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ABI PRISM™ 377

DNA Sequencer

User's Manual

Part Number 903433
Rev. A
January, 1995

PERKIN

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1 Introduction

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How to Use This Manual

ABI Prism™ 377 Manual Sets

The ABI Prism 377 DNA Sequencer manual set varies with the co
the instrument. The following table shows the manuals you should
different configurations (this manual is listed in boldface type).

Table 1-1. ABI Prism 377 Reference Manuals

For Sequencing Analysis	For GeneScan analysis	Manual descr
ABI Prism 377 DNA Sequencer User's Manual	ABI Prism 377 DNA Sequencer User's Manual	**Preparing ge Running san Data collecti Instrument h Macintosh m**
ABI Prism DNA Sequencing Analysis Software User's Manual	ABI Prism GeneScan™ Analysis Software User's Manual	Software that collected fron
ABI Prism 377 DNA Sequencing Analysis Chemistry and Safety Guide	ABI Prism 377 GeneScan™ Analysis Chemistry and Safety Guide	Chemistry pr sample prepa (Protocols tha with Sequenc kits are not in Safety inform Troubleshoot User Bulletins

Information Provided in This Manual

This manual describes how to prepare gels and run samples, and
information about the data collection software and the instrumen
hardware. The manual is equipped with an extensive index and tal
to help you find information quickly. Even if you do not normally
these features should help you use it as a reference.

To get started quickly, see *How to Get Started Quickly* on page 1-7 a
recommended to the appropriate *ABI Prism 377 Chemistry and Saf*
Sections 2 and 3 of this manual. Appendix B, *Quick Reference*, cont

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Safety Information

The Safety Summary provided in the *ABI Prism 377 DNA Sequencin Chemistry and Safety Guide* includes detailed information about ope Prism™ 377 safely. It also contains Material Safety Data Sheets (M chemicals that are commonly used with the instrument. After you Summary, file it behind the safety tab provided in the back of the cl Always keep the Safety Summary available for reference.

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

WARNING	***ELECTRICAL SHOCK HAZARD. The ABI Prism 377 voltage power supply. Although the instrument has with safety features in the door to disconnect the when the door is open, please follow procedures as with any electrophoresis apparatus, be careful du operation and when handling electrodes and liqui***

WARNING	***LASER HAZARD. The ABI Prism 377 contains a la this instrument without due regard to the precauti manner that is not in compliance with procedures here, may be unsafe and can cause physical harm exposure.***

WARNING	***CHEMICAL HAZARD. Some chemicals used with t are considered hazardous. Hazard warnings are p displayed on the bottle labels of all hazardous ch Safety Summary for this manual contains materia sheets (MSDSs) that provide information about pl characteristics, health hazards, safety precaution cleanup and disposal procedures. Please familiari the information contained in the MSDSs before op instrument or using the reagents. Additional copie are available from Perkin-Elmer at no extra cost.***

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How to Get Started Quickly

Be sure your ABI Prism™ 377 system is completely installed befor preparing for a run. After it is installed:

- Refer to the *ABI Prism 377 DNA Sequencing Analysis Chemist Guide* as you prepare your DNA template and choose a me performing the sequencing or labelling reactions.
- Read through Section 2 of this manual as you prepare your Note that the use of a cassette to hold the gel plates has ch pouring procedure substantially from the traditional gel cas used with the ABI Prism 373 instrument.
- Use the instructions in Section 3 for step-by-step operation instrument, from instrument setup to the analysis results. S extensively cross-referenced, so you can easily refer to Sect necessary.

Note	*Appendix B, Quick Reference, contains a laminated c summarizes the steps described in Sections 2 and 3. tables of frequently used information.*

Registering Your Software Programs

When you send in the registration cards for your software prograr become eligible to receive upgrade notices. Often you also becom technical support and to purchase software upgrades at lower price cost to purchase the new upgraded programs. These privileges mi depending on the company that provides the software.

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

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You can use various sizes of gel plates for electrophoresis, depend application. The gel cassette supports plates providing 36 cm and well-to-read lengths for sequencing, and gel plates providing 12 c well-to-read lengths for GeneScan analysis.

Note	*This manual describes only how to collect raw data o For information about sequencing analysis or GeneS applications, refer to the* Sequencing Analysis Softwar *or the* GeneScan™ Analysis Software User's Manual,

Sequencing versus GeneScan analysis on the ABI P

The ABI Prism 377 functions the same way for both sequencing a analysis. It performs electrophoretic separation and spectral dete dye-labeled DNA fragments. The main differences come in the pr chemistries and the analysis performed on the resulting data.

As described above, either of two software programs analyzes the depending on your application. The analysis software programs a described on page 1-13, and more completely described in the se software user's manuals.

To prepare DNA template, you can use standard laboratory meth example, you can clone the DNA into a vector such as M13 or pU vector, then purify the template by standard methods. To prepare running on the ABI Prism 377, you must incorporate fluorescent chemistries for doing so are different, depending on whether you sequencing or GeneScan analysis.

Preparatory Chemistry for Sequencing Applications

For sequencing applications on the ABI Prism 377, you perform s reactions with fluorescent labels, instead of the usual radioactive l different dyes identify the A, C, G, and T extension reactions. You c the dye labels using either 5'-dye labeled primers or 3'-dye labeled dideoxynucleotide terminators.

Use commonly employed polymerases, such as AmpliTaq® and T polymerase, for primer extension (both are provided in kits with terminator and the dye primer chemistries from The Perkin-Elmer The labeling method you use depends on the sequencing strategy

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Real-Time Status and Data Observation

The data collection program stores the digitized output signal int
Macintosh hard disk. The computer screen displays the informati
from the ABI Prism 377 in real time.

Note	*Real-time viewing of the first peaks as they pass the la occurs within 25 minutes of loading the samples on the throughput (200 bases per hour or 2400 scans per hc 36-cm separation distance.*

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

- The Status window displays current instrument conditions
- The Electrophoresis History window displays the set and a the electrophoresis power supply and gel temperature.
- The Scan window displays the raw data; it shows the sweeps across the gel with a different colored line representing ea
- The Gel window displays a reconstruction of actual data as i on an autoradiogram.

Data Analysis

At the end of collection, the computer can automatically analyze t data and print electropherograms on a color printer. Depending application, this is performed by either the Sequencing Analysis or Analysis software. Both programs are briefly described on page 1-1 completely described in the separate analysis software user's manu

You can display the raw and analyzed data on the computer screei print it at any time.

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Software for Data Analysis

Analysis of Raw Data

Two standard software programs are available to analyze raw data
Prism 377: Sequencing Analysis and GeneScan Analysis. The instr
shipped with one of them. Since the program provided with the ins
be either one (or both, if you ordered both), both are listed here
programs.

DNA Sequencing Analysis Software

The Sequencing Analysis software analyzes raw sequencing data c
data collection program on the ABI Prism genetic analysis instrum

The analysis software processes data using a multicomponent anal
subtraction and scaling. After processing, it detects the peaks and
sequence. Automatic base calling with the sequencing software is
98.5% accurate (for Taq standard ss M13 DNA), based on sequen
and read lengths as listed in Table 1-2. The four-dye, one-lane seq
approach used with the ABI Prism 377 makes this level of accurac
One-lane sequencing eliminates electrophoresis variables and red
to a consistent pattern that can be accurately determined by the c

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

The Sequencing Analysis program comes bundled with two other
GelDoc and DataUtility. GelDoc removes tracking data from a Gel
removes and restores the gel image from a Gel file. The DataUtilit
performs two functions. It makes matrices for use with the Sequenc
and Analysis software, and monitors noise levels for troubleshootir
Perkin-Elmer technical specialists.

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- GeneAssist Analysis allows you to analyze data from ABI Pr sequence files, files processed in AutoAssembler, text files, You can use this program to rapidly search biological datab sequences or motifs, and to interactively work with the resu lists. In addition to sequence lists the program can create (alignments, and restriction maps of sequence data.

AutoAssembler™ Sequence Assembly Software

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

Sequence Navigator™ Sequence Comparison Software

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

Genotyper™ Fragment Analysis Software

Genotyper™ is a data analysis and transformation tool that conve GeneScan™ results files into user application results. Genotyper cc into the format required by downstream applications, such as link programs, databases, or spreadsheets. The Genotyper software the create seamless automation between the ABI Prism 377 DNA Seq final experimental results.

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when it is loaded in the instrument. Many gels are run at e temperatures.

- *Spectrograph/CCD detector.* These replace the filter wheel/PM providing more reliability and higher sensitivity.
- *New user's manual.* The new manual is a smaller, more conve contains a glossary, a quick reference procedure, and an e

Chemistry

- *High speed runs.* Run your sample at approximately 200 bas using increased voltage and cooled CCD camera technolog detection of all four dyes with a single pass of the laser.
- *Load less sample.* The high speed runs use thinner gels and sample. You load between one fourth and one sixth the amo required and obtain the same resolution.
- *Gel reagent concentrations.* All length sequencing runs now u acrylamide, 6M urea, 1X TBE solution.
- *Use less urea.* Because less urea is required for the gel soluti without application of heat.
- *More reactions from Sequencing kits.* The kits are unchanged. less DNA per gel, your sequencing kits produce a larger nu

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Addresses –North and South America

The Perkin-Elmer Corporation
Applied Biosystems Division
850 Lincoln Centre Drive
Foster City, California 94404

Telephone: (415) 570-6667
Toll Free: (800) 831-6844
Facsimile: (415) 638-5891

Canada
6535 Millcreek Drive, Unit #74
Mississauga, Ontario, L5N 2M2

Telephone: (905) 821-8183
(800) 668-6913
Facsimile: (905) 821-8246

Addresses – Asia/Australia

Japan, Asia, Pacific Rim
Business Court Shin-urayasu 6F
1-9-2 Mihama
Urayasu, Chiba, 279
Japan

Telephone: 81-4-7380-8502
Facsimile: 81-4-7380-8505

Australia
26 Harker Street
Burwood, Victoria 3125

Telephone: 61-3-808-7777
Facsimile: 61-3-887-1469

Addresses – Eu

United Kingdom
Kelvin Close
Birchwood Science
Warrington, Chesh

Telephone: 44-925-
Facsimile: 44-925-8

Germany
European Life Scie
Paul Ehrlich Str 17
D-68255 Langen
Germany

Telephone: 49-610
Facsimile: 49- 610

France
Paris Nord II - BP 5
95948 Roissy C. de

Telephone: 33-1-49
Facsimile: 33-1-48

Italy
Via Tiepolo, 24
1-20052 Monza (Mi

Telephone: 39-392
Facsimile: 39-39238

Netherlands (Benelux,
Hanzeweg 16
Post Bus 490
2800 AL Gouda

Telephone: 31-1820
Facsimile: 31-1820-7

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2 Preparing Gels

This section describes properties of polyacrylamide gels, and how for use with the ABI Prism™ 377 DNA Sequencer using the gel ca details about placing the gel cassette on the instrument refer to S *the ABI Prism™ 377.*

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Polyacrylamide Gels and DNA Sequencing
- Mechanism of Polymerization
- Effect of Reagent Purity on Gel Quality
  - Acrylamide
  - Bis-acrylamide
  - Ammonium Persulfate (APS)
  - TEMED
  - Urea
  - Buffers and Gel Additives
- Other Factors that Affect Gel Quality
  - Rate of Polymerization
  - Air bubbles
  - Using an Old Gel

Preparing Acrylamide Denaturing Gels
- Reagents and Supplies for Stock Solutions
  - 10X TBE Stock Solution
  - 40% (19:1) Acrylamide Stock Solution
- Reagents and Supplies for Gels
- Gel Concentrations and Volumes
- Preparing the Plates and the Gel Cassette
  - About the Gel Cassette
  - Cleaning the Plates
  - Mounting the Plates in the Gel Cassette
  - Attaching the Gel Injection Device

(continued on next page)

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Polyacrylamide Gels and DNA Sequencin

One of the most important variables that determines the number o read when sequencing DNA is the polyacrylamide gel. Your seque only be as good as the quality of your sequencing gel.

The quality of your sequencing gel is even more important with the than it is with manual sequencing, since much more DNA (over 60(through a gel in a single loading on the ABI Prism 377. Therefore paramount importance to produce a first-rate gel. Some of the fac gel quality are described below.

Mechanism of Polymerization

Polyacrylamide gels are formed by the copolymerization of acrylar bis-acrylamide. The process begins when TEMED (tetramethyleth reacts with APS (ammonium persulfate) to yield a TEMED radical c radical and a sulfate ion. The sulfate radical adds an unpaired ele acrylamide monomer, converting it to a free radical which then re monomer, causing polymer chain elongation. The polymer chains 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 rel Optimal sequencing results depend on gel quality and therefore, quality.

Acrylamide

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

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

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Ammonium Persulfate (APS)

APS begins to decompose at once when dissolved in water. The re activity. For reproducible results, therefore, you should prepare A fresh daily. It is vital that this chemical be fresh since it affects the polymerization and thus the gel properties. Store solid APS tightly is hygroscopic. If tightly sealed, it can be stored at 4 °C for up to 1

Note	*Store solid APS in an airtight container with desiccant, avoid water condensation problems, let the bottle of A temperature before opening it.*

TEMED

TEMED (N, N, N', N'-tetramethylethylenediamine) is by nature ve prone to oxidation. The oxidized form is yellow and less active. U TEMED slows gel polymerization time, thereby significantly alteri characteristics. Because it is hygroscopic, this initiator gradually a water, which increases the rate of oxidation. Water-free TEMED (stored in a tightly sealed container at room temperature, should l to six months.

WARNING	***CHEMICAL AND FIRE HAZARD. TEMED is extrem and can be very destructive to the skin, eyes, nos respiratory system. Keep it in a tightly closed con inhalation and contact with skin, eyes and clothin under a hood and wear chemical resistant gloves TEMED solutions. Read the MSDS in the Safety Su with your ABI Prism 377 Chemistry Guide.***

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

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

Decreasing the concentration of initiators has a desirable effect, e also causes slower polymerization. When polymerization is too slo enter the monomer solution during the process and inhibit polyn

If you use degraded TEMED or APS, the concentration of initiato that recommended in the protocol.

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

- Polymerization must be fast enough to prevent too much c dissolving into the gel solution during polymerization. As l fresh, high-quality reagents and follow the protocol in this n should not cause a problem during polymerization.
- Minimize the amount of oxygen dissolved in the gel solutic casting the gel, since it can interfere with the rate of polymer degassing is accomplished during the vacuum filtration ste protocol. It is important to keep the vacuum strength and t during this step for run-to-run reproducibility. Since cold s greater capacity for dissolved oxygen, perform vacuum filte casting) with the solution at room temperature. Also, be ge stirring and pouring steps of gel casting so that oxygen is no

Air bubbles

Air bubbles can distort the sample path, which affects lane trackin carefully and gently so bubbles never form. If bubbles are trapped comb and the gel solution, carefully remove and reinsert the com bubbles are trapped.

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Preparing Acrylamide Denaturing Gels

This protocol for the preparation of sequencing gels follows gene procedures; however, there are some important distinctions to re throughout the process. The ABI Prism 377 DNA Sequencer is a r electrophoresis detector. A laser scans across a region of the gel d electrophoretic process, detecting the fluorescently labeled DNA p that region. The glass and the gel must therefore be non-fluorescer careful to eliminate any particulate or fluorescent matter from the solutions. In addition, keep the glass plates extremely clean and fi Always use ultra-pure reagents and high-grade, distilled, deionizec prepare solutions, and wear clean laboratory gloves. Filtration of a essential to remove any particulate matter that may fluoresce or sc

To maximize gel resolution, high quality acrylamide gels are essent when you are sequencing inserts of greater than 350 bases in leng prepare acrylamide solutions with low-quality starting materials or excessively, breakdown products might detract from the quality of a deionizing resin and preparing carefully helps to avoid problems breakdown activity.

WARNING	***CHEMICAL HAZARD. Acrylamide and bis-acrylam neurotoxins. Avoid inhalation and skin contact. We times and work in a fume hood when handling acr solutions, and use appropriate precautions to avo crystalline acrylamide.***

Reagents and Supplies for Stock Solutions

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

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5. Transfer to a 100-mL graduated cylinder and adjust volume dH_2O.

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

Reagents and Supplies for Gels

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

- Urea
- TEMED

WARNING ***CHEMICAL HAZARDS. Urea is a potential mutage extremely flammable and can be very destructive t nose, and respiratory system. Do not store, handl any chemicals or hazardous materials unless you appropriate safety training and have read and unc related Material Safety Data Sheets. Comply with a and local laws related to chemical storage, handlin***

- 10% Ammonium persulfate (w/v) in dH_2O

  Prepare fresh stocks daily, and store refrigerated.
- Volumetric pipets and tips
- 0.2-µm cup filters
- Acrylamide gel casting equipment

  The gel cassette and gel injection device are provided with t Optically clear glass plates, spacers, and combs are also avail Perkin-Elmer Corporation. Part numbers are listed after th Appendix A.
- Alconox™ detergent
- Methanol (high grade quality)

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Preparing the Plates and the Gel Cassette

Note *Be sure you have a level working area for preparing t*

About the Gel Cassette

The ABI Prism 377 DNA Sequencer comes with a gel cassette for
positioning the gel on the instrument (Figure 2-1). It is also a help
gel pouring and transportation of the plates. Using the gel cassette
need for taping the gel or using clips to prevent leakage while you
horizontal gels. The structure of the cassette allows you to carry y
from one place to another.

