

# User Bulletin

## ABI PRISM<sup>®</sup> 377 DNA Sequencer

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December 2002

**SUBJECT:   Protocols for Processing AmpF $\mathcal{L}$ STR<sup>®</sup> PCR Amplification Kit PCR Products with the ABI PRISM<sup>®</sup> 377 DNA Sequencer and Windows NT OS**

### **In This User Bulletin**

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### **Overview**

AmpF $\mathcal{L}$ STR PCR Amplification Kit products are run on 36-cm well-to-read plates on the ABI PRISM<sup>®</sup> 377 DNA Sequencer (ABI PRISM 377), the ABI PRISM 377 DNA Sequencer with XL Upgrade (ABI PRISM 377XL), or the ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade (ABI PRISM 377-96). Protocols for analyzing samples on these configurations with the Microsoft<sup>®</sup> Windows NT<sup>®</sup> operating system are included in this user bulletin.

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# Safety

## Safety Conventions Used in This Document

### Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

**IMPORTANT!** Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.



**CAUTION** Indicates a potentially hazardous situation that, if not avoided, can result in minor or moderate injury. It can also alert against unsafe practices, damage to an instrument, or loss of data.



**WARNING** Indicates a potentially hazardous situation that, if not avoided, can result in serious injury or death.



**DANGER** Indicates an imminently hazardous situation that, if not avoided, will result in serious injury or death. This signal word is to be limited to the most extreme situations.

## Chemical Safety

### Chemical Hazard Warning



**WARNING CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

## Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See “About MSDSs.”
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

## About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

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## Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **<https://docs.appliedbiosystems.com/msdssearch.html>**.
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
  - **Open** – To view the document
  - **Print Target** – To print the document
  - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

## Chemical Waste Safety

### Chemical Waste Hazard



**WARNING**

**CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

### Chemical Waste Guidelines

To minimize the hazards of chemical waste:

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers.
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.

- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

## Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Waste profiles help you plan for the handling and disposal of waste generated by operation of the instrument. Read the waste profiles and all applicable MSDSs for your instrument before handling or disposing of chemical waste.

## Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

**Note:** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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## Biological Hazard Safety



### **WARNING**

**BIOHAZARD.** Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4, <http://bmbl.od.nih.gov>)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030, [http://www.access.gpo.gov/nara/cfr/waisidx\\_01/29cfr1910a\\_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html))

Additional information about biohazard guidelines is available at:  
**<http://www.cdc.gov>**

# Before You Begin

## Software Requirements

This user bulletin is written for:

- ABI PRISM® Data Collection Software v3.0 (PN 4326986)
- ABI PRISM® GeneScan® Analysis Software Version 3.7.1 or higher

Before using the ABI PRISM 377, ABI PRISM 377XL, or ABI PRISM 377-96, you must install the appropriate software. The associated documentation is listed below.

**Table 1 Software documentation**

Software	Part Number
<i>ABI PRISM® 377 DNA Sequencer User Guide For Data Collection Software on the Windows NT® Platform</i>	4325703
<i>ABI PRISM® GeneScan® Analysis Software Version 3.7 for the Windows NT® Platform User Guide</i>	4308923
<i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i>	4335617

## AmpFℓSTR Kits

The protocols in this user bulletin are applicable to all AmpFℓSTR kits.

**Table 2 AmpFℓSTR Kits and Part Numbers**

AmpFℓSTR Kits	Source
AmpFℓSTR® Blue™ PCR Amplification Kit (PN 402800)	Applied Biosystems
AmpFℓSTR® COfiler® PCR Amplification Kit (PN 4305246)	Applied Biosystems
AmpFℓSTR® Green™ I PCR Amplification Kit (PN 402902)	Applied Biosystems
AmpFℓSTR® Identifiler® PCR Amplification Kit (PN 4322288)	Applied Biosystems

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**Table 2 AmpF $\ell$ STR Kits and Part Numbers**

<b>AmpF<math>\ell</math>STR Kits</b>	<b>Source</b>
AmpF $\ell$ STR <sup>®</sup> Profiler <sup>®</sup> PCR Amplification Kit (PN 403038)	Applied Biosystems
AmpF $\ell$ STR <sup>®</sup> Profiler Plus <sup>™</sup> PCR Amplification Kit (PN 4303326)	Applied Biosystems
AmpF $\ell$ STR <sup>®</sup> SEfiler <sup>™</sup> PCR Amplification Kit (PN 4335129)	Applied Biosystems
AmpF $\ell$ STR <sup>®</sup> SGM Plus <sup>®</sup> PCR Amplification Kit (PN 4307133)	Applied Biosystems

## **Equipment and Supplies**

The equipment and supplies necessary or recommended for running AmpF $\ell$ STR kit data on the ABI PRISM 377 instruments are listed in the tables under “Equipment and Supplies Required” on page 10.

## **Related Documentation**

Refer to the documents listed below for more detailed information on the instrument and software used with these protocols:

- ABI PRISM<sup>®</sup> 377 DNA Sequencer with 96-Lane Upgrade User Bulletin (4313688)
- ABI PRISM<sup>®</sup> 377 DNA Sequencer 96-Lane Upgrade User’s Manual (4305423)
- ABI PRISM<sup>®</sup> 377 Gel Processor Software User Guide (PN 4317592)
- AmpF $\ell$ STR<sup>®</sup> Blue<sup>™</sup> PCR Amplification Kit User’s Manual (PN 402827)
- AmpF $\ell$ STR<sup>®</sup> COfiler<sup>®</sup> PCR Amplification Kit User Bulletin (PN 4305469)
- AmpF $\ell$ STR<sup>®</sup> Green<sup>™</sup> PCR Amplification Kit User’s Manual (PN 402944)
- AmpF $\ell$ STR<sup>®</sup> Identifiler<sup>®</sup> PCR Amplification Kit User’s Manual (PN 432391)
- AmpF $\ell$ STR<sup>®</sup> Profiler<sup>®</sup> PCR Amplification Kit User’s Manual (PN 402945)
- AmpF $\ell$ STR<sup>®</sup> Profiler Plus<sup>™</sup> PCR Amplification Kit User’s Manual (PN 4303501)



- AmpFSTR® SEfiler™ PCR Amplification Kit User's Manual (PN 4337410)
- AmpFSTR® SGM Plus® PCR Amplification Kit User's Manual (PN 4309589)

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# Equipment and Supplies Required

## Dedicated Equipment and Supplies

Follow the guidelines for dedicated equipment and supplies to ensure that exogenous DNA and PCR products are confined to designated areas:

- Designate a work area and dedicated equipment and supplies used for amplified DNA. Do not remove amplified DNA, equipment, or supplies from this work area.
- Samples that are not amplified should never come into contact with supplies and equipment in the amplified DNA work area.

## Required Equipment

The equipment necessary or recommended for running AmpF $\mathcal{L}$ STR kit data on the ABI PRISM 377, 377XL, and 377-96 DNA Sequencer instruments is listed in the table below. You can order the part numbers listed below from Applied Biosystems. If no part number is listed, you can order from any major laboratory supplier (MLS).

**Table 3 Equipment required**

Equipment	Source
<ul style="list-style-type: none"><li>• ABI PRISM<sup>®</sup> 377 DNA Sequencer</li><li>• ABI PRISM<sup>®</sup> 377 DNA Sequencer with XL Upgrade, or</li><li>• ABI PRISM<sup>®</sup> 377 DNA Sequencer with 96-Lane Upgrade</li></ul>	Applied Biosystems
Thermal cycler: <ul style="list-style-type: none"><li>• GeneAmp<sup>®</sup> PCR System 9700</li><li>• GeneAmp<sup>®</sup> PCR System 9600</li><li>• GeneAmp<sup>®</sup> PCR System 2400</li><li>• DNA Thermal Cycler 480</li></ul>	Applied Biosystems For ordering information, see your Applied Biosystems sales representative.
Autoclave	MLS
Benchkote absorbent protector sheets	MLS
Conductivity meter	MLS
Freezer, –15 to –25 °C, non-frostfree	MLS
Glassware	MLS

Table 3 Equipment required (*continued*)

Equipment	Source
Gloves, disposable, powder-free	MLS
Ice bucket	MLS
Lab coat	MLS
Lamp, 27-inch gooseneck with magnetic base	Sunnex, PN 701-27, or office supply store
Lint-free tissues	MLS
Magnetic stir plate and stir bar	MLS
Microcentrifuge	MLS
Microtube racks	MLS
Nalgene filter apparatus, 150-mL, 0.2- $\mu$ m CN filter	MLS
Needles for the two-pitch, eight-channel loader, 0.01-in., 0.25-mm point	Kloehn (PN 18663, 8 needles)  World Precision Instruments (PN 67126, 10 needles)
pH meter	MLS
Pipet bulb	MLS
Pipets, serological	MLS
Pipet tips for gel loading, 0.2 mm flat tips (Rainin, PN GT1514)	MLS
Pipet tips, sterile, disposable hydrophobic filter-plugged	MLS
Pipettors, adjustable, 0.5–10 $\mu$ L, 2–20 $\mu$ L, 20–200 $\mu$ L, and 200–1000 $\mu$ L	MLS
Plastic wrap	MLS
Razor blades	MLS

**Table 3 Equipment required (*continued*)**

<b>Equipment</b>	<b>Source</b>
Refrigerator	MLS
Repeat pipettor and Combitips that dispense 1–5 µL (optional)	MLS
Sink	MLS
Syringe, 20- or 35-cc (optional)	MLS
Tacks	Office supplier
Tube decapper, autoclavable	MLS
Two-pitch, eight-channel loader, 0.25-mm	Kloehn Company (PN 18597)  World Precision Instruments (PN GelMate96)
Vortexer	MLS
Water bath, 37 °C	MLS

## Required Supplies

The supplies necessary or recommended for running AmpF $\mathcal{L}$ STR kit data on the ABI PRISM 377, 377XL, and 377-96 DNA Sequencer instruments are listed in the table below. You can order the part numbers listed below from Applied Biosystems. If no part number is listed, you can order from any major laboratory supplier (MLS).

**Table 4 Supplies required**

Supplies	Source
ABI PRISM 377 DNA Sequencer Accessories: <ul style="list-style-type: none"> <li>• 36-cm well-to-read plates:               <ul style="list-style-type: none"> <li>– 36-cm rear glass plate (PN 401839)</li> <li>– 36-cm front glass plate (PN 401840)</li> <li>– 36-cm step plate for 96 wells (PN 4305384)</li> <li>– 36-cm gel spacers, 0.2 mm thick (PN 401836)</li> </ul> </li> </ul>	Applied Biosystems
Combs: <ul style="list-style-type: none"> <li>• 34-well square tooth comb, 0.2 mm thick (PN 401907)</li> <li>• 50-well square tooth comb, 0.2 mm thick (PN 402053)</li> <li>• 96-lane Mylar shark's tooth comb, 0.4 mm (PN 4305385)</li> <li>• optional: 24-well square tooth comb, 0.2 mm thick (PN 401904)</li> </ul>	Applied Biosystems
GeneAmp $\text{\textsuperscript{®}}$ Thin-Walled Reaction Tubes with Caps, 0.5-mL (PN N8010737)	Applied Biosystems
MicroAmp $\text{\textsuperscript{®}}$ Caps, 8 caps/strip (PN N8010535)	Applied Biosystems
MicroAmp $\text{\textsuperscript{®}}$ 8-Strip Reaction Tubes (PN N8010580)	Applied Biosystems
MicroAmp $\text{\textsuperscript{®}}$ Optical 96-Well Reaction Plate (PN N8010560)	Applied Biosystems

**Table 4 Supplies required (*continued*)**

<b>Supplies</b>	<b>Source</b>
MicroAmp® 96-Well Full Plate Covers (PN N8010550)	Applied Biosystems
Stainless steel gel clamp (large), 1 each (PN 4305386)	Applied Biosystems
Filter units, Nalgene (150-mL, 0.2-µm CN filter)	MLS
Filter units, Nalgene (150-mL, 0.2-µm or 0.45-µm nylon filter)	MLS
Lint-free tissues or dedicated wash cloth	MLS

## Required Chemicals and Reagents

The chemicals and reagents necessary or recommended for running AmpF<sub>STR</sub> kit data on the ABI PRISM 377, 377XL, and 377-96 DNA Sequencer instruments are listed in the table below. You can order the part numbers listed below from Applied Biosystems. If no part number is listed, you can order from any major laboratory supplier (MLS).

