurs again, call technical support (see Chapter 1.)

Using Calibration File Make

Use the Calibration File Make function from the Manual Control window to create the ABI 377 Calibrations file and store the CCD pixel position value in it. You can add the instrument serial number to the file as described below. The ABI 377 Calibrations file is placed in the Preferences folder inside the System folder.

IMPORTANT You must have the correct CCD pixel position value in instrument memory before using Calibration File Make.

Creating an ABI 377 Calibrations File

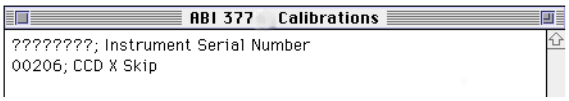
To create an ABI 377 Calibrations file:

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu in data collection software.
3	Open the Fxn Name pop-up menu, and select Calibration File Make.
4	Click Execute, and select Save from the File menu.

Adding the Serial Number to the ABI 377 Calibrations File

You can add the instrument serial number to the ABI 377 Calibrations file using Simple Text, which is provided with the Macintosh® operating system.

To enter the instrument serial number:

Step	Action
1	Open the System Folder, the Preferences Folder inside it, and the ABI 377 Calibrations file.
2	Select the series of question marks displayed, and type the instrument serial number in their place. (The serial number is located on the back of the instrument.) 
3	Select Save from the File menu.
4	Close the ABI 377 Calibrations file.
5	Quit the SimpleText program.

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Using Calibration File Send

Use Calibration File Send from the Manual Control window to send the CCD pixel position value and instrument serial number to the instrument. The serial number is included in all Sample files created by the instrument and can be viewed in the annotative view of the sample file. This is useful for instrument identification, particularly if you operate more than one instrument.

To send a Calibration file to the instrument:

Step	Action
1	Turn power on to the instrument.
2	Select Manual Control from the Window menu.
3	Open the Fxn Name pop-up.
4	Select Calibration File Send.
5	Click Execute.

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Computer Maintenance Recommendations

How To Maintain Maximum Operating Efficiency

Computers require regular maintenance to operate efficiently and consistently. Because the ABI PRISM 377 software works with large files and accesses the hard disk often, it is important to follow the procedures described in this section. Adherence to these recommendations will minimize the occurrence of errors during operation, and will help maintain maximum operating efficiency.

Recommendation	For more information see …
Restart the Macintosh before each run.	"Restarting the Computer" on page 8-18
Rebuild the desktop: ♦ Once a month ♦ After installing new software ♦ After running Norton Utilities™	"Rebuilding the Desktop" on page 8-18
Delete (or store on another medium and then delete) data files and other non-essential files from the hard disk at least once a week. Data files are gel files*, sample sheets, run sheets, and sample files. This practice will ensure that enough disk space is always available to run the instrument. *Because gel files are so large (20–70 MB), we recommend deleting them as soon as satisfactory sample files are generated.	♦ "Deleting Data Files" on page 8-18 ♦ "Archiving Data from Runs" on page 8-23 Includes a short description of each type of data file.
Optimize the hard disk when fragmentation is severe. Check the level of fragmentation once a month using Norton Speed Disk. **IMPORTANT** Always backup important files before optimizing the hard disk.	♦ "Optimizing the Hard Disk" on page 8-19 ♦ "Norton Utilities" on page 8-22
Backup all programs and important files regularly. Examples include: ♦ Analysis and data collection software ♦ Matrix/instrument files ♦ Customized files such as custom modules and analysis parameters ♦ Size standard files	♦ "Backing Up Important Files" on page 8-20
Do not add other software programs to the Macintosh that runs the instrument.	"Adding Non-Applied Biosystems Software" on page 8-28
Install only one System file per hard disk.	"System File" on page 8-29
Configure SAM™ to scan the desktop only.	See "SAM" on page 8-21
Disable Norton's automatic optimization features.	See "Norton Utilities" on page 8-22

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Restarting the Computer

Overview Restart the Macintosh once each day or before every run. Restarting the computer clears the random access memory (RAM). This helps ensure enough memory is available to begin data collection.

Rebuilding the Desktop

Overview Rebuilding the desktop cleans up the Desktop system file. This file:

- Is invisible to users
- Keeps track of where everything is located on the hard disk
- Grows larger over time if it is not rebuilt

If the Desktop system file grows too large, the computer's response time will slow to unacceptable levels. Therefore, we recommend rebuilding the desktop:

- Once a month
- After installing new software
- If you get a system error message with a bomb after inserting a disk

Procedure To rebuild the desktop:

Step	Action
1	Hold down the Command and Option keys, and choose Restart from the Special menu.
2	Continue to hold down the two keys until the message "Are you sure you want to rebuild the desktop file …?" appears on the screen.
3	Click OK.

Deleting Data Files

Overview We recommend removing unnecessary files from the hard disk before starting the data collection program. For example, the ABI PRISM 377 data collection and analysis software programs create large data files (gel files, sample files, sample sheets, and run sheets) that accumulate on the hard disk. Data files include gel files, sample files, sample sheets, and run sheets. These files can quickly fill all the available memory on the computer.

Backup data files and other files that are not used regularly, and then delete the original files from the hard disk to reclaim memory for future work. This practice will ensure that enough disk space is available for new data files.

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Procedure To delete a file from the hard disk:

Step	Action
1	If you wish to save the file, store it on a floppy disk or other storage medium.
2	Drag the file into the trash can.
3	Open the Special menu and choose Empty Trash.
4	Click OK.

Optimizing the Hard Disk

Why Optimize the Hard Disk

Over time, files stored on the hard disk become *fragmented*—stored in pieces in various locations on the disk. As the amount of fragmentation increases, computer performance slows because it takes the computer longer to find all the pieces.

Optimizing the hard disk:

- Reduces fragmentation
- Frees up disk space
- Helps maintain optimum computer performance

Recommendations

We recommend:

- Checking the fragmentation level of the hard disk once a month using Norton Speed Disk™.
- Optimizing the hard disk when the fragmentation level is severe.
- Turning off automatic optimization features. Enabling these features can result in loss of data if the utility decides to optimize the hard disk while a run is in progress and data is being collected.

Refer to the Norton Utilities user's manual for instructions on using Norton Speed Disk.

Before Optimizing the Hard Disk

Before optimizing the hard disk, back up all important files onto floppy disks or another storage medium. Make sure you know where all the original software program disks (or copies of the programs) are located, should it become necessary to reload all the system software. See "Backing Up Important Files" on page 8-20, and "System Software" on page 8-25.

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Backing Up Important Files

Overview

Backing up means making a copy of the programs and files present on the hard disk, and storing the copies in a location other than the hard disk of the computer that runs the instrument. Various methods can be used to backup files, such as:

- Copying the files onto a portable disk, such as a Zip™ disk
- Copying the files to a server on a network
- Copying the files onto a hard drive other than the one used to run the instrument
- Using Disk Copy to make image files of software programs from the original floppy disks directly onto a hard drive or other storage media

Recommendations

To avoid losing data files and software programs in the event of a hard disk failure, we strongly recommend:

- Backing up all important data files (gel files, sample files, run sheets, and sample sheets) once each day
- Making a copy of each important software program using an application such as Disk Copy
- Backing up other important files the same day they are created (*e.g.* custom modules, matrix/instrument files, size standard files, analysis parameter files)
- Before optimizing the hard disk:
  - Backing up all important files
  - Making sure the original or a copy of all the system software is available should it become necessary to reinstall the software

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SAM

Product Overview

Symantec AntiVirus for Macintosh (SAM™) is comprehensive virus prevention, detection, and elimination software. Computer viruses are programs that replicate themselves in the computer system. They can spread throughout the programs on your hard disk, or spread over a network, eventually causing severe damage.

SAM performs the following functions:

- Checks applications for viruses when they are run
- Checks Microsoft Word documents and Excel worksheets for macro viruses
- Checks floppy disks for viruses when inserted into the hard drive
- Checks files downloaded from the Internet, a BBS, or email
- Monitors the Macintosh for activity that might indicate the presence of a virus

All of the software supplied with the ABI PRISM 377 DNA Sequencer is free of computer viruses. However, any additional software installed on the computer could have a virus.

Recommendations

We recommend:

- Keeping SAM activated on the Macintosh at all times.
- Setting up SAM so that it scans the desktop only. Otherwise, it may scan the hard drive while data is being collected, resulting in lost data.

Refer to the SAM user's manual for more detailed information.

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Norton Utilities

Product Overview

Norton Utilities™ is a collection of software utilities designed for data protection and recovery.

Use the following utilities to repair or recover disks, folders, or files:

- Norton Disk Doctor—repairs hard disks
- Volume Recover—restores accidentally initialized (but not formatted) hard disks or badly damaged disks using FileSaver information
- UnErase—Recovers files that have been thrown away or are not visible due to disk damage
- Norton Disk Editor—Edits data directly on the hard disk

Use the following utilities to prevent disk problems from becoming serious, and to minimize the effects of unavoidable damage:

- FileSaver
  - Checks disks for problems while the Macintosh is idle, and alerts the user to problems as they develop
  - Tells users when to perform backups or optimization
  - Saves valuable information about your disks and files that other Norton Utilities use
- Startup Disk Builder—creates three types of custom startup disks used to start up the Macintosh and run Norton Disk Doctor, Norton Disk Editor, and Speed Disk
- Norton Fastback—Makes and restores backup copies of your files
- Wipe Info—Overwrites file contents so that they can never be recovered
- DiskLight—Alerts you to when the disk is being accessed

Use the following utilities to improve the performance of your Macintosh:

- Speed Disk—Optimizes (defragments) files on the hard disk to improve computer performance
- System Info—Rates the performance of your Macintosh
- Fast Find—Searches disks to find files by name or category
- Floppier—Makes exact copies of your disks that can be copied to new disks, or stored to disk as image files

Refer to the Norton Utilities user's manual for more detailed information on this product.

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Archiving Data from Runs

Recommendations

For optimal computer performance, we recommend the following:

- Keep as few files as possible on the hard disk.
- Do not store gel files and run folders on the hard disk.
- Copy all files you want to save onto a backup medium before each run to ensure adequate disk space is always available.

Files Created During a Run

When you set up a run, the data collection software creates a Run folder and a run file. During the run, the program automatically creates and saves two files: the Gel file and the Log file. After the run, the analysis program creates Sample and sequence (.Seq) files.

Run Files

Each run file contains information that associates specific samples with specific lane positions. It also stores information about the sample sheet and module associated with the run. Run files are small enough to store on floppy disk.

Gel Files

Gel files contain the raw data acquired by the data collection program. They are typically very large (20–70 MB.) Because Gel files are so large, we recommend deleting them from the hard disk after you have obtained satisfactory Sample files. You do not usually need to keep gel files once lane tracking is adjusted and sample files are created.

Use magnetic tapes, removable cartridge drives, or optical drives to archive gel files. Gel files are too large to fit on floppy disks.

IMPORTANT Do not discard a gel file until lane tracking is adjusted (if necessary), and verified.

Log Files

Log files provide a good record of your runs. Individually, Log files are small enough to archive on floppy disks.

Sample files

Use floppy disks, a magnetic tape drive, a removable cartridge drive, or an optical drive to archive sample files. A Sample file is 150-200 KB in size, depending on the length of the run. A 1.4 MB, high-density disk holds about six files. Archive Sample files once the channel selections (tracking) are correct.

.Seq files

For sequencing applications only, .Seq files show the base letter sequence only (or a header, depending on the format you choose). You can open them from word processing programs and print them. You can also save the files in several formats that can be read by other software programs. The Preferences dialog box in the specific analysis program allows you to choose the file format.

Other Files

Other files provide information for a run that you might have customized and should therefore save. These include the sample sheets, analysis settings, or the matrix/instrument files you create.

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Sample Sheet Files

Sample sheets can be a good reference after data collection. Individually, sample sheet files are small enough to archive on floppy disks.

Custom Analysis Settings and Matrix Files

A backup copy of all custom analysis settings and matrix/instrument files should be kept on a separate storage medium. Each of these types of files alone is small enough to store on a floppy disk.

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Reinstalling ABI PRISM 377 Software

System Software

The following software is supplied with the ABI PRISM 377 and is installed onto the computer by the Applied Biosystems Field Service Specialist during system installation.

- ABI PRISM® Data Collection Software, version 2.1 or higher
- Data analysis software—one or both of the following:
  - ABI PRISM® GeneScan® Analysis Software, version 2.1 or higher
  - ABI PRISM® DNA Sequencing Analysis Software, version 3.0 or higher
- Symantec AntiVirus for Macintosh (SAM™), version 4.0 or greater
- Norton Utilities™

Separate disks for each software program are included. Store these disks in a safe place.

Installation Procedure

The following general procedure can be used to reinstall ABI PRISM 377 instrument software, such as sequencing or GeneScan analysis software. More specific instructions for downloading and installing data collection software from our website are listed under “Downloading Data Collection Software from Our Website” on page 8-26.

To install ABI PRISM 377 software:

Step	Action
1	Quit any currently running applications.
2	Hold down the Shift key and choose Restart from the Special menu to restart the computer with extensions turned off. Continue holding down the shift key until you know the extensions are disabled. Some Macintosh’s will display a message that extensions are turned off. All Macintosh’s display extension icons that are X’d over. **IMPORTANT** If extensions are not turned off, you may not be able to complete the installation.
3	Insert the Install disk into the appropriate drive.
4	If the Installer window does not open automatically, double-click the disk icon.
5	Open the Readme file and read it.
6	Double-click the Installer icon.
7	Respond to the prompts as appropriate.
8	Click Quit when installation is complete.
9	Restart the computer to turn the extensions back on, and rebuild the desktop. (See “Rebuilding the Desktop” on page 8-18.)

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Downloading Data Collection Software from Our Website

Overview

The most current version of ABI PRISM Data Collection Software for this instrument can be downloaded from our website. The procedure we recommend is as follows.

1. Save the existing software under a new name. This is recommended as a safeguard just in case something goes wrong when installing the new software.
2. Delete the existing Preferences file by saving it under a new name.
3. Download the new software from our website onto a floppy disk.
4. Load the new software from the floppy disk onto the hard drive.
5. Launch the new software.
6 Reset the Folder Locations preferences and any other preferences you use.

Renaming the Existing Software and Preferences

During installation, the new data collection software automatically overwrites the older version currently installed on the hard drive. We recommend saving the older version under a different name before the new version is installed. The old version can be deleted once you have verified the new version is successfully installed. We also recommend deleting the existing Preferences file prior to installing the new software.

To rename the old software and existing Preferences file:

Step	Action
1	Rename the ABI PRISM 377 folder. We suggest renaming the folder “ABI PRISM 377 <version number>”. For example, ABI PRISM 377 v2.1.
2	Open the Preferences folder located inside the System folder.
3	Select the file named ABI 377, ABI 377XL, ABI 377-96, or ABI 377-18 as appropriate, and rename it. We suggest adding the suffix “v <version number>”. For example, Preferences v2.1.
4	Proceed to “Downloading Data Collection Software from Our Website”.

Downloading from the Website

To download the software from our website:

Step	Action
1	Access the ABI PRISM 377 DNA Sequencer website at: www.appliedbiosystems.com/techsupport
2	Click Download Software.
3	Click the 377 folder icon.
4	If not already on the hard drive, click on the DiskCopy.<version number>.hqx file. This utility program is required to create a floppy disk.
5	Click the appropriate data collection software .hqx file. For example: ABI PRISM 377 v2.1.image.hqx

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Step	Action
6	To copy the software onto a floppy disk: a. Double-click the DiskCopy icon. b. Open the Utilities menu and select Make A Floppy. c. Select the data collection software image from the menu, and click Open. d. When prompted, insert a blank floppy disk into the hard drive. e. When finished, open the File menu and select Quit.
7	Quit the internet connection, and continue the installation by following the instructions listed under “Loading from Floppy Disk to Hard Drive” below.

Loading from Floppy Disk to Hard Drive

To load the software from the floppy disk onto the hard drive:

Step	Action
1	Quit any currently running applications.
2	Hold down the Shift key and choose Restart from the Special menu to restart the computer with extensions turned off. Continue holding down the shift key until you see the extensions are all disabled. **IMPORTANT** If extensions are not turned off, you may not be able to complete the installation.
3	Insert the disk with the software into the floppy drive of the Macintosh connected to the instrument.
4	If the Installer window does not open automatically, double-click the disk icon.
5	Open the Readme file and read it.
6	Double-click the Installer icon.
7	Respond to the prompts by clicking Continue or Install.
8	Click Quit when installation is complete.
9	Close the installer window, and drag the disk icon to the trash to eject the disk.
10	Store the disk for future use.
11	Restart the Macintosh to turn the extensions back on, and rebuild the desktop. (See “Rebuilding the Desktop” on page 8-18.)
12	Launch the new software. If the old Preferences file was renamed as recommended under “Renaming the Existing Software and Preferences” on page 8-26, you will now be asked to specify various folder locations. See “Folder Location Preferences” in Chapter 5, “Setting Preferences.”

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Adding Non-Applied Biosystems Software

Recommendations

IMPORTANT We strongly recommend that you do not install other programs on the Macintosh computer that runs the instrument. Some programs can interfere with instrument operation.

If additional software is installed, keep SAM activated to check for viruses (described on page 8-21).

Additional recommendations:

- Do not install games or screen savers onto the hard disk.
- Do not install programs that require additional control panels (CDEVs) or extensions (INITs). This includes games with custom sound or graphics. Unnecessary INITs can be turned off using the Extensions Manager.

INITs, Extensions, and CDEVs reside in the extensions and control panel folders in the System folder, and alter the programming code of the system. Compatibility is guaranteed only for the INITs shipped with the ABI PRISM 377 DNA Sequencer. Even seemingly innocuous INITs like SuperClock! and After Dark can interfere with system operation. If you suspect there is a problem with a specific INIT, follow the troubleshooting procedure listed below.

INIT Troubleshooting Procedure

If you suspect there is a problem with a specific INIT, follow this procedure.

To turn off specific INITs:

Step	Action
1	Open the Extensions Manager window as follows: a. Open the Apple menu. b. Select Control Panels and open the control panels menu. c. Select Extensions Manager.
2	Click the suspect INIT to remove the checkmark and turn it off.
3	Open the Special menu and select Restart to restart the Macintosh.
4	Try running the system without the INIT to see if this solves the problem.

To turn off all INITs:

Step	Action
1	Restart the Macintosh while holding down the Shift key to turn off all INITs.

To turn all INITs back on:

Step	Action
1	Restart the computer.

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System File

Overview

Only one file named “System” is required to operate the Macintosh computer. The System file is located in the System Folder on the hard disk, and is essential for all operations on the Macintosh. The System folder and file are set up on the computer during system installation.

Other software programs sold by Applied Biosystems that can be used on the ABI PRISM 377 DNA Sequencer do not include a System file. However, applications sold by other manufacturers might include a System file. We strongly recommend that you do not install other System files if additional software is installed. See “Adding Non-Applied Biosystems Software” on page 8-28 for more information.

Note Store the System floppy disks or CDs in a safe place. We also recommend making a copy of each disk. A utility such as Disk Copy can be used.

For more information on the System file and folder, refer to the Macintosh system reference manual.

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Data Collection Software 9

Chapter Contents

In this Chapter The following topics are discussed in this chapter:

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System Software Overview

Overview

The ABI PRISM® 377 DNA Sequencer is shipped with two types of software:

- ABI PRISM® Data Collection Software
- One of the following data analysis software programs:
  - ABI PRISM® DNA Sequencing Analysis Software
  - ABI PRISM GeneScan® Analysis Software

This chapter describes the ABI PRISM Data Collection Software. This software:

- Controls the instrument by sending it commands that are contained in files called modules
- Collects data from the instrument and stores it in a gel file
- Transfers data to either sequencing or GeneScan software for analysis

The data collection software is installed on the Macintosh® computer hard drive during system installation.

For more information on the data analysis software programs and the chemistries available for use with this system, refer to the following manuals:

- *ABI PRISM GeneScan® Analysis Software User's Manual*
- *GeneScan Reference Guide: ABI™ 373 and ABI PRISM 377 DNA Sequencers,* P/N 4303188
- *ABI PRISM® DNA Sequencing Software User's Manual*
- *Automated DNA Sequencing Chemistry Guide: ABI 373 and ABI PRISM 377 DNA Sequencers,* P/N 4305080

Also refer to the chemistry kit protocols, and to our website at: www.appliedbiosystems.com/techsupport.

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Data Collection Software Files and Folders

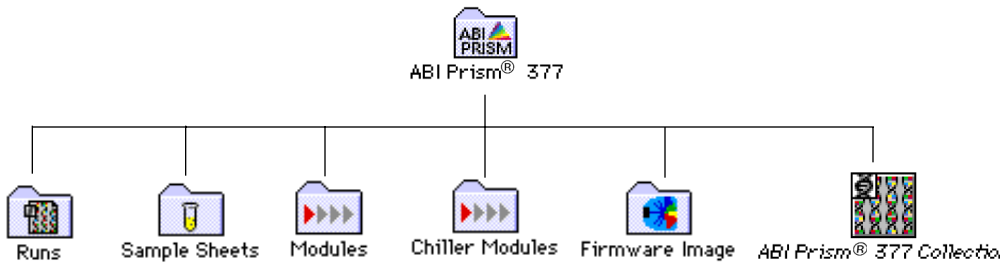
Organizational Structure

The data collection software program, and the various ancillary files and folders required to perform runs, generate sample files for data analysis, and store data are organized inside two folders on the Macintosh hard drive:

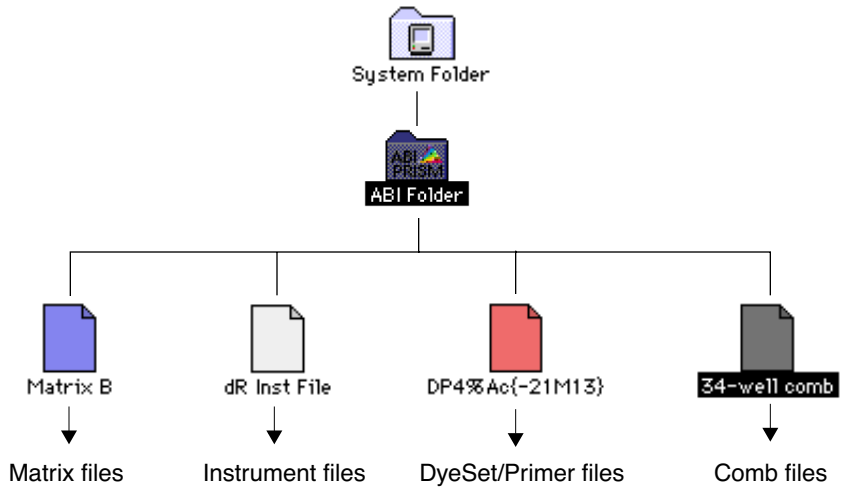
- ABI PRISM 377 Folder — located directly on the hard drive
- ABI Folder — located inside the System folder (also referred to as the *Settings Folder*)

The folder and file structure is shown below.

