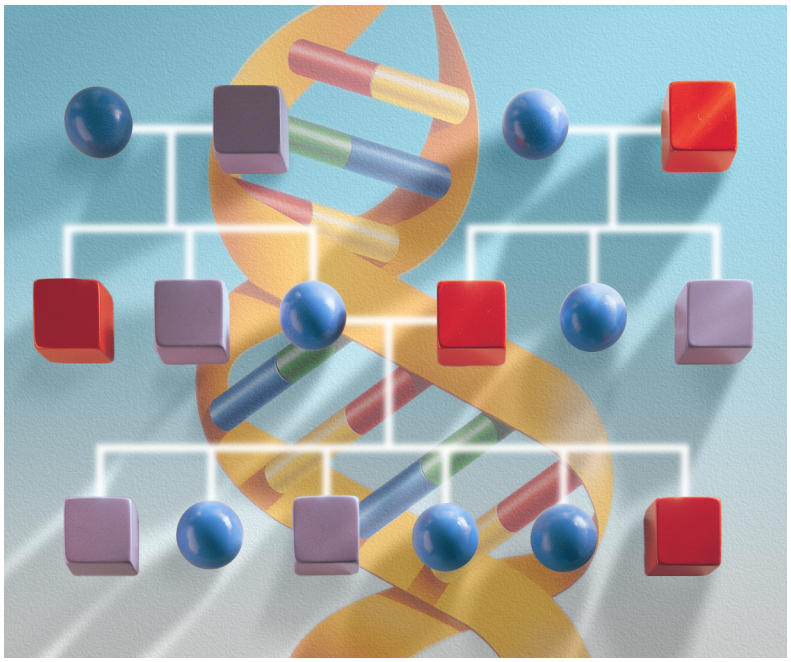

ABI PRISM® Linkage Mapping Set

Version 2.5

User Guide

Applied Biosystems

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1 Introduction

Overview

In This Chapter

The following topics are covered in this chapter:

About This User Guide

Intended Use of User Guide

This user guide provides information on the use, analysis, and troubleshooting of the ABI PRISM® Linkage Mapping Set v2.5.

What You Should Be Familiar With

To follow the procedures in this manual, you should be familiar with:

- The operation of your instrument(s)
  - ABI PRISM® 3700 DNA Analyzer
  - ABI PRISM® 3100 Genetic Analyzer
  - ABI PRISM® 377 DNA Sequencer
  - ABI PRISM® 310 Genetic Analyzer
- Thermal cycler operation
  - GeneAmp® PCR System 9600 or 9700
- Microsoft® Windows NT® operating system
- ABI PRISM® GeneScan® Analysis Software, version 3.7
- ABI PRISM® Genotyper® Software, version 3.7

Note These versions are for the Microsoft Windows NT operating system only. For the Macintosh® computer, use GeneScan software version 3.1 or higher and Genotyper software version 2.5.

-or-

- ABI PRISM® GeneMapper Software, version 1.0

IMPORTANT This user guide is not intended as a substitute for the detailed information contained in the instrument and software user guides.

For More Information

The Linkage Mapping Set v2.5 is organized into panels that can be ordered as kits, panels, or individual primer pairs.

Refer to the *ABI PRISM Linkage Mapping Set v2.5 Panel Guide* (P/N 4330150) for more information regarding the:

- Selected markers
- Panel layout
- Lane-loading information

Product Description

Overview

The ABI PRISM Linkage Mapping Set v2.5 comprises 811 fluorescently labeled PCR primer pairs (markers) optimized to amplify highly informative microsatellite loci selected from the Généthon human linkage map (Weissenbach, *et.al.*, 1992; Gyapay, *et.al.*, 1994; Dib, *et. al.*, 1996).

Product Configurations

The Linkage Mapping Set v2.5 is available in the following configurations:

Set Name	Set Properties	Markers
Linkage Mapping Set v2.5-HD5	♦ High density ♦ 5-cM Resolution	811 Markers total: ♦ 400 markers from the Linkage Mapping Set-MD10, plus ♦ 411 additional markers
Linkage Mapping Set v2.5-MD10	♦ Medium density ♦ 10-cM Resolution	The 400 original markers from the ABI PRISM® Linkage Mapping Set Version 2

Markers

The markers are provided as a single tube containing forward and reverse PCR primer pairs used to generate PCR products that can be pooled (combined) and detected in a single gel lane or capillary injection. Each Linkage Mapping Set v2.5 has the markers organized into panels that contain 3 to 20 fluorescent dye-labeled primer pairs.

Markers have been optimized and tested with 6-FAM™, VIC™, and NED™. The choice of dye with a particular loci has been predetermined. Markers are available only with the specified dye and cannot be changed. For more information about dye labels, please refer to the *ABI PRISM Linkage Mapping Set Version 2.5 Panel Guide* (P/N 4330150).

Common Features of All Markers

All 811 markers share the following features:

- DS33, which uses 6-FAM, VIC, NED, PET™, and LIZ™ for improved spectral resolution and strong signal strength. Linkage Mapping Set v2.5 markers are labeled only with 6-FAM, VIC, or NED. PET can be used to label custom primers specified by the user. LIZ is used to label the size standard.
- Reverse primer chemistry (tailing) (Brownstein, *et al.*, 1996) to promote the nontemplate directed nucleotide addition during amplification, resulting in consistent allele calls and streamlined data analysis.
- Amplification using one set of PCR conditions and a single PCR master mix (True Allele® PCR Premix).
- Rigorous quality control during primer manufacturing and use testing to verify amplification of target alleles and confirm performance of the markers.

Materials Included in Each Panel

Each panel contains 3 to 20 primer pairs (a fluorescently labeled forward primer and unlabeled reverse primer) combined in one tube.

Each tube contains a 5-μM solution of forward and reverse primers (10 μM total primer concentration) in TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. The locus name for each marker is printed on the tube.

Fluorescent Labels for Markers

Markers are labeled with one of the following four fluorescent dyes.

Dye	Color
6-FAM	Blue
VIC	Green
NED	Yellow
PET[a]	Red

a. Available to label custom-synthesized markers specified by the user.

The cap color on the tube corresponds to the display color of each dye when detected on the ABI PRISM instruments using default settings for Dye Set G5.

Marker Storage

Linkage Mapping Set v2.5 markers can be stored for one year after date of receipt at –15 to –25 °C. Freeze thawing should be avoided.

Product Quality and Performance

All of the primers in the Linkage Mapping Set v2.5 were tested on CEPH family 1347 and various sample DNAs to confirm PCR conditions and verify allele size ranges. In addition, a final use test is performed on manufactured lots of primers using individual CEPH 1347-02.

The PCR conditions and optimized protocols described in this manual were developed using:

- GeneAmp PCR System 9600
- True Allele PCR Premix
- CEPH control DNA and DNA extracted from whole blood

Note Optimization may be required when using other instruments, reagents, or DNA.

All of the markers in each panel can be detected in a single gel lane or capillary. This is made possible by using multicolor detection to distinguish overlapping alleles labeled with the fluorescent dyes 6-FAM, VIC, and NED, and the use of an internal-lane size standard labeled with LIZ. This results in more information per lane and better precision.

Product Features

Dye Set Composition

The ABI PRISM Linkage Mapping Set v2.5 uses the dye set consisting of:

Dye	Color
6-FAM	Blue
VIC	Green
NED	Yellow
PET[a]	Red
LIZ (the size standard)	Orange

a. Available to label custom-synthesized markers specified by the user.

This dye set is more "spectrally resolved" than the dye set used with the original ABI PRISM Linkage Mapping Set—6-FAM, HEX, NED, and ROX.

Note Spectral resolution is the degree of separation between the emission spectra of dyes used together as a set. The more spectrally resolved the dyes in a set are, the less overlap there is at the wavelength ranges where light is collected.

Dye Set Advantages

In addition to being more spectrally resolved, this dye set:

- Provides strong signal strength for data analysis
- Reduces the potential for matrix-related problems greatly
- Yields cleaner data

Module Requirements

The following modules are required for using the Linkage Mapping Set v2.5:

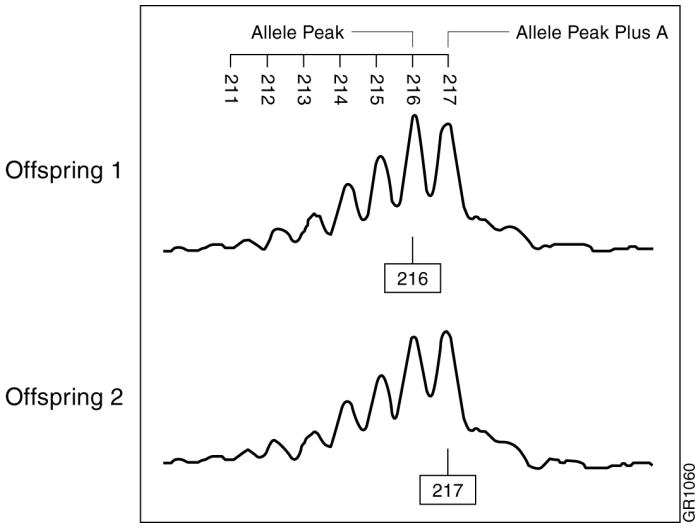
If using the …	Then use the…
3700 DNA Analyzer or the 3100 Genetic Analyzer	♦ GS default spectral run module and G5 parameter file for matrix generation ♦ Appropriate GS run module
377 DNA Sequencer	GS 36G5-2400 run module
310 Genetic Analyzer	GS STR POP4 (1 mL) G5 run module

About the Reverse-Primer Tailing Chemistry

The patented reverse-primer tailing chemistry improves allele calling efficiency by eliminating problems associated with nontemplate nucleotide addition. Before tailing, one of the main difficulties in semiautomated, microsatellite-based genotyping was the "plus A artifact"—the tendency of *Taq* polymerase to add a nontemplated nucleotide (usually an A) to the 3' end of double-stranded DNA (Brownstein, *et al.*, 1996). For a given marker, this addition was not absolute, and only a certain fraction of amplicons received the additional nucleotide.

Why the Ambiguity in Allele Calling Resulted

Ambiguity in allele calling resulted when the allele and allele plus A peaks were of near equal height. This occurred for approximately 5 to 10% of markers in a given laboratory. For example, the following illustration shows two individuals with the same genotype analyzed with the same dinucleotide repeat marker. Genotyper software will not correctly call the alleles even though the pattern is visible for both offspring because the allele peak (216) is the highest peak for offspring 1, while the allele plus A peak (217) is the highest peak for offspring 2. Data of this type requires manual editing to avoid missed or incorrect allele calls.



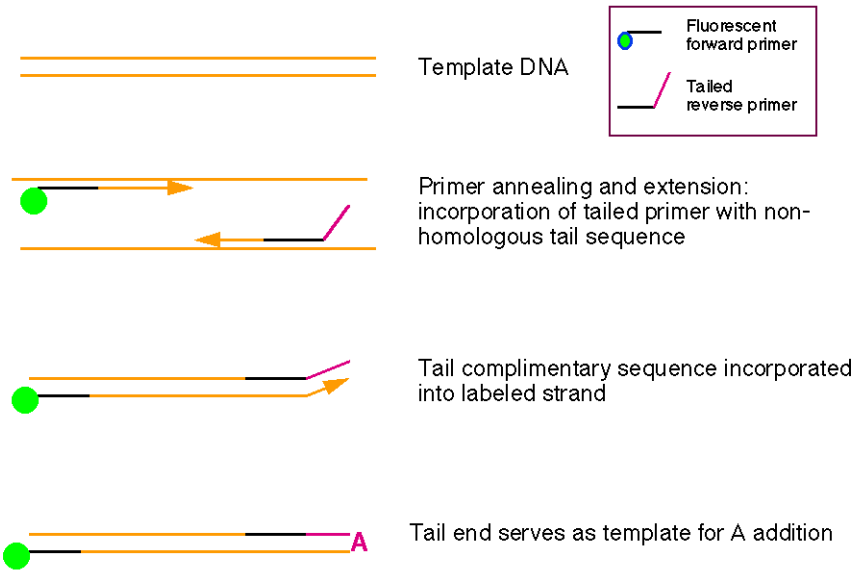
Why Primer Tailing Was Developed

Primer tailing was developed to address the plus A problem in automated genotyping and has the following advantages:

- It is a relatively universal fix
- The method works well
- No additional experimental steps are required

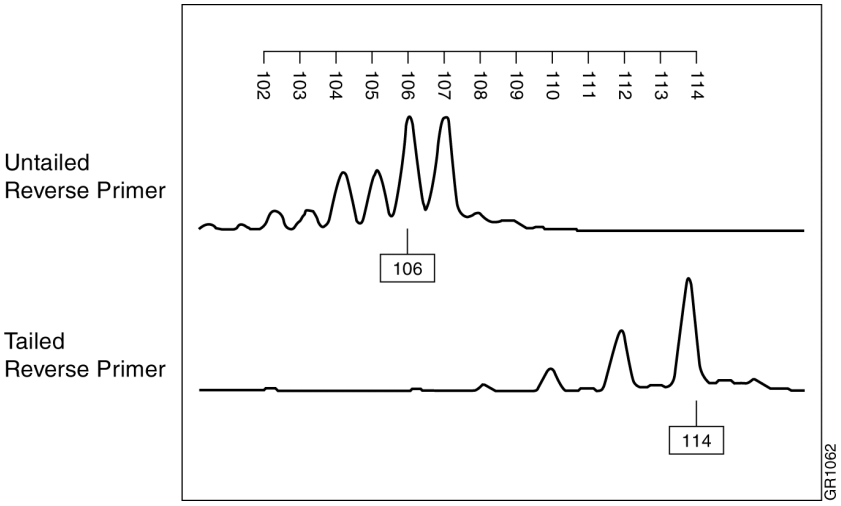
How Tailing Works

As shown below, tailing works by controlling the sequence context where the polymerase binds to the end of double-stranded DNA and adds the nontemplated nucleotide.



By controlling this sequence context with the addition of a seven-base tail, the plus A addition can be either promoted or inhibited. Promotion of the plus A addition was found to be the most effective method for generating consistent allele peak patterns, as the rich PCR conditions used to generate ample amounts of PCR products also favor the plus A addition.

As shown below, dinucleotide repeats tend to generate complex patterns due to the combination of stutter and the plus A artifact. In this example, the 106 peak is the allele peak in the untailed product. The 114 peak is the allele peak plus A in the tailed product. It is eight bases longer because it includes the seven-base tail and the additional A. Since Genotyper software filters out stutter peaks automatically, elimination of the plus A problem results in easily called alleles.



Genotyping Support Reagents

Premixed Reagents Simplify PCR Setup

True Allele PCR Premix contains an optimized solution of the following reagents for amplification of microsatellite loci.

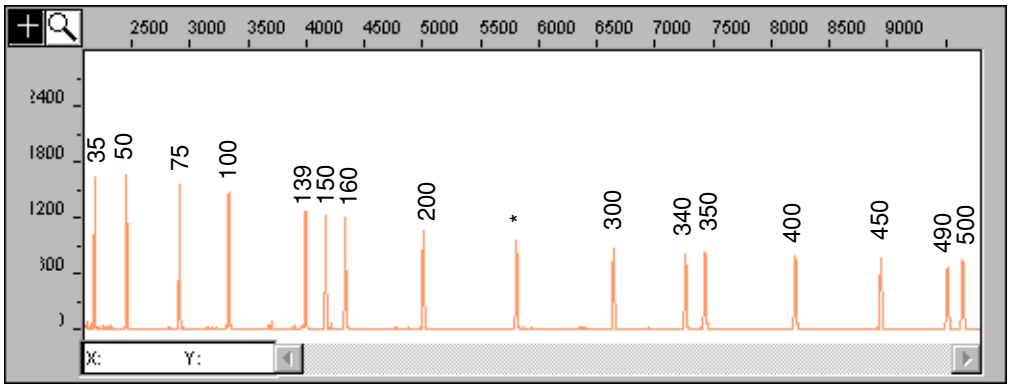
- AmpliTaq Gold® DNA Polymerase
- dNTPs
- Buffer
- Magnesium

Having all reagents premixed simplifies PCR reaction setup and increases consistency.

Size Standard

Multicolor detection ensures precise sizing of PCR products through the use of an internal lane size standard—GeneScan™-500 LIZ™ Size Standard. This size standard includes 16 evenly spaced fragments labeled with LIZ. Because it is labeled with a different color dye than the samples, the GeneScan-500 LIZ Size Standard can be loaded into each gel lane or capillary and run with your samples to:

- Minimize gel-to-gel and capillary-to-capillary variability
- Provide precise sizing of alleles



GeneScan-500 LIZ Size Standard fragment sizes (bp) appear above the peaks.

IMPORTANT An asterisk (*) for the 250-bp peak denotes a peak resulting from abnormal migration of double strands that did not completely separate under denaturing conditions. Do not use this peak to size samples. This peak shows varying smaller values than the actual size of the fragments.

Uses for Control DNA

The use of control DNA is recommended for optimal genotyping results. Control DNA can be used to:

- Monitor PCR amplification efficiency
- Control gel-to-gel or capillary-to-capillary variation
- Aid in allele binning
- Correlate allele sizes with data from external sources such as the CEPH database

Reasons to Use CEPH Individual 1347-02

The use of CEPH individual 1347-02 as the control DNA is recommended because:

- It is the reference individual used by Généthon
- DNA from this individual has been widely genotyped, so allele information in databases is very accurate

Control DNA Tube Contents

Each tube contains:

- Cell line DNA from CEPH individual 1347-02
- 180 µL DNA at a concentration of 50 ng/µL in 10 mM Tris HCl, 0.1 mM EDTA (pH 8.0)

When to Use Control DNA

We recommend using one sample of control DNA:

- In every plate of samples amplified
- With every set of markers used
- Whenever the capillary, buffer, or polymer is changed on the instrument
- When running under new conditions
- When making changes to an experimental design

Platform Differences

Because of the small variations in sizing between different platforms, it is highly recommended that control samples are run for each panel. This will allow historical data run on one platform to be compared to data run on a new platform.

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

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

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.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or 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 (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or 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 (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, 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.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current MSDS before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

To order documents by automated telephone service:

Step	Action
1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

To order documents by telephone:

In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	♦ To order in English, dial **1.800.668.6913** and press **1**, then **2**, then **1.** ♦ To order in French, dial **1.800.668.6913** and press **2**, then **2**, then **1.**
From any other country	See the specific region under "To Contact Technical Support by Telephone or Fax (Outside North America)."

To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS.**
3	Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a PDF version of the MSDS.

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Performing PCR 2

Overview

In This Chapter

The following topics are covered in this chapter:

DNA Isolation Recommendations

Puregene Isolation Kits

A variety of DNA isolation kits were used to help develop the ABI PRISM® Linkage Mapping Set v2.5. Puregene DNA Isolation Kits provided the most satisfactory results. We recommend using the kits designed for isolating DNA from whole blood drawn in Vacutainer lavender-top EDTA tubes.

IMPORTANT Do not freeze blood samples before DNA isolation. Freezing can lyse red blood cells, and increase the concentration of PCR inhibitors in prepared samples.

Supplier	Suggested Puregene Kit Catalog Numbers	Tubes for Blood Collection
Gentra Systems, Inc. 13355 10th Ave. N Suite 120 Minneapolis, MN 55441 USA Toll-free: 888.ISOLATE (888.476.5283) or 800.866.3039 Telephone: 612.543.0678 Fax: 612.543.0699	♦ D-5000 ♦ D-50K	Vacutainer lavender-top liquid EDTA (K3) tubes

Preventing Sample Contamination

Minimizing PCR Product Carryover and Sample Contamination

To minimize PCR product carryover and sample cross-contamination:

- Prepare all reaction mixes in a room or laboratory that is separate from where thermal cycling, PCR product analysis, and post-PCR sample preparation is performed.
- Use dedicated or disposable vessels, solutions, and pipettors (positive displacement pipettors with disposable or aerosol resistant tips) for DNA preparation, reaction assembly, and sample analysis.
- Aliquot reagents into daily use amounts.
- Spin down PCR tubes before opening to collect solutions at bottom of tubes.
- Keep a dedicated lab coat in each area.
- Use disposable gloves.
- Treat PCR products with the same level of care that you would treat radioactive products.

Before Screening Samples

Primer Pairs Have Been Optimized

Linkage Mapping Set v2.5 primer pairs have been optimized using:

- CEPH family 1347 DNAs
- DNA extracted from blood using commercially available preparation kits
  - See “DNA Isolation Recommendations” on page 2-2 for kit recommendations.
- Applied Biosystems reagents and instruments

Variation in any of these components could alter the efficiency and specificity of the PCR reaction.

Purpose of the PCR Verification Protocol

The PCR Verification Protocol tests multiple DNA samples with each primer pair in a panel.

Only one PCR product with each dye color is electrophoresed and detected in a single gel lane or capillary injection. This allows for evaluation of each primer pair’s performance by displaying the ABI PRISM® GeneScan® Analysis Software electropherogram in full-view display for each dye color.

Once the performance of the panel has been verified, study samples can be amplified for that particular panel.

When to Perform the PCR Verification Protocol

Prior to pooling PCR products, we highly recommend performing the following PCR Verification Protocol to verify the integrity of the PCR reaction:

- Before performing large-scale experiments
- When using Linkage Mapping Set v2.5 panels for the first time
- Any time DNA samples are:
  - Isolated using a new procedure
  - Stored under new conditions

PCR Verification Protocol

DNA Sample Recommendations

Select multiple DNA samples representative of your study samples including all DNA isolation methods utilized. Analysis of only a single DNA sample for each primer pair could result in the erroneous interpretation of results caused by pipetting errors or variations in sample preparation.

Materials Required

Use the “Materials Required” lists on page 2-11.

Setting Up the Reaction Tray

To set up each reaction tray:

Step	Action
1	Place the reaction tray in the base, so that the well numbered A1 is located in the upper left corner of the tray.
2	Place MicroAmp® reaction tubes in the tray or a MicroAmp® 96-Well Reaction Plate.
3	Layout recommendations: a. Draw a layout (grid) of the tubes in the tray b. Specify on the grid what each tube will contain c. Arrange: – A single DNA sample in each row – A single primer pair in each column
4	Secure the retainer onto the tray and base.

Loading the Reaction Tray

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

To load the reaction tray:

Step	Action
1	Thaw and gently mix each reagent, and briefly centrifuge to collect contents at the bottom of each tube.
2	Pipet the following into each tube: ♦ 9.0 µL True Allele® PCR Premix ♦ 3.8 µL sterile deionized water
3	Add 1.0 µL of each primer pair to the appropriate tubes, one primer pair per tube.
4	Add 1.2 µL of DNA sample to the appropriate tubes, one sample per tube.
5	Cap all tubes, making sure they are tightly sealed.
6	Mix gently, and spin down the tray to collect solutions at the bottom of each tube.

Performing PCR

To program the GeneAmp® PCR System 9600 or 9700 thermal cycler and start PCR:

Step	Action
1	Program the thermal cycler using the conditions listed in the following table. **Repetitions of Each Cycle** / **Cycle Conditions** 1 / 95 °C for 12 min 10 / Melt at 94 °C for 15 sec Anneal at 55 °C for 15 sec Extend at 72 °C for 30 sec 20 / Melt at 89 °C for 15 sec Anneal at 55 °C for 15 sec Extend at 72 °C for 30 sec 1 / Final Extension at 72 °C for 10 min HOLD / 4°C (forever)
2	Place the loaded tray into the thermal cycler. Make sure the tray is properly oriented (position A1 in upper left corner).
3	Close and tighten the cover.
4	Start PCR.

Note The Linkage Mapping Set v2.5 was developed and optimized using the GeneAmp PCR System 9600. If using the GeneAmp PCR System 9700, additional optimization may be required. Refer to the appropriate instrument user guide for instructions on how to emulate the 9600 system.

Pooling PCR Products

Pool the PCR products into clean MicroAmp® tubes so that only one product with each dye is included in a single pool.

To pool the PCR products:

Step	Action
1	Mix: ♦ 5 µL of the first 6-FAM™ dye-labeled product ♦ 5 µL of the first VIC™ dye-labeled product ♦ 10 µL of the first NED™ dye-labeled product ♦ 80 µL of deionized water 6-FAM dye- and VIC dye-labeled products are diluted 1:20, while NED dye-labeled products are diluted 1:10.
2	Repeat this pooling ratio for all of the markers in the panel. **Note** If not pooling all three dyes, replace the pooled volume of the absent dye color with an equal volume of water. This will keep the dilution ratio for each dye constant.
3	Cap the tubes.
4	Vortex briefly, and spin down to collect samples at the bottom of each tube.

Performing Electrophoresis or Analysis Run

Perform electrophoresis or an analysis run by following the instructions for your instrument(s) in the following chapters:

Topic	See Chapter
Performing a Run on the 3700 Instrument	3
Performing a Run on the 3100 Instrument	4
Electrophoresis on the 377 Instrument	5
Electrophoresis on the 310 Instrument	6

Optimizing PCR

Overview

The Linkage Mapping Set v2.5 microsatellite markers have been optimized and use-tested to work under a common set of reaction conditions. However, variations in DNA sample quality and lab conditions or procedures can sometimes affect PCR. Various methods for optimizing marker performance are listed below.

Optimizing Pooling Ratios

We recommend testing the pooling ratios for a given panel on a small subset of representative DNA samples (4–8) to determine the appropriate adjustments for optimal detection of multiple markers from a panel in one lane. In general, heterozygote peak heights should fall between 200 and 1000 fluorescent units.

To optimize pooling ratios:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>After PCR, pool the reaction products for a panel of markers at a 1:1:2 ratio (6-FAM:VIC:NED).</td></tr>
<tr><td>2</td><td>Determine the average heterozygote peak height for each marker using this formula:

Sum of all allele peaks / (2 x number of DNAs tested)</td></tr>
<tr><td>3</td><td>Adjust the pooling ratio of individual markers as follows to achieve even peak heights across all loci.

<table>
<tr><th>If the...</th><th>Then...</th></tr>
<tr><td>marker symbol is too weak</td><td>increase the pooling volume of that particular marker.</td></tr>
<tr><td>marker symbol is too strong</td><td>decrease the pooling volume of that particular marker.</td></tr>
<tr><td>overall signal is too high</td><td>dilute the pooled PCR products with deionized water.</td></tr>
<tr><td>overall signal is too low</td><td>concentrate the sample by following the procedure in “Concentrating Samples to Increase Signal Strength” on page 2-8.</td></tr>
</table>
</td></tr>
</table>

Concentrating Samples to Increase Signal Strength

Before following this procedure, optimize the pooling ratios for the markers being used by following the procedure in “Optimizing Pooling Ratios” on page 2-7.

To concentrate samples:

Step	Action
1	Prepare samples as suggested for the ABI PRISM® instrument of choice.
2	Centrifuge briefly to collect samples at the bottom of each tube.
3	Leaving the caps off the tubes and the cover off the thermal cycler, denature the samples for up to 15 min at 95 °C.

Increasing Signal Strength

To increase signal strength, modify the standard protocol as suggested below. Perform these modifications in the order in which they appear, one at a time.

- Increase the amount of a particular marker used by adjusting the pooling ratio for that marker.
- Increase the number of PCR cycles from 30 to 33–35 by increasing the second set of melt/anneal/extend cycles.
- Increase the magnesium chloride concentration by performing a titration as described in “Optimizing the Magnesium Ion Concentration” on page 2-10.
- Decrease the annealing temperature 2 to 3 degrees at a time. Background may increase.

Decreasing Background

To decrease background (nonspecific amplification), modify the standard protocol as suggested below. Perform these modifications in the order in which they appear, one at a time.

- If background is interfering with allele calls of other markers, decrease the amount of the marker used by adjusting the pooling ratio for that marker.
- Increase the annealing temperature 2 to 3 degrees at a time. Overall signal may decrease.

Optimizing Thermal Cycling Conditions

Applied Biosystems thermal cyclers calculate the sample temperature based on tube type and volume of contents. If other thermal cyclers are used, optimization will be required to determine optimal conditions for marker amplification in terms of hold temperatures, hold times, ramp times, and number of cycles.

Adding Cycles

Greater amplification can sometimes be obtained by adding more cycles to the thermal cycling conditions listed earlier in this chapter. If the PCR has not reached plateau after the standard 30 cycles, adding 3 to 5 cycles may improve the PCR yield. This may help when the DNA template concentration is lower than expected.

To compare the effect of cycle number on the PCR yield:

Step	Action
1	Prepare master cocktails with all reagents.
2	Subject aliquots of these cocktails to different numbers of cycles.
3	Run the PCR products from the different cycling conditions on the same gel or capillary.
4	Compare the results.

Changing the Annealing Temperature

In general, changes to the annealing temperature can have the following effects:

AnnealingTemperature Change	Positive Effects	Negative Effects
Decreased	Increased PCR product yield	Increased amplification of nonspecific products (background)
Increased	Increased PCR specificity	Reduced PCR yield

To perform a comparison of annealing temperatures, begin with a representative set of 4 to 8 DNA samples and follow these steps:

Step	Action
1	Prepare master cocktails containing all reagents.
2	Subject aliquots of the master cocktails to different thermal cycling protocols where all parameters are held constant except the annealing temperature. Vary the annealing temperature in 2 to 3 °C increments.
3	Run all products on the same gel or capillary.
4	Compare the results.

Optimizing the Magnesium Ion Concentration

Increasing the $MgCl_2$ concentration may increase the yield for some markers, but may also increase the amplification of nonspecific products (background).

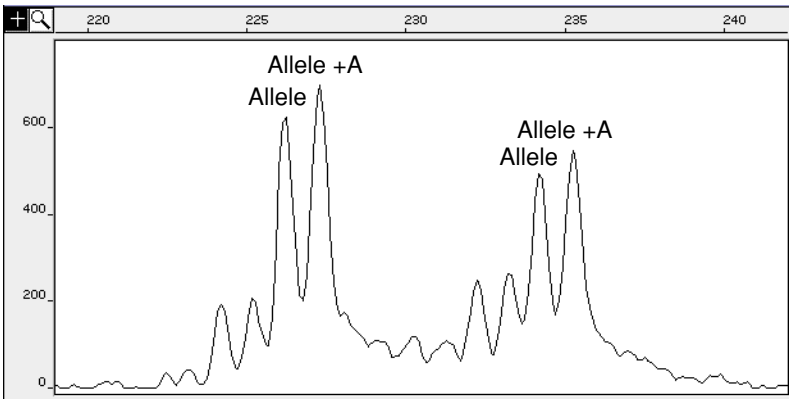
True Allele PCR Premix contains all the reaction components necessary for PCR, except DNA and primers. The premix contains AmpliTaq Gold® DNA Polymerase and a 2.5 mM $MgCl_2$ concentration (final). All of the markers in the Linkage Mapping Set v2.5 were developed with this premix.

To determine the benefit of increasing the $MgCl_2$ concentration:

Step	Action
1	Prepare master cocktails with all reagents (including DNA and primers), but do not bring to the final volume with water.
2	Aliquot equal amounts of master cocktail to each tube.
3	Add $MgCl_2$ to yield an increase in final $MgCl_2$ concentration in 0.5-mM increments.
4	Bring all reactions up to final volume with water.
5	Perform PCR.
6	Run all products on the same gel or capillary.
7	Compare the results.

Promoting the Plus A Addition

All Linkage Mapping Set v2.5 markers are tailed to promote complete nontemplated nucleotide addition (plus A addition). If reaction conditions are inhibitory, however, plus A addition may not be complete. Incomplete A addition is not a problem unless the percentage of fragments without the A addition approaches the percentage of fragments with the A addition (*i.e.*, the peak height of the allele and allele plus A peaks are of near equal height as shown below).



It is unlikely that any Linkage Mapping Set v2.5 markers will show a problematic plus A pattern if the protocols and recommendations in this user guide are followed. If these conditions are modified and a plus A problem occurs, increasing the final hold at 72 °C from 10 minutes to 20 to 40 minutes may help restore an easily called allele pattern.

Performing PCR Using the GeneAmp PCR System 9600 or 9700

Process Overview

The PCR process is as follows:

- Prepare the master mixes
- Set up and load the reaction tray(s)
- Program the thermal cycler and perform PCR
- Pool the PCR products

Control DNA Recommendations

We strongly recommend including Control DNA CEPH 1347-02:

- In every plate of samples amplified
- With every set of markers used
- For every run
- Whenever the capillary, buffer, or polymer is changed on the instrument
- When running new conditions
- When making changes to an experimental design

Materials Required

Materials Available from Applied Biosystems

Material	Applied Biosystems Part Number
Control DNA CEPH 1347-02	403062
Linkage Mapping Set v2.5 PCR products	Assorted part numbers
True Allele PCR Premix	403061
For the 9700 Instrument	
GeneAmp PCR System 9700	N8050001
MicroAmp® 9700 tray/retainer set	403081
MicroAmp® 9700 base	N8010531
For the 9600 Instrument	
GeneAmp® PCR System 9600	N8010001, N8010002, N8010003
MicroAmp® 9600 tray/retainer set	403081
MicroAmp® 9600 base	N8010531
For All Instruments	
MicroAmp® reaction tubes, 0.2 mL with caps	N8010540
MicroAmp 96-Well Reaction Plate	N8010560

Materials Available from Major Laboratory Suppliers

Material	Source
Gloves, disposable, powder-free	Major Laboratory Supplier (MLS)
Ice bucket with lid	MLS
Microcentrifuge or centrifuge adapted for spinning microtiter plates	MLS
Pipet and tips	Rainin Instruments

Materials Available from Major Laboratory Suppliers *(continued)*

Material	Source
Vortex	MLS
Water, sterile, deionized	MLS

Reaction Volumes

The primers in the Linkage Mapping Set v2.5 are optimized for a total reaction volume of 15 µL. If desired, reaction volumes may be reduced by one-half or one-third, but performance may be affected.