**Table 5 Chemicals and reagents required**

<b>Equipment</b>	<b>Supplier</b>
Blue dextran “Loading Buffer” (contained in GeneScan-500 LIZ or GeneScan-500XL ROX Size Standard Kit) (PN 402055)	Applied Biosystems
GeneScan-500 LIZ Size Standard Kit (PN 4322682)	Applied Biosystems
GeneScan-500XL ROX Size Standard Kit (PN 403039)  <b>Note</b> This kit includes blue dextran loading buffer.	Applied Biosystems
Hi-Di™ Formamide, 24 mL (PN 4311320)	Applied Biosystems

Table 5 Chemicals and reagents required (*continued*)

Equipment	Supplier
Either: Matrix Standard Set DS-32 for the 377 and 310 instrument platforms (PN 4312131), or Matrix Standard Set DS-33 for the 377 and 310 instrument platforms (PN 4318159)	Applied Biosystems
TBE, 5X (see "Preparation of Required Reagents" on page 24)	Applied Biosystems
AG501 X8 ion exchange resin	BioRad
Alconox detergent	MLS
Ammonium persulfate (APS)	MLS
Disodium ethylenediaminetetraacetate, dihydrate (Na <sub>2</sub> EDTA•2H <sub>2</sub> O) (MLS)	MLS
Long Ranger, 50% stock solution (BioWhittaker Molecular Applications, PN 50610, 50611)	FMC Bioproducts
N,N,N',N'-Tetramethylethylenediamine (TEMED)	MLS
Sodium hydroxide pellets (NaOH)	MLS
Tris base (MLS)	MLS
Urea	MLS
Water, glass distilled, deionized	MLS

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# Preparing 36-cm Well-to-Read Gel Plates

**Preparing Plates** The 36-cm well-to-read gel plates must be cleaned and prepared by following the instructions below.

ABI PRISM 377 and the ABI PRISM 377-96

To prepare 36-cm well-to-read plates for the 377 and the 377-96:

1.	Clean 36-cm well-to-read gel plates with Alconox detergent using a dedicated wash cloth or lint-free tissues. To obtain the MSDS for Alconox, go to: <b><a href="http://www.jtbaker.com/asp/Catalog.asp">http://www.jtbaker.com/asp/Catalog.asp</a></b>
2.	Rinse the plates with deionized water, and allow the plates to air dry.
3.	Place the larger, unnotched plate with the center etch facing down on a covered bench top. <b>Note:</b> The narrow width is the bottom portion of the plate.
4.	Place 0.2-mm spacers on either side of the plate so that: <ul style="list-style-type: none"><li>• The notched end of the spacer is at the top of the plate</li><li>• The notch faces the center of the plate</li></ul>
5.	Place the notched, “rabbit-eared” plate over spacers. Make sure that the external side of the plate: <ul style="list-style-type: none"><li>• Is the one that was previously in contact with the silicone rubber gasket of the upper buffer chamber</li><li>• Contains etched writing</li></ul>



**To prepare 36-cm well-to-read plates for the 377 and the 377-96:**

6.	Clamp plates together with four medium binder clips (for the 377 and 377XL formats) or four large stainless steel gel clamps (for the 377-96 lane format) on each side: <ul style="list-style-type: none"> <li>Place the clamps directly over the spacers.</li> <li>Distribute the clamps for even tightening across the gel.</li> </ul>
7.	Elevate the top end of plates approximately 1.0 cm. <b>Note:</b> A pipette-tip box top can be used to elevate the plates.

**ABI PRISM 377XL**

When preparing plates for the ABI PRISM 377 analyzer with the XL Upgrade, you must silanize the plates. Treat the well-forming region of the notched (rabbit-eared) glass plate with Bind-Silane to immobilize the wells and facilitate subsequent loading of the gel by following the instructions in the table below.

**To prepare 36-cm well-to-read plates for the 377XL:**

1.	Clean 36-cm well-to-read gel plates with Alconox detergent using a dedicated wash cloth or lint-free tissues. Rinse with deionized water and air dry.  To obtain the MSDS for Alconox, go to: <b><a href="http://www.jtbaker.com/asp/Catalog.asp">http://www.jtbaker.com/asp/Catalog.asp</a></b>
2.	Place the cleaned, notched (rabbit-eared) glass plates face up on a covered benchtop.
3.	Dip the end of a cotton swab into a bottle of Bind-Silane to wet the tip.  Obtain the MSDS for Bind-Silane from the manufacturer.  <b>IMPORTANT!</b> Apply a fresh coating of Bind-Silane every time you clean gel plate.

**To prepare 36-cm well-to-read plates for the 377XL: (continued)**

4.	Transfer the Bind-Silane to the well-forming region of the notched glass plate (approximately the top 1.0 cm of the plate) by swiping the swab across the plate three times.  <b>Note:</b> Do not treat the side that had been previously in contact with the silicon rubber gasket of the upper buffer chamber.
5.	Allow the Bind-Silane to dry on the plate for 1 minute.
6.	Dry the treated region with a lint-free tissue by wiping three times using moderate pressure.
7.	Assemble the plates. Place the larger, unnotched plate with the center etch facing down on a covered bench top.  <b>Note:</b> The narrow width is the bottom portion of the plate.
8.	Place 0.2-mm spacers on either side of the plate so that: <ul style="list-style-type: none"> <li>• The notched end of the spacer is at the top of the plate</li> <li>• The notch faces the center of the plate</li> </ul>
9.	Place the notched, “rabbit-eared” plate over spacers. Make sure that the external side of the plate: <ul style="list-style-type: none"> <li>• Is the one that was previously in contact with the silicone rubber gasket of the upper buffer chamber</li> <li>• Contains etched writing</li> </ul>
10.	Clamp plates together with four medium binder clips on each side: <ul style="list-style-type: none"> <li>• Place the clamps directly over the spacers.</li> <li>• Distribute the clamps for even tightening across the gel.</li> </ul>
11.	Elevate the top end of plates approximately 1.0 cm.  <b>Note:</b> A pipette-tip box top can be used to elevate the plates.
12.	After running the gel, clean the plates using Alconox detergent. It may be necessary to scrape the gel off the notched plate where it has stuck to the Bind-Silane-treated region.

## Preparing Gels

Applied Biosystems recommends using Long Ranger<sup>®</sup> gel solutions instead of 4% acrylamide for 36-cm well-to-read gels. Long Ranger gels form better wells around the comb, facilitating loading and potentially enhancing results. The following procedure for preparing 50 mL of 5% Long Ranger/6.0 M urea gel mixture makes enough gel mixture for two 36-cm well-to-read gels.

Read the warnings listed below before proceeding.



**WARNING CHEMICAL HAZARD.** Urea is a possible mutagen. Do not breathe the dust. Urea can be harmful by inhalation, skin contact, and ingestion. Refer to the MSDS for proper protective equipment.



**WARNING CHEMICAL HAZARD.** TBE 5X buffer causes eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING CHEMICAL HAZARD.** Long Ranger gel solution contains acrylamide. Acrylamide is a neurotoxin. Avoid skin contact with Long Ranger gel solution because acrylamide can be absorbed through the skin. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.



**DANGER CHEMICAL HAZARD.** Ammonium persulfate is an oxidizer, and contact with other materials may cause a fire. Exposure causes burns to the eyes, skin, and respiratory tract. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING CHEMICAL HAZARD.** TEMED is extremely flammable, and can be very destructive to the skin, eyes, nose, and respiratory system. Keep TEMED in a tightly closed container. Avoid inhalation and contact with the skin, eyes, and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.

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**To prepare the gel mixture:**

1.	Combine the following:  Urea 18.0 g Deionized water 21.5 mL 5X TBE (Maniatis formulation)* 10.0 mL 50% Long Ranger stock solution 5.0 mL *See “Preparation of Required Reagents” on page 24.
2.	Warm the mixture in a 37 °C water bath, stirring occasionally, to dissolve the urea. Once the urea is dissolved, allow the gel mixture to equilibrate to room temperature. This gel mixture should be used within 12 h. Keep the gel mixture covered during this step to prevent evaporative loss.
3.	Filter the gel mixture using a 150-mL Nalgene filter apparatus with a 0.2 µm CN filter. Attach to a vacuum source (approximately 20 inches Hg) to pull liquid through filter. This step removes any particulates that may fluoresce or scatter light.
4.	Let stand for 5 min, swirling occasionally, with the vacuum on to degas the mixture.
5.	Turn off the vacuum, remove the top of the filter apparatus, and discard.  The filter apparatus can be rinsed thoroughly with deionized water and air-dried to be reused up to five times.
6.	Add 250 µL of freshly prepared 10% APS and 35 µL TEMED to the gel mixture. Swirl gently to mix.  Immediately proceed to “Pouring Gels” on page 21.

## Pouring Gels

Follow the steps in the table below to effectively pour gels onto gel plates. For the 96-lane upgrade follow the steps in “Pouring Gels for the 377-96” on page 22.



**WARNING CHEMICAL HAZARD.** TBE 1X buffer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### Pouring gels for the 377 or 377XL

#### To pour gels:

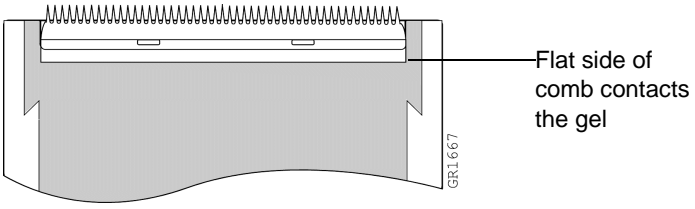
1.	Using a 35-cc syringe, draw up gel mixture.
2.	<p>Slowly inject the gel mixture into the top center area between the plates.</p> <p><b>Note:</b> Tap the gel plates firmly with your palm while pouring, to prevent bubbles and to facilitate movement of gel mixture to the bottom of the plates.</p>
3.	<p>Stop injecting when the gel mixture reaches close to the bottom of the plates, and immediately lay plates flat on a level surface.</p> <p><b>Note:</b> Each gel requires approximately 20 to 25 mL of Long Ranger gel mixture.</p>
4.	<p>Allow gel to polymerize for at least 2.0 h.</p> <p>The polymerized gel can be stored for up to 24 h at room temperature. After polymerization, place a lint-free tissue wetted with deionized water over the top and bottom edges of the gel plates. Do not remove the gel comb. Wrap the top and bottom edges tightly with clear plastic wrap and store at room temperature.</p>
5.	Prepare 1.5 L of 1X TBE (Maniatis formulation) running buffer (89 mM Tris, 89 mM borate, 2 mM EDTA).

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## Pouring Gels for the 377-96

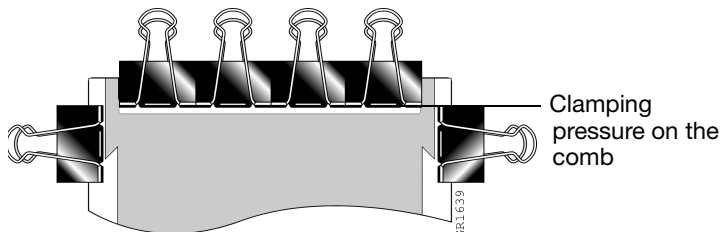
Follow the steps below to pour gels onto gel plates for the 96-lane upgrade.

### To pour gels for the 377 96-Lane upgrade:

1.	Using a 35-cc syringe, draw up gel mixture.
2.	<p>Slowly inject the gel mixture into the top center area between the plates.</p> <p><b>Note:</b> Tap the gel plates firmly with your palm while pouring, to prevent bubbles and to facilitate movement of gel mixture to the bottom of the plates.</p>
3.	<p>Stop injecting when the gel mixture reaches close to the bottom of the plates, and immediately lay plates flat on a level surface.</p> <p><b>Note:</b> Each gel requires approximately 20 to 25 mL of Long Ranger gel mixture.</p>
4.	<p>Insert a 96-lane Mylar 0.4-mm shark's-tooth comb into gel, with the flat side of the comb contacting the gel.</p>  <p>Flat side of comb contacts the gel</p> <p>GR1667</p> <p><b>Figure 1 A 96-lane Mylar 0.4-mm shark's-tooth comb</b></p>

## To pour gels for the 377 96-Lane upgrade: (continued)

5. Clamp the comb into place with four large stainless steel gel clamps for even tightening across the gel.



**Figure 2** Gel clamps on the shark's tooth comb

6. Allow the gel to polymerize for at least 2.0 h.
- The polymerized gel can be stored for up to 24 h at room temperature. After polymerization, place a lint-free tissue wetted with deionized water over the top and bottom edges of the gel plates. Do not remove the gel comb. Wrap the top and bottom edges tightly with clear plastic wrap and store at room temperature.

