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Introduction and Safety 1

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ABI PRISM® 3100-Avant Genetic Analyzer

Definition

The ABI PRISM® 3100-Avant Genetic Analyzer is an automated capillary electrophoresis system that can separate, detect, and analyze fluorescently labeled DNA fragments in one run.

System Components

The 3100-Avant Genetic Analyzer system includes the following components:

- ABI PRISM® 3100-Avant Genetic Analyzer
- Computer workstation with Microsoft® Windows NT operating system
- ABI PRISM® 3100-Avant Genetic Analyzer software
- ABI PRISM® DNA Sequencing Analysis and/or ABI PRISM® GeneScan® Analysis software
- Capillary array
- Reagent consumables

To Get Started Quickly

Important Safety Information

Before using the instrument, read the safety information starting on page 1-4 and in the *ABI PRISM® 3100 and 3100-Avant Genetic Analyzer Site Preparation and Safety Guide* (P/N 4315835).

What You Should Know

This manual is written for principle investigators and laboratory staff who are planning to operate and maintain a 3100-Avant Genetic Analyzer.

Before attempting the procedures in this manual, you should be familiar with the following topics:

- Windows NT operating system
- General techniques for handling DNA samples and preparing them for electrophoresis. Detailed information about preparing samples for sequencing and fragment analysis is given in other Applied Biosystems' manuals (see the table below).
- Networking, which is needed if you want to integrate the 3100-Avant Genetic Analyzer into your existing laboratory data flow system

Documentation

List of User Documents

The following table lists the complete ABI PRISM® 3100-Avant Genetic Analyzer document set for users:

Title	Contents	P/N
	Instrument	
ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer Site Preparation and Safety Guide	♦ Laboratory requirements for installation ♦ Instrument and chemical safety	4315835
ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide	♦ Theory of operations ♦ System management ♦ Troubleshooting	4335393
ABI PRISM® 3100-Avant Genetic Analyzer User Guide	User procedures for using and maintaining the instrument	4333549
	Software	
ABI PRISM® DNA Sequencing Analysis Software v. 3.7 NT User Guide	Detailed procedures for analyzing sequencing data	4308924
ABI PRISM® GeneScan Analysis Software v. 3.7 NT User Guide	Detailed procedures for analyzing fragment analysis data	4308923
	Chemistry	
ABI PRISM® 3100 Genetic Analyzer Sequencing Chemistry Guide	♦ Detailed chemistry procedures specific for the 3100 and 3100-Avant Genetic Analyzers ♦ Chemistry troubleshooting for the 3100 and 3100-Avant Genetic Analyzers	4315831
ABI PRISM® Automated DNA Sequencing Chemistry Guide	♦ A description of DNA sequencing instruments, chemistries, and software ♦ Detailed procedures for preparing DNA templates, performing cycle sequencing, and preparing extension products	4305080

About User Bulletins

User bulletins inform you of technical information, product improvements, and related new products and laboratory techniques.

Applied Biosystems will mail user bulletins related to the use of this instrument to you. We recommend storing the bulletins in this manual. A tab labeled “User Bulletins” has been included for this purpose.

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, accurate chemistry kit use, or safe use of a chemical.

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

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

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

Chemical Hazard Warning

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

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

Chemical Waste Hazard Warning

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

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Site Preparation and Safety Guide

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

About MSDSs

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

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

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

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

Ordering MSDSs

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

To order documents by automated telephone service:

Step	Action
1	From the U.S. or Canada, dial **1.800.487.6809**.
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 automated telephone service:

1	From the U.S. or Canada, dial **1.800.487.6809**.
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 automated telephone service:

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

To obtain documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	In the **SEARCH** field at the top of the page, type in the chemical of interest.
3	Select **MSDS** from the **IN** drop-down list to the right, then click **GO**.
4	When the **Search Results** page opens, find the document you want in the language of your choice and click on it to open a PDF of the document.

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 and 3100-Avant 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.

Computer Workstation Safety

Correct ergonomic configuration of your computer workstation can prevent stress-producing effects such as fatigue, pain, and strain. Minimize or eliminate these effects on your body by designing your workstation to promote neutral or relaxed working positions.

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

- ♦ Use equipment that comfortably supports the user in neutral working positions and maintains adequate accessibility to the keyboard, monitor, and mouse.
- ♦ Position keyboard, mouse, and monitor to promote relaxed body and head postures.

Electric Shock

⚠ 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.

Lifting/Moving

⚠ WARNING **PHYSICAL INJURY HAZARD**. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Two or three people are required to lift the instrument, depending upon instrument weight.

Performing a Run

2

In This Chapter

The following topics are covered in this chapter:

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The following topics are covered in this chapter: *(continued)*

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Section: Introduction

In This Section

The following topics are covered in this section:

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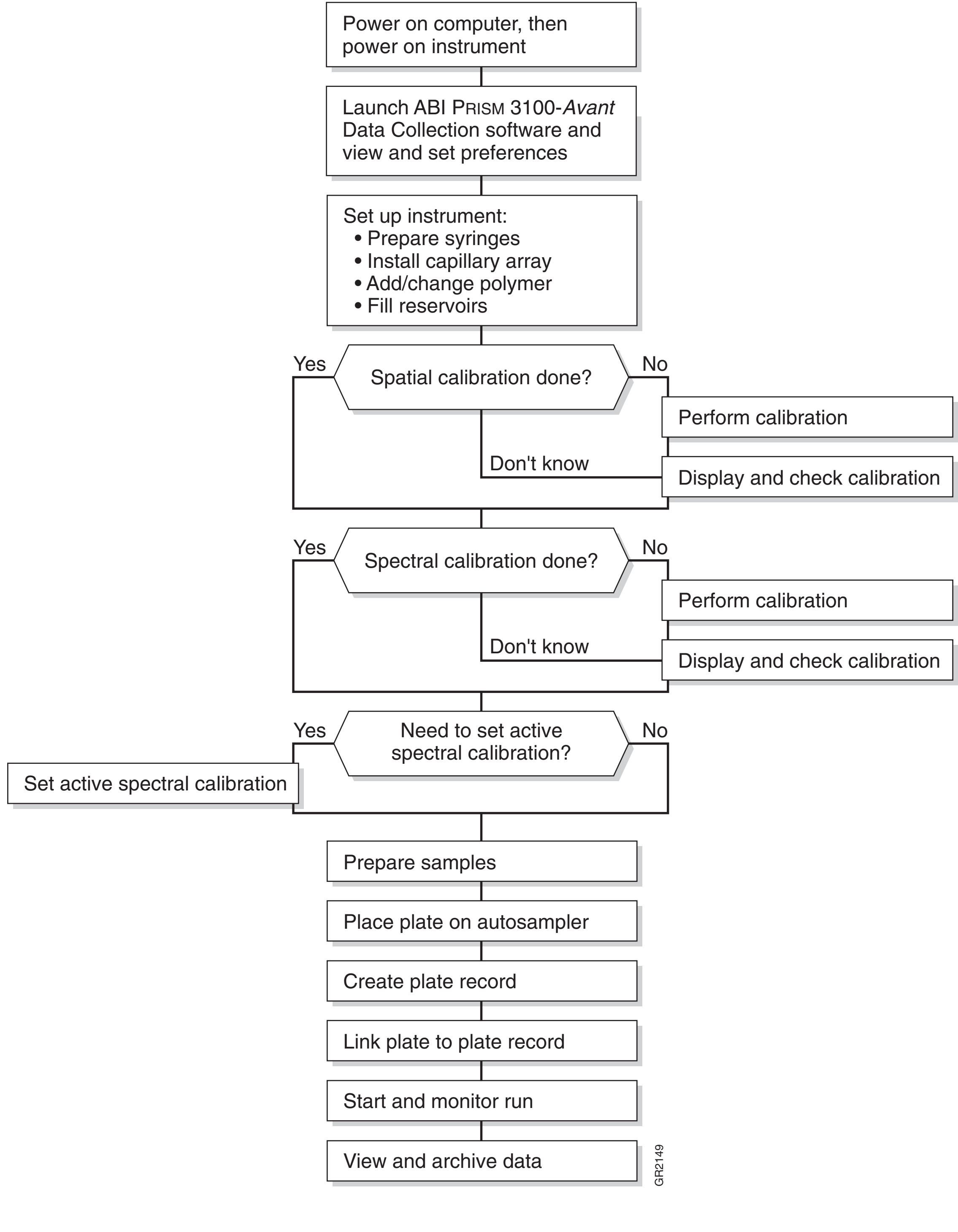
Planning Your Runs	2-5

Summary Flowchart

Flowchart of a Typical Run

This flowchart provides an overview of the steps required to perform a run on the ABI PRISM® 3100-Avant Genetic Analyzer.



Planning Your Runs

Decisions to Make

The main decisions you will need to make when preparing for a run are listed below.

Decision Table

Decision	Comments
Analysis application	Either: ♦ ABI PRISM® GeneScan® Analysis Software for fragment analysis ♦ ABI PRISM® DNA Sequencing Analysis Software for DNA sequencing
Type of polymer	Either: ♦ ABI PRISM® 3100 POP-4™ polymer for fragment analysis, long read sequencing or ultra rapid sequencing ♦ ABI PRISM® 3100 POP-6™ polymer for standard sequencing or rapid sequencing
Length of capillary array	Either: ♦ 22-cm capillary array for microsatellites and SNP genotyping ♦ 36-cm capillary array for microsatellites, SNP genotyping, rapid sequencing or ultra rapid sequencing ♦ 50-cm capillary array for standard sequencing ♦ 80-cm capillary array for long read sequencing
Type of plate	Either a: ♦ 96-well plate ♦ 384-well plate
Method of creating plate records	There are six different ways to create plate records.
Analysis module	Either: ♦ Select one of the supplied analysis modules ♦ Create your own analysis module
Run module	Either: ♦ Select one of the supplied run modules ♦ Edit one of the supplied run modules to change the conditions used for a run
Number of times to run your samples	To run your samples only once, use only one run module column and one analysis module column when creating the plate record. To run each sample up to five times, use the same or different run module columns and the same or different analysis module columns.
Single or batch run	Either: ♦ A single run that electrophoreses up to four samples ♦ A batch run that performs several sequential runs without needing operator attention

Decision Table *(continued)*

Decision	Comments
Where to save data	Data are saved as ABIF sample files. Sample files can be stored locally or on a network. The storage location is defined in the Preferences or the default location: D:\AppliedBio\3100-Avant\Data Extractor
Spatial calibration	A spatial calibration must be performed after each time you: ♦ Install or replace a capillary array ♦ Temporarily remove the capillary array from the detection block
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/replaced by a service engineer ♦ If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks) ♦ For fragment analysis only, if you change capillary array lengths

Section: Starting the 3100-Avant System

In This Section

The following topics are covered in this section:

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Starting the Instrument	2-9
Starting the 3100-Avant Data Collection Software	2-10
Setting Software Preferences	2-12

Starting the Computer

Starting the Computer Workstation

IMPORTANT You must start the computer workstation before starting the instrument.

To start the computer workstation:

Step	Action
1	Power on the monitor.
2	Power on the computer. The computer boots and then the Begin Logon dialog box opens.
3	Press **Ctrl + Alt + Delete** and enter the user name and, if applicable, a password. ♦ The default user name for the workstation is 3100-AvantUser. Do not change this user name. ♦ There is no default password. If you would like to use a password, your system administrator can create one. ♦ If the computer is connected to a network, you do not need to log on to the network before starting the instrument. ♦ OrbixWeb™ Daemon and AEServer will launch automatically. If they do not launch, double-click on the OrbixWeb Daemon and AEServer shortcuts on the Desktop. – OrbixWeb Daemon must be running for the ABI PRISM® 3100-Avant Data Collection software to run – AEServer must be running for data extraction and analysis to occur

Starting the Instrument

Starting the Instrument

To start the 3100-Avant Genetic Analyzer:

Step	Action
1	On the instrument, ensure that the: ♦ Oven door is closed and locked ♦ Instrument doors are closed **Note** If the doors are open during power on, the red failure light will illuminate.
2	On the computer, ensure that the: ♦ Computer is powered on (see “Starting the Computer” on page 2-8) ♦ Microsoft® Windows NT® operating system has loaded **Note** The computer must be on and running the Windows NT operating system because the instrument must copy the firmware from the computer.
3	Turn on the instrument by pressing the on/off button on the front of the instrument. TRAY Status lights GR1770 Press the on/off button to start the instrument **Note** While the instrument is booting up and performing self-checks, the yellow status light will blink.
4	Ensure the green status light is on and constant before proceeding. **Note** If the green light does not come on, start the data collection software and view the event log. The pathway to the event log is: D:\AppliedBio\3100-Avant\Data Collection

Starting the 3100-Avant Data Collection Software

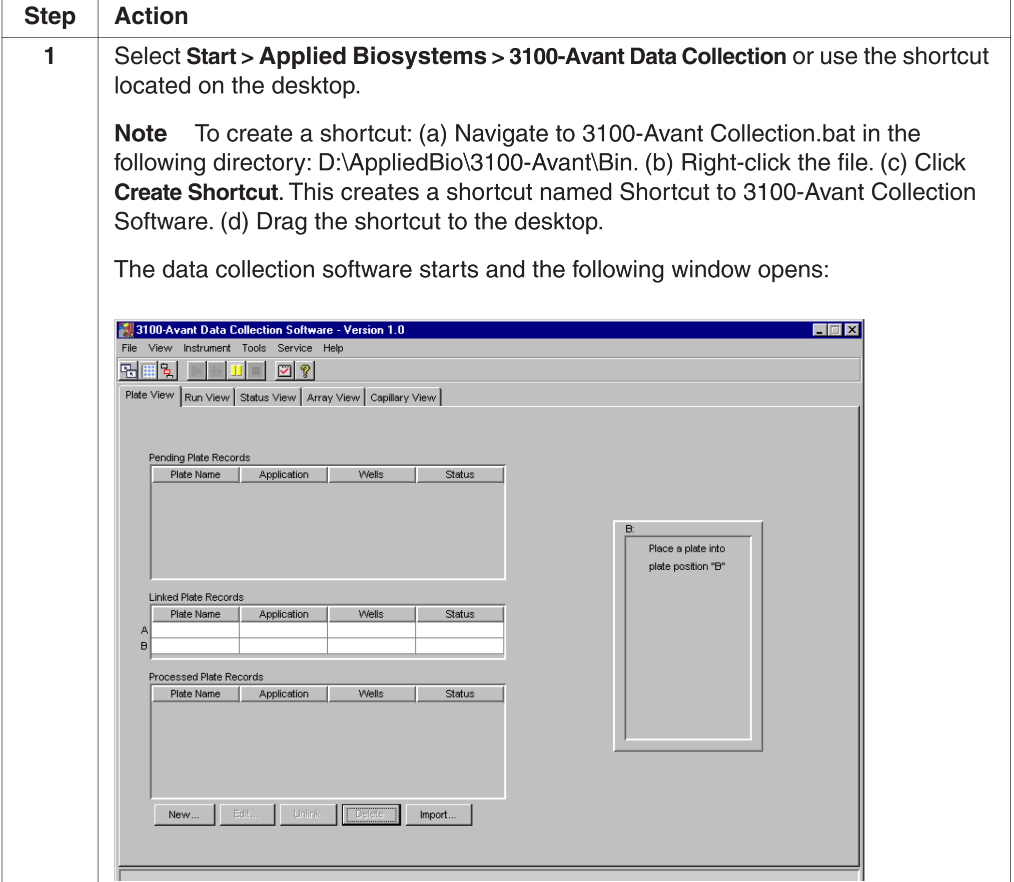
Before You Begin

To prepare to start the data collection software:

Step	Action
1	Ensure that the instrument is powered on and that the green status light is on solid (not flashing).
2	Ensure that AEServer and OrbixWeb Daemon are running by finding their buttons on the Windows NT taskbar. ♦ If AEServer is not running, select **Start** > Applied Biosystems > **3100-Avant Utilities** > **AEServer**. ♦ If OrbixWeb Daemon is not running, select **Start** > **Applied Biosystems** > **OrbixWeb Daemon**. **IMPORTANT** AEServer and OrbixWeb Daemon must be started before the data collection software can run.

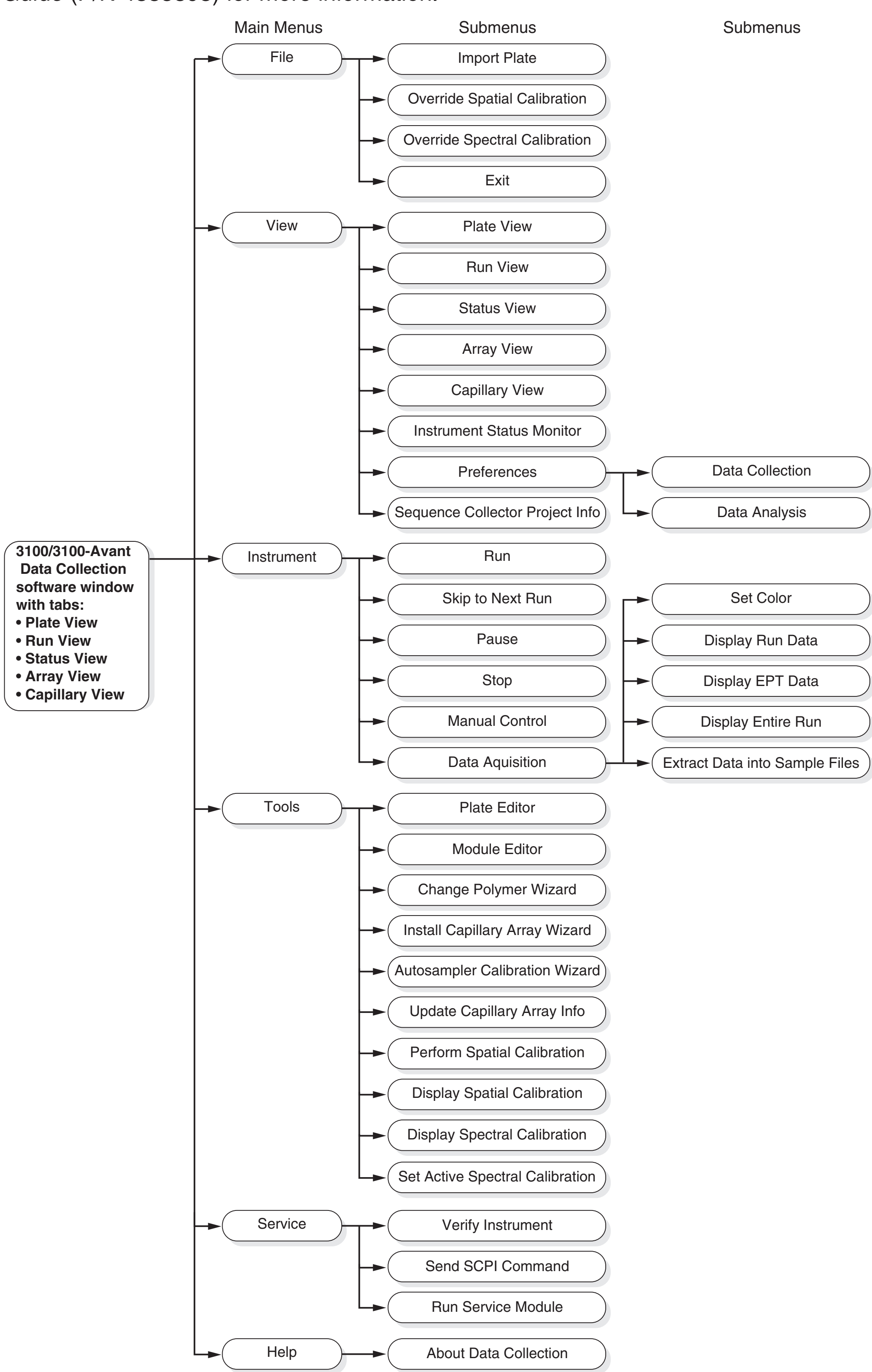
Starting the Data Collection Software

To start the data collection software:

Step	Action
1	Select **Start** > **Applied Biosystems** > **3100-Avant Data Collection** or use the shortcut located on the desktop. **Note** To create a shortcut: (a) Navigate to 3100-Avant Collection.bat in the following directory: D:\AppliedBio\3100-Avant\Bin. (b) Right-click the file. (c) Click **Create Shortcut**. This creates a shortcut named Shortcut to 3100-Avant Collection Software. (d) Drag the shortcut to the desktop. The data collection software starts and the following window opens: 

Software Menus

Below is a flowchart of the software menus in the data collection software. Refer to the *ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (P/N 4335393) for more information.