Standard Volume—15.0 µL

The standard 15.0-µL reaction volume contains 5 pmol each of forward (labeled) primer and reverse (unlabeled) primer.

Recommended components for 15-µL reactions

Reaction Components	Volume for 15-µL Reaction (µL)
Primer pair mix (5 µM each primer)	1.0
DNA (50 ng/µL)	1.2
True Allele PCR Premix	9.0
Sterile, deionized water	3.8
Total Reaction Volume	**15.0**

Recommended components for 15-µL reactions if not using True Allele PCR Premix

Reaction Components	Volume for 15-µL Reaction (µL)
Primer pair mix (5 µM each primer)	1.00
DNA (50 ng/µL)	1.20
GeneAmp dNTP mix (2.5 mM)	1.50
10X GeneAmp PCR Buffer II	1.50
AmpliTaq Gold DNA Polymerase (5 units/µL) **⚠ CAUTION CHEMICAL HAZARD.** **AmpliTaq Gold DNA Polymerase** may cause eye and skin irritation. It may cause discomfort if swallowed or inhaled. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.	0.12
25 mM $MgCl_2$	1.50
Sterile, deionized water	8.18
Total Mix Volume	**15.00**

One-Half Volume—7.5 µL

To decrease the total reaction volume to 7.5 µL, decrease the volume of all reagents proportionally. Reducing the volume by one-half usually requires little optimization.

One-Third Volume—5.0 µL

To decrease the total reaction volume to 5.0 µL, decrease the volume of all reagents proportionally. In most cases signal is reduced, and additional optimization is required.

When working with low reaction volumes, we recommend:

- Preparing master mixes
- Pipetting volumes of 1.0 µL or greater
- Using well-calibrated pipets

Performing the PCR Verification Protocol

Instructions for performing the PCR Verification Protocol are provided on pages 2-4 to 2-6. If optimization of PCR is needed, see pages 2-7 to 2-10.

Preparing Master Mixes

For large experiments, prepare a master mix for each primer pair with the True Allele PCR Premix and sterile, deionized water based on the following ratio of ingredients.

Ingredient	Volume for 1 Reaction (µL)[a]	Volume for 100 Reactions (µL)[a]
True Allele PCR Premix[b]	9.0	900.0
Sterile, deionized water	3.8	380.0
Primer pair	1.0	100.0

a. Volumes listed in this table are based on using the standard 15.0-µL reaction volume. Refer to "Reaction Volumes" on page 2-12 for information on reducing reaction volumes.

b. We highly recommend using True Allele PCR Premix, an optimized solution of reagents designed to simplify PCR setup and increase consistency of results. If you are not using the premix, then refer to the table on page 2-12 to prepare master mixes.

To prepare a master mix for each primer pair:

Step	Action
1	Gently mix the appropriate volume of: ♦ True Allele PCR Premix ♦ Sterile, deionized water ♦ Primer solution
2	Gently vortex the mixture for 3 to 5 sec.
3	Use immediately or store as directed below.

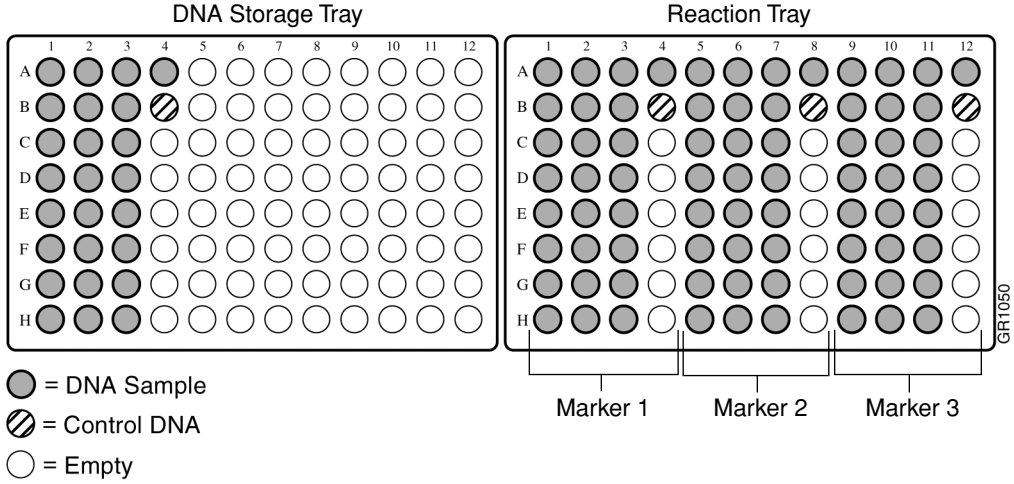
Storing Reagents and Master Mixes

Store reagents and stock solutions at –15 to –25 °C in a constant-temperature, non–frost-free freezer. If reagents are used more than once every 2 days, they can be stored at 2 to 8 °C between uses.

Setting Up the Reaction Tray

To set up each reaction tray:

Step	Action
1	Place the MicroAmp reaction tray base on a flat surface.
2	Place the reaction tray in the base so the well numbered A1 is in the upper left corner of the tray.
3	Place the MicroAmp reaction tubes in the reaction tray(s).
4	Secure the retainer onto the tray and base.
5	Sample loading suggestion: A common practice is to setup experiments so that a given DNA is always in the same position in each reaction tray. To identify samples you may want to: ♦ Draw a layout (grid) of the tubes in the tray ♦ Specify on the grid what each tube will contain An example is shown below.



This example illustrates a convenient way to organize a reaction tray to amplify 25 sample DNAs and 1 control DNA with 3 microsatellite markers. The tray on the left (DNA Storage Tray) is used to store the bulk solutions for the 25 sample DNAs and 1 control. Note how the placement of samples in the reaction tray on the right reflects the sample positions in the storage tray.

Loading the Reaction Tray When Primer Master Mixes Were Used

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

To load the reaction tray(s) when primer master mixes have been prepared:

Step	Action
1	Thaw and vortex gently each DNA sample, and centrifuge briefly to collect the contents at the bottom of each tube.
2	Add 1.2 µL of each DNA sample (50 ng/µL) to the appropriate tubes.
3	Pipet 13.8 µL of each primer pair master mix into the appropriate tubes.
4	Cap all tubes, making sure they are tightly sealed.
5	Mix gently, and spin down the tray to collect solutions at the bottom of each tube.
6	Proceed to “Performing PCR” on page 2-16.

Loading the Reaction Tray When No Primer Master Mixes Were Used

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

To load the reaction tray(s) when no primer master mixes were prepared:

Step	Action
1	Pipet the following into each tube: ♦ 9.0 µL True Allele PCR Premix ♦ 3.8 µL sterile, deionized water
2	Thaw and gently vortex each primer pair and DNA sample, and centrifuge briefly to collect the contents at bottom of the tube.
3	Add 1.0 µL of each primer pair to the appropriate tubes.
4	Add 1.2 µL sample or control DNA (50 ng/µL) to the appropriate tubes.
5	Cap all tubes, making sure they are tightly sealed.
6	Mix gently, and spin down the tray to collect solutions at the bottom of each tube.
7	Proceed to “Performing PCR” on page 2-16.

About Thermal Cycling Conditions

The following thermal cycling conditions have been optimized for the Linkage Mapping Sets v2.5 used with Applied Biosystems instruments and consumables and for Control DNA CEPH 1347-02. Additional optimization may be required if other instruments or consumables are used.

Performing PCR

To program the GeneAmp PCR System 9600 or 9700 thermal cycler and start PCR:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Program the thermal cycler using the conditions listed in the following table.

<table>
<tr><th>Repetitions of Each Cycle</th><th>Cycle Conditions</th></tr>
<tr><td>1</td><td>95 °C for 12 min</td></tr>
<tr><td>10</td><td>Melt at 94 °C for 15 sec
Anneal at 55 °C for 15 sec
Extend at 72 °C for 30 sec</td></tr>
<tr><td>20</td><td>Melt at 89 °C for 15 sec
Anneal at 55 °C for 15 sec
Extend at 72 °C for 30 sec</td></tr>
<tr><td>1</td><td>Final Extension at 72 °C for 10 min</td></tr>
<tr><td>HOLD</td><td>4 °C (forever)</td></tr>
</table></td></tr>
<tr><td>2</td><td>Place the loaded tray into the thermal cycler. Make sure the tray is properly oriented (position A1 in upper left corner).</td></tr>
<tr><td>3</td><td>Close and tighten the cover.</td></tr>
<tr><td>4</td><td>Start PCR.</td></tr>
<tr><td>5</td><td>When PCR is finished, proceed to the pooling instructions, “Pooling PCR Products Produced on the GeneAmp 9600 or 9700” on page 2-17.</td></tr>
</table>

Note The Linkage Mapping Set v2.5 was developed and optimized using the GeneAmp PCR System 9600. If using the GeneAmp PCR System 9700, additional optimization may be required. Refer to the appropriate instrument user guide for instructions on how to emulate the 9600 system.

Pooling PCR Products Produced on the GeneAmp 9600 or 9700

Overview

The microsatellite markers in each panel of the Linkage Mapping Set v2.5 are optimized so that reactions can be combined (pooled) without precipitation, and loaded onto a single gel lane or capillary injection to yield even, recognizable allele peaks for all markers.

IMPORTANT We highly recommend using the pooling ratios listed below as a starting point only, and that you optimize these ratios based on your experimental results. Optimization will yield improved performance—more even peaks from locus to locus. Refer to "Optimizing Pooling Ratios" on page 2-7 for further information.

Pooling PCR Products

To pool PCR products:

Step	Action
1	Thaw and spin down the contents of each tube before opening.
2	Label the appropriate quantity of MicroAmp reaction tubes (one per DNA sample or control per panel), and place them in a reaction tray.
3	Transfer the following volume of each reaction product to the appropriate tube. (6-FAM:VIC:NED = 1:1:2) ♦ 5.0 µL each 6-FAM dye-labeled product ♦ 5.0 µL each VIC dye-labeled product ♦ 10.0 µL each NED dye-labeled product
4	Cap all reaction tubes.
5	Invert the reaction tray to mix the samples.
6	Centrifuge samples briefly to collect solutions at bottoms of tubes.
7	If samples will be loaded for electrophoresis the same day, keep chilled at 4 °C until ready to load. Otherwise, store the pooled samples and remaining reaction products at –15 to –25 °C.

Performing Electrophoresis or Analysis Run

Perform electrophoresis an analysis run by following the instructions for your instrument(s) in the following chapters.

Topic	See Chapter
Performing a Run on the 3700 Instrument	3
Performing a Run on the 3100 Instrument	4
Electrophoresis on the 377 Instrument	5
Electrophoresis on the 310 Instrument	6

Performing a Run on the 3700 Instrument 3

Overview

In This Chapter

The following topics are covered in this chapter:

Before You Begin

Before Performing a Run

Before performing a run, make sure that:

- The fluid levels are appropriate
- The ABI PRISM® 3700 DNA Analyzer has been calibrated and that spectral and spatial calibrations have been successfully run
- There is sufficient space on the hard drive to store the runs
- The software has been started
- The hardware has been checked (see the *ABI PRISM 3700 DNA Analyzer User Guide*, P/N 4306152)

Preparing the Samples

Overview

This section briefly discusses changes in sample preparation for the ABI PRISM® 3700 Data Collection Software v2.0. For complete information about sample preparation, see the *ABI PRISM 3700 DNA Analyzer User Guide* (P/N 4306152).

Dye Set

The ABI PRISM® Linkage Mapping Set v2.5 uses Dye Set G5 with the following dyes:

- 6-FAM™
- VIC™
- NED™
- PET™

  PET is available to label custom-synthesized markers specified by the user.
- LIZ™

Pooling Ratios

The pooling ratio is the amount of each dye-labeled product added with respect to the other products in the pool. Because the fluorescent dyes are detected with different efficiencies, the pooling ratio must be adjusted to ensure appropriate detection of all the loci.

Pooling Ratios for the Linkage Mapping Set v2.5

Linkage Mapping Set v2.5	Dye-Labeled Product	Pooling Ratio
HD5	6-FAM:VIC:NED	1:1:1
MD10		

For each Linkage Mapping Set v2.5 panel, pool 1 µL of each PCR product in a microcentrifuge tube. If necessary, bring the total volume to 20 µL with deionized water.

Suggested Loading Volume

Use these ratios of pooled PCR products and size standards as a starting point only. Optimize these ratios as necessary, based on your experimental data.

To obtain the suggested loading volume:

Step	Action
1	Prepare the formamide:size standard mixture using: ♦ 50 µL of GeneScan™-500 LIZ™ Size Standard ♦ 900 µL of Hi-Di™ formamide (P/N 4311320) or similar quality formamide **⚠ WARNING CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Mix 0.5 µL of pooled PCR product with 9 µL of the formamide: size standard mixture.

Denaturing Samples

To denature the samples:

Step	Action
1	Heat the samples at 95 °C for 5 min.
2	Place on ice immediately for at least 5 min before loading.

Creating a Plate Record

Overview

The plate record is similar to the sample sheet or the injection list that you might have used with other ABI PRISM® instruments. It contains the following information about the samples:

- Name
- Position of the sample on the plate
- Comments about the plate and about individual samples
- Dye set information
- Name of the analysis module

Additional Ways to Create Plate Records

There are a number of ways to create and import plate records.

The most straightforward method is using the Plate Editor, presented in this section. After you have created and saved some plate records as described here, you may want to try other ways to create plate records.

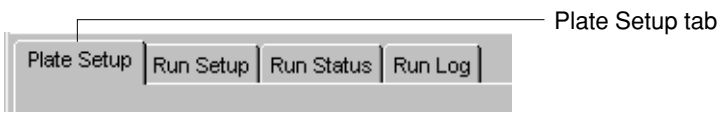
For example, if you have Microsoft® Excel or similar spreadsheet software, you can create new plate records by:

- Exporting a sample plate record from the ABI PRISM 3700 Data Collection Software. (Use the Export button in the Plate Editor.)
- Editing the exported plate record in the spreadsheet program. (You must change the name of the plate record in the file and save the file as tab-delimited text with the filename extension of .plt)
- Importing the edited plate record into the 3700 Data Collection software using the Import button on the Plate Setup page.

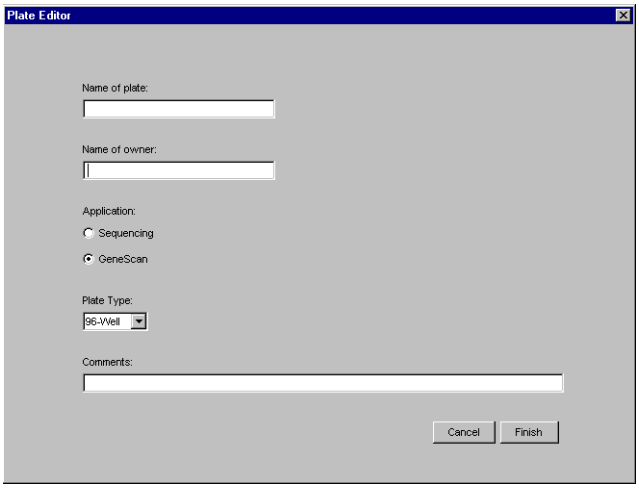
Details about how to export and import plate records and other ways to create plate records are described in the *ABI PRISM 3700 DNA Analyzer User Guide* (P/N 4306152).

Opening the Plate Editor

To open the Plate Editor dialog box and enter plate record data:

Step	Action
1	Click the **Plate Setup** tab to go to the **Plate Setup** page. 

To open the Plate Editor dialog box and enter plate record data: *(continued)*

Step	Action
2	Click the **New** button in the bottom left of the page to open the **Plate Editor** dialog box. 
3	Type a name for the plate record in the **Name of plate** field. **Note** Use letters, numbers, and the following punctuation only: - _ ( ) { } # . + Do not use spaces.
4	Type a name in the **Name of owner** field. This might be your name or the name of the owner of the samples.
5	Under **Application**, choose **GeneScan**.
6	Select **96-Well** or **384-Well** from the **Plate Type** pull down menu.
7	Optional: Type comments about the plate in the **Comments** field.
8	Check that everything is entered correctly, then click **Finish**. The **Plate Editor** dialog box opens.

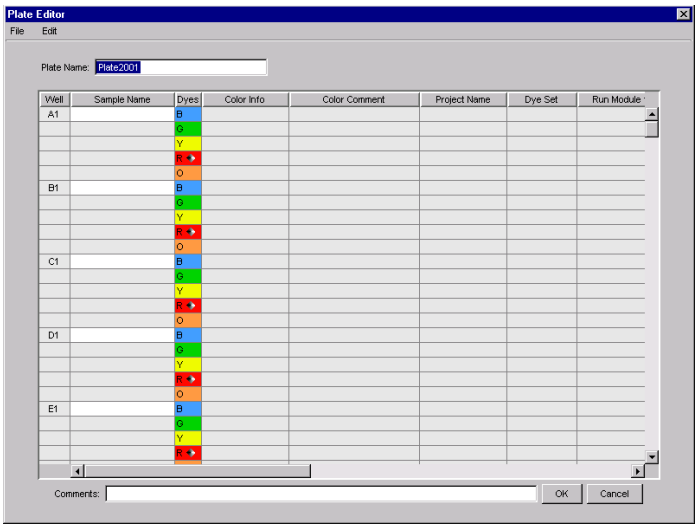
Tips for Entering Sample Information

Use the following tips to enter sample information quickly:

- Use the Fill Down command whenever a field is the same for all samples.
- Use the keyboard shortcuts for cutting (Ctrl+X), copying (Ctrl+C), and pasting (Ctrl+V) in text fields.
- Use the new copy/paste feature to copy whole columns.

Entering Sample Information

To enter sample information:

Step	Action
1	In the **Plate Editor** sample sheet, type the names of all the samples in the **Sample Name** column. **IMPORTANT** Be sure that sample file names are not longer than 59 characters. There is no automatic error checking for sample names that exceed this limit. Sample files with long names cannot be opened by the DNA Sequencing Analysis software. 
2	For each sample, select a size standard dye in the **Dyes** column. For a five-dye sample sheet, select **O** (orange).
3	Optional: For each sample, type any desired information in the **Color Info** and **Color Comment** fields. **Note** If using GeneScan software or Genotyper software, the **Sample Name** field is not used. It is recommended that you put the sample name in the **Color Info** or **Color Comment** fields.
4	For each sample, select a project name from the drop-down list. **IMPORTANT** A project name is required for every sample, even if you are not using the Applied Biosystems Sequence Collector (formerly BioLIMS®) program. For more information about projects, see the *ABI PRISM 3700 DNA Analyzer User Guide* (P/N 4306152).
5	For each sample, select the appropriate GeneScan dye set from the **Dye Set** drop-down list.

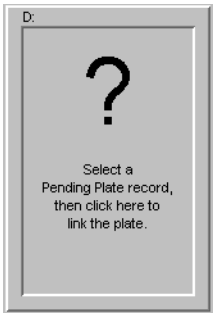
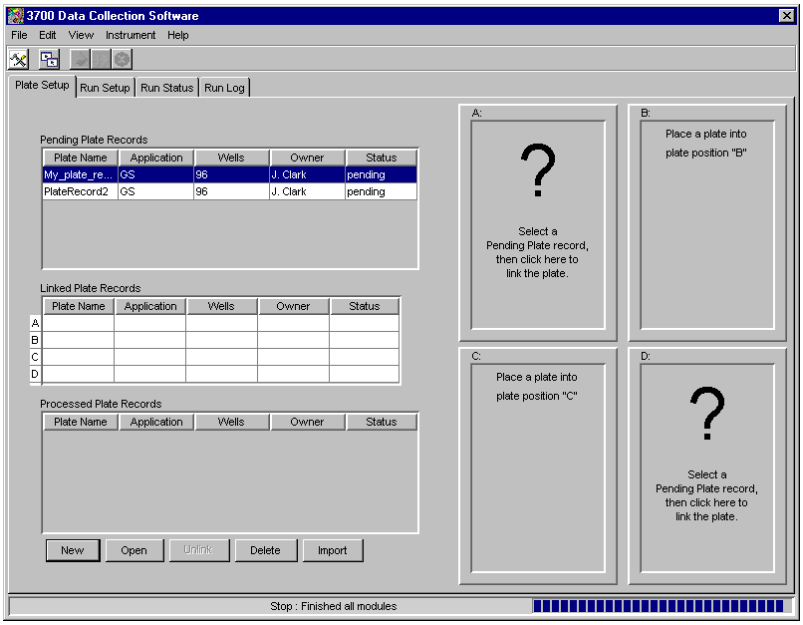
To enter sample information: *(continued)*

Step	Action
6	For each sample, select a run module from the drop-down list. **Note** If you select different modules for different samples, the samples will be automatically grouped so that all samples with the same run module are run at the same time. Runs are scheduled alphanumerically by run module name, not by the order indicated in the plate record nor by plate letter.
7	For each sample, select the appropriate analysis module from the drop-down list. **Note** The analysis module should match the size standard being used.
8	If you want to run the same sample again under the same or different run conditions, choose a second run module and a second analysis module. You can run a sample in a linked plate up to five times, provided there is enough sample in the well.

Linking a Reaction Plate

Note The procedures below show an example of linking plate records to plate positions A and D, but you can link your plate records to any plate positions (A to D).

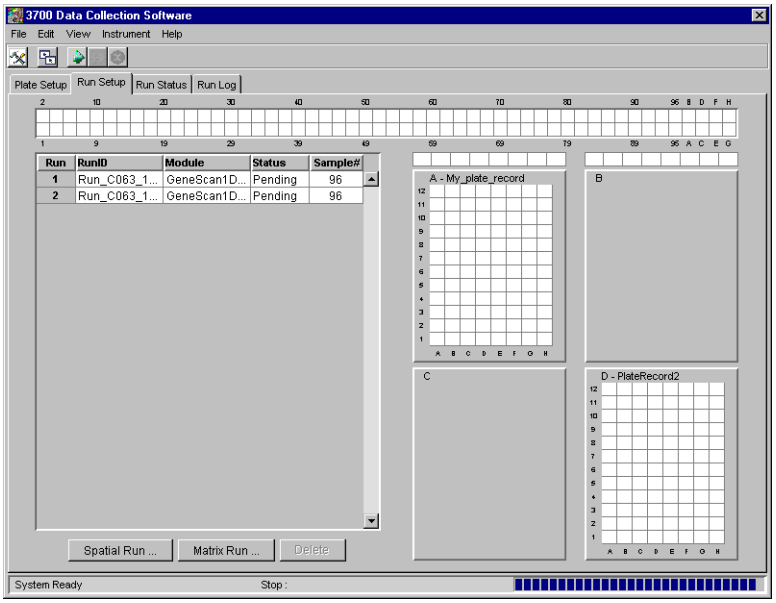
To link a reaction plate:

Step	Action
1	In the 3700 Data Collection software, click the **Plate Setup** tab. The plate position indicator corresponding to your reaction plate (placed in the previous procedure) should look like this: 
2	In the **Pending Plate Records** table, click the plate record for plate A. 
3	Click the plate position indicator for plate A. When the plate is linked, the plate record moves from the **Pending Plate Records** table to the **Linked Plate Records** table.
4	Repeat steps 2 and 3 for any other reaction plates. You can link up to four reaction plates at a time.

Starting and Monitoring a Run

Starting a Run

To start a run:

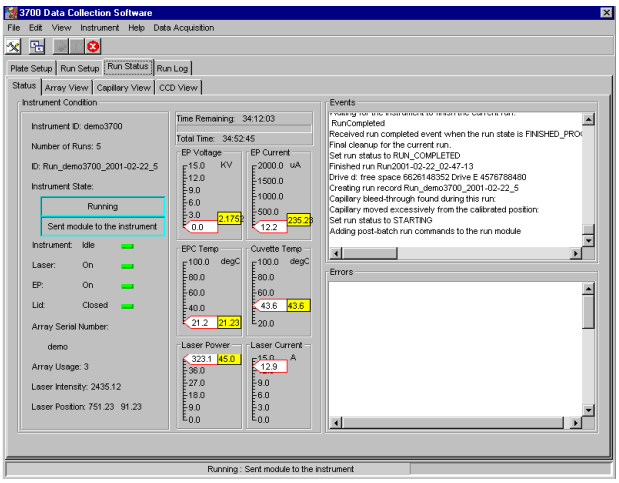
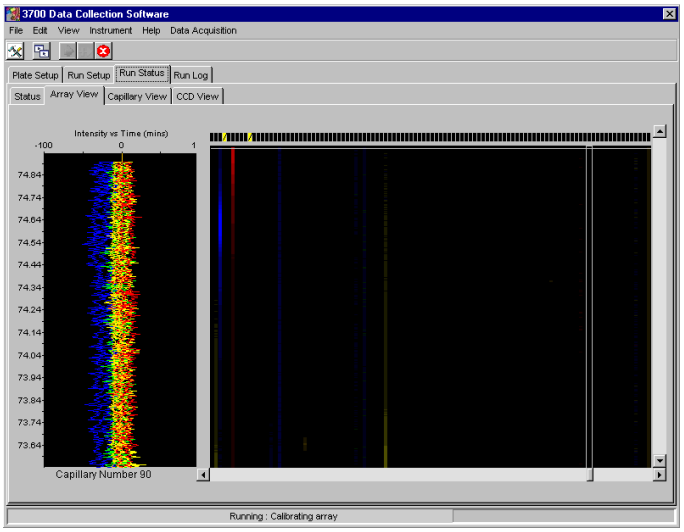
Step	Action
1	In the 3700 Data Collection software, click the **Plate Setup** tab. Check that the plates you want to run are listed in the **Linked Plate Records** table.
2	Click the **Run Setup** tab. Check that the run setup is correct.  Plates are scheduled according to: ♦ Run module name (numerically, then alphabetically) if run modules are the same ♦ The order they were linked **If the run setup is…** / **Then…** correct: the **Run** button in the toolbar is green (active). not correct: the plate is not linked properly. Relink the plate.
3	Click the Run button to start the run. 

Monitoring a Run

To monitor a run:

Step	Action
1	In the **Run Status** page, click the **Status** sub-tab.

To monitor a run: *(continued)*

Step	Action
2	Watch the **Instrument Condition** pane (for voltage, current, temperature, etc.), **Events** pane, and **Error** pane. 
3	After about 1.5 hours, click the **Array View** sub-tab to see data. **Note** Do not leave the **Array View** page open overnight. This will result in a scrambled screen. 
4	Click the **Capillary View** sub-tab to view each capillary.
5	Click the **CCD View** sub-tab to view the CCD image.
6	If any of the data is unsatisfactory, click the **Stop Run** button. **Note** When the run is complete, you may view the data in GeneScan Analysis software. See Chapter 8, “Data Analysis,” for more information.

Performing a Run on the 3100 Instrument

4

Overview

In This Chapter

The following topics are covered in this chapter:

Before You Begin

Before Performing a Run

Before performing a run, make sure:

- The computer and the ABI PRISM® 3100 Genetic Analyzer have been correctly configured.
- The instrument has been calibrated: spatial and spectral calibrations have been successfully run.
- There is sufficient space on the computer hard drive to store the data that will be generated.
- There is sufficient polymer, water, and buffer to carry out the required runs.

Preparing Samples

Overview

This section briefly discusses changes in sample preparation for the ABI PRISM® 3100 Data Collection Software. For complete information about sample preparation, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834).

Dye Set

The ABI PRISM Linkage Mapping Set v2.5 uses Dye Set G5 with the following dyes:

- 6-FAM™
- VIC™
- NED™
- PET™

  PET is available to label custom-synthesized markers specified by the user.
- LIZ™

Pooling Ratios

The pooling ratio is the amount of each dye-labeled product added with respect to the other products in the pool. Because the fluorescent dyes are detected with different efficiencies, the pooling ratio must be adjusted to ensure appropriate detection of all the loci.

Pooling Ratios for the Linkage Mapping Set v2.5

Linkage Mapping Set v2.5	Dye-Labeled Product	Pooling Ratio
HD5	6-FAM:VIC:NED	1:1:2
MD10		

For each Linkage Mapping Set v2.5 panel, pool 1 µL of each PCR product in a microcentrifuge tube. If necessary, bring the total volume to 20 µL with deionized water.

Preparing Samples for Loading

Use these ratios of pooled PCR products and size standards as a starting point only. Optimize these ratios, as necessary, based on your experimental results.

To prepare samples for loading:

Step	Action
1	Prepare the formamide:size standard mix using: ♦ 900 µL of Hi-Di™ formamide (P/N 4311320) or similar quality formamide ♦ 50 µL of GeneScan™-500 LIZ™ Size Standard **⚠ WARNING CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	For loading, mix 0.5 µL of pooled PCR products with 10 µL of formamide:size standard mix.

Denaturing Samples

To denature the samples:

Step	Action
1	Heat samples at 95 °C for 5 min. There are several acceptable options for covering samples during denaturation:
2	Place immediately on ice for at least 5 min before loading.

Part	P/N
MicroAmp® Clear Adhesive Films	4306311
MicroAmp® Caps (12-Strip)	N8010534
MicroAmp Caps (8-Strip)	N8010535
MicroAmp® Optical 96-Well Reaction Plates	N8010560
MicroAmp® 384-Well Reaction Plates	4305505

Creating a Plate Record

About Plate Records

Plate records are data tables in the instrument database that store information about the plates and the samples they contain.

Note A plate record is similar to a sample sheet or an injection list that you may have used with other ABI PRISM instruments.

Using the Plate Editor to Create a Plate Record

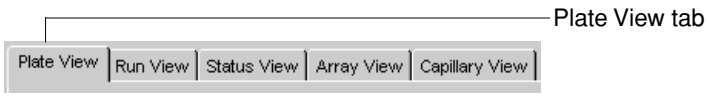
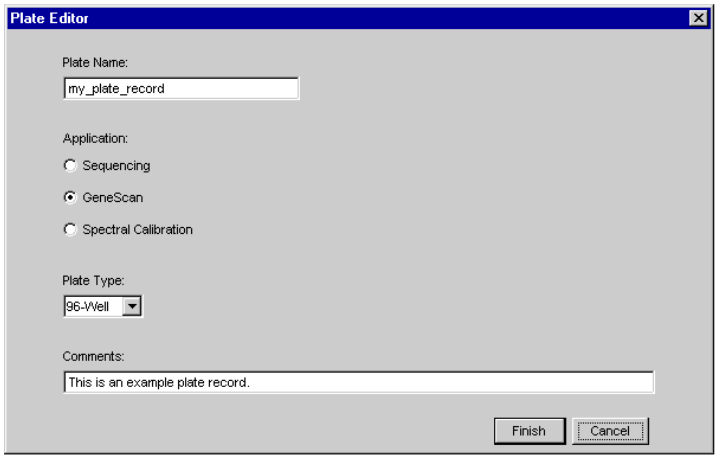
Follow the two procedures below to create a plate record with the Plate Editor.

See the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834) for other ways to create plate records and for information about importing and exporting plate records.

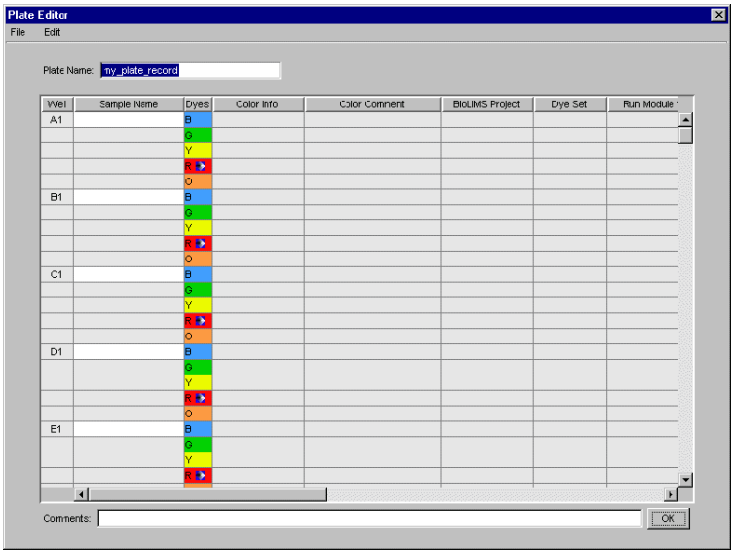
Entering Plate Record Information

Note You cannot create a plate record while a run is in progress.

To enter plate record information:

Step	Action
1	Click the **Plate View** tab on the **3100 Data Collection Software** window to go to the **Plate View** page. 
2	In the **Plate View** page, click **New**. Or, double-click the **Plate Editor** button on the toolbar.  The **Plate Editor** dialog box opens. 

To enter plate record information: *(continued)*

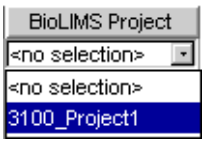
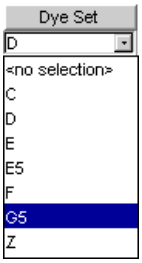
Step	Action
3	Use the **Plate Editor** dialog box to name your plate and to specify the application and plate type. Entering comments is optional. In the **Plate Editor** dialog box: a. Name your plate. b. Specify the application. c. Select the plate type. d. Enter any comments (optional). **IMPORTANT** When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces.
4	When done, click **Finish**. The **Plate Editor** spreadsheet displays. 

