ABI Prism® 373 DNA Sequencer

With XL Upgrade

User's Manual

AB Applied Biosystems

Contents

Applied Biosystems

1 Introduction

Contents

How to Use This Manual

IMPORTANT	*This manual replaces your previous Model 373 User's Manual. Transfer all User Bulletins, the Safety Summary, and personal notations to this manual before discarding the old manual.*

ABI PRISM 373 DNA Sequencer with XL Upgrade Manual Sets

The ABI PRISM 373 DNA Sequencer with XL Upgrade manual set varies with the configuration of the instrument. The following table shows the manuals you should receive for the different configurations (this manual is listed in boldface type).

Table 1-1. ABI PRISM 373 DNA Sequencer with XL Upgrade Reference Manuals

For Sequencing Analysis	For GeneScan Analysis	Manual Describes:
ABI PRISM 373 DNA Sequencer with XL Upgrade User's Manual	**ABI PRISM 373 DNA Sequencer with XL Upgrade User's Manual**	Preparing gels Running samples Data collection software Instrument hardware Macintosh maintenance
ABI PRISM DNA Sequencing Analysis Software User's Manual	ABI PRISM GeneScan® Analysis Software User's Manual	Software that analyzes data collected from the instrument
DNA Sequencing Chemistry Guide	672 GeneScan User's Manual	Chemistry protocols for sample preparation (Protocols that are supplied with Sequencing chemistry kits are not included) Safety information Troubleshooting information User Bulletins

Information Provided in This Manual

Overview

This manual describes how to prepare gels and run samples. It provides information about the data collection software and the instrument and computer hardware. The manual is equipped with an extensive index and tables of contents to help you find information quickly. Even if you do not normally read manuals, these features should help you use it as a reference.

To get started quickly, see *How to Get Started Quickly* on page 1-8 and refer as recommended to the appropriate *ABI PRISM DNA Sequencing Chemistry Guide* and Sections 2 and 3 of this manual. Appendix B, *Quick Reference*, contains copies of useful tables outlining the process of pouring gels and performing runs.

To learn about the instrument and the collection software, read Sections 5 through 7. These sections describe the hardware and software in more depth.

The appendixes contain specialized information, such as the instrument warranty, a consumables parts list, and details regarding matrices and data files.

This manual is written with the assumption that you have previously used a Macintosh computer and know how to use the mouse and select items from menus. If you are new to the Macintosh computer, refer to the *Macintosh System 7 Reference* manual for information about using it.

Model-Specific Notation

This manual applies to three configurations of Model 373 DNA Sequencers:

- **Classic.** This configuration includes 370s with Macintosh upgrade and the 373A. Classic models have electrophoresis chambers which support 24-cm WTR distances.

- **Leon.** This configuration has an electrophoresis chamber which supports 6-, 12-, and 24-cm WTR distances.

- **Stretch.** This configuration supports 6-, 12-, 24-, 34-, and 48-cm well-to-read (WTR) distances and is easily identified by its extended door.

Note	*All three instrument configurations must have the five-filter wheel capability.*

Most of the information in this manual applies to all three configurations, however, some sections of the manual apply only to one or two of these configurations. These sections are preceded by the name of the configuration (Classic, Leon, or Stretch) to which they apply.

Special Text Usage

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

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.***

Safety Information

Refer to your original Safety Summary for information about operating the ABI PRISM 373XL safely. The Safety Summary also contains Material Safety Data Sheets (MSDSs) for chemicals that are commonly used with the instrument. Always keep the Safety Summary available for reference.

Safety warnings appear throughout the manual at relevant locations. These warnings address chemical, high voltage, and laser safety and appear in the format shown below. Please heed the following general warnings:

WARNING   **ELECTRICAL SHOCK HAZARD. The ABI PRISM 373 DNA Sequencer with XL Upgrade 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. The ABI PRISM 373 DNA Sequencer with XL Upgrade contains a laser. Operating this instrument without due regard to the precautions, or in any manner that is not in compliance with procedures recommended here, may be unsafe and can cause physical harm from radiation exposure.**

WARNING   **CHEMICAL HAZARD. The acrylamide and bis-acrylamide used to prepare sequencing gels for the ABI PRISM 373 DNA Sequencer with XL Upgrade are toxic chemicals whose use requires utmost attention to safety. Be sure to read the Material Safety Data Sheets (MSDSs)for emergency and first-aid procedures. MSDSs are in the ABI PRISM 373 DNA Sequencer with XL Upgrade Pre-Installation Manual, P/N 901173. Acrylamide is a neurotoxin that can be absorbed through the skin. Wear a laboratory coat, eye protection, and gloves. The handling and disposal of chemical waste is the responsibility of the user, and not the Applied Biosystems service representative.**

User Bulletins

User Bulletins are updates to this manual that provide a quick way to ensure that you have current information and new protocols. They contain technical information that is essential to ABI PRISM 373 DNA Sequencer with XL Upgrade operation and related laboratory techniques.

How to Get Started Quickly

Be sure your ABI PRISM 373 DNA Sequencer with XL Upgrade is completely installed before you begin preparing for a run. After it is installed:

- Refer to the *ABI PRISM DNA Sequencing Chemistry Guide* or the *672 GeneScan User's Manual* as you prepare your DNA template and choose a method for performing the sequencing or labeling reactions.

- Use the instructions in Section 3 for step-by-step operation of the instrument in sequencing mode. Section 3 is extensively cross-referenced so you can easily refer to Section 5 for more detailed information on the ABI PRISM 373 DNA Sequencer with XL Upgrade collection software.

- Use the instructions in Section 4 for step-by-step operation of the instrument in GeneScan mode. Section 4 is extensively cross-referenced so you can easily refer to Section 5 for more detailed information on the ABI PRISM 373XL collection software.

Registering Your Software Programs

When you send in the registration cards for your software programs you become eligible to receive upgrade notices. Often you also become eligible for technical support and to purchase software upgrades at lower prices than it would cost to purchase the new upgraded programs. These privileges might vary, depending on the company that provides the software.

To register your software, fill out the registration card included in the program package and return it to the company, as indicated.

Description of ABI PRISM 373 DNA Sequencer with XL Upgrade

The ABI PRISM 373 DNA Sequencer with XL upgrade is a high-throughput, automated instrument system (see Figure 1-1) that is capable of determining base sequence, fragment size, or relative quantity of fluorescent dye-labeled nucleotide fragments. It consists of an electrophoresis instrument, a Power Macintosh Computer that includes software for data collection and data analysis, a Translation Interface Processor, and an optional printer. Accessories for standard runs such as combs and spacers are included.

ABI PRISM 373A

Power Macintosh

Translation Interface processor
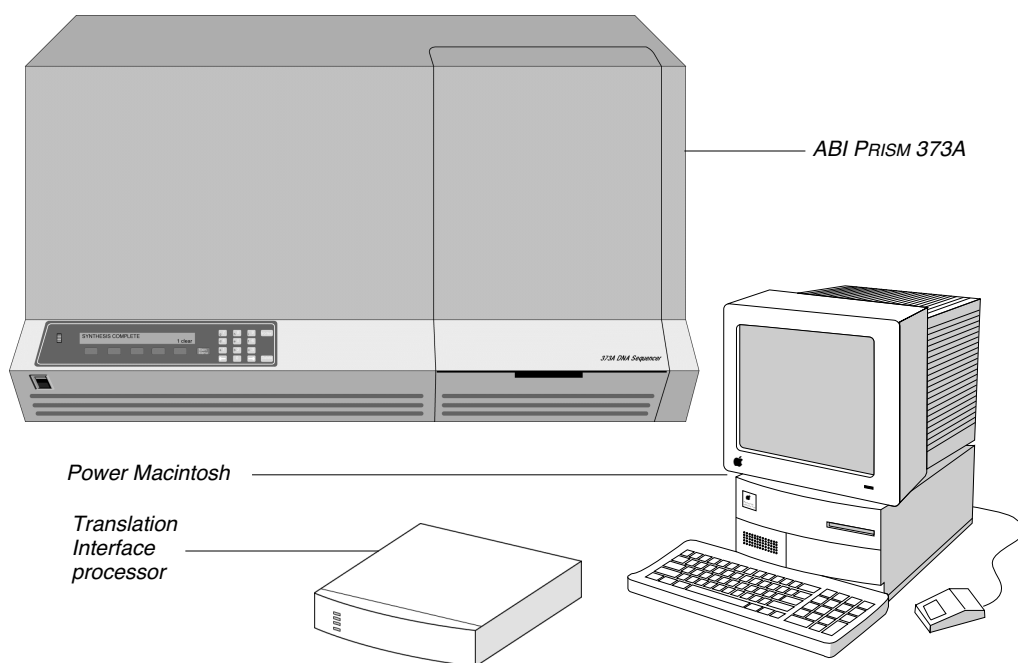
Figure 1-1. The ABI PRISM 373 DNA Sequencer with XL upgrade

Two software applications are available for analyzing the raw data produced by the instrument. With the Sequencing Analysis software you can analyze DNA sequence data. The GeneScan® Analysis software allows you to size and quantitate

DNA fragments. Either or both of these programs is shipped with the instrument, depending on your order.

Note	*This manual describes only how to collect raw data on the instrument. For information about Sequencing Analysis or GeneScan Analysis applications, refer to the* Sequencing Analysis Software User's Manual *or the* GeneScan Analysis Software User's Manual*, respectively.*

Sequencing and GeneScan Analysis on the ABI PRISM 373 DNA Sequencer with XL Upgrade

IMPORTANT	*Analysis of raw data from the ABI PRISM 373 DNA Sequencer with XL Upgrade requires version 3 or higher of Sequencing Analysis, or version 2.1 or higher of GeneScan Analysis. The correct version of the software should be included in your ABI PRISM 373 DNA Sequencer with XL Upgrade DNA Sequencer upgrade package.*

The ABI PRISM 373 DNA Sequencer with XL Upgrade operates the same way for both Sequencing and GeneScan Analysis modes: it performs electrophoretic separation and spectral detection of dye-labeled DNA fragments. The difference between the two modes is in the preparatory chemistries and in the analysis performed on the resulting data.

Sequencing and GeneScan Analysis software programs each analyze the resulting data for your application. The analysis software programs are briefly described on page 1-16, and more completely described in the separate analysis software user's manuals.

To prepare a DNA template, you can use standard laboratory methods. For example, you can use PCR or clone the DNA into a vector such as M13 or pUC, amplify the vector, then purify the template by standard methods. To prepare samples for running on the ABI PRISM 373 DNA Sequencer with XL Upgrade, you must incorporate fluorescent labels. The chemistries for incorporating fluorescent labels are different, depending on whether your application is Sequencing or GeneScan Analysis.

The Sequencing Applications

For sequencing applications on the ABI PRISM 373 DNA Sequencer with XL Upgrade, the sequencing reaction involves the incorporation of fluorescent labels, instead of radioactive isotopes. Four different dyes identify the A, C, G, and T extension reactions. You can incorporate the dye labels using either 5'-dye labeled primers or 3'-dye labeled dideoxynucleotide terminators.

You can use standard polymerases, such as AmpliTaq® and Sequenase T7 DNA polymerase, for primer extension. Both are available in kits with the dye terminator and the dye primer chemistries from Applied Biosystems. The labeling method you use depends on the sequencing strategy and on your personal preference. Refer to the *ABI PRISM DNA Sequencing Chemistry Guide.*

The GeneScan Applications

GeneScan analysis of Sample files includes establishing a baseline, adjusting for spectral overlap of the dyes, peak detection, and size calling.

When you use the GeneScan system on the ABI PRISM 373 DNA Sequencer with XL Upgrade, you can label DNA fragments with up to three different fluorescent dye colors, and then use a fourth dye color to label an internal lane or size standard. When you use an internal size standard, the GeneScan software performs precise size calling without band-shift artifacts and run-to-run variation, problems often encountered using other techniques. GeneScan Analysis software can also be used for relative quantitation when a dye-labeled fragment of known quantity is included in the same lane as the DNA fragments.

You can display the results of an experiment as electropherograms, tabular data, or a combination of both. Electropherograms are a graphical display of peaks detected during collection. Each electropherogram represents a single lane for ABI PRISM 373 DNA Sequencer with XL Upgrade data. The tabular data provides detailed sizing and quantitative information.

Fluorescent labels are incorporated in the form of 5'-end labeling using NHS-esters or phosphoramidites. A second labeling strategy incorporates internal dye labels in the form of fluorescent dNTPs

How the ABI PRISM 373 DNA Sequencer with XL Upgrade Works

After your DNA fragments are labeled, the ABI PRISM 373 DNA Sequencer with XL Upgrade automatically separates and detects them, providing data for analysis by the Sequencing Analysis or GeneScan Analysis software program.

Electrophoresis and Detection

DNA templates labeled with four different fluorescent dyes are combined and loaded into one lane on a vertical acrylamide slab gel. You can analyze up to 66 lanes simultaneously on one gel.

The dye-labeled DNA fragments electrophorese through the acrylamide gel and separate according to size. At the lower portion of the gel they pass through a region 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 data collection software collects the light intensities using the 5-filter wheel and stores them as electrical signals for eventual processing.

Real-Time Status and Data Observation

The data collection program stores the digitized output signal into files on the Macintosh hard disk. The computer screen displays the information received from the ABI PRISM 373 DNA Sequencer with XL Upgrade in real time.

Note	*Real-time viewing of the first peaks as they pass the laser detection site occurs within 90 minutes of loading the samples on the gel during a run using 24- or 34-cm separation distance.*

You can view instrument status and data in several windows on the computer screen by simply choosing the window name from the Window menu.

- The Status window displays current instrument conditions and the set values for the electrophoresis power supply.

- The Electrophoresis History window displays the actual values for the electrophoresis power supply and gel temperature.

- The Scan window displays the raw data; it shows the sweeps of the laser across the gel with a different colored line representing light emission through each filter.

- The Gel window displays a reconstruction of actual data as it would appear on an autoradiogram.

Data Analysis

At the end of collection, the computer can automatically analyze the collected data and print electropherograms on a color printer. Depending on your application, this is performed by either the Sequencing Analysis or the GeneScan Analysis software. Both programs are briefly described on page 1-10, and more completely described in the separate analysis software user's manuals.

You can display the raw and analyzed data on the computer screen, or edit and print it at any time.

ABI PRISM 373 DNA Sequencer with XL Upgrade Throughput

You can use the ABI PRISM 373 DNA Sequencer with XL Upgrade in different ways for various combinations of accuracy, read length, and number of channels.

Table 1-2 summarizes the throughput for typical analysis runs.

Table 1-2. ABI PRISM 373 DNA Sequencer with XL Upgrade Throughput for Sequencing

Instrument Model	WTR Length (cm)	Gel Concen-tration (%)	Gel Thickness (mm)	Scan Mode	Power (W)	Collec-tion Time (hr)	Length of Read (bases resolved)
Classic	24	6.0	0.4	XL or Full scan	30	12–14	450
Classic	24	4.75	0.4	BaseSprinter	40	6.5	450
Leon	24	6.0	0.4	XL or Full Scan	30	12–14	450
Leon	24	4.75	0.4	BaseSprinter	40	6.5	450
Stretch	24	6.0	0.4	XL or Full Scan	26[1]	12–14	450
Stretch	24	4.75	0.4	BaseSprinter	35	7	450
Stretch	34	4.75	0.4	XL or Full Scan	32[1]	14–16	550
Stretch	34	4.25	0.4	BaseSprinter	45	8	550
Stretch	48	4.0	0.3	XL or Full Scan	40[1]	17–18	700

1. If necessary, adjust the power to achieve spacing of 9.5 to 12 for analyzed data.

Table 1-3. ABI PRISM 373 DNA Sequencer with XL Upgrade Throughput for GeneScan

Application	Resolution (bp)	Size Standard	Gel Type (Acrylamide)	Gel Length (cm)	Run Time (hr)
Microsatellite Genotyping (Linkage Mapping Set, dinucleotide tepeats)	1	GS-350 or GS-500	6.0% denaturing	24	6
Microsatellite Genotyping (tri- and tetranucleotides)	1–2	GS-500	6.0% denaturing	12	4
VNTRs	1–2	GS-350 or GS-500	8.0% denaturing	12	4
Large Fragments (0.5–2.5 kb)	3–4	GS-1000 or GS-2500	8.0% denaturing	6	2
AFLP™ Plant Mapping Kit	1	GS-500	6.0% denaturing	24	11
Stock Marks™ for Cattle	1	GS-350	6.0% denaturing	24	6
AmpF*l*STR™ (tetranucleotides)	1	GS-500	6.5% denaturing	24	6

Software for Data Analysis

Two standard software programs are available to analyze raw data from the ABI PRISM 373XL: Sequencing Analysis and GeneScan Analysis.

DNA Sequencing Analysis Software

The Sequencing Analysis software analyzes raw sequencing data collected by the data collection program on the ABI PRISM genetic analysis instruments.

The analysis software processes data using a multicomponent analysis, baseline subtraction and scaling. After processing, it detects the peaks and determines the sequence. The four-dye, one-lane sequencing approach eliminates electrophoresis variables and reduces lane-to-lane variations.

You can reanalyze and edit the sequence data in a format called a Sample file. The same Sample file can be used by other Applied Biosystems software programs for additional data processing. Also, Sample files are in formats that you can export to commercially available or user-generated programs on the Macintosh and on other compatible computers for further analysis.

The Sequencing Analysis program comes bundled with three other programs, GelDocII, DataUtility, and Factura. GelDocII removes tracking data from a Gel file and removes and restores the gel image from a Gel file. The DataUtility program performs two functions. It makes matrices for use with the ABI PRISM 373 DNA Sequencer with XL Upgrade Collection and Sequencing Analysis software, and monitors noise levels for troubleshooting by Applied Biosystems technical specialists. Factura performs additional processing on Sample files to clarify the target DNA sequence.

GeneScan Analysis Software

The GeneScan® Analysis software enables you to use the automated fluorescence detection capabilities of the ABI PRISM genetic analysis instruments to determine accurate sizing and relative quantitation of DNA fragments. You can size PCR products (STRs, VNTRs, and RAPDs) and perform quantitative gene analysis (COP assays, mRNA) faster than with traditional methods, yet with sufficient resolution to accurately analyze molecular fragment lengths in the 50–500 nucleotide size range. The software uses internal lane size standards to reduce gel-to-gel and lane-to-lane variation.

The software automates the entire process of separating, quantifying, and sizing DNA fragments. The data can be displayed as a gel image, an electropherogram, tabular data, or a combination of electropherograms and the corresponding tabular data.

Further Analysis of Processed Data

The Inherit Sequence Analysis System

Inherit is a multi-user system used to edit, assemble, and analyze DNA and protein sequence data. The Inherit 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.

Inherit has three Macintosh programs:

- Factura prepares 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 overlapping pieces of DNA into larger segments of DNA, using ABI PRISM 373 DNA Sequencer with XL Upgrade 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 allows you to analyze data from ABI PRISM 373 DNA Sequencer with XL Upgrade sequence files, files processed in AutoAssembler, text files, or a database. You can use this program to rapidly search biological databases for sequence similarity or patterns, and to interactively work with the resulting sequence lists. In addition to searching results, the program can create dot plots, alignments, and restriction maps of sequence data.

AutoAssembler Sequence Assembly Software

AutoAssembler is available as a separate program from the Inherit system. You can purchase it for use with a Macintosh only, and perform the same functions as described above, using a local algorithm. Upgrade to the more powerful assembly capabilities of the Inherit system by adding the client-server option (which runs on a UNIX server). This provides a multi-user environment and allows you to use an assembly algorithm that handles repeat sequences and large projects more efficiently than the local algorithm.

Sequence Navigator Sequence Comparison Software

Sequence Navigator™ software is specifically designed to address the needs of researchers performing comparative sequencing, as it accurately aligns sequences to a standard or a population and identifies sequence variants.

Sequence Navigator runs on the Macintosh and can be used for many applications, such as mutation identification/heterozygote screening of sequences such as p53 and HIV, and mitochondrial DNA. The program incorporates five powerful algorithms for pairwise or multiple alignment of DNA and protein sequences. It is bundled with the Factura program described above, allowing identification of heterozygous base positions and quick cleanup of sequences before alignment.

Genotyper Genetic Analysis Software

Genotyper™ is a data analysis and transformation tool that converts data from GeneScan into user application results. Genotyper converts data into the format required by downstream applications, such as linkage analysis programs, databases, or spreadsheets. The Genotyper software thereby helps create seamless automation between the ABI PRISM 373 DNA Sequencer with XL Upgrade and the final experimental results.

Primer Express Software

Primer Express™ is a primer design program with an easy-to-use interface. The software is applications oriented, taking into consideration the most updated criteria for primer design.

EditView Software

EditView is a free, DNA Sequence viewer that allows you to view and print sequence data from an ABI PRISM 373 DNA Sequencer with XL Upgrade, an ABI PRISM 377 DNA Sequencer, or an ABI PRISM 310 Genetic Analyzer. Using EditView on your Macintosh computer, you can open an analyzed sample file and view the sequence data either as an electropherogram (traces), or in text format. You can then edit individual bases, export the data to a text file, or print. To receive a copy of EditView, visit our Web site at www.appliedbiosystems.com/techsupport.

Differences between the ABI PRISM 373 DNA Sequencer with XL Upgrade and the Model 373

Instrument and Accessories

- *More samples per instrument run.* The Model 373 allows 36 samples to be analyzed simultaneously. With the ABI PRISM 373 DNA Sequencer with XL Upgrade, you can analyze up to 64 samples at one time. This increased rate of throughput is due to the redesign of the rate at which the laser detects data.

- *Instrument Performance.* While more samples can be analyzed simultaneously, parameters such as length of sequence detected, peak sizing and quantitation, and time allocated to run the instrument is not expected to change after the ABI PRISM 373 DNA Sequencer with XL Upgrade upgrade is installed.

- *New spacers and combs.* The XL combs (48- and 64-well sharks-tooth and 50- and 66-well square-tooth) are made of a material called Mylar. In addition to the combs, glass plate spacers, casting combs, and overlays are also available in Mylar. The other combs supplied by Applied Biosystems for use on the ABI PRISM 373 DNA Sequencer with XL Upgrade are made of a material called Valox.

IMPORTANT	*Always use combs, glass plate spacers, and casting combs as a matched set. Do not mix and match material types or thicknesses within a set. Mismatching can cause loose-fitting combs, and can result in sample leaking and/or damage to the gel.*

- *New glass plate spacer design.* New glass plate spacers are made of Mylar to be compatible with the XL combs. The new spacers are available in only one length but are marked to indicate where to cut them in order to fit each size of glass plate. Offering spacers in a single length reduces the chances of making errors during order entry. It also allows us to offer them at a lower price than previous custom-cut spacers.

  Notches are cut out of one end of the spacers. When you assemble the plates, be sure that the notches are at the top of the plates and facing

toward the well. These new spacers help reduce buffer leaks between the gel and spacer.

- *Power Macintosh control of instrument.* The instrument is operated entirely from the Power Macintosh computer. While the Model 373 instrument was controlled by a combination of the keypad on the front of the instrument and the computer, the keypad on the front of the instrument is now used only for instrument diagnostics.

- *New user's manual.* The new manual is a smaller, more convenient size, and contains a glossary, a quick reference procedure, and an expanded index.

- *Translation Interface Processor.* This hardware acts as a translator and memory buffer between the ABI PRISM 373 DNA Sequencer with XL Upgrade and the Power Macintosh. This enables the instrument to be controlled by the Power Macintosh. In addition, the Translation Interface Processor increases the amount of memory buffer available with the Model 373.

Chemistry

- *Load less sample.* Smaller well size requires less sample. See Table 3-3 on page 3-40 for Sequencing sample volumes. See Table 4-2 on page 4-43 for GeneScan application load volumes.

Software

- *Streamlined data collection software.* Input of information and viewing of real-time data/instrument status is simplified. All data for a run can be viewed in the Gel window during data collection.

- *Compatibility with Analysis software.* Raw data generated by the ABI PRISM 373 DNA Sequencer with XL Upgrade can be analyzed using only version 3 or higher of Sequencing Analysis software or version 2.1 or higher of GeneScan Analysis software.

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

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

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	apisupport@sciex.com or api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

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

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

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

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844,** then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844,** then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844,** then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844,** then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments)	**1-800-831-6844,** then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844,** then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS®, BioMerge™, and SQL GT™ applications)	**1-800-831-6844,** then press **25**	**1-505-982-7690**

Product or Product Area	Telephone Dial...	Fax Dial...
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**
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858**, then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858**, then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
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Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
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2   Preparing Gels

Contents

Polyacrylamide Gels

For sequencing, the polyacrylamide gel is one of the most important variables that determines the number of bases you can read. For fragment analysis, the polyacrylamide gel is critical for the resolving capabilities. In short, your data is only as good as the quality of your gel.

The quality of your gel is even more important with the ABI PRISM 373XL than it is with manual sequencing, since much more DNA (over 600 bands) passes through a gel in a single loading on the ABI PRISM 373XL. Therefore, it is of paramount importance to produce a first-rate gel. Some of the factors that affect gel quality are described below.

Mechanism of Polymerization

Polyacrylamide gels are formed by the copolymerization of acrylamide and bis-acrylamide. The process begins when TEMED (tetramethylethylenediamine) reacts with APS (ammonium persulfate) to yield a TEMED radical cation, a sulfate radical and a sulfate ion. The sulfate radical adds an unpaired electron to the acrylamide monomer, converting it to a free radical which then reacts with more monomer, causing polymer chain elongation. The polymer chains are randomly cross-linked with bis-acrylamide to form the gel matrix.

Effect of Reagent Purity on Gel Quality

It is extremely important that you use pure gel reagents from a reliable source. Optimal results depend on gel quality and therefore, on gel reagent quality.

Acrylamide
Impurities in this reagent (or in bis-acrylamide) can cause:

- Irreproducible gel porosity
- Deviant mobility
- Inhibition of polymerization
- Poor resolution

all of which lead to poor results, wasted time, and frustration.

These problems lead to compromised reproducibility. Some possible contaminants that can cause them include the following:

- Acrylic acid - The hydrolysis product of acrylamide. It copolymerizes with acrylamide and bis-acrylamide. Its presence causes the DNA to migrate slowly, resulting in broad, diffuse bands and consequently, poor resolution.

- Linear polyacrylamide - Caused by catalytic contaminants in the dry acrylamide monomer. It decreases the effective concentration of the acrylamide, causing the DNA to migrate faster.

- Ionic contaminants - Mostly metals, such as iron or copper. These can inhibit or accelerate polymerization.

Good quality solid form/dry acrylamide can be stored at room temperature for up to one year. You can store a 40% stock solution of acrylamide and bis-acrylamide (19:1) at 4 °C for up to one month.

WARNING	**CHEMICAL HAZARD. Acrylamide and bis-acrylamide 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. Read the MSDS in the Safety Summary provided with your original user's manual.**

Bis-acrylamide
Impure bis-acrylamide contains some of the same contaminants as acrylamide. Store it dry at room temperature for up to one year. You can store a 40% stock solution of acrylamide and bis-acrylamide (19:1) at 4 °C for up to one month.

Note	*You can either make 40% acrylamide stock solution from solid acrylamide and bis-acrylamide, or buy high-quality premixed, preweighed powders and dilute them to a 40% solution. Use distilled or deionized water only, and store at 4 °C for up to one month. Using the pre-made powders saves time and minimizes exposure to the solid form of the neurotoxins acrylamide and bis-acrylamide.*

Ammonium Persulfate (APS)

APS (ammonium persulfate) begins to decompose at once when dissolved in water. The result is loss of activity. For reproducible results, therefore, you should prepare APS solutions fresh daily. It is vital that this chemical be fresh since it affects the rate of polymerization and thus the gel properties. Store solid APS tightly sealed, since it is hygroscopic. If tightly sealed, it can be stored at 4 ˚C for up to 1 year.

Note	Store solid APS in an airtight container with desiccant, to keep it dry. To avoid water condensation problems, let the bottle of APS warm to room temperature before opening it.

TEMED

TEMED (N, N, N', N'-tetramethylethylenediamine) 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. 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. Read the MSDS in the Safety Summary provided with your original user's manual.**

Urea

Use urea in the crystalline form, and weigh it fresh each time you use it. Old urea has breakdown products that affect sample migration.

WARNING	*CHEMICAL HAZARD. Urea is a potential mutagen. Avoid inhalation and contact with skin, eyes and clothing. Read the MSDS in the Safety Summary provided with your original user's manual.*

Note	*Do not make up a stock solution containing the urea. Prepare the components fresh.*

Buffers and Gel Additives

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 the polymerization in various ways, causing run-to-run irreproducibility. Non-buffer ions tend to inhibit DNA mobility and also cause run-to-run irreproducibility. To help avoid these problems, work only with high quality Tris, borate and urea, and use only distilled or deionized water for diluting reagents.

Other Factors that Affect Gel Quality

Rate of Polymerization

The properties of the gel depend on the rate of polymerization. The rate of polymerization is affected by temperature, initiator concentration, and oxygen. Each parameter is discussed in detail in this section for academic reasons only. The protocol in this section has already been optimized with regard to temperature, initiator concentration and oxygen content, therefore, you do not need to experiment with these variables.

Temperature: Controlling the temperature is crucial for achieving reproducible gels because it directly affects the polymerization time and thus affects the gel properties. A gel formed in a cold environment (at 4 °C) is turbid, porous, and inelastic, and the run-to-run reproducibility is greatly compromised. Polymerization in a 20–23 °C room ("normal" room temperature) is optimal. It is important that the gel solution and glass plates as well as the room temperature

be at 20-23 °C. Gels formed at this temperature are transparent, less porous, more elastic, and more reproducible. If the temperature is too high, the polymer chains are shorter and the resulting gel is inelastic, again resulting in non-reproducibility from run to run.

Initiator Concentration: Increasing the concentration of TEMED or APS decreases the average polymer chain length, and increases gel turbidity. Decreasing the concentration of TEMED or APS increases the average polymer chain length, and decreases gel turbidity.

Decreasing the concentration of initiators has a desirable effect, except that this also causes slower polymerization. When polymerization is too slow, oxygen can enter the monomer solution during the process and inhibit polymerization.

If you use degraded TEMED or APS, the concentration of initiators is less than that recommended in the protocol.

Oxygen: Oxygen acts as a free radical trap, thereby inhibiting polymerization. The result is a porous gel. To prevent the problems caused by oxygen, be careful to meet the following conditions.

- Polymerization must be fast enough to prevent too much oxygen from dissolving into the gel solution during polymerization. As long as you use fresh, high-quality reagents and follow the protocol in this manual, oxygen should not cause a problem during polymerization.

- Minimize the amount of oxygen dissolved in the gel solution prior to casting the gel, since it can interfere with the rate of polymerization. Partial degassing is accomplished during the vacuum filtration step of this protocol. It is important to keep the vacuum strength and time constant during this step for run-to-run reproducibility. Since cold solutions have a greater capacity for dissolved oxygen, perform vacuum filtering (and gel casting) with the solution at room temperature. Also, be gentle during the stirring and pouring steps of gel casting so that oxygen is not introduced.

Air bubbles

Air bubbles can distort the sample path, which affects lane tracking. Pour gels carefully and gently so bubbles do not form. If bubbles are trapped between the comb and the gel solution, carefully remove and reinsert the comb until no bubbles are trapped.

Using an Old Gel

For consistent results, use gels between two and six hours after casting. Be sure to wait at least two hours after casting the gel to ensure complete polymerization, but do not wait longer than six hours. 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.

WARNING **CHEMICAL HAZARD. The acrylamide and bis-acrylamide used to prepare sequencing gels for the ABI PRISM 373 DNA Sequencer with XL Upgrade are toxic chemicals whose use requires utmost attention to safety. Be sure to read the Material Safety Data Sheets (MSDSs)for emergency and first-aid procedures. MSDSs are in the ABI Prism 373 DNA Sequencer with XL Upgrade Pre-Installation Manual, P/N 901173. Acrylamide is a neurotoxin that can be absorbed through the skin. Wear a laboratory coat, eye protection, and gloves. The handling and disposal of chemical waste is the responsibility of the user, and not the Applied Biosystems service representative.**

Preparing Acrylamide Denaturing Gels

This protocol for the preparation of acrylamide gels follows generally accepted procedures; however, there are some important distinctions to remember throughout the process. The ABI PRISM 373XL DNA Sequencer is a real-time electrophoresis detector. A laser scans across a region of the gel during the electrophoretic process, detecting the fluorescently labeled DNA passing through that region. The glass and the gel must be non-fluorescent. Be especially careful to:

- Eliminate any particulate or fluorescent matter from the acrylamide gel solutions.

- Keep the glass plates extremely clean and free of dust.

- Always use ultra-pure reagents and high-grade, distilled, deionized water to prepare solutions

- Wear clean laboratory gloves.

- Filter all solutions to remove any particulate matter that may fluoresce or scatter light.

To maximize gel resolution, high quality acrylamide gels are essential, particularly when you are sequencing templates of greater than 350 bases in length. If you prepare acrylamide solutions with low-quality starting materials or heat them excessively, breakdown products might detract from the quality of the run. Using a deionizing resin and preparing carefully helps to avoid problems caused by such breakdown activity.

WARNING   **CHEMICAL HAZARD. Acrylamide and bis-acrylamide 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.**

Reagents and Supplies for Stock Solutions

You can either make 40% acrylamide stock solution from solid acrylamide and bis-acrylamide, or buy high-quality premixed, preweighed powders and dilute them to a 40% solution. To dilute them use distilled or deionized water only. Using the pre-made powders saves time and minimizes exposure to the solid form of the neurotoxins acrylamide and bis-acrylamide. You can also purchase ready-made stock solutions of the 10X TBE buffer. The concentration must be 0.89 M Tris-base, 0.89 M Borate, 0.02 M EDTA, pH 8.3 ± 0.2.

If you prefer to prepare your own stock solutions, the recipes follow. Be careful to use ultra-pure reagents and high-grade, distilled, deionized water to prepare the solutions.

10X TBE Stock Solution
Per liter:

- Tris base   108.0 g
- Boric Acid   55.0 g
- $Na_2EDTA \bullet 2H_2O$ 8.3 g

Working solution (1X) is 89 mM Tris-base, 89 mM Boric acid, 2 mM EDTA; pH≈ 8.3 at ambient temperatures.

IMPORTANT   *Discard the working solution if the pH is not 8.3 (± 0.2), and make a fresh solution. Do not attempt to adjust the pH.*

To prepare the 1X TBE working solution (Classic and Leon):

1.   Add 150 mL 10X TBE stock solution to a large graduated cylinder.

2.   Dilute with dH_2O to a total volume of 1500 mL.

To prepare the 1X TBE working solution (Stretch):

1.   Add 200 mL 10X TBE stock solution to a large graduated cylinder.

2.   Dilute with dH_2O to a total volume of 2000 mL.

40% (19:1) Acrylamide Stock Solution

Per 100 mL:

- Acrylamide   38.0 g
- Bis-Acrylamide   2.0 g

To prepare the acrylamide solution:

1. Dissolve the crystalline acrylamide and bis-acrylamide in enough dH$_2$O to bring the total volume up to 90 mL.

2. Add 10 g of mixed-bed, ion-exchange resin.

3. Stir at room temperature until all crystals dissolve, then continue to stir for an additional 5-10 minutes.

4. Filter through a 0.2-µm cellulose nitrate cup filter assembly.

5. Transfer to a 100-mL graduated cylinder and adjust volume to 100 mL with dH$_2$O.

The finished solution will be 19:1, acrylamide:bis-acrylamide. Solutions are stable for approximately 1 month when refrigerated at 4 °C.

Reagents and Supplies for Gels

WARNING	**CHEMICAL HAZARDS. Urea is a potential mutagen. TEMED is extremely flammable and can be very destructive to the skin, eyes, nose, and respiratory system. Do not store, handle, or work with any chemicals or hazardous materials unless you have received appropriate safety training and have read and understood all related Material Safety Data Sheets. Comply with all federal, state, and local laws related to chemical storage, handling, and disposal.**

Ensure that you have each item on the following list ready before beginning to mix a sequencing gel.

- Urea
- TEMED

- 10% Ammonium persulfate (w/v) in dH$_2$O

  Prepare fresh stocks daily, and store refrigerated.

- Volumetric pipets and tips

- 0.2-µm cellulose nitrate cup filters

- Plates, spacers, and combs

  Optically clear glass plates, spacers, and combs are available from Applied Biosystems.

- Alconox detergent

- Mixed bed, ion-exchange resin

- Level gauge and a level working surface

Preparing the Plates (all sizes except 48 cm)

Cleaning the Plates

Rinse both sides of the glass plates with warm water, then wash with a detergent such as Alconox that will not leave a residue. Be careful not to scratch or abrade the glass surfaces.

Caution **Avoid contacting the glass plates with metal. Scratches caused by metal objects, such as drying racks or spatulas weaken the glass and reduce its usable life.**

Rinse the plates well with warm water followed by distilled, deionized water. Stand the plates up and allow them to air dry.

Note *If you are in a hurry, dry the plates gently with lint free towels rather than with an organic solvent which might contain fluorescent residues. Fluorescent residues tend to cause a green haze on your gel image.*

Notched plate   *Plain plate*   *Spacers*
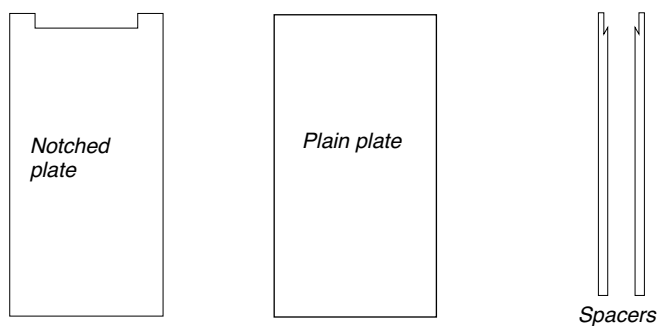
Figure 2-1. Glass plates for casting gel

Note	Do not touch the cleaned surfaces of the glass plates and wear gloves to handle the plates.

Each time you use the glass plates, use them with the same side of each plate on the inside. After the first use, the front plate has a hydrophobic area where the buffer chamber gasket made contact with the plate. You must use the front plate in the same configuration each time to avoid difficulty pouring the gel. You may mark the outside of each plate the first time you use it. Mark the plates with a tiny scratch at the top that doesn't interfere with the read region or the upper buffer chamber seal.

To clean plates that have been previously used:

This cleaning is usually done immediately after the previous run is complete. Refer to page 3-47 for instructions.

Applying the Bind-Silane Compound

Note	Bind-Silane use is optional with any of the sharks-tooth combs. However, it must be used with all square-tooth combs with more than 36 wells.

Silating the unnotched glass plate in the well area (upper 3.5 cm) with a solution like Pharmacia's Bind-Silane compound helps eliminate deformed wells. Follow the instructions below for performing the silation. This must be repeated every time a new gel is poured. Remove the old gel from the silated area with 0.1 N HCl or NaOH, or scrape off gently.

To apply the Bind-Silane compound:

1. Wash the unnotched glass plate with Alconox detergent, rinse well with distilled water, and leave to dry.

2. Adjust 200 mL H_2O to pH 3.5 with acetic acid. Add 800 μL Bind-Silane and stir until clear, approximately 15 minutes.

3. Set the unnotched plate in a tub with the Bind-Silane solution covering the top 3.5 cm of the glass. Leave immersed at room temperature for 10–60 minutes.

IMPORTANT   *Do not get Bind-Silane on the laser-scanning region of the gel; it will fluoresce.*

---

4.   Rinse the glass plate with warm water, and then with dH$_2$O, and let dry.

Assembling the Plates

To use your time efficiently, you might want to mix the acrylamide-urea gel solution and, while it is being stirred, clean and prepare the plates. To mix the acrylamide-urea solution, refer to page 2-18.

To assemble the plates:

1.   Clean the plates, as described on page 2-13.

2.   Clean the 0.4-mm gel spacers in a similar manner, and make sure they are the proper length for the glass plates.

     If the spacers are longer than the full length of the glass plates, clip them to the proper size. A paper cutter works well for trimming spacers. The XL spacers are marked with lines to make cutting easier.

IMPORTANT   *Because of tolerance issues, verify the combs, spacers, and casting comb you are using are compatible (made of the same material) and are the same thickness.*

---

3.   Place the plain plate on a protected bench top.

4.   Place the gel spacers on the plain plate with the notches at the top of the plate and facing in.

     You may use a pipet to apply small water droplets to the edge of the plate at several spots along the length so the water flows between the spacers and the glass to keep the spacers in position.

Place water droplets here to hold spacers in position

Spacers notched facing in

Spacers

Plain plate
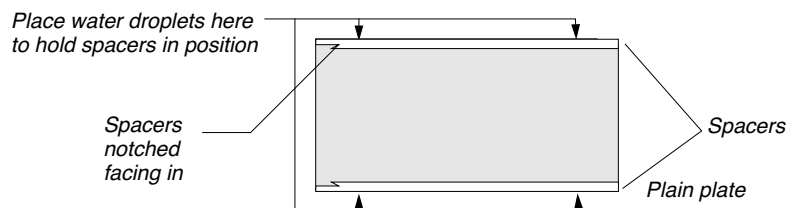
Figure 2-2. Securing spacers on glass plates (top view)

5. Align the notched plate (inside down) on top of the spacers. The edges of the glass plates should be flush.

   Again, be sure the inside of the plate is free of any particles that might fluoresce.

6. Temporarily clamp one side of the plates.

Notched plate

Plain plate
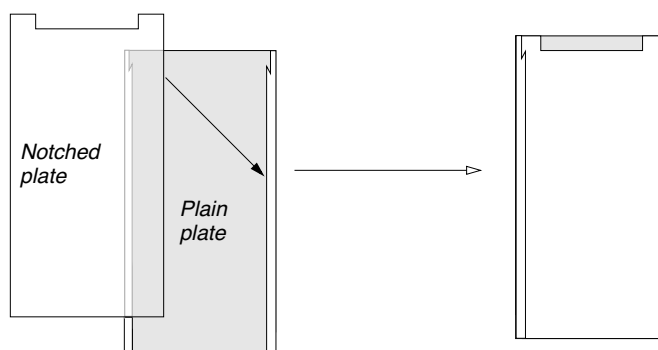
Figure 2-3. Plates aligned

7. Apply 1.5-inch wide tape tightly along the edge opposite the clamp. Wrap tape around both the top and bottom corners.

8. Remove the clamp and apply tape along the second long edge. Wrap tape around both the top and bottom corners.

9. Apply tape along the bottom edge of the plates, wrapping around both corners.

10. Eliminate any air pockets under the tape and reinforce points of assembly that are likely to leak acrylamide solution.

Note   *Using a commercially available gel pouring device can simplify gel casting by eliminating the need to tape the glass plates.*

Preparing the Gel (all sizes except 48 cm)

Preparing Acrylamide-Urea Gel Solutions

The ABI PRISM 373XL DNA Sequencer gels are very thin. Use only high purity reagents to prepare them. You can scale the recipe for double or half the amount as necessary. The recipes can be used for both Sequencing and GeneScan 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 bis-acrylamide 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.*

Preparing 4.25%, 4.75%, or 6% Acrylamide-urea Gel Solutions

Use the following three recipes for all gels except 48 cm gels (for 48-cm gels see *Preparing the Plates (48 cm only)* on page 2-22). The volume of 40% acrylamide stock solution varies between the different gel recipes, and the resulting water needed to adjust to the total volume differ; everything else remains the same.

1. Combine the following components in a 150-mL beaker to prepare a final volume of 80 mL of one of the three gel solutions:

   - 40 g urea

   - 27 mL dH_2O

   - 1 g (less for smaller volumes) mixed-bed, ion-exchange resin

2. Customize the gel solution by adding the appropriate amount of 40% acrylamide stock, as follows:

   • For a 4.25% acrylamide, 8.3 M urea, 1X TBE (34 cm BaseSprinter) add 8.5 mL 40% acrylamide stock solution.

   • For a 4.75% acrylamide, 8.3 M urea, 1X TBE (24 cm BaseSprinter or 34 cm Full or XL Scan) add 9.5 mL 40% acrylamide stock solution.

   • For a 6% acrylamide, 8.3 M urea, 1X TBE (12 cm and 24 cm Full or XL Scan) add 12 mL 40% acrylamide stock solution.

3. Stir the solution while gently warming it until the urea crystals begin to dissolve and the flask is slightly warm to the touch. Continue to stir the solution with no additional heating for 5-10 minutes longer, or until all the urea crystals have dissolved.

   While the solution is being stirred, you can prepare the plates, if you have not already done so.

4. Filter the acrylamide solution through a 0.2 µm cellulose nitrate filter, de-gas for 5 minutes, and transfer to a 100-mL graduated cylinder.

Note   *The degas time should be constant for all gels, to ensure a reproducible polymerization rate for all runs.*

---

5. Add 8 mL filtered 10X TBE buffer and adjust the volume to 80 mL with dH$_2$O, if necessary.

IMPORTANT   *Never add the TBE buffer before removing the mixed bed, ion-exchange resin by filtration.*

Casting the Gel (all sizes except 48 cm)

To cast 48-cm gels, see page 2-26.

1. Gently pour the acrylamide solution into a 150 mL beaker.

2. Add 400 µL 10% ammonium persulfate (freshly made) and gently swirl. Avoid adding air bubbles.

3. Add 45 µL TEMED and gently swirl. Avoid adding air bubbles.

4. Immediately and carefully cast the gel by holding the plates at an angle to the bench top, and resting the lip of the solution vessel against the edge of the notched plate. Pour the solution between the plates and fill to about 3–5 cm from the top edge of the notched plate (the level of gel solution must be high enough so that the "ears" of the plates are filled when the plates are laying flat). The solution can also be dispensed using a 60 mL plastic disposable syringe.

5. Allow all air bubbles to rise. Gently tap the plates to help trapped bubbles rise to the surface.

6. *For sharks-tooth combs:* Insert the gel casting comb. Wet the gel casting comb with the acrylamide solution. Be sure there are no bubbles trapped at the gel solution/casting comb interface.

   For square-tooth combs: Insert the square-tooth comb. Be sure there are no bubbles trapped at the gel solution/comb interface.

IMPORTANT	Because of tolerance issues, verify the combs, spacers, and casting comb you are using are compatible (made of the same material) and are the same thickness.

Note	Ensure that the casting comb is completely dry before wetting it with acrylamide. Using a wet casting comb can make the surface of the acrylamide wavy, which can change the way samples migrate through the gel.

IMPORTANT   *Be sure there are no air bubbles trapped at the gel solution/comb interface after the comb is inserted. Bubbles result in depressions on the well surface, leading to sample leakage (for sharks-tooth combs) and/or decreased resolution in affected lanes.*

---

7. Lay the plates in the horizontal position and secure the casting or square-tooth comb with three large binder clamps. Place the clamps directly over the region of the gel casting comb that is sandwiched between the glass plates.

IMPORTANT   *Use a level gauge to ensure that the plates are on a level surface while the gel is polymerizing.*

---

8. Wait at least two hours to ensure complete polymerization prior to using the gel.

    For sequencing and GeneScan runs, refer to Section 3 and Section 4, respectively, the Step-by-Step procedure, for details on how to prepare and place the gel and glass plates on the instrument.

Preparing the Plates (48 cm only)

The 48 cm run uses a different gel recipe and set up procedures than any of the other runs. The following section provides the necessary information for preparing the gel solution, preparing and setting up the glass plates, and pouring the gel.

Preparing Gel Casting Equipment for a 48 cm Run

Rinse both sides of the notched and plain glass plates with warm water. Then wash the plates with a detergent such as Alconox that will not leave a residue and warm water. Take care not to scratch or abrade the glass surfaces.

Caution	**Avoid contacting the glass plates with metal. Scratches caused by metal objects, such as clamps, drying racks, or spatulas cannot be detected by eye and will weaken the glass and reduce its usable life.**

Rinse the plates well with warm water followed by distilled, deionized water. Stand the plates up and allow them to air dry.

Note	*Do not touch the cleaned surfaces of the glass plates and wear gloves to handle the plates. Always try to use the glass plates with the same side of each plate on the inside.*

Preparing Glass Plates for 48 cm Gels

To achieve the high resolution of a 48 cm run, a very flat well surface is required. This is more difficult with a 4.0% acrylamide gel, which is less rigid than gels with higher percentages. Silating the unnotched glass plate in the well area (upper 3.5 cm) with a solution like Pharmacia's Bind-Silane compound will help to ensure a flat well surface. Follow the instructions below for performing the silation. This must be repeated every time a new gel is poured. Remove the old gel from the silated area with 0.1 N HCl or NaOH, or scrape off gently.

Applying the Bind-Silane Compound

1. Wash the unnotched glass plate in detergent, rinse well with distilled water, and leave to dry.

2. Adjust 200 mL H_2O to pH 3.5 with acetic acid. Add 800-µL Bind-Silane and stir until clear, approximately 15 minutes.

3. Set the unnotched plate in a tub with the Bind-Silane solution covering the top 3.5 cm of the glass. Leave immersed at room temperature for 10–60 minutes.

4. Rinse the glass plate with warm water, and then with dH_2O, and let dry.

IMPORTANT   Do not get Bind-Silane on the laser-scanning region of the gel; it will fluoresce.

Assembling the Glass Plates for a 48 cm Run

1. Make sure to use the gel spacers and gel casting comb labeled 0.3 mm. Wash the comb and spacers with warm water, and then with dH_2O, and then with reagent quality ethanol, and let dry.

IMPORTANT   The spacers used for 48-cm WTR gels have notches cut out of one end. When assembling the plates, ensure that the notches are at the top of the plates, and facing in, towards the well.

2. Place the unnotched plate on a protected benchtop and arrange the spacers on the outer edges of the plate. Lay the notched plate on top of the unnotched plate and spacers. Align the plates and spacers and clamp one side to temporarily hold plates in position.

3. Apply 1.5-inch wide tape tightly along the edge opposite the clamp. Wrap tape around both the top and bottom corners.

4. Remove the clamp and apply tape along the second long edge. Wrap tape around both the top and bottom corners.

5. Apply tape along the bottom edge of the plates, wrapping around both corners.

Note	*Using a commercially available gel pouring device can simplify gel casting by eliminating the need to tape the glass plates.*

6. Eliminate any air pockets under the tape and reinforce points of assembly that are likely to leak acrylamide solution.