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Preparation of  
Required  
Reagents

0.5 M EDTA, pH 8.0



**CAUTION**

**CHEMICAL HAZARD.** EDTA may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**DANGER**

**CHEMICAL HAZARD.** Sodium hydroxide (NaOH) causes severe eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare 0.5 M EDTA, pH 8.0:

1.	Slowly add 186.1 g disodium ethylenediaminetetraacetate dihydrate ( $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ ) to 800 mL glass-distilled or deionized water.
2.	Stir vigorously on a magnetic stirrer.
3.	Adjust to $\text{pH } 8.0 \pm 0.2$ by adding NaOH pellets (approximately 20 g). <b>Note:</b> EDTA will not go into solution without pH adjustment.
4.	Adjust the final volume to 1 L with glass-distilled or deionized water.
5.	Autoclave the solution or filter it through a 0.2 $\mu\text{m}$ Nalgene filter. Store at room temperature.



**5X TBE**

**WARNING CHEMICAL HAZARD.** TBE 5X buffer causes eye, skin, and respiratory tract irritation. Exposure may cause central nervous system depression and kidney damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING CHEMICAL HAZARD.** Boric acid is a hazardous chemical that is harmful if ingested, inhaled, or absorbed through the skin and can be irritating to the eyes, respiratory system, and skin. Handling boric acid while pregnant brings possible risk to the unborn child. Prolonged or repeated exposure can potentially impair fertility. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To prepare 5X TBE (Maniatis formulation: 445 mM Tris, 445 mM borate, 10 mM EDTA):**

1.	Add approximately 900 mL of deionized water to 20 mL of 0.5 M EDTA, pH 8.0.
2.	Add 54 g Tris base and 27.5 g boric acid to the diluted EDTA solution. Stir vigorously on a magnetic stir plate.
3.	Adjust the volume to 1 L with deionized water and mix thoroughly.
4.	Filter the mixture using a 0.2 µm or 0.45 µm Nalgene filter unit to remove particulate matter and prevent formation of a precipitate.
5.	Store in a glass container to facilitate visual inspection of precipitates. If a precipitate forms, discard the 5X TBE buffer and remake it.

---

## Deionized Formamide



**WARNING CHEMICAL HAZARD.** Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and safety glasses when handling. Refer to the supplier's MSDS for more details on handling and storage.

**Note:** Hi-Di Formamide (PN 4311320) is the recommended denaturant. However, if you choose to prepare your own deionized formamide, follow the directions below.

### To prepare Deionized Formamide:

1.	Mix 50 mL of formamide and 5 g of AG501 X8 ion-exchange resin.
2.	Using a magnetic stirrer and stirbar, stir for 1–3 h at room temperature.
3.	<p>Filter the Formamide through a 150 mL Nalgene filter apparatus with a 0.2 <math>\mu</math>m nylon filter.</p> <p>Alternatively, if a filter apparatus is not available, allow the beads to settle to the bottom of the beaker. Remove the supernatant (formamide), taking care not to disturb the beads.</p> <p><b>Note:</b> The conductivity of the solution should be approximately 30 micro siemens or less.</p>
4.	Dispense the deionized formamide into aliquots of 500 $\mu$ L and store for up to 3 months at –15 to –25 °C.
5.	Use one aliquot per set of samples. Discard any unused deionized formamide.

## Filter Sets

After samples are prepared, they are run on the 377, 377XL or 377-96-lane configurations. In the following sections, directions are given for setting up the instrument for either Filter Set G5 or Filter Set F. See “Setting Up the Instrument for Filter Set G5” on page 27 or “Setting Up the Instrument for Filter Set F” on page 39.

# Setting Up the Instrument for Filter Set G5

## Setting up the Sample Sheet

A sample sheet for analyzing AmpFISTR kit PCR products is required to start the run module. The following instructions describe setting up the sample sheet for either the 377/377XL or the 377-96 instrument.

**Note:** If you are running Filter Set F, see “Setting Up the Instrument for Filter Set F” on page 39.

### 377 or 377XL Sample Sheet Setup

To prepare a sample sheet for the 377 or 377XL:

1.	Launch the Data Collection Software by selecting <b>Start</b> , then select the data collection appropriate for your 377 instrument.
2.	If a sample sheet needs to be created, select <b>File &gt; New &gt; GeneScan Sample</b> . Otherwise skip to step 1 in the procedure, “Creating Run Files for the 377, 377XL and 377-96” on page 31.
3.	Select five dyes from the sample sheet template window in the upper right corner.
4.	Enter sample names/numbers for each lane in the Sample Name column.

---

To prepare a sample sheet for the 377 or 377XL: (continued)

5.	<p>Enter the sample description.</p> <ol style="list-style-type: none"><li>Enter the sample description for each row in the Sample Info column (for Blue, Green, Yellow, and Red for each sample). This is necessary for the AmpF<math>\mathcal{L}</math>STR Genotyper Template File to build tables containing the genotypes for each sample.</li><li>Type the word <b>ladder</b> for the Blue, Green, Yellow and Red rows for the AmpF<math>\mathcal{L}</math>STR Allelic Ladder lane.</li><li>Alternatively, using the copy feature under the Edit menu, copy all sample names at one time by highlighting the “Sample Name” header and paste by highlighting the “Sample Info” header. The sample name will appear in the blue, green, yellow, red and orange sample information columns for each sample.</li></ol>
6.	<p>Be sure that the diamond symbol in the Std column indicates the orange as the standard in each lane.</p>
7.	<p>Save and close the sample sheet in the Sample Sheets folder.</p> <p><b>Note:</b> For more information on how to create and edit a sample sheet, refer to the <i>ABI PRISM<sup>®</sup> 377 DNA Sequencer User Guide</i>.</p>

**377-96 Sample Sheet Setup**

To prepare a sample sheet for the 377-96:

1.	<p>Launch the ABI PRISM 377-96 Collection Software.</p>
2.	<p>From the File menu select <b>New</b>, and click <b>GeneScan Sample</b>.</p> <p>An untitled Sample Sheet template appears.</p>
3.	<p>Select five dyes from the sample sheet template window in the upper right corner.</p>

**To prepare a sample sheet for the 377-96: (continued)**

4.	<p>Enter sample names and numbers for each lane in the Sample Name column and assign four lanes to each AmpF<math>\mathcal{L}</math>STR kit allelic ladder.</p> <ul style="list-style-type: none"> <li>Two lanes of each allelic ladder in odd-numbered lanes</li> <li>Two lanes of each allelic ladder in even-numbered lanes</li> </ul> <p><b>IMPORTANT!</b> See “Lane Assignment for the 96-Lane Upgrade” on page 30 for the gel lane assignments.</p>
5.	<p>For each sample, enter a unique sample description in the Blue, Green, Yellow, and Red rows in the Sample Info column.</p> <p>Alternatively, using the copy feature under the Edit menu, copy all sample names at one time by highlighting the “Sample Name” header and paste by highlighting the “Sample Info” header. The sample name will appear in the blue, green, yellow, red and orange sample information columns for each sample.</p> <p><b>IMPORTANT!</b> Sample descriptions are necessary for the AmpF<math>\mathcal{L}</math>STR Kit Template File to build tables containing the genotypes for each sample.</p>
6.	<p>Be sure that the diamond symbol in the Std column indicates orange as the standard in each lane.</p>
7.	<p>For each sample, verify that the diamond symbol in the Std column is shown on the orange row.</p>
8.	<p>Save the sample sheet in the Sample Sheets folder in the <i>ABI PRISM 377-96</i> folder.</p>
9.	<p>Close the sample sheet.</p>

# Lane Assignment for the 96-Lane Upgrade

The figure below shows lane assignments for 96 samples when samples are stagger loaded using a two-pitch, eight-channel loader.

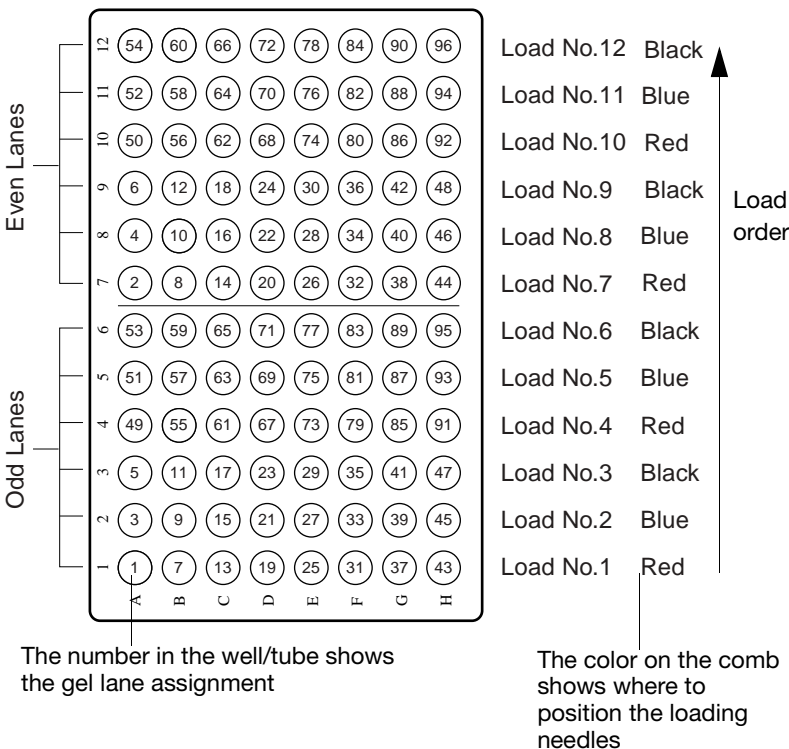


Figure 3 Lane assignments for the 96-lane upgrade

## Creating Run Files for the 377, 377XL and 377-96

To create run files:

1.	Select <b>File &gt; New</b> and click <b>GeneScan Run</b> .
2.	Select a five-dye sample sheet that has been prepared previously. The run module cannot be started without a sample sheet.  <b>Note:</b> You must select a five-dye sample sheet to view and select five-dye modules.
3.	Select <b>Plate Check Module &gt; Plate Check G5.md5</b> as shown in Figure 4.
4.	Select <b>PreRun Module &gt; GS PR 36G5-2400.md5</b> .
5.	Select <b>Run Module &gt; GS Run 36G5-2400.md5</b> .
6.	Make sure the settings are the same as shown in the following example.  Click the document icon to the right of the Run Module to verify the electrophoresis settings for the particular run.  <b>Note:</b> Select the appropriate number of lanes.