Entering Sample Information

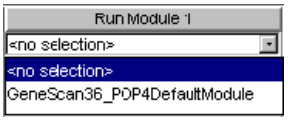
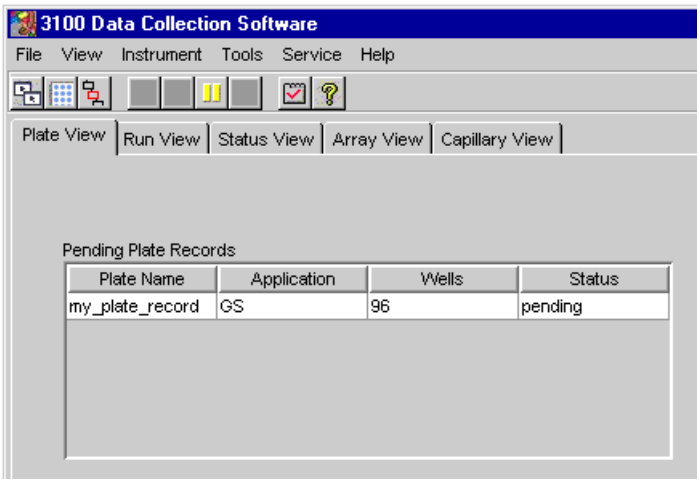
To enter sample information and save the plate record:

Step	Action
1	In the **Plate Editor** spreadsheet, type the names of all the samples in the **Sample Name** column. (Use **Edit/Copy** and **Edit/Fill Down** whenever a field is the same for all samples in the plate record.) **IMPORTANT** When naming the samples, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces. **IMPORTANT** Be sure that sample file names are not longer than 55 characters. An underscore separates each preference selected, so be sure to count the underscore in the total number of characters. There is no automatic error checking for sample names that exceed this limit. Sample files with long names cannot be opened by the analysis software. **Note** When running LIZ as the size standard, be sure to select **Orange** in the dyes box.

To enter sample information and save the plate record: *(continued)*

Step	Action
2	*Optional:* For each sample, enter **Color Info** and **Color Comment** text. **Note** If you are using ABI PRISM® GeneScan® Analysis Software or ABI PRISM® Genotyper® Software, the **Sample Name** field is not used. It is recommended that you put the sample name in the **Color Info** or **Color Comment** field**.**
3	Enter a BioLIMS® project. **IMPORTANT** A BioLIMS project is required for every sample, even if a BioLIMS database is not used. a. Click in the **BioLIMS Project** cell for Well A1. b. Select a project name from the drop-down list.  **Note** For more information about setting up a BioLIMS project, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834). c. To assign the same project name to each sample in the plate record: – Click the column header to select the whole column. – Press **Ctrl+D** or select **Edit/Fill Down**. **Note** Press **Ctrl+D** or select **Edit/Fill Down** whenever a field is the same for all samples in the plate record.
4	For each sample, select the appropriate **Dye Set** from the drop-down list. Linkage Mapping Set v2.5 requires the G5 matrix.  **IMPORTANT** Be sure to select the correct dye set for your run(s). Data collected with the incorrect dye set selected cannot be saved, and the runs will have to be repeated because multicomponenting is applied during collection.

To enter sample information and save the plate record: *(continued)*

Step	Action
5	For each sample, select the appropriate **Run Module** from the drop-down list.  For Linkage Mapping Set v2.5, use the default module. **Note** If you need to view or edit a run module file, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834). **Note** If you select different modules for different samples, the samples will be automatically grouped so that all samples with the same run module are run at the same time. **IMPORTANT** Runs are scheduled alphanumerically by run module name, not by the order indicated in the plate record, nor by sample name.
6	For each sample, select the appropriate **Analysis Module** from the drop-down list. **IMPORTANT** The **AutoAnalysis ON** preference must be selected if analysis is to take place automatically after the run.
7	If you want to run the same sample again, select a second run module and a second analysis module. You can run a sample in a linked plate up to five times. Samples will be automatically grouped so that all samples with the same run module are run sequentially.
8	Make sure the plate record is correct, and then click **OK**. **Note** It may take a moment for the new plate record to be saved to the database and added to the **Pending Plate Records** table as shown below. **Note** The plate record must be deleted from the database first, in order to use the same name for another plate record. 

Linking and Unlinking a Plate

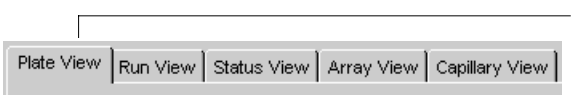
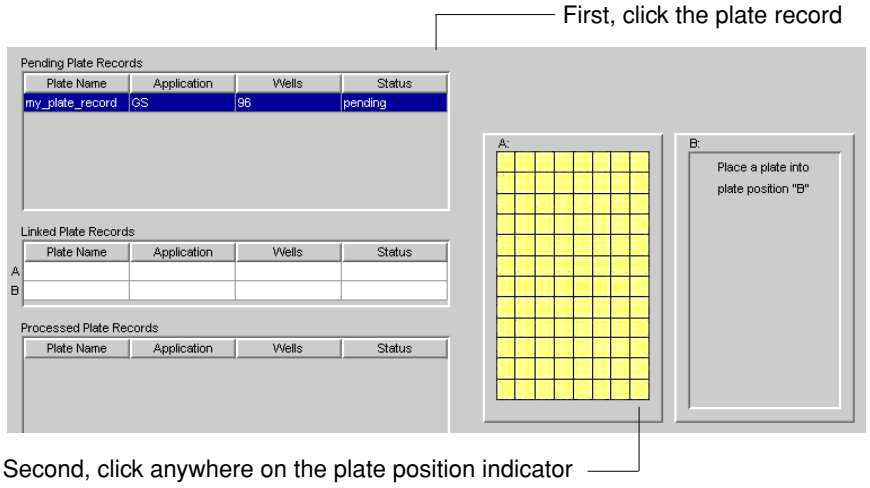
Overview

The procedure below describes how to link a plate on the autosampler to the plate record you have created. This must be done before a plate can be run.

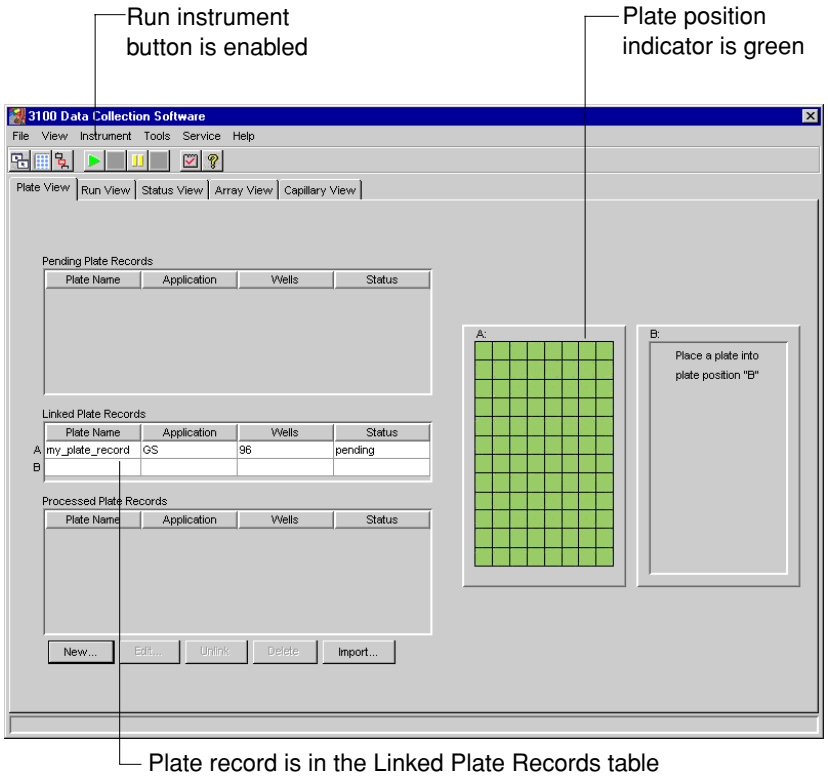
IMPORTANT A plate can be linked even if there are no run modules selected for its samples. In this case, there is no error message, and runs for samples in the plate will not be scheduled.

Linking a Plate to a Plate Record

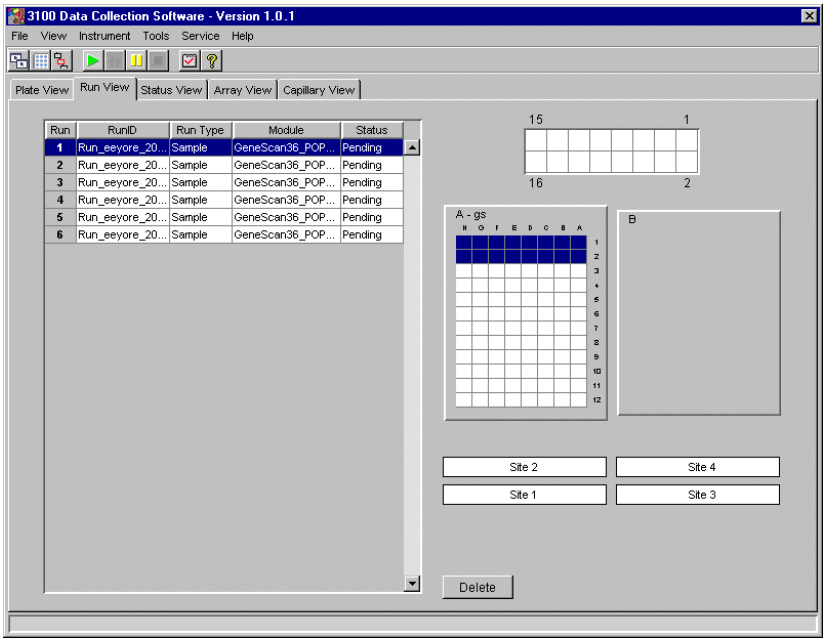
To link a plate to a plate record:

Step	Action
1	Click the **Plate View** tab on the **3100 Data Collection Software** window to go to the **Plate View** page. 
2	On the **Plate View** page: a. In the **Pending Plate Records** table, click the plate record for the plate you are linking. b. Click the plate position indicator that corresponds to the plate you are linking. 

To link a plate to a plate record: *(continued)*

Step	Action
3	Verify that the plate has been linked. Once the plate has been linked: ♦ The **Run Instrument** button on the toolbar is enabled, meaning that the instrument is ready to run. ♦ The plate position indicator for the linked plate becomes green. ♦ The plate record moves from the **Pending Plate Records** table to the **Linked Plate Records** table. 
4	Repeat steps 1 to 3 to link a second plate, if applicable.

To link a plate to a plate record: *(continued)*

Step	Action
5	Click the **Run View** tab to view the run schedule. **Note** Although individual runs can be deleted, the order in which the runs are scheduled cannot be altered. Run scheduling depends upon a number of factors; see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834) for information. 

Unlinking a Plate Record

To unlink a plate record:

Step	Action
1	In the **Linked Plate Records** table of the **Plate View** page, select the plate record that you want to unlink.
2	Click **Unlink**. **If the plate record is...** / **Then the plate record will...** completed — go to the **Processed Plate Records**. not completed — return to the **Pending Plate Records** table, and the plate position indicator will return to yellow.

Starting and Monitoring the Run

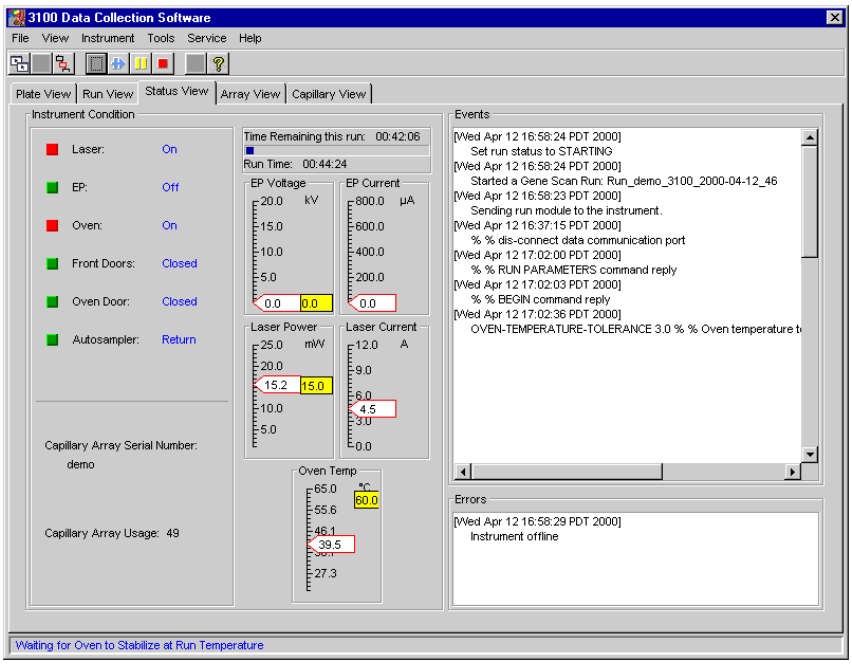
Starting a Run

To start a run:

Step	Action
1	Click the green **Run Instrument** button to begin the scheduled runs.  A run using GeneScan_POP4DefaultModule takes approximately 45 min.

Monitoring a Run

To monitor a run:

Step	Action
1	Click the **Status View** tab to monitor the status of the instrument during the run. 
2	During the run, you can view the data using the **Array View** and **Capillary View** pages. **IMPORTANT** Always exit from the **Array View** and the **Capillary View** windows. Do not leave these windows open for extended periods during a run because unrecoverable screen update problems will occur. Leave the **Status View** window open. For more information about the Array and Capillary views, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834). **Note** When the run is completed you may view the data in GeneScan Analysis software. See Chapter 8, "Data Analysis," for more information.

Electrophoresis on the 377 Instrument

5

Overview

In This Chapter

The following topics are covered in this chapter:

List of Procedures

To perform electrophoresis on pooled PCR products using the ABI PRISM® 377 DNA Sequencer, you need to:

- Prepare a gel
- Set up the sequencer
- Create a sample sheet and run file
- Pre-run the gel
- Denature the pooled PCR products
- Prepare matrix standard samples (if required)
- Load PCR products onto the gel

Site Preparation and Safety

Safety Information

For information on the safe operation of the 377 DNA Sequencer, refer to the *ABI PRISM 377 DNA Sequencer Site Preparation and Safety Guide* (P/N 903393).

A few of the sections covered in the site preparation and safety guide are noted below.

Site Preparation

- Preinstallation checklist
- Items shipped with the instrument

Instrument Safety

- Instrument safety user attention words
- Safety alert symbols

Chemical Safety

- Chemical hazard warnings
- MSDSs for chemicals manufactured or distributed by Applied Biosystems
- Waste profiles

Software Requirements

Software for the 377 Instrument

Standard ABI PRISM 377 DNA Sequencer

- ABI PRISM® 377 Data Collection Software version 3.0 or later
- ABI PRISM® GeneScan® Analysis Software version 3.7 or later
- G5 modules

Software for the 377-XL Instrument

ABI PRISM® 377 DNA Sequencer with XL Upgrade

- ABI PRISM® 377 XL Data Collection Software version 3.0 or later
- ABI PRISM GeneScan Analysis Software version 3.7 or later
- G5 modules

Software for the 377-96 Instrument

ABI PRISM® 377 DNA Sequencer with 96-Lane Upgrade

- ABI PRISM 377 Data Collection Software version 3.0 or later
- ABI PRISM GeneScan Analysis Software version 3.7 or later with Gel Processor.
- G5 modules

Preparing a Gel

Overview

Prepare the gel, plate, and cassette as described in the following documents:

- *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307164)
- *ABI PRISM 377 DNA Sequencer User Guide for Data Collection Software on the Windows NT Platform* (P/N 4325703)
- *ABI PRISM GeneScan Analysis Software for the Windows NT Platform User Guide* (P/N 4308923)

Gel Recommendations

The gel should be:

- 36-cm well-to-read
- 5.0% Long Ranger gel solution
- Cast with either a square or shark's-tooth comb
- Allowed to polymerize for at least 2 hours

Note Although we recommend using a 5.0% Long Ranger gel, a 4.25% polyacrylamide gel can also be used.

⚠ WARNING CHEMICAL HAZARD. Long Ranger gel solution (containing acrylamide) is harmful if in contact with the skin or if swallowed. Acrylamide may cause eye, skin and respiratory tract irritation. It may also cause an allergic reaction. Exposure may cause damage to the nervous system, kidneys and reproductive system. Acrylamide is a possible cancer and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Setting Up the ABI PRISM 377 Instrument

Overview

This section contains instructions for setting up the sequencer. More detailed instructions are located in the instrument user guide.

- Preparing and mounting the gel
- Creating a sample sheet
- Configuring the Run window to create a Run file
- Performing a plate check
- Completing instrument setup
- Prerunning the gel

Preparing and Mounting the Gel

To set up the 377 instrument:

Step	Action
1	Install the lower buffer chamber.
2	Prepare the gel for mounting onto the instrument as follows: a. Remove the casting comb. b. Remove any polymerized gel from the notch between the plates above the wells. c. If applicable, insert a shark's-tooth comb approximately 2 mm into the gel. d. Clean the glass plates thoroughly, particularly the read region, with deionized water and dry with lint-free wipes.
3	Mount the gel cassette in the electrophoresis chamber as described in the instrument user guide. **Note** If properly positioned, you will see the two positioning pins through the plates and spacers of the cassette.
4	Launch the ABI PRISM 377 Data Collection software.
5	Create a Sample Sheet by following the instructions that follow.

Sample Sheet Overview

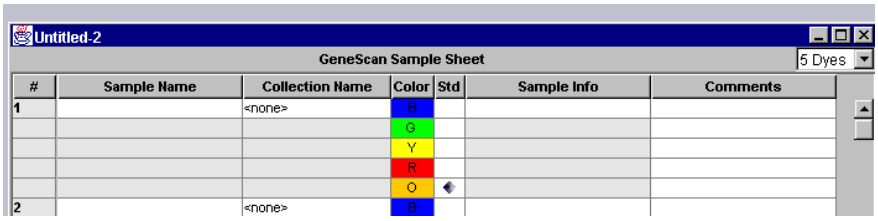
Before beginning a GeneScan run on the ABI PRISM 377 DNA Sequencer, you must create a Sample Sheet and configure a Run window to generate a Run file. The Run file associates sample information with each lane in the gel as it has been entered onto the Sample Sheet.

If matrix standard samples are also being run, information for these samples must be included in the Sample Sheet as well. Instructions for preparing matrix standard samples are listed in Chapter 7, "Matrix Sample and Matrix File Guidelines." Instructions for creating matrix files are located in the following documents:

- Your instrument user guide
- *GeneScan Reference Guide for the ABI PRISM 377 and ABI 373 DNA Sequencers* (P/N 4303188)
- *ABI PRISM GeneScan Analysis Software for the Windows NT Platform User Guide* (P/N 4308923)

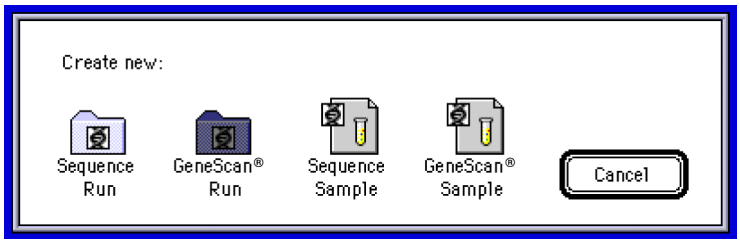
Creating a Sample Sheet

To create a sample sheet:

Step	Action
1	From the **File** menu select **New**.
2	Click the **GeneScan Sample** icon.
3	Complete the sample sheet as follows. For tracking to be as accurate as possible, enter data for the lanes that will be used for that particular run only.  a. Enter the sample names in the **Sample Name** column in the exact order the samples will be loaded onto the gel. An entry must be made in this column for the sample to appear in the **Run** file. The sample name is assigned to the entire lane, so it includes all four sample/dye combinations in the lane. b. For DNA samples only, specify orange **(O)** in the **standard (Std)** column by clicking in the box to the right of letter **O** in the **Color** column. When selected, a diamond is displayed in the box. Do not specify O for matrix standard samples. c. Enter the sample name for each dye used, and any additional information into the **Sample Info** column. Making an entry automatically selects the corresponding check box in the **Pres** (Present) column. **Note** If these fields are left blank, only the words "sample file" will appear in the GeneScan data imported into Genotyper software. The sample info and sample comment fields will be copied into Genotyper software and can be useful for sample identification and sorting. d. If not selected automatically in step c above, select each check box in the **Pres** (Present) column for which a sample with that dye color will be loaded. (B = blue; G = green; Y = yellow; R = red; O = orange) e. If matrix standard samples will be included in the run, proceed to step 4. If not, proceed to step 5.
4	If matrix standard samples will be included in the run, enter a name for each matrix standard sample in the **Sample Name** column field that corresponds to the lane in which each matrix sample will be loaded. ♦ Leave at least one empty lane between the DNA samples and the matrix standard samples. ♦ Load the matrix standard samples into every other lane.
5	From the **File** menu, select **Save As**. Name the sample sheet, and press **Return** to save it in the **Sample Sheets** folder.

Configuring the Run Window

To configure the run window:

Step	Action
1	From the **File** menu select **New**. The following box of icons is displayed. 
2	Click **GeneScan Run** to display a new Run window and create a Run file.
3	Set the parameters in the Run window. **Note** The default settings are for four-dye runs. To select five-dye modules, you must import a five-dye sample sheet.

Parameter	Setting
Plate Check Module	Plate Check G5
PreRun Module	GS PR 36G5-2400
Run Module	GS 36G5-2400
Collect time	2.5 hr
Sample Sheet	The sample sheet created for this run
Well-to-Read distance	36 cm
Gel's Matrix File	Either the matrix file created for this instrument using these modules and the matrix standard kits listed on page 7-2, or **`Bogus matrix`** if a matrix file must be created
Operator	Your name
Lanes	Open the pop-up menu, and select the appropriate number of lanes.
Matrix File	Same as Gel's Matrix File above
Auto Analyze	Turn Auto Analyze off by deselecting the boxes in the Auto Analyze column.
Run Mode (XL Upgrade only)	Open the pop-up menu, and select the appropriate run mode.

Performing a Plate Check

To perform a plate check:

Step	Action
1	Click **Plate Check**. The Scan window is displayed. Watch the scan for approximately 1 min. If the plates are clean, the scan lines will be flat and above the baseline with no spikes in any colors.
2	**If the plates are...** / **Then...** clean — go to the next step. not clean — perform the following before continuing a. Clean the plates as described in the *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307164). b. Repeat the Plate Check. c. Continue to the next step when the plates are clean.
3	Click **Cancel**, and select **Terminate** when prompted to cancel the plate check.

Completing the Instrument Setup and Prerunning the Gel

To complete instrument setup and pre-run the gel:

Step	Action
1	Install the upper buffer chamber.
2	Fill the buffer chambers and check for leaks.
3	Connect the front heat-transfer plate and electrode cables.
4	Flush the wells with buffer loaded in a syringe to remove air bubbles. **Note** A flat gel loading tip attached to the end of the syringe works well.
5	If you are using a square-tooth comb, pipet approximately 10.0 µL blue dextran/EDTA across the top of the lanes. This will mark the wells and make sample loading easier.
6	Close the instrument door.
7	Click **PreRun**.
8	Open the **Window** menu and select **Status**. Check the information in the **Status** window to make sure the PreRun is executing as expected.
9	Pre-run the gel a minimum of 10 min, a maximum of 1 hr. The gel temperature must be 38 °C or higher before you load the samples.

Preparing the Loading Cocktail and Denaturing the Samples

About Preparing the Loading Cocktail

To improve reproducibility of results for all samples, we recommend preparing a larger volume of loading cocktail using the 5:1:1 ratio of reagents listed in the table below for four reactions.

IMPORTANT The loading cocktail for the ABI PRISM 310 instrument differs from the one prepared for the ABI PRISM 377 instrument, and should not be used on this instrument.

Preparing the Loading Cocktail

⚠ WARNING CHEMICAL HAZARD. Formamide is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Loading Cocktail Ingredients

Ingredient	Volume for 1 Reaction (µL)	Volume for 100 Reactions (µL)
Deionized formamide	2.5	250
Blue dextran/EDTA Loading Buffer	0.5	50
GeneScan™-500 LIZ™ Size Standard	0.5	50

To prepare the loading cocktail:

Step	Action
1	Based on the list of ingredients shown above, mix the appropriate amounts of: ♦ Deionized formamide ♦ Blue dextran/EDTA loading buffer ♦ GeneScan–500 LIZ Size Standard
2	Gently vortex the mixture for 3 to 5 sec.
3	Store at 2 to 8 °C for up to 2 weeks. Use for sample loading as needed.

Denaturing the Samples

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

To denature your samples:

Step	Action
1	Label the reaction tubes (optional), and place them in the reaction tray.
2	Load 3.0 µL of loading cocktail to each tube. **IMPORTANT** Do **not** add loading cocktail to matrix standard samples.
3	Load 2.0 µL of each pooled PCR product into a reaction tube, one pooled product per tube.
4	If you are running matrix standard samples, prepare the samples now by following the instructions in Chapter 7, “Matrix Sample and Matrix File Guidelines,” or on the product inserts, and place them in the reaction tray.
5	Cap each tube and vortex briefly.
6	Spin down the contents of the tubes, and load them onto the thermal cycler or heat block.
7	Denature the samples at 95 °C for 5 min. **Note** Matrix standard samples can be denatured using these conditions even though they differ from the conditions listed on the matrix standard samples product insert. Product performance will not be affected.
8	Remove samples from the thermal cycler or heat block and snap cool them in an ice water bath before loading them onto the gel. **IMPORTANT** To prevent samples from re-annealing, we recommend loading the gel as soon as the samples have cooled.

Loading the Gel and Starting the Run

Overview

In this section, two sets of loading instructions are provided:

- Loading the standard 377 instrument
- Loading the 377 instrument with the XL upgrade

Loading the Gel on the Standard 377 Instrument

To load samples onto the standard 377 instrument:

Step	Action
1	Once the gel temperature is 38 °C or higher, either open the instrument door, or click **Pause** in the **Run** window. By pausing the PreRun rather than cancelling it, the gel temperature is maintained.
2	Flush the wells with buffer.
3	<table><tr><th>If using a…</th><th>Then…</th></tr><tr><td>square-tooth comb</td><td>load 2.5 µL of sample into each well, one sample per well.</td></tr><tr><td>shark's-tooth comb</td><td>select Loading Method 1 or 2 below. **Loading Method 1** Load 2.5 µL of sample into every other lane, one sample per lane. **Loading Method 2** a. Load 2.5 µL of sample into the odd numbered lanes, one sample per lane. b. Start the run as described below. c. After 3 min, click **Pause** to pause the PreRun. d. Load 2.5 µL of the remaining samples into the even lanes, one sample per lane.</td></tr></table>
4	If loading matrix standard samples, we recommend that you: ♦ Leave at least one empty lane between other samples and the matrix standard samples ♦ Load the matrix standard samples into every other lane This loading technique will help ensure a clean matrix is produced. The quality of the matrix file directly effects the quality of the analyzed data.
5	Click **Cancel** to cancel the PreRun. **IMPORTANT** Do **not** click **Resume**. If you click **Resume**, the PreRun will continue, and no data will be collected.
6	Proceed to "Starting the Run" on page 5-12.

Loading the Gel on the 377-XL Instrument

To load samples onto the 377 instrument with the XL upgrade:

Step	Action
1	Once the gel temperature is 38 °C or higher, click **Pause** in the **Run** window. By pausing the PreRun rather than cancelling it, the gel temperature is maintained.
2	Flush the wells with buffer.
3	Follow this table for load volumes: **If loading…** / **Then load…** 50 wells / 1.0 to 1.5 µL sample into each lane. 66 wells / 0.5 to 1.0 µL sample into each lane.
4	If loading matrix standard samples, we recommend that you: ♦ Leave at least one empty lane between other samples and the matrix standard samples ♦ Load the matrix standard samples into every other lane This loading technique will help ensure a clean matrix is produced. The quality of the matrix file directly effects the quality of the analyzed data.
5	Click **Cancel** to cancel the PreRun. **IMPORTANT** Do not click **Resume**. If you click **Resume**, the PreRun will continue, and no data will be collected.
6	Proceed to "Starting the Run" below.

Starting the Run

To start the run:

Step	Action
1	If the PreRun has not already been cancelled, click **Cancel** to cancel the PreRun. **IMPORTANT** Do not click **Resume**. If you click **Resume**, the PreRun will continue, and no data will be collected.
2	Click **Run** to start the GeneScan run.
3	Enter a name for the gel file and click **OK**.
4	Open the **Window** drop-down menu, and select **Status** to open the **Status** window.
5	Review the information in the **Status** window to confirm the run is proceeding correctly. If an error is found: a. Cancel the run. b. Refer to the troubleshooting section for the ABI PRISM 377 DNA Sequencer in the *GeneScan Reference Guide for the* ABI PRISM *377 and ABI 373 DNA Sequencers* (P/N 4303188), and correct any problems. c. Reset the Run parameters if necessary. d. Restart the run. **Note** When the run is completed you may review your data in GeneScan Analysis software. See Chapter 8, "Data Analysis," for more information.

Electrophoresis on the 310 Instrument

6

Overview

In This Chapter

The following topics are covered in this chapter:

List of Procedures

To perform electrophoresis on pooled samples using the ABI PRISM® 310 Genetic Analyzer, you need to:

- Set up the genetic analyzer
- Prepare matrix standard samples (if necessary)
- Denature the samples
- Load samples onto the autosampler
- Start the run by:
  - Selecting or creating a sample sheet
  - Setting up an injection list

Software Required

Software for the 310 Instrument

To determine which software to use, see the table below:

Software	Macintosh Version	NT Version
ABI PRISM® 310 Firmware	1.0.3 or later	1.0.3 or later
ABI PRISM® 310 Data Collection Software	2.1	3.0
ABI PRISM® GeneScan® Analysis Software	3.1.2 or later	3.7
G5 modules	GS STR POP4 (1 mL) G5	GS STR POP4 (1 mL) G5

Note This chapter uses the Microsoft® Windows NT® operating system as the reference. However, when using the Macintosh® operating system, the steps are the same. For specific information and examples for the Macintosh operating system, refer to the *ABI PRISM 310 Genetic Analyzer User Guide* (P/N 4317588).

Preparing the Genetic Analyzer

Overview

The following general guidelines and instructions are for setting up the Genetic Analyzer. Detailed instructions are included in the ABI PRISM® 310 Genetic Analyzer user bulletin, *POP-4 Polymer Sequencing Protocols for the 310 Genetic Analyzer* (P/N 4327927), and in the *ABI PRISM 310 Genetic Analyzer User Guide* (P/N 4317588).

Replacement and Cleaning Guidelines

Perform the following procedures every 100 injections or as otherwise noted:

- Clean the syringe
- Clean the pump block every 2 to 3 days or when the polymer is changed
- Reload syringe with fresh polymer
- Replace the capillary
- Recalibrate the autosampler if:
  - the electrode is replaced or removed from the instrument to clean
  - the capillary is replaced

Replacing and Cleaning Parts

Refer to “Replacement and Cleaning Guidelines” above for recommendations on when to replace and clean parts.

To replace and clean parts:

Step	Action
1	If necessary, replace the syringe on the instrument with a 2.5-mL GeneScan glass syringe or a 1.0-mL glass syringe.
2	Clean the syringe with deionized water if necessary. We recommend cleaning the syringe every time fresh polymer is loaded.
3	Clean the pump block with deionized water and dry thoroughly, particularly the metallic screws.
4	Reset the Syringe Max Travel parameter if the syringe has been replaced and is a different size (2.5-mL to 1.0-mL or vice versa).
5	Install a new capillary if necessary.
6	If a new capillary is installed, reset the capillary injection counter.
7	Recalibrate the autosampler if: ♦ The electrode was replaced. ♦ The electrode was removed from the instrument for cleaning. ♦ The capillary was replaced.

Loading the Reagents

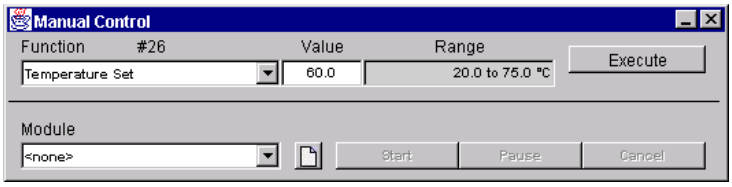
To load the reagents:

Step	Action
1	Load the syringe with polymer.
2	Dilute 1.5 mL of 10X Genetic Analyzer buffer with EDTA to a 1X concentration (15.0 mL) with deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Prime the pump block to remove air bubbles.
4	Fill the anode buffer reservoir to the red line with 1X Genetic Analyzer buffer and install it on the pump block. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
5	Label one of the buffer vials as **`Buffer`** and fill it to the line with 1X Genetic Analyzer buffer. Cap the vial, insert the septum, and place it in position 1 on the autosampler.
6	Label the other glass buffer vial as **`H2O`** and fill it to the line with deionized water. Cap the vial, insert the septum, and place it in position 2 on the autosampler.
7	If the 1.5-mL microcentrifuge tube has a lid attached, cut off the lid. Fill the tube with deionized water and place it in position 3 on the autosampler.