Folders and Files Inside the ABI PRISM 377 Folder



Files Inside the ABI (Settings) Folder



The names of the ABI Folder files shown here are examples only, and do not represent the entire contents of the ABI Folder. Refer to the following tables for a description of the folders and files in the ABI PRISM 377 and ABI folders.

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Folders Inside the ABI PRISM 377 Folder

Description of folder in the ABI PRISM 377 folder:

Folder Name	Description of Contents
Runs	Contains an individual run folder for each run sheet created. At the end of a run, the individual run folder will typically contain a run, gel, and log file (generated by data collection software), and sample files (generated by the analysis software).
Sample Sheets	Contains each sample sheet created.
Modules	Contains the standard module files. These files contain the commands used to operate the instrument for plate checks, preruns, and runs.
Chiller Modules	Contains the module files used for plate checks, preruns, and runs for subambient temperature operation of the instrument.
Firmware Image	Contains the firmware that resides on the instrument.
ABI PRISM 377 Collection	The data collection software program file.

The ABI folder (also referred to as the Settings folder) is located in the System Folder on the Macintosh hard drive. The ABI folder contains the following files:

File Type	Description
DyeSet/Primer	Also referred to as mobility files. Contain the information used to compensate for differences (shifts) in sample mobility. See “DyeSet/Primer Files” on page 9-49 for more information.
Comb	No longer used.
Instrument	For sequencing applications only. A set of mathematical matrices used to compensate for the spectral overlap that occurs between the dyes used together as a set. Sometimes referred to as a matrix file.
Matrix	For GeneScan applications only. A mathematical matrix used to compensate for the spectral overlap that occurs between the dyes used together as a set.

Data collection software uses files from both the ABI PRISM 377 and ABI folders:

- For the preparation of sample and run sheets
- To control the instrument and collect data

As such, data collection software must always know where the folders containing these files are located on the hard drive. During system installation, the Applied Biosystems Field Service Specialist specifies the location of these folders by setting the Folder Locations Preference to the recommended defaults. Periodically you may have to reset these preferences. Refer to Chapter 5, “Setting Preferences,” on page 5-5 for more detailed information and instructions.

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Setting Folder Location Preferences

Overview

To complete sequencing sample sheets and run sheets, you must select files from various pop-up menus. If one of these pop-up menus lists <none> only, software cannot find the folder that contains the pertinent files. To correct this, you must reset the appropriate Folder Locations Preference.

Guidelines are provided in the tables below. The information listed reflects the default folder names and locations as assigned automatically when ABI PRISM Data Collection Software is installed.

IMPORTANT To avoid problems setting up sample and run sheets, we strongly recommend not changing the names or locations of these folders.

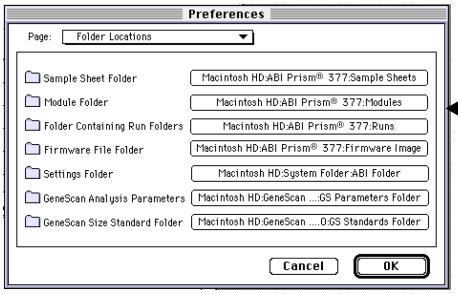
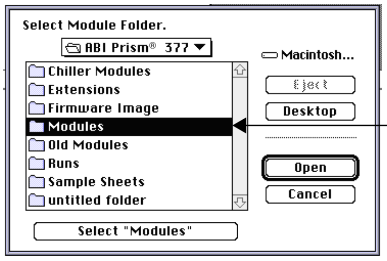
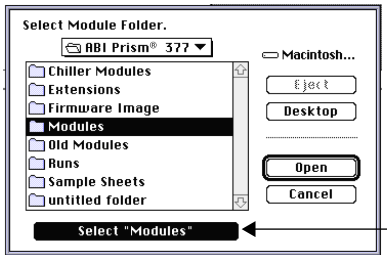
Instructions for setting Folder Location Preferences are listed on page 9-6. For more information, see "Data Collection Software Files and Folders" on page 9-3, and Chapter 5, "Setting Preferences."

Sequencing Sample Sheet Pop-Up Menus	Folder Location Preference Dialog Box Field	Folder Name and Location
DyeSet/Primer	Settings Folder	ABI folder inside the System folder
Instrument File	Settings Folder	ABI folder inside the System folder

Run Sheet Pop-Up Menus	Folder Location Preference Dialog Box Field	Folder Name and Location
Plate Check, PreRun and Run Modules	Module Folder	Modules folder (or Chiller Modules folder if appropriate) inside the ABI PRISM 377 folder
Sample Sheet	Sample Sheet Folder	Sample Sheets folder inside the ABI PRISM 377 folder
Instrument File (sequencing only)	Settings Folder	ABI folder inside the System folder
Gel's Matrix File and Matrix File (GeneScan only)	Settings Folder	ABI folder inside the System folder
Analysis Parameters (GeneScan only)	GeneScan Analysis Parameters	GS Parameters folder inside the GeneScan Analysis software folder
Size Standard (GeneScan only)	GeneScan Size Standard Folder	GS Standards folder inside the GeneScan Analysis software folder

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Procedure To set folder location preferences:

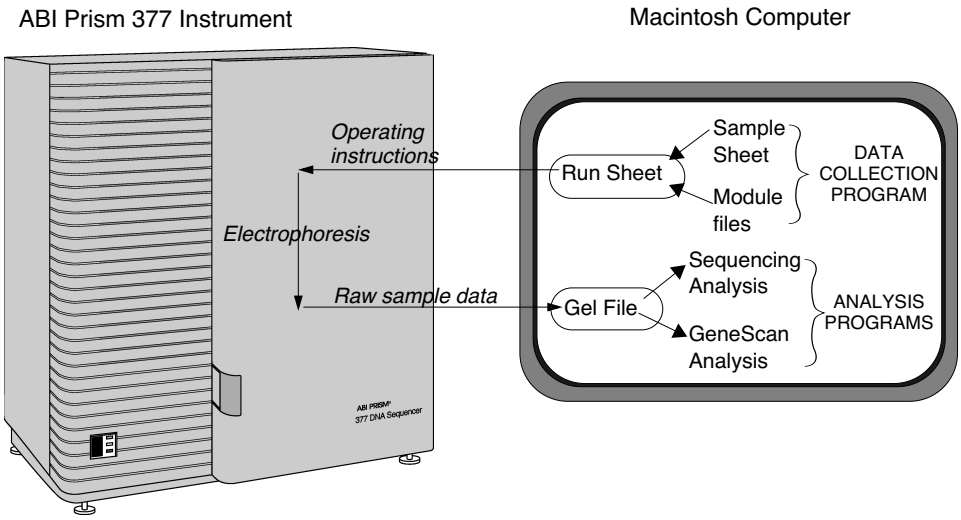
Step	Action
1	Open the Windows menu, select Preferences, and then select Folder Locations.
2	Click the field that corresponds to the folder location you wish to set. 
3	Using the menus in the dialog box, locate and select the appropriate folder. 
4	Click the “Select” field at the bottom of the dialog box to set the folder location. Do **not** click Open. 
5	Return to step 2 to set additional folder location preferences, or click OK if finished.

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Data Flow Between the Computer and Instrument

Overview of Data Flow

Data flows between the Macintosh computer and the ABI PRISM 377 instrument as shown below.



Data collection software sends the operating instructions specified in modules selected on the run sheet to the instrument. As the run is performed, the instrument sends raw sample data back to the computer. This data is stored in a Gel file. Data is then automatically or manually extracted from the gel file, and analyzed by sequencing or GeneScan analysis software.

Input Files

Information from the following files is used by data collection software to operate the instrument, and identify and store the data collected. These files are located in the ABI PRISM 377 Folder.

File Type	Location*	Description
Sample Sheet	Sample Sheets folder inside the ABI PRISM 377 folder	Contain information used for: ♦ sample identification and tracking ♦ data analysis
Module or Chiller Module	Modules or Chiller Modules folder inside the ABI PRISM 377 folder	Contain instrument settings for the various plate checks, pre-runs, and runs that can be performed. The modules to be used for a run are specified on a run sheet.
Run Sheet	Individual run folders inside the Runs folder inside the ABI PRISM 377 folder	Contain: ♦ Sample information imported from the sample sheet ♦ Module designations and other pertinent information used to direct instrument operation and data collection

* Locations valid only if folder location preferences are not changed. See “Setting Folder Location Preferences” on page 9-5 and Chapter 5, “Setting Preferences,” for more information.

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Sample and run sheets both require the selection of additional information for their completion. Refer to the following for more information:

- "About Sample Sheets for Sequencing Applications" on page 9-14, or "About Sample Sheets for GeneScan Applications" on page 9-20
- "About Run Sheets" on page 9-28
- "Modules" on page 9-42

Output Files

The following files are created automatically by data collection software for each run.

File Type	Location*	Description
Gel	All three files are located in an Individual Run folder inside the Runs folder inside the ABI PRISM 377 folder	Contains the data collected during a run.Gel files are typically very large (20–70 MB.) The default naming convention used for gel files is: •Gel File•<date>•<time>
Log		Contains a date and time-stamped comprehensive history of actions taken, instrument and computer conditions, and any errors received during a run. The default naming convention used for log files is: Log
Run		This file is a copy of the run sheet prepared for the run. The run sheet is also an input file. The default naming convention used for run files is: •Run File•<date> <time>

* Locations valid only if folder location preferences are not changed. See "Setting Folder Location Preferences" on page 9-5 and Chapter 5, "Setting Preferences," for more information.

Temporary Files Created in the System Folder

Software creates a number of temporary files in the System folder. Do not delete these files. They are deleted automatically when no longer needed.

Launching the Data Collection Software Program

How to Launch

To launch the data collection program.

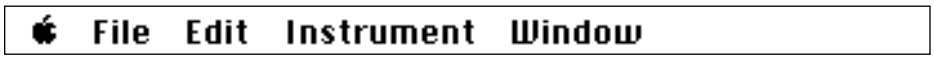
Step	Action	
1	From the Finder, open the Special menu and select Restart. We recommend restarting the computer once a day or before each run to reduce memory fragmentation, and to quit any applications running in the background.	
2	Double-click the data collection software program icon or the alias.  	
3	**If …**	**Then …**
	the software launches properly	proceed with preparing the instrument and software for a run.
	a dialog box asking for folder locations appears	you must set the Folder Locations Preference. Instructions are located on page 9-5, and in Chapter 5, "Setting Preferences.".

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Menu Commands

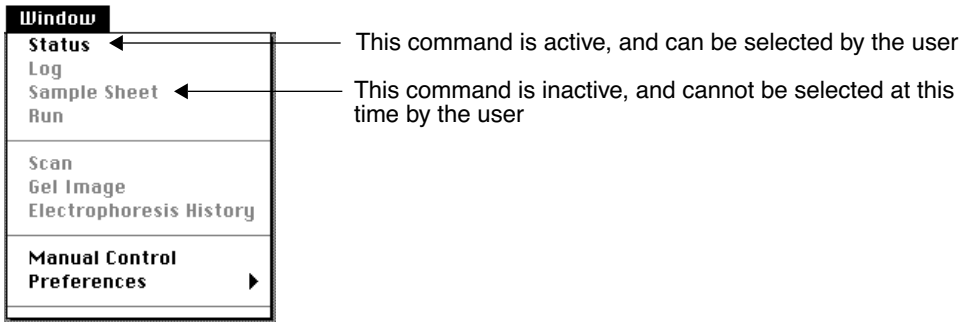
Overview

Data collection software has its own menu bar (shown below) located at the top of the computer screen after the software is launched. Pull-down menus and the point-and-click technique are used to execute commands. As you become familiar with this software, you can also use shortcut keyboard commands when available. Shortcut keyboard commands are listed opposite the corresponding command on the pull-down menu (see the File Menu illustration on page 9-10).

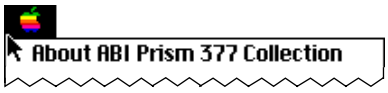


Active versus Inactive Menu Commands

Commands in the pull-down menus can be active or inactive. Active commands are shown in bold print and can be selected by the user. Inactive commands are shown in grey and cannot be selected at that particular time. You will see inactive commands become active at various times throughout the procedures you perform.



Apple Menu



The Apple menu is a standard Macintosh feature. It displays and provides access to the desktop accessories installed on the system.

The first item in the Apple menu, About ABI Prism 377 Collection, opens the data collection software splash screen, which shows general information about the software including the version. Click anywhere on the splash screen to close it.

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File Menu



The File menu contains commands for:

- Creating, opening, closing, and saving files
- Importing and exporting data to other file types
- Printing
- Quitting the application

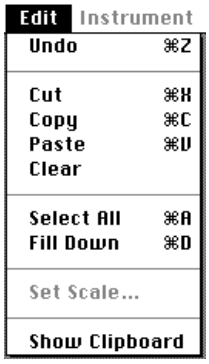
Keyboard shortcut commands

File menu commands

Command	Description	For more detail see
Close, Save, Save As …, Page Setup …, Print …, Quit	Standard Macintosh commands.	Macintosh manual
New … Open …	Opens run and sample sheets. Use New to create a new file. Use Open to open an existing file.	—
Save a Copy In …	Use to make an extra copy of an existing file for backup or modification. The copy can be renamed and stored in a different location from the original.	—
Import …	Imports text from files such as those generated by a database into a sample sheet.	page 9-27
Export …	Exports data from a sample sheet in tab-delimited text format for database applications.	page 9-27
Print One	Prints one copy of the active window. Bypasses the standard print dialog box.	—

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Edit Menu



The Edit menu contains:

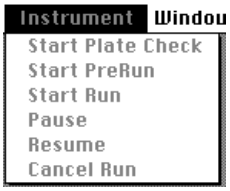
- Standard editing and selecting commands
- Spreadsheet editing commands
- Command for changing the scale in the Electrophoresis History window

Edit menu commands

Command	Description
Undo (Undo Typing, Undo Paste, Can't Undo), Cut, Copy, Paste, Clear, Show Clipboard	Standard Macintosh commands. Refer to the Macintosh manual for more information.
Select All	Selects all the cells of a sample sheet.
Fill Down	Inserts text into all the cells selected in a column on a sample sheet.
Set Scale...	Sets the scale of the panels in the Electrophoresis History window.
Show Clipboard	The information selected when using the Copy command is saved to the clipboard. Use this command to see what you have copied to the clipboard prior to using the Paste command.

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Instrument Menu



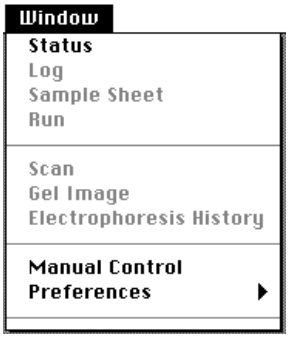
The Instrument menu contains commands for starting, pausing, and stopping instrument operation.

Instrument menu commands

Command	Description	For more detail see
Start Plate Check	Starts a plate check using the settings of the plate check module specified on the run sheet. The gel and plates are scanned without electrophoresis before loading samples to ensure the plates are clean and the gel is not contaminated.	Chapters 1 and 3
Start PreRun	Starts a prerun using the settings of the prerun module specified on the run sheet.	
Start Run	Starts a run using the settings of the run module specified on the run sheet.	
Pause	Temporarily halts the scanner. No functions are performed. The data in instrument memory continues to be stored, and the gel temperature increases or is maintained as appropriate.	
Resume	Resumes instrument operation. This command becomes active after instrument operation has been paused using the Pause command.	
Cancel Run	Active only when a module is running. Allows you to immediately stop the run in progress. The run is ended, and the run sheet is closed. A run cannot be resumed once it is cancelled.	

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Window Menu



The Window menu opens:

- The standard windows associated with a run (Status, Log, Sample Sheet, Run Sheet, Scan, Gel Image, and Electrophoresis History)
- Manual Control window
- Preferences menu
- Any other windows listed at the bottom of the menu

Choosing any window from this menu brings that window to the front and makes it the active window.

Window menu commands

Command	Description	For more detail see
Status	Displays the Status window.	page 9-53
Log	Displays the Log file. Command is active only when a module is running.	page 9-54
Sample Sheet	Command active only if a sample sheet is open in the background while a module is running. Sample information can be changed. However, the changes will not affect the run in progress.	page 9-14 and 9-20
Run	Command active only if a run sheet is open in the background while a module is running. Information cannot be changed once the Run button is clicked.	page 9-28
Scan	Displays the Scan window. Command is active only when a module is running.	page 9-55
Gel Image	Displays the Gel File. Command is active only when a module is running.	page 9-55
Electrophoresis History	Displays the Electrophoresis History window. Command is active only when a module is running.	page 9-56
Manual Control	Displays a window that allows you to manually control certain instrument functions.	page 9-57
Preferences	Opens the Preferences menu.	Chapter 5, “Setting Preferences”

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About Sample Sheets for Sequencing Applications

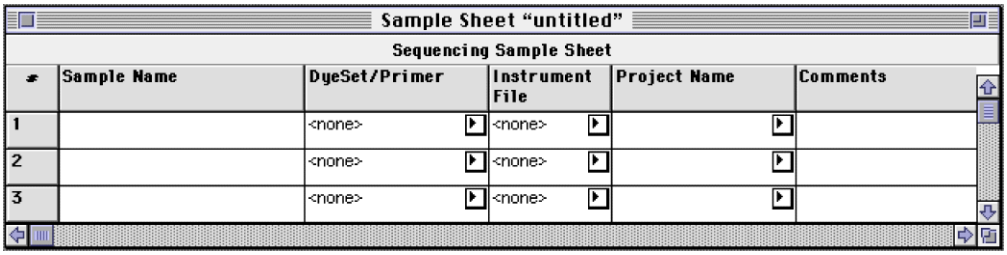
What is a Sequencing Sample Sheet

A sequencing sample sheet (shown below) is a file that contains information used for:

- Sample identification and tracking
- Data analysis

There are two types of sample sheets: one for sequencing, and one for GeneScan analysis applications. The information entered on the sample sheet is imported to the sequencing run sheet (described on page 9-28).

IMPORTANT Do not mix sequence and GeneScan analysis samples on the same sample sheet or in the same run.



Description of Fields on the Sample Sheet

The information specified on sequencing sample sheets is described below:

Name of Field	Description
Sample Name	Name assigned to a sample. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters*. Do not use colons, slashes, or symbols in sample names.
DyeSet/Primer	Also referred to as a mobility file. Contains the information used to: ♦ Compensate for differences (shifts) in sample mobility ♦ Interpret what each dye color in a set represents A dye set/primer file must be selected for data to be analyzed automatically at the end of a run. For more information see: ♦ "About Automatic Data Analysis" on page 9-38 ♦ "DyeSet/Primer Files" on page 9-49
Project Name (for BioLIMS® software users only)	Used to identify data transferred to BioLIMS software. If a project name is not specified, the data transferred to BioLIMS is identified by the gel file name only. Project names must be entered in the Project Info Preference prior to preparing a sample sheet. Refer to "Project Information Preferences" in Chapter 5 for instructions and more information.

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Name of Field	Description
Instrument File	Also referred to as a matrix file, an instrument file is a set of up to three mathematical matrices used to compensate for the spectral overlap that occurs between the dyes used together as a set. During system installation, the engineer installing the system creates a default instrument file that is given the instrument serial number as a file name. Depending on the chemistries used, you may have to create additional instrument files. An instrument file must be selected for data to be analyzed automatically at the end of a run. For more information, see: ♦ "About Automatic Data Analysis" on page 9-38 ♦ Chapter 6, "Making Matrix Files for GeneScan" ♦ The chemistry kit protocol ♦ *Automated DNA Sequencing Chemistry Guide,* P/N 4305080
Comments	Any additional information.

General Sample Sheet Information

The following general information will help you prepare sample sheets.

- Sample sheet information is used by the software to identify each individual sample when Sample Files (described on page 9-60) are generated after a run. Therefore, it is critical that each sample name be entered in the field whose number corresponds to the gel lane in which the sample is loaded.
- If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up sequencing sample sheets by *setting sequencing sample sheet default preferences*. See "Save Time by Setting Sample Sheet Preferences" on page 9-16, and Chapter 5, "Setting Preferences," for more information.
- An instrument file is specified on both the sample and run sheets. The same file should be specified on both sheets. However, if different files are selected, the instrument file specified on the sample sheet is the one used for automatic data analysis. If <none> is selected on the sample sheet, the instrument file selected on the run sheet is used.
- Information can be imported from tab-delimited text files (for instance, files generated by a database). The sample sheet can be exported as a tab-delimited text file to database, spreadsheet, or word processing programs. Refer to "Importing and Exporting Sample Sheet Information" on page 9-27 for more information.
- The same sample sheet can be used for more than one run if the information required is the same. An existing sample sheet can also be used as a template to create new sample sheets by opening the file, saving it under a different name ("Save As" command under the File menu), and then modifying the new file.
- If the wrong dye set/primer or instrument file is specified, the data can be reanalyzed using the correct file. Refer to the *ABI Prism DNA Sequencing Analysis Software User's Manual* for more information.
- Sample sheets can be printed.

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Preparing a Sequencing Sample Sheet

IMPORTANT Do not mix sequence and GeneScan analysis samples on the same sample sheet or in the same run.

Save Time by Setting Sample Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up sample sheets by *setting sequencing sample sheet default preferences*. Setting sample sheet preferences means setting the default value of certain fields to the values used most often. The following fields on sequencing sample sheets can be set as preferences:

- DyeSet/Primer
- Instrument File

Once these preferences have been set, the preferred values appear automatically on each new sequence sample sheet. Preferences can be changed as often as necessary either by setting new preference values, or by opening the pop-up menus and manually selecting new values. Instructions for setting sequencing sample sheet default preferences are located in Chapter 5, “Setting Preferences.”

Entering Information

See “How to Enter Information on Sample Sheets” on page 9-26. Procedures include:

- Applying the same parameter to all fields in a column
- Copying information from one field or row to another
- Moving information from one lane to another

Opening a New Sample Sheet

To open a new sequencing sample sheet:

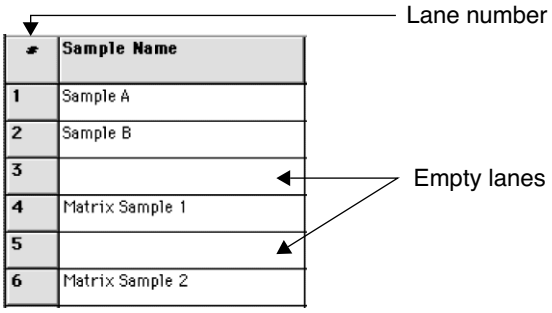
Step	Action
1	If necessary, launch the data collection software. (Instructions are listed under “Launching the Data Collection Software Program” on page 9-8.)
2	Open the File menu and select New.
3	Click the icon named Sequence Sample.