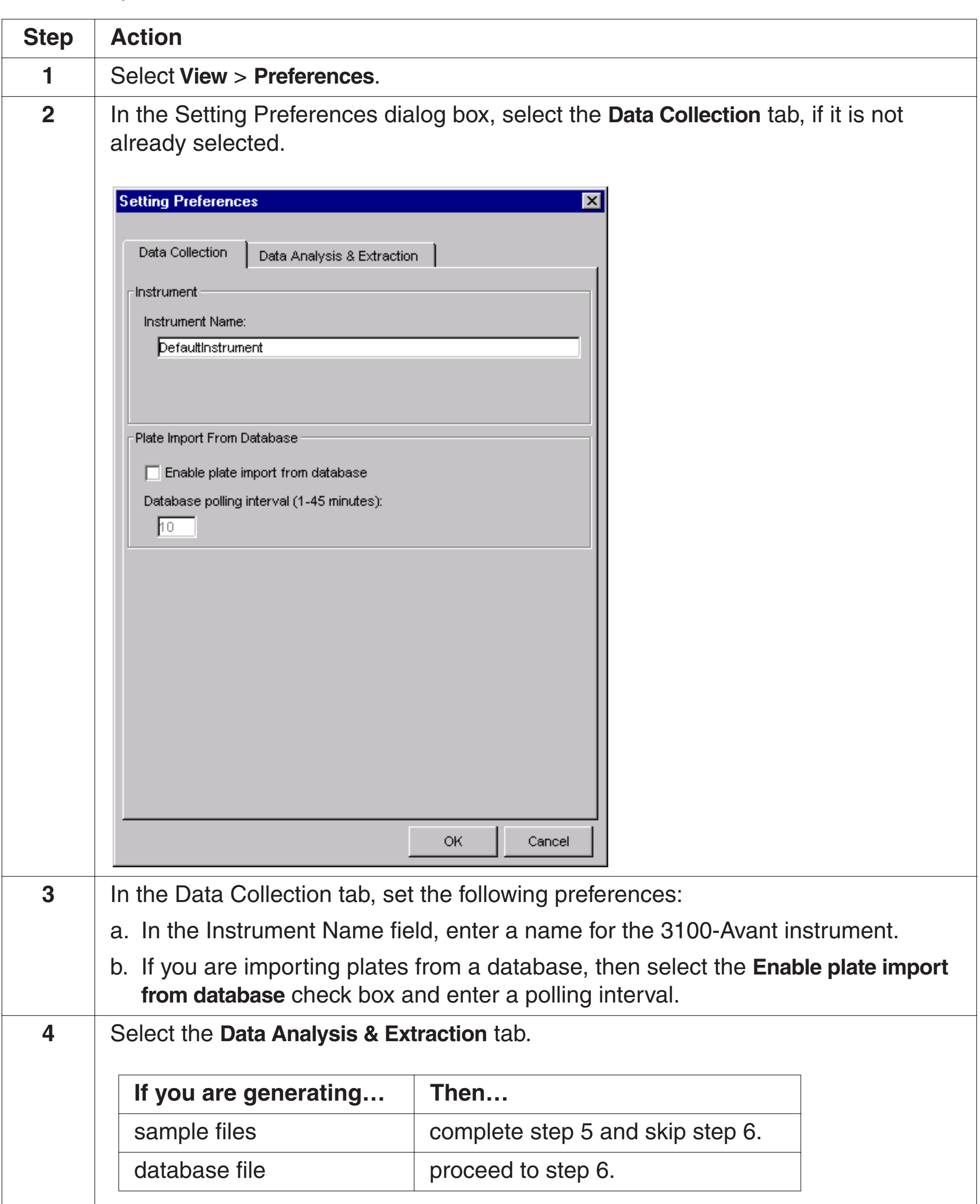
Setting Software Preferences

Overview

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

Defining the Setting Preferences Dialog Box

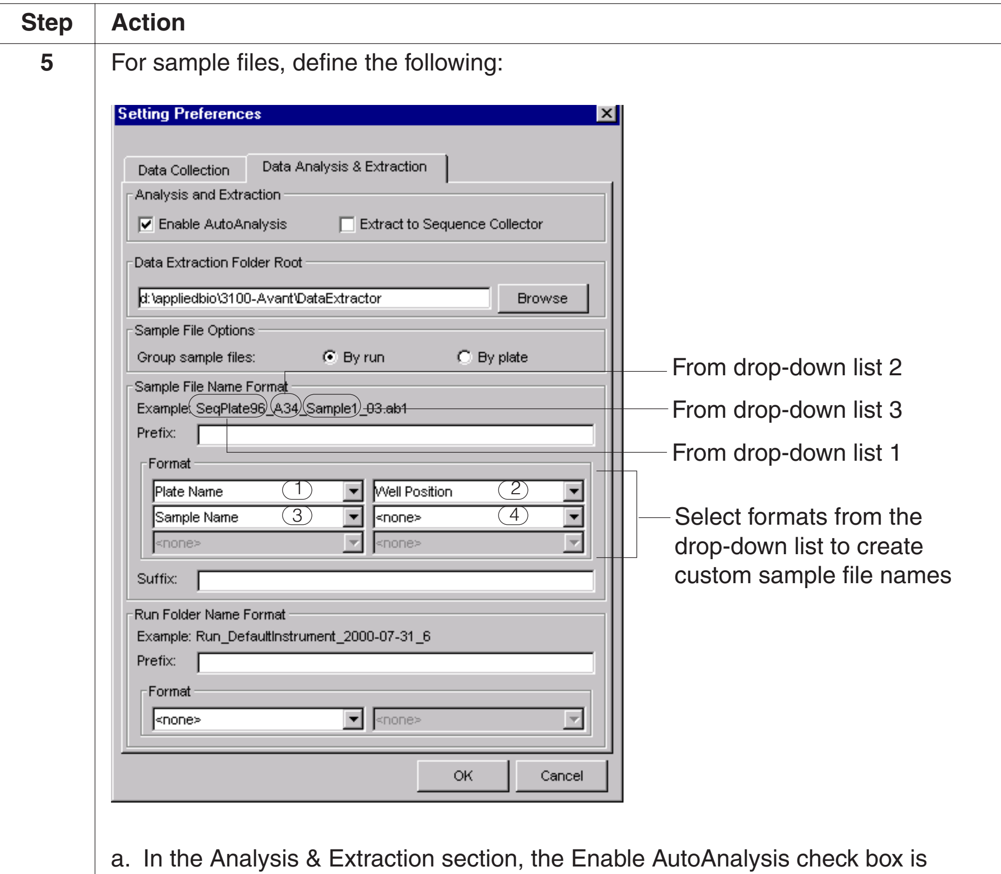
To set the preferences:

Step	Action
1	Select **View** > **Preferences**.
2	In the Setting Preferences dialog box, select the **Data Collection** tab, if it is not already selected. 
3	In the Data Collection tab, set the following preferences: a. In the Instrument Name field, enter a name for the 3100-Avant instrument. b. If you are importing plates from a database, then select the **Enable plate import from database** check box and enter a polling interval.
4	Select the **Data Analysis & Extraction** tab.

If you are generating…	Then…
sample files	complete step 5 and skip step 6.
database file	proceed to step 6.

To set the preferences: *(continued)*

Step	Action
5	For sample files, define the following:



a. In the Analysis & Extraction section, the Enable AutoAnalysis check box is selected as default. Clear the box if you do not want your samples autoanalyzed.

b. In the Data Extraction Folder Root section, use the default or click **Browse** to select a folder location for all generated data.

c. In Sample File Options section, select how you want your sample files grouped. Select the **By run** option button to group by individual run or select the **By plate** option button to group by the entire plate.

d. In the Sample File Name Format section, use the drop-down lists to define the sample file name format. A prefix and/or suffix can be added as needed.

Identifier	Origin
Run ID	Generated by the data collection software
Sample Name	Taken from the Plate Editor spreadsheet entry
Well Position	Taken from the sample's position on the plate (column letter and row number, *e.g.*, C3)
Plate Name	Taken from the Plate Editor dialog box entry
Instrument ID	Taken from the Data Collection page preferences entry
Array ID	Taken from the Install Capillary Array or Update Capillary array wizard entry

e. In the Run Folder Name Format section, use the drop-down lists to define the sample file name format. A prefix can be added as needed.

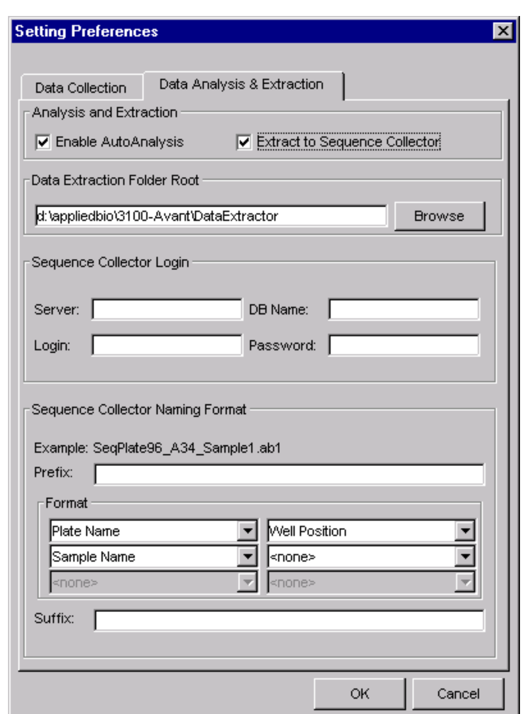
f. Click **OK**.

To set the preferences: *(continued)*

Step	Action
6	For database files, define the following:

a. In the Analysis & Extraction section, select **Extract to Sequence Collector**.

The **Enable AutoAnalysis c**heck box is selected as default. Clear the box if you do not want your samples autoanalyzed.



b. In the Data Extraction Folder Root section, use the default or click **Browse** to select a folder location for all generated data.

c. In the Sequence Collector Login section, define the server, DB name, login, and password for the database you are using.

d. In the Sequence Collector Naming Format section, use the drop-down lists to define the sample file name format. Add a prefix and/or suffix as needed.

Identifier	Origin
Run ID	Generated by the data collection software
Sample Name	Taken from the Plate Editor spreadsheet entry
Well Position	Taken from the sample's position on the plate (column letter and row number, *e.g.*, C3)
Plate Name	Taken from the Plate Editor dialog box entry
Instrument ID	Taken from the Data Collection page preferences entry
Array ID	Taken from the Install Capillary Array or Update Capillary array wizard entry

e. Click **OK**.

Section: Preparing the Instrument

In This Section

The following topics are covered in this section:

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Calibrating the Instrument	2-22

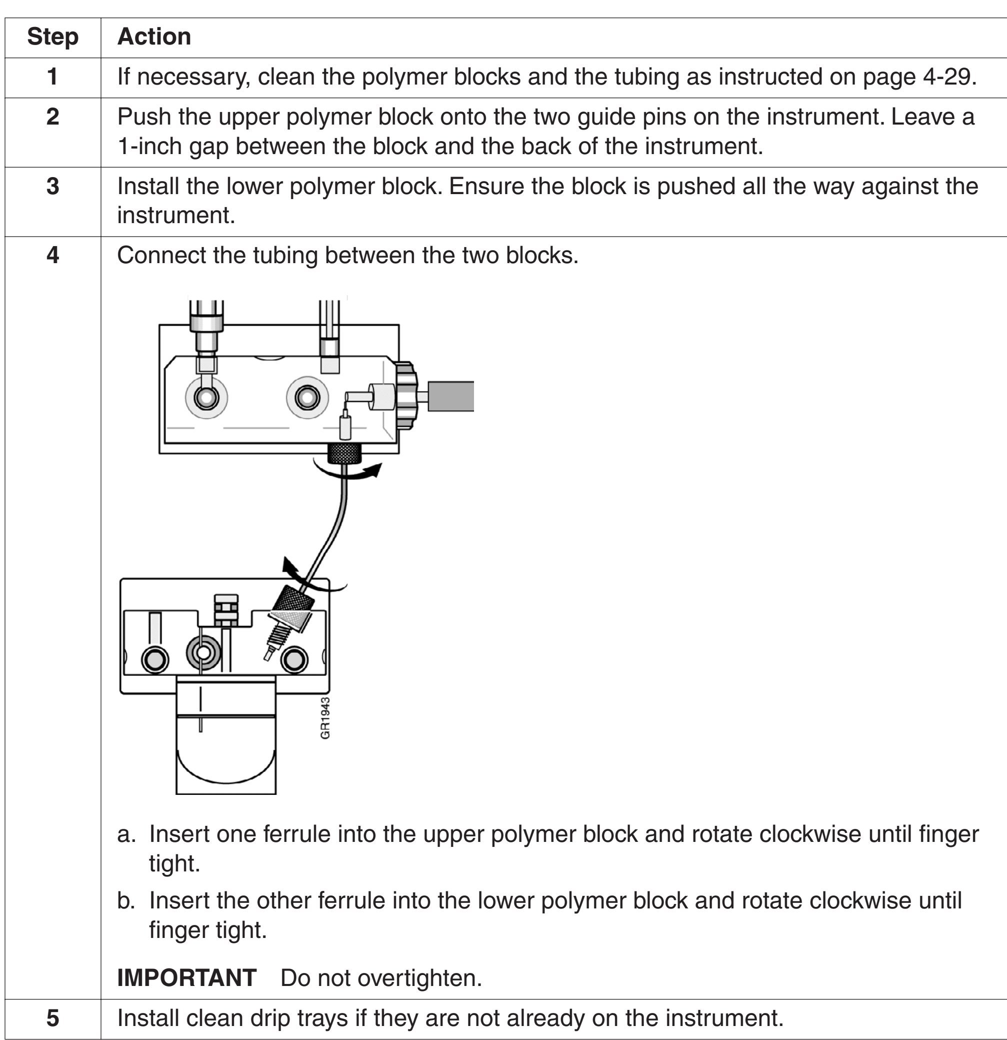
Setting Up the Instrument

Using Manual Control Commands

While you are setting up the instrument, you may find manual control useful. For example, you can use manual control commands to move the syringe plungers up and down, open or close the pin valve, and turn on the oven before starting your run. Refer to the *ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (P/N 4335393) for more information.

Attaching the Polymer Blocks

To attach the polymer blocks to the instrument:

Step	Action
1	If necessary, clean the polymer blocks and the tubing as instructed on page 4-29.
2	Push the upper polymer block onto the two guide pins on the instrument. Leave a 1-inch gap between the block and the back of the instrument.
3	Install the lower polymer block. Ensure the block is pushed all the way against the instrument.
4	Connect the tubing between the two blocks.  a. Insert one ferrule into the upper polymer block and rotate clockwise until finger tight. b. Insert the other ferrule into the lower polymer block and rotate clockwise until finger tight. **IMPORTANT** Do not overtighten.
5	Install clean drip trays if they are not already on the instrument.

Selecting a Capillary Array

Use the table below to select the correct capillary array length for your application.

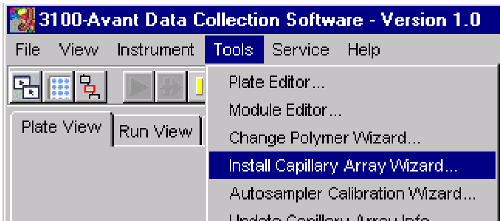
Application or Kit	Capillary Array Length (cm)
Fragment Analysis	
ABI PRISM® SNaPshot® Multiplex System	22
	36
♦ ABI PRISM® Linkage Mapping Set v2.5 ♦ ABI PRISM® Mouse Mapping Set v1.0 ♦ Custom oligos	22 36
DNA Sequencing	
Ultra rapid sequencing	36
Rapid sequencing	36
Standard sequencing	50
Long read sequencing	80

IMPORTANT Fragment Analysis: For optimal resolution, as in the case of fine mapping, Applied Biosystems recommends using the 36-cm capillary array. However, the 22-cm capillary array can be used to rapidly scan the genome when using markers less than 360 bp. Refer to *ABI PRISM® 3100 22-cm Capillary Array for High Throughput Microsatellite and SNP Genotyping User Bulletin* for more information.

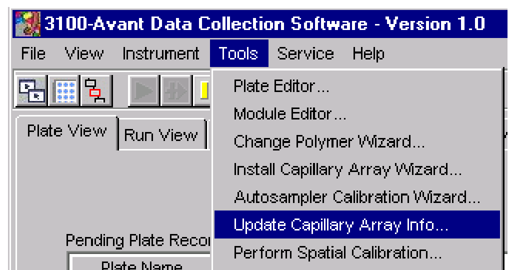
Installing or Replacing the Capillary Array

IMPORTANT The capillary array length that you are using must match the capillary array length in the instrument database. If they do not match then data quality could be compromised.

If necessary, install a capillary array using the Install Capillary Array wizard. For instructions, see “Installing and Removing the Capillary Array” on page 4-15.



Alternatively, you can install the capillary array without using the wizard. Update the capillary array information using the Update Capillary Array Info utility. For instructions, see “Installing the Capillary Array Without Using the Install Wizard” on page 4-16.



Selecting the Polymer

Use the following table to select the correct polymer for your application.

Application	Capillary Length	Polymer
Fragment analysis	all lengths	POP-4
Ultra rapid sequencing	36	
Long read sequencing	80	
Rapid sequencing	36	POP-6
Standard sequencing	50	

Preparing and Installing the 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 prepare and install the syringes:

Step	Action
1	If necessary, clean and inspect the syringes as instructed on page 4-22.
2	Prime and fill the syringes with the correct polymer as instructed on page 4-24.
3	Install the syringes as instructed on page 4-25.