Preheating the Genetic Analyzer

Preheating the genetic analyzer at this point in the procedure is optional and is suggested to save time. The instrument must preheat to 60 °C before the first injection occurs. Preheating can take up to 20 min.

To preheat the genetic analyzer:

Step	Action
1	From the **Window** drop-down menu, choose **Manual Control**. The **Manual Control** window is displayed. 
2	Choose T**emperature Set** from the **Function** menu, and set the temperature to **60** in the **Value** window.
3	Click **Execute**. The instrument preheats to 60 °C. **Note** The door on the ABI PRISM 310 instrument must be closed for the instrument to preheat. You can perform the next procedure, “Preparing the Samples for Loading” on page 6-5, while the instrument preheats.

Preparing the Samples for Loading

About Preparing the Formamide–Size Standard Mix

To improve reproducibility of results for all samples, prepare a larger volume of formamide–size standard mix using the 24:1 ratio of reagents stated in the procedure below.

IMPORTANT The formamide–size standard mix for the ABI PRISM 310 instrument differs from the loading cocktail prepared for the ABI PRISM 377 instrument. The loading cocktail for the 377 instrument should not be used on 310 instruments.

Preparing the Formamide–Size Standard Mix

⚠ WARNING CHEMICAL HAZARD. Formamide is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Ingredient	Vol. for 1 Reaction (µL)	Vol. for 50 Reactions (µL)	Vol. for 100 Reactions (µL)
Deionized formamide	12.0	600	1200
GeneScan™–500 LIZ™ Size Standard	0.5	25.0	50.0

To prepare the formamide–size standard mix:

Step	Action
1	Based on the list of ingredients above, mix the appropriate amounts of formamide and size standard.
2	Vortex gently for 3 to 5 sec, and centrifuge briefly.
3	Store at 2 to 8 °C for up to 2 weeks. Use for sample loading as required.

Denaturing the Samples

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

To denature your samples:

Step	Action
1	Load 1.0 µL of pooled PCR product into a reaction tube, one product per tube.
2	Add 12.0 µL formamide-size standard mix to each tube. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **IMPORTANT** Do not add size standard mix to matrix standard samples.
3	If you are running matrix standard samples, prepare the samples now by following the instructions in Chapter 7, “Matrix Sample and Matrix File Guidelines,” or on the product inserts, and place them in the reaction tray.
4	Cap each tube. **IMPORTANT** If using a 96-well tray, do not seal the tubes with the septa because the septa will melt inside the thermal cycler.

To denature your samples: *(continued)*

Step	Action
5	Spin down the contents of the tubes, and load them onto a thermal cycler or heat block.
6	Denature the samples as follows: Heat to 95 °C for 5 min. **Note** PCR products in 0.5-mL reaction tubes can be sealed with septa and denatured on the GeneAmp® PCR System 9600, or 9700 thermal cycler even though most of the tube will stick up out of the tray. **Note** Matrix standard samples can be denatured using these conditions even though the conditions differ from those listed on the product insert. Product performance will not be affected.
7	Remove samples from the thermal cycler or heat block, and snap cool them in an ice water bath.

Performing a Run

About Creating a Sample Sheet

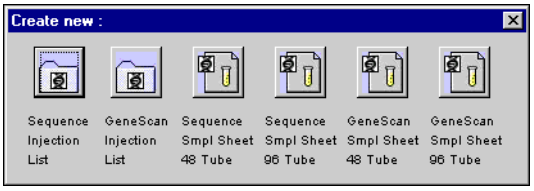
Before beginning a GeneScan run on the 310 Genetic Analyzer, you must create a Sample Sheet and Injection List to generate a Run file. The Injection List associates sample information with each tube position in the autosampler as it is entered on the Sample Sheet. Therefore, the position number assigned to a sample on the Sample Sheet (Figure 6-1 on page 6-9) must be the same as the location of the sample on the autosampler.

If matrix standard samples are also being run, information for these samples must be included on the Sample Sheet. Instructions for preparing matrix standard samples are listed in Chapter 7, “Matrix Sample and Matrix File Guidelines.” Instructions for creating matrix files are located in the following documents:

- Your instrument user guide
- *GeneScan Reference Guide for the ABI PRISM 310 Genetic Analyzer* (P/N 4303189)
- *ABI PRISM GeneScan Analysis Software Version 3.7 for the Windows NT Platform User Guide* (P/N 4308923)

Creating a Sample Sheet

To create a sample sheet:

Step	Action
1	Open the **File** menu and select **New**.
2	Click **GeneScan Smpl Sheet 48** or **96 Tube** as appropriate. 

To create a sample sheet: *(continued)*

Step	Action
3	Complete the Sample Sheet (Figure 6-1 on page 6-9) as follows: a. Enter sample names in the **Sample Name** column. The number of the sample in the list (A1, A3, etc.) must correspond to the sample position in the autosampler. **IMPORTANT** The position number assigned to a sample on the Sample Sheet must correspond to the location of the sample on the autosampler. The instrument will pause if the autosampler finds an empty position designated by the sample sheet to contain a sample. The instrument will remain paused until the error is corrected. b. For DNA samples only (not matrix standard samples), specify orange (O) in the size **standard column (Std)** by clicking in the box to the right of letter O in the **Color** column. **Note** Size standard selection can be preset in **Preferences** under GeneScan Sample Sheet Defaults. c. Select the boxes in the **Pres** (Present) column that reflect the dyes present in that sample position (B = blue; G = green; Y = yellow; R = red; O = orange). Check boxes must be selected for samples to be analyzed automatically. d. If desired, enter additional information in the **Sample Info** column. The **Sample Info** field will be transferred to Genotyper® software and can be very useful for sample identification and sorting. e. If matrix standard samples are to be included in the run, proceed to step 4. If not, proceed to step 5.
4	If matrix standard samples are to be included in the run, enter them onto the sample sheet following these guidelines: a. Enter a name for each matrix standard sample in the **Sample Name** column in the field that corresponds to the tube position in the autosampler. b. Select only the box in the **Pres** column that corresponds to the color of the matrix standard loaded in that position (*e.g.* for 6-FAM™ select the **Pres** box that corresponds to B [blue] only).
5	Open the **File** pull-down menu, and select **Save As**. Name the sample sheet, and press **Return** to save it in the **Sample Sheets** folder.

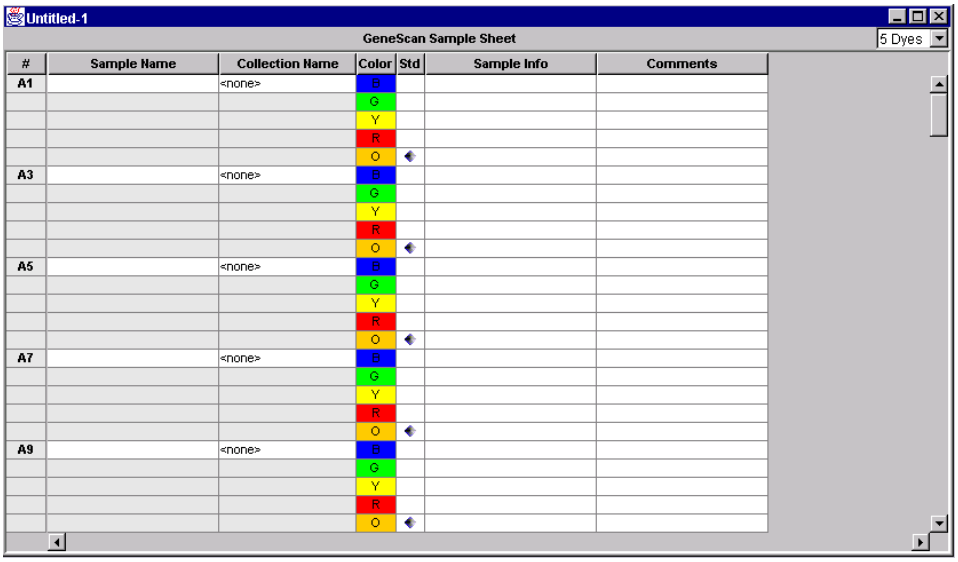
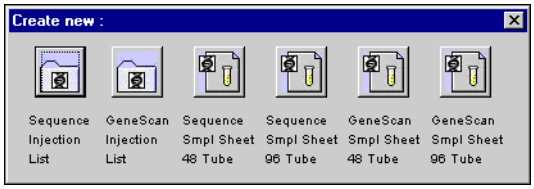


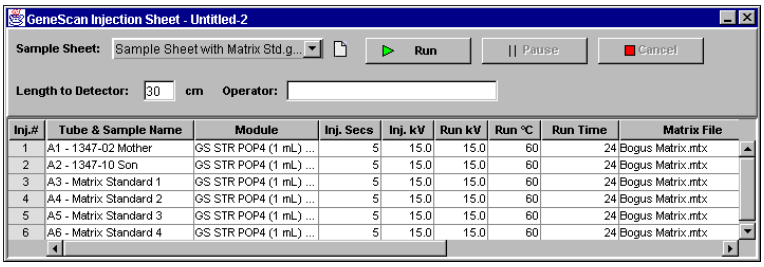
Figure 6-1 Example of a 48-Tube Sample Sheet

Starting the Run

To start the run:

Step	Action
1	If necessary, transfer the denatured samples to a 48- or 96-well tray. **IMPORTANT** The tube arrangement and order of the samples on the sample sheet must be the same.
2	Seal each tube with a septum, and place the tray into the autosampler.
3	Launch the ABI PRISM 310 Data Collection Software.
4	From the **File** menu, select **New** to display the following icons. 
5	Click **GeneScan Smpl Sheet 48** or **96 Tube** as appropriate.
6	Create a sample sheet.
7	From the **File** menu select **New**.
8	Click **GeneScan Injection List**.

To start the run: *(continued)*

Step	Action
9	Complete the Injection List as follows: a. Open the Sample Sheet pop-up menu, and select the sample sheet created for this run. b. Enter your name in the **Operator** field. c. Open the Module pop-up menu for each sample, and select the appropriate run module GS STR POP4 (1 mL) G5. **Note** Five-dye modules are available only after a five-dye sample sheet has been imported. d. Open the Matrix file pop-up menu and select the appropriate matrix file. e. Leave the remaining parameters at their default settings. f. If a matrix file, size standard and analysis parameters have not been created/defined for the run conditions you are using, then deselect **Auto Analyze** for every sample. Otherwise, leave the **Auto Analyze** boxes selected. **Note** To automate completion of the Injection List, preset the GeneScan Analysis Run defaults. 
10	Click **Run**. Once the run is completed you may view your data in GeneScan Analysis software. See Chapter 8, “Data Analysis,” for more information.

Matrix Sample and Matrix File Guidelines 7

Overview

In This Chapter

The following topics are covered in this chapter:

Sample Kits

Required Kits

The following matrix standard sample kits are required for the ABI PRISM® Linkage Mapping Set v2.5:

DS33 (6FAM, VIC, NED, PET, LIZ)	P/N
Matrix standard set DS33 for the 3700 instrument	43275691 with CD[a] 43182541 without CD
Matrix standard set DS33 for the 3100 instrument	4323016
Matrix standard Set DS33 for the 310/377 instrument	4318159

a. The CD is five-dye updated for ABI PRISM® 3700 DNA Analyzer Data Collection Software versions 1.0, 1.1, and 1.1.1. The CD contains the necessary instructions and files for running five-dye chemistry (6-FAM™, VIC™, NED™, PET™, and LIZ™) on the 3700 DNA Analyzer.

Storage Conditions

- Store matrix standard sample kits at 2 to 8 °C for up to 6 months.
- Avoid repeated freeze/thaw cycles.

Matrix Files

Purpose of a Matrix File

The most intense fluorescence emitted by an ABI PRISM® dye will fall within a small wavelength detection range. However, some fluorescence emission in the detection ranges of the other dyes in a set always occurs. This is referred to as "spectral overlap." The multicomponent correction matrix (matrix file) compensates for this overlap. The matrix file subtracts from each dye detection range (collection window) all overlapping signal from the other dyes.

When a Matrix File Must Be Made

Matrix standard samples must be run and a matrix file must be created:

- The first time the Linkage Mapping Set v2.5 is used
- When any of the run conditions change

When a Matrix File Can Be Reused

Once a matrix file is created, it can be stored and used again for subsequent runs performed:

- On the same instrument
- Using the same run conditions as follows:
  - Run module
  - Dye set
  - Gel or polymer

Factors Affecting Matrix Quality

Environmental fluctuations can affect the quality of a matrix file. Examples of minor environmental fluctuations include:

- Changes in the polymer/buffer composition due to aging reagents
- Fluctuations in temperature when running the instrument at ambient temperature

Other factors that can affect matrix quality are:

- Minor misalignment in the instrument's optical detection system
- Imperfections in, or misalignment of, glass plates when using a gel

Preparing and Loading Matrix Standard Samples

Preparing Samples for the 3700 Instrument

To prepare matrix standards for the ABI PRISM® 3700 DNA Analyzer:

Step	Action
1	Vortex the contents of the matrix standard tube and spin down the liquid.
2	Combine 50 µL of the matrix standard with 350 µL of Hi-Di™ formamide. **⚠ WARNING CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Vortex briefly and spin down the liquid.
4	Separate the mixture into two tubes (200 µL per tube).
5	Denature for 5 min at 95 °C, and chill on ice.
6	Refer to the product insert for information on loading.

Preparing Samples for the 3100 Instrument

To prepare matrix standards for the ABI PRISM® 3100 Genetic Analyzer:

Step	Action
1	Vortex the contents of the matrix standard tube and spin down the liquid.
2	Combine 5 µL of the matrix standard with 195 µL of Hi-Di formamide. **⚠ WARNING CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Vortex briefly and spin down the liquid.
4	Aliquot 10 µL of the standard into each of 16 wells in a microtiter plate.
5	Denature for 5 min at 95 °C and chill on ice.
6	Refer to the product insert for information on loading.

Loading Volumes for the 377 Instrument

Loading volumes for the ABI PRISM® 377 DNA Sequencer:

System Platform	Hi-Di Formamide (µL)	Matrix Standard (µL)	Total Load Volume (µL)
377	1.5	1.5	2.0
377 XL-48/50	1.0	1.0	1.5
377 XL-64/66	1.0	1.0	1.0
377 96 Lanes	1.5	1.5	1.0

Preparing Samples for the 377 Instrument

To prepare matrix standard samples for the 377 instrument:

Step	Action
1	Vortex the contents of each matrix sample tube, and spin down to collect the contents at the bottom of each tube.
2	Using the table above, load the appropriate volume of sample and Hi-Di formamide into each tube, one sample per tube. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Vortex briefly and spin down the contents of each tube.
4	Denature at 95 °C for 5 min. **Note** Matrix standard samples can also be denatured using the same denaturing conditions listed in "Denaturing the Samples" on page 5-10. Product performance will not be affected.
5	Snap cool samples in an ice water bath before loading.
6	Refer to the next section, "Loading Matrix Standards on the 377 Instrument," and the product insert for information on loading.

Loading Matrix Standards on the 377 Instrument

When loading matrix standard samples, we recommend:

- Leaving at least one empty lane between matrix standard samples and all other samples
- Loading the matrix standard samples into every other lane, one per lane

This loading technique will help ensure that a clean matrix is produced. The quality of the matrix file directly effects the quality of the analyzed data.

Preparing Samples for the 310 Instrument

Use a separate tube and injection for each matrix standard sample.

To prepare matrix standard samples for the ABI PRISM® 310 Genetic Analyzer:

Step	Action
1	Vortex the contents of each matrix sample tube, and spin down to collect the contents at the bottom of each tube.
2	Load 1 µL of sample into each tube, one sample per tube.
3	Add 12 µL Hi-Di formamide to each tube. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Vortex briefly and spin down the contents of each tube.
5	Denature at 95 °C for 5 min. **Note** Matrix standard samples can also be denatured using the same denaturing conditions listed in "Denaturing the Samples" on page 6-5. Product performance will not be affected.
6	Snap cool samples in an ice water bath before loading.
7	Refer to the product insert for information on loading.

Matrix File References

Creating a Matrix File

Instructions for creating matrix files are located in the specific instrument user's manual.

Evaluating the Quality of Matrix Files for the 377 and 310 Instruments

Matrix files must be remade from time-to-time for a variety of reasons. "Evaluating the Quality of a Matrix File" on page 9-31 in the Troubleshooting chapter of this guide discusses the following topics:

- Purpose of a matrix file
- Why matrices must be remade
- How to recognize matrix problems
- What to do if you have a matrix problem

8 Data Analysis

Overview

In This Chapter

The following topics are covered in this chapter:

Introduction

Objective The objective of data analysis is to arrive at a final set of called alleles which can be exported for further analysis.

The recommended solution for users of ABI PRISM® Linkage Mapping Set v2.5 is ABI PRISM® GeneMapper™ Genotyping Software. Other alternatives are ABI PRISM® GeneScan® Analysis Software and ABI PRISM® Genotyper® Software. The different routes for data analysis are shown in Figure 8-1:

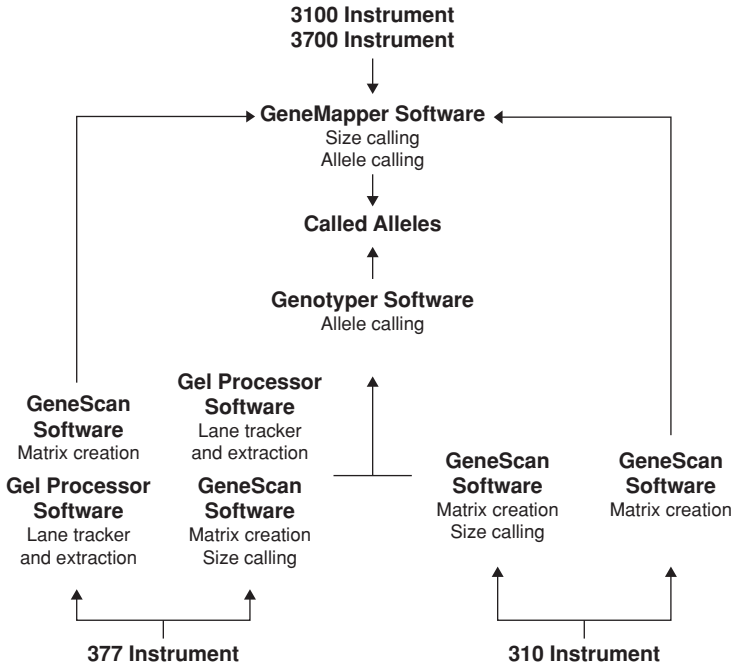


Figure 8-1 Potential Workflows

- GeneMapper software can read all .fsa files. This means the ABI PRISM® 377 data needs to be extracted from the Gel File using ABI PRISM® Gel Processor Software (Microsoft® Windows NT® operating system) or GeneScan software (Macintosh operating system).
- If you are not using GeneMapper software:
  - ABI PRISM® 3700 DNA Analyzer and the ABI PRISM® 3100 Genetic Analyzer data require Genotyper Software for allele calling and GeneScan Analysis Software for size calling if size calling was not carried out on auto extraction.
  - ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer data require GeneScan Analysis Software for size calling and Genotyper software for allele calling.

Analyzing Data with GeneMapper Analysis Software

Overview

To analyze data with GeneMapper software:

Step	Action
1	Import samples.
2	Select an analysis method.
3	Select a size standard.
4	Select a panel.
5	Analyze the samples.
6	View the quality values and edit any problem calls.
7	Export sample results for further analysis.

Importing Samples

To import samples:

Step	Action
1	Open GeneMapper software.
2	Go to **File**/**Open Project** to select a project.
3	Select **File**/**Add Samples** to add sample files to the project.

Panels and Bins

A kit is a set of markers used for a particular set of experiments. The Linkage Mapping Set v2.5 is an example of a kit. The markers in a kit are arranged into a hierarchy of panels. Each panel consists of the markers which will be run together in one capillary. Each kit can have multiple bin sets. Each bin set lists the allele characteristics when markers are run under a set of conditions. For example, a user may have two bin sets, one to analyze data generated on a 3700 instrument and the other to analyze data generated on a 377 instrument.

Creating Panels and Bins

Panels and bins can be created in two ways:

- Interactively in GeneMapper software
- By importing text files

For details of creating panels and bins, see the *ABI PRISM GeneMapper Genotyping Software User's Manual* (P/N 4318910).

Creating an Analysis Method

If you have previously set up GeneMapper software for your analysis, then you will only need to select the following:

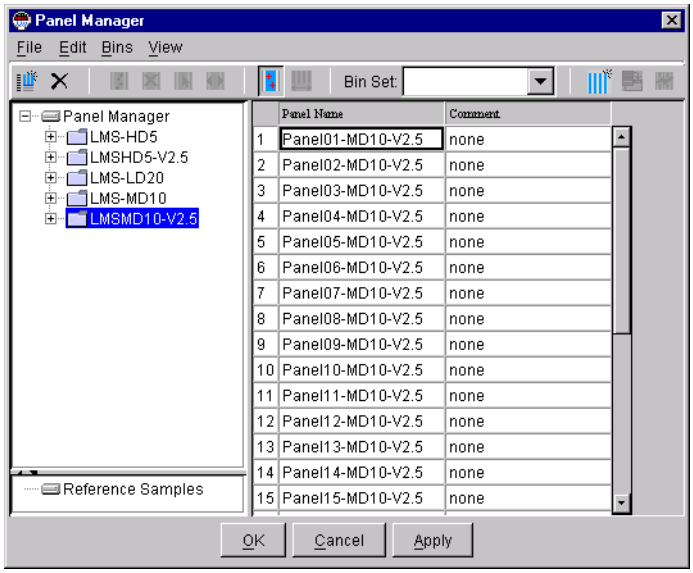
- Sample—select the sample or control
- Analysis—select the appropriate analysis software
- Panel—select the appropriate panel
- Size Standard—select the appropriate size standard

Working with Panels and Bins in GeneMapper Software

Overview

Selecting the Panel Manager icon or text in the navigation pane (left pane shown below) shows a table of the current Panel Manager folders or kits in the right portion of the window. Selecting a particular kit produces a list of panels in the right portion of the window. This list is called the Panel Table view.

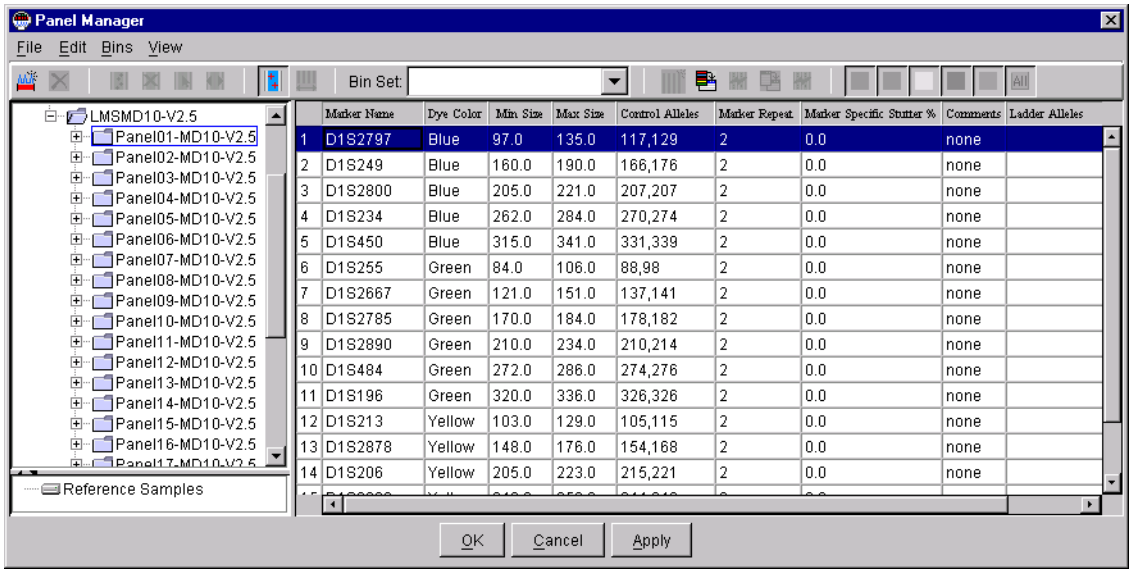
IMPORTANT Before editing or deleting an existing kit, be aware that changing panel data previously used for analysis can prevent you from displaying the results of such analysis.



Note A Warning alert is shown whenever kits or panels are deleted to remind you that this reference data may have been used previously in Sample analysis.

Marker Table View

Overview Selecting a panel in the navigation pane displays the Marker table for that panel. The Marker table, shown below, enables you to view, create, and edit marker names and associated data.



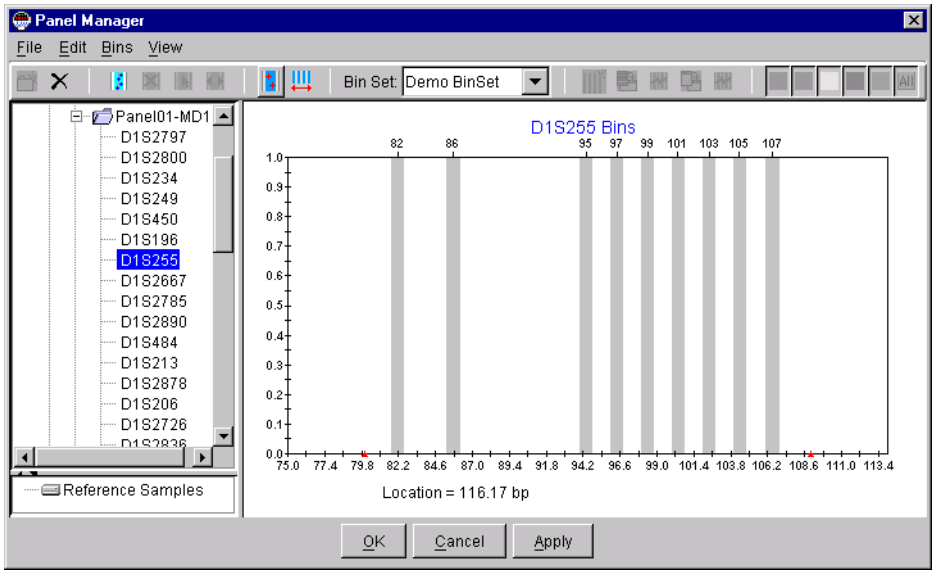
	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker Repeat	Marker Specific Stutter %	Comments	Ladder Alleles
1	D1S2797	Blue	97.0	135.0	117,129	2	0.0	none	
2	D1S249	Blue	160.0	190.0	166,176	2	0.0	none	
3	D1S2800	Blue	205.0	221.0	207,207	2	0.0	none	
4	D1S234	Blue	262.0	284.0	270,274	2	0.0	none	
5	D1S450	Blue	315.0	341.0	331,339	2	0.0	none	
6	D1S255	Green	84.0	106.0	88,98	2	0.0	none	
7	D1S2667	Green	121.0	151.0	137,141	2	0.0	none	
8	D1S2785	Green	170.0	184.0	178,182	2	0.0	none	
9	D1S2890	Green	210.0	234.0	210,214	2	0.0	none	
10	D1S484	Green	272.0	286.0	274,276	2	0.0	none	
11	D1S196	Green	320.0	336.0	326,326	2	0.0	none	
12	D1S213	Yellow	103.0	129.0	105,115	2	0.0	none	
13	D1S2878	Yellow	148.0	176.0	154,168	2	0.0	none	
14	D1S206	Yellow	205.0	223.0	215,221	2	0.0	none	

Bin View

Overview

The Bin view enables you to view, create, and edit bins.

Bins allow the user to average the minor variations in size that occur run-to-run and capillary-to-capillary.



Creating and Editing Kits and Panels

The table below shows how to perform a number of common tasks in the Panel Manager involving kits and panels.

Panel Manager Tasks

To do this...	Do the following...	Result
view the Panel table	Select a kit folder.	A Panel table is displayed.
add a new kit	a. Select the Panel Manager icon. b. Choose **New Kit** (**File** menu) or **Ctrl+N** to present the **New Kit** dialog box. c. Enter the name for the kit into the dialog box. d. Click **OK**.	A new kit folder is created under the Panel Manager icon.

Panel Manager Tasks *(continued)*

To do this...	Do the following...	Result
create a new panel	a. Select a kit folder icon in the Navigation pane. b. Choose **New Panel** (**File** menu) or Ctrl+N, or click on New Panel icon. – This presents a new line in the table with a default New Panel name and Comment field. c. Select the panel name that appears (“New Panel”) and type in the desired name. d. Repeat steps b and c until you have the desired number of new panels assigned to the kit folder. e. Click **Apply**, if you will be performing other tasks, or click **OK** to save your last change and close the Panel Manager.	A Panel icon is added to the Navigation pane each time you click **Apply** after step d, with the following conditions applying: ♦ Panel names must be unique. You cannot have the same panel name in two kit folders. ♦ When entering new panel names, the table is constrained so that only one empty row is enabled at a time.
add markers to new panels	a. Select a new panel in the navigation pane. b. Choose **New Marker** (**File** menu) or click on **New Marker** icon. This presents a new line in the table, allowing you to enter a name and other values for the marker. c. Select each field in the new marker line and make appropriate entries. d. Repeat steps b and c for each new marker to be added. e. Click **Apply,** if you will be performing other tasks, or click **OK** to save your last change and close the Panel Manager.	This operation is required if you have created a new panel rather than imported it or to add new markers to existing panels.

Panel Manager Tasks *(continued)*

To do this...	Do the following...	Result
import a kit	a. Select **Panel Manager**. b. Choose **Import Panels** (**File** menu). This presents a file directory dialog box. c. Choose the text file containing the kit information and import the kit by clicking **Import**. d. Save your new kit by clicking **Apply** (if you will be making other changes) or click **OK** (to close the Panel Manager)**.**	The panels you import will become available in the chosen kit. GeneMapper software includes the panel information for the Linkage Mapping Sets v2.5: MD10 and HD5.
delete a panel	Select the Panel icon in the navigation pane and then choose **Clear Panel** (**Edit** menu).	The selected panel is deleted. If the panel has been used to analyze data, the following alert message is displayed: *Delete the selected panel?* *If the panel was used to analyze data your results might become obsolete and you may not be able to restore an analysis.* *[OK] [Cancel]*
rename a panel	a. Select the name of the panel. b. Type the new name.	A new name is displayed in the cell.

Performing Marker Table Tasks

The table below shows how to view, create, and edit markers.

Marker Table Tasks

To do this...	Do the following...	Result
view the Marker table	Select a Panel icon.	The Marker table is displayed.
create a new marker	a. Make sure the Markers table is visible. b. Choose **New Marker** (**File** menu) or **Ctrl+N**, or click **New Marker** icon. c. Enter the name of the marker and its data into the bottom table cell labeled **New Marker**. d. Repeat steps b and c for each new marker to be added. e. Click **Apply**, if you will be performing other tasks, or click **OK** to save your last change and close the Panel Manager.	Marker icon is added to the Navigation pane, with the following condition applying: ♦ The table is constrained so that only one empty row is enabled as markers are being entered.
select a marker	Select the Marker row in the table.	The row is highlighted.
delete a marker	Select a Marker icon in the navigation pane, and then click **Clear Marker** (**Edit** menu).	The selected marker is deleted. If the marker was used to analyze data, the following alert message is displayed: *Do you want to delete the selected marker? If the marker was used to analyze data your results might become obsolete and you may not be able to restore an analysis. [OK] [Cancel]*
rename a marker	a. Select the name of the marker. b. Type the new name.	The new name is displayed in the cell.
display the Bin view	Click a marker name icon.	The bin data view is displayed.
view a different panel	Click the panel icon.	The Marker table for that panel is displayed.

Performing Bin View Tasks

The table below provides instructions for performing a number of tasks in the Bin view. The first column lists a number of common tasks, the second column lists the action(s) required to perform the task, and the third column of the table lists the result.

Bin View Tasks

To do this...	Do the following...	Result
view bin set data	Click on a marker in the navigation pane.	Bin view of selected data is displayed.
add a bin set	a. Select a kit in the navigation pane. b. Choose the **New Bin Set** command (**Bins** menu), or click **Create New Bin Set** icon on the toolbar.	A dialog box is opened enabling a new (unique) bin set name to be assigned. Clicking **OK** after typing a name creates the new bin set. **Note** No bins will be associated with the bin set until after autobinning.
import a bin set	a. Select a kit in the navigation pane. b. Choose the **Import Bin Set** command (**Bins** menu). This presents a file directory dialog box. c. Choose the bin set name in the dialog box (text file containing the Bin set information) and import the bin set by clicking **Import**. d. Save by clicking **Apply** (if you will be making other changes) or click **OK** (to close the Panel Manager).	The bin set you import will become available in the navigation pane.
select bin(s)	Click on a bin.	The color of the selected bin inverts to show it is selected.