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Entering Sample Names

To import sample names from tab-delimited text files, follow the instructions listed under “Importing and Exporting Sample Sheet Information” on page 9-27. Otherwise, follow this procedure.

To enter sample names:

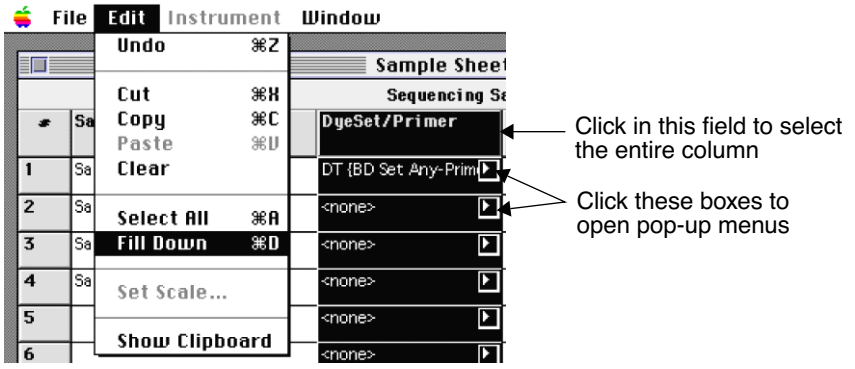
Step	Action
1	Enter sample names in the Sample Name column by clicking in the Sample Name field, and typing the sample name. Enter names in the exact order the samples will be loaded onto the gel. The numbers to the left of the Sample Name column represent the gel lane numbers. Leave fields blank that correspond to empty lanes. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters.* Do not use colons, slashes, or symbols in sample names. **Note** More text can be entered than is visible. Text automatically shifts as the information is entered. Use the keyboard arrow keys to scroll through long entries.
2	If matrix standard samples are being run, enter a name for each matrix sample. To help ensure a robust matrix is produced, we strongly recommend you follow these guidelines: ♦ Leave at least one empty lane between non-matrix standard samples and matrix standard samples. ♦ Leave one empty lane between each matrix standard sample. 

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Selecting the DyeSet/Primer File

Different dye set/primer files can be used for the same run as long as the virtual filter set is the same for all samples. Dye set/primer file names for dRhodamine Terminators are similar to those for BigDye™ Terminators, and can easily be mistaken for one another. If the wrong file is selected, base spacing in the data will not be noticeably affected. C and T bases will be miscalled. If you are not sure which file to select, refer to the chemistry kit protocol and to “DyeSet/Primer Files” on page 9-49.

To select a dye set/primer file:

Step	Action
1	Open the DyeSet/Primer pop-up menu and select the appropriate file for the first sample. You must select a file if you want the data to be analyzed automatically at the end of the run. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the dye set/primer files. To correct this, you must set the Settings Folder Preference to the ABI Folder. See “Setting Folder Location Preferences” on page 9-5.
2	If the file is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate DyeSet/Primer file for each sample individually. 

Selecting the Instrument File

The instrument file must be the same for all the samples.

To select the instrument file:

Step	Action
1	Open the Instrument File pop-up menu for the first sample, and select the appropriate file. You must select a file if you want the data to be analyzed automatically at the end of the run. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the instrument files. To correct this, you must set the Settings Folder Preference to the ABI Folder. See “Setting Folder Location Preferences” on page 9-5.
2	Click in the column heading to select the entire column, open the Edit menu, and select Fill Down to enter the same instrument file for the remaining samples.

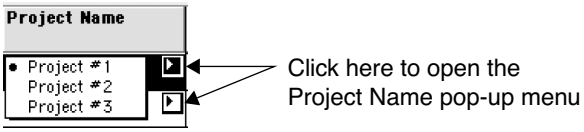
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Selecting Project Names and Entering Comments

The Project Name field is for BioLIMS software users only. Otherwise, the field can be left blank. If a project name is not identified, the data transferred to BioLIMS is identified by the gel file name only.

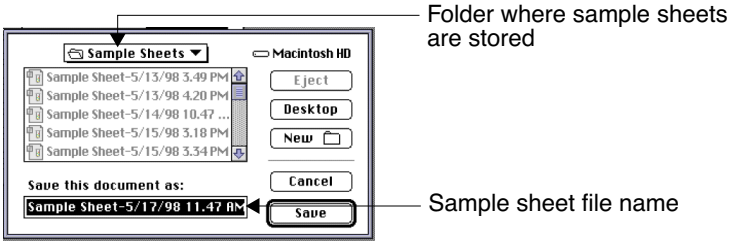
Project names must be defined prior to setting up the sample sheet as Project Info Preferences. Refer to “Project Information Preferences” in Chapter 5 for instructions and more information.

To select project names and enter comments:

Step	Action
1	Open the Project Name pop-up menu for each sample, and select the appropriate project name. 
2	If the Project Name is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate Project Name for each sample individually.
3	To enter a comment, click in the Comments field, and type the information.

Saving and Closing the Sample Sheet

To save and close the sample sheet:

Step	Action
1	Do one of the following: ♦ Open the File menu and select Close. ♦ Click the box in the upper left-hand corner of the window, and then click Save.
2	Click Save.
3	A dialog box showing the default sample sheet file name, and the location where the sample sheet will be stored is displayed. a. Change the file name now if desired. b. Click Save. **IMPORTANT** Although it is an option, we do not recommend changing the storage location of the sample sheet. If the location is changed, software will not be able to locate the sample sheet when you set up the run sheet.  **Note** You can also save the sample sheet by opening the File menu and selecting Save, Save As, or Save A Copy In.

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About Sample Sheets for GeneScan Applications

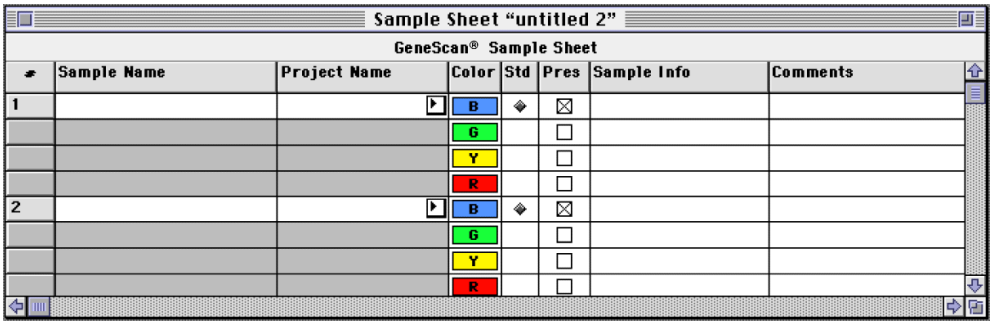
What is a GeneScan Sample Sheet

A GeneScan sample sheet (shown below) is a file that contains information used for:

- Sample identification and tracking
- Data analysis

There are two types of sample sheets: one for GeneScan and one for sequencing analysis applications. The information entered on the sample sheet is imported to the GeneScan run sheet (described on page 9-28).

IMPORTANT Do not mix GeneScan and sequence analysis samples on the same sample sheet or in the same run.



Description of Fields on the Sample Sheet

The information specified on GeneScan sample sheets is described below:

Name of Field	Description
Sample Name	Name assigned to a sample. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters*. Do not use colons, slashes, or symbols in sample names.
Project Name (for BioLIMS software version 2.0 and up only)	Used to identify data transferred to BioLIMS software. If a project name is not specified, the data transferred to BioLIMS is identified by the gel file name only. Project names must be entered in the Project Info Preference prior to preparing a sample sheet. Refer to "Project Information Preferences" in Chapter 5 for instructions and more information.
Color and Pres	Used to indicate the dye colors run in each lane on the gel. Required for data to be analyzed automatically. See "About Automatic Data Analysis" on page 9-38 for more information.
Std	Used to indicate the color of the size standard. Required for data to be analyzed automatically. See "About Automatic Data Analysis" on page 9-38 for more information.
Sample Info	Additional sample identification information used by ABI PRISM Genotyper® DNA Fragment Analysis Software.
Comments	Any additional information.

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General Sample Sheet Information

The following general information will help you prepare sample sheets.

- Sample information is used by the software to identify each individual sample when Sample Files (described on page 9-60) are generated after a run. Therefore, it is critical that each sample name be entered in the field whose number corresponds to the gel lane in which the sample is loaded.
- If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up GeneScan sample sheets by *setting GeneScan sample sheet default preferences*. See "Save Time by Setting Sample Sheet Preferences" on page 9-22, and Chapter 5 for more information.
- Information can be imported from tab-delimited text files (for instance, files generated by a database). The sample sheet can be exported as a tab-delimited text file to database, spreadsheet, or word processing programs. Refer to "Importing and Exporting Sample Sheet Information" on page 9-27 for more information.
- The same sample sheet can be used for more than one run if the information required is the same. An existing sample sheet can also be used as a template to create new sample sheets by opening the file, saving it under a different name ("Save As" command under the File menu), and then modifying the new file.
- Sample sheets can be printed.

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Preparing a GeneScan Sample Sheet

IMPORTANT Do not mix GeneScan and sequence analysis samples on the same sample sheet or in the same run.

Save Time by Setting Sample Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up sample sheets by *setting GeneScan sample sheet default preferences*. Setting sample sheet preferences means setting the default value of certain fields to the values used most often. The following field can be set as a preference:

- Std (size standard dye color)

Once you have set this preference, the preferred value appears automatically on each new GeneScan sample sheet. This preference can be changed as often as necessary either by setting a new preference value, or by manually selecting a different value on the sample sheet. Instructions for setting GeneScan sample sheet default preferences are located in Chapter 5, “Setting Preferences.”

Entering Information

See “How to Enter Information on Sample Sheets” on page 9-26. Procedures include:

- Applying the same parameter to all fields in a column
- Copying information from one field or row to another
- Moving information from one lane to another

Opening a New Sample Sheet

To open a new GeneScan sample sheet:

Step	Action
1	If necessary, launch the data collection software. (Instructions are listed under “Launching the Data Collection Software Program” on page 9-8.)
2	Open the File menu and select New.
3	Click the icon named GeneScan Sample.

Entering Sample Names

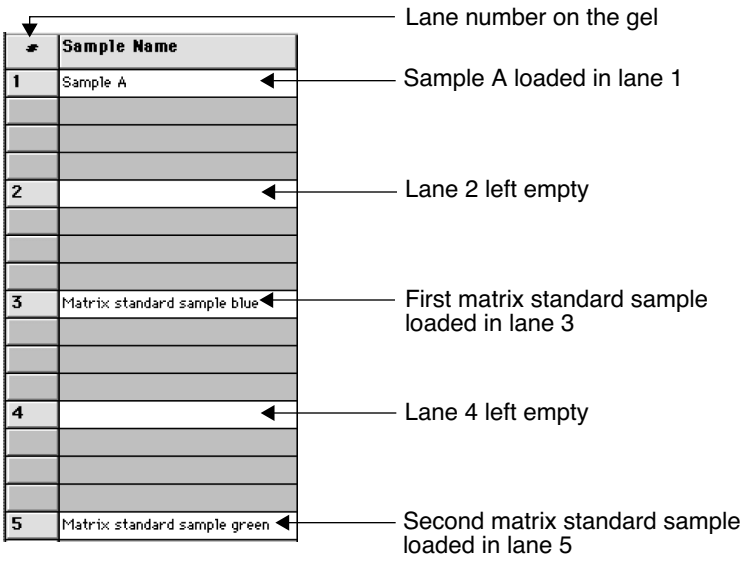
To import sample names from tab-delimited text files, follow the instructions listed under “Importing and Exporting Sample Sheet Information” on page 9-27. Otherwise, follow this procedure.

To enter sample names:

Step	Action
1	Enter sample names in the Sample Name column by clicking in the appropriate Sample Name field, and typing the sample name. Enter names in the exact order the samples will be loaded onto the gel. The numbers to the left of the Sample Name column represent the gel lane numbers. Leave fields blank that correspond to empty lanes. **IMPORTANT** Each sample must have a unique name. Limit sample names to 27 characters *including the default characters*. Do not use colons, slashes, or symbols in sample names. **Note** More text can be entered than is visible. Text automatically shifts as the information is entered. Use the keyboard arrow keys to scroll through long entries.

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To enter sample names: *(continued)*

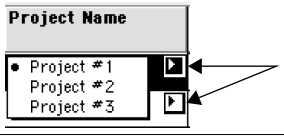
Step	Action
2	If matrix standard samples are being run, enter a name for each matrix sample. To help ensure a robust matrix is produced, we strongly recommend you follow these guidelines: ♦ Leave at least one empty lane between non-matrix standard samples and matrix standard samples. ♦ Leave one empty lane between each matrix standard sample. **IMPORTANT** Matrices are dye set, instrument, and run condition dependent. As such, matrices must be remade when any of these conditions change. For more information, refer to Chapter 6, "Making Matrix Files for GeneScan.". 

Selecting Project Names

The Project Name field is for BioLIMS software version 2.0 and up users only. Otherwise, the field can be left blank. If a project name is not identified, the data transferred to BioLIMS is identified by the gel file name only.

Project names must be defined prior to setting up the sample sheet as Project Info Preferences. Refer to "Project Information Preferences" in Chapter 5, "Setting Preferences," for instructions and more information.

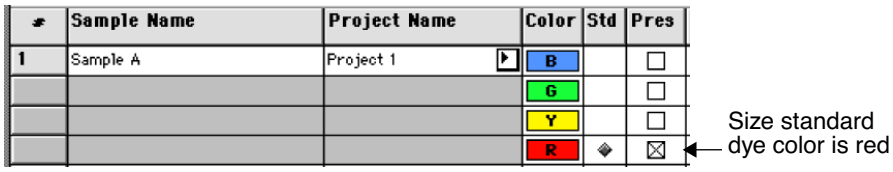
To select project names:

Step	Action
1	Open the Project Name pop-up menu for each sample, and select the project name. 
2	If the Project Name is the same for all remaining samples, click in the column heading to select the entire column, open the Edit menu, and select Fill Down. Otherwise, select the appropriate Project Name for each sample individually.

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Specifying the Size Standard Dye Color

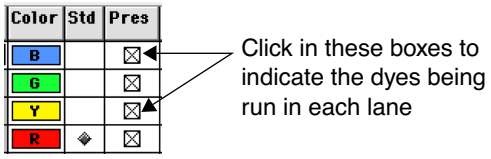
To specify the size standard dye color:

Step	Action
1	For all non-matrix standard samples, designate the color of the size standard by clicking in the box to the right of the appropriate letter in the Color column (B = blue; G = green; Y = yellow; R = red). When selected, a diamond is displayed in the Std column. This must be specified for data to be analyzed automatically. 
2	Leave the Std column blank for matrix standard samples. If necessary, remove diamonds for matrix standard samples by clicking on the diamond.

Specifying the Dyes Run in Each Lane

The dye colors run together in a lane must be specified for each sample.

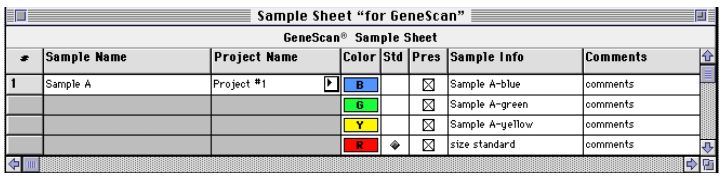
To specify the dye colors run together in each lane:

Step	Action
1	Click in the appropriate boxes in the Pres (present) column to select the dye colors that will be run in each lane for each sample. Codes for the colors are displayed in the Color column (B = blue; G = green; Y = yellow; R = red). Dyes be specified for data to be analyzed automatically. 
2	To deselect a color, click in the appropriate box to remove the X.

Entering Sample Info and Comments

The information in the Sample Info and Comments fields is imported into ABI PRISM Genotyper® DNA Fragment Analysis Software, and is used for sample identification and sorting. If these fields are left blank, only the words “sample file” will appear when sample information is displayed in Genotyper.

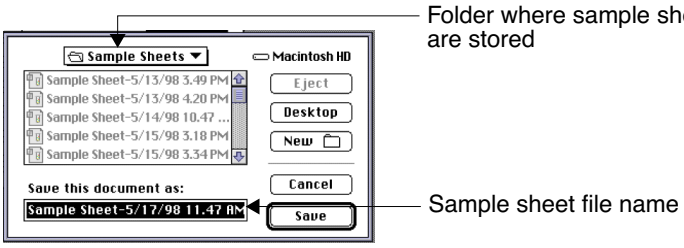
To enter Sample Info and Comments:

Step	Action
1	To enter Sample Info, click in the Sample Info field and type the information. 
2	To enter a comment, click the Comments field, and type the information.

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Saving and Closing the Sample Sheet

To save and close the sample sheet:

Step	Action
1	Do one of the following: a. Open the File menu and select Close. b. Click the box in the upper left-hand corner of the window, and then click Save.
2	Click Save.
3	A dialog box showing the default sample sheet file name, and the location where the sample sheet will be stored is displayed. a. Change the file name now if desired. b. Click Save. **IMPORTANT** Although it is an option, we do not recommend changing the storage location of the sample sheet. If the location is changed, software will not be able to locate the sample sheet when you set up the run sheet.  **Note** You can also save the sample sheet by opening the File menu and selecting Save, Save As, or Save A Copy In.

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How to Enter Information on Sample Sheets

Entering Sample Names, Sample Info, and Comments

Click in the Sample Name, Sample Info (GeneScan sample sheets only), or Comments field. Type the desired information. More text can be entered in a field than is visible. Text automatically shifts as it is entered. Use the keyboard arrow keys to scroll through long entries.

Moving From One Field to Another

Several methods can be used to move from one field to another:

- Click in the field
- Press the directional arrow keys
- Press Tab to move to the next field to the right
- Press Return to move to the next field down

Applying Same Parameter to all Fields in a Column

To apply the same parameter to all fields in a column:

Step	Action
1	Select the parameter in the top field of the column.
2	Click the column title to select the entire column.
3	Open Edit menu and choose Fill Down.

Copying Information

To copy information from one field to another:

Step	Action
1	Click the field to be copied.
2	Open the Edit menu and choose Copy.
3	Click the new field.
4	Open the Edit menu and choose Paste.

To copy an entire row of information to another row:

Step	Action
1	Click the lane number in the left-most column to select the entire row.
2	Open the Edit menu and choose Copy.
3	Click the lane number of the row you wish to copy into.
4	Open the Edit menu and choose Paste.

Moving Information

To move information to a different lane:

Step	Action
1	Hold down the Option key, and click the lane number of the row containing the information you want to move.
2	Holding down Option key and the mouse button, drag the lane number to another lane number. When you release the mouse button, the sample information appears in the new location.

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Importing and Exporting Sample Sheet Information

Importing Information

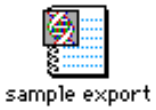
To import information into a sample sheet:

Step	Action
1	Export the information to be imported to the sample sheet into a tab-delimited text file. Format the file based on the following guidelines: ♦ The file should contain only the information you wish to import. It should not have a header. ♦ The information imported to the first field in the sample sheet is everything up to the first tab in the text file. ♦ Each row in the text file should contain the information for one row in the sample sheet. Rows should ordered as you wish them to appear on the sample sheet.
1	Open the Edit menu and choose Select All to select all fields in the sample sheet.
2	Open the Edit menu and choose Import.
3	In the dialog box, choose the file you wish to import.
4	Click OK.

Exporting Information

Exporting information from a completed sample sheet creates a tab-delimited text file. The format of this file is the same as the format discussed in step 1 of the import procedure listed above.

To export information from the sample sheet to a text file:

Step	Action
1	Activate the sample sheet window by clicking in it.
2	Open the Edit menu and choose Export.
3	In the dialog box, enter a name for the text file.
4	Click OK. The icon used for exported files is shown below. 

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About Run Sheets

What is a Run Sheet

A run sheet is a file that contains:

- The software modules and other pertinent information used to direct instrument operation during a run. A typical run consists of the:
  - Plate check
  - Prerun
  - Run

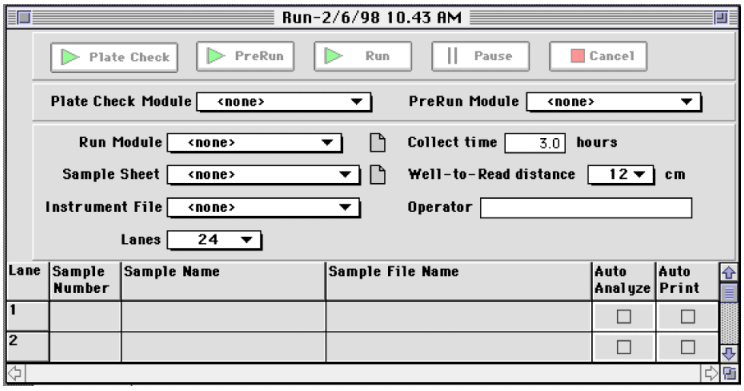
  (See Chapters 1 and 3 for more information on the plate check, prerun and run.)
- Sample identification information
- Gel comb configuration
- Information required for automatic data analysis (see “About Automatic Data Analysis” on page 9-38 for more information)

The run sheet is also used to control the instrument. In this context it is referred to as the *run window.* The instrument is controlled by clicking the buttons at the top of the run window (Plate Check, PreRun, Run, Pause/Resume, and Cancel.)

There are two types of run sheets: one for sequencing, and one for GeneScan analysis applications.

Sequencing Run Sheets

The following is a new sequencing run sheet for standard ABI PRISM 377 instruments. The run sheet for instruments with the XL or 96-lane upgrade differs slightly as noted in the following table.



Run sheets for sequencing analysis applications contain the following information:

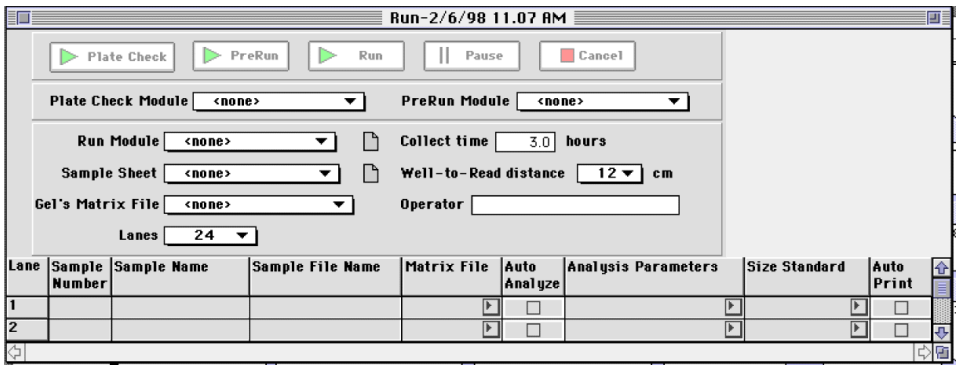
Parameter	Description
Plate Check Module*	File containing the instrument settings for the plate check.
PreRun Module*	File containing the instrument settings for the prerun.
Run Module*	File containing the instrument settings for the run.
Collect time*	The length of time in hours that data will be collected.
Sample Sheet	The sample sheet prepared for the run. When selected, the information from the sample sheet is imported to the sample information fields on the run sheet.
* Can be set as Preferences. See “Setting Run Sheet Preferences” on page 9-31.	