7. Place three binder clips over each spacer (on top of the tape), spaced evenly between the top and bottom of the plates. Be careful to keep the clips over the spacer and not in the region where the acrylamide will be poured. Although using binder clips is not recommended with the shorter gel plates, it does help keep the gel thickness uniform with the longer glass plates.

Preparing the Gel (48 cm only)

Preparing 4% Acrylamide-urea Gel Solutions

The 48-cm plates hold a larger volume than the other plates. Therefore, this recipe is for 100 mL and the volume of TBE, APS, and TEMED are greater than for the other gels also.

To prepare 4% gel solutions:

1. Combine the following components in a 150 mL beaker to prepare a final volume of 100 mL of a 4.0% acrylamide, 8.3 M urea, 1X TBE:

    *   50 g urea

    *   37 mL dH$_2$O

    *   10 mL 40% acrylamide stock solution

    *   1 g mixed bed, ion-exchange resin

2. Stir the solution while gently warming it until the urea crystals begin to dissolve and the flask is slightly warm to the touch. Continue to stir the solution with no additional heating for 5-10 minutes longer, or until all of the urea crystals have dissolved.

3. Filter the acrylamide solution through a 0.2 µm cellulose nitrate filter, de-gas for 5 minutes, and transfer to a 100-mL graduated cylinder.

Note   *The degas time should be constant for all gels, to ensure a reproducible polymerization rate for all runs.*

---

4. Add 10 mL 10X TBE, and bring to 100 mL with dH$_2$O, if necessary.

IMPORTANT   *Never add the TBE buffer before removing the mixed bed, ion-exchange resin by filtration.*

Casting the 48-cm Gel

To cast the 48-cm gel:

1. Gently pour the acrylamide solution into a 250-mL beaker.

2. Add 500 µL 10% ammonium persulfate (freshly made) and gently swirl. Avoid adding air bubbles.

3. Add 50 µL TEMED and gently swirl. Avoid adding air bubbles.

Note   *Adjust the volume of TEMED as needed to ensure complete polymerization in 5 to 10 minutes.*

---

4. Holding the plates at an angle, immediately and carefully pour the solution between the plates and fill to about 3–5 cm from the top edge of the notched plate.

   The level of gel solution must be high enough so that the "ears" of the plates are filled when the plates are laying flat. You can also use a plastic disposable syringe to dispense the solution.

IMPORTANT   *Pour the solution very carefully. It is difficult to remove air bubbles out from between the longer plates, and any air bubbles can significantly reduce resolution. Small air bubbles are difficult to see.*

---

5. Allow all air bubbles to rise. Gently tap the plates to help trapped bubbles rise to the surface.

   Avoid tapping plates in the laser scan region with a hard object. Scratches or nicks on the plate surface can interfere with data collection.

6. Wet the 0.3-mm gel casting comb with the acrylamide solution and insert it between the two glass plates.

Note   *Ensure that the casting comb is completely dry before wetting it with acrylamide. Using a wet casting comb can make the surface of the acrylamide wavy and change the way samples migrate through the gel.*

| IMPORTANT | Be sure there are no air bubbles between the glass plates and the acrylic casting comb after the casting comb has been inserted into the gel. Bubbles will be wicked into the acrylamide and result in depressions on the well surface, leading to decreased resolution in affected lanes. |

7. Lay the plates in the horizontal position and secure the comb with three large binder clamps. Place the clamps directly over the region of the gel casting comb that is sandwiched between the glass plates.

| IMPORTANT | Use a level gauge to ensure that the plates are on a level surface while the gel is polymerizing. |

3   Using the Instrument in Sequencing Mode

Contents

```
                    ┌─────────────────────────────┐
                    │   Prepare and label samples │
                    └─────────────────────────────┘
                                   │
                    ┌─────────────────────────────┐
                    │          Pour gel           │
                    └─────────────────────────────┘
                                   │
  ╭──────────╮                ╭─────────────────╮
  │Polymerize│                │ Restart Macintosh│
  │  ~2 hr   │                │ Set up software  │
  ╰──────────╯                ╰─────────────────╯
                                   │
                    ┌─────────────────────────────┐
                    │    Load gel on instrument   │
                    │      Run plate check        │
                    └─────────────────────────────┘
                                   │
                    ┌─────────────────────────────┐
                    │     Cancel plate check      │
                    │  Add buffer, Start pre-run  │
                    └─────────────────────────────┘
                                   │
  ╭──────────╮                ╭─────────────────╮
  │ Pre-run  │                │  Resuspend DNA  │
  │ ~5 min   │                ╰─────────────────╯
  ╰──────────╯                     │
                    ┌─────────────────────────────┐
                    │       Pause pre-run         │
                    │  Load first set of samples  │
  ╭──────────╮      │       Resume pre-run        │
  │Run samples│     └─────────────────────────────┘
  │ into gel │                    │
  │ ~2 min   │      ┌─────────────────────────────┐
  ╰──────────╯      │       Pause pre-run         │
                    │  Load second set of samples │
                    └─────────────────────────────┘
                                   │
                    ┌─────────────────────────────┐
                    │      Cancel pre-run         │
                    │        Start Run            │
                    └─────────────────────────────┘
                                   │
  ╭──────────╮                ╭─────────────────╮
  │   Run    │                │ Observe status  │
  │ ~7–16 hr │                ╰─────────────────╯
  ╰──────────╯                     │
                    ┌─────────────────────────────┐
                    │ Analyze and print (automatic)│
                    └─────────────────────────────┘
                                   │
                    ┌─────────────────────────────┐
                    │         Clean up            │
                    └─────────────────────────────┘
```

Figure 3-1. Flow chart for electrophoresing/collecting data on ABI PRISM 373 DNA Sequencer with XL Upgrade

---

Introduction

This section guides you through the process of electrophoresing your DNA samples and collecting the resulting sequence data on the ABI PRISM 373XL DNA Sequencer. Figure 3-1 shows the recommended flow of the process.

Although this section is detailed, it does not provide complete details for each procedure. Use it as a guide, and refer as necessary to the cross-referenced pages for more detail.

Note	*Even if you have previous experience using the Model 373, you should read through these steps. The data collection software has been streamlined. You no longer need to enter any commands on the instrument itself.*

The information in this section applies to Sequencing applications. Instrument setup consists of the same steps for either Sequencing or GeneScan applications. The only differences are in completing the Sample Sheet and Run file.

For further information about the analysis performed for each application, refer to the Sequencing and GeneScan analysis software manuals.

Before You Set Up the Instrument

Preparing Samples

Make sure you have prepared your samples as described in the *ABI PRISM DNA Sequencing Chemistry Guide*, or in the appropriate kit protocol. Before you set up your instrument, perform the following procedures:

- Purify and quantitate the template DNA.

- Complete the appropriate sequencing reactions with dye primer or dye terminator chemistry.

- Prepare a polyacrylamide gel.

It is very important that you purify the DNA thoroughly and have a sufficient quantity of DNA to get the best results. Optimal quantities for single-stranded and double-stranded templates are shown in the chemistry guides and protocol manuals for appropriate kits.

Setting up the Software

While the gel is polymerizing, you can set up the Sample Sheet and Run file for the data collection software. Refer to *Setting Up the Software* on page 3-16.

If you want automatic analysis after data collection, set up the conditions that control data analysis before you start a run. To do so, refer to the user's manual provided with your analysis software.

IMPORTANT   *If you choose automatic analysis and want the Sequencing Analysis software to use a setting other than the default, customize the analysis preferences before you start data collection.*

If you specify automatic analysis in the data collection program, the analysis program starts automatically at the end of the run to process the collected data.

Setting Up the Instrument

Pour your gel as described in Section 2, and allow it to polymerize for two hours before proceeding with this section.

IMPORTANT	*Make sure to use the appropriate gel concentration for the gel length and run type.*

Preparing the Gel for Loading

Note	*Wear clean laboratory gloves throughout this procedure both for your protection, and to avoid transferring fluorescent contaminants from your hands to the glass plates.*

Reagents and supplies needed for preparing the glass plates

- A single-edged razor blade or a second casting comb

- 1.5 L 1X Tris Borate EDTA buffer (2 L for 34-cm gels), pH 8.3 (from 10X TBE stock solution, Section 2)

- A clean, 10–50 mL syringe and 22 G needle

To prepare the plates for loading on the instrument:
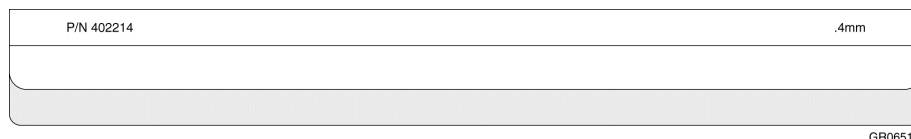
Figure 3-2. Casting comb

1. Remove the clamps on the gel casting (single-well) comb, and the tape from the edges of the plates.

2. Carefully remove the gel casting comb from between the plates. Remove any acrylamide in the well region with the razor blade.

Note   *To avoid cutting the comb, do not use the razor blade while the casting comb is in place.*

3. Rinse the glass plates with cold water.

    a. Rinse both sides and edges of the glass plates thoroughly with cold tap water to remove dried acrylamide and urea.

    b. Rinse the well and plates with dH_2O and allow the plates to air dry.

Note   *Wash the gel casting comb and allow it to air dry, so it will be ready when you pour the next gel.*

Preparing the Electrophoresis Chamber

WARNING   **ELECTRICAL SHOCK HAZARD. The Model 373 contains a high voltage power supply. This instrument has been designed with safety features in the door to disconnect the power supply when the door is open. Although it is designed for safe operation, please follow procedures as prescribed. As with any electrophoresis apparatus, care should be taken during instrument operation and when handling electrodes and liquids.**

WARNING   **ELECTRICAL SHOCK HAZARD. Wait at least 5 seconds after opening the door before touching the electrodes in the electrophoresis chamber. Capacitors hold an electrical charge even after the power supply is disconnected.**

Model-Specific Notation

This manual applies to three configurations of Model 373 DNA Sequencers:

- **Classic.** This configuration includes 370s with Macintosh upgrade and the 373A. Classic models have electrophoresis chambers which support 24-cm WTR distances.

- **Leon.** This configuration has an electrophoresis chamber which supports 6-, 12-, and 24-cm WTR distances.

- **Stretch.** This configuration supports 6-, 12-, 24-, 34-, and 48-cm WTR distances and is easily identified by its extended door.

Note   *All three instrument configurations must have five-filter wheel capability.*

Most of the information in this manual applies to all three configurations, however, some sections of the manual apply to only one or two of these configurations. These sections are preceded by the name of the configuration (Classic, Leon, or Stretch) to which they apply.

To prepare the electrophoresis chamber:

Classic 1. Open the door to the Model 373 and make sure the heating plate is dry.

2. Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.

Upper buffer chamber

Glass plates

Beam-stop bar

Lower buffer chamber

Model 373 (Classic), side view
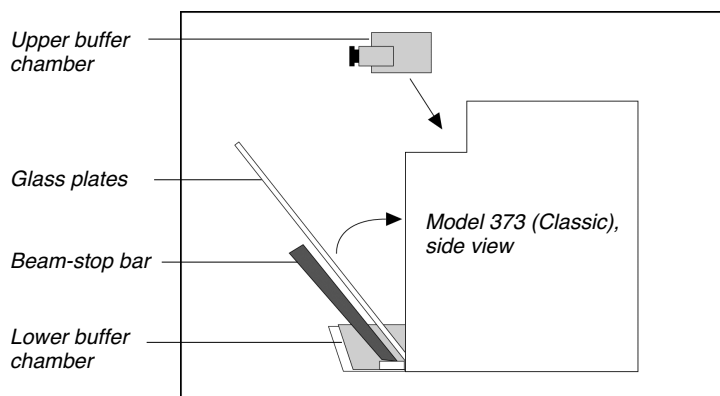
Figure 3-3. Placement of the Buffer Chambers, glass plates, and beam-stop bar in the electrophoresis chamber of the Model 373 (Classic)

3. Pull the black beam-stop bar in the electrophoresis chamber toward you until it stops (Figure 3-3). Rest the bottom edge of the plates on the notched supports in the bottom of the lower buffer chamber, with the notched plate facing away from you. Stand the plates in a vertical position. Press them firmly against the heating plate, and then push the beam-stop bar up against the plates. Center the plates in relation to the beam-stop bar, and then lock the beam stop bar in place by sliding the latches down along the outer edges.

IMPORTANT *Center the plates to ensure that each sample migrates within the read region of the laser.*

Note *You can remove the beam-stop bar. In normal operation you do not need to remove it. To re-install the beam-stop bar, line up the alignment pins in the alignment grooves and slide the beam-stop bar into place.*

Leon

To prepare the electrophoresis chamber:

1.   Open the door to the Model 373.

Upper buffer chamber support

Heat plate reset

Beam-stop bar

Beam-stop swivel clamps

Glass plate support

Lower buffer chamber
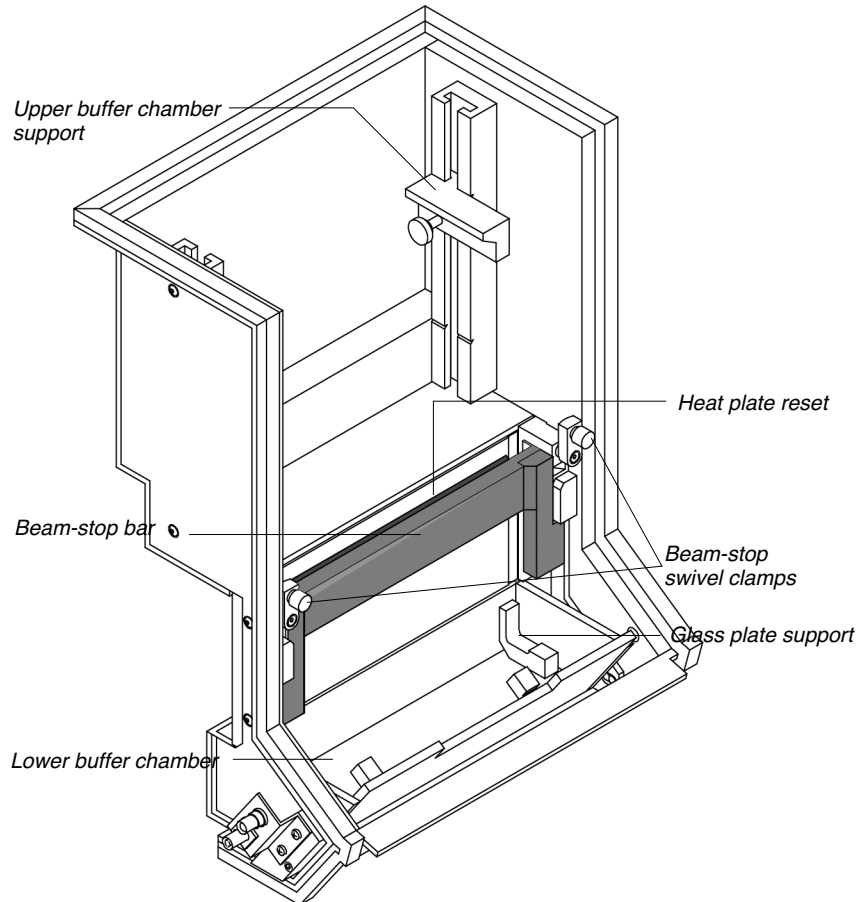
Figure 3-4. Model 373 (Leon) Electrophoresis Chamber

2.   Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.

3. Adjust the upper buffer chamber supports to 24 cm. Loosen the screws and slide the brackets to the correct position as indicated by the well-to-read length printed on the supports, and then tighten the screws.

Note   *You must use an acrylamide gel with a 24-cm well-to-read distance for sequencing on the Model 373.*

4. Place the glass plates into the instrument as follows:

   a. Pull the black beam-stop bar in the electrophoresis chamber towards you until it stops (Figure 3-4 on page 3-10).

   b. Rest the bottom edge of the plates on the notched supports in the bottom of the lower buffer chamber, with the notched plate facing away from you.

   c. Stand the plates up in the vertical position. Press them firmly against the back of the chamber (avoid touching the laser read region, and then push the beam-stop bar up against the plates.

   d. Center the plates with relation to the beam-stop bar and then latch the bar in place.

To Set Up the Electrophoresis Chamber:

Stretch   1.   Open the door to the Model 373. The electrophoresis chamber is shown in Figure 3-5.

48-cm WTR riser shelf

34-cm WTR

24-cm WTR

Upper buffer chamber supports and shelf

Beam-stop bar

Gel plate rests (ABS block)

Lower buffer chamber
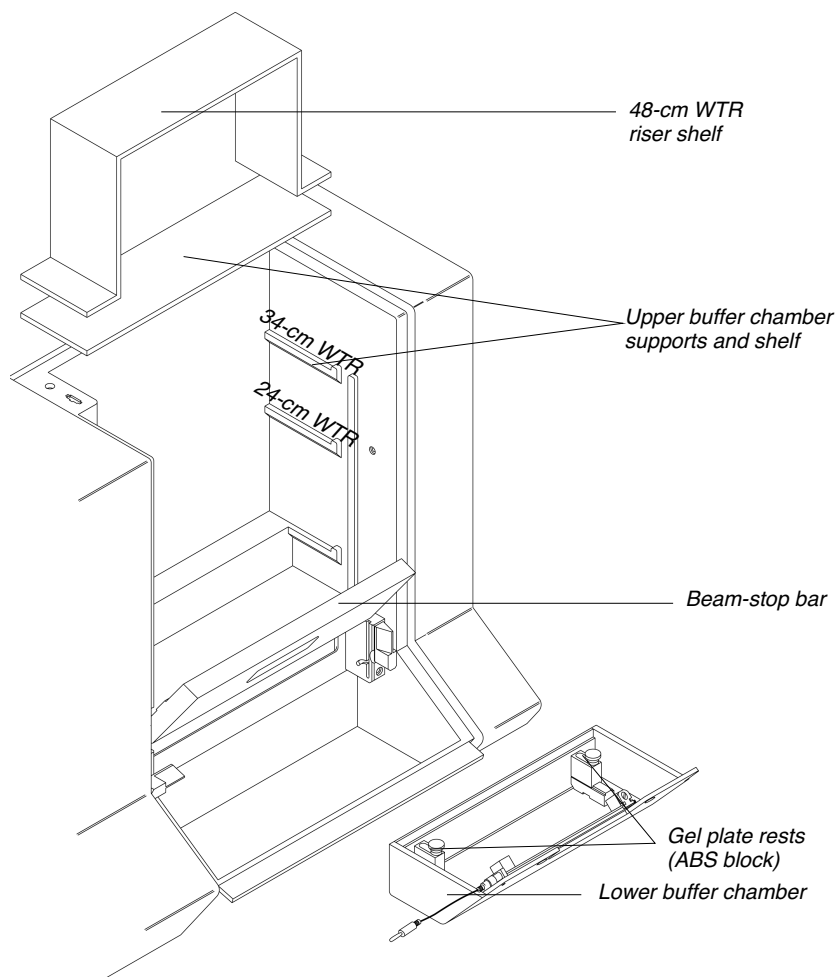
Figure 3-5. Model 373 (Stretch) Electrophoresis Chamber

2.   *For 24- or 34-cm gel:* place the straight white upper buffer chamber shelf on the appropriate support on the inside of the electrophoresis chamber (see Figure 3-5).

For 48-cm gel: Place the straight white plastic upper chamber shelf on the 34-cm support. Place the 48-cm WTR extension shelf on top of the straight shelf. The upper buffer chamber is placed on this shelf.

3. Slide the heat plate into place, as follows:

   a. Choose the correct heat plate for the glass plates you are using.

   b. Make sure the electrical wires for the temperature sensor are at the top, with the plate clamps toward the front. Align the grooves on either side of the heat plate with the heat plate guides on the chamber.

   c. Slide the heat plate down until it rests on the bottom of the chamber.

   d. Plug the temperature sensor on the heat plate into the socket at the top left of the chamber.

   e. Plug the heat plate ground into the socket at the top right of the chamber.

IMPORTANT   *Make sure the heat plate ground is connected before continuing.*

---

4. Adjust the gel plate rests in the lower buffer chamber, if necessary (see Figure 3-6). The gel plates rests are located in the bottom of the buffer chamber and serve to elevate the glass plates. The 34-cm plates must sit higher in the buffer chamber to rest in the correct position. All other plates rest in the lower position.

   Each gel plate rest consists of several parts, a large ABS block on the bottom, a shorter ABS block on top of it, and an adjustable thumb screw. When the thumb screw is loose, the small block can be moved forward and back, so that the plates rest either on one block, or on two blocks (see Figure 3-6).

   a. Loosen the thumb screws.

   b. Slide the blocks toward the back of the chamber if you are using 34-cm plates, so the plates rest in the *high* position. Slide the blocks toward the front of the chamber for all other gel lengths so the plates rest in the *low* position.

   c. Tighten the thumb screws.

Thumb screw

a. For all run lengths except 34 cm. *With the movable blocks toward the front of the electrophoresis chamber, the glass plates rest lower in the buffer chamber.*

Front of electrophoresis chamber

Glass plates rest here

Thumb screw

b. For 34-cm runs only. *With the movable blocks toward the back of the electrophoresis chamber, the glass plates rest higher in the buffer chamber.*

Front of electrophoresis chamber
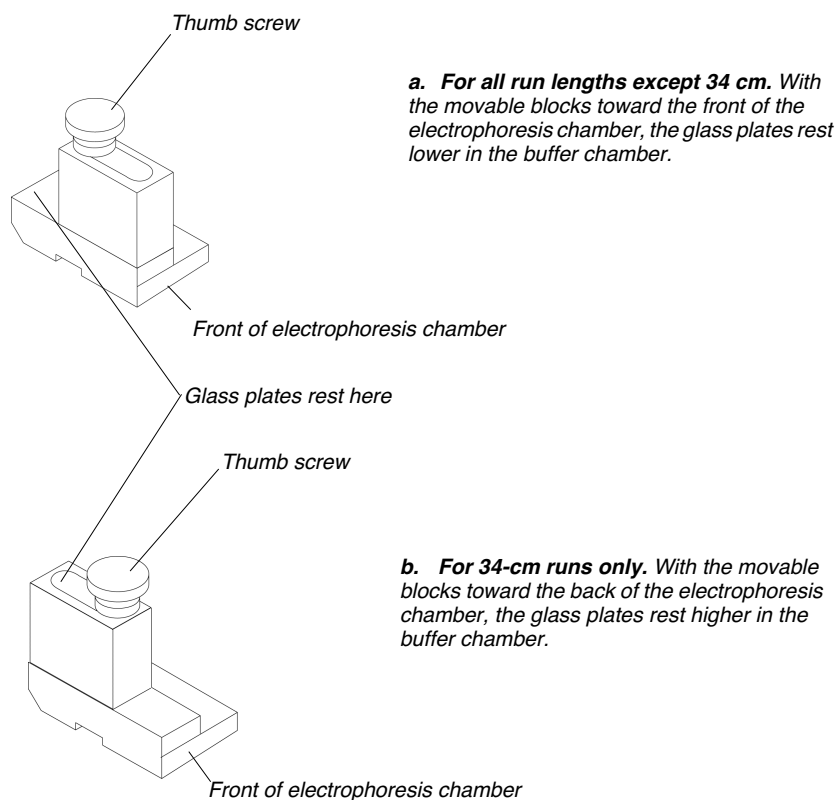
Figure 3-6. Adjustable gel plate rests

5. Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.

6. Unfasten the latches on either side of the beam-stop bar and swing the beam-stop bar forward, toward you.

7. Place the glass plates in the electrophoresis chamber in front of the heat plate and read window, with the bottom of the plates resting on the gel plate rests.

a. Center the glass plates carefully, or the beam-stop bar will not fasten.

b. Press the glass plates against the heat plate and close the beam-stop bar.

c. Fasten the latches at either side of the beam-stop bar.

IMPORTANT   *Do not touch the gel plates within the read region.*

Automatic Analysis

If you want automatic analysis after data collection, set up the conditions that control data analysis before you start a run. To do so, refer to the user's manual provided with your analysis software.

IMPORTANT   *If you choose Automatic Analysis and want the Sequencing Analysis software to use settings other than the default, customize the analysis preferences before you start data collection. Define all preferences from the Edit pulldown menu.*

If you specify automatic analysis in the data collection program, the analysis program starts automatically at the end of the run to process the collected data.

Setting Up the Software

Before starting electrophoresis, ensure there is sufficient space on the hard disk and restart the Macintosh. After deleting files, you must restart the Macintosh. You must also set up certain files in the data collection software:

- Set preferences
- Record sample information in the Sequence Sample Sheet
- Create a Sequence Run file

Note	*These tasks can be performed while you are waiting for the gel to polymerize.*

You can simplify creation of the files by setting preferences for certain parameters in each file.

The procedures described here do not include detailed descriptions of the data collection software. They are intended to give you a guide for steps you perform and the order in which to perform them. For specific information about the software and how to complete specific fields, refer to Section 5.

You may not need to change these default parameters for future runs, if you always run the same or similar parameters. However, it is important to verify these defaults, since they determine the names and locations of your data.

For more detail, refer to the specific pages in Section 5.

Setting up the Analysis Software

If you want automatic analysis after data collection, set up the conditions that control data analysis before you start a run. To do so, refer to the User's Manual provided with your analysis software.

IMPORTANT	*The default Base Caller used by the Sequencing Analysis software is SemiAdaptive. If you want to use the ABI BaseCallers or Adaptive Base Caller, change the analysis preferences before you start data collection.*

Starting the Data Collection Software

1. Choose Restart from the Special menu in the Finder to restart your Macintosh.

2. The data collection software should automatically open upon restarting the Macintosh. If it does not open, double-click the icon to open the program. Refer to *To make the data collection program open at startup:* on page 5-13 for instructions to open the collection program at startup.

Note   When you start the ABI PRISM 373 DNA Sequencer with XL Upgrade data collection program, the program searches for the current version of firmware on the Translation Interface Processor (TIP). If none is found, the program automatically copies the firmware image file (stored on the Macintosh) to the TIP. If the TIP's version of firmware is different from the collection software version, a dialog box appears, allowing you to continue or send a new firmware to the TIP. Click Send New Firmware to update your firmware version.

Creating User-Specific Module Files

The ABI PRISM 373 DNA Sequencer with XL Upgrade is shipped with module file templates for plate check, prerun, sequencing, and GeneScan runs. Table 3-1 shows the default parameters for the module file templates.

IMPORTANT   The parameters in Table 3-1 apply only to 24-cm WTR Classic or Leon 6% acrylamide gels.

Table 3-1.   Module File Default Parameters

Module	Field (Volts)	Power (Watts)	Current (mAmps)	Laser Power (mWatts)	Run Time (Hours)
Plate Check	--	--	--	40	0.50
Pre Run	2500	30	40	40	0.33
Seq Run	2500	30	40	40	12
GS Run	2500	30	40	40	12

You must create user-specific module files the first time the instrument is used and for each different run condition. Once you modify a module file template, you can save it and use it each time a similar run is performed. Refer to Table 1-2 on page 1-14 and Appendix B for suggested module file and Run parameters.

To modify a module file template:

1. Choose New from the File menu.

2. Click the Sequence Run or GeneScan icon in the window that appears.

3. Choose the module file you wish to modify from the Run Module pop-up menu.

Note   *You do not need to complete the other information in the Run window to modify a module file.*

---

4. Click the small document icon next to the Run module pop-up menu.


```
                        Seq Run

  Electrophoresis Voltage    2500  V      Collection Time    12.0  Hours

  Electrophoresis Current    40.0  mA     Scan Delay            0  Hours

  Electrophoresis Power        32  W      Laser Power        40.0  mW

  Filter Set            ⦿ A  ○ B

      Save As Default          Cancel   Save Copy in...    Save
```
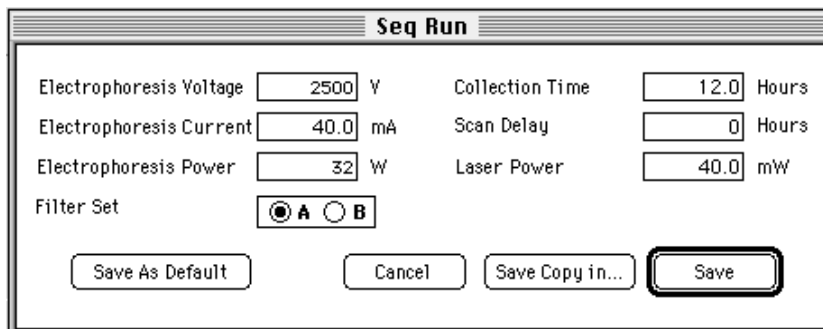
Figure 3-7. Module settings dialog box

5. Type a new number in the applicable entry fields. Refer to Table 1-2 on page 1-14 for recommended run parameters.

6. Save the changes.

   • *Save as Default:* To save the changed parameters as defaults for the module without changing the module name, click Save as Default. The

new settings are permanently saved and the name of the module file remains unchanged.

- *Save Copy in…:* To save the changed parameters as defaults, click Save Copy in. Type in a new name for the module file and save the file in the Modules Folder. The new settings are permanently saved and the name of the module file is changed.

Note	You will not be able to select your new module file in the pop-up menu until you save and close the Run window, then reopen the saved Run file or create a new Run file.

- *Save:* To use the changes as a one-time override for the current run, click Save.

Note	Custom module files are stored on your hard disk and can be used for any subsequent run. If you reload your collection software, however, you must re-create your custom modules.

Setting Default Parameters (Preferences)

You might only need to set default parameters prior to the first run. However, if more than one person uses the instrument, we recommend you set or verify all preferences before starting the run. If you are satisfied with the defaults stored in the instrument, skip to page 3-21 to create a Sample Sheet and a Run file.

You can set several types of preferred defaults:

- *Folder locations:* The ABI PRISM 373XL data collection software uses specified folders for data storage. This option allows you to specify the folders it uses. For information about changing folder locations, refer to *To change one of the data storage folders:* on page 5-17.

- *Default File names:* The data collection program automatically names new files and folders created during a run. You can specify the prefix and suffix of each type of file (see *Setting File Name Preferences* on page 5-18).

- *Sequence Sample Sheet defaults:* You can set defaults for certain parameters on the Sample Sheet. Since the Sample Sheets differ for Sequence Analysis

and GeneScan (fragment) Analysis, the parameters for which you can set defaults also differ. To set defaults for the sequence Sample Sheet, refer to *To set Sample Sheet defaults for Sequencing Analysis applications:* on page 5-21 .

- *Sequence Run defaults:* You can also set defaults for certain parameters in the Run file. As with the Sample Sheet defaults, separate defaults apply for sequence runs and GeneScan analysis runs. To set defaults for sequence Run files, refer to *Setting Defaults for Sequencing Analysis Applications* on page 5-21.

- *General settings:* The ABI PRISM 373XL instrument is normally attached to the Modem port of the Macintosh computer. If you need to change the connection, you can specify the change using this preference choice. Also use this choice to reset the global serial number (refer to *General Settings* on page 5-27). To change general settings, refer to *To change the general settings:* on page 5-28.

- *Dye indicators:* Each of the four dyes used during a run has a preset code and color. You can change the color assigned to each dye, and change the color that appears in the plot when it is printed. To do so, refer to *To set dye indicator preferences for the analysis applications:* on page 5-29.

To set preferences:

1. Choose Preferences from the Window menu and choose the appropriate option from the submenu that appears.
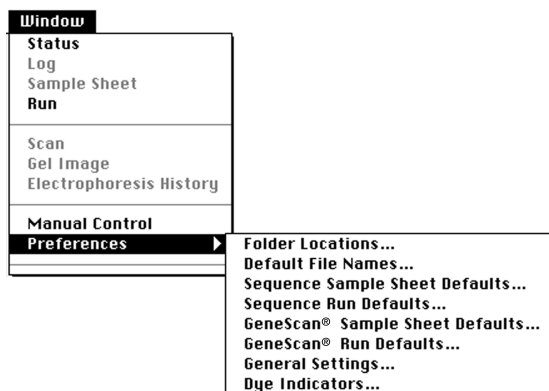
Figure 3-8. Preferences submenu

2. Enter or change the defaults, as described on the pages noted above.

3. Click OK to accept the changes.

Creating the Sample Sheet

Before starting a run you must record sample information in the Sequence Sample Sheet and the Sequence Run file. Once you have set up the Sample Sheet, you can automatically import the Sample Sheet information into the Run file, which associates sample information (name and type of analysis) with each lane position in the gel.

Separate Sample Sheets exist for sequencing and GeneScan analysis applications (see Figure 3-9). Be sure to choose Sequence Sample Sheet.

If a Sample Sheet containing the correct information already exists, you can use it. In most cases, you need to create a new Sample Sheet for each run. You can enter the information directly or export information from a database in tab-delimited format and import it into the Sample Sheet.

To create a Sample Sheet:

IMPORTANT	Although the Sample Sheet has 72 lanes available, fill in the sample information only for the number of lanes you are using.
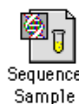
1. Choose New (⌘-N) from the File menu.

2. Click the Sequence Sample Sheet icon in the window that appears.

Sequence Sample

IMPORTANT	Do not mix sequence analysis and GeneScan analysis samples on the same Sample Sheet or the same Run.

The Sample Sheet is similar to a spreadsheet or ledger. It accepts more text than fits in the visible fields and automatically scrolls as you type your entry. To view the beginning of a long entry, use the keyboard arrow keys. Many of the cells have default values that are automatically filled in for each sample.

3. Enter the necessary information. For details about completing the Sample Sheets (including information about importing from a database), refer to *Creating and Editing a Sample Sheet* on page 5-32.

4. When you are finished, choose Save from the File menu.

   The dialog box that appears shows the default file name and storage location as defined in the File Names and Folders locations preferences, respectively. You can change the file name if you wish.

5. Click Save.

6. Choose Close from the File menu, or click the close box.

Click a title to select the entire column

Click an arrow to display a pop-up menu of options

Enter Sample name here.

When you set defaults for mobility and matrix files, the columns are filled in automatically
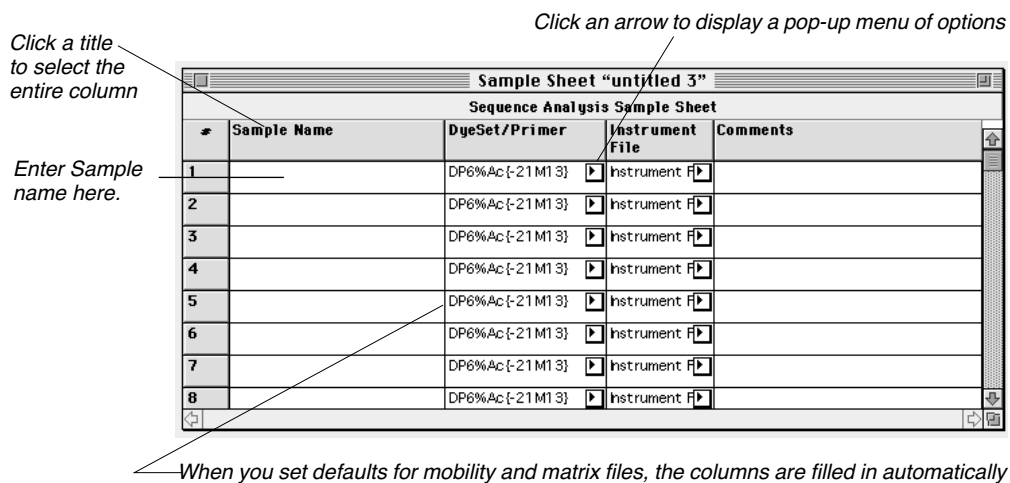
Figure 3-9. Blank Sequence Sample Sheet

Creating a Run File

In the Run file, you specify the lanes in which the samples will run and parameters to be used for both the pre-run and the run. If a Run file exists that contains the proper parameters, you can copy it to a new folder and use it. In most cases you need to create a Run file for each new run.

Separate Run windows exist for sequencing and GeneScan analysis applications. They are very similar; the GeneScan analysis version has an additional (Analysis Settings) column. Be sure to choose the correct type for your application.

To create a Run file:

1. Choose New from the File menu.

2. Click the sequence Run icon in the window that appears.

Small document icons open the selected module or Sample Sheet for review

Specify length of run

Run control buttons

Pop-up menus in these panels allow you to choose the modules (parameters), the associated Sample Sheet, the number of lanes, the separation distance (WTR), and the instrument file for the run

Lane numbers cannot be edited

Select filter set
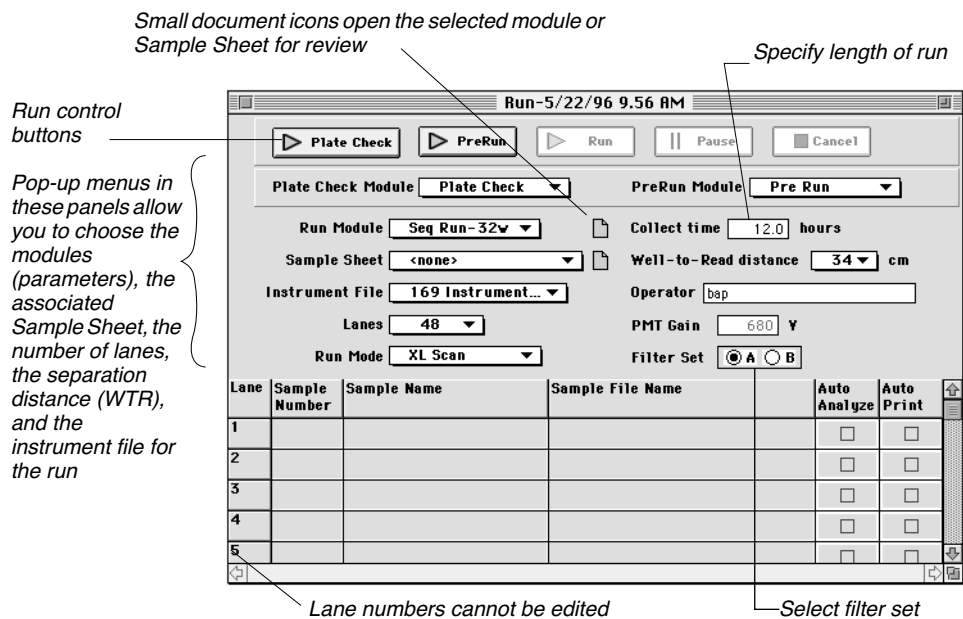
Figure 3-10. Sequence Run window

3. Choose the parameters for the run from the pop-up menus.

| *Note* | *Any changes made in your preferences will not take effect until you open a new Run file.* |

a. Choose modules to be used for the plate check, the pre-run and the run in the Module pop-up menus.

b. Choose the Sample Sheet from the Sample Sheet pop-up menu.

    When you choose the Sample Sheet, the information about the lanes in which the samples are to run is imported automatically.

IMPORTANT *If you select number of lanes less than the number of lanes you filled out in the in your Sample Sheet, the information will be truncated. To fix this:*
1. Select correct number of lanes and Run mode.
2. Select <none> on the Sample Sheet pop-up menu and reselect the Sample Sheet you want to use.

---

  c. Choose an instrument file from the Instrument File pop-up menu.

    Appendix C describes Sequencing Instrument Files.

  d. Choose the number of lanes and the separation distance from the lanes pop-up menu and the WTR pop-up menu, respectively.

  e. Choose XL Scan, Full Scan, or BaseSprinter in the Run Mode pop-up menu. Use the table below to determine the appropriate run mode for the lane configuration you are using.

Lanes	Comb	Run Mode
18	Sharks-tooth	BaseSprinter
24	Sharks-tooth	Full Scan
32	Sharks-tooth	Full Scan
36	Sharks-tooth	Full Scan
48	Sharks-tooth	XL Scan
64	Sharks-tooth	XL Scan

Note *If you select a run mode which is incompatible with the number of lanes selected in Step d, the lane pop-up menu will display <none>.*

---

4. Enter the length of the run in the Collect Time entry field. Following are some suggested run times.

Plate Length (cm)	Type of Run	Typical Collection Time (hr)
24	XLScan or Full Scan	12
24	BaseSprinter	6.5
34	XLScan or Full Scan	12
34	BaseSprinter	7
48	XLScan or Full Scan	16

5. Enter your name in the Operator entry field.

As noted, the sample information for each lane is entered automatically in the grid at the bottom of the Run window when you choose a Sample Sheet.

Note   *You cannot edit the Sample Sheet information in the Run window. Make any changes in the Sample Sheet, save the changes, select <none> and then select the Sample Sheet again in the Run window to make the changes effective.*

6. Choose the appropriate filter set:

    For Sequenase T7 Terminator chemistry: Select filter set B.

    For all other sequencing chemistries: Select filter set A.

IMPORTANT   *Failure to select the correct filter set will result in the loss of data.*

7. Select the Auto Analyze and Auto Print checkboxes if you want the analysis program to analyze and print the data.

When you finish completing the information in the Run file, do not close the Run file window. You can use it to perform a plate check, as described in the next section.

The options available to you at this time are:

• Save the file for future use, then click the close box to close it.

- Start a plate check by clicking the Plate Check button.

To save the file, choose Save from the File menu. To perform a plate check, see page 3-27.

For more information about creating the Run file, refer to page 5-40.

Checking the Plates

Scan the gel and plates before adding buffer and loading samples, to ensure that no peaks are produced by fluorescent particles on the glass plates or in the gel.

Click Plate Check after you choose the Plate Check module
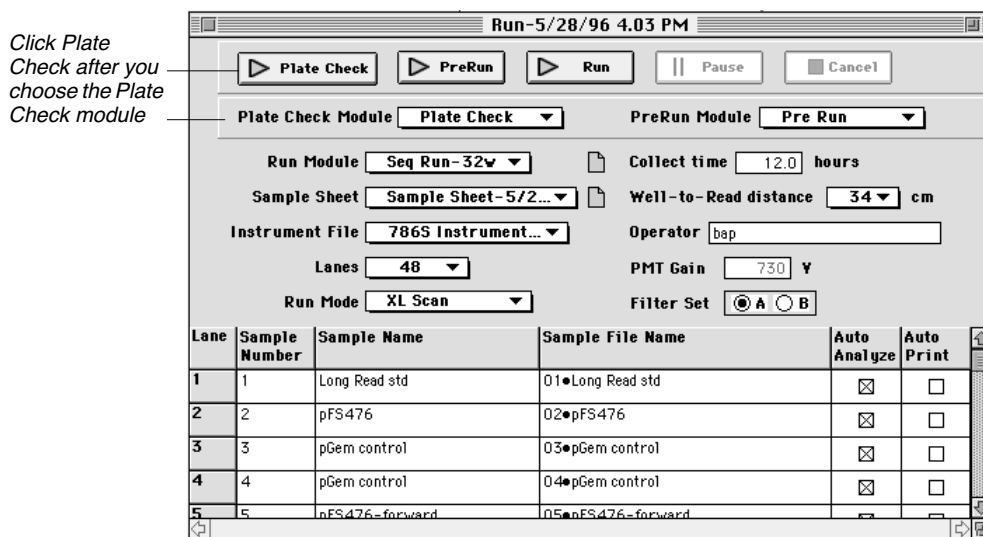
Figure 3-11. Completed Sequencing Run window

To check the plates:

1. Click Plate Check in the Run window.

Note	If a dialog box appears indicating you do not have enough room on your hard disk to save the files created by a run, delete files (particularly Gel files) to create enough space, making sure to back them up on a floppy disk or other storage device first, then click Plate Check again.

The laser scans the plates without electrophoresis to detect any unwanted fluorescent material in the read region.

2. Observe the Scan window that appears.

The Scan window should show a relatively flat line across the screen in each of the four colors. Typically the lowest line is blue, followed by the green, yellow (black), and red lines. If the scan line is flat, then the plates are clean. Proceed to *Check the PMT Gain Setting* on page 3-32.

If you see peaks in the Scan window, clean the glass plates again, as described below.

WARNING   LASER HAZARD. Do not look directly into the laser beam or allow a reflection of the beam to enter your eyes. Exposure to direct or reflected laser light at 40 mW for 0.1 seconds can burn the retina of the eye and leave a permanent blind spot.

Cleaning the Plates or Skipping Lanes

Peaks in the scan lines could indicate dirt on the glass or contaminating fluorescence in the gel. To eliminate dirt on the glass, clean the plates and scan them again. If peaks still appear and you wish to use the gel, avoid loading samples in the lanes where the peaks appear.

To clean the plates and re-scan:

1. Click Pause to pause the plate check.

2. Open the door of the instrument and unlatch the beam stop bar and remove the plates.

3. Carefully re-clean both sides of the glass in the laser read region with dH_2O and a lint-free tissue or ultra-pure pressurized air.

4. Replace the plates, center, and latch the beam stop bar in the electrophoresis chamber.

5. Click Resume and check the plates again.

If the peaks do not disappear after cleaning the glass, the gel mixture or buffer might contain particles. If you wish to use the gel, avoid loading samples in the lanes where the peaks appear.

Note	Occasionally, peaks in the scan window are caused by a dirty beam stop bar, rather than by dust or particulates in the gel. Clean the beam stop bar with dH_2O and (optionally) a thin layer of oil, if the peaks do not disappear after cleaning the glass.

Skipping Lanes

If, even after cleaning, a plate check shows peaks in some lanes of a gel, use Table 3-2 to determine which lanes are contaminated. Do not load samples in those lanes.

Table 3-2 shows scan numbers that correspond to the center of each lane for five different sharks-tooth combs. The exact positions of the channels may vary slightly from instrument to instrument, and with each comb placement.

If the contaminant peaks are close to the edge of a lane, you may wish to skip two lanes to ensure that there is no contamination in the lanes you load.

Note	Table 3-2 is based on the premise that all lanes run straight and do not deviate from the "expected" path, so channel/lane center may be an approximation, depending on the quality of your gel.

Table 3-2. Channel Numbers at Center of Each Lane for Sharks-tooth Combs

24-well comb		32-well comb		36-well comb		48-well comb		64-well comb	
Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel
1	7	1	5	1	4	1	7	1	5
2	15	2	11	2	9	2	15	2	11
3	23	3	17	3	14	3	23	3	17
4	30	4	23	4	20	4	30	4	23
5	38	5	29	5	25	5	38	5	25
6	46	6	35	6	30	6	46	6	35
7	54	7	41	7	36	7	54	7	41
8	62	8	47	8	41	8	62	8	47
9	69	9	53	9	46	9	69	9	53
10	77	10	58	10	52	10	77	10	58
11	85	11	64	11	57	11	85	11	64
12	93	12	70	12	62	12	93	12	70

Table 3-2. Channel Numbers at Center of Each Lane for Sharks-tooth Combs (continued)

24-well comb		32-well comb		36-well comb		48-well comb		64-well comb	
Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel
13	101	13	76	13	68	13	101	13	76
14	107	14	82	14	73	14	107	14	82
15	116	15	88	15	78	15	116	15	88
16	124	16	94	16	84	16	124	16	94
17	132	17	100	17	89	17	132	17	100
18	140	18	106	18	94	18	140	18	106
19	148	19	112	19	100	19	148	19	112
20	155	20	117	20	105	20	155	20	117
21	163	21	123	21	110	21	163	21	123
22	171	22	129	22	116	22	171	22	129
23	179	23	135	23	121	23	179	23	135
24	187	24	141	24	126	24	187	24	141
		25	147	25	132	25	195	25	147
		26	153	26	137	26	203	26	153
		27	159	27	142	27	211	27	159
		28	165	28	148	28	218	28	165
		29	171	29	153	29	226	29	171
		30	177	30	158	30	234	30	177
		31	183	31	164	31	242	31	183
		32	189	32	169	32	250	32	189
				33	174	33	257	33	194
				34	180	34	265	34	200
				35	185	35	273	35	206
				36	190	36	281	36	212
						37	289	37	218
						38	297	38	224
						39	306	39	230
						40	314	40	236
						41	322	41	242
						42	330	42	248
						43	338	43	258
						44	345	44	264
						45	353	45	264

Table 3-2. Channel Numbers at Center of Each Lane for Sharks-tooth Combs (continued)

24-well comb		32-well comb		36-well comb		48-well comb		64-well comb	
Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel	Lane	Channel
						46	361	46	270
						47	369	47	276
						48	377	48	282
								49	288
								50	294
								51	300
								52	305
								53	311
								54	317
								55	323
								56	329
								57	335
								58	341
								59	347
								60	353
								61	359
								62	365
								63	371
								64	377

Check the PMT Gain Setting

Verify Run Mode
The PMT gain setting differs for each of the run modes (XL Scan, Full Scan, and BaseSprinter). Verify that you have selected the correct run mode in the Run window before checking the PMT gain setting.

First Time Run
The PMT gain settings are stored in a Calibration file. If this is the first time the instrument is being used, you should make and send a Calibration file. Refer to Section 5 for instructions on how to make and send a Calibration file.

Recommended PMT Gain

The PMT gain should be set such that the lowest scan line (usually the blue line) is positioned at approximately 900 on the y-axis in the Scan window. For subsequent runs, the PMT gain will need to be adjusted only if the y-axis value is out of the range of 800–1000 on the y-axis. Different PMT gains are required for the three run modes (XL Scan, Full Scan, and BaseSprinter).

To check the PMT gain:

1. While the Plate Check is running, view the four base lines (each line represents one of the four filters) in the Scan window of the 373XL collection program.

2. Position the cursor so the tip of the arrow just touches a flat section of the lowest scan line (usually the blue line), as in Figure 3-12.

Figure 3-12. Cursor positioned correctly to measure PMT gain

3. Read the y-axis value displayed in the upper-left hand corner of the scan window.

   • If the lowest scan line is positioned between 800–1000 on the y-axis, cancel the Plate Check, and then proceed to *Inserting the Sharks-tooth Comb.*

   • If the lowest scan line is outside the recommended range, adjust the PMT gain.