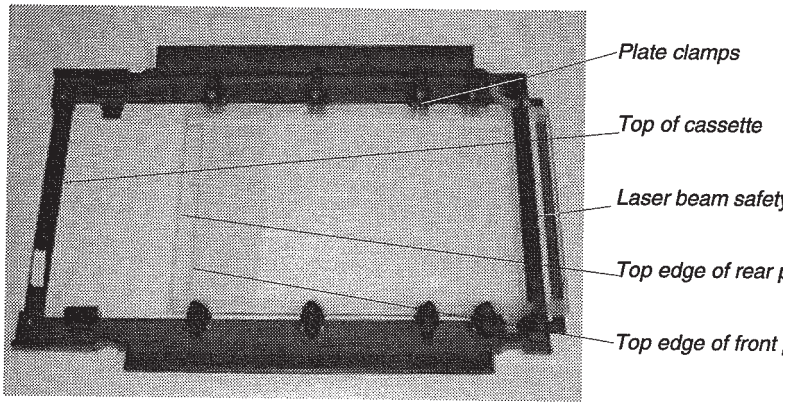


Figure 2-1. Gel cassette

The clamps along the sides of the cassette are multifunctional (see
Use them to:

- Hold only the gel plate
- Attach the upper electrophoresis buffer chamber
- Hold the front heat transfer plate, when you use one
- Hold the upper (front and back) and lower gel injection fi
(see page 2-19)
- Hold the laser beam safety stop (beam stop) in the up or d

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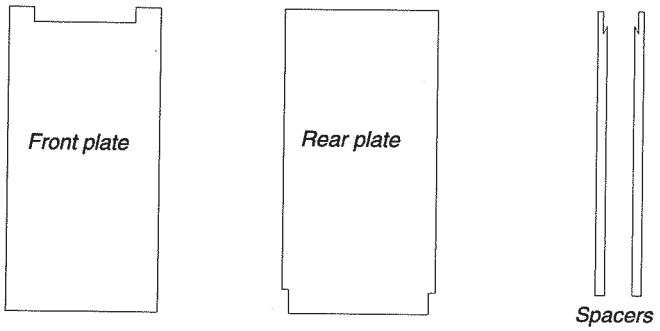


Figure 2-3. Glass plates for casting gel

Note	*Do not touch the cleaned surfaces of the glass plates to handle the plates.*

Each time you use the glass plates, use them with the same side of the inside. After the first use, the front plate has a hydrophobic ar buffer chamber gasket made contact with the plate. You must ther front plate in the same configuration each time to avoid difficulty p It is a good idea to mark the outside of each plate the first time yo the plates with a tiny scratch at the top that doesn't interfere with t 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 Refer to page 3-32 for instructions.

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7. Place the 0.2-mm gel spacers on the rear plate.

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

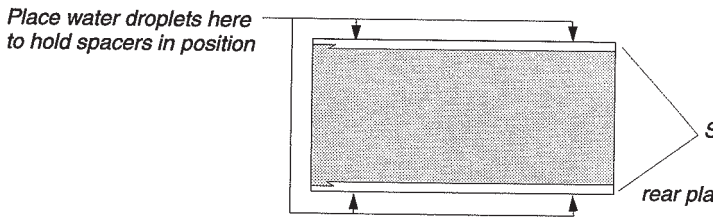


Figure 2-4. Securing spacers on glass plates (top view)

8. Align the front plate (inside down) on top of the spacers. Tl of the plates should be flush and the notch of the front pla oriented toward the top of the cassette.

   Again, be sure the inside of the plate is free of any particle: fluoresce.

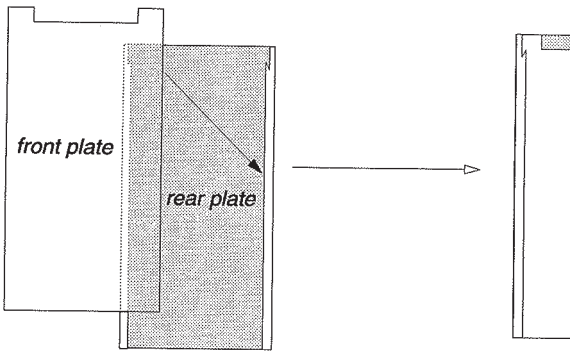


Figure 2-5. Plates aligned for placement in the cassette

9. Push the plates together toward the bottom of the cassette.

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Attaching the Gel Injection Device

IMPORTANT	*If your plates are not identical in length, you must ins the bottoms of the plates are aligned, to avoid leakag solution. Align the bottoms of the plates and attach the described in step 4, then attach the top fixture.*

1. Place the assembly on a level raised support.

IMPORTANT	*Support the cassette on the cassette frame, rather th glass plates on a central platform. This provides more thickness. Two 2" X 4" pieces of wood on each side r support.*

2. Attach the clear brace of the top fixture.
   a. Slide the side pegs under the bottom edge of the top
   b. Lower the fixture and slide the top pegs over the top ec glass plate in the ear region.
   c. Tighten the adjusting screws until they just contact th front glass plate.

   Do not close the top plate clamps until after the comb (described on page 2-27).

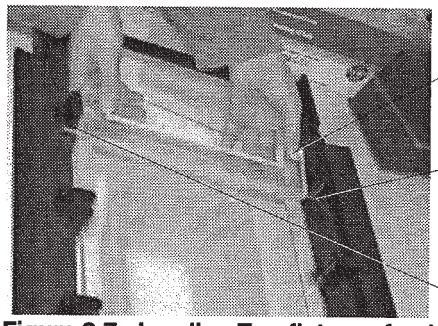


Figure 2-7. Loading Top fixture of gel injection device

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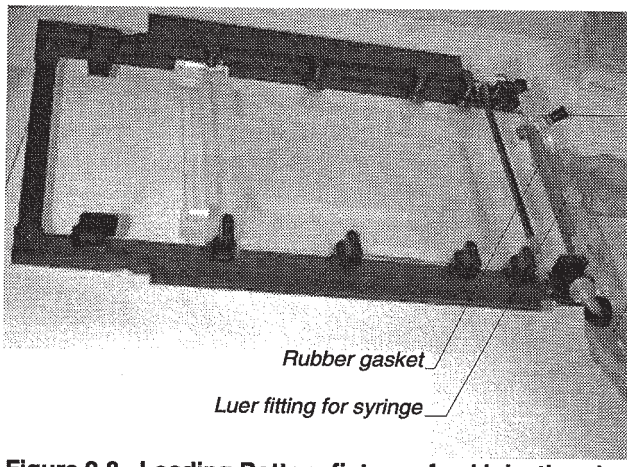


Figure 2-8. Loading Bottom fixture of gel injection dev

5. Place absorbent paper on the table at each end of the casse gel solution that spills during injection.

The cassette is now ready for you to inject the gel. If you performe preparation while your acrylamide-urea solution was being stirred, page 2-23 to continue.

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1. Combine the following components in a 150-mL beaker to volume of gel solution as shown:

Reagent	To prepare 50 mL for 36-cm run	To p for
Urea	18 g	
40% Acrylamide stock	5 mL	
dH2O	25 mL	
mixed-bed ion exchange resin	0.5 g	

2. Stir the solution until all the urea crystals have dissolved.

   While the solution is being stirred, you can prepare the cass if you have not already done so. The steps start on page 2-1

3. Filter the acrylamide solution through a 0.2-µm cellulose n degas for 2–5 minutes, and transfer to a 100-mL graduated

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

4. Add filtered 10X TBE buffer and adjust the volume with dI

Reagent	To prepare 50 mL for 36-cm run	To pre 4
10X TBE buffer	5 mL	
dH20	To 50 mL	1

IMPORTANT	*Never add the TBE buffer before removing the mixed ion-exchange resin by filtration. Presence of the resin effectiveness of the buffer.*

Before the next steps, if you have not already done so, you need to clean the plates and mount the plates in the gel cassette. See the in beginning on page 2-14 to clean the plates and prepare the cassett

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Casting the Gel

This manual describes two methods of pouring gels. The first des
the gel while the plates are mounted in the cassette. The second
pouring with the plates unmounted. The first method (*Using the*
Device) is the recommended method.

IMPORTANT *You need to thoroughly clean the plates and mount th*
cassette before pouring the gel. The instructions are

Adding Polymerizing Reagents

1. Gently add freshly made 10% APS solution to the gel solut
careful not to introduce air bubbles.

For 36-cm run	For 48-cm run
250 µL	400 µL

Swirl to mix. Avoid introducing air bubbles.

2. Gently add TEMED (distilled) to the gel solution, being ca
introduce air bubbles.

For 36-cm run	For 48-cm run
35 µL	55 µL

Swirl gently and thoroughly to mix and initiate polymeriza
introducing air bubbles.

Work quickly and carefully at this point to cast the gel, usin
methods described below.

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Note	*If you do need to refill the syringe, be careful not to intr the luer fitting. Keep any excess solution in the beake polymerization.*

4. Remove the syringe.
5. Insert the comb.

   For sequencing applications, use the straight edge of the s comb, which has a stop bar to help you gauge the depth of forms a single slot into which you later insert the teeth of t

   For GeneScan analysis applications, use the square tooth co wells.

Note	*Make sure the casting comb is completely dry. Using comb can make the surface of the acrylamide wavy, the way samples migrate through the gel.*

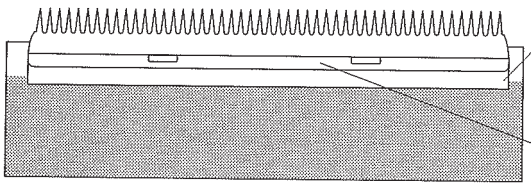


Figure 2-10. Inserting the casting comb

IMPORTANT	*Be sure no air bubbles are trapped between the glass mylar casting comb after the casting comb has been in air bubbles cause depressions at the surface, possibly wells or depressions on the well surface. This leads to resolution in affected lanes.*

6. Turn the top plate clamps to hold the top fixture against th

   This clamps the comb between the plates.

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5. Pour about 35 mL (for a 36-cm gel) of the prepared gel so lower 1/3 of the rear plate.

   The solution is held by surface tension at the lower edge o along the spacers.

6. Lift the front plate, inside face down, and lay it onto the re follows:

   a. Start by aligning the lower edges at a shallow angle, th lower the front plate onto the rear plate.

      Surface tension quickly traps the solution between the front of liquid should move easily up the plates to fill t between them as the front plate is lowered. The liquid over the spacers and out at all edges during this opera

   b. If bubbles or empty spots form as you are laying down raise the front plate to draw the liquid back to the prob carefully lower the plate again as described above.

   c. Once the plates are together, adjust their relative posi of the spacers as necessary.

7. Clamp the edges of the plates with five small clamps on eac

   Inserting the comb might be easier if you leave the topmos unattached.

8. Insert the casting comb, then attach three large clamps at th over the place where the comb meets the gel.

9. If you did not attach the top two side clamps in step 7, attac inserting the comb.

10. Allow the gel to polymerize for at least two hours before prep

Note	*While the gel is polymerizing you can prepare the San Run file for data collection. Refer to page 3-17.*

After the gel is polymerized, clean the plates and mount the cassette.

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3 Using the ABI Prism™ 377

Introduction
Before You Set Up the Instrument
- Preparing Samples
- Setting up the Software

Setting Up the Instrument
- Preparing the Gel for Loading
- Mounting the Gel Cassette in the Electrophoresis Chamber
- Checking the Plates
- Filling the Buffer Chambers and Checking for Leaks
- Connecting the Front Heat-transfer Plate and Electrode Cat

Setting Up the Software
- Setting up the Analysis Software
- Starting the Data Collection Software
- Setting Default Parameters (Preferences)
- Creating the Sample Sheet
- Creating a Run File

Preparing and Loading Samples
- Pre-running the Gel
- Resuspending the DNA
- Loading the Samples and Starting the Run

Observing Instrument Status and Data
- Data Collection Status Windows
- Real-time Data Windows

Cleaning Up After the Run
- Removing the Gel from the Instrument
- Cleaning the Plates, Comb, and Spacers

Analyzing the Data
- Color of Data Display and Output
- Reading an Electropherogram

Archiving the Data
- Files Created During a Run
- Other Files

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Introduction

This section guides you through the process of electrophoresing samples and collecting the resulting sequence data on the ABI Pr Sequencer. Figure 3-1 shows the recommended flow of the proce

Although this section is detailed, it does not provide complete de procedure. Use it as a guide, and refer as necessary to the cross-re for more detail. As you become familiar with the procedures, you c to the Quick Reference laminated card in Appendix B.

Note	*If you have previous experience using the Model 373, to read through these steps. Loading the gel onto the changed because of the addition of the gel cassette, collection software has been streamlined. You no lon any commands on the instrument itself.*

The information in this section applies to both Sequencing and D applications. Instrument setup consists of the same steps for eithe The only differences are in completing the Sample Sheet and Rur

For further information about the analysis performed for each ap to the separate analysis software manuals: the *ABI Prism DNA Seque Software User's Manual* (P/N 903436) covers sequencing applicatio *Prism GeneScan Analysis Software User's Manual* (P/N 903434) cover analysis/sizing applications.

To read details about the software and the instrument itself refer t and 6.

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Setting Up the Instrument

Pour your gel as described in Section 2, and allow it to polymeriz before proceeding with this section. While the gel is polymerizing the Sample Sheet and Run file for the data collection software. T *Setting Up the Software* on page 3-17.

Preparing the Gel for Loading

Note	*Wear clean laboratory gloves throughout this proced protection, and to avoid transferring fluorescent contan hands to the glass plates.*

Reagents and supplies needed for preparing the glass plate

- A single-edged razor blade
- 1.2 L 1X Tris Borate EDTA buffer, pH 8.3 (from 10X TBE refer to page 2-10)
- A clean, 60-cc syringe and 16- to 18-G needle

To prepare the plates for loading on the instrument:

1. Open the top two plate clamps of the cassette and remove t of the gel injection device.
2. Carefully remove the casting comb from between the plate

   Use the razor blade to clear any acrylamide from the notch plate. Remove small pieces of acrylamide from the well regi that none fall into the square wells or into the slot when yo shark's-tooth comb.

Note	*Do not use the razor blade while the casting comb is in cut the comb.*

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IMPORTANT *The glass must be clean, particularly right around the beam stop. This is where the laser reads the gel.*

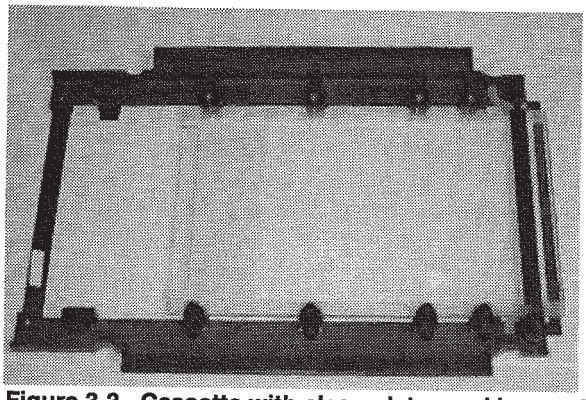


Figure 3-3. Cassette with clean plates and beam stop in pla

6. If you are using the square-tooth comb, skip to step 7 on p sequencing run, make sure the shark's-tooth comb is clean clean it with a Kimwipe and dH_2O) and insert it.

   a. Carefully align the center registration line on the com registration mark in the notch of the glass plates and between the plates.

      Make sure all of the teeth on the comb enter the spac plates at the same time to avoid bending or breaking t

   b. Slide the comb down until the tips of all the teeth just tc depress the surface of the gel (see Figure 3-4).

      Make sure the teeth just barely indent the surface. If the gel is not completely flat in the loading region, you mi insert some of the teeth below the surface of the gel (so all of the teeth touch the gel surface. If a tooth has p gel surface, do not attempt to withdraw the comb or sa into adjacent wells.

*If you use the square-tooth comb, the wells are formed during polymerization.

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3. Place the lower buffer chamber in the bottom shelf of the chamber.

WARNING ***ELECTRICAL SHOCK HAZARD. The ABI Prism 37 high voltage power supply. Although the instrum designed with safety features in the door to disco supply when the door is open, please follow proc prescribed. As with any electrophoresis apparatu during instrument operation and when handling e liquids.***

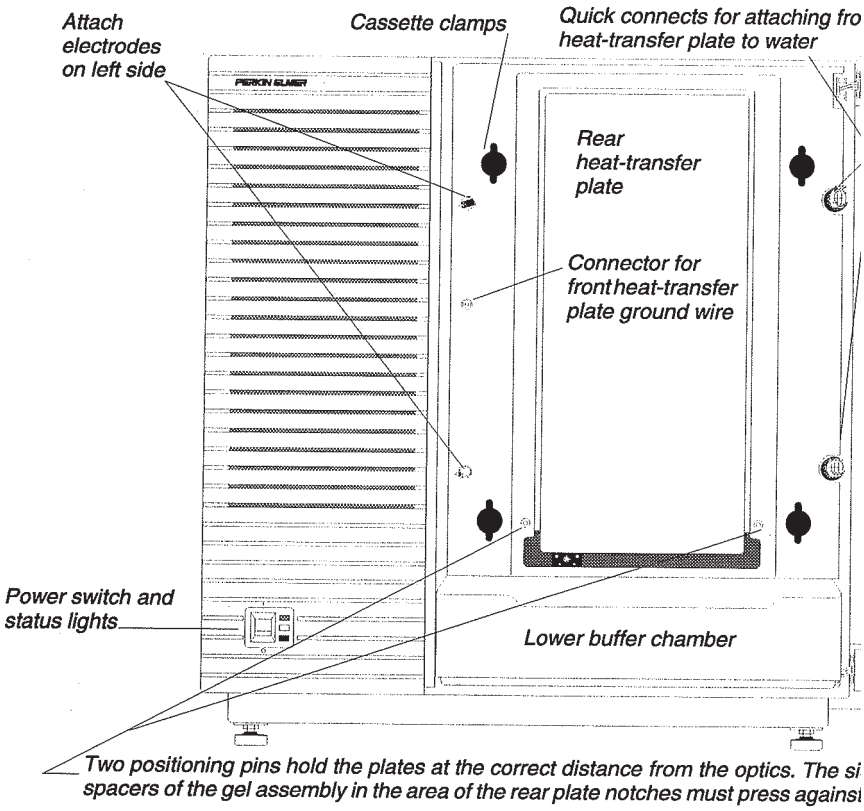


Figure 3-5. ABI Prism 377 Electrophoresis Chamber

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Checking the Plates

Scan the gel and plates before adding buffer and loading samples no peaks are produced by fluorescent particles on the glass plates

To check the plates:

1. If you opened a Run window to create a Run file while the polymerizing, display the window by choosing Run from th menu.

   If you did not create a run file while the gel was polymerizir from the File menu, and click the Run folder icon to open window.