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Parameter	Description
Well-to-Read Distance*	The well-to-read distance of the glass plates in cm.
Instrument File*	Also referred to as a matrix file, an instrument file is a set of mathematical matrices used to compensate for the spectral overlap that occurs between the dyes used together as a set. The instrument file created by the service engineer during system installation is given the instrument serial number as its name. Depending on the chemistries used, you may have to create additional instrument files. An instrument file must be selected for data to be analyzed automatically at the end of a run. For more information, refer to: ♦ "About Automatic Data Analysis" on page 9-38 ♦ Chapter 7, "Making Instrument Files for Sequencing" ♦ *Automated DNA Sequencing Chemistry Guide*, P/N 4305080 ♦ Chemistry kit protocol
Lanes*	Number of lanes in the gel.
Run Mode* (XL or 96-lane upgrade instruments only)	This field is displayed for ABI PRISM 377 XL or 96-lane upgrade instruments only. It is used to designate the scan mode: 96 Lane Scan, XL Scan, or Full Scan.
Operator*	Operator's name.
Sample Number, Sample Name, Sample File Name	These fields are filled in automatically when the Sample Sheet is selected.
Auto Analyze	When selected, data is automatically transferred to sequencing analysis software and analyzed at the end of a run. See "About Automatic Data Analysis" on page 9-38 for more information.
Auto Print	When selected, analyzed data is printed automatically.
* Can be set as Preferences. See "Setting Run Sheet Preferences" on page 9-31.	

GeneScan Run Sheets

The following is a new GeneScan run sheet for standard ABI PRISM 377 instruments. The run sheet for instruments with the XL or 96-lane upgrade differs slightly as noted in the following table.



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Run sheets for GeneScan analysis applications contain the following information:

Parameter	Description
Plate Check Module*	File containing the instrument settings for the plate check.
PreRun Module*	File containing the instrument settings for the prerun.
Run Module*	File containing the instrument settings for the run.
Collect time*	The length of time in hours data will be collected.
Sample Sheet	The sample sheet prepared for the run. When selected, the information from the sample sheet is imported to the sample information fields on the run sheet.
Well-to-Read Distance*	The well-to-read distance of the glass plates in cm.
Gel's Matrix File*	A mathematical matrix used to compensate for the spectral overlap that occurs between the dyes used together as a set. Matrices are dye set, instrument, and run condition dependent. As such, matrices must be remade when any of these conditions change. A matrix file must be selected for data to be analyzed automatically. For more information, refer to: ♦ "About Automatic Data Analysis" on page 9-38 ♦ Chapter 6, "Making Matrix Files for GeneScan" ♦ *ABI PRISM GeneScan Reference Guide: ABI 373 and ABI PRISM 377 DNA Sequencers*, P/N 4303188
Lanes*	Number of lanes in the gel.
Run Mode* (XL or 96-lane upgrade instruments only)	This field is displayed for ABI PRISM 377 XL or 96-lane upgrade instruments only. It is used to designate the scan mode: 96 Lane Scan, XL Scan, or Full Scan.
Operator*	Operator's name.
Sample Number, Sample Name, Sample File Name	These fields are filled in automatically when the Sample Sheet is selected.
Matrix File	The same as the Gel's Matrix File listed above. Required only if data is to be analyzed automatically at the end of the run.
Auto Analyze	When selected, data is automatically transferred to GeneScan analysis software and analyzed at the end of a run. See also "About Automatic Data Analysis" on page 9-38.
Analysis Parameters	A set of analysis parameters predefined in GeneScan analysis software. Selecting analysis parameters is required only if the Auto Analyze feature is selected. Otherwise, this field can be left blank. See also "About Automatic Data Analysis" on page 9-38.
Size Standard	A file that contains information on the size standard run with the samples. Selecting a size standard is required only if the Auto Analyze feature is selected. Otherwise, this field can be left blank. See also "About Automatic Data Analysis" on page 9-38.
Auto Print	When selected, analyzed data is printed automatically. See also "About Automatic Data Analysis" on page 9-38.
* Can be set as Preferences. See "Setting Run Sheet Preferences" on page 9-31.	

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Preparing a Run Sheet

Setting Run Sheet Preferences

If the same type of run is performed repeatedly on the same instrument, you can reduce the time spent setting up run sheets by *setting sequencing or GeneScan run sheet default preferences*. Setting run sheet preferences means setting the default value of certain fields on the run sheet template to the values used most often. The following fields can be set as preferences:

- Operator
- Lanes
- Well-to-read distance
- Prerun and run modules
- Software program to be used for automatic data analysis
- Auto print
- Analysis parameters (GeneScan run sheets only)
- Gel's matrix file (GeneScan run sheets only)
- Size standard (GeneScan run sheets only)

Once these preferences are set, the preferred values appear automatically on each new run sheet. Preferences can be changed as often as necessary either by setting new preference values, or by manually selecting new files/values on the run sheet. Instructions for setting run sheet preferences are located in Chapter 5, "Setting Preferences.".

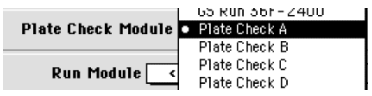
Opening a New Run Sheet

To open a new run sheet:

Step	Action
1	If necessary, launch the data collection software. (Instructions are listed under "Launching the Data Collection Software Program" on page 9-8.)
2	Open the File menu and select New.
3	Click either the Sequence Run or GeneScan Run icon as appropriate. A new Run folder is created automatically in the Runs folder inside the ABI PRISM 377 folder.

Selecting the Plate Check, PreRun, and Run Modules

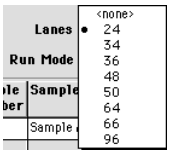
To select the plate check, prerun, and run modules:

Step	Action
1	Open the Plate Check Module pop-up menu, and select the a plate check module.  **IMPORTANT** If the pop-up menus list <none> only, software cannot find the folder that contains the modules. See "Setting Folder Location Preferences" on page 9-5.
2	Open the PreRun Module pop-up menu, and select a prerun module.
3	Open the Run Module pop-up menu, and select a run module.

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Selecting the Number of Lanes

To select the number of lanes:

Step	Action
1	Open the lanes pop-up menu and select the appropriate value.  For GeneScan applications: Full Scan–24, 34, 36 lanes XL Scan–48, 50, 64, 66 lanes 96 Lane Scan–96 lanes For Sequencing applications: Full Scan–24, 32, 36 lanes XL Scan–48, 64 lanes 96 Lane Scan–96 lanes **Note** If this is an XL or 96-lane upgrade instrument, the appropriate value for the Run Mode field is selected automatically when this parameter is set. The run sheet for the standard ABI PRISM 377 instrument does not have a Run Mode field.

Selecting the Sample Sheet

IMPORTANT Select the number of lanes before selecting the sample sheet. If the default number of lanes is less than the number of samples, the sample information on the run sheet will be truncated. For example, if you entered 36 samples on the sample sheet, but the default number of lanes on the run sheet is 24, only the information for the first 24 samples will be imported to the run sheet if the sample sheet is selected before the number of lanes is changed to 36.

To select a sample sheet:

Step	Action
1	Open the sample sheet pop-up menu, and select the sample sheet prepared for this run. Once selected, the information on the sample sheet is imported into the run sheet. **IMPORTANT** If the pop-up menu lists <none> only, software cannot find the folder that contains the sample sheets. To correct this, see “Setting Folder Location Preferences” on page 9-5.
2	The information imported from the sample sheet cannot be changed on the run sheet. If changes are made to the sample sheet after it has been selected on the run sheet, you must reimport the sample sheet data. To make changes to the sample sheet after selecting it on the run sheet: a. Open the sample sheet by clicking the icon next to the sample sheet pop-up menu on the run sheet.  b. Make changes to the sample sheet. c. Close and save the sample sheet. d. Open the sample sheet pop-up menu on the run sheet, and select <none>. e. Open the sample sheet pop-up menu on the run sheet, and reselect the sample sheet. You cannot make changes to the sample sheet while a module is running.

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Selecting the Instrument or Gel's Matrix File

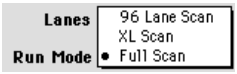
For GeneScan runs in particular, matrices are dye set, instrument, and run condition dependent. As such, matrices must be remade when any of these conditions change. For more information, refer to Chapter 6, "Making Matrix Files for GeneScan," and the *ABI PRISM GeneScan Reference Guide,* P/N 4303188.

To select an instrument or gel matrix file:

If this is a …	Then …
sequencing run	open the Instrument File pop-up menu, and select the appropriate file. If one does not yet exist, leave the field set to <none>. A file must be selected for automatic data analysis.
GeneScan run	open the Gel's Matrix File pop-up menu, and select the appropriate file. If one does not yet exist, leave the field set to <none>. Required for automatic data analysis.
IMPORTANT If the pop-up menu lists <none> only, software cannot find the folder that contains the instrument/matrix files. To correct this, see "Setting Folder Location Preferences" on page 9-5.	

Selecting the Run Mode, Well-to-Read Distance, and Operator

To select the run mode, well-to-read distance, and operator:

Step	Action	
1	Open the Well-to-Read distance pop-up menu and select the appropriate value.	
2	**If this is …**	**Then …**
	a standard instrument	proceed to the next step.
	an XL or 96-lane upgrade instrument	verify that the correct mode is displayed in the Run Mode field. 
3	Click in the Operator field and type your name.	

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Setting the Collection Time

To set the collection time:

<table>
<tr><th>Step</th><th colspan="4">Action</th></tr>
<tr><td rowspan="8">1</td><td colspan="4">Enter the duration of the run in the Collect time field. Use the run time recommended in your protocol. The following are suggested run times only.</td></tr>
<tr><td rowspan="2">Well-to-Read Length in cm</td><td rowspan="2">Type of Run Scans per Hour/Application</td><td colspan="2">Suggested Collection Time in Hours</td></tr>
<tr><td>19:1 Acrylamide Gels</td><td>Long Ranger (LR), PAGE-PLUS (PP), 29:1 Acrylamide Gels</td></tr>
<tr><td>12</td><td>1200/GS</td><td>1.0</td><td>1.0</td></tr>
<tr><td>12</td><td>2400/GS</td><td>1.0</td><td>1.0</td></tr>
<tr><td>36</td><td>1200/GS & Seq</td><td>7.0</td><td>9.0</td></tr>
<tr><td>36</td><td>2400/GS
2400/Seq</td><td>2.0
3.5</td><td>4.0
4.0</td></tr>
<tr><td>48</td><td>1200/Seq</td><td>10.0</td><td>11.0 (29:1 & LR)
12.0 (PP)</td></tr>
<tr><td colspan="4">GS = GeneScan application; Seq = Sequencing Application

Note The number of scans that can be collected is limited by the software.</td></tr>
<tr><td rowspan="4">2</td><td colspan="2">If ...</td><td colspan="2">Then ...</td></tr>
<tr><td colspan="2">you do not want the data to be analyzed automatically</td><td colspan="2">a. deselect the boxes in the Auto Analyze column by clicking in each box to remove the X.
b. Proceed to “Starting the Run or Closing the Run Sheet” on page 9-37.</td></tr>
<tr><td colspan="2">this is a sequencing run and you want the data to be analyzed automatically</td><td colspan="2">proceed to “Auto Analysis for Sequencing Runs” on page 9-35.</td></tr>
<tr><td colspan="2">this is a GeneScan run and you want the data to be analyzed automatically</td><td colspan="2">proceed to “Auto Analysis for GeneScan Runs” on page 9-36.</td></tr>
</table>

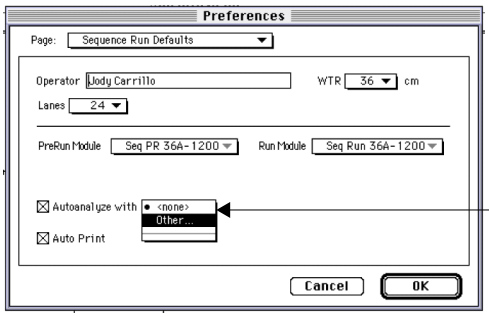
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Auto Analysis for Sequencing Runs

See “About Automatic Data Analysis” on page 9-38 for more information.

IMPORTANT Do not analyze matrix standard samples.

To set up the run sheet for automatic data analysis:

Step	Action
1	Verify that the data analysis software is selected in Preferences. To do this: a. Open the Window menu, and select Preferences. b. Select Sequence Run Defaults … c. Look at the “Autoanalyze with …” field. 
2	**If the analysis software is …** / **Then …** selected: click OK to return to the run sheet. not selected: a. Open the “Autoanalyze with” pop-up menu, and select Other. b. In the dialog box, locate the ABI PRISM DNA Sequencing Analysis Software and click Open. c. Close the current run sheet, and create a new run sheet. **Note** Changing the preference has no affect on run sheets created prior to the change.
3	On the run sheet, verify that the Auto Analyze boxes are selected for each non-matrix standard sample. If the boxes are not selected: a. Reopen the sample sheet by clicking the icon next to the sample sheet pop-up menu.  b. Select a dye set/primer file for each sample that you want analyzed automatically. c. Close and save the sample sheet. d. Open the sample sheet pop-up menu, and select <none>. e. Open the sample sheet pop-up menu, and reselect the sample sheet to update the information.
4	If matrix standard samples are being run, deselect Auto Analyze for all matrix standard samples.
5	Select Auto Print for analyzed data to be printed automatically.
6	Proceed to “Starting the Run or Closing the Run Sheet” on page 9-37.

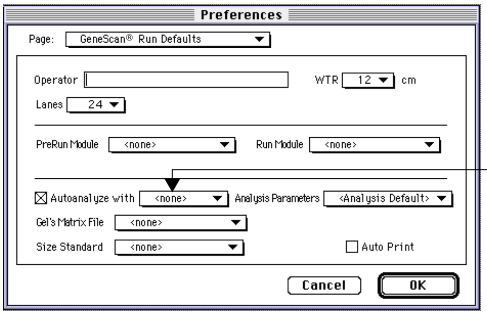
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Auto Analysis for GeneScan Runs

See “About Automatic Data Analysis” on page 9-38 for more information.

IMPORTANT Do not analyze matrix standard samples.

To set up the run sheet for automatic data analysis:

Step	Action	
1	Verify that the data analysis software is selected in Preferences. To do this: a. Open the Window menu, and select Preferences. b. Select GeneScan Run Defaults … c. Look at the “Autoanalyze with …” field. 	
2	**If the analysis software is …**	**Then …**
	selected	click OK to return to the run sheet.
	not selected	a. Open the “Autoanalyze with” pop-up menu, and select Other. b. In the dialog box, locate the ABI PRISM DNA Sequencing Analysis Software and click Open. c. Close the current run sheet, and create a new run sheet. **Note** Changing the preference has no affect on run sheets created prior to the change.
3	Verify that the Auto Analyze boxes are selected for each non-matrix standard sample (Figure 9-1 on page 9-37). Selecting Auto Analyze allows you to select analysis parameters, a size standard, and the auto print option.	
4	If matrix standard samples are being, run, deselect Auto Analyze for all matrix standard samples.	
5	If not already selected, or if you wish to change the preference settings, set the remaining fields as follows: a. Open the Analysis Parameters pop-up menu, and select an analysis parameters file. Analysis parameters must be set up in the analysis software program prior to completing the run sheet. Refer to *ABI PRISM GeneScan Analysis Software User's Manual* for instructions. b. Open the Size Standard pop-up menu, and select the appropriate size standard file. c. Select the Auto Print boxes for analyzed data to be printed automatically. **Note** If the pop-up menus list <none> only, software cannot find the folders that contain the analysis parameters and size standard files. To correct this, see “Commands Used to Perform a Plate Check, PreRun, and Run” on page 9-39.	

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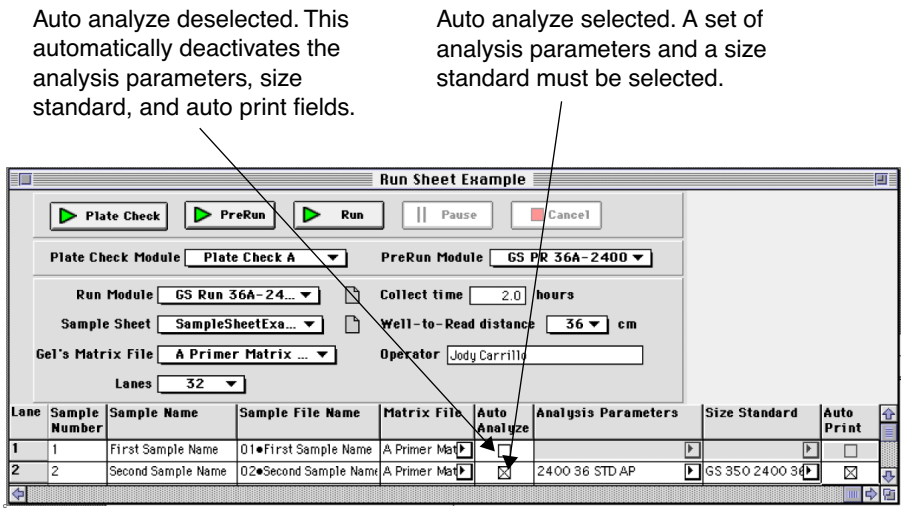
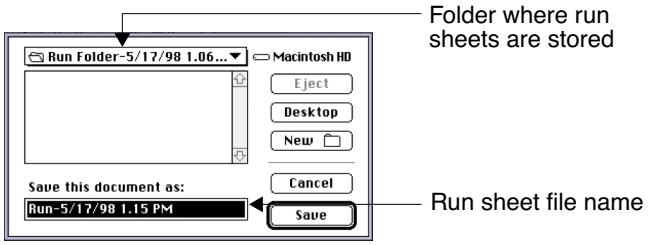


Figure 9-1 Auto analyze selected and deselected on a GeneScan run sheet

Starting the Run or Closing the Run Sheet

To start the run or close the run sheet:

If …	Then …
you are ready to proceed with instrument operation	a. Open the File menu and choose Save. A dialog box showing the default run sheet name, and the location where the file will be saved is displayed. Change the file name and storage location now if desired.  b. Click Save. c. Click the appropriate button on the run sheet to start a plate check, prerun, or run. For more information on the run sheet buttons and other commands used for instrument operation, see “Commands Used to Perform a Plate Check, PreRun, and Run” on page 9-39.
you wish to close the run sheet	a. Open the File menu and choose Close. b. Click Save. A dialog box showing the default run sheet name, and the location where the file will be saved is displayed (shown in previous step). Change the file name and storage location now if desired. c. Click Save.

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About Automatic Data Analysis

Overview

Data can be analyzed and printed automatically or manually after a run. When the software is set up for automatic data analysis, the data collected during the run and all other information required for analysis, is transferred to the analysis software program automatically at the end of the run. The analysis software then automatically analyzes the data and prints the results.

The information that must be specified for automatic data analysis is application specific. Requirements are listed below.

Auto analyze must be selected to use the Stop & Analyze feature during the run. (Described on page 9-39.)

For Sequencing Applications

To perform automatic data analysis after a sequencing run, the following information must be selected on sequencing sample and run sheets:

- On the sequencing sample sheet:
  - A dye set/primer file

    Selecting a dye set/primer file on the sample sheet automatically places an X in the corresponding Auto Analyze box on the run sheet.
  - An instrument file
- On the sequencing run sheet:
  - Auto print (optional)

In addition, the analysis program—ABI PRISM DNA Sequencing Analysis Software—must be selected on the Sequence Run Defaults Preference. This tells the data collection software where to send the data when the run is finished. Instructions are listed under “Auto Analysis for Sequencing Runs” on page 9-35.

For GeneScan Applications

To perform automatic data analysis after a GeneScan run, the following information must be selected on GeneScan sample and run sheets:

- On the GeneScan sample sheet:
  - Size standard designation (Std column)
  - Dye colors run in each lane (Pres column)
- On the GeneScan run sheet:
  - Gel’s matrix file
  - Matrix file
  - Analysis parameters
  - Size standard
  - Auto analyze
  - Auto print (optional)

In addition, the analysis program—ABI PRISM GeneScan Analysis Software—must be selected on the GeneScan Run Defaults Preference. This tells the data collection software where to send the data when the run is finished. Instructions are listed under “Auto Analysis for GeneScan Runs” on page 9-36.

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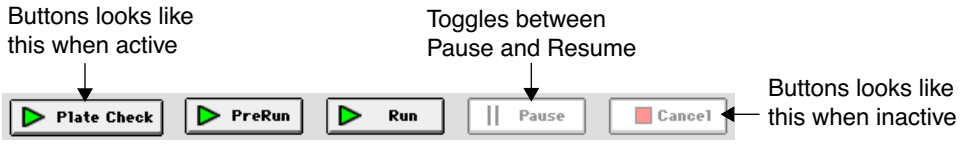
Commands Used to Perform a Plate Check, PreRun, and Run

Overview

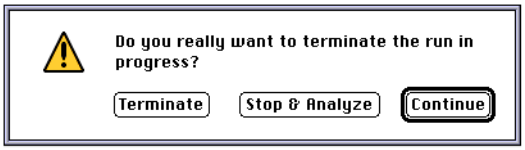
A plate check, prerun, and run can be started by using the buttons on the run sheet, or by using commands from the Instrument menu.

Using the Buttons on the Run Sheet

A button must be “active” to be selected.

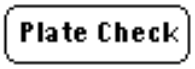
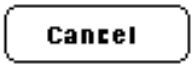
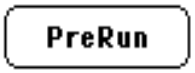
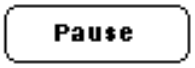
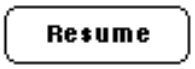
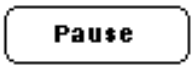
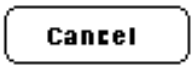
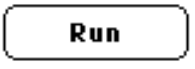


The following is a description of each button and its function.

