ABI PRISM® 3100 Genetic Analyzer

Quick Start Guide for Fragment Analysis

Applied Biosystems | HITACHI

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1 Introduction

Overview

In This Chapter

This chapter includes the following topics:

About This Manual

Purpose The purpose of this manual is to give users basic instructions on how to:

- Do a fragment analysis run
- Analyze the resulting data
- Calibrate and perform routine maintenance on the ABI PRISM® 3100 Genetic Analyzer

For More Information

Where to Find More Information Other manuals and guides that relate to the 3100 Genetic Analyzer are listed below.

If you want…	Refer to the…	Part Number
safety information and information about preparing your lab for the 3100 Genetic Analyzer	*ABI PRISM 3100 Genetic Analyzer Site Preparation and Safety Guide*	4315835
detailed information about the 3100 Genetic Analyzer	*ABI PRISM 3100 Genetic Analyzer User's Manual*	4315834
detailed information about analyzing and viewing fragment data using the ABI PRISM® GeneScan® Analysis Software or the ABI PRISM® GeneMapper® Analysis Software	*ABI PRISM GeneScan Analysis Software v. 3.7 User Guide*	4308923
an abbreviated procedure for how to do a typical sequencing run, view and analyzer run data, and perform common maintenance operations	*ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Sequencing*	4315833
information on a procedure for block cleaning	*ABI PRISM 3100 Genetic Analyzer Block Cleaning Procedure*	4322930

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:

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 of the MSDS.

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

Instrument Safety Labels

Safety labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (*e.g.,* CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
- A message panel, which explains the hazard and any user action required.
- A safety alert symbol, which indicates a potential personal safety hazard. See the *ABI PRISM 3100 Genetic Analyzer Site Preparation and Safety Guide* for an explanation of all the safety alert symbols provided in several languages.

About Waste Disposal

As the generator of potentially hazardous waste, it is your responsibility to perform the actions listed below.

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

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

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories
- Received instruction in specific safety practices for the instrument
- Read and understood all related MSDSs

⚠ CAUTION Avoid using this instrument in a manner not specified by Applied Biosystems. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

Safe and Efficient Computer Use

Operating the computer correctly prevents stress-producing effects such as fatigue, pain, and strain.

To minimize these effects on your back, legs, eyes, and upper extremities (neck, shoulder, arms, wrists, hands and fingers), design your workstation to promote neutral or relaxed working positions. This includes working in an environment where heating, air conditioning, ventilation, and lighting are set correctly. See the guidelines below.

⚠ CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by the following potential risk factors which include, but are not limited to, repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

- Use a seating position that provides the optimum combination of comfort, accessibility to the keyboard, and freedom from fatigue-causing stresses and pressures.
  - The bulk of the person's weight should be supported by the buttocks, not the thighs.

  - Feet should be flat on the floor, and the weight of the legs should be supported by the floor, not the thighs.
  - Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
  - The proper height to position the forearms horizontally and upper arms vertically.
  - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
  - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
  - Positioning the screen to minimize reflections from ambient light sources.
  - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.
- When considering the user's distance from the screen, the following are useful guidelines:
  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

Electrical Shock Warnings

⚠ WARNING ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high-voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

⚠ WARNING ELECTRICAL SHOCK HAZARD. To reduce the chance of electrical shock, do not remove covers that require tool access. No user serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.

Laser Warning

⚠ WARNING LASER BURN HAZARD. An overheated laser can cause severe burns if it comes in contact with the skin. DO NOT operate the laser when it cannot be cooled by its cooling fan. Always wear laser safety goggles.

Moving and Lifting the Instrument

⚠ CAUTION PHYSICAL INJURY HAZARD. Improper lifting can cause painful and sometimes permanent back injury.

Use proper lifting techniques when lifting or moving the instrument. Safety training for proper lifting techniques is recommended.

Do not attempt to lift or move the instrument without the assistance of others. Depending on the weight of the instrument, this action may require two or more people.

Performing a Fragment Analysis Run 2

Overview

In This Chapter This chapter includes the following topics:

Before You Begin

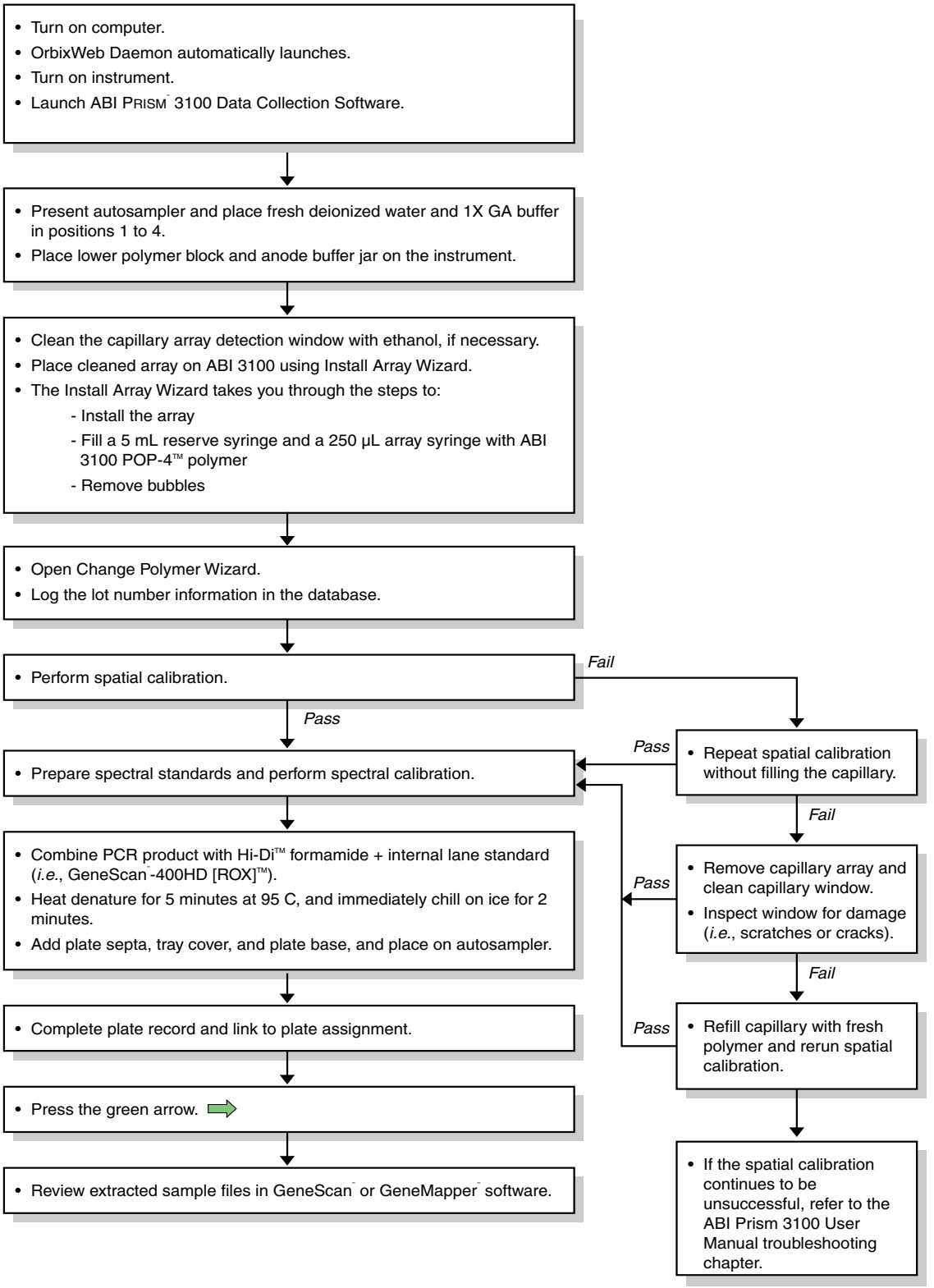
Assumptions

The procedures in this chapter make the following assumptions:

- The computer and the instrument have been correctly configured.
- The instrument has been calibrated: spatial and spectral calibrations have been successfully run. If necessary, refer to Chapter 4 of this guide.
- There is sufficient space on the computer hard drive to store the data that will be generated. If necessary, refer to Chapter 5 of this guide.

ABI PRISM® 3100 Genetic Analyzer - User Flowchart for Fragment Analysis

User Flowchart



Sample Preparation

Dye Set

The ABI PRISM® 3100 Data Collection Software version 1.0.1 supports Dye Set DS-30, DS-31, DS-32, DS-33, DS-02, and the ABI PRISM® Linkage Mapping Sets-LD20, -MD10, and -HD5.

Dye Sets, Dye Chemistry, and Applications:

Dye Set	Dye Chemistry	Application Kit
DS-30 (D)	6-FAM™ (blue), HEX™ (green), NED™ (yellow), ROX™ (red)	♦ Linkage Mapping Sets-LD20, -MD10, and -HD5 ♦ Custom Oligos
DS-31 (D)	New 4-Dye Chemistry: 6-FAM (blue), VIC™ (green), NED (yellow), ROX (red)	♦ Linkage Mapping Custom Oligos ♦ Mouse Mapping Markers ♦ Custom Oligos
DS-32 (F)	5-FAM™, JOE™, NED, ROX	♦ AmpFℓSTR® products ♦ Stockmarks™
DS-33 (G5) 5-dye chemistry for high throughput genotyping	6-FAM, VIC, NED, PET™, LIZ™	♦ AmpFℓSTR® Identifiler™ ♦ Custom Oligos
DS-02 (E5)	dR110, dR6G, dTAMRA™, dROX™, LIZ	SNAPshot™ Multiplex Kit

Pooling Ratios

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

IMPORTANT Follow the suggested protocol for each application. See the Linkage Mapping Sets example below.

Pooling Ratios for the ABI PRISM Linkage Mapping Sets

For Linkage Mapping Sets-LD20, -MD10, and -HD5, a ratio of 1:1:1 (6-FAM:HEX:NED- labeled products) gives acceptable balance across most loci. For each Linkage Mapping Set panel, pool 1 µL of each PCR product in a microcentrifuge tube. If necessary, bring the total volume to 10–20 µL with deionized water.

Suggested Loading Volumes

⚠ 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.

Prepare the formamide:size standard mix using:

- 1000 µL of Hi-Di™ Formamide (P/N 4311320) or similar quality formamide
- 50 µL of GS500 ROX, GS500 LIZ, GS120 LIZ, or GS400 HD

Note We recommend that you use Hi-Di Formamide, but if you prefer to prepare your own formamide, see Appendix A, “Preparing Formamide,” for important information.

Note 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.

For loading, mix 1µL 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 3–5 min. There are several acceptable options for covering samples during denaturation. ♦ MicroAmp® Clear Adhesive Films (P/N 4306311) ♦ MicroAmp Caps (12-Strip) (P/N 801-0534) ♦ MicroAmp Caps (8-Strip) (P/N 801-0535) ♦ MicroAmp Optical 96-well Reaction Plates (P/N N801-0560) ♦ MicroAmp 384-well Reaction Plates (P/N 4305505)
2	Place immediately on ice for at least 5 min before loading.

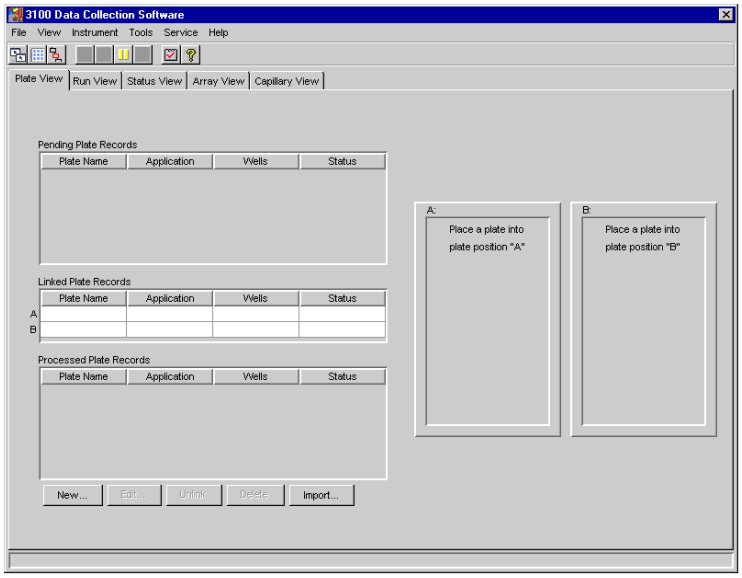
Starting the Data Collection Software

Before You Begin Before starting the ABI PRISM® Data Collection Software:

Step	Action
1	Ensure the computer and monitor are powered on. **IMPORTANT** The computer must be powered on before the instrument. The default user name is “3100User” and the default password is blank.
2	Ensure the ABI PRISM® 3100 Genetic Analyzer is powered on and the green status light is on solid (not flashing).
3	Ensure OrbixWeb Daemon is running by finding its button on the Windows NT taskbar.  If OrbixWeb Daemon is not running, go to the **Start** menu, point to **Applied Biosystems**, and select **OrbixWeb Daemon**. **Note** To create a shortcut: (a) Navigate to orbixd.exe in the following directory: D:\dbtools\iona\orbixweb3.2\bin. (b) Right-click the file. (c) Click **Create Shortcut**. This creates a shortcut named **Shortcut to orbixd.exe**. (d) Drag the shortcut to the desktop. **IMPORTANT** OrbixWeb Daemon must be started before the 3100 Data Collection software can run.

Starting the Data Collection Software

To start the Data Collection software:

Step	Action
1	From the **Start** menu, point to **Applied Biosystems**, and select **3100 Data Collection Software**. **Note** To create an Applied Biosystems shortcut: (a) Navigate to 3100Collection.bat in the following directory: D:\appliedbio\3100\Bin. (b) Right-click the file. (c) Click **Create Shortcut**. This creates a shortcut named **Shortcut to 3100 Collection Software**. (d) Drag the shortcut to the desktop. The 3100 Data Collection software opens and the Plate View window displays as shown below. 

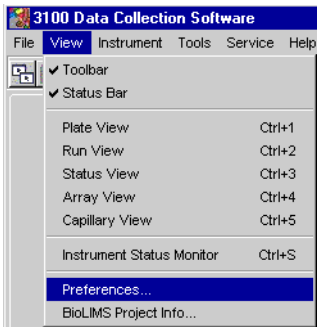
Setting Software Preferences

Introduction

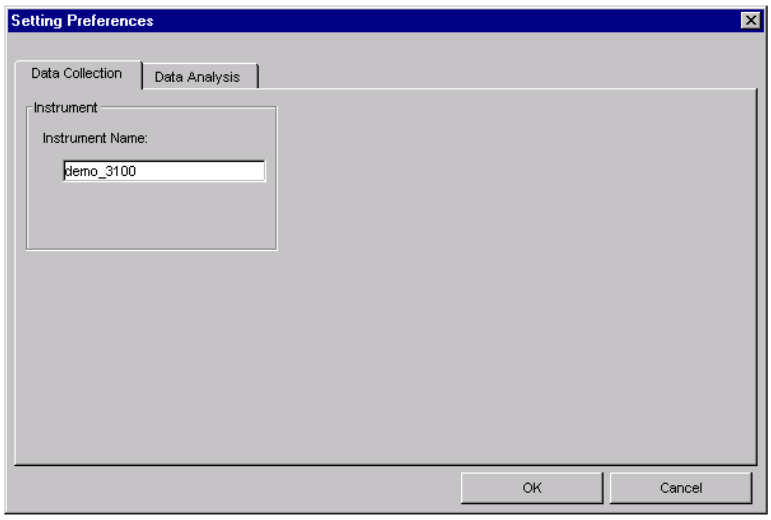
The Data Collection software preferences are set during instrument installation; however, you can view or change these preferences in the Setting Preferences dialog box.

Viewing the Setting Preferences Dialog Box

To view the Setting Preferences dialog box:

Step	Action
1	From the **View** menu, select **Preferences** or click the Preferences button on the toolbar.   The dialog box has two pages and are described below.

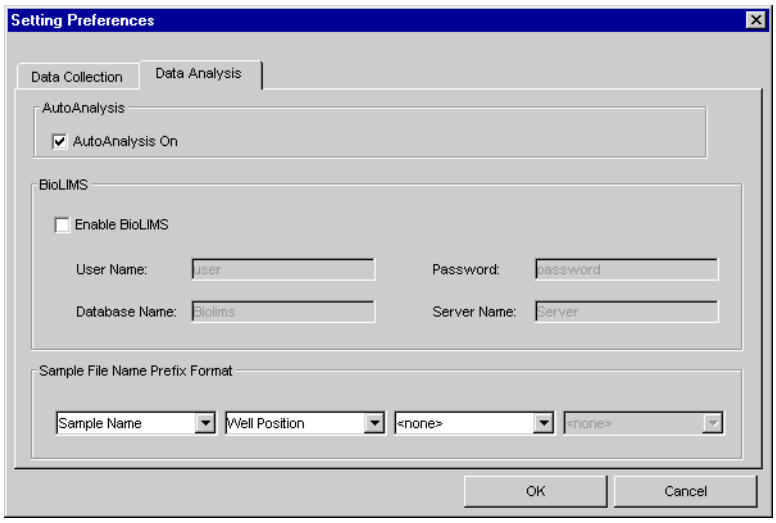
Data Collection Page



The table below describes the preferences that can be set within this page.

Preference	Description
Instrument Name	This field automatically populates with **demo_3100.** You can change it to any name (*e.g.,* the instrument's serial number). **Note** Avoid spaces between words or any special characters.

Data Analysis Page



The table below describes the preferences that can be set within this page.

Data Analysis Page Settings:

Preference	Description
AutoAnalysis On	Select **AutoAnalysis ON** to have samples automatically analyzed by the analysis software after the run. **Note** You will still be able to reanalyze your sample data at a later time.
BioLIMS	Use these settings to have data extracted to a BioLIMS database instead of to sample files on the hard drive.