Bin View Tasks *(continued)*

To do this...	Do the following...	Result
create a bin manually	a. Choose **New Bin** (**Bins** menu) or click the **Add Bin** icon on the toolbar. b. Position the cursor in the bin display in the location where you want the new bin and click.	Enters "New Bin mode." The cursor becomes a crosshair (+) with a blue tracking vertical line reporting base pair location when the cursor is in the plot area. The **Bin Properties** dialog box opens; change defaults as necessary. ♦ Click **OK** to create the new bin. ♦ Click **Cancel** to close the dialog without creating a bin, exiting from the New Bin mode. **Note** Bins may not overlap. If you click in an existing bin, a new bin will not be created. If you click between bins and there is not enough space for the initial default bin size, a new bin will not be created.
create bins manually for new allele calls	a. Choose the **Show Alleles** command (**Bins** menu), or click the **Show Project Alleles** icon. b. Create or edit bins as needed to include the new alleles.	All allele calls for the selected marker from the currently open project are shown as blue asterisks in the bin view. These allele calls are not reference data. The alleles displayed by this command are not saved as part of the Bin view for this marker/bin set.
view the properties of a bin	Either: ♦ Select a bin. or ♦ Choose **Edit Bin** (**Bins** menu) or click the **Edit Bins** icon.	The **Bin Properties** dialog box is displayed.

Bin View Tasks *(continued)*

To do this...	Do the following...	Result
edit the properties of a bin	Same as above for “view the properties” except changes are made to the properties.	bin properties are changed. **IMPORTANT** Be aware that if the panel the bin is associated with has been used to analyze data, editing the bin may make all previously analyzed data invalid. **Note** Bins may not overlap. **Note** Bin boundaries are validated by clicking **OK**; the dialog box remains open if any bin property is not valid.
zoom in on data	Shift+drag within the Bin view to outline a region. The Bin view zooms instantly. (The mouse must move a few pixels to cause a zoom; Shift+click with no move behaves like click.)	Bin view is scaled to the range of the selected scale region (X-coordinates of the zoom rectangle).
zoom out	Choose **Full View** (**View** menu) or click the Full View icon.	Bin data is scaled to 100%.
select a bin	Click a bin.	The selected bin turns yellow.
move a bin	a. Choose **Move Bin** (**Bins** menu) or click the Move Bin icon. b. Select a bin and drag it into position.	Bins are moved to the specified position. If the panel the bin is associated with has been used to analyze data, the following alert message is displayed after the moved Bin is deselected: *Move this bin? Moving it may make all previously-analyzed data invalid. You cannot undo this action. [No] [Yes]* **Note** Bins may not overlap.

Bin View Tasks *(continued)*

To do this...	Do the following...	Result
name or rename a bin	a. Select a bin. b. Click the **Edit Bin** icon.	The **Bin Properties** dialog box is displayed, enabling you to edit the name. **Note** Bin names must be unique. An alert message is displayed if the name is not unique when field is closed.
clear a bin	a. Select a bin. b. Choose **Remove Bin** (**Bins** menu) or click the **Remove Bin** icon.	The selected bin is deleted. **Note** If the bin was used to analyze data, your results may become corrupted.

Adding a Bin Manually

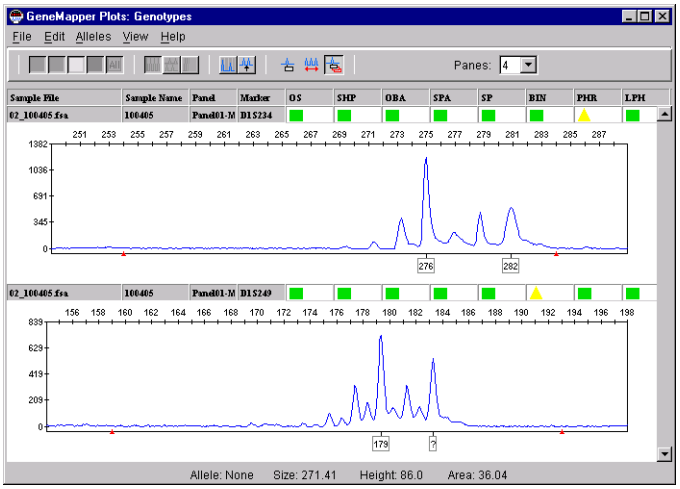
To add a Bin:

Step	Action	Result
1	Samples are imported into the Project window and analyzed.	One or more markers in the Markers table displays a “yellow” status indicator in the Bin column of the Genotypes table.
2	Select the markers showing the yellow flag in the Bin column, then click the **Display Plot** icon to display the plots for the markers.	The Plot window opens, showing the electropherograms for the markers. Here you must decide whether the marker is an outlier, or if a new bin must be created to handle the uncalled allele.
3	Close the **Plot** window and open the **Panel Manager** window. Using the Panel Manager tree, navigate to the marker that had the yellow flag.	The bin view is displayed.
4	Verify that the sample is not within a bin and: a. Click the **Add Bin** icon. b. Drag the insertion point over the Sample and click. This opens the **Add Bin Properties** dialog box. c. Name your bin.	A bin is inserted at the insertion point. Repeat the action to the left for the other yellow flagged bins.
5	Click **OK**.	The **Panel Manager** window closes and the pending changes are updated.
6	Reanalyze the data.	The markers with the yellow flags should now show green flags in the **Bin** column.

Viewing Allele Calls in GeneMapper Software

Purpose of Plot Window

The Plot window displays graphical data associated with the samples and genotypes (results), as shown below.



You can use this window to assess the following as well as perform other visual data assessment tasks:

- Quality of size standards
- Quality of peak calling
- Quality of allele calling
- Relationship between controls, such as an allelic ladder, and samples

Displaying the Plot Window

You display the Plot window from the GeneMapper Project window as follows.

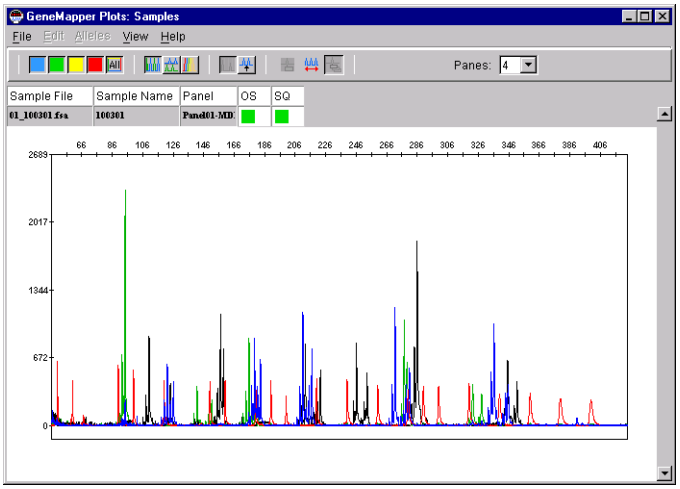
Plot Window Display Tasks

To do this...	Do the following...	Result
display a full length electropherogram in the **Plot** window	a. Click the **Samples** tab (Project window). b. Select the samples you want to display. After selecting the first sample, multiple samples are selected as follows: – Shift+click to select samples in a continuous range. – Hold down the **Ctrl** key and individually click samples to be selected in a discontinuous group. c. Click the **Display Plots** icon on the toolbar.	The electropherograms of samples you selected are displayed in the Plot window. Since samples contain all four or five dyes, each electropherogram pane displays traces for all dyes and color icons on the Plot window are enabled.
display Genotypes (or results) records in the Plot window	a. Click the **Genotypes** tab (Project window). b. Select the results records you want to display (see procedure above for multiple selections.) c. Click the **Display Plots** icon on the toolbar.	The electropherograms results records you selected are displayed in the **Plot** window, one marker in each panel. The Marker Plot view is automatically zoomed into the allele size range for each marker (±5 bp on either side), similar to the Bin view in the Panel Manager. Since results records contain data from only one dye, each electropherogram is a single color.
Note The Plot window cannot contain electropherograms from both the **Samples** tab and **Genotypes** tab at the same time.		

Electropherogram Pane Interaction

General Features

Each electropherogram provides a profile of the selected sample or genotype record it represents.



The electropherogram is displayed as follows:

- The Y-axis represents the relative fluorescence of the detected fragments as they occurred over time.
- The X-axis represents time and can be displayed by scan lines or base pairs.
- The line just above the X-axis scale provides information for the sample.
- Below the Genotype electropherogram baseline are the allele call labels.

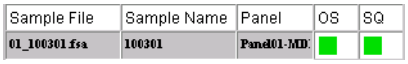
Note You can choose to have the horizontal tick marks on the X-axis represent size in base pairs instead of scan lines only if you run an internal size standard with the sample.

Interaction Elements

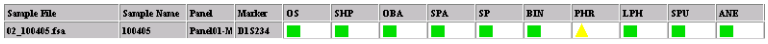
Electropherogram Pane Elements

Element	Description
Sample Data/Genotypes Data row	Above each electropherogram pane is data for the Sample or Genotype record that matches the columns displayed in the Project window. This data is read-only.
Cursor depiction	Arrow = within the electropherogram pane and at the top of the window. Crosshair = whenever the cursor is in the two information rows just below the toolbar. Its function, in this area, is to display tooltips.
X-axis scale	Scan lines or base pairs (bp).

Sample view



Genotypes view



Electropherogram Pane Elements *(continued)*

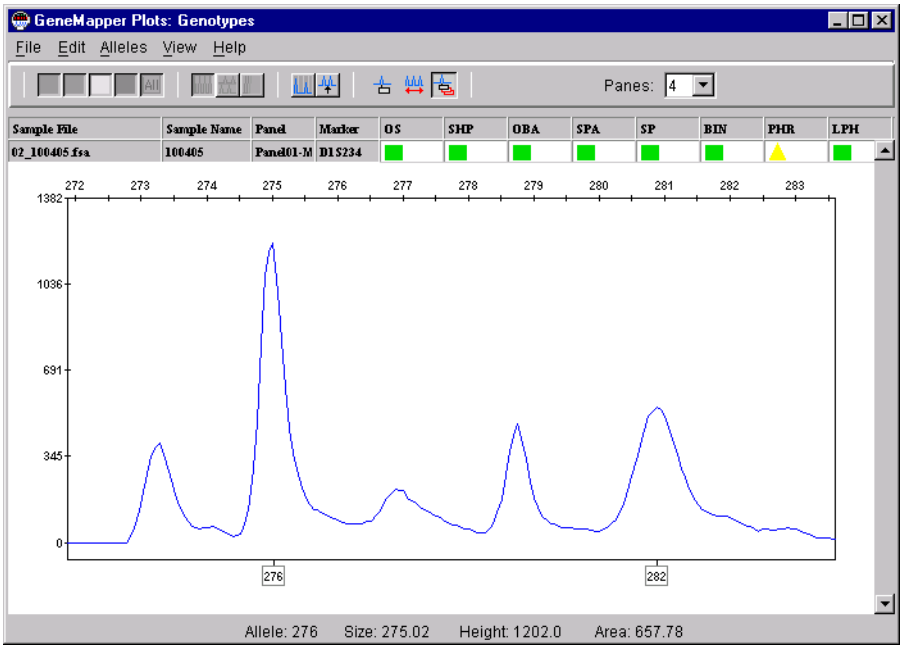
Element	Description
Y-axis scale	Peak height. Scale can be changed from the View menu.
Allele call labels	The labels are attached to a single peak and indicate the allele call for that peak. You can label peaks using the Add Allele Call Label tool.

Interacting with an Electropherogram

You may perform the actions listed in the following table with an electropherogram pane in a Plot window.

Electropherogram Actions

To do this...	Do the following...
display the X- and Y-axis positions of a peak	When the **Plot** window displays Genotypes, position the cursor within the electropherogram pane. A vertical line (called a locator) snaps to the peak nearest the cursor. The X- and Y-coordinates are displayed on the Plot window's status bar. **Note** In addition to the X- and Y-axis values, the area and height are also listed on the status bar. Allele: 179 Size: 179.37 Height: 730.0 Area: 409.97
zoom in	Shift-drag a region within the electropherogram. The electropherogram zooms to the X-width of the rectangle instantly. **Samples view**: Zooming affects the X-scale of all electropherograms in the Plot window. **Genotypes view**: Zooming affects the X-scale of only one electropherogram in the Plot window. Zooms may be nested. Multiple zooms are allowed.



Electropherogram Actions *(continued)*

To do this...	Do the following...
zoom out	a. Press **Ctrl+]** (zooms to 100%). b. Click the **Full View** icon. **Note** Zoom out affects the X-scale of all electropherograms in the **Plot** window. Panes scaled to 100% are not changed.
highlight (select) peak	a. Click within a peak (can only select and highlight one peak at a time). b. The selected peak is filled with the dye color. **Note** Selected peaks are highlighted in the dye color of the plot. A vertical dashed line follows the cursor and snaps to the peak positions.
scroll the display	The display is scrolled using the standard scroll bar on the right side.
change the number of electropherograms shown in the visible part of the Plot window.	Choose the number you want from the **Panes** drop-down menu on the toolbar.
show off-scale data	Select **Off Scale Indicator** (**View** menu) or press **Ctrl+9**.

Note Creating, modifying, and clearing allele call labels effects the allele calls in the Marker table, and vice versa.

Interacting with Allele Call Labels

The following procedure list describes how to perform various allele call label actions.

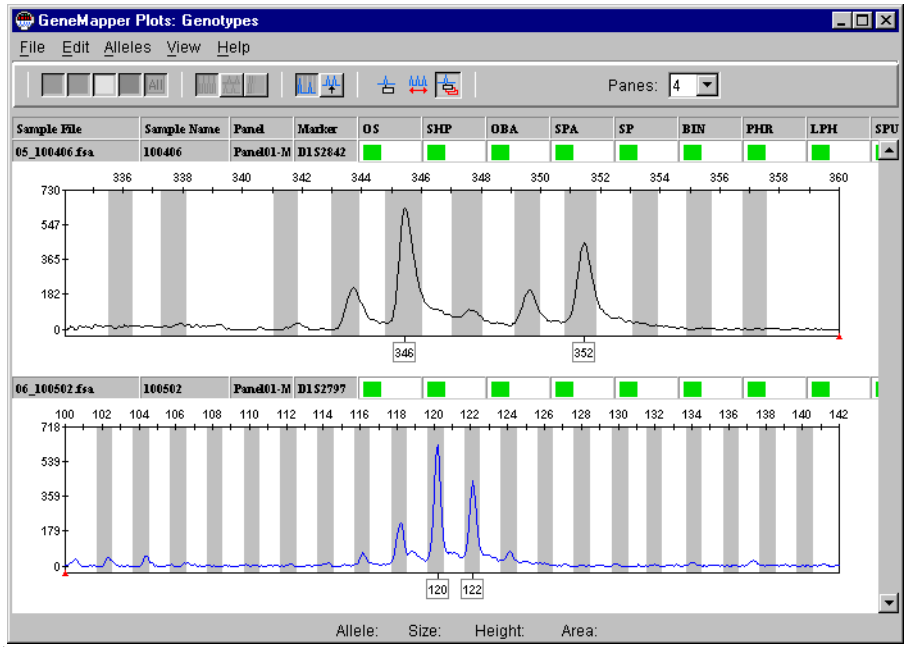
Allele Call Label Actions

To do this...	Do the following...
select allele call labels	Click an allele call label to select it. A selected label is marked in magenta.
Note Renaming a label, clearing a call, and adding a call can be done as described later in this table and can also be done by right-clicking on a peak. ♦ Right-clicking on a peak without a label presents the Add Allele Call option. – When you click the command, a dialog box is presented that enables you to type a comment about the edit. Click **OK** to add a label. ♦ Right-clicking on an existing label presents a pop-up menu that allows you to delete or change the allele call. The history command is enabled only after the editing operation is complete.	
change the label of an allele	a. Select a labeled peak. b. Choose **Change Allele Call** (**Alleles** menu), and make the appropriate selection. c. Add a comment, if necessary. **Note** Editing an allele call label changes the color of the label content.
delete a call from a peak	Select the allele label, and choose **Delete Allele Call** (**Alleles** menu) or press the **Delete** key. **Note** With “Show allele edit history” enabled (when this tooltip is presented for the toolbar button), deleted alleles are displayed as small black boxes.
add a call to a peak that does not have a label	a. Select the peak. b. Click the **Add Allele Call** icon. This displays a dialog box enabling you to assign a call to the selected peak. (Default label is “?”.)
move an allele call to a different peak	a. Left-click the allele call label to select it. b. Drag it to another peak. The drag is constrained so that the allele call label jumps from peak to peak. **Note** Alleles that have been moved show a small black box at the old peak when “show allele edit history” is enabled (this tool tip is presented for the toolbar button) and an allele call label is placed at the new peak.

Note Creating, modifying, and clearing allele call labels affects the allele calls in the Genotypes table, and vice versa.

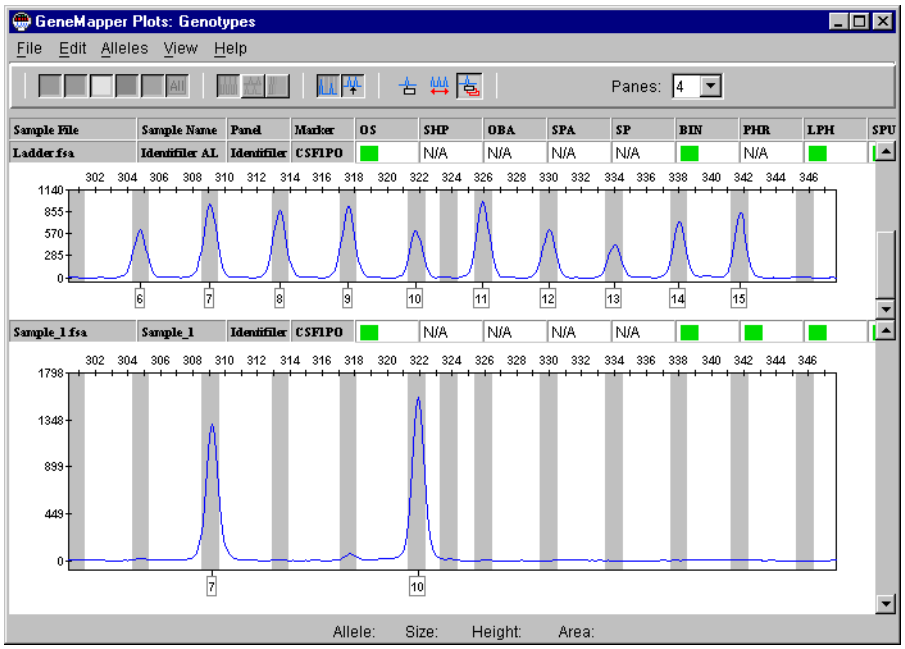
Overlay Bins View

When the Overlay Bins icon is enabled, a transparent gray overlay representing the bins for the Genotype record appears over each electropherogram.



Controls to Top View

When the Controls to Top icon is enabled, a new pane appears at the top of the Genotypes Plot window. Only samples that have the sample type of control or allelic ladder appear in this pane. If there are multiple controls, you may use the scroll bars to scroll through the different controls.



The Panes list box reflects the number of electropherograms in both the Controls and Sample regions of the window.

Exporting Data

Once all allele calls have been analyzed, they can be exported as a text file for further analysis. See the *ABI PRISM GeneMapper Genotyping Software User's Manual* (P/N 4318910) for a complete description of this function.

Analyzing Data with GeneScan Analysis Software

Overview for the 3700 Instrument

The following table lists the steps required to analyze your data collected on the 3700 DNA Analyzer with GeneScan Analysis Software. For more information, refer to the *ABI PRISM 3700 DNA Analyzer User Guide* (P/N 4306152).

3700 Instrument Data Analysis Overview

Step	Action
1	Open the project file.
2	Check to see if the samples have autoanalyzed. ♦ If the samples have not autoanalyzed, proceed to step 3. ♦ If the samples have autoanalyzed, proceed to step 6
3	Define the size standard
4	Set the analysis parameters.
5	Analyze the data.
6	Review the analysis results.

Overview for the 3100 Instrument

The following table lists the steps required to analyze your data collected on the 3100 Genetic Analyzer with GeneScan Analysis Software. For more information, refer to the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834)

3100 Instrument Data Analysis Overview

Step	Action
1	Open the project file.
2	Check to see if the samples have autoanalyzed. ♦ If the samples have not autoanalyzed, proceed to step 3. ♦ If the samples have autoanalyzed, proceed to step 6
3	Define the size standard
4	Set the analysis parameters.
5	Analyze the data.
6	Review the analysis results.

Overview for the 377 Instrument

The following table lists the steps required to analyze your data collected on the 377 DNA Sequencer with Gel Processor and GeneScan Analysis Software. For more information, refer to the *ABI PRISM 377 DNA Sequencer User Guide for Data Collection Software on the Windows NT Platform* (P/N 4325703) or the *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307164)

377 Instrument Data Analysis Overview

Step	Action
1	Open the Gel Processor software. Open your gel file.
2	Adjust the gel contrast and track lanes.
3	Extract the lanes. This will create a GeneScan project.

377 Instrument Data Analysis Overview *(continued)*

Step	Action
4	Apply the matrix file to the sample files in GeneScan software or create a new matrix and click **Apply**.
5	Define the size standard.
6	Set the analysis parameters.
7	Analyze the data.
8	Review the analysis results.

Overview for the 310 Instrument

The following table lists the steps required to analyze your data collected on the 310 Genetic Analyzer with GeneScan Analysis Software. For more information, refer to the *ABI PRISM 310 Genetic Analyzer User Guide* (P/N 4317588)

310 Instrument Data Analysis Overview

Step	Action
1	Open the project file.
2	Apply the matrix file to the sample files or create a new matrix and click **Apply**.
3	Define the size standard.
4	Set the analysis parameters.
5	Analyze the data.
6	Review the analysis results.

Applying the Matrix File

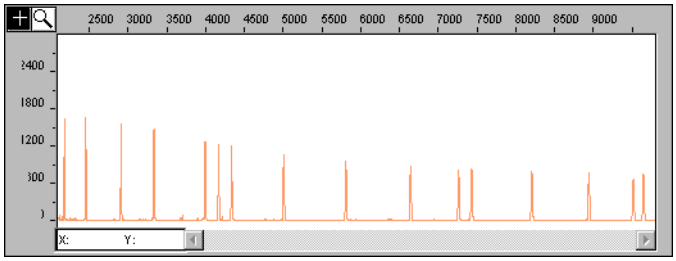
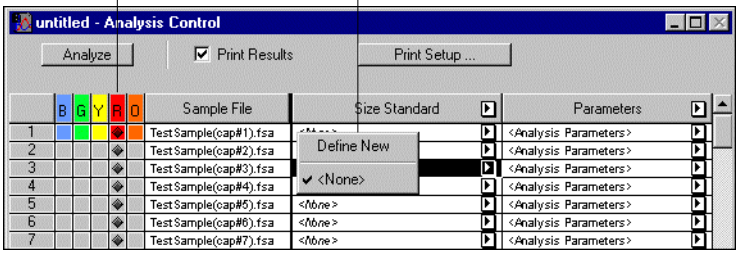
Note Matrix files can be applied only to 377 and 310 instrument sample files.

To apply the matrix file:

Step	Action
1	Open GeneScan software. Import the sample files.
2	**If a matrix file...** / **Then...** already exists — go to step 3. does not yet exist — create a matrix file now. Instructions for creating matrix files are located in the instrument user's manual and *ABI PRISM GeneScan Analysis Software for the Windows NT Platform User Guide* (P/N 4308923).
3	Click the **Sample File** to highlight all the samples files at once.
4	From the **Sample** drop-down menu, select **Install New Matrix**.
5	Select the appropriate matrix file and click **Open**. The matrix is automatically applied to all the sample files.

Defining and Selecting the Size Standard

To define and select the size standard:

Step	Action
1	Select one of the sample files in the **Analysis Control** window.
2	If a size standard file already exists, proceed to step 8 on page 8-25. Otherwise, continue to step 3 and create a size standard file now.
3	From the **Size Standard** pop-up menu for the highlighted sample, select **Define New**.
4	Define the size standard. Below is an electropherogram of the GeneScan™–500 LIZ™ Size Standards run on the 310 instrument under denaturing conditions.  The following table lists the GeneScan–500 LIZ Size Standard fragment sizes: 35 | 139 | (250 *) | 400 50 | 150 | 300 | 450 75 | 160 | 340 | 490 100 | 200 | 350 | 500 **IMPORTANT** Do not include the 250-bp peak in the size standard definition. The peak results from abnormal migration of double strands that did not completely separate under denaturing conditions. This peak shows variably smaller values than the actual size of the fragments. Refer to the size standard product insert for more information.
5	From the **File** drop-down menu, select **Close**.
6	Click **Save**.
7	Name the size standard file, and click **Save**.
8	Open the **Size Standard** pop-up menu at the top of the **Size Standard** column and select the size standard as defined above in step 4. A diamond in this column indicates that the size standard will be applied to the corresponding sample Size Standard pop-up menu 

To define and select the size standard: *(continued)*

Step	Action
9	The size standard must be selected for all the samples *except* matrix standard samples. The size standard is selected if a diamond appears in the **R** (red) column for a particular sample. To select or deselect the size standard, hold down the command key and click in the appropriate square. **Note** For four-dye chemistry, select **(R)**. For five-dye chemistry, select **(0)**.

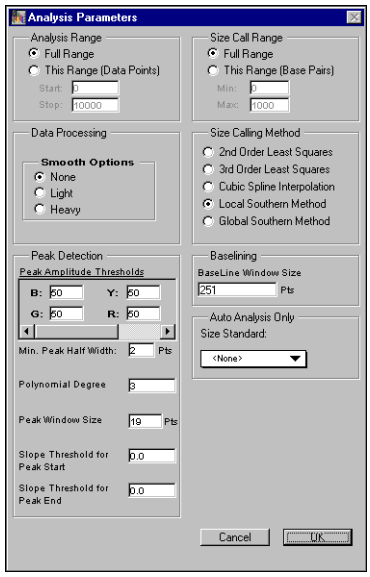
Configuring the Analysis Parameters and Analyzing the Data

To configure the analysis parameters and analyze the data:

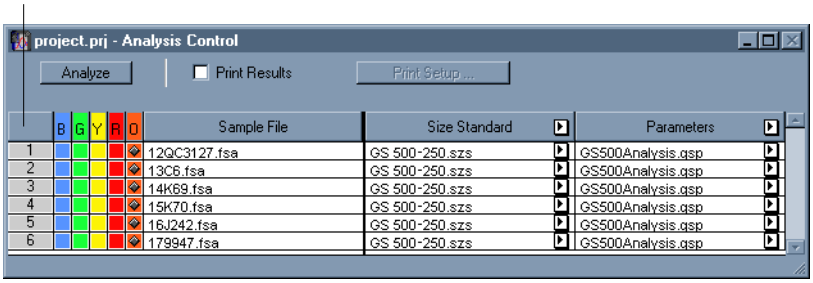
Step	Action
1	If an analysis parameter file for this kit has not been created, create one as follows: a. From the **File** menu select **New**. b. Click **Analysis Parameters**. c. Change the values listed under **Analysis Range** as follows:

If using the…	Then …
3700, 3100, and 310 instruments	look at the raw data to determine the analysis range and enter those values. **Note** Scan ranges vary between the 377 and 310 instruments.
377 instrument	either: ♦ select Full Range if the primer peak was excluded ♦ enter new values that exclude the primer peak. **Note** See the *ABI PRISM 377 DNA Sequencer User Guide for Data Collection Software on the Windows NT Platform* (P/N 4325703) or the *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307164) for more information about regenerating the gel file to exclude the primer peak.

d. Enter the values as shown for the remaining parameters.



To configure the analysis parameters and analyze the data: *(continued)*

Step	Action
2	From the **File** menu select **Save As**. Name the file and click **Save** to save it in the **Parameters** folder.
3	Open the pop-up menu for the **Parameters** column heading, and select the analysis parameters file for this kit.
4	Highlight (select) all the colors (B,G,Y, R, O) for each sample file by clicking in the grey box located to the left of B and above number 1. Click in this box to select all the colors for all the sample files 
5	Click **Analyze**. The files are analyzed, and an Analysis Log is displayed.

Viewing the Data After Analysis

To view the data after analysis:

Step	Action
1	From the **Windows** drop-down menu, select **Results Control**.
2	Select the data you want to look at by clicking in the boxes to the left of the **Sample File** column. For more information on viewing options, refer to the *ABI PRISM GeneScan Analysis Software for the Windows NT Platform User Guide* (P/N 4308923).
3	Click **Display**.
4	If the data is not already aligned by size, open the **View** menu and select **Align By Size**. **Note** If the data is already aligned by size, the **Align By Size** option is not listed. Instead, the option **Align By Scan** is displayed.
5	If the data does not look correct, check the following: a. Matrix—Was the proper matrix file applied? b. Sizing—Was the correct size standard file used? c. Was the data aligned by size?
6	If the data still looks incorrect: a. Make a new matrix file, and apply it to the data. b. Make sure the size standard file was properly made. If necessary, make a new size standard file, and apply it to the data. c. For further information refer to Chapter 9, “Troubleshooting.”

Examples of Analyzed Data

Overview

Successful amplification will yield one or two allele peaks with the associated PCR stutter bands within a maximum range of 6 to 8 bp from the allele peak. The number of allele peaks depends on whether the individual tested is a heterozygote or homozygote.

Example	Description	See Page
1	Dinucleotide repeat marker from a homozygous individual with a 2-bp stutter pattern	8-28
2	Dinucleotide repeat marker from a heterozygous individual where alleles differ by 8 bp	8-29
3	Dinucleotide repeat marker from a heterozygous individual where alleles differ by 4 bp	8-29
4	Dinucleotide repeat marker from a heterozygous individual where alleles differ by 2 bp	8-30
5	Untailed dinucleotide repeat marker from a heterozygous individual showing 1-bp ladder	8-30

Example 1

Figure 8-2 is the electropherogram of a dinucleotide repeat marker from a homozygous individual (genotype: 190 bp, 190 bp).

The peaks at 188, 186, and 184 bp are the typical 2 bp stutter pattern seen with dinucleotide repeats. They represent the 2-bp, 4-bp, and 6-bp stutters from the true 190-bp allele.

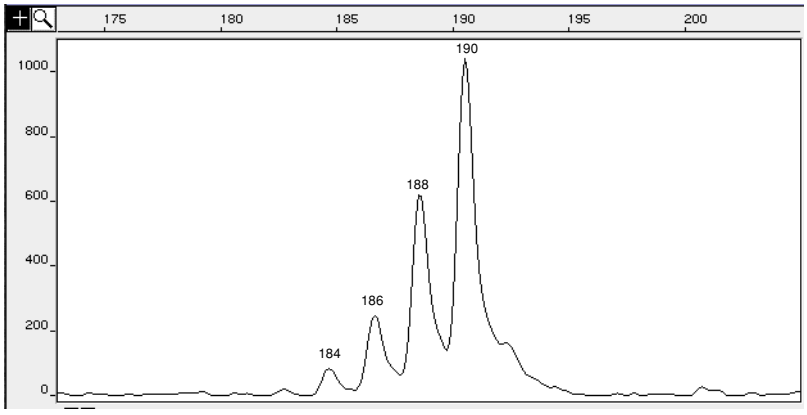


Figure 8-2 Typical pattern for a dinucleotide repeat marker from a homozygous individual

Example 2 Figure 8-3 is the electropherogram of a dinucleotide repeat marker from a heterozygous individual (allele 1–139 bp; allele 2–147 bp).

The 2-bp stutter peak to the left of each allele peak is of lower intensity than the allele peak itself. The 147-bp allele peak is of lower intensity than the 139-bp allele. In heterozygotes, the higher molecular weight allele often produces a fluorescent signal of lower intensity than the lower molecular weight peak.

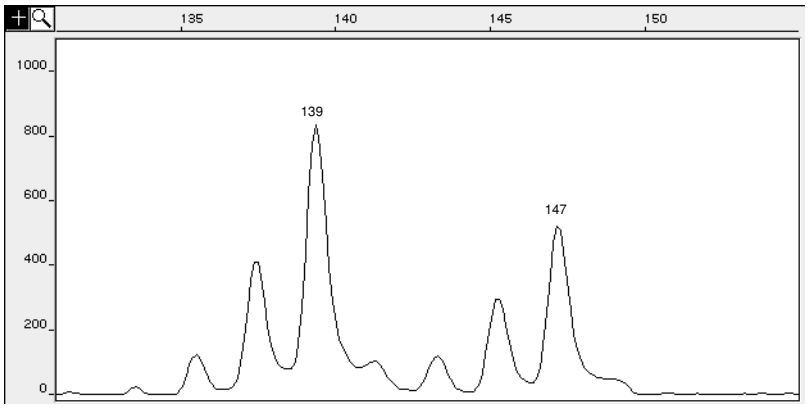


Figure 8-3 Typical pattern for dinucleotide repeat marker from a heterozygous individual where alleles differ by 8 bp

Example 3 Figure 8-4 is the electropherogram of a dinucleotide repeat marker from a heterozygous individual (allele 1–185 bp; allele 2–189 bp).

When the difference between allele sizes is 4 bp or less, a shift occurs in the height ratio between the two allele peaks (compare with Figure 8-3). The fluorescent signal from the 4-bp stutter of the 189-bp allele is added to the signal from the 185-bp allele.