⚠ CAUTION **CHEMICAL HAZARD**. **POP polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

When to Add or Change Polymer

If syringes containing polymer are on the instrument, use the table below to determine whether to add or change the polymer 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 that there are no air bubbles, and then proceed with instrument preparation.
less than 1 week old, and insufficient in quantity to complete your runs	Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard (see page 4-10).
more than 1 week old	a. Remove and clean the polymer blocks and syringes (see “Polymer Blocks” on page 4-27 and “Syringes” on page 4-21). b. Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard (see page 4-10).
wrong type (changing between POP-4 and POP-6 polymers)	

a. A run uses ~20 μL of polymer. A minimum of 100 μL of polymer is required for the instrument to operate.

IMPORTANT Always replace polymer that is older than 1 week.

IMPORTANT Ensure that there are no air bubbles in the upper polymer block and polymer block tubing before proceeding. To remove any air bubbles, see page 4-32.

Preparing Buffer and Filling the Reservoirs

Required Materials

The following materials are required to prepare 1X running buffer:

- 10X Genetic Analyzer Buffer (P/N 401884)
- Quality deionized water

⚠ CAUTION **CHEMICAL HAZARD**. **10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Making Buffer for a Single Run

To prepare 50 mL of 1X running buffer:

Step	Action
1	Add 5 mL of 10X Genetic Analyzer buffer into a graduated cylinder.
2	Add deionized water to bring the total volume up to 50 mL.
3	Mix well.

Storing the Buffer

The 1X running buffer can be stored at 2 to 8 °C for up to 1 month.

Replacing the Buffer

Replace the 1X running 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 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.

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 and lock the oven door and then close the instrument doors.
2	Press the Tray button on the outside of the instrument to bring the autosampler to the forward position. [Figure: TRAY; Tray button; GR1770]
3	Wait until the autosampler has stopped moving, and then open the instrument doors.
4	Remove the cathode buffer reservoir and water reservoirs from the instrument.

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

Step	Action
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 running buffer, and then fill to the line with 1X running buffer (about 16 mL).
7	Fill the water reservoirs to the line with quality deionized water (about 16 mL).
8	Place a clean reservoir septa on each reservoir, and dry the outside of the reservoirs using a lint-free wipe. **⚠ CAUTION** Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips. Septa is lying flat on the reservoir Fill line GR1657
9	Place the reservoirs into position on the autosampler as shown below. 2 Water reservoir (rinse) 4 Water reservoir (waste) 1 Cathode reservoir (1X running buffer) 3 Water reservoir (spare)
10	Close the instrument doors. **Note** Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in water.

Filling the Anode Buffer Reservoir

Change the anode buffer:

- ♦ Before each run, 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 running 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.

To fill the anode buffer reservoir to the fill line with 1X running buffer: *(continued)*

Step	Action
4	Fill the reservoir to the fill line with fresh 1X running buffer (about 9 mL). Fill line
5	Put the anode buffer reservoir on the instrument. **Note** The meniscus should line up with the fill line.
6	If the reservoir fills with fluid, repeat this procedure to discard and replace the running buffer. **Note** The reservoir could fill during bubble clearing.

Calibrating the Instrument

Spatial Calibration

If necessary, perform a spatial calibration.

A spatial calibration must be performed after each time you:

- ♦ Install a capillary array
- ♦ Replace a capillary array with a new one
- ♦ Move or remove the capillary array temporarily from the detection block

For instructions, see “Spatial Calibration” on page 3-3.

Spectral Calibration

If necessary, perform a spectral calibration.

A spectral calibration must be performed:

- ♦ Whenever you use a new dye set on the instrument
- ♦ After the laser has been realigned by a service engineer
- ♦ After the CCD camera has been realigned/replaced by a service engineer
- ♦ If you begin to see pull-up and/or pull-down peaks consistently
- ♦ For fragment analysis only, if you change the capillary array length

For instructions, see “Spectral Calibration” on page 3-13.

Affect of Capillary Array Length

The affect of the capillary array length on spectral calibrations vary between sequencing and fragment analysis applications.

- ♦ Sequencing analysis

  Typically, for each dye set, a single spectral calibration can be used for all capillary array lengths and polymer combinations.

- ♦ Fragment analysis

  For each dye set, a separate spectral calibration must be used for the 22- and 36-cm capillary arrays. Refer to “Activating a Spectral Calibration for a Dye Set” on page 3-31, for information on how to switch calibrations.

Section: Working with Samples

In This Section

The following topics are covered in this section:

Topic	See Page
Plate Mapping	2-24
Preparing and Loading Samples	2-26

Plate Mapping

Introduction

Below is information on how samples are scheduled for injection based on the plate configuration. This is helpful for arranging samples with high priority or running a plate that is partially filled.

The instrument injects samples using an alphanumeric system that schedules runs based on the following criteria:

- Order the plates are linked and then,
- Run module name with numerics scheduled first (0...9) followed by upper case then lower case

If all run module names are identical, runs are scheduled as outlined below.

Selecting a Plate Type

The 96- and 384-well plates can be used for fragment analysis and sequencing applications.

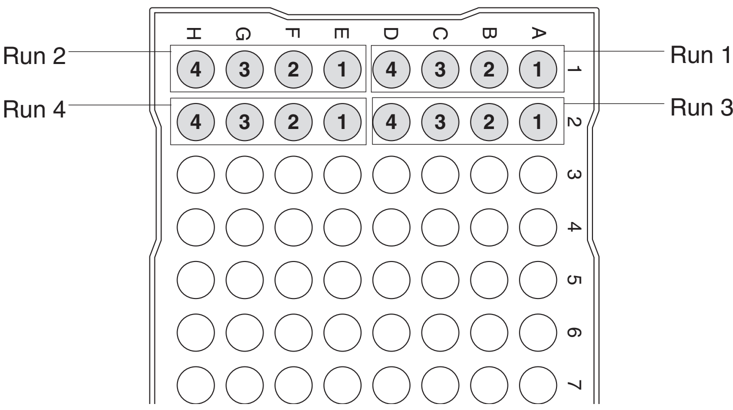
IMPORTANT The 384-well plate can be used for sequencing applications, but sample quality may be affected. Hi-Di™ formamide exposed to air for more than 24 hours begins to break down. The breakdown of Hi-Di formamide can compromise sample quality.

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.

96-Well Plate Mapping

For a 96-well plate, injections are made from four consecutive wells. A full plate of 96 sample requires 24 runs to inject all samples once.

Below is an example of a 96-well plate. The gray circles represent samples, and the number in the well indicates capillary number. It takes four runs to inject 16 samples.

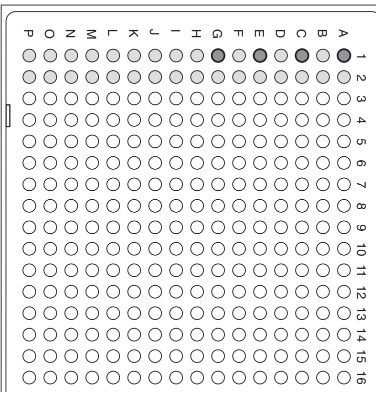


384-Well Plate Mapping

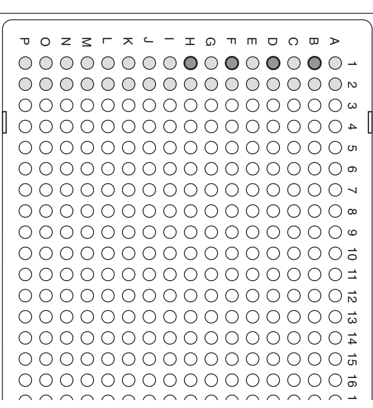
For a 384-well plate, injections are made from every other well. A full plate of 384 sample requires 96 runs to inject all samples once.

Below is an example of the injection pattern for the first six injections. The light gray circles represent samples and the dark gray circles indicate the pick up pattern for injection.

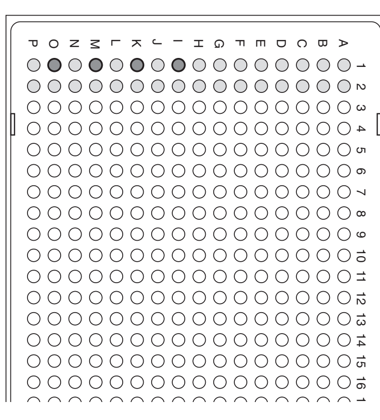
Run 1



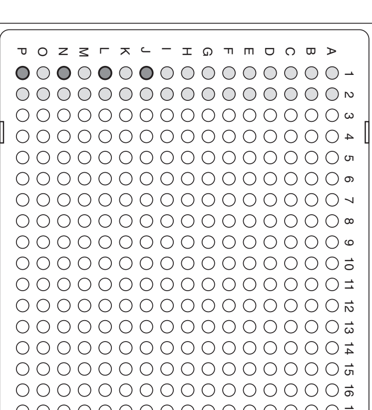
Run 2



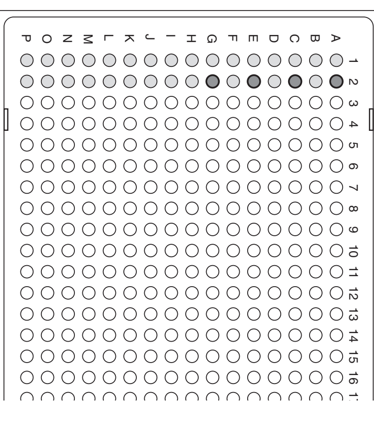
Run 3



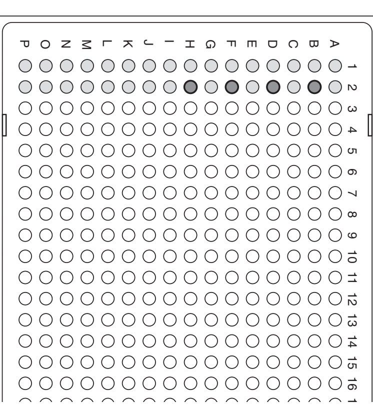
Run 4



Run 5



Run 6



Preparing and Loading Samples

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD**. All chemicals on the instrument, including liquid in the lines, are potentially hazardous. Please read the MSDS, and follow the handling instructions. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

References for Sample Preparation

For information on required materials, sample preparation, and plate centrifugation, refer to the appropriate guide as follows:

For...	Refer to the...
DNA sequencing samples	*ABI PRISM® Automated DNA Sequencing Chemistry Guide* (P/N 4305080) or kit protocol
Fragment analysis samples	Kit protocol

Loading the Samples

To load the samples:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Dispense 10 μL of the denatured samples into the wells of a plate.</td></tr>
<tr><td>2</td><td>Tap down or centrifuge the plate so that each sample is positioned at the bottom of its well. Your samples should:
<table>
<tr><th>Look like this...</th><th>Not look like this...</th><th>Not look like this...</th></tr>
<tr><td>The sample is positioned correctly in the bottom of the well.</td><td>The sample lies on the side wall because the plate was not centrifuged.</td><td>An air bubble lies at the bottom of the well because the plate was not centrifuged with enough force or time.</td></tr>
</table>
</td></tr>
<tr><td>3</td><td>Leave the plate on ice until you are ready to prepare the plate assembly and place the assembly on the autosampler.</td></tr>
</table>

Section: Working with Plate Records

In This Section

The following topics are covered in this section:

Topic	See Page
About Plate Records	2-28
Creating a Plate Record for Fragment Analysis	2-29
Creating a Plate Record for DNA Sequencing Analysis	2-35
Using a Previous Plate Record to Complete a New Plate Record Automatically	2-41

About Plate Records

Overview

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

Plate records are data tables in the instrument database that store information about the plates and the samples they contain. Specifically, a plate record contains the following information:

- Plate name, type, and owner
- Position of the sample on the plate (well number)
- Sample name
- Dye color of size standard (fragment analysis only)
- Mobility file (DNA sequencing analysis only)
- Comments about the plate and about individual samples
- Dye set information
- Project name (this entry is mandatory, even when Sequence Collector is not used)
- Name of the run module (run modules specify information about how samples are run)
- Name of the analysis module (analysis modules specify how raw data is autoanalyzed at the end of the run)

When to Create a Plate Record

A plate record must be created for each plate of samples for the following types of runs:

- Fragment analysis
- DNA sequencing
- Spectral calibrations

Note For fragment analysis and sequencing runs, there is no need to re-create a plate record for a plate that has failed. Simply edit the plate record to add a run module and an analysis module column to the rows that need to be rerun. This will move the existing plate record from the Processed window to the Pending window.

Plate records can be created before or after placing the plates on the instrument. New plate records can be created while a run is in progress.

About Creating Plate Records

The next three sections cover the most common method for creating a plate record. Other options exist for creating plate records from tab delimited files.

Refer to the *ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (P/N 4335393) for more information.

Plate Records for Spectral Runs

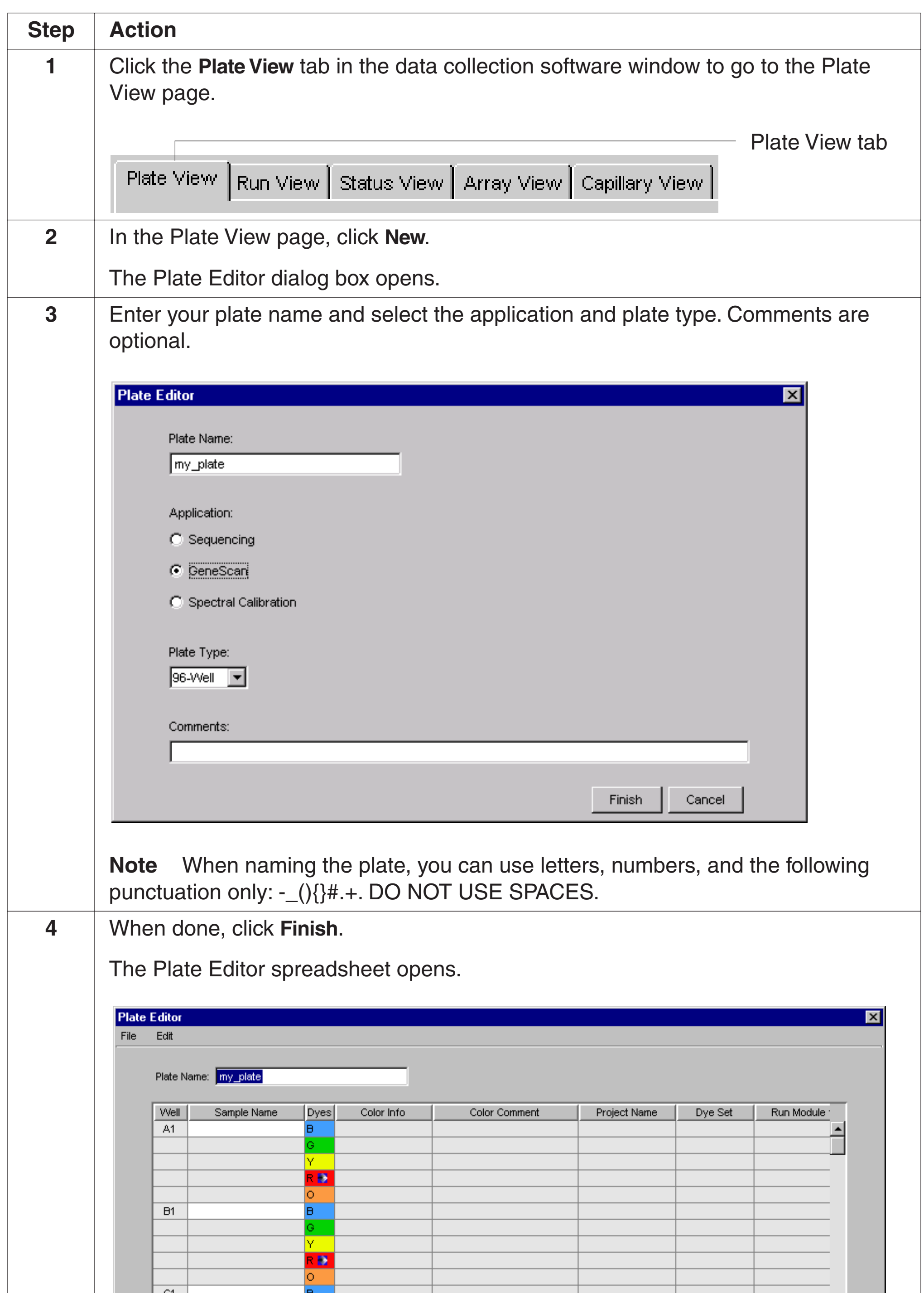
For information on creating plate records for spectral calibration runs, see “Creating a Plate Record” on page 3-20.

Creating a Plate Record for Fragment Analysis

Entering Plate Record Information

Note You can create a plate record while a run is in progress.

To enter plate record information:



Step	Action
1	Click the **Plate View** tab in the data collection software window to go to the Plate View page.
2	In the Plate View page, click **New**. The Plate Editor dialog box opens.
3	Enter your plate name and select the application and plate type. Comments are optional. **Note** 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 opens.

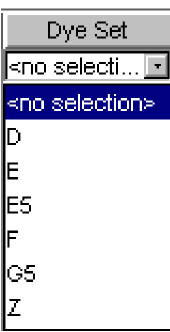
Entering Sample Information

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. ♦ Sample names are limited to a maximum of 32 characters. ♦ When naming the samples, you can use letters, numbers, and the following punctuation only: -_(){}#.+. DO NOT USE SPACES. ♦ In the default naming convention, the sample name you type is incorporated into the sample file name. For example: **PlateName_A01_MySample__01.fsa** — Fragment analysis file extension Capillary position Sample name you type Well position Plate name you type **Note** The sample file naming convention used can be changed in the **Preferences** dialog box. See page 2-12 for details.
2	Change the size standard dye color, if necessary. The default is red. Use red for 4-dye applications and orange for 5-dye applications. a. Click on the dye color you want to use. b. Select all the samples. c. Select **Edit > Fill down**.
3	Type in Color info and Color Comment, if needed. **Note** Color Info and Color Comment information is the same as Sample Info and Sample Comment in the ABI PRISM® Genotyper® software. Refer to the *ABI PRISM® Genotyper® Software User's Manual* for more information.
4	Enter a Project name. **Note** A project name is required for every sample, even if a Sequence Collector database is not used. a. Click in the **Project Name** cell for Well A1. b. Select a project name in the drop-down list. Project Name <no selection> <no selection> 3100-Avant_Project1 **Note** You must select a project. 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**. **Note** Press Ctrl+D to fill down whenever a field is the same for all samples in the plate record.

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

Step	Action
5	For each sample, select the appropriate **Dye Set** in the drop-down list.



The following table shows the dye set to select based on your application.

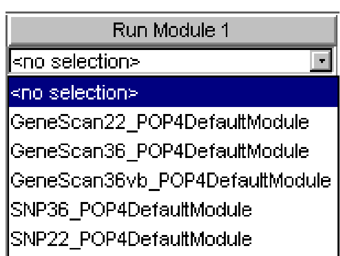
Application or Kit	Dye Set	Matrix Standard Set
Custom oligos	D	DS-30
♦ ABI PRISM Mouse Mapping Set v1.0 ♦ Custom oligos	D	DS-31 (DS-30 + VIC™ Matrix Standard)[a]
ABI PRISM® SNaPshot® Multiplex System	E5	DS-02
♦ ABI PRISM® Linkage Mapping Set v2.5 ♦ Custom Oligos	G5	DS-33

a. Replace the HEX™ matrix standard in DS-30 set with the VIC matrix standard.