Data Analysis Page Settings: *(continued)*

<table>
<tr><th>Preference</th><th>Description</th></tr>
<tr><td>Sample File Name Prefix Format</td><td>Specify the format for the sample file names by using the drop-down lists to reorder the identifiers.

<table><tr><th>Identifier</th><th>Origin</th></tr><tr><td>Run ID</td><td>Generated by the Data Collection software and contains the capillary number and the date.</td></tr><tr><td>Sample Name</td><td>Taken from the Plate Editor spreadsheet entry. See page 2-18.</td></tr><tr><td>Well Position</td><td>Taken from the sample's position on the plate (column letter and row number, e.g., C3).</td></tr><tr><td>Plate Name</td><td>Taken from the Plate Editor dialog box entry.</td></tr><tr><td>Instrument ID</td><td>Taken from the Data Collection page preferences entry.</td></tr><tr><td>Array ID</td><td>Taken from the Install Capillary Array Wizard entry.</td></tr></table>
Note In addition to the four identifiers you set with the drop-down lists, all names are automatically appended with the capillary number and a file extension. This is also called the Run ID. Therefore, in the Data Analysis page example shown above, the sample name will be:

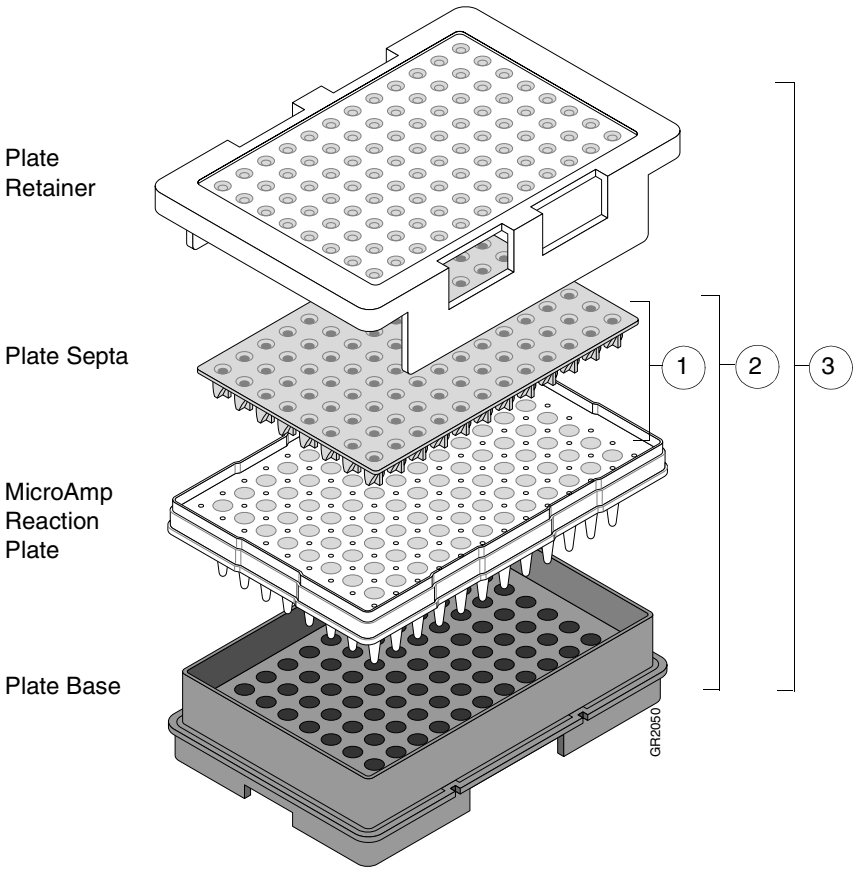
Sample Name_Well Position_Capillary Number.fsa

IMPORTANT Using additional filters will create very long file names which may affect down-stream software analysis.</td></tr>
</table>

Working with Plate Assemblies

Plate Assembly Components

The plate assembly components are assembled as follows:



The table below contains ordering information for the plate assembly components.

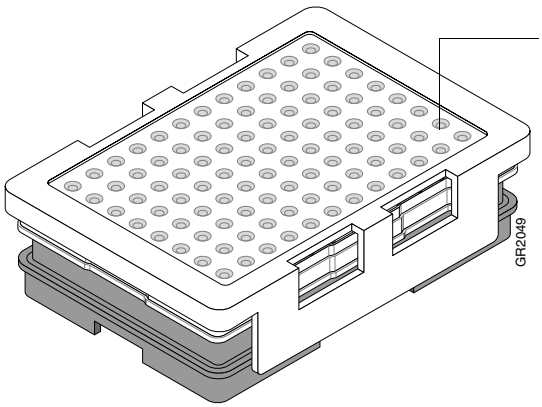
Component	P/N 384-Well	P/N 96-Well
Plate Retainer	**4317240**	**4317241**
Plate Septa	**4315934**	**4315933**
MicroAmp Reaction Plate	**4305505**	**N801-0560**
Plate Base	**4317236**	**4317237**

Preparing a Plate Assembly

To prepare a plate assembly:

Step	Action
1	Secure a clean and dry septa strip on the micro amp optical plate. **IMPORTANT** Never use warped plates. **IMPORTANT** Ensure the septa strip lies flat on the plate.
2	Place the sample plate into the plate base.
3	Snap the plate retainer onto the plate and plate base.

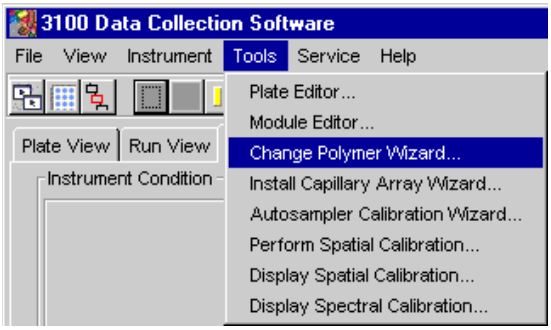
To prepare a plate assembly: *(continued)*

Step	Action
4	Ensure the plate retainer holes are aligned with the holes in the septa strip. **IMPORTANT** Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.  The plate retainer holes must align with the holes in the septa strip.

Checking and Refilling Fluids

Adding or Changing Polymer

Determine whether to add or change the polymer on the instrument before proceeding with instrument preparation.

If polymer on the instrument is...	Then...
less than 1 week old, and sufficient in quantity to complete your runs[a]	Ensure there are no air bubbles, and then proceed with instrument preparation. **Note** To remove any air bubbles, see page 5-4.
greater than 1 week old, or insufficient in quantity to complete your runs	Fill the syringes and the upper polymer block with polymer by following the **Change Polymer Wizard**. For instructions, see page 5-12. **⚠ CAUTION CHEMICAL HAZARD. POP polymers** may cause eye, skin, and respiratory tract irritation. Please 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. 

a. A run uses 50–80 µL of polymer depending on the length of the array. This is equivalent to 60–100 runs from one 5-mL syringe. A minimum of 100µL of polymer is required for the instrument to operate.

IMPORTANT Always replace polymer that is older than 1 week.

IMPORTANT Ensure there are no air bubbles in the upper and lower polymer blocks before proceeding. To remove any air bubbles, see page 5-4.

When to Replace the Buffer

Replace the 1X Genetic Analyzer buffer in the anode buffer reservoir and the cathode buffer reservoir daily, or before each batch of runs.

IMPORTANT Failing to replace buffer may lead to loss of resolution, precision and data quality.

IMPORTANT Replenishing buffer and placing the plate requires that the autosampler be in the forward position, with the capillary tips removed from the buffer solution. Do not leave the autosampler in this position for an extended time because the capillaries can dry out.

Making Buffer for a Single Run

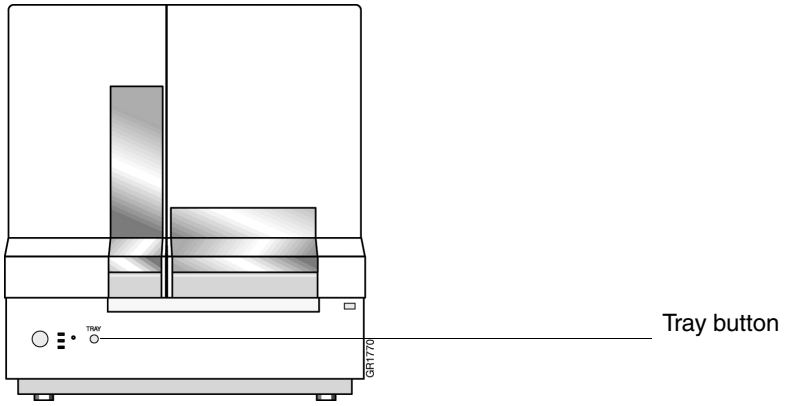
To prepare 50 mL of 1X Genetic Analyzer buffer:

Step	Action
1	Add 5.0 mL of 10X Genetic Analyzer buffer with EDTA into a graduated cylinder. **⚠ CAUTION CHEMICAL HAZARD. Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Add deionized water to bring the total volume up to 50 mL.
3	Mix well.

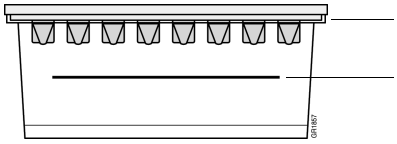
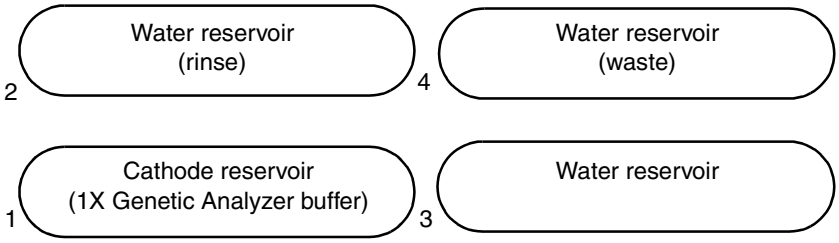
Filling the Water and Cathode Buffer Reservoirs

IMPORTANT Wear gloves while performing the following procedure, and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

To fill the water and cathode buffer reservoirs:

Step	Action
1	Close the instrument doors.
2	Press the Tray button on the outside of the instrument to bring the autosampler to the forward position. 
3	Wait until the autosampler has stopped moving, then open the instrument doors.
4	Remove the cathode buffer reservoir and water reservoirs from the instrument.
5	Dispose of remaining fluids and rinse out the reservoirs with deionized water. **Note** The waste is very dilute; however, you should follow your company's waste disposal practices for appropriate disposal procedures.
6	Rinse the cathode reservoir with 1X Genetic Analyzer buffer, and fill to the line with 1X Genetic Analyzer buffer (about 16 mL).
7	Fill the water reservoirs to the line with quality deionized water (about 16 mL).

To fill the water and cathode buffer reservoirs: *(continued)*

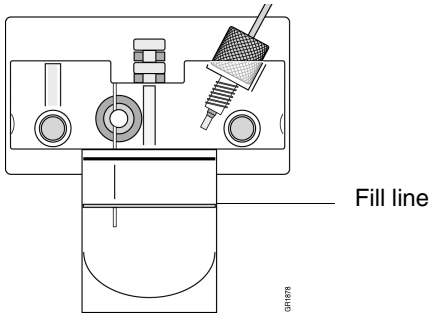
Step	Action
8	Place a clean septa strip on each reservoir, and dry the outside of the reservoirs using a lint-free wipe. **Note** We suggest labeling the reservoirs to prevent mixing them up. **⚠ CAUTION** Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips. 
9	Place the reservoirs into position on the autosampler as shown below. 

Filling the Anode Buffer Reservoir

Change the anode buffer:

- Before each batch of runs, or at least every 24 hours
- Every time you fill the polymer block with new polymer

To fill the anode buffer reservoir to the fill line with 1X Genetic Analyzer buffer:

Step	Action
1	Remove the anode buffer reservoir by firmly pulling down and twisting slowly.
2	Discard the used buffer appropriately.
3	Clean and rinse the reservoir with deionized water, and then rinse with buffer.
4	Fill the reservoir to the fill line with fresh 1X Genetic Analyzer buffer (about 9 mL). 
5	Put the anode buffer reservoir on the instrument. **Note** The meniscus should line up with the fill line.

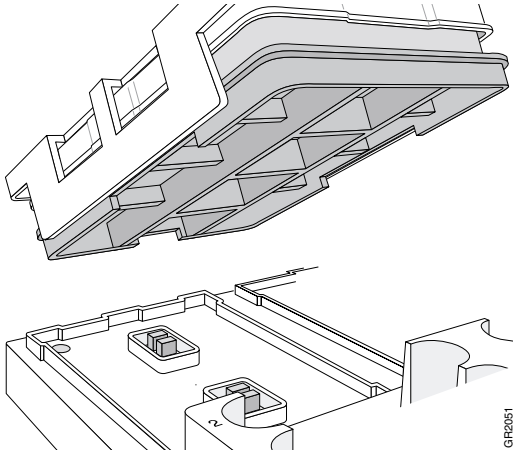
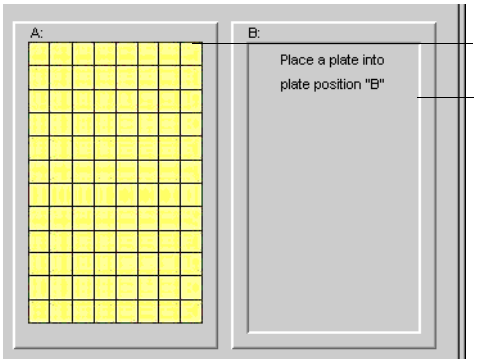
To fill the anode buffer reservoir to the fill line with 1X Genetic Analyzer

Step	Action
6	If the reservoir fills with fluid, repeat this procedure to discard and replace the Genetic Analyzer buffer. **Note** The reservoir could fill during bubble clearing.

Placing the Plate onto the Autosampler

Placing the Plate onto the Autosampler

To place the plate onto the autosampler:

Step	Action
1	Place the plate assembly on the autosampler as shown below. **Note** There is only one orientation for the plate, with the notched end of the plate base away from you.  **IMPORTANT** Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off of the autosampler.
2	When the plate is correctly positioned, the plate position indicator on the **Plate View** page changes from gray to yellow. Check to ensure this has happened. 
3	Close the instrument doors. **Note** Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in buffer.

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

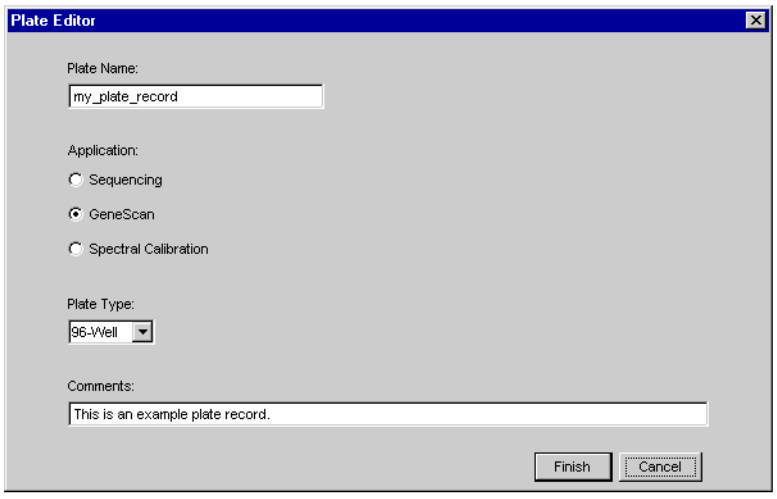
Follow the two procedures below to create a plate record with the Plate Editor.

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.

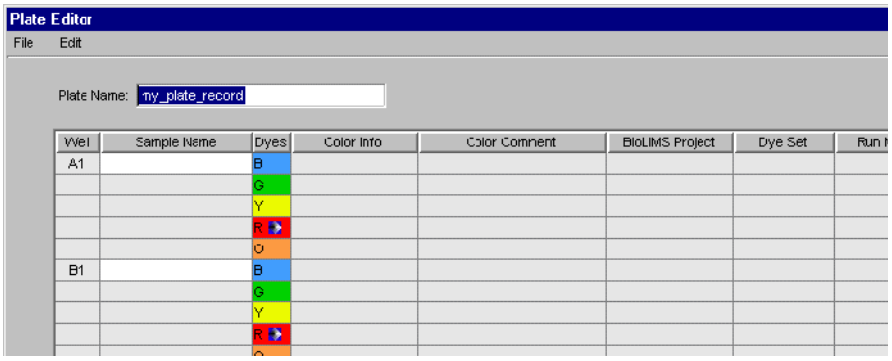
Entering Plate Record Information

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)*

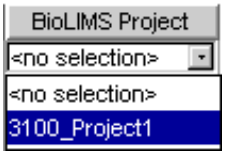
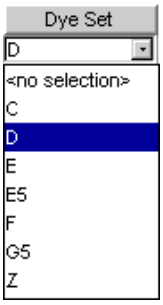
Step	Action
3	Use the **Plate Editor** dialog box to name your plate and to specify the application and plate type. Entering comments is optional. In the Plate Editor dialog box: a. Name your plate. b. Specify the application. c. Select the plate type. d. Enter any comments (optional). **IMPORTANT** 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:

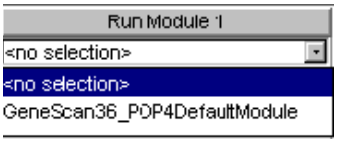
Step	Action
1	In the **Plate Editor** spreadsheet, type the names of all the samples in the **Sample Name** column. (Use **Edit/Copy** and **Edit/Fill Down** whenever a field is the same for all samples in the plate record.) **Note** In the default naming convention, the sample name you type is incorporated into the sample file name. For example: **MySample_A01_01.fsa** Capillary position Well position Sample name you type The sample file naming convention used can be changed in the **Preferences** dialog box. See page 2-9 for details. **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 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.

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

Step	Action
2	*Optional* For each sample, enter **Color Info** and **Color Comment** text.
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*. 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.  **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. This is called a *chemometric process*.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>For each sample, select the appropriate Run Module from the drop-down list.