To adjust the PMT gain:

1. While the Plate Check is running, choose Manual Control from the Window menu.

2. Adjust the size and position of the Scan and Manual Control windows so you can see both windows without any overlap.

3. In the Manual Control window, choose PMT Gain from the Fxn Name pop-up menu.

4. Type a new value, then click Execute.

Note   *If you enter a larger number than the one displayed, the lines in the Scan window will shift up; a smaller number causes them to shift down.*

5. Check the y-axis value in the Scan window. (First click on the Scan window to make it active.)

6. Repeat steps 3–5 until the PMT gain is within the recommended range.

7. Close the Manual Control window.

8. Cancel the Plate Check in the Run window.

Note   *If you select a different run mode, you must change the PMT gain.*

Inserting the Sharks-tooth Comb

IMPORTANT   *Because of tolerance issues, be sure that the combs, spacers, and casting comb you are using are compatible (made of the same material) and are the same thickness.*

1. Insert the sharks-tooth comb (see Figure 3-13).

    a. Center the sharks-tooth comb in the glass notch and carefully slide it between the glass plates. Make sure all of the points on the comb enter the space between the plates at the same time to avoid bending or breaking the teeth. Carefully align the center registration line with the registration mark on the plates.

    b. Slide the comb down until the tips of all the teeth just touch or slightly depress the surface of the gel. Do not deform the wells. Make sure the teeth just barely indent the well surface. All of the teeth must touch the surface of the gel. If the surface of the gel is not completely flat in the loading region, some of the teeth may have to be inserted below the surface of the gel (up to 0.5 mm) so all of the teeth touch the gel surface. If a tooth has penetrated the gel surface, do not attempt to withdraw the comb or sample will leak into adjacent wells.

Figure 3-13. Sharks-tooth comb just touching the gel

Completing Instrument Setup

Classic

1. After performing a plate check, place the upper buffer chamber at the top of the gel plates by placing it on the upper shelf (Figure 3-3 on page 3-9) and sliding the front brackets of the chamber over the glass plates. The brackets should sit over the spacers on the outside edges of the plate.

2. Clamp the glass plates to the upper buffer chamber by placing the sharks-tooth alignment brace (the rectangular acrylic brace) against the front of the glass plates and inside the brackets of the buffer chamber.

3. Tighten the screws on each end of the alignment brace equally on both sides to maintain even pressure and to ensure that the brace does not slip up at one end. Turn the screws until you see the gasket compress completely against the glass. Finger-tighten only—do not overtighten. You may need to apply light pressure on the top of the brace to prevent it from moving during tightening.

Note   *The main function of the alignment brace is to maintain a firm, even pressure on the sharks-tooth comb with the glass plates, to prevent leakage of the sample around the teeth.*

---

4. Fill the upper buffer chamber with the 1X TBE buffer prepared earlier to at least half-way up the notched part of the plate (~ 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

Note   *If there is a leak, tighten the brackets slightly. If the leak continues, siphon the upper buffer, then clean the gasket area on the chamber and the area of the plate that contacts the gasket. Reassemble as before.*

---

5. Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full.

Note   *Avoid splashing buffer against the back of the chamber since it will spill over into the liner. Remove any bubbles that are under the bottom edge of the gel plates using a clean needle and syringe. Bend the needle at an angle to guide the bubbles out.*

---

6. Attach the top and bottom electrode leads located on the left side of the electrophoresis chamber and close the door gently.

Leon 1. After performing a plate check, slide the heat plate into place against the glass plates, as follows (see Figure 3-4 on page 3-10):

   a. Rest the lower edge of the heat plate (the edge with the supports protruding on both sides) on the upper inside ledge of the beam-stop bar where it meets the glass plate. The supports rest in the notches.

   b. Stand the heat plate vertically against the glass plates.

   c. Secure the beam-stop swivel clamps.

2. Place the upper buffer chamber at the top of the gel plates, on the supports. Slide the clamps over the plates.

3. Put the alignment brace in place against the front of the glass plates, inside the brackets of the buffer chamber, and resting on top of the heat plate.

   The alignment brace is made of highly polished acrylic so the numbers and the comb are readily visible through the plastic. Do not mark the alignment brace with felt-tipped pens, as the use of most organic solvents to remove the ink will dull the surface of the brace and reduce its clarity. Some ink may also fluoresce.

4. Tighten the screws on both ends of the upper buffer chamber equally to maintain even pressure across the alignment brace, and to ensure that the brace does not slip up at one end. Turn the screws just until you see the gasket compress completely against the glass—do not overtighten. You may need to apply light pressure on the top of the brace to prevent it from moving during tightening.

   The main function of the alignment brace is to maintain a firm, even pressure on the sharks-tooth comb with the glass plates to prevent leakage of the sample around the teeth.

5. Fill the upper buffer chamber with the 1X TBE buffer prepared earlier to at least 2/3 up the notched part of the plate (greater than 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

If there is a leak, slightly tighten the brackets. If the leak continues, siphon the buffer out of the upper buffer chamber and clean the gasket area on the chamber. Also, clean the area of the plate that contacts the gasket. Reassemble as before.

6.   Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full. Avoid splashing buffer against the back of the chamber because it will spill over into the liner. Bubbles should be removed if they are under the bottom edge of the gel plates. This can be done with a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

7.   Attach the top and bottom electrode leads located on the left side of the electrophoresis chamber and gently close the door.

Stretch

1.   After performing a plate check, place the upper buffer chamber at the top of the gel plates, on the white plastic shelf. Slide the clamps over the plates but do not tighten the clamps. To ensure a tight seal between the buffer chamber and the glass plates, make sure the gasket on the upper buffer chamber is dry.

IMPORTANT   *If you have two different upper buffer chambers, use the larger of the two for 34-cm and 48-cm runs. Use the small upper buffer chamber for 24-cm runs.*

2.   Slide the plain acrylic alignment brace over the comb (the one without the temperature sensor), between the upper buffer chamber clamps and the glass plates. Finger-tighten the clamps; be careful not to overtighten.

The alignment brace is made of highly polished plastic so the numbers and the comb are readily visible through the clear plastic. Do not mark the alignment brace or glass plates with felt-tipped pens, as the use of most organic solvents to remove the ink will dull the surface reduce its clarity. Some ink may also fluoresce.

3.   Plug the electrode lead on the upper buffer chamber into one of the two jacks. For 34-cm and 48-cm gels, use the top (rear) socket, and for all other gels, use the lower (front) socket.

4. Fill the upper buffer chamber to at least two-thirds up the notched part of the plate (greater than 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

   If there is a leak, slightly tighten the brackets. If the leak continues, siphon the buffer out of the upper buffer chamber and clean the gasket area on the chamber. Also, clean the area of the plate that contacts the gasket. Reassemble as before.

5. Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full. For 34-cm runs, fill the lower buffer chamber until the bottom of the glass plates is covered. Avoid splashing buffer against the back of the chamber because it will spill over into the liner. Bubbles should be removed if they are under the bottom edge of the gel plates. This can be done with a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

IMPORTANT   *The buffer must completely cover the bottom of the gel plates. You will need to prepare 2 L of buffer for 34-cm and 48-cm gels. For all other lengths, the standard volume of 1.5 L is sufficient.*

---

6. Attach bottom electrode lead located on the left side of the electrophoresis chamber.

Preparing and Loading Samples

Pre-running the Gel

Pre-run the gel for 5 minutes to make sure all connections and components of the electrophoresis system are working properly.

While the pre-run is in progress, resuspend the DNA in preparation for loading.

To pre-run the gel:

1. Flush all of the wells with running buffer. Direct the 22 G needle of a syringe filled with running buffer at the space between adjacent comb teeth, and gently flush the well.

    Remove any bubbles under the bottom edge of the gel plates in the lower buffer chamber. Use a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

2. If you have not done so, choose the PreRun module from the PreRun pop-up menu.

3. Click the PreRun button in the Run window.

    The instrument performs electrophoresis without collecting data during a pre-run.

4. Pre-run the gel for 5 minutes.

5. Click Cancel in the Run window.

Resuspending the DNA

Resuspend the DNA in loading buffer, as described below. After the pre-run (about 5 minutes), load the sample and start the run. When using 48- and 64-well combs, you can obtain optimum accuracy by loading 1/4 to 1/8 of the total resuspension volume, e.g., resuspend your dry sample in 8–12 µL loading buffer and load only 1.5 µL.

IMPORTANT	*Do not overload your lanes. Both volume and quantity excess lead to a decrease in base calling accuracy. Best results are obtained when the average signal strengths for G, A, T, and C are all below 500 fluorescent units/base. More signal is no guarantee of better results; with 48 and 64 wells, it actually translates to less accuracy. You may need to optimize what volume in this range is best for you.*

WARNING	**CHEMICAL HAZARD. Formamide is a known teratogen (can cause birth defects). Before using formamide, read the MSDS found in the Safety Summary provided previously. Wear protective eyewear, gloves, and safety clothing when working with formamide. Wash thoroughly after handling formamide.**

To resuspend the DNA:

1. Resuspend your DNA in loading buffer (5:1 deionized formamide: 25–50 mM EDTA with 25–50 mg/mL Blue Dextran).

   Use the following table to determine the appropriate resuspension and loading volumes for the comb you are using.

   Table 3-3. Sequencing Resuspension and Loading Volumes

	Comb Configuration (number of wells)				
	18	24	32/36	48	64
Resuspension volume	4 µL	4–6 µL	4 µL	8–10 µL	8–12 µL
Loading volume	4 µL	4–6 µL	4 µL	1.5–2 µL	1–1.5 µL

2. Vortex the samples and centrifuge briefly.

3. Heat samples at 90 °C for two minutes.

4. Immediately place samples on ice until ready to load.

5. Resuspend samples within 2 hours of loading.

Loading the Samples and Starting the Run

IMPORTANT	*Be sure you load samples in the lanes specified in the Run window. The tracker refers to the sample information to perform accurate lane tracking. The sample information in the Run window when you start the run is stored in the Gel file. Changes you make in the Run window after you start the run are not implemented.*

When you are loading many samples using a sharks-tooth comb and need to run them in adjacent lanes, it is important that you load samples in alternate wells and electrophorese the gel for 2 minutes, then rinse all the wells (step 1 on page 3-39) and load the rest of the samples in the remaining unused wells. The automatic lane tracker in the analysis software needs to have discrete spaces between the samples to identify the lanes properly. Loading samples in adjacent lanes can blur the definition between the lanes. Be sure to load strong (control) samples in the outside (left-most and right-most) lanes to orient the tracker during analysis. For best results, follow the procedure described below.

Note	*In the following procedures, each button in the Run window has a corresponding menu choice in the Instrument menu. You can either click the button in the Run window or choose the command from the Instrument menu.*

For 48- and 64-well gels, make sure to deliver the samples to the bottom of the wells. Use either a single- or multi-channel pipettor/syringe equipped with a flat tip less than 0.3 mm in diameter (we recommend 0.17 to 0.20 mm). Do not overload the lanes. Follow the recommendations in Table 3-3 for the allowable loading volumes. If you use too much sample volume or quantity, you will experience poor resolution.

To load samples:

1. If you are pre-running the gel, click Cancel in the Run window.

2. Carefully flush all of the wells with running buffer.

Tip	*Place formamide or loading buffer in the well to the left of your first sample lane, and in the well to the right of your last sample lane (the comb creates wells to either side of the numbered wells). The formamide in the buffer helps focus the bands in the first and last lanes.*

3. Immediately load the odd-numbered samples into each of the odd-numbered wells.

    If you are loading fewer than half the samples allowed by the comb, skip steps 6–10 and proceed to step 11. If you are loading a square-tooth comb, you can load the wells consecutively and skip steps 6–10 and proceed to step 11.

IMPORTANT	*Be sure to load strong (control) samples in the outside (left-most and right-most) lanes. Avoid skipping more than one lane between samples. It is better to leave extra empty lanes at the right end of the gel. The lane tracker may not work properly if there are many empty lanes between samples.*

4. Click Run in the Run window to start the run.

    A dialog box appears, allowing you to change the name and location of the gel file created by the run.

5. Click OK when you are satisfied with the name and location for the gel file.

Note	*If a dialog box appears indicating you do not have enough room on your hard disk to save the data generated by a run, delete files (particularly Gel files) to create enough space, making sure to back them up on a floppy disk or other storage device first, then click Run again.*

6. Electrophorese for two minutes so the entire sample enters the gel.

7. Click Pause in the Run window.

8. Carefully flush *all* of the wells with running buffer to remove any residual formamide from the previously loaded wells.

9. Immediately load the even-numbered wells.

10. Click Resume in the Run window.

11. Choose Status from the Window menu to verify that electrophoresis and scanning resume (refer to *The Status Window* on page 5-50).

   If either electrophoresis or scanning does not resume, cancel the run and restart it.

Figure 3-14 summarizes the buttons you click in the Run window for a typical run. They are listed from top to bottom in the order you use them.

Note   *The tasks described in Figure 3-14 are detailed in the preceding pages.*

Button to click	Module in effect	Task to perform
Plate Check	Plate Check module	Check plates and gel for fluorescence
Cancel	n/a	(Stops plate check scan) Add buffer and check for leaks Mount front heat-transfer plate—Leon only
PreRun	Pre-run module	(Electrophorese gel without data collection to equilibrate temperature)
Pause	Pre-run module	Load first set of samples during pause
Resume	Pre-run module	(Electrophorese samples into gel without data collection)
Pause	Pre-run module	Load second set of samples during pause
Cancel	n/a	(Stops pre-run)
Run	Run module	(Starts electrophoresis and data collection)

Figure 3-14. Run window buttons used during a typical run

Observing Instrument Status and Data

After you begin electrophoresis and data collection, data appears on the computer screen as it is collected. You can open up to four windows to view the data and the instrument status. Only one window can be active at any given time, but status windows still update when they are not active.

Note	*If they are displayed, status windows continually update during a run. They do so even when another application is running.*

Open the data collection and status windows by choosing the name of the window of interest from the Window menu.

Data Collection Status Windows

To observe instrument status, choose one of the following commands from the Window menu. The page reference next to a window name indicates where you can find detailed information about that window.

- Status (refer to page 5-50)

- Electrophoresis History (refer to page 5-57)

- Log (refer to page 5-51)

Note	*After the run is completed, you can choose Log from the Window menu to display a comprehensive record of all error and status messages generated during the run.*

Real-time Data Windows

The data displayed in the real-time data windows (the Scan window and the Gel window) represents light intensities collected by the data collection software from the filter wheel and the PMT (photomultiplier tube) in the instrument. The colors in the raw data windows represent the wavelengths of the dyes being detected, rather than the bases being detected. This is explained in *Filters and Color of Data Display* on page 5-52.

To observe the data as it is being generated, choose one of the following from the Window menu:

- Scan (refer to page 5-55)

- Gel (refer to page 5-56)

Cleaning Up After the Run

After your run is complete, perform the following general cleanup steps:

- Remove the gel from the instrument.
- Clean the plates, comb, and spacers.

Removing the Gel from the Instrument

Classic and Leon

1. Disconnect the electrode leads from both buffer chambers.

2. Unlatch the beam-stop bar and pull it forward.

3. Carefully remove the upper buffer chamber, glass plates, and heat plate (Leon only) as one unit. Discard the buffer.

   You can also set up a siphon system to drain the upper and lower buffer chambers before removing them.

4. Remove the lower buffer chamber and discard the buffer.

5. Clean up any liquid left in the electrophoresis chamber.

6. Unclamp the plates from the upper buffer chamber.

7. Rinse the buffer chambers with dH$_2$O and allow them to air dry.

Stretch

1. Disconnect the electrode leads from both buffer chambers.

2. Unlatch the beam-stop bar and pull it forward.

3. Carefully remove the upper buffer chamber and glass plates as one unit. Discard the buffer.

4. Remove some of the buffer from the lower buffer chamber before removing it.

   This is especially important for 34-cm runs, as the buffer level is very high. You can also set up a siphon system to drain the upper and lower buffer chambers before removing them.

5. Remove the lower buffer chamber and discard any remaining buffer.

6. Clean up any liquid left in the electrophoresis chamber.

7. If you plan to change plate length next time, unplug the temperature sensor, and then remove the heat plate, otherwise leave both in place.

8. Unclamp the plates from the upper buffer chamber.

Note   *If the unnotched plate has been silated with a bind-silane compound, use 0.1 N HCl or NaOH to remove the gel from the silated area, or scrape it off gently.*

9. Rinse the buffer chambers with dH$_2$O and allow them to air dry.

Cleaning the Plates, Comb, and Spacers

Caution   ***Avoid contacting the glass plates with metal. Scratches caused by metal objects, such as drying racks or spatulas, weaken the glass and reduce its usable life.***

1. Gently push a plastic wedge almost all the way in at the bottom of the plates (avoid the top notches) and pry the plates apart. Do not use too much force or the plates might chip. Remove the sharks-tooth comb and gel spacers.

2. Lay a large Kimwipe on the gel and roll it up, lifting the gel off the plate, and discard appropriately.

3. Rinse the plates with water. Rub with a gloved hand to dislodge remaining gel bits, then rinse again with water.

4. Wash with a detergent such as Alconox that will not leave a residue, rinse with dH$_2$O, and allow to dry.

5. Clean the comb and spacers and store them.

| Note | Do not expose combs or spacers to moisture any longer than is necessary to clean them. Store combs and spacers carefully to avoid adding blemishes. Even very small bumps or bends can ruin the combs and spacers. |

---

6. Wipe any spilled buffer off the heat plate.

Analyzing the Data

If you choose Auto Analyze for your samples in the Sequence Run window, the data collected on the ABI PRISM 373XL is automatically analyzed by the Sequencing Analysis program. For complete instructions on using the analysis program, refer to the *ABI PRISM DNA Sequencing Analysis Software User's Manual* that came with the software.

Automatic data analysis occurs approximately 6.5 to 16 hours after you start data collection (the amount of time depends on the module you chose). The analysis software prints electropherograms (if you selected AutoPrint in the Sample Sheet) and displays the gel image on the computer screen.

Color of Data Display and Output

The colors in the raw data windows represent the wavelengths of the dyes being detected, rather than the bases being detected (see *Filters and Color of Data Display* on page 5-52).

The analysis program converts the information collected by the data collection program so that after analysis the colors are the same for each base. The colors on all displays of analyzed data, including printed electropherograms, are as follows:

C = Blue   A = Green   G = Yellow   T = Red

Note	When printed and when shown against white on the screen, G is black, so it is easier to see.

Reading an Electropherogram

- You can view the electropherograms in the analysis program as Raw or Analyzed data (data is also printed in these formats). Raw and analyzed data in a Sample file correspond to data/scan points collected.

- Information from the Sample Sheet appears at the top of printed electropherograms.

- The colors of the peaks and the letters above the peaks correspond to the appropriate bases.

- An N above a peak means that the software could not confirm the identity of that base.

- In the analyzed data, the numbers above the colored peaks indicate analyzed base positions, counted from the first analyzed data point.

Archiving the Data

For best performance, keep as few files as possible on your hard disk. Do not archive gel files and run folders on your hard disk. It is important that you copy all files you want to save onto a backup medium before beginning another sequencing run, both to preserve the files and to free disk space.

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 Run Log file. After the run, the analysis program creates Sample and sequence (.Seq) files.

Run Files

Each Run file stores information associating 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 a disk.

Gel Files

Gel files contain the raw data acquired by the data collection program and typically take up 10–55MB of disk space. A separate Gel file is created in the Run folder each time you perform a run. Because Gel files take a substantial amount of space, you should delete them from your hard disk after you have obtained satisfactory Sample files. You do not usually need to keep gel files once the lane tracking is verified 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 any gel file until you have verified the tracking and taken corrective action if necessary.*

Run Log Files

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

Sample files

Use disks, a magnetic tape drive, a removable cartridge drive, or an optical drive to archive sample files. A 1.4 MB, high-density disk holds about six files. A Sample file is 150–200KB in size, depending on the length of the sequencing run; however, Sample files for 388 runs are twice as large. Save a Sample file when you feel confident that the channel selections (tracking) are correct for the sample.

.Seq files

.Seq files show the base letter sequence only (or might contain a header, depending on the format you choose). You can open .Seq files from word processing programs and print them. The Preferences dialog box in the Sequencing analysis program allows you to choose the file format before analysis.

Other Files

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

Sample Sheet Files

You can reuse the Sample Sheet, and it is a good reference after data collection. Individually, Sample Sheet files are small enough to archive on disks.

Custom Analysis Settings and Instrument Files

If you have created customized files, you should keep backup copies of the files on a separate medium. Each of these types of files alone is small enough to store on a disk.

4   Using the Instrument in GeneScan Mode

Contents

Prepare and label samples

↓

Pour gel

↓

Polymerize
~2 hr

Restart Macintosh
Set up software

↓

Load gel on instrument
Run plate check

↓

Cancel plate check
Add buffer,
Start pre-run

↓

Pre-run
~5 min

Mix sample with loading buffer size std
Heat sample

↓

Run samples
into gel
~ 2 min

Cancel Pre-run
Load samples

↓

Start Run

↓

Run
~ 3–8 hr

Observe status

↓

Analyze and print (automatic)

↓

Clean up

Figure 4-1. Flow chart for electrophoresing/collecting data on ABI PRISM 373 DNA Sequencer with XL Upgrade

Introduction

This section guides you through the process of electrophoresing your DNA samples and collecting the resulting GeneScan data on the ABI PRISM 373XL DNA Sequencer. Figure 4-1 shows the recommended flow of the process.

Although this section is detailed, it does not provide complete details for each procedure. Use it as a guide and refer, as necessary, to the cross-referenced pages for more detail.

Note	*If you have previous experience using the Model 373, you should read through these steps. Data collection software has been streamlined. You no longer need to enter any commands on the instrument itself.*

The information in this section applies to GeneScan applications. Instrument setup consists of similar steps for both Sequencing and GeneScan applications. The main differences are in completing the Sample Sheet and the Run file.

For further information about the analysis performed for each application, refer to the GeneScan Analysis software manual.

To read details about the data collection software and the instrument hardware refer to Sections 5, 6, and 7.

Before You Set Up the Instrument

Preparing Samples

Make sure you have prepared your samples as described in the *GeneScan 672 User's Manual*, or in the appropriate kit protocol. Before you set up your instrument, perform the following procedures:

- Purify and quantitate DNA samples.

- Label DNA fragments with fluorescent dyes.

- Prepare the appropriate gel.

Experimental Design Considerations

Fluorescent labeling enables you to analyze many independent fragments in the same lane using either color or fragment length to distinguish between fragments.

Some factors to consider when you design your experiments are:

- Working with overlapping allele size ranges

- Multiplexing the Polymerase Chain Reaction (PCR)

- Optimizing salt concentrations

- Sizing and quantifying sample fragments

Working with Overlapping Allele Size Ranges

If the expected size ranges of PCR products from different primer pairs overlap, you can do one of the following to differentiate between them:

- Label primer pairs with different dye colors

- Choose new primer sites to alter PCR product fragment lengths

- Load overlapping products in different gel lanes

For more information on labeling techniques, see the *GeneScan 672 Software User's Manual.*

Multiplexing the Polymerase Chain Reaction

You can multiplex the Polymerase Chain Reaction (PCR) by adding more than one pair of primers into the same reaction tube, and then performing the PCR. If you multiplex, you should first check primers for compatibility considerations, such as excessive regions of complementarity, or similar melting temperatures.

Once you have chosen compatible primer pairs, you should then test the pairs for successful co-amplification. Multiplexing often requires that you optimize reaction conditions, and sometimes requires that you redesign primers. For more information, refer to Appendix E, Optimizing PCR.

Optimizing Salt Concentrations

Multiplexing by co-loading several PCR products in a single lane improves throughput, but can also increase the salt concentration in your samples. High salt concentration in your samples can inhibit complete denaturation of double-stranded DNA. As a result, the ABI PRISM 373 DNA Sequencer with XL Upgrade detects less DNA, weakening the signal. To prevent high salt concentrations from negatively impacting your run, make sure you use at least equal volumes of formamide and PCR solution in each of your samples.

To increase the signal intensity, do one of the following:

- Combine the samples, precipitate the product, and resuspend the samples

- Use a spin column

- Add 1 or 2 extra PCR cycles in your protocol to produce more product per µL of PCR reaction

Merely evaporating your samples increases the concentration of salt, which makes it more difficult to denature the DNA in subsequent steps.

Multiplexing Samples in a Single Lane

In addition to multiplexing the PCR, you can multiplex samples on the ABI PRISM 373 DNA Sequencer with XL Upgrade by performing multiple PCRs in separate reaction tubes, pooling the PCR products together, and co-loading multiple samples in a single lane of the gel.

If you multiplex samples, consider the following when designing your experiment:

- Use different colored ABI PRISM dyes for labeling each sample you co-load in a single lane, if they overlap in size.

- Because each dye has a different absorption maximum, the intensity of fluorescent signal emitted differs for each dye:

Filter Set A	Filter Set B
ROX (lowest signal intensity)	TAMRA (lowest signal intensity)
TAMRA	HEX
JOE	TET
5-FAM (highest signal intensity)	6-FAM (highest signal intensity)

When labeling fragments, use a greater concentration of dye for dyes of lower intensity. For example, you would need to use approximately eight times as much ROX dye as 6-FAM or TET dye to generate signals of equal intensity. Also, when choosing combinations of dyes for sample labeling, choose dyes that can be detected by the same filter set: A or B.

Sizing and Quantifying Sample Fragments

When preparing samples, consider how choice of dye labels and size standard affects analysis of fragment size and quantity. For the same study, use the same size standard and dye label for all samples.

When analyzing your results, compare only peak heights, peak sizes, or peak areas of fragments labeled with the same dye label. For example, compare HEX-labeled fragments only to other HEX-labeled fragments.

Applied Biosystems

Setting Up the Software

While the gel is polymerizing, you can set up the Sample Sheet and the Run file for the data collection software. Refer to *Setting Up the Software* on page 4-19.

If you are running GeneScan applications or wish to use custom analysis settings, you should also set up the conditions that control data analysis before you start a run. To do so, refer to the user's manual provided with your GeneScan analysis software.

IMPORTANT	*If you choose automatic analysis and want the GeneScan Analysis software to use settings other than the default, customize the analysis settings before you start data collection.*

If you specify automatic analysis in the data collection program, the GeneScan Analysis program starts automatically at the end of the run to process the collected data.

Setting Up the Instrument

Pour your gel as described in Section 2, and allow it to polymerize for two hours before proceeding with this section.

IMPORTANT	*Make sure to use the appropriate gel concentration for the gel length and run type.*

Preparing the Gel for Loading

Note	*Wear clean laboratory gloves throughout this procedure, both for your protection and to avoid transferring fluorescent contaminants from your hands to the glass plates.*

Reagents and Supplies Needed For Preparing the Glass Plates

- A single-edged razor blade or a second casting comb

- 1.5-L, 1X Tris Borate EDTA buffer (2 L for 34-cm gels), pH 8.3 (refer to *10X TBE Stock Solution* on page 2-10)

- A clean, 10–50 mL syringe and a 22 G needle

To clean the plates:

1. Remove the clamps on the gel casting (single-well) comb or square-tooth comb, and the tape from the edges of the plates.

2. Rinse the glass plates with cold water.

    a. Rinse rinse both sides and edges of the glass plates thoroughly with cold tap water to remove dried acrylamide and urea.

    b. Rinse the well and plates with dH_2O and allow the plates to air dry.

To remove the square-tooth comb:

1. Before you remove the square-tooth comb (Figure 4-2), lay the appropriate plastic overlay strip on the clean glass. Align the well outlines on the overlay strip with the white teeth of the comb.
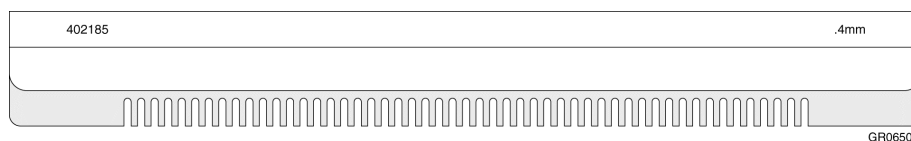
Figure 4-2. Square-tooth comb

2. Carefully lift the edge of the square-tooth comb.

    To avoid suction, pry gently with a razor blade or plastic wedge, or squirt deionized water over and around the comb.

3. Using both hands, apply gentle, even pressure and slowly pull out the comb.

4. Clean the comb area with deionized water and Kimwipes.

Note   *If you are using the Sharks-tooth comb: refer to To prepare the plates for loading on the instrument: on page 3-7 for removing the casting comb; refer to Inserting the Sharks-tooth Comb on page 3-34.*

Preparing the Electrophoresis Chamber

WARNING	*ELECTRICAL SHOCK HAZARD. The Model 373 contains a high voltage power supply. This instrument has been designed with safety features in the door to disconnect the power supply when the door is open. Although it is designed for safe operation, please follow procedures as prescribed. As with any electrophoresis apparatus, care should be taken during instrument operation and when handling electrodes and liquids.*

WARNING	*ELECTRICAL SHOCK HAZARD. Wait at least 5 seconds after opening the door before touching the electrodes in the electrophoresis chamber. Capacitors hold an electrical charge even after the power supply is disconnected.*

Model-Specific Notation

This manual applies to three configurations of Model 373 DNA Sequencers: Classic, Leon, and Stretch.

- **Classic.** This configuration includes 370s with Macintosh upgrade and the 373A. Classic models have electrophoresis chambers which support 24-cm WTR distances.

- **Leon.** This configuration has an electrophoresis chamber which supports 6-, 12-, and 24-cm WTR distances.

- **Stretch.** This configuration supports 6-, 12-, 24-, 34-, and 48-cm WTR distances and is easily identified by its extended door.

Note	*All three instrument configurations must have the five-filter wheel capability.*

Most of the information in this manual applies to all three configurations, however, some sections of the manual apply to only one or two of these configurations. Those sections are preceded by the name of the configuration (Classic, Leon, or Stretch) to which they apply.

To prepare the electrophoresis chamber:

Classic 1. Open the door to the Model 373 and make sure the heating plate is dry.

2. Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.
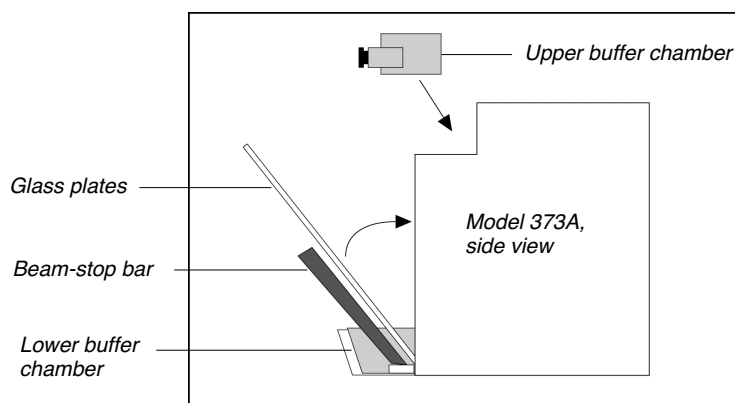
Figure 4-3. Placement of the Buffer Chambers, glass plates, and beam-stop bar in the electrophoresis chamber of the Model 373 (Classic)

3. Pull the black beam-stop bar in the electrophoresis chamber toward you until it stops (Figure 4-3). Rest the bottom edge of the plates on the notched supports in the bottom of the lower buffer chamber, with the notched plate facing away from you. Stand the plates in a vertical position. Press them firmly against the heating plate, and then push the beam-stop bar up against the plates. Center the plates in relation to the beam-stop bar, and then lock the beam stop bar in place by sliding the latches down along the outer edges.

IMPORTANT   *It is very important to center the plates. Centering the plates ensures that each sample migrates within the read region of the laser.*

Note	The beam-stop bar is removable. In normal operation you should not need to remove it. To install the beam-stop bar if it is removed, line up the alignment pins in the alignment grooves and slide the beam-stop bar into place.

Leon   1.   Open the door to the Model 373.

Upper buffer chamber support

24 cm

12 cm

6 cm

Beam-stop bar

Beam-stop swivel clamps

Glass plate support

Lower buffer chamber
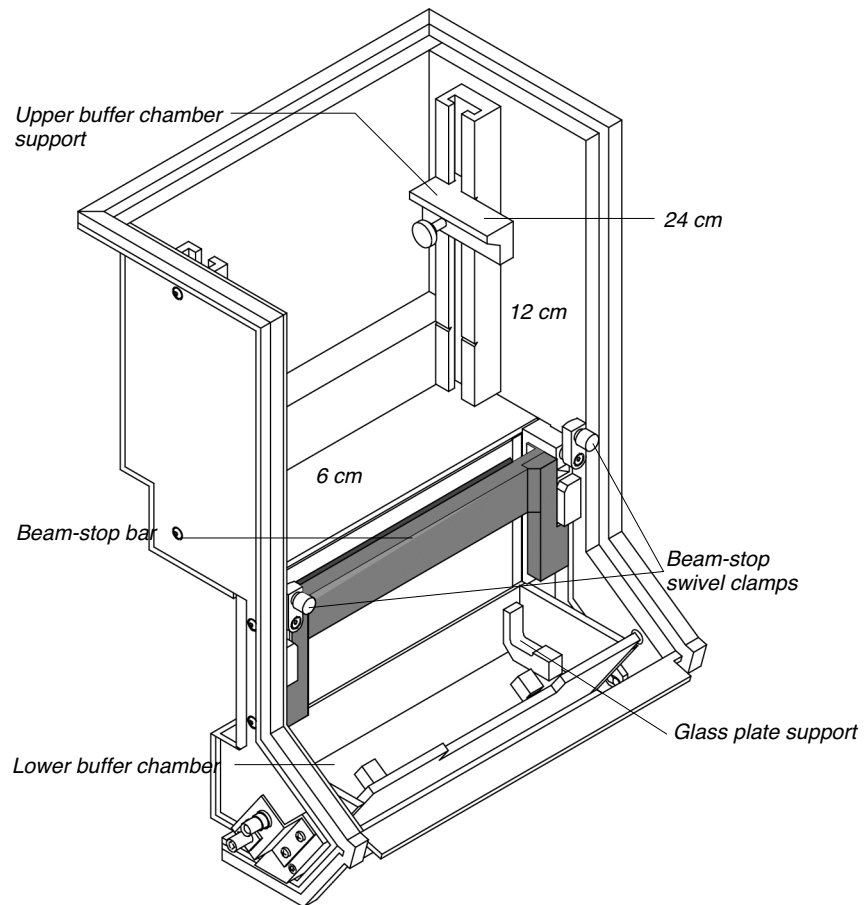
Figure 4-4. Model 373 (Leon) Electrophoresis Chamber

2. Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.

3. Adjust the upper buffer chamber supports to accommodate the different plate lengths (6, 12, and 24 cm) (refer to Figure 4-4 on page 4-13. Loosen the screws and slide the brackets to the correct position as indicated by the well-to-read length printed on the supports, and then tighten the screws.

4. Place the glass plates into the instrument as follows:

    a. Pull the black beam-stop bar in the electrophoresis chamber towards you until it stops (Figure 4-4 on page 4-13).

    b. Rest the bottom edge of the plates on the notched supports in the bottom of the lower buffer chamber, with the notched plate facing away from you.

    c. Stand the plates up in the vertical position. Press them firmly against the back of the chamber (avoid touching the laser read region, and then push the beam-stop bar up against the plates.

    d. Center the plates with relation to the beam-stop bar and then latch the bar in place.

Stretch 1. Open the door to the Model 373. The electrophoresis chamber looks like the one shown in Figure 4-5.
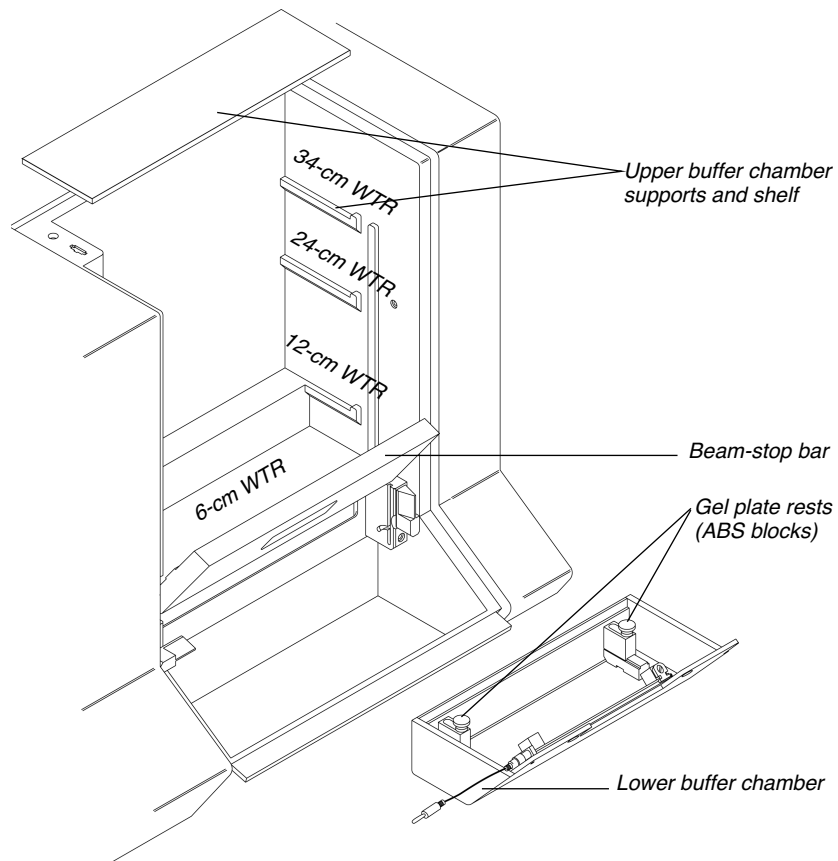
Figure 4-5. Model 373 (Stretch) Electrophoresis Chamber

2. If you are running a 24- or 34-cm gel, place the white plastic upper buffer chamber shelf on the appropriate support on the inside of the electrophoresis chamber (see Figure 4-5).

    If you are running a 12-cm gel, place *two* white plastic upper buffer chamber shelves on the support.

If you are running a 6-cm gel, then no shelves are required.

3. For 34- and 24-cm gels, slide the heat plate into place, as follows:

    a. Choose the correct heat plate for the glass plates you are using.

    b. Make sure the electrical wires for the temperature sensor are at the top, with the plate clamps toward the front. Align the grooves on either side of the heat plate with the heat plate guides on the chamber.

    c. Slide the heat plate down until it rests on the bottom of the chamber.

    d. Plug the temperature sensor on the heat plate into the socket at the top left of the chamber.

    e. Plug the heat plate ground into the socket at the top right of the chamber.

    For 6 and 12 cm WTR gels, use the temperature sensor alignment brace.

IMPORTANT   *Make sure the heat plate ground is connected before continuing.*

---

4. Adjust the gel plate rests in the lower buffer chamber, if necessary (see Figure 4-6). The gel plates rests are located in the bottom of the buffer chamber and serve to elevate the glass plates. In order for the 34 cm plates to rest in the correct position, they must sit higher in the buffer chamber. All other plates rest in the lower position.

    Each gel plate rest consists of several parts, a large ABS block on the bottom, a shorter ABS block on top of it, and an adjustable thumb screw. When the thumb screw is loose, the small block can be moved forward and back, so that the plates rest either on one block, or on two blocks (see Figure 4-6).

    a. Loosen the thumb screws.

    b. Slide the blocks toward the back of the chamber if you are using 34 cm plates, so the plates rest in the *high* position. Slide the blocks toward the front of the chamber for all other gel lengths so the plates rest in the *low* position.

c. Tighten the thumb screws.

Thumb Screw

a. For all run lengths except 34 cm. *With the movable blocks toward the front of the electrophoresis chamber, the glass plates rest lower in the buffer chamber.*

Front of electrophoresis chamber

Glass Plates rest here

Thumb Screw
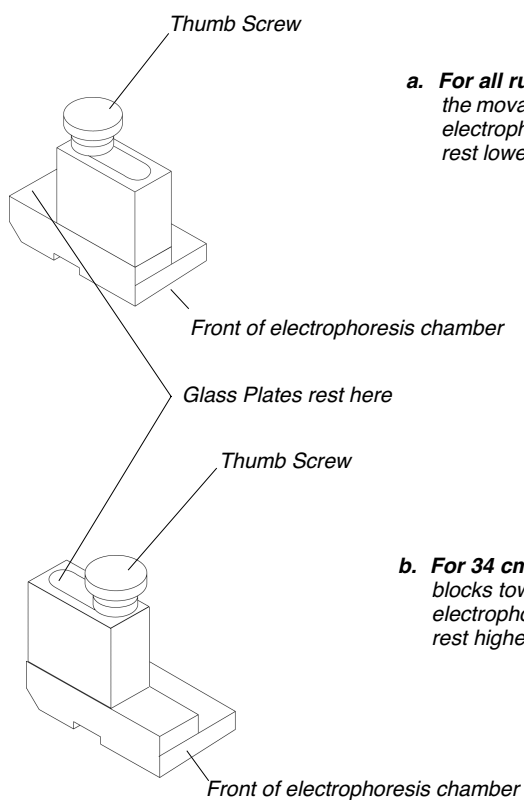
b. For 34 cm runs only. *With the movable blocks toward the back of the electrophoresis chamber, the glass plates rest higher in the buffer chamber.*

Front of electrophoresis chamber

Figure 4-6. Adjustable gel plate rests

5. Place the lower buffer chamber in the bottom of the electrophoresis chamber with the electrode to the front. The upper chamber will not be needed until later.

6. Unfasten the latches on either side of the beam-stop bar and swing the beam-stop bar forward, toward you.

7. Place the glass plates in the electrophoresis chamber in front of the heat plate and read window, with the bottom of the plates resting on the gel plate rests.

a.   Center the glass plates carefully, or the beam-stop bar will not fasten.

b.   Press the glass plates against the heat plate and close the beam-stop bar.

c.   Fasten the latches at either side of the beam-stop bar.

IMPORTANT   *Do not touch the gel plates within the read region.*

Setting Up the Software

Before starting electrophoresis, you must ensure there is sufficient space on your hard disk and restart the Macintosh. If you delete files, you must restart the Macintosh. You must also set up certain files in the data collection software:

- Set preferences
- Record sample information in the GeneScan Sample Sheet
- Create a GeneScan Run file

Note	*These tasks are best performed while you are waiting for the gel to polymerize.*

You can simplify creation of the files by setting preferences for certain parameters in each file.

The procedures described here do not include detailed descriptions of the data collection software. They are intended to give you a guide for steps you perform and the order in which to perform them. For specific information about the software and how to complete specific fields, refer to Section 5.

You may not need to change these default parameters for future runs, if you always run the same or similar parameters. However, it is important to verify these defaults, since they determine the names and locations of your data.

If you desire more detail, the steps described here refer you to the specific pages in Section 5 where you can find related information.

Setting up the Analysis Software

If you are running GeneScan applications or wish to use custom analysis settings, you should set up the conditions that control data analysis before you start a run. To do so, refer to the User's Manual provided with your GeneScan analysis software.

Starting the Data Collection Software

1. Choose Restart from the Special menu in the Finder to restart your Macintosh.

2. The data collection software should automatically open upon restarting the Macintosh. If it does not open, double-click the icon to open the program. Refer to *To make the data collection program open at startup:* on page 5-13 for instructions to open the collection program at startup.

Note	*When you start the ABI PRISM 373 DNA Sequencer with XL Upgrade data collection program, the program searches for the current version of firmware on the Translation Interface Processor (TIP). If none is found, the program automatically copies the firmware image file (stored on the Macintosh) to the TIP. If the TIP's version of firmware is different from the collection software version, a dialog box appears, allowing you to continue or send a new firmware to the TIP. Click Send New Firmware to update your firmware version.*

Creating User-Specific Module Files

The ABI PRISM 373 DNA Sequencer with XL Upgrade DNA Sequencer upgrade is shipped with module file templates for plate check, prerun, sequencing and GeneScan runs. Table 4-1 shows the default parameters for the modules file templates.

IMPORTANT	*The parameters in Table 4-1 apply only to 24-cm WTR Classic or Leon denaturing 6% acrylamide gels. You must make appropriate modules for all other, i.e., 6-, 12-, and 34-cm plate lengths and for native applications.*

Table 4-1. Module File Default Parameters

Module	Field (Volts)	Power (Watts)	Current (mAmps)	Laser Power (mWatts)	Run Time (Hours)
Plate Check	--	--	--	40	0.50
Pre Run	2500	30	40	40	0.33
Seq Run	2500	30	40	40	12

Module	Field (Volts)	Power (Watts)	Current (mAmps)	Laser Power (mWatts)	Run Time (Hours)
GS Run	2500	30	40	40	12

You must create user-specific module files the first time the instrument is used and for each different run condition. Once you modify a module file template, you can save it and use it each time a similar run is performed. Refer to Table 1-3 on page 1-15 and Appendix B for suggested module file and Run parameters.

To modify a module file:

1. Choose New from the File menu.

2. Click the Sequence Run or GeneScan icon in the window that appears.

3. Choose the module file you wish to modify from the Run Module pop-up menu.

Note	*You do not need to complete the other information in the Run window to modify a module file.*

4. Click the small document icon next to the Run module pop-up menu.
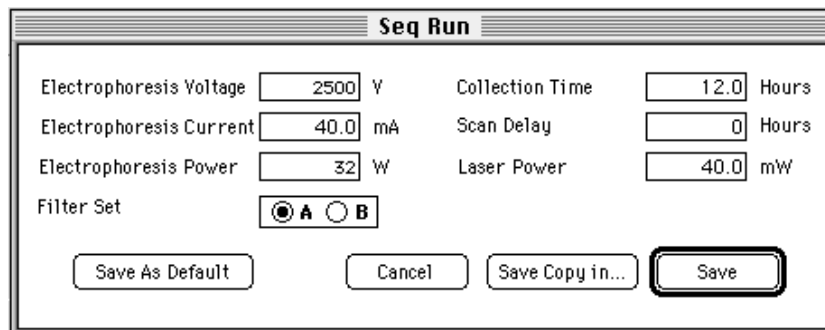
Figure 4-7. Module settings dialog box

5. Type new number in the applicable entry fields. Refer to Table 1-2 on page 1-14 for recommended run parameters.

6. Save the changes.

- *Save as Default:* To save the changed parameters as defaults for the module without changing the module name, click Save as Default. The new settings are permanently saved and the name of the module file remains unchanged.

- *Save Copy in…:* To save the changed parameters as defaults, click Save Copy in. Type in a new name for the module file and save the file in the Modules Folder. The new settings are permanently saved and the name of the module file is changed.

Note	You will not be able to select your new module file in the pop-up menu until you save and close the Run window, then reopen the saved Run file or create a new Run file.

- *Save:* To use the changes as a one-time override for the current run, click Save.

Note	Custom module files are stored on your hard disk. and can be used for any subsequent run. If you reload your collection software, however, you must re-create your custom modules.

Setting Default Parameters (Preferences)

You might only need to set default parameters prior to the first run. If you are satisfied with the defaults stored in the instrument, skip to page 4-24 to create a Sample Sheet and a Run file. However, if more than one person uses the same instrument, we recommend you set or verify all preferences prior to starting a run.

You can set several types of preferred defaults:

- *Folder locations:* The ABI PRISM 373XL data collection software uses specified folders for data storage. This option allows you to change the folders it uses. For information about changing folder locations, refer to *To change one of the data storage folders:* on page 5-17.

- *Default File names:* The data collection program automatically names new files and folders created during a run. You can specify the prefix and suffix of each type of file (see *Setting File Name Preferences* on page 5-18).

- *GeneScan Sample Sheet defaults:* You can set defaults for certain parameters on the Sample Sheet. Since the Sample Sheets differ for Sequence Analysis and GeneScan (fragment) Analysis, the parameters for which you can set defaults also differ somewhat. To set defaults for the GeneScan Sample Sheet, refer to *To set Sample Sheet defaults for GeneScan analysis applications:* on page 5-25.

- *GeneScan Run defaults:* You can also set defaults for certain parameters in the Run file. As with the Sample Sheet defaults, separate defaults apply for sequence runs and GeneScan analysis runs. To set defaults for sequence Run files, refer to *Setting Defaults for GeneScan Analysis Applications* on page 5-25.

- *General settings:* The ABI PRISM 373XL instrument is normally attached to the Modem port of the Macintosh computer. If you need to change the connection, you can specify the change using this preference choice. Also use this choice to reset the global serial number, which is a number used by the file naming defaults (refer to *General Settings* on page 5-27). To change general settings, refer to *To change the general settings:* on page 5-28.

- *Dye indicators:* Each of the four dyes used during a run has a preset code and color. You can change the color assigned to each dye, and change the color that appears in the plot when it is printed. To do so, refer to *To set dye indicator preferences for the analysis applications:* on page 5-29.

To set preferences:

1. Choose Preferences from the Window menu and choose the appropriate option from the submenu that appears.
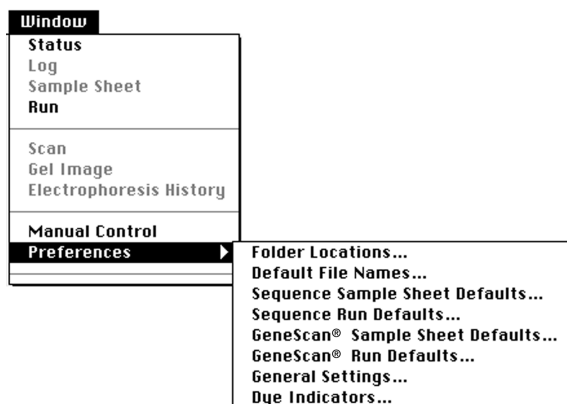
Figure 4-8. Preferences submenu

2.   Enter or change the defaults, as described on the pages noted above.

3.   Click OK to accept the changes.

Creating the GeneScan Sample Sheet

Before starting a run you must record sample information in the GeneScan Sample Sheet and the Run file. Once you have set up the Sample Sheet, you can automatically copy sample information into the Run file, which associates sample information (name and type of analysis) with each lane position in the gel.

Separate Sample Sheets exist for sequencing and GeneScan analysis applications (see Figure 4-9). Be sure to choose the correct type for your application.

If a Sample Sheet containing the correct information already exists, you can use it. In most cases, you need to create a new Sample Sheet for each run. You can enter the information directly or export information from a database in tab-delimited format and import it into the Sample Sheet.

To create a GeneScan Sample sheet:

1.   Choose New (⌘-N) from the File menu.

2.   Click the GeneScan Sample Sheet icon in the window that appears.

      The Sample Sheet is similar to a spreadsheet or ledger. It accepts more text than fits in the visible fields and automatically scrolls as you type your entry.

To view the beginning of a long entry, use the keyboard arrow keys. Many of the cells have default values that are automatically filled in for each sample.

3. Enter the necessary information. For details about completing the Sample Sheets (including information about importing from a database), refer to *Creating and Editing a Sample Sheet* on page 5-32.