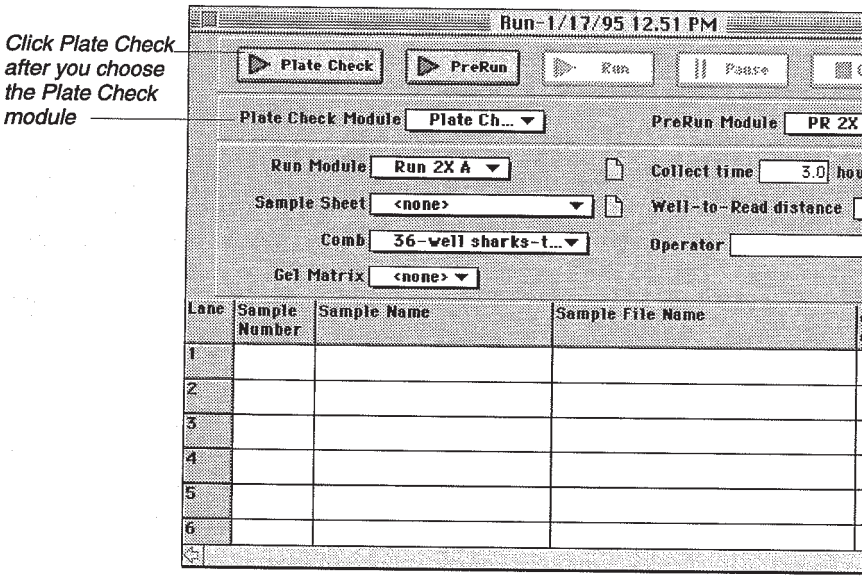


Figure 3-7. New Run window

1. Choose the Plate Check module from the Plate Check pop-

Note *You do not need to complete the other information for*

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3. Lift the beam stop bar and carefully reclean both sides of laser read region with dH_2O and a lint-free tissue or ultra-p air (see Figure 3-2 on page 3-6 for the location of the read

4. Replace the beam-stop bar, and replace the cassette in the chamber.

5. Click Resume and check the plates again.

If the peaks do not disappear after cleaning the glass, the gel mix might contain particles. If you wish to use the gel, avoid loading s lanes where the peaks appear.

Skipping Lanes

If a plate check shows peaks in some lanes of a gel even after clea window indicates which channels have contaminating fluorescenc Table 3-1 to determine which lanes are contaminated and do not l those lanes.

Table 3-1 shows scan numbers that correspond to the center of eacl different sharks-tooth combs. The exact positions of the channels n from instrument to instrument, and with each comb placement.

If the contaminant peaks are close to the edge of a lane, you may v lanes to ensure that there is no contamination in the lanes you lo

Table 3-1. Channel numbers at center of each lane

24-well comb		32-well comb		36-well comb	
Lane	Channel	Lane	Channel	Lane	Channel
1	14	1	14	1	14
2	21	2	19	2	19
3	29	3	25	3	24
4	36	4	30	4	29
5	43	5	36	5	34
6	50	6	41	6	39
7	58	7	46	7	44
8	65	8	52	8	48
9	72	9	57	9	53
10	79	10	62	10	58

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Filling the Buffer Chambers and Checking for Lea

IMPORTANT *Fill the buffer chambers and check for leaks before yo heat-transfer plate. Leaks are not visible with the plat*

1. Fill the upper buffer chamber with 580–600 mL 1X TBE bu for leaks.

   If you find a leak, siphon the buffer out of the upper buffe clean the gasket area on the chamber. Also, clean the area c contacts the gasket. Reassemble as before.

2. Carefully fill the lower buffer chamber with 1X TBE buffer bottoms of the glass plates are covered. Avoid splashing.

   Fill the lower buffer chamber to the top edge of the dam tha chamber from the overflow reservoir in front of it.

IMPORTANT *Make sure the lid is in place in the upper buffer chambe your run. This prevents excess buffer loss from evapo*

Connecting the Front Heat-transfer Plate and Electr

1. Attach the front heat-transfer plate for 36-cm runs.

IMPORTANT *If you are preparing for a 48-cm run, skip to step 2.*

   a. Open the plate clamps between the upper buffer char beam stop.

   b. Push the front heat-transfer plate against the front of th rests on the platform that is part of the beam stop bar.

   c. Secure it with the plate clamps and attach the quick-co lines and the ground cable.

2. Plug in the electrode cables extending from the buffer cha

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Setting Up the Software

Before starting electrophoresis, you must restart the Macintosh an files in the data collection software:

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

Note	*These tasks are best performed while you are waiting polymerize.*

You can simplify creation of the files by setting defaults for certain each file.

The procedures described here do not include detailed descriptic collection software. They are intended to give you a guide for step and the order in which to perform them. For specific information software and how to complete specific fields, refer to Section 4.

Setting default parameters is not necessary for each individual rur need to perform it before the first run and occasionally thereafter, the run. If you have already set defaults, skip to page 3-20 to compl run information.

If you desire more detail, the steps described here refer you to the in Section 4 where you can find related information.

Setting up the Analysis Software

If you are running GeneScan applications or wish to use custom ar you should set up the conditions that control data analysis before y To do so, refer to the User's Manual provided with your analysis sc

IMPORTANT	*The default Base Caller used by the Sequencing Anal ABI 100. If you choose automatic analysis for a high sp to use the SemiAdaptive or Adaptive Base Caller, cha preferences before you start data collection.*

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- *Dye indicators:* Each of the four dyes used during a run has and color. You can change the color assigned to each dye, color that appears in the plot when it is printed. To do so, 4-59.

- *General settings:* The ABI Prism 377 instrument is normally Modem port of the Macintosh computer. If you need to ch connection, you can specify the change using this preferen use this choice to reset the global serial number, which is a by the file naming defaults (refer to page 4-50). To change g refer to page 4-61.

To set preferences:

1. Choose Preferences from the Window menu and choose th option from the submenu that appears.

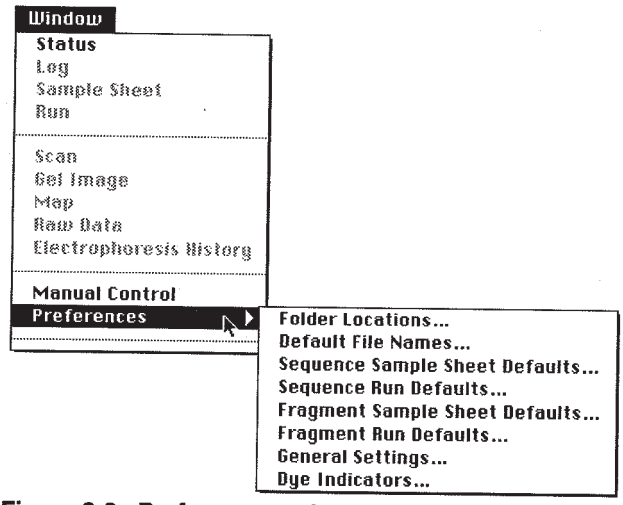
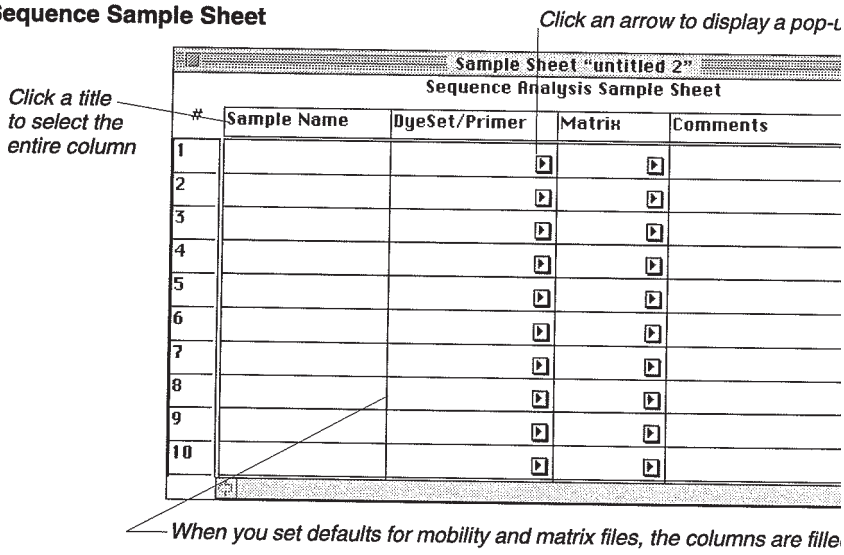


Figure 3-9. Preferences submenu

2. Enter or change the defaults, as described on the pages not
3. Click OK to accept the changes.

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Sequence Sample Sheet



Fragment Sample Sheet

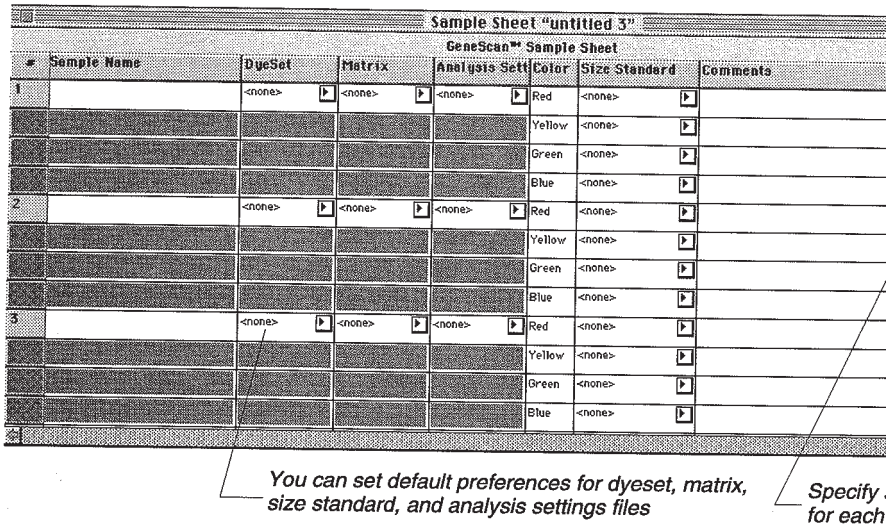


Figure 3-10. Blank Sample Sheets

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2. Choose the parameters for the run from the pop-up menu

Note *Most of the fields in steps 2, 4, and 4 are completed au set preferences for them.*

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

Refer to *Choosing and Changing Modules* on page 4-25 f about choosing and running the modules.

b. Choose the Sample Sheet from the Sample Sheet pop

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

c. Choose the type of comb and the separation distance f pop-up menu and the WTR pop-up menu, respectivel

d. Choose a matrix file from the Gel Matrix pop-up men

Appendix D describes using and creating matrices.

3. Enter the length of the run in the Collect Time entry field. some suggested run times.

Plate length	Type of Run	Typical C
36 cm	100 bph (1200 scans/hr)	7.(
36 cm	200 bph (2400 scans/hr)	3.!
48 cm	100 bph(1200 scans/hr)	10.

Note *Be aware that running for more than 10 hours or at tem than 51 °C can cause more rapid buffer evaporation.*

4. Enter your name in the Operator entry field.

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

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Preparing and Loading Samples

Pre-running the Gel

Pre-run the gel to equilibrate the temperature and to make sure a
and components of the electrophoresis system are working prope

While the pre-run is in progress, resuspend the DNA in preparati

IMPORTANT	*It is particularly important that you pre-run the gel prio (200 bph or 2400 scans/hr) run. The gel must be at ru when you load samples to ensure appropriate denatu Pre-running also removes mobile ions from the gel ar power surge that might occur if you suddenly apply hi*

To pre-run the gel:

1. Flush all of the wells with running buffer. Direct the needl filled with running buffer at the space between adjacent cc gently flush the well.

   Remove any bubbles under the bottom edge of the gel plat buffer chamber. Use a clean needle and syringe, with the ne angle to guide the bubbles out.

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

3. Click the PreRun button.

   The instrument performs electrophoresis without starting during a pre-run, so no windows appear.

Prepare your DNA samples while the pre-run is in progress.

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Formamide in the loading buffer helps narrow the lane width, bu adjacent wells are required. Loading samples in adjacent lanes ca definition between the lanes. Whenever possible, load a sample ir help orient the tracker during analysis. For best results, follow the described here.

WARNING	***CHEMICAL HAZARD. Formamide is a known terat birth defects). Before using formamide, read the M the Safety Summary. Wear protective eyewear, glo clothing when working with formamide. Wash tho handling formamide.***

Note	*In the following procedures, each button in the Run w corresponding menu choice in the Instrument menu. ' click the button in the Run window or choose the com Instrument menu.*

To load samples:

1. If you are pre-running the gel, click Pause in the Run wind

   Pausing stops electrophoresis, but maintains the temperatu while you are loading.

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

Tip	*Place formamide or loading buffer in the well to the lef sample lane, and in the well to the right of your last sa comb creates wells to either side of the numbered wel formamide in the buffer helps focus the bands in the fir*

3. Immediately load 1.5 µL of sample into each of the odd-nu

   If you are loading fewer than half the samples allowed by th steps 4–8 and perform step 9. If you are loading a square to GeneScan analysis, you can load the wells consecutively and

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Button to click	Module in effect	Task to
Plate Check	Plate Check module	Check plates a fluorescence
Cancel	n/a	(Stops plate ch Add buffer and Mount front he
PreRun	Pre-run module	(Electrophores data collection temperature) Prepare sampl pre-running
Pause	Pre-run module	Load first set of pause
Resume	Pre-run module	(Electrophorese gel without data
Pause	Pre-run Module	Load second se during pause
Cancel	n/a	(Stops pre-run)
Run	Run module	(Starts electropl collection)

Figure 3-12. Run window buttons used during a typical run

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

- Scan (refer to page 4-38)
- Gel (refer to page 4-39)

You can change the colors that appear in the real-time displays by
Dye Indicator Defaults (refer to page 4-59).

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Cleaning the Plates, Comb, and Spacers

1. Gently push a single-edge razor blade almost all the way in of the plates (avoid the top notches) and pry the plates ap too much force or the plates might chip. Remove the well spacers.

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

Note	*Alternatively, wash the gel from the plate with a strea*

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

4. Wash with a detergent such as Alconox™ that will not leave with dH_2O, and allow to dry.

5. Rinse the buffer chambers with dH_2O and allow them to a

6. Clean the comb and spacers and store them.

WARNING	***CHEMICAL WASTE HAZARD. Some chemicals us instrument might cause hazardous waste. Hazard prominently displayed on the bottle labels of all h chemicals. In addition, material safety data sheets provide information about physical characteristic hazards, safety precautions, first aid, spill cleanu procedures. The MSDSs are included in the Safety the User Manual. Please familiarize yourself with t contained in these documents before attempting t instrument or use the reagents. Additional copies are available from The Perkin-Elmer Corporation a***

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Reading an Electropherogram

- You can view the electropherograms in the analysis progra
  Analyzed data (data is also printed in these formats). Raw
  data in a Sample file appear in the direction of electropho
- Information from the Sample Sheet appears at the top of
  electropherogram.
- The peaks and the letters above the peaks are colored to r
  appropriate bases.
- An N above a peak means that the software could not conf
- The numbers indicate base positions, counted from the be
  sequenced data.

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Sample files

Use floppy disks, a magnetic tape drive, a removable cartridge driv drive to archive sample files. A 1.4 MB, high-density disk holds ab Sample file is 150-200 KB in size, depending on the length of the s Save a Sample file when you feel confident that the channel select are correct for the sample.

.Seq files

.Seq files show the base letter sequence only (or might contain a l depending on the format you choose).You can open them from w programs and print them. You can also save the files in several forn read by other software programs. The Preferences dialog box in t analysis program allows you to choose the file format.

Other Files

Other files provide information for a run that you might have cus should therefore save. These include the Sample Sheet, analysis set files you create.

Sample Sheet Files

You can reuse the Sample Sheet, and it is a good reference after d Individually, Sample Sheet files are small enough to archive on flc

Custom Analysis Settings and Matrix Files

If you have created customized files, you should keep backup copie a separate medium. Each of these types of files alone is small enou a floppy disk.

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4 Using the Data Collection Software

The standard software provided with the ABI Prism™ 377 DNA S consists of two programs: one for data collection and one for sub analysis. You can order instrument configurations with either ana (Sequencing Analysis or GeneScan™ Analysis). If you wish, you c second analysis program separately. Each analysis program has a and chemistry guide associated with it.

This section describes the data collection software in detail. The s already be loaded on your hard disk. If it is not, or if you need to to *Installing ABI Prism 377 Software* on page 6-4.

Contents

Overview of Data Collection Menu Commands
- Apple Menu
- File Menu
- Edit Menu
- Instrument Menu
- Window Menu

Using the Data Collection Program
- Starting the Data Collection Program

Creating and Editing a Sample Sheet
- Importing and Exporting Sample Sheet Information
- Creating a Sequence Sample Sheet
- Creating a GeneScan Analysis Sample Sheet
- DyeSet/Primer File Naming Conventions
- Editing a Sequence or GeneScan Analysis Sample Sheet

Using the Run Window
- Opening the Run Window
- Checking the Plates
- Pre-Running a Gel or Running Samples
- Starting a Pre-run or a Run

(Continued on next page)

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Overview of Data Collection Menu Comr

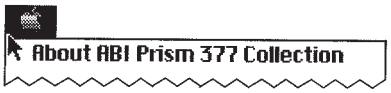
ABI Prism 377 software programs are designed with the easy-to-us menus and the point-and-click technique commonly used in Maci compatible programs. The menu bar at the top of the screen give functions. You can also use keyboard commands in many cases. If available, keyboard commands are listed opposite their correspond on the pull down menus.

This section briefly describes each of the four menus on the data program main menu bar.



Figure 4-1. Data collection program main menu bar

Apple Menu



At the left end of the menu bar is the Apple menu, a standard fea Macintosh applications. It displays desk accessories installed on yo

The top command, About ABI Prism 377, opens the splash screen general information about the data collection program. Click OK

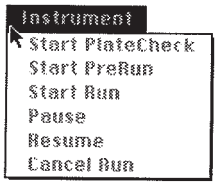
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Table 4-1. File Menu Commands

Command	Description
Close, Save, Save As..., Revert, Page Setup..., Print..., Quit	Standard Macintosh commands. See the *Macintosh® System 7 Reference* manual
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.
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.
Import...	Imports text from files such as those generated by a database into the grid of the Sample Sheet or Run window.
Export...	Exports data from the grid of the Sample Sheet or Run window in tab-delimited text format for database applications.
Print One	Prints one copy of the active window, bypassing the standard print dialog box.

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Instrument Menu



The Instrument menu contains commands for starting, pausing ar
ABI Prism 377.

Table 4-3. Instrument Menu Commands

Command	Description
Start Plate Check	Allows you to scan the gel and plates before loadinç samples to ensure that no peaks are produced by fluorescent particles on the glass plates or in the ge
Start PreRun	Starts a pre-run of the gel, using parameters in the pre-run module specified in the Run window.
Start Run	Begins automatic execution of a run using parameters in the run module specified in the Run window.
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, and temperature is maintained.
Resume	Resumes a run that has been paused.
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.

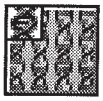
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Table 4-4. Window Menu

Command	Description
Status	Displays the status of hardware and status of the run. During a run, it displays current instrument conditions.
Log	Text file that contains date- and time-stamped status and error messages in chronological order.
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.
Run	Allows you to specify the Sample Sheet and module information for an automatic run.
Scan	Displays electropherogram of raw data as it is being collected during the run.
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.
Electrophoresis History	Displays target and actual values for the electrophoresis power supply and gel temperature throughout the course of an electrophoresis.
Manual Control	Displays a window for manual control of certain functions during a run.
Preferences...	Allows you to specify folder locations, file name conventions, and default settings.