IMPORTANT Be sure to select the correct dye set for your run(s). Data collected with the incorrect dye set selected cannot be saved and the runs will have to be repeated because multicomponenting is applied during collection.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>
For each sample, select the appropriate Run Module in the drop-down list.



The following table shows the run module to select based on your run type:

<table>
<tr><th>Application or Kit</th><th>Capillary Array Length</th><th>Run Module</th></tr>
<tr><td rowspan="2">SNaPshot Multiplex System</td><td>22 cm</td><td>SNP22_POP4DefaultModule</td></tr>
<tr><td>36 cm</td><td>SNP36_POP4DefaultModule</td></tr>
<tr><td rowspan="2">♦ LMS v2.5
♦ ABI PRISM Mouse Mapping Set v1.0
♦ Custom oligos</td><td>22 cm</td><td>GeneScan22_POP4DefaultModule</td></tr>
<tr><td>36 cm</td><td>GeneScan36_POP4DefaultModule</td></tr>
<tr><td>LMS v2.5</td><td>36 cm</td><td>GeneScan36vb_POP4DefaultModule
Note This module supports specific fragment analysis applications using the G5 chemistry</td></tr>
</table>

Note If you select different modules for different samples, the samples will be automatically grouped so that all samples with the same run module are run at the same time. Runs are scheduled alphanumerically by run module name, not by the order indicated in the plate record, nor by sample name.
</td></tr>
</table>

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

Step	Action
7	For each sample, select the appropriate **Analysis Module** from the drop-down list.

IMPORTANT The Enable AutoAnalysis preference must be selected if analysis is to take place automatically after the run (see page 2-12).

Analysis Module 1

<no selection>

<no selection>
GS120Analysis.gsp
GS350Analysis.gsp
GS400CubicAnalysis.gsp
GS400HDAnalysis.gsp
GS400Ord2Analysis.gsp
GS500Analysis.gsp

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

If using size standard...	Select this analysis module...
GeneScan™120	GS120Analysis.gsp
GeneScan™ 400HD	GS400HDAnalysis.gsp
GeneScan™ 350	GS350Analysis.gsp
GeneScan™ 500	GS500Analysis.gsp
GeneScan™ 400 *(see footnote)*	GS400CubicAnalysis.gsp[a]
	GS400Ord2Analysis.gsp[a]

a. These modules are for advanced users with specific sizing needs. See the *ABI PRISM® GeneScan® Analysis Software v. 3.7 NT User Guide.*

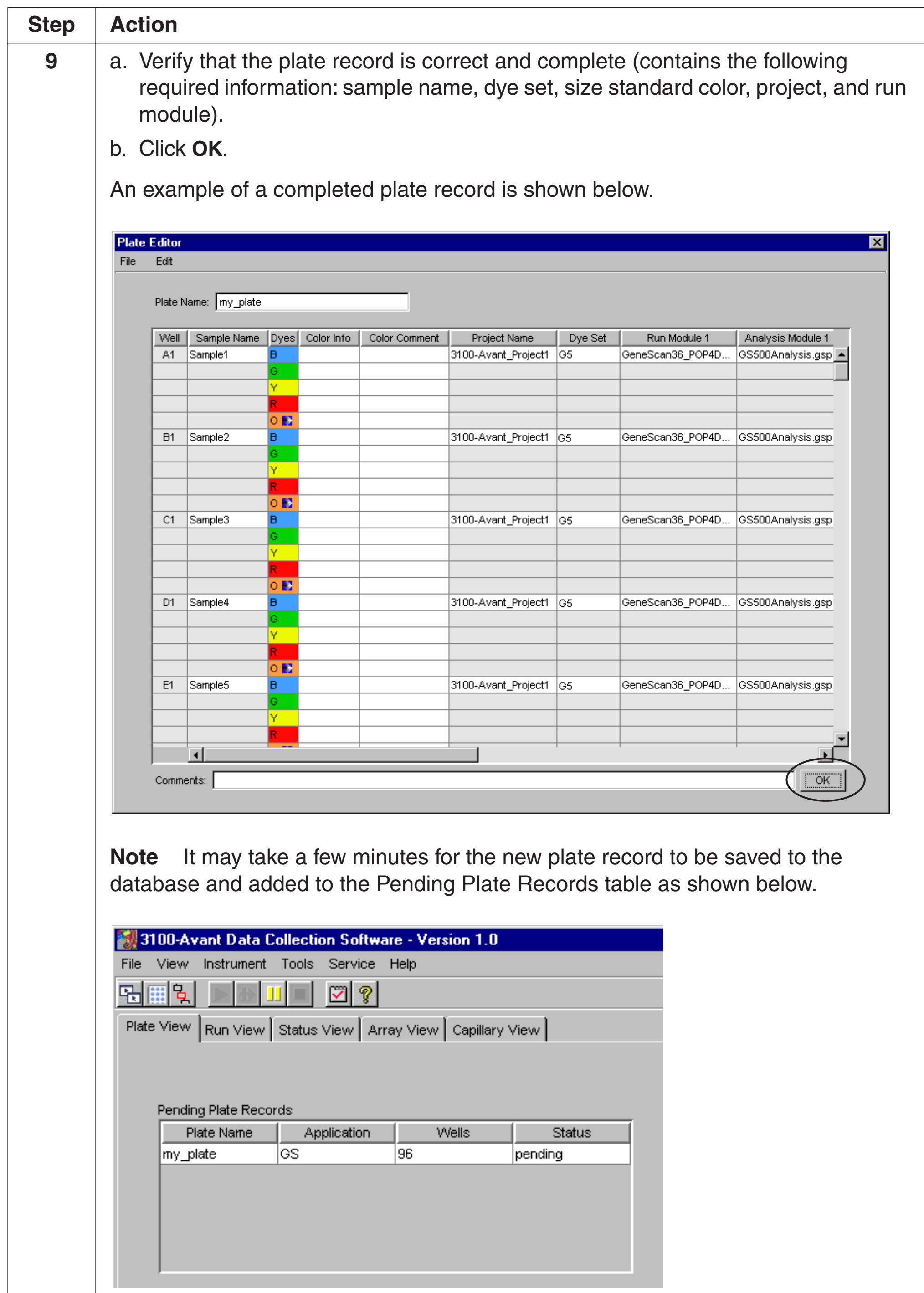
Note You can examine the settings for each of these files using GeneScan Analysis Software. The meanings of the settings are described in the *ABI PRISM® GeneScan® Analysis Software v. 3.7 NT User Guide*.

Step	Action
8	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.

Run Module 2 | Analysis Module 2

Note Samples will be automatically grouped so that all samples with the same run module are run sequentially.

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

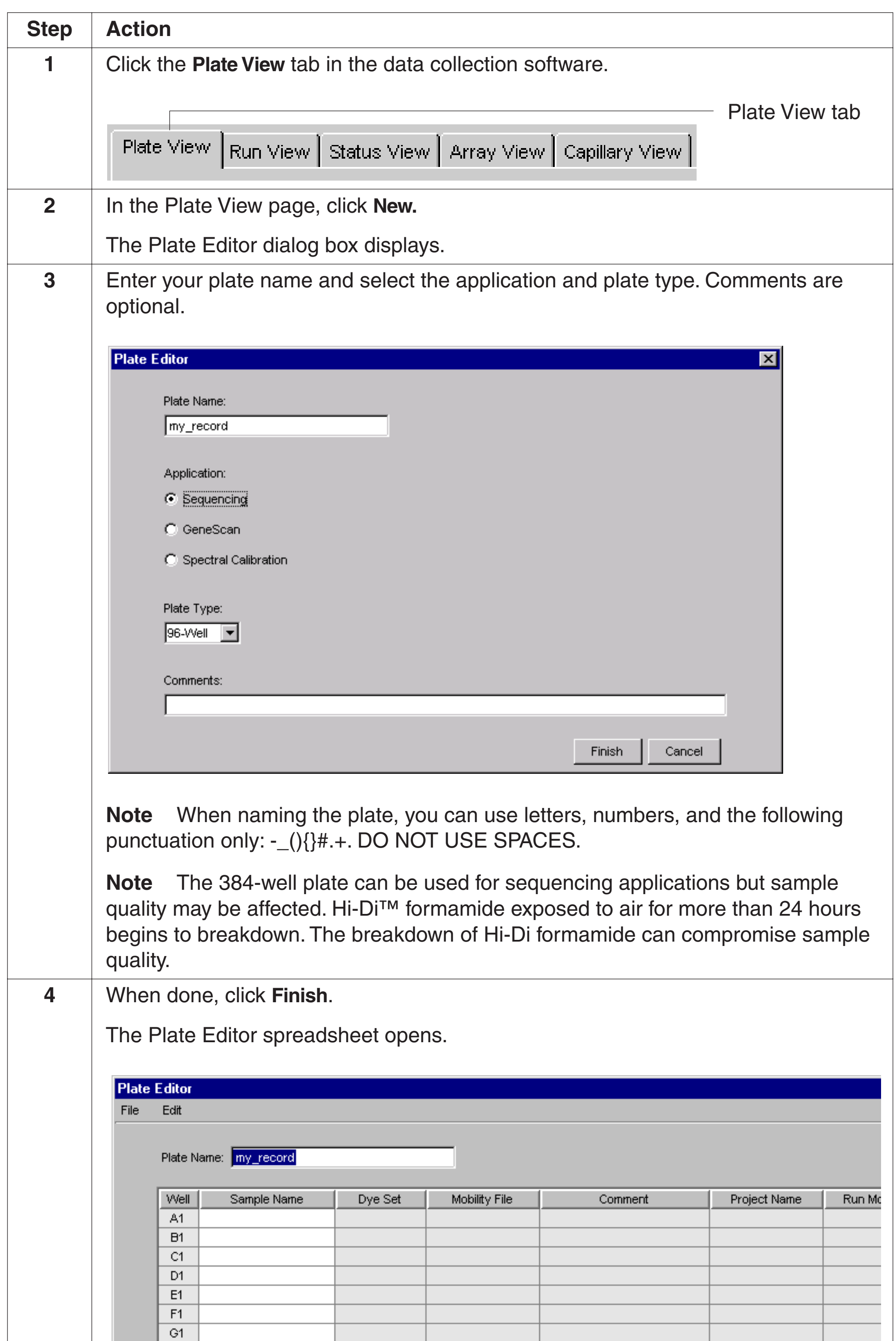
Step	Action
9	a. Verify that the plate record is correct and complete (contains the following required information: sample name, dye set, size standard color, project, and run module). b. Click **OK**. An example of a completed plate record is shown below.  **Note** It may take a few minutes for the new plate record to be saved to the database and added to the Pending Plate Records table as shown below.

Creating a Plate Record for DNA Sequencing Analysis

Entering Plate Record Information

Note You can create a plate record while a run is in progress.

To enter plate record information:



Step	Action
1	Click the **Plate View** tab in the data collection software.
2	In the Plate View page, click **New.** The Plate Editor dialog box displays.
3	Enter your plate name and select the application and plate type. Comments are optional. **Note** When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. DO NOT USE SPACES. **Note** The 384-well plate can be used for sequencing applications but sample quality may be affected. Hi-Di™ formamide exposed to air for more than 24 hours begins to breakdown. The breakdown of Hi-Di formamide can compromise sample quality.
4	When done, click **Finish**. The Plate Editor spreadsheet opens.

Entering Sample Information

To enter sample information and save the plate record:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>In the Plate Editor spreadsheet, type the names of all the samples in the Sample Name column.
♦ Sample names are limited to a maximum of 32 characters.
♦ When naming the samples, you can use letters, numbers, and the following punctuation only: -_(){}#.+. DO NOT USE SPACES.
♦ In the default naming convention, the sample name you type is incorporated into the sample file name. For example:

PlateName_A01_MySample__01.ab1 — Sequencing analysis file extension
Capillary position
Sample name you type
Well position
Plate name you type

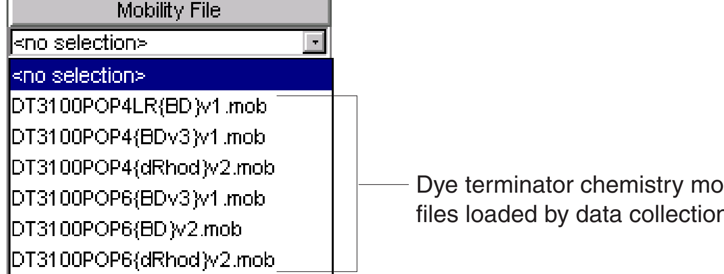
♦ The sample file naming convention used can be changed in the Preferences dialog box. See page 2-12 for details.</td></tr>
<tr><td>2</td><td>For each sample, select the appropriate Dye Set in the drop-down list.

Dye Set
<no selecti...
<no selection>
D
E
E5
F
G5
Z

Use the table below to identify the correct dye set for the chemistry you are using.
<table><tr><th>Chemistry</th><th>Dye Set</th></tr><tr><td>ABI PRISM® BigDye™ Terminator</td><td rowspan="2">E</td></tr><tr><td>ABI PRISM® dRhodamine Terminator</td></tr><tr><td>ABI PRISM® BigDye™ v3.0 Terminator</td><td>Z</td></tr></table>
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.</td></tr>
</table>

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

Step	Action
3	For each sample, select the appropriate dye terminator chemistry **Mobility File** in the drop-down list.



IMPORTANT Mobility files for dye primer chemistry also display in the drop-down list, if ABI PRISM® Sequencing Analysis Software v3.7 is loaded on the computer. Dye terminator files start with “DT” and dye primer files start with “DP”. Do not use the “DP” files.

You may need to resize the column to see the whole file name. To do this, place the pointer between the column headers (it will become a double-headed arrow) and drag right.

The following table shows which mobility file to select based on your sequencing chemistry:

DNA Sequencing Chemistry	Capillary Array Length (cm)	Mobility File
ABI PRISM BigDye Terminator	36: ultra rapid	DT3100POP4LR{BD}v1.mob
	80: long read	
	36: rapid read	DT3100POP6{BD}v2.mob
	50: std run	
ABI PRISM dRhodamine Terminator	36: ultra rapid	DT3100POP4{dRhod}v2.mob
	80: long read	
	36: rapid read	DT3100POP6{dRhod}v2.mob[a]
	50: std run	
ABI PRISM BigDye v3.0 Terminator	36: ultra rapid	DT3100POP4{BDv3}v1.mob
	80: long read	
	36: rapid read	DT3100POP6{BDv3}v1.mob
	50: std run	

a. If Sequencing Analysis software is on the computer, then two versions of the DT3100POP6{dRhod} mobility file exist. Use the newest version, DT3100POP6{dRhod}v2.mob, instead of DT3100POP6{dRhod}v1.mob.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>
Enter a Project Name.

Note A project name is required for every sample, even if a Sequence Collector database is not used.

a. Click in the Project Name cell for Well A1.
b. Select a project name in the drop-down list.

Project Name
<no selection>
<no selection>
3100-Avant_Project1

Note You must select a project name.

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

Note Press Ctrl+D to fill down whenever a field is the same for all samples in the plate record.
</td></tr>
<tr><td>5</td><td>
For each sample, select the appropriate Run Module from the drop-down list.

Run Module 1
<no selection>
<no selection>
RapidSeq36_POP6DefaultModule
UltraSeq36_POP4DefaultModule
StdSeq50_POP6DefaultModule
LongSeq80_POP4DefaultModule

The following table shows the run module to select based on your run type.

<table>
<tr><th>DNA Sequencing Run Type</th><th>Capillary Array Length (cm)</th><th>Run Module</th></tr>
<tr><td>Ultra rapid</td><td>36</td><td>UltraSeq36_POP4DefaultModule</td></tr>
<tr><td>Rapid</td><td>36</td><td>RapidSeq36_POP6DefaultModule</td></tr>
<tr><td>Standard</td><td>50</td><td>StdSeq50_POP6DefaultModule</td></tr>
<tr><td>Long read</td><td>80</td><td>LongSeq80_POP4DefaultModule</td></tr>
</table>
Note If you select different modules for different samples within the same plate record, the samples will be automatically grouped so that all samples with the same run module are run at the same time. Runs are scheduled alphanumerically by run module name, not by the order indicated in the plate record, nor by sample name. To see the scheduled order of the runs, select the Run View tab.
</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 Enable AutoAnalysis preference must be selected if analysis is to take place automatically after the run (see page 2-12).

Analysis Module 1
<no selection>
<no selection>
BC-3100APOP4UR_SeqOffFtOff.saz
BC-3100APOP4_80cm_SeqOffFtOff.saz
BC-3100APOP6RR_SeqOffFtOff.saz
BC-3100APOP6SR_SeqOffFtOff.saz

The following table shows the analysis module to select based on your run type:

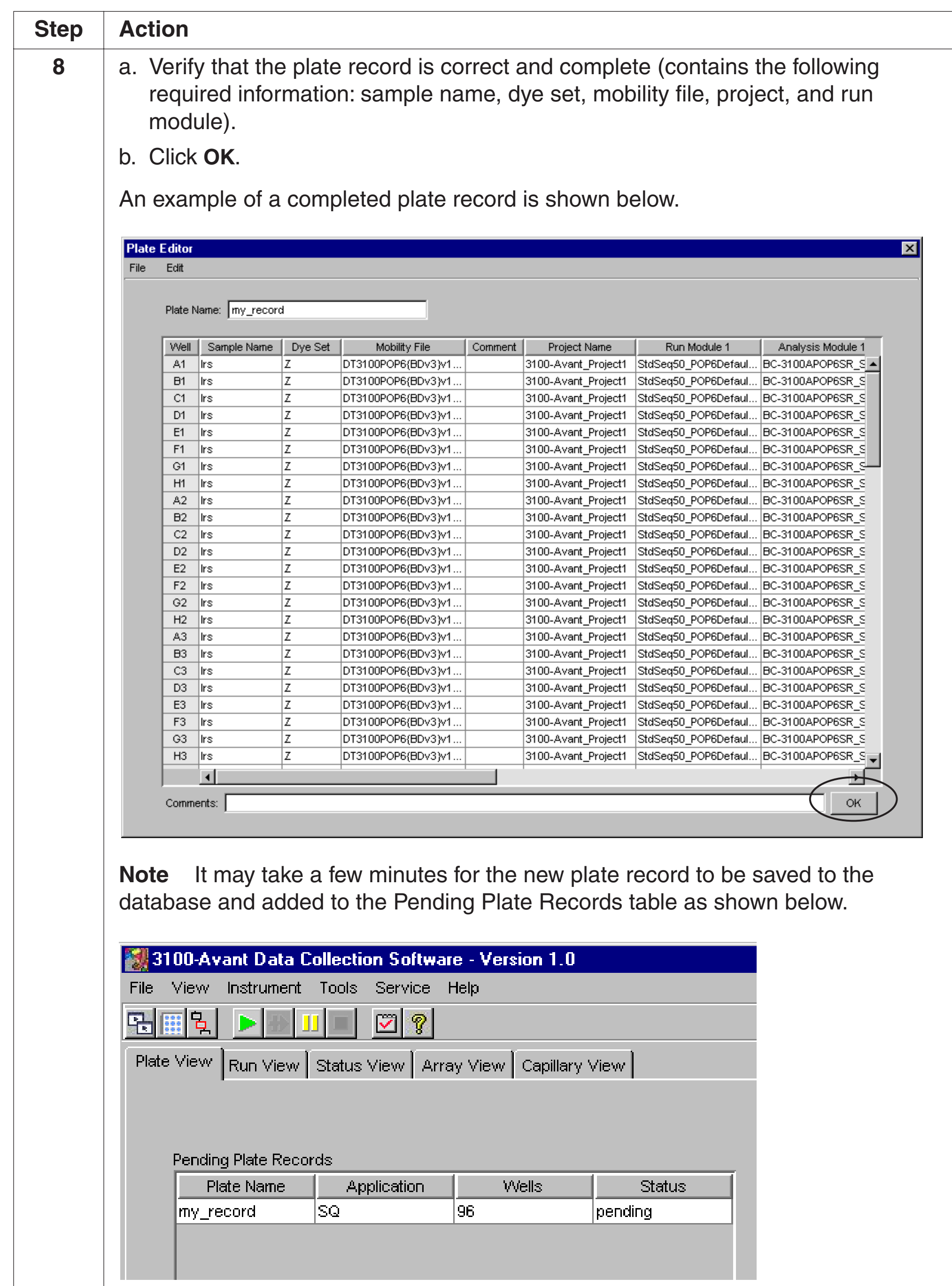
<table>
<tr><th>DNA Sequencing Run Type</th><th>Analysis Module</th></tr>
<tr><td>Ultra rapid</td><td>BC-3100APOP4UR_SeqOffFtOff.saz</td></tr>
<tr><td>Rapid DNA</td><td>BC-3100APOP6RR_SeqOffFtOff.saz</td></tr>
<tr><td>Standard</td><td>BC-3100APOP6SR_SeqOffFtOff.saz</td></tr>
<tr><td>Long read</td><td>BC-3100APOP4_80cm_SeqOffFtOff.saz</td></tr>
</table>
Note You can examine the settings for each of these files using DNA Sequencing Analysis software. The meanings of the settings are described in the ABI PRISM® DNA Sequencing Analysis Software v. 3.7 NT User’s Manual.
</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.