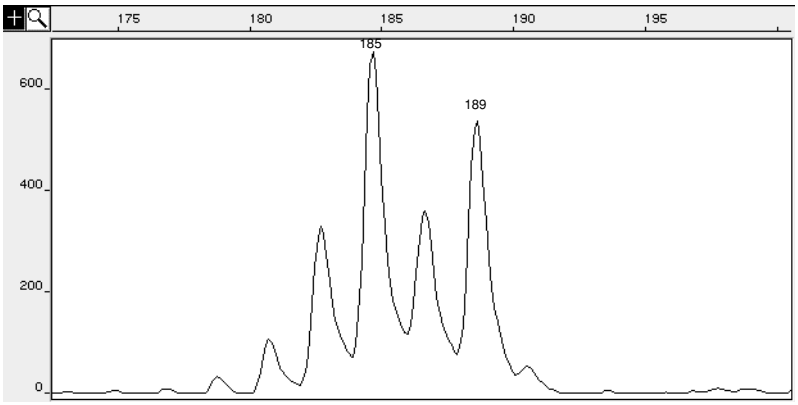


Figure 8-4 Typical pattern for a dinucleotide repeat marker from a heterozygous individual where alleles differ by 4 bp

Example 4 Figure 8-5 is the electropherogram of a dinucleotide repeat marker from a heterozygous individual where alleles differ by 2 bp (allele 1–193 bp; allele 2–195 bp).

The fluorescent signal from the 2-bp stutter of the 195-bp allele is added to the signal of the 193-bp allele. The signal from the 4-bp stutter band of the 195-bp allele is added to the signal from the 2-bp stutter band of the 193-bp allele. A dinucleotide repeat marker for heterozygous individuals when alleles differ by 2 bp has this typical triangle pattern.

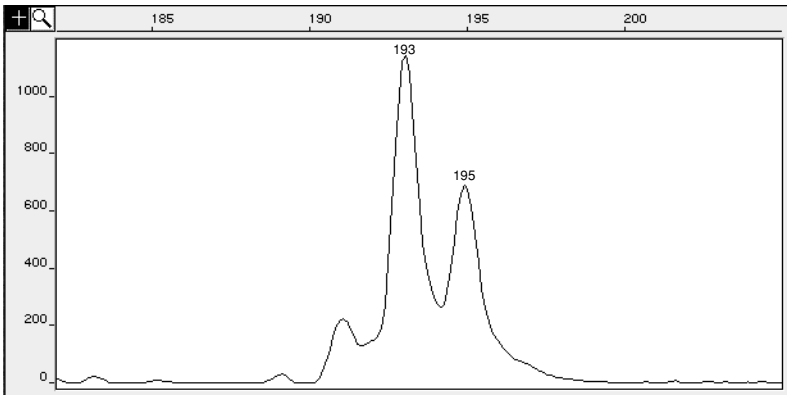


Figure 8-5 Typical pattern for a dinucleotide repeat marker from a heterozygous individual where alleles differ by 2 bp

Example 5 Figure 8-6 on page 8-31 is an electropherogram for a dinucleotide repeat marker where peaks are seen at 1 bp intervals. AmpliTaq® DNA Polymerase tends to add a non-templated A to the end of a PCR product during amplification. This is referred to as a “plus A artifact.”

A seven-base tail has been added to the reverse primers in the Linkage Mapping Set v2.5 to promote complete A addition. However, if PCR reaction conditions are inhibitory, or if the protocols and recommendations in this user guide are modified, the A addition may not go to completion. If this occurs, a ladder of peaks differing by 1 bp may be seen for PCR products when both the allele and allele-plus-A products show 2-bp stutter bands. One allele in Figure 8-6 is labeled to indicate the origin of the peaks seen. The pattern produced is a combination of both the 2-bp stutter peaks from the allele, and the allele-plus-A. The resulting peaks differ by 1 bp.

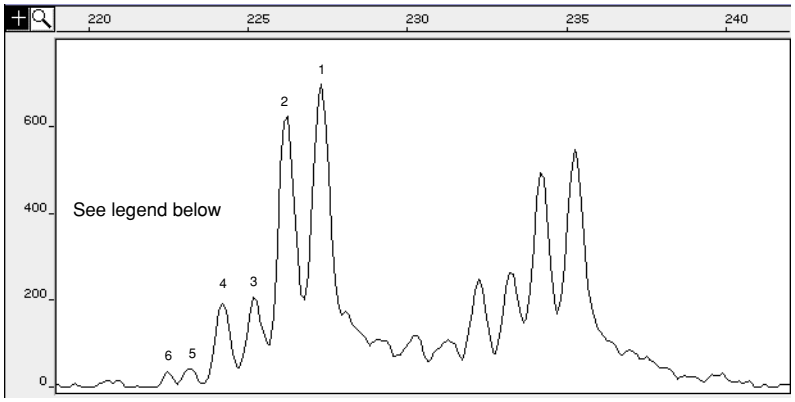


Figure 8-6 Untailed dinucleotide repeat marker from a heterozygous individual with a 1 bp ladder

Legend for Figure 8-6

Peak	Origin of 1-bp Peak Patterns in Dinucleotide Repeat Markers
1	Allele-plus-A
2	Allele peak based on DNA sequence
3	2-bp stutter plus A
4	2-bp stutter
5	4-bp stutter plus A
6	4-bp stutter

Preferential Allele Amplification and Null Alleles

As noted in “Example 2” on page 8-29, lower molecular weight alleles are often preferentially amplified over larger alleles in heterozygous individuals. Sometimes, however, a much greater difference in amplification efficiency between alleles occurs due to a polymorphism in one of the primer binding sites. The result can be one of the following:

- Preferential amplification of one allele
- Failure to amplify one allele (referred to as a “null” allele)

Null alleles are usually detected as a Mendelian inheritance problem. While relatively rare, null alleles can occur depending on the markers and populations under study.

The Genotyper software filtering algorithm can be adjusted to avoid filtering out the less intense allele in cases of preferential amplification. Total failure to amplify an allele generally requires detection by an inheritance check.

Nonspecific Amplification (Background Peaks)

Background peaks are the result of nonspecific (nontarget) amplification. If the background peaks are small, and are outside the size range of other markers for the panel, they present no problem for genotyping.

However, if the background peaks are large enough, and fall within the size range of allele products of the same dye color, they can interfere with allele calling in Genotyper software.

Background peaks can be dealt with as follows:

- A peak or peaks that always fall in the same place can be removed by using the “Remove Labels” feature in Genotyper software.
- If background is interfering with allele calls of other markers, decrease the amount of marker used by adjusting the pooling ratios. Increase the annealing temperature one degree at a time. Overall signal may decrease.

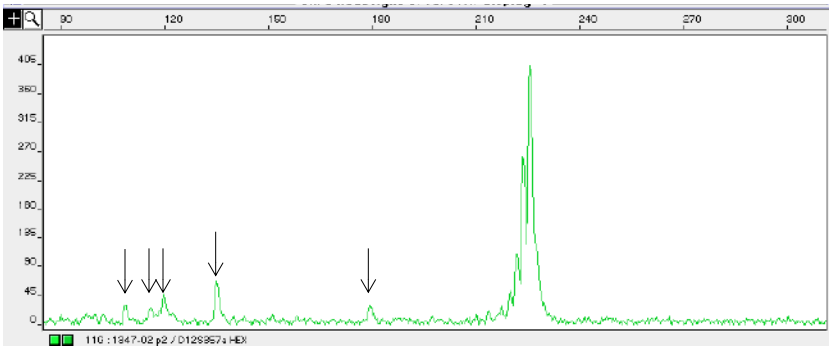


Figure 8-7 Background peaks indicated by the arrows

Using Control DNA

Benefits of Using a Control

Using a control has several benefits. The control:

- Functions as a control template to monitor PCR amplification.
- Serves as a sizing reference for controlling any gel-to-gel or capillary-to-capillary variation.
- Aids in allele binning (the comparison of allele sizes from different gels), since it sustains the same effects as the sample DNA.
- Enables correlation with allele frequency data from external sources such as databases and other labs. See “Converting Allele Frequency Data from External Sources” on page 8-50 for more information.

We highly recommend using a control with the Linkage Mapping Set v2.5 and have selected CEPH 1347-02 as our standard control. This DNA was selected because Généthon has generated its microsatellite marker sequences from this individual and uses CEPH 1347-02 for its reference genotypes.

CEPH 1347-02 DNA can be purchased from Applied Biosystems as follows:

Part Number	Description
403062	One tube containing 180 µL of DNA at a concentration of 50 ng/µL in 10 mM Tris-HCL, 0.1 mM EDTA (pH 8.0)

Recommended Use

We recommend using a control:

- In every tray of samples prepared for PCR
  - Include control DNA in every tray or group of samples prepared for the same PCR amplification.
- On every run
- Each time a new set of markers is run

Comparing Allele Sizes Within and Across Instrument Platforms

Overview

ABI PRISM instruments are highly precise within a gel or set of injections if variations in run conditions are carefully controlled. However, the called size for the same fragment will still differ between instrument platforms. Cross-platform and cross-run sizing differences occur as a result of various factors including differences in:

- Type and concentration of gel or capillary polymer
- Well-to-read or time-to-read
- Electrophoresis conditions (*e.g.*, the electric field)
- Temperature
  - The actual temperature of the gel or capillary polymer can vary from the designated run temperature. To ensure consistent results, ambient temperature should not vary more than ±2 °C.
- Sizing methods or GeneScan size standards
  - Always use the same sizing method and size standard definition when comparing sizes across runs or injections.

Precision Comparison

A comparison of precision within and between instrument platforms for a typical data set is presented in the following table.

Allele	3700 Instrument		3100 Instrument		377 Instrument		310 Instrument	
	Mean	S.D.[a]	Mean	S.D.	Mean	S.D.	Mean	S.D.
Locus 1								
Allele A	114	0.05	112.1	0.06	116.6	0.04	112.4	0.03
Allele B	120	0.05	118.1	0.06	122.7	0.04	118.7	0.04
Locus 2								
Allele A	239.4	0.03	237.8	0.06	240.2	0.04	238.1	0.04
Allele B	241.4	0.04	239.8	0.06	242.1	0.05	240.1	0.04
Locus 3								
Allele A	84.8	0.06	82.3	0.05	87.7	0.07	82.9	0.05
Allele B	96.9	0.06	94.1	0.06	99.5	0.06	94.5	0.04
Locus 4								
Allele A	218.7	0.06	216.4	0.07	219.3	0.04	217.1	0.05
Allele B	220.6	0.07	218.3	0.07	221.2	0.04	219.0	0.05
Locus 5								
Allele A	139.9	0.04	137.6	0.06	141.3	0.07	137.9	0.03
Allele B	147.7	0.04	145.3	0.06	149.1	0.07	145.7	0.04
Locus 6								
Allele A	305.4	0.06	303.1	0.07	306.2	0.07	303.8	0.04
Sample Size	480		768		96		32	

a. S.D. = standard deviation

For example, consider allele A of locus 1. On all four platforms, twice the standard deviation is less than 0.5 base pairs. Yet, the mean called size for this allele differs by more than four base pairs when run on the ABI PRISM 377 and 310 instruments.

Handling Run-to-Run Differences

Setting up allele bins rather than simply using the fragment size is a much more effective way of handling slight run-to-run differences.

- With Genotyper software, you can use the category offset feature for data sets that have size discrepancies or were obtained from different platforms.
- With GeneMapper software, use different bin sets for different platforms.

Binning Alleles for Microsatellite Markers in Genotyper Software

Allele Binning Overview

Allele definitions for microsatellite markers are based on the fragment length (size) of the PCR products estimated by gel or capillary electrophoresis. The fragment length is expressed in base pairs or mobility units as compared to a known size standard. Because of the inherent nature of DNA mobility under electrophoresis, allele sizes tend to "shift" slightly between runs. Such variation in sizing has led to the practice of "binning" alleles—grouping allele fragments belonging to a particular size into a range (bin) centered around the average size with a tolerance limit. A typical allele definition would look like this: 101.5 ± 0.5 bp.

Benefits of Allele Binning

Allele binning has several benefits:

- As sample size increases for a particular marker or set of markers, new or previously undefined alleles may appear. Allele binning makes it easier to accommodate undefined alleles.
- Allele sizes tend to shift between runs as a result of differences in gels or capillaries and electrophoretic conditions. Allele binning allows you to set tolerances for these shifts.
- Alleles are defined more precisely when binning is based on sample size.
- If using a reference individual on every run, allele definitions can be adjusted against the reference set of alleles automatically by binning alleles using ABI PRISM Genotyper software. (The control recommended for the ABI PRISM Linkage Mapping Set v2.5 is CEPH 1347-02.)

Methods Used to Bin Alleles

Several methods are available for binning alleles using Genotyper v2.0 software. Each method uses one of the following Genotyper v2.0 software features:

- Histogram Window
- Plot Window
- Make from Labels
- Add Multiple Categories
- Offset/Calculate Offset

Although several methods are presented in this section for your convenience, we recommend using the Histogram window for binning alleles. This method works best when the full data set from a study is available for each marker before the allele bins are determined.

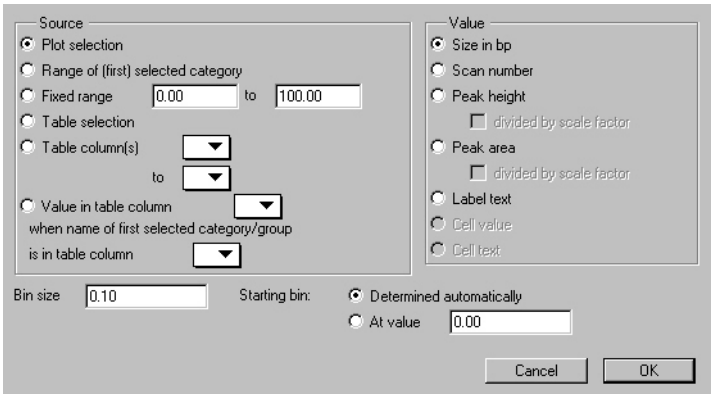
Getting Started

To familiarize yourself with the allele binning methods described in this section, use the Genotyper Applications Tutorials supplied with the software.

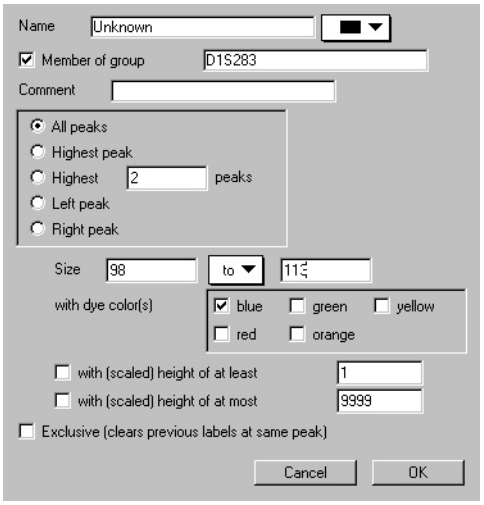
Using the Histogram Window

We recommend using the Histogram window for binning alleles. This method works best when the full data set from a study is available for each marker before the bins are determined.

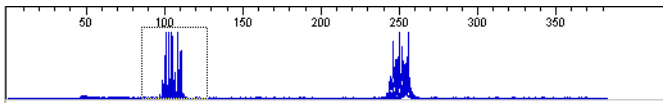
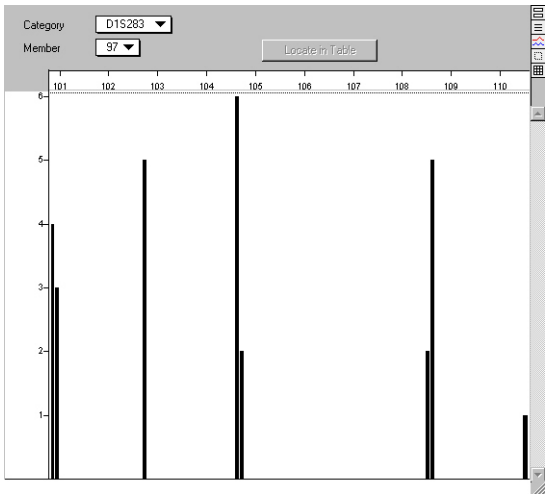
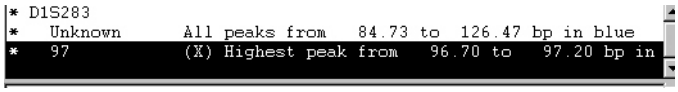
To bin alleles using the Histogram window in Genotyper software:

Step	Action
1	Define the bin size as follows: a. From the **Analysis** menu choose **Set Statistics Options**… b. Select the following buttons as shown below: – **Plot selection** – **Size in bp** – **Starting bin: Determined automatically** c. Enter **0.10** in the **Bin size** field. **Note** A bin size of 0.1 bp results in the most precise allele binning. If enough data is not available, however, an error message stating the bin size is too small will be displayed. If this occurs, increase the bin size accordingly. 
2	Open the **Categories** window.

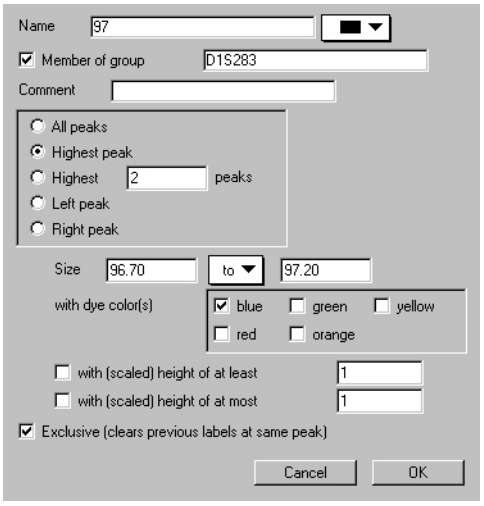
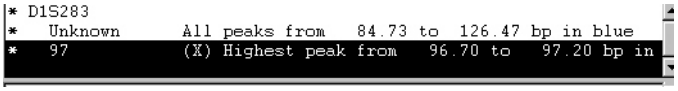
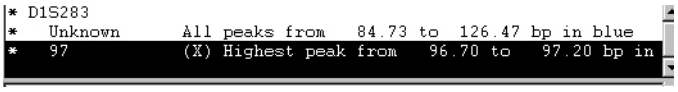
To bin alleles using the Histogram window in Genotyper software: *(continued)*

Step	Action
3	Follow these steps to set up a Category (Group) for each marker. a. From the **Analysis** menu select **Clear Category List**. b. From the **Category** menu select **Add Category**. c. Enter the locus (marker) name, size range, and dye color for the first marker as shown below. Note that `Unknown` is entered in the **Name** field, the locus name is entered in the **Member of group** field, and the box to the left of the **Member of group** field is selected with an '**x**'. d. Click **OK**. e. Repeat these steps for the remaining markers: – From the **Category** menu select **Add Category**. – Enter the marker name, size range, and dye color. – Click **OK**. 
4	From the **Analysis** menu choose **Label Peaks.** Label peaks with Size in bp only.
5	From the **Analysis** menu choose **Filter Labels.** Filter labels using the default settings (best for dinucleotide repeat markers).

To bin alleles using the Histogram window in Genotyper software: *(continued)*

Step	Action
6	Working with one dye color at a time in the **Main** window: a. Click **B** to choose all blue dye/lanes. b. Draw a box in the Plot window that covers all of the peaks associated with a single marker.  c. From the **Views** menu choose **Show Histogram Window**.  d. Make sure the correct marker name is displayed in the **Category** field. Leave the **Member** field blank. All labeled peaks in the selected range for a given marker are displayed as vertical bars in the histogram window. Each bar represents a particular size (x-axis, value). The height represents the number of labeled peaks found for that size (y-axis, counts). When the cursor is placed on a particular peak/bar, the corresponding value and counts are displayed in the status box.
7	Draw a box around a bar or group of bars that represent one allele. The area inside the box is the allelic bin. The size range for the bin and the number of peaks found in that range are displayed in the status box at the bottom of the window. 

To bin alleles using the Histogram window in Genotyper software: *(continued)*

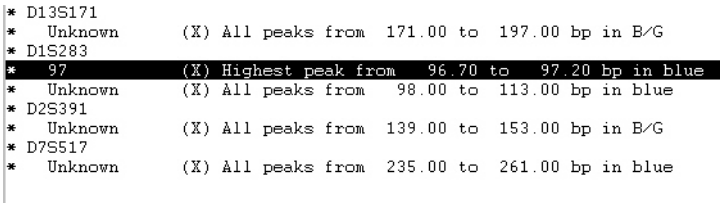
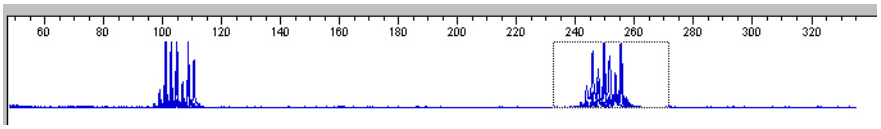
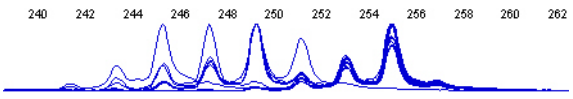
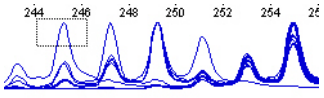
Step	Action
8	From the **Category** menu choose **Add Category**. Genotyper software automatically enters the: ♦ **Name** of the allele (member) set to the rounded size in bp ♦ **Member of group** name (marker name) ♦ **Highest peak** button ♦ **Size** range of the allele (from x to y bp) ♦ Color of dye ♦ **Exclusive** check box Check the information entered automatically by Genotyper software for accuracy. **Note** Genotyper software will not allow the addition of a new group if a group or category with the same name already exists. 
9	Click **OK**. A category member (allele bin) such as the one shown here is generated. 
10	*Optional*: The bin shown in the preceding step was created with the size as a range. You can also create a bin centered around the median size of the range with a tolerance (*i.e.*, 104.68 ± 0.5 bp) as follows: a. Hold down the **Shift** key while choosing **Add Category** from the **Category** menu. b. Edit the bin tolerance as desired. The size is displayed in the dialog box as shown here.  The category member generated will appear as follows: 

To bin alleles using the Histogram window in Genotyper software: *(continued)*

Step	Action
11	Repeat these steps to continue adding categories for each allele. Remember to: ♦ Select the markers by color ♦ Make sure the correct marker name is displayed in the **Histogram** Window
12	Now that the bins for the marker have been created, you can use the Change Labels command in the **Analysis** menu to change labels to the name of the category.

Binning Alleles Using the Plot Window

To bin alleles using individual allele plots directly:

Step	Action
1	Open the **Categories** window and set up the main categories (Groups) for each marker as shown here. 
2	From the **Analysis** menu choose **Label Peaks… Label peaks with Size in bp only**.
3	From the **Analysis** menu choose **Filter Labels… Filter labels using the default settings** (best for dinucleotide repeat markers).
4	Working with one dye color at a time in the **Main** window: a. Choose all blue dye/lanes by clicking on the **Blue** color button to the left of the dye/lanes window. b. Draw a box in the plot window that covers all of the peaks associated with a single marker.  c. From the **Views** menu choose **Zoom In** (Selected Range), or press **Ctrl+R** to display the plots for the individual alleles. 
5	Draw a box around the first tall peak from the left. 

To bin alleles using individual allele plots directly: *(continued)*

Step	Action
6	From the **Category** menu choose **Add Category**. Genotyper software automatically enters the size information for the category definition. Enter the following information: ♦ **Name** of the allele (member) set to the rounded size in bp ♦ **Member of group** name (marker name) ♦ **Highest peak** button ♦ **Size** range of the allele (from x to y bp) ♦ Color of dye ♦ **Exclusive** check box **Note** Genotyper software will not allow you to add a new group if a group or category with the same name already exists.
7	Click **OK** to add the category (bin).
8	Continuing to move from left to right, repeat steps 4, 5, and 6 for the remaining peaks.
9	*Optional:* To create a bin centered around the median size of the range with a tolerance (*i.e.*, 104.68 ± 0.5 bp), follow these steps: a. Hold down the **Shift** key while choosing **Add Category** from the **Category** menu. b. Edit the bin tolerance as desired. The size is displayed in the dialog box as shown here. 
10	Now that the bins for the marker have been created, you can use the Change Labels command in the **Analysis** menu to change labels to the name of the category.

Binning Alleles Using the Make from Labels Feature

The Make from Labels feature in Genotyper software can be used to generate category members (allele bins) automatically.

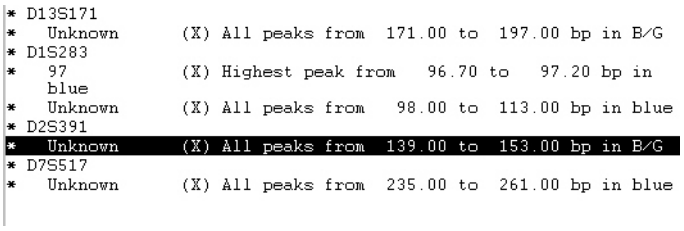
This method is ideal for linkage mapping projects:

- Involving a single family/pedigree typed with a number of markers
- Where all the markers for all individuals can be loaded onto a single gel

Unlike the other binning methods presented in this manual, using this method requires:

- Working with one marker at a time to make categories from labels
- Clearing all labels between markers/categories

To bin alleles using the Make from Labels feature:

Step	Action
1	Open the **Categories** window and set up the main categories (groups) for your markers as follows: 
2	Open the **Categories** window or activate the Category window in the **Main** window.
3	Press **Ctrl+A** (or open the **Edit** menu and choose **Select All**) to select all categories.
4	Press **Ctrl+U** (or open the **Edit** menu and choose **Unmark**) to unmark the categories.
5	Select the first category in the list. Press **Ctrl+M** (or open the **Edit** menu and choose **Mark**).
6	**If the first category…** / **Then…** is currently being defined — proceed directly to step 7. has already been defined — open the **Analysis** menu and select **Clear All Labels**.
7	Select the appropriate dye/lanes by clicking the appropriate color button.
8	From the **Analysis** menu choose **Label Peaks**. Label peaks with Size in bp only.
9	From the **Analysis** menu choose **Filter Labels**. Filter labels using the default settings (best for dinucleotide repeat markers).

To bin alleles using the Make from Labels feature: *(continued)*

Step	Action
10	From the **Category** menu choose **Make from Labels** to display the **Make Categories from Labels** dialog box. Set the parameters as follows: a. Select **"Unmark overlapping categories"**, and deselect **"Skip overlapping categories."** If two or more category members overlap in size based on the tolerance, these members will be automatically unmarked by Genotyper software so that you can correct for the overlaps. b. In the **Name** box: – Either leave the **Prefix** field blank (Figure 8-8 on page 8-45), or enter a name for the allele in the **Prefix** field which will become part of the name of the alleles (Figure 8-9 on page 8-45). – In the **First number** box, enter the number of the first allele (the smallest allele expected in the data, *e.g.*, 101) for the marker, or the starting number (*e.g.*, 1) if using a prefix. – In the **Number increment** box, enter a numeric value. This is the value by which software automatically increases each successive allele number. For example, enter 2 for dinucleotide markers if alleles are expected every two base pairs. Enter 1 to number alleles sequentially (*e.g.*, A1, A2, A3, etc.). c. Select the **With** check box and the **group name** button. d. Enter the group/marker name in the field to the right of the **group name** parameter. This indicates that the category members created belong to the group/marker that you are currently working with. e. The appropriate dye color box should have been selected automatically by Genotyper software. If not, check the appropriate box. f. Select the **Exclusive** check box if not automatically selected by Genotyper software. g. Click **OK**.
11	Return to step 4 on page 8-43 to define the remaining categories.
12	When all the categories (markers) have been defined, press **Ctrl+A** or open the **Edit** menu and choose **Select All** to select all categories. Press **Ctrl+M** to mark all the categories.
13	From the **Analysis** menu choose **Clear All Labels**. Alleles can now be labeled with the newly defined bin names. Remember to label with the category name rather than the size once categories have been defined.

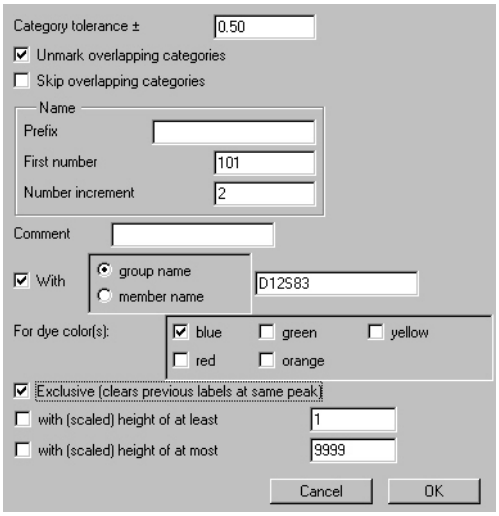


Figure 8-8 Make Categories from Labels dialog box configured to use allele sizes as allele names

```
* D12S83
*   101          (X) Highest peak at  100.82 ±    0.50 bp in blue
*   103          (X) Highest peak at  102.80 ±    0.50 bp in blue
*   105          (X) Highest peak at  104.75 ±    0.50 bp in blue
*   107          (X) Highest peak at  108.61 ±    0.50 bp in blue
*   109          (X) Highest peak at  110.60 ±    0.50 bp in blue
*   111          (X) Highest peak at  245.24 ±    0.50 bp in blue
*   113          (X) Highest peak at  247.13 ±    0.50 bp in blue
*   115          (X) Highest peak at  249.11 ±    0.50 bp in blue
*   117          (X) Highest peak at  251.07 ±    0.50 bp in blue
*   119          (X) Highest peak at  254.88 ±    0.50 bp in blue
```

Figure 8-9 Example of allelic bin names generated from the Make Categories from Labels dialog box configured as shown in Figure 8-8

Using the Add Multiple Categories Feature

The Add Multiple Categories feature can be used to automatically make a defined set of category members (allele bins) that are equally spaced (*e.g.*, 2 bp apart) with a set tolerance. Once such categories are made, you can:

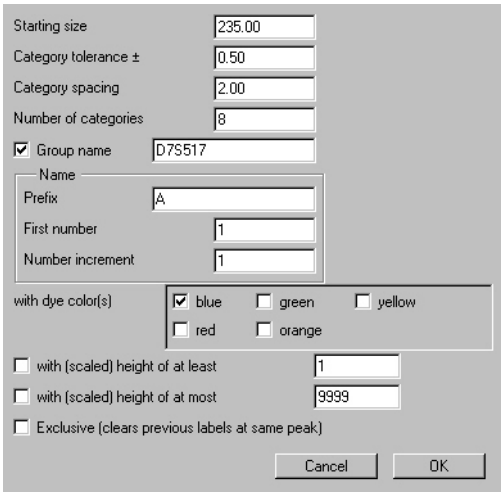
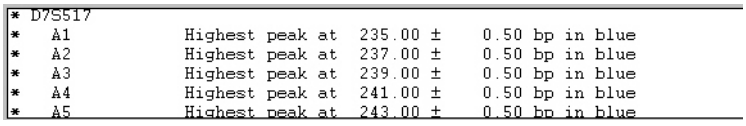
- Label and filter peaks
- Use the histogram window to fine tune category definitions to change allele definitions automatically.

To make a defined set of equally spaced allelic bins with a set tolerance:

Step	Action

1	Set up the main categories (groups) for your markers as follows:
	
	`* D13S171`
	`*  Unknown  (X) All peaks from  171.00 to  197.00 bp in B/G`
	`* D1S283`
	`*  97  (X) Highest peak from  96.70 to  97.20 bp in blue`
	`*  Unknown  (X) All peaks from  98.00 to  113.00 bp in blue`
	`* D2S391`
	`*  Unknown  (X) All peaks from  139.00 to  153.00 bp in B/G`
	`* D7S517`
	`*  Unknown  (X) All peaks from  235.00 to  261.00 bp in blue`

To make a defined set of equally spaced allelic bins with a set tolerance: *(continued)*

Step	Action
2	From the **Category** menu choose **Add Multiple Categories**. Choose the appropriate settings for the first marker as follows: 
3	Click **OK** to generate a set of categories for the marker as follows: 
4	Repeat this for the rest of the markers making sure that the appropriate starting size, dye color and marker name is entered in the **Add Multiple Categories** dialog box.
5	From the **Analysis** menu choose **Label Peaks**. **Label peaks with Size in bp only**.
6	From the **Analysis** menu choose **Filter Labels**. **Filter lab<$startrange>data analysis:allele binning for microsatellite markers:making defined set of allelic bins;allele binning:making defined set of allelic binsels using the default settings** (best for dinucleotide repeat markers).

Offsetting Allele Shifts (Category Offset) and Creating Macros in Genotyper Software

Overview

Using control DNA can greatly assist in comparing data across instrument platforms and between laboratories. Even on the same instrument, allele sizes can shift from run-to-run if conditions are not kept consistent.

The size of an unknown DNA fragment is derived from its mobility relative to the migration of GeneScan size standard fragments. Factors such as gel polymer concentration and the ionic strength of the buffer can alter the migration speed (mobility) of the same fragment from run-to-run, resulting in size variations. Consequently, the allele bins defined using data from one run may be slightly different (shifted) when compared to data from subsequent runs.

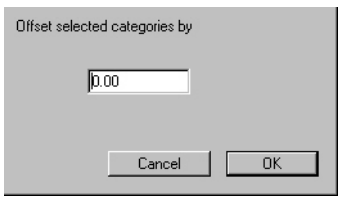
To compensate for these shifts, linkage mapping researchers include the same control DNA sample (*e.g.*, CEPH 1347-02) with every run. This allows them to check for variations in sizing from run to run. The “Calculate Category Offsets” and “Offset Categories” features in Genotyper can be used to automatically shift allele bins based on any sizing variations of the control DNA that may occur.