Button	Function
Plate Check	Click the Plate Check button to start a plate check. The Plate Check button becomes active when the plate check module is selected on the run sheet. The duration of the plate check is specified in the module. However, the plate check can be paused or cancelled by clicking the Pause or Cancel button.
PreRun	Click the PreRun button to start a prerun. The PreRun button becomes active when the prerun module is selected on the run sheet. The duration of the prerun is specified in the module. A prerun can be paused or cancelled by clicking the Pause or Cancel button.
Run	Click the Run button to start the run. The Run button becomes active when the run module and sample sheet are selected on the run sheet.
Pause/ Resume	The Pause button becomes active when a plate check, prerun, or run is started. Click Pause to temporarily halt instrument operation. Pausing a prerun or run halts electrophoresis, while maintaining gel temperature. When the Pause button is selected, it changes to Resume. Click Resume to resume instrument operation.
Cancel	The Cancel button becomes active when a plate check, prerun, or run is started. Click Cancel to cancel a plate check, prerun, or run. If cancel is selected during a run, the following dialog box is displayed.  ♦ Clicking *Terminate* terminates the run without automatic data analysis. ♦ Clicking *Stop & Analyze* terminates the run, and starts automatic data analysis of the data collected to that point in the run. The run cannot be resumed. To use this feature, the run sheet must be configured for automatic data analysis prior to starting the run. ♦ Clicking *Continue* resumes the run and data collection.

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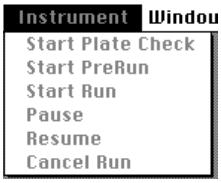
The following table is an example of a typical series of commands that would be used for a sequencing run. This example is written as if a shark's-tooth comb is being used, and samples are being loaded using a staggered load method. Refer to Chapter 3, "Instrument Operation," for more information on sample load methods and recommendations.

Button Clicked	Module Affected	Action
	Plate Check module	Starts the plate check. Plates are scanned without electrophoresis to check for fluorescent contaminants.
	N/A	Terminates the plate check. Buffer chambers are then filled and checked for leaks. Front heat-transfer plate mounted if applicable.
	PreRun module	Starts the prerun. Gel is electrophoresed without data collection to equilibrate gel temperature.
	PreRun module	Pauses the prerun. Wells flushed and samples loaded into the odd numbered lanes (this is the staggered load method). Gel temperature is maintained.
	PreRun module	Prerun is resumed. Samples are electrophoresed into the gel without data collection for approximately two minutes.
	PreRun module	Pauses the prerun. Wells flushed again, and remainder of samples loaded into the even numbered lanes. Gel temperature is maintained.
	N/A	Terminates the prerun.
	Run module	Starts the run (electrophoresis and data collection).

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Using Commands from the Instrument Menu

The Instrument menu contains commands that perform the same functions as the run sheet buttons.



The functions of the commands on the Instrument menu are summarized below.

To …	Select this command from the Instrument menu …
Start a plate check	Start Plate Check
Start a prerun	Start PreRun
Start a run	Start Run
Temporarily halt a prerun or run so it can be resumed later	Pause
Resume a prerun or run after it has been paused	Resume
Terminate a plate check, prerun, or run	Cancel Run

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Modules

Overview

Modules are software files that contain the various settings (electrophoresis voltage, gel temperature, etc.) required for instrument operation (the plate check, prerun, and run). ABI PRISM Data Collection Software version 2.1 modules can be grouped into two general categories:

- Standard modules

  Provide gel temperature control from 10°C above ambient to a maximum of 60°C. Located in the Modules folder.

- Chiller modules

  Modules used when an external cold water bath is attached to the instrument, and temperatures below 10°C above ambient are required. Located in the Chiller Modules folder. See “Chiller Modules” on page 9-44 for more information.

Within each of these categories are prerun and run modules specific for sequencing or GeneScan analysis applications. The application is designated by the module name (see “Module Naming Conventions” below).

Choosing a module automatically selects a virtual filter set. (Virtual filter sets, dye sets, and the various chemistries available are described under “Virtual Filter Sets” on page 9-51 and “DyeSet/Primer Files” on page 9-49.) If the wrong run module (and therefore virtual filter set) is chosen for a particular chemistry, the data will be poor or useless. For sequencing applications, bases will be miscalled, or the data will be meaningless. We recommend running the samples again using the correct run module.

See page 9-47 for a list of ABI PRISM Data Collection Software version 2.1 modules.

Module Naming Conventions

The naming conventions used for module files are described in the table below.

Name	Definition
Seq	Module is for ABI PRISM DNA Sequencing Analysis Software applications
GS	Module is for ABI PRISM GeneScan Analysis Software applications
Plate Check	Plate check module
PR	Prerun module
Run	Run module
12, 36, or 48	The well-to-read distance of the gel plates in centimeters
A, B, C, D, E, or F	The virtual filter set used by the module
1200	1200 scans/hour (approximately 100 bph for sequencing)
2400	2400 scans/hour—high speed (approximately 200 bph for sequencing)
CHILLER	Modules designed for use with an external cold water bath

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The following examples show how to interpret a module name.

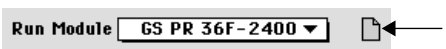
Module	Definition
GS Run 36D-2400 CHILLER	GS = GeneScan
	Run = Run
	36 = 36 cm well-to-read glass plates
	D = virtual filter set D
	2400 = 2400 scans/hour
	CHILLER = chiller module
Seq PR 48A-1200	Seq = Sequencing
	PR = prerun
	48 = 48 cm well-to-read glass plates
	A = virtual filter set A
	1200 = 1200 scans/hour
Plate Check A	Plate Check = plate check
	A = virtual filter set A

To View the Settings in a Module

To determine the instrument settings specified by a particular module, open the module settings dialog box from a run sheet (instructions listed below). The module settings window shows the following information.

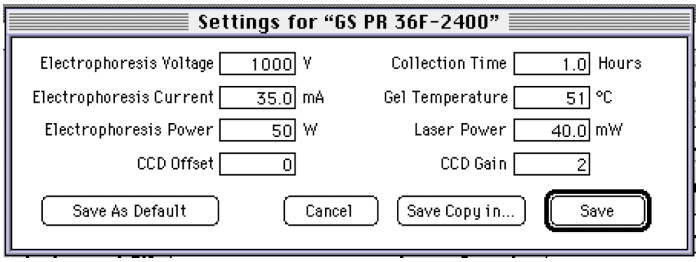
Electrophoresis Voltage	Collection Time
Electrophoresis Current	Gel Temperature (also referred to as the run temperature)
Electrophoresis Power	Laser Power
CCD Offset	CCD Gain

To open a Module Settings dialog box:

Step	Action
1	Open the File menu and choose New.
2	Click Sequence Run or GeneScan Run to open a new run sheet.
3	Open the Run Module pop-up menu and choose the module file you wish to view.
4	Click the small document icon next to the Run module pop-up menu to open the Module Settings dialog box.  Click this icon to view the settings of the module selected

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To open a Module Settings dialog box: *(continued)*

Step	Action
5	The Module Settings dialog box also allows you to change module settings. Module settings can be modified temporarily for one run without changing the original module. You can also use existing modules as templates for creating new modules, or you can change the default settings of an existing module. For more information and instructions, see “Modifying and Creating Modules” on page 9-45.  Shown above is the Module Settings dialog box for the module named GS PR 36F-2400.
6	Close the run sheet when finished.

Chiller Modules

Chiller modules and an external cold water bath operating at 22°C and below allow you to perform PCR Single-stranded Conformation Polymorphism (SSCP) analysis.

For more information on using chiller modules and an external cold water bath, refer to Appendix B, “Subambient Temperature Operation.”

For more information on the PCR SSCP technique, refer to *PCR SSCP Analysis: A Guide to Fluorescent PCR Single-stranded Conformation Polymorphism Analysis on the ABI PRISM 377 DNA Sequencer* (P/N 904413).

Installing Chiller Modules

Chiller modules are installed by moving them into the Modules folder with the standard modules, or by setting the Folder Locations Preference to allow the software to access them directly in the Chiller Modules folder.

Moving Chiller Modules to the Modules Folder

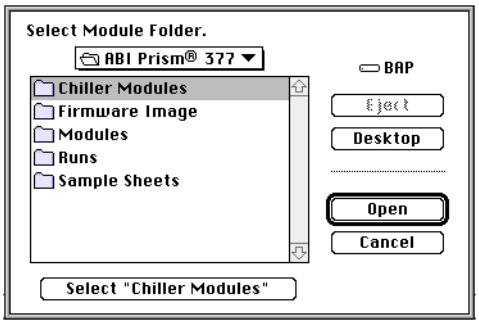
To move chiller modules to the Modules folder:

Step	Action
1	Double-click the hard drive icon on the Macintosh to access the Finder and open the hard drive directory.
2	Open the ABI PRISM 377 folder.
3	Open both the Modules folder and the Chiller Modules folder.
4	Click in the Chiller Modules window so it becomes the active window.
5	Open the Edit menu, and choose Select All to select all the files in the Chiller Modules folder.
6	Click and drag all the chiller module files into the Modules folder.
7	Close both folders.

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Setting Preferences to Access Chiller Modules Directly in the Chiller Modules Folder

To set preferences to access chiller modules in the Chiller Modules folder:

Step	Action
1	Open the ABI PRISM Data Collection Software version 2.1.
2	Open the Window menu, select Preferences, and then select Folder Locations.
3	Click the Module Folder to open the Select Module Folder dialog box. 
4	Click once on Chiller Modules to highlight the folder.
5	Click the Select “Chiller Modules” button.
6	Click OK to save this setting.

IMPORTANT To run the instrument using standard conditions, change the Module folder preferences setting from the Chiller Modules folder back to the standard Modules folder.

Modifying and Creating Modules

ABI PRISM 377 Data Collection Software version 2.1 module files can be:

- Permanently modified
- Temporarily modified for one run
- Used as templates to create custom module files without changing the original module

Module files are created and modified by opening an existing module file that has been selected on a run sheet. How the file is saved determines whether a new module is created, or an existing module is temporarily or permanently modified.

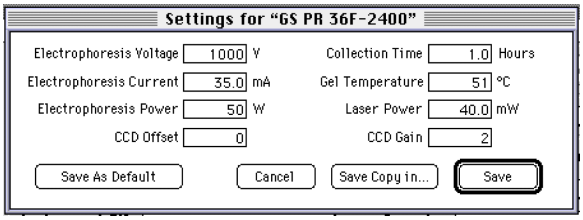
Note Rather than permanently modifying existing modules, we strongly recommend using the modules supplied with the software as templates to create new modules. This ensures you will always have all the modules designed for use with your instrument.

To modify an existing module or create a new one:

Step	Action
1	Open the File menu and choose New.
2	Click Sequence Run or GeneScan Run to open a new run sheet.
3	Open the Run Module pop-up menu and choose the module file you wish to work with.
4	Click the small document icon next to the Run module pop-up menu to display the Module Settings dialog box. 

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To modify an existing module or create a new one: *(continued)*

Step	Action	
5	Enter new values in the respective fields as desired. 	
6	**Save the changes by clicking …**	**To …**
	Save	use the changes as a one-time override for the current run.
	Save As Default	save the changes as the new defaults in the existing module. **Note** Rather than permanently modifying existing modules, we recommend using the Save Copy In command to create a new module. This ensures you will always have all the modules designed for use with your instrument.
	Save Copy In …	create a new module. Enter a name for the new module in the dialog box displayed, and then click Save.
7	Close the run sheet.	

Unused Modules

We recommend moving modules that are not used to a folder named Unused Modules. This will limit the number of modules displayed in the module pop-up menus on the run sheet.

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List of Modules

The table below lists all the modules supplied with ABI PRISM 377 Data Collection Software version 2.1. To keep the number of modules displayed in the pop-up menus on the run sheet to a minimum, we recommend moving all unused module files to a folder named Unused Modules on the Macintosh hard drive.

ABI PRISM 377 Data Collection Software version 2.1 module files:

Standard Modules	Chiller Modules
N/A	N/A
Plate Check A	Plate Check A CHILLER
Plate Check B	Plate Check B CHILLER
Plate Check C	Plate Check C CHILLER
Plate Check D	Plate Check D CHILLER
Plate Check F	Plate Check F CHILLER
Seq PR 36A-1200	Seq PR 36A-1200 CHILLER
Seq PR 36B-1200	Seq PR 36B-1200 CHILLER
Seq PR 36A-2400	Seq PR 36A-2400 CHILLER
Seq PR 36B-2400	Seq PR 36B-2400 CHILLER
Seq Run 36A-1200	Seq Run 36A-1200 CHILLER
Seq Run 36B-1200	Seq Run 36B-1200 CHILLER
Seq Run 36A-2400	Seq Run 36A-2400 CHILLER
Seq Run 36B-2400	Seq Run 36B-2400 CHILLER
Seq Run 36E-1200	Seq Run 36E-1200 CHILLER[1]
Seq Run 36E-2400	Seq Run 36E-2400 CHILLER
Seq Run 48A-1200	Seq Run 48A-1200 CHILLER
Seq Run 48B-1200	Seq Run 48B-1200 CHILLER
Seq Run 48E-1200	Seq Run 48E-1200 CHILLER
GS PR 12A-1200	GS PR 12A-1200 CHILLER
GS PR 12C-1200	GS PR 12C-1200 CHILLER
GS PR 12D-1200	GS PR 12D-1200 CHILLER[1]
GS PR 12F-1200	GS PR 12F-1200 CHILLER[1]
GS PR 12A-2400	GS PR 12A-2400 CHILLER
GS PR 12C-2400	GS PR 12C-2400 CHILLER
GS PR 12D-2400	GS PR 12D-2400 CHILLER
GS PR 12F-2400	GS PR 12F-2400 CHILLER
GS PR 36A-1200	GS PR 36A-1200 CHILLER
GS PR 36C-1200	GS PR 36C-1200 CHILLER
GS PR 36D-1200	GS PR 36D-1200 CHILLER
GS PR 36F-1200	GS PR 36F-1200 CHILLER

1. Use virtual filter set E modules with ABI PRISM dRhodamine Terminator Cycle Sequencing Kits or the dRhodamine Matrix Standards Kit only. Use virtual filter set D and F modules with GeneScan and NED dyes.

2. These chiller modules are recommended for PCR Single-stranded Conformation Polymorphism Analysis (SSCP).

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ABI PRISM 377 Data Collection Software version 2.1 module files: *(continued)*

GS PR 36A-2400	GS PR 36A-2400 CHILLER
GS PR 36D-2400	GS PR 36D-2400 CHILLER
GS PR 36C-2400	GS PR 36C-2400 CHILLER
GS PR 36F-2400	GS PR 36F-2400 CHILLER
GS Run 12A-1200	GS Run 12A-1200 CHILLER
GS Run 12C-1200	GS Run 12C-1200 CHILLER
GS Run 12D-1200	GS Run 12D-1200 CHILLER
GS Run 12F-1200	GS Run 12F-1200 CHILLER
GS Run 12A-2400	GS Run 12A-2400 CHILLER
GS Run 12C-2400	GS Run 12C-2400 CHILLER
GS Run 12D-2400	GS Run 12D-2400 CHILLER
GS Run 12F-2400	GS Run 12F-2400 CHILLER
GS Run 36A-1200	GS Run 36A-1200 CHILLER
GS Run 36C-1200	GS Run 36C-1200 CHILLER
GS Run 36D-1200	GS Run 36D-1200 CHILLER
GS Run 36F-1200	GS Run 36F-1200 CHILLER
GS Run 36A-2400	GS Run 36A-2400 CHILLER
GS Run 36C-2400	GS Run 36C-2400 CHILLER
GS Run 36D-2400	GS Run 36D-2400 CHILLER
GS Run 36F-2400	GS Run 36F-2400 CHILLER
	GS Run 60W A CHILLER[2]
	GS Run 60W C CHILLER[2]
	GS Run 60W D CHILLER[2]
	GS Run 2140V A CHILLER[2]
	GS Run 2140V D CHILLER[2]
	GS Run 2140V C CHILLER[2]

1. Use virtual filter set E modules with ABI PRISM dRhodamine Terminator Cycle Sequencing Kits or the dRhodamine Matrix Standards Kit only. Use virtual filter set D and F modules with GeneScan and NED dyes.

2. These chiller modules are recommended for PCR Single-stranded Conformation Polymorphism Analysis (SSCP).

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DyeSet/Primer Files

What are DyeSet/Primer Files

For sequencing applications only, dye set/primer files:

- Must be specified on sample sheets for data to be analyzed automatically
- Contain the information used to:
  - Compensate for differences (shifts) in sample mobility
  - Interpret what each dye color in a set represents

Each of the dyes used in a set affects the electrophoretic mobility of cycle sequencing extension products differently. The relative mobility of the dye-labeled fragments is specific to each sequencing chemistry. Under the same set of run conditions, the mobilities are very reproducible.

ABI PRISM DNA Sequencing Analysis Software compensates for these mobility differences by applying the mobility shift information contained in dye set/primer files. As a result, evenly spaced peaks are presented in the analyzed data.

Dye set/primer files also tell the software which matrix in the instrument file to use for data analysis.

DyeSet/Primer File Naming Conventions

Dye set/primer file names indicate the primer, chemistry, type and concentration of gel, and sometimes the filter set used. The abbreviations used for these file names are as follows:

Abbreviation	Definition
DP	Dye primer
DT	Dye terminator
x%	The approximate percent concentration of the gel used
Ac	Acrylamide gel
LR	Long Ranger gel
BD	BigDye
dR	dRhodamine
{x x x}	Any combination of virtual filter set, primer, and chemistry

Note The percentages (4%, 5%, and 6%) are approximates. For example, if the gel is 4.25% acrylamide, use the corresponding 4% acrylamide dye set/primer file. Also, 5% Long Ranger equals 4% acrylamide when selecting these files.

The following examples show how to interpret a dye set/primer file name.

DyeSet/Primer File Name	Definition
DP4%Ac{-21M13}	DP = dye primer
	4%Ac = 4% acrylamide gel
	{-21M13} = the primer
DP5%LR{BD M13 FWD & REV}	DP = dye primer
	5%LR = 5% Long Ranger gel
	BD = Big Dye
	M13 FWD & REV = m13 forward and reverse primers

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DyeSet/Primer File Name	Definition
DT {dR Set Any-Primer}	DT = dye terminator
	dR = dRhodamine
	dR Set = virtual filter set E
	Any Primer = works with any primer
DT4%Ac{B Set–AnyPrimer}	DT = dye terminator
	4%Ac = 4% acrylamide gel
	B Set = virtual filter set B
	Any Primer = works with any primer

Choosing a DyeSet/ Primer File

The dye set/primer files listed below are recommended for the following sequencing chemistries.

Dye Set/Primer Files and DNA Sequencing Chemistries

Dye Primer	BigDye Primer	Dye Terminator	dRhodamine Terminator	BigDye Terminator
DP4%Acv2{M13Rev}	DP5%LR{BD M13 FWD & REV}	DT4%Ac{A Set-AnyPrimer}	DT {dR Set Any-Primer}	DT {BD Set Any-Primer}
DP4%Ac{-21M13}				
DP4%Ac{KS}				
DP4%Ac{SK}				
DP4%Ac{SP6}				
DP4%Ac{T3}				
DP4%Ac{T7}				

If the wrong dye set/primer file is specified on the sequencing sample sheet and is used for automatic data analysis, the data can be reanalyzed using the correct file. Refer to the *Automated DNA Sequencing Chemistry Guide* for more information.

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Virtual Filter Sets

What Are They

Virtual filter sets are used to detect light intensity in four non-overlapping, light-collecting regions on a CCD camera located inside the instrument. Each region corresponds to a wavelength range that contains or is close to the emission maximum of an ABI PRISM dye. Data collection software color-codes the intensity displays from the four regions. These appear as the blue, green, black (yellow on gel images), and red peaks in the raw data.

This process is similar to using a physical filter to separate light of different wavelengths. However, the filter sets are “virtual” because the instrument uses no physical filtering hardware to perform the separation. Each virtual filter set has been optimized to provide the maximum possible separation among the centers of detection for the different dyes used together as a set, while maintaining an excellent signal-to-noise ratio. The virtual filter used during data collection is designated by the run module selected on the run sheet.

The exact positions of the CCD regions and the dye combinations appropriate to these positions depend upon the virtual filter set used. For example, with Virtual Filter Set E the instrument records the light intensity in four regions, or “windows,” centered at 540 nm, 570 nm, 595 nm, and 625 nm. The window positions in each virtual filter set have been optimized to provide the maximum possible separation among the centers of detection for the different dyes while maintaining good signal strength.

The data collection software color-codes the intensity displays from the four light-collection regions. These appear as the blue, green, black (yellow on gel images), and red peaks in the raw data.

The Sequencing Analysis Software uses the same four colors to color-code analyzed data from all dye/virtual filter set combinations. The display colors represent the relative, not the actual, detection wavelengths. For consistency, the software always displays analyzed data with A as green, C as blue, G as black, and T as red in the electropherogram view.

The wavelengths of the “windows” in the virtual filter sets used in cycle sequencing applications are listed in the following table.

Virtual Filter Set	Color	Wavelength Range of Virtual Filter (nm)
A	blue	530–541
	green	554–564
	yellow/black	581–591
	red	610–620
E	blue	535–545
	green	565–575
	yellow/black	590–600
	red	620–630

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Filter Sets Available

All the ABI PRISM 377 instruments use six virtual filter sets designated A through F.

For GeneScan applications

Virtual filter sets A,C, D, and F are used.

For Sequencing Applications

Virtual filter sets A and E are used.

For More Information

For more information, see:

- "About Run Sheets" on page 9-28
- "Modules" on page 9-42
- *GeneScan Reference Guide*
- *Automated DNA Sequencing Chemistry Guide*

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Viewing Data and Instrument Status

Windows Available

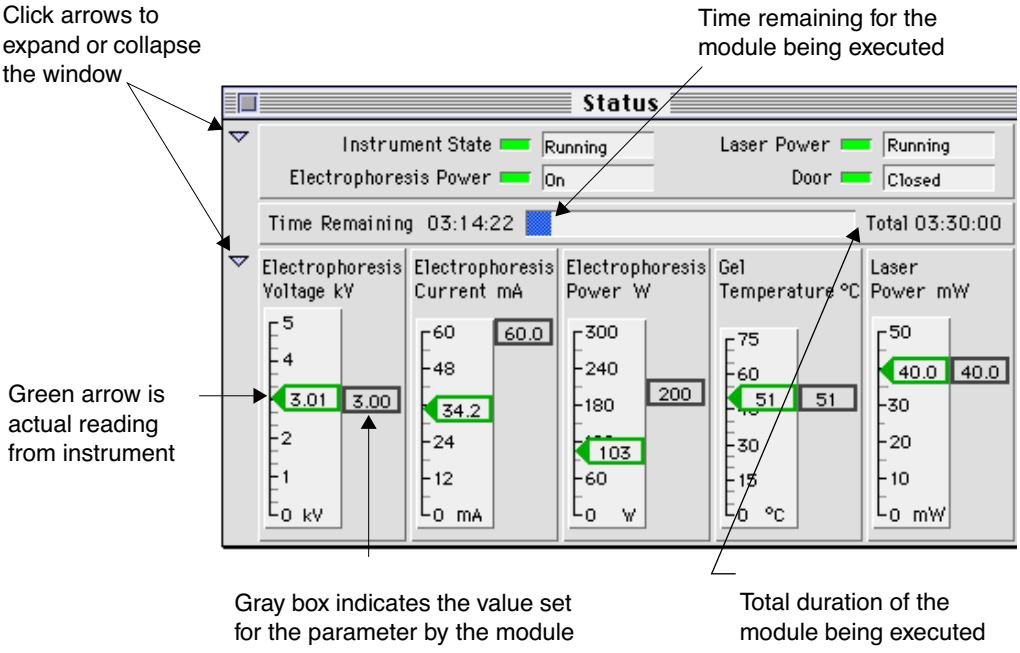
Four different windows are available for viewing real-time data and instrument status.