The table below shows the run module to select based on your run type.
<table>
<tr><th>Analysis Type</th><th>Run Module</th></tr>
<tr><td>GeneScan</td><td>GeneScan36_POP4DefaultModule</td></tr>
<tr><td>SNP</td><td>SNP36_POP4DefaultModule</td></tr>
</table>

Note If you need to view or edit a run module file, see page 2-29.

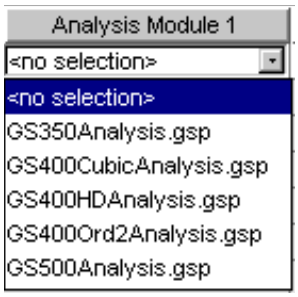
Note If you select different modules for different samples, the samples will be automatically grouped so that all samples with the same run module are run at the same time.

IMPORTANT Runs are scheduled alphanumerically by run module name, not by the order indicated in the plate record, nor by sample name.</td></tr>
</table>

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>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 (see page 2-9).

The table below shows which analysis module to select based on the number of fragments in your size standard.

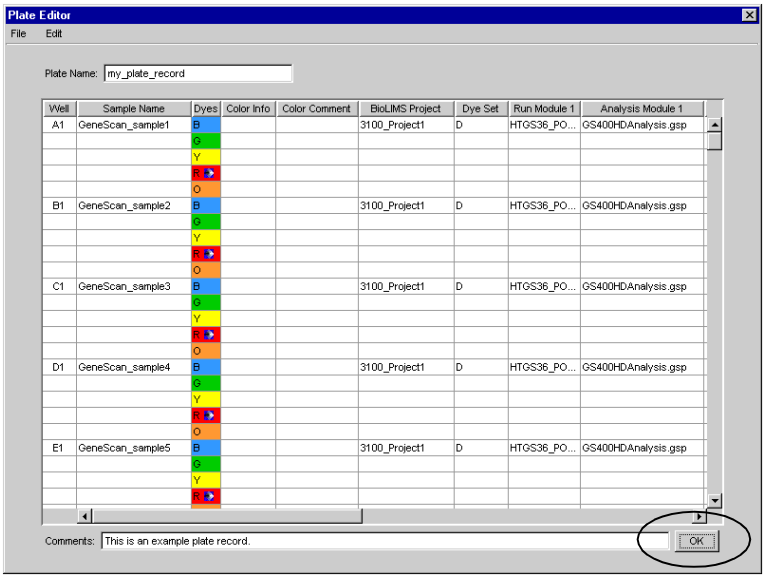
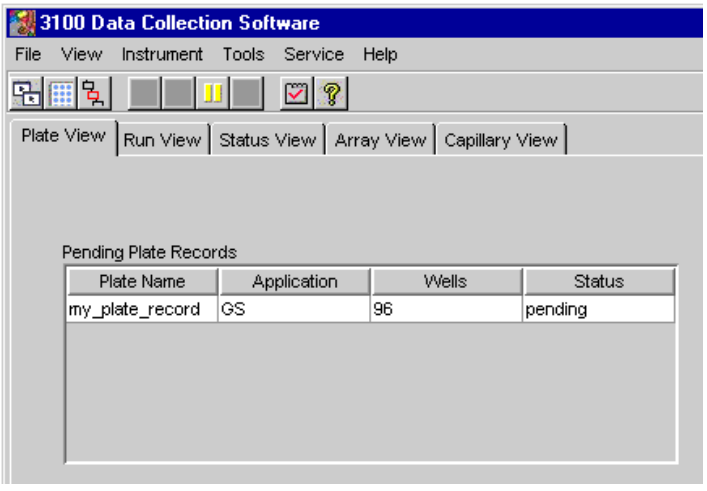
<table>
<tr><th>If using size standard...</th><th>Select this analysis module...</th></tr>
<tr><td>GS120</td><td>GS120Analysis.gsp</td></tr>
<tr><td>GS400HD</td><td>GS400HDAnalysis.gsp</td></tr>
<tr><td>GS350</td><td>GS350Analysis.gsp</td></tr>
<tr><td>GS500</td><td>GS500Analysis.gsp</td></tr>
<tr><td rowspan="2">GS500</td><td>GS400CubicAnalysis.gsp[a]</td></tr>
<tr><td>GS400Ord2Analysis.gsp[a]</td></tr>
</table>
a. These modules are for advanced users with specific sizing needs. See the ABI PRISM GeneScan Analysis Software User Guide.

Note You can examine the settings for each of these files using GeneScan Analysis software. The meanings of the settings are described in the ABI PRISM GeneScan Analysis Software User Guide (P/N 4308923).</td></tr>
<tr><td>7</td><td>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.</td></tr>
</table>

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

Step	Action
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

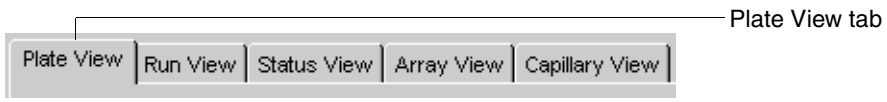
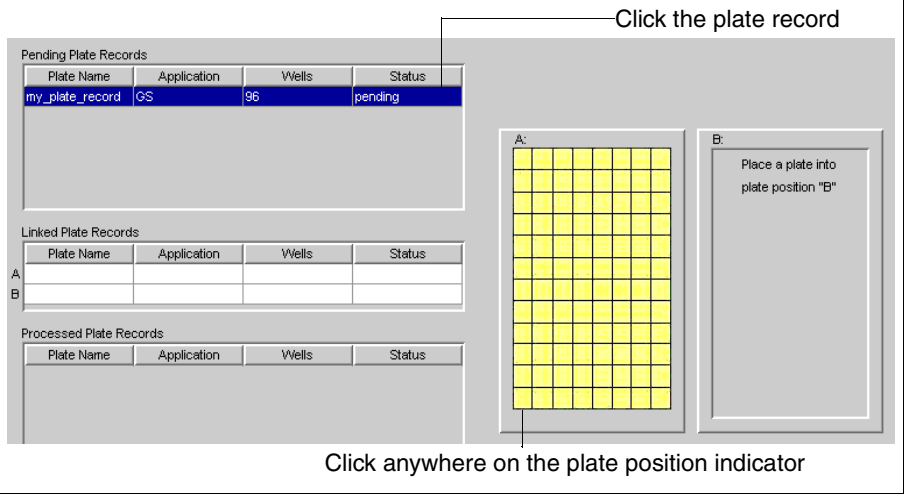
Introduction

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.

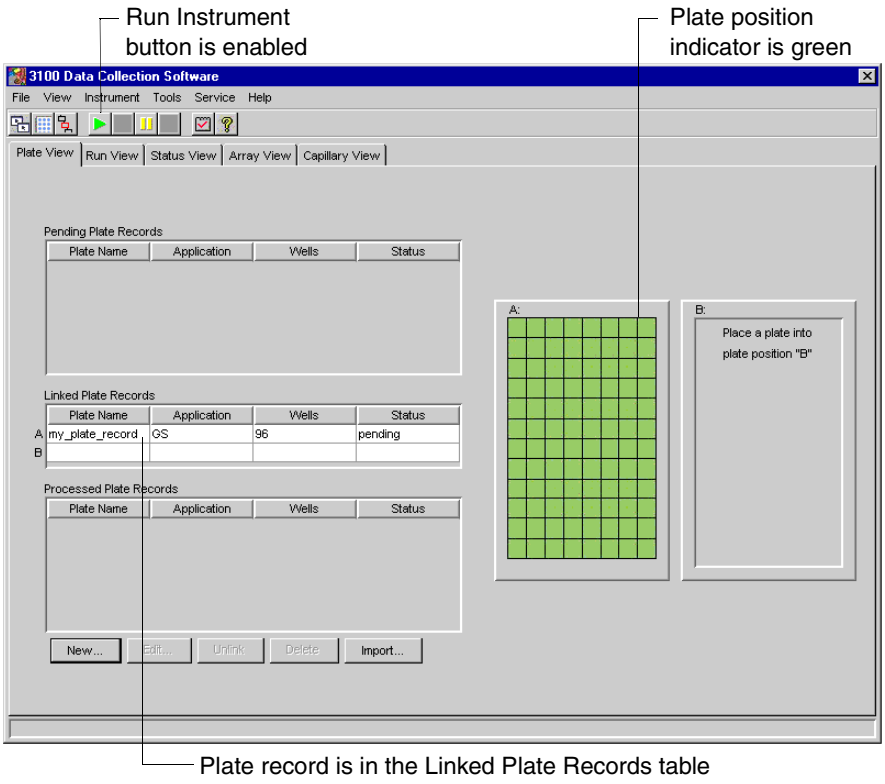
IMPORTANT A plate can be linked even if there are no run modules selected for its samples. In this case, there is no error message and runs for samples in the plate will not be scheduled.

Linking a Plate to a Plate Record

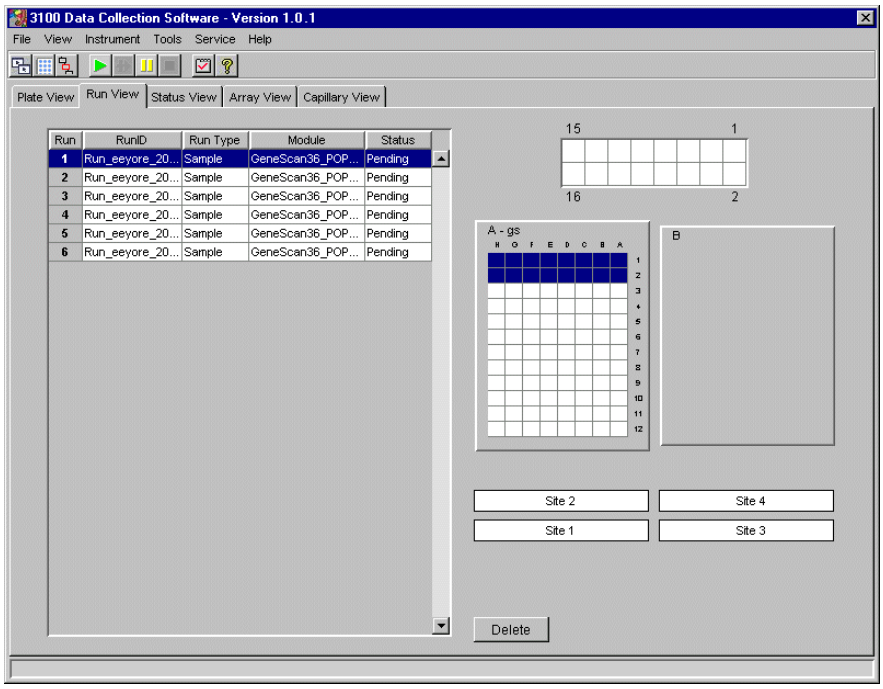
To link a plate to a plate record:

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: ♦ The **Run Instrument** button on the toolbar is enabled, meaning that the instrument is ready to run. ♦ Plate position indicator for the linked plate becomes green. ♦ Plate record moves from the **Pending Plate Records** table to the **Linked Plate Records** table. 
4	Repeat steps 1–3 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* 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

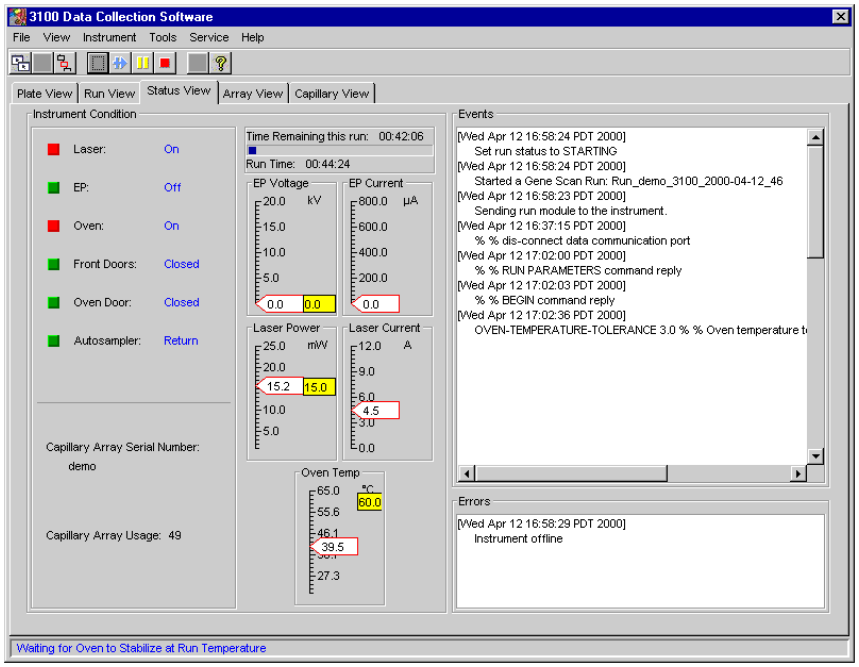
Starting a Run

To start a run:

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

Monitoring a Run

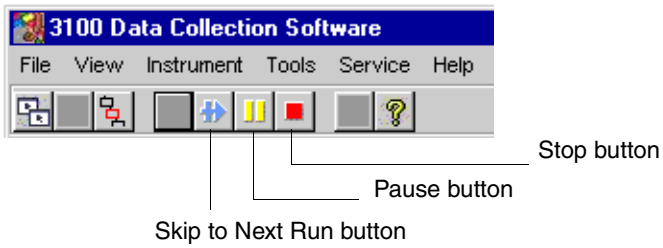
To monitor a run:

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 “Viewing Raw Data” on page 3-2.

Stopping a Run and Recovering the Data

Stopping or Skipping a Run

When a run is in progress, the Skip, Pause, and Stop buttons on the toolbar are visible.

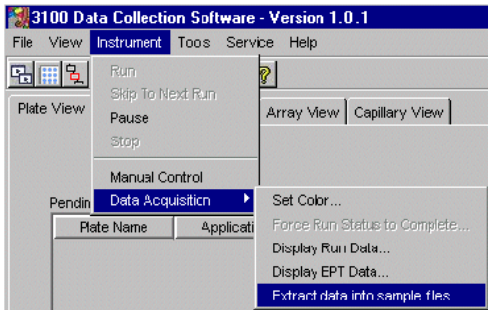


To stop the current run and…	Click…
continue the other scheduled runs	the **Skip** button.
stop the other scheduled runs	a. the **Stop** button. b. **Now** in the **Question** dialog box. 

If Autoextraction Fails

The auto extractor should have automatically extracted your data from the stopped run. If it did not, use the Extract data into sample files commands as described below.

To recover data from a stopped run:

Step	Action
1	From the **Instrument** menu, point to **Data Acquisition** and select **Extract data into sample files**.  Look for the message “Sample Files Successfully Extracted” in the Status bar. **Note** The extracted data is unanalyzed. Use GeneScan Analysis software to analyze the sample files.

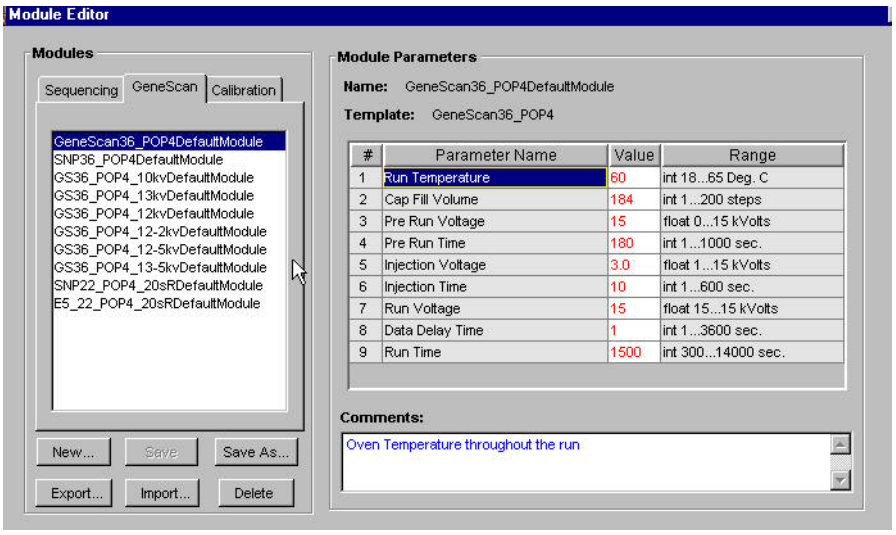
Viewing, Editing, or Creating a Run Module

Introduction

The run module specifies information about how the sample is run (*e.g.,* the duration of the run, the run temperature, and the injection time).

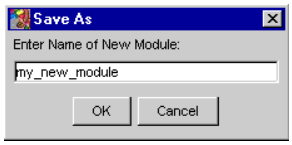
Viewing a Run Module

To view a run module:

Step	Action
1	Click the **Module Editor** button on the toolbar.  The **Module Editor** dialog box opens.
2	In the **Modules** group box, click the **GeneScan** tab.
3	To view the parameters for a particular module, select the name of the module from the list. All the parameters for the run module are displayed. 

Editing or Creating a Run Module

To edit an existing run module or to create a new run module:

Step	Action
1	Click the **Module Editor** button on the toolbar.  The **Module Editor** dialog box opens.
2	Select a run module to use as a template.
3	Edit the parameter values that you want to change. **IMPORTANT** Only whole numbers are accepted. **IMPORTANT** Be sure that all values are red. Values in black are not saved.
4	Click **Save As** to create a new run module. Enter a unique descriptive name and click **OK**.  **Note** **Save** cannot be applied to default run modules. Modules are saved in the database and are accessed through the module editor for viewing.
5	When you are finished, click the Close button ( ) to exit the Module Editor.