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Starting the Data Collection Program



1. Choose Restart from the Special menu in the Finder to res Macintosh.
2. Double-click the data collection program icon.

If any of the data collection folders are not in the default location appears asking you to specify where they are located.

The data collection program allows you to indicate preferences fo locations, file name conventions, and default settings. To do so, r *Preferences* on page 4-45.

Note	*If communications between the Macintosh and the ins seem to be working properly, refer to Clearing Corrup page 7-6.*

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Importing and Exporting Sample Sheet Informati

If you wish to import information from a database into the Sampl export the information from the database into a tab-delimited tex following:

- The file should contain only the information you wish to im not have a header.
- The information imported to the first field in the Sample S everything up to the first tab in the text file.
- Each row in the text file should contain the information for Sample Sheet, in the same order as the Sample Sheet colu

Exporting a Sample Sheet creates a tab-delimited text file, with th 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 fi Sample Sheet grid.
2. Choose Import from the File menu.
3. In the dialog box that appears, choose the file you wish to i
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, cl
2. Choose Export from the File menu.
3. In the dialog box that appears, enter a name for the text fil
4. Click OK.

The exported file has an icon like that of a Log file:



sample export

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- 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, se*

a. In the second column, enter the sample names corre each lane number.

b. To choose a DyeSet/Primer mobility file or a multicon other than the default, click the arrow in the field you and choose the new file from the pop-up menu that a

The DyeSet/Primer files and a default matrix file (th file) are provided with your instrument. When you cli DyeSet/Primer column, the pop-up menu shows only DyeSet/Primer files.

The DyeSet/Primer file names indicate information t choose the correct file. See *DyeSet/Primer File Naming* page 4-18.

For most sequencing chemistries the default matrix s your instrument is appropriate. To read about matric Appendix D, *Making and Copying Matrices.*

Note *To apply the same parameter to all fields in a column, parameter in the top field of the column, click the colu the entire column, and choose Fill Down from the Edi*

c. To enter a comment, click the comment field. An ins appears, allowing you to type in your comment.

4. Click the close box to accept the entries.

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- 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.

Note *To import information from tab-delimited text files, se*

a. To choose a DyeSet/Primer mobility file, a multicomp size standard, or analysis settings other than the defa arrow in the field you wish to change and choose the n pop-up menu that appears. The pop-up menus are fil only the applicable files.

   The DyeSet/Primer files and a default matrix file are your instrument. You must create your size standards to the *ABI Prism 377 GeneScan Analysis Chemistry and S* you wish to view or customize the analysis settings, yo the analysis software program.

   The DyeSet/Primer file names indicate information t choose the correct file. See *DyeSet/Primer File Naming* page 4-18.

   If you are performing GeneScan analysis applications different buffers and gels, you must create matrix file different chemistry. For more information, refer to A *Making and Copying Matrices.*

b. To enter a comment, click the Comments field. A text appears, allowing you to type in your comment.

4. Click the close box to accept the entries.

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Editing a Sequence or GeneScan Analysis Sample

When you edit Sample Sheets the general procedure is the same, Sequence Sample Sheet or a GeneScan Analysis Sample Sheet. Fol procedure described here, and refer as necessary to the instructic the individual Sample Sheets.

Note	*The Sample Sheet can contain up to 96 rows. If you in that, the last row in the sheet is dropped off.*

You can use the standard Edit menu commands for the File Name, or Comments fields.

To modify an existing Sample Sheet:

1. Choose Open (⌘- O) from the File menu, and choose the 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 n

   Be sure to save the file under a different name, so you prese file.

Note	*You cannot change a run in progress by editing the Sa can change the Sample Sheet, but cannot re-select it in while the run is in progress. The changes are therefore 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

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Using the Run Window

In the Run window you specify the lane in which each sample will
modules containing the parameters to be used for the run. You al
window to start a plate check, a pre-run, or a run.

Separate Run files exist for sequencing and GeneScan analysis app
look almost the same; the GeneScan analysis version has an additi
Settings) column. Be sure to choose the correct type for your app

Opening the Run Window



1. Choose New from the File menu and click the appropriate

   This automatically creates a new Run folder, and opens the

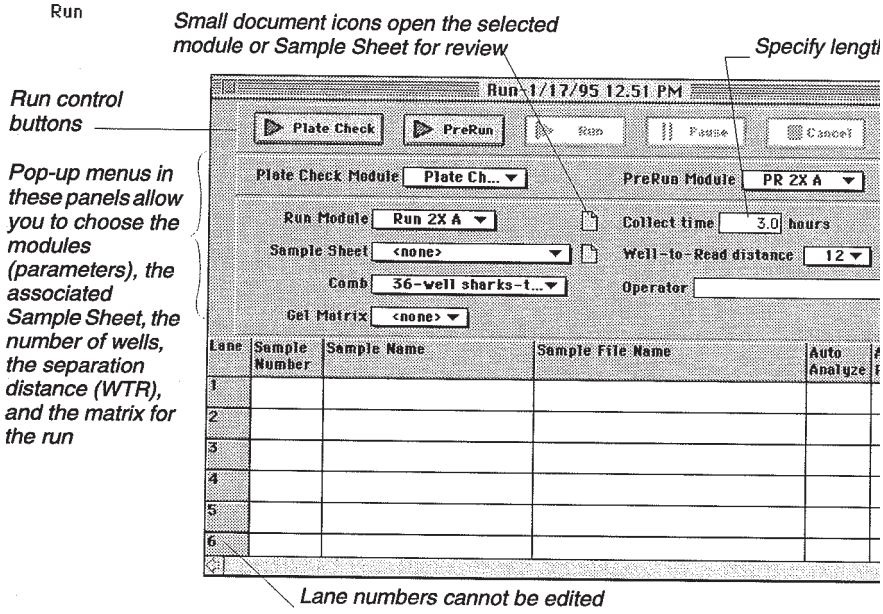


Figure 4-4. Run window

You need not complete the entire window to perform a pla
must do so to pre-run the gel or to run samples.

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Pre-Running a Gel or Running Samples

You must complete the information in the Run window before pr
or running samples.

To complete the Run window for a pre-run or a run:

1. Choose the parameters for the run from the pop-up menu

Note	*Most of the fields in steps 1, 2, and 3 are completed au set preferences for them.*

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

      See *Choosing and Changing Modules* on page 4-25 for in
      about the modules to use for various runs, the param
      module, and how to change certain parameters.

   b. Choose the Sample Sheet from the Sample Sheet pop

      By default, when you choose the Sample Sheet, the infc
      the lanes in which the samples are to run fills in autor

   c. Choose the type of comb and the separation distance f
   pop-up menu and the WTR pop-up menu, respectivel

   d. Choose a matrix file from the Gel Matrix pop-up men

      Appendix D describes using and creating matrices.

2. Enter the length of the run in the Collect Time entry field.
   some suggested run times.

Plate length	Type of Run	Typical Co
36 cm	100 bph (1200 scans/hr)	7.0
36 cm	200 bph (2400 scans/hr)	3.5
48 cm	90 bph(1200 scans/hr)	10.0

3. Enter your name in the Operator entry field.

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Choosing and Changing Modules

In the Run window three pop-up menus allow you to choose modu
instructions about conditions of operation to the instrument.

- The Plate Check module scans the laser across the plates t
  peaks are caused by fluorescent particles on the plates. Dat
  you can view it in the Scan window, but it is not stored per
- The PreRun module governs instrument conditions while
  gel. The instrument performs electrophoresis without star
  collection, so no windows appear during a pre-run.
- The Run module governs conditions after you start the ru
  the data as it is being collected during the run.

Note *The PreRun modules use lower voltage than the Run*

Table 4-5 lists the modules to use for each type of run and Table 4
describes the parameters of each module.

Table 4-5. Modules to use for various conditions

To run this chemistry	At this run speed	Plate Check Module	Pre-Mod
Taq and T7 primers, *Taq* terminators	100 bph (1200 scans/hr)	Plate Check	PR 2
	200 bph (2400 scans/hr)	Plate Check	PR 4
	48 cm – 90 bph (1200 s/hr)	Plate Check	PR 2
T7 terminators	100 bph (1200 scans/hr)	Plate Check	PR 2
	200 bph (2400 scans/hr)	Plate Check	PR 4
	48 cm – 90 bph (1200 s/hr)	Plate Check	PR 2

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Module Parameters

Table 4-7. ABI Prism 377 module parameters

Module Name	Virtual Filter Set	Field (Volts)	Power (Watts) *	Current (mAmp) *	Laser Power (mW)	Scan Rate (Scans/Hr)
Plate Check	–	–	–	–	40	1200
PR 2X A	A	1000	50	35	40	1200
PR 2X B	B	1000	50	35	40	1200
PR 4X A	A	1000	50	35	40	2400
PR 4X B	B	1000	50	35	40	2400
Run 2X A	A	1680	150	50	40	1200
Run 2X B	B	1680	150	50	40	1200
Run 4X A	A	3000	200	60	40	2400
Run 4X B	B	3000	200	60	40	2400
Run 48 A	A	2400	200	50	40	1200
Run 48 B	B	2400	200	50	40	1200

* These parameters are limited by the Field parameter.

† Can be set in the Run window

IMPORTANT *The parameters shown in Table 4-7 have been set ba acrylamide, 5% cross-linked, 6M urea gels with a gel t 0.2 mm. You should be thoroughly familiar with the pr electrophoresis before using a different gel compositio changing electrophoresis conditions.*

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To save changes to a locked module file:

1. Find the file in the Modules folder inside the ABI Prism™
2. Select the file and choose Duplicate (⌘-D) from the File r
3. Click the duplicate file name once to select it, and choose the File menu.
4. In the lower left corner of the dialog box that appears, des checkbox labelled "Locked".
5. Close the dialog box.
6. Override the parameters of the file and save the changes a above.

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- Click the Cancel button to stop a pre-run or run. If you ca the module that is running stops and you can start a run. I run, data collection stops. You cannot continue the run fr which you cancelled it.

Figure 4-6 shows a typical series of commands for a plate check, pr on the ABI Prism 377.

Button to Click	Module in effect	Task to
Plate Check	Plate Check module	Check plates a fluorescence
Cancel	n/a	(Stops plate che Add buffer and Mount front hea
PreRun	Pre-run module	(Electrophorese data collection t temperature) Prepare sample pre-running
Pause	Pre-run module	Load first set of during pause
Resume	Pre-run module	(Electrophorese gel without data
Pause	Pre-run module	Load second se during pause
Cancel	n/a	(Stops pre-run)
Run	Run module	(Starts electroph data collection)

Figure 4-6. Run window buttons used during a typical run

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Viewing the Data

The Data Collection Program Windows

In the collection program you can open all windows at one time, ; number might be limited by the available RAM on your Macintosl window can be active at any given time. Using these windows you

- Monitor the instrument status and error occurrence durin
- Observe the data as it is generated, in the Scan or Gel view the electrophoresis power supply
- Record or change sample information on the Sample Shee
- Set up and start a run
- Set default settings and parameters for automatic analysis

Viewing the data and the instrument status is described here. Desc other functions are found on the following pages:

To perform this function	See this page
Record sample information	Page 4-12
Set up and start a run	Page 4-21
Set defaults	Page 4-45

Following is some general information about working with windov

- To move a window around on the screen, click the top bor mouse button down, and drag the window to another locat
- To change the size of a window, click the size box in the low of the window, hold the mouse button down, and drag the m or shrink the window.
- To close a window, click the close box at the upper left corı window or click in the window to make it active and choose from the File menu.

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Table 4-8. Readings under normal running conditions

Run Speed	Separation Distance	Gel Thickness	Parameters Set Voltage	Temp.	Approxi Watts* Start	Er
200 bph (2400 scans/hr)	36 cm	0.2 mm	3000	51 °C	150	9(
100 bph (1200 scans/hr)	36 cm	0.2 mm	1680	51 °C	45	3(
90 bph (1200 scans/hr)	48 cm	0.2 mm	2400	51 °C	75	5

* These values drop over the course of the run from the start value to the end value.

The Log File

A Run Log file is created for each run and stored in the Run folder the run. To view the Log file, choose Log from the Window menu

The Log file contains a comprehensive record of all error and sta generated by the data collection program during a sequencing ru

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

These messages can be helpful to ABI Field Service Engineers.

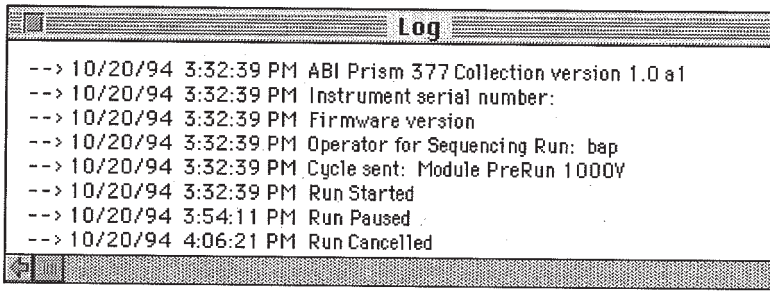


Figure 4-8. Log window example

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represent the wavelengths of the dyes being detected, rather than detected.

Different virtual filter sets use the same four colors to represent d wavelengths, so the colors do not represent actual wavelengths. T the *relative* wavelengths of the four dyes in each dye set. Virtual fil four colors to represent wavelengths within Dye Set 1 and Dye Set B uses the four colors to represent wavelengths within Dye Set 3.

Each of the three chemistries used for preparing DNA is associate the dye sets. Each dye set labels the four bases differently, so the r wavelength, and therefore the color, associated with each base vai chemistry used to label it. Because of this, the four colors on the re represent different bases, depending on the chemistry used for la

Table 4-9 describes the colors that represent each of the four base real-time displays.

Table 4-9. Color guide for data collection program raw data displa

	Virtual Filter A		V
Color	*Taq* and T7 Primers (Dye Set 1)	*Taq* Terminators (Dye Set 2)	T
Blue	C	G	
Green	A	A	
Yellow	G	T	
Red	T	C	

Note *The analysis program converts the information collect collection program, so after analysis the colors are the base: Blue for C, Green for A, Yellow* for G, and Red*

* When printed, G is black, so it is easier to see.

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The Gel Window

To display the Gel window, choose Gel Image from the Window n

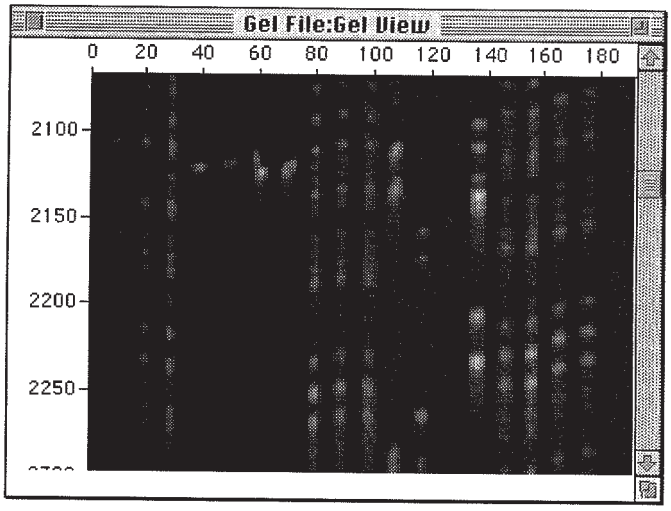


Figure 4-10. Gel window example

This window is a reconstruction of actual data similar to the way it as an autoradiogram, but in color. The first fragments passing the the bottom of the screen and move toward the top of the screen a recorded throughout the sequencing run. By the end of the run, t appears similar to an autoradiogram with the smaller DNA fragme bottom and the larger fragments near the top. Remember, howev window is a re-creation of the data through time and does not indic position of the fragments.

Note *You can scroll up or down the Gel window to see part visible.*

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Note	*The information in the Electrophoresis History Windo Gel file.*

To adjust the scale:

1. Double-click one of the panels in the window, or click a pa choose Scale from the Edit menu.

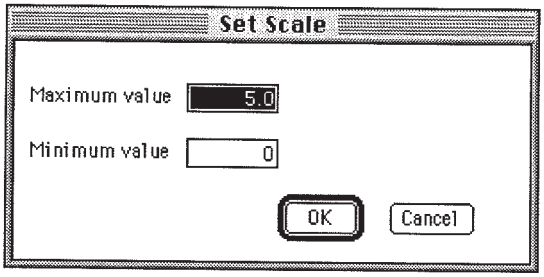


Figure 4-12. Set Scale dialog box

2. In the entry fields of the dialog box that appears, type the 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 ru Sample Sheet from the Window menu. This option is available on Sheet is listed in a currently open Run window. See page 4-12 for creating or editing a Sample Sheet.

The Run File

To display the Run file for the current run, choose Run from the V See page 4-21 for information about creating the Run file.

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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 corresp
If you click Pause, the text on the button changes to read Resume
click it again to resume the function or module.

Table 4-10. Manual Control Functions

Function	Range of Values
Electrophoresis On	n/a
Electrophoresis Off	n/a
Electrophoresis Volts*	0.00 to 5.00 kV
Electrophoresis Current*	0.00 to 60.0 mA
Electrophoresis Power*	0 to 300 W
Laser On	n/a
Laser Off	n/a
Laser Standby	n/a
Laser Run	n/a
Laser Power	0.00 to 40.0 mW (in 10ths of mW)
Temperature On	20 to 60 °C
Temperature Off	n/a
CCD Gain†	1, 2, 4, or 8 CCD Output
CCD Offset	0 to 4096 CCD Output
CCD Pixel Position‡	1 to 512 pixels

* 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.

† This parameter amplifies the signal. You should not need to adjust it.

‡ Use this only if instrument memory has been reset and the value has been lost.

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Setting Preferences

The data collection program allows you to indicate preferences fc locations, file name conventions, and default settings.

You can choose several types of preferences to set (see Figure 4-1

- 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 w apply to all samples. Since separate Sample Sheets and Run files e sequencing and GeneScan analysis, the defaults you can set are als different.

Although the defaults you set appear when you open a new Sample file, you can change the fields as required for the individual situati

Some default settings also appear in the Page Setup dialog box for y printer. They are discussed in the section on printing (see page 4-

Note	*The preferences you set are saved automatically whe Preferences window. You do not need to save each pa*

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Setting Folder and File Preferences

Setting Folder Location Preferences

The data collection program automatically uses several specific fo storage. You can specify that it use different folders by changing t location preferences.