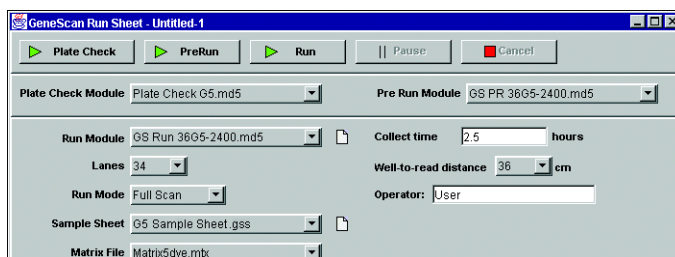


Figure 4 Filter set G5 run settings for a 34-lane gel

To create run files: *(continued)*

7.	<p>Make selections from the Lanes pop-up menu:</p> <table border="1" data-bbox="490 286 1189 581"> <thead> <tr> <th data-bbox="490 286 997 355">If using the...</th><th data-bbox="997 286 1189 355">Select...</th></tr> </thead> <tbody> <tr> <td data-bbox="490 355 997 413">ABI PRISM 377 DNA Sequencer</td><td data-bbox="997 355 1189 413">34-Lane</td></tr> <tr> <td data-bbox="490 413 997 494">ABI PRISM 377 DNA Sequencer with XL Upgrade</td><td data-bbox="997 413 1189 494">50-Lane</td></tr> <tr> <td data-bbox="490 494 997 581">ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade</td><td data-bbox="997 494 1189 581">96-Lane</td></tr> </tbody> </table>	If using the...	Select...	ABI PRISM 377 DNA Sequencer	34-Lane	ABI PRISM 377 DNA Sequencer with XL Upgrade	50-Lane	ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade	96-Lane
If using the...	Select...								
ABI PRISM 377 DNA Sequencer	34-Lane								
ABI PRISM 377 DNA Sequencer with XL Upgrade	50-Lane								
ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade	96-Lane								
8.	<p>Select the appropriate matrix file from the gel's <b>Matrix File</b> pop-up menu. The matrix file must be previously prepared and saved in the matrix folder located in the D drive so the matrix file can be applied automatically to the gel image:</p> <p><i>D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix</i></p> <p><b>Note:</b> A matrix must be selected for every run. For runs where a matrix has not yet been generated, a “<i>Bogus 5-dye matrix</i>” file has been supplied.</p> <p><b>IMPORTANT!</b> The matrix file must be one that was created using the DS-33 matrix standards (6-FAM, VIC, NED, PET, and LIZ<sup>®</sup>) and Filter Set G5 module files.</p>								
9.	<p>In addition, select the appropriate matrix file for each sample in the Sample Sheet area of the run window, so that the matrix is applied automatically to the sample files. To do so:</p> <ol style="list-style-type: none"> <li>Click the arrow in the <b>Matrix File</b> column for the first sample/lane to view the pop-up menu and select the appropriate matrix file.</li> <li>After selecting a matrix file for the first sample/lane, select the entire matrix column by clicking the column heading and selecting <b>Edit &gt; Fill Down</b>.</li> </ol>								
10.	<p>Verify settings:</p> <ol style="list-style-type: none"> <li>Verify that the collection time is 2.5 h.</li> <li>Set the well-to-read distance to 36 cm for the GS Run G5-2400 module.</li> </ol>								



To create run files: *(continued)*

11.	Make selections from the Run Mode pop-up menu:	
	<b>If using the...</b>	<b>Select...</b>
	ABI PRISM® 377 DNA Sequencer with comb size of 34 lanes	Full Scan
	ABI PRISM® 377 with XL Upgrade DNA Sequencer and comb size of 50 lanes	XL Scan
	ABI PRISM® 377 with 96-Lane Upgrade DNA Sequencer and comb size of 96 lanes	96-Lane Scan
12.	Select <b>File</b> > <b>Save</b> to save all collection settings.	

---


## Running the Plate Check on the 377 or 377XL

The user must run the plate check and prerun modules before running any samples on the ABI PRISM 377 or the ABI PRISM 377XL. Prior to the plate check, follow the steps below to correctly place the gel plates on the instrument. Then follow the steps for running the plate check. (For the 377-96, see “Running the Plate Check on the 377-96” on page 36.)



**WARNING** **CHEMICAL HAZARD.** TBE 1X buffer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To place the gel plates on the instrument:**

1.	Remove the comb.  <b>IMPORTANT!</b> Remove the comb slowly while lubricating with 1X TBE for the best results.
2.	Remove any gel that remains around the top of the wells.
3.	Clean the outside of the gel plates with deionized water and wipe dry.   <b>WARNING</b> Do not touch the plates in the laser scanning region from this point forward.
4.	Place the gel plates into the ABI PRISM 377 DNA Sequencer cassette. Place the cassette into the instrument, making sure that the lower buffer chamber is already in place.
5.	Lock the plate into place.
6.	Make sure the gel plate spacers are pressing against the two positioning pins on the instrument.
7.	Close the instrument door.

**To run the plate check for 377 or 377XL:**

1.	<p>Click the <b>Plate Check</b> button. Five colored, horizontal lines should appear in the scan window after approximately 30 sec.</p> <table border="1" data-bbox="525 348 1232 1039"> <thead> <tr> <th data-bbox="525 348 794 440">If the lines in the scan window...</th><th data-bbox="794 348 1232 440">Then...</th></tr> </thead> <tbody> <tr> <td data-bbox="525 440 794 552">are relatively flat and level across the screen</td><td data-bbox="794 440 1232 552">The plates are clean. Click <b>Cancel</b> to cancel plate check.</td></tr> <tr> <td data-bbox="525 552 794 743">show peaks</td><td data-bbox="794 552 1232 743">The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.</td></tr> <tr> <td data-bbox="525 743 794 1039">are flat but not level across the screen</td><td data-bbox="794 743 1232 1039">The cassette is not positioned properly in the instrument (<i>i.e.</i>, the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.</td></tr> </tbody> </table>	If the lines in the scan window...	Then...	are relatively flat and level across the screen	The plates are clean. Click <b>Cancel</b> to cancel plate check.	show peaks	The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.	are flat but not level across the screen	The cassette is not positioned properly in the instrument ( <i>i.e.</i> , the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.
If the lines in the scan window...	Then...								
are relatively flat and level across the screen	The plates are clean. Click <b>Cancel</b> to cancel plate check.								
show peaks	The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.								
are flat but not level across the screen	The cassette is not positioned properly in the instrument ( <i>i.e.</i> , the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.								
2.	<p>After determining that the plates are clean and positioned correctly, clamp the upper buffer chamber onto the gel plates.</p> <p><b>Note:</b> For 96-lane gels, go to “Running the Plate Check on the 377-96” on page 36.</p>								
3.	Add 1X TBE buffer to the upper buffer chamber and check for leaks.								
4.	Clamp on and connect the heat transfer plate.								

---

To run the plate check for 377 or 377XL: *(continued)*

5.	Add 1X TBE buffer to the lower buffer chamber for 34- and 50-lane gels.  <b>Optional:</b> To facilitate the subsequent loading of the gel, drop 15 µL of blue dextran loading buffer (contained in the GeneScan®-500 LIZ Size Standard Kit) over the wells in a sweeping motion using a P10 or P20 pipet tip. The dye outlines the walls and bottom of the wells.
6.	Plug in the electrodes and close the instrument door.

**Note:** Prepare samples while you prerun the gel.

## Running the Plate Check on the 377-96

The user must run the plate check and prerun modules before running any samples on the ABI PRISM 377-96. Prior to the plate check, follow the steps below to correctly place the gel plates on the instrument. Then follow the steps for running the plate check.



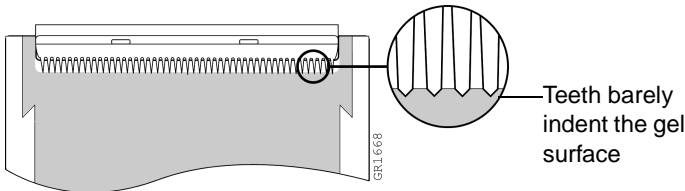
### **WARNING**

**CHEMICAL HAZARD. TBE 0.5X buffer** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### To run the plate check for the 377-96:

1.	Add 0.5X TBE buffer to the upper buffer chamber and check for leaks.
2.	Clamp on and connect the heat transfer plate.
3.	Add 0.5X TBE buffer to the lower buffer chamber.
4.	Rinse the well region thoroughly with 0.5X TBE buffer using a 0.17-mm flat pipette tip attached to a 35-cc syringe.  <b>Note:</b> It may be necessary to cut off the top end of the pipette tip with a razor blade for it to fit on the end of the luer-lock syringe without leaking. (See “Modifying 20-cc Syringes for the 96-Lanes Upgrade” on page 57.)

## To run the plate check for the 377-96: (continued)

5.	To facilitate shark's-tooth comb insertion, drop 15 $\mu$ L of blue dextran loading buffer over the well area in a sweeping motion using a pipettor with a 20- $\mu$ L pipette tip. The dye outlines the well area.
6.	Carefully insert the 96-lane Mylar shark's-tooth comb into the gel. <ol style="list-style-type: none"> <li>Align the center registration line on the comb with the registration mark on the back plate.</li> <li>Slide the comb between the plates.</li> </ol> <p><b>IMPORTANT!</b> To avoid bending or breaking the teeth of the comb, ensure that all teeth enter the space between the plates at the same time. Do not force the comb into the gel because bending the teeth may cause leaking.</p>
7.	Continue to slide the comb down until the tips of the teeth just touch or slightly depress the gel surface.  <p><b>Note:</b> If the surface of the gel is not completely flat in the loading region, insert some of the teeth below the surface of the gel (up to 0.5 mm) so that all of the teeth touch the gel surface.</p> <p>Once a tooth has penetrated the gel surface, do not attempt to withdraw the comb, because samples may leak into adjacent wells.</p>
8.	Plug in the electrodes and close the instrument door.

**Note:** Prepare samples while you prerun the gel.

---

## Performing the Prerun on the 377 or 377XL

To perform the prerun on the 377 or 377XL:

1.	Click the <b>PreRun</b> button in the Run window. Prerun the gel for approximately 15 min.
2.	Click <b>Pause</b> in the Run window to pause the prerun. Pausing stops the electrophoresis, but continues to heat the gel to 51 °C and then maintains that temperature.  Samples can be loaded onto the gel during this pause. See page 52 for sample loading information.

## Performing the Prerun on the 377-96

To perform the prerun on the 377-96:

1.	Click the <b>PreRun</b> button in the Run window.
2.	Immediately click <b>Pause</b> in the Run window to pause the prerun.  <b>Note:</b> Pausing stops the electrophoresis but allows the gel to heat to 51 °C and then maintains that temperature.
3.	From the Window menu, select <b>Status</b> , and verify that the electrophoresis power is turned off.

# Setting Up the Instrument for Filter Set F

## Setting up the Sample Sheet

A sample sheet for analyzing AmpF $\mathcal{L}$ STR kit PCR products is required to start the run module. The following instructions describe setting up the sample sheet for either the 377/377XL or the 377-96 instrument.

**Note:** If you are running Filter Set G5, see “Setting Up the Instrument for Filter Set G5” on page 27.

### 377 or 377XL Sample Sheet Setup

To prepare a sample sheet for the 377 or 377XL:

1.	Launch the Data Collection Software by selecting <b>Start</b> , then select the data collection appropriate for your 377 instrument.
2.	If a sample sheet needs to be created, select <b>File &gt; New &gt; GeneScan Sample</b> . Otherwise skip to step 1 of the next procedure, on page 43.
3.	Select four dyes from the sample sheet template window in the upper right corner.
4.	Enter sample names/numbers for each lane in the Sample Name column.
5.	Enter the sample descriptions. <ol style="list-style-type: none"> <li>Enter the sample description for each row in the Sample Info column (for Blue, Green, and Yellow for each sample). This is necessary for the AmpF<math>\mathcal{L}</math>STR Genotyper Kit Template File to build tables containing the genotypes for each sample.</li> <li>Type the word <b>ladder</b> for the Blue, Green, and Yellow rows for the AmpF<math>\mathcal{L}</math>STR Allelic Ladder lane.</li> <li>Using the copy feature under the Edit menu, copy all sample names at one time by highlighting the “Sample Name” header and paste by highlighting the “Sample Info” header. The sample name will appear in the blue, green, yellow, and red sample information columns for each sample.</li> </ol>

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**To prepare a sample sheet for the 377 or 377XL: (continued)**

6.	Be sure that the diamond symbol in the Std column indicates the red sample as the standard in each lane.
7.	Save the sample sheet in the Sample Sheets folder.
8.	Close the sample sheet.  <b>Note:</b> For more information on how to create and edit a sample sheet, refer to the <i>ABI PRISM® 377 DNA Sequencer User Guide</i> .

**377-96 Sample Sheet Setup**

**To prepare a sample sheet for the 377-96:**

1.	Launch the ABI PRISM 377-96 Collection Software.
2.	Select <b>File &gt; New</b> , then click <b>GeneScan Sample</b> . An untitled Sample Sheet template appears.
3.	Select four dyes from the sample sheet template window in the upper right corner.
4.	Enter sample names and numbers for each lane in the Sample Name column and assign four lanes for each AmpF $\mathcal{L}$ STR kit allelic ladder. <ul style="list-style-type: none"><li>• Two lanes of each allelic ladder in odd-numbered lanes</li><li>• Two lanes of each allelic ladder in even-numbered lanes</li></ul> <b>IMPORTANT!</b> See “Lane Assignment for the 96-Lane Upgrade” on page 42 for the gel lane assignments.



## To prepare a sample sheet for the 377-96: (continued)

5. For each sample, enter a unique sample description in the Blue, Green, and Yellow rows in the Sample Info column.
- IMPORTANT!** Sample descriptions are necessary for the AmpF $\mathcal{L}$ STR Kit Template File to build tables containing the genotypes for each sample.