Run Module 2 | Analysis Module 2

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	a. Verify that the plate record is correct and complete (contains the following required information: sample name, dye set, mobility file, project, and run module). b. Click **OK**. An example of a completed plate record is shown below. **Note** It may take a few minutes for the new plate record to be saved to the database and added to the Pending Plate Records table as shown below.

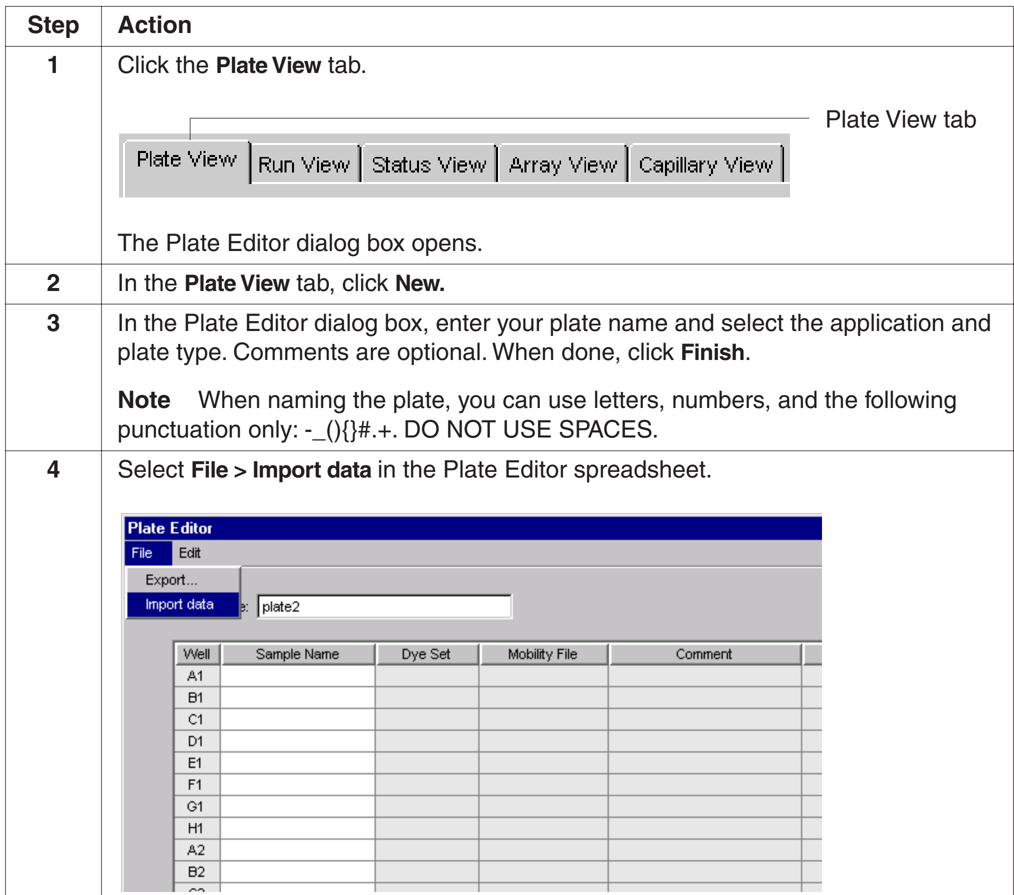


Using a Previous Plate Record to Complete a New Plate Record Automatically

Reusing Plate Records

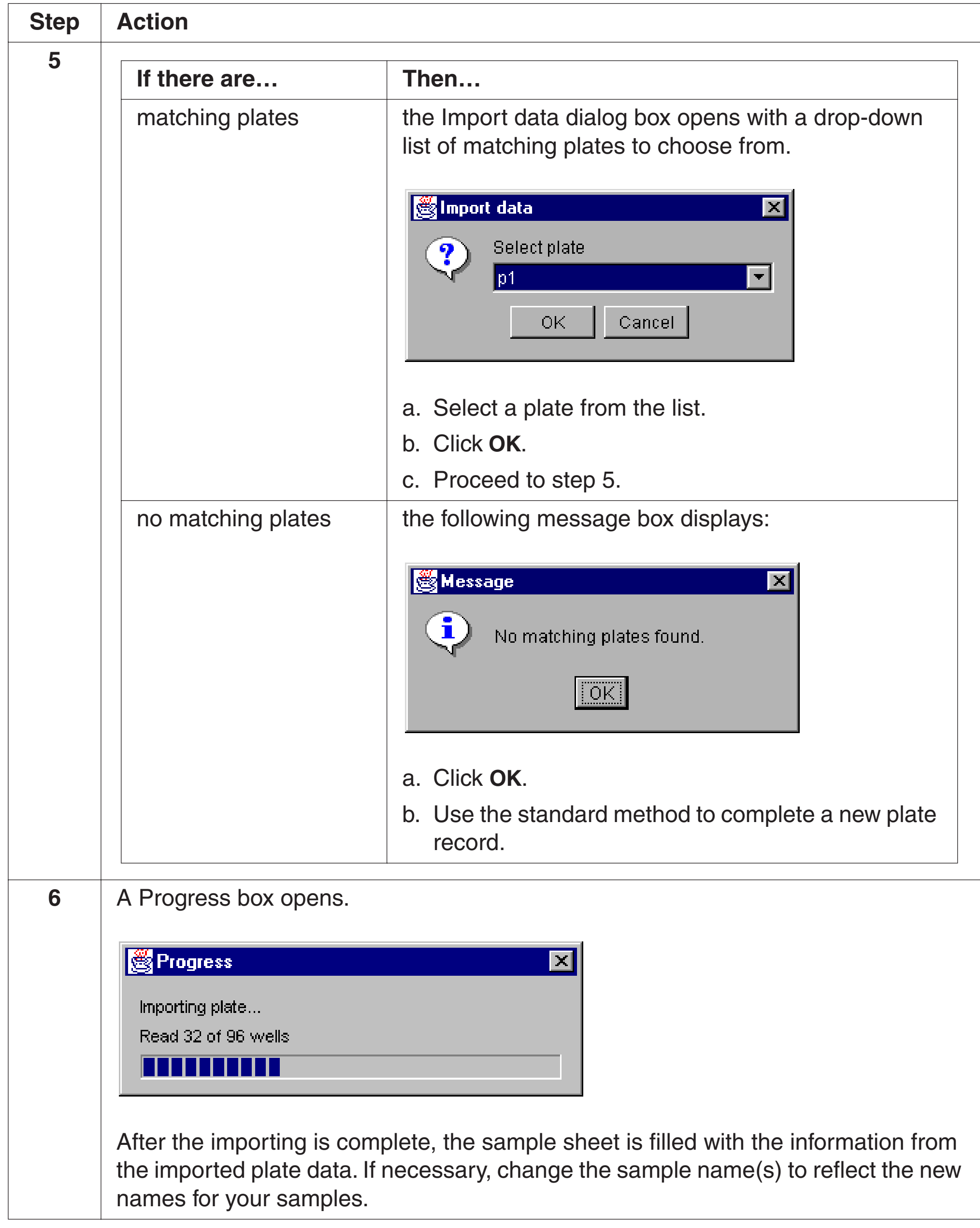
You can reuse plate records by importing data from an existing plate into the current plate. The imported plate must match the configuration (application type and number of wells) of the current plate.

To import plate data:



Step	Action
1	Click the **Plate View** tab. The Plate Editor dialog box opens.
2	In the **Plate View** tab, click **New.**
3	In the Plate Editor dialog box, enter your plate name and select the application and plate type. Comments are optional. When done, click **Finish**. **Note** When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. DO NOT USE SPACES.
4	Select **File > Import data** in the Plate Editor spreadsheet.

To import plate data: *(continued)*



Step	Action
5	<table><tr><th>If there are…</th><th>Then…</th></tr><tr><td>matching plates</td><td>the Import data dialog box opens with a drop-down list of matching plates to choose from. a. Select a plate from the list. b. Click **OK**. c. Proceed to step 5.</td></tr><tr><td>no matching plates</td><td>the following message box displays: a. Click **OK**. b. Use the standard method to complete a new plate record.</td></tr></table>
6	A Progress box opens. After the importing is complete, the sample sheet is filled with the information from the imported plate data. If necessary, change the sample name(s) to reflect the new names for your samples.

Section: Working with Plates and Run Scheduling

In This Section

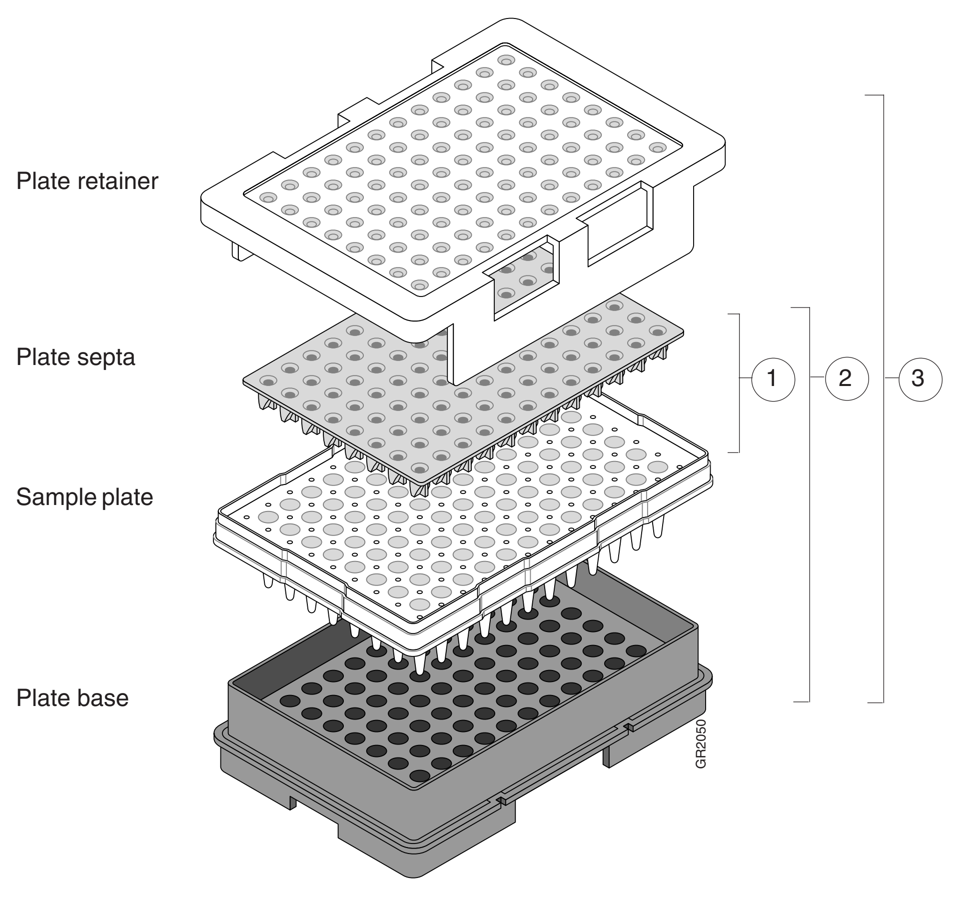
The following topics are covered in this section:

Topic	See Page
Working with Plate Assemblies	2-44
Placing the Plate onto the Autosampler	2-46
Linking and Unlinking a Plate	2-47
Run Scheduling	2-49

Working with Plate Assemblies

Plate Assembly Components

The plate assembly components are assembled as follows:



Preparing a Plate Assembly

To prepare a plate assembly:

Step	Action
1	Secure a clean and dry plate septa on the sample plate. **IMPORTANT** Never use warped plates. **IMPORTANT** Ensure that the plate septa 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.
4	Ensure that 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 plate septa. GR1853 b

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 in position B. **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. Verify that 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.

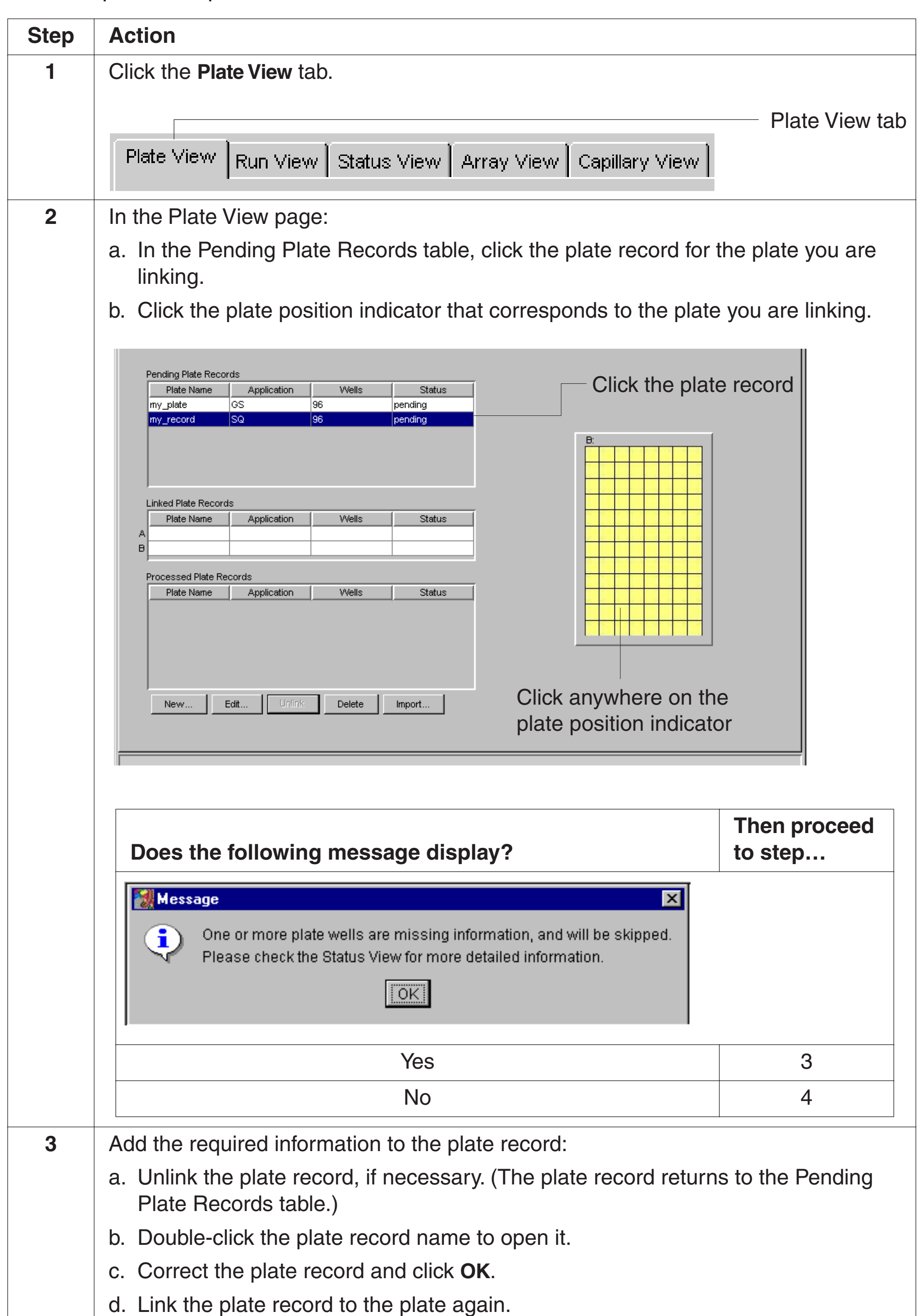
Linking and Unlinking a Plate

Overview

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

Linking a Plate to a Plate Record

To link a plate to a plate record:

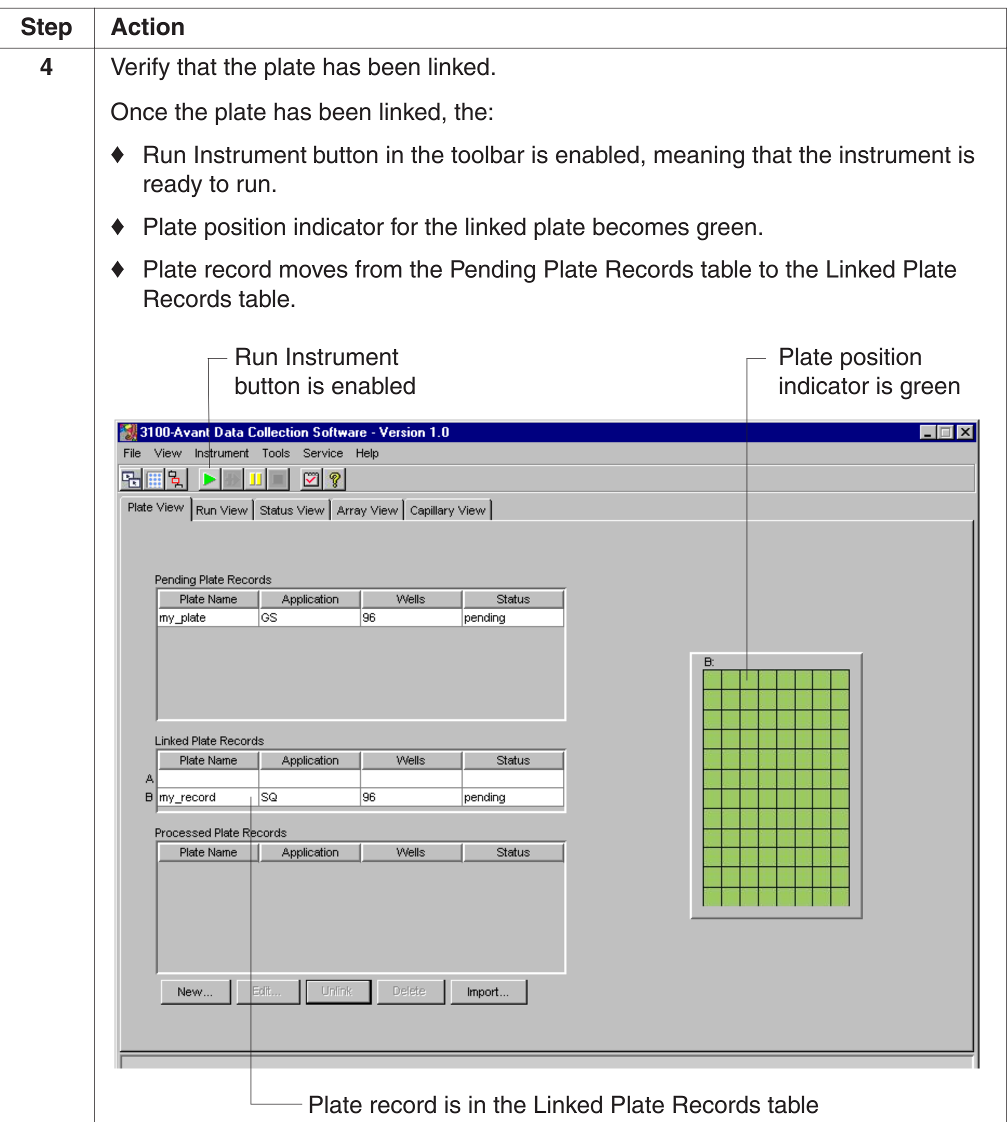


Step	Action
1	Click the **Plate View** tab.
2	In the Plate View page: a. In the Pending Plate Records table, click the plate record for the plate you are linking. b. Click the plate position indicator that corresponds to the plate you are linking.
3	Add the required information to the plate record: a. Unlink the plate record, if necessary. (The plate record returns to the Pending Plate Records table.) b. Double-click the plate record name to open it. c. Correct the plate record and click **OK**. d. Link the plate record to the plate again.