The Folder Locations page of the preferences dialog box shows tl folders that store Sample Sheets, modules, runs, the firmware ima (see Figure 4-15).

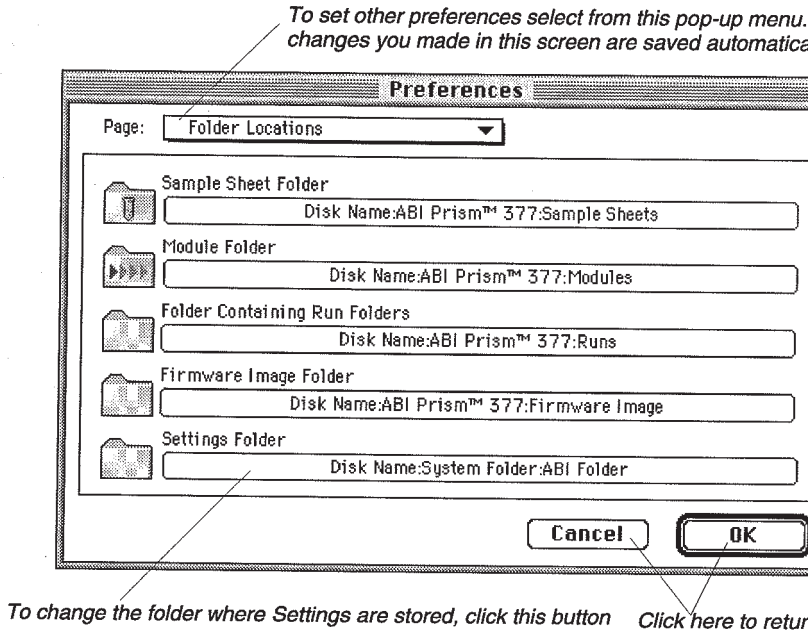


Figure 4-15. Preferences Folder Locations window

The locations initially shown in this screen are the folders where tl files are normally stored. If you store them in other folders, it is imp specify in this screen where you store them. When you create Sam modules, and Run files, the pop-up menus that allow you to choos options refer to the folders indicated in this screen.

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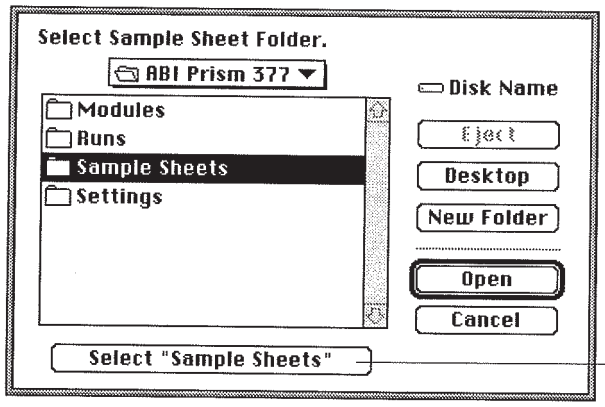


Figure 4-16. Folder selection dialog box

3. Find the new location and click the Select button at the bo selection dialog box (Figure 4-16).

IMPORTANT *The data collection software allows you to specify a S location. If you analyze your data with the ABI Prism Analysis program on the computer that you use for da Settings folder must always be specified as the ABI fol in the System folder.*

4. When you have changed all the desired folders, click OK ir Locations Preferences dialog box (Figure 4-15 on page 4-4 another preference from the Page pop-up menu.

These folder locations are normally on your local hard disk, but c AppleShare server volume as well.

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To change automatically generated file names:

1. Choose Preferences from the Window menu and choose Fi
   the submenu that appears.

   If you are already in the Preferences window, choose File N
   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 th

3. Specify a suffix by choosing from the pop-up menu to the r
   entry field.

4. When you are finished specifying file name defaults, click
   another preference from the Page pop-up menu.

Note *If you choose the "global serial number" option for a suf
the global serial number if necessary. To do so, see p*

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To set Sample Sheet defaults for Sequencing Analysis applications:

1. Choose Preferences from the Window menu and choose Se
   Sheet Defaults from the submenu that appears.

   If you are already in the Preferences window, choose Sequ
   Sheet Defaults from the pop-up menu labeled "Page:".

2. Choose parameters for the two defaults by selecting from t
   menus.

   The files listed in the menu are all the applicable files in the
   (filtered according to type). If you wish to choose a file tha
   choose Other, and a file dialog box allows you to choose any
   in any other folder.

3. Click OK or choose another preference from the Page pop

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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 to-read) pop-up menu.

      The well- to-read (WTR) distance for sequencing runs or 48 cm.

   c. Click the Comb button and choose a comb file from th that appears.

      The comb files listed in the pop-up are those stored i folder.

   d. Choose default modules from the PreRun Module an Module pop-up menus.

      The menu shows all Module files in the Module folde 4-48).

   e. To specify automatic transfer of sample information fr Sheet to the Run file, select the checkbox labeled "Au allocate one lane per sample".

   f. To specify that the data be automatically analyzed by t Analysis program, select the checkbox labeled "Autoan choose Other from the pop-up menu.

   g. In the dialog box that appears, find the analysis progr Open.

      This specifies the location of the analysis program.

   h. Select the checkbox at the bottom of the window to spe printing of the data after analysis.

3. Click OK or choose another preference from the Page pop

---

* If you are running Sequencing Analysis applications, this folder should be set as your System folder (see page 4-48).

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Each item has a pop-up menu that appears when you click t
to it. The choices in the DyeSet, Matrix, and Size Standard p
in the Settings folder (see page 4-48 for a description of th

a. Choose defaults for the size standard color and name

b. Choose a multicomponent matrix from the Matrix pc

c. Choose the DyeSet.

2. Click OK or choose another preference from the Page pop

Setting GeneScan Run Defaults

To set Run file preferences for GeneScan Analysis applications:

1. Choose Preferences from the Window menu and choose Ge
Defaults from the submenu that appears.

   If you are already in the Preferences window, choose Gene
Defaults from the pop-up menu labeled "Page:".

   A dialog box appears.

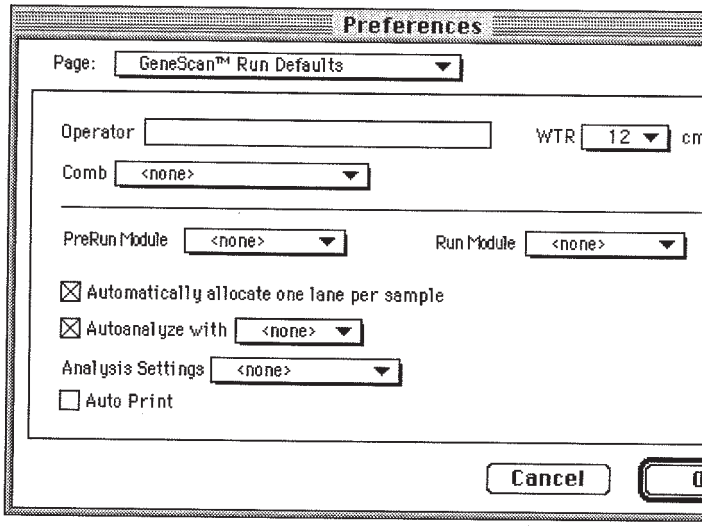


Figure 4-21. Preferences GeneScan Run Defaults window

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Setting Dye Indicators Defaults

To change the colors that represent the dyes when data is displaye
Dye Indicators defaults. This is especially useful if you are color bl
default settings shipped with the software are not appropriate for

To set dye indicator preferences for the analysis applications:

1. Choose Preferences from the Window menu and choose D
from the submenu that appears.

   If you are already in the Preferences window, choose Dye I
the pop-up menu labeled "Page:".

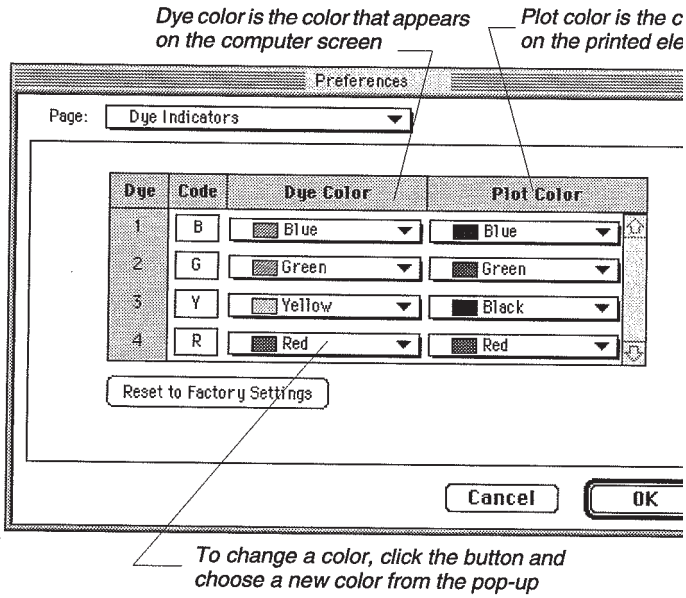


Figure 4-22. Preferences Dye Indicators window

2. Make changes as desired.

   - To change a code, type a different character in the appr
   field in the Code column.
   - To change a color, click the appropriate color button anc
   color from the pop-up menu that appears. The Display (

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General Settings

The General Settings window allows you to specify:

- A global serial number that increments across runs and car names of automatically generated files (see page 4-50).
- The module to be used for checking the plates.
- The Macintosh port to which the instrument is attached.

  The Macintosh computer has two standard connection po port and a Printer port. Normally, the instrument is attach computer by means of the Modem port, and a printer or n connection is attached to the Printer port. If, however, you the instrument connection to the Printer port, specify that the change in the general settings. A third option allows you collection software when no instrument is attached to the
- The minimum number of scan lines in a gel.

  The size of a gel file is calculated according to the collect tir the Run window, assuming the fastest collection rate of 20((2400 scans/hr). The number in the General Settings spec minimum number of scan lines for a gel file.

IMPORTANT *Do not change the minimum number of scan lines in a it smaller, the data collection software might not alloc disk space to collect all of your data.*

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3. Click OK or choose another preference from the Page po

Note	*The data collection software always performs at leas number of scans specified in the General Settings wi more than the minimum specified if necessary to com on the collection time specified in the Run window.*

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Archiving Files

Gel Files

Gel files contain the raw data acquired by the data collection prog
typically take up 10–20 MB of disk space. A separate Gel file is crea
folder each time you perform a run. Because Gel files take a subst
of space, you should delete them from your hard disk after you ha
satisfactory Sample files. You do not usually need to keep gel files
tracking is verified and sample files are created.

If you do wish to archive Gel files, use magnetic tapes, removable c
or optical drives. Gel files are too large to fit on floppy disks.

IMPORTANT *Do not discard any gel file until you have verified the tra
corrective action if necessary.*

Sample files

Use floppy disks, a magnetic tape drive, a removable cartridge drive
drive to archive sample files. A 1.4 MB, high-density disk holds abo
sample file is 150-200 KB in size, depending on the length of the se
Save a sample file when you feel confident that the channel selecti
are correct for the sample.

Printing Files

You can print the contents of any of the editable windows (Sample
window, Module window).

Setting Up the Page for Printing

Before you print, you can specify certain parameters about the pri
the paper size, the orientation of the page, and whether or not the
be reduced from actual size. To do so, choose Page Setup from the

The appearance of the Page Setup dialog box depends on the prin
using. For information about the other default settings, refer to yo
manual.

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Importing and Exporting Files

Exporting

Use the Export command to save the contents of a window grid (f
from the Sample Sheet or the Run window) into a tab-delimited te
format is useful with most database, word processing, or spreadsh

To export information to tab-delimited text format:

1. Click the window containing the information you wish to e
   it active.

   The window must be one of the windows containing a grid
   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
Sheet and Run windows. Each row in the text file should contain th
for one row in the grid, in the same order as the columns of the g

To import tab-delimited text files into the data collection program windo

1. Open the window into which you wish to import informatio
   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
   text you wish to import.

3. Click OK.

Note *Everything up to the first tab in the text file is imported i*
in the grid.

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About Data Collection Program Files

Various files appear on the computer that runs the ABI Prism 377 file and various ancillary files are organized in folders on your har in Figure 4-25.

The ABI Prism 377 Folder contains the program file and subfolde Sheets, modules, runs, and settings.

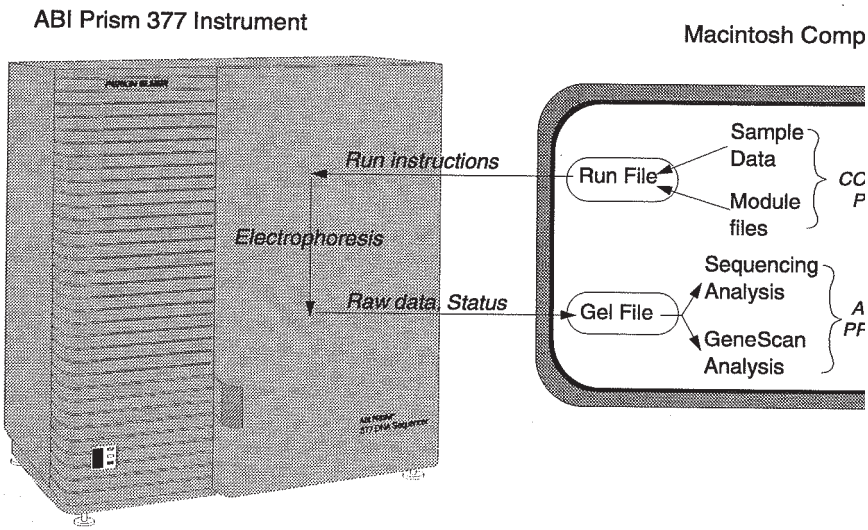
Other files are located in the ABI folder (see Figure 4-25). This AB always be in the System folder on your hard disk. It is the default for the data collection software.

IMPORTANT	*Although you can change the location specified as the the data collection software, the Settings folder must a folder if you use the Sequencing Analysis program to a*

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ABI Prism 377 Data Flow

Figure 4-26 shows the flow of data between the Macintosh compu
ABI Prism 377 instrument during data collection.



*The Run file associates a list of samples with an electrophoresis module. The instrun
run data and sends back raw data to a Gel file, which is passed to the appropriate a*

Figure 4-26. Data collection program data flow

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Output Files

The instrument automatically places two output files in the Run f
individual run: a Run file and a status log. Table 4-12 details all of
that are not located in the System folder and their locations. The
be located in the System folder are described in Table 4-13.

Table 4-12. ABI Prism 377 Data collection program output files

File type	Location	Description
Gel file	Individual Run folder inside the Runs folder inside the ABI Prism 377 folder	A large file created by the data collectio typical run a gel file can be very large (c file contains all raw data acquired by the program during the run. It is created in t when you perform a run.
Run Logs	Individual Run folder inside the Runs folder inside the ABI Prism 377 folder	Created in the Run folder at the start of e file contains date and time-stamped stat messages that maintain a comprehensi actions taken, instrument and computer any errors received. You can view and p any time while the application is running
Sample (data) files (Analysis program output)	Individual Run folder inside the Runs folder inside the ABI Prism 377 folder	When you analyze the raw data, the gel The Sequencing Analysis and GeneSca programs* extract sample information fr create sample files. Each file contains th sample, or lane, including information yc Sample Sheet; raw data from the instrum stop times; voltage, temperature and pov the run; analyzed data; and base calls.
Sequence files (Analysis program output)	Individual Run folder inside the Runs folder inside the ABI Prism 377 folder	The Sequencing Analysis program also c files called ".Seq" files (the file names al ".Seq"). These files show the base letter s might contain a header, depending on th choose).You can open them from word p programs and print them. You can also s several formats that can be read by othe programs. The Preferences dialog box ir analysis program allows you to choose t

* The Analysis programs are described in detail in separate manuals. Refer to the ABI Prism *Software User's Manual* or the *ABI Prism GeneScan Analysis Software User's Manual*, as

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5 ABI Prism 377 Instrument Hardware

The ABI Prism™ 377 DNA Sequencing System consists of two sep components:

- The electrophoresis scanning unit (the Instrument)
- The Apple® Macintosh® Computer

The components are connected by an asynchronous RS-232C seri

This section describes the instrument hardware components. For about the Macintosh computer, refer to Section 6. For informatio computer software, refer to Section 4.

Contents

About the ABI Prism™ 377 Instrument Hardware
System Overview
The Loading System
The Gel Cassette
The Injection Device
The Electrophoresis Chamber
Replaceable Parts
Glass Plates
Spacers
Combs
The Separation System
The Electrophoresis Power Supply
The Buffer Chambers and Electrodes
The Temperature Control System
Heat-transfer Plates
Water Reservoir, Pump, Heater, and Sensor

(Continued on next page)

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About the ABI Prism™ 377 Instrument H

The ABI Prism 377 instrument is a microprocessor-controlled ele
and fluorescence detection system.

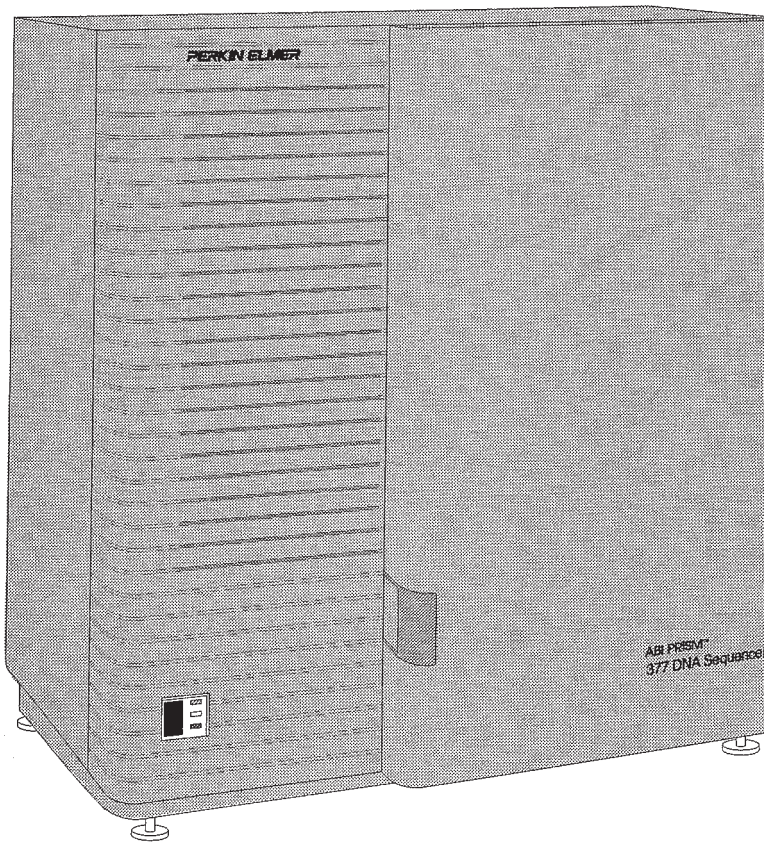


Figure 5-1. ABI Prism 377 instrument

It is an integrated system in which all the components have been c
engineered to provide maximum performance.