**Note:** If you are using Profiler Plus and COfiler PCR Amplification Kits together with Genotyper<sup>®</sup> Software v3.7 and the combined COPP (COfiler and Profiler Plus) Template Files, enter the information as follows:

AmpF $\mathcal{L}$ STR Kit Used	Sample Info *	Comments
Profiler Plus PCR Amplification Kit	Unique sample description	Profiler Plus
COfiler PCR Amplification Kit		COfiler

\*For each sample, enter the same Sample Info for both kits used.

6. For each AmpF $\mathcal{L}$ STR Allelic Ladder, type **ladder** in the Blue, Green, and Yellow rows in the Sample Info column.

**Note:** If you are using the Profiler Plus and COfiler PCR Amplification Kits together with Genotyper software v3.7 and the combined COPP (COfiler and Profiler Plus) Template Files, then enter the information as follows:

AmpF $\mathcal{L}$ STR Allelic Ladder Used	Sample Info	Comments *
Profiler Plus	ladder	PPL
COfiler		COL

\*Entries are case sensitive.

Using the copy feature under the Edit menu, copy all sample names at one time by highlighting the “Sample Name” header and paste by highlighting the “Sample Info” header. The sample name will appear in the blue, green, yellow, and red sample information columns for each sample.

To prepare a sample sheet for the 377-96: (continued)

7.	Be sure the diamond symbol in the Std column indicates the red sample as the standard in each lane.
8.	Save the sample sheet in the Sample Sheets folder in the <i>ABI PRISM 377-96</i> folder.
9.	Close the sample sheet.

## Lane Assignment for the 96-Lane Upgrade

The figure below shows lane assignments for 96 samples when samples are stagger loaded using a two-pitch, eight-channel loader.

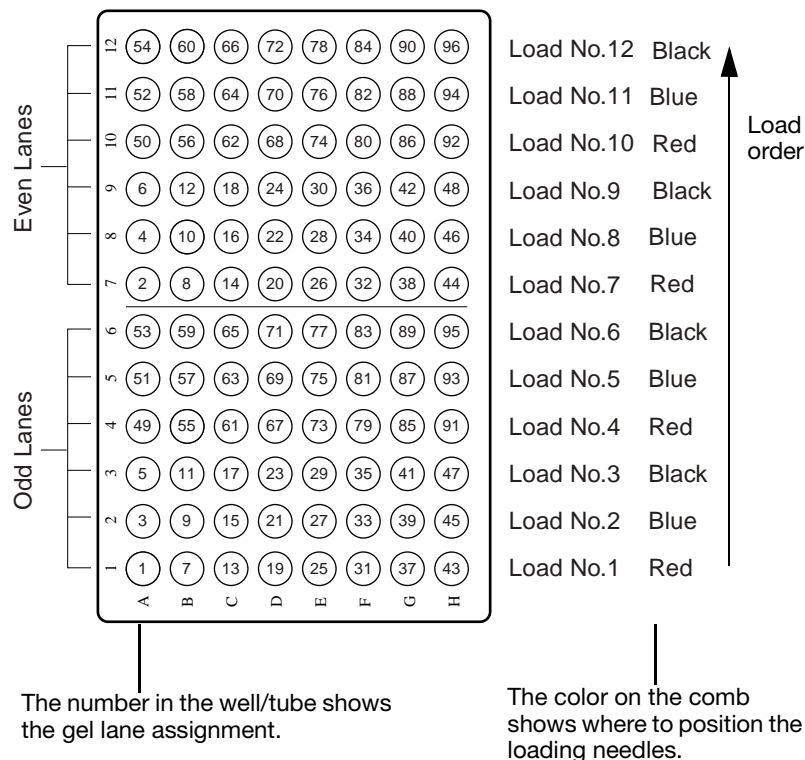


Figure 5 Lane assignments for the 96-lane upgrade

## Creating Run Files for the 377, 377XL and 377-96

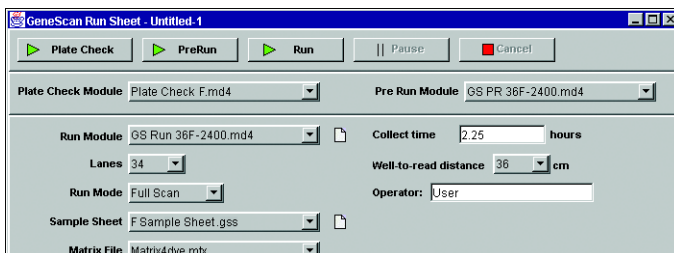
To create run files:

1. Select **File > New**, then click **GeneScan Run**.
2. Select a sample sheet that has been prepared previously. The run module cannot be started without a sample sheet.
3. Select **Plate Check Module > Plate Check F.md4** as shown in Figure 6.
4. Select **PreRun Module > GS PR 36F-2400.md4**.
5. Select **Run Module > GS Run 36F-2400.md4**.

Make sure the settings are the same as shown in the following example.

Click the document icon to the right of the Run Module to verify the electrophoresis settings for the particular run.

**Note:** Select the appropriate number of lanes.



**Figure 6** Filter Set F run settings for a 34-lane gel

7. Make selections from the Lanes pop-up menu:

If using the...	Select...
ABI PRISM® 377 DNA Sequencer	34-Lane
ABI PRISM® 377 DNA Sequencer with XL Upgrade	50-Lane
ABI PRISM® 377 DNA Sequencer with 96-Lane Upgrade	96-Lane

---

To create run files: *(continued)*

8.	<p>Select the appropriate matrix file from the gel's Matrix File pop-up menu. The matrix file must be previously prepared and saved in the matrix folder located in the D drive so the matrix file can be applied automatically to the gel image:</p> <p><i>D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix</i></p> <p><b>Note:</b> A matrix must be selected for every run. For runs where a matrix has not yet been generated, a “<i>Bogus 4-dye matrix</i>” file has been supplied.</p> <p><b>IMPORTANT!</b> The matrix file must be one that was created using the DS-32 Matrix Standards (5-FAM, JOE, NED, and ROX) and Filter Set F module files.</p>
9.	<p>In addition, select the appropriate matrix file for each sample in the Sample Sheet area of the run window. This is necessary to have the matrix applied automatically to the sample files. To do so:</p> <ol style="list-style-type: none"><li>Click the arrow in the <b>Matrix File</b> column for the first sample/lane to view the pop-up menu and select the appropriate matrix file.</li><li>After selecting a matrix file for the first sample/lane, select the entire matrix column by clicking the column heading and selecting <b>Edit &gt; Fill Down</b>.</li></ol>
10.	<p>Verify settings:</p> <ol style="list-style-type: none"><li>Verify that the collection time is 2.25 h.</li><li>Set the well-to-read distance to 36 cm for the GS Run F-2400 module.</li></ol>

## To create run files: (continued)

11.	Make selections from the Run Mode pop-up menu: <table><tr><th>If using the...</th><th>Select...</th></tr><tr><td>ABI PRISM® 377 DNA Sequencer with comb size of 34 lanes</td><td>Full Scan</td></tr><tr><td>ABI PRISM® 377 with XL Upgrade DNA Sequencer and comb size of 50 lanes</td><td>XL Scan</td></tr><tr><td>ABI PRISM® 377 with 96-Lane Upgrade DNA Sequencer and comb size of 96 lanes</td><td>96-Lane Scan</td></tr></table>	If using the...	Select...	ABI PRISM® 377 DNA Sequencer with comb size of 34 lanes	Full Scan	ABI PRISM® 377 with XL Upgrade DNA Sequencer and comb size of 50 lanes	XL Scan	ABI PRISM® 377 with 96-Lane Upgrade DNA Sequencer and comb size of 96 lanes	96-Lane Scan
If using the...	Select...								
ABI PRISM® 377 DNA Sequencer with comb size of 34 lanes	Full Scan								
ABI PRISM® 377 with XL Upgrade DNA Sequencer and comb size of 50 lanes	XL Scan								
ABI PRISM® 377 with 96-Lane Upgrade DNA Sequencer and comb size of 96 lanes	96-Lane Scan								
12.	Select <b>File</b> > <b>Save</b> to save all collection settings.								


### Running the Plate Check on the 377 or 377XL

The user must run the plate check and prerun modules before running any samples on the ABI PRISM 377 or ABI PRISM 377XL. Prior to the plate check, follow the steps below to correctly place the gel plates on the instrument. Then follow the steps for running the plate check. (For the 377-96, see “Running the Plate Check on the 377-96” on page 48.)



**WARNING** **CHEMICAL HAZARD.** TBE 1X buffer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### To place the gel plates into the instrument:

1.	Remove the comb.  <b>IMPORTANT!</b> Remove it slowly while lubricating with 1X TBE for the best results.
2.	Remove any gel that remains around the top of the wells.
3.	Clean the outside of the gel plates with deionized water and wipe dry.   <b>WARNING</b> Do not touch the plates in the laser scanning region from this point forward.

**To place the gel plates into the instrument:**

4.	Place the gel plates into the ABI PRISM 377 cassette. Place the cassette into the instrument, making sure that the lower buffer chamber is already in place.
5.	Lock the plate into place.
6.	Make sure the gel plate spacers are pressing against the two positioning pins on the instrument.
7.	Close the instrument door.

**To run the plate check for 377 or 377XL:**

1.	Click the <b>Plate Check</b> button. Four colored, horizontal lines should appear in the scan window after approximately 30 sec.								
	<table> <tr> <th>If the lines in the scan window...</th><th>Then...</th></tr> <tr> <td>are relatively flat and level across the screen</td><td>The plates are clean. Click the <b>Cancel</b> button to cancel plate check.</td></tr> <tr> <td>show peaks</td><td>The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.</td></tr> <tr> <td>are flat but not level across the screen</td><td>The cassette is not positioned properly in the instrument (<i>i.e.</i>, the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.</td></tr> </table>	If the lines in the scan window...	Then...	are relatively flat and level across the screen	The plates are clean. Click the <b>Cancel</b> button to cancel plate check.	show peaks	The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.	are flat but not level across the screen	The cassette is not positioned properly in the instrument ( <i>i.e.</i> , the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.
If the lines in the scan window...	Then...								
are relatively flat and level across the screen	The plates are clean. Click the <b>Cancel</b> button to cancel plate check.								
show peaks	The plates are not clean. 1. Pause the plate check, remove the cassette, and clean the plates again. 2. Resume plate check.								
are flat but not level across the screen	The cassette is not positioned properly in the instrument ( <i>i.e.</i> , the plates are not flush against the back heat transfer plate and/or the two positioning pins that set the plates the correct distance from the optics). 1. Pause the plate check and reposition the cassette. 2. Resume the plate check.								

**To run the plate check for 377 or 377XL: (continued)**

2.	After determining that the plates are clean and positioned correctly, clamp the upper buffer chamber onto the gel plates.  <b>Note:</b> For 96-lane gels, go to “Running the Plate Check on the 377-96” on page 48.
3.	Add 1X TBE buffer to the upper buffer chamber and check for leaks.
4.	Clamp on and connect the heat transfer plate.
5.	Add 1X TBE buffer to the lower buffer chamber for 34- and 50-lane gels.  <b>Optional:</b> To facilitate the subsequent loading of the gel, drop 15 µL of blue dextran loading buffer (contained in the GeneScan <sup>®</sup> -500 ROX Size Standard Kit) over the wells in a sweeping motion using a P10 or P20 pipet tip. The dye outlines the walls and bottom of the wells.
6.	Plug in the electrodes and close the instrument door.

**Note:** Prepare samples while you prerun the gel.

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## Running the Plate Check on the 377-96

The user must run the plate check and prerun modules before running any samples on the ABI PRISM 377-96. Prior to the plate check, follow the steps below to correctly place the gel plates on the instrument. Then follow the steps for running the plate check.