- Data windows are:
  - Scan
  - Gel
- Instrument windows are:
  - Status
  - Electrophoresis History

In addition to these windows, a chronological, comprehensive record of all significant system events including error and status messages is kept in a Log file. All of these windows can be open at the same time, however, only one window can be active at any given time.

The Status Window

During a run, the current status of the instrument is viewed in the Status window. The information in this window is updated approximately every three seconds. Open the Status window by choosing Status from the Window menu.



IMPORTANT The electrophoresis voltage, gel temperature and laser power are setpoints. Electrophoresis current and power are limits.

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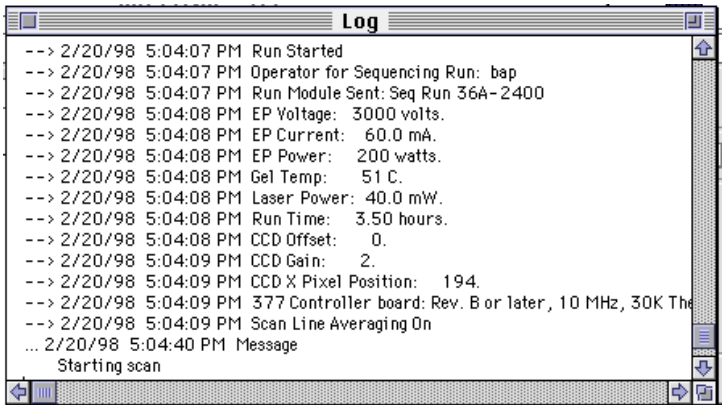
The Log File

A Log file is created automatically for each run when the run is started. Log files contain a comprehensive, chronological record of all error and status messages generated by the data collection program during a run. This information includes:

- Start and stop times of the run
- Instrument and Macintosh errors

Each Log file is stored in its respective Run folder inside the Runs folder. These files can be very large, and only the last 32K of information can be viewed using the data collection program. To view the Log file using the data collection software program, open the Window menu and choose Log. Use Microsoft® Word or Simple Text to view the entire Log file stored in a Run folder.

The following is an example of the kinds of information stored in a Log file.



The information in the file is formatted as follows:

```
xxx   mm/dd/yy hh:mm:ssdescription
```

The entry in the first column, xxx, is variable. Possible entries are:

```
...   information (system start or stop, file created)
 - -> message sent to instrument
<- -  message received from instrument
***   warning
###   error
```

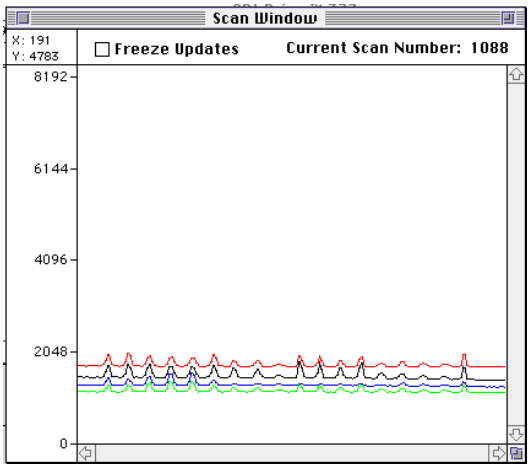
The second column shows the month/day/year and hours:minutes:seconds. The third column is a brief description of the event.

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The Scan Window

The Scan window (below) shows real-time raw data as sweeps of the laser across the gel. The different colored lines represent each dye color in the dye set. Data is updated every few seconds.

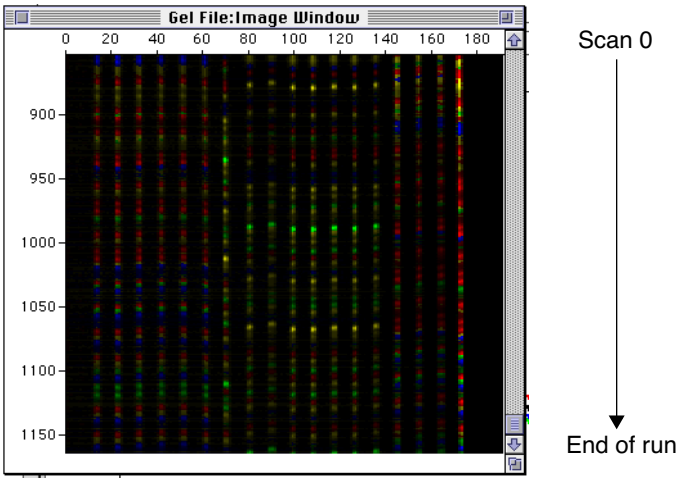
To display the Scan window, open the Window menu and choose Scan.To set the scale of the Scan window, open the Edit menu and choose Set Scale. Enter minimum and maximum values for the scale in the dialog box that is displayed.



The Gel Window

The Gel window is a reconstruction of actual data. The first fragments passing the laser appear at the top of the window and move toward the bottom as new data is collected. You can scroll up or down the Gel window to see parts that are not visible. Remember, however, that this window is a re-creation of the data through time, and does not indicate the physical position of the fragments.

To display the Gel window, choose Gel Image from the Window menu.

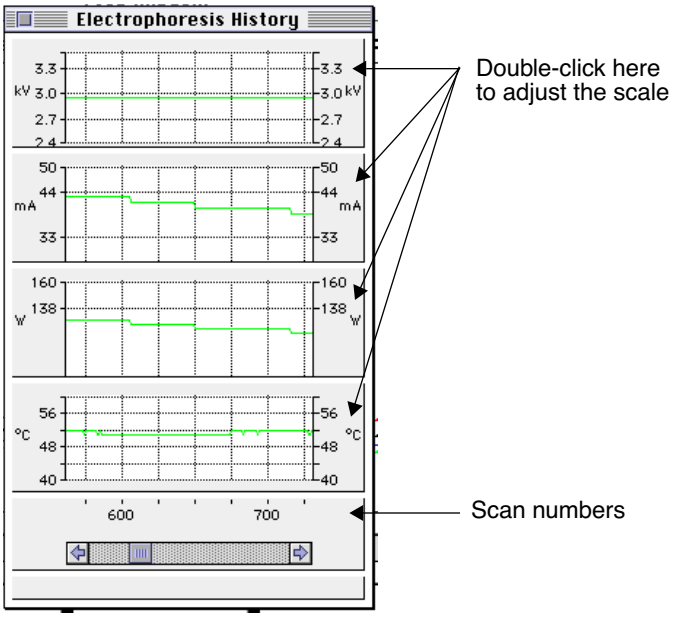


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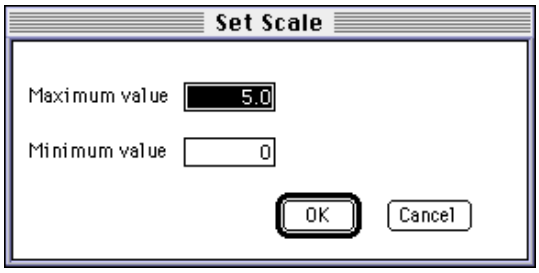
The Electrophoresis History Window

The Electrophoresis History window displays the set and actual values for the electrophoresis power supply and gel temperature throughout the course of a run. The scale for each panel is adjustable.

The information in the Electrophoresis History window is also stored in the Gel file. To display the electrophoresis history window, open the Window menu and choose Electrophoresis History.



To adjust the scale:

Step	Action
1	Double-click one of the panels in the window, or click a panel once and choose Set Scale from the Edit menu. The Set Scale dialog box is displayed. 
2	Type in the minimum and maximum values you wish to display.
3	Click OK.

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Viewing Other Run-Related Information

The Sample Sheet

To display the sample sheet associated with the current run, choose Sample Sheet from the Window menu. This option is available only if a sample sheet is selected in a Run window that is open. For more information, see:

- "About Sample Sheets for Sequencing Applications" on page 9-14
- "About Sample Sheets for GeneScan Applications" on page 9-20
- Sequencing or GeneScan Analysis Software user's manual as appropriate

The Run Sheet

To display the Run sheet for the current run, choose Run from the Window menu. For more information see:

- "About Run Sheets" on page 9-28

Opening and Saving Files

Opening Files

Most files are opened using the Open command in the File menu. Other files, such as Log and Gel files, are opened by clicking the file icon from the data collection software program.

Saving Files

Files are saved by choosing one of the Save commands from the File menu. Sample and run sheets are automatically named and saved based on the default settings entered for the Default File Names and Folder Locations Preferences. Refer to Chapter 5, "Setting Preferences," for more information.

When modifying existing sample sheets, run sheets, or Module Settings dialog boxes, we recommend saving the changes under a new file name to preserve the original information.

To save a modified file under a new name:

Step	Action
1	Open the File menu and choose Save As.
2	Enter a name for the modified file, and select a storage location for it.
3	Click Save.

To save a backup copy of the file you are working with:

Step	Action
1	Open the File menu and choose Save a Copy In.
2	Enter a name for the file, and select a storage location for it.
3	Click Save. A backup copy of the file is saved with the new file name, and the original file remains on the computer screen.

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Archiving Files

Gel Files

A gel file is automatically created for each run, and contains the raw data collected by the data collection software. Gel files typically require 10–70 MB of disk space. Because gel files are so large, they can be deleted from the hard disk once satisfactory sample files have been generated. It is typically not necessary to save gel files once the tracking has been verified or adjusted, and sample files are created. (See page 9-60.)

To archive Gel files, use magnetic tapes, removable cartridge drives, CD ROMs, or optical drives. Gel files are too large to fit on floppy disks.

Note If a gel file is deleted, the data can be reanalyzed, but cannot be retracked.

Sample Files

Use floppy disks, a magnetic tape drive, a removable cartridge drive, or an optical drive to archive sample files (described on page 9-60). A 1.4 MB, high-density disk holds about six files. A sample file is 150-200 KB in size, depending on the length of the run. Save a sample file when you feel confident that the channel selections (tracking) are correct for the sample.

Printing Files

Setting Up the Page for Printing

The contents of any of the editable window can be printed (for example, sample sheets, run sheets, module settings dialog boxes).

Before printing, certain parameters can be specified, such as the paper size, the orientation of the page, and whether or not the print should be reduced from actual size. To set up a page for printing, choose Page Setup from the File menu and make the appropriate selections.

The appearance of the Page Setup dialog box depends on the printer you are using. Refer to the manual for your particular printer for information on page set up.

Printing Procedure

To print the contents of a window:

Step	Action
1	Make sure the printer is on and loaded with paper.
2	Click the window you wish to print to make it active.
3	Choose Print, or Print One from the File menu. Print One prints the window immediately, bypassing the standard print dialog box. If you choose Print, you can set the number of copies and the pages that you want to print. The appearance of the print dialog box depends on the printer you are using.
4	Click Print.

Note If you choose Auto Analyze and Auto Print on the run sheet, electropherograms are automatically printed after the collected data is analyzed.

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Quitting the Data Collection Program

Closing Open Windows

To close open windows:

Step	Action
1	Click a window to make it active.
2	Close the window using one of the following methods: ♦ Click the close box in the upper left corner of the window. ♦ Open the File menu and choose Close. The current settings in the window are saved.

Quitting the Program

To quit the data collection program:

Step	Action
1	Open the File menu and choose Quit.

Note If a run is in progress, a dialog box asks you to verify that you want to quit. Quitting the data collection software program while a run is in progress cancels the run. Whatever data has been collected to that point is saved. You can then use either analysis program to open the Gel file containing the collected data.

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Data Analysis Overview

Overview

When the run is finished, data is analyzed automatically or manually using one of two data analysis software programs:

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

The following information is a brief overview of the data analysis process up through the generation of sample files. For more information on these software programs and how to use them to analyze your data, refer to the following publications:

- *ABI PRISM DNA Sequencing Analysis Software User's Manual*
- *ABI PRISM GeneScan Analysis Software User's Manual*

Sample Files

When a run is finished, the gel file is processed by sequencing or GeneScan analysis software either automatically or manually. The Auto Analyze feature and associated parameters on the run sheet must be properly configured for data analysis to occur automatically at the end of a run. (See "About Run Sheets" on page 9-28 for more information.) Gel file processing includes:

- Examining and adjusting the gel image
- Adjusting the tracking, and marking lanes for extraction
- Extracting sample data for each lane marked, and generating sample files

One sample file is created for each lane of data extracted from the gel file. Each sample file contains:

- Information entered on the sample and run sheets
- Raw and analyzed data
- Run start and stop times
- Voltage, temperature, and power values during the run
- A record of analysis settings
- Peak locations and size calling values
- Base calls (for sequencing applications only)

Sample files are stored in the corresponding run folder along with the Gel, Log, and run sheet files.

<File Name>.seq Files

Created by ABI PRISM DNA Sequencing Analysis Software only, these files show the base letter sequence of the data. The files can also contain a header, and are stored in the corresponding run folder along with the Gel, Log, run sheet, and sample files.

.seq files can be opened from word processing programs and printed. They can also be saved in several formats that can be read by other software programs. The Preferences dialog box in the sequencing analysis software program allows you to choose a file format.

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ABI PRISM® 377 DNA Sequencer

Appendix A — Gel Recipes

Quick Reference Guide

Applied Biosystems

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Gel Recipes

A

Appendix Contents

In this Appendix The following information is provided in this appendix:

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List of Gel Recipes and Recommendations

List of Recipes

The following gel recipes for sequencing applications are provided in this appendix.

Gel Formulation		See page
Polyacrylamide	Generic 19:1	A-19
	29:1—4.5%	A-11
	29:1—4.25%	
	for SSCP	
Long Ranger ™	5.0%	A-15
	4.75% (Long Ranger Singel)	
	for SSCP	
PAGE-PLUS	4.8%	A-13
	5.25%	
Mutation Detection gel (MDE) for SSCP	—	A-18
SSCP = Single-strand Conformation Polymorphism		

Recommendations for Longest Read Length

Gel formulations that provide longer read lengths when compared to read lengths from 19:1 polyacrylamide gels of the same plate size and run speed are listed in the following table.

Plate Size and Run Speed	Recommended Gel Formulations	Expected Read Length Ranges
♦ 36-cm well-to-read (WTR) plates ♦ 1200 scans/hr	♦ 4.5% 29:1 polyacrylamide ♦ 4.8% PAGE-PLUS ♦ 5.0% Long Ranger (concentrate or Singel™ gel forms)	650–800
♦ 36-cm WTR plates ♦ 2400 scans/hr	♦ 4.5% 29:1 polyacrylamide	550–700
♦ 48-cm WTR plates ♦ 1200 scans/hr	♦ 4.25% 29:1 polyacrylamide ♦ 4.75% Long Ranger Singel ♦ 5.25% PAGE-PLUS	750–900

Note The products required to make the gels presented in this appendix are not manufactured by Applied Biosystems. The quality assurance of these products is regulated by the respective suppliers.

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Sequencing Chemistries Released in 1997

New Chemistries

During 1997, three new sequencing chemistries were released:

Chemistry	Part Numbers
ABI PRISM® dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS	403044 for the 100 reaction kit
	403045 for the 1000 reaction kit
ABI PRISM® BigDye™ Primer Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS	403051 for the 100 reaction kit
	403049 for the 1000 reaction kit
ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS	4303149 for the 100 reaction kit
	4303150 for the 1000 reaction kit

When these new chemistries are used with the gel formulations listed under "Recommendations for Longest Read Length" on page A-2, read lengths commonly fall at the upper end of the ranges listed in the table. The new chemistries produce longer read lengths because they have:

- Greater signal-to-noise ratios
- More even peak heights in both dye terminator chemistries
- More intense signal with the BigDye™ primers and terminators

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Factors that Affect Gel Quality and Read Lengths

Factors

One of the most critical variables that determines the success or failure of both sequencing and GeneScan® analysis software runs is the gel. The use of consistently prepared, high quality gels will help ensure the best experimental results and minimize time spent troubleshooting gel problems. Poor quality gels often cause problems that are mistaken for instrument problems.

For sequencing runs, the quality of the gel directly effects the number of bases that can be called. For GeneScan runs, the quality of the gel effects the mobility of DNA fragments from run-to-run, reproducibility of sizing, signal strength, and resolution.

Factors that affect gel quality include:

- Purity and freshness of reagents
- Rate of polymerization
- Presence of air bubbles
- Age of the gel

Other important variables that can significantly impact read lengths include:

- Template
  - quality
  - base composition
  - quantity
- Primer design
- Amount of sequencing reaction loaded on the gel
- Quality of formamide used in loading buffer
- Type of chemistry used

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Reagent Purity

Always use ultra-pure reagents obtained from a reliable source and high-grade, distilled, deionized water to prepare solutions, and wear clean laboratory gloves. Filtration of all solutions is essential to remove any particulate matter that may fluoresce or scatter light.

Reagent purity and associated problems:

Reagent	Problems That Can Occur When Fresh Reagents are Not Used
Acrylamide and bisacrylamide	Impurities in these reagents can cause: ♦ Irreproducible gel porosity ♦ Deviant mobility ♦ Inhibition of polymerization ♦ Poor resolution These problems lead to compromised reproducibility. Possible contaminants include: ♦ Acrylic acid – The hydrolysis product of acrylamide – Copolymerizes with acrylamide and bisacrylamide – Causes DNA to migrate slowly, resulting in broad, diffuse bands (poor resolution) ♦ Linear polyacrylamide – Caused by catalytic contaminants in the dry acrylamide monomer. – Decreases the effective concentration of the acrylamide, causing the DNA to migrate faster ♦ Ionic contaminants – Mostly metals, such as iron or copper – Can inhibit or accelerate polymerization **IMPORTANT** Storage guidelines for these reagent are listed under "Storing Reagents and Stock Solutions" on page A-23. Proper storage is critical for the production of high quality gels. **! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are neurotoxins. Avoid inhalation and skin contact. Wear gloves at all times and work in a fume hood when handling acrylamide solutions, and use appropriate precautions to avoid inhalation of crystalline acrylamide.**
Ammonium Persulfate (APS)	APS begins to decompose immediately when dissolved in water. The result is loss of activity. Therefore, it is vital that this chemical be prepared fresh daily, since it affects the rate of polymerization and thus the gel properties. Store solid APS at room temperature in an airtight container with desiccant to keep it dry.

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Reagent purity and associated problems: *(continued)*

Reagent	Problems That Can Occur When Fresh Reagents are Not Used
TEMED	N, N, N', N'-tetramethylethylenediamine (TEMED) is by nature very reactive and prone to oxidation. The oxidized form is yellow and less active. Using oxidized TEMED slows gel polymerization time, thereby significantly altering the gel characteristics. Because it is hygroscopic, this initiator gradually accumulates water, which increases the rate of oxidation. **! WARNING ! CHEMICAL AND FIRE HAZARD. TEMED is extremely flammable and can be very destructive to the skin, eyes, nose, and respiratory system. Keep it in a tightly closed container. Avoid inhalation and contact with skin, eyes and clothing. Always work under a hood and wear chemical resistant gloves when handling TEMED solutions.**
Urea	Old urea has breakdown products that affect sample migration. Guidelines for using urea are as follows: ♦ Use urea in the crystalline form ♦ Weigh urea fresh each time it is used ♦ Prepare solutions containing urea fresh each time ♦ Do not prepare stock solutions containing urea **! WARNING ! CHEMICAL HAZARD. Urea is a potential mutagen. Avoid inhalation and contact with skin, eyes and clothing.**
Buffers and Other Gel Components	Metals, non-buffer ions, and decomposition products are contaminants commonly found in reagents such as Tris, borate and urea. Transition metal ions tend to affect gel polymerization in various ways, causing run-to-run irreproducibility. Non-buffer ions tend to inhibit DNA mobility, also causing run-to-run irreproducibility. To help avoid these problems: ♦ Use only high quality Tris, borate and urea ♦ Use only distilled, deionized water to dilute reagents ♦ Do **not** use 10X TBE buffer which has precipitated. The change in ion concentrate affects sample migration.

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Rate of Polymerization

The properties of the gel depend on the rate of polymerization. The rate of polymerization is affected by:

- Temperature
- Oxygen
- Initiator concentration

Each parameter is discussed in detail below for academic purposes only. The protocols contained in this appendix have already been optimized. Therefore, experimentation with these variables is not necessary.

Factors that affect the rate of polymerization:

Factor	Affect on Rate of Polymerization
Temperature	Temperature directly affects the rate of polymerization. The rate of polymerization determines the properties of the gel. Therefore, temperature control is crucial for the production of high-quality gels. Optimal room temperature for gel production and polymerization is 20 to 23°C. The gel solution and glass plates should be the same temperature. Gels produced within this temperature range are transparent. They are not too porous, and more elastic than gels produced in colder or warmer temperatures. These gels are also the most reproducible. Gels produced in cold environments such as 4°C are turbid, porous, and inelastic. Run-to-run reproducibility is greatly compromised. Gels produced in environments that are too warm are inelastic because the polymer chains are too short. The result is non-reproducibility from run to run.
Oxygen	Oxygen acts as a free radical trap, thereby inhibiting polymerization. The result is a porous gel. Therefore, polymerization must be fast enough to prevent too much oxygen from dissolving into the gel solution during polymerization. Follow these guidelines to minimize the amount of oxygen that dissolves in the gel solution during polymerization. ♦ Gently stir gel solutions to avoid the introduction of air bubbles ♦ Handle the gel solution gently when casting the gel to avoid the introduction of air bubbles ♦ Keep the vacuum strength and time constant during the vacuum filtration step in the protocols provided in this Appendix for run-to-run reproducibility. ♦ Perform vacuum filtering and gel casting with the solution at room temperature, since cold solutions have a greater capacity for dissolving oxygen ♦ Use fresh, high-quality reagents ♦ Carefully follow the gel preparation protocols in this manual

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Factors that affect the rate of polymerization: *(continued)*

Factor	Affect on Rate of Polymerization
Initiator Concentration	TEMED and APS initiate gel polymerization. Too much TEMED or APS decreases the average polymer chain length, and increases gel turbidity. Too little TEMED or APS can slow polymerization to the point where oxygen enters the monomer solution and inhibits further polymerization. The concentration of initiators in degraded TEMED and APS may be too low for the protocols presented in this manual.