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At the end of collection, the analysis software can automatically p
and translate the collected data into either base sequence, fragme
information, or relative concentrations, depending on your applic
it on a color printer.

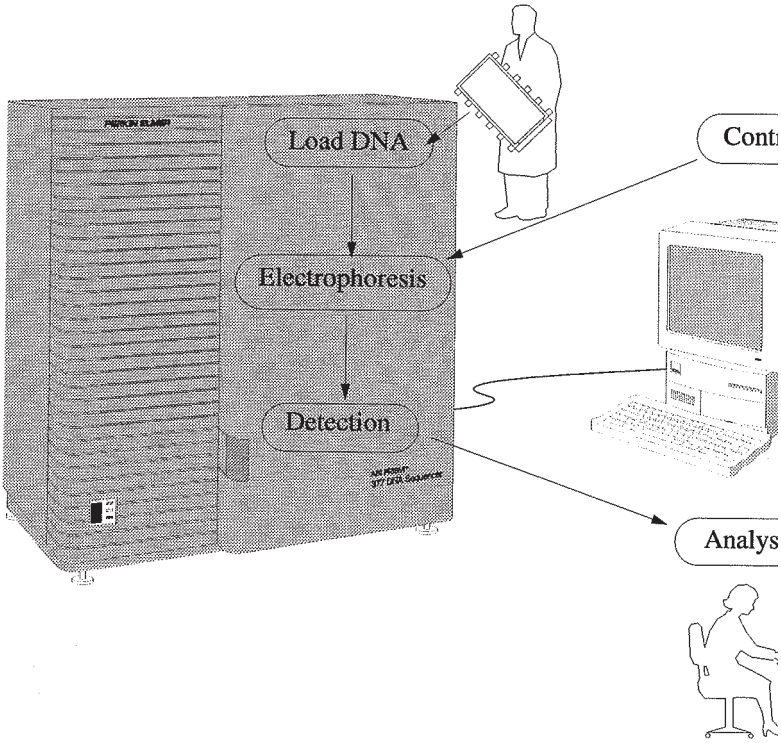


Figure 5-2. ABI Prism 377 system overview

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The Injection Device

The gel injection device allows you to use a standard luer lock to solution between glass plates that are secured in the gel cassette.T consists of two fixtures, which attach to the gel cassette after the g spacers have been inserted.

The top fixture has two pieces. The front clear brace has two side under the plate clamps of the gel cassette, and rear pegs that fit ove front glass plate to stabilize it and align it in the cassette. The bac raised brackets that fit through notches on the cassette and stops th align the rear glass plate. When the two pieces are clamped at the plates by the plate clamps on the cassette, they hold the casting co eliminating the need for binder clamps.

The bottom fixture accommodates the syringe, and has a rubber tension springs to ensure an effective seal during gel injection.

Note	*Some leakage may occur as you inject the gel.*

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The back of the chamber is a heat-transfer plate (see Figure 5-4). clamps allow you to attach the gel cassette, which holds the gel pl buffer chamber, to the instrument. The lower buffer chamber sits the rear heat-transfer plate.

Electrophoresis connections and receptacles for quick-disconnec circulating water that cools the gel are also located in this chamb

Positioning pins beside the base of the rear heat-transfer plate po the focal point of the instrument optics.

Replaceable Parts

Glass Plates

Two specially designed glass plates are used to hold the gel throu electrophoreses.

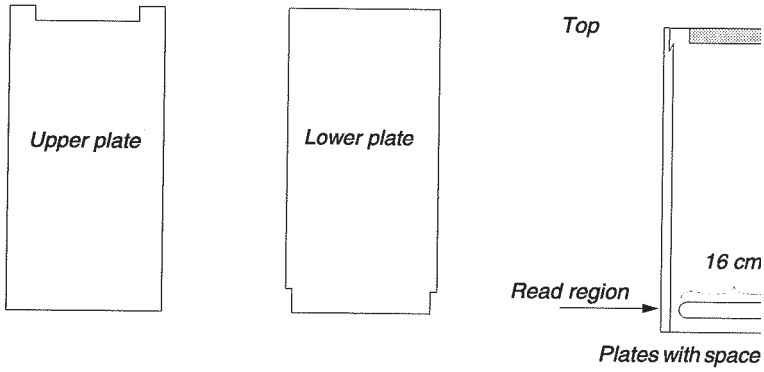


Figure 5-5. Glass plates and example of read region

The plates are specially shaped to facilitate use with the gel cassette, to accommodate uniform 0.2-mm gels. They are specially manufa free as possible from any fluorescent materials.

The region of the glass where the laser scans is called the read reg located behind the laser beam safety stop (in the down position) w mounted in the gel cassette. It is approximately 16 cm wide in the glass, and is located from approximately 2.5 cm to approximately 4.. bottom of the glass (see Figure 5-5).

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Combs

ABI Prism 377 combs are used to create the individual wells at the into which you load your DNA samples. Two different combs are a two general applications on the ABI Prism 377. Each has varying nu

The shark's-tooth comb (Figure 5-7) is provided with the Sequenc configuration of the ABI Prism 377. This comb is available with 24 36 lanes.

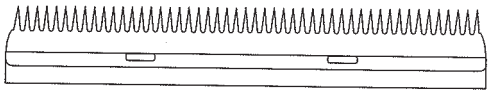


Figure 5-7. Shark's-tooth comb

This comb serves two purposes. During gel polymerization, it is a After injecting the gel solution, insert the straight edge of the com the gel to create a single well or slot. Remove the comb after polyr

The comb also forms loading wells. Before loading samples, insert t comb into the slot formed during polymerization. This creates the i into which you load the DNA. The comb is marked with lane numb loading.

The square tooth comb (Figure 5-8) is provided with the GeneScan of the ABI Prism 377. It is available with 34 lanes.



Figure 5-8. Square tooth comb

You need only use the square tooth comb during gel polymerizatic injecting the gel solution, insert the teeth of the square tooth com solution so the comb creates individual wells at the top of the gel c polymerization. After polymerization, remove the comb. You do n re-insert it when you load samples.

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Heat-transfer Plates

The back of the electrophoresis chamber is a built-in coated alum heat-transfer plate, against which the gel plates are mounted. Thi plate contains channels through which water circulates, drawing l gel as it builds up during electrophoresis, and producing uniform

A second heat-transfer plate, also containing water channels, is m front of the 36-cm gel plates. Using both heat-transfer plates on a reduce formation of temperature gradients both across the width a thickness of the gel.

The gel cassette is spring-loaded against the rear heat-transfer pla positioning pins, to ensure proper thermal contact and optical ali

Water Reservoir, Pump, Heater, and Sensor

A pump circulates water from a built-in reservoir through the heat- The water acts as a heat exchange fluid, adding or removing heat liquid-to-air heat exchanger and fan facilitate heat removal. The f when heat must be removed. A heater allows addition of heat to tl temperature sensors on the back heat-transfer plate and one on th provide feedback for active temperature control.

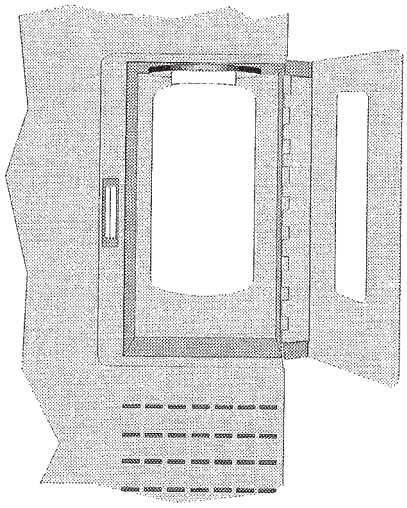


Figure 5-9. Water reservoir (located on side of ABI Prism 377)

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The Scanning/Detection System

Detection on the ABI Prism 377

When DNA fragments pass through the read region of a gel, an a
excites the fluorescent dye labeled fragments. Positioning pins in
electrophoresis chamber hold the gel at the focal point of the las

A series of lenses collect the emitted light and focus it into a spec
spectrograph separates the light (based on wavelength) into a pre
pattern across a charge coupled device (CCD) camera.

The data collection software contains information about which dy
used and their emission light wavelengths, and collects the light i
the appropriate areas of the CCD camera. The analysis software tl
analyzes, and translates the information into either base sequenc
sizing information, or relative concentrations, depending on the

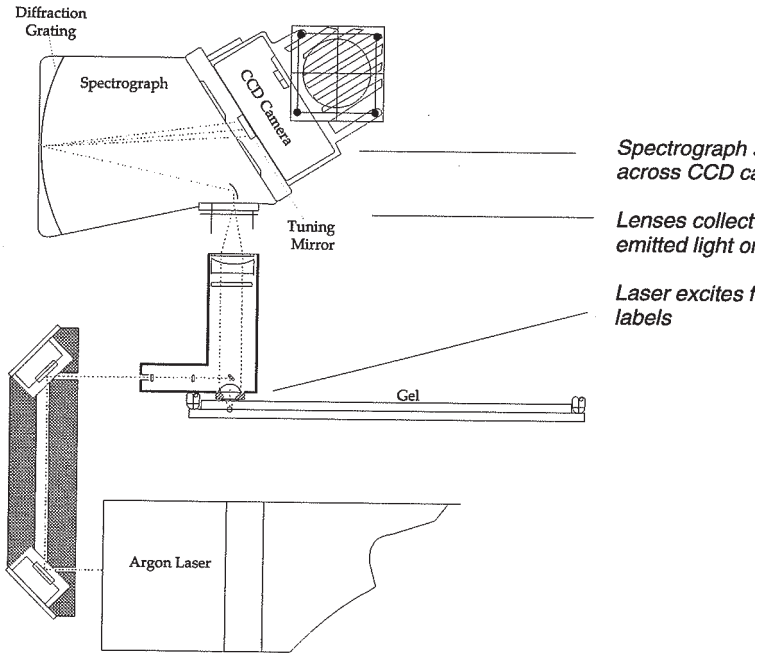


Figure 5-10. The ABI Prism 377 scanning/detection system

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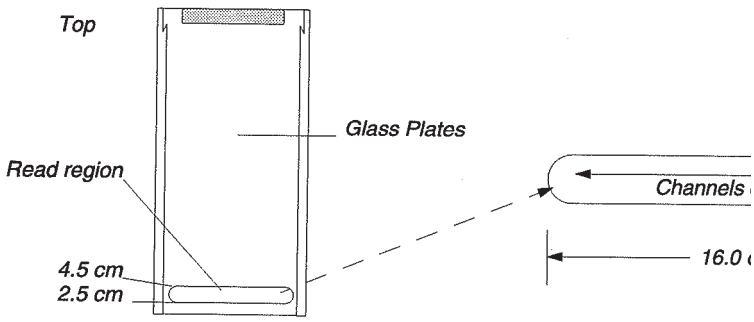


Figure 5-11. The read region

The read region of the gel is theoretically divided into 194 chann numbered 0–193. For a 36-well loading comb, approximately 5 ch one sample lane.

The Spectrograph and CCD Camera

The scanning spectrograph-CCD system allows detection of multi emissions with a single pass of the scanner. The spectrograph splits gel image by wavelength onto the CCD camera for intensity detec camera is cooled to –15 °C by a Peltier device. It is optimized for s applications and is specifically designed for integration into Perki instruments.

Correcting Emission Spectrum Overlap

All ABI Prism 377 chemistries use fluorescent dye labels that are ex Although the dye fluoresces at different wavelengths, the emission s to some degree. It is necessary to correct for this overlap (or filter before analyzing data. A mathematical matrix file is stored in the S (refer to page 4-74). When data is analyzed the matrix is applied t correct for any emission overlap.

The Settings* folder must contain a matrix file for each chemistry Instrument. For Sequencing Analysis applications, the Instrument contains three matrices, is sufficient. If you are running GeneScan

* If you are running Sequencing Analysis applications, this folder should be set as your System folder (see page 4-48).

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Instrument Status and Control

Instrument Control

The power switch for the ABI Prism 377 DNA Sequencer and thre status lights are located on the front of the instrument. The status described on page 5-21.

The first time you turn the instrument on and start the data colle the program copies a firmware image to the instrument (refer to p turn off the instrument between runs, a battery backup holds the memory, so you do not need to copy it again when you restart the

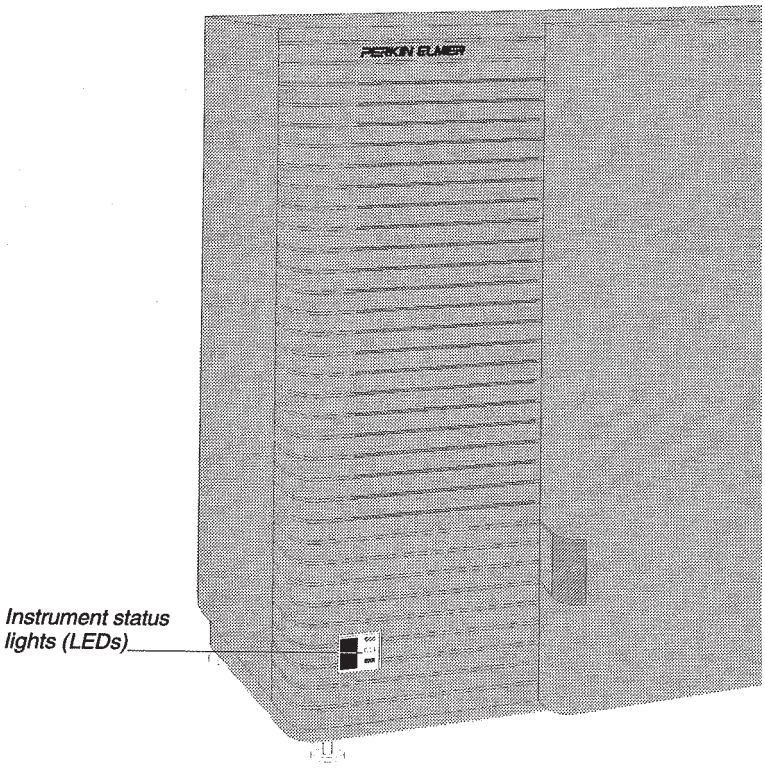


Figure 5-12. ABI Prism 377 front view

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Status Indicators

Several instrument status lights (LEDs) are located on the front a ABI Prism 377. See Figure 7-2 on page 7-6 for the location of the back. You need only observe them if the instrument's memory ap corrupted and you need to clear it.

Three LEDs are located on the front of the instrument (see Figur indicate instrument status as follows:

- Green indicates that the system is operational or operating comes on after completion of the power-on self tests and b run unless a special condition causes the amber or red LED end of a run, it stops blinking and a blinking amber light ap indicate that the instrument is operational, but is paused b finished the run. When you open the doors (breaking the remove the gel, the green light goes out and the amber ligh you close the doors again and the system verifies that the i engaged, the amber LED goes out and the green LED ligh
- Amber indicates that the instrument is operating but paus blinks as the system goes through self-test when you turn th also blinks if you open the instrument door (this breaks th electrophoresis safety interlocks), or if you pause the instru selecting Pause from the Macintosh. At the end of a run, it with a stable green light to indicate that the instrument is o is paused because it has finished the run.
- Red indicates an error condition that must be corrected fo run properly. This LED lights if an error is detected during lights if any failures are detected during a run (for exampl electrophoresis power, laser, or temperature system fails). D indicated by the red LED appear in the log file on the Maci LED also lights if communication between the instrument Macintosh fails.

Table 5-1 shows some examples of how the LEDs indicate status du and special instrument states.

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6 ABI Prism 377 Macintosh Computer

The ABI Prism™ 377 DNA Sequencing System includes an electrophoresis/scanning unit (the instrument) and the Macintos This section provides some basic information about the Macintosh information about the data collection software installed on the con Section 4. For information about the instrument hardware, refer t

Contents

About the ABI Prism™ 377 Computer System
- System Requirements
- Installing ABI Prism 377 Software
- Special Software for Data Collection and Analysis
- Other Software Provided with Your Computer
- What You Need to Know About Using the Macintosh

Maintaining and Caring for Your Macintosh
- Hard Disk Maintenance
  - Install Only One System per Hard Disk
  - Back Up all Programs and Files Regularly
  - Restart the Macintosh Before Each ABI Prism 377 Run
  - Remove Non-essential Files from the Hard Disk
  - Rebuild the Desktop Regularly
  - Use a Hard Disk Maintenance Program Regularly
  - Use Discretion When Adding Software Programs.
- Error Messages
- Hard Disk Maintenance Programs
- Backing Up Important Files Before Optimizing Your Disk

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About the ABI Prism™ 377 Computer Sy

The ABI Prism 377 computer system performs three primary func

- Instrument control
- Data collection
- Data analysis

The Macintosh computer provided with your instrument runs spe
(described on page 6-5) to perform these functions.

System Requirements

The ABI Prism 377 electrophoresis instrument is shipped with a Pc
computer. An optional printer is also available. If you need to rep
computer, or did not order a printer with the instrument, followi
specifications for the type of computer system you should use with

- *Macintosh Model:* Power Macintosh
- *Monitor:* Apple Power Macintosh-compatible color monitor,
- *Disk Drive:* Hard disk with a minimum of 250 MB storage (5
recommended)
- *RAM:* Minimum 16 MB random access memory

Note	*About 8 MB of RAM should be available at any given t the data collection and analysis programs open togethe available RAM, choose About this Macintosh from the*

IMPORTANT	*Do not run data collection and analysis at the same tim programs can be open, only one should be operating a*

- *Printer:* Color printer (contact Technical Support or your s
representative for a recommendation)
- *System Software:* Macintosh system software version 7.1.2 or l

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Special Software for Data Collection and Analysis

The ABI Prism 377 comes with software programs that perform ra collection and data analysis.

The Data Collection software communicates instructions to firmw instrument that controls instrument function. It also records and instrument status and raw data during a run. You can specify that i open the analysis program for data analysis after the run is compl

To analyze the data collected, two other software packages are ava depending on your application. Normally, one is supplied with yo ABI Prism 377, depending on the configuration you ordered, althc have ordered both.