IMPORTANT   *Although the Sample Sheet has 72 lanes available, fill in the sample information only for the number of lanes you are using.*

IMPORTANT   *Do not mix sequence analysis and GeneScan analysis samples on the same Sample Sheet or the same run.*

---

4. When you are finished, choose Save from the File menu.

    The dialog box that appears shows the default file name and storage location as defined in the File Names and Folders locations preferences, respectively. You can change the file name if you wish.

5. Click Save.

    Make sure you are saving the file into the Sample Sheet folder, so the name will appear in the pop-up menu.

6. Choose Close from the File menu, or click the close box.
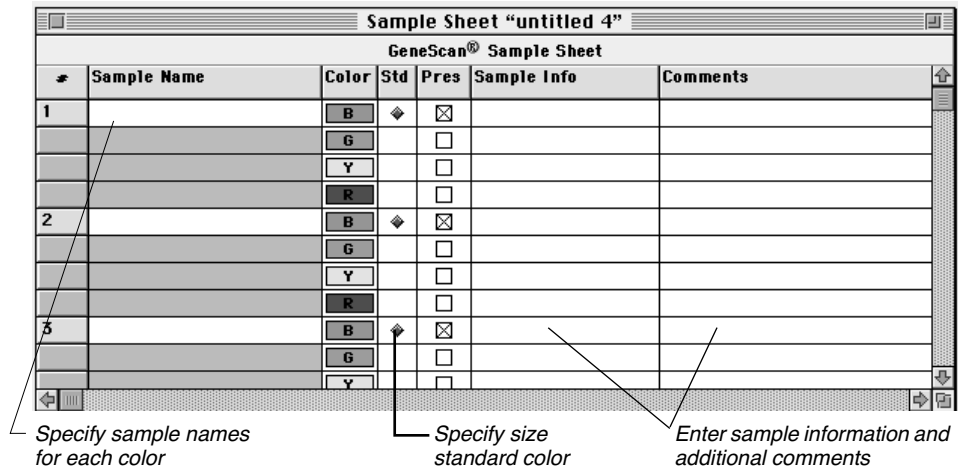
Figure 4-9. Blank GeneScan Sample Sheet

Creating a Run File

In the Run file you specify the lanes in which the samples will run and parameters to be used for both the pre-run and the run. If a Run file exists that contains the proper parameters, you can copy it to a new folder and use it. In most cases you need to create a Run file for each new run.

Separate Run windows exist for sequencing and GeneScan analysis applications. They look almost the same; the GeneScan analysis version has an additional (Analysis Settings) column. Be sure to choose the correct type for your application.

To create a Run file:

1. Choose New from the File menu.

2. Click the GeneScan Run icon in the window that appears.

GeneScan®
Run

Small document icons open the selected
*module or Sample Sheet for review

Specify length of run

Run control buttons

Pop-up menus in these panels allow you to choose the modules (parameters), the associated Sample Sheet, the number of wells, the separation distance (WTR), and the matrix for the run

Lane numbers cannot be edited
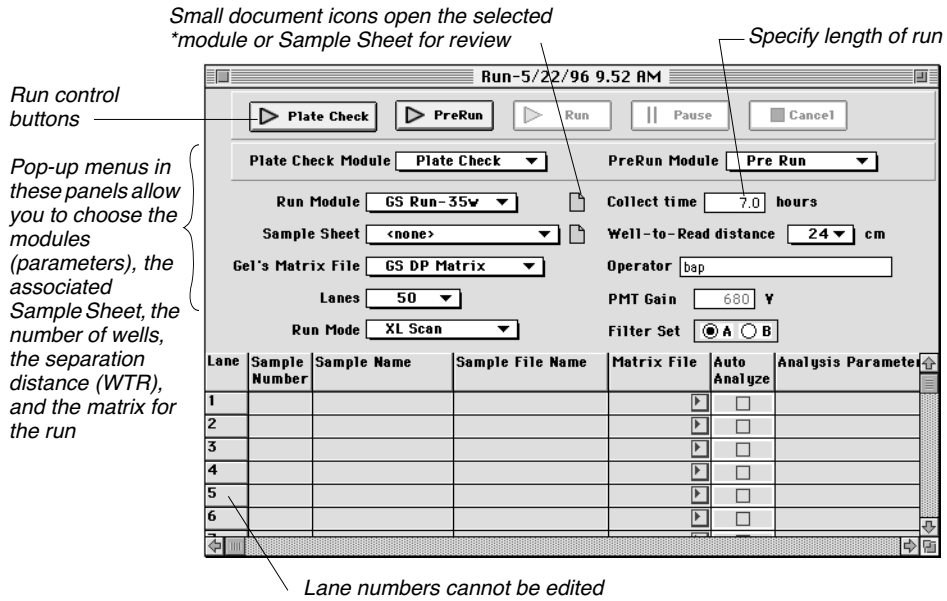
Figure 4-10. GeneScan Run window

3. Choose the parameters for the run from the pop-up menus.

| Note | Any changes you make in your preferences setting will not take effect until you open a new Run window. |

a. Choose modules to be used for the plate check, the pre-run and the run in the Module pop-up menus.

b. Choose the Sample Sheet from the Sample Sheet pop-up menu.

When you choose the Sample Sheet, the information about the lanes in which the samples are to run fills in automatically.

c. Choose the number of lanes and the separation distance from the Lanes pop-up menu and the WTR pop-up menu, respectively.

d. Choose XL Scan or Full Scan in the Run Mode pop-up menu. Use the table below to determine the appropriate run mode for your lane configuration.

Lanes	Comb	Run Mode
24	Sharks-tooth	Full Scan
24	Square-tooth	Full Scan
32	Sharks-tooth	Full Scan
34	Square-tooth	Full Scan
36	Sharks-tooth	Full Scan
36	Square-tooth	Full Scan
48	Sharks-tooth	XL Scan
50	Square-tooth	XL Scan
64	Sharks-tooth	XL Scan
66	Square-tooth	XL Scan

Note   *If you select a run mode that is incompatible with the selected lane configuration, the lane popup menu displays <none>.*

e. Choose a matrix file from the Gel Matrix pop-up menu.

4. Enter the length of the run in the Collect Time entry field.

Refer to Application Protocols in Appendix C of the 672 User's Manual for collection times.

5. Enter your name in the Operator entry field.

The sample information for each lane is entered automatically in the grid at the bottom of the Run window when you choose a Sample Sheet.

Note	You cannot edit the Sample Sheet information in the Run window. Make any changes in the Sample Sheet, save it, select <none>, and then select the Sample Sheet again in the Run window to make the changes effective.

6. Choose the appropriate filter set: A or B.

IMPORTANT	Failure to select the appropriate filter set results in the loss of data.

7. Select the Auto Analyze and Auto Print checkboxes if you want the analysis program to analyze and send data to the printer.

When you finish completing the information in the Run file, do not close the Run file window. You can use it to perform a plate check, as described next.

The options available to you at this time are:

- Save the file for future use, then click the close box to close it.

- Start a plate check by clicking the Plate Check button.

To save the file, choose Save from the File menu. To perform a plate check, see page 4-30.

For more information about creating the Run file, refer to page 5-40.

Checking the Plates

Scan the gel and plates before adding buffer and loading samples, to ensure that no peaks are produced by fluorescent particles on the glass plates or in the gel.

Click Plate Check after you choose the Plate Check module
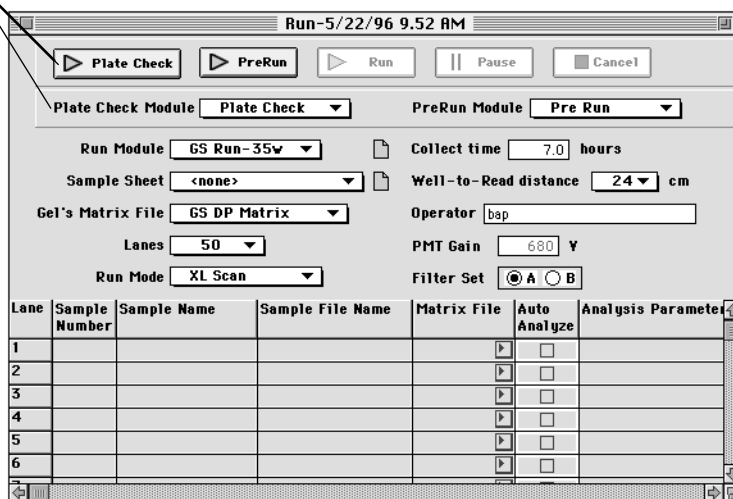
Figure 4-11. Completed GeneScan Run window

To check the plates:

Note	For 6-cm and 12-cm runs on Stretch-configured instruments, the temperature sensor alignment brace must be connected before runing the plate check.

1. Click Plate Check in the Run window.

Note	If a dialog box appears indicating you do not have enough room on your hard disk to save the files created by a run, delete files (particularly Gel files) to create enough space, making sure to back them up on a disk or other storage device first, then click Plate Check again.

The laser scans the plates without electrophoresis to detect any unwanted fluorescent material in the read region.

2. Observe the Scan window that appears.

   The Scan window should show a relatively flat line across the screen in each of the four colors. Typically, the lowest line is blue, followed by the green, yellow (black), and red lines. If the scan line is flat, then the plates are clean. Proceed to *Checking the PMT Gain Setting* on page 4-32.

   If you see peaks in the Scan window, clean the glass plates again, as described below.

WARNING   *LASER HAZARD. Do not look directly into the laser beam or allow a reflection of the beam to enter your eyes. Exposure to direct or reflected laser light at 40 mW for 0.1 seconds can burn the retina of the eye and leave a permanent blind spot.*

Cleaning the Plates or Skipping Lanes

Peaks in the scan lines could indicate dirt on the glass or contaminating fluorescence in the gel. To eliminate dirt on the glass, clean the plates and scan them again. If peaks still appear and you wish to use the gel, avoid loading samples in the lanes where the peaks appear.

To clean the plates and re-scan:

1. Click Pause to pause the plate check.

2. Open the door of the instrument and unlatch the beam stop bar and remove the plates.

3. Carefully reclean both sides of the glass in the laser read region with dH_2O and a lint-free tissue or ultra-pure pressurized air.

4. Replace the plates, center them, and latch the beam stop bar in the electrophoresis chamber.

5. Click Resume and check the plates again.

If the peaks do not disappear after cleaning the glass, the gel mixture or buffer might contain particles. If you wish to use the gel, avoid loading samples in the lanes where the peaks appear.

Note	*Occasionally, peaks in the scan window are caused by a dirty beam stop bar, rather than by dust or particulates in the gel. Clean the beam stop bar with dH$_2$O and (optionally) a thin layer of oil, if the peaks do not disappear after cleaning the glass.*

Skipping Lanes

If a plate check shows peaks in some lanes of a gel even after cleaning, the Scan window indicates which channels have contaminating fluorescence in them. Refer to Table 3-2 on page 3-29 as a guide for estimating the channel number/lane assignment correlations. This table should be used only as an approximation, as it determines the center of each lane for sharks-tooth comb configurations and may differ slightly for square-tooth combs.

If the contaminant peaks are close to the edge of a lane, you may want to skip two lanes to ensure that there is no contamination in the lanes you load.

Checking the PMT Gain Setting

Verify Run Mode

The PMT gain setting differs for each of the run modes (XL Scan and Full Scan). Verify that you have selected the correct run mode in the Run window before checking the PMT gain setting.

First Time Run

The PMT gain settings are stored in a Calibration file. If this is the first time the instrument is being used, you must make and send a Calibration file. Refer to Section 5 for instructions on how to make and send a Calibration file.

Recommended PMT Gain

The photomultiplier (PMT) gain should be set such that the lowest scan line (usually the blue line) is positioned at approximately 900 on the y-axis in the Scan window. For subsequent runs, the PMT gain will need to be adjusted only if the y-axis value is out of the range of 800–1000 on the y-axis. Different PMT gains are required for the three run modes (XL Scan, Full Scan, and BaseSprinter).

To check the PMT gain:

1. While the Plate Check is running, view the four base lines (each line represents one of the four filters) in the Scan window of the 373XL collection program.

2. Position the cursor so the tip of the arrow just touches a flat section of the lowest scan line (usually the blue line), as in Figure 4-12.

Figure 4-12. Cursor positioned correctly to measure PMT gain

3. Read the y-axis value displayed in the upper-left hand corner of the scan window.

    •   If the lowest scan line is positioned between 800–1000 on the y-axis, cancel the Plate Check, and then proceed to *Inserting the Sharks-tooth Comb* on page 4-34.

- • If the lowest scan line is outside the recommended range, adjust the PMT gain.

To adjust the PMT gain:

1. While the Plate Check is running, choose Manual Control from the Window menu.

2. Adjust the size and position of the Scan and Manual Control windows so you can see both windows without any overlap.

3. In the Manual Control window, choose PMT Gain from the Fxn Name pop-up menu.

4. Type a new value, then click Execute.

Note	*If you enter a larger number than the one displayed, the lines in the Scan window will shift up; a smaller number causes them to shift down.*

5. Check the y-axis value in the Scan window. (First click on the Scan window to make it active.)

6. Repeat steps 3–5 until the PMT gain is within the recommended range.

7. Close the Manual Control window.

8. Cancel the Plate Check in the Run window.

Note	*After selecting a different run mode, you must change the PMT gain.*

Inserting the Sharks-tooth Comb

Note	*If you are using a square-tooth comb, skip this section and proceed to Completing Instrument Setup on page 4-36.*

To insert the sharks-tooth comb:

1. Insert the sharks-tooth comb (see Figure 4-13).

   a. Center the sharks-tooth comb in the glass notch and carefully slide it

between the glass plates. Make sure all of the points on the comb enter the space between the plates at the same time, to avoid bending or breaking the teeth. Carefully align the center registration line with the registration mark on the plates.

b. Slide the comb down until the tips of all the teeth just touch or slightly depress the surface of the gel. Do not deform the wells. Make sure the teeth just barely indent the well surface. All of the teeth must touch the surface of the gel. If the surface of the gel is not completely flat in the loading region, some of the teeth may have to be inserted below the surface of the gel (up to 0.5 mm), so all of the teeth touch the gel surface. If a tooth has penetrated the gel surface, do not attempt to withdraw the comb, or sample will leak into adjacent wells.

Figure 4-13. Sharks-tooth comb just touching the gel

Completing Instrument Setup

After completing Plate Check, do the following:

Classic 1. *If you are using a square-tooth comb*, attach the D-clamps to the upper buffer chamber, if they are not already attached.

      a.   Remove the screws at either side of the upper buffer chamber.

      b.   Place D-clamps so that the screw holes are aligned.

      c.   Insert the screws and turn them a few times.

2. Place the upper buffer chamber at the top of the gel plates by placing it on the upper shelf (Figure 4-3 on page 4-12) and sliding the front brackets of the chamber over the glass plates. The brackets should sit over the spacers on the outside edges of the plate.

3. *For square-tooth comb runs*, clamp the glass plates to the upper buffer chamber by placing the D-clamp against the front of the glass plates and inside the brackets of the buffer chamber.

    The brace is not used for square-tooth comb runs.

4. *For sharks-tooth runs*, put the alignment brace in place against the front of the glass plates, inside the brackets of the buffer chamber, and resting on top of the heat plate.

    The alignment brace is made of highly polished acrylic so the numbers and the comb are readily visible through the plastic. Do not mark the alignment brace with felt-tipped pens, as the use of most organic solvents to remove the ink will dull the surface of the brace and reduce its clarity. Some ink may also fluoresce.

5. Tighten the screws on the buffer chamber equally on both sides to maintain even pressure across the D-clamp or alignment brace. Turn the screws until you see the gasket compress completely against the glass. Finger-tighten only—do not overtighten. You may need to apply light pressure on the top of the brace to prevent it from moving during tightening.

Note   *The D-clamps are used in place of the alignment brace to prevent deformation of the wells formed by the acrylamide square teeth.*

---

6. Fill the upper buffer chamber with the 1X TBE buffer prepared earlier to at least half-way up the notched part of the plate (~ 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

Note	*If there is a leak, tighten the brackets slightly. If the leak continues, siphon the upper buffer, then clean the gasket area on the chamber and the area of the plate that contacts the gasket. Reassemble as before.*

7. Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full.

Note	*Avoid splashing buffer against the back of the chamber since it will spill over into the liner. Remove any bubbles that are under the bottom edge of the gel plates using a clean needle and syringe. Bend the needle at an angle to guide the bubbles out.*

8. Attach the top and bottom electrode leads located on the left side of the electrophoresis chamber and close the door gently.

Leon

1. *If you are using a square-tooth comb,* attach the D-clamps to the upper buffer chamber, if they are not already attached.

   a. Remove the screws at either side of the upper buffer chamber.

   b. Place D-clamps so that the screw holes are aligned.

   c. Insert the screws and turn them a few times.

2. *For 24-cm runs only,* slide the heat plate into place against the glass plates, as follows (see Figure 4-4 on page 4-13):

   a. Rest the lower edge of the heat plate (the edge with the supports protruding on both sides) on the upper inside ledge of the beam-stop bar where it meets the glass plate. The supports rest in the notches.

   b. Stand the heat plate vertically against the glass plates.

   c. Secure the beam-stop swivel clamps.

3. Place the upper buffer chamber at the top of the gel plates, on the supports. Slide the clamps over the plates.

4. *For square-tooth comb runs,* clamp the glass plates to the upper buffer chamber by placing the D-clamps against the front of the glass plates and inside the brackets of the buffer chamber.

   The brace is not used for square-tooth comb runs.

5. *For sharks-tooth runs,* put the alignment brace in place against the front of the glass plates, inside the brackets of the buffer chamber, and resting on top of the heat plate.

   The alignment brace is made of highly polished acrylic so the numbers and the comb are readily visible through the plastic. Do not mark the alignment brace with felt-tipped pens, as the use of most organic solvents to remove the ink will dull the surface of the brace and reduce its clarity. Some ink may also fluoresce.

Note   *To use the D-clamps with 24-cm WTR, you must omit the heat plate.*

---

6. Tighten the screws on both ends of the upper buffer chamber equally to maintain even pressure across the alignment brace or D-clamps. Turn the screws just until you see the gasket compress completely against the glass—do not overtighten.

Note   *The D-clamps are used in place of the alignment brace to prevent deformation of the wells formed by the acrylamide square teeth.*

---

7. Fill the upper buffer chamber with the 1X TBE buffer prepared earlier to at least 2/3 up the notched part of the plate (greater than 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

   If there is a leak, slightly tighten the brackets. If the leak continues, siphon the buffer out of the upper buffer chamber and clean the gasket area on the chamber. Also, clean the area of the plate that contacts the gasket. Reassemble as before.

8. Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full. Avoid splashing buffer against the back of the chamber

because it will spill over into the liner. Bubbles should be removed if they are under the bottom edge of the gel plates. This can be done with a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

9. Attach the top and bottom electrode leads located on the left side of the electrophoresis chamber and gently close the door.

Stretch

1. *If you are using a square-tooth comb,* attach the D-clamps to the upper buffer chamber, if they are not already attached.

  a. Remove the screws at either side of the upper buffer chamber.

  b. Place D-clamps so that the screw holes are aligned.

  c. Insert the screws and turn them a few times.

1. Place the upper buffer chamber at the top of the gel plates, on the white plastic shelf. Slide the clamps over the plates but do not tighten the clamps. (To ensure a tight seal between the buffer chamber and the glass plates, make sure the gasket on the upper buffer chamber is dry.)

IMPORTANT   *If you have two different upper buffer chambers, use the larger of the two for 34 cm runs. Use the small upper buffer chamber for all other GeneScan runs.*

2. Follow the instructions for the WTR and comb configuration you are using:

  • *For 24-cm and 34-cm runs with a sharkstooth comb:* Slide the plain acrylic alignment brace over the comb, between the upper buffer chamber clamps and the glass plates. Finger-tighten the clamps; be careful not to over tighten.

  • *For 24-cm and 34–cm runs with a square-tooth comb:* Tighten the screws on the D-clamps; be careful not to over tighten.

  • *For 6-cm and 12-cm runs with a square-tooth comb:* Slide the acrylic alignment brace with the temperature sensor over the comb, between the upper buffer chamber clamps and the glass plates. Finger-tighten the clamps; be

careful not to over tighten. Plug the temperature sensor into the socket at the top left of the chamber.

Note   *You must have the temperature sensor from either a heat plate or the acrylic brace connected for the instrument to run.*

The alignment brace is made of highly polished plastic so the numbers and the comb are readily visible through the clear plastic. Do not mark the alignment brace or glass plates with felt-tipped pens, as the use of most organic solvents to remove the ink will dull the surface reduce its clarity. Some ink may also fluoresce.

3.   Plug the electrode lead on the upper buffer chamber into one of the two jacks. For 34 cm gels, use the top (rear) socket, and for all other gels, use the lower (front) socket (see Figure 4-5 on page 4-15).

4.   Fill the upper buffer chamber to at least two-thirds up the notched part of the plate (greater than 1.3 cm). Check for leaks. Close the lid of the buffer chamber.

If there is a leak, slightly tighten the brackets. If the leak continues, siphon the buffer out of the upper buffer chamber and clean the gasket area on the chamber. Also, clean the area of the plate that contacts the gasket. Reassemble as before.

5.   Carefully fill the lower buffer chamber with 1X TBE buffer to about two-thirds full. For 34 cm runs, fill the lower buffer chamber until the bottom of the glass plates is covered. Avoid splashing buffer against the back of the chamber because it will spill over into the liner. Bubbles should be removed if they are under the bottom edge of the gel plates. This can be done with a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

IMPORTANT   *The buffer must completely cover the bottom of the gel plates. You will need to prepare 2 L of buffer for 34 cm gels. For all other lengths, the standard volume of 1.5 L is sufficient.*

6.   Attach bottom electrode lead located on the left side of the electrophoresis chamber.

Preparing and Loading Samples

Pre-running the Gel

Pre-run the gel for 5 minutes to make sure all connections and components of the electrophoresis system are working properly.

To pre-run the gel:

1. Flush all of the wells with running buffer. Direct the needle of a syringe filled with running buffer at the space between adjacent comb teeth, and gently flush the well.

   Remove any bubbles under the bottom edge of the gel plates in the lower buffer chamber. Use a clean needle and syringe, with the needle bent at an angle to guide the bubbles out.

2. If you have not done so, choose the PreRun module from the PreRun pop-up menu.

3. Click the PreRun button.

   The instrument performs electrophoresis without collecting data during a pre-run.

4. Pre-run the gel for 5 minutes.

5. Click Cancel in the Run window.

Preparing Samples for Electrophoresis

Add your samples to loading cocktail, as described here. After the pre-run (~5 minutes), load the samples and start the run.

WARNING	**CHEMICAL HAZARD. Formamide is a known teratogen and can cause birth defects. Before using formamide, read the MSDS found in the Safety Summary. Wear protective eyewear, gloves, and safety clothing when working with formamide. Wash thoroughly after handling formamide.**

To prepare samples for electrophoresis:

1. Prepare loading cocktail by mixing (5:1:1)

   - 2.5 µL deionized formamide

   - 0.5 µL Blue Dextran EDTA [1 mg Blue Dextran per 1 mL EDTA (25–50 mM, pH 8.0)]

   - 0.5 µL appropriate GeneScan Size Standard

   Store at 4° C for up to 1 week.

2. Determine the appropriate dilution of your fluorescently-labeled PCR products.

   The dilution used is dependent on the volume of your PCR reaction labeling strategy, and efficiency of label incorporation.

3. Prepare sample for loading.

   Use the following table to determine the appropriate sample, cocktail, and loading volumes for the comb you are using.

Table 4-2. Sample, Cocktail, and Loading Volumes

	Comb Configuration (number of wells)			
	24	34/36	50	66
Sample volume* (µL)	1.5	1.5	1.5	1.5
Cocktail volume (µL)	3.5	3.5	2.5	2.5
Loading volume (µL)	5	4	1.5–2.5	0.5–1.5

*User must determine the correct concentration, such that the signal is not >2000 fluorescent units.

Note	*In the 66-well configuration, the best resolution is achieved with 0.5 µL load volumes. It might be necessary to concentrate your PCR product by vacuum drying or evaporation. Perform concentration before adding the loading cocktail.*

4. Vortex and centrifuge briefly.

5. Heat samples at 95°C for 5 minutes.

6. Immediately place samples on ice until ready to load.

Loading the Samples and Starting the Run

IMPORTANT	*Be sure you load samples in the lanes specified in the Run window. The sample information in the Run window when you start the run is the information stored in the Gel file. Changes you make in the Run window after you start the run are not implemented.*

For GeneScan applications, we recommend you use a square-tooth comb. Sharks-tooth combs may be used only if there is a vacant lane between samples. Loading every other lane for a sharks-tooth prevents even the smallest amount of sample bleed-through from samples in adjacent lanes and is imperative for GeneScan analysis accuracy.

Note	If you are doing GeneScan analysis and have formed the wells using the square tooth comb, you can load the samples in consecutive lanes.

Whenever possible, load a sample in lane one to help orient the tracker during analysis. For best results, follow the procedure described here.

Note	In the following procedures, each button in the Run window has a corresponding menu choice in the Instrument menu. You can either click the button in the Run window or choose the command from the Instrument menu.

Make sure you deliver the samples to the bottom of the wells. Use either a single- or a multi-channel pipetter/syringe equipped with a flat tip less than 0.3 mm in diameter (we recommend one with a diameter of 0.17 to 0.20 mm). Do not overload the lanes. Follow the recommendations in Table 3-3 on page 3-40 for the allowable loading volumes. If you use too much sample volume or quantity, you will experience poor resolution.

To load samples from a square-tooth comb:

1. If you are pre-running the gel, click Cancel in the Run window.

2. Carefully flush all of the wells with running buffer.

Tip	Place formamide or loading buffer in the well to the left of your first sample lane, and in the well to the right of your last sample lane (the comb creates wells to either side of the numbered wells). The formamide in the buffer helps focus the bands in the first and last lanes.

3. If you are loading a square tooth comb for GeneScan analysis, you can load the wells consecutively.

IMPORTANT	Whenever possible, load a sample in lane one. Avoid skipping more than one lane between samples. It is better to leave extra empty lanes at the right end of the gel. The lane tracker may not work properly if there are many empty lanes between samples.

4. Click Run to start the run.

   A dialog box appears, allowing you to change the name and location of the gel file created by the run.

5. Click OK when you are satisfied with the name and location for the gel file.

Note   *If a dialog box appears indicating you do not have enough room on your hard disk to save the files created by a run, delete files (particularly Gel files) to create enough space, making sure to back them up on a floppy disk or other storage device first, then click Run again.*

To load samples from a sharks-tooth comb:

1. If you are pre-running the gel, click Cancel in the Run window.

2. Carefully flush all of the wells with running buffer.

Tip   *Place formamide or loading buffer in the well to the left of your first sample lane, and in the well to the right of your last sample lane (the comb creates wells to either side of the numbered wells). The formamide in the buffer helps focus the bands in the first and last lanes.*

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3. Immediately load the sample into each of the odd-numbered wells.

   If you are loading fewer than half the samples allowed by the comb, skip steps 6–10 and perform step 11. If you are loading a square tooth comb, you can load the wells consecutively and skip steps 4–9.

IMPORTANT   *Whenever possible, load a sample in lane one. Avoid skipping more than one lane between samples. It is better to leave extra empty lanes at the right end of the gel. The lane tracker may not work properly if there are many empty lanes between samples.*

---

4. Click Run in the Run window to start the run.

   A dialog box appears, allowing you to change the name and location of the gel file created by the run.

5. Click OK when you are satisfied with the name and location for the gel file.

Note   *If a dialog box appears indicating you do not have enough room on your hard disk to save the files created by a run, delete files (particularly Gel files) to create enough space, making sure to back them up on a floppy disk or other storage device first, then click Run again.*

6. Choose Status from the Window menu to verify that electrophoresis and scanning resume (refer to page 5-50). If either electrophoresis or scanning does not resume, cancel the run and restart it.

Figure 4-14 summarizes the buttons you click in the Run window from the time you start the plate check for a typical run. They are listed from top to bottom in the order you use them.

Note   *The tasks described in Figure 4-14 are detailed in the preceding pages.*

Button to click	Module in effect	Task to perform
Plate Check	Plate Check module	Check plates and gel for fluorescence
Cancel	n/a	(Stops plate check scan) Add buffer and check for leaks Mount front heat-transfer plate
PreRun	Pre-run module	(Electrophoreses gel without data collection to equilibrate temperature) Prepare samples while pre-running
Pause	Pre-run module	Load first set of samples during pause
Resume	Pre-run module	(Electrophoreses samples into gel without data collection)
Pause	Pre-run module	Load second set of samples during pause
Cancel	n/a	(Stops pre-run)
Run	Run module	(Starts electrophoresis and data collection)

Figure 4-14. Run window buttons used during a typical run

Observing Instrument Status and Data

After you begin electrophoresis and data collection, data appears on the computer screen as it is collected. You can open up to four windows to view the data and the instrument status. Only one window can be active at any given time, but status windows still update when they are not active.

Note	*If they are displayed, status windows continually update during a run. They do so even when another application is running.*

Open the data collection and status windows by choosing the name of the window of interest from the Window menu.

Data Collection Status Windows

To observe instrument status, choose one of the following commands from the Window menu. The page reference next to a window name indicates where you can find detailed information about that window.

- Status (refer to page 5-50)

- Electrophoresis History (refer to page 5-57)

- Log (refer to page 5-57)

Note	*After the run is completed, you can choose Log from the Window menu to display a comprehensive record of all error and status messages generated during the run.*

Real-time Data Windows

The data displayed in the real-time data windows (the Scan window and the Gel window) represents light intensities collected by the data collection software from the filter wheel and PMT in the instrument. The colors in the raw data windows represent the wavelengths of the dyes being detected, rather than the bases being detected. This is explained in *The Electrophoresis History Window* on page 5-57.

To observe the data as it is being generated, choose one of the following from the Window menu:

- Scan (refer to page 5-55)

- Gel (refer to page 5-56)

Cleaning Up After the Run

After your run is complete, perform the following general cleanup steps:

- Remove the gel from the instrument.
- Clean the plates, comb, and spacers.

Removing the Gel from the Instrument

Classic and Leon

1. Disconnect the electrode leads from both buffer chambers.

2. Unlatch the beam-stop bar and pull it forward.

3. Carefully remove the upper buffer chamber, glass plates, and heat plate (Leon only) as one unit. Discard the buffer.

   You can also set up a siphon system to drain the upper and lower buffer chambers before removing them.

4. Remove the lower buffer chamber and discard the buffer.

5. Clean up any liquid left in the electrophoresis chamber.

6. Unclamp the plates from the upper buffer chamber.

7. Rinse the buffer chambers with dH_2O and allow them to air dry.

Stretch

1. Disconnect the electrode leads from both buffer chambers.

2. Unlatch the beam-stop bar and pull it forward.

3. Carefully remove the upper buffer chamber and glass plates as one unit. Discard the buffer.

4. Remove some of the buffer from the lower buffer chamber before removing it.

   This is especially important for 34-cm runs, as the buffer level is very high. You can also set up a siphon system to drain the upper and lower buffer chambers before removing them.

5. Remove the lower buffer chamber and discard any remaining buffer.

6. Clean up any liquid left in the electrophoresis chamber.

7. If you plan to change plate length next time, unplug the temperature sensor, and then remove the heat plate, otherwise leave both in place.

8. Unclamp the plates from the upper buffer chamber.

Note   *If the unnotched plate has been silated with a bind-silane compound, use 0.1 N HCl or NaOH to remove the gel from the silated area, or scrape it off gently.*

---

9. Rinse the buffer chambers with dH_2O and allow them to air dry.

Cleaning the Plates, Comb, and Spacers

1. Gently push a plastic wedge almost all the way in at the bottom of the plates (avoid the top notches) and pry the plates apart. Do not use too much force or the plates might chip. Remove the sharks-tooth comb and gel spacers.

2. Lay a large Kimwipe on the gel and roll it up, lifting the gel off the plate, and discard appropriately.

3. Rinse the plates with water. Rub with a gloved hand to dislodge remaining gel bits, then rinse again with water.

4. Wash with a detergent such as Alconox that will not leave a residue, rinse with dH_2O, and allow to dry.

5. Clean the comb and spacers and store them.

WARNING **CHEMICAL HAZARD. The acrylamide and bis-acrylamide used to prepare sequencing gels for the ABI P**RISM** 373 DNA Sequencer with XL Upgrade are toxic chemicals whose use requires utmost attention to safety. Be sure to read the Material Safety Data Sheets (MSDSs)for emergency and first-aid procedures. MSDSs are in the ABI P**RISM** 373 DNA Sequencer with XL Upgrade Pre-Installation Manual, P/N 901173. Acrylamide is a neurotoxin that can be absorbed through the skin. Wear a laboratory coat, eye protection, and gloves. The handling and disposal of chemical waste is the responsibility of the user, and not the Applied Biosystems service representative.**

Analyzing the Data

If you choose Auto Analyze for your samples in the GeneScan Run window, the data collected on the ABI PRISM 373XL is automatically analyzed by the GeneScan Analysis program. For complete instructions on using the analysis program, refer to the *ABI PRISM GeneScan Analysis Software User's Manual.*

Automatic data analysis occurs approximately 1 to 10 hours after you start data collection (the amount of time depends on the run conditions and WTR you chose). Three channels of data are analyzed for each lane containing a sample on the gel. The analysis software prints electropherograms (if you selected AutoPrint in the Sample Sheet) and displays the gel window on the computer screen.

Evaluation Checklist

Before evaluating the quality of the data in your Samples files, check the following:

- After analysis, always view the Gel file on the computer screen. Check tracking and look for any atypical characteristics of the gel that may indicate a problem. For more information on fine tuning the gel file, refer to the *ABI PRISM GeneScan Analysis Software User's Manual.*

- Examine data obtained with control reactions. If you obtain good data with control reactions, you can eliminate the possibility of problems with reagents, equipment, or general technique.

- Study the analyzed data. Take note of the peak shape, the amount of noise, any obscured, crowded peaks, or other peaks that appear under peaks.

- Review *Appendix F* for strategies you can employ to optimize PCR performance.

- For forensics applications, run a positive control of the analyst's DNA to check for possible contamination of unknown samples and a negative control.

- Examine the standard curve for the sizing standard in each lane. Ensure the curve is linear and the proper peaks have been identified. If not, reanalyze the lane defining a new standards file for that lane. Refer to the *ABI PRISM GeneScan Analysis Software User's Manual.*

Characteristics of Good Data

An electropherogram from a Sample file that contains good data has sharp, well-defined peaks.
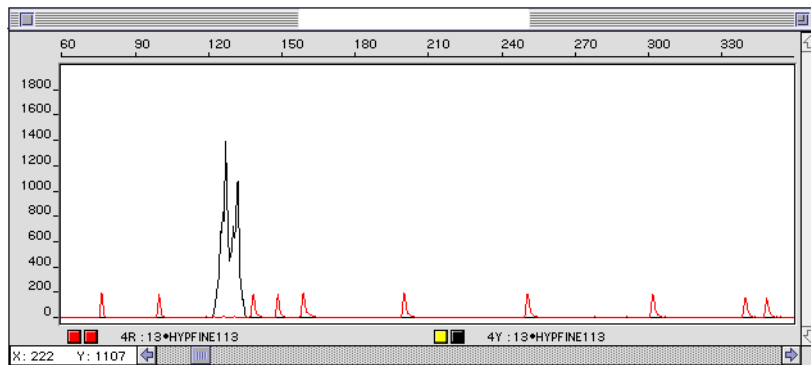
Figure 4-15. Example of good data with sharp, well-defined peaks

Characteristics of Poor Data

No Peaks

The absence of peaks in your results file could be due to no PCR amplification. To determine if this is the case, run another gel and include a lane of *Taq* Standard PCR Control or another reaction you know that has run successfully before. If you have no bands in your sample, but the size standard appears to be good, there was no amplification. For tips on optimizing PCR, see Appendix E.

Too Many Peaks— Noisy Data

If artifact bands, extra peaks, or peaks under peaks appear in your Sample files, try one or several of the following:

- Check for possible PCR carryover.
- Check PCR technique for practices that may have led to sample contamination.
- Check primer sequences for 3' complementarity.
- Design longer primers.
- Increase the amount of target DNA.
- Reduce the primer concentration.
- Reduce the number of cycles.
- Raise the annealing temperature.
- Decrease $MgCl_2$ concentration in your reaction.

Smeared or Fuzzy Bands

If you see smeared or fuzzy bands, try one or several of the following:

- Reduce the concentration of AmpliTaq DNA Polymerase.

- Create a Master Mix of PCR reagents to ensure each reaction is getting the correct amount of all reagents.

- Increase the annealing temperature and consider a two-temperature PCR protocol.

- Reduce the magnesium concentration.

- Minimize the incubation times of the annealing and extension steps.

- Decrease the number of cycles.

- Perform electrophoresis at lower voltage.

Troubleshooting Table

Table 4-3. Troubleshooting for PCR and Electrophoresis Conditions

Observation	Possible Causes	Potential Solution
PCR Conditions		
Unsuccessful amplification (faint or no peaks).	Impure DNA template. PCR inhibitors may exist in the DNA sample.	Try different extraction procedures combined with filtration of DNA through a spin column before amplification.
	Insufficient template DNA for human identification experiments.	Use recommended amount of template DNA. Use a procedure such as QuantiBlot™ Human DNA Quantitation Kit to accurately quantitate human DNA samples. If DNA is stored in water, check water purity.
	Insufficient enzyme activity.	Use the recommended amount of AmpliTaq DNA Polymerase.
	$MgCl_2$ solution not added to PCR control DNA reaction.	Add appropriate amount of $MgCl2$ solution to amplification reaction.
	Incorrect thermal cycling parameters.	Check protocol for correct thermal cycling parameters.
	GeneAmp PCR instrument system failure.	Choose correct amplification program for each primer pair.

Observation	Possible Causes	Potential Solution
Unsuccessful amplification (faint or no peaks).	High salt concentrations of K^+, Na^+, or Mg^{2+}.	Incorrect volume of DNA can alter pH: use correct amount of DNA, and buffer.
	High concentration of EDTA.	
	Incorrect pH.	
	Tubes not seated tightly in the thermal cycler during amplification.	Push reaction tubes firmly into contact with block before first cycle.
Unsuccessful amplification (faint or no peaks).	Wrong style tube.	Use Applied Biosystems GeneAmp Thin-Walled Reaction Tubes for the DNA Thermal Cycler 480, or MicroAmp Reaction Tubes with Cap for the GeneAmp PCR System 9600.
	Primer concentration too low.	Use recommended primer concentration.
Weak signal.	For multiplex PCR, lack of 2 minute down ramp time between denaturation and annealing.	Between denaturation and annealing, add 2 minute down ramp time to thermal cycling program. For multiplex reactions, the fastest ramp time is *not* necessarily the best.

Observation	Possible Causes	Potential Solution
Weak signal.	Incorrect Mg^{2+} concentration.	Store DNA in H$_2$O. The DNA volume should not be more than one fifth of the total reaction volume. EDTA in TE storage buffer affects the MgCl$_2$ concentration.
		Add correct amount of Mg^{2+} to reaction mix. Use correct dilution (1X) of 10X PCR buffer.
		Check dilution of dNTP stocks concentrations higher than recommended may lower the free Mg^{2+}.
		Perform additional organic extractions with EtOH precipitations and /or filter DNA through a spin column before amplification to remove any potential PCR inhibitors.
Inconsistent results with control DNA.	Incorrect PCR thermal profile program.	Choose correct temperature control parameters for PCR thermal profile (See *GeneAmp PCR System 9600 User's Manual*).
	System 9600 misaligned lid.	Align 9600 lid properly so that white stripes align after twisting the top portion clockwise.
	For DNA Thermal Cycler 480, Improper tube placement in block.	Refer to *DNA Thermal Cycler 480 User's Manual*.
	Pipetting errors.	Calibrate pipettes, attach tips firmly, check technique. Make a master mix of reagents.
	Combined reagents not spun to bottom of tube.	Place all reagents in apex of tube, spin briefly after combining.
	Combined reagents left at room temperature or on ice for extended periods of time.	Put tubes in block immediately after reagents are combined.

Observation	Possible Causes	Potential Solution
Unbalanced signals with control DNA.	Pipetting errors.	Calibrate pipets, attach tips firmly, check technique.
Unbalanced signals for samples.	PCR inhibitors may be present in DNA sample.	Try different extraction procedures. Filter DNA through a spin column before amplification.
Extra peaks visible when sample(s) is known to contain DNA from a single source.	Contamination with exogenous DNA.	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Renaturation of denatured samples.	Load sample immediately following denaturation, or store on ice until you are ready to load.
	Too much DNA in reaction, promoting renaturation.	Use recommended amount of template DNA. Use a procedure such as QuantiBlot Human DNA Quantitation Kit to accurately quantitate DNA sample.
	Too much DNA amplified and/or loaded resulting in crossover between color channels.	Check if any peak, particularly a homozygote, is over 6000 peak height. Re-run PCR using less DNA, or load less sample during electrophoresis.
For Stock-Marks For Cattle protocol, weak signal for TGLA53.	During thermal cycling, temperature ramps too quickly.	For GeneAmp instrument system, program one minute ramp rates.

Observation	Possible Causes	Potential Solution
Peaks are split into two peaks that are 1 bp different in size.	Partial non-template addition of an extra nucleotide to the blunt end of the PCR product. High Mg^{2+} concentrations can lower the degree of nucleotide addition.	Add correct amount of Mg^{2+} to reaction mix.
Signal continually gets weaker.	Outdated or mishandled reagents.	Check expiration dates on reagents and store and use according to manufacturers instructions. Compare with fresh reagents.
Electrophoresis Conditions		
Inconsistent sizing of known DNA sample.	Inadvertent change in analysis parameters.	Check settings for GeneScan analysis parameters.
	Incorrect internal standard.	Check internal size standard peaks against definition used.
	Incorrect gel composition.	Check if gel composition matches protocol requirements.
		Check urea concentration.
	Incorrect sample sheet.	Check Sample Sheet selection in data collection program, or check sample sheet embedded in Gel file.
Data was not automatically analyzed.	Sample sheet not completed.	Correctly complete sample sheet.

Observation	Possible Causes	Potential Solution
Crossover of one or more colors different than the primary color.	Incorrect matrix chosen.	Check matrix selection in Run window of data collection program or Gel file.
	Sample overloaded.	Load sample volume recommended in application protocol.
Samples run faster than usual with decreased resolution.	Incorrect gel composition.	Check whether gel composition matches protocol requirements.
Continued weakening of signal over time.	Degraded primers.	Store primers at -20°C when not in use. Keep on ice during set-up.
Additional colors displayed underneath the position of one strong peak.	Off-scale data not matrixed correctly.	Repeat electrophoresis; load less sample.
Peaks appearing in a dye color that should not be present.	Bleed through from other colors due to off-scale data.	Repeat electrophoresis; load less sample.
Allele peaks seen in correct molecular weight range, with additional peaks seen outside this range.	Bleed through from other colors due to off-scale data. Primers not optimized.	Repeat electrophoresis; load less sample. Refer to Appendix E.

Observation	Possible Causes	Potential Solution
With allele peaks of high intensity, GeneScan sites many small peaks.	Background above minimum peak height.	Adjust minimum peak height; reanalyze (See the *GeneScan Analysis Software User's Manual)*.
	Too much PCR product loaded on gel.	Repeat electrophoresis; load less sample.
No current.	Incorrect TBE buffer concentrations.	Check concentrations of TBE buffer: 10X in gel 1X in running buffer.
	Instrument not assembled correctly.	Check if leads are secure, and make sure that the bottom and top of gel immersed in running buffer.
	Instrument parts broken.	Check for breaks in leads, buffer chambers, or platinum wire.

Archiving the Data

For best performance, keep as few files as possible on your hard disk. Do not archive gel files and run folders on your hard disk. It is important that you copy all files you want to save onto a backup medium before beginning another sequencing run, both to preserve the files and to free disk space.

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 Run Log file. After the run, the analysis program creates Sample files.

Run Files

Each Run file stores information associating 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 and typically take up 30–50MB of disk space. A separate Gel file is created in the Run folder each time you perform a run. Because Gel files take a substantial amount of space, you should delete them from your hard disk after you have obtained satisfactory Sample files. You do not usually need to keep gel files once the lane tracking is verified 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 any gel file until you have verified the tracking and taken corrective action if necessary.

Run Log Files

Run logs provide a good record of your runs. Individually, Run 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 1.4 MB, high-density disk holds about six Sample or Sequence files. A Sample file is 150–200KB in size, depending on the length of the sequencing run. Save a Sample file when you feel confident that the channel selections (tracking) are correct for the sample.

Other Files

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

Sample Sheet Files

You can reuse the Sample Sheet, and it is a good reference after data collection. Individually, Sample Sheet files are small enough to archive on floppy disks.

Custom Analysis Settings and Matrix Files

If you have created customized files, you should keep backup copies of the files on a separate medium. Each of these types of files alone is small enough to store on a floppy disk.

5 Using the Data Collection Software

Contents

Overview of Data Collection Menu Commands

ABI PRISM 373 DNA Sequencer with XL Upgrade software programs are designed with the easy-to-use pull-down menus and the point-and-click technique commonly used in Macintosh-compatible programs. The menu bar at the top of the screen gives access to all functions. You can also use keyboard commands in many cases. If they are available, keyboard commands are listed opposite their corresponding commands on the pull down menus.

This section briefly describes each of the four menus on the data collection program main menu bar.
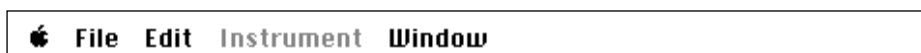
Apple Menu
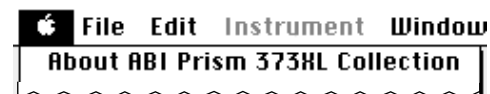
At the left end of the menu bar is the Apple menu, a standard feature of Macintosh applications. It displays desk accessories installed on your system.

The top command, About ABI PRISM 373 DNA Sequencer with XL Upgrade Collection, opens the splash screen, which shows general information about the data collection program. Click on it to close it.
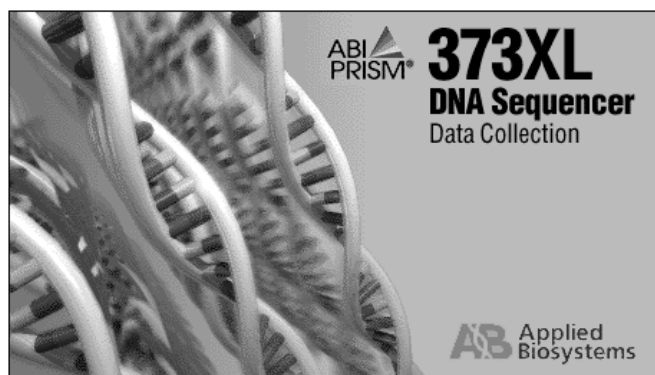
Figure 5-1. Splash screen

File Menu

```
┌─────────────────────────────┐
│ File                        │
│ New...              ⌘N      │
│ Open...             ⌘O      │
│                             │
│ Close               ⌘W      │
│ Save                ⌘S      │
│ Save As...                  │
│ Save a Copy In...           │
│                             │
│ Import...                   │
│ Export...                   │
│                             │
│ Page Setup...               │
│ Print One                   │
│ Print...            ⌘P      │
│                             │
│ Quit                ⌘Q      │
└─────────────────────────────┘
```

The File menu contains commands for creating, opening, closing, and saving windows, importing and exporting data to other file types, printing, and quitting from the application.

Table 5-1. File Menu Commands

Command	Description	See for Detail
Close, Save, Save As..., Revert, Page Setup..., Print..., Quit	Standard Macintosh system commands. See the *Macintosh System 7 Reference* manual	Page 5-65
New... and Open...	Open Run files or Sample Sheets for Sequencing or GeneScan applications. Use New to create a new file, or use Open to open an existing file.	Pages 5-34 to 5-40
Save a Copy In...	Saves a backup copy in a file other than the original. The name of the file with which you are working does not change.	Page 5-65

Table 5-1. File Menu Commands (continued)

Command	Description	See for Detail
Import...	Imports text from files such as those generated by a database into the grid of the Sample Sheet or Run window.	Page 5-33
Export...	Exports data from the grid of the Sample Sheet or Run window in tab-delimited text format for database applications.	Page 5-33
Print One	Prints one copy of the active window, bypassing the standard print dialog box.	Page 5-66

Edit Menu

The Edit menu contains standard clipboard commands, commands for selecting and searching, spreadsheet editing commands (Insert and Fill Down), and a command for changing the time scale in the Electrophoresis History window.

Table 5-2. Edit Menu Commands

Command	Description	See for Detail
Undo (Redo), Cut, Copy, Paste, Clear, and Show Clipboard	Standard Macintosh system commands. See the *Macintosh System 7 Reference* manual	pages 5-32, 5-38
Select All	Selects all entries in the open window.	
Fill Down	Applies a parameter change to all cells in a column of a Sample Sheet or Run window.	Page 5-38
Set Scale...	Sets the scale of the panels in the Electrophoresis History window.	Page 5-58

Applied Biosystems

Instrument Menu

```
Instrument
Start Plate Check
Start PreRun
Start Run
Pause
Resume
Cancel Run
```

The Instrument menu contains commands for starting, pausing and stopping the
ABI PRISM 373XL.

Table 5-3. Instrument Menu Commands

Command	Description	See for Detail
Start Plate Check	Allows you to scan the gel and plates before loading samples to ensure that no peaks are produced by fluorescent particles on the glass plates or in the gel.	Page 5-48
Start PreRun	Starts a pre-run of the gel, using parameters in the pre-run module specified in the Run window.	Page 5-48
Start Run	Begins automatic execution of a run using parameters in the run module specified in the Run window.	Page 5-48
Pause	Temporarily interrupts a run. During a pause, no additional functions are performed. The laser stops scanning, but any data remaining in instrument memory continues to be collected.	Page 5-48
Resume	Resumes a run that has been paused.	Page 5-48
Cancel Run	Only available if a module is currently running. Allows you to immediately stop the current module on the instrument. The run is ended and the Run file is closed. You cannot resume the run.	Page 5-48

Window Menu

Window
Status
Log
Sample Sheet
Run
Scan
Gel Image
Electrophoresis History
Manual Control
Preferences ▶
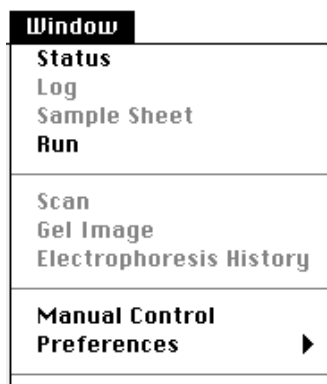
The Window menu contains a list of the default windows: instrument Status, Log file, Sample Sheet, Run file, Scan, Gel Image, and Electrophoresis History. At the bottom it also contains a list of any other open windows. Choosing any window from this menu brings that window to the front and makes it the active window.