Air bubbles

Air bubbles can distort the sample path, which affects lane tracking. Pour gels carefully and gently so air bubbles never form.

If air bubbles are trapped between the comb and the gel solution, carefully remove and reinsert the comb until the bubbles are gone.

Age of the Gel

Follow these guidelines for consistent results:

- To ensure complete polymerization, wait a full two hours after casting the gel before use
- Use gels within two to six hours after casting

After six hours, resolution begins to noticeably deteriorate. Gels that stand overnight can show significantly slower DNA migration due to the slow hydrolysis of urea to ammonium carbonate. Since the amide groups of the polymer slowly hydrolyze into acid groups, gels that stand 48 hours can also show significant loss in resolution beyond 350 bases.

Also, the exposed areas (top and bottom) of the thin gels commonly used with the ABI Prism® 377 DNA Sequencer dry out quickly.

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Chemical Abbreviations Used

Chemical Abbreviations Used

Chemical abbreviations used in the following gel recipes:

Abbreviation	Chemical Substance
APS	ammonium persulfate
EDTA	ethylenediaminetetraacetic acid
TEMED	N,N,N',N'-tetramethylelthylenediamine
TBE	tris-borate-ethylenediaminetetaacetic acid buffer

Preparing TBE Buffer

10X TBE

To make 50 mL of 10X TBE:

Step	Action
1	To a 50-mL screw cap tube, add the following: ♦ 5.4 g Tris ♦ 2.8 g Boric acid ♦ 0.4 g Na_2EDTA ♦ Distilled, deionized water to 50 mL **IMPORTANT** Be sure to use disodium EDTA to make 10X TBE stock. Some major laboratory suppliers provide monosodium EDTA or tetrasodium EDTA.
2	Mix ingredients thoroughly by vortexing.
3	Verify that the pH is between 8.2 and 8.3.

Note 10X TBE stored at room temperature should be used within 1 month. Do not use if a precipitate is present.

IMPORTANT Discard if the pH is not 8.3 (± 0.2), and make a fresh solution. Do not attempt to adjust the pH.

IMPORTANT Do not use 10X TBE buffer which has precipitated. The change in ion concentrate affects sample migration.

1X TBE

Working solution (1X) is 89 mM Tris-base, 89 mM Boric acid, 2 mM Na_2EDTA; pH is approximately 8.3 at ambient temperatures.

To prepare a 1X TBE working solution:

Step	Action
1	Add 120 mL 10X TBE stock solution to a large graduated cylinder
2	Dilute with deionized water to a total volume of 1200 mL.

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Deionizing Formamide

Procedure

IMPORTANT Always use deionized formamide. Over time, formamide hydrolyzes to formic acid and formate. Deionized formamide stock lasts for 3 months at –15 to –25°C.

Step	Action
1	Mix 50 mL of formamide and 5 g of AG501 X8 ion-exchange resin. **! WARNING ! CHEMICAL HAZARD. Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and safety glasses when handling.**
2	Stir for 30 minutes at room temperature.
3	Check that the pH is greater than 7.0 using pH paper. If the pH is not greater than 7.0, decant the formamide into a beaker containing another 5 g of ion-exchange resin and repeat 30-minute stirring at room temperature.
4	When the pH is greater than 7.0, allow the beads to settle to the bottom of the beaker. Remove the supernatant (formamide), taking care not to disturb the beads.
5	Dispense the deionized formamide into aliquots of 500 µL and store for up to 3 months at –15 to –25°C.
6	Use one aliquot per set of samples. Discard any unused deionized formamide.

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29:1 Polyacrylamide Gels—Protocol and Run Conditions

Ingredients and Run Conditions

For 36-cm well-to-read runs—4.5% 29:1 Polyacrylamide Gel, 6 M Urea

Ingredient	For 50 mL	Run Conditions
urea	18.0 g	For runs at 1200 scans/hr: ♦ Use standard 36-cm, 1200 scans/hr run modules ♦ Increase run time to 9 hr For runs at 2400 scans/hr: ♦ Use standard 36-cm, 2400 scans/hr run modules ♦ Increase run time to 4 hr
40% acrylamide stock	5.625 mL	
deionized water	25.0 mL	
Mixed-bed ion exchange resin	0.5 g	
Filter and degas the above ingredients before adding TBE.		
10X TBE	5.0 mL	
10% APS	250 µL	
TEMED	30.0 µL	

For 48-cm well-to-read runs—4.25% 29:1 Polyacrylamide Gel, 6 M Urea

Ingredient	For 50 mL	Run Conditions
urea	18.0 g	♦ Use standard 48-cm run modules ♦ Increase run time to 11 hr
40% acrylamide stock	5.31 mL	
deionized water	25.0 mL	
Mixed-bed ion exchange resin	0.5 g	
Filter and degas the above ingredients before adding TBE.		
10X TBE	5.0 mL	
10% APS	250 µL	
TEMED	30.0 µL	

Protocol for 29:1 Polyacrylamide Gels

! WARNING ! CHEMICAL HAZARD. Urea causes eye, skin, and respiratory irritation. Lab experiments have shown mutagenic effects. Avoid contact. Wear chemical resistant gloves, safety goggles, and other protective clothing.

! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are neurotoxins. Avoid inhalation and skin contact. Wear gloves at all times, and work in a fume hood when handling acrylamide solutions. Use appropriate precautions to avoid inhalation of crystalline acrylamide.

! WARNING ! CHEMICAL AND FIRE HAZARD. TEMED is extremely flammable and can be very destructive to the skin, eyes, nose, and respiratory system. Keep it in a tightly closed container. Avoid inhalation and contact with skin, eyes and clothing. Always work under a hood and wear chemical resistant gloves when handling TEMED solutions. Read the MSDS in the Safety Summary included with your instrument user's manual.

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To prepare the acrylamide-urea solution:

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, “Pouring Gels.” Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Prepare all stock solutions per the appropriate list of ingredients on page A-11.
3	Combine urea, 40% acrylamide stock, 25.0 mL deionized water, and mixed-bed ion exchange resin in a 150 mL beaker.
4	Stir the solution until all the urea crystals have dissolved.
5	Filter the solution through a 0.2-μm cellulose nitrate filter.
6	Degas for 2–5 minutes. **Note** Degas time for all gels should be constant to ensure a reproducible polymerization rate for all gels.
7	Transfer the solution to a 100-mL graduated cylinder.
8	Add filtered 10X TBE buffer. **IMPORTANT** Always remove the mixed-bed ion exchange resin by filtration (step 5 above) before adding the TBE buffer. Resin will destroy the effectiveness of the buffer.
9	Adjust the volume to 50.0 mL with deionized water. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to the gel solution. Instructions are listed in Chapter 2, “Pouring Gels.”

Adding the polymerizing reagents:

Step	Action
1	Add freshly made 10% APS, and swirl carefully to mix without introducing air bubbles. **Note** Be as accurate and reproducible as possible when making the 10% APS solution. Significant variation in this reagent can produce changes in data quality.
2	Add TEMED, and swirl carefully to mix without introducing air bubbles.
3	Immediately pour the gel.
4	Allow the gel to polyermize a minimum of 2 hours before use. **Note** 29:1 polyacrylamide gels take a minimum of 2 hours to polymerize, sometimes longer.

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PAGE-PLUS Gels—Protocol and Run Conditions

Overview

To obtain the longest read length, 5.0% Long Ranger (from concentrate or Singel) and 4.8% PAGE-PLUS gels are recommended for 36-cm runs at 1200 scans/hr only. These gel formulations have not been shown to consistently increase read lengths on 36-cm runs at 2400 scans/hr, or 48-cm gel runs.

For 48-cm gel runs, 5.25% PAGE-PLUS and 4.75% Long Ranger Singel gels give longer read lengths than 19:1 polyacrylamide gels.

For 36-cm gel runs at 2400 scans/hr, use a 4.5% 29:1 polyacrylamide gel.

Ingredients and Run Conditions

For 36-cm well-to-read runs—4.8% PAGE-PLUS Gel, 6 M Urea

Ingredient	For 50 mL	Run Conditions
urea	18.0 g	♦ Use standard 36-cm, 1200 scans/hr run modules ♦ Increase run time to 9 hr
40% gel stock solution	6.0 mL	
10X TBE	5.0 mL	
deionized water	to 50.0 mL	
10% APS	300 µL	
TEMED	30.0 µL	

For 48-cm well-to-read runs—5.25% PAGE-PLUS Gel, 6 M Urea

Ingredient	For 50 mL	Run Conditions
urea	18.0 g	♦ Use 48-cm run module ♦ Increase run time to 12 hr
40% gel stock solution	6.6 mL	
10X TBE	5.0 mL	
deionized water	to 50.0 mL	
10% APS	250 µL	
TEMED	25.0 µL	

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Protocol for PAGE-PLUS Gels

To prepare 4.8 and 5.25% PAGE-PLUS gels:

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, “Pouring Gels.” Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Prepare all stock solutions per the appropriate list of ingredients on page A-13.
3	Weigh out the urea and carefully transfer it to a stoppered, graduated cylinder.
4	Using a pipette, add the appropriate amount of gel stock solution and 10X TBE buffer to the cylinder.
5	Adjust the volume to 49.5 mL by slowly adding deionized water, and tapping the cylinder to release air bubbles trapped by the urea.
6	Stopper the cylinder and invert to dissolve the urea.
7	Allow the solution to warm to room temperature.
8	Add deionized water to bring the solution to a final volume of 50 mL.
9	Stopper the cylinder and mix the contents thoroughly.
10	Filter the solution through a 0.2-μm cellulose nitrate filter.
11	Degas for 2–5 minutes, and transfer the solution to a wide-mouthed container. **Note** Degas time for all gels should be constant to ensure a reproducible polymerization rate for all gels. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to your solution. Instructions are listed in Chapter 2, “Pouring Gels.”

Adding the polymerizing reagents:

Step	Action
1	Add freshly made 10% APS, and swirl carefully to mix without introducing air bubbles. **Note** Be as accurate and reproducible as possible when making the 10% APS solution. Significant variation in this reagent can produce changes in data quality.
2	Add TEMED, and swirl carefully to mix without introducing air bubbles.
3	Immediately pour the gel.
4	Allow the gel to polymerize for 2 hours before using.

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Long Ranger Gel Solution Protocols

Overview

To obtain the longest read length, 5.0% Long Ranger™ (from concentrate or Singel) and 4.8% PAGE-PLUS gels are recommended for 36-cm runs at 1200 scans/hr only. These gel formulations have not been shown to consistently increase read lengths on 36-cm runs at 2400 scans/hr, or 48-cm gel runs.

For 48-cm gel runs, 5.25% PAGE-PLUS and 4.75% Long Ranger Singel gels give longer read lengths than 19:1 polyacrylamide gels.

For 36-cm gel runs at 2400 scans/hr, use a 4.5% 29:1 polyacrylamide gel.

Ingredients and Run Conditions

For 48-cm well-to-read runs—4.75% Long Ranger Singel

Ingredients	Run Conditions
As supplied by the manufacturer.	♦ Use 48-cm run module ♦ Increase run time to 11 hr

For 36-cm well-to-read runs—5.0% Long Ranger Singel

Ingredients	Run Conditions
As supplied by the manufacturer.	♦ Use standard 36-cm run modules with a run speed of 1200 scans/hr ♦ Increase run time to 9 hr

For 36-cm well-to-read runs—5.0% Long Ranger/6 M Urea Gel

Ingredient	For 50 mL	Run Conditions
urea	18.0 g	♦ Use standard 36-cm run modules with a run speed of 1200 scans/hr ♦ Increase run time to 9 hr
50% gel stock solution	5.0 mL	
10X TBE	5.0 mL	
deionized water	to 50.0 mL	
10% APS	250 µL	
TEMED	25.0 µL	

10% Ammonium Persulfate

IMPORTANT Use fresh ammonium persulfate. Prepare the 10% ammonium persulfate solution no more than two hours before pouring the gel. Crystals should crackle as dissolved.

Step	Action
1	Weigh out 0.50 ± 0.005 g of ammonium persulfate into a 15-mL polypropylene tube. ! WARNING ! **CHEMICAL HAZARD. Always wear appropriate safety attire (full-length laboratory coat, protective glasses, gloves, etc.) when handling and mixing hazardous chemicals. Always work under a chemical fume hood when handling and mixing hazardous chemicals. The room in which you work must have proper ventilation and a waste-collection system.**
2	Using a P-5000 Pipetman or equivalent, add 5 mL of deionized water to the tube.
3	Vortex until all crystals dissolve.
4	The 10% APS solution can be stored in tightly capped tubes for up to 1 month.

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4.75% Long Ranger Singel

Follow the manufacturer's instructions to prepare the 4.75% Long Ranger Singel gel solution.

5.0% Long Ranger Gel (not for SSCP)

For SSCP applications, follow the instructions listed under "5.0% Long Ranger Gel for SSCP" on page A-17.

IMPORTANT The 5.0% Long Ranger (FMC Corporation, P/N 50610) gel solution is hazardous waste. Dispose of the unused working solution in accordance with all applicable federal, state, and local health and safety regulations. After you finish pouring the gel, thoroughly clean all work surfaces with water.

To prepare a 36-cm, 5.0% Long Ranger/6 M urea gel:

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, "Pouring Gels." Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Weigh out 18.0 g of urea and transfer it carefully to a graduated cylinder. **CHEMICAL HAZARD. Urea is a mutagen. Do not breathe the dust. Refer to the Material Safety Data Sheet for the proper protective equipment that should be worn when working with this reagent.**
3	Using a pipette, add the following: ♦ 5.0 mL 50% Long Ranger gel solution concentrate ♦ 5.0 mL 10X TBE ! WARNING ! **CHEMICAL HAZARD. Long Ranger gel solution contains acrylamide. Acrylamide is a potent neurotoxin and is absorbed through the skin. Effects are cumulative. Always wear appropriate safety attire (a full-length laboratory coat, protective glasses, chemical-resistant gloves, etc.) and work under a chemical fume hood when handling powders, solutions, and gels. Unpolymerized acrylamide sublimes. Use in a well-ventilated area and clean up spills immediately.**
4	Slowly add distilled, deionized water to bring the liquid level to approximately 45 mL. **Note** Gently tap the cylinder while adding the water to release any air bubbles trapped by the urea.
5	Stopper and then invert the cylinder to dissolve the urea. **Note** Although the cylinder and its contents become very cold, the urea dissolves rapidly.
6	Allow the solution to warm to room temperature.
7	Add distilled, deionized water to a final volume of 50 mL.
8	Stopper the cylinder and then mix the contents thoroughly.
9	Use a 0.2-μm cellulose nitrate filter to filter the solution.
10	Degas the filtrate by one of the following methods: ♦ Purging with argon or helium ♦ Applying a vacuum for at least five minutes **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to the gel solution. Instructions are listed in Chapter 2, "Pouring Gels."

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Adding the polymerizing reagents:

Step	Action
1	Add fresh 10% APS to the gel solution. Swirl gently to mix. Be careful to avoid introducing air bubbles.
2	Add 25 µL of TEMED (tetramethylethylenediamine) to the solution. Swirl gently to mix. Be careful to avoid introducing air bubbles. **! WARNING ! TEMED (Tetramethylethylenediamine) is a flammable liquid and is extremely corrosive. Vapors may travel considerable distance to sources of ignition and flash back. TEMED is harmful by inhalation, contact with the skin, and if swallowed. Use only in a chemical fume hood. When handling, wear lab coat, safety glasses, and chemical-resistant gloves.**
3	Immediately pour the gel.
4	Allow the gel to polymerize for at least 2 hours before use.

5.0% Long Ranger Gel for SSCP

The protocol for making a non-denaturing, 8.1% Long Ranger gel solution for SSCP differs from the preceding protocol in two places:

- Step 2 on page A-16—Do not add urea.
- Step 3 on page A-16:

  Add the following:

  - 8.1 mL 50% Long Ranger gel solution concentrate
  - 1.25 g glycerol
  - 5.0 mL 10X TBE

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MDE Gel Protocol for SSCP

Preparation Procedure

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, “Pouring Gels.” Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Combine the following: ♦ 10.0 mL 2X Mutation Detection Gel Solution (MDE) ♦ 4.0 mL 10X TBE ♦ 2.0 g glycerol **! WARNING ! MDE (Mutation Detection Gel Solution) contains Acrylamide. Acrylamide is a poison, neurotoxin, irritant, carcinogen, and possible teratogen. Acrylamide sublimes (the solid releases toxic vapor) and is harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.**
3	Add sufficient distilled, deionized water to bring the total volume to 40.0 mL. Mix well to obtain a homogenous solution. **Note** This step is critical because glycerol is viscous, and it is difficult to obtain a homogeneous solution.
4	Use a 0.2-μm filter unit to vacuum filter the MDE solution containing glycerol and 1X TBE for 5 minutes. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to the gel solution. Instructions are listed in Chapter 2, “Pouring Gels.”

Adding the polymerizing reagents:

Step	Action
1	With the filtered MDE solution at room temperature, add 200 μL of 10% APS. Swirl gently to mix. Be careful to avoid air bubbles.
2	Add 25 μL of TEMED to the solution. Swirl gently to mix. Be careful to avoid air bubbles.
3	Immediately pour the gel.
4	Allow the gel to polymerize for at least 2 hours before use.

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Polyacrylamide Gel Solution Protocols

Preparing the 40% Polyacrylamide Stock Solution

To make 150 mL of 40% polyacrylamide stock solution, use:

Application	Acrylamide (g)	Bisacrylamide (g)
SSCP	58.5	1.56
All other applications	57	3

Note For SSCP, always use a low cross-linker concentration (≥37.5:1, acrylamide: bisacrylamide) in the polyacrylamide stock solution. For other applications, typically use a 19:1 stock solution.

To prepare the 40% polyacrylamide stock solution:

Step	Action
1	Working in a fume hood, combine the appropriate amounts acrylamide and bisacrylamide in a glass beaker. **! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are poisons, neurotoxins, irritants, carcinogens, and possible teratogens. Acrylamide and bisacrylamide sublime (the solids release toxic vapor) and are harmful if swallowed, inhaled, or absorbed through the skin. Effects are cumulative. When handling, always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) and use in a well-ventilated area. On a routine basis, thoroughly clean surfaces subject to contamination.**
2	Dissolve the crystalline acrylamide and bisacrylamide in enough distilled, deionized water to bring the total volume to 135 mL.
3	Add 15 g of mixed-bed, ion-exchange resin.
4	Stir at room temperature until all crystals dissolve. Continue stirring for 5–10 minutes.
5	Filter the mixture through 0.2-μm cellulose nitrate filter.
6	Transfer the filtrate to a graduated cylinder and bring the total volume to 150 mL with distilled, deionized water.
7	Store at 2–6°C.

Note 40% polyacrylamide stock lasts for 1 month at 2–6°C.

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For All Applications Except SSCP

To prepare 40 mL of an **N**% polyacrylamide/6 M urea gel:

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, "Pouring Gels." Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Combine the following: ♦ **N** mL 40% polyacrylamide stock (19:1) **Note** **N** is the desired gel percentage. ♦ 14.4 g urea ♦ 1.0 g mixed-bed, ion-exchange resin ♦ 25 mL distilled, deionized water
3	Stir well until all the urea crystals have dissolved.
4	Use a 0.2-μm cellulose nitrate filter to filter the polyacrylamide solution for 5 minutes. **Note** During this step you both filter and degas the solution.
5	Add 4.0 mL of filtered 10X TBE and sufficient distilled, deionized water to bring the total volume to 40 mL. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to the gel solution. Instructions are listed in Chapter 2, "Pouring Gels."

Adding the polymerizing reagents:

Step	Action
1	With the filtered polyacrylamide solution at room temperature, add 200 μL of 10% APS (ammonium persulfate). Swirl gently to mix. Be careful to avoid air bubbles.
2	Add 25 μL of TEMED to the solution. Swirl gently to mix. Be careful to avoid air bubbles. **! WARNING ! TEMED (tetramethylethylenediamine) is a flammable liquid and is extremely corrosive. Vapors may travel considerable distance to sources of ignition and flash back. TEMED is harmful by inhalation, contact with the skin, and if swallowed. Use only in a chemical fume hood. When handling, wear lab coat, safety glasses, and chemical-resistant gloves.**
3	Immediately pour the gel.
4	Allow the gel to polymerize for at least 2 hours before use.

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For SSCP Applications Only

To prepare a standard 6.5% nondenaturing, polyacrylamide gel for SSCP:

Step	Action
1	Instructions for preparing glass plates and two gel pouring methods are located in Chapter 2, “Pouring Gels.” Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Combine the following: ♦ 6.5 mL 40% polyacrylamide stock (37.5:1) ♦ 1.0 g mixed-bed, ion-exchange resin ♦ 1.25 g glycerol ♦ 25 mL distilled, deionized water
3	Mix well to obtain a homogenous solution. **Note** This step is critical because glycerol is viscous, and it is difficult to obtain a homogeneous solution.
4	Use a 0.2-μm cellulose nitrate filter to filter the solution for 5 minutes. **Note** During this step you both filter and degas the solution.
5	Add 4.0 mL of filtered 10X TBE and sufficient distilled, deionized water to bring the total volume to 40.0 mL. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to the gel solution. Instructions are listed in Chapter 2, “Pouring Gels.”

Adding the polymerizing reagents:

Step	Action
1	With the filtered polyacrylamide solution at room temperature, add 200 μL of 10% APS (ammonium persulfate). Swirl gently to mix. Be careful to avoid air bubbles.
2	Add 25 μl of TEMED to the solution. Swirl gently to mix. Be careful to avoid air bubbles. **! WARNING ! TEMED (Tetramethylethylenediamine) is a flammable liquid and is extremely corrosive. Vapors may travel considerable distance to sources of ignition and flash back. TEMED is harmful by inhalation, contact with the skin, and if swallowed. Use only in a chemical fume hood. When handling, wear lab coat, safety glasses, and chemical-resistant gloves.**
3	Immediately pour the gel.
4	Allow the gel to polymerize for at least 2 hours before use.

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Supplier Information

Item	Supplier
♦ 29:1 acrylamide stock solutions ♦ PAGE-PLUS stock solutions	Amresco, Inc. 30175 Solon Industrial Parkway Solon, OH 44139-4300 800-829-2805 216-349-2805
♦ Long Ranger Singel ♦ Long Ranger Concentrate (5% solution, P/N 50610) ♦ Mutation Detection (MDE) Gel Solution (P/N 50621)	FMC BioProducts 191 Thomaston St Rockland, ME 04841 800-341-1574 207-594-3495

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Storing Reagents and Stock Solutions

Acrylamide

- Good quality acrylamide can be stored dry at room temperature for up to one year.
- 40% stock solution of acrylamide and bisacrylamide (19:1 or 29:1) can be stored at 4°C for up to one month. Use distilled, deionized water only.
- Using the premixed powders saves time, and minimizes your exposure to the solid form of the neurotoxins acrylamide and bisacrylamide.