- The Sequencing Analysis software analyzes the raw sequen collected by the data collection program and calls the base program comes bundled with two other programs, GelDoc a GelDoc allows you to perform special tasks relating to Gel i DataUtility program enables you to make and copy matrice the spectral overlap of the fluorescent dye labels. Refer to A instructions on using the DataUtility program. It can also b Perkin-Elmer technical specialists to check noise levels.
- The GeneScan Analysis software enables you to use ABI Pri automated fluorescence detection to size and quantify DN/ The program automates the entire process of separating, qu sizing DNA fragments. It also provides flexibility to interact and fine-tune the data analysis, and allows you to display th experiment in several different ways.

Each analysis program is described in detail in a separate manual: *DNA Sequencing Analysis Software User's Manual* and the *ABI Prism Ge Software User's Manual*, respectively. For information about the Mac operating system, refer to the *Macintosh System 7 Reference* manual.

The data collection software and the analysis program(s) you orde installed on the Power Macintosh by your field service specialist wl instrument is installed, so you do not need to install them yourself

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Maintaining and Caring for Your Macint

Computers require regular attention and maintenance to operate consistently. Because the ABI Prism 377 software on the Macintos large files and accesses the hard disk often, it is especially importa follow the procedures described here to minimize the occurrence o operation. These procedures should help keep your Macintosh co operating at maximum efficiency.

The following describes procedures recommended by Apple Com The Perkin-Elmer Corporation for regular maintenance of Macint and includes some hints specifically aimed at keeping your ABI Pr software running smoothly.

Hard Disk Maintenance

The following general principles can benefit all Macintosh system these guidelines for optimal performance of the ABI Prism 377 pro Macintosh.

- Install only one System per hard disk.
- Back up all programs and files regularly.
- Restart the Macintosh before each ABI Prism 377 run.
- Remove non-essential files from the hard disk.
- Use a hard disk maintenance program regularly.
- Use discretion when adding software programs, especially c (CDEV) and extension (INIT) files.

Each of these guidelines is discussed in more detail below.

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Remove Non-essential Files from the Hard Disk

It is a good idea to remove any unnecessary files from the hard disk the data collection program. This ensures that you have enough 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

Rebuild the Desktop Regularly

Rebuild the desktop monthly and after installing any new softwar

To rebuild the desktop:

1. Hold down the Command (⌘) and Option keys and choo the Special menu.
2. Continue to hold down the two keys until the message "Ar 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 then open and rewrite t physical location on the disk can change. This causes the compute become "fragmented." If a significant amount of fragmentation oc might run more slowly, and files might be lost. It is extremely imp protect your data by running a disk optimizer program regularly.

Optimize the hard disk monthly with Disk Express II, the Speed D Norton Utilities®, or a similar program, to prevent the hard disk f too fragmented. Be sure to back up custom matrix files (in the fol as the Settings folder—refer to page 4-48) and any important data optimize the hard disk. *Hard Disk Maintenance* on page 6-7 describ recommended programs for hard disk maintenance.

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Error Messages

Abiding by the procedures and precautions described above redu
probability of errors occurring during ABI Prism 377 runs. Unfor
might not prevent errors from occurring altogether, so you still n
messages after performing a run. A table in Section 7 lists some S
Analysis error messages that might appear on the screen, and des
recommended methods of correcting them.

Note	*If you experience recurring errors, use Norton Utilities MacCHECK, or a similar program, to check and repai the hard disk. After it is repaired, follow all of the prev maintenance procedures described in this document.*

Hard Disk Maintenance Programs

Following is a limited list of maintenance programs that you can u
maintain your hard disk.

- Disk Express II — AlSoft
- Norton Utilities® — Symantec
- Public Utilities — Fifth Generation
- SilverLining™ — La Cie
- CP Optimizer — Central Point Software

IMPORTANT	*Do not use automatic optimization features that might disk in the background while collecting ABI Prism 377 cause loss of data.*

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7 ABI Prism 377 Maintenance/Troubles

Contents

ABI Prism™ 377 Maintenance
- Cleaning Instrument Accessories
- Refilling the Water Reservoir and Replenishing Algicide
- Replacing Electrophoresis Cables and Electrodes
- Backing Up Important Files Before Optimizing Your Disk
- Other Macintosh Computer Maintenance

Troubleshooting
- Hardware/Firmware
  - Clearing Corrupted Memory
  - Finding the Firmware Image File
- Problems During a Run
- Troubleshooting Gels
- Software Troubleshooting
  - Data Collection Run Log Errors

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ABI Prism™ 377 Maintenance

Most maintenance procedures on the ABI Prism 377 DNA Sequer performed by a trained Perkin-Elmer service technician. You can p procedures yourself, however, as described below.

Cleaning Instrument Accessories

Be sure to clean the following regularly, as described on the page:

- Glass plates (pages 2-14 and 3-33)
- Heat transfer plates (pages 3-8 and 3-32)
- Spacers and comb (page 3-33)
- Gel cassette (wipe with lint-free towel before each use to er contact with positioning pins)
- Positioning pins in the electrophoresis chamber (pages 3-8

Refilling the Water Reservoir and Replenishing Alg

The water reservoir is located in a compartment on the right side instrument (refer to Figure 5-9 on page 5-13). Visually check the wa and refill the water reservoir when it is between one-third and one Replenish the algicide every month, regardless of whether you ne reservoir.

To refill the water reservoir or replenish the algicide:

1. Make sure the pump is not running.

IMPORTANT *Do not remove the water reservoir while a run is in pr*

2. Open the water reservoir compartment on the right side of i by pulling gently on the door.

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To change the electrophoresis cable/electrode assembly:

1. Disconnect the electrophoresis cable from the instrument a buffer chamber from the instrument.

2. Remove the two screws on the back left end of the buffer c hold the electrode assembly in place, and remove the asse buffer chamber.

   The assembly in the lower buffer chamber is held under a h end of the chamber. Lift the electrode assembly from the l pull it from beneath the hook at the right end of the buffe

3. Insert the new assembly into the buffer chamber.

   Be sure the assembly in the lower buffer chamber is under right end of the chamber.

4. Replace the two screws at the left end of the buffer chambe assembly in place.

Backing Up Important Files Before Optimizing Yo

Before optimizing your hard drive, back up all important files onto another storage medium, and locate all of the original disks neces reloading the whole system. Refer to page 6-12.

Other Macintosh Computer Maintenance

Section 6, *ABI Prism 377 Macintosh Computer*, describes regular mair for the Macintosh computer. Please refer to it.

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- The instrument does not run even after pushing the reset rear of the instrument AND
- The rear panel LEDs (see Figure 7-2) do not blink in their pattern but are stuck in one pattern, which might be all or
- The Mac software might not be able to load a new image o instrument, OR
- The Mac software appears to have loaded a new image onto but the instrument still does not run and the rear panel LE changing.

To clear the instrument's memory:

1. Press the reset button on the back of the instrument.

   Use the eraser end of a pencil or something similar.

2. Watch the back panel LEDs and wait for them to blink in a off sequence.

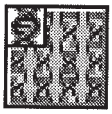
   In this state, four consecutive lights are on at one time, ther and the other four go on together.

3. While the four on, four off blink sequence is occurring pre button a second time. You should do so within about three the sequence.

   The LEDs change to the "every other one on" state. In this lights blink on and off. This indicates that the instrument n and the instrument is ready for the Macintosh to copy over a image. The next time you start the data collection program, new image to the instrument.

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3. Double-click the ABI Prism 377 Collection icon to start the



If, after step 1, the program does not locate the firmware i box appears asking you to identify its location.

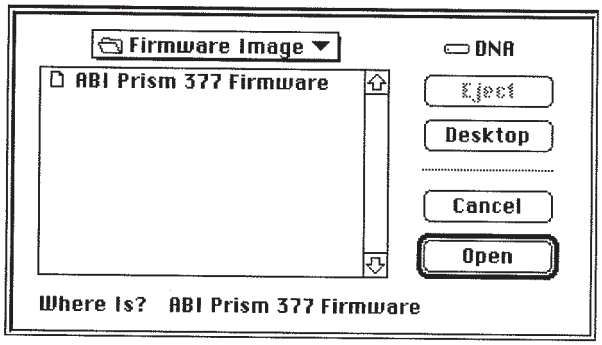


Figure 7-3. Find Image dialog box

4. Locate the ABI Prism 377 Firmware file and click Open.
5. Click Quit when it is finished and restart the ABI Prism 37 program.

Note *If you click Cancel in the Find Image dialog box, the pr you return to the Macintosh Finder.*

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Troubleshooting Gels

Table 7-2 shows some problems you might encounter relating to g
recommends some solutions.

Table 7-2. Gel Troubleshooting

Problem	Possible Cause	S
Inconsistent mobilities from gel to gel	total% polymer wrong	follow prot
	wrong concentration of Bis	follow prot
	wrong buffer concentration	follow prot
	poor quality reagents	remake 10 acrylamide using high reagents. be ultra pu
	dissolved O_2 concentration	keep vacu strength/ti stir/pour g gently; filte 20-23 °C
	variation in spacers	use space sets that a thickness
	temperature of room, gel solution or glass varies	20-23° C is
Slow mobility	old gel	remake 10 acrylamide using high reagents. be ultra pu pour a fres
Poor resolution	poor quality reagents (especially acrylamide)	Try fresh r reliable so
	small bubble between load and read region	cast gel as protocol

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Table 7-2. Gel Troubleshooting (continued)

Problem	Possible Cause	S
Gel extrudes from between plates into upper buffer chamber during electrophoresis		After clea wash brie wash with clean as c page 2-14

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The Perkin-Elmer Corporation

A PE Limited Warranty

The Perkin-Elmer Corporation, by and through its Applied Biosy hereinafter jointly referred to as "PE/ABD", warrants to the Custc period ending on the earlier of one year from completion of install (15) months from the date of shipment to the Customer (the "War the ABI PRISM™ 377 DNA Sequencer purchased by the Custome "Instrument") will be free from defects in material and workmans perform in accordance with the performance specifications conta Instrument User's manual that accompanies the instrument (the "Specifications"). During the Warranty Period, if the Instrument f in accordance with such specifications, PE/ABD will repair or rep Instrument at no charge to the Customer, subject to the conditio

This Warranty does not apply to the Instrument's valves, reagent l performance, unless the Customer uses only reagents and solvent PE/ABD or expressly recommended by PE/ABD, or to damages c reagents or solvents not supplied by PE/ABD, even though recom PE/ABD. This Warranty does not extend to any Instrument or pa that has been the subjected of misuse, neglect or accident, (ii) tha modified or repaired by any party other than PE/ABD or (iii) that in a manner not in accordance with the instructions contained in t User's Manual. This Warranty does not cover the customer-installat parts for the Instrument that are listed in the Instrument User's M

PE/ABD's obligation under this Warranty is limited to repairs or r that PE/ABD deems necessary to correct covered defects or failur PE/ABD is notified prior to expiration of the Warranty Period. Al replacements under this Warranty shall be performed by PE/ABD Customer's location at PE/ABD's expense.

No agent, employee, or representative of PE/ABD has any author PE/ABD to any affirmation, representation, or warranty concerni Instrument that is not contained in the printed product literature o Statement. Any such affirmation, representation or warranty mad employee, or representative of PE/ABD shall not be binding on P

PE/ABD shall not be liable for any incidental, special, or consequ damage or expense directly or indirectly arising from the purchas

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ABI Prism 377 System Accessories (sold s

Kits

Note	*The three Operating Mode kits include Software, Soft Chemistry Guide, and Combs.*

672-70	Genescan Software Module (Square-tooth Combs)
677-70	Sequencing Software Module (two Shark's-tooth C

Note	*The Glass/Spacer kits include two back Plates, two fr two sets of Spacers.*

401876	36-cm WTR plates/0.2-mm Spacers kit
401877	12-cm WTR/0.2-mm Spacers kit
401878	48-cm WTR/0.2-mm Spacers kit

Gel Cassette

603827	Gel Cassette (one size accommodates all plate sizes
603947	Top and Bottom Gel Pouring Fixtures

Heat Plates/Buffer Chambers

603833	Front 36-cm WTR Heat Transfer Plate
603873	Upper Buffer Chamber
603875	Lower Buffer Chamber
603822	Upper Electrode Assembly
603823	Lower Electrode Assembly

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Software

	377 GeneScan™ Analysis (see *Kits* on page A-3)
	Sequencing Analysis (see *Kits* on page A-3)
401614	Genotyper™ Fragment Analysis
670-11	GeneAssist Sequence Analysis
401725	AutoAssembler™ DNA Sequence Assembly
401615	Sequence Navigator™ DNA and Protein Sequence

Manuals

903433	ABI Prism™ 377 DNA Sequencer User's Manual
903436	ABI Prism™ DNA Sequencing Analysis Software M:
903437	ABI Prism™ 377 DNA Sequencing Analysis Chemis Guide
903434	377 GeneScan™ Analysis Software Manual
903435	377 GeneScan Analysis Chemistry Guide

Other

401892	Algicide Concentrate

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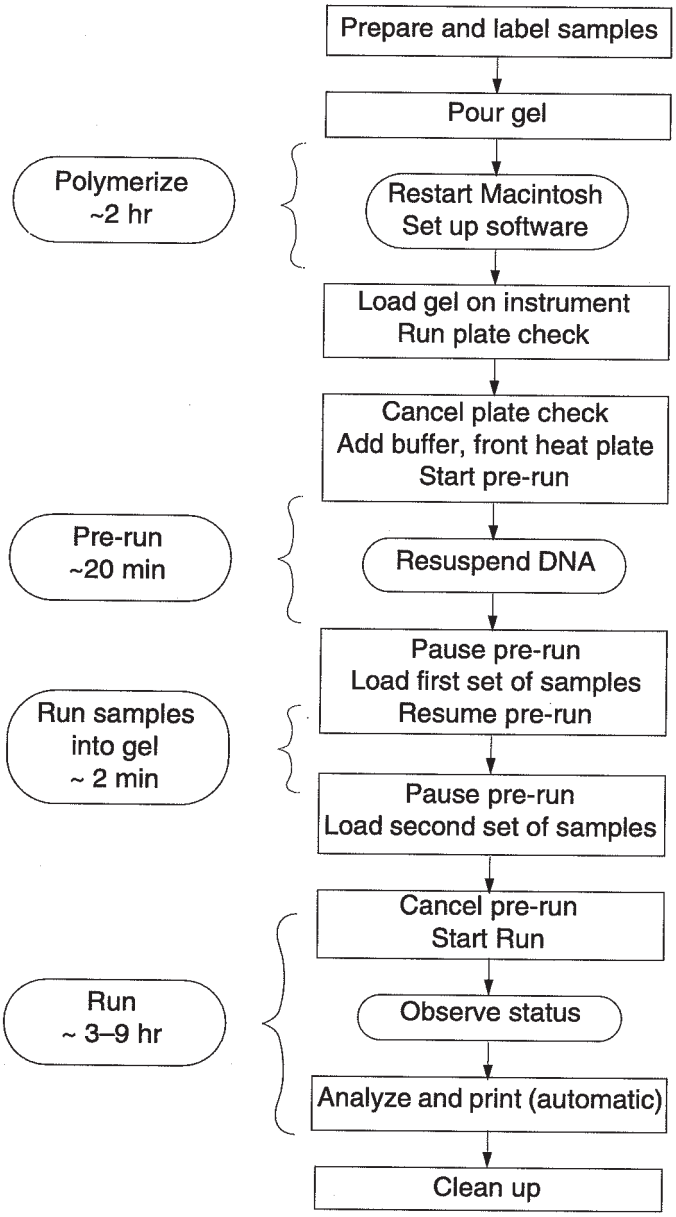
B Quick Reference

Contents

Flow Chart of Typical Run Process
Acrylamide Gel Recipes
Modules
Run Times and Filter Sets
ABI Prism™ 377 Run Quick Reference
Table of Abbreviations (alphabetical)

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Flow Chart of Typical Run Proc



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Modules

Modules to Use For Various Conditions

To run this chemistry	At this run speed	Plate Check Module	Pre-ru Modu
Taq and T7 primers, *Taq* terminators	100 bph (1200 scans/hr)	Plate Check	PR 2X
	200 bph (2400 scans/hr)	Plate Check	PR 4X
	48 cm – 90 bph (1200 s/hr)	Plate Check	PR 2X
T7 terminators	100 bph (1200 scans/hr)	Plate Check	PR 2X
	200 bph (2400 scans/hr)	Plate Check	PR 4X
	48 cm – 90 bph (1200 s/hr)	Plate Check	PR 2X

ABI Prism™ 377 Module Parameters

Module Name	Virtual Filter Set	Field (Volts)	Power (Watts) †	Current (mAmp) *	Laser Power (mW)	Scan Rate (Scans/Hr)	Te (
Plate Check	–	–	–	–	40	1200	
PR 2X A	A	1000	50	35	40	1200	5
PR 2X B	B	1000	50	35	40	1200	5
PR 4X A	A	1000	50	35	40	2400	5
PR 4X B	B	1000	50	35	40	2400	5
Run 2X A	A	1680	150	50	40	1200	5
Run 2X B	B	1680	150	50	40	1200	5
Run 4X A	A	3000	200	60	40	2400	5
Run 4X B	B	3000	200	60	40	2400	5
Run 48 A	A	2400	200	50	40	1200	5
Run 48 B	B	2400	200	50	40	1200	5

† These parameters are limited by the Field parameter.

‡ Can be set in the Run window

**The parameters shown in this table have been set based on 4% acrylamide, 5% cross-linked, a gel thickness of 0.2 mm. You should be thoroughly familiar with the principles of electrophore different gel composition or thickness.*

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Table of Abbreviations (alpha

APS	Ammonium persulfate
bp	Base pairs
CCD	Charge coupled device
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, -tetraace
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
P/N	Part number
q. s.	Quantity sufficient
RAPD	Random Amplified Polymorphic DNA
RF DNA	Restriction Fragment DNA
s	Seconds
ss	Single stranded

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Appendix C Glossary

Note	*This section defines special terminology used in the A DNA Sequencer User's Manual. The terms are listed order. Many terms are defined in the text of the User's do not find a term here, check the index to see if you c manual.*

base spacing – number of scans from one peak to the next. This va determining the effectiveness of the base calling performed by th Analysis software. Spacing between 9 and 14 (the lower the better Spacing of –12 indicates a problem. You can view the base spacing sample in Annotation View of the Sample File window.

beam stop – laser beam safety stop. This is the bar across the bott cassette that prevents outside exposure to the laser beam when it gel.

bottom fixture – one of the two components of the gel injection c bottom fixture aligns the bottom edges of the plates, accommoda and has a rubber gasket and tension springs to ensure an effective injection.

cassette clamps – four built-in clamps in the electrophoresis cham ABI Prism 377 that hold the gel cassette in place.