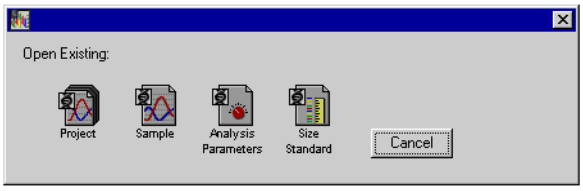
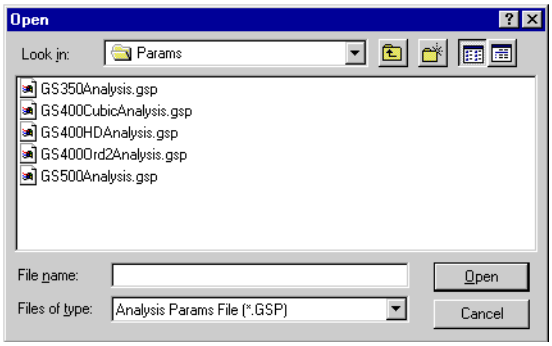
About Viewing and Editing Modules for GeneScan Analysis

Introduction

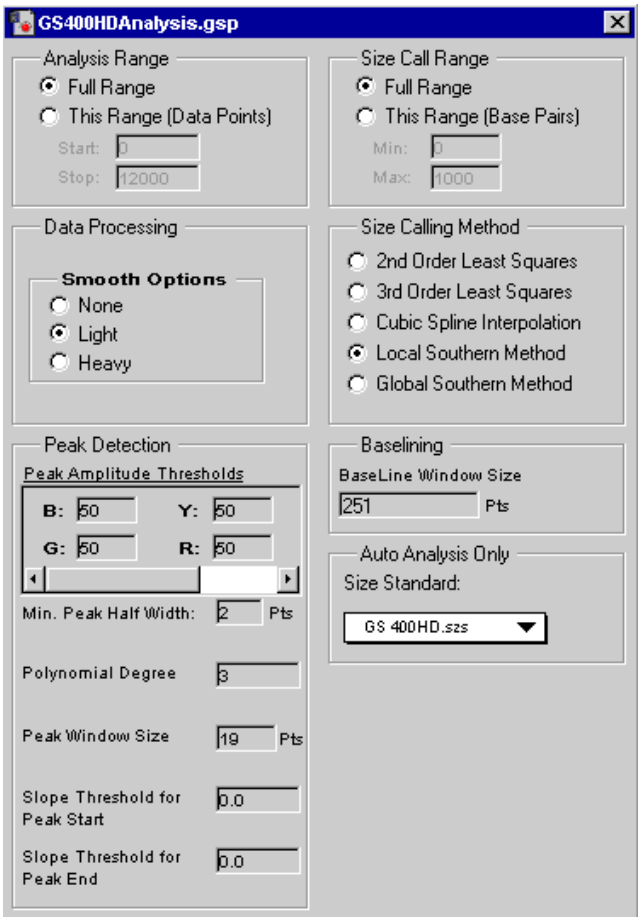
The analysis module specifies how the raw data is autoanalyzed at the end of the run (*e.g.*, analysis range and size standard parameters).

Viewing and Editing Modules for GeneScan Analysis

To view or edit a GeneScan analysis module (.gsp file):

Step	Action
1	Start the GeneScan Analysis software. You may have a program icon for the GeneScan Analysis software on the **Start** menu or a shortcut icon on your desktop. If not, you can find the application (GeneScan.exe) in the following directory: D:\appliedbio\GeneScan\Bin
2	From the **File** menu, select **Open**.
3	Select the **Analysis Parameters** icon. 
4	Select the analysis module you want to view or edit. The analysis modules are stored in the following directory: D:\appliedbio\Shared\Analysis\Sizecaller\Params 
5	Click **Open**. This opens the analysis module.

To view or edit a GeneScan analysis module (.gsp file): *(continued)*

Step	Action
6	If you want, you can make changes to the analysis module. For more information about the parameters, see the *ABI PRISM GeneScan Analysis Software User Guide*. 
7	<table><tr><th>If you have made changes to the analysis module and you want to...</th><th>Then...</th></tr><tr><td>save the changes as a new analysis module</td><td>we recommend that you select **Save As** from the **File** menu, assign a new name, and click **OK**. **Note** The analysis modules must be stored in the following folder: D:\appliedbio\Shared\Analysis\Sizecaller\Params</td></tr><tr><td>discard the changes</td><td>click the Close button to close the window.</td></tr></table>

Viewing and Analyzing Data 3

Overview

In This Chapter

This chapter includes the following topics:

Note This chapter assumes that run data has been extracted into sample files. If you are using the ABI PRISM® 3100 Genetic Analyzer in conjunction with the BioLIMS® database system, you may want to refer to the *ABI PRISM GeneScan Analysis Software User Guide* (P/N 4308923) for information about accessing the database using the analysis program.

Viewing Raw Data from a Completed Run in the Data Collection Software

Introduction

Raw data is data that has been multicomponented (corrected for spectral overlap) but mobility correction has not been applied. There are two formats for viewing the raw data within the ABI PRISM® 3100 Data Collection Software.

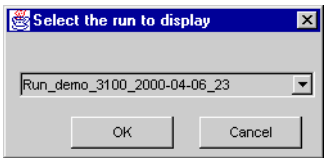
- In the Array View page, the review data accesses all 16 capillaries.
- In the Capillary View page, the data is accessed capillary-by-capillary.

Note Only current run data can be viewed during a run. You cannot view data from previous runs while the instrument is running.

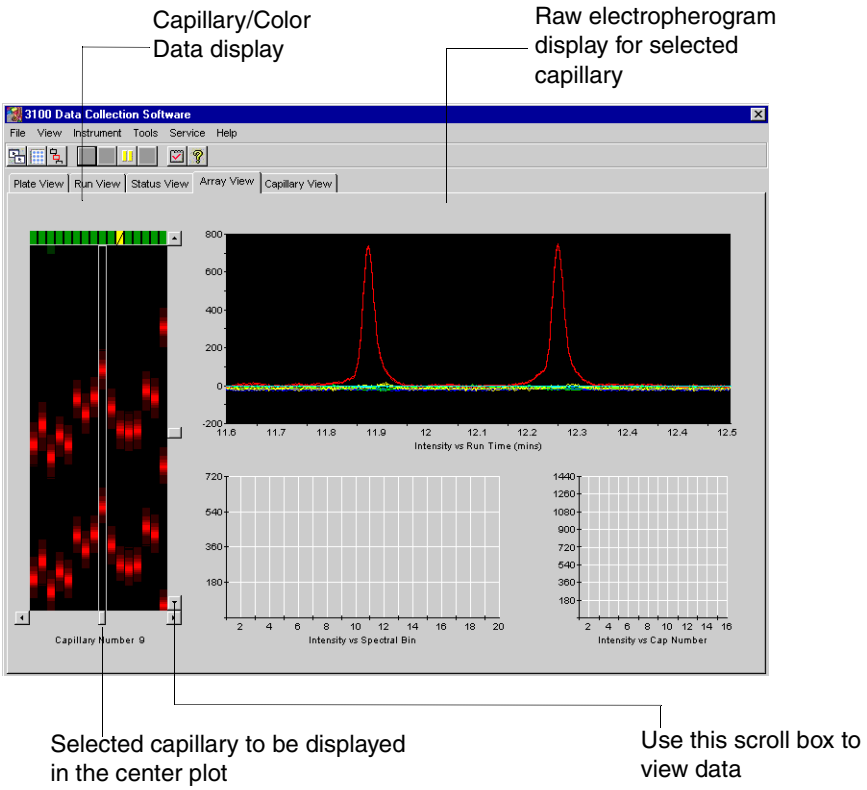
IMPORTANT Always exit from the Array View and the Capillary View windows. During a run, do not leave these pages open for extended periods. This may cause unrecoverable screen update problems. Leave the Status View window open.

Viewing Raw Data

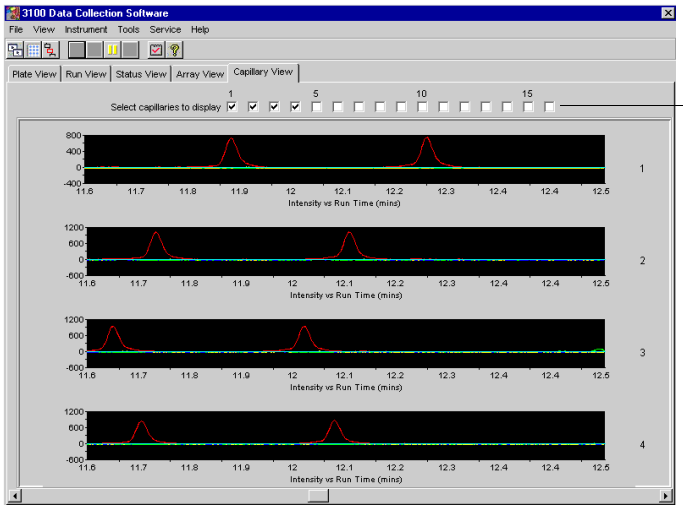
To view raw data from a completed run:

Step	Action
1	In the 3100 Data Collection software, click the **Array View** tab to display the **Array View** page.
2	From the **Instrument** menu, point to **Data Acquisition**, and choose **Display Run Data**. The **Select the run to display** dialog box opens. 
3	From the drop-down list, select the run that you want to display and click **OK**. **Note** You can view any completed run data in the instrument database. **Note** It may take a few moments to retrieve the data.

To view raw data from a completed run: *(continued)*

Step	Action
4	Use the scroll features on the **Array View** page to view the data. **IMPORTANT** Always exit from the **Array View** and the **Capillary View** windows. During a run, do not leave these pages open for extended periods. This may cause unrecoverable screen update problems. Leave the **Status View** window open. 

To view raw data from a completed run: *(continued)*

Step	Action
5	Alternatively, to view electropherogram data from several capillaries at once, click the **Capillary View** tab to display the **Capillary View** page. **IMPORTANT** Always exit from the **Array View** and the **Capillary View** windows. During a run, do not leave these pages open for extended periods. This may cause unrecoverable screen update problems. Leave the **Status View** window open. 

Viewing Analyzed Data in GeneScan Software

Introduction

After a run has been extracted to sample files, you can use the ABI PRISM® GeneScan® Analysis Software to view the electropherogram data, both raw and analyzed.

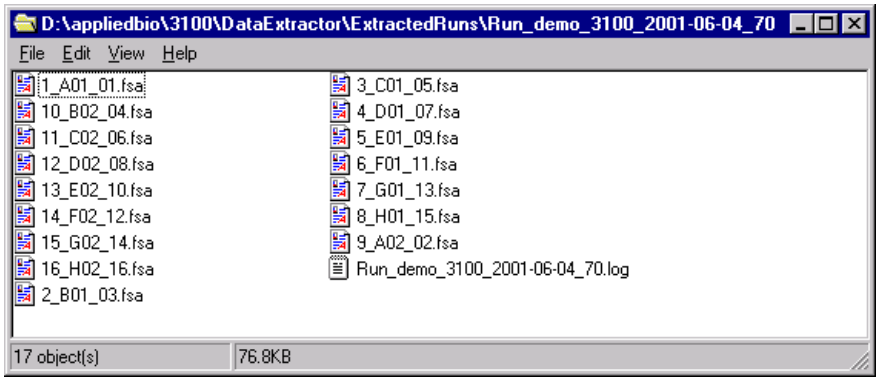
Refer to the *ABI PRISM GeneScan Analysis Software User Guide* for details on viewing and analyzing GeneScan data.

Locating Sample Files

When a run is finished, the analyzed sample files are extracted into a run folder, along with a run log, in the following directory:

D:\appliedbio\3100\DataExtractor\ExtractedRuns

An example of the run folder and its contents is shown below.

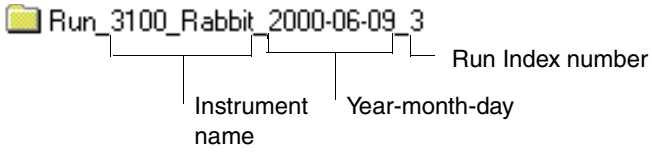


Run Folder Default Name

The default name of the run folder is:

Run_<Instrument name>_<date>_<runID>

An example of a run folder name is shown below.

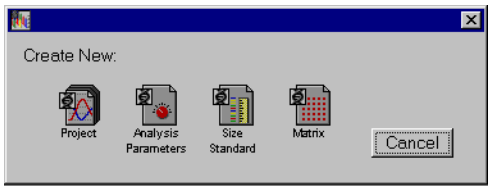
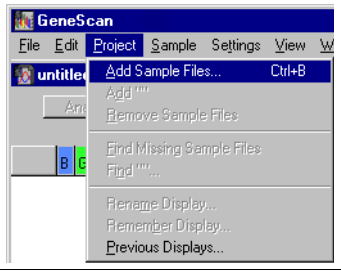


Note The counter for the Run Index number does not reset each day.

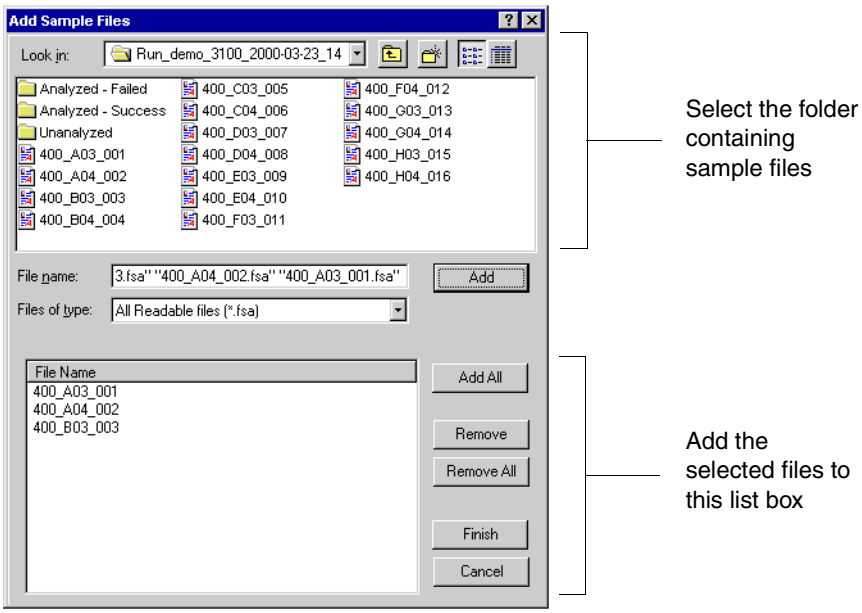
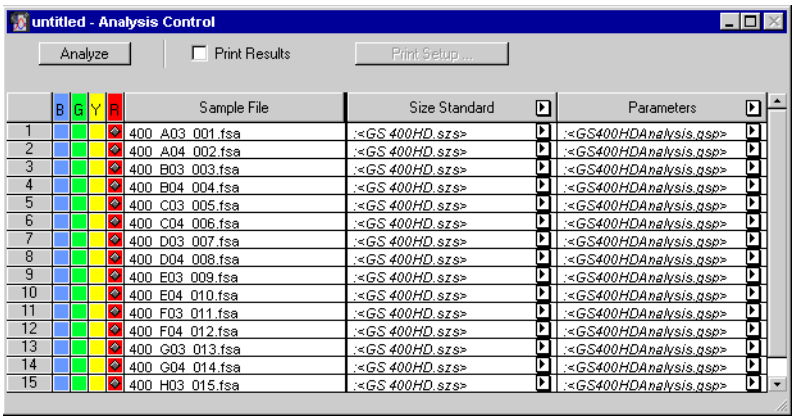
Viewing Individual Sample Files

Note All the features of the software are described in the *ABI PRISM GeneScan Analysis Software User Guide*.

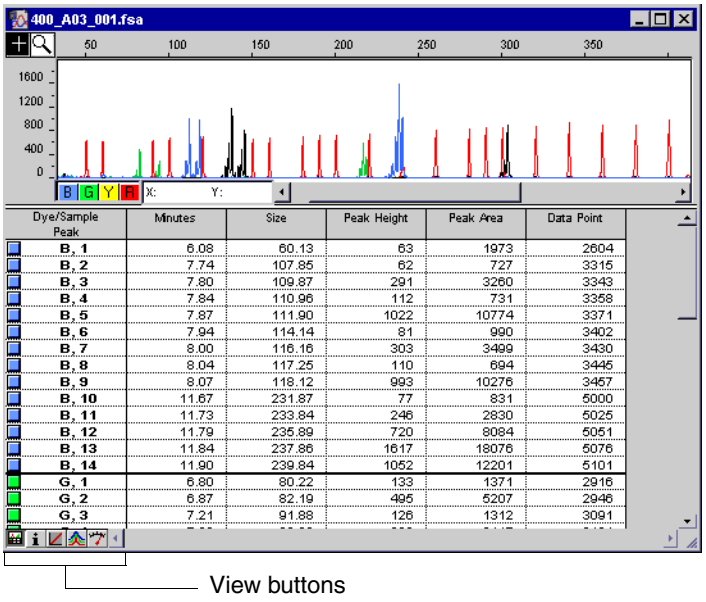
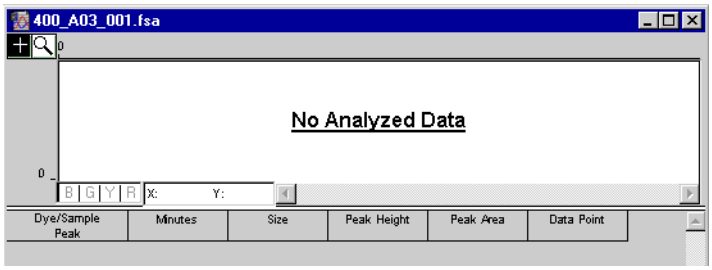
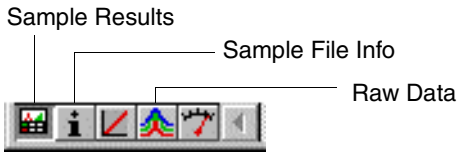
To create a new project and view sample files:

Step	Action
1	Start the GeneScan Analysis software. You may have a program icon for the GeneScan Analysis software on the **Start** menu or a shortcut icon on your desktop. If not, you can find the GeneScan Analysis program (GeneScan.exe) in the following directory: D:\appliedbio\Genescan\Bin 
2	From the **File** menu, select **New**. 
3	Click the **Project** icon to create a new project.  An untitled **Analysis Control** window opens.
4	From the **Project** menu, select **Add Sample Files** to open the **Add Sample Files** dialog box. 