- To move a window around on the screen, click the top border, hold the mouse button down, and drag the window to another location on the screen.

- To change the size of a window, click the size box in the lower right corner of the window, hold the mouse button down, and drag the mouse to stretch or shrink the window.

- To close a window, click the close box at the upper left corner of that window or click in the window to make it active and choose Close (⌘-W) from the File menu.

- To expand a window so that it displays the entire contents within the window, click the zoom box in the upper right corner.

Table 5-4. Window Menu

Command	Description	See for Detail
Status	Displays the status of hardware and status of the run. During a run, it displays current instrument conditions.	Page 5-50
Log	Text file that contains date- and time-stamped status and error messages in chronological order.	Page 5-51
Sample Sheet	Allows you to specify sample information for each lane position in the gel. The information you enter depends on the analysis program you are using.	Page 5-58
Run	Allows you to specify the Sample Sheet and module information for an automatic run.	Page 5-58
Scan	Displays electropherogram of raw data as it is being collected during the run.	Page 5-55
Gel Image	Displays a reconstruction of actual data as it would appear on an autoradiogram. It is updated as data is collected from the instrument.	Page 5-56
Electrophoresis History	Displays target and actual values for the electrophoresis power supply and gel temperature throughout the course of an electrophoresis run.	Page 5-57
Manual Control	Displays a window for manual control of certain functions during a run.	Page 5-59
Preferences...	Allows you to specify folder locations, file name conventions, and default settings.	Page 5-16

Using the Data Collection Program

Before you start an ABI PRISM 373XL run, the following conditions should apply:

- You have set or verified Preferences

- You have created the Sample Sheet and the Run file

- The instrument is ready and samples are loaded

- The Macintosh hard disk has adequate free space to store the collected data (It needs a minimum of 40 to 60MB free disk space for an average run.)

To learn about preparing gels and setting up the instrument, refer to Sections 2 and 3. For information about choosing a proper sequencing chemistry, refer to the *ABI PRISM DNA Sequencing Analysis Chemistry and Safety Guide.* For information about GeneScan experimental design, refer to the *GeneScan 672 User's Manual.*

This section describes how to use the data collection software to create and edit Sample Sheets and Run files, to perform a run, and to view the resulting data.

To prepare the software for a run, perform the following general steps:

- Restart the computer

- Start the data collection program

- Set or verify preferences

- Create a Sample Sheet*

- Create a Run file

When you start the data collection program and begin a run, the software displays data as it is received from the ABI PRISM 373XL, and you can view the data in different views, described on page 5-49. The data is stored on the computer.

The data collection program typically runs for approximately 3.5–16 hours (1–6 hours for GeneScan) from the time you start the run to the time data analysis begins.

---

[*] If a Sample Sheet already contains information that applies to your samples, you do not need to create a new one. Specify lane position for each sample when you create the Run file.

Starting the Data Collection Program

- Choose Restart from the Special menu in the Finder to restart your computer.

  The data collection software should automatically open when you restart the computer. If it does not, double-click the icon to open the program. Refer to the instructions below to have the 373XL collection program open at startup.

If any of the data collection folders are not in the default locations, a dialog box appears asking you to specify where they are located.

The data collection program allows you to indicate preferences for folder locations, file name conventions, and default settings. To do so, refer to *Setting Preferences* on page 5-14.

Note   *If communications between the computer and the instrument do not seem to be working properly, refer to Total Reset of the Instrument on page 8-8.*

To make the data collection program open at startup:

1. Locate and select (single-click) the 373XL data collection program icon.

2. Choose Make Alias (⌘-M) from the File menu.

3. Open the System folder and locate the Startup Items folder.

4. Drag the 373XL collection alias into the Startup Items folder.

5. Choose Restart from the Special menu.

Setting Preferences

The data collection program allows you to indicate preferences for folder locations, file name conventions, and default settings.

You can choose several types of preferences to set (see Figure 5-3):

- Folder Locations
- File Names

For Sequencing analysis {
- Sequence Sample Sheet Defaults
- Sequence Run Defaults

For GeneScan analysis {
- GeneScan Sample Sheet Defaults
- GeneScan Run Defaults

- General Settings
- Dye Indicators

The defaults are global parameters that appear in the data entry windows so they apply to all samples. Since separate Sample Sheets and Run files exist for sequencing and GeneScan analysis, the defaults you can set are also somewhat different.

Although the defaults you set appear when you open a new Sample Sheet or Run file, you can change the fields as required for the individual situation.

Some default settings also appear in the Page Setup dialog box for your particular printer. They are discussed in the section on printing (see *Printing* on page 5-67).

| Note | The preferences you set are saved automatically when you close the Preferences window. You do not need to save each page individually. |

To set preferences:

1. Choose Preferences from the Window menu and choose the appropriate option from the submenu that appears.
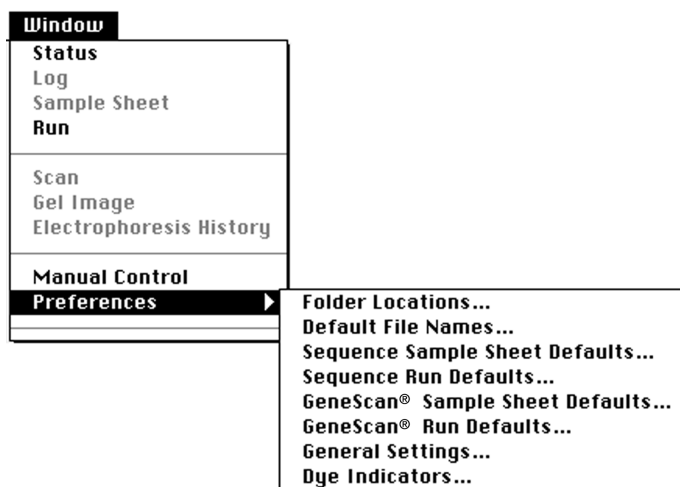
Figure 5-2. Preferences submenu

2. Enter or change the defaults, as described on the pages below.

3. Click OK to accept the changes.

Note	*You can switch from one preferences window to another by using the pop-up menu labeled "Page:" in the upper left corner of each preferences window.*

Setting Folder and File Preferences

Setting Folder Location Preferences

The data collection program automatically uses several specific folders for data storage. You can specify that it use different folders by changing the folder location preferences.

The Folder Locations page of the preferences dialog box shows the current folders that store Sample Sheets, modules, runs, the firmware image, and settings (see Figure 5-3).

To set other preferences select from this pop-up menu. Any changes you made in this screen are saved automatically

To change the folder where Settings are stored, click this button   Click here to return to the main menu
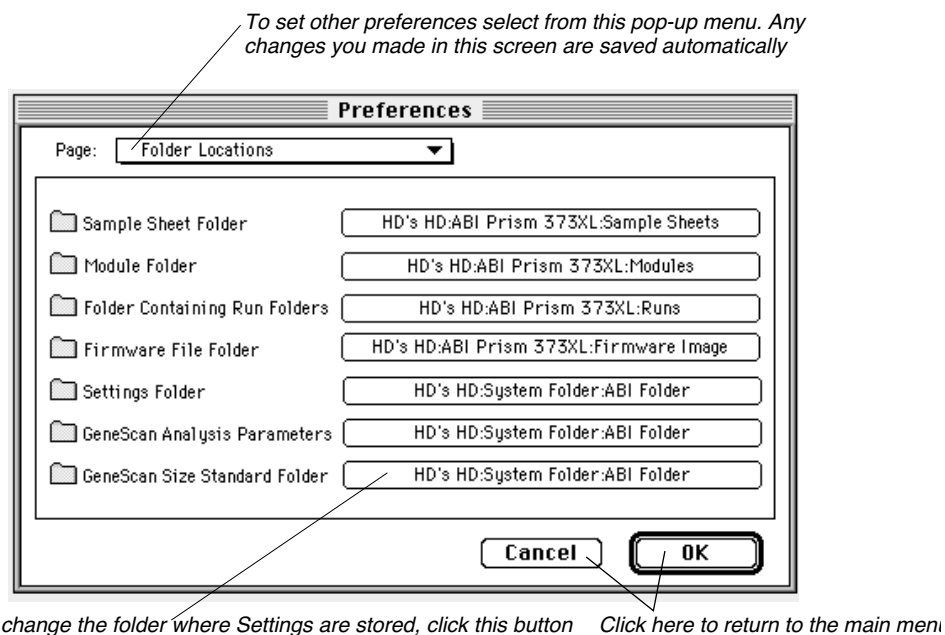
Figure 5-3. Preferences Folder Locations window

The locations initially shown in this screen are the folders where the indicated files are normally stored. If you store them in other folders, it is important that you specify in this screen where you store them. When you create Sample Sheets, modules, and Run files, the pop-up menus that allow you to choose various options refer to the folders indicated in this screen.

- The Sample Sheet Folder button shows the name and path of the folder in which you store your Sample Sheets. The Run window pop-up menus that allow you to choose a Sample Sheet file refer to this folder.

- The Module Folder button shows the name and path of the folder where you store modules. This is referred to by the Run window pop-up menus for plate check, pre-run, and run modules.

- The Folder containing Run folders button indicates the name and path of the folder in which Run folders are created when you start a new run.

- The Firmware Image folder button indicates the name and path of the folder that contains the Firmware Image file. The first time you turn on the ABI PRISM 373 DNA Sequencer with XL Upgrade and open the data collection program, the program looks in this folder for the image file, which it automatically copies to the instrument.

- The Settings Folder button indicates the folder containing mobility files, matrix files, analysis settings files, fragment size standard files, and comb files. The choices in other Preferences dialog boxes and in the Run windows refer to this folder for their pop-up menus. The Settings folder is initially set to be the ABI folder inside your System folder.

IMPORTANT   *If you plan to analyze your data using the Sequencing Analysis software program after data collection, the Settings folder must be set as the ABI folder.*

To change one of the data storage folders:

1. Choose Preferences from the Window menu and choose Folder Locations from the submenu that appears.

    If you are already in the Preferences window, choose Folder Locations from the pop-up menu labeled "Page:".

2. Click the button showing the folder's path name to display a dialog box (Figure 5-4).
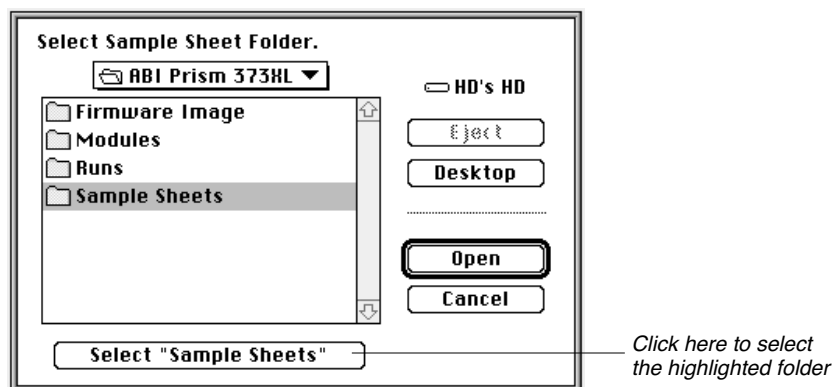
Figure 5-4. Folder selection dialog box

3. Find the new location and click the Select button at the bottom of the file selection dialog box (Figure 5-4).

IMPORTANT   *The data collection software allows you to specify a Settings folder location. If you analyze your data with the ABI PRISM Sequencing Analysis program on the computer that you use for data collection, the Settings folder must always be specified as the ABI folder that is located in the System folder.*

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4. When you have changed all the desired folders, click OK in the Folder Locations Preferences dialog box (Figure 5-3 on page 5-16) or choose another preference from the Page pop-up menu.

These folder locations are normally on your local hard disk, but can be on any AppleShare server volume as well.

Setting File Name Preferences

The data collection program automatically names new files and folders created during a run (Sample Sheet files, Run folders, Run files, gel files). The Default File Names page of the preferences dialog shows the method used for automatically creating file and folder names (see Figure 5-5).

pop-up menus allow you to select suffixes

Enter text in the entry fields to change names
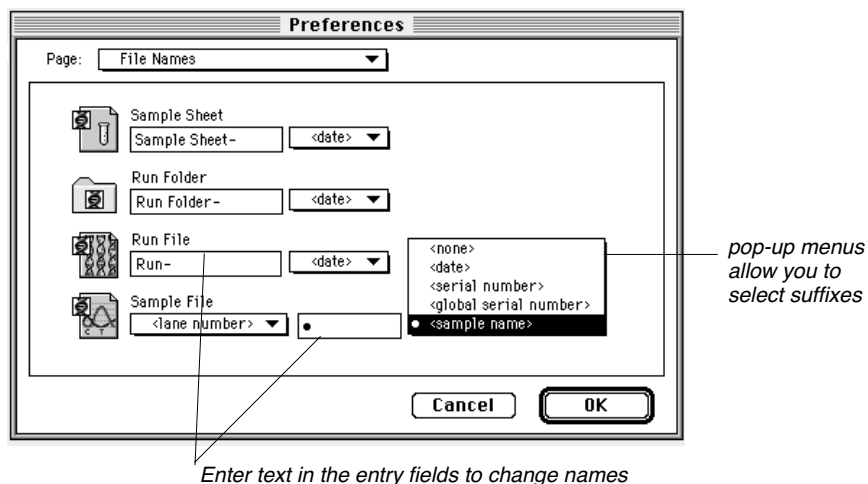
Figure 5-5. Preferences File Names window

A file name can be any text string or nothing at all. By default, the software adds the date as a suffix, but you can choose to not display a suffix. A Sample file can have a prefix and a suffix. The prefix can be the lane number or the sample name. You can choose the suffix from a pop-up menu. The choices are:

- Nothing <none>

- Today's date and the current time <date>

- A serial number reset to one 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>

If you manually save any of these types of files, the name as specified in this dialog box appears automatically in the Save dialog box. You can change it if you wish.

To change automatically generated file names:

1. Choose Preferences from the Window menu and choose File Names from the submenu that appears.

    If you are already in the Preferences window, choose File Names from the pop-up menu labeled "Page:".

2. Enter the desired text in the entry field for each type of automatically-named file or folder.

    The text you enter will be the prefix for all file names of that type.

3. Specify a suffix by choosing from the pop-up menu to the right of the text entry field.

4. When you are finished specifying file name defaults, click OK or choose another preference from the Page pop-up menu.

Note   *If you choose the "global serial number" option for a suffix, you can reset the global serial number if necessary. To do so, see page 5-29.*

Applied Biosystems

Setting Defaults for Sequencing Analysis Applications

The following two headings discuss default settings for Sequencing Analysis applications only. If you are running GeneScan applications, see *Setting Defaults for GeneScan Analysis Applications* on page 5-25.

Setting Sequence Sample Sheet Defaults

If you are running sequencing analysis applications, you can set defaults for two of the choices in the Sample Sheet (Figure 5-6).

The pop-up menu options are files in the Settings* folder and are filtered for the correct file type
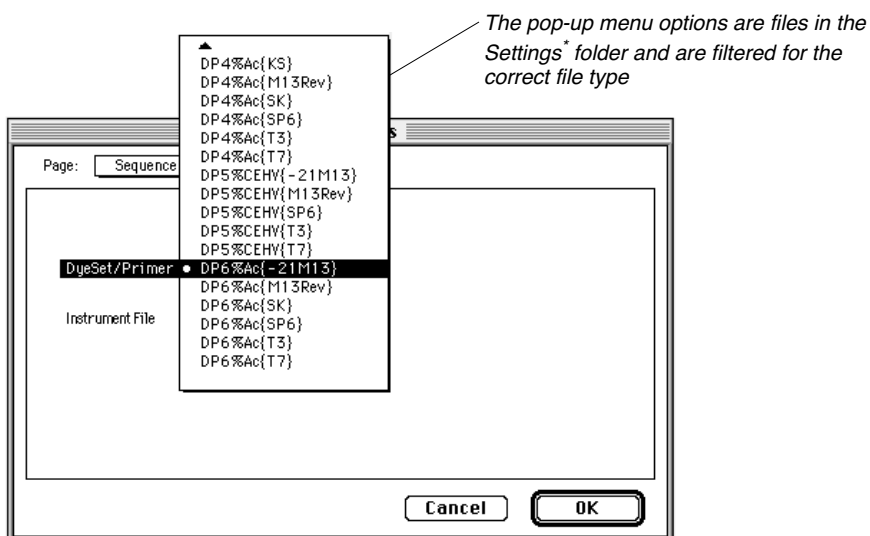
Figure 5-6. Sequence Sample Sheet Defaults window

Both the DyeSet/Primer and the Instrument file parameters have pop up menus that allow you to choose none or any dye/set and Instrument file in the Settings* folder.

To set Sample Sheet defaults for Sequencing Analysis applications:

1. Choose Preferences from the Window menu and choose Sequence Sample Sheet Defaults from the submenu that appears.

* If you are running Sequencing Analysis applications, this folder should be set as the ABI folder in your System folder (see page 5-17).

If you are already in the Preferences window, choose Sequence Sample Sheet Defaults from the pop-up menu labeled "Page:".

2. Choose parameters for the two defaults by selecting from the pop-up menus.

    The files listed in the menu are all the applicable files in the Settings folder (filtered according to type). If you wish to choose a file that is not listed, choose Other, and a file dialog box allows you to choose any applicable file in any other folder.

3. Click OK or choose another preference from the Page pop-up menu.

Setting Sequence Run Defaults

You can set several defaults for the Run file of a Sequencing Analysis run (see Figure 5-7).

To set Run file preferences for Sequencing Analysis applications:

1. Choose Preferences from the Window menu and choose Sequence Run Defaults from the submenu that appears.

    If you are already in the Preferences window, choose Sequence Run Defaults from the pop-up menu labeled "Page:".

Pop-up menus allow you to choose from filtered lists of files. The procedure below lists where the files are located. To choose a file that is not listed, choose Other. A file dialog box allows you to choose files from other folders
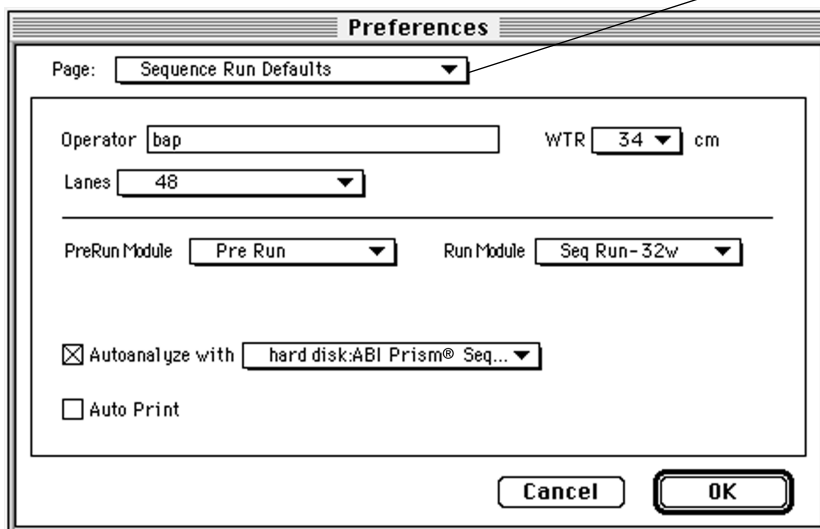
Figure 5-7. Preferences Sequence Run Defaults window

2. Set the defaults as described below.

   a. Enter your name in the Operator entry field.

   b. Specify the separation distance by choosing from the WTR (well-to-read) pop-up menu.

      The well- to-read (WTR) distance for sequencing runs is usually 24, 34, or 48 cm.

   c. Click Lanes and choose the number of lanes from the pop-up menu that appears.

   d. Choose default modules from the PreRun Module and the Run Module pop-up menus.

      The menu shows all Module files in the Module folder (see page 5-17).

   e. To specify that the data be automatically analyzed by the Sequencing Analysis program, select the checkbox labeled "Autoanalyze with" and choose Other from the pop-up menu.

      In the dialog box that appears, find the analysis program and click Open.

      This specifies the location of the analysis program.

   f. Select the checkbox at the bottom of the window to specify automatic printing of the data after analysis.

3. Click OK or choose another preference from the Page pop-up menu.

Setting Defaults for GeneScan Analysis Applications

The following three headings discuss default settings for GeneScan Analysis applications only. If you are running sequencing applications, see *Setting Defaults for Sequencing Analysis Applications* on page 5-21.

Setting GeneScan Sample Sheet Defaults

If you are running GeneScan analysis applications, you can set defaults for four Sample Sheet entries.

To set Sample Sheet defaults for GeneScan analysis applications:

1. Choose Preferences from the Window menu and choose GeneScan Sample Sheet Defaults from the submenu that appears.

    If you are already in the Preferences window, choose GeneScan Sample Sheet Defaults from the pop-up menu labeled "Page:".
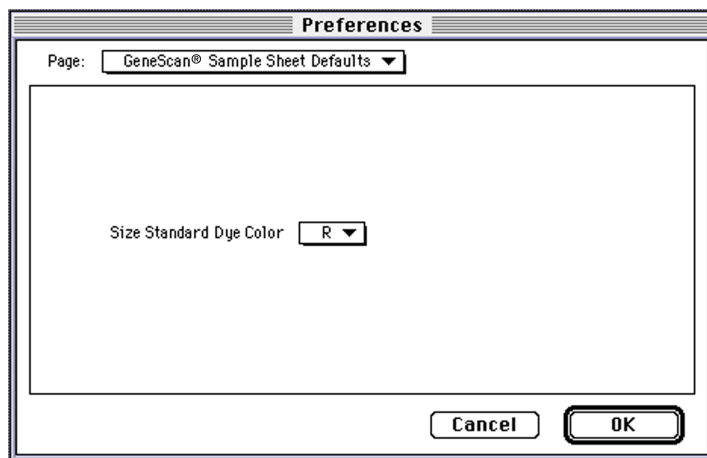
Figure 5-8. Preferences GeneScan Sample Sheet Defaults window

- Choose the color for the size standard color you are using.

2. Click OK or choose another preference from the Page pop-up menu.

Setting GeneScan Run Defaults

To set Run file preferences for GeneScan Analysis applications:

1. Choose Preferences from the Window menu and choose GeneScan Run Defaults from the submenu that appears.

   If you are already in the Preferences window, choose GeneScan Run Defaults from the pop-up menu labeled "Page:".

   A dialog box appears.
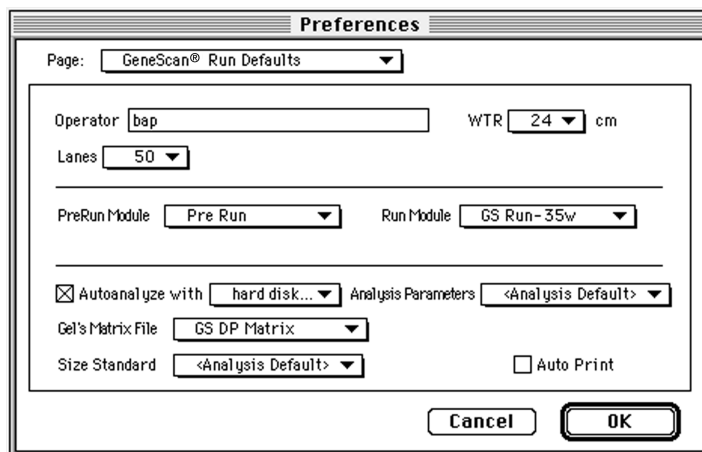
Figure 5-9. Preferences GeneScan Run Defaults window

The default options are the same as for sequencing runs, but the filtered choices might be different for GeneScan analysis.

2. Set the defaults as described below.

    a. Enter your name in the Operator entry field.

    b. Specify the separation distance by choosing from the WTR (well-to-read) pop-up menu.

       The well to read (WTR) distance for GeneScan analysis is usually 6, 12, 24, or 34 cm.

    c. Choose the number of lanes from the Lanes pop-up menu.

    d. Choose default modules for the pre-run and the run from the Module pop-up menus.

       The menu shows all Module files in the Modules folder (see page 5-17).

    e. To specify that the data be automatically analyzed by the GeneScan Analysis program, select the checkbox labeled "Autoanalyze with" and choose Other from the pop-up menu.

       In the dialog box that appears, find the analysis program and click Open.

       This specifies the location of the analysis program.

    f. Select the checkbox at the bottom of the window to specify automatic printing of the data after analysis.

3. Click OK or choose another preference from the Page pop-up menu.

General Settings

The General Settings window allows you to specify:

- A global serial number that increments across runs and can be used in the names of automatically generated files (see page 5-19).

- The module to be used for checking the plates.

- The computer port to which the instrument is attached. Set to modem port.

   The Macintosh computer has two standard connection ports: a Modem port and a Printer port. Normally, the instrument is attached to the

computer by means of the Modem port, and a printer or network connection is attached to the Printer port. If, however, you need to move the instrument connection to the Printer port, specify that you have made the change in the general settings. A third option allows you to use the data collection software when no instrument is attached to the computer.

• The minimum number of scan lines in a gel.

The size of a gel file is calculated according to the collect time you enter in the Run window. The number in the General Settings specifies the minimum number of scan lines for a gel file.

IMPORTANT *Do not change the minimum number of scan lines in a gel. If you make it smaller, the data collection software might not allocate enough hard disk space to collect all of your data.*

To change the general settings:

1. Choose Preferences from the Window menu, and then choose General Settings from the submenu that appears.

    If you are already in the Preferences window, choose General Settings from the pop-up menu labeled "Page:".
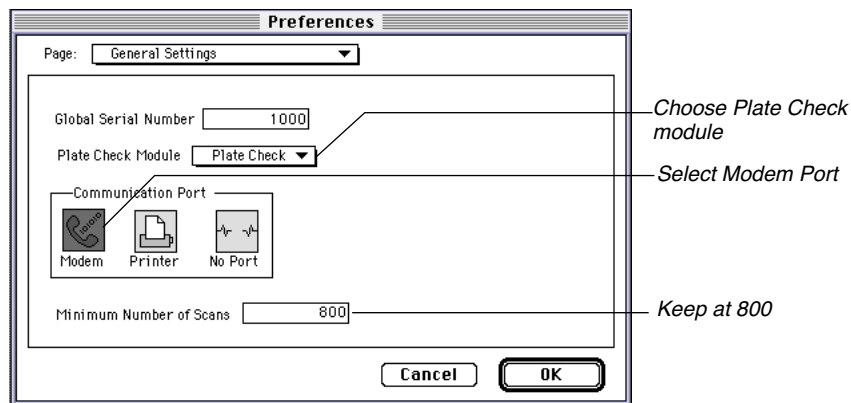
Figure 5-10. General settings window

2. Make changes as desired.

- To change the global serial number, select the number in the entry field and type the new number.

- Choose a plate check module from the pop-up menu.

- If you changed the instrument connection, click the name of the port to which the instrument is now attached. If you wish to use the data collection software off-line (not connected to the instrument), click No Port.

- To change the initial file size of the Gel file, select the number in the Number of Scans entry field and type a new one. You should not need to change this setting (see note below).

3. Click OK or choose another preference from the Page pop-up menu.

Note   *The data collection software always performs at least the minimum number of scans specified in the General Settings window. It performs more than the minimum specified if necessary to complete a run, based on the collection time specified in the Run window.*

Setting Dye Indicators Defaults

To change the colors that represent the dyes when data is displayed, you can set Dye Indicators defaults. This is especially useful if you are color blind and the default settings shipped with the software are not appropriate for your needs.

To set dye indicator preferences for the analysis applications:

1. Choose Preferences from the Window menu and choose Dye Indicators from the submenu that appears.

If you are already in the Preferences window, choose Dye Indicators from the pop-up menu labeled "Page:".

Dye Color is the color that appears on the computer screen

Plot color is the color that appears on the printed electropherogram

To change a color, click the button and choose a new color from the pop-up
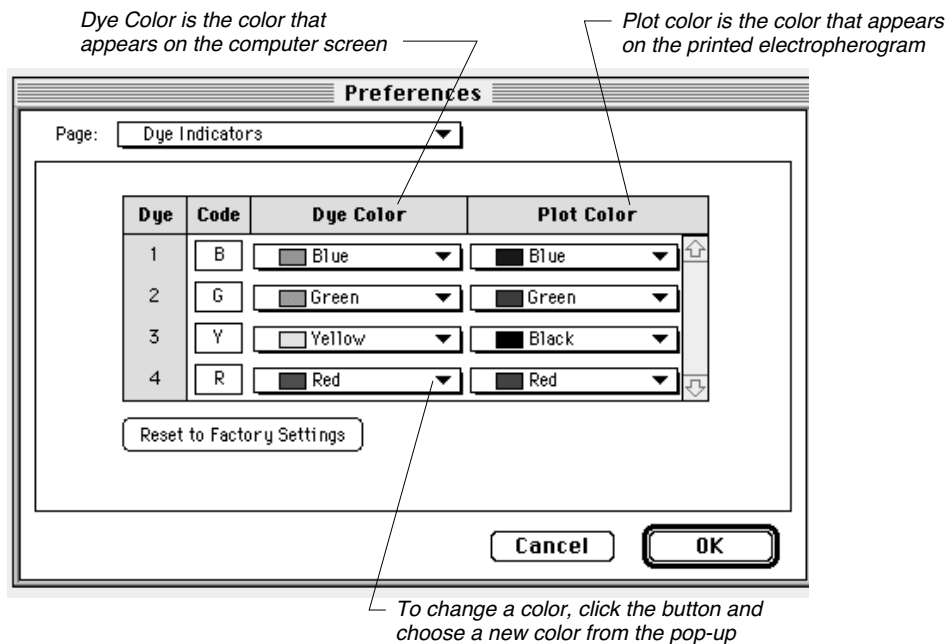
Figure 5-11. Preferences Dye Indicators window

2. Make changes as desired.

- To change a code, type a different character in the appropriate entry field in the Code column.

- To change a color, click the appropriate color button and choose a new color from the pop-up menu that appears. The Display Color column shows the colors used on the computer screen. The Print Color column shows the colors used for printing.

  If you choose Other from the pop-up menu, a color picker appears (Figure 5-12).

The upper box shows the selected color

The lower box shows the previous color

Click a scrolling arrow or the color wheel to change colors

Hue	19287
Saturation	4771
Brightness	56683
Red	53523
Green	56683
Blue	52556

Cancel   OK

Use the scroll bar to quickly change brightness
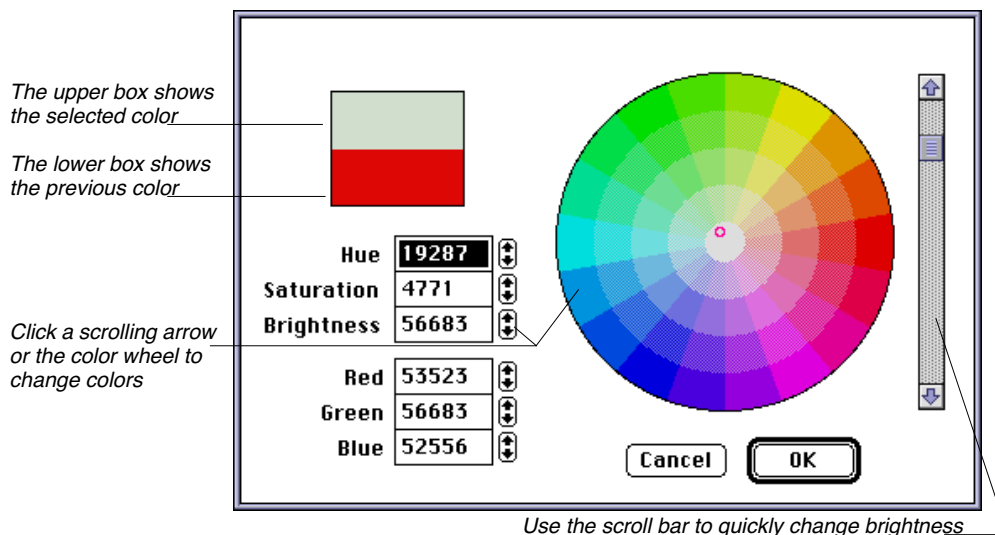
Figure 5-12. Color picker

You can choose a new color by using either the scrolling fields to the left or the color wheel to the right.

To use the scrolling fields, click one of the up or down arrows and hold down the mouse key. The upper half of the color box shows the colors as the parameters change. The lower half retains the original color until you click OK to accept a new color.

To use the color wheel, click the desired color in the wheel. The new color appears in the upper half of the color box. Move the insertion point and click again until you find the desired color.

Click OK when you are satisfied with the color.

3. When you are finished changing dye indicator preferences, click OK or choose another preference from the Page pop-up menu.

Creating and Editing a Sample Sheet

Before starting a run you must record sample information in the Sample Sheet and the Run file. Once you have set up the Sample Sheet, you can automatically copy sample information into the Run file, which associates sample information (name and type of analysis) with each lane position in the gel.

You can choose from two different Sample Sheets (typing or templates), depending on your application (Sequencing or GeneScan analysis). The only difference between them is in the entry fields. The entry fields in each are described under *Creating a Sequence Sample Sheet* on page 5-34 and *Creating a GeneScan Analysis Sample Sheet* on page 5-36. This general information applies to both.

IMPORTANT	Do not mix sequence analysis and GeneScan analysis samples on the same Sample Sheet or the same run.

The information you record in the Sample Sheet becomes the sample identification and other sample-related information in the sample file when the data is analyzed. The information that you record is critical to the accuracy of automatic analysis, and is a good reference for you after data collection.

The Sample Sheet is a two-dimensional array, similar to a spreadsheet or ledger. It consists of rows and columns of fields, each field separated by horizontal and vertical lines (see Figure 5-13). It accepts more text than fits in the visible fields and automatically scrolls as you type your entry. To view the beginning of a long entry, use the keyboard arrow keys. Many of the cells have default values that are automatically filled in for each sample.

You can use the standard File menu and Edit menu commands in the Sample Sheet window. You can import from tab-delimited text files (for instance, files generated by a database) and print the window. You can reuse a Sample Sheet as often as you wish.

You can export the Sample Sheet as a tab-delimited text file for database, spreadsheet, or word processing programs (choose Export from the File menu).

Importing and Exporting Sample Sheet Information

If you wish to import information from a database into the Sample Sheet, first export the information from the database into a tab-delimited text file. Note the following:

- 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, in the same order as the Sample Sheet columns.

Exporting a Sample Sheet creates a tab-delimited text file, with the same format as a file created for import.

To import a text file to the Sample Sheet:

1. Choose Select All (⌘-A) from the Edit menu to select all fields in the Sample Sheet grid.

2. Choose Import from the File menu.

3. In the dialog box that appears, choose the file you wish to import.

4. Click OK.

To export from the Sample Sheet to a text file:

1. Make sure the Sample Sheet window is active. If it is not, click in it.

2. Choose Export from the File menu.

3. In the dialog box that appears, enter a name for the text file, and then choose where to save the file.

4. Click OK.

The exported file has an icon like that of a Log file:

sample export

Creating a Sequence Sample Sheet

In the Sequence Sample Sheet you specify dyeset/primer and matrix information for each sample you plan to run. You can specify default values for both fields by setting preferences. The defaults appear automatically for each sample, but you can change them by editing the Sample Sheet. See *Setting Defaults for Sequencing Analysis Applications* on page 5-21.

To create a new Sequence Sample Sheet:
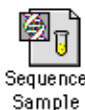
Sequence
Sample

1. Choose New (⌘- N) from the File menu.

2. Click the Sequence Sample Sheet icon in the window that appears.

    If you set default values they appear in a new Sample Sheet automatically.

Click an arrow to display a pop-up menu of options

Click a title to select the entire column

Sample lane numbers

When you set defaults for mobility and Instrument files, the columns are filled in automatically
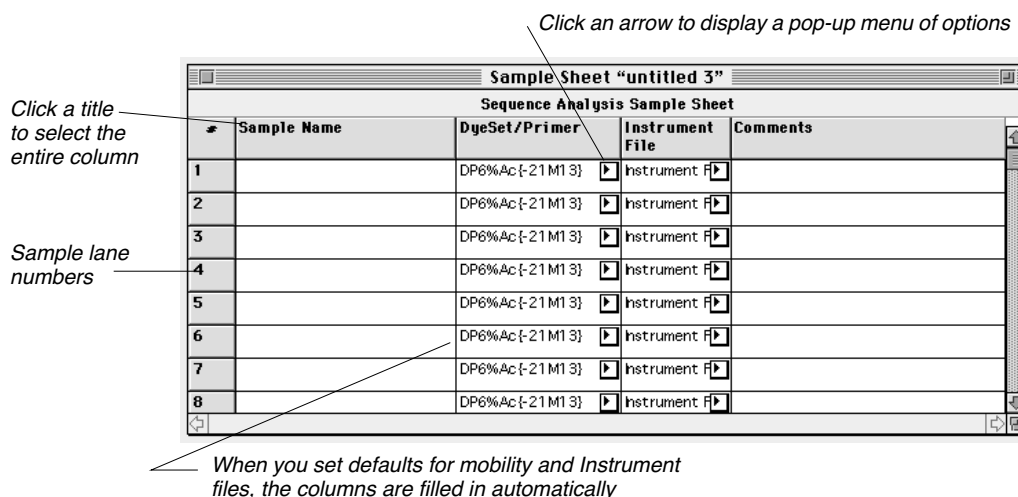
Figure 5-13. The Sequence analysis Sample Sheet

To move from one field to another:

- Click a desired field

- Press the directional arrow keys on the keyboard

- Press Tab to move to the next field to the right

- Press Return to move to the next field down

3. Enter/change information as necessary.

Note To import information from tab-delimited text files, see page 5-33.

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a. In the second column, enter the sample names corresponding to each lane number.

b. To choose a DyeSet/Primer mobility file or a multicomponent matrix other than the default, click the arrow in the field you wish to change and choose the new file from the pop-up menu that appears.

The DyeSet/Primer files and a default matrix file (the Instrument file) are provided with your instrument. When you click a field in the DyeSet/Primer column, the pop-up menu shows only available DyeSet/Primer files.

The DyeSet/Primer file names indicate information that helps you choose the correct file. See DyeSet/Primer File Naming Conventions in your Sequence Analysis User's Manual.

For most sequencing chemistries the default matrix supplied with your instrument is appropriate. To read about matrices refer to Appendix C, *Sequencing Instrument File.*

Note To apply the same parameter to all fields in a column, select the parameter in the top field of the column, click the column title to select the entire column, and choose Fill Down from the Edit menu.

---

c. To enter a comment, click the comment field. An insertion point appears, allowing you to type in your comment.

4. Click the close box to accept the entries.

Note If you analyze with the wrong dye/primer set, you can use manual analysis to re-analyze the data against the correct set. Refer to the ABI PRISM DNA Sequencing Analysis Software User's Manual.

Creating a GeneScan Analysis Sample Sheet

In the GeneScan Sample Sheet, in addition to Matrix defaults, you can specify preferences for the size standard and the dye color used for the standard (see *Setting Defaults for GeneScan Analysis Applications* on page 5-25). The defaults appear automatically for each sample, but you can edit them in the Sample Sheet.

To create a new GeneScan Sample Sheet:

1. Choose New (⌘- N) from the File menu.

2. Click the GeneScan Sample Sheet icon in the window that appears.

Figure 5-14. GeneScan Sample Sheet

To move from one field to another:

- Click a desired 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

3. Enter/change information as necessary.

    a. Type a descriptive sample name in the Sample Name column.

    b. Select a size standard dye color.

       The size standard marker (X) appears in the Std field next to the specified standard dye. You can mark only one size standard for each sample, and each sample can include up to four dyes per sample.

    c. Enter dye/sample information in the Sample Info column.

       The checkbox labeled Used becomes selected so the sample can be analyzed automatically.

    d. To enter a comment, click the Comments field.

       A text entry box appears, allowing you to type in your comments.

Note	*To import information from tab-delimited text files, see page 5-33.*

IMPORTANT	*For downstream analysis and sorting, you must fill out both the Sample Sheet and the Sample Information window when you create a new GeneScan Sample Sheet.*

4. Choose Save from the File menu.

Editing a Sequence or GeneScan Analysis Sample Sheet

When you edit Sample Sheets the general procedure is the same, whether it be a Sequence Sample Sheet or a GeneScan Analysis Sample Sheet. Follow the general procedure described here, and refer as necessary to the instructions for creating the individual Sample Sheets.

Note	*The Sample Sheet can contain up to 72 rows. If you insert rows beyond that, the last row in the sheet is dropped off.*

You can use the standard Edit menu commands for the File Name, Sample Name, or Comments fields.

To modify an existing Sample Sheet:

1. Choose Open (⌘- O) from the File menu, and choose the file you want from the file dialog box that appears.

2. Edit fields as described for the individual Sample Sheets.

3. Save the Sample Sheet by choosing Save As from the File menu.

   Be sure to save the file under a different name, so you preserve the original file.

Note	You cannot change a run in progress by editing the Sample Sheet. You can change the Sample Sheet, but cannot re-select it in the Run window while the run is in progress. The changes are therefore not effective for the run in progress.

To move between fields in the Sample Sheet:

- Click a desired 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

To apply the same parameter to all fields in a column:

1. Select the parameter in the top field of the column.

2. Click the column title to select the entire column.

3. Choose Fill Down (⌘-D) from the Edit menu.

To copy information from one field to another:

1. Click the field to be copied.

2. Choose Copy (⌘-C) from the Edit menu.

3. Click the new field.

4. Choose Paste (⌘-V) from the Edit menu.

To copy an entire row of information to another row:

1. Click the lane number in the left-most column to select the entire row.

2. Choose Copy (⌘-C) from the Edit menu.

3. Click the lane number of the row you wish to copy into.

4. Choose Paste (⌘-V) from the Edit menu.

To move sample information to a different lane:

• Hold down the Option key and click the lane number of the row containing the information you want to move, and drag the lane number to another lane number.

When you release the mouse button the sample information appears in the new location. The lane number does not move.

Using the Run Window

In the Run window you specify the lane in which each sample will run and the modules containing the parameters to be used for the run. You also use this window to start a plate check, a pre-run, or a run.

Separate Run files exist for sequencing and GeneScan analysis applications. Be sure to choose the correct type for your application.

Opening the Run Window

Sequence Run

1. Choose New from the File menu and click the appropriate Run icon.

    This automatically creates a new Run folder, and opens the Run window.

GeneScan® Run

Figure 5-15. Sequence Run window

Small document icons open the selected
module or Sample Sheet for review

Specify length of run

Run control
buttons

Pop-up menus in
these panels allow
you to choose the
modules
(parameters), the
associated
Sample Sheet, the
number of wells,
the separation
distance (WTR),
and the matrix for
the run

Lane numbers cannot be edited
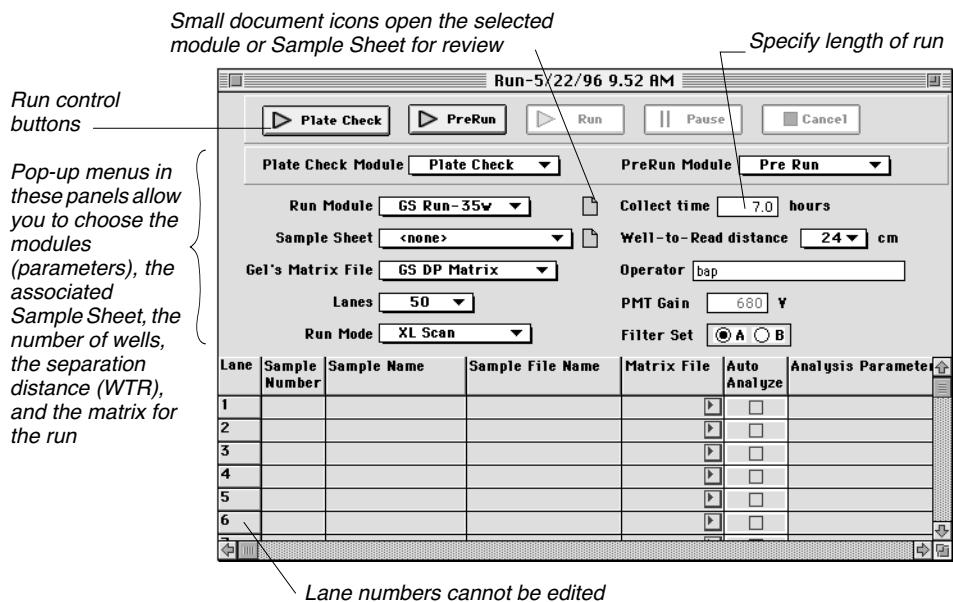
Figure 5-16. GeneScan Run window

You need not complete the entire window to perform a plate check. You
must do so to run samples.

Completing a Run Window

You must complete the information in the Run window before pre-running a gel
or running samples.

To complete the Sequence Run window:

1. Choose the parameters for the run from the pop-up menus.

    a. Choose modules to be used for the plate check, the pre-run and the
        run in the Module pop-up menus.

    b. Choose the Sample Sheet from the Sample Sheet pop-up menu.

        When you choose the Sample Sheet, the information about the lanes
        in which the samples are to run fills in automatically.

c. Choose the number of lanes and the separation distance from the Lanes pop-up menu and the WTR pop-up menu, respectively.

d. Choose an instrument file from the Instrument File pop-up menu.

   Appendix D describes creating and using matrices.

e. Choose XL Scan or Full Scan in the Run Mode pop-up menu. Use the table below to determine the appropriate run mode for your lane configuration.

Lanes	Comb	Run Mode
24	Sharks-tooth	Full Scan
32	Sharks-tooth	Full Scan
36	Sharks-tooth	Full Scan
36	Square-tooth	Full Scan
48	Sharks-tooth	XL Scan
64	Sharks-tooth	XL Scan

2. Enter the length of the run in the Collect Time entry field. Following are some suggested run times for Sequencing runs.

Plate length (cm)	Type of Run	Typical Collection Time (hours)
24	Full Scan or XL Scan	12
24	BaseSprinter	6.5
34	Full Scan or XL Scan	14
34	BaseSprinter	7
48	Full Scan or XL Scan	16

3. Enter your name in the Operator entry field.

   The sample information for each lane is entered automatically in the grid at the bottom of the Run window when you choose a Sample Sheet.

Note   *You cannot edit the Sample Sheet information in the Run window. Make changes in the Sample Sheet, and select the Sample Sheet again in the Run window to make the changes effective.*

4. Choose the appropriate filter set:

For Sequenase T7 Terminator chemistry: Select filter set B.

For all other sequencing chemistries: Select filter set A.

5. Select the Auto Analyze and Auto Print checkboxes if you want the analysis program to analyze and print the data.

To complete the GeneScan Run window:

1. Choose the parameters for the run from the pop-up menus.

Note | Any changes you make in your preferences setting will not take effect until you open a new Run window.

a. Choose modules to be used for the plate check, the pre-run and the run in the Module pop-up menus.

b. Choose the Sample Sheet from the Sample Sheet pop-up menu.

When you choose the Sample Sheet, the information about the lanes in which the samples are to run fills in automatically.

c. Choose the number of lanes and the separation distance from the Lanes pop-up menu and the WTR pop-up menu, respectively.

d. Choose XL Scan or Full Scan in the Run Mode pop-up menu. Use the table below to determine the appropriate run mode for your lane configuration.

Lanes	Comb	Run Mode
24	Sharks-tooth	Full Scan
24	Square-tooth	Full Scan
32	Sharks-tooth	Full Scan
34	Square-tooth	Full Scan
36	Sharks-tooth	Full Scan
36	Square-tooth	Full Scan
48	Sharks-tooth	XL Scan
50	Square-tooth	XL Scan
64	Sharks-tooth	XL Scan
66	Square-tooth	XL Scan

Note   *If you select a run mode that is incompatible with the selected lane configuration, the lane popup menu displays <none>.*

e. Choose a matrix file from the Gel Matrix pop-up menu.

    Appendix D describes using and creating matrices.

2. Enter the length of the run in the Collect Time entry field. Following are some suggested collection times.

Plate length (cm)	Typical Collection Time (hr)
6	1.5
12	4
24	8
34	10

3. Enter your name in the Operator entry field.

    As noted, the sample information for each lane is entered automatically in the grid at the bottom of the Run window when you choose a Sample Sheet.

Note	You cannot edit the Sample Sheet information in the Run window. Make any changes in the Sample Sheet, select <none>, and then select the Sample Sheet again in the Run window to make the changes effective.

4. Choose the appropriate filter set: A or B.

IMPORTANT	Failure to select the appropriate filter set results in the loss of data.

5. Select the Auto Analyze and Auto Print checkboxes if you want the analysis program to analyze and print the data.

6. Complete the information in the Run window, save the file, and start a plate check or start a pre-run or run, as described in *Starting a Pre-run or a Run* on page 5-45.

Starting a Pre-run or a Run

You can start a pre-run or a run either from the Run window or by using menu commands from the Instrument menu.

IMPORTANT	When starting a run, be sure the sample lanes specified in the Run window correspond to the lanes you loaded. The sample information in the Run window is the information stored in the Gel file when you start the run. Changes you make in the Run window after you start the run are not implemented.

Starting from the Run Window

When you are finished completing the Run window information, you can start the run directly from the Run window, as noted above. Instrument control buttons allow you to start, pause, and cancel the run (see Figure 5-15 on page 5-40).

- Click the PreRun button to pre-run the gel. The button becomes active when you have entered a module file name for the Pre-run. The Pre-run lasts for the period of time specified in the pre-run module listed in the Run window, unless you click the Pause button or the Cancel button.

Clicking Pre-run causes the instrument to perform electrophoresis, but no data is collected.

- Click the Run button to start the run. The button becomes active when you have entered enough information in the window to execute a run. Clicking Run causes the instrument to perform both electrophoresis and data collection.