Does the following message display?	Then proceed to step…
Yes	3
No	4

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

Step	Action
4	Verify that the plate has been linked. Once the plate has been linked, the: ♦ Run Instrument button in 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.



Unlinking a Plate Record

To unlink a plate record:

Step	Action
1	In the Linked Plate Records table in 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 table and the plate position indicator will return to yellow.
not completed	return to the Pending Plate Record table, and the plate position indicator will return to yellow.

Run Scheduling

Sample Run Order

The 3100-Avant Run Scheduler follows an alphanumeric system that schedules runs based on the run module name with numerics scheduled first (0...9) followed by upper case then lower case letters.

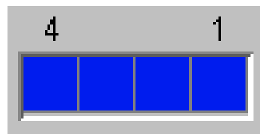
Note The analysis module of a sample plays no part in the order in which it will be run.

If all run module names are identical, runs are scheduled as outlined below.

Capillary Array Map

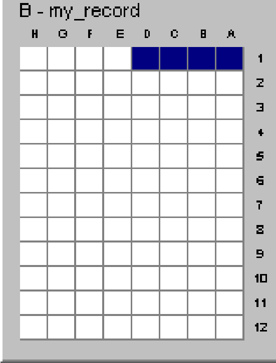
Below is the layout of the capillary numbers in the capillary array. The capillary array layout is the same for both 96- and 384-well plates.

Capillary numbers:

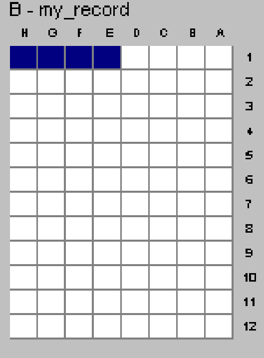


96-Well Plate Mapping

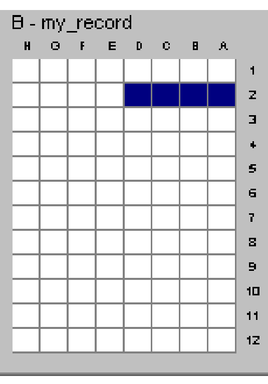
For a 96-well plate, injections are made from four consecutive wells. A full 96-well plate requires 24 runs to inject all samples once.



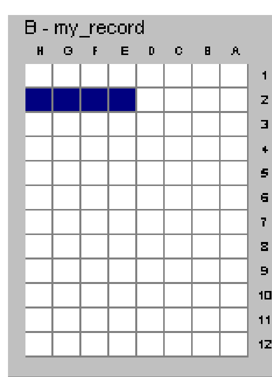
Run 2



Run 3



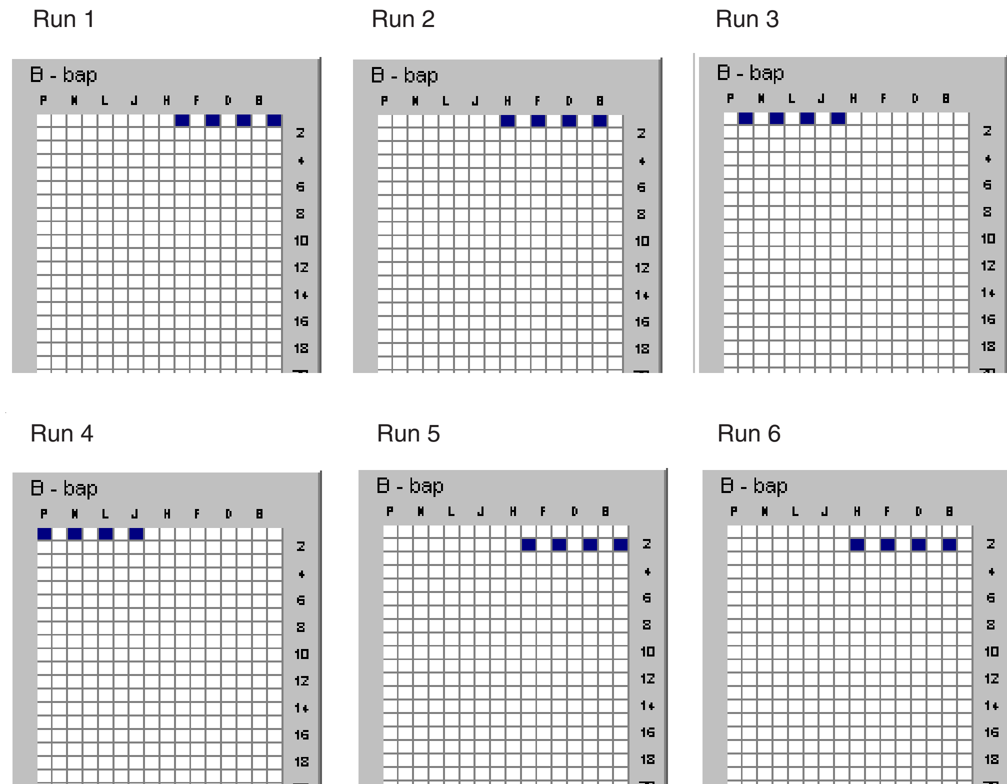
Run 4



Note Multiple injections (Run Module 2 to 5) from the same well are run before moving to the next set of wells.

384-Well Plate Mapping

For a 384-well plate, injections are made from every other well. A full 384-well plate requires 96 runs to inject all the samples once.



Note Multiple injections (Run Module 2 to 5) from the same well are run before moving to the next set of wells.

Viewing the Run Schedule

After a plate is linked, use the Run View page to verify that runs are scheduled correctly.

To view the scheduled runs:

Step	Action
1	Click the **Run View** tab. Plate View tab Plate View | Run View | Status View | Array View | Capillary View
2	Select a row for any run. The corresponding wells to be injected for that run becomes highlighted in the plate diagram on the left. For more information about the Run View page, see page 2-62.

Note Although you can delete individual runs, you cannot alter the order in which the runs are scheduled.

Continuous Operation

If you wish to continuously run the instrument using different run module names, you must adhere to a strict run module naming convention that ensures a correct alphanumeric order for the duration of the continuous run period.

Deleted Runs Rescheduled

The run scheduler re-initializes every time you use the plate editor to link or unlink a plate, create a new plate record, or edit an existing plate record.

If you delete runs associated with a plate and then use the plate editor, the deleted runs will be rescheduled. Remove any unwanted runs in the Run View window.

Section: Running the Instrument

In This Section

The following topics are covered in this section:

Topic	See Page
Launching the Run	2-54
Automatic Checking of Available Space on Drives D and E	2-55
Run Times	2-56
Controlling the Run	2-57
Continuous Runs	2-58
Using the Same Array for Sequencing and Fragment Analysis	2-59

Launching the Run

Starting the Run

To start a run:

Step	Action
1	If you want to review the run schedule before beginning the run, click the **Run View** tab.
2	Click the green **Run Instrument** button in the toolbar. File View Instrument Tools Service Help
3	The software will automatically check if the database and/or drive D are full.

If the database or drive D are…	Then…
full	a. proceed to "Automatic Checking of Available Space on Drives D and E" on page 2-55. b. Make more space. c. Click the green **Run Instrument** button.
not full	the run will start.

Startup Time

When the run starts, the following module steps are performed.

Module Steps	Approximate Time
Turn Oven On	N/A
Wait for oven to equilibrate Initialize autosampler Fill syringes	1 min 40 sec
Fill Array	2-3 min
PreRun	3 min
Inject samples	30 sec
Start separation Ramp voltage	10 min
Collect Data	Variable
Run ends: Leave oven on Laser to idle	Until next run starts
Total time prior to separation: ♦ Cold start: ~16.5 min ♦ 2nd run: ~6.5 min	

Note A PostBatch Utility, which runs automatically, turns off the oven and the laser at end of a batch of runs.

Automatic Checking of Available Space on Drives D and E

Overview

Before a run or batch of runs, the data collection software automatically checks the available space to ensure sufficient space to store the database and sample file data that will be created.

3100-Avant Files Drive D

The data collection software sends the following warning message to remove data when drive D is full.

Runs cannot be started until the data is removed from the drive. For information on archiving data, refer to “Creating a Data CD” on page 4-37.

Database Drive E

The data collection software sends the following warning message when the database is getting full (~75% of 9 GB).

Runs cannot be started until the database is cleaned up. For information on using the Cleanup DB utility, refer to “Deleting Processed Frame Data” on page 4-39.

Run Times

DNA Sequencing Run Times

The following table lists the approximate run times of common DNA sequencing analysis runs:

Type of Run	Run Module	Run Time
Ultra rapid DNA sequencing	UltraSeq36_POP4DefaultModule	40 min
Rapid DNA sequencing	RapidSeq36_POP4DefaultModule	1 h
Standard DNA sequencing	StdSeq50_POP6DefaultModule	2 h 30 min
Long read DNA sequencing	LongSeq80_POP4DefaultModule	3 h 45 min

Fragment Analysis Run Times

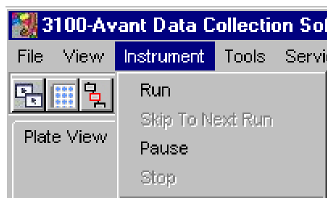
The following table lists the approximate run times of common fragment analysis runs:

Type of Analysis	Run Module	Run Time
Fragment analysis	GeneScan22_POP4DefaultModule	20 min
Fragment analysis	GeneScan36_POP4DefaultModule	45 min
Fragment analysis	GeneScan36vb_POP4DefaultModule	45 min
SNP analysis	SNP22_POP4DefaultModule	15 min
SNP analysis	SNP36_POP4DefaultModule	30 min

Controlling the Run

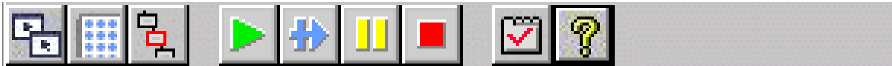
Controlling the Run Using the Instrument Menu

You can use the Instrument menu to start, skip, pause, or stop a run.



Controlling the Run Using the Toolbar

You can also use the toolbar at the top of the data collection software window to control the run.



To...	Click...	Comment
Start the run	**Run Instrument**	♦ This begins all scheduled runs. ♦ The run starts only when set temperature is reached.
Pause the run	**Pause**	Pausing the instrument for too long, especially after sample injection, will affect data quality. The best time to pause is before sample injection.
♦ Complete the current run, and ♦ Stop the other scheduled runs	a. **Stop** b. **After run** in the **Question** dialog box Question Stop now or after current run? Now After run Cancel	
♦ Stop the current run, and ♦ Stop the other scheduled runs	a. **Stop** b. **Now** in the **Question** dialog box Question Stop now or after current run? Now After run Cancel	When you click **Now**, the run files extract automatically. The files will be automatically analyzed if the AutoAnalysis preference is enabled. To recover data from a stopped run, see “Recovering Data If Autoextraction Fails” on page 2-70.
♦ Stop the current run, and ♦ Continue the other scheduled runs	**Skip to Next Run**	To recover data from a stopped run, see “Recovering Data If Autoextraction Fails” on page 2-70.

Continuous Runs

Running Continuously

The continuous run feature allows you to create, import, and link a plate during a run. This feature gives you the capability of running one plate, removing the plate once samples have run, and then linking and running additional plates.

- ♦ Plates can only be mounted or unmounted when the instrument is paused.
- ♦ Plate records can be created before or after a pause.
- ♦ New plates are linked after a run has resumed.

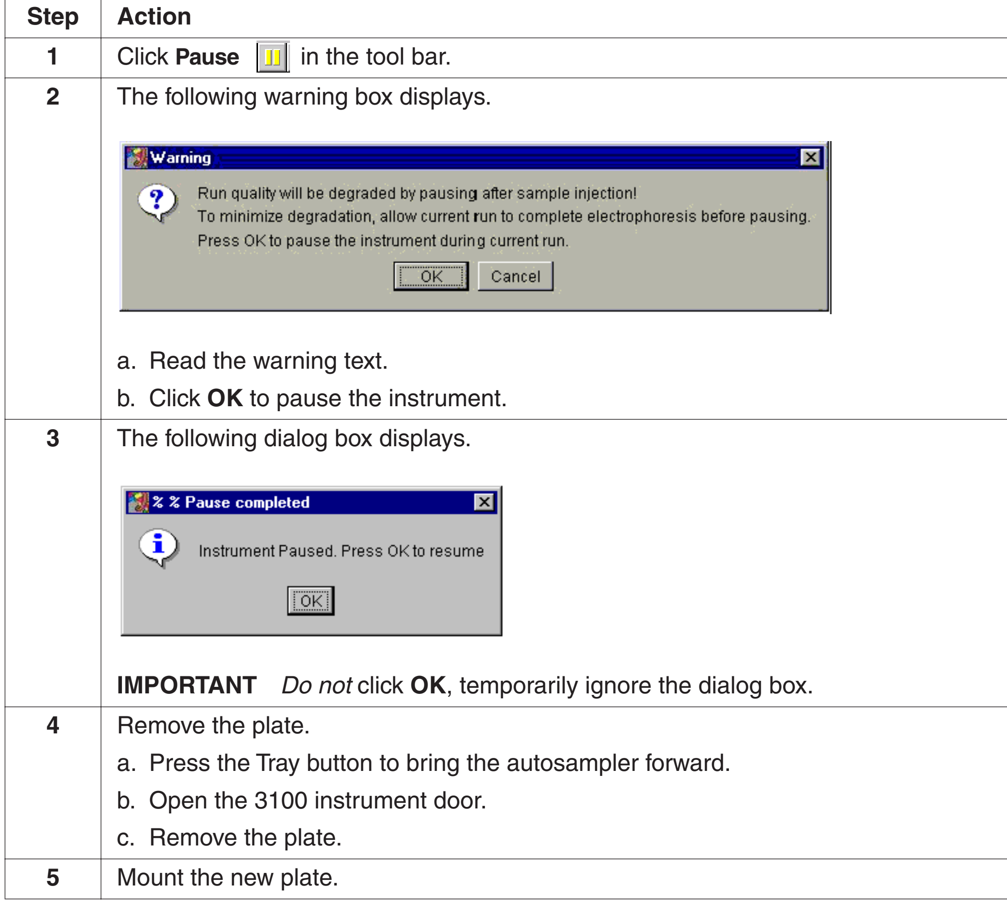
Note The run scheduler re-initializes every time you use the plate editor to link or unlink a plate, create a new plate record, or edit an existing plate record. If you delete runs associated with a plate and then use the plate editor, the deleted runs will be rescheduled. Remove any unwanted runs in the Run View window.

Replacing a Plate in Use

During a run, you can mount and unmount plates while the instrument is paused. The plate record can be created and then linked after the run has resumed.

IMPORTANT Pausing the instrument for too long, especially after sample injection, will affect data quality. The best time to pause is before sample injection

To replace a plate that is currently in use:



Step	Action
1	Click **Pause** in the tool bar.
2	The following warning box displays. a. Read the warning text. b. Click **OK** to pause the instrument.
3	The following dialog box displays. **IMPORTANT** *Do not* click **OK**, temporarily ignore the dialog box.
4	Remove the plate. a. Press the Tray button to bring the autosampler forward. b. Open the 3100 instrument door. c. Remove the plate.
5	Mount the new plate.

To replace a plate that is currently in use: *(continued)*

Step	Action
6	Close the door. The instrument resumes when the autosampler completes the initialization and returns to the home position.
7	If the 3100 instrument does not resume automatically, open and close the door again.
8	a. Click **OK** in the Paused completed dialog box. The following message appears: “Unlinking will cause current batch of runs to end after the current run finishes. Continue unlinking?” b. Click **OK** to continue. The following message appears: “Current batch of runs will end after current run finishes.” d. Click **OK**.
9	Create a plate record and link the new plate.
10	Click **Skip** on the toolbar. This stops the run from the plate that was removed but continues the remaining scheduled runs. **Note** Any time you link or unlink a plate, the run scheduler re-initializes. If you delete run(s) associated with a plate and then link or unlink a plate, the deleted runs will be rescheduled. Remove any unwanted runs in the Run View window.

Using the Same Array for Sequencing and Fragment Analysis

Running Sequencing and Fragment Analysis Samples

The new ultra rapid sequencing module provides the opportunity to run sequencing and fragment analysis samples using the same 36-cm capillary array and POP-4 polymer. Alternating runs of sequencing and fragment analysis samples does not affect the data quality.