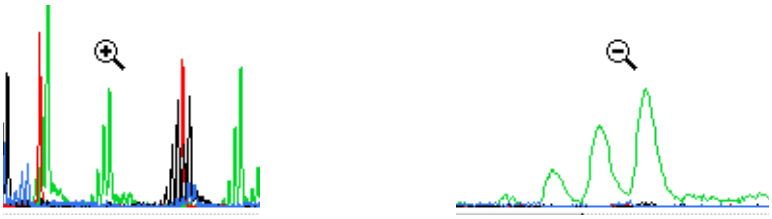
To create a new project and view sample files: *(continued)*

Step	Action
5	In the **Add Sample Files** dialog box: a. Select the folder containing the sample files of interest. b. Click **Add All** to add all the files in the folder to the project list, or **Add** to add only selected files. c. Use the **Add All**, **Remove**, or **Remove All** buttons as necessary to list all the files that you want in the **File Name** list box. These are the files that will be added to the Sample Manager. **Note** If you hold down the **SHIFT** key and press **ENTER**, you can select all continuous files (*i.e.*,1, 2, 3, 4, etc.). If you hold down the **CONTROL** key and press **ENTER**, you can select files out of order (*i.e.*,1, 3, 7, 12, etc.). 
6	Click **Finish** to close the **Add Sample Files** dialog box. The sample files are added to the **Analysis Control** window. **Note** Only the first 20 characters of the sample file name are displayed in the **Analysis Control** window. 

To create a new project and view sample files: *(continued)*

Step	Action
7	Save the project: a. From the **File** menu, select **Save Project**. b. Enter a file name for the project, and click **Save**.
8	Double-click the sample file name in the **Analysis Control** window to view a sample file. This automatically displays the **Sample Results** view. 
9	If the window looks like the one below, the sample files have not been analyzed. Refer to “Analyzing or Reanalyzing Data in GeneScan Software” on page 3-12 for information on how to analyze data. 
10	If your window does not look like either step 8 or step 9 of above, click the **Sample Results** button in the corner of the window. 

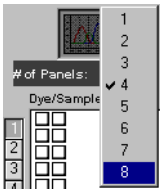
To create a new project and view sample files: *(continued)*

Step	Action
11	Use the Magnifying tool to change the scale of the plot. a. Click on the tool to select it. ( ) . b. Click on the plot to zoom in or ALT-click to zoom out.  Alternatively, use the commands on the **View** menu to adjust the plot scale.
12	To identify a peak, click it. That peak's row in the GeneScan table is highlighted.

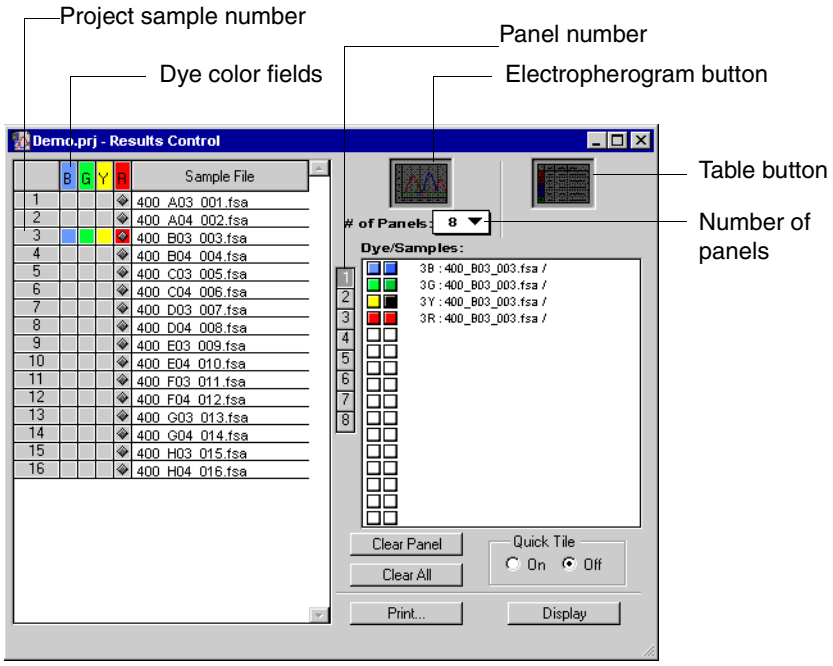
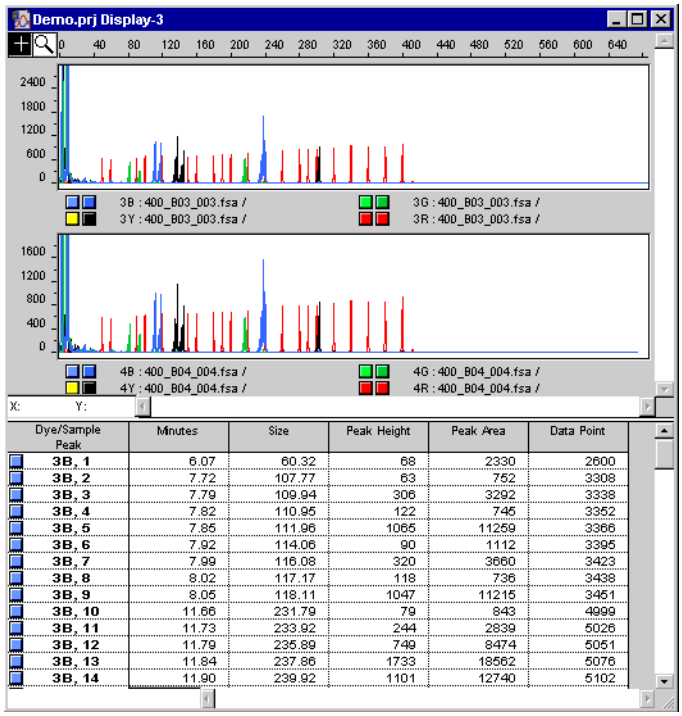
Viewing Multiple Files

The procedure below introduces you to the Results Control window. A complete description of the Results Control window is provided in the *ABI PRISM GeneScan Analysis Software User Guide*.

To view multiple data sets using the Results Control window:

Step	Action
1	If not already open, start the GeneScan Analysis software.
2	Create a project as described in steps 2 to 6, beginning on page 3-6. Or open an existing project by selecting **Open** from the **File** menu.
3	From the **Windows** menu, select **Results Control**.
4	From the **# of Panels** menu, select **8**. 

To view multiple data sets using the Results Control window: *(continued)*

Step	Action
5	Click on the project sample number to select all colors for that sample. 
6	Configure the next panel by clicking the panel number button for panel 2 and repeating step 5 above.
7	Click the display button to display the panels and table. 

To view multiple data sets using the Results Control window: *(continued)*

Step	Action
8	To print the display, from the **File** menu, choose **Print**.
9	Use the **Clear Panel** or **Clear All** buttons on the **Results Control** window to clear panels.

Analyzing or Reanalyzing Data in GeneScan Software

Introduction

Note For more information about analyzing data using GeneScan Analysis software, see the *ABI PRISM GeneScan Analysis Software User Guide*.

When to Analyze Data with GeneScan Analysis Software

The sample file will not contain analyzed data if you did not specify an analysis module in the plate record.

If the sample file does not contain analyzed data, you need to analyze the file as described in the procedure below.

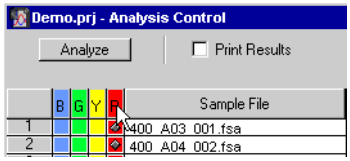
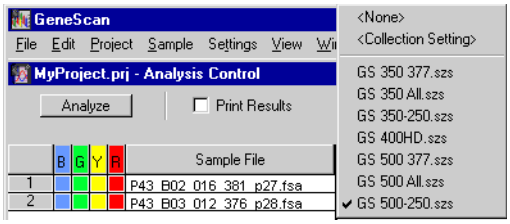
When to Reanalyze Data with GeneScan Analysis Software

Reanalyze the sample files using GeneScan Analysis software when you:

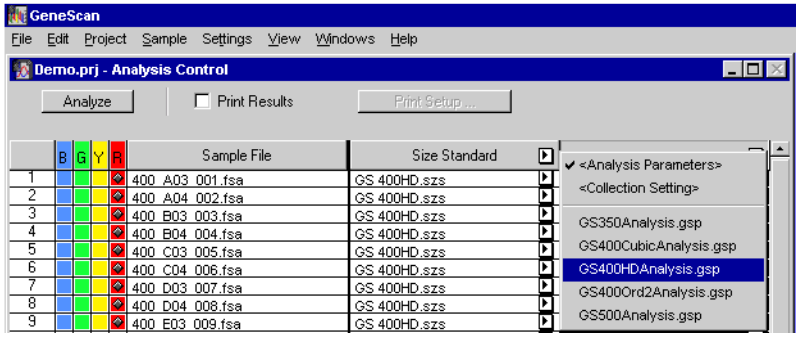
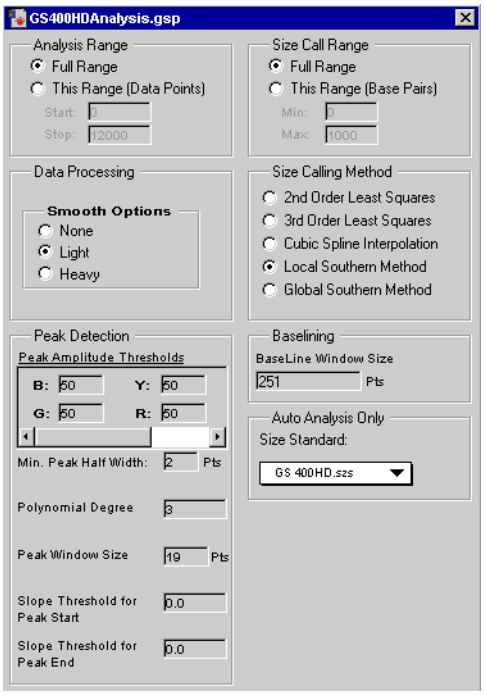
- Chose the wrong analysis module file in the plate record
- Want to see the effect of changing analysis parameters on your data

Analyzing or Reanalyzing Sample Files

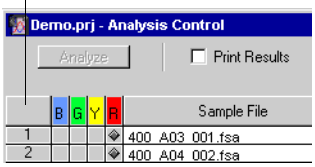
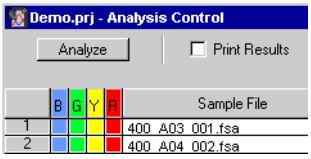
To analyze or reanalyze sample files:

Step	Action
1	If not already open, start the GeneScan Analysis software.
2	Create a project as described in steps 2 to 6, beginning on page 3-6. Or open an existing project by selecting **Open** from the **File** menu.
3	CTRL-click the dye color field to set the size standard color.  The diamond (lozenge) in the red color field indicates that the red size standard will be used for the analysis.
4	From the **Size Standard** pop-up list, select the correct size standard (.szs) file for the sample files in the table. 

To analyze or reanalyze sample files: *(continued)*

Step	Action
5	From the **Parameters** pop-up list, select the correct analysis parameters (.gsp) file for the sample files in the table. 
6	To review or edit the analysis parameters, double-click the parameters file name. The **Analysis Parameters** dialog box opens. Suggested values for parameters are shown below. Refer to the *ABI PRISM GeneScan Analysis Software User Guide* for more information about analysis parameters. 
7	If you make changes to the analysis parameters: a. Select **Save As** from the **File** menu. b. Choose a new name for the analysis parameters (.gsp) file.

To analyze or reanalyze sample files: *(continued)*

Step	Action
8	Select all dye lanes by clicking in the upper-left corner of the dye color fields bar. Click here to select all lanes and all colors  *Before clicking*  *After clicking*
9	Click the **Analyze** button. The analyzed data is automatically saved to the sample files. If analyzed data already existed in the sample file, it is overwritten. As the lanes are analyzed, they are deselected in the dye color fields.

Spatial and Spectral Calibrations 4

Overview

In This Chapter

This chapter includes the following topics:

Performing a Spatial Calibration

When to Do a Spatial Calibration

A spatial calibration must be performed each time after you:

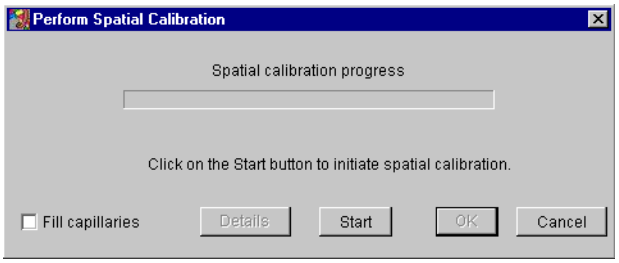
- Install or replace a capillary array
- Temporarily remove the capillary array from the detection block

What a Spatial Calibration Tells You

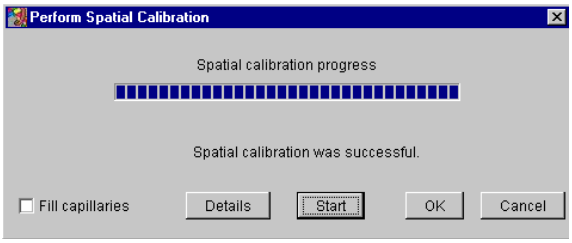
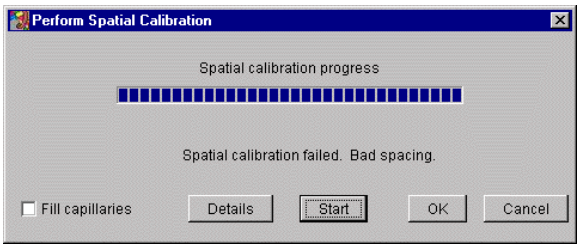
A spatial calibration provides information about the position of the fluorescence from each capillary on the CCD camera. It does not provide information about the performance of the capillaries.

Performing a Spatial Calibration

To perform a spatial calibration:

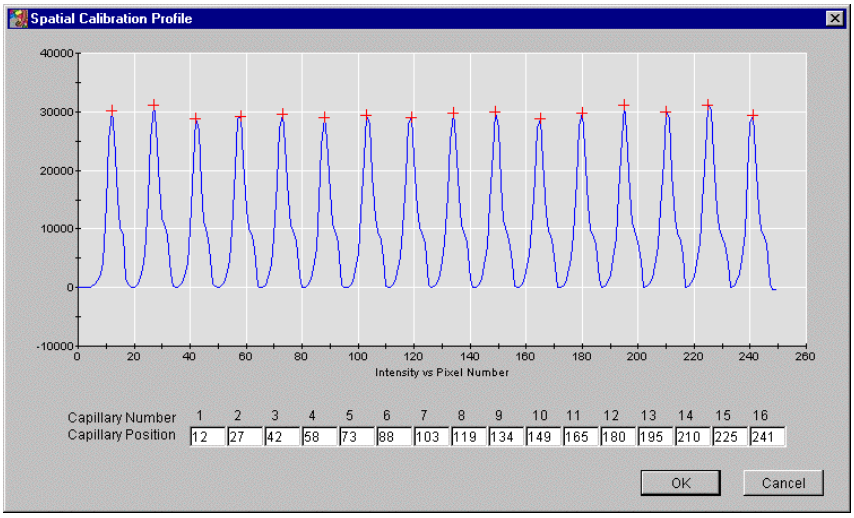
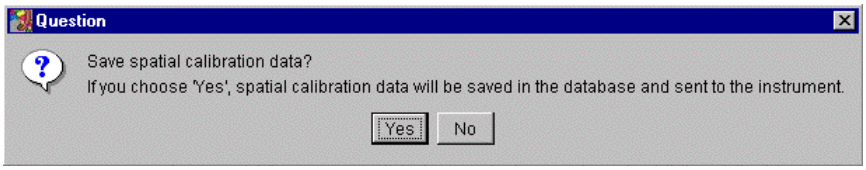
Step	Action
1	From the **Tools** menu, select **Perform Spatial Calibration**. The **Perform Spatial Calibration** dialog box opens. 
2	Select the **Fill capillaries** check box if the: - Capillaries have no polymer (*i.e.,* a new capillary array), or - Polymer in the capillaries has been used in a run **Note** You do not need to fill the capillaries each time you perform a spatial calibration.
3	Click **Start**. The calibration takes approximately: - 2 min without filling the capillaries - 6 min with filling the capillaries

To perform a spatial calibration: *(continued)*

Step	Action	
4	**If the calibration...**	**Then...**
	succeeded	the following dialog box opens:  a. Click **Details** to view the **Spatial Calibration Profile** window. b. Continue on to "Viewing Successful Results and Saving the Data" below.
	failed	an error message box opens, providing some information about the reason for the failure.  a. Click **Details** to view the **Spatial Calibration Profile** window. b. Do one of the following: – Click **Cancel**, and then click **Start** to repeat the calibration. – Take corrective action as outlined on page 4-5.

Viewing Successful Results and Saving the Data

To view the spatial calibration results and save the data:

Step	Action
1	Evaluate the spatial calibration profile. **Note** For information about evaluating the profile, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834).  When you are finished, click **OK** to close the **Spatial Calibration Profile** box.
2	**If the spatial calibration profile is...** / **Then...** satisfactory: Continue on to step 3. unsatisfactory: a. Click **Cancel** to close the **Details** box, and then click **Start** to repeat the calibration, or b. Reposition one or more of the red crosses. To move a cross, change the value in the Capillary Position box, and then click outside of that box. c. Override the data with data from a previous run, see the *ABI PRISM 3100 Genetic Analyzer User's Manual*. If the calibration continues to provide unsatisfactory results, see "If the Calibration Fails" on page 4-5.
3	Click **OK** to close the **Perform Spatial Calibration** window and to send the passing calibration to the instrument. The **Question** dialog box opens. 