CDEVs – Macintosh system extensions that customize Control Pan stored in the control panel folder inside the System folder. Do not INITs other than those shipped with your system.

channels – divisions across the read region of a gel where the data software samples the data. The number of wells in the loading cor determines the approximate number of channels assigned per lane instance, with a 36-well comb, one lane is approximately five chann Sequencing Analysis software tracks a gel, it places the tracker line in the channel showing the strongest fluorescent signal. The data channel and the adjacent channel on each side (total of three) ar determine the raw data for the sample file.

cycle – see *module.*

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heterozygote – "a diploid or polyploid individual that has inherite alleles at one or more loci and therefore does not breed true" (K Stansfield, W.D. *A Dictionary of Genetics*; Oxford University: New Yor In this manual, the term describes a position at which the ABI pri electropherogram displays more than one nucleotide peak, indic possible presence of different alleles.

hygroscopic - readily absorbing moisture

image – *see firmware image, gel image.*

INIT – a Macintosh system extension that expands the capabilitie software. Examples are printer drivers and programs that add fea Finder or the system software. INITs are stored in the extensions fc System folder. Do not use CDEVs and INITs other than those shi system.

IUB code – alphabetic character representing the occurrence of n given position in a sequence. Originally defined by the Internatio Biochemistry. Table 1 contains a table of IUB codes and the mixe represent, and a listing of the complements.

Table C-1. IUB Codes and their complements

IUB Codes		Complem	
A = Adenosine	R = A or G (puRine)	A	T
C - Cytidine	Y = C or T (pYrimidine)	C	G
G = Guanosine	K = G or T (Keto)	G	C
T = Thymidine	M = A or C (aMino)	T	A
B = C,G, or T	S = G or C (Strong— 3 H bonds)		
D = A, G, or T	W = A or T (Weak—2 H bonds)	R	Y
H = A, C, or T	N = aNy base	Y	R
V = A, C, or G		K	M

IUPAC – International Union of Pure and Applied Chemistry. IUB referred to with this acronym, since IUPAC adopted the codes as a

length – the length of a sequence is the number of characters it cc

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separation distance – the length from the wells of the gel to the rea gel. Also called the well-to-read or WTR.

sequence – a linear series of characters. The characters are displaye left to right. More specifically, a sequence is a series of nucleotide that represent a linear DNA sequence, or a series of amino acid c represent a protein sequence.

sequencing reactions – the reactions performed to incorporate flu labels into DNA extension products. The chemistries and kits sup Perkin-Elmer Corporation for performing such reactions are descr *Prism 377 Sequencing Analysis Chemistry and Safety Guide.*

settings – choices that you specify in the data collection software a parameters used for data collection. Refer to page 4-45.

sharks-tooth comb – mylar piece inserted into a gel for sequencin edge of the comb is used to form a well during gel polymerization edge is inserted to form wells into which samples are loaded.

signal numbers – numbers that appear at the top of printed electr Compare these numbers to see relative signal strengths of the dye each base.

software code – the programming code that the instrument execu run.

spacing – see *base spacing.*

tared – counterbalanced.

text files – type of simple file produced by all Macintosh word pro programs, and many other programs.

top fixture – one of the two components of the gel injection devic fixture consists of two pieces: a clear brace that stabilizes and aligns plate and a back support that aligns and stabilizes the rear plate. V onto the plates in the gel cassette, the two pieces also serve as a clai casting comb in place during gel polymerization.

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Appendix D Making and Copying Matrices

Contents

Introduction
- Matrix Files
- Virtual Filters and Data Display

Creating a Matrix File
- Matrix Standards
- Preparing, Loading and Electrophoresing Matrix Standards
- Making the First Matrix for a New Instrument File
- Adding or Replacing a Matrix in the Instrument File

Correcting Errors in Matrix Creation
- Signal Size Too Small
- Incorrect Files or Chemistry

Viewing and Copying Matrices
- Viewing a Matrix in the Instrument File
- Copying Matrices from Source to Destination Files

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Introduction

Matrix Files

Three chemistries are currently available to prepare DNA samples on the ABI Prism 377: Dye Primers, *Taq* DyeDeoxy Terminators, a Terminators. Each chemistry has a specified dye set: a set of dye la fluorescence when excited by a laser. Each dye label in the set emi at a different wavelength and, during data collection, the wavelen separated by a spectrograph into a predictably spaced pattern acr camera.

Although the dyes fluoresce at different wavelengths, there is some spectra. To correct for this overlap before analyzing data, a mathe is created for each chemistry type (dye set) and stored in a file cal Instrument file. During data analysis the appropriate matrix is app any spectral overlap.

The Instrument file must contain a matrix for each chemistry that instrument. When the Gel and Sample files are created, the Instru attached to each to allow subsequent data analysis. Each ABI Prism Sequencer must have an Instrument file inside the ABI folder loca System Folder.

IMPORTANT *Due to slight variations in the CCD cameras, the Instru on your ABI Prism 377 is not valid on other ABI Prism*

Each ABI Prism 377 DNA Sequencer is shipped with an Instrument created for that instrument. Create a new Instrument file only if th is replaced, or if you should need a new matrix for a type of chemis the three mentioned above.

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The analysis program converts the information collected by the d
program, so after analysis the colors are the same for each base. T
displays of analyzed data, including printed electropherograms, a

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

*When printed, and when shown against a white background on screen, G is bla
easier to see.

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Preparing, Loading and Electrophoresing Matrix S

After obtaining the correct matrix standards, follow the procedure run matrix standards for Dye Primers and *Taq* DyeDeoxy terminat gel, using Filter Set A. Run standards for T7 Terminators on a sep Filter Set B.

1. Prepare 4.0% acrylamide gel for a 36-cm, 200 bph (2400 s sequencing run.
2. Set up the instrument according to Section 3 of this manu
3. Restart the Macintosh (choose Restart from the Special m Finder).



4. Start the data collection program.



5. Choose New from the File menu and click the Sequence S icon.
6. Fill in the Sample Sheet as described in Section 6 of the *ABI Sequencer User's Manual.*
   a. For convenience, name the matrix standards accordin base (A, C, G, or T), followed by the specific sequenci (Primers, *Taq* Terminator, or T7 Terminator).
   b. Choose Save As from the File menu to save the Sampl

      Be sure to save it in the folder designated as the Sampl in the data collection program preferences.



7. Choose New from the File menu and click the Sequence R
8. In the Run window, choose the proper files from the pop-u

   The Sample Sheet should be the one you completed in Ste

   The Gel Matrix should be the current instrument file or ar applicable matrix file.

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a. Briefly vortex the matrix standards to ensure the solu mixed. Spin the solution briefly to collect the fluid at the tube.

b. For each standard (one tube for each base), combine with 1 µl of deionized formamide.

c. Briefly vortex each of the tubes prepared above to en sample is well mixed. Spin the solution briefly to colle the bottom of the tube.

d. Heat all matrix samples at 90 °C for 2 minutes. Place s until loaded.

12. Load the matrix standards in the lanes you designated in th

13. Click the Run button in the Run window to start the sequen instrument.

14. Proceed to either *Making the First Matrix for a New Instrumen or Replacing a Matrix to an Instrument File* after the sequenci 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 t 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) a new instrument.

Note	*If you lose your Instrument file and do not have a back floppy disk refer to Viewing and Copying Matrices on p. re-creating the entire Instrument file.*

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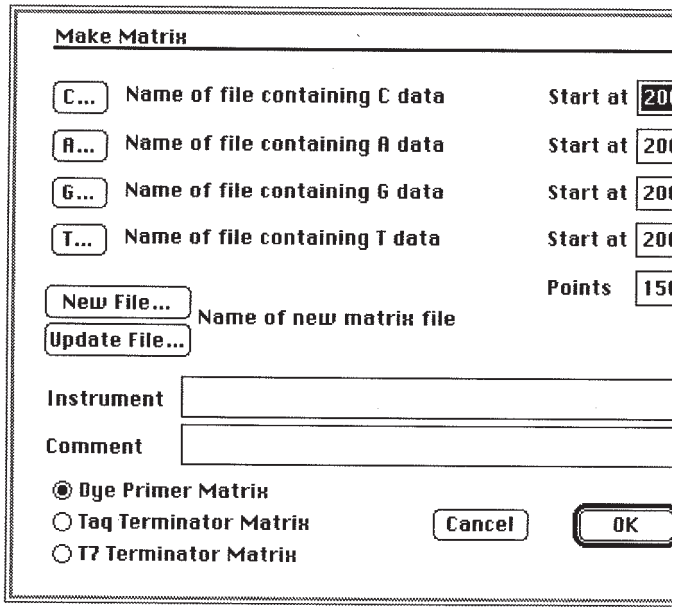


Figure D-2. Make Matrix dialog box

Note *The files you select for the four nucleotides are the Sa named on the Sample Sheet when you electrophores Standards.*

3. Complete the Matrix dialog box as follows:

   a. Click the C... button. In the box that appears, select t contains the data from the C standard.

      Click the A... button. In the box that appears, select t contains the data from the A standard.

      Click the G... button. In the box that appears, select t contains the data from the G standard.

      Click the T... button. In the box that appears, select t contains the data from the T standard.

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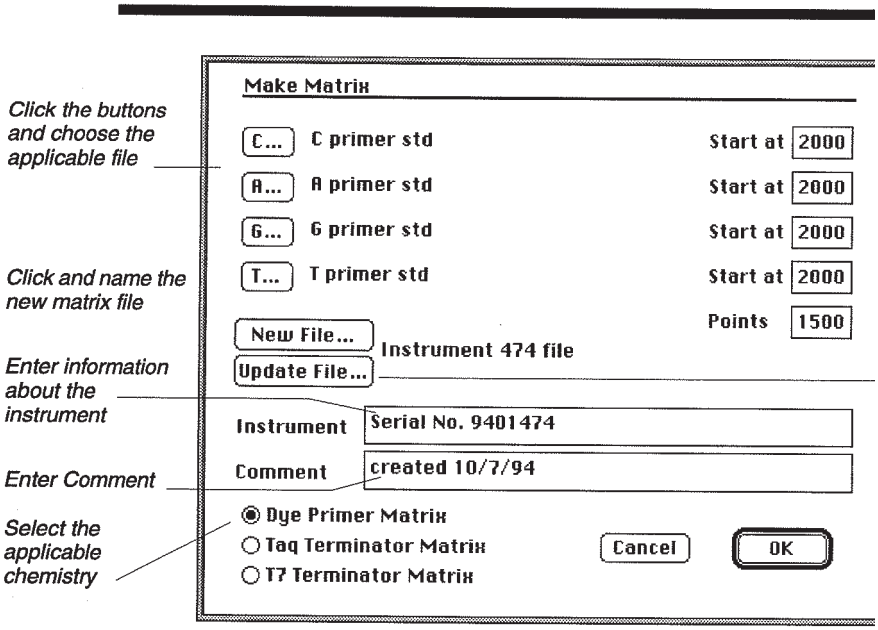


Figure D-3. Example of a completed Make Matrix dialog box

4. Click OK to initiate the matrix calculation.

   The calculation takes about one minute. When the matrix is message "Make matrix successfully completed" appears.

5. Click OK or wait about 20 seconds for the dialog box to dis

   If an error message appears and the matrix is not made, se *Errors in Matrix Creation* on page D-17.

To verify accuracy of the matrix file:

1. Choose Open from the File menu.
2. Locate and select the Sample file you wish to check and cli
3. Choose Analyzed data from the Window menu.

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IMPORTANT *Make a backup copy of the Instrument file and store*

Once you have a made the first matrix for your Instrument file, rep
until you have created a matrix for each chemistry. Use the proce
in *Adding or Replacing a Matrix in the Instrument File* below.

Adding or Replacing a Matrix in the Instrument Fi

Use this procedure under the following circumstances:

- To add matrices to an incomplete Instrument file.
- If you want to run new standards for a chemistry that alrea
in the Instrument file either to replace the existing file, or
additional Instrument file for testing purposes. Be sure to
copy of the original Instrument file before making the nev

This procedure is similar to making the first matrix on a system. C
steps are different.

1. Verify the lane tracking and peaks as described on page D-
2. Open the DataUtility program, choose Make Matrix from th
and specify the files containing the standards, as described
b on page D-12.
3. Click the Update File button (see Figure D-2).
   a. In the dialog box that appears, choose the name of the
   you want to update or to which you want to add the n
   click Open.
   b. Click the button for the appropriate matrix chemistry
   of the Make Matrix dialog box.

IMPORTANT *If you make a matrix that already exists in the Instrum*
overwritten.

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Correcting Errors in Matrix Creation

Signal Size Too Small

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

To correct for small signal size:

1. Open the sample file.
2. Choose Raw Data from the Window menu.
3. Find a data range with about 1500 points with reasonable s
4. After determining a range containing enough usable data, Matrix from the Utility menu.
5. In the Make Matrix dialog box enter a Start Point for each Points value that encompasses the usable data range in the

If the file does not contain enough good data, repeat the procedu *Preparing, Loading and Electrophoresing Matrix Standards* on page D-7 set of Matrix Standards.

Incorrect Files or Chemistry

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

To correct file or chemistry selection:

1. Repeat the Make Matrix process, selecting the correct chem the correct set of matrix sample files.
2. Use the gel file to verify that the matrix sample files contai the file indicates.

IMPORTANT *The gel file in the data collection program shows uncor so the colors displayed represent different bases, dep chemistry. See Virtual Filters and Data Display on pag*

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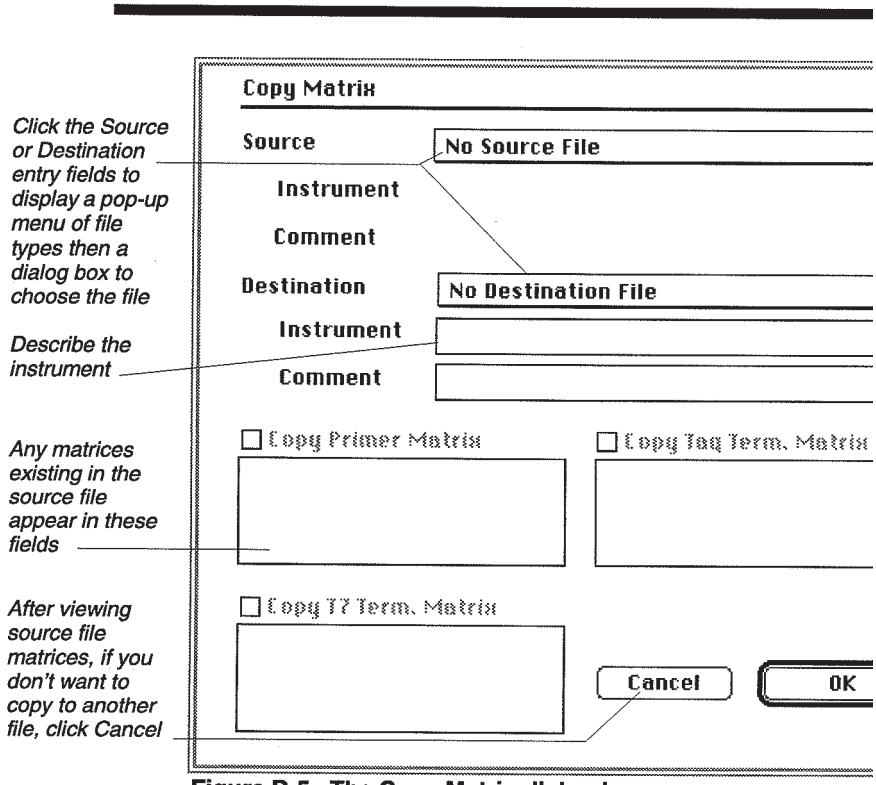


Figure D-5. The Copy Matrix dialog box

3. Click the Source pop-up menu and choose Instrument File that appears.

4. In the dialog box that appears, locate and select the Instru contains the matrix information you wish to view.

5. Click Open.

   The information fields at the bottom of the Copy Matrix dia the scale factors that are contained in the selected matrix fil factors are listed with dyes in columns and filters in rows. Th can be useful for troubleshooting.

6. Click Cancel to leave the dialog box unchanged.

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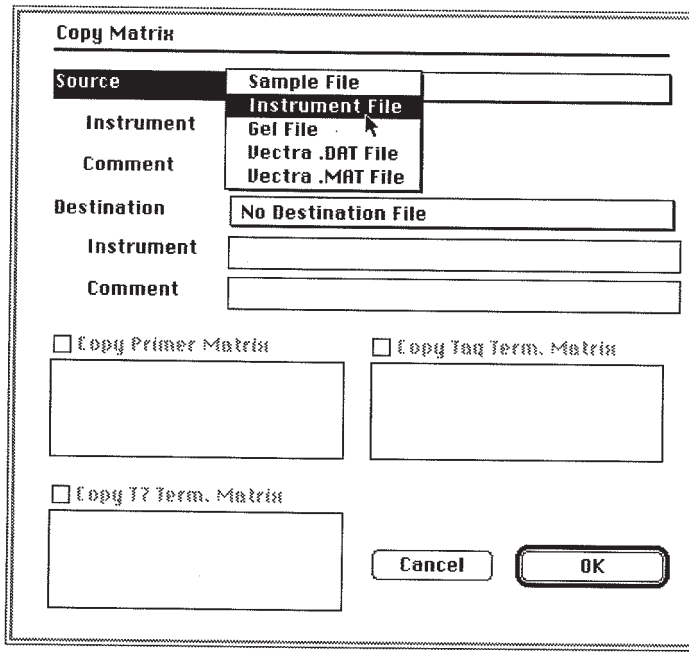


Figure D-6. Source Pop-up Menu

4. Choose the type of file that contains the matrices you wish

5. In the dialog box that appears, locate and select the file tha matrix information you wish to copy.

6. Click Open.

   The information boxes at the bottom of the Copy Matrix dial the scale factors that are contained in the selected matrices factors are listed with the dyes in columns and filters in row D-7 for an example). This information can be useful for trc

7. Click the Destination pop-up menu (Figure D-7).

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9. Verify that all displayed information is correct and click OI copy matrix procedure.

Note	*Any matrix already associated with the destination file you copy the new matrix.*

10. Use the Copy Matrix dialog box to view the destination files the matrices were successfully copied.

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