Section: Monitoring a Run

In This Section

The following topics are covered in this section:

Topic	See Page
Run View Page	2-62
Status View Page	2-63
Array View Page	2-65
Capillary View Page	2-67
Instrument Status Monitor	2-68

Introduction

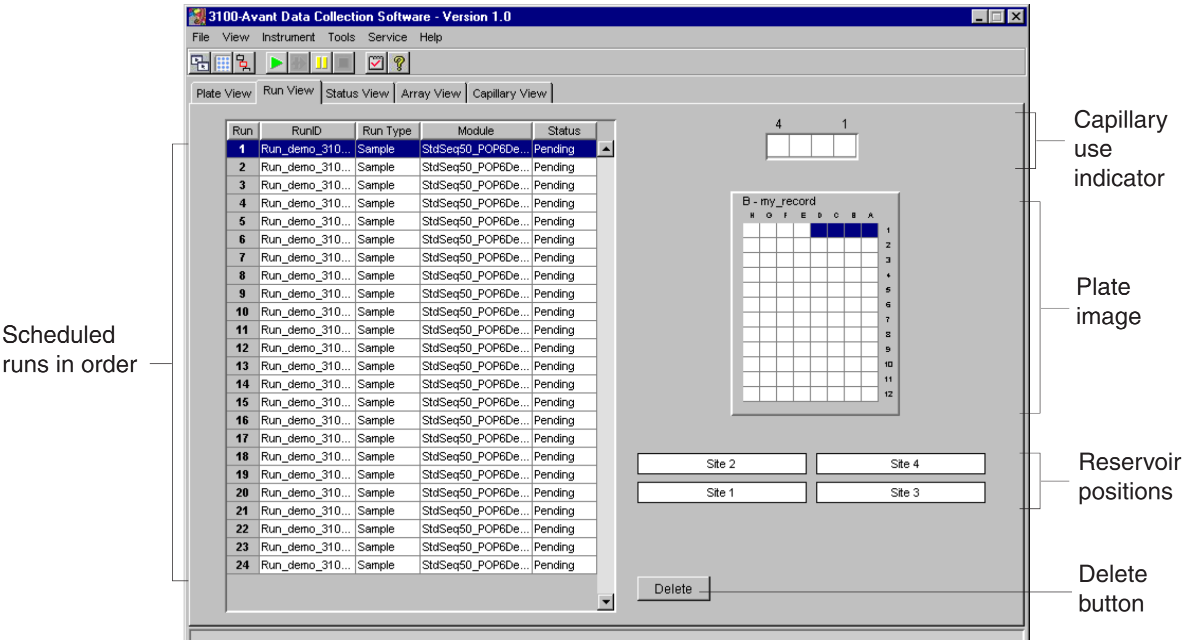
This section describes the functions and features of the:

- Data collection software views that are used to monitor a run
- Instrument Status Monitor, which provides a summary of the current run conditions

Run View Page

Function Click the Run View tab to monitor the status of the scheduled runs.

Features This is an example of the Run View page.



Run Schedule Each row in the table provides information about a scheduled run. A run can be selected by single-clicking on a row.

Note Although you can delete individual runs, you cannot alter the order in which the runs are scheduled. For more information on run scheduling, see page 2-49.

Capillary Use Indicator This grid displays the capillaries in use during a run and the name of the sample that will be injected into a specific capillary.

Each cell in the grid represents a specific capillary. Once a run has started, the cells representing capillaries in use will turn blue. Position the pointer over an individual cell to display the name of the sample to be injected in that capillary.

Plate Image Indicators The plate images provide a visual representation of the physical sample layout for a selected run.

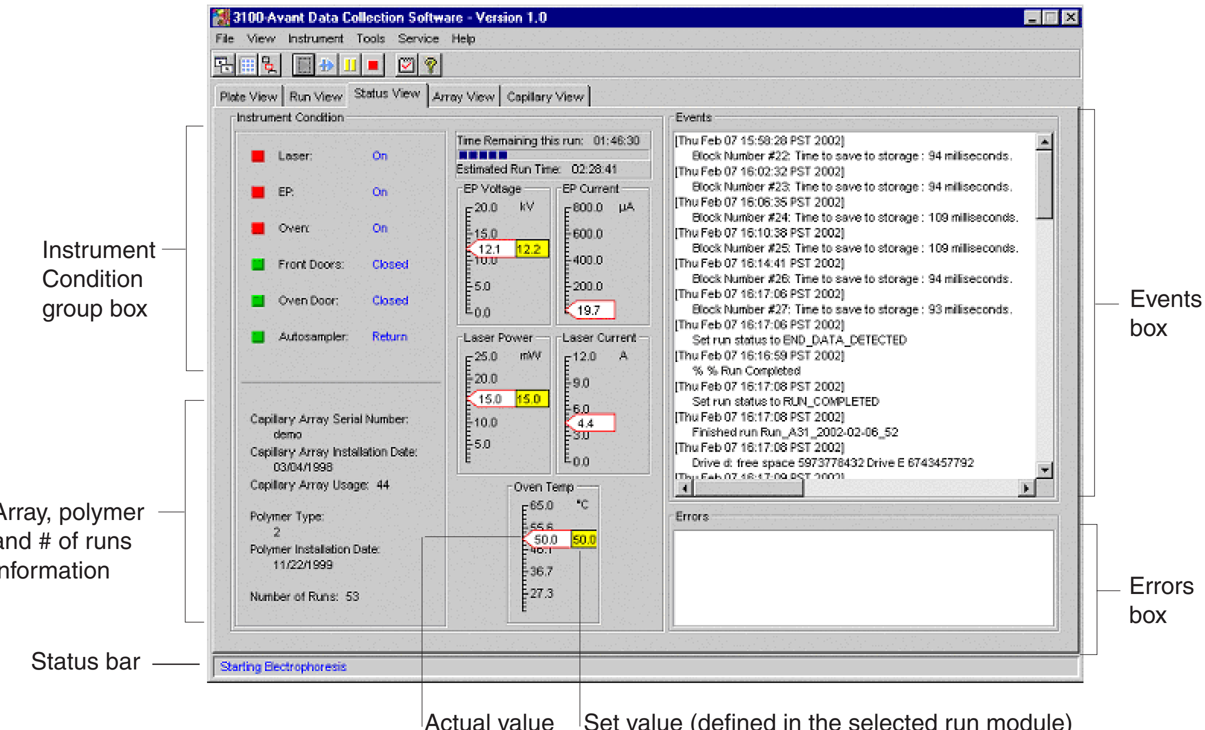
Delete Button The Delete button removes a run from the list of scheduled runs. First select the run in the Run Schedule window on the left, and then click the Delete button.

Note The Delete button does not delete the samples from the plate record. The samples can be run later, if desired.

Status View Page

Function Click the Status View tab to monitor the status of the instrument during a run.

Features This is an example of the Status View page.



Instrument Condition Group Box The color of the box provides a quick way to check the status of the item to the right. See the table below for a definition of each color.

For...	A green box indicates...	A red box indicates...	A yellow box indicates...
Laser	Laser is off	Laser is on	Laser is idle
EP	Electrophoresis is off	Electrophoresis is on	—
Oven	Oven is off	Oven is on	—
Front Doors	Doors are closed	Doors are open	—
Oven Door	Door is closed	Door is open	—
Autosampler	Autosampler is homed	Autosampler is forward	—

Events Box

The Events box lists the:

- ♦ Instrument's recent actions
- ♦ Status of each capillary as passed or failed at the end of a spectral calibration
- ♦ Calibration data at the end of a spatial calibration

Some of the events listed in the Events box provide information for service engineers.

Errors Box

The Errors box lists errors that have occurred during the current run.

Some of the error messages provide information for service engineers. A "fatal" error usually requires that you restart the data collection software.

Status Bar

The Status bar indicates the instrument's current state or operation.

Array View Page

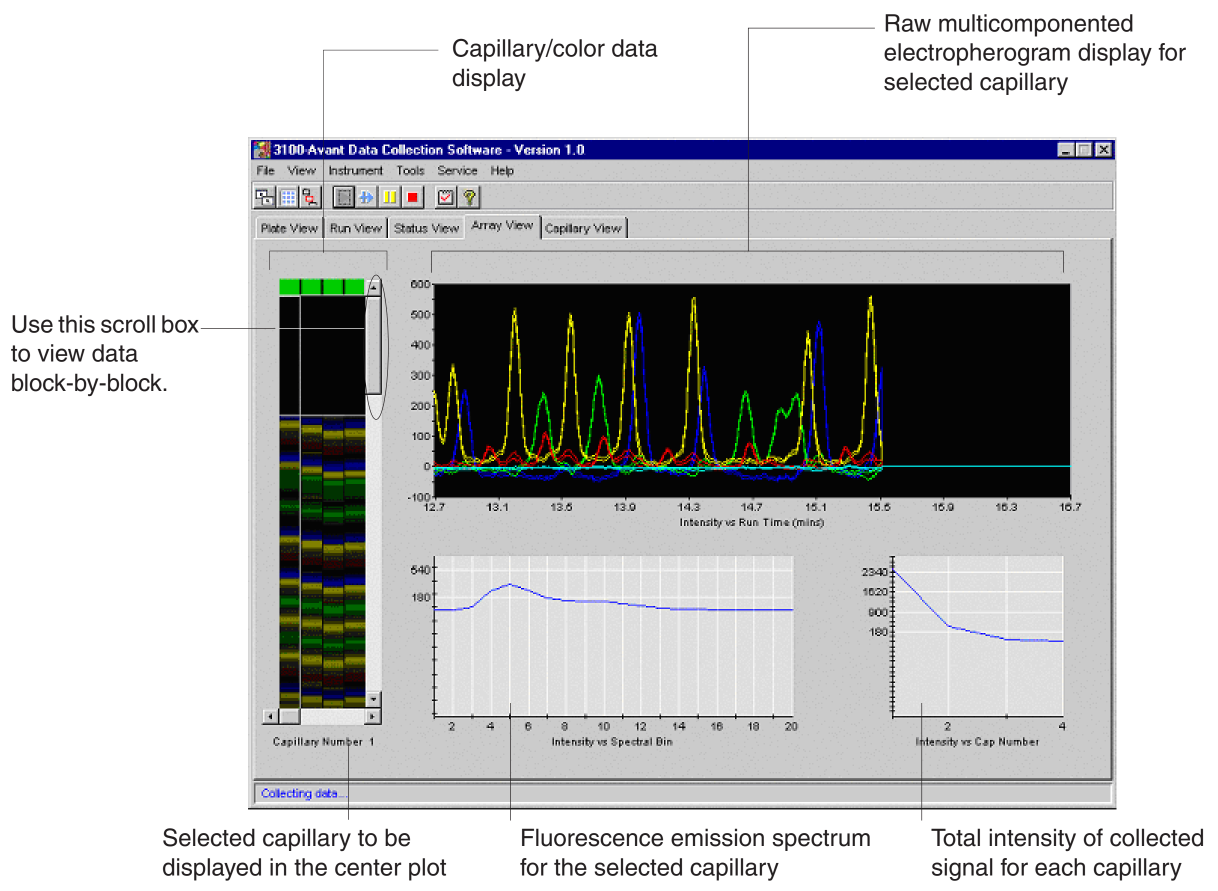
Function

Click the Array View tab during or after a run to examine the quality of your data, which is displayed as individual electropherograms and as color data for the entire capillary array.

IMPORTANT During a run, do not leave this page open for extended periods. This may cause unrecoverable screen update problems,

Features

This is an example of the Array View page.



Capillary/Color Data Display

Each cell in the capillary/color data display represents one capillary. The status of that capillary is indicated by the color of the cell (see below).

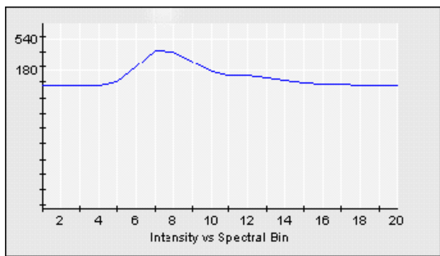
Cell Color	Status of the Capillary	Comment
Green	Operational	Passed spectral calibration.
Yellow	Failed Spectral	This capillary did not pass the spectral calibration and has been assigned the spectral profile of its nearest passing neighbor. There may be a problem with data collected from this capillary.
Red	Nonoperational	All capillaries will have a red cell until a spatial calibration is performed.

Capillary Display

During data collection, the capillary window displays the signal intensity by capillary number.

Fluorescence Emission Spectrum

The fluorescence emission spectrum displays the real-time fluorescence emission spectrum of the dye-labeled fragments from the capillary selected. The spectrum is plotted against the CCD bin number instead of wavelength.

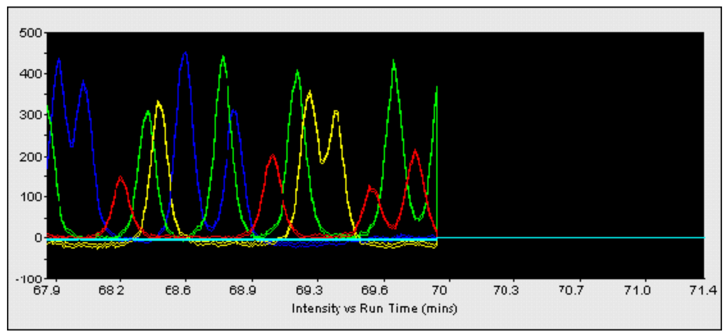


Note This window works only during data collection. This window is updated each time you select a different capillary in the Capillary Display window during data collection.

Electropherogram Display

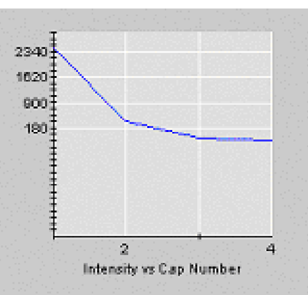
An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The data displayed has been multicomponented. The relative dye concentration is determined by applying chemometric algorithms to the collected fluorescence data.

There are two plots for each dye. The plots represent the upper and lower confidence limits associated with the measured fluorescence intensity.



Total Intensity Graph

The total intensity graph is a graph of the total intensity detected for each capillary.



Note This window works only during data collection. This window is updated each time you select a different capillary in the Capillary Display window during data collection.

Capillary View Page

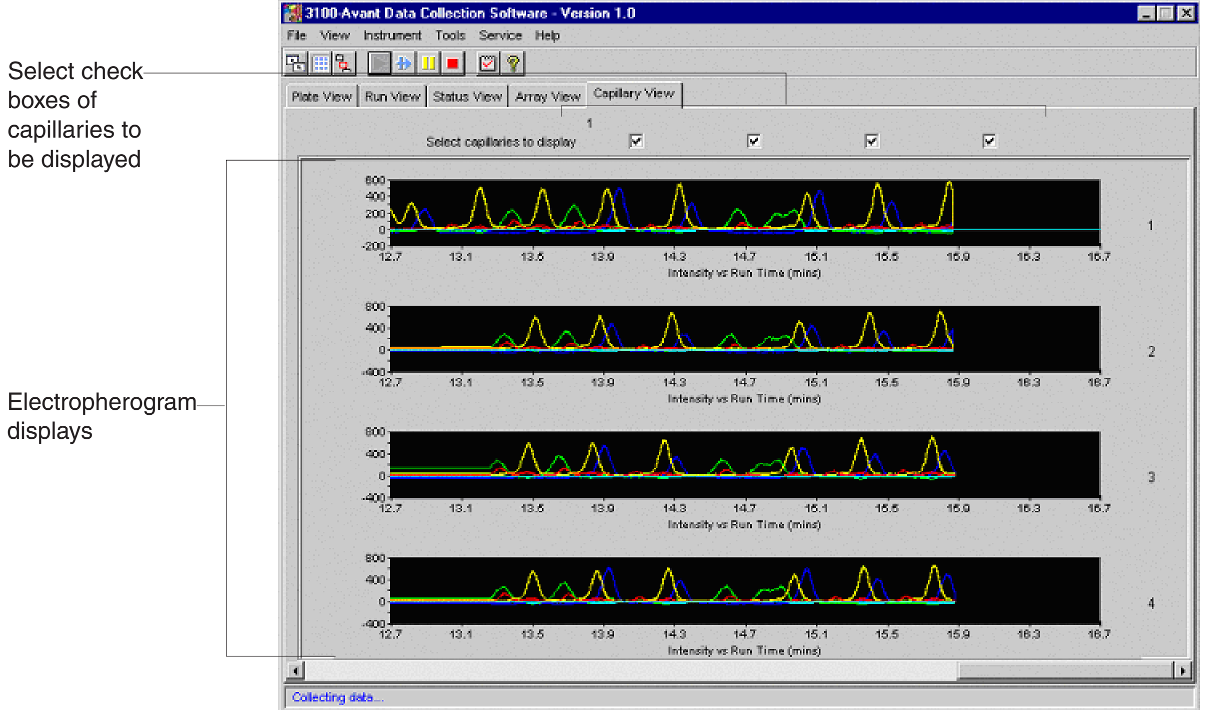
Function

Click the Capillary View tab to examine the quality of electropherogram data for several capillaries at once.

IMPORTANT During a run, do not leave this page open for extended periods. This may cause unrecoverable screen update problems.

Features

This is an example of the Capillary View page for a sequencing run.



Check Boxes

Select the check boxes of the capillaries for which you want electropherograms displayed. The capillaries are displayed in the order in which the boxes are checked.

Electropherogram Displays

An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The data displayed has been corrected for spectral overlap (multicomponented). The relative dye concentration is determined by applying chemometric algorithms to the collected fluorescence data.

There are two plots for each dye. The plots represent the upper and lower confidence limits associated with the measured fluorescence intensity.

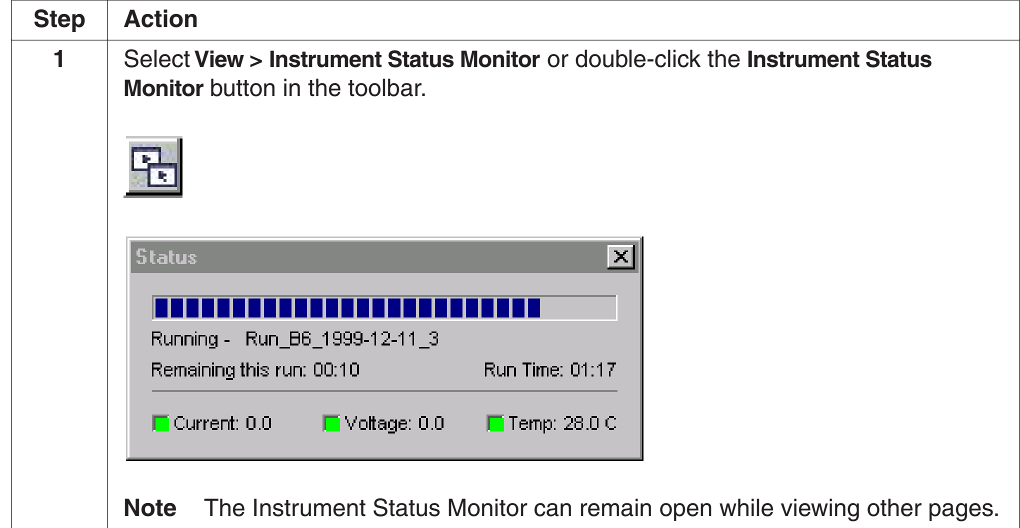
Instrument Status Monitor

Function

The Instrument Status Monitor displays the current run conditions.

Viewing the Instrument Status Monitor

To view the Instrument Status Monitor:

Step	Action
1	Select **View > Instrument Status Monitor** or double-click the **Instrument Status Monitor** button in the toolbar.  **Note** The Instrument Status Monitor can remain open while viewing other pages.