- The Pause button becomes active when you start a run or a pre-run. Click it to temporarily halt the run (for instance, to load samples). When you do, the instrument stops electrophoresis. When the run is paused, the Pause button changes to read Resume. When you are ready to resume the run or pre-run, click the button again.

Note   *If you click Pause during a pre-run to load samples, after all samples are loaded, click Cancel to stop the pre-run, then click Run to start the run.*

- Click the Cancel button to stop a pre-run or run. If you cancel a pre-run, the module that is running stops and you can start a run. If you cancel a run, data collection stops. You cannot continue the run from the point at which you cancelled it.

Figure 5-17 shows a typical series of commands for a plate check, pre-run, and run on the ABI PRISM 373XL.

Button to Click	Module in effect	Task to perform
Plate Check	Plate Check module	Check plates and gel for fluorescence
Cancel	n/a	(Stops plate check scan) Add buffer and check for leaks Mount front heat-transfer plate
PreRun	Pre-run module	(Electrophoreses gel without data collection to equilibrate temperature) Prepare samples while pre-running
Pause	Pre-run module	Load first set of samples during pause
Resume	Pre-run module	(Electrophoreses samples into gel without data collection)
Pause	Pre-run module	Load second set of samples during pause
Cancel	n/a	(Stops pre-run)
Run	Run module	(Starts electrophoresis and data collection)

Figure 5-17. Run window buttons used during a typical run

Starting from the Instrument Menu

The Instrument menu contains commands that perform the same functions as the Run window buttons (Plate Check, Start PreRun, Start Run, Pause, Resume, and Cancel Run).

To check the plates for contamination:

Choose Start Plate Check from the Instrument menu.

To start a pre-run:

Choose Start PreRun from the Instrument menu.

To start a run:

Choose Start Run from the Instrument menu.

To temporarily stop a pre-run or run (and resume it later):

Choose Pause from the Instrument menu.

To resume a pre-run or run that has been paused:

Choose Resume from the Instrument menu.

To stop a run before it is finished:

Choose Cancel Run from the Instrument menu. This stops the module that is running and closes the Run file. You cannot continue the run from the point at which you cancelled it.

Note	*If you are familiar with running the Model 373 before this ABI PRISM 373XL DNA Sequencer upgrade, please note the data collection program no longer has a controller panel; you do not need to select the Collect button.*

Viewing the Data

Using the Data Collection Program Windows

In the collection program you can open all windows at one time, although the number might be limited by the available RAM on your Macintosh computer. Only one window can be active at any given time. Using these windows you can:

- Monitor the instrument status and error occurrence during a run

- Observe the data as it is generated, in the Scan or Gel views, and monitor the electrophoresis power supply

- Record or change sample information on the Sample Sheet

- Set up and start a run

- Set default settings and parameters for automatic analysis

Viewing the data and the instrument status is described here. Descriptions of the other functions are found on the following pages:

To perform this function:	See this page:
Record sample information	Page 5-32
Set up and start a run	Page 5-40
Set defaults	Page 5-21 (Sequencing)
	Page 5-25 (GeneScan)

Following is some general information about working with windows:

- To move a window around on the screen, click the top border, hold the mouse button down, and drag the window to another location.

- To change the size of a window, click the size box in the lower right corner of the window, hold the mouse button down, and drag the mouse to stretch or shrink the window.

- To close a window, click the close box at the upper left corner of that window or click in the window to make it active and choose Close (⌘-W) from the File menu.

Observing Instrument Status During and After a Run

During a run, you can observe the current status of the ABI PRISM 373XL in a status window, which is updated approximately every three seconds. After the run is finished, you can view a chronological record of significant system events in the Log file.

The Status Window

Open the Status window by choosing Status from the Window menu.

Click arrows to collapse /expand parts of window

Time remaining in the function being executed

Green arrow on scale indicates actual reading from the instrument

Gray box to right and red arrow on scale indicate value set for this parameter by the chosen module

The total time selected for the function currently being executed
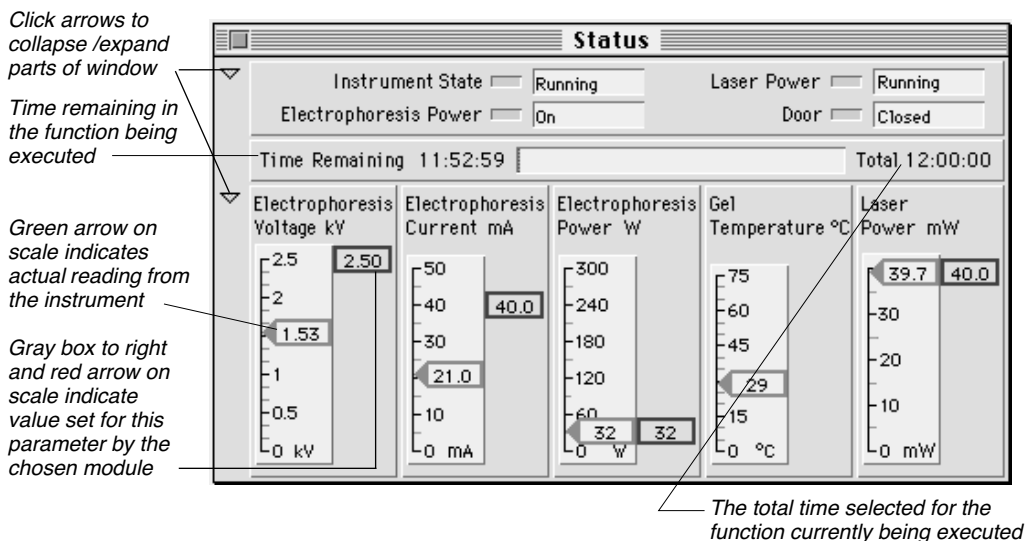
Figure 5-18. Status Window example

Note	The actual power and current readings during electrophoresis change gradually throughout the run. Table 5-5 lists the approximate readings you should see for power and current under normal running conditions.

Table 5-5. Readings Under Normal Running Conditions

			Approximate Readings (Volts)	
Gel (%)	WTR (cm)	Watts	Start	End
6	24	30	1200–1500	1800
4.75	34	32	1200–1500	1800
4.0	48	40	1500–1700	2100

The Log File

A Run Log file is created for each run and stored in the Run folder when you start the run. To view the Log file, choose Log from the Window menu.

The Log file contains a comprehensive record of all error and status messages generated by the data collection program during a sequencing run, including:

- Start and stop times of the run

- Instrument errors and computer errors

These messages can be helpful to Applied Biosystems Field Service Engineers.


```
                          Log
--> 5/22/96  7:15:46 PM  Run Started
--> 5/22/96  7:15:46 PM  Operator for Sequencing Run:
--> 5/22/96  7:15:46 PM  Run Module Sent: Seq Run-32w
--> 5/22/96  7:15:47 PM  EP Voltage:  2500 volts.
--> 5/22/96  7:15:47 PM  EP Current:   40.0 mA.
--> 5/22/96  7:15:47 PM  EP Power:     32 watts.
--> 5/22/96  7:15:47 PM  Laser Power:  40.0 mW.
--> 5/22/96  7:15:47 PM  Run Time:    12.00 hours.
--> 5/22/96  7:15:47 PM  Filter Set:   0 .
--> 5/22/96  7:15:47 PM  PMT Gain:    740 Volts.
--> 5/22/96  7:15:47 PM  Scan Delay:  0.0 Hours.
--> 5/22/96  7:21:04 PM  Run Paused
--> 5/22/96  7:21:06 PM  Instrument Paused
--> 5/22/96  7:29:48 PM  Run Resumed
--> 5/22/96  7:29:49 PM  Instrument Resumed
```

Figure 5-19. Log window example

The information in the file is formatted as follows:

      xxx   mm/dd/yy hh:mm:ss   description

The entry in the first column, xxx, is variable. The possible entries are:

...	information (system start or stop, file created)
- ->	message sent to instrument
<- -	message received from instrument
***	warning
###	error

The information in the second column shows month/day/year and hours:minutes:seconds, formatted as shown. The third column has a brief description of the event.

A Log file can get as big as your available disk space, although you can only view the last 32K of information using the data collection program. You can use a word processing program to view larger Log files.

Viewing Data

You can view information from a run in real time during data collection. The Scan window and the Gel window display the data being collected, and the Electrophoresis History window displays the set and actual values for the temperature and the electrophoresis power supply.

The real-time data display differs from the display of data after analysis, as described next.

Filters and Color of Data Display

On the real-time displays (the Scan window and the Gel window), the data collection program displays these intensities, color-coded according to wavelength. Blue, green, yellow, and red (in that order) represent the wavelengths of the dye emissions within each dye set. Blue represents the shortest wavelength, and red represents the longest. The colors on the real-time displays therefore represent the wavelengths of the dyes being detected, rather than the bases being detected.

Each of the three chemistries used for preparing DNA is associated with one of the dye sets. 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. Because of this, the four colors on the real-time displays represent different bases, depending on the chemistry used for labeling.

Table 5-6 describes the colors that represent each of the four bases on the real-time displays.

Table 5-6. Color Guide for Data Collection Program Raw Data Sequencing Displays

	Filter A		Filter B
Color	*Taq* and T7 Primers (Dye Set 1)	*Taq* Terminators (Dye Set 2)	T7 Terminators (Dye Set 3)
Blue	C	G	T
Green	A	A	C
Yellow	G	T	A
Red	T	C	G

Note	*The Sequencing Analysis program converts the information collected by the data collection program, so after analysis the colors are the same for each base: Blue for C, Green for A, Yellow* for G, and Red for T.*

Table 5-7 shows the filter and its band pass wavelength for each filter set, and which dyes are detected with each filter. For GeneScan applications, it is important to have a multi-component matrix for every combination of dye sets you use. In some instances, you may need to make matrices specific for the gel type as well, as the spectral properties of the dyes are different under denaturing and native conditions. Because no two band pass filters (of the same wavelength) are exactly the same, it is necessary to make matrices for each instrument used.

---

[*] When printed, G is black, so it is easier to see.

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Table 5-7. GeneScan Filter Set Table

Filter Set	Band Pass Filters				
	531	545	560	580	610
	Blue		Green	Yellow	Red
A	5-FAM		HEX	TAMRA	ROX
	R-110	--			
	6-FAM		R6G		
	Blue	Green	Yellow	Red	
B	5-FAM	TET	HEX	TAMRA	
	6-FAM		JOE		

The Scan Window

Viewing the Scan window is one of three methods of viewing real-time data. Raw data appears in the Scan window, always in real time. To display the Scan window, choose Scan from the Window menu.

The Scan display shows the sweeps of the laser across the gel, with a different colored line representing each filter. The computer simultaneously updates the Scan window with four lines every few seconds during instrument operation.
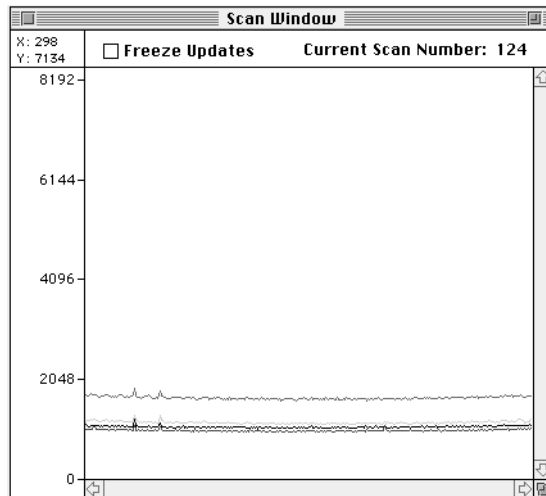
Figure 5-20. Scan window example

Note   *You can set the scale of the Scan window by choosing Set Scale from the Edit menu. A dialog box allows you to enter minimum and maximum values for the scale.*

The Gel Window

To display the Gel window, choose Gel Image from the Window menu.
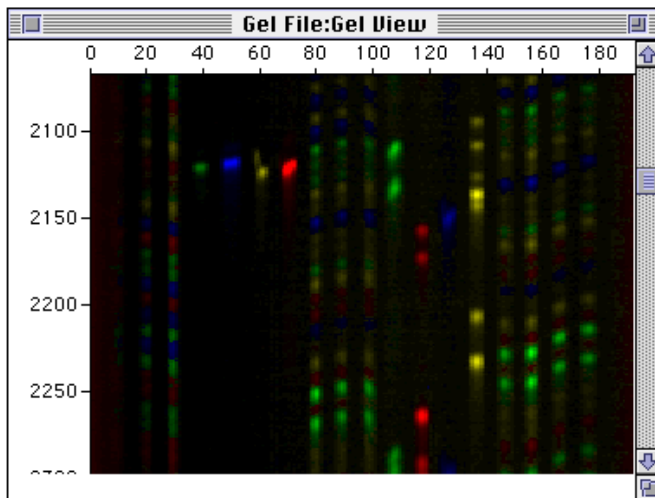
Figure 5-21. Gel window example

This window is a reconstruction of actual data similar to the way it would appear as an autoradiogram, but in color. The first fragments passing the laser appear at the bottom of the screen and move toward the top of the screen as new data is recorded throughout the run. By the end of the run, the window appears similar to an autoradiogram with the smaller DNA fragments near the top and the larger fragments near the bottom. Remember, however, that this window is a re-creation of the data through time and does not indicate the physical position of the fragments.

Note	You can scroll up or down or resize the Gel window to see the entire gel image representation.

The Electrophoresis History Window

To display the electrophoresis history window, choose Electrophoresis History from the Window menu.

This window displays the set and actual values for the electrophoresis power supply and gel temperature throughout the course of an electrophoresis run. The scale for each panel is adjustable (see below).
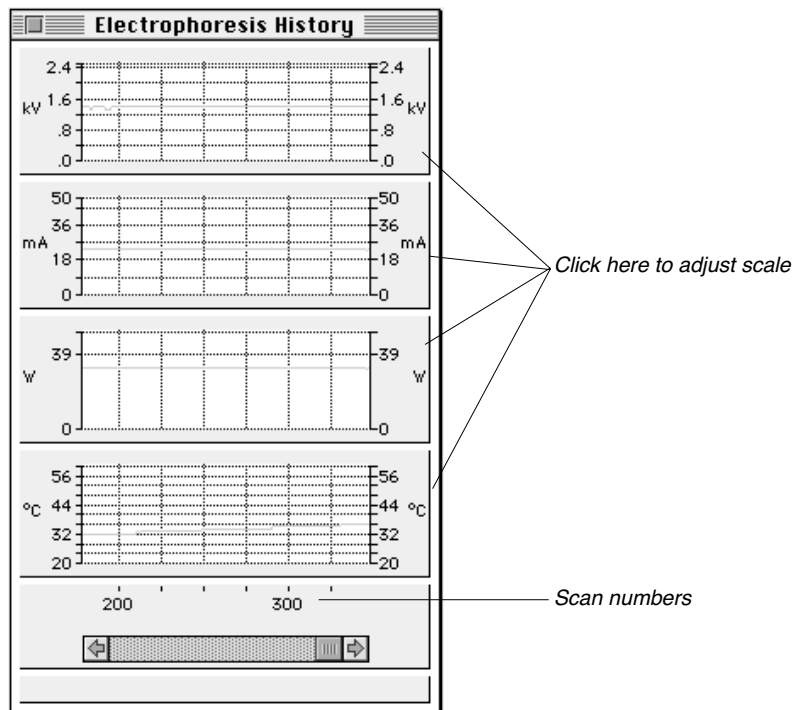
Figure 5-22. Electrophoresis History window example

A green graph represents the actual value; a red graph represents the target. When the actual and target are identical, the green covers the red. This allows you to quickly check the status of the instrument visually: red alerts can indicate potential problems, although some fluctuation is normal.

Note	The information in the Electrophoresis History window is stored in the Gel file.

To adjust the scale:

1. Double-click one of the panels in the window, or click a panel once and choose Scale from the Edit menu.
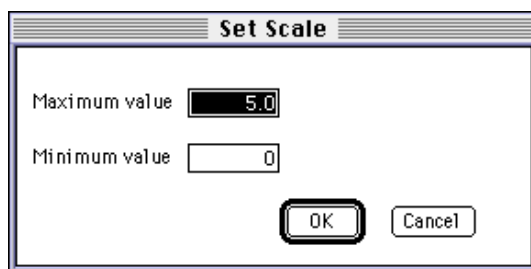
Figure 5-23. Set Scale dialog box

2. In the entry fields of the dialog box that appears, type the minimum and maximum values you wish to display.

3. Click OK.

Viewing Other Run-Related Information

The Sample Sheet

To display the Sample Sheet that is associated with the current run, choose Sample Sheet from the Window menu. This option is available only if a Sample Sheet is listed in a currently open Run window. See page 5-32 for details about creating or editing a Sample Sheet.

The Run File

To display the Run file for the current run, choose Run from the Window menu. See page 5-40 for information about creating the Run file.

Controlling the ABI PRISM 373 DNA Sequencer with XL Upgrade Manually

In Manual Control mode, you can change the instrument settings in real time. Functions you specify from the Manual Control Function pop-up menu are executed immediately when you click the Execute button, and remain in effect unless functions in a running module override them. You can run a module manually only when the instrument is not running any other module.

To control the instrument manually:

1.   Choose Manual Control from the Window menu.

To control individual functions, choose a function from the Fxn pop-up menu, and enter a value that is within the specified range
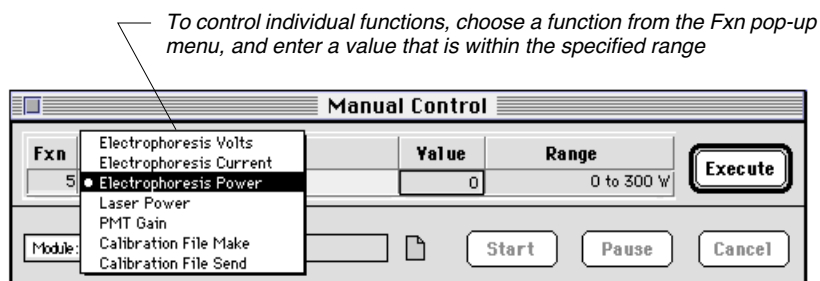
Figure 5-24. Manual Control window

2.   Choose a function or a module to run. Table 5-8 on page 5-60 lists the options.

To choose individual functions:

a.   Click the Fxn Name field to choose a function from a pop-up menu.

b. Enter a value, if necessary, in the Value column of the grid.

The right-most column of the grid lists the range allowed. If you choose a function that does not take a parameter, No Value appears in the Value field.

Table 5-8. Manual Control Functions

Function	Range of Values
Electrophoresis Volts*	0.00 to 2.50 kV
Electrophoresis Current*	0.00 to 50.0 mA
Electrophoresis Power*	0 to 300 W
Laser Power	0.00 to 40.0 mW (in 10ths of mW)
PMT Gain	0 to 999 V
Calibration File Make	no value
Calibration Send Make	no value

* You can manually control these parameters to make any of them limiting, but you should be thoroughly familiar with the principles of electrophoresis before doing so.

To choose a module:

a. Click the Module field to choose the module from a pop-up menu.

3. Start the chosen function or module.

• *To start an individual function:* Click the Execute button.

• *To start a module:* Click the Start button.

To pause or cancel a currently running module, click the corresponding button. If you click Pause, the text on the button changes to read Resume, and you can click it again to resume the function or module.

PMT Gain and Calibration File

Checking and Adjusting the PMT Gain

This section describes, in detail, how to check and adjust the PMT gain. Before checking or adjusting the PMT gain, you must have

- placed a gel in the instrument, and

- defined the Collection software preferences

Recommended PMT Gain

The PMT gain should be set such that the lowest scan line (usually the blue line) is positioned at approximately 900 on the y-axis in the Scan window display. For subsequent runs, the PMT gain needs to be adjusted only if the y-axis value is out of the range of 800–1000 on the y-axis. Different PMT gains are required for the three run modes (XL Scan, Full Scan, and BaseSprinter).

Checking the PMT

Use the procedure below to check and adjust the PMT gain for a one-time use or as part of the procedure to make a calibration file.

To check the PMT gain:

1. Choose New from the File menu.

2. Click the Sequence Run icon in the window that appears.

3. Complete the following information in the Run window.

    a. Choose Plate Check from the Plate Check Module pop-up menu.

    b. Choose the appropriate run mode from the Run Mode pop-up menu.

Note	You do not need to complete the other information in the Run window to perform a plate check or create Calibration File.

4. Click the Plate Check button.

5. Allow time for the Translation Processor to update the scan window and then view the four scan lines in the Scan window.

6. Position the mouse so the tip of the arrow just touches a flat section of the lowest scan line (usually the blue line).

Read y-axis here ———→
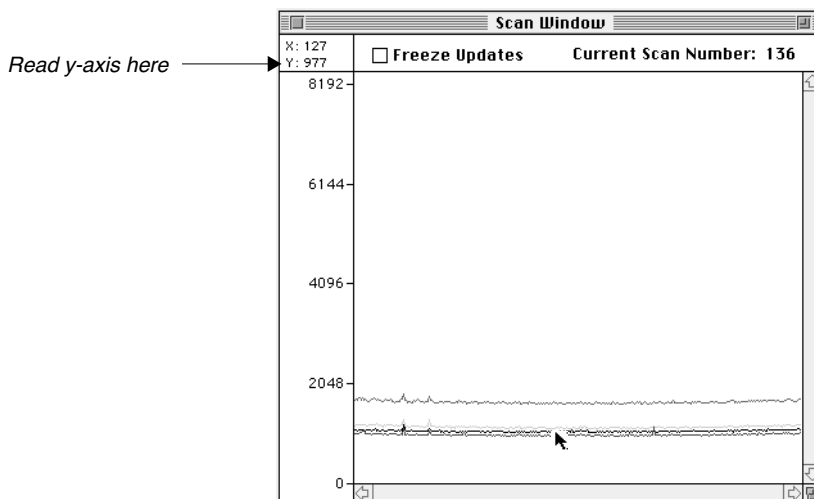
Figure 5-25. Scan lines at the recommended range

Note	You can set the scale of the Scan window by choosing Set Scale from the Edit menu. A dialog box allows you to enter minimum and maximum values for the scale.

7. Read the y-axis value displayed in the upper-left hand corner of the scan window.

- If the lowest scan line is positioned between 800–1000 on the y-axis, cancel the plate check. Proceed to step 8.

- If the lowest scan line is outside of the recommended range (800—1000 on the y-axis), then proceed to *Adjusting the PMT Gain.*

8. Cancel plate check in the run window. If you wish to make a calibration file for this PMT gain setting, then proceed to *Making and Sending a Calibration File* on page 5-63.

Adjusting the PMT Gain

To adjust the PMT gain:

1. While the plate check is running, choose Manual Control from the Window menu.

2. Adjust the size and position of the Scan and Manual control windows so that you can see both windows (no overlap).

3. In the Manual Control window, choose PMT Gain from the Fxn Name pop-up menu.

4. Enter a new value then click Execute.

   Entering a larger number than displayed in the value box will shift the scan lines up in the scan window. A smaller number will shift them down.

5. Click on the scan window to make it active. Allow time for the Translation Processor to update the scan window , and then check the y-axis value.

6. Repeat steps 4 and 5 until the PMT gain is within the recommended range.

7. Cancel the Plate Check. If you wish to make a calibration file for this PMT gain setting, then proceed to *Making and Sending a Calibration File* on page 5-63.

Making and Sending a Calibration File

This section describes how to make and send a Calibration file. Before making a calibration file, you must:

- have a gel loaded in the instrument and

- know how to performed a plate check to obtain a valid PMT gain for each of the run modes

Calibration File

A calibration file is a single file which stores the PMT gain settings for the three run modes (XL Scan, Full Scan and BaseSprinter). When a calibration file is created, the file is named ABI 373XL Calibrations and is stored in the Preferences folder inside the System folder of the Macintosh. This file can be opened and read with the SimpleText application.

Making a Calibration File

The function Calibration File Make in manual control is used store a valid PMT gain value for each run mode into the ABI 373XL Calibrations file.

To make a calibration file:

1. Perform a plate check in the XL Scan mode; adjust the PMT gain, if necessary.

2. Perform a plate check in the Full Scan mode; adjust the PMT gain, if necessary.

3. Perform a plate check in the BaseSprinter mode; adjust the PMT gain, if necessary.

4. Choose Manual Control from the Window menu.

5. Choose Calibration File Make from the Fxn Name pop-up menu.

6. Click Execute.

7. The PMT gain values for the three run modes are saved in the ABI 373XL Calibrations file.

Sending a Calibration File

The function Calibration File Send sends a copy of the information (PMT gains) in the ABI 373XL Calibrations file to the Translation Processor's non-volatile memory. The Translation Processor uses these stored PMT gain values for subsequent runs.

To send a calibration file:

1. Open the Manual Control window, if it is not already open.

2. Choose Calibration File Send, then click Execute.

3. A copy of the calibration values now reside in non-volatile memory in the Translation Processor.

Saving and Printing Files

Saving Files

The log file created during a run is automatically saved in the corresponding Run folder. When you close Sample Sheet, run, or preferences windows, the settings in those windows are stored in the appropriate files. The files are named according to the default File Names preferences (see *Setting File Name Preferences* on page 5-18), and are stored in the default folder locations specified in preferences.

You can open most files using the Open command in the File menu, and save them by choosing Save from the File menu. If you open and change a Sample Sheet, Run file, or Module window, you might want to save it under a separate file name to preserve the original information.

To save an altered file under a new name:

1. Choose Save As… from the File menu.

2. When the standard file dialog box appears, type a name for your file in the entry field, and select a location for it.

3. Click Save.

To save a backup copy of the file you are working with:

1. Choose Save a Copy In from the File menu.

2. When the standard file dialog box appears, type a name for your file in the entry field, and select a 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 your screen.

Archiving Files

Gel Files

Gel files contain the raw data acquired by the data collection program and typically take up 10–55MB of disk space. A separate Gel file is created in the Run folder each time you perform a run. Because Gel files take a substantial amount of space, you should delete them from your hard disk after you have obtained satisfactory Sample files. You do not usually need to keep gel files once the tracking is verified and sample files are created.

If you do wish to archive Gel files, use magnetic tapes, removable cartridge drives, or optical drives. Gel files are too large to fit on disks.

IMPORTANT   *Do not discard any gel file until you have verified the tracking and taken corrective action if necessary.*

Sample files

Use disks, a magnetic tape drive, a removable cartridge drive, or an optical drive to archive sample files. A 1.4MB, high-density disk holds about six files. A sample file is 150–200KB in size, depending on the length of the sequencing run. Save a sample file when you feel confident that the channel selections (tracking) are correct for the sample.

Printing Files

You can print the contents of any of the editable windows (Sample Sheet, Run window, Module window).

To specify the location of the printer and the desired printer driver, choose Chooser from the Apple menu, and then highlight the printer and driver.

Setting Up the Page for Printing

Before you print, you can specify certain parameters about the printout, such as the paper size, the orientation of the page, and whether or not the print should be reduced from actual size. To do so, choose Page Setup from the File menu.

The appearance of the Page Setup dialog box depends on the printer you are using. For information about the other default settings, refer to your printer manual.

You must also designate the format of your printed analyzed data through the appropriate analysis program. Refer to the Sequence Analysis and/or the GeneScan Analysis preferences.

Printing

Note	*If you choose Auto Print for your samples in the Run window, electropherograms are automatically printed for them after the collected data is analyzed.*

To print the contents of a window:

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 (⌘-P) 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.

Importing and Exporting Files

Exporting

Use the Export command to save the contents of a window grid (for instance, from the Sample Sheet or the Run window) into a tab-delimited text format. This format is useful with most database, word processing, or spreadsheet programs.

To export information to tab-delimited text format:

1. Click the window containing the information you wish to export, to make it active.

   The window must be one of the windows containing a grid. The information in the grid is exported.

2. Choose Export from the File menu.

3.   Type a file name in the dialog box that appears.

4.   Click OK.

Importing

You can also import from tab-delimited text files into the grids of the Sample Sheet and Run windows. Each row in the text file should contain the information for one row in the grid, in the same order as the columns of the grid.

To import tab-delimited text files into the data collection program windows:

1.   Open the window into which you wish to import information and click the top left column of the grid.

1.   Choose Import from the File menu.

2.   In the dialog box that appears, choose the name of the file containing the text you wish to import.

3.   Click OK.

Note   Everything up to the first tab in the text file is imported into the first field in the grid.

Quitting the Data Collection Program

Be careful not to quit the data collection program while a run is in progress. If you do, however, a dialog box appears asking you if you want to quit.

To close any open windows:

Click a window to make it active, then close it in one of the following ways:

•   Click the close box in the upper left corner of the window, or

•   Choose Close from the File menu.

The current settings in the window are saved.

To quit the data collection program:

Choose Quit (⌘-Q) from the File menu.

Note	*If a run is in progress, a dialog box asks you to verify that you want to quit. Quitting while a run is in progress cancels the run. If data collection is in progress, the collection is stopped, but collected data is not lost. You can use either analysis program to open the Gel file containing the collected data.*

About Data Collection Program Files

Numerous files are stored on the computer that runs the ABI PRISM 373XL. The application program and various ancillary files are organized in folders on your hard disk as shown in Figure 5-26 on page 5-71.

The ABI PRISM 373 DNA Sequencer with XL Upgrade folder contains the program file and folders for Sample Sheets, modules, runs, and settings.

Other files are located in the ABI folder (see Figure 5-26 on page 5-71). This ABI folder should always be in the System folder on your hard disk. It is the default Settings folder for the data collection software.

IMPORTANT	*Although you can change the location specified as the Settings folder in the data collection software, the Settings folder must always be the ABI folder if you use the Sequencing Analysis program to analyze your data.*

Folders and Files in the ABI PRISM 373 DNA Sequencer with XL Upgrade Folder

ABI Prism®373XL

Runs — Sample Sheets — Modules — Firmware Image

The Runs folder contains an individual folder for each run. Each individual run folder contains the Run file, the Gel file, the Run Log, and, after analysis, the Sample files

Sample Sheets you create are stored in the Sample Sheets folder

The Module folder contains the module files that provide instructions to the instrument during plate check, pre-run and run

The first time you start the data collection program the software copies the Image file to the instrument firmware

Files in the ABI folder in the System Folder

System Folder

ABI Folder

The Settings folder can contain preferences and special files used by the data collection and analysis programs. The default settings folder is the ABI folder in the System folder.
If you use the Sequencing Analysis software, you must leave the settings files in the ABI folder.

Figure 5-26. ABI PRISM 373XL file locations on the computer

ABI PRISM 373 DNA Sequencer with XL Upgrade Data Flow

Figure 5-27 shows the flow of data between the Macintosh computer and the ABI PRISM 373 instrument during data collection.
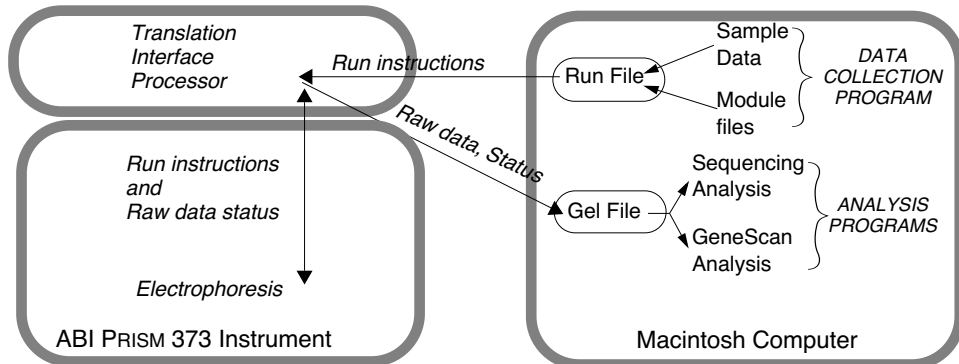
The Run file associates a list of samples with an electrophoresis module. The instrument receives the run data and sends back raw data to a Gel file, which is passed to the appropriate analysis program.

Figure 5-27. Data collection program data flow

Input and Output Files

The following describes the files that contribute information for software operation (input), the files created by the software (output), and the files located in the ABI folder (which contains both input and output files).

Input Files

The ABI PRISM 373XL input files contribute to operation of the instrument. Table 5-9 details the input files that are not located in the System folder. Output files are described in Table 5-10 on page 5-74. The files that must be located in the System folder are described in Table 5-11 on page 5-76.

Table 5-9. Input Files in ABI PRISM 373XL Data Collection Program

File Type	Location	Description
ABI PRISM 373 DNA Sequencer with XL Upgrade Firmware file (input)	Firmware Image folder in the ABI PRISM 373XL folder	Contains an unalterable copy of firmware code that the instrument executes in order to run. This file is copied from the computer to the instrument the first time you start the ABI PRISM 373XL or if the instrument memory is cleared after a major power failure.
Program files	ABI PRISM 373XL folder	Provide the primary input that runs the instrument and analyzes data. The data collection program collects and stores the data sent from the ABI PRISM 373XL during the run.The analysis program (Sequencing Analysis or GeneScan Analysis) analyzes this data after the run is complete.
Run files	Individual Run folders inside the Runs folder inside the ABI PRISM 373XL folder	Each stores information associating specific samples with specific lane positions for a particular run. It also stores information about the Sample Sheet and modules associated with the run. Files associated with the run, such as the Run Log, the Gel file, and the Sample files, are stored in the Run folder.

Table 5-9. Input Files in ABI PRISM 373XL Data Collection Program (continued)

File Type	Location	Description
Sample Sheet files	Sample Sheets folder inside the ABI PRISM 373XL folder	Contain the header information that you entered into the data collection program Sample Sheet window. You can export Sample Sheet files in a format that Microsoft Excel can read.
Module files	Modules folder inside the ABI PRISM 373XL folder	Each contains a list of functions that are used by the instrument during a plate check, a pre-run, or a run. Module files containing commonly used functions are provided with the instrument.

Output Files

The instrument automatically places two output files in the Run folder for an individual run: a Run file and a status log. Table 5-10 details all of the output files that are not located in the System folder and their locations. The files that must be located in the System folder are described in Table 5-11.

Table 5-10. Output Files in ABI PRISM 373XL Data Collection Program

File type	Location	Description
Gel file	Individual Run folder inside the Runs folder inside the ABI PRISM 373XL folder	A large file created by the data collection program. For a typical run a gel file can be very large (over 20MB). This file contains all raw data acquired by the data collection program during the run. It is created in the Run folder when you perform a run.
Run Logs	Individual Run folder inside the Runs folder inside the ABI PRISM 373XL folder	Created in the Run folder at the start of each run. This text file contains date and time-stamped status and error messages that maintain a comprehensive history of actions taken, instrument and computer conditions, and any errors received. You can view and print the log file at any time while the application is running.

Table 5-10. Output Files in ABI PRISM 373XL Data Collection Program (continued)

File type	Location	Description
Sample (data) files (Analysis program output)	Individual Run folder inside the Runs folder inside the ABI PRISM 373XL folder	When you analyze the raw data, the gel file is not altered. The Sequencing Analysis and GeneScan Analysis programs* extract sample information from the gel file to create sample files. Each file contains the data for a single sample, or lane, including information you entered in the Sample Sheet; raw data from the instrument; run start and stop times; voltage, temperature and power values during the run; analyzed data; and base calls. Extracted data depends on tracking: if the tracking is incorrect, the Sample files are incorrect.
Sequence files (Analysis program output)	Individual Run folder inside the Runs folder inside the ABI PRISM 373XL folder	The Sequencing Analysis program also creates document files called ".Seq" files (the file names always end with ".Seq"). These files show the base letter sequence only (or might contain 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.

* The Analysis programs are described in detail in separate manuals. Refer to the ABI PRISM *Sequencing Analysis Software User's Manual* or the *ABI PRISM GeneScan Analysis Software User's Manual*, as necessary.

Files Located in the System Folder

The System folder on your Macintosh computer contains both input and output files. Some are in the ABI subfolder. Others are in the Preferences subfolder. Table 5-11 lists the ABI PRISM 373XL files located in the System folder.

Table 5-11. Input and Output Files in the System Folder

File type	Subfolder	Description
DyeSet Primer files* (input)	ABI folder	Contain dye and primer mobility tables. Applied Biosystems supplies these files, which are used by both the data collection and the Sequencing analysis programs.
Matrix files* (input)	ABI folder	Contain mathematical matrices that are applied to data to correct for spectral overlap.
Preferences file (input)	Preferences folder	Maintains information about folder locations and default file name and settings data.

* Installed by the analysis program

Temporary Files Created in the System Folder

The software programs create a number of temporary files in the System folder. If you happen to see them, leave them alone. The programs delete them when they no longer need them.

6 Instrument Hardware

Contents

About the ABI PRISM 373 DNA Sequencer With XL Upgrade

The ABI PRISM 373 DNA Sequencer with XL upgrade includes a microprocessor-controlled electrophoresis and fluorescence detection instrument, a Power Macintosh computer, and a Translation Interface Processor.
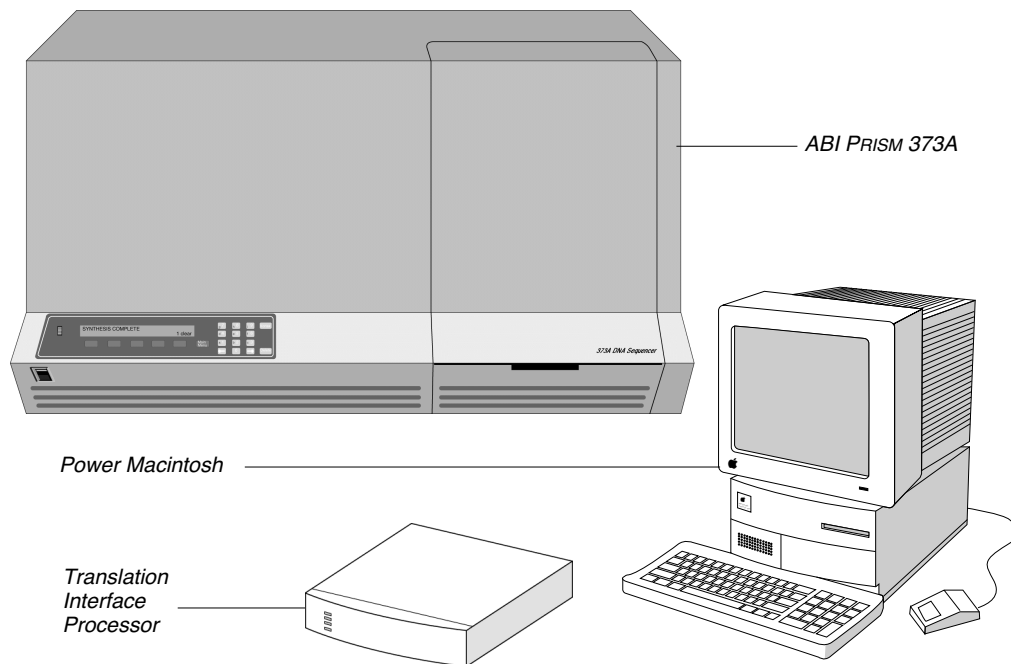
Figure 6-1. ABI PRISM 373 DNA Sequencer with XL Upgrade

It is an integrated system in which all the components have been carefully engineered to provide maximum performance.

The components of the instrument fall into five major categories.

- Loading:
  Gel plates
  Spacers
  Casting comb
  Sharks-tooth comb
  Square-tooth comb
  Comb overlay

- Separation:
  Electrophoresis power supply
  Buffer chambers, electrodes
  Heat-transfer plates (Leon and Stretch)

- Detection:
  Argon laser and scanning stage
  Filter wheel and PMT

- Control:
  Instrument firmware
  Macintosh computer and software.
  Main power supply
  Translation Interface Processor

System Overview

Using a vertical slab gel, dye-labeled DNA fragments are loaded onto the instrument. Voltage is applied, causing the fragments to electrophorese through acrylamide gel and separate according to size.

When the fragments reach a fixed position (the read region) above the lower buffer chamber, light from the laser excites the dyes, causing them to fluoresce. The laser beam scans back and forth across the read region of the gel. The emitted light is collected and focused onto a 5-filter wheel and photomultiplier tube (PMT). The computer screen displays information received from the Translation Interface Processor in real time.

At the end of collection, the analysis software can automatically process, analyze, and translate the collected data into either base sequence, fragment sizing information, or relative concentrations, depending on your application. The software also allows you to print the collected data.
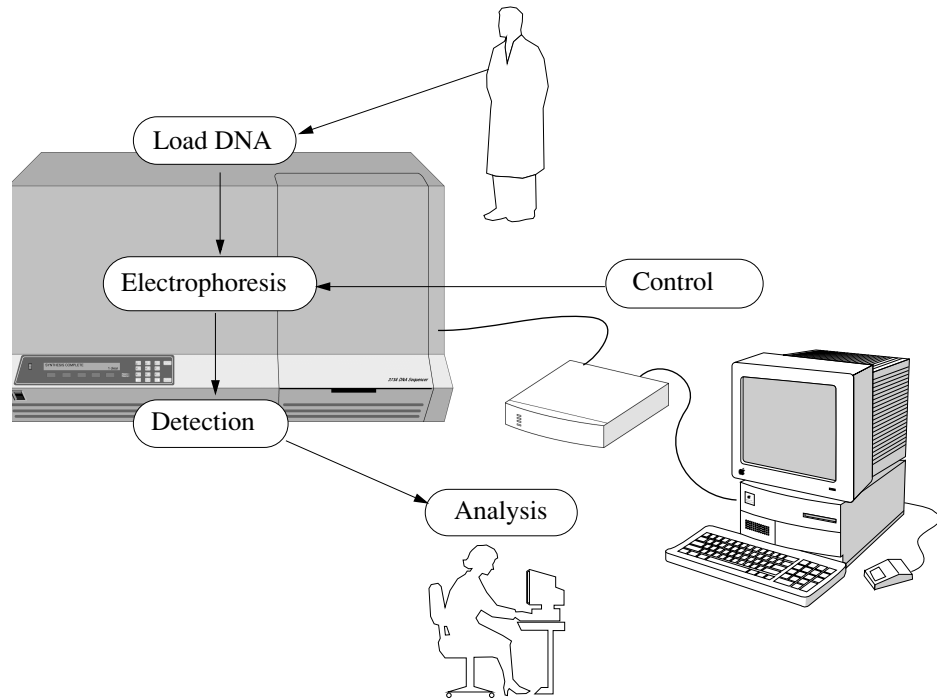
Figure 6-2. ABI PRISM 373 DNA Sequencer with XL upgrade overview

The Loading System

Components

Glass Plates

Two specially designed glass plates are used to hold the gel through which DNA electrophoreses.
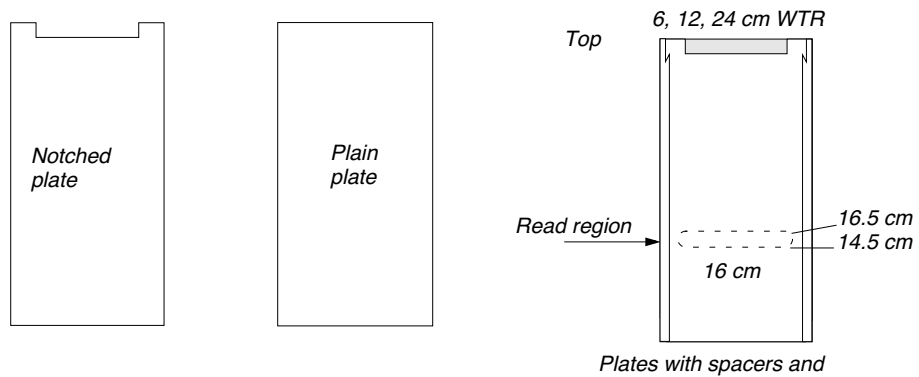
Figure 6-3. Glass plates and position of read region (Classic and Leon)
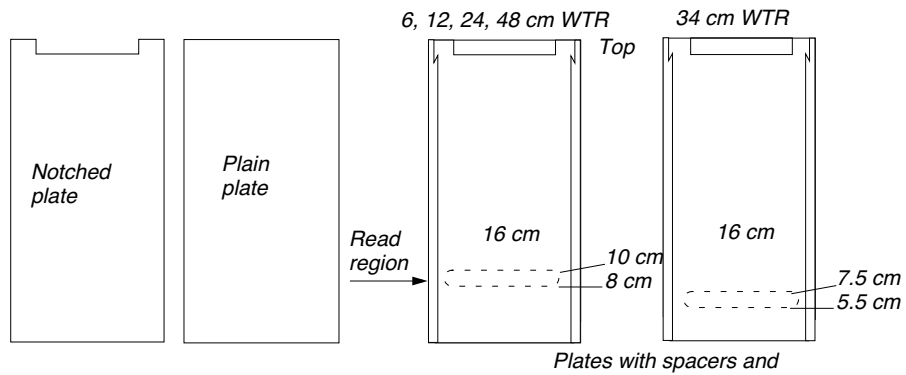
Figure 6-4. Glass plates and position of read region (Stretch)

The region of the glass where the laser scans is called the read region, and is the located behind the laser beam safety stop when the glass is mounted in the ABI PRISM 373 DNA Sequencer with XL Upgrade. It occupies an area of approximately 16 cm x 2 cm located in the lower center area of the plates, and its location varies, depending on the instrument configuration: Classic, Leon, or Stretch (see Figure 6-3 and Figure 6-4 on page 6-6).

Each time you use the glass plates, use them with the same side of each plate on the inside. After the first use, the notched plate has a hydrophobic area where the buffer chamber gasket made contact with the plate. You must therefore use the notched plate in the same orientation each time to avoid difficulty pouring the gel. It is a good idea to mark the outside of each plate the first time you use it. Mark the plates with a tiny scratch at the top that doesn't interfere with the read region or the upper buffer chamber seal.

Note	*Do not touch the cleaned surfaces of the glass plates and wear gloves to handle the plates.*

Spacers

The spacers are small straight plastic pieces that determine the thickness of the gel. Positioning of the spacers is described in detail in Section 2.
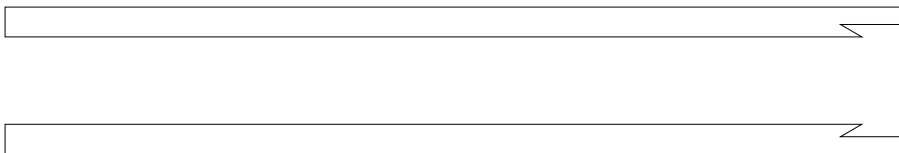
Figure 6-5. Spacers

Combs

ABI PRISM 373 DNA Sequencer with XL Upgrade lane-forming combs are used to create the individual wells at the top of the gel into which you load your DNA samples. The Sharks-tooth and square-tooth combs are available for the two general applications on the ABI PRISM 373 DNA Sequencer with XL Upgrade. A variety of well numbers is available.

IMPORTANT   *Always use combs, glass plate spacers, and casting combs as a matched set. Do not mix and match material types or thicknesses within a set. Mismatching can cause loose-fitting combs, and can result in sample leaking and/or damage to the gel.*

The sharks-tooth comb and casting comb (Figure 6-6) are used with the Sequencing application of the ABI PRISM 373 DNA Sequencer with XL Upgrade. The sharks-tooth comb is available with 18, 24, 36, 48, or 64 lanes (wells).

Sharks-tooth comb
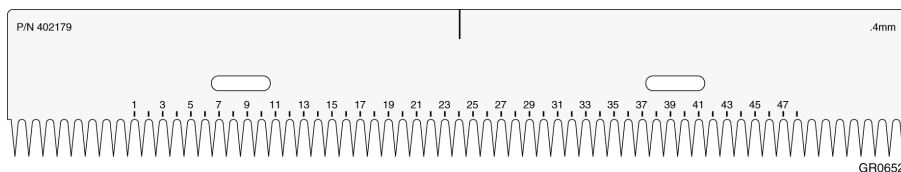
Casting comb
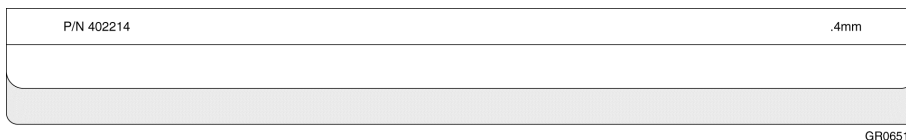
Figure 6-6. Sharks-tooth comb and casting comb

After pouring the gel solution, insert the casting comb at the top of the gel to create a single well or slot. Remove the comb after polymerization.

The sharks-tooth comb is used to form loading wells. Before loading samples, insert the teeth of the comb into the slot formed by the casting comb during polymerization. This creates the individual wells into which you load the DNA. The comb is marked with lane numbers to facilitate loading.

The square tooth comb (Figure 6-7) is used with the GeneScan application of the ABI PRISM 373 DNA Sequencer with XL Upgrade. It is available with 34, 36, 50, and 66 lanes.
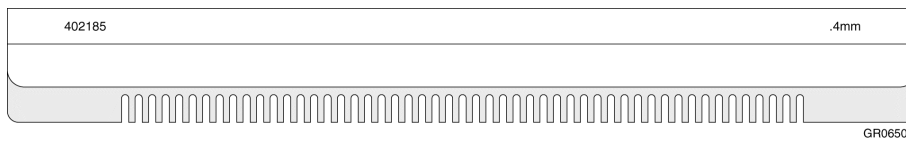
Figure 6-7. Square tooth comb

You need to use only the square tooth comb during gel polymerization. After pouring the gel solution, insert the teeth of the square tooth comb into the solution so the comb creates individual wells at the top of the gel during gel polymerization. After polymerization, remove the comb. You do not need to re-insert it when you load samples.

The Separation System

The Electrophoresis Power Supply

The electrophoresis power supply provides the electrophoresis field across the gel. The wattage and voltage are determined by the module you choose in the data collection software when you start a run. The electrophoresis supply has a maximum output of 3000 volts, 80 mAmps.

Note	*For Sequencing runs and some GeneScan runs, the ABI PRISM 373 DNA Sequencer with XL Upgrade uses constant power, rather than constant voltage, in order to control temperature.*

The Buffer Chambers and Electrodes

The ABI PRISM 373 is supplied with upper and lower buffer chambers. Color-coded electrophoresis cables, attached to electrodes installed in the properly filled buffer chambers, attach at the top and bottom of the electrophoresis chamber, and carry the current that facilitates electrophoresis.