This practice is commonplace in DNA-based forensic studies where an “Allelic Ladder” is generated for each of the markers and loaded onto a single lane in the gel. The alleles found in the “unknown” samples are compared to the corresponding alleles in the Allelic Ladder, and the bins are adjusted accordingly.

Using the Offset Categories Feature

Use the Offset Categories feature to make changes to the bins automatically when you know the variation in allele sizes between gels is a constant number (*i.e.*, the new size is ±0.8 bp from the previously defined bin).

To use the Offset Categories feature:

Step	Action
1	From the **Category** window, select the **Category Groups** or **Members** that require adjustment. **Note** Use the Shift key to select a continuous range of categories, or Command-click to select categories randomly.
2	From the **Category** menu choose **Offset Categories**.
3	Enter the value to offset the selected categories. This number can be positive or negative (*e.g.*, 0.8 or -0.8). 

To use the Offset Categories feature: *(continued)*

Step	Action

4	Click **OK**.
	
	The new definitions of the categories that were offset will be displayed as shown below. The new bin definition for allele A10 in this example can be read as “Highest peak at 252.2 + 0.5 bp in blue”.
	
	`*  A1  Highest peak at  235.00 ±  0.50 bp (+0.80) in blue`
	`*  A2  Highest peak at  237.00 ±  0.50 bp (+0.80) in blue`
	`*  A3  Highest peak at  239.00 ±  0.50 bp (+0.80) in blue`
	`*  A4  Highest peak at  241.00 ±  0.50 bp (+0.80) in blue`
	`*  A5  Highest peak at  243.00 ±  0.50 bp (+0.80) in blue`

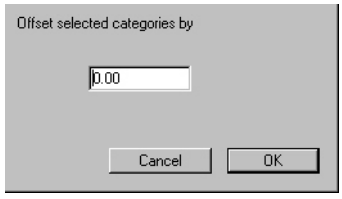
Using the Calculate Offset Feature to Create Macros

Use the Calculate Offset feature to make adjustments if the shift in allele sizes between runs is random *(i.e.*, each allele or some of the alleles shift by different amounts). The Calculate Offset feature:

- Creates a macro that compares the new sizes to the reference ladder
- Automatically calculates the difference
- Adjusts the bins accordingly

A separate macro must be created for each set of markers or Linkage Mapping Set v2.5 panel, and stored in the respective Genotyper template. To create a macro, you must first perform a minimum of two runs with the control DNA sample loaded in the first lane of every run. Loading the control in the first lane of every run automates the macro so that it shows up as the first sample file in the dye/lane list. Detailed instructions for creating macros are listed in the *ABI PRISM Genotyper NT Software User’s Manual* (P/N 4309947).

To use the Calculate Offset feature:

Step	Action
1	Define the category members for the alleles in the reference sample on the first run. Let us call the alleles a1, a2, a3, and so on for simplicity.
2	Import the data for the control DNA from the second run into Genotyper software.
3	Select and mark the first category member (a1) only. Unmark all others.
4	Select the dye/lane containing data for the reference sample.
5	Label category peaks with category name.
6	In the **Category** window, Shift-click to select the rest of the categories (members) that belong to that marker/group.
7	From the **Category** menu choose **Offset Categories**. As shown below, enter the median value of the category as defined in the **Category** window, and choose the name of the marked category from step 5 above in the pop-up menu. 

To use the Calculate Offset feature: *(continued)*

Step	Action
8	Click **OK**. All selected categories are offset by the difference between 100.23 and the new size of the same allele (a1) from the second run.
9	Now select all categories for that marker/group and unmark them.
10	Select and mark the second allele (category member a2) for the marker.
11	Repeat steps 4 to 8. In the **Offset Categories** dialog box, enter the median value of the second allele (category member a2) as defined in the **Category** window and choose the name of the marked category in the pop-up menu.
12	Follow steps 9 to 11 for the remaining alleles.
13	Once all the bins for a particular marker are offset, open the **Views** menu and choose **Show Step Window**. Examine the steps, delete any unwanted steps, and save the macro. Name the macro (*e.g.*, “Offset Marker 1”), and assign a Control-key to run the macro.
14	Repeat steps 3 to 13 for the rest of the markers in the Genotyper template, and save the macros under their appropriate marker names.

Converting Allele Frequency Data from External Sources

Overview

The use of control DNA facilitates the conversion of allele sizes and frequency data from external sources such as the CEPH database to allele sizes generated from Applied Biosystems instruments.

Once the allele sizes of a control DNA for each marker are generated on your Applied Biosystems instrument, they can be correlated to the allele sizes for that same DNA and markers from external sources. A conversion table can then be made and used to relate allele frequencies to your sample data.

See “Using Control DNA” on page 8-33 for more information on the use of control DNA.

Creating a Conversion Table

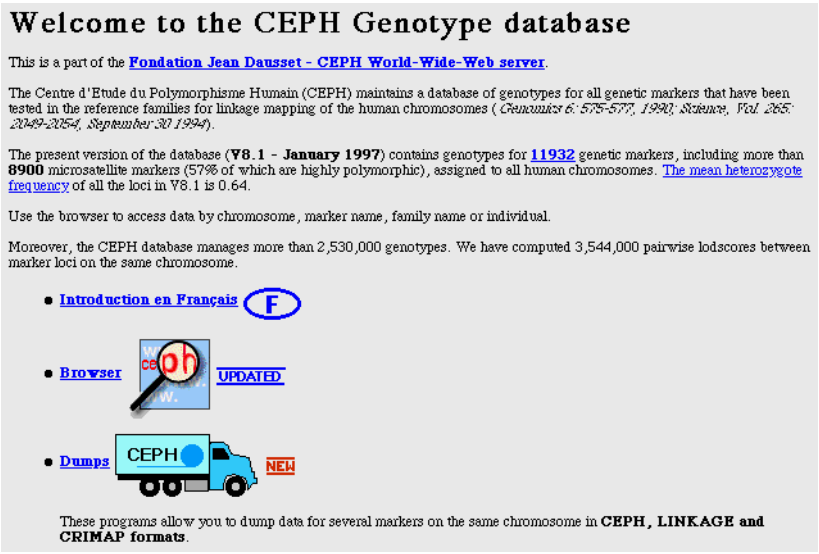
Use the following procedures to create a conversion table. These procedures consist of:

- Generating allele sizes for a particular marker using the recommended control DNA, CEPH 1347-02.
- Looking up the allele sizes and frequencies for that marker and control DNA in the CEPH Genotype database on the World Wide Web.
- Calculating the difference between the allele sizes from the CEPH Genotype database and those from your Applied Biosystems instrument, and making a conversion table.
- Including the control DNA with each sample run with that particular marker, and using the conversion table to calculate allele frequency data for your samples.

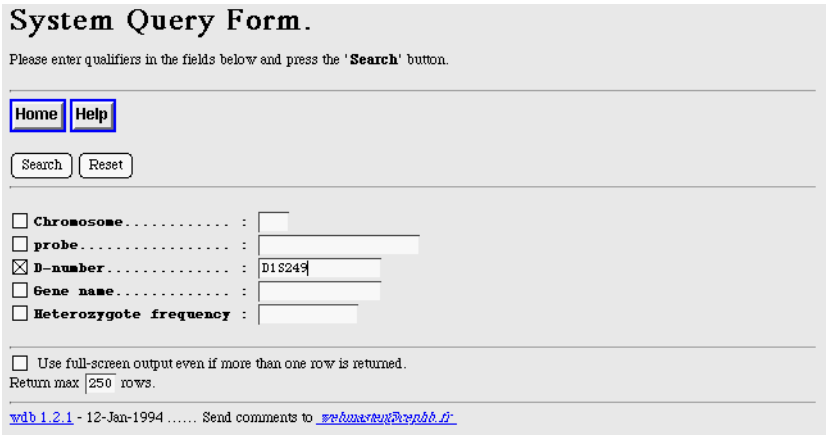
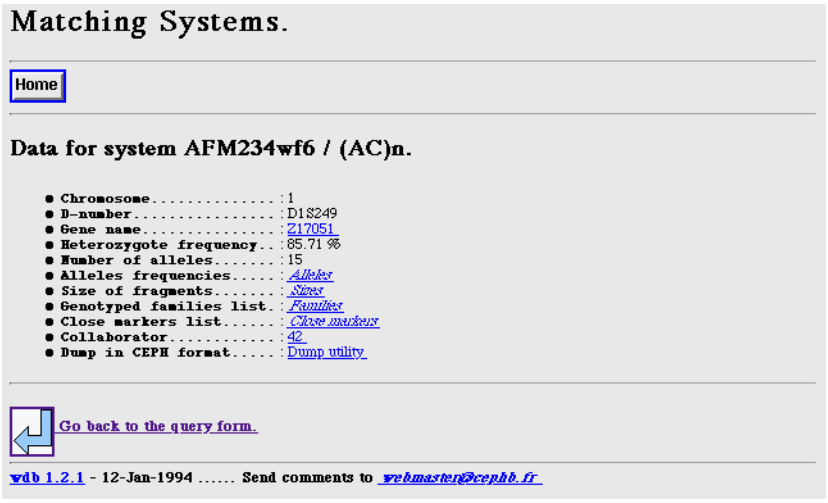
Looking Up the Genotype Software Template for a Particular Marker

To look up the genotype for a particular marker:

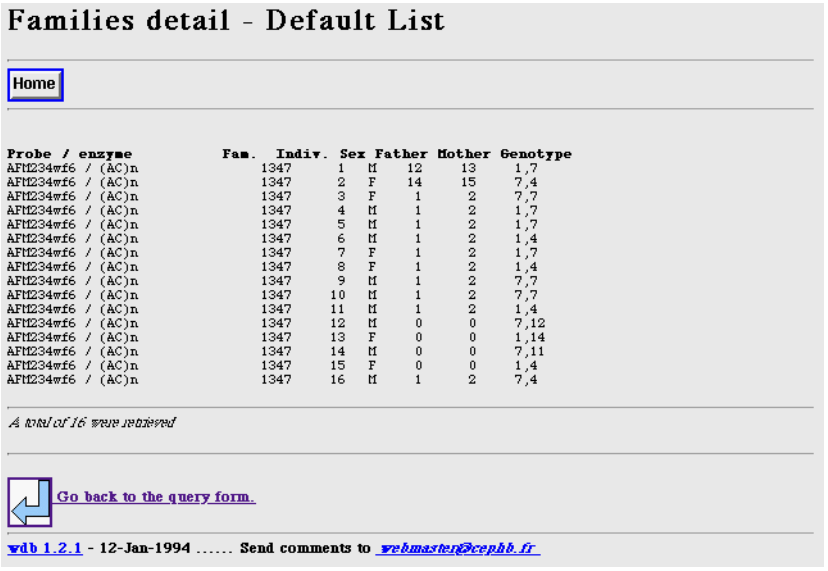
Step	Action
1	Go to the CEPH Genotype database on the World Wide Web. The address is: http://www.cephb.fr/cephdb



To look up the genotype for a particular marker: *(continued)*

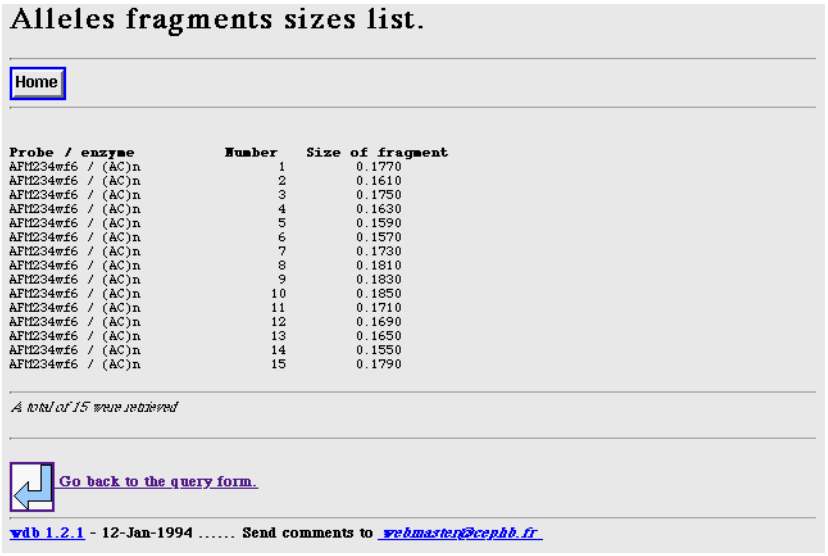
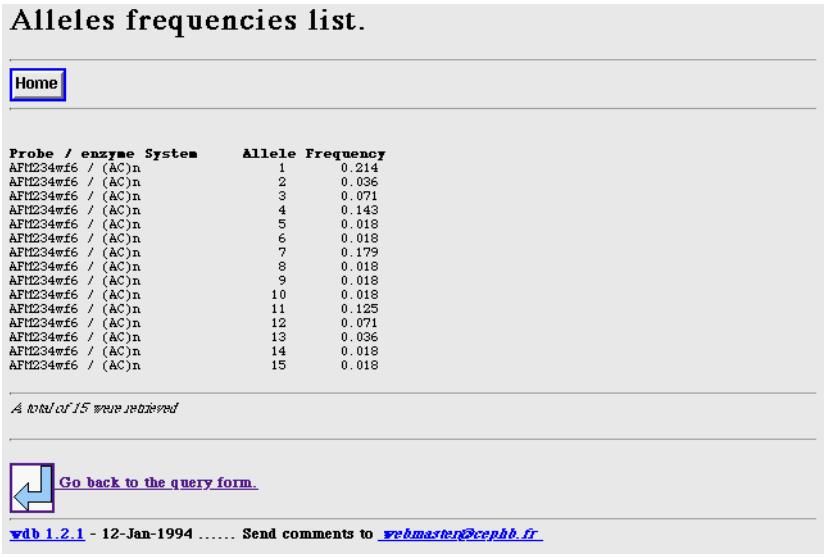
Step	Action
2	Click **Browser** to go to the **System Query Form** page. 
3	Type the name of your marker in the **D-number** field.
4	Click **Search** to go to the **Matching Systems** page, then click **Families** to go to the **Families — Default List** page. 

To look up the genotype for a particular marker: *(continued)*

Step	Action
5	Click the appropriate CEPH family to go to the **Families detail – Default List** page. *Example:* If you are using the recommended control DNA, click **1347**. 
6	Look up the genotype for the CEPH individual used as a control. *Example:* For CEPH individual 1347-02, the genotype for marker D1S249 is 7,4. 
7	Click **Back** twice to return to the **Matching Systems** page.

Looking Up the Fragment Size and Allele Frequencies for the Genotype

To look up the fragment sizes and allele frequencies for the genotype:

Step	Action
1	Click **Sizes** to go to the **Alleles fragments sizes list** page.
2	Using the allele number(s), look up the corresponding fragment size(s). *Example: A*llele 7 is 0.173 kb; allele 4 is 0.163 kb 
3	Click **Back** to return to the **Matching Systems** page.
4	Click **Alleles** to go to the **Alleles frequencies list** page. 
5	Using the allele number, find the corresponding frequency. *Example:* Allele 4 has a frequency of 0.143; allele 7 has a frequency of 0.179.
6	Match up the alleles of the control DNA run on your Applied Biosystems instrument with the alleles from the CEPH database.

To look up the fragment sizes and allele frequencies for the genotype: *(continued)*

Step	Action
7	Calculate the difference in bases between the CEPH database sizes and the sizes generated from your Applied Biosystems instrument. **Note** Sizes in the CEPH database are in kb; sizes generated on Applied Biosystems instruments are in bases.
8	For convenience, make a conversion table that lists the allele sizes and frequencies as listed in the CEPH database, along with the corresponding sizes as run on your Applied Biosystems instrument.
9	Use the conversion table to determine allele frequencies for future sample genotypes.

9 Troubleshooting

Overview

In This Chapter

The following topics are covered in this chapter.

General Troubleshooting

General Troubleshooting

Observation	Possible Causes	Recommended Actions
Faint or no signal from DNA samples and control DNA (CEPH 1347-02) at all loci	Incorrect volume or absence of: ♦ True Allele® PCR Premix ♦ Primers	Repeat amplification, carefully following the appropriate protocol in Chapter 2.
	No activation of AmpliTaq Gold® DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95 °C for 12 min.
	Non-Applied Biosystems thermal cycler used	Optimize thermal cycling conditions as described in “Optimizing PCR” on page 2-7.
	Samples and/or reagents not thawed completely before vortexing and pipetting	Completely thaw samples and reagents before use.
	Reagents not vortexed thoroughly before aliquoting	Vortex reagents thoroughly before aliquoting.
	Primers exposed to too much light	Protect primers from light while in storage.
	Thermal cycler malfunction	Refer to the thermal cycler user guide and check instrument calibration.
	Incorrect thermal cycler parameters	Check parameters used against the protocol in this user guide.
	Tubes not tightly seated in thermal cycler during amplification	Make sure all caps are firmly closed on the reaction tubes.
	GeneAmp® PCR System 9600 or 9700 cover misaligned	Seat cover properly before tightening.
	Wrong PCR reaction tubes used	Use Applied Biosystems MicroAmp® Reaction Tubes with caps or microtiter plates for the GeneAmp 2400, 9600, and 9700 thermal cyclers.
	MicroAmp base used with tray/retainer set during thermal cycling	Remove MicroAmp® Base from tray/retainer set before thermal cycling.

General Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
Faint or no signal from DNA samples and control DNA (CEPH 1347-02) at all loci *(continued)*	Insufficient PCR product loaded onto gel or injected into capillary	**⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
		For the ABI PRISM® 3700 or ABI PRISM® 3100 instruments, mix: ♦ 0.5 µL pooled PCR product ♦ 9.5 µL Hi-Di™ formamide–size standard mix
		For the ABI PRISM® 377 instrument, mix: ♦ 2.0 µL pooled PCR product ♦ 3.0 µL Loading Cocktail Load 2.5 µL onto each lane.
		For the ABI PRISM® 310 instrument, mix: ♦ 1.0 µL pooled PCR product ♦ 12.0 µL of Hi-Di formamide-size standard mix Increase volume of PCR product to 2.0 µL if 1.0 µL is not enough.
Positive signal from Control DNA (CEPH 1347-02), but no signal from DNA samples	Quantity of DNA sample is below assay sensitivity	Quantitate DNA and use 60 ng of DNA per 15 µL reaction. Repeat test.
	Sample contains PCR inhibitor (*e.g.*, heme compounds)	Quantitate DNA and use minimum volume necessary. Repeat test.
		Wash the sample in a Centricon-100. Repeat test.
	Sample DNA degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, re-amplify with an increased amount of DNA or remake the template.
	Sample DNA diluted in wrong buffer (*e.g.*, wrong EDTA concentration)	Dilute DNA again using 0.1 mM EDTA in TE buffer.
Elevated baseline	Poor or incorrect matrix file	♦ For the 377 or the 310 instrument, reanalyze using the correct matrix. ♦ Run matrix standards, make a new matrix, and reanalyze the data. The matrix cannot be corrected for the 3700 or 3100 instrument.

General Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
Bleedthrough peaks	♦ Poor or incorrect matrix file ♦ Signal from one color is offscale	For matrix problems: ♦ For the 377 or 310 instrument, reanalyze using the correct matrix. ♦ Run matrix standards, make a new matrix, and reanalyze the data. For offscale signal: ♦ Dilute samples and rerun.
More than two alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling (*e.g.*, previously amplified products or another genomic DNA source).
	Too much DNA in reaction	Use less DNA per 15-µL reaction.
	Lack of 100% 3' A base addition (n-1 bp position)	Be sure to include the final PCR extension step of 72 °C for 10 min.
	Signal exceeds dynamic range of instrument (off-scale data) resulting in bleedthrough from another color	Dilute the pooled PCR products with deionized water.
	Poor spectral separation (bad matrix)	Make a new matrix file.
		For the 377 and 310 instruments, confirm that virtual filter set G5 modules are installed and used for analysis.
	Spillover between gel comb lanes	Repeat the procedure. Use extreme care if loading a shark's tooth comb.
Some but not all loci visible on the electropherogram	Pooling ratio not optimized	See "Optimizing Pooling Ratios" on page 2-7.
	Sample DNA is degraded	If possible, evaluate DNA sample quality by running an agarose gel. If DNA is degraded, re-amplify with an increased amount of DNA, or remake the template.
	Sample contains PCR inhibitor (*e.g.*, heme compounds)	Quantitate DNA and use minimum volume necessary. Repeat test.
		Wash the sample in a Centricon-100. Repeat test.
	Random variation in reaction setup	♦ Avoid pipetting volumes less than 1.0 µL when setting up PCR reactions. ♦ Use well-calibrated, small-volume pipets.

3700 Instrument Troubleshooting

3700 Instrument Troubleshooting

Observation	Possible Cause	Recommended Action
Fragments from a specific capillary are detected later than fragments from adjacent capillaries. Fragment resolution is good for 50 to 100 bp, but is poor after that.	A bubble is in the loading well	Rerun sample.
	Too much DNA was injected	Dilute the sample and rerun.
Low relative dye concentration (fluorescence intensity, signal strength).	Salt concentration in the samples is too high	Desalt the samples. Refer to the *ABI PRISM 3700 DNA Analyzer Sequencing Chemistry Guide* (P/N 4309125) for directions.
	Pellets are not completely resuspended	♦ Do not overdry samples. ♦ Vortex well during resuspension.
	Detergent or excess RNA is in the samples	Purify the template more rigorously to remove detergent and/or RNA. Refer to the *ABI PRISM 3700 DNA Analyzer Sequencing Chemistry Guide* (P/N 4309125) for directions.
	Injection time is too short so that insufficient DNA is loaded into the capillary	Increase the injection time.
Low fragment resolution.	Too much DNA was loaded into the capillary	♦ Use a run module with a shorter injection time or lower voltage. ♦ Use samples with a lower DNA concentration.
	Old polymer or buffer is present	Check the age of the polymer and replace if it has been on the instrument for more than 5 days. Check the age of the 1X Genetic Analyzer buffer and replace it if it is more than 1 week old.

3700 Instrument Troubleshooting *(continued)*

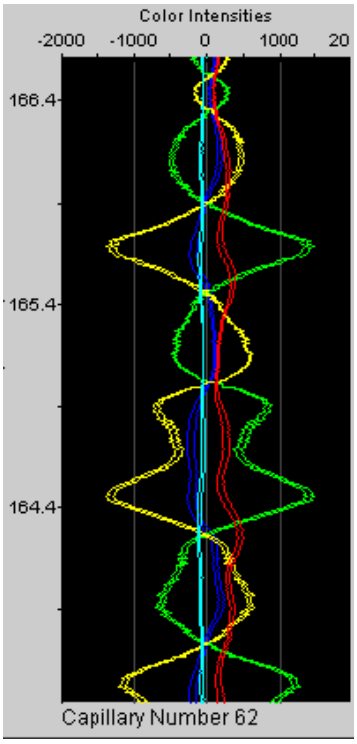
Observation	Possible Cause	Recommended Action
No fluorescence detected	Autoloader tips are bent or not seated correctly in the autoloader, so the samples were not loaded	♦ Replace tips if bent. ♦ Adjust tips if not seated correctly.
	Laser shutter did not open	Push the reset button to reset the power to the instrument. If this does not solve the problem, call Applied Biosystems Technical Support.
	There is a problem with interlocks	♦ Check the position of the electrophoresis chamber lid, and restart the instrument. ♦ Call Applied Biosystems Technical Support if the problem persists.
	CCD camera did not turn on	Restart instrument and computer.
	Back or side panels are off, activating the interlock	Replace the panels. **IMPORTANT** Never remove the back or side panels.
Cannot find sample files	Automatic extraction is not selected	Click the **Data Analysis** tab in Data Collection preferences and then select the **Enable AutoAnalysis** check box.
	Analysis failed	Read the Auto analysis report log.
Cannot find samples in Sequence Collector database	Automatic extraction was not selected	Click the **Data Analysis** tab in **Data Collection** preferences and then select the **Extract to Sequence Collector** check box.
Cannot find samples in Sequence Collector database Data Extractor logs show failure to extract run		a. Open **Sequence Collector**. b. Click the **Customize** button. c. Verify login information.
	Instrument database settings for BioLIMS are incorrect	a. Click the **Start** menu. b. Point to **Programs** and select **Oracle for Windows NT®/SQL Net Easy Configuration**. c. Verify the presence of an alias to the BioLIMS database.
	Connection between instrument database and BioLIMS® database is lost	a. Re-establish connection. b. From the **Data Acquisition** menu, select **Force Run Status to Complete**.
	BioLIMS database is full	♦ Database administrator to add space to BioLIMS. ♦ Remove unwanted samples from BioLIMS using BioLIMS Manager.
	Network problems are preventing connection to BioLIMS	Try to connect using BioLIMS Manager
When starting a run soon after restarting the instrument, a message appears stating “Instrument is not idle”	Instrument reboot is not complete.	Wait 15 min, and then restart the run.

3700 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Run will not start following an unexplained abort of the previous run	Instrument is not in an idle state	Run the PreRun Check service module to reset the instrument to an idle state.
No signal in selected lanes	Incorrect loading	♦ Verify that there are no air gaps in the autoloader's fluid lines. ♦ Centrifuge plates before placing them on the plate deck. ♦ Verify that loading-end tips of capillaries are fully submerged in injection wells.
	No samples	Repeat the run with samples loaded.
Small white dots are randomly dispersed across the capillary display of the Array View sub-page of the ABI PRISM® 3700 Data Collection Software	Particles present in the sheath-flow polymer	Replace the inline filter.

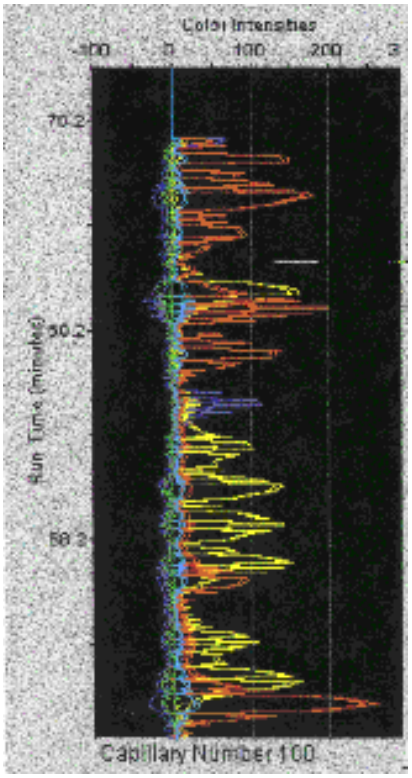
Run Profile Examples

Example 1



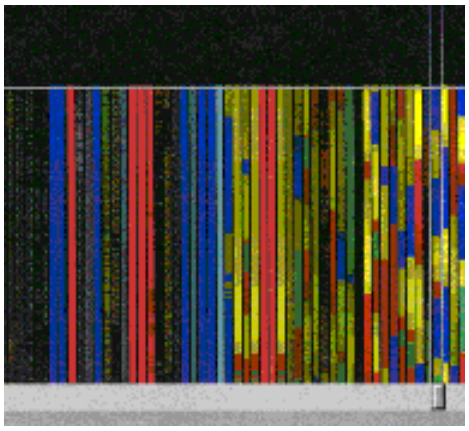
Observation	Possible Causes	Recommended Action
Mirror-image plots are in an electropherogram	Wrong dye set was selected for the run	Repeat the run with the correctly chosen dye set.
	Bad matrix was chosen for the capillary	Either: ♦ Override the matrix used for the capillary with a successful matrix from a capillary no more than four capillaries away. ♦ Repeat the run.

Example 2



Observation	Possible Causes	Recommended Action
Electropherogram shows “filled-in” peaks The plume hop plot in the spatial calibration graph of the Capillary View sub-page shows during a run that the fluorescence image on the CCD does not correspond to the expected position based on the current spatial calibration	Bubbles in the cuvette	Run the **CuvetteFlush.mod** service module.
	Array has been removed/replaced without performing a spatial calibration run	♦ Confirm cause by checking the plume hop plot in the spatial calibration graph of the Capillary View sub-page. ♦ Rerun spatial calibration and then samples.
	Syringe problem	Check for leakage around the syringe. If there is evidence of leakage, replace the sheath syringe.
	Spatial Calibration is no longer valid	Rerun the spatial calibration.

Example 3



Observation	Possible Causes	Recommended Action
Either or both: ♦ Long bands of one color are in the capillary display of the Array View sub-page ♦ Electropherogram shows extremely broad peaks or waves (not pictured)	There is insufficient buffer in injection wells	♦ Stop the run if possible to prevent the capillary ends from drying out and damaging the capillary array. ♦ Make sure that there is enough buffer in the buffer reservoir. ♦ Check that the sinker lies at the bottom of the buffer reservoir.
	Old buffer or polymer was used	Rerun calibration with new buffer and polymer.
	1X Genetic Analyzer buffer prepared incorrectly	Prepare new buffer.
	Sample is heavily overloaded (usually accompanied by delayed start point)	Rerun with less sample or resuspend sample in a greater volume of injection solution.
	Bubbles are in capillary array (if it occurs only in some capillaries)	♦ Check that there is sufficient buffer in the buffer reservoir. ♦ Check that the sinker lies at the bottom of the buffer reservoir.
	No sheath flow or slow sheath flow	Check to make sure that: ♦ There are no bubbles in the sheath-flow syringe. ♦ The sheath-flow pump is dispensing fluid. If this problem persists, call Technical Support.

3700 Capillary Array Troubleshooting

Observation	Possible Cause	Recommended Action
Poor resolution	Protein or other contaminant interacting with the walls of the capillaries and causing short capillary lifetime	Run the regenerate part of the Change Array wizard every 100 runs or when loss or resolution is noticed.