### **WARNING** CHEMICAL HAZARD. TBE 0.5X buffer

causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

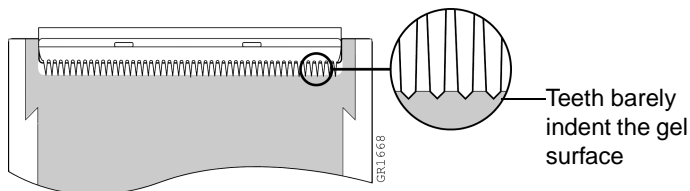
#### To run the plate check for 377-96:

1.	Add 0.5X TBE buffer to the upper buffer chamber and check for leaks.
2.	Clamp on and connect the heat transfer plate.
3.	Add 0.5X TBE buffer to the lower buffer chamber.
4.	<p>Rinse the well region thoroughly with 0.5X TBE buffer using a 0.17-mm flat pipette tip attached to a 35-cc syringe.</p> <p><b>Note:</b> It may be necessary to cut off the top end of the pipette tip with a razor blade for it to fit on the end of the luer-lock syringe without leaking. (See “Modifying 20-cc Syringes for the 96-Lanes Upgrade” on page 57.)</p>
5.	<p>To facilitate shark’s-tooth comb insertion, drop 15 <math>\mu</math>L of blue dextran loading buffer over the well area in a sweeping motion using a pipettor with a 20-<math>\mu</math>L pipette tip.</p> <p>The dye outlines the well area.</p>
6.	<p>Carefully insert the 96-lane Mylar shark’s-tooth comb into the gel.</p> <ul style="list-style-type: none"><li>a. Align the center registration line on the comb with the registration mark on the back plate.</li><li>b. Slide the comb between the plates.</li></ul> <p><b>IMPORTANT!</b> To avoid bending or breaking the teeth of the comb, ensure that all teeth enter the space between the plates at the same time. Do not force the comb into the gel because bending the teeth may cause leaking.</p>



## To run the plate check for 377-96: (continued)

7. Continue to slide the comb down until the tips of the teeth just touch or slightly depress the gel surface.



**Figure 7** 96-lane comb teeth indent the gel surface

**Note:** If the surface of the gel is not completely flat in the loading region, insert some of the teeth below the surface of the gel (up to 0.5 mm) so that all of the teeth touch the gel surface.

Once a tooth has penetrated the gel surface, do not attempt to withdraw the comb, because samples may leak into adjacent wells.

8. Plug in the electrodes and close the instrument door.

**Note:** Prepare samples while you prerun the gel.

---

**Perform the  
Prerun on the 377  
or 377XL**

**To perform the prerun on the 377 or 377XL:**

1.	Click the <b>PreRun</b> button in the Run window. Prerun the gel for approximately 15 min.
2.	After 15 min, click <b>Pause</b> in the Run window to pause the prerun. Pausing stops the electrophoresis, but continues to heat the gel to 51 °C and then maintains that temperature.  Samples can be loaded onto the gel during this pause. See page 52 for sample loading information.

**Performing the  
Prerun on the  
377-96**


**To perform prerun on the 377-96:**

1.	Click the <b>PreRun</b> button in the Run window.
2.	Immediately click <b>Pause</b> in the Run window to pause the prerun.  <b>Note:</b> Pausing stops the electrophoresis but allows the gel to heat to 51 °C and then maintains that temperature.
3.	From the Window menu, select <b>Status</b> , and verify that the electrophoresis power is turned off.

# Electrophoresis

## Preparing Samples and AmpF $\ell$ STR Allelic Ladder

To prepare samples and allelic ladder:

1.	<p>Prepare formamide loading solution (FLS) by combining:</p> <ul style="list-style-type: none"> <li>• Blue dextran “Loading Buffer” (contained in GeneScan-500 LIZ or GeneScan-500 ROX Size Standard Kit or GeneScan-500XL ROX Size Standard Kit), 100 <math>\mu</math>L</li> <li>• Deionized formamide, 500 <math>\mu</math>L (Refer to “Deionized Formamide” on page 26.)</li> </ul> <p> <b>WARNING</b> <b>CHEMICAL HAZARD. Formamide</b> is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and safety glasses when handling.</p>
2.	Vortex to mix. FLS can be stored at 2–6 °C for up to 2 weeks.
3.	<p>Combine the necessary amount of FLS and GeneScan-500 Size Standard in a single microcentrifuge tube as shown:</p> <ul style="list-style-type: none"> <li>• (Number of samples) <math>\times</math> 5.0 <math>\mu</math>L FLS denaturant</li> <li>• (Number of samples) <math>\times</math> 0.55 <math>\mu</math>L GeneScan-500 Size Standard</li> </ul> <p><b>Note:</b> The above formulation provides a slight overfill to allow for volume lost in pipetting. Be sure to include the appropriate number of lanes of AmpF<math>\ell</math>STR Allelic Ladder per gel in the calculations.</p>
4.	Vortex the tube to mix and spin briefly in a microcentrifuge.
5.	Aliquot 5.0 $\mu$ L of FLS/GeneScan-500 mixture into 0.5-mL GeneAmp $^{\text{®}}$ Thin-walled Reaction Tubes with caps (for use in the DNA Thermal Cycler 480) or 0.2-mL MicroAmp $^{\text{®}}$ tubes with caps (for use in the GeneAmp PCR Instrument System 2400, 9600, or 9700). Label one tube per sample.

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To prepare samples and allelic ladder: *(continued)*

6.	<p><b>For 34- and 50-lane gels:</b></p> <p>Add 4.0 µL PCR product or AmpF<math>\mathcal{L}</math>STR Allelic Ladder per tube and close the caps.</p> <p><b>For 96-lane gels:</b></p> <p>Add 5.0 µL PCR product or AmpF<math>\mathcal{L}</math>STR Allelic Ladder per tube and close the caps.</p> <p><b>Note:</b> You may need to increase the amount of allelic ladder to achieve greater signal intensity.</p>
7.	<p>Heat samples in a thermal cycler for 2 min at 95 °C to denature them.</p>
8.	<p>Chill for at least 3 min in an ice-water bath. Keep on ice until ready to load. Proceed to either “Loading Samples on the 377 or 377XL” on page 52 or “Loading Samples on the 377-96” on page 55.</p>

### Loading Samples on the 377 or 377XL

Use the following instructions to correctly load samples on the 377 or 377XL instrument. For the 377-96, see “Loading Samples on the 377-96” on page 55.



**WARNING** **CHEMICAL HAZARD.** TBE 0.5X buffer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING** **CHEMICAL HAZARD.** Urea may cause eye, skin and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING** **CHEMICAL WASTE HAZARD.** Dispose of it in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

**To load samples on the 377 or 377XL:**

1.	<p>Rinse the urea out of each sample well with 0.5X TBE, using a 0.17-mm flat pipet tip attached to a 35-cc syringe.</p> <p><b>Note:</b> It may be necessary to cut off the top end of the pipet tip with a razor blade for it to fit on the end of the Luer lock syringe without leaking.</p>								
2.	<p>From the Window menu, select <b>Status</b> to verify that the gel has heated to 51 °C.</p> <p><b>IMPORTANT!</b> The samples should be loaded very shortly after the gel heats to 51 °C.</p>								
3.	<p>Access the sample wells.</p> <ol style="list-style-type: none"> <li>Open the instrument door.</li> <li>Remove the upper buffer chamber lid.</li> </ol>								
4.	<p>Load the sample for the appropriate instrument as shown in the table below:</p> <table border="1"> <thead> <tr> <th>If using the...</th><th>Then...</th></tr> </thead> <tbody> <tr> <td>ABI PRISM® 377 DNA Sequencer</td><td>Load 1.5 µL of denatured sample or allelic ladder per well. Load the AmpF<math>\zeta</math>STR Allelic Ladder into at least two wells on the gel.</td></tr> <tr> <td>ABI PRISM® 377 DNA Sequencer with XL Upgrade</td><td>Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF<math>\zeta</math>STR Allelic Ladder into at least two wells on the gel.</td></tr> <tr> <td>ABI PRISM® 377 DNA Sequencer with 96-Lane Upgrade</td><td>Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF<math>\zeta</math>STR Allelic Ladder into at least two wells on the gel.</td></tr> </tbody> </table>	If using the...	Then...	ABI PRISM® 377 DNA Sequencer	Load 1.5 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.	ABI PRISM® 377 DNA Sequencer with XL Upgrade	Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.	ABI PRISM® 377 DNA Sequencer with 96-Lane Upgrade	Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.
If using the...	Then...								
ABI PRISM® 377 DNA Sequencer	Load 1.5 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.								
ABI PRISM® 377 DNA Sequencer with XL Upgrade	Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.								
ABI PRISM® 377 DNA Sequencer with 96-Lane Upgrade	Load 1.0 µL of denatured sample or allelic ladder per well. Load the AmpF $\zeta$ STR Allelic Ladder into at least two wells on the gel.								

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To load samples on the 377 or 377XL: *(continued)*

5.	Prepare the instrument for electrophoresis. <ul style="list-style-type: none"><li>a. Attach the upper buffer chamber lid, and plug in the cathode.</li><li>b. Close the instrument door.</li></ul> Wait 30 sec before proceeding.
6.	Run samples into the gel. <ul style="list-style-type: none"><li>a. Click the <b>PreRun</b> button in the Run window.</li><li>b. After 90 sec, click <b>Cancel</b> to stop the prerun.</li></ul> Proceed to “Sample Electrophoresis for the 377 or 377XL.”

**Sample  
Electrophoresis  
for the 377 or  
377XL**

To run sample electrophoresis:

1.	Select <b>Run</b> to start gel electrophoresis.
2.	Name the <b>Run</b> file and the <b>Gel</b> file, and click <b>Save</b> .
3.	Select <b>Window &gt; Status</b> to check that the run settings are correct and to monitor the run.  <b>Note:</b> Real time data collection can be viewed at any time during the run by selecting either Scan or Gel Image from the Window menu.  <b>IMPORTANT!</b> The Gel Image window should not be left open for long periods of time during the run, as it occupies a large block of memory.  Proceed to “Analyzing the Data” on page 59.

## Loading Samples on the 377-96

When loading samples on the 377-96:

- Use a two-pitch, eight-channel loader.
- Load the samples in a staggered format, odd lanes first.
- Refer to the comb color coding to locate odd-lane loading positions.



**WARNING CHEMICAL HAZARD. TBE 0.5X buffer** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



**WARNING CHEMICAL HAZARD. Urea** may cause eye, skin and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To load samples on the 377-96:

1.	From the Window menu, select <b>Status</b> to verify that the gel has heated to 51 °C.  <b>IMPORTANT!</b> The samples should be loaded very shortly after the gel heats to 51 °C.
2.	Access the sample wells. <ol style="list-style-type: none"> <li>Open the instrument door.</li> <li>Remove the upper buffer chamber lid.</li> </ol>
3.	Using a modified 20-cc syringe, rinse the urea out of each sample well with 0.5X TBE. (See “Modifying 20-cc Syringes for the 96-Lanes Upgrade” on page 57.)
4.	Load the odd lanes using a two-pitch, eight-channel loader. <ol style="list-style-type: none"> <li>Position the needles close to the bottom of the wells (at the gel surface).</li> <li>Very slowly dispense 1 µL of each sample or AmpF<math>\mathcal{L}</math>STR Allelic Ladder into the wells, withdrawing the needles as the samples are expelled.</li> </ol> <p><b>Note:</b> Load each AmpF<math>\mathcal{L}</math>STR Allelic Ladder into any two odd-numbered lanes.</p>

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To load samples on the 377-96: *(continued)*

5.	<p>Prepare the instrument for electrophoresis.</p> <ol style="list-style-type: none"><li>Attach the upper buffer chamber lid, and plug in the cathode.</li><li>Close the instrument door.</li></ol> <p><b>IMPORTANT!</b> Wait 30 sec before proceeding.</p>
6.	<p>Run samples into the gel.</p> <ol style="list-style-type: none"><li>Click the <b>PreRun</b> button in the Run window.</li><li>After 120 sec, click <b>Pause</b> in the Run window.</li></ol>
7.	<p>Access the sample wells.</p> <ol style="list-style-type: none"><li>Open the instrument door.</li><li>Remove the upper buffer chamber lid.</li></ol>
8.	<p>Load the even lanes using a two-pitch, eight-channel loader.</p> <ol style="list-style-type: none"><li>Position the needles close to the bottom of the well (at the gel surface).</li><li>Very slowly dispense 1 µL of each sample or AmpF<math>\mathcal{L}</math>STR Allelic Ladder into the wells, withdrawing the needles as the samples are expelled.</li></ol> <p><b>Note:</b> Load each AmpF<math>\mathcal{L}</math>STR Allelic Ladder into any two even-numbered lanes.</p>
9.	<p>Prepare the instrument for electrophoresis.</p> <ol style="list-style-type: none"><li>Attach the upper buffer chamber lid.</li><li>Close the instrument door.</li></ol> <p><b>IMPORTANT!</b> Wait 30 sec before proceeding.</p>
10.	<p>Run samples into the gel.</p> <ol style="list-style-type: none"><li>Click the <b>PreRun</b> button in the <b>Run</b> window.</li><li>After 90 sec, click <b>Cancel</b> to stop the prerun.</li></ol>



## Modifying 20-cc Syringes for the 96-Lanes Upgrade

To modify a 20-cc syringe, you need the following materials:

- 20-cc syringe
- Tack

Needle, 0.01-in., 0.25 mm point (Kloehn)

To modify a 20 cc syringe:

1.	Remove the cap from a 20-cc syringe.
2.	Using a tack, poke a small hole in the center of the tip of the cap.
3.	Carefully remove the white cap from the base of a 0.01-in. needle.
4.	Insert the 0.01-in. needle through the hole in the 20-cc syringe cap.
5.	Aspirate buffer solution with the 20-cc syringe.
6.	Firmly attach the modified cap and needle to the syringe.
7.	Apply pressure to the plunger to ensure the seal is tight and there are no leaks.