To view the spatial calibration results and save the data: *(continued)*

Step	Action
4	

To...	Then...
save this calibration data to the Data Collection software database	Click **Yes.**
delete this data and use data from a previous run	a. Click **No.** b. Override the current spatial calibration map.

If the Calibration Fails

If the calibration failed, or if you do not like the appearance of the passed calibration profile, try one or more of the following corrective actions.

- Repeat the calibration.
- Fill the capillaries with polymer, and then repeat the calibration.
- Clean the detection cell, and then repeat the calibration.
- Reposition the array window in the detection cell, and then repeat the calibration.

Performing a Spectral Calibration

Introduction

Performing a spectral calibration can be divided into three main tasks:

- Setting up the standards
- Starting the spectral calibration
- Checking the spectral calibration

Note This section describes spectral calibration using the Matrix Standard Set DS-30 of Dye Set D. For information about performing spectral calibration for another dye set, see the *ABI PRISM 3100 Genetic Analyzer User's Manual* .

A spectral calibration is performed to create a matrix to correct for the overlapping of fluorescence emission spectra of the dyes. Application of this matrix to the raw data is called multicomponenting. The multicomponenting occurs as the data is collected; therefore it is important to make good quality matrices for each capillary and ensure each is unique. For a more detailed explanation of spectral calibration, see the *ABI PRISM 3100 Genetic Analyzer User's Manual*.

When to Perform a Spectral Calibration

A spectral calibration must be performed:

- Whenever you use a new dye set on the instrument
- After the laser or CCD camera has been realigned by a service engineer
- If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks)

Instructions for Preparing the Matrix Standards

To prepare the Matrix Standards (Dye Set D Matrices as an example).

Step	Action
1	Thaw and mix thoroughly the four DS-30 (P/N 4316100) matrix standard tubes.
2	Spin the tubes briefly in a microcentrifuge.
3	**⚠ 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. Prepare the Matrix Standard Set DS-30 for Dye Set D by combining the following in a labeled 1.5-mL microcentrifuge tube:
4	Vortex thoroughly.
5	Spin the mixture briefly in a microcentrifuge.

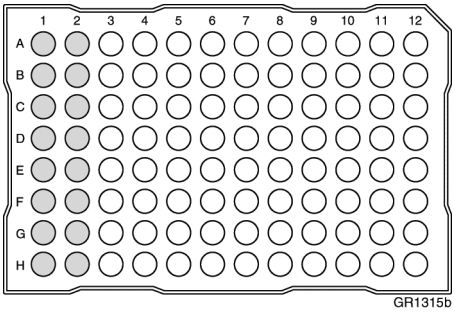
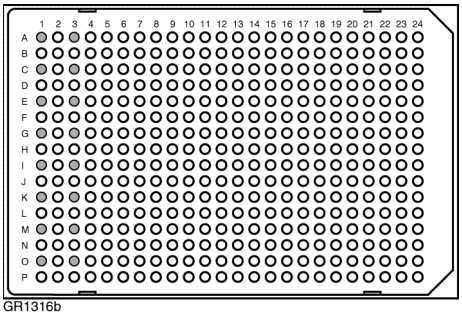
Reagent	Volume (µL)
6FAM	2.5
HEX	2.5
NED	2.5
ROX	2.5
Hi-Di™ Formamide (P/N 4311320)	190
Final Volume	**200**

To prepare the Matrix Standards (Dye Set D Matrices as an example). *(continued)*

Step	Action
6	Heat the standard tube at 95 °C for 5 min to denature the DNA.
7	Immediately place the tubes on ice for 2 min.

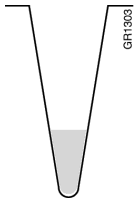
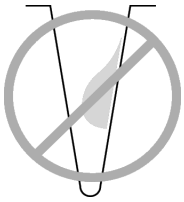
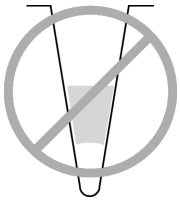
Loading the Standards

To load the standards:

Step	Action
1	Dispense 10 µL of the denatured matrix standard into a: ♦ 96-well plate, wells A1 through H2, as shown below  GR1315b ♦ 384-well plate, wells A1, A3, C1, C3, E1, E3, etc., as shown below  GR1316b

To load the standards: *(continued)*

Step	Action
2	Centrifuge the plate so that the each standard is positioned at the bottom of its well. Your samples should:

Look like this...	Not look like this...	Not look like this...
 The sample is positioned correctly in the bottom of the well.	 The sample lies on the side wall because the plate was not centrifuged.	 An air bubble lies at the bottom of the well because the plate was not: ♦ Centrifuged with enough force, or ♦ Centrifuged for enough time

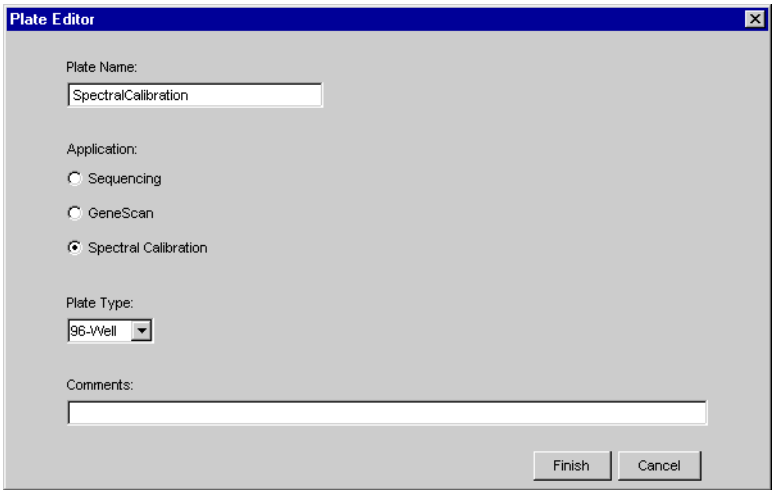
Preparing the Plate and Instrument

Follow the instructions on pages 2-11 through 2-17 to:

- Assemble the plates
- Check and refill the fluids on the instrument
- Place the plate on the autosampler

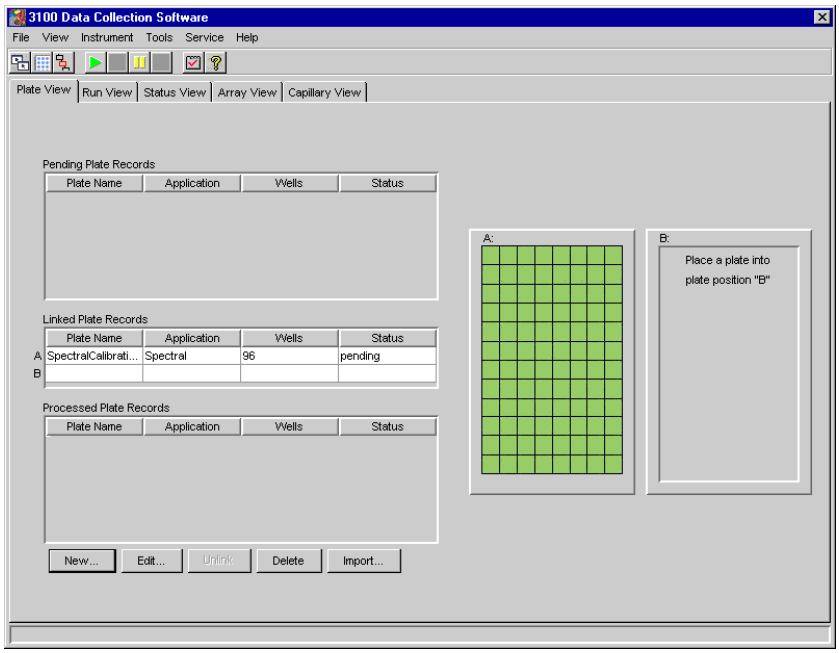
Creating a Plate Record

To create a plate record for the denatured matrix standards:

Step	Action
1	On the **Plate View** page of the Data Collection software, click **New**. The **Plate Editor** dialog box opens.
2	In the **Plate Editor** dialog box: a. Name the plate. b. Select **Spectral Calibration**. c. Make sure that the appropriate plate size is selected. d. Click **Finish**.  The **Plate Editor** spreadsheet opens.
3	Complete the Plate Editor spreadsheet for the wells you have loaded: a. Type a name for the samples. b. Select **Dye Set D**. c. Select the run module **Spect36_POP4DefaultModule**. d. Select the spectral parameter **MtxStd{GeneScan-SetD}.par**. e. Click **OK**. **IMPORTANT** Make sure the correct spectral parameter file has been selected for the type of dyes you are running. Selecting the incorrect parameter file will cause the spectral calibration to fail. This creates a plate record for the calibration run in the database. After a few seconds, the entry for the plate record appears in the **Pending Plate Records** table of the **Plate Setup** page.

Linking the Plate

To link the plate record to the plate:

Step	Action
1	In the **Pending Plate Records** table, select the plate record that you just created.
2	Click the plate graphic that corresponds to the plate on the autosampler.  **Note** When a plate is linked, the: – Plate graphic changes from yellow to green. – Plate record moves from the **Pending Plate Records** table to the **Linked Plate Records** table. (This may take up to 30 sec.) – The **Run Instrument** button on the toolbar is enabled, meaning that the instrument is ready to run.

Starting the Calibration

To start the calibration:

Step	Action
1	To review the run schedule before beginning the run, click the **Run View** tab.
2	Click the **Run Instrument** button on the toolbar to begin the run. The spectral calibration run takes approximately 30 min.

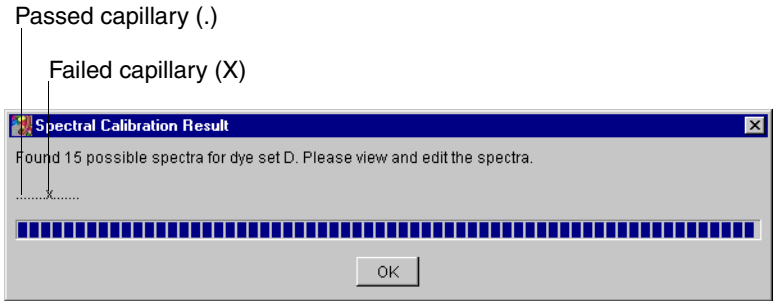
Run Times

The table below lists the spectral calibration run times.

Application	Capillary Array Length (cm)	Approximate Run Time (min)
Fragment analysis	36	30
DNA sequencing	36	40
	50	65

Spectral Calibration Result Box

At the end of the run, while the data is being analyzed, the Spectral Calibration Result dialog box opens to indicate which capillaries have passed and which have failed. The example below for Dye Set D shows failed capillaries, which are represented by “x” and passed capillaries which are represented by “.” dots.



To acknowledge the completed calibration run:

Step	Action
1	In the **Spectral Calibration Result** box, click **OK**.

IMPORTANT Review and evaluate the spectral calibration profile for each capillary, even if the Spectral Calibration Results box indicated that they all passed. See the *ABI PRISM 3100 Genetic Analyzer User’s Manual*.

When a Capillary Fails

If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it will be assigned the profile of the nearest passing capillary to the right. These capillaries are marked yellow instead of green in the Array View (*e.g.,* page 3-3).

IMPORTANT For applications where pull-ups and pull-downs will cause critical errors, we recommend that you repeat the spectral calibration and use a unique spectral for each capillary.

When the Calibration Fails

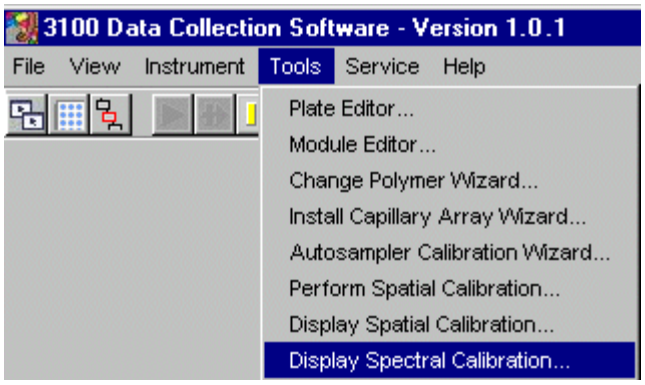
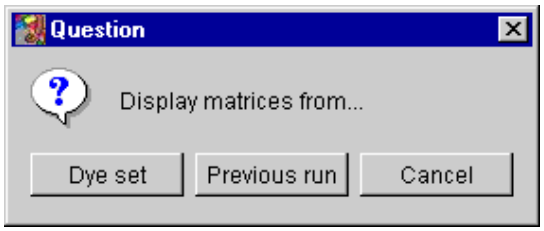
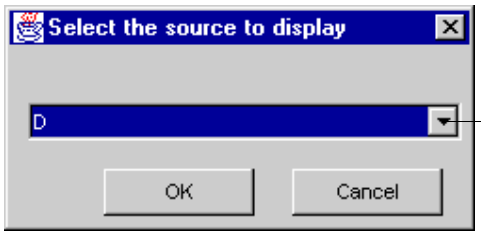
If the spectral calibration failed, or if you do not like the appearance of the passed calibration, try one or more of the following:

- Verify that the correct parameter file and run module were selected. If not, correct, and then repeat the run.
- Verify the freshness of the reagents used.
- Verify that all peaks were detected. A slow running system can result in the blue peak being partially or totally cut off. Add time to the run, or change the reagents if they are suspect, and then repeat the run.

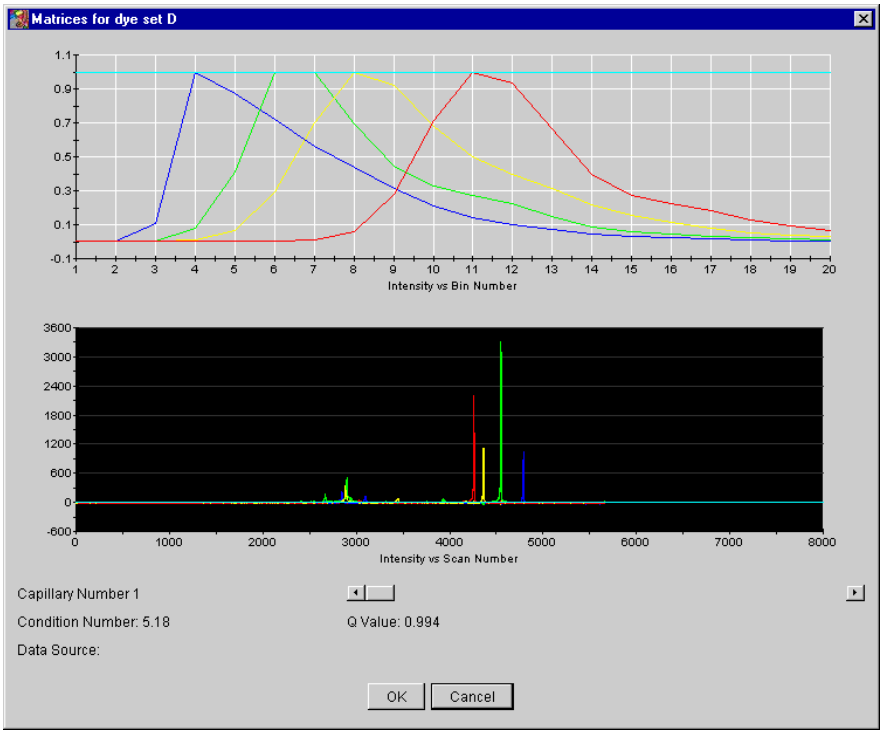
Examining a Spectral Calibration Profile for Dye Set D

After completing a spectral calibration, it is good practice to check the quality of the spectral data for each capillary.

To display a current spectral calibration profile stored for a dye set:

Step	Action
1	From the **Tools** menu, select **Display Spectral Calibration**.  The **Question** dialog box opens. 
2	Click **Dye set**. The **Select the source to display** dialog box opens.  Drop-down list of dye sets

To display a current spectral calibration profile stored for a dye set: *(continued)*

Step	Action
3	From the drop-down list, select Dye Set **D** and click **OK**. The **Matrices for dye set D** dialog box opens. 
4	Use the arrow buttons or the slider to review the data for each capillary. For a good-quality calibration, each capillary should have a: ♦ Q value above 0.95 ♦ Condition number from 4–7 **Note** This condition number is for dye set D only. The condition number is specific for each dye set.
5	Click **Cancel** to close the dialog box. **Note** The software automatically overrides failed capillaries. However, if you are dissatisfied with a particular profile, you can override it with a preferred profile. For more information, see the *ABI PRISM 3100 Genetic Analyzer User's Manual*.

Maintaining the Instrument

5

Overview

In This Chapter

This chapter contains information about the things you should do to maintain your ABI PRISM® 3100 Genetic Analyzer.

Maintenance Task Lists

Overview

This section lists common tasks required to maintain your Genetic Analyzer in good working condition. The lists are divided into tables based on how often you should perform each task.

IMPORTANT Wear gloves anytime you handle the capillary array, glass syringes, septa, or buffer reservoirs.

Daily Tasks

Perform tasks listed below at least once per day.