3100 Instrument Troubleshooting

3100 Instrument Troubleshooting

Observation	Possible Cause	Recommended Action
No data in all capillaries	♦ Bubbles in the system ♦ No sample injection	Visually inspect the polymer block and the syringes for bubbles. Remove any bubbles using the Change Polymer Wizard. If bubbles still persist, perform the following: a. Remove the capillary array. b. Clean out the polymer block and syringes. c. Replace polymer with fresh polymer. Make sure to draw the polymer into the syringe very slowly. **⚠ CAUTION CHEMICAL HAZARD. POP polymers** may cause eye, skin, and respiratory tract irritation. Read the MSDS for the polymer you are using, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.
No signal	Autosampler calibration is not optimal	Check the injection with 20-µL samples. If the injection is OK, recalibrate the autosampler using the Autosampler Calibration Wizard. Pay particular attention to the Z-axis. If the injection is not OK, perform the procedures below.
	Dead space at bottom of sample tube	Centrifuge the sample tubes.
	Bent capillary array	Replace the capillary array and recalibrate the autosampler using the Calibrate Autosampler Wizard.
	Failed reaction	Repeat reaction.
	Cracked or broken capillary	Visually inspect the capillary array, including the detector window area for signs of breakage.
Signal too high	Sample concentration is too high	Dilute the sample.
		Decrease the injection time.
	Too much DNA added to the reaction, resulting in uneven signal distribution	Optimize chemistry.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Low signal strength	Poor quality formamide	Use a fresh lot of Hi-Di™ formamide. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Pipetting error; not enough sample	Increase the amount of DNA added.
		Recalibrate the pipets.
	Sample has high salt concentration	Dilute in high-quality water.
		Desalt using a column purification method.
	Insufficient mixing	Vortex the sample thoroughly, and then centrifuge the tube to condense the sample to the bottom of the tube.
	Autosampler out of calibration	Check the injection with 20-µL samples. If the injection is OK, recalibrate the autosampler using the Autosampler Calibration Wizard. Pay particular attention to the Z-axis.
	Weak amplification of DNA	Re-amplify the DNA.
		Check DNA quality.
Elevated baseline	Possible contaminant in the polymer path	Wash the polymer block with hot water. Pay particular attention to the upper polymer block, the ferrule, the ferrule screw, and the polymer tubing. Dry the parts with compressed air before replacing them onto the instrument. Do not wash syringes in hot water because the Teflon plungers will get damaged.
	Possible contaminant or crystal deposits in the polymer	Bring the polymer to room temperature, swirl to dissolve any deposits. Replace the polymer if it has expired. **⚠ CAUTION CHEMICAL HAZARD. POP polymers** may cause eye, skin, and respiratory tract irritation. Read the MSDS for the polymer you are using, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.
	Poor spectral calibration	Perform new spectral calibration.
	Detection cell is dirty	Place a drop of methanol onto the detection window and dry with compressed air. Use only light air force. **⚠ WARNING CHEMICAL HAZARD.** **Methanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Loss of resolution	Too much sample injected	Dilute the sample and re-inject.
	Poor quality water	Use high-quality, ultra-pure water.
	Poor quality or dilute running buffer	Prepare fresh running buffer from 10X Genetic Analyzer Buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Poor quality or breakdown of polymer	Use a fresh lot of polymer.
	Capillary array used for more than 100 injections	Replace with new capillary array.
	Degraded formamide	Use fresh Hi-Di formamide and ensure correct storage conditions.
	High salt concentration in samples	Use a recommended protocol for salt removal. Dilute salts with water.
Poor resolution in some capillaries	Insufficient filling of array	Refill array and look for cracked or broken capillaries. If problem persists contact Technical Support.
		Re-inject the same samples.
	Poor quality samples	Check the sample preparation.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
No current	Poor quality water	Use only high-quality ultra-pure water.
	Water placed in buffer reservoir position 1	Replace with fresh 1X Genetic Analyzer buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Not enough buffer in anode reservoir	Add buffer up to the fill line.
	Buffer too dilute	Prepare 1X Genetic Analyzer Buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Add 3 mL10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Bubble(s) present in the polymer block and/or the capillary and/or polymer tubing	Pause run and inspect for the instrument for bubbles. They may be hidden in the polymer tubing. Remove any bubbles according to the remove bubble procedure in the Replace Polymer Wizard.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Elevated current	Decomposed polymer	Open fresh lot of polymer and store at 4 °C.
	Incorrect buffer dilution	Prepare 1X Genetic Analyzer Buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Add 3 mL 10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Arcing in the gel block	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.
Fluctuating current	Bubble in polymer block	Pause the run, check the polymer path for bubbles, and remove them if present.
	A slow leak may be present in the system	Check polymer blocks and syringes for leaks. Tighten all fittings.
	Incorrect buffer concentration	Prepare 1X Genetic Analyzer Buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Add 3 mL 10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Not enough buffer in anode reservoir	Add buffer up to the fill line.
	Clogged capillary	Refill capillary array and check for clog.
	Arcing	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Poor performance of capillary array used for fewer than 100 runs	Poor quality samples, possible cleanup problems	Desalt samples using a recommended purification protocol.
	Poor quality formamide	Prepare fresh Hi-Di formamide and reprep samples. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Incorrect buffer	Use 10X Genetic Analyzer Buffer with EDTA to prepare 1X Genetic Analyzer Buffer with EDTA. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Migration time becomes progressively slower	Leak in system	Tighten all ferrules, screws, and check valves. Replace any faulty parts.
	Improper filling of polymer block	Check polymer pump force. If the force needs to be adjusted, call a service representative.
	Expired polymer	Check expiration of polymer. If necessary, change the lot.
Migration time becomes progressively faster	Water in syringe resulting in diluted polymer	Clean the syringe and dry it with compressed air.
Extra peaks in the electropherogram	Data off scale	Dilute the sample and re-inject the sample.
	Possible contaminant in sample	Re-amplify the DNA.
	Sample renaturation	Heat-denature the sample in Hi-Di formamide and immediately place on ice. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

3100 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Peaks exhibit a shoulder effect in GeneScan applications	Sample renaturation	Heat-denature the sample in Hi-Di formamide and immediately place on ice. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Purging of polymer from the polymer reserve syringe	Arcing in the anode gel block	Replace the lower polymer block.
	Bubbles in syringes	Remove bubbles.
Leaking polymer at the top of either syringe	Insufficient seal around the TEFLON® tip of the plunger	Make sure to wet the TEFLON before filling the syringe with polymer. If the leaking persists, replace the syringe. **Note** Do not mix and match barrels and plungers
Leaking polymer at the bottom of the polymer-reserve syringe	Improper tightening of the array ferrule knob to the syringe or/and to the polymer block	Ensure the array ferrule knob is tightened.
Error message, “Leak detected” appears. The run aborts	Air bubbles in the polymer path	Check for bubbles and remove if present. Then, look for leaks.
Buffer jar fills very quickly with polymer	Air bubbles in the polymer path	Check for bubbles and remove if present. Bubbles can cause polymer to fill the jar.
Detection window pops out while replacing the capillary array. Replacing the window in the correct orientation is difficult	Tightening of the array ferrule knob at the gel block causes high tension	Loosen the array ferrule knob to allow the secure placement of the window. Retighten and close the detection door.
Detection window stuck. It is difficult to remove when changing the capillary array		To loosen the detection window: a. Undo the array ferrule knob and pull the polymer block towards you to first notch. b. Remove the capillary comb from the holder in oven. c. Hold both sides of the capillary array around the detection window area, and apply gentle pressure equally on both sides. d. Release.

377 Instrument Troubleshooting

377 Instrument Troubleshooting

Observation	Possible Cause	Recommended Actions
Misshapen wells	Suction when removing comb	Lay gel flat, pour 1X TBE over comb, and remove comb slowly.
Severely bowed gel image	Clamping bottom of gel plates	Clamp plates as recommended in instrument user guide.
	Gel extruded between plates into upper buffer reservoir	After cleaning plates, wash briefly in 3 M HCl, then rinse with water. Remake gel. **⚠ DANGER CHEMICAL HAZARD**. **Hydrochloric acid (HCl)** causes severe eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Fuzzy or smeared bands in electropherogram	Dirty gel plates	Clean plates with Alconox detergent and a soft cloth. Rinse thoroughly with deionized water.
	Old or low quality reagents used to make the gel	Use only the highest quality, fresh reagents to make gels.
	Excess urea in wells before loading	Flush wells immediately before loading.
	Old formamide	Use fresh, deionized formamide. **⚠ WARNING CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Wrong TBE buffer formulation	Remake buffer, carefully following the protocol.
Signal gets weaker over time	Outdated or mishandled reagents	♦ Check expiration dates on reagents. ♦ Store and use according to manufacturer's instructions. ♦ Compare with fresh reagents.
Red or green smearing on gel	Gel dried out before running	Wrap gel ends with damp lint-free wipes and plastic wrap before storing. Use gel within 24 hr.

377 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Actions
Inconsistent mobilities from gel to gel	Total polymer percentage wrong	Remake gel, carefully following protocol.
	Wrong TBE buffer concentration	Remake buffer, carefully following protocol.
	Poor quality reagents	Remake TBE and gel solution stock using fresh reagents from a reliable source. Use ultrapure urea. **⚠ CAUTION CHEMICAL HAZARD. TBE 10X liquid concentrate** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION 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.
	Gel not properly degassed	When degassing the acrylamide solution, keep vacuum strength at ~20 inches Hg and leave under vacuum for 5 min. Stir and pour gel solutions gently. Filter and pour gels at 20 to 23 °C.
	Variations in spacers	Use spacers and combs that are of equal thickness. Keep together as dedicated sets.
Poor resolution	Poor quality or old reagents	Use fresh reagents from a reliable source.
	Small bubble between load and read region	Cast gel as described in protocol, and avoid introducing air bubbles.
	Well shape not flat	Ensure that no air is trapped by comb. Remove comb carefully, and only load in flat wells.
	Wrong TBE buffer formulation	Remake buffer, carefully following protocol.
Gel image not showing 50 and/or 400 bp bands	Gel ran slower or faster than expected	Under **Gel** Menu, select **Regenerate Gel Image**. Adjust the scan range. Click **OK.**
Size standard peaks not recognized when defining size standard	Height of size standard peak <50 fluorescent units	Rerun sample, adding recommended volume of size standard.
	Peak threshold set too high	Reset peak threshold to **50** fluorescent units, and reanalyze.
	Minimum peak half-width set too high	Set minimum peak half-width to **3** and reanalyze.
	Size standard not properly defined	Redefine size standard.
	Gel image does not display all analysis peaks	Under **Gel** menu, select **Regenerate Gel Image**. Adjust the scan range to full range. Click **OK**.

377 Instrument Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Actions
GeneScan-500 LIZ electropherogram displays extraneous peaks	Samples not fully denatured	Make sure samples are heated at 95 °C in a thermal cycler for 5 min and cooled in an ice water bath prior to loading.
	Renaturation of denatured samples	Load sample immediately following denaturation, or store on ice no longer than 1 hr before loading.
	Bleedthrough peaks due to off-scale peaks in another color.	♦ Check raw data. ♦ Dilute PCR products.
Lower than usual peak heights for size standard and samples	Cassette not flush with back heat transfer plate and alignment pins	Place cassette flush against back heat transfer plate. The plate must touch the alignment pins.
	Optics/detector misalignment	Call Applied Biosystems Technical Support.
	Insufficient PCR product loaded onto the gel	Mix 2.0 µL of PCR product and 3.0 µL of Loading Cocktail. Denature and load appropriate volume.
Low signal	Insufficient PCR product loaded onto the gel	Mix 2.0 µL of PCR product and 3.0 µL of Loading Cocktail. Denature and load appropriate volume.
		Concentrate samples by following the procedure in “Concentrating Samples to Increase Signal Strength” on page 2-8.

Gels

Gels Troubleshooting

Problem	Possible Cause	Solution
Leaking wells	Loose combs	Sequencing: Electrophorese immediately, then after each three loads with the eight-channel loader. GeneScan: Leaking wells are not tolerated in GeneScan applications. If a well leaks, it is best to run another gel. At the very least, do not use the wells around the leaking lane.
	Bad clamps	Be sure to use three “bulldog” clamps (P/N 4305386) with 10 to 12 lbs. clamping pressure.
	Burrs or bent teeth on comb	Remove the burrs or replace the comb.
	Bent, kinked, or damaged spacers	Replace the spacers.
Error: “Your CCD offset is too high. I will reset it to zero.”	The CCD reading is below zero during calibration scan	Reset the CCD offset value: Open **377-96 Collection**. In the **Run** window select the **Run** module. Double-click the small document icon next to the **Run Module** drop-down menu. Change the CCD offset value to **zero**. Click **Save as Default**.
Comb is difficult to insert	Using a different comb	Be sure to use same comb for loading that was used for casting.
	Clamps are too tight	♦ Insert comb slowly. Fix any misaligned teeth with a syringe before they touch the gel. ♦ Use looser clamps on future gels.

Run Results

Run Results Troubleshooting

Problem	Possible Cause	Solution
Odd and even lanes overlap	Running too long between staggered loadings	Shorten the run time between loadings.
	Too much salt in the sample	♦ Resuspend samples in formamide only. ♦ Perform extra 70% ethanol rinse of samples if precipitated (may lead to slight loss in signal). **⚠ WARNING CHEMICAL HAZARD.** **Ethanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Signal showing up in neighboring lanes	Leaky lanes	Check clamps and comb fit.
	Signal intensity very high and signal is being detected in neighboring lanes due to closeness of spacing	♦ Move tracker lane position from center of band to the edge of the band away from the strong signal and extract as usual. ♦ Use one or two lane averaging to extract lanes. ♦ Load less volume.
Signal too weak	Multiple	♦ Resuspend the samples in less volume (concentrate). ♦ Increase the CCD gain to four: a. Open 377-96 Collection. b. In the **Run** window select the **Run** module. c. Double-click the small document icon next to the **Run Module** drop-down menu. d. Change the CCD gain to four.

377-96 Instrument Error Messages

377-96 Instrument Error Messages

Message	Possible Cause	Solution
A Valid 96 Lane Firmware Image is Required!	A non-96 collection software has tried to establish communications with a 377 instrument that has the 96-lane option installed	Install the 96-lane collection software and firmware.
EP Voltage Deviation Exceeds Tolerance	The EP voltage deviated outside its tolerance range. The instrument operation is paused	Call Applied Biosystems Technical Support.

377-96 Instrument Error Messages *(continued)*

Message	Possible Cause	Solution
Err: Coolant Flow Failure!	Occurs after the pump was turned on and off three times to see if coolant flow was detected	Open the **Manual Control** window and try to turn on the pump manually. If the problem persists call Technical Support.
Flow Detected With Pump Off –External Cooling In Use!	Either: The wrong module is being used for a run where an external cooling device is attached, or The internal coolant system valve is stuck on or in the open position	If an external cooling device is in use: ♦ Check the modules selected on the run sheet. Use Chiller modules. If no external cooling system is in place: ♦ Try to start a run as follows: a. Click **OK** in the error message box and try to start the run. b. Open the **Manual Control** window and try to turn on the pump manually. ♦ Call Applied Biosystems Technical Support.
No flow detected! Attempted Pump Restart	Indicates the coolant pump was turned on, but no coolant flow was detected by the flow switch	Check the reservoir to see if there is liquid in the cooler.
Scanner Did Not Find Its Home Position	Indicates the scanner did not find its home position prior to collecting data for a plate check, prerun, or run	Reset by pressing the **Reset** button once on the back of the 377 instrument. Click the **Resume** button in the **Collection Run** window.
Warning: Plate Out. Thermistor P43/J43 Open/Short Circuit Warning: Plate In. Thermistor P44/J44 Open/Short Circuit Warning: Possible Heater Thermistor Open/Short Circuit	Indicates one of the following: ♦ Possible open or short circuit exists with the thermistor/cable connected to J43 or J44 ♦ Temperature of the plate in an instrument with the 100k ohm thermistors is 21.9 °C or less	One of the thermistors is not functioning properly. Schedule a service call, and continue to operate the instrument as usual. This message may appear when you launch data collection software and start a plate check, prerun, or run.

310 Instrument Troubleshooting

310 Instrument Troubleshooting

Observation	Possible Causes	Recommended Actions
Data not automatically analyzed	Sample sheet not completed	Complete sample sheet as described in “Performing a Run” on page 6-7.
	Injection list not completed	Complete injection list as described in “Performing a Run” on page 6-7.
	Preferences not set correctly in ABI PRISM® 310 Data Collection Software	In **Preferences** under the **Window** menu, select **Injection List Defaults** and the **Autoanalyze** check box. Also specify directory path to GeneScan Analysis.
Extra peaks visible when sample is known to contain DNA from a single source	Incomplete denaturation before loading onto instrument	Follow the protocol in Chapter 6 to denature the samples.
Current too high	Decomposition of urea in the POP-4™ polymer solution	Add fresh POP-4 polymer solution to the syringe. **⚠ CAUTION CHEMICAL HAZARD. POP-4 polymer** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.
	Incorrect buffer concentration	Replace buffer with freshly made 1X Genetic Analyzer Buffer. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

310 Instrument Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
No current	No 1X Genetic Analyzer buffer	Refill buffer vials with 1X Genetic Analyzer buffer. **⚠ CAUTION CHEMICAL HAZARD. 1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Pump block channel blockage	Remove and clean block.
	Loose valve fittings or syringe	Tighten valve fittings and syringe.
	Capillary not flush with electrode	Tape capillary securely to heat plate. Refer to the *ABI PRISM 310 Genetic Analyzer User Guide* (P/N 4317588).
	Electrode bent	Recalibrate the autosampler.
	Instrument problem	♦ Reset tray button. ♦ Call Applied Biosystems Technical Support.
Low signal	PCR product added to non-deionized formamide	Always use deionized formamide for sample preparation. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	PCR product not mixed well with formamide-size standard mix	♦ Mix PCR product with Hi-Di formamide-size standard mix by pipetting up and down several times. ♦ Increase the injection time in 1 sec increments. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Insufficient PCR product loaded	Increase the amount of PCR product loaded to 2.0 µL.
Signal too high		♦ Decrease the voltage. ♦ Decrease the injection time in 1 sec increments.

310 Instrument Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
No signal	Autosampler not calibrated correctly	Calibrate autosampler in X, Y, and Z directions.
	No PCR product added	Add 1.0 µL of each pooled PCR product to the Hi-Di formamide-size standard mix. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Capillary bent out of sample tube	Align capillary and electrode. Recalibrate autosampler.
	Capillary misaligned with electrode	Align capillary and electrode. Recalibrate autosampler.
	PCR product not at bottom of tube	Spin sample tube in microcentrifuge.
	Air bubble at bottom of sample tube	Spin tube in microcentrifuge to remove air bubbles.
	Sealed sample tube septum	Replace septum.
	Instrument problem	♦ Reset tray button. ♦ Call Applied Biosystems Technical Support.
Loss of resolution after 100 bp	Excess salt in sample	Do not concentrate PCR product by evaporation. Use Centricon-100 if necessary.
	Too much DNA in sample	Dilute pooled PCR products with sterile, deionized water. (See “Optimizing Pooling Ratios” on page 2-7.)
	Bad water	Use sterile, deionized water.
	Incorrectly prepared and/or old solutions	Replace buffer and polymer with fresh solutions. **⚠ CAUTION CHEMICAL HAZARD.** **POP polymers** may cause eye, skin, and respiratory tract irritation. Read the MSDS for the polymer you are using, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.
Runs get progressively slower, *i.e.*, size standard peaks come off at higher and higher scan numbers	♦ Leaking syringe	Clean syringe thoroughly.
	♦ Polymer not filling capillary before every injection	Replace syringe. Check for leakage around the teflon seal of the syringe plunger. If leakage, have the seal replaced.

Observation	Possible Causes	Recommended Actions
Runs get progressively faster, *i.e.*, size standard peaks come off at lower and lower scan numbers	Water in syringe	Prime syringe with small volume of polymer and discard. Fill syringe with polymer. **⚠ CAUTION CHEMICAL HAZARD**. **POP polymers** may cause eye, skin, and respiratory tract irritation. Read the MSDS for the polymer you are using, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.
High baseline	Dirty capillary window	Clean capillary window with 95% ethanol. **⚠ WARNING CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Capillary moved out of position in laser window	Position capillary in front of laser window.

No Occurrence of Amplification

If No Amplification Occurs

For PCR failures, repeat PCR on the control DNA (CEPH 1347-02; Applied Biosystems P/N 403062) using the recommended protocol in this user guide, Applied Biosystems reagents, consumables, and thermal cyclers. Make sure that pipets are calibrated, and that reagents have been stored properly.

If the control DNA is amplified, the problem may lie with the sample DNA. We suggest you try the following:

- Use the DNA isolation kit recommended in “Performing PCR.”
- Increase the pooling ratio of that marker.
- Perform a DNA titration with:
  - 1/5 less DNA than the original concentration
  - 1/2 less DNA than the original concentration
  - Twice as much DNA as the original concentration
  - Five times as much DNA as the original concentration
- Increase the number of PCR cycles from 30 to 33–35 by increasing the second set of melt/anneal/extend cycles.

If amplification occurs using samples containing less DNA, inhibitors might be present. Washing the samples in a Centricon-100 may help remove inhibitors.

If amplification occurs using samples containing more DNA, the original concentration of DNA in the sample may not have been high enough, or the sample may be degraded.

Optimizing Marker Performance

For More Information	Refer to Chapter 2, “Performing PCR,” for more detailed information and for protocols for the following suggestions.
Increasing Signal Strength	♦ Increase the amount of a particular marker in your sample by adjusting the pooling ratios for that marker. ♦ Increase the number of PCR cycles from 30 to 33–35 by increasing the second set of melt/anneal/extend cycles. ♦ Increase the magnesium chloride concentration by performing a titration. Background may increase as well. ♦ Decrease the annealing temperature 2 to 3 degrees at a time. Background may increase.
Decreasing Background (Nonspecific Amplification)	♦ Decrease the amount of the marker used by adjusting the pooling ratios if background is interfering with allele calls of other markers. ♦ Increase the annealing temperature 2 to 3 degrees at a time. Overall signal may decrease.

Evaluating the Quality of a Matrix File

Purpose of a Matrix File

While the most intense fluorescence emitted by an ABI PRISM® dye falls within a small wavelength detection range, some fluorescence emission in the detection ranges (the collection windows) of the other dyes in a set always occurs. This is referred to as "spectral overlap." The multicomponent correction matrix (matrix file) is used to correct the spectral overlap that occurs on that particular instrument when the same dyes, gel or polymer, and run modules are used. The matrix file subtracts all overlapping signal from each collection window.

Why Matrix Files Must Be Remade

Because the emission spectra of the dyes vary with the physical environment, matrix files must be remade whenever the following run conditions change:

- Run module
- Dye set
- Type of gel or polymer
- The instrument
- The instrument is upgraded

Factors Affecting Matrix File Quality

Matrix file quality is affected by minor environmental fluctuations including:

- Changes in the polymer/buffer composition due to aging reagents
- Fluctuations in temperature when running the instrument at ambient temperature

Other factors that can affect matrix quality are:

- Minor misalignment in the instrument's optical detection system
- Imperfections in, or misalignment of glass plates when using a gel

How to Recognize Matrix Problems

A poor or incorrect matrix results in too much or too little spectral overlap correction. Each scenario causes one of the following recognizable electropherogram anomalies.

- Bleedthrough peaks (also referred to as pull-ups)
- Elevated baseline

Bleedthrough Peaks

Bleedthrough peaks are small peaks of one color that lay directly under larger peaks of another color even though there is no PCR product corresponding to the smaller peak. Bleedthrough peaks occur when:

- Not enough of one color is subtracted from another color
- Signal from one color is offscale and saturates the detector

In Figure 9-1 on page 9-33, bleedthrough occurred because not enough of the fluorescence from the blue dye-labeled product was subtracted from the green channel, resulting in the appearance of a false peak (the green peak).

When bleedthrough occurs because the signal from one color is offscale and saturates the detector, software is unable to subtract the correct amount of signal from the other colors.

Elevated Baseline

An elevated baseline occurs when too much of one color is subtracted from another color. Note in Figure 9-2 on page 9-33 how the green baseline between the two large black peaks (yellow signal) is elevated. This occurred because too much of the yellow signal was subtracted from the green signal, resulting in abnormally low green data points. ABI PRISM® GeneScan® Analysis Software erroneously interpreted these low data points as the "zero-points", or baseline, for the green signal. The true baseline is elevated.

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

If you suspect that an elevated baseline is being caused by a bad matrix file, inspect the data:

- With the matrix file applied
- Without baselining

Figure 9-3 on page 9-33 represents the same data shown in Figure 9-2 before baselining. Note how the abnormally low green data points appear as troughs beneath the black peaks (yellow signal).

What to Do If You Have Matrix Problems

If matrix-related problems occur with any regularity:

- Run matrix standard samples again.
  - If using a gel, follow the loading guidelines listed below
- Make a new matrix file.
- Reanalyze sample data with the new matrix file for the 377 and 310 instruments only).

Instructions for creating matrix files are located in the following documents:

- Instrument user guide
- *ABI PRISM GeneScan Analysis Software for the Windows NT Platform User Guide* (P/N 4308923)

Preparing Matrix Standard Samples

Refer to Chapter 7, "Matrix Sample and Matrix File Guidelines," or follow the instructions listed on the product inserts to prepare matrix standard samples.

Loading Matrix Standard Samples Onto a Gel

When loading matrix standard samples, we recommend that you:

- Leave at least one empty lane between matrix standard samples and all other samples
- Load the matrix standard samples into every other lane, one per lane

This loading technique will help ensure that a clean matrix is produced. The quality of the matrix file directly effects the quality of the analyzed data.

Matrix File Examples

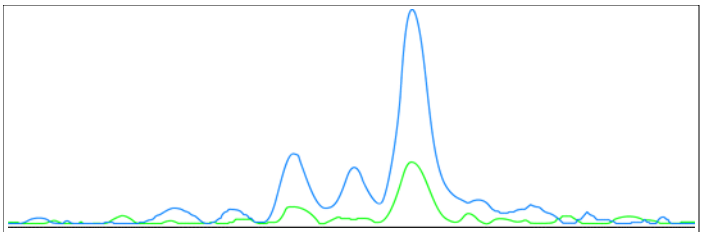


Figure 9-1 Bleedthrough peaks

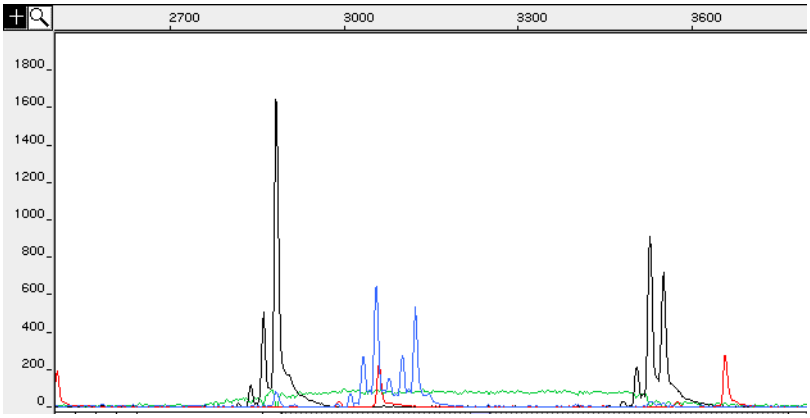


Figure 9-2 Example of an elevated baseline caused by a bad or incorrect matrix

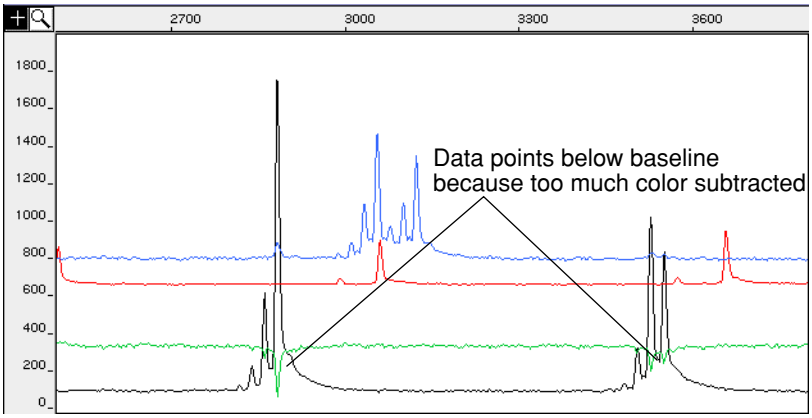


Figure 9-3 Same data as shown in Figure 9-2 after applying a matrix file without baselining

Obtaining Technical Support

A

Services and Support

Applied Biosystems Web Site

To access the Applied Biosystems Web site, go to:

http://www.appliedbiosystems.com

At the Applied Biosystems Web site, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Applied Biosystems Web site provides a list of telephone and fax numbers that can be used to contact Technical Support.

How to Order

B

Overview

In This Appendix

The following topics are covered in this appendix.

Ordering Complete Linkage Mapping Sets

Linkage Mapping Set v2.5-MD10

Use the appropriate part number from the following table to order complete sets of 28 panels in the ABI PRISM® Linkage Mapping Set v2.5-MD10:

Number of Reactions per Tube (15-µL reactions)	Part Number[a]	Phone Number
50	4329186	1.800.327.3002
300	4329185	1.800.327.3002
1200	4329184	1.800.327.3002
User-defined large-scale	4329183	1.650.638.5660

a. The *ABI PRISM Linkage Mapping Set Version 2.5 User Guide* (P/N 4330202) is included with each of these parts.

Linkage Mapping Set v2.5-HD5

Use the appropriate part number from the following table to order complete sets of 86 panels in the Linkage Mapping Set v2.5-HD5:

Number of Reactions per Tube (15-µL reactions)	Part Number[a]	Phone Number
300	4329189	1.800.327.3002
1200	4329188	1.800.327.3002
User-defined large-scale	4329187	1.650.638.5660

a. The *ABI PRISM Linkage Mapping Set Version 2.5 User Guide* (P/N 4330202) is included with each of these parts.

Ordering Individual Panels

Linkage Mapping Set v2.5-MD10

Use the appropriate part number from the following table to order all 28 panels in the ABI PRISM Linkage Mapping Set v2.5-MD10. Call 1.800.327.3002 to order.

Chromosome	Panel No.	50 Rxn P/N	300 Rxn P/N
1	1	4329306	4329334
	2	4329307	4329335
2	3	4329308	4329336
	4	4329309	4329337
3	5	4329310	4329338
	6	4329311	4329339
	7	4329312	4329340
4	5	4329310	4329338
	6	4329311	4329339
	7	4329312	4329340
5	8	4329313	4329341
	9	4329314	4329342
	10	4329315	4329343
6	8	4329313	4329341
	9	4329314	4329342
	10	4329315	4329343
7	11	4329316	4329344
	12	4329317	4329345
8	11	4329316	4329344
	12	4329317	4329345
9	13	4329318	4329346
	14	4329319	4329347
	15	4329320	4329348
	16	4329321	4329349
10	13	4329318	4329346
	14	4329319	4329347
	15	4329320	4329348
	16	4329321	4329349
11	13	4329318	4329346
	14	4329319	4329347
	15	4329320	4329348
	16	4329321	4329349
12	17	4329322	4329350
	18	4329323	4329351
	19	4329324	4329352
13	17	4329322	4329350
	18	4329323	4329351
	19	4329324	4329352
14	20	4329325	4329353
15	21	4329326	4329354
	22	4329327	4329355
16	21	4329326	4329354
	22	4329327	4329355
17	23	4329328	4329356
	24	4329329	4329357
18	23	4329328	4329356
	24	4329329	4329357
19	25	4329330	4329358
	26	4329331	4329359
	27	4329332	4329360
20	25	4329330	4329358
	26	4329331	4329359
	27	4329332	4329360
21	25	4329330	4329358
	26	4329331	4329359
	27	4329332	4329360
22	25	4329330	4329358
	26	4329331	4329359
	27	4329332	4329360
X	28	4329333	4329361

Linkage Mapping Set v2.5-HD5: 300 Reaction Size Panels

Call 1.800.327.3002 to order:

Chromosome	Panel No.	P/N
1	1	4329334
	2	4329335
	29	4329362
	30	4329363
	31	4329364
	32	4329365
2	3	4329336
	4	4329337
	33	4329366
	34	4329367
	35	4329368
	36	4329369
3	5	4329338
	6	4329339
	7	4329340
	37	4329370
	38	4329371
	39	4329372
	40	4329373
4	5	4329338
	6	4329339
	7	4329340
	41	4329374
	42	4329375
	43	4329376
5	8	4329341
	9	4329342
	10	4329343
	44	4329377
	45	4329378
	46	4329379
6	8	4329341
	9	4329342
	10	4329343
	47	4329380
	48	4329381
	49	4329382

Chromosome	Panel No.	P/N
7	11	4329344
	12	4329345
	50	4329383
	51	4329384
	52	4329385
8	11	4329344
	12	4329345
	53	4329386
	54	4329387
	55	4329388
9	13	4329346
	14	4329347
	15	4329348
	16	4329349
	56	4329389
	57	4329390
	58	4329391
10	13	4329346
	14	4329347
	15	4329348
	16	4329349
	59	4329392
	60	4329393
	61	4329394
11	13	4329346
	14	4329347
	15	4329348
	16	4329349
	62	4329395
	63	4329396
12	17	4329350
	18	4329351
	19	4329352
	64	4329397
	65	4329398
	66	4329399

Chromosome	Panel No.	P/N
13	17	4329350
	18	4329351
	22	4329355
	72	4329405
	73	4329406
	19	4329352
	67	4329400
14	20	4329353
	68	4329401
	69	4329402
15	21	4329354
	22	4329355
	70	4329403
	71	4329404
16	21	4329354
17	23	4329356
	24	4329357
	74	4329407
	75	4329408
18	23	4329356
	24	4329357
	76	4329409
	77	4329410

Chromosome	Panel No.	P/N
19	25	4329358
	26	4329359
	27	4329360
	78	4329411
	79	4329412
20	25	4329358
	26	4329359
	27	4329360
	80	4329413
21	25	4329358
	26	4329359
	27	4329360
	81	4329414
22	25	4329358
	26	4329359
	27	4329360
	82	4329415
X	28	4329361
	83	4329416
	84	4329417
	85	4329418
	86	4329419

User-Defined Large-Scale Panels

For user-defined large-scale sets and panels, please inquire by calling 1.800. 327.3002.

Ordering Individual Markers

Individual Markers

Specify the locus when placing an order with this part number.

Number of Reactions per Tube (15-µL reactions)	Part Number	Phone Number
300	4329191	1.800.327.3002
User-defined large-scale	4329190	1.650.638.5660

IMPORTANT Markers are available only with their specified dye and cannot be changed.

Ordering True Allele PCR Premix with AmpliTaq Gold DNA Polymerase

True Allele True Allele™ PCR Premix with AmpliTaq Gold® DNA Polymerase

Number of Reactions per Kit	Part Number	Phone Number
2000	403061	1.800.327.3002

Ordering Control DNA CEPH 1347-02

Control DNA Control DNA individual CEPH 1347-02

Description	Number of Reactions	Part Number	Phone Number
Each tube contains: Cell line DNA from CEPH individual 1347-02 180 µL DNA at a concentration of 50 ng/µL in 10 mM Tris HCl, 0.1 mM EDTA (pH 8.0)	150 (based on the standard, 15-µL reaction protocol)	403062	1.800.327.3002

Ordering GeneScan–500 LIZ Size Standard

GeneScan Standard GeneScan–500 LIZ Size Standard

Description	Part Number	Phone Number
Kit includes: ♦ 2 tubes of size standard, 200 µL/tube ♦ 1 tube of Loading Buffer, 400 µL/tube [25 mM EDTA (ph 8.0) with blue dextran (50 mg/mL)]	4322682	1.800.327.3002

Matrix Standard Kits

Matrix Kits

The following matrix standard sample kits are required for this product:

DS33 (6-FAM, VIC, NED, PET, LIZ)	P/N
Matrix standard set DS33 for the 3700 instrument	43275691 with CD[a] 43182541 without CD
Matrix standard set DS33 for the 3100 instrument	4323016
Matrix standard set DS33 for the 310/377 instrument	4318159

a. The CD is five-dye updated for ABI PRISM® 3700 DNA Analyzer Data Collection Software versions 1.0, 1.1, and 1.1.1. The CD contains the necessary instructions and files for running five-dye chemistry (6-FAM™, VIC™, NED™, PET™, and LIZ™) on the 3700 DNA Analyzer.

User Documentation for the Linkage Mapping Set v2.5

Documents

Title	Part Number	Phone Number
ABI PRISM Linkage Mapping Set Version 2.5 User Guide	4330202	1.800.327.3002
ABI PRISM Linkage Mapping Set v2.5 Panel Guide	4330150	

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C

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