---

**Sample  
Electrophoresis  
for 377-96**

To run electrophoresis for 96-lane upgrade:

1.	Select <b>Run</b> to start gel electrophoresis.
2.	Name the <b>Run</b> file and the <b>Gel</b> file, and click <b>Save</b> .
3.	<p>Select <b>Window &gt; Status</b> to check that the run settings are correct and to monitor the run.</p> <p><b>Note:</b> Real time data collection can be viewed at any time during the run by selecting either Scan or Gel Image from the Window menu.</p> <p><b>IMPORTANT!</b> The Gel Image window should not be left open for long periods of time during the run, as it occupies a large block of memory.</p> <p>Proceed to “Analyzing the Data” on page 59.</p>

# Analyzing the Data

## Analysis of Filter Set Data

Analyze the data using the Gel Processor 1.0 software. There are separate procedures for Filter Set G5 and Filter Set F. Follow the instructions below for each set.

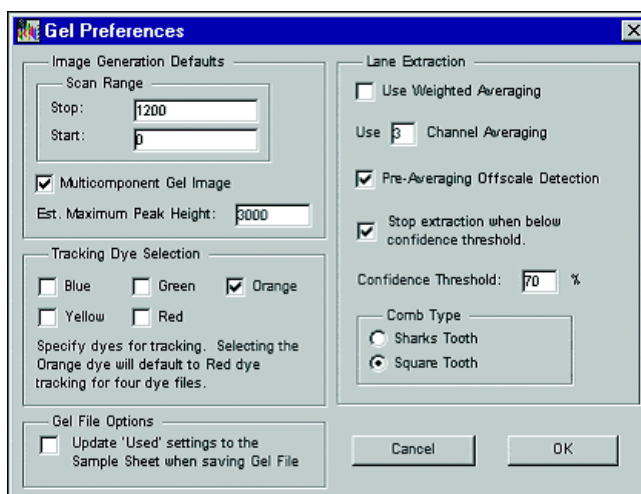
**Note:** For more information on data analysis, refer to the *ABI PRISM® 377 Gel Processor Software User Guide*.

### Filter Set G5

To set up the gel preferences for Filter Set G5:

1. Launch the Gel Processor 1.0 Software by selecting **Start > Applied Biosystems**.
2. Select **Edit > Gel Preferences**.
3. Fill in the dialog box with the settings shown in the following example.

**Note:** Choose the appropriate comb type.



**Figure 8** Gel Preferences dialog box

**Note:** You should extract lanes from the full scan range of gel. Primer peaks can be excluded using GeneScan software, not the gel processor.

To set up the gel preferences for Filter Set G5: *(continued)*

4.	Click <b>OK</b> when done.
5.	Proceed to set up for analysis parameters.

To set up the analysis parameters for Filter Set G5:

1.	Launch the GeneScan Analysis Software v3.7.1 by selecting <b>Start</b> .						
2.	<p>Select <b>Settings &gt; Analysis Parameters</b>.</p> <p><b>Note:</b> A more detailed discussion for each of the six analysis parameters is in the <i>ABI PRISM® GeneScan® Analysis Software Version 3.7 User Guide</i>.</p>						
3.	<p>Enter parameters in the dialog box and click OK when you have set the parameters properly.</p> <table border="1"> <thead> <tr> <th>Parameter</th><th>Entry</th></tr> </thead> <tbody> <tr> <td>Analysis Range</td><td> <p>1. Click the <b>This Range (Data Points)</b> radio button.</p> <p>2. Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 450 bp size standard peak.</p> <p>3. Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</p> </td></tr> <tr> <td>Smooth Options</td><td> <p>The default parameter for Smooth Options is light.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software V3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.</p> </td></tr> </tbody> </table>	Parameter	Entry	Analysis Range	<p>1. Click the <b>This Range (Data Points)</b> radio button.</p> <p>2. Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 450 bp size standard peak.</p> <p>3. Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</p>	Smooth Options	<p>The default parameter for Smooth Options is light.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software V3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.</p>
Parameter	Entry						
Analysis Range	<p>1. Click the <b>This Range (Data Points)</b> radio button.</p> <p>2. Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 450 bp size standard peak.</p> <p>3. Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</p>						
Smooth Options	<p>The default parameter for Smooth Options is light.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software V3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.</p>						

## To set up the analysis parameters for Filter Set G5: (continued)

3.	(continued)
Parameter	Entry
Peak Detection	<ol style="list-style-type: none"> <li>1. Select a Peak Amplitude Threshold (PAT) for each dye color.</li> <li>2. Use the active scroll bar to enter the PATs for each of the five colors.</li> <li>3. After analysis, the GeneScan table contains data for all peaks with a height above that specified by the PAT.</li> </ol> <p><b>Note:</b> We suggest that you determine the PATs appropriate for your analysis. Sensitivity experiments should be conducted in your laboratory with each instrument to evaluate the PATs used for analysis.</p>
Min. Peak Half Width	<p>The default parameter for Min. Peak Half Width is 2 Pts.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for Min. Peak Half Width.</p>
Polynomial Degree	<p>The default parameter for polynomial degree is 3.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for the polynomial degree.</p>
Peak Window Size	<p>The default parameter for peak window size is 15.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for Peak Window Size.</p>

To set up the analysis parameters for Filter Set G5: *(continued)*

3.	<i>(continued)</i>	
	<b>Parameter</b>	<b>Entry</b>
	Slope Threshold	The default parameter for slope threshold for peak start and peak end should be 0.0.  Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for slope threshold.
	Size Call Range	Click the <b>This Range (Base Pairs)</b> radio button and enter the values of <b>75</b> for Min and <b>450</b> for Max.
	Size Calling Method	Click the <b>Local Southern Method</b> radio button for sizing of the AmpFtSTR products. This method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.
	Baselining	The default setting for the baseline window size is 51 pts.  Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for the baseline window size.
	Auto Analysis Only	Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information.
4.	Click <b>OK</b> when done.	
5.	Proceed to using the Gel Processor Software.	

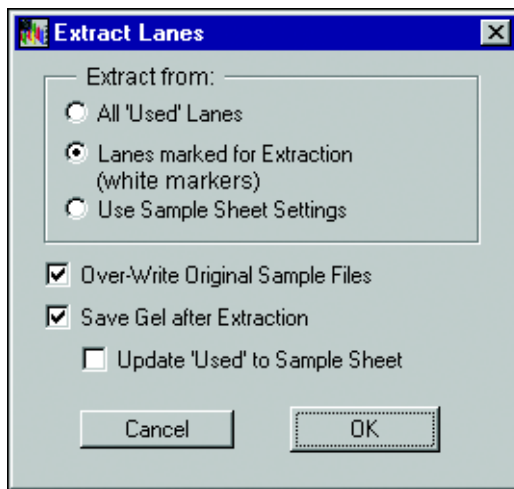
**To use gel processor software for Filter Set G5:**

1.	For filter set G5, examine the orange GeneScan-500 LIZ Size Standard bands on the gel picture and check that the 75-bp and 450-bp bands are present.
2.	<p>Verify the automatic lane tracking. It may be necessary to adjust the gel contrast for blue, green, yellow, red and orange to see all of the bands clearly. Zoom in on the gel image by selecting <b>View &gt; Zoom In</b>.</p> <ul style="list-style-type: none"><li>a. Move the lane tracking line over each sample lane, using the arrow keys on the computer keyboard or the mouse. Change the channel and introduce nodes as necessary. When modifications are made to a lane, the associated diamond lane marker turns from blue to white.</li><li>b. Mark all used lanes for extraction from the Gel menu (white diamond lane marker) before proceeding to step c.</li><li>c. Save the changes that have been made by selecting <b>File &gt; Save</b>.</li></ul>

---

To use gel processor software for Filter Set G5: *(continued)*

3. Select **Control > Extract Lanes**, to create the sample files. Make selections in the dialog box as shown in the following example.



**Figure 9** Extract Lanes dialog box

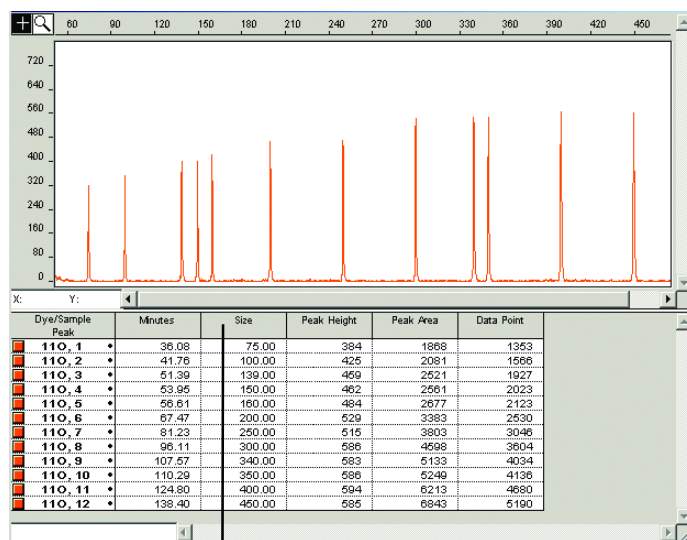
4. Click **OK** when done.
5. Open the GeneScan 3.7.1 software.
6. Create a new project and import the sample files.



## To use gel processor software for Filter Set G5: (continued)

7. Assign a size standard:
  - a. Ctrl-click the orange box column in the Analysis Control window to indicate **Orange** as the standard (a diamond symbol should appear in the orange boxes).
  - b. Click the arrow in the **Size Standard** box for the first sample to view the pop-up menu and select **Define New**, or select a correct standard already stored.

For more information on defining a size standard, refer to the *ABI PRISM® GeneScan® Analysis Software Version 3.7 User Guide*. Refer to the figure below for the sizes of the peaks in the GeneScan-500 LIZ Size Standard. The following example shows the markers from 75–450 bp. Tabular data is shown below the electropherogram.



Click here to select the Size column

**Figure 10** GeneScan-500 LIZ Size Standard

- c. To apply one size standard to all lanes, select the **Size Standard** column.
- d. Copy the size standard to other rows by using **Ctrl+C**, then **Ctrl+V** to paste.