Applied Biosystems

The Scanning/Detection System

The fluorescent dye labeled fragments passing through the read region (refer to Figure 6-9 on page 6-12) of the gel are excited by the argon ion laser. The laser beam is directed into the gel at a constant angle by means of the gel optics, which are attached to the moving stage. The collection optics are aligned directly in front of the point at which the laser beam contacts the gel medium. The collected fluoresced light is then passed in turn through four of the five filters. Each filter cuts a narrow band of light frequencies corresponding to the emission maxima of each of the four dyes. Behind the filter wheel is the photomultiplier tube (PMT). The signal from the PMT is amplified, integrated, and converted to a digital signal for transmission to the data acquisition unit (refer to Figure 6-8).
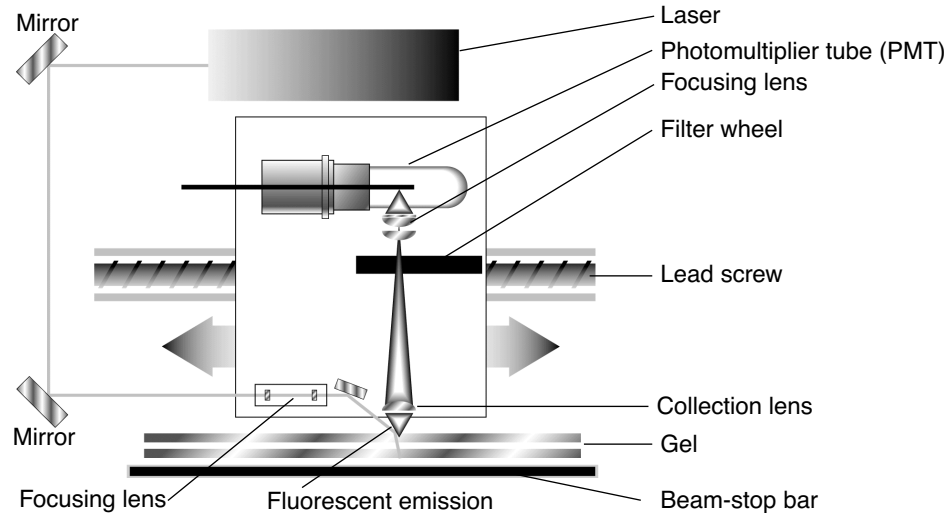
Figure 6-8. Diagram of the laser/scanner assembly (top view)

The Read Region and Run Modes

The read region is the section of the glass plates and gel where the laser scans (refer to Figure 6-9). This region is composed of channels at which data is collected. The ABI PRISM 373 DNA Sequencer with XL Upgrade is equipped with three data sampling or run modes: XL Scan, Full Scan, and BaseSprinter.
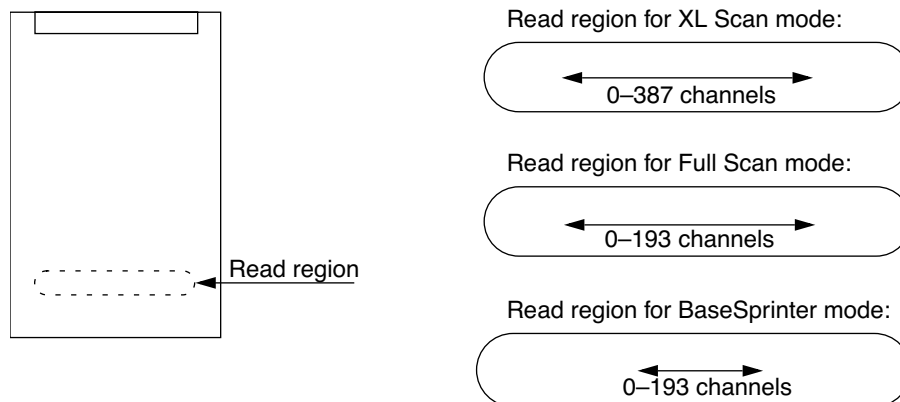
Figure 6-9. The Read region

XL Scan Mode

Data is collected along the entire read region (approximately six inches). The read region is divided into 388 channels or points where data acquisition occurs. A single scan of the gel plates with one filter takes 1.5 seconds. A complete scan with four filters takes six seconds.

Full Scan Mode

Data is collected along the entire read region (approximately six inches) in the Full Scan mode. The read region is divided into 194 channels or points where data acquisition occurs. A single scan of the gel plates with one filter takes 1.5 seconds. A complete scan with four filters takes six seconds. A complete scan equals one data point or one scan number. Either 194 or 388 channels can be used for the Full Scan mode.

BaseSprinter Mode

BaseSprinter is available only for Sequencing runs. Data is collected only along the center section of the read region (approximately three inches). A single scan of the gel plates with one filter takes 0.75 seconds. A complete scan with four filters

takes three seconds. A complete scan equals one data point or one scan number. BaseSprinter mode defaults to 388 channels, although only the middle 194 channels are used.
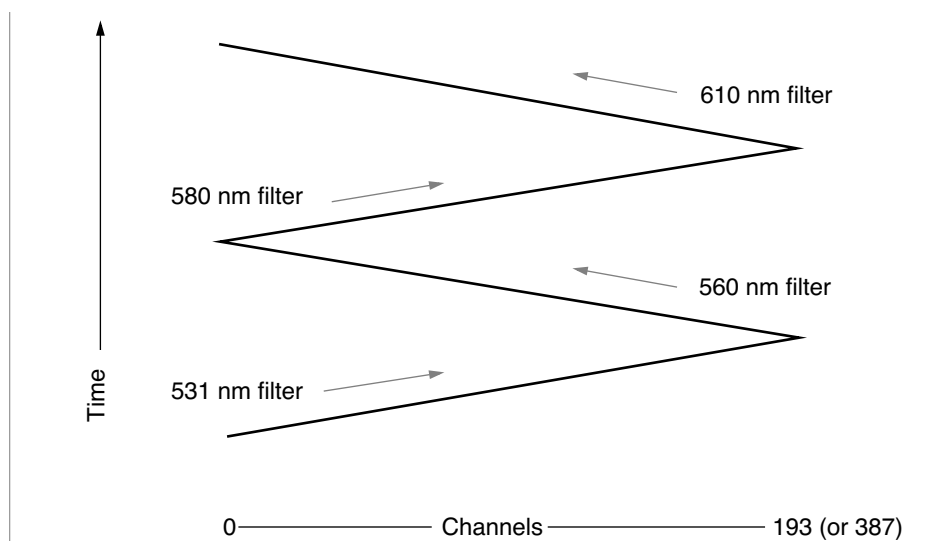
Figure 6-10. Pictorial representation of sequencing scan mode (filter set A).

Filter Wheel and Data Display

The five bandpass filters contained in the filter wheel are unique to your instrument. The center bandpasses of the individual filters are 531, 545, 560, 580 and 610 nm. The five filters are split into two sets of four, which are called filter set A and filter set B. The filters used in filter set A are 531, 560, 580, and 610 nm. The filters used in filter set B are 531, 545, 560, and 580 nm. The two filter sets cannot be accessed at the same time.

The filter wheel separates the wavelengths emitted by the fluorescent dyes. On the real time displays (the Scan and Gel window), the data collection program displays these intensities, color-coded according to wavelength. Blue, green, yellow, and red (in that order) represent the wavelengths of the dyes' emissions within each dye set. Blue represents the shortest wavelength, and red represents the longest. Different filter sets use the same four colors to represent different wavelengths, so the colors do not represent the relative wavelengths of the four dyes in each dye set.

Sequencing

Four filters are used for each sequencing run, one for each dye. Filter set A is used with the fluorescent Dye Primer and *Taq* Terminator chemistries. Filter set B is used for the T7 Sequenase Terminator chemistry.

The colors shown for raw data in the Gel and Scan windows (in the data collection program) reflect the filter used to detect each dye. Since base calling has not occurred, the colors used do not reflect the bases. Table 6-1 summarizes the relationship between the filters, dyes, and color display of raw data. In all analyzed data displays and printed sequencing analysis electropherograms, the colors correspond to the bases, as follows:

C - Blue, A - Green, G - Yellow (black when printed), T - Red

Table 6-1. Relationship Between Filters, Sequencing Dyes and Data Display

Filter Center Band (nm)	Filter Set A *Taq* Primers		Filter Set A *Taq* Terminators		Filter Set B T7 Terminators	
	Color	Base	Color	Base	Color	Base
531	Blue	C	Blue	G	Blue	T
545	--	--	--	--	Green	C
560	Green	A	Green	A	Yellow	A
580	Yellow	G	Yellow	T	Red	G
610	Red	T	Red	C	--	--

GeneScan

Nine ABI PRISM dyes are available for labeling DNA fragments. The filter set you choose determines what color peaks appear for a particular dye. Each dye is detected within its optimum wavelength range through either filter set A or B. Table 6-2 summarizes the relationship between the filters, dyes, and color display.

Table 6-2. Relationship Between Filters, GeneScan Dyes and Data Display

Dyes		Filter Set			
		A		B	
		Color Display	Filter Center Band (nm)	Color Display	Filter Center Band (nm)
Dye Phosphoramidites	HEX	Green	560	Yellow	560
	6-FAM	Blue	531	Blue	531
	TET	*	*	Green	545
NHS Esters	TAMRA**	Yellow	580	Red	580
	ROX	Red	610	*	*
	5-FAM***	Blue	531	Blue	531
	JOE***	Green	560	Yellow	560
[F]dNTPs	TAMRA	Yellow	580	Red	580
	RG6	Green	560	Yellow	560
	R110	Blue	531	Blue	531

* Not recommended; the filter is not set at the emission maxima
** TAMRA is available as an NHS-Ester or as an [F]dNTP.
*** 5-FAM and JOE are available only as labeled primers in select reagent kits.

Instrument and Matrix Files

All three sequencing chemistries and GeneScan applications use fluorescent dye labels that are excited by an argon ion laser. Although the four dyes that are used fluoresce at different wavelengths, there is some overlapping in the emission spectra. It is necessary to correct for this overlap (or filter cross-talk) before analyzing data.

To accomplish this, a mathematical matrix is created for each chemistry type or dye set, and stored in a special file. For sequencing, this file is called an Instrument file and contains a separate matrix for each chemistry (dye set) that you run on

the instrument. For GeneScan, the file is called a matrix file. A different matrix is required for each combination of the four dyes used, as well as for each type of gel.

When data is analyzed the appropriate matrix is applied to the data to subtract out any emission overlap. The Instrument file or matrix file is attached to a Gel file or Sample file when it is created to allow subsequent data analysis. Due to slight variations in the filters used in the filter wheels, the instrument and matrix files created on one ABI PRISM 373 DNA Sequencer with XL Upgrade instrument are not valid on other ABI PRISM 373 DNA Sequencer with XL Upgrade instruments.

Translation Interface Processor Status and Control

Translation Interface Processor Control

The Translation Interface Processor, which sits on top of the computer's CPU, serves as the communication link and data buffer/translator between the ABI PRISM 373 DNA Sequencer with XL Upgrade and the Power Macintosh.

The power switch is located on the back of the Translation Interface Processor. Three instrument status lights and a power light are located on the front of the Translation Interface Processor (see Figure 6-11). The status lights are described on page 6-19.

The first time you turn on the instrument and start the data collection program, the program copies a firmware image to the Translation Inteface Processor (refer to *Restarting the Macintosh and Loading the Firmware Image* on page 8-10). If you turn off the instrument between runs, a battery backup holds the image in memory, so you do not need to copy it again when you restart the system.

Note	*In order to maintain the optimum charge on the battery, we recommend you leave the Tranlation Interface Processor on when it is not in use.*

Front View

Status LEDs (3)
Power light

Green
Amber
Red

GR0692

Back View

Status LEDs (9)
Power connector
Power switch

GR0693

373XL connection
Diagnostics connection
Macintosh connection
Reset button
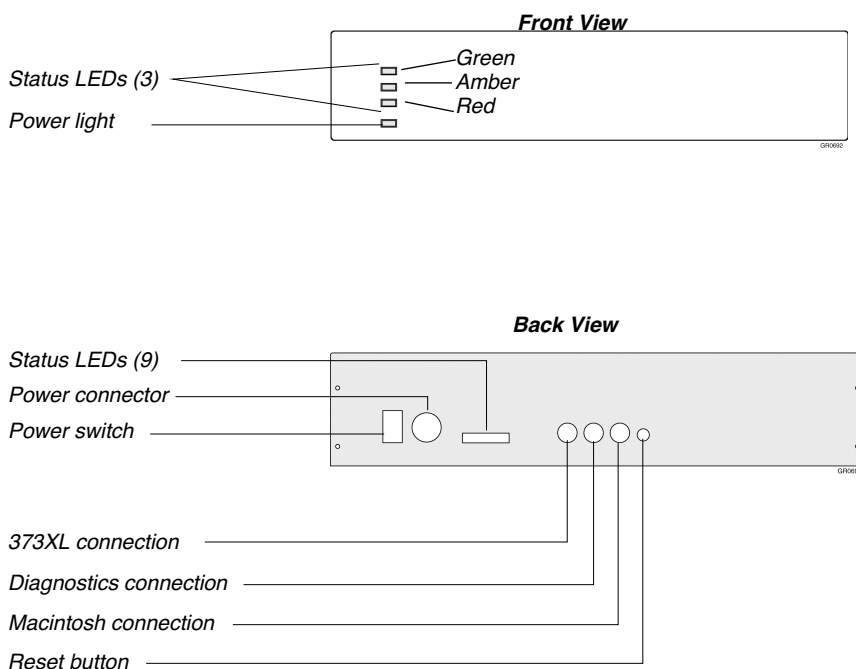
Figure 6-11. Translation Interface Processor

Translation Interface Processor Firmware

Firmware programmed into the Translation Interface Processor itself controls the basic functions of the instrument. The Macintosh software communicates instructions to the firmware, which then operates the hardware.

If the firmware memory becomes corrupted, you might need to clear the Translation Information Processor's memory so that the Macintosh copies a new firmware image into the Translation Interface Processor's memory the next time it connects to the Translation Interface Processor. This process is called Resetting the Firmware Image.

ABI PRISM 373 DNA Sequencer with XL Upgrade Data Collection Software

The ABI PRISM 373 DNA Sequencer with XL Upgrade data collection software allows you to control and collect data from the instrument. The software

communicates instructions to the instrument firmware, which controls the hardware operation. In addition to displaying data as it is collected, the software allows you to display instrument status during a run.

After data collection, the data collection software can automatically start the correct analysis program, if selected.

For more detailed information about the data collection software, refer to Section 5. For information about the Macintosh computer hardware, refer to Section 7. Each of the analysis programs has a separate user's manual.

Status Indicators

Three instrument status lights (LEDs) are located on the front and back of the Translation Interface Processor. See Figure 8-3 on page 8-9 for the location of the lights on the back. The LEDs report the status of instrument and indicate if the instrument's memory appears to be corrupted.

Three LEDs are located on the front of the instrument (see Figure 6-11 on page 6-18). They indicate instrument status as follows:

- Green indicates that the system is operational or operating. This LED comes on after completion of the power-on self tests and blinks during a run unless a special condition causes the amber or red LED to light. At the end of a run, it stops blinking but remains lit and a blinking amber light appears with it to indicate that the run is completed and the instrument is operational. When you open the doors (breaking the interlocks) to remove the gel, the green light goes steady and the amber light blinks. When you close the doors again and the system verifies that the interlocks are engaged, the amber LED goes out and the green LED lights again.

- Blinking amber indicates that the instrument is operating but paused, or has lost the firmware image. This LED blinks as the Translation Interface Processor goes through self-test when you turn the power on. It also blinks if you open the instrument door (this breaks the laser and electrophoresis safety interlocks), or if you pause the instrument by selecting Pause from the Macintosh. At the end of a run, it blinks along with a stable green light to indicate that the instrument is operational, but is paused because it has finished the run. A blinking amber light, in the absence of of all others means the Firmware Image has been lost.

- Red indicates an error condition that must be corrected for the system to

run properly. This LED lights if an error is detected during self-test. It also lights if any failures are detected during a run (for example, if the electrophoresis power or laser fails). Details of errors indicated by the red LED appear in the log file on the Macintosh. The red LED also lights if communication between the instrument and the Macintosh fails.

Table 6-3 shows some examples of how the LEDs indicate status during common and special instrument states.

Table 6-3. System states indicated by status lights

Indicator	State	Notes
All off	Instrument off	
Green (steady)	Operational	Awaiting Run (no module running) Passed self-test Interlocks connected (door closed)
Green (blinking)	Operational	Run in progress Run resumed after pause
Green (steady)/ Amber (blinking)	End of run	Once instrument door is opened or a new module is started, the amber light goes off
Amber (blinking)/ Green (steady)/ Red (steady)	Power-up Self test in progress	When completed successfully, Red and Amber goes off and Green stays lit
Green (blinking)/ Amber (blinking)/	Run paused Door open	Awaiting Resume Awaiting door closure
Red (blinking)	Self-test failed Instrument Failure	Check Macintosh Error Log
Amber (blinking)	Firmware Image lost	Re-load Firmware Image

7 Computer System

Contents

About the ABI PRISM 373 DNA Sequencer with XL Upgrade Computer System

The ABI PRISM 373 DNA Sequencer with XL Upgrade computer system performs three primary functions:

- Instrument control

- Data collection

- Data analysis

The Macintosh computer provided with your instrument runs special software (described on page 7-5) to perform these functions.

System Requirements

The ABI PRISM 373 DNA Sequencer with XL Upgrade electrophoresis instrument is shipped with a Power Macintosh computer. An optional printer is also available. If you need to replace the computer, or did not order a printer with the instrument, following are specifications for the type of computer system you should use with the instrument.

- *Macintosh Model:* Power Macintosh (contact an Applied Biosystems representative for specific models)

- *Monitor:* Apple Power Macintosh-compatible color monitor, 15-inch display

- *Disk Drive:* Hard disk with a minimum of 250MB storage (500MB is recommended)

- *RAM:* Minimum 24MB random access memory (32MB is recommended)

Note   About 16MB of RAM should be available at any given time to have both the data collection and analysis programs open together. To check your available RAM, choose About This Macintosh… from the Apple (🍎) menu.

- *Printer:* Color printer (contact an Applied Biosystems representative for a recommendation)

- *System Software:* Macintosh system software version 7.5 or higher

Installing ABI PRISM 373 DNA Sequencer with XL Upgrade Software

Your field service specialist installs the ABI PRISM 373 DNA Sequencer with XL Upgrade collection software and analysis software on the Power Macintosh while installing the instrument.

If you need to re-load the software at some point, this section describes how to load and configure the programs needed to perform data collection and analysis.

IMPORTANT	Disable the virus protection software on your Macintosh during the installation process. After installation is complete, you can re-enable virus protection.

To install the ABI PRISM 373 DNA Sequencer with XL Upgrade software:

Each ABI PRISM 373 DNA Sequencer with XL Upgrade special software program installed on your computer (data collection and one or two analysis programs, as described below) is shipped on a separate disk. Follow these steps to install the ABI PRISM 373 DNA Sequencer with XL Upgrade Data Collection software.

1. Quit any currently running applications.

2. Disable any virus protection software and all other inits by holding down the shift key while choosing Restart from the Special menu.

3. Insert the Data Collection Install disk into the 3.5-inch disk drive of your computer.

4. Click the Installer icon.

5. Respond to prompts, as appropriate.

The installer script prompts you if you need to insert any other disks, and notifies you when installation is completed.

Refer to the individual analysis user's manual for instructions on installing that application.

When you have completed the software installation, we recommend you rebuild the desktop.

To rebuild the desktop:

1. Hold down both the command key (⌘) and Option key 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.

Special Software for Data Collection and Analysis

The ABI PRISM 373 DNA Sequencer with XL Upgrade comes with software programs that perform raw data collection and data analysis.

The Data Collection software communicates instructions to firmware on the instrument that controls instrument function. It also records and displays instrument status and raw data during a run. You can specify that it automatically open the analysis program for data analysis after the run is completed.

To analyze the data collected, two other software packages are available, depending on your application. Normally, one is supplied with your ABI PRISM 373XL, depending on the configuration you ordered, although you might have ordered both.

*   The Sequencing Analysis software analyzes the raw sequencing data collected by the data collection program and calls the base sequence. This program comes bundled with two other programs, GelDocII and DataUtility. GelDocII allows you to perform special tasks relating to Gel files. The DataUtility program enables you to make and copy matrices to correct for the spectral overlap of the fluorescent dye labels. Refer to Appendix C for instructions on using the DataUtility program. It can also be used by Applied Biosystems technical specialists to check noise levels.

*   The GeneScan Analysis software enables you to use ABI PRISM 373 DNA Sequencer with XL Upgrade automated fluorescence detection to size and quantify DNA fragments. The program automates the entire process of separating, quantifying, and sizing DNA fragments. It also provides

> flexibility to interactively confirm and fine-tune the data analysis, and allows you to display the results of an experiment in several different ways.

Each analysis program is described in detail in a separate manual: the *ABI PRISM DNA Sequencing Analysis Software User's Manual* and the *ABI PRISM GeneScan Analysis Software User's Manual*, respectively. For information about the Macintosh operating system, refer to the *Macintosh System 7 Reference* manual.

The data collection software and the analysis program(s) you ordered are installed on the Power Macintosh by your field service specialist when the instrument is installed, so you do not need to install them yourself.

What You Need to Know About the Macintosh Computer

To run the ABI PRISM 373 DNA Sequencer with XL Upgrade Sequencing System, you should be familiar with basic Macintosh computer vocabulary and operations, such as the following:

- *Using the mouse:* clicking and double-clicking, selecting and dragging

- *Choosing commands:* using menus, dialog boxes, radio buttons, checkboxes

- *Working with windows:* opening and closing, re-sizing and repositioning, scrolling, understanding the active window

- *Using the Macintosh hierarchical file system:* finding files and creating folders

Maintaining and Caring for Your Macintosh Computer

Computers require regular attention and maintenance to operate efficiently and consistently. Because the ABI PRISM 373 DNA Sequencer with XL Upgrade software on the Macintosh computer works with large files and accesses the hard disk often, it is especially important that you follow the procedures described here to minimize the occurrence of errors during operation. These procedures should help keep your Macintosh computer operating at maximum efficiency.

The following describes procedures recommended by Apple Computer, Inc. and Applied Biosystems for regular maintenance of Macintosh computers, and includes some hints specifically aimed at keeping your ABI PRISM 373 DNA Sequencer with XL Upgrade software running smoothly.

Hard Disk Maintenance

The following general principles can benefit all Macintosh system users. Follow these guidelines for optimal performance of the ABI PRISM 373 DNA Sequencer with XL Upgrade programs and the Macintosh computer.

- Install only one System per hard disk.

- Back up all programs and files regularly.

- Restart the Macintosh computer before each ABI PRISM 373 DNA Sequencer with XL Upgrade run.

- Remove non-essential files from the hard disk.

- Use a hard disk maintenance program every 2–4 weeks (see *Use a Hard Disk Maintenance Program Regularly* on page 7-9).

IMPORTANT   With the large size of gel files and the constant reading and writing to the hard disk, defragmentation with DiskTools ((included on the Mac Operating System CD-ROM) or Norton Utilities (from Symantec) is required every 2 to 4 weeks.

- Use discretion when adding software programs, especially extension files.

Install Only One System Per Hard Disk

The Macintosh computer requires only one System file to operate. This System file is located in the System Folder on your computer's hard disk, and is essential for all operations on the Macintosh computer. The file contains critical information necessary to monitor and direct all interactions between you and the computer.

Note	*Be sure to safely store the System and Utilities disks that came with your Macintosh computer. Because they are useful if you have problems starting the hard disk, you should make an extra copy of each of these important disks.*

You do not receive a System file with the ABI PRISM 373 DNA Sequencer with XL Upgrade programs, but some other applications might furnish an additional System file. Whenever you copy a program from a disk to your hard disk, avoid copying additional System files.

For more complete information about the System file and the System Folder, refer to the *Macintosh System 7 Reference* manual.

Back Up All Programs and Files Regularly

Although the hard disk is extremely reliable, always back up, or copy onto disks, the data files on your computer's hard disk so you do not lose any data. The time you spend making copies of your files is minimal compared to the time you might spend if your hard disk fails and you must completely re-create all your files. You should also keep the original program disks for your software programs in a safe place, so you can reinstall the programs if necessary.

When you use the ABI PRISM 373 DNA Sequencer with XL Upgrade data collection and analysis programs regularly, several large data files might accumulate on your hard disk. These large files can eventually use up all your available Macintosh storage. Make copies of the data files you do not use regularly and remove the originals from your hard disk to reclaim storage space for future work.

Restart the Macintosh computer Before Each ABI PRISM 373 DNA Sequencer with XL Upgrade Run

Restarting clears the random access memory (RAM) and provides access to the necessary amount of memory to start data collection.

Remove Non-essential Files From the Hard Disk

It is a good idea to remove any unnecessary files from the hard disk before starting the data collection program. This ensures that you have enough disk space to store the new Gel file and Sample files for a new run.

To remove a Gel file from your hard disk:

1. Drag the Gel file into the trash can.

2. Choose Empty Trash from the Special menu.

The files are not removed from the hard disk until you empty the trash.

Rebuild the Desktop Regularly

Rebuild the desktop monthly and after installing any new software.

To rebuild the desktop:

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.

Use a Hard Disk Maintenance Program Regularly

Whenever you write files to the hard disk, and then open and rewrite them, their physical location on the disk can change. This causes the computer memory to become "fragmented." If a significant amount of fragmentation occurs, the system might run more slowly, and files might be lost. It is extremely important that you protect your data by running a disk optimizer program regularly.

Optimize the hard disk monthly with Disk Express II, the Speed Disk utility in Norton Utilities (from Symantec), or a similar program, to prevent the hard disk from becoming too fragmented. Be sure to back up custom matrix files (in the folder designated as the Settings folder—refer to *Setting Folder Location Preferences* on page 5-16) and any important data before you optimize the hard disk.

Use Discretion When Adding Software Programs

- Keep SAM Intercept (from Symantec) loaded on your hard disk to check any disks you insert, and use it to inspect your hard disk either when you start up or shut down the disk (at least once a day). Make sure you have a current version of SAM (version 4.08 or later).

  Viruses can simply be annoying, but they can also, in the worst case, destroy all information stored on your hard disk. This could include the System software, ABI PRISM 373 DNA Sequencer with XL Upgrade software, and data files.

- Prevent program conflicts before they occur. Do not load games or other software programs onto your hard disk. Use the Macintosh computer only for ABI PRISM 373 DNA Sequencer with XL Upgrade software.

- Do not use any programs that require the installation of additional extensions. This includes games that might have custom sounds or graphics. Use only those that came on your original System Disks, or on the ABI PRISM 373 DNA Sequencer with XL Upgrade software disks.

  Extensions reside in your extension folder in your system folder. They actually alter the programming code of the system. Compatibility with the ABI PRISM 373 DNA Sequencer with XL Upgrade software is only guaranteed for the extensions shipped with your system. Even seemingly innocuous extensions like After Dark, a screen saver, can interfere with operations.

  While it is unlikely, you might experience conflicts from some extensions that you think you must use. For example, different printers might have extensions that access them. If you think a conflict exists, turn off specific extensions, or all extensions, restart your Macintosh computer, then try running without the suspect extensions.

  You can turn off all extensions by holding down the Shift key while you restart your computer. You will need to restart it again to turn them back on.

- If your System is ≥ 7.5, you have Extensions Manager. Do not keep the programs Init Picker, used previously with your Model 373, and Extensions Manager on the same volume.

Error Messages

Following the procedures and precautions described above reduces the probability of errors occurring during ABI PRISM 373 DNA Sequencer with XL Upgrade runs. Unfortunately, it might not prevent errors from occurring altogether, so you still might see error messages after performing a run.

Note　　　*If you experience recurring errors, use a hard disk utility program to check and repair any damage on the hard disk. After it is repaired, follow all of the preventative maintenance procedures described in this document.*

Hard Disk Maintenance Programs

Following is a limited list of maintenance programs that you can use to check or maintain your hard disk.

- Disk Express II　　AlSoft
- Norton Utilities　　Symantec
- Public Utilities　　Fifth Generation
- SilverLining　　La Cie
- CP Optimizer　　Central Point Software
- DiskTools　　Included on the Mac Operating System CD-ROM

IMPORTANT　　*Do not use automatic optimization features that might optimize the hard disk in the background while collecting ABI PRISM 373 DNA Sequencer with XL Upgrade data. This could cause loss of data.*

Backing Up Important Files Before Optimizing Your Disk

Before optimizing your hard drive, back up all important files onto disks, or another storage medium, and locate all of the original disks necessary for reloading the whole system. Proceed as follows:

1. Back up Instrument and/or matrix files.

    a. Insert a disk into your computer.

    b. Open the System folder.

Note   *If a different folder is designated in Preferences as the Settings folder, open it instead.*

---

  c. Open the ABI folder.

  d. Drag the Instrument and/or matrix file icons to the disk icon.

2. Make backup copies of your data.

3. Optimize your hard drive according to the instructions included with your software.

8 Maintenance/Troubleshooting

Contents

Maintenance

Most maintenance procedures on the ABI PRISM 373 DNA Sequencer with XL Upgrade should be performed by a trained Applied Biosystems service technician. However, you can perform certain procedures yourself, as described below.

Cleaning Instrument Accessories

Be sure to clean the following regularly, as described on the pages noted:

- Glass plates (pages 2-13 and 3-48)

- Spacers and combs (page 3-48)

Replacing Electrophoresis Cables and Electrodes

The electrophoresis cables and electrodes are built as assemblies that simplify installation in the buffer chambers. Change the entire electrode assembly when you need to replace either a cable or an electrode.

Figure 8-1 shows a drawing of the upper buffer chamber. Figure 8-2 refers to the lower buffer chamber. Refer to both figures as you perform the following procedures.

Upper Buffer Chamber Replacement

Remove the clamps from the old upper buffer chamber and install them onto the new upper buffer chamber using the following steps (see Figure 8-1).
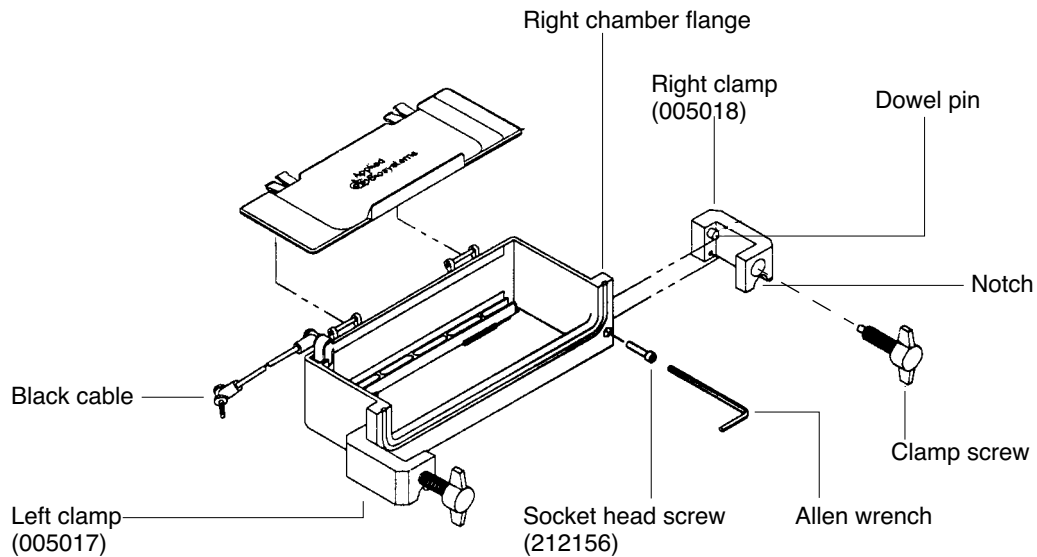
Figure 8-1. Attach the clamps to the upper buffer chamber

Note	*The buffer chamber should be opaque with the ABI logo stamped on the lid. If your buffer chamber looks different, contact Applied Biosystems.*

1. Remove the upper buffer chamber from the instrument and place it on a work area.

2. Remove the clamp screw from the right clamp by unwinding the head from the base of the screw.

3. Insert the provided Allen wrench through the notch of the right clamp and loosen the socket head screw by turning it counter-clockwise. If there is no

notch on the clamp, use the shorter end of the Allen wrench to loosen the socket head screw.

Caution   ***If the clamp cannot be removed because it is attached with adhesive or the screw is frozen, contact Applied Biosystems for further instructions.***

---

4. Remove the right clamp from the flange of the old upper buffer chamber.

5. Install the right clamp onto the new upper buffer chamber. Make sure that the dowel pin of the clamp has been inserted into the hole of the right flange of the new chamber. Use the Allen wrench to tighten the socket head screw.

6. Replace the head of the clamp screw on the base.

7. Repeat steps 2–6 to remove the left clamp from the old upper buffer chamber

8. Place the new buffer chamber in the instrument.

9. Connect the new black cable to the instrument.

WARNING   ***Use ONLY the opaque upper buffer chamber with the ABI logo on the lid. If you do have extra upper buffer chambers that are made of the transparent material do not use them. If you need replacement chambers contact Applied Biosystems.***

Lower Electrode Replacement

Replace the electrode of the lower buffer chamber using the following steps (see Figure 8-2).

Nylon screw 1
(212160)
(do not loosen this screw

Nylon washer
(223071)

Red cable

Cable clip
(200442)
(do not remove cable
clip from the chamber)

Connector

Nylon screw 2
(212179)

Tie holder
(200629)

Feet

Electrode
(604175)

Right support
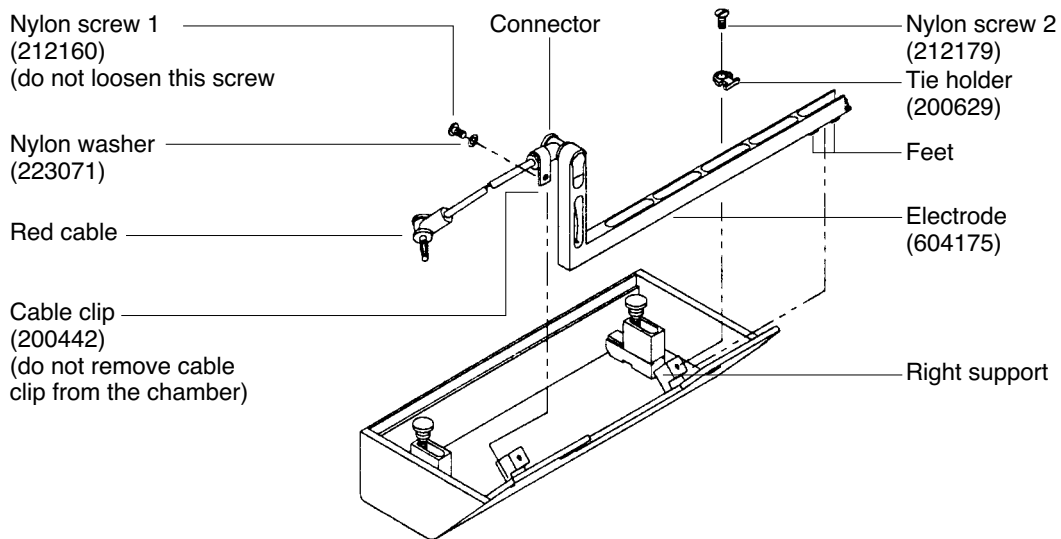
Figure 8-2. Attach the electrode to the lower buffer chamber

1. Lift the lower buffer chamber from the instrument and place it on your work area.

2. Remove the red cable from the lower buffer chamber and discard.

3. Partially loosen nylon screw 2 using the screw driver and turn the tie holder 180 degrees.

    Nylon screw 2 is located on the inside bottom of the buffer chamber on the right side when the chamber is facing you.

    It is not necessary to loosen or remove nylon screw 1, which is the smaller nylon screw that attaches the curled cord clip on the left side of the electrode.

4. Remove the old electrode from the lower buffer chamber.

5.   Place the new electrode onto the lower buffer chamber.

     The right support of the chamber should be between the electrode's two feet.

6.   Insert the cable under the cable clip.

     The cable clip stretches around the top of the cable. Rotate the tie holder to its original position, and use the screw driver to tighten nylon screw 2. The tie holder and the cable clip should now secure the electrode in place.

7.   Replace the chamber in the instrument.

     Plug the new red cable into the instrument. The cable lies on the instrument between the chamber and the wall of the instrument.

     Make a final check that both electrode cables are stored away so that they won't interfere with the operation of the instrument door. The new electrode cables are stronger than the old cables, however, they are also less flexible so storing the cables may be slightly more difficult.

Note	*If in the future either the upper or lower electrode should need to be replaced, the entire electrode unit will be replaced by ordering a new electrode from Applied Biosystems. The upper electrode part number is P/N 604174. The lower electrode part number is P/N 604175.*

Backing Up Important Files Before Optimizing Your Disk

Before optimizing your hard drive, back up all important files onto disks or another storage medium, and locate all of the original disks necessary for reloading the whole system. Refer to page 7-12.

Other Macintosh Computer Maintenance

Refer to Section 7, *Computer System*, for routine maintenance issues for the Macintosh computer.

Troubleshooting

If you have problems with the instrument, Translation Interface Processor, or the Macintosh computer, perform a Total Reset. Total Reset consists of several parts:

- Performing a Total Reset through the keyboard on the instrument

- Resetting the Firmware Image and restarting the Macintosh computer and Translation Interface Processor to load the Firmware Image

Total Reset of the Instrument

To perform a total reset of the instrument:

1. Verify the electrophoresis chamber door is closed.

2. Press the Main Menu key, and then press the Delete key.

3. Press Total Reset.

   The screen goes blank, then the message, "Calibration in Progress Please Wait" appears. Total Reset takes about 60 seconds. The instrument is now ready for use.

Resetting the Firmware Image and Restarting the Macintosh Computer

If the firmware memory becomes corrupted, you might need to clear the instrument's memory so that the computer copies a new firmware image into memory the next time it connects to the instrument. Figure 8-3 shows the reset button and status indicators (LEDs) on the back of the Translation Interface Processor.
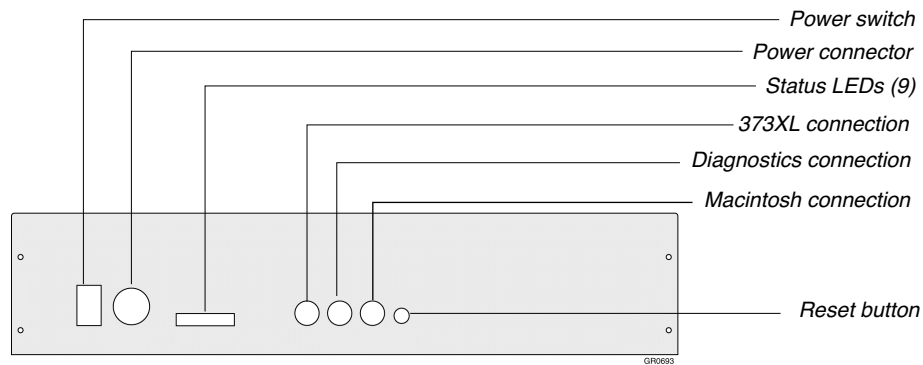
Figure 8-3. ABI PRISM 373XL Translation Interface Procesor (back view)

Clearing the Firmware Image

Possible symptoms of corrupted memory

- The computer does not communicate with the Translation Interface Processor or the instrument does not appear to run properly (flat or no scan lines in the Scan window, or no instrument response to computer commands).

- The Translation Interface Processor does not communicate with the instrument or the Macintosh after pressing the reset button, located on the rear of the Translation Interface Processor.

- The Translation Interface Processor LEDs (see Figure 8-3 on page 8-9) do not blink in their characteristic pattern but are stuck in one pattern, which might be all on or all off AND

- The Translation Interface Processor might not be able to load a new Firmware Image on the instrument, OR

- The Translation Interface Processor appears to have loaded a new Firmware Image onto the instrument but the instrument still does not run and the Translation Interface Processor LEDs are still not changing.

To clear the Translation Interface Processor's memory:

1. Press the reset button on the back of the Translation Processor (refer to Figure 8-3 on page 8-9.

   Use the eraser end of a pencil or something similar.

2. Watch the back panel LEDs and wait for them to blink in a four-on, four-off sequence.

   In this state, four consecutive lights are on at one time, then all four go off and the other four go on together.

3. While the four-on, four-off blink sequence is occurring, press the reset button a second time within about three seconds after the sequence.

   The LEDs change to the "every other one on" state. This indicates that the Translation Interface Processor's memory is clear and the Translation Interface Processor is ready for the computer to copy over a new firmware image. The next time you start the data collection program, the new Firmware Image will be copied to the instrument.

Restarting the Macintosh and Loading the Firmware Image

The first time you turn on the ABI PRISM 373 DNA Sequencer with XL Upgrade and start the data collection program, the program searches for a current version of firmware on the instrument. If none is located, it automatically copies the Firmware Image file from the computer to the instrument.

If you turn off the instrument between runs, a battery backup holds the image in memory, so you do not need to copy it again when you restart the instrument. Occasionally, however, you might need to copy the image file again, for instance, if a power outage clears the instrument memory or the memory becomes corrupted.

To restart the Macintosh to load the Firmware Image:

1. Choose Restart from the Special menu in the Finder to restart your Macintosh.

2. The data collection software should automatically open upon restarting the Macintosh. If it does not open, double-click the icon to open the program. Refer to To make the data collection program open at startup: on page 5-13 for instructions to open the collection program at startup.

   If the program locates the firmware image file it automatically displays a progress window and starts copying the firmware image file to the instrument.
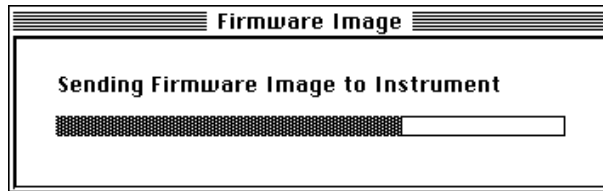
   When the file is copied, an alert box notifies you to start the data collection program again.
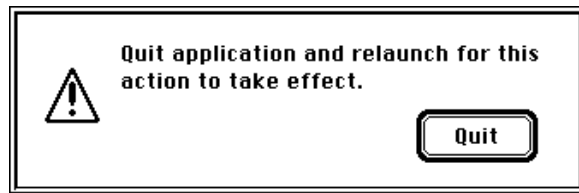
3. Click Quit.

4. Double-click the ABI PRISM 373XL Collection icon to start the program again.
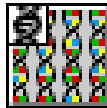
   If, after Step 1, the program does not locate the Firmware Image, a dialog box appears asking you to identify its location.
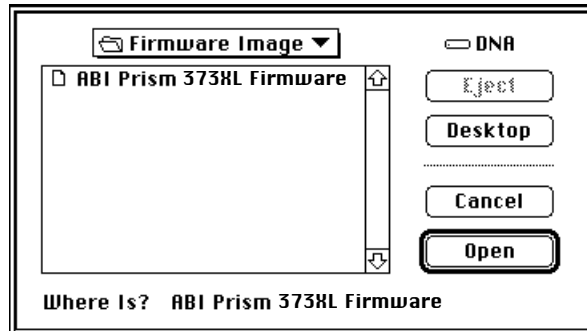
Figure 8-4. Find Image dialog box

5.   Locate the ABI PRISM 373XL Firmware file and click Open.

6.   Click Quit when it is finished and restart the ABI PRISM 373XL Collection program.

Note   *If you click Cancel in the Find Image dialog box, the program quits and returns you to the Macintosh Finder. You cannot start a run without an ABI PRISM 373XL Firmware Image file.*

Problems During a Run

Table 8-1 contains a brief list of problems you might encounter during a run and what to do about them.

Table 8-1. Problems That Might Occur During Runs

Problem	What to do
Dialog box appears indicating there is not enough room to store files from run.	Delete files (particularly Gel files) from the hard disk to make room. Gel files are stored in each individual Run folder when you perform a run, and take up a substantial amount of room on the hard disk.
Buffer level in upper chamber is getting lower, but no external leaks apparent	Siphon the buffer out of the upper buffer chamber, dry the corners of the notched area at the top of the front plate, then apply a small amount of molten agarose to the corners of the notches. Refill the upper buffer chamber. If still I leaking, you may need to replace the gasket.
Buffer splashes and crystallizes around the read region	Be careful to not overfill the lower buffer chamber.
Communication problems between the instrument and the Manintosh	• Check for loose or improperly connected cables • Collection software preference set to No Port. Change preference to Modem Port. • Corrupted Firmware. Perform a Total Reset.

Troubleshooting Gels

Table 8-2 shows some problems you might encounter relating to gels, and recommends some solutions.

Table 8-2. Gel Troubleshooting

Problem	Possible Cause	Solution
Inconsistent mobilities from gel to gel	Wrong total percentage polymer	Follow protocol carefully.
	Wrong concentration of Bis	Follow protocol carefully.
	Wrong buffer concentration	Follow protocol carefully.
	Poor quality reagents	Remake 10X TBE and 40% acrylamide stock solution using highest grade reagents. Urea must also be ultra pure.
	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 that are of equal thickness
	Temperature of room, gel solution or glass varies	20–23 ˚C is optimal
Slow mobility	Old gel	Remake 10X TBE and 40% acrylamide stock solution using highest grade reagents. Urea must also be ultra pure.
		Pour a fresh gel
Poor resolution	Poor quality reagents (especially acrylamide)	Use fresh reagents from a reliable source.
	Small bubble between load and read region	Cast gel as described in protocol.
	Well shape not flat	Assure no oxygen trapped by comb; remove comb carefully; only load in flat wells.

Table 8-2. Gel Troubleshooting (continued)

Problem	Possible Cause	Solution
	Old buffer	Make 1X TBE fresh daily.
	Old gel	Use within 6 hours; do not refrigerate
Catastrophic loss of resolution (bands tilted and poorly resolved)	Gel extruding from between plates into upper buffer chamber	After cleaning plates, wash briefly in 3M HCl, wash with water, then clean as described on page 2-13.
Swirls in gel	Excessive TEMED or APS	Follow protocol
	Temperature too high	Polymerize at 20–23 ˚C
Polymerization too slow	Excessive dissolved O_2	Keep vacuum filter strength and time constant; stir and pour solutions gently; filter and pour gels at room temperature
	Not enough TEMED or APS (or degraded)	Use fresh high-quality reagents; use as indicated in protocol
	Temperature too low	Polymerize at 20–23 ˚C
	Did not use dH_2O	Use only distilled or deionized water for making all solutions

Software Troubleshooting

Data Collection Run Log Errors

If an error message appears on the screen during collection, it also appears in the Run Log file. The Run Log lists both computer errors and instrument firmware errors.

Computer errors are individually created and listing each possibility individually is too massive a task for this manual. Such errors are usually due to problems related to:

- Memory

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

- File searching

  For example, a file might be missing or in a different location so it is not found when the data collection program tries to open or save it, or a file might be corrupted.

- Disk problems

  Any problems with your hard disk could cause computer error messages. Refer to Use a Hard Disk Maintenance Program Regularly on page 7-9.

Check these areas if the Run log lists computer errors.

One instrument firmware error might appear:

- The AD converter is not functioning.

If you see this message, turn off the power switch, then turn it on again. If the message appears again, run a plate check to see if a signal is displayed. If a signal appears, the irregularity is not severe, and you should be able to run samples and collect data.

Appendix A   Applied Biosystems Limited Warranty

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 373 DNA Sequencer with XL Upgrade purchased by the Customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the performance specifications contained in the Instrument user's manual that accompanies the instrument (the "Specifications"). During the Warranty Period, if the Instrument fails to perform in accordance with such specifications, Applied Biosystems will repair or replace the Instrument at no charge to the Customer, subject to the conditions below.

This Warranty does not apply to the Instrument's valves, reagent lines, or performance, unless the Customer uses only reagents and solvents supplied by Applied Biosystems or expressly recommended by Applied Biosystems, or to damages caused by reagents or solvents not supplied by Applied Biosystems, even though recommended by Applied Biosystems. This Warranty does not extend to any Instrument or part thereof (i) that has been the subjected of misuse, neglect or accident, (ii) that has been modified or repaired by any party other than Applied Biosystems or (iii) that has been used in a manner not in accordance with the instructions contained in the Instrument User's Manual. This Warranty does not cover the customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual.

Applied Biosystems obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct covered defects or failures of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty shall be performed by Applied Biosystems on-site at the Customer's location at Applied Biosystems expense.

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

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; such products or parts will be subject to the warranties, if any, of their respective manufacturers.