Maintenance Task	Frequency	See...
Ensure the reservoir septa are firmly seated and flat.	Before each run	—
Ensure the plate assemblies were put together properly. **IMPORTANT** The holes in the plate retainer must align with the holes in the septa or the capillary tips will be damaged.	Before each run	page 2-11
Ensure the plate assemblies are positioned on the plate deck properly. Plates should sit snugly on the deck. **IMPORTANT** Never use warped plates.	Before each run	—
Replenish the water and 1X Genetic Analyzer buffer reservoirs on the instrument.	Daily	page 2-13
Check for bubbles in the polymer block and polymer block channels and remove. **IMPORTANT** Ensure that all bubbles have been pushed through the peek tubing.	Daily	page 5-4
Check the loading-end header to ensure the capillary tips are not crushed or damaged.	Daily	—
Check the level of polymer in the polymer-reserve syringe to ensure there is at least 1 mL.	Daily or before each run	—
Check the polymer block to ensure it fits securely on the instrument.	Daily	—
Clean the instrument surfaces.	Daily	—
Check for dried polymer around the polymer block and clean as necessary.	Daily	—
Check for leaks inside the syringe around the plunger and capillary array ferrule.	Daily	—
Check database space. Delete plate records from the instrument database and archive sample files.	Daily	page 5-6

Weekly Tasks

Perform tasks listed below at least once per week.

Maintenance Task	Frequency	See...
Clean the syringes.	Weekly	page 5-8
Clean the water and buffer reservoirs with warm water.	Weekly	—
Clean the upper and lower polymer blocks.	Weekly	page 5-11
Replace the polymer in the syringes, upper polymer block, and capillary array.	Weekly	page 5-12
Check the storage conditions of the used arrays.	Weekly	—

As Needed Tasks

Perform tasks listed below as needed.

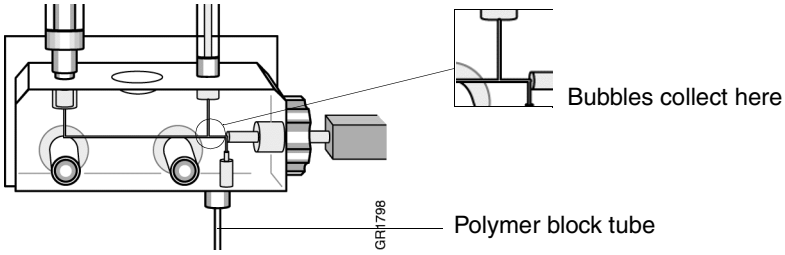
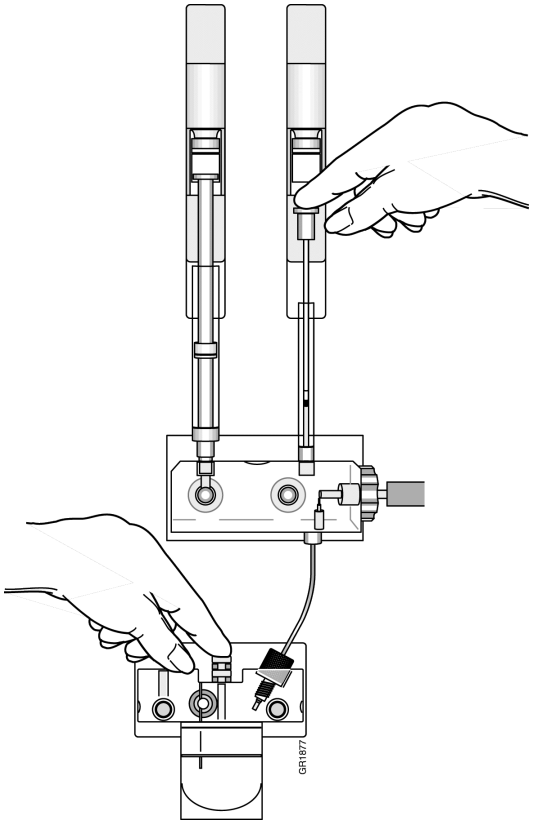
Maintenance Task	Frequency	See...
Clean the drip trays.	As needed	—
Change the array.	As needed	page 5-15
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed	—
Calibrate the autosampler.	Very rarely	User's Manual

Removing Air Bubbles from the Upper Polymer Block

Removing Air Bubbles

Note For information about the Change Polymer wizard, see the *ABI PRISM 3100 Genetic Analyzer User's Manual (P/N 4315834)*.

To clear air bubbles from the upper polymer block use the Change Polymer Wizard in the steps below:

Step	Action
1	Push down on the polymer-reserve syringe to move bubbles through to the lower right of the block. Push slowly (or tap) to minimize the amount of polymer used.
2	Push down slowly on the array-fill syringe to move bubbles down the channel. The bubbles will collect where the channels join. 
3	a. Hold down the anode buffer pin valve and simultaneously push down on the array-fill syringe to build pressure in the channels. b. Release the buffer pin valve (while still pressing down on the array-fill syringe) to expel bubbles into the polymer block tube. 

To clear air bubbles from the upper polymer block use the Change Polymer Wizard in the steps below: *(continued)*

Step	Action
4	Repeat step 3 as necessary. **IMPORTANT** Make sure all air bubbles are pushed out of the tubing assembly into the lower buffer reservoir before proceeding. There should be no bubbles in the tubing or channel of the lower polymer block.

Checking the Available Disk Space

Introduction

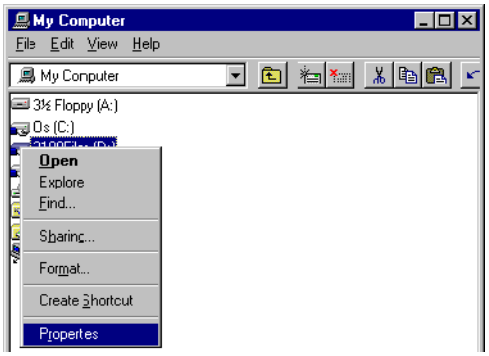
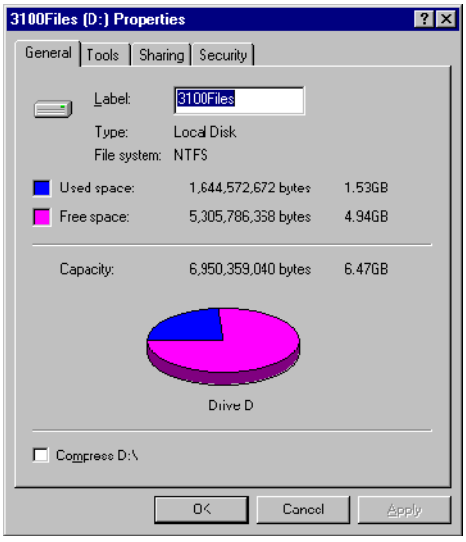
Before a run or a batch of runs, check the available space to ensure there is sufficient space to store the data you will create. Every week, delete records in the database.

The procedures that follow tell you:

- How to check the available space on the hard drive (typically located on drive D) for the extracted sample files
- How to check the available space in the instrument database (typically located on Drive E) for the raw data
- Where to find the procedures for deleting database records

Checking Hard Drive Space

To check the hard drive for space for sample files:

Step	Action
1	Double-click the **My Computer** icon on the desktop to view the drives.
2	Right-click the D drive and select **Properties**.  The **Properties** dialog box opens displaying the used and free space. 

To check the hard drive for space for sample files: *(continued)*

Step	Action
3	Estimate how much free space you need by using the information provided below. **File Type** / **Approximate Space Required Per File (kB)[a]** Analyzed sample file for fragment analysis: 500 Unanalyzed sample file: 100 a. The values provided are estimates only. The actual file size depends on the run module selected. For example, 400 runs x 16 samples equals approximately 6.4 MB.
4	If there is insufficient space: a. Archive the sample files to another volume. b. Delete the original files from the drive.

Checking Database Space

Note The instrument database automatically expands from 2 to 9 GB, depending on the amount of data that needs to be stored.

To check the database space:

Step	Action
1	Run the Diskspace utility. For instructions, see the *ABI PRISM 3100 Genetic Analyzer User's Manual*.
2	If the used space is more than 8 GB, purge the database of some or all data. For instructions, see the *ABI PRISM 3100 Genetic Analyzer User's Manual.*

Cleaning and Inspecting Syringes

When to Clean Syringes

Thoroughly clean the syringes:

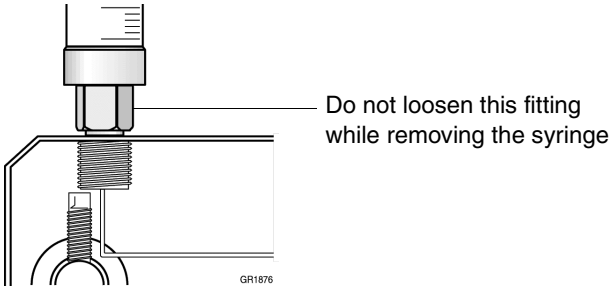
- Whenever they are removed from the instrument, or at least once per week
- Each time the polymer is replaced, including when switching to a new type or lot of polymer

Note For more information about cleaning syringes, see the *ABI PRISM 3100 Genetic Analyzer User's Manual*.

IMPORTANT Wear gloves while performing the following procedure and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

Removing Syringes

To remove the syringes from the instrument:

Step	Action
1	Remove the syringe guard.
2	Grasp the polymer-reserve syringe just above the fitting or at the base (not the glass barrel) and rotate the syringe counterclockwise.  **IMPORTANT** Be careful not to remove the fitting. There are several rings and check valves that could come out if this fitting is removed.
3	Grasp the array-fill syringe and rotate the syringe counterclockwise.
4	Properly dispose of any remaining polymer.
5	Proceed to table below.

Cleaning Syringes

IMPORTANT Wear gloves while performing the following procedure and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

To clean a syringe:

Step	Action
1	Clean the syringe thoroughly by rinsing the inside and outside of the syringe barrel and the syringe tip with warm water. For more information about cleaning syringes, see the *ABI PRISM 3100 Genetic Analyzer User's Manual.* **⚠ CAUTION** Do not use hot water. Hot water can distort the Teflon in the syringe tip. **IMPORTANT** Be sure there is no dried polymer left in the syringes.

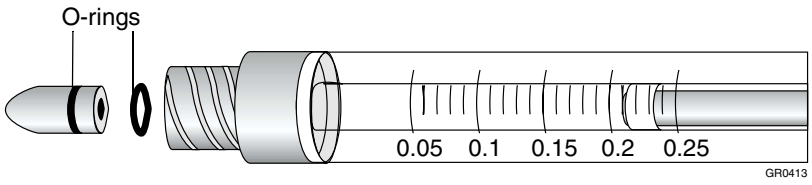
To clean a syringe: *(continued)*

Step	Action
2	Rinse the syringe barrel and tip with deionized water.
3	Blow dry with compressed air.
4	Reassemble the syringe and then inspect it as described below.

Inspecting a Syringe

IMPORTANT After cleaning a syringe, always inspect it for missing O-rings to avoid leaks during your run.

To inspect the syringe:

Step	Action
1	Inspect the syringe for two O-rings (P/N 221102): one behind the ferrule and one around the ferrule. 
2	Verify that the ferrule is firmly seated in the end of the syringe.

Removing the Polymer Blocks

Removing the Upper Polymer Block

To remove the upper polymer block:

Step	Action
1	Remove the syringe guard.
2	Remove the syringes as described on page 5-8.
3	Disconnect the capillary array from the polymer block: a. Press the **Tray** button. b. Open the instrument, oven, and detection block doors. c. Loosen the capillary array nut. d. Pull out the polymer block part way. e. Remove the detection cell from the detection block. f. Remove the capillary array sleeve from the polymer block. g. If the capillary array is to be reused, store it as described on page 5-16.
4	Disconnect the lower polymer block by unscrewing the polymer block tube fitting on the upper polymer block's under right side.
5	Grasp the upper polymer block with two hands and pull it straight out.
6	The upper polymer block rides on two steel shafts and slides out easily after a spring moves past a check point.

Removing the Lower Polymer Block

To remove the lower polymer block:

Step	Action
1	Remove the anode reservoir and properly dispose of the buffer.
2	Inspect the O-rings.
3	Grasp the lower polymer block and pull it straight out.
4	Disconnect the polymer block tube fitting.

Cleaning the Polymer Blocks

When to Clean the Polymer Blocks

Clean the upper and lower polymer blocks once a week:

- Before replacing the polymer on the instrument.
- When the polymer has been on the instrument for longer than 1 week.

Note Polymer older than 1 week may cause a transient increase in current during electrophoresis due to urea decomposition.

Cleaning the Polymer Blocks

IMPORTANT Do not expose the polymer blocks to any organic solvents.

To wash the upper and lower polymer blocks:

Step	Action
1	Remove the polymer blocks from the instrument as described on page 5-10.
2	Use running water or a squirt bottle to rinse the upper polymer block thoroughly with hot water.
3	Visually inspect the channels for white residue (dried polymer). Continue washing the channels until the residue is gone.
4	Rinse the block and its channels with deionized water.
5	Remove residual water from the polymer block and fittings to ensure that the running polymer is not diluted. Force air through the channels, using canned compressed air, until the channels are dry.

Note To purchase a 3100 Polymer Cleaning Kit (P/N 4322931), visit our web site at **http://www.appliedbiosystems.com** and select **Store**.

Putting Fresh Polymer on the Instrument

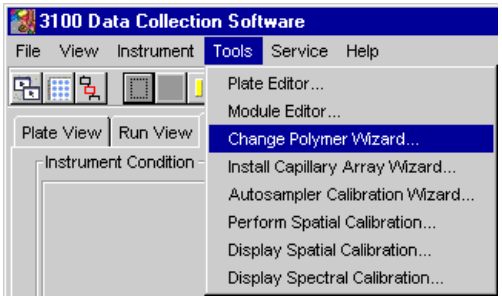
When to Change the Polymer

We recommend that you change the polymer weekly. The polymer is good at 25 °C for about 7 days.

Adding or Changing Polymer

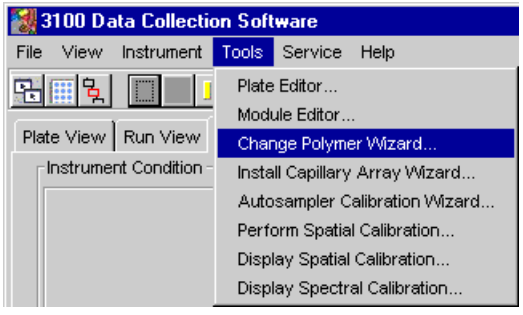
Determine whether to add or change the polymer on the instrument before proceeding with instrument preparation.

⚠ CAUTION **CHEMICAL HAZARD. POP polymers** may cause eye, skin, and respiratory tract irritation. Please 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.

If polymer on the instrument is...	Then...
less than 1 week old, and sufficient in quantity to complete your runs	Ensure there are no air bubbles, and then proceed with instrument preparation. **Note** For a procedure to remove any air bubbles, see *ABI PRISM 3100 Genetic Analyzer User's Manual*.
greater than 1 week old, or insufficient in quantity to complete your runs	Fill the syringes and the upper polymer block with polymer by following the instructions under **Change Polymer Wizard**. 

Changing Polymer

To put fresh polymer on the instrument:

Step	Action
1	From the **Tools** menu, select **Change Polymer Wizard**.  A warning message displays.

To put fresh polymer on the instrument: *(continued)*

Step	Action
2	If the length of the array on the instrument is the same as the length given in the warning message, click **OK** to begin the **Change Polymer Wizard**. 
3	Follow the directions given in the wizard to put fresh polymer on the instrument.

Before Installing a Previously Used Capillary Array

Introduction

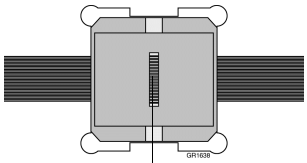
Before you reinstall a capillary array, it is recommended that you:

- Clean the front of the detection cell
- Check that the cathode bar is dry

Cleaning the Detection Cell

This procedure is unnecessary for new arrays unless you have accidently touched the detection cell.

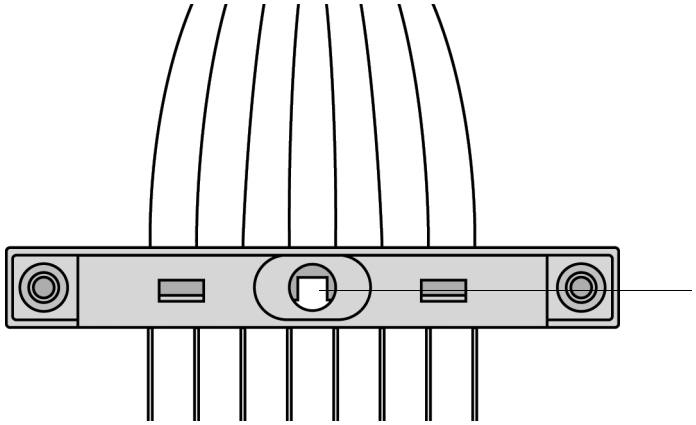
To clean the detection cell:

Step	Action
1	Put one drop of methanol on the front surface of the detection cell. **⚠ 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. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.  Front surface of detection cell
2	Blow dry the cell using clean pressurized air.

Checking the Cathode Bar

When putting a used array back on the instrument, be sure that the cathode bar is dry. A wet bar could lead to arcing.

⚠ WARNING ELECTRICAL SHOCK/FIRE HAZARD. Do not leave liquid in the cathode bar. This can lead to electric shock or even fire if not properly maintained.



Installing and Removing the Capillary Array

When to Change a Capillary Array

A capillary array should last approximately 100 runs.