---

To use gel processor software for Filter Set G5: *(continued)*

8.	<p>Analyze the sample files:</p> <ol style="list-style-type: none"><li>Highlight the blue, green, yellow, red and orange columns.</li><li>Click the <b>Analyze</b> button.</li></ol> <p>This action calculates the base pair size of each fragment and fills the sample files with the analyzed data from each lane.</p>
9.	<p>After the analysis is complete, confirm that the sizes for the peaks in the GeneScan-500 LIZ Size Standard have been correctly assigned.</p> <ol style="list-style-type: none"><li>Select <b>Window &gt; Results Control</b> and examine the orange GeneScan-500 peaks in overlapping groups (Quick Tile Off). Use Ctrl+ to enlarge the view window for more careful examination; use Ctrl– to reduce it to full range. Be sure to select <b>View &gt; Align By Size</b>.</li><li>Scroll through the tables to verify correct peak assignments (see Figure 10 on page 65). Check the remaining lanes, taking note of which lanes (if any) have incorrect peak assignments.</li><li>If the size standard peak assignments are incorrect in one lane, define a new size standard for that sample using the peaks in that lane. To do so, select the <b>Define New</b> option in the Size Standard row for that sample (see step 7b).</li><li>Reanalyze any incorrectly sized lanes (select the blue, green, yellow, red, and orange boxes) using the newly defined GeneScan-500 LIZ Size Standard file for that lane. Reanalyzing creates a new standard file for each of these lanes, replacing the previous analysis results for those lanes only.</li><li>Confirm that the GeneScan-500 LIZ Size Standard peaks are now correctly assigned in the reanalyzed lanes.</li></ol>

## To use gel processor software for Filter Set G5: (continued)

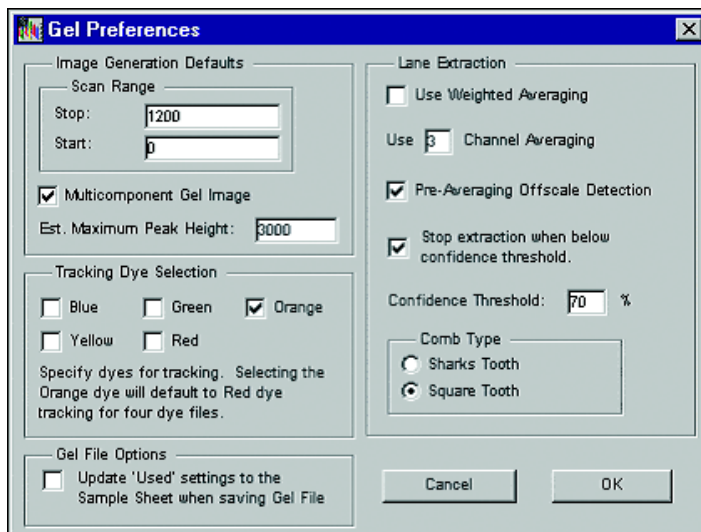
10. View the AmpF $\mathcal{L}$ STR kit results (using the Results Control window) and print. Refer to the *ABI PRISM $\mathcal{R}$  GeneScan $\mathcal{R}$  Analysis Software Version 3.7 User Guide* for printing options.

## Filter Set F

## To set up the gel preferences for Filter Set F:

1. Launch the Gel Processor 1.0 Software by selecting **Start > Applied Biosystems**.
2. Select **Edit > Gel Preferences**.
3. Fill in the dialog box with the settings shown in the following example.

**Note:** Choose the appropriate comb type.



**Figure 11** Gel Preferences dialog box

**Note:** You should extract lanes from the full scan range of gel. Primer peaks can be excluded using GeneScan software not the gel processor.

---

To set up the gel preferences for Filter Set F: *(continued)*

4.	Click <b>OK</b> when done.
5.	Proceed to set up for analysis parameters.

## To set up the analysis parameters for Filter Set F:

1.	Launch the GeneScan Analysis Software v3.7.1 by selecting <b>Start</b> .						
2.	Select <b>Settings &gt; Analysis Parameters</b> .  <b>Note:</b> A more detailed discussion for each of the six analysis parameters is in the <i>ABI PRISM® GeneScan® Analysis Software Version 3.7 User Guide</i> .						
3.	Enter parameters in the dialog box: <table border="1"> <thead> <tr> <th>Parameter</th><th>Entry</th></tr> </thead> <tbody> <tr> <td>Analysis Range</td><td> <ol style="list-style-type: none"> <li>Click the <b>This Range (Data Points)</b> radio button.</li> <li>Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 400 bp size standard peak.</li> <li>Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</li> </ol> </td></tr> <tr> <td>Smooth Options</td><td>           The default parameter for Smooth Options is light.             Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.         </td></tr> </tbody> </table>	Parameter	Entry	Analysis Range	<ol style="list-style-type: none"> <li>Click the <b>This Range (Data Points)</b> radio button.</li> <li>Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 400 bp size standard peak.</li> <li>Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</li> </ol>	Smooth Options	The default parameter for Smooth Options is light.  Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.
Parameter	Entry						
Analysis Range	<ol style="list-style-type: none"> <li>Click the <b>This Range (Data Points)</b> radio button.</li> <li>Enter Start and Stop data point numbers in the entry fields. Select the <b>Start</b> data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the <b>Stop</b> data point just after the last peak of interest, the 400 bp size standard peak.</li> <li>Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display.</li> </ol>						
Smooth Options	The default parameter for Smooth Options is light.  Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for smooth options.						

To set up the analysis parameters for Filter Set F: *(continued)*

3.	<i>(continued)</i>
Parameter	Entry
Peak Detection	<ol style="list-style-type: none"> <li>1. Select a Peak Amplitude Threshold (PAT) for each dye color.</li> <li>2. Use the active scroll bar to enter the PATs for each of the four colors.</li> <li>3. After analysis, the GeneScan table contains data for all peaks with a height above that specified by the PAT.</li> </ol> <p><b>Note:</b> We suggest that you determine the PATs appropriate for your analysis. Sensitivity experiments should be conducted in your laboratory with each instrument to evaluate the PATs used for analysis.</p>
Min. Peak Half Width	<p>The default parameter for Min. Peak Half Width is 2 Pts.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for Min. Peak Half Width.</p>
Polynomial Degree	<p>The default parameter for polynomial degree is 3.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for the polynomial degree.</p>

To set up the analysis parameters for Filter Set F: *(continued)*

3.	<i>(continued)</i>
Parameter	Entry
Peak Window Size	<p>The default parameter for peak window size is 15.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information on how to set the appropriate value for Peak Window Size.</p>
Slope Threshold	<p>The default parameter for slope threshold for peak start and peak end should be 0.0.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> (PN 4335617) for more information on how to set the appropriate value for slope threshold.</p>
Size Call Range	<p>Click the <b>This Range (Base Pairs)</b> radio button and enter the values of <b>75</b> for Min and <b>400</b> for Max.</p>
Size Calling Method	<p>Click the <b>Local Southern Method</b> radio button for sizing of the AmpF<math>\mathcal{L}</math>STR products. This method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.</p>
Baselining	<p>The default setting for the baseline window size is 51 pts.</p> <p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> (PN 4335617) for more information on how to set the appropriate value for the baseline window size.</p>
Auto Analysis Only	<p>Refer to the <i>ABI PRISM® GeneScan Analysis Software Version 3.7.1 User Bulletin</i> for more information.</p>

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To set up the analysis parameters for Filter Set F: *(continued)*

4.	Click <b>OK</b> when done.
5.	Proceed to using the Gel Processor Software for Filter Set F.

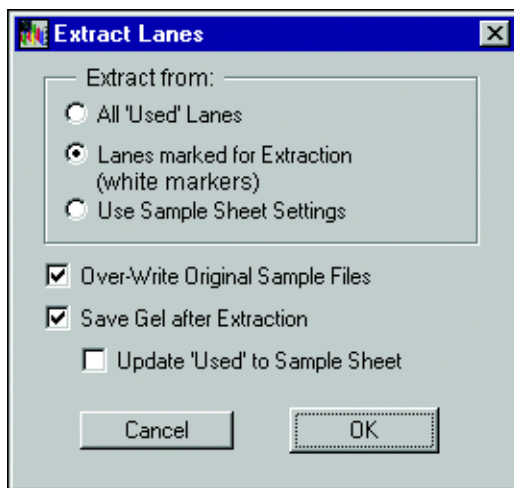
To use gel processor software for Filter Set F:

1.	For filter set F, examine the red GeneScan-500 ROX Size Standard bands on the gel picture and check that the 75-bp and 400-bp bands are present.
2.	<p>Verify the automatic lane tracking. It may be necessary to adjust the gel contrast for blue, green, yellow and red to see all of the bands clearly. Zoom in on the gel image by selecting <b>View &gt; Zoom In</b>.</p> <ul style="list-style-type: none"><li>a. Move the lane tracking line over each sample lane, using the arrow keys on the computer keyboard or the mouse. Change the channel and introduce nodes as necessary. When modifications are made to a lane, the associated diamond lane marker turns from blue to white.</li><li>b. Mark all used lanes for extraction from the Gel menu (white diamond lane marker) before proceeding to step c.</li><li>c. Save the changes that have been made by selecting <b>File &gt; Save</b>.</li></ul>



To use gel processor software for Filter Set F: *(continued)*

3. Select **Control > Extract Lanes**, to create the sample files. Make selections in the dialog box as shown in the following example.



**Figure 12** Extract Lanes dialog box

4. Click **OK** when done.
5. Open the GeneScan 3.7.1 software.
6. Create a new project and import the sample files.

To use gel processor software for Filter Set F: (continued)

7. Assign a size standard:
  - a. Ctrl-click the red box column in the Analysis Control window to indicate **Red** as the standard (a diamond symbol should appear in the red boxes).
  - b. Click the arrow in the **Size Standard** box for the first sample to view the pop-up menu and select **Define New**, or select a correct standard already stored.

For more information on defining a size standard, refer to the *ABI PRISM® GeneScan® Analysis Software Version 3.7 User Guide*. Refer to the figure below for the sizes of the peaks in the GeneScan-500 ROX Size Standard. The example below shows the markers from 75–400 bp. Tabular data is shown below the electropherogram.

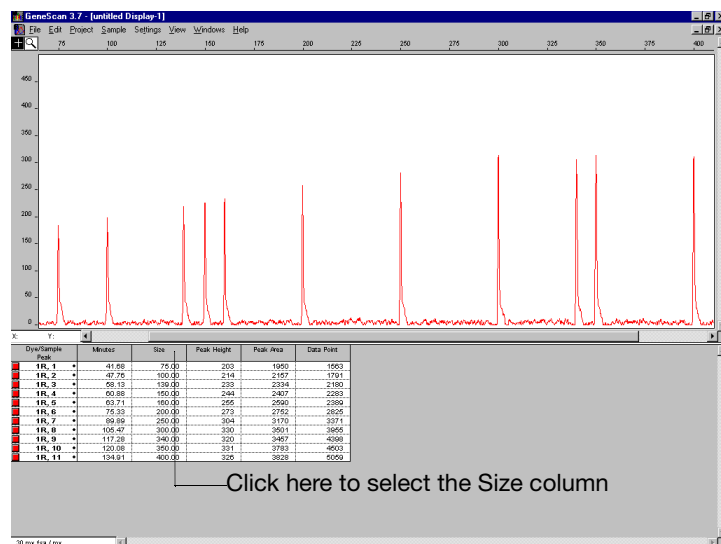


Figure 13 GeneScan-500 ROX size standard

- c. To apply one size standard to all lanes, select the **Size Standard** column.
- d. Copy the size standard to other rows by using **Ctrl+C**, then **Ctrl+V** to paste.

## To use gel processor software for Filter Set F: (continued)

8.	<p>Analyze the sample files:</p> <ol style="list-style-type: none"> <li>Highlight the blue, green, yellow, and red columns.</li> <li>Click the <b>Analyze</b> button.</li> </ol> <p>This action calculates the base pair size of each fragment and fills the sample files with the analyzed data from each lane.</p>
9.	<p>After the analysis is complete, confirm that the sizes for the peaks in the GeneScan-500 ROX Size Standard have been correctly assigned.</p> <ol style="list-style-type: none"> <li>Select <b>Window &gt; Results Control</b> and examine the red GeneScan-500 peaks in overlapping groups (Quick Tile Off). Use Ctrl+ to enlarge the view window for more careful examination; use Ctrl– to reduce it to full range. Be sure to select <b>View &gt; Align By Size</b>.</li> <li>Scroll through the tables to verify correct peak assignments (see Figure 13 on page 74). Check the remaining lanes, taking note of which lanes (if any) have incorrect peak assignments.</li> <li>If the size standard peak assignments are incorrect in one lane, define a new size standard for that sample using the peaks in that lane. To do so, select the <b>Define New</b> option in the Size Standard row for that sample (see step 7b).</li> <li>Reanalyze any incorrectly sized lanes (select the blue, green, yellow and red boxes) using the newly defined GeneScan-500 ROX Size Standard file for that lane. Reanalyzing creates a new standard file for each of these lanes, replacing the previous analysis results for those lanes only.</li> <li>Confirm that the GeneScan-500 ROX Size Standard peaks are now correctly assigned in the reanalyzed lanes.</li> </ol>
10.	<p>View the AmpF<math>\mathcal{L}</math>STR kit results (using the Results Control window) and print. Refer to the <i>ABI PRISM<sup>®</sup> GeneScan<sup>®</sup> Analysis Software Version 3.7 User Guide</i> for printing options.</p>

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**Headquarters**

850 Lincoln Centre Drive  
Foster City, CA 94404 USA  
Phone: +1 650.638.5800  
Toll Free (In North America): +1 800.345.5224  
Fax: +1 650.638.5884

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Printed in USA, 12/2002  
Part Number 4340648 Rev. A, Stock Number 112UB03-01-UB

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