This Warranty is limited to the original Customer and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

Appendix B   Quick Reference

Contents

Flow Chart of Typical Sequence Run Process

Prepare and label samples

↓

Pour gel

↓

Polymerize ~2 hr ⎧ Restart Macintosh
Set up software

↓

Load gel on instrument
Run plate check

↓

Cancel plate check
Add buffer
Start pre-run

↓

Pre-run ~5 min ⎧ Resuspend DNA

↓

Pause pre-run
Load first set of samples
Resume pre-run

↓

Run samples into gel ~ 2 min ⎧ Pause pre-run
Load second set of samples

↓

Cancel pre-run
Start Run

↓

Run ~ 2–16 hr ⎧ Observe status

↓

Analyze and print (automatic)

↓

Clean up

Flow Chart of Typical GeneScan Run Process

Pour gel

Polymerize
~2 hr

Restart Macintosh
Set up software

Load gel on instrument
Run plate check

Cancel plate check
Add buffer,
Start pre-run

Pre-run
~5 min

Mix sample with loading buffer size std
Heat sample

Run samples
into gel
~ 2 min

Cancel Pre-run
Load samples

Start Run

Run
~ 3–8 hr

Observe status

Analyze and print (automatic)

Clean up

Acrylamide Gel Recipes

10% Ammonium Persulfate (APS)	**10X TBE**	**25 mM EDTA 50 mg/mL Blue Dextran**
• 0.1 g APS • 1.0 mL dH$_2$O	• 108 g Tris • 55 g boric acid • 8.3 g EDTA (Na$_2$) • Bring to 1 L with dH$_2$O	• 0.93 g EDTA • Add 90 mL dH$_2$O • Adjust to pH 8.0 • Bring to 100 mL with dH$_2$O • Add 50 mg Blue Dextran to 1 mL EDTA solution

40% Acrylamide Stock

• 38 g acrylamide
• 2 g bis-acrylamide •
Bring to 100 mL with dH$_2$O
• Deionize
• Filter

4.0% Gel (100 mL)

• 10 mL 40% acrylamide stock
• 50 g urea
• 37 mL dH$_2$O
• 1.0 g mixed-bed resin

4.25% Gel (80 mL)

• 8.5 mL (40%)
• 40 g urea
• 27 mL dH$_2$O
• 1 g resin

4.75% Gel (80 mL)

• 9.5 mL (40%)
• 40 g urea
• 27 mL dH$_2$O
• 1 g resin

6.0% Gel (80 mL)

• 12 mL (40%)
• 40 g urea
• 27 mL dH$_2$O
• 1 g resin

• Stir to dissolve
• Filter and degas
• Add 10 mL 10X TBE
• Bring to 100 mL with dH$_2$O

• Stir to dissolve
• Filter and degas
• Add 8 mL 10X TBE
• Bring to 80 mL with dH$_2$O

To Polymerize 4.0% Gel

• Add 500μL 10% APS
• Add 50 μL TEMED
• Mix gently
• Inject quickly

To Polymerize 4.25%, 4.75%, and 6% Gels

• Add 400μL 10% APS
• Add 45 μL TEMED
• Mix gently
• Inject quickly

To Prepare Sequencing Sample

• Prepare solution:
  · 1 part 25 mM EDTA with 50 mg/mL Blue Dextran
  · 5 parts deionized formamide
• Add to samples
• Mix well
• Heat at 90 °C for 2 min
• Place on ice

To Prepare Matrix Standards

• Prepare sample:
  · 3 μL standard
  · 3 μL formamide
• Mix well
• Heat at 90 °C for 2 min
• Place on ice

To Prepare GeneScan Sample

• Add loading cocktail to sample: Blue EDTA; formamide, size standard (see Chapter 4)
• Mix well
• Heat at 95 °C for 5 min
• Place on ice

Modules

ABI PRISM 373 DNA Sequencer with XL Upgrade Module Default Values[a]

Module Name	Field (volts)	Power (watts)[b]	Current (mAmp)	Laser Power (mW)	Run Time[c] (hr)
Plate Check	--	--	--	40	0.50
PreRun	2500	30	40	40	0.33
Seq Run	2500	30	40	40	12
GS Run	2500	30	40	40	12

a. These default values should be changed according to conditions appropriate to the gel you are running.

b. These parameters are limited by the Field parameter.

c. Can be set in the Run window

IMPORTANT   The Seq Run and GS Run default parameters are applicable only to 24-cm WTR Classic and Leon 6% acrylamide gels.

Throughputs and Filter Sets

ABI PRISM 373 DNA Sequencer with XL Upgrade Throughputs for Sequencing

Instrument Model	WTR Length (cm)	Gel Concen-tration (%)	Gel Thickness (mm)	Scan Mode	Power (W)	Collec-tion Time (hr)	Length of Read (bases resolved)
Classic	24	6.0	0.4	XL or Full scan	30	12–14	450
Classic	24	4.75	0.4	BaseSprinter	40	6.5	450
Leon	24	6.0	0.4	XL or Full Scan	30	12–14	450
Leon	24	4.75	0.4	BaseSprinter	40	6.5	450
Stretch	24	6.0	0.4	XL or Full Scan	26[1]	12–14	450
Stretch	24	4.75	0.4	BaseSprinter	35	7	450
Stretch	34	4.75	0.4	XL or Full Scan	32[1]	14–16	550
Stretch	34	4.25	0.4	BaseSprinter	45	8	550
Stretch	48	4.0	0.3	XL or Full Scan	40[1]	17–18	700

1. If necessary, adjust the power to achieve spacing of 9.5 to 12 for analyzed data.

ABI PRISM 373 DNA Sequencer with XL Upgrade Throughputs for GeneScan

Application	Resolution (bp)	Size Standard	Gel Type (Acrylamide)	Gel Length (cm)	Run Time (hr)
Microsatellite Genotyping (Linkage Mapping Set, dinucleotide tepeats)	1	GS-350 or GS-500	6.0% denaturing	24	6
Microsatellite Genotyping (tri- and tetranucleotides)	1–2	GS-500	6.0% denaturing	12	4
VNTRs	1–2	GS-350 or GS-500	8.0% denaturing	12	4
Large Fragments (0.5–2.5 kb)	3–4	GS-1000 or GS-2500	8.0% denaturing	6	2
AFLP™ Plant Mapping Kit	1	GS-500	6.0% denaturing	24	11
Stock Marks™ for Cattle	1	GS-350	6.0% denaturing	24	6
AmpF*l*STR™ (tetranucleotides)	1	GS-500	6.5% denaturing	24	6

Dye Sets and Filter Sets by Chemistry

Chemistry	Dye Set	Filter Set
Taq and T7 Primers	1	Filter Set A
Taq Terminators	2	Filter Set A
T7 Terminators	3	Filter Set B

Color Guide for Data Collection Program Raw Data and Gel Image Displays

	Filter A		Filter B
Color	*Taq* and T7 Primers (Dye Set 1)	*Taq* Terminators (Dye Set 2)	T7 Terminators (Dye Set 3)
Blue	C	G	T
Green	A	A	C
Yellow	G	T	A
Red	T	C	G

Combs and Run Modes

Lanes	Comb	Run Mode
18	Sharks-tooth	BaseSprinter
24	Sharks-tooth	Full Scan
24	Square-tooth	Full Scan
32	Sharks-tooth	Full Scan
34	Square-tooth	Full Scan
36	Sharks-tooth	Full Scan
36	Square-tooth	Full Scan
48	Sharks-tooth	XLScan
50	Square-tooth	XLScan
64	Sharks-tooth	XLScan
68	Square-tooth	XLScan

Table of Abbreviations

APS	Ammonium persulfate
bp	Base pairs
CEHV	Capillary Electrophoresis High Viscosity
COP assay	Competitive Oligonucleotide Priming assay
CsCl	Cesium chloride
dH_2O	Deionized, distilled water
DNA	Deoxyribonucleic acid
ddNTP	Dideoxynucleoside triphosphate
dNTP	Deoxynucleoside triphosphate
dITP	2'-deoxyinosine-5'- triphosphate
ds	Double stranded
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid, -tetracetate, -tetraacetato
HCl	Hydrochloric acid
IPTG	Isopropyl-ß-D-thio-galactopyranoside
KH_2PO_4	Potassium phosphate
kV	Kilovolts
mA	MilliAmps
min	Minutes
mM	Milli molar
mL	Milliliter
mRNA	Messenger RNA
nt	Nucleotide
µg	Microgram
µL	Microliter
µm	Micron or micrometer
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol 8000
PMT	Photomultiplier tube
P/N	Part number
q. s.	Quantity sufficient
RF DNA	Restriction Fragment DNA
s	Seconds
ss	Single stranded

Table of Abbreviations

STR	Short tandem repeat
SDS	Sodium dodecyl sulfate
TBE	Tris-Borate-EDTA (pH 8.3)
TE	10 mM Tris-Cl (pH 8.0), 1 m M EDTA (pH 8.0)
TEMED	N, N, N', N'-tetramethylethylenediamine
Tris-Cl	Tris hydrochloride
VNTR	Variable number of tandem repeats
w	Watts
WTR	*Well-to-read* or separation length; the distance the sample travels through the acrylamide before detection, this equals the distance from the bottom of the well to the laser-reading region.
X-GAL	5-bromo-4-chloro-3-indolyl-ß-galactoside

Appendix C   Sequencing Instrument File

Contents

Sequencing Instrument File

Sequencing Matrix Files in an Instrument File

Three chemistries are currently available to prepare DNA samples for sequencing on the ABI PRISM 373 DNA Sequencer with XL Upgrade: Dye Primers, *Taq* DyeDeoxy Terminators, and T7 Terminators. Each chemistry has a specified dye set: a set of dye labels that emit fluorescence when excited by a laser. Each dye label in the set emits fluorescence at a different wavelength and, during data collection, the wavelengths are detected. The wavelengths are separated by physical filters.

Although the dyes fluoresce at different wavelengths, there is some overlap in the spectra. To correct for this overlap before analyzing data, a mathematical matrix is created for each chemistry type (dye set) and stored in a file called the Instrument file. During data analysis the appropriate matrix is applied to remove any spectral overlap.

The Instrument file must contain a matrix for each chemistry that you run on the instrument. When the Gel and Sample files are created, the Instrument file is attached to each to allow subsequent data analysis. Each ABI PRISM 373 DNA Sequencer with XL Upgrade must have an Instrument file inside the ABI folder located in the System Folder.

IMPORTANT   *Due to slight variations in the filter wheels, the Instrument file created on your ABI PRISM 373 DNA Sequencer with XL Upgrade is not valid on other ABI PRISM 373 DNA Sequencer with XL Upgrade instruments.*

Create a new Instrument file only if the filter wheel is replaced, or if you should need a new matrix for a type of chemistry other than the three mentioned above.

Filter Wheel and Data Display

A filter wheel separates the wavelengths emitted by the fluorescent dyes. On the real-time displays (the Scan window and the Gel window), the data collection program displays these intensities, color-coded according to wavelength. Blue, green, yellow, and red (in that order) represent the wavelengths of the dye emissions within each dye set. Blue represents the shortest wavelength, and red

represents the longest. The colors on the real-time displays therefore represent the wavelengths of the dyes being detected, rather than the bases being detected.

Different 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. Filter A uses the four colors to represent wavelengths within Dye Set 1 and Dye Set 2. Filter B uses the four colors to represent wavelengths within Dye Set 3.

Each of the three chemistries used for preparing DNA is associated with one of the dye sets. 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. Because of this, the four colors on the real-time displays represent different bases, depending on the chemistry used for labeling. Table C-1 describes the colors that represent each of the four bases on the real-time displays.

Table D-1. Color guide for data collection program raw data displays

| Color | Filter Band Pass Center (nm) | Filter A | | Filter Band Pass Center (nm) | Filter B |
		Taq and T7 Primers (Dye Set 1)	*Taq* Terminators (Dye Set 2)		T7 Terminators (Dye Set 3)
Blue	531	C	G	531	T
Green	560	A	A	545	C
Yellow	580	G	T	560	A
Red	610	T	C	580	G

The analysis program converts the information collected by the data collection program, so after analysis the colors are the same for each base. The colors on all displays of analyzed data, including printed electropherograms, are as follows:

C = Blue   A = Green   G = Yellow   T = Red

Note   *When printed, and when shown against a white background on screen, G is black so it is easier to see.*

Creating a Matrix File

Note	If a valid Instrument file exists in the ABI folder (inside the System folder) on your Macintosh, you need not create one. If you lose your Instrument file and do not have a backup copy on a floppy disk, refer to the *Sequencing Analysis User's Manual*.

This section describes, in detail, how to make a matrix. The steps you need to perform are:

1. Obtain the proper matrix standards for your purpose.

2. Prepare an acrylamide gel.

3. Prepare, load and electrophorese the matrix standards.

4. Refer to the *Sequencing Analysis User's Manual.*

Matrix Standards

To make a matrix, you must electrophorese and analyze specific matrix standards. A separate standard exists for each of the four reactions in each of the three dye sets. Load only one matrix standard per lane. The matrix standards are sold in kits, one for each chemistry.

Preparing, Loading and Electrophoresing Matrix Standards

After obtaining the correct matrix standards, follow the procedure below. You can run matrix standards for Dye Primers and *Taq* DyeDeoxy terminators on the same gel, using Filter Set A. Run standards for T7 Terminators on a separate gel using Filter Set B.

1. Prepare either a:

    • 4.75% acrylamide gel (for Stretch) or a

    • 6% acrylamide gel (for Classic and Leon)

2. Set up the instrument according to Section 3 of this manual.

3. Restart the Macintosh (choose Restart from the Special menu in the Finder).

ABI PRISM 373XL Collection

4. Start the data collection program.

5. Verify or define the data collection preferences.

Sequence Sample Sheet

6. Choose New from the File menu and click the Sequence Sample Sheet icon.

7. Fill in the Sample Sheet, as described in Section 3 of this manual.

    a. For convenience, name the matrix standards according to the labeled base (A, C, G, or T), followed by the specific sequencing chemistry (Primers, *Taq* Terminator, or T7 Terminator).

    b. Choose Save As from the File menu to save the Sample Sheet.

        Be sure to save it in the folder designated as the Sample Sheets folder in the data collection program preferences.
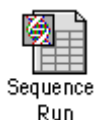
Sequence Run

8. Choose New from the File menu and click the Sequence Run icon.

9. In the Run window, choose the proper files from the pop-up menus.

    The Sample Sheet should be the one you completed in Step 7 above.

    The Gel Matrix should be the current instrument file or any other applicable matrix file.

10. Choose Off from the pop-up menus for Auto Analyze and Auto Print.

    a.   Choose Off in the top pop-up menu of the column.

    b.   Click the heading of the column to select the entire column.

    c.   Choose Fill Down (⌘-D) from the Edit menu to fill in the other fields.

11. Click the PreRun button to prerun the gel while you are preparing the standards. Pre-run for 5 minutes, then click Pause in the Run window.
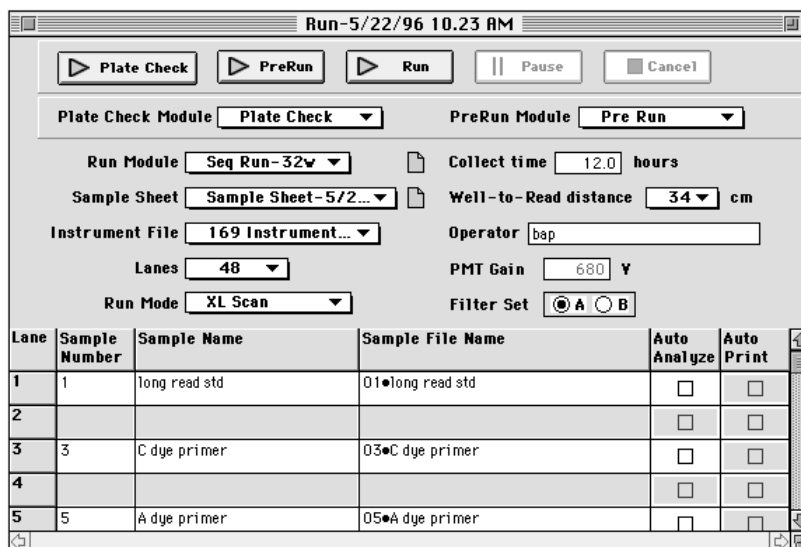
Figure D-1. Completed Sequencing Run window

12. Prepare the Matrix Standards as follows:

Note	Do not prepare the matrix standard more than 3 hours in advance of loading.

Note	As a precaution, prepare and run a duplicate set of matrix standards, in case one lane is inadequate or the data is lost.

a. Briefly vortex the matrix standards to ensure the solutions are well mixed. Spin the solution briefly to collect the fluid at the bottom of the tube.

b. For each standard (one tube for each base), combine 3 µL of standard with 3 µL of deionized formamide.

c. Briefly vortex each of the tubes prepared above to ensure that the sample is well mixed. Spin the solution briefly to collect the fluid at the bottom of the tube.

d. Heat all matrix samples at 90˚C for 2 minutes. Immediately place samples on ice until loaded.

13. Load the matrix standards in the lanes you designated in the Sample Sheet.

	Comb Configuration				
	18	24	32/36	48	64
Loading Volume (µL)	4	4–6	4	1.5–2	1–1.5

14. Click Run to start the run.

    A dialog box appears, allowing you to change the name and location of the gel file created by the run.

15. Proceed to either *Making the First Matrix for a New Instrument File,* or *Adding or Replacing a Matrix to an Instrument File* after the sequencing run is complete.

Making the First Matrix for a New Instrument File

If you need to re-create a matrix file for any reason, you should:

• Run the appropriate matrix standards

• Check the gel file to verify that lane tracking is correct and that peaks exist in the raw data.

• Make the matrix file, using the DataUtility program.

• Verify the accuracy of the matrix file, and its location.

Follow these steps (usually along with your Service Representative) when you get a new instrument.

Note	*If you lose your Instrument file and do not have a backup copy on a floppy disk refer to the Sequencing Analysis User's Manual before re-creating the entire Instrument file.*

Now that the matrix standards have been electrophoresed, switch to the matrix making instructions in the *Sequencing Analysis User's Manual.*

Appendix D   GeneScan Matrices

Contents

Introduction

Although the dyes used to label your fragments fluoresce at different wavelengths, there is some overlap in the spectra. To correct for this overlap before analyzing data, the software uses a matrix file, which contains a mathematical matrix.

You create one matrix for each chemistry and gel type. During data analysis the appropriate matrix is applied to remove any spectral overlap.

A specifically created matrix file provides the best results for your particular instrument and the dye set and run conditions used in the experiment. In most cases, you should not need to modify the matrix file once you have created it.

You may want to create a new matrix to accommodate these conditions:

- If you use gel materials or buffers with pH values that differ greatly from one another (in such a case you need different matrices)

- If you change dyes in a dye set, e.g., changing from 5-FAM to 6-FAM. Even though the dyes represent the same color, their spectral characteristics are different.

- If you use dyes other than those provided by Applied Biosystems

- If you see problems that appear to be the result of spectral overlap

Once you create a matrix file, you can apply that file to any run made under the same conditions, using the same dye set, and on the same instrument.

Applied Biosystemss provides Matrix Standard kits that are designed to test and define the multicomponent matrices for specific dye sets. To work with a matrix file, use the Matrix Standard kit that was designed for the specific dye set you plan to use. Run the matrix standards of different types in different lanes to make the matrix and to test whether the spectral overlap of the dyes is corrected by the matrix.

IMPORTANT	To specify matrix file in the data collection program so you can perform automatic analysis, you must store the matrix files in the ABI folder inside your System folder.

Filter Wheel and Data Display

The band pass filters on the filter wheel separate the wavelengths emitted by the fluorescent dyes. On the real-time displays (the Scan window and the Gel window), the data collection program displays these intensities, color-coded according to wavelength. Blue, green, yellow, and red (in that order) represent the wavelengths of the dye emissions within each dye set. Blue represents the shortest wavelength, and red represents the longest. The colors on the real-time displays therefore represent the wavelengths of the dyes being detected, rather than the fragments being detected.

Different 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.

The filter set you choose determines what color peaks appear for a particular dye (see Table D-1). Through either filter set A or B, each dye is detected within its optimum wavelength range. It is important to have a matrix for every combination of dye sets you use.

Applied Biosystems

Table D-1. ABI PRISM dyes and filter set color displays

| Dyes | | Filter Set | | | |
| | | A | | B | |
		Color Display	Filter Center Band (nm)	Color Display	Filter Center Band (nm)
Dye phosphoramidites	HEX	Green	560	Yellow	560
	6-FAM	Blue	531	Blue	531
	TET	*	*	Green	545
NHS Esters	TAMRA**	Yellow	580	Red	580
	ROX	Red	610	*	*
	5-FAM***	Blue	531	Blue	531
	JOE***	Green	560	Yellow	560
[F]dNTPs	TAMRA	Yellow	580	Red	580
	RG6	Green	560	Yellow	560
	R110	Blue	531	Blue	531

* Not recommended; the filter is not set at the emission maxima
** TAMRA is available as an NHS-Ester or as an [F]dNTP.
*** 5-FAM and JOE are available only as labeled primers in select reagent kits.

Creating a Matrix File

To make a matrix:

1. Obtain the appropriate matrix standards.

2. Prepare an acrylamide gel.

3. Prepare, load, and electrophorese the matrix standards.

4. Follow the procedure on page D-6.

Matrix Standards

To make a matrix, you must electrophorese and analyze specific matrix standards. Separate standards exist for each dye. Each dye used must be recognized as a different color by a single filter (Table D-1 on page D -5). Table D-2 lists the kits and their part numbers.

Table D-2. Matrix Standard Dyes and Part Numbers

Matrix Standards	Part Number
Dye Primer (5-FAM, JOE, TAMRA, ROX)	401114
Fluorescent Amidite (6-FAM, HEX, TET, TAMRA, ROX)	401456

Preparing, Loading and Electrophoresing Matrix Standards

To set up the instrument:

1. Prepare the appropriate gel.

2. Set up the instrument according to Section 4 of this manual.

3. Restart the computer (choose Restart from the Special menu in the Finder).

To set up the GeneScan Analysis software:

1. Start the GeneScan Analysis program.

2. Define the gel processing parameters.

    a. Choose Gel Processing Parameters from the Settings menu.

b.   Select both boxes for Auto-Launch Processing.

c.   Set the Scan Range from 0 to 10000.

d.   Deselect Multicomponent.

e.   Click OK.

3.   Define the analysis parameters.

a.   Choose Analysis Parameters from the Settings menu.

b.   Define all the appropriate parameters.

c.   Select Baseline and deselect Multicomponent boxes.

d.   Click OK.

4.   Define the project options.

a.   Choose Project Options from the Settings menu.

b.   Complete the each of the submenus.

To complete the set up:

1.   Start the data collection program.

2.   Choose Preferences from the Window menu to verify or set the data collection preferences.

3.   Choose New from the File menu, and then click the GeneScan Sample icon.

4.   Fill in the Sample Sheet as described in Section 4 of this manual.

a.   In the sample name column, type in a name appropriate to the matrix standard loaded.

b.   Choose Save As from the File menu to save the Sample Sheet.

Be sure to save it in the folder designated as the Sample Sheets folder in the data collection program preferences.

5.   Choose New from the File menu, and then click the GeneScan Run icon.

6. In the Run window, choose the proper files from the pop-up menus.

   The Sample Sheet should be the one you completed in Step 15, above.

   The Gel Matrix should be <none>.

7. Choose Off from the pop-up menus for Auto Analyze and Auto Print.

   a. Choose Off in the top pop-up menu of the column.

   b. Click the heading of the column to select the entire column.

   c. Choose Fill Down (⌘-D) from the Edit menu to fill in the other fields.

8. Click Plate Check.

   a. Check the plates for extra peaks. Reclean, if necessary.

   b. Set or verify the PMT setting.

9. Click PreRun to prerun the gel while you are preparing the standards. Prerun for 5 minutes, and then click Cancel in the Run window.
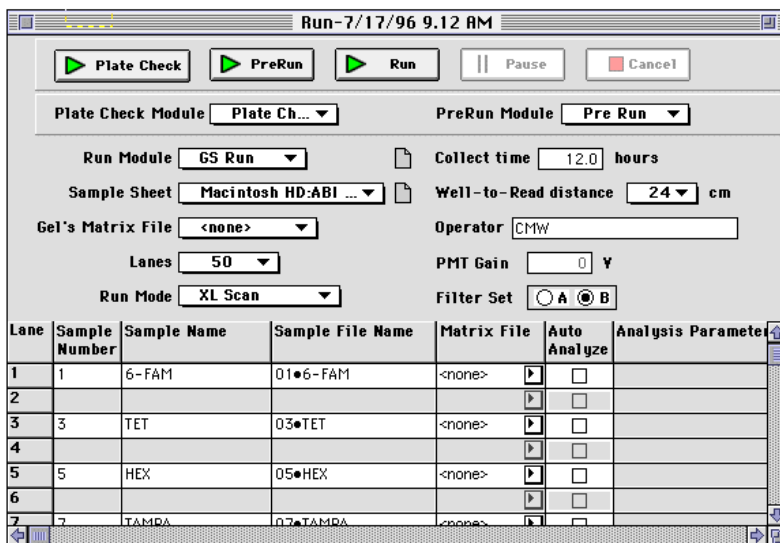
Figure D-1 Completed GeneScan Run window

10. Prepare the Matrix Standards as follows:

Note	Do not prepare the matrix standard more than 3 hours in advance of loading.

Note	As a precaution, prepare and run a duplicate set of matrix standards, in case one lane is inadequate or the data is lost.

    a.   Briefly vortex the matrix standards to ensure the solutions are well mixed. Spin the solution briefly to collect the fluid at the bottom of the tube.

    b.   For each standard (one tube for each base), combine 1 part standard with 1 part deionized formamide.

    c.   Briefly vortex each of the tubes prepared above to ensure that the sample is well mixed. Spin the solution briefly to collect the fluid at the bottom of the tube.

    d.   Heat all matrix samples at 90˚C for 2 minutes. Immediately place samples on ice until loaded.

11.   Flush the wells with loading buffer.

12.   Load the matrix standards in the lanes you designated in the Sample Sheet. Use the following table to determine the load volumes for your matrix run.

Number of Wells	Load Volume (µL)
24	5
34/36	4
50	1.5–2
66	1–1.5

13.   Click Run to start the run.

A dialog box appears, allowing you to change the name and location of the gel file created by the run. Click Save.

Creating the Matrix File

After preparing, loading, and electrophoresing matrix standards, you verify lane tracking and peaks in the raw data, and then create the matrix.

To verify lane tracking and peaks in the raw data:

1. Start the GeneScan Analysis program.

2. Choose Open Gel from the File menu and open the gel containing the matrix standards.

3. Check the Gel File window to see if the lane tracking was successful for each standard, and correct any gel tracking problems.

Note   *If you are unfamiliar with how to retrack a gel file and view data, refer to the* ABI PRISM DNA GeneScan Analysis User's Manual *for detailed instructions.*

To create the matrix and print the values:

1. Determine the range of scan points to be used for making the matrix.

    a. Click the name of the four matrix standard sample files in the Analysis Control window and choose Raw Data (⌘-R) from the Project menu.

    b. Select a starting data point that starts at a flat baseline in the middle of the run, well after the primer peaks. The range needs to contain a minimum of three well-defined peaks or 1000 data points.

        Typically, the same start point and range is used on all four matrix standard sample files.

    c. Close the raw data windows.

2. Choose New from the File menu, and then click the Matrix icon in the

window that appears.
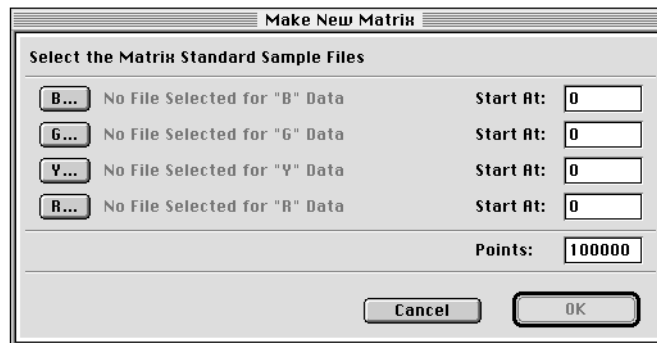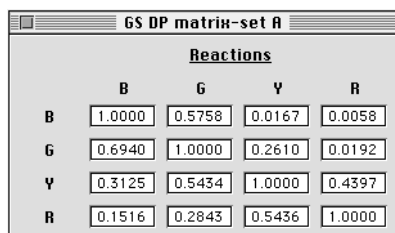
Figure D-2. Make New Matrix dialog box

a.   Click the B… button.

b.   In the file dialog box that appears, choose the Sample file containing your blue dye matrix standard.

c.   Enter the Start At point as determined in step 1.

d.   Repeat steps a through c for the other three dyes.

e.   Enter the number of data points to include, at least three well-defined peaks and 1000 points.

f. Click OK.

The Matrix Values window appears, showing the values used to calculate the overlap correction.

GS DP matrix-set A			
Reactions			
	B	G	Y	R

	B	G	Y	R
B	1.0000	0.5758	0.0167	0.0058
G	0.6940	1.0000	0.2610	0.0192
Y	0.3125	0.5434	1.0000	0.4397
R	0.1516	0.2843	0.5436	1.0000

Figure D-3. Matrix window example

For each dye, the value where the dye fluorescence is read by the appropriate filter is 1.000. The adjacent colors show the amount of overlap for which the system needs to compensate.

3. Choose Save from the File menu, and give the new matrix file a unique, descriptive name.

Note   *Save all matrix files in the ABI folder in the System folder and name matrix files by instrument serial number and type of gel used, for example, "#273 TBE den urea."*
It is also helpful to include "GS" in the name of the matrix files to differentiate between sequencing and GeneScan applications.

4. Choose Print (⌘-P) or Print One from the File menu to print the matrix values.

You can use this printout for documentation.

5. Click the Close box to close the window.

Applying and Verifying the Matrix

To apply and verify the matrix:

Use either of the following two methods to check the quality of the generated matrix.

Method 1

1. Apply the new matrix to your matrix sample files within a project.

    a. Choose Analysis Control from the Windows menu.

    b. Select the matrix standard sample files.

       Shift-click to select multiple consecutive files or ⌘-click to select multiple files that are not consecutive.

    c. Choose Assign New Matrix from the Project menu.

    d. In the directory box that appears, find and select the newly created matrix.

    e. Click Open.

2. Verify the matrix.

    a. Choose Analysis Parameters from the Settings menu.

    b. Select Baseline and Multicomponent (and any other applicable parameters).

    c. Select the matrix sample files and the colors to be analyzed (B, G, Y, and R buttons).

    d. Click Analyze.

    e. Choose Results Control from the Windows menu.

    f. Display the analyzed data.

       For each sample file, the only visible peaks should represent the dye loaded in that lane. All other lines should be relatively flat. This indicates that the matrix properly compensated for the spectral overlap.

A matrix that does not compensate for the other dyes (see Figure D-1) is unacceptable and must be regenerated. If necessary, the gel and matrix standards must be rerun. Examples of good and poor matrices applied to the same 5-FAM data (12-cm run) are shown in Figure D-1 and Figure D-1, respectively.
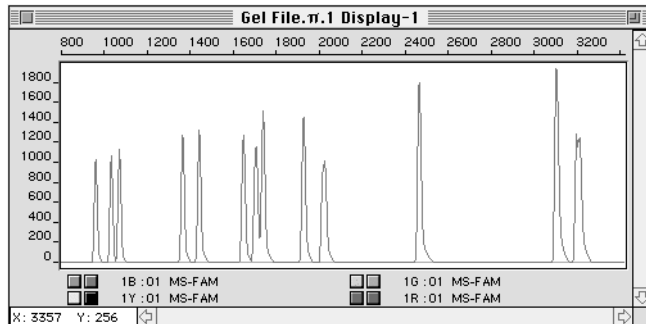
Figure D-1Good matrix—only blue peaks are present
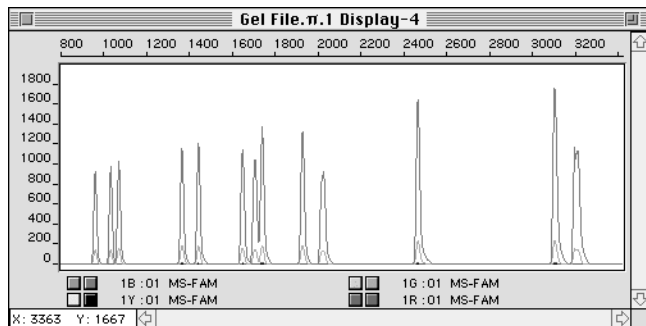
Figure D-1Poor matrix—note the other peaks at the same scan number as the blue peaks

Method 2

1. Apply the new matrix to your gel file.

    a. With the collection gel open, choose Attach New Gel Matrix from the Gel menu.

    b. Find and select the newly created matrix in the dialog box that appears.

    c. Click Open.

2. Define the gel processing parameters.

    a. Choose Gel Processing Parameters from the Settings menu.

    b. Select the appropriate Scan range (excluding excess primer peak).

    c. Select Multicomponent and baseline.

3. Choose Regenerate Gel Images from the Gel menu.

4. Use the "slice view" to examine the multicomponented sample files.

    a. On the gel display, click the triangle above the lane which represents one of the matrix standards.

    b. To the left of the gel image is a slice view. Use this view to examine the data.

    c. Verify that the only visible peaks correspond to dye-labeled fragments loaded in the lane. All other lines should be relatively flat. This indicates that the matrix properly compensated for the spectral overlap.

    d. Repeat steps a through c for the remaining sample files.

5. Apply the new matrix to the sample files within a project. Refer to page D-4 and page D-5 for help interpreting data.

Appendix E   Optimizing PCR

Contents

Preparing Reaction Mixtures

A reaction mixture consists of all the components you put into a reaction tube before loading the tubes in a thermal cycler and running a PCR reaction. When preparing reaction mixtures, consider the following factors that can affect overall yield of specific DNA target sequences:

- Reaction volume and tube types

- Deoxynucleoside triphosphate (dNTP) concentration

- Magnesium ion concentration

- Primer concentration

- Template concentration

- Enzyme concentration

Reaction Volume and Tube Types

When using an Applied Biosystems PCR instrument system, reaction volumes may cover the range of 5–100 µL, but should not exceed 100 µL. If you use volumes larger than100 µL, you may need to program longer incubation times to assure adequate thermal equilibration of the reaction mixture.

Table E-1 on page E -4 shows the types of reaction tubes that you can use with each PCR instrument system.

Table E-1. Reaction tube types for PCR instrument systems

PCR Instrument System	Reaction Tube Type	Uses
DNA Thermal Cycler	GeneAmp® PCR Reaction Tubes	Optimized for fast PCR amplification of reaction volumes between 25 μL and 100 μL.
DNA Thermal Cycler 480	0.5 mL GeneAmp Thin-Walled Reaction Tubes[a]	Allows you to program shorter hold times (45 sec. or more) at each temperature in the PCR cycle.
GeneAmp PCR System 9600	0.2 mL Thin-Walled MicroAmp® Reaction Tubes	Optimized for fast PCR amplification of reaction volumes between 5 μL and 100 μL. Can be used without mineral oil.
	0.5 mL Thin-Walled MicroAmp Reaction Tubes	Optimized for fast PCR amplification of 100 μL reaction volumes. Use with either an oil overlay or AmpliWax PCR Gem beads.
GeneAmp PCR System 2400	0.2 mL MicroAmp Reaction Tubes	Optimized for fast PCR amplification of reaction volumes between 5 μL and 100 μL.

a. Can also be used with the DNA Thermal Cycler if you set hold times of at least 60 seconds.

Although reaction tubes usually do not need to be sterilized or siliconized, you should autoclave tubes when dealing with small quantities of starting DNA template.

Deoxynucleoside Triphosphate (dNTP) Concentration

In the standard GeneAmp PCR protocol, the concentration of each deoxynucleoside triphosphate (dNTP) is 200 µM. For best base incorporation, keep the four dNTP concentrations balanced and above the estimated K_m of each dNTP (10–15 µM).

Note	*Some applications may deviate from these standards for dNTP concentrations.*

Lower dNTP concentrations should not significantly affect yield of PCR amplification product.

Magnesium Ion Concentration

For most PCR amplifications, you can relate yield and enzyme fidelity to the free Mg^{++} concentration (free Mg^{++} concentration is the concentration of Mg^{++} minus the total dNTP concentration added to the reaction solution). When using AmpliTaq DNA Polymerase, too little free magnesium ion results in little or no PCR product, and too much free magnesium ion can produce a variety of unwanted by-products.

Magnesium ion concentration generally has the most significant effect on amplification yield and specificity. To identify the optimal magnesium ion concentration; that which produces the highest yield of a specific PCR product, you can perform the following experiment. In the presence of 0.8 mM total dNTP concentration, run a series of 0.5 mM magnesium chloride concentration increments over a 1–4 mM range and identify the optimal concentration.

Primer Concentration

PCR primers are oligonucleotides, typically 15–30 bases long, hybridizing to opposite strands and flanking the region of interest in the target DNA. When choosing two PCR primers, never choose primers that contain bases complementary to themselves or with each other. Especially avoid primers that show complementarity at the 3' and 5' ends. These often promote formation of product artifacts called primer-dimers or primer-oligomers.

To avoid problems with the internal secondary structure of primers or long stretches of any one base, choose primers with a 40–60% G+C content. Also, avoid using primers that bind to a target region of secondary structure For instance, RNA sequences often have regions of looped secondary structures. Primers will have difficulty annealing to these target sequence.

By adding non-template complementary 5' extensions to primers you can facilitate a variety of useful post-amplification manipulations of the PCR product without adversely affecting the amplification process. These 5' extensions can be restriction sites, or promoter sequences.

Template Concentration

PCR templates may be single or double-stranded DNA or RNA. If your starting sample is RNA, then your template can be:

- Total cellular RNA
- Poly (A+) RNA
- Viral RNA
- tRNA
- rRNA

To prepare first-strand cDNA prior to conventional PCR amplification, you can include a reverse transcriptase such as MuLV or recombinant *Thermus thermophilus* (r*Tth*) DNA Polymerase. For RNA templates with high G+C content or complex secondary structure, the high-temperature reverse transcriptase activity of thermostable r*Tth* DNA Polymerase is effective.

If your starting sample is DNA, use nanogram amounts of cloned template, microgram amounts of genomic DNA, or up to 20,000 target copies to start optimization trials.

Even very low template concentrations (mRNA from tens of cells, DNA from single cells or individual genomes) are often sufficient for PCR amplification.

Enzyme Concentration

AmpliTaq DNA Polymerase is the recombinant form of Taq DNA Polymerase. It is obtained by expressing the Taq DNA Polymerase gene in an *E. Coli* host. Like

native Taq DNA Polymerase, it lacks endonuclease and
3'-5' exonuclease activities, but has a 5'-3' exonuclease activity. AmpliTaq DNA Polymerase is the enzyme used for the majority of PCR applications.

For most PCR applications with a 100 µL reaction volume, 2.0–2.5 units of AmpliTaq DNA Polymerase are recommended. The enzyme can be added conveniently to a fresh Master Mix prepared for a number of reactions, thereby avoiding the tedium and possible accuracy problems associated with adding individual 0.5 µL enzyme aliquots to each tube.

Recombinant 94-kDa *Thermus thermophilus (rTth)* DNA Polymerase is able to quickly and efficiently reverse transcribe RNA to cDNA in the presence of $MgCl_2$, after chelation of the magnesium ion with EGTA. R*Tth* DNA Polymerase can thus be used as both a thermostable reverse transcriptase and as a thermostable DNA polymerase in successive reactions in the same tube.

Performing Hot Start PCR

Competing side reactions can become a major obstacle to routine, sensitive and specific PCR amplification. Examples of side reactions include amplification of non-target sequences in background DNA (mis-priming) and primer oligomerization. These reactions usually occur during pre-PCR setup when you mix together all reactants at room temperature.

In the Hot Start™ technique, you add reagents to the reaction tubes so that all reactants do not mix until they reach a temperature high enough to suppress primer annealing to non-target sequences. You can perform either a manual Hot Start or an AmpliWax™ PCR Gem-mediated Hot Start.

To perform a manual Hot Start:

Note   Although manual Hot Start can increase specificity and yield, it is inconvenient and you may encounter reproducibility and contamination problems.

1.   At room temperature, mix all reactants except AmpliTaq DNA polymerase below a mineral oil cap.

2.   Load all tubes into a GeneAmp PCR instrument system.

3.   Define temperature control parameters so that the temperature rises to 70–80°C.

4.   Add AmpliTaq DNA polymerase enzyme to each tube, changing pipet tips after each sample.

To perform AmpliWax PCR Gem mediated Hot Start:

1.   At room temperature mix all reactants except AmpliTaq DNA polymerase in each reaction tube.

2.   Add a single AmpliWax PCR Gem to each reaction tube.

3.   Define temperature control parameters so that the temperature rises to 70–80°C for 5 to 10 minutes, then cools to room temperature. This creates a wax barrier over the aqueous layer.

4. Add the omitted reagent(s) and the test sample above the solid wax layer.

5. Create a PCR thermal profile as follows:

    a. Rapidly heat samples to the first denaturation temperature.

       This melts the wax layer, denatures the DNA template, and creates enough thermal convection to assure complete mixing of all PCR components under the melted wax. The wax also serves as a vapor barrier during cycling.

    b. Program temperature control parameters for conventional thermal cycling.

6. Run the PCR.

   After thermal cycling ends, the wax forms a solid shield, preventing spillage and evaporation.

7. For post-PCR analysis, you can penetrate the wax layer with a pipet tip and withdraw the PCR product.

8. Reheat to seal for long-term storage.

Note	*AmpliTaq Gold DNA polymerase is a modified form of AmpliTaq DNA polymerase specifically designed for Hot Start PCR. AmpliTaq Gold is provided in an inactive state and regains activity through a pre-PCR heat step. Contact Applied Biosystems for more information about AmpliTaq Gold.*

Setting Temperature Control Parameters

Temperature control parameters are the temperature values and hold times you specify for a PCR thermal profile. Table E-2 shows guidelines you can follow when considering the effects of different PCR parameters on the outcome of a PCR reaction.

Table E-2. How Changing Temperature Control Parameters Affects the PCR

Temperature Control Parameter	PCR Performance
Raising the denaturation temperatures (up to 96°C)	Amplifies GC-rich templates
Lowering annealing temperatures	Increases yield, but may reduce specificity
Varying the annealing or denaturation temperatures in each run. For example, six runs with annealing temperatures that vary by 2°C per run.	Increases chances of annealing
Setting each denaturation, annealing, and extension step to at least 15 seconds (preferably 30 seconds).	Allows complete extension of PCR product
Using the autoextension function of a thermal cycler, to vary the parameters to allow longer extension times in the later cycles.	Increases yield

DNA denaturation is the critical step in the GeneAmp PCR process and is often the focus of attention if PCR experiments fail. The practical range of effective denaturation temperatures for most samples is 94–96°C.

Always allow sufficient time for thermal equilibration of the sample at this high-temperature plateau.

Avoiding Contamination

PCR protocols can be very sensitive to contaminants in the DNA, as well as to DNA degradation that occurs before or during the purification process.

Note	*Although many protocols have been published recently that describe "simple" or "fast" extraction or purification methods, you should carefully evaluate any changes or improvements in extraction or purification methods. Also, be sure that the physical and chemical condition of the sample itself is adequate for the intended labeling and assay method.*

Sample contamination when preparing or analyzing samples can impair the PCR process, and lead to poor results.

Preventing PCR Product Carryover

Carryover of PCR products from previous amplifications contaminates samples, resulting in false–positive amplifications. Adopting the following laboratory practices can help you minimize the likelihood of PCR product carryover:

- If possible, use positive displacement pipets.

- Always run a negative control to ensure that target DNA from previous experiments does not contaminate your samples.

Note	*A negative control contains no DNA. The negative control contains only the DNA diluent (usually water or buffer).*

- For each run, use the same reaction composition and samples in the same tubes, but after each run transfer the product to a unique product tube.

- Physically separate reactions prior to and following amplification. For each reaction, use separate sets of devices such as automatic pipetters, disposable pipet tips, microfuge tubes, and gloves.

- Use identical pipetting techniques for each run, but handle pre- and post-PCR solutions with separate dedicated pipetters.

- Aliquot reaction reagents so as to minimize the number of times you use a particular stock solution.

- Use AmpErase® UNG in reaction mixtures to prevent the subsequent re-amplification of DU-containing PCR products.

Cleaning Up Spills on Work Surfaces

If you do spill samples on your work surface, use a cloth soaked with 50% bleach to clean your bench top, thermal cycler sample blocks, or any other work surfaces contaminated with nucleic acids.

IMPORTANT	*When cleaning the sample block of a thermal cycler, refer to the instrument manual for proper cleaning instructions.*

Analyzing Post-PCR Products

If your PCR does not produce enough product to analyze, check the following:

- Did you add the right amount of enzyme to your reaction mixture?

- Did you use the right size GeneAmp or MicroAmp Reaction tubes for the Applied Biosystems thermal cycler you are using?

- Did you check the chemical integrity of the primers?

Suggestions for improving yield:

- Pre–incubate samples at 95°C for 5 to 10 minutes in the absence of enzyme. This inactivates harmful proteases or nucleases in the sample. Pre-incubation also ensures complete denaturation of complex starting templates.

- Consider performing the Hot Start technique (See *Performing Hot Start PCR* on page E-8).

Glossary

This glossary defines special terminology used in the ABI PRISM 373 DNA Sequencer with XL Upgrade User's Manual. The terms are listed in alphabetical order. Many terms are defined in the text of the user's manual, so if you do not find a term here, check the index.

beam stop – laser beam safety stop. This is the bar that prevents outside exposure to the laser beam when it is scanning the gel.

channels – divisions across the read region of a gel where the data collection software samples the data. The number of wells in the loading comb used determines the approximate number of channels assigned per lane of the gel (for instance, with a 36-well comb, one lane is approximately five channels). When the Sequencing Analysis software tracks a gel, it places the tracker line for each lane in the channel showing the strongest fluorescent signal. The data from that channel and the adjacent channel on each side (total of three) are averaged to determine the raw data for the sample file.

Classic – This configuration includes 370s with Macintosh upgrade and the 373A. Classic models have electrophoresis chambers which support 24-cm WTR distances.

data point – The data collection software samples data 194 or 388 times as it scans across the gel. Each "sampling" is stored as a data point. A data point is also represented by one line on the Map or Gel display.

electropherogram – a four-color representation of a sequence, showing peaks that represent the bases. The term is used interchangeably with chromatogram in this manual. An electropherogram is generated by the Sequence or GeneScan Analysis applications.

exporting – storing the contents of selected sequences in a file other than the associated data file.

Firmware Image – a file containing the executable software needed to run the Translation Processor (TP). This code is automatically sent to the TP by the ABI PRISM 373XL collection software running on the Macintosh, if the TP indicates it has no firmware.

gel casting comb – a specially formed piece of Mylar or Valox used to create wells or a single slot at the top of the gel during polymerization. For Sequencing applications, use the sharks-tooth comb after the single slot is formed by the gel casting comb.

gel image – the colored display of the Gel file that appears on the computer screen. To decrease the size of the file, you can use the GelDocII program to remove the image from the file. If you do so, you cannot display the gel image on the screen. If you remove the image from the file, you can also use the GelDocII program to rebuild the image.

grid - a spreadsheet-like display used for entering data in tabular format. The Sample

Sheet and several data collection windows display grids for entering Sample information.

hygroscopic - readily absorbing moisture

image – *see firmware image, gel image.*

Instrument file – a file used to adjust for the spectral overlap between the fluorescent dyes used on the ABI PRISM 373 DNA Sequencer with XL Upgrade. A mathematical matrix of the spectral overlaps is created and the inverse matrix is used to correct the data during analysis. The file is stored in the ABI folder in the System folder.

INIT – a Macintosh system extension that expands the capabilities of the system software. Examples are printer drivers and programs that add features to the Finder or the system software. INITs are stored in the extensions folder inside the System folder. Do not use CDEVs and INITs other than those shipped with your system.

IUB code – alphabetic character representing the occurrence of mixed bases at a given position in a sequence. Originally defined by the International Union of Biochemistry. The following table contains a list of IUB codes, the mixed bases they represent, and a listing of the complements.

IUB Codes and their complements

IUB Codes		Complements			
A = Adenosine	R = A or G (puRine)	A	T	S	W
C - Cytidine	Y = C or T (pYrimidine)	C	G	W	S
G = Guanosine	K = G or T (Keto)	G	C		
T = Thymidine	M = A or C (aMino)	T	A	B	V
B = C,G, or T	S = G or C (Strong— 3 H bonds)			D	H
D = A, G, or T	W = A or T (Weak—2 H bonds)	R	Y	H	D
H = A, C, or T	N = aNy base	Y	R	V	B
V = A, C, or G		K	M	N	N

IUPAC – International Union of Pure and Applied Chemistry. IUB codes are also referred to with this acronym, since IUPAC adopted the codes as a standard.

length – the length of a sequence is the number of characters it contains.

LED – light emitting diode. In this manual, this term refers to the status-indicator lights on the front and the back of the instrument. In general, this refers to a "semiconductor diode that converts applied voltage to light and that is used in digital displays", such as calculator and some computer displays (*The American Heritage Dictionary, Second College Edition*, Houghton Mifflin Company: Boston/New York,1991; p 721).

Leon – this Model 373 configuration has an electrophoresis chamber which supports 6-, 12-, and 24-cm WTR distances.

matrix file/multicomponent matrix – a file used to adjust for the spectral overlap between the fluorescent dyes used on the ABI PRISM 373 DNA Sequencer with XL Upgrade. A mathematical matrix of the spectral overlaps is created and the inverse matrix is used to correct the data during analysis. The file is stored in the ABI folder the System folder.

module – a file that provides instructions about conditions of operation to the instrument. You might use three different modules during a typical run to specify conditions for plate check, pre-run, and the run itself.

PMT – photomultiplier tube.

read region – The region of the glass plates where the laser scans. The location of this area depends on the instrument. See Figure 6-3. and Figure 6-3. on page 2-6.

sample files – data files produced by the Analysis Software associated with the ABI PRISM 373XL DNA Sequencer. These files contain data produced by the instrument: a sequence of base calls, peak locations, and an electropherogram. The original data in sample files is always maintained in its pristine state (as it came from the ABI PRISM 373 DNA Sequencer with XL Upgrade). When you save changes you have made to the sequence, they are stored to a copy of the original data called the editable data.

separation distance – the length from the wells of the gel to the read region of the gel. Also called the well-to-read or WTR.

sequence – a linear series of characters. The characters are displayed in rows from left to right. More specifically, a sequence is a series of nucleotide base characters that represent a linear DNA sequence, or a series of amino acid characters that represent a protein sequence.

sequencing reactions – the chemistry performed to incorporate fluorescent dye labels into DNA extension products. The chemistries and kits supplied by Applied Biosystems for performing such reactions are described in the *ABI PRISM 300Sequencing Chemistry Guide.*

settings – choices that you specify in the data collection software about the parameters used for data collection.

sharks-tooth comb – Mylar or Valox device used to form wells for a sequencing gel. It is inserted after the gel has polymerized with the gel casting comb in place. The casting comb is removed, leaving a slot into which the sharks-tooth comb is inserted.

square-tooth comb – specially formed piece of Mylar or Valox used to create square-shaped wells at the top of a gel during casting.

Stretch - this Model 373 configuration supports 6-, 12-, 24-, 34-, and 48-cm WTR distances and is easily identified by its extended door.

tared – counterbalanced.

text files – type of simple file produced by all Macintosh word processing programs, and many other programs.

Translation Interface Processor– this device, which sits on top of the computer's CPU, serves as the communication link and data buffer/translator between the ABI PRISM 373 DNA Sequencer with XL Upgrade and the Power Macintosh.

well-to-read length (WTR) – the distance from the wells of the gel to the laser scanning (read) region of the gel; separation distance for a run. The length of the glass plates is usually expressed as the well-to-read length (for instance, when you prepare a gel using 34-cm plates, the separation distance between the sample loading wells and the read region of the gel is 34 cm).

Index

Symbols

A

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AB Applied Biosystems

an Applera business