Bisacrylamide

- Store bisacrylamide dry at room temperature for up to one year.
- 40% stock solution of acrylamide and bisacrylamide (19:1 or 29:1) at 4°C for up to one month.

! WARNING ! CHEMICAL HAZARD. Acrylamide and bisacrylamide are neurotoxins. Avoid inhalation and skin contact. Wear gloves at all times and work in a fume hood when handling acrylamide solutions, and use appropriate precautions to avoid inhalation of crystalline acrylamide.

TEMED

Because it is hygroscopic, this initiator gradually accumulates water, which increases the rate of oxidation. Water-free TEMED (>99% pure), stored in a tightly sealed container at room temperature, should be good for up to six months.

! WARNING ! CHEMICAL AND FIRE HAZARD. TEMED is extremely flammable and can be very destructive to the skin, eyes, nose, and respiratory system. Keep it in a tightly closed container. Avoid inhalation and contact with skin, eyes and clothing. Always work under a hood and wear chemical resistant gloves when handling TEMED solutions.

Urea

Store at room temperature.

Ammonium Persulfate (APS)

APS is hygroscopic. Store solid APS at room temperature in an airtight container. Store with desiccant if the environment is humid. If tightly sealed, it can be stored at 4°C for up to 1 year.

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Subambient Temperature Operation

B

Appendix Contents

In this Appendix

The information in this appendix describes how to modify your ABI PRISM® 377 DNA Sequencer to run with an external gel cooling system (water bath) for run temperatures below the standard temperature. The standard run temperature is ambient plus 10°C up to 60°C.

The following topics are discussed in this appendix:

Topic	See page
Purpose of an External Cooling System	B-1
Hardware and Software Requirements	B-2
How the External Water Bath Functions	B-3
Installing the External Water Bath	B-5
Operating at Subambient Temperatures	B-6
Avoiding Condensation Inside the Instrument	B-7

For More Information

Refer to the *GeneScan® Reference Guide : ABI™ 373 and ABI PRISM® 377 DNA Sequencers* (P/N 4303188) for more information on Single-stranded Conformation Polymorphism (SSCP) and operating the instrument at subambient temperatures.

Purpose of an External Cooling System

Performing SSCP

The ABI PRISM 377 DNA Sequencer controls gel temperature from 10°C above ambient temperature to a maximum of 60°C. To perform PCR Single-stranded Conformation Polymorphism (SSCP) protocols at lower temperatures (below 10°C above ambient), a water bath must be attached to the instrument.

For more information on performing SSCP, refer to *PCR SSCP Analysis: A Guide to Fluorescent PCR Single-stranded Conformation Polymorphism Analysis on the ABI PRISM 377 DNA Sequencer* (P/N 904413).

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Hardware and Software Requirements

Overview

The following hardware and software is required to operate the instrument at subambient temperatures:

- An external water bath connected to the instrument
- 30 k-ohm thermistors installed on the instrument
- ABI PRISM® 377 Data Collection Software version 2.1 or higher

Hardware Requirements

Water Bath

The water bath, polyurethane tubing, and tubing connectors required for instrument modification must meet the following minimum requirements.

Hardware	Minimum Requirements
Water Bath	Cooling capacity: 250 watts at 20°C
	Heating capacity: 500 watts up to 60°C
	Pump flow rate: 1 gal/min or 4 L/min at pressure head of 3 m
	Automatic Shutdown: At high temperature and low liquid-level
Polyurethane Tubing	6 ft. (1/4-in. i.d., 3/8-inch o.d.)
Tubing Connectors	1/4-in. female NPT, 1/4-in. male barbed fittings (NESLAB P/N 126000000007)

Any external water bath that meets these requirements should work properly. The NESLAB Model RTE111 meets these requirements and has been tested with the instrument.

Refer to the user manual for the water bath you select, follow proper procedures, and ensure that all product safety and regulatory requirements applicable to your location are met.

Thermistors

To perform SSCP at temperatures less than 22°C, the instrument must have 30 k ohm thermistors installed. Some instrument may have 100 k ohm thermistors which must be replaced to use an external water bath.

To determine which thermistors are installed on the instrument, check a Log file from a recent run using ABI PRISM Data Collection Software version 2.1 or higher. The configuration information in the Log lists the type of thermistors installed.

If necessary, schedule a service call with your Applied Biosystems service engineer to replace the 100 k thermistors with 30 k thermistors.

Software Requirements

ABI PRISM Data Collection Software version 2.1 and above contain the features required to accommodate the external water bath, including chiller modules. Performing runs at subambient temperatures with an external water bath requires the specification of chiller modules on the run sheet.

Standard modules are located in a folder called Modules. At system installation, the software is typically set up so that the standard modules are the ones displayed in the

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pop-up menus on run sheets. Chiller modules are stored in a folder called Chiller Modules. You must either move the chiller modules to the Modules folder, or change the Folder Locations Preference for Modules to the Chiller Modules folder for the software to access the chiller modules. Refer to “Installing Chiller Modules” on page 9-44 in Chapter 9, “Data Collection Software,” and Folder Location Preferences in Chapter 5, “Setting Preferences,” for instructions on how to access the chiller modules.

Requirements for Specific Run Conditions

The following table lists the requirements for standard and SSCP operation of the instrument.

Run Conditions	Data Collection Software Version	Thermistor (ohms)	External Bath
>10°C above ambient to 60°C	1.1 or 2.1	100 k	not required
SSCP from 22°C to ambient +10°C	2.1	100 k	required
SSCP at <22°C	2.1	30 k	required

How the External Water Bath Functions

Disengages Internal Pump System

The instrument configuration with the external water bath attached is similar to Figure 1 when viewed from the back. Instructions in the chiller modules disengage the instrument’s internal pump system and switch to the external water bath. When the external valve is turned so the arrow points down, access to the internal gel temperature control unit is closed off.

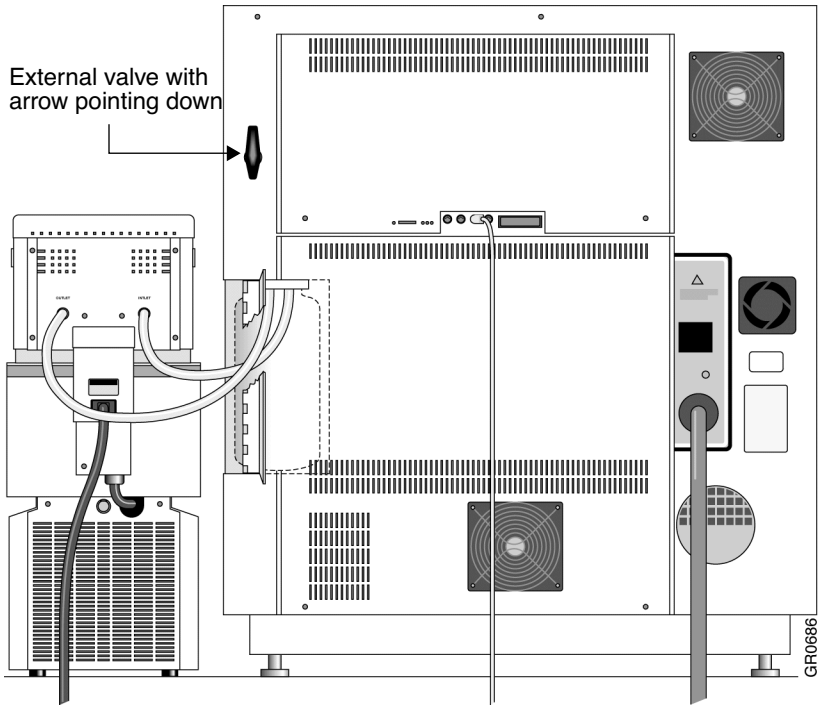


Figure 1. Rear view of the instrument with external water bath attached

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Water Bypasses the Gel Temperature Control Unit

With the external water bath attached, water passes directly into the front and rear heat-transfer plates in the instrument, bypassing the gel temperature control unit.

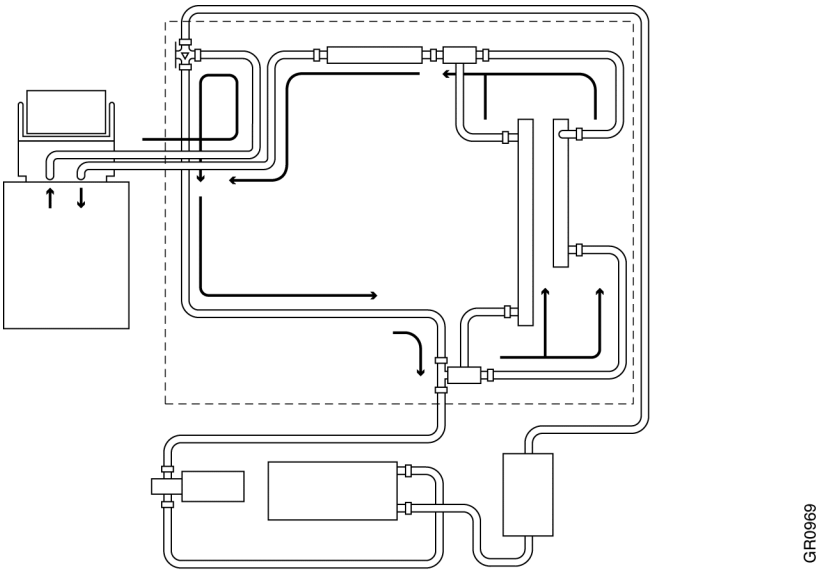


Figure 2. Instrument interior view with arrows showing water flow. Dotted line shows instrument components used with the external water bath.

External Water Bath Attached for Standard Operating Conditions

If the external water bath used has a heating capacity to 60°C, the instrument can be run under standard operating conditions (ambient temperature plus 10°C—60°C) with the external water bath attached.

Frequent monitoring of the water level is required when the external bath is used during standard runs. For long periods of use, disconnect the external water bath, reinstall the water bottle and tubes, and use the standard run modules rather than the chiller modules.

At system installation, the software is typically set up so that the standard modules are the ones displayed in the pop-up menus on run sheets.Chiller modules are stored in a folder called Chiller Modules. You must either move the chiller modules to the Modules folder, or change the Folder Locations Preference for Modules to the Chiller Modules folder for the software to access the chiller modules. Refer to “Installing Chiller Modules” on page 9-44 in Chapter 9, “Data Collection Software,” and Folder Location Preferences in Chapter 5, “Setting Preferences,” for instructions on how to access standard and chiller modules.

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Installing the External Water Bath

Positioning the Machines

To position the instrument and external water bath:

Step	Action
1	Position the ABI PRISM 377 so the rear of the instrument can be accessed easily.
2	Facing the ABI PRISM 377, position the water bath on the right side of the instrument and at the same height.
3	Connect the instrument and water bath to an external power supply.

Determining Intake and Outlet

To identify the intake and outlet flow tubes on the instrument:

Step	Action
1	Turn on the instrument.
2	Open the right panel of the instrument where the water bottle is mounted.
3	Unscrew the water bottle, and slowly pull it down to expose the ends of the tubes, but do not remove the bottle.
4	Observe the direction of water flow from the tubes into and out of the bottle to identify the intake and outlet tubes. **Note** Water coming into the bottle is from the instrument outlet, and water drawn out of the bottle goes into the instrument intake. Some instruments have a small cutout at the bottom of the water intake tube.
5	Turn off the instrument.

Disconnecting the Internal Pump

To disconnect the internal pump:

Step	Action
1	Remove the water bottle, and pull down the intake and outlet tubes to remove them.
2	Store the tubes and water bottle for later use.
3	Step to the rear of the instrument, and turn the external valve so that the arrow points down Figure 1 on page B-3).
4	Cut two pieces of polyurethane tubing approximately three feet (one meter) long.
5	Insert one end of a piece of tubing into the intake port on the instrument.
6	Repeat step 5 with the second piece of tubing in the outlet port.

Attaching the External Water Bath

To attach the external water bath to the instrument:

Step	Action
1	Wrap the threads of the intake and outlet ports on the external water bath with Teflon tape.
2	Attach a metal tubing connector to each port.
3	Connect the free end of the intake tube to the outlet fitting on the back of the external water bath.
4	Connect the free end of the outlet tube to the intake fitting on the back of the external water bath.

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Operating at Subambient Temperatures

Performing a Run at Subambient Temperatures

Follow these steps to perform runs at subambient temperatures:

Step	Action
1	Follow all instructions in the external water bath user manual.
2	Fill the external water bath with cold, deionized distilled water, and add antifreeze as recommended by the manufacturer. **! WARNING ! CHEMICAL HAZARD. Antifreeze may cause respiratory tract, skin, and eye irritation. Wear chemical-resistant gloves and safety glasses when handling, and always use in a well-ventilated area.**
3	Check that all tubing is tightly connected.
4	Turn on power to the external water bath, start the circulating pump, and add water as needed to maintain the proper level. **IMPORTANT** If the water level drops below the level recommended by the manufacturer, the circulation pump shuts off, and the temperature is not maintained.
5	Set the external water bath temperature at 3–4°C below the desired gel temperature. Open the Status window and monitor the temperature.
6	Place a lid on the bath to minimize evaporation.
7	Add water and antifreeze during operation as needed to maintain the manufacturer's recommended water level.
8	Monitor the temperature and humidity in the lab (see "Avoiding Condensation Inside the Instrument" on page B-7). **CAUTION Condensation can cause decreased performance and severe damage to the instrument. Monitor the room temperature and humidity, consult the following condensation tables, and maintain the water bath temperature setting above the corresponding dew point.**

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Avoiding Condensation Inside the Instrument

How To Avoid Severe Instrument Damage

Operating the ABI PRISM 377 DNA Sequencer at subambient temperatures may cause condensation to form inside the instrument. This can cause decreased performance and severely damage the instrument. To avoid condensation, keep the water temperature above the dew point (temperature at which water vapor condenses into liquid), or lower the humidity in the room.

Refer to the following table to determine the dew point of water at various relative humidities and ambient temperatures in your lab.

CAUTION **Condensation can cause decreased performance and severe damage to the instrument. Monitor the room temperature and humidity, consult the following condensation tables, and maintain the water bath temperature setting above the corresponding dew point.**

Dew Point Table

Ambient Temp. 15°C		Ambient Temp. 20°C		Ambient Temp. 25°C		Ambient Temp. 30 °C		Ambient Temp. 35°C	
Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)
100	15	100	20	100	25	100	30	100	35
90	14	90	18	90	23	90	29	90	33
80	12	80	16	80	21	80	26	80	31
70	10	70	14	70	19	70	24	70	29
		60	12	60	17	60	21	60	26
		50	10	50	14	50	19	50	23
				40	10	40	15	40	19
						30	10	30	15
								20	9

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Parts and Accessories

C

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In this Appendix Part numbers for the following are provided in this appendix:

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Part Number Updates

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

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Glass Plates, Spacers, and Clamps

cm	Description	Part Number
48	Stepped Glass Plate and Spacer Kit, 48-cm Includes two sets of 0.4 mm 48-cm well-to-read stepped glass plates and 48 cm spacers.	4305810 (for 96-lane upgrade only)
	Front Stepped Glass Plate, 48-cm	4305387 (for 96-lane upgrade only)
	Glass Plate and Spacer Kit Includes two sets of 48-cm well-to-read glass plates and 48-cm spacers.	401878
	Rear Glass Plate, 48-cm	401835
	Front Glass Plate, 48-cm	401838
	Spacers, two 48-cm, 0.2 mm thick	401837
36	Stepped Glass Plate and Spacer Kit, 36-cm Includes two sets of 0.4 mm 36-cm well-to-read stepped glass plates and 48 cm spacers that must be cut to size before use.	4305693 (for 96-lane upgrade only)
	Stepped glass plates only, 36-cm, one set	4305384 (for 96-lane upgrade only)
	Clamps, three, 2 inch (use with 36-cm stepped glass plates, P/N 4305693)	4305386
	Glass Plate and Spacer Kit Includes two sets of 36-cm well-to-read glass plates and 36-cm spacers.	401876
	Rear Glass Plate, 36-cm	401839
	Front Glass Plate, 36-cm	401840
	Spacers, two 36-cm, 0.2 mm thick	401836
12	Glass Plate and Spacer Kit Includes: two sets of 12-cm well-to-read glass plates and spacers	401877
	Front Glass Plate, 12-cm	401834
	Rear Glass Plate, 12-cm	401833

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Combs and Overlays

Wells	Description	Part Number
100	Sharktooth Comb, 0.4 mm thick, 1.8 mm center	4305385 (96 lane)
66	Squaretooth Comb, 0.2 mm thick, 66-well	402183
	Plastic Overlay, 66-well	402187
64	Sharktooth Comb, 0.2 mm thick, 64-well	402180
50	Squaretooth Comb, 0.2 mm thick, 50-well	402053
	Plastic Overlay, 50-well	402186
48	Sharktooth Comb, 0.2 mm thick, 48-well	402177
36	Sharktooth Comb, 0.2 mm thick, 36-well	401828
	Squaretooth Comb, 0.2 mm thick, 36-well	401910
32	Sharktooth Comb, 0.2 mm thick, 32-well	401922
	Squaretooth Comb, 0.2 mm thick, 32-well	401907
24	Squaretooth Comb, 0.2 mm thick, 24-well	401904
	Sharktooth Comb, 0.2 mm thick, 24-well	401827
18	Sharktooth Comb, 0.2 mm thick, 18-well	402168

Gel Cassette and Pouring Fixtures

604297	Gel Cassette
401991	Gel Pouring Fixture Kit (contains top and bottom fixtures, clamps, and syringe)
401969	Top Pouring Fixture
604014	Bottom Pouring Fixture

Buffer Chambers and Gasket Kits

4304406	Upper Buffer Chamber
604078	Upper Buffer Chamber (obsolete; gasket kit available as P/N 604524))
603875	Lower Buffer Chamber
4304409	Gasket Replacement Kit (for upper buffer chamber P/N 4304406)
604524	Gasket Replacement Kit for upper buffer chamber P/N 604078

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Electrophoresis Cable and Electrode Assemblies

603822	Upper Buffer Chamber Electrode Assembly
603823	Lower Buffer Chamber Electrode Assembly

Front Heat-Transfer Plate

603833	Front 36-cm Well-to-Read Heat-Transfer Plate

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Two Pitch, Eight-Channel Loader Suppliers

Introduction

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

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

Suppliers Inside the United States

Supplier	Supplier Headquarters	Product	Part No.
Kloehn Company	10000 Banburry Cross Dr. Las Vegas, NV 89134 USA Voice: (702) 243-7727 Fax: (702) 243-6036 World Wide Web: http://www.kloehn.com **Outside U.S. offices are listed on the following page.**	Loader, 0.25-mm	18597
		Loader, 0.3-mm	18663
		Needle, 0.25-mm (8)	18597
		Needle, 0.3-mm (8)	18628
World Precision Instruments, Inc.	Sarasota International Trade Center 175 Sarasota Center Blvd. Sarasota, FL 34240-9258 USA Voice: (941) 371-1003 Fax: (941) 377-5428 World Wide Web: http://www.wpiinc.com **Outside U.S. offices are listed on the following page**.	Loader	Gel Mate 96
		Needle, 0.25-mm (10)	67124

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

Suppliers Outside the U.S.

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

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Supplier	Supplier Contact	Geographic Areas Served
World Precision Instruments, Inc. Germany	Liegnitzer Str. 15 D-10999 Berlin, Germany Voice: 49 (0) 30-6188845 Fax: 49 (0) 30-6188670 E-mail: wpi@wpi.sireco.de	♦ Austria ♦ Bulgaria ♦ Czechoslovakia ♦ Germany ♦ Greece ♦ Holland (Netherlands) ♦ Hungary ♦ Italy ♦ Poland ♦ Rumania ♦ Russia ♦ Switzerland ♦ Yugoslavia
World Precision Instruments, Inc. Japan	1-4-2-702 Naka-Meguro, Meguro Tokyo 153-0061, Japan Voice: 81 (0) 3-3760-5050 Fax: 81 (0) 3-3760-5055 E-mail: wpi@tkb.att.ne.jp	♦ Japan
World Precision Instruments, Inc. United Kingdom	Astonbury Farm Business Centre Aston, Stevenage Hertfordshire SG2 7EG England Voice: 44 (0) 1438-880025 Fax: 44 (0) 1438-880026 E-mail: wpi@piuk.demon. co.uk	♦ Belgium ♦ Denmark ♦ England ♦ Finland ♦ France ♦ Ireland ♦ Norway ♦ Portugal ♦ Scotland ♦ Spain ♦ Sweden
World Precision Instruments, Inc. Other world-wide areas	Sarasota International Trade Center 175 Sarasota Center Blvd. Sarasota, FL 34240-9258 USA Voice: (941) 371-1003 Fax: (941) 377-5428 E-mail wpi@wpiinc.com	♦ Areas not listed above

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User's Manuals

Instrument

4303613	*ABI PRISM® 377 DNA Sequencer User's Manual*
904412	*ABI PRISM® 377 DNA Sequencer XL Upgrade User's Manual*
4305423	*ABI PRISM® 377 DNA Sequencer 96-lane Upgrade User's Manual*

Software

4303242	*ABI PRISM GeneScan® Analysis Software User's Manual* (version 3.0)
902842	*GeneScan 672 Software User's Manual*
4303188	*GeneScan Reference Guide: ABI™ 373 and ABI PRISM® 377 DNA Sequencers*
4305080	*Automated DNA Sequencing Chemistry Guide: ABI 373 and ABI PRISM 377 DNA Sequencers*
904532	*ABI PRISM® DNA Sequencing Software User's Manual* (version 3.0)
4304075	*ABI PRISM® DNA Sequencing Software User's Manual* (version 3.2)

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ABI PRISM DNA Fragment Analysis Kits and Reagents

GeneScan Size Standards

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

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

Fluorescent dNTPs

For fluorescent labeling of DNA during PCR amplification:

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

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

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Fluorescent dNTP PCR Kits

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

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

Fluorescent Phosphoramidites

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

401527	[6-FAM] Phosphoramidite	85 mg
401533	[TET] Phosphoramidite	100 mg
401526	[HEX] Phosphoramidite	105 mg

Fluorescent NHS-Esters

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

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

Matrix Standard Sets

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

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Fluorescent Genotyping Demonstration Kits A and B

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

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ABI PRISM Linkage Mapping Set Version 2

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

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

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Applied Biosystems Limited Warranty

D

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® 377 DNA Sequencer purchased by the Customer (the “Instrument”) will be free from defects in material and workmanship, and will perform in accordance with the specifications for performance set forth at the time of sale in the Instrument Specification Sheet published and maintained by Applied Biosystems (the “Specifications”).

During the Warranty Period, if the Instrument’s hardware fails to perform in accordance with the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems expense.

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

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 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.

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

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