Section: Working with Data

In This Section

The following topics are covered in this section:

Topic	See Page
Recovering Data If Autoextraction Fails	2-70
Viewing Raw Data from a Completed Run in the Data Collection Software	2-71
Viewing Analyzed Data	2-74

Recovering Data If Autoextraction Fails

Overview

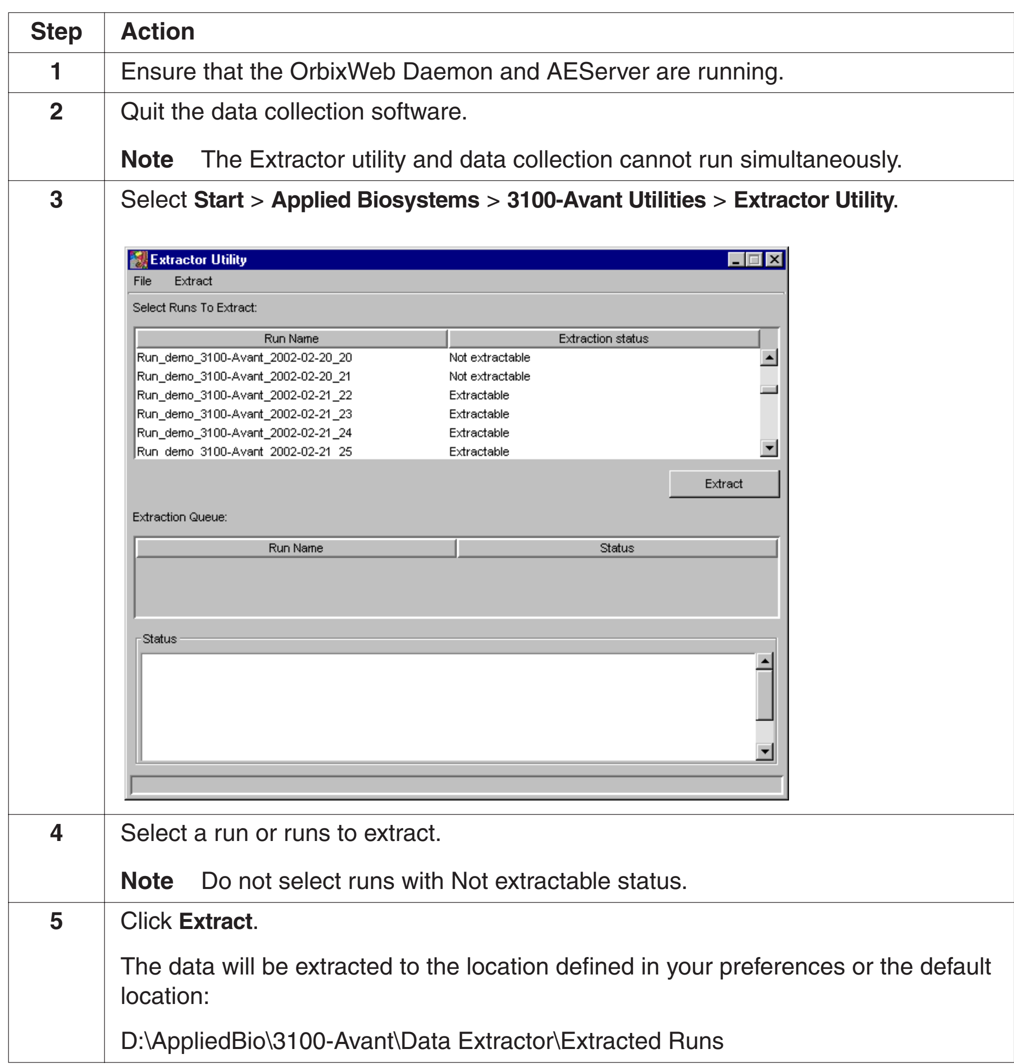
Runs that are stopped before completion display the status Completed in the run table on the Run View page.

The auto extractor software should automatically extract data from stopped runs. However, if autoextraction fails, use the Extractor utility as described below.

Selecting and Queuing Runs for Extraction

You can queue runs for extraction. This is especially useful for extracting failed runs or batches of runs.

To select and queue runs for extraction:

Step	Action
1	Ensure that the OrbixWeb Daemon and AEServer are running.
2	Quit the data collection software. **Note** The Extractor utility and data collection cannot run simultaneously.
3	Select **Start** > **Applied Biosystems** > **3100-Avant Utilities** > **Extractor Utility**. 
4	Select a run or runs to extract. **Note** Do not select runs with Not extractable status.
5	Click **Extract**. The data will be extracted to the location defined in your preferences or the default location: D:\AppliedBio\3100-Avant\Data Extractor\Extracted Runs

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

Overview

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 data collection software:

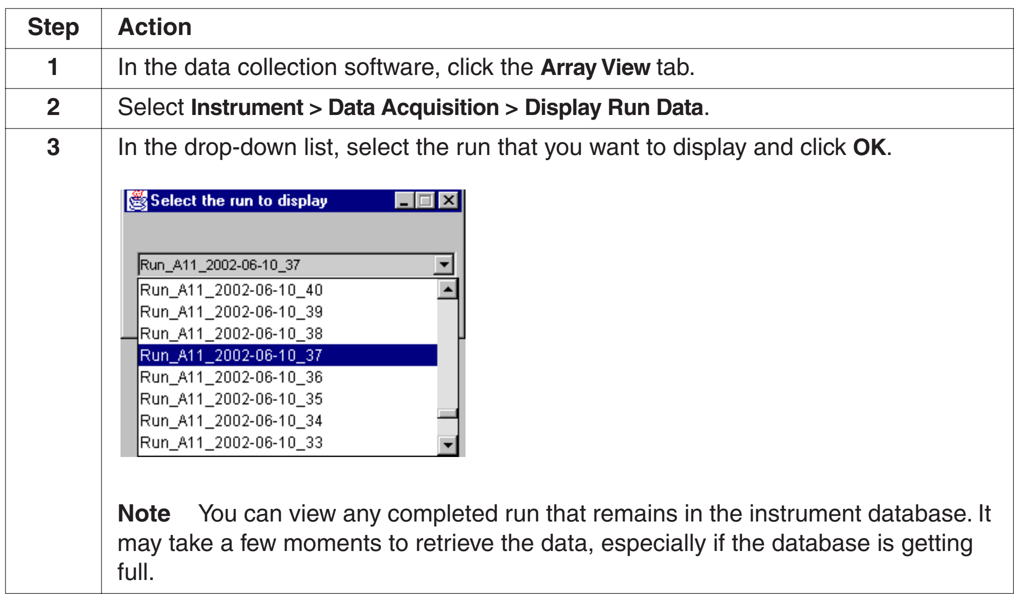
- ♦ In the Array View page (similar to the gel file output from an ABI PRISM slab gel instrument)
- ♦ In the Capillary View page, capillary-by-capillary

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.

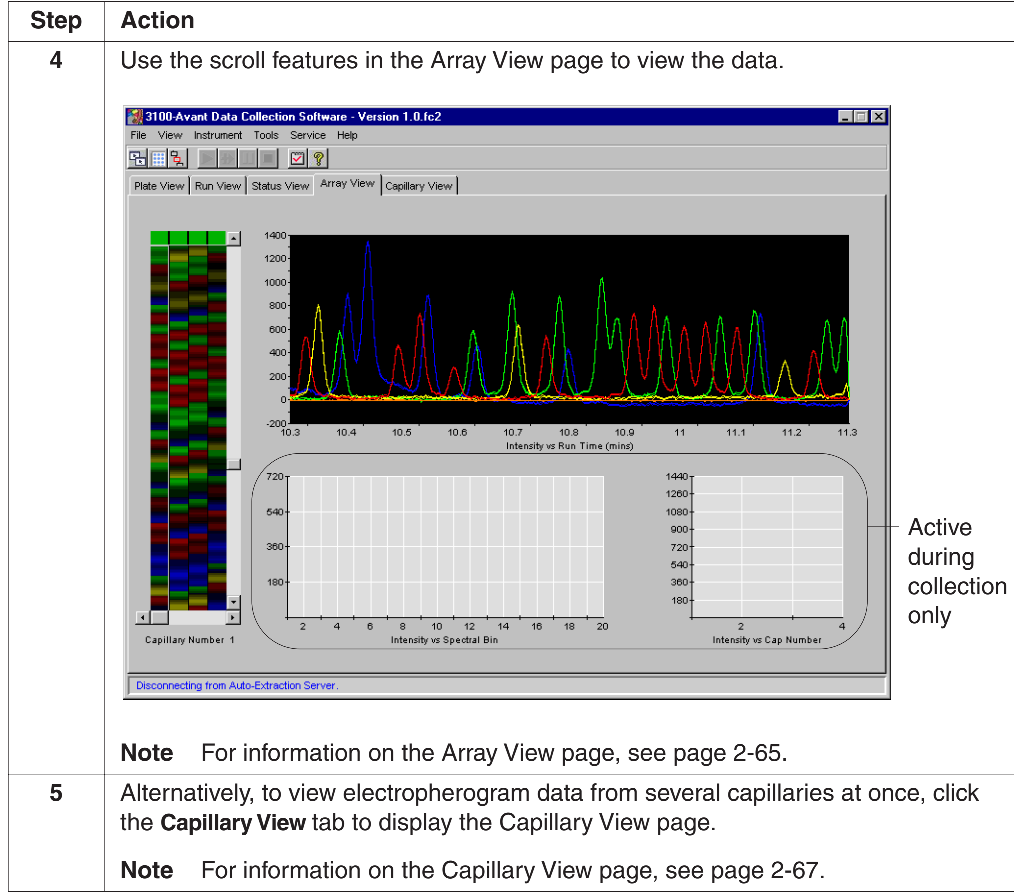
Viewing Raw Data

To view raw data from a completed run:

Step	Action
1	In the data collection software, click the **Array View** tab.
2	Select **Instrument > Data Acquisition > Display Run Data**.
3	In the drop-down list, select the run that you want to display and click **OK**.  **Note** You can view any completed run that remains in the instrument database. It may take a few moments to retrieve the data, especially if the database is getting full.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>Use the scroll features in the Array View page to view the data.

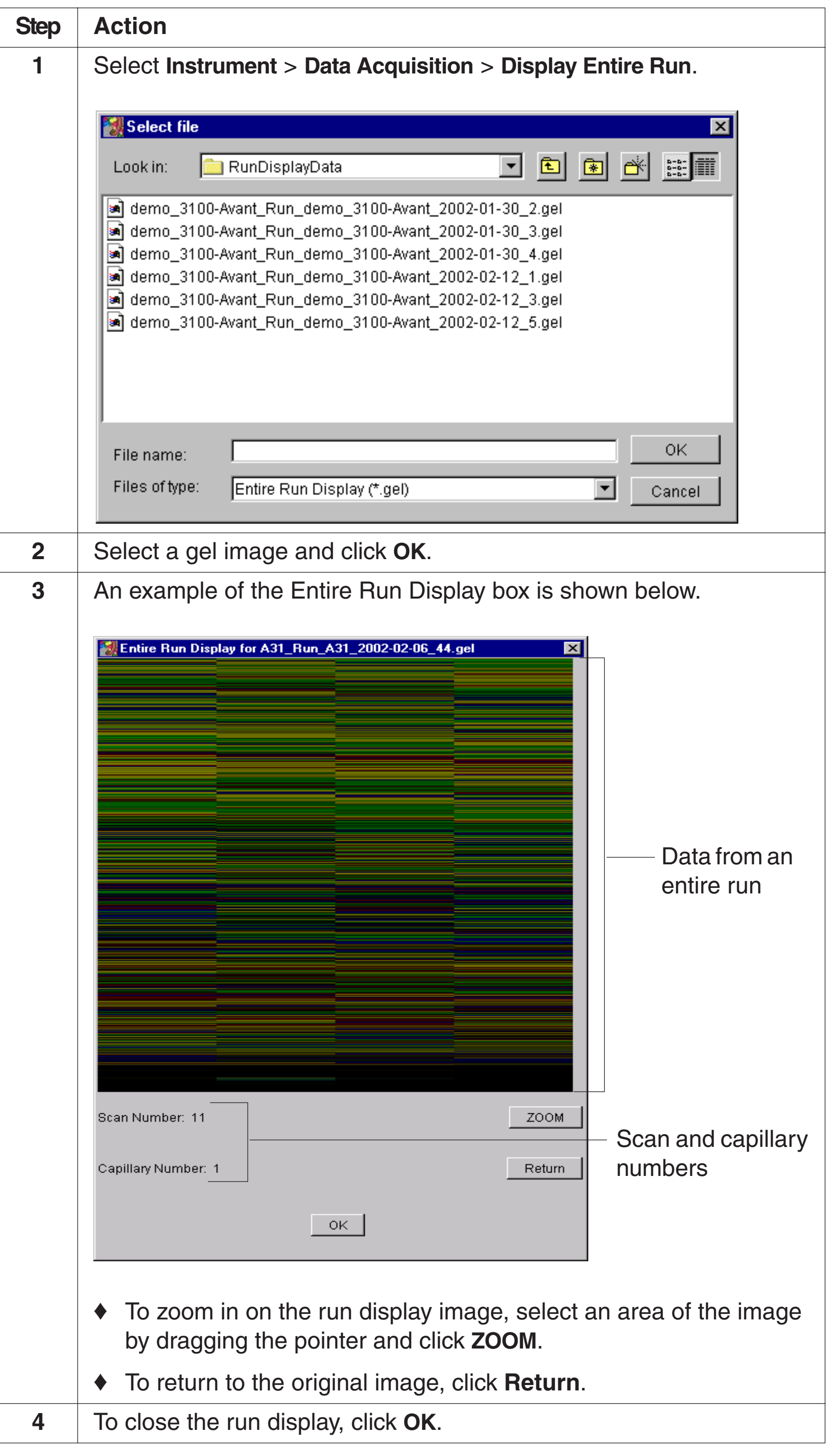


Note For information on the Array View page, see page 2-65.</td></tr>
<tr><td>5</td><td>Alternatively, to view electropherogram data from several capillaries at once, click the Capillary View tab to display the Capillary View page.

Note For information on the Capillary View page, see page 2-67.</td></tr>
</table>

Displaying an Entire Run

To display an entire previous run (gel image):



Step	Action
1	Select **Instrument** > **Data Acquisition** > **Display Entire Run**.
2	Select a gel image and click **OK**.
3	An example of the Entire Run Display box is shown below. ♦ To zoom in on the run display image, select an area of the image by dragging the pointer and click **ZOOM**. ♦ To return to the original image, click **Return**.
4	To close the run display, click **OK**.

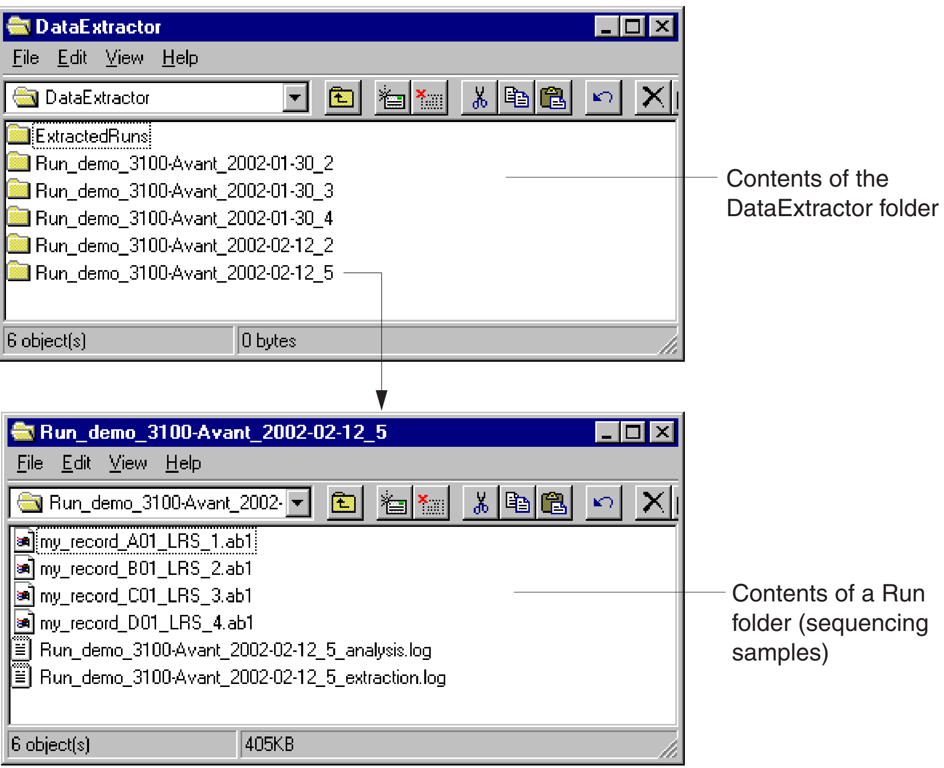
Viewing Analyzed Data

Locating Sample Files

When a run is finished, the analyzed sample files are extracted into a run folder, along with a run log, to a location defined in your preferences or the default location:

D:\AppliedBio\3100-Avant\DataExtractor

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



If the data has been re-extracted, the data is in the location defined in your preferences or the default location:

D:\AppliedBio\3100-Avant\Data Extractor\Extracted Runs

DNA Sequencing Sample Files

After a run has been extracted to sample files, you can use the DNA Sequencing Analysis software to view the electropherogram data, both raw and analyzed. All sequencing sample files contain the .ab1 extension.

Refer to the *ABI PRISM® DNA Sequencing Analysis Software v. 3.7 NT User Guide* (P/N 4308924) for details on viewing and analyzing sequencing data.

Fragment Analysis Sample Files

After a run has been extracted to sample files, you can use the analysis software to view the electropherogram data, both raw and analyzed. All fragment analysis sample files contain the .fsa extension.

Refer to the *ABI PRISM® GeneScan®Analysis Software v. 3.7 NT User Guide* (P/N 4308923) for details on viewing and analyzing GeneScan data.

Spatial and Spectral Calibrations

3

In This Chapter The following topics are covered in this chapter:

Topic	See Page
Calibrating the Instrument	3-2
Section: Spatial Calibration	3-3
About Spatial Calibration	3-4
Performing a Spatial Calibration	3-5
Displaying a Spatial Calibration Profile	3-9
Overriding the Current Spatial Calibration Map	3-10
Section: Spectral Calibration	3-13
About Spectral Calibrations	3-14
Preparing and Loading Spectral Calibration Standards	3-16
Performing a Spectral Calibration Using Default Processing Parameters	3-19
Displaying a Spectral Calibration Profile	3-27
Activating a Spectral Calibration for a Dye Set	3-31
Overriding a Spectral Calibration Profile	3-33

Calibrating the Instrument

Required Calibrations

A charge-coupled device (CCD) camera is used to collect the fluorescent signal as samples electrophorese through the detection cell. Before running samples on the ABI PRISM® 3100 Genetic Analyzer, the CCD must be calibrated in two dimensions:

- Spatial dimension (y-axis), which is used to detect each capillary position
- Spectral dimension (x-axis), which is used to detect the dyes