The following problems may indicate that a new capillary array is required:

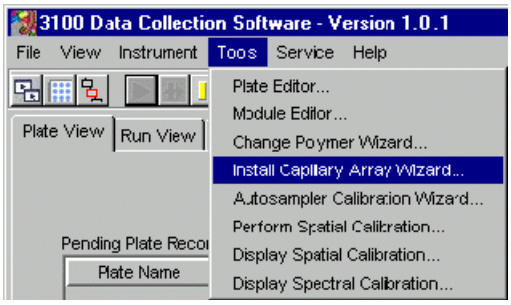
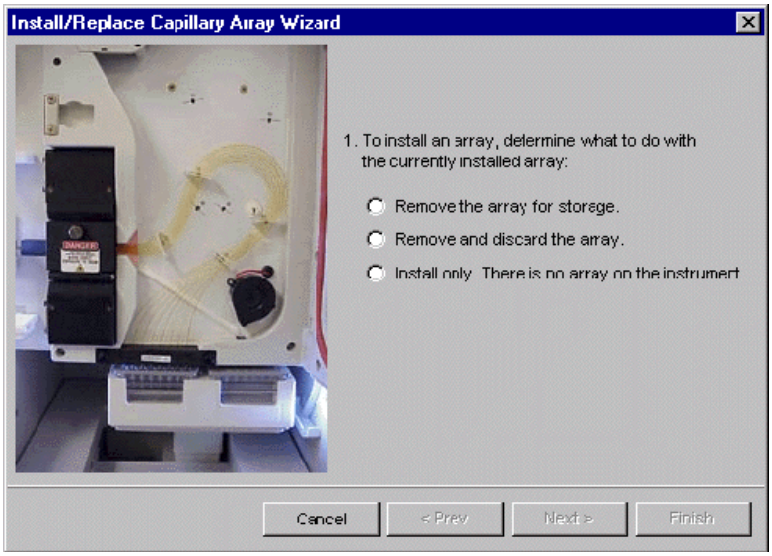
- Poor sizing precision or allele calling
- Poor resolution and/or decreased signal intensity

Installing or Removing the Capillary Array Using the Wizard

IMPORTANT Wear gloves while performing the following procedure, and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

⚠ CAUTION CHEMICAL HAZARD. POP polymers may cause eye, skin, and respiratory tract irritation. Please 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.

To replace a capillary array or to install a capillary array on an instrument:

Step	Action
1	Close the oven and instrument doors, and then press the Tray button.
2	From the **Tools** menu, select **Install Capillary Array Wizard**.  The **Install/Replace Capillary Array Wizard** opens. 
3	Follow the directions given in the wizard to replace or install an array.

Storing a Capillary Array

When to Store off the Instrument

Store the capillary array off of the instrument when the capillary array will be unused for longer than 1 week.

Before storing the capillary array for long periods, we recommend filling the capillaries with fresh polymer.

Storing the Capillary Array off the Instrument

IMPORTANT If you intend to reuse the capillary array, do not let the capillaries dry out. Store the capillary array with both ends in 1X Genetic Analyzer buffer.

IMPORTANT Wear gloves while performing the following procedure, and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

To store the capillary array off of the instrument:

Step	Action
1	Fill the capillary array with fresh polymer using the Change Polymer Wizard or manual control commands.
2	Remove the syringe guard.
3	Remove both syringes from the upper polymer block and properly dispose of any remaining polymer.
4	Wash the syringes.
5	Remove the capillary array from the instrument using the **Install/Replace Capillary Array Wizard**. For instructions see, “Installing and Removing the Capillary Array” on page 5-15.
6	Replace the cover over the detection cell.
7	a. Fill a buffer reservoir with fresh 1X Genetic Analyzer buffer and cover with a septa strip. b. Insert the capillary tips into the buffer.
8	a. Fill a storage cap from a new array or a 5 mL ABI 310 buffer vial (P/N 401955) with septa and caps (P/N 401956) with 1X Genetic Analyzer buffer. b. Insert the rod end of the capillary array into the cap/vial.
9	Wrap the tube with laboratory film (such as Parafilm) to prevent evaporation.
10	Store the capillary array upright.
11	Check the 1X Genetic Analyzer buffer level in the reservoir and tube weekly.

When to Store on the Instrument

Store the capillary array on the instrument when the capillary array will be unused for less than one month.

Storing a Capillary Array on the Instrument

To store the capillary array on the instrument, follow the instructions to perform a short-term shutdown.

Shutting Down the Instrument

When to Perform Each Shut-Down Procedure

Perform the appropriate shut-down procedure as follows:

If the instrument will be unattended for...	Perform this shut-down procedure...
no more than 1 week with a full buffer reservoir	Short-term **IMPORTANT** The key to a successful short-term shutdown is keeping the capillary array tip in 1X Genetic Analyzer buffer. This prevents the polymer from drying in the capillaries.
for more than 1 week	Long-term

Performing a Short-Term Shutdown

To perform a short-term shutdown:

Step	Action
1	Fill the capillaries with fresh polymer using manual control commands.
2	Push the Tray button to move the autosampler forward.
3	Fill the buffer reservoir with 1X Genetic Analyzer buffer to the black line of the reservoir. **⚠ CAUTION CHEMICAL HAZARD. Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Fill the other reservoirs with fresh deionized water.
5	Secure a septa onto each reservoir and place on the autosampler.
6	Close the instrument doors. The autosampler will move to position 1, leaving the capillary tips in the buffer reservoir.
7	Shut down the computer and turn off the instrument.

Performing a Long-Term Shutdown

To perform a long-term shutdown:

Step	Action
1	Follow the procedure on page 5-16 to remove and store the capillary array off of the instrument.
2	Remove from the instrument: ♦ Syringes from the upper polymer block. For instructions see page 5-8. ♦ Upper polymer block. For instructions see page 5-10. ♦ Lower polymer block. For instructions see page 5-10.
3	Remove from the autosampler: ♦ Plate assemblies ♦ Reservoirs
4	Wipe the autosampler and drip trays with lint-free tissue dampened with water.
5	Close the instrument doors.
6	Shut down the computer and turn off the instrument.

To perform a long-term shutdown: *(continued)*

Step	Action
7	Wash the syringes, polymer blocks, and reservoirs with warm water. Rinse with deionized water. **IMPORTANT** Make sure all parts of the array are completely dry before long-term storage.

Preparing Formamide A

Deionizing and Storing Formamide

About Formamide: Denaturation Agent

Formamide is used to denature the DNA samples before placing them on the ABI PRISM® 3100 Genetic Analyzer.

IMPORTANT High quality formamide is essential for reproducible data.

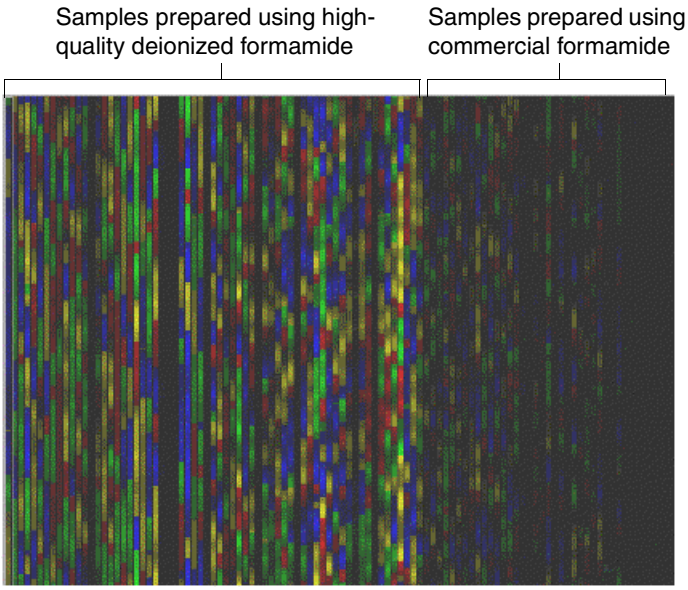
Problems with Commercial Formamide

Formamide purchased from commercial suppliers is often contaminated with variable amounts of water and undesirable organic and inorganic ions. In addition, formamide is often supplied in glass bottles, which, when opened, exposes the formamide to the air and allows it to absorb water.

Water reacts slowly with formamide to produce formic acid (methanoic acid) and ammonia. The ionic products of this reaction cause two problems:

- They compete significantly with the larger DNA ions for injection into the capillary, resulting in weaker signals.
- They react with the DNA, causing degradation of the sample.

The figure below shows the effect of the ionic products from formamide on electrokinetic injection.



Deionized formamide containing an alkaline stabilizer prevents these problems.

Materials Required

The following materials are recommended for this procedure:

Material	Description
Formamide	The raw (prior to deionization) formamide should be: ♦ 99.5% purity or greater, with low water content ♦ Packed under an inert gas ♦ Have a conductivity of approximately 100 μSiemens/cm or less **Note** Siemens, formerly called mho, are the units of measurement for specific conductance or conductivity.
Ion-exchange resin	♦ Mixed-bed resin containing the following strong ion exchange functional groups: $R\text{-}SO_3^-$ (as H^+ form) (cation) $R\text{-}CH_2N^+(CH_3)_3$, (as OH^- form) (anion) – These groups are attached to a styrene divinylbenzene matrix with 8% cross-linkage. ♦ The minimum wet capacity is 1.5 meq/mL with 20–50 dry mesh size (AG501 X8, molecular biology grade mixed-bed resin) ♦ Available from Bio-Rad Laboratories (P/N 143-6424) or equivalent
Conductivity meter	A commercial conductivity meter, or pH meter with an external conductivity cell, is sufficient to measure the conductivity of formamide if it has a cell constant of 1.0.
Na_2EDTA	♦ Dihydrate (M_r 372.2) ♦ ACS reagent, 99% purity or greater ♦ Available from Sigma (P/N E4884) or equivalent
Container for storing formamide	Use a polypropylene screw-cap container **Note** Glass containers are not recommended because of potential contamination from minerals.

Ion-Exchange Resin

The raw formamide is deionized with cationic and anionic mixed resins to remove impurities such as ammonium and formate ions. Deionization occurs at a slow mass-transfer rate in the equilibrium ion exchange kinetics due to:

- Physical changes in the resin in the presence of formamide
- Differences in molecular size and selectivity between the impurity ions and the H^+ and OH^- counterions

Therefore, the conductivity of formamide must be monitored over time to determine the extent of deionization by the resin.

Calibrating the Conductivity Meter

A conductivity meter and cell are needed to measure the effectiveness of the deionization process. The more deionized the formamide, the lower its conductivity.

Within the range or measurement, the conductivity meter should be routinely calibrated (to 10 μSiemens/cm or less). Calibrate the meter using standard potassium chloride solutions that are traceable to the National Institute of Standards and Technology (NIST). Because temperature affects conductivity, samples must be brought to room temperature before measuring the conductivity.

Preparing EDTA

Alkaline EDTA (ethylenediaminetetraacetic acid) is added to the deionized formamide to stabilize it and to facilitate the electrokinetic injection of DNA. To minimize the amount of water added to the formamide, a concentrated (200-mM) stock solution of the EDTA is added.

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

To prepare the 200-mM EDTA stock solution:

Step	Action
1	Add 7.44 g of Na_2EDTA to 70 mL of deionized water and stir.
2	While stirring, slowly adjust to pH 8.0–8.8 by dropwise addition of a concentrated solution of sodium hydroxide. **Note** This helps the EDTA to dissolve over time, because the EDTA has a limited solubility until the pH is increased.
3	Dilute to 100 mL with deionized water.
4	Store at 4 °C.

Preparing the 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.

To begin purification and measure conductivity:

Step	Action
1	Calibrate the conductivity meter cell, and rinse the cell with distilled water.
2	In a polypropylene screw-cap container, wash 10 g of Bio-Rad AG501 X8 ion-exchange resin by swirling the sample with 10–20 mL of formamide for 1 minute.
3	Either decant off or filter through a course nylon or teflon filter, and discard the formamide.
4	Repeat steps 2 and 3 twice.
5	Add 100 mL of formamide to the washed resin.
6	Cap the mixture, ensuring that it is well sealed.
7	Stir the mixture rapidly with a magnetic stirrer, or mix with an electric shaker, ensuring that the resin is suspended for adequate mixing and ion exchange. Stir at room temperature for approximately 2 hr.

To begin purification and measure conductivity: *(continued)*

Step	Action
8	Remove a small aliquot of the mixture, and measure the conductivity at room temperature.
9	Rinse the conductivity cell with distilled water.
10	<table><tr><th>If the conductivity is...</th><th>Then...</th></tr><tr><td>>5 µSiemens/cm</td><td>Return to step 7, and stir for an additional 30 min.</td></tr><tr><td><5 µSiemens/cm</td><td>Continue with “To complete purification of deionized formamide:” below.</td></tr></table> **Note** If the conductivity is not <5 µSiemens/cm after about 4.5 hr of mixing, repeat the entire procedure using a new lot of formamide and new resin. **Note** Starting formamide with a higher purity and lower conductivity deionizes more efficiently.

To complete purification of deionized formamide:

Step	Action
1	Vacuum-filter the deionized formamide using a 0.2- or 0.4-µm nylon or teflon filter.
2	Measure the final volume of deionized formamide.
3	Add the required volume of 200-mM EDTA to the deionized formamide to achieve a final concentration of approximately 0.3-mM EDTA. **Note** After adding the EDTA, the final conductivity of the formulation is increased to approximately 30 µSiemens/cm. Use the equation below to calculate the volume of EDTA to add. $V_{EDTA\ (\mu L)} = 1.5V_{\ Form(mL)}$ Where, $_{VEDTA(\mu L)}$ = volume of EDTA to add in microliters $V_{FORM(mL)}$= measured volume of formamide in milliliters Sample calculation with a final volume of 90-mL formamide: $V_{EDTA(\mu L)} = 1.5 \times 90 = 135\ \mu L$
4	Immediately aliquot the formamide into smaller polypropylene tubes and store at –15 to –20 °C for up to about 6 mos.

Using the Formamide

When ready for use, thaw and completely use one tube at a time before opening and exposing another tube. Store the tubes at 4 °C during the day for intermittent use. Otherwise, refreeze them.

Getting Help

B

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems web site. (Please see the section “To Obtain Technical Documents” following the telephone information below)

To Contact Technical Support by E-Mail

You can contact Applied Biosystems Technical Support by e-mail for help in the following product areas:

Product/Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems (Real-Time PCR) and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com

Product/Product Area	E-mail address
♦ Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products) ♦ Expedite™ 8900 Nucleic Acid Synthesis Systems ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ PNA Custom and Synthesis ♦ Pioneer™ Peptide Synthesizers ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent ♦ FMAT™ 8100 HTS Systems ♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

To Contact Technical Support by Telephone or Fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

Note To schedule a service call for other support needs, or in case of an emergency, dial **1.800.831.6844**, then press **1**.

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1.800.831.6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1.800.831.6844**, press **2**, then press **1**[a]	1.650.638.5981
Fluorescent DNA Sequencing	**1.800.831.6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1.800.831.6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1.800.831.6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1.800.831.6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43x Systems)	**1.800.831.6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1.800.831.6844**, press **3**, then press **2**[a]	1.650.638.5981
Sequence Detection Systems (Real-Time PCR) and PCR	**1.800.762.4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI Prism, 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1.800.831.6844**, then press **5**[a]	1.240.453.4613
♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent	**1.800.899.5858**, press **1**, then press **3**[b]	1.508.383.7855

Product/Product Area	Telephone	Fax
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1.800.899.5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ 8900 Nucleic Acid Synthesis Systems	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
Pioneer™ Peptide Synthesizers	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
♦ FMAT™ 8100 HTS Systems ♦ CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 AM to 5:00 PM Pacific time.

b. 8:00 AM to 6:00 PM Eastern time.

c. 9:00 AM to 5:00 PM Eastern time.

To Contact Technical Support by Telephone or Fax (Outside North America)

To contact Applied Biosystems Technical Support or Field Service outside North America, use the telephone or fax numbers below.

Region	Telephone	Fax
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608** or **86 800 8100497**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
India (New Delhi)	**91 11 653 3743/3744**	**91 11 653 3138**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 79588268**	**60 3 79549043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 532 4484**	**32 (0)2 582 1886**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0)6150 101 0**	**49 (0)6150 101 101**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**

Region	Telephone	Fax
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Portugal (Lisboa)	**351.(0)22.605.33.14**	**351.(0)22.605.33.15**
Spain (Tres Cantos)	**34.(0)91.806.1210**	**34.(0)91.806.12.06**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 392400**	**31 (0)180 392409** or **31 (0)180 392499**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	**27 11 478 0411**	**27 11 478 0349**
Africa, French speaking (Paris, France)	**33 1 69 59 85 11**	**33 1 69 59 85 00**
India (New Delhi)	**91 11 653 3743** **91 11 653 3744**	**91 11 653 3138**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 22 866 40 10**	**48 22 866 40 20**
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia)	**44 1925 282481**	**44 1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Caribbean countries, Mexico, and Central America	**52 55 35 3610**	**52 55 66 2308**
Brazil	**0 800 704 9004** or **55 11 5070 9654**	**55 11 5070 9694/95**
Argentina	**800 666 0096**	**55 11 5070 9694/95**
Chile	**1230 020 9102**	**55 11 5070 9694/95**
Uruguay	**0004 055 654**	**55 11 5070 9694/95**

To Reach Technical Support Through the Applied Biosystems Web Site

At the Applied Biosystems web site, you can search through frequently asked questions (FAQs) or a solution database, or you can submit a question directly to Technical Support.

Search FAQs

To search for FAQs:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Click you geographic region for the product area of interest.
4	Follow the instructions under the **Frequently Asked Questions** section (1) to display a list of FAQs for your area of interest.

Search the Solution Database

To search for solutions to problems using the Solution Database:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Follow the instructions under the **Search the Solution Database** section (2) to find a solution to your problem.

Submit a Question

To submit a question directly to Technical Support:

1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	In the **Personal Assistance – E-Mail Support** section (3), click **Ask Us RIGHT NOW**.
4	In the displayed form, enter the requested information and your question, then click **Ask Us RIGHT NOW**. Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

To Obtain Technical Documents

You can obtain technical documents, such as Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents for free, 24 hours a day. You can obtain documents:

- By telephone
- Through the Applied Biosystems web site

Ordering Documents by Telephone

To order documents by telephone:

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.

Obtaining Documents Through the Web Site

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, then click **Documents on Demand**.
3	In the search form, enter and select search criteria, then click **Search** at the bottom of the page.
4	In the results screen, do any of the following: ♦ Click the pdf icon to view a PDF version of the document. ♦ Right-click the pdf icon, then select **Save Target As** to download a copy of the PDF file. ♦ Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you. ♦ Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you. **Note** There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.

To Obtain Customer Training Information

To obtain Applied Biosystems training information:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Training**.

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Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our web site at www.appliedbiosystems.com or to the Technical Support appendix in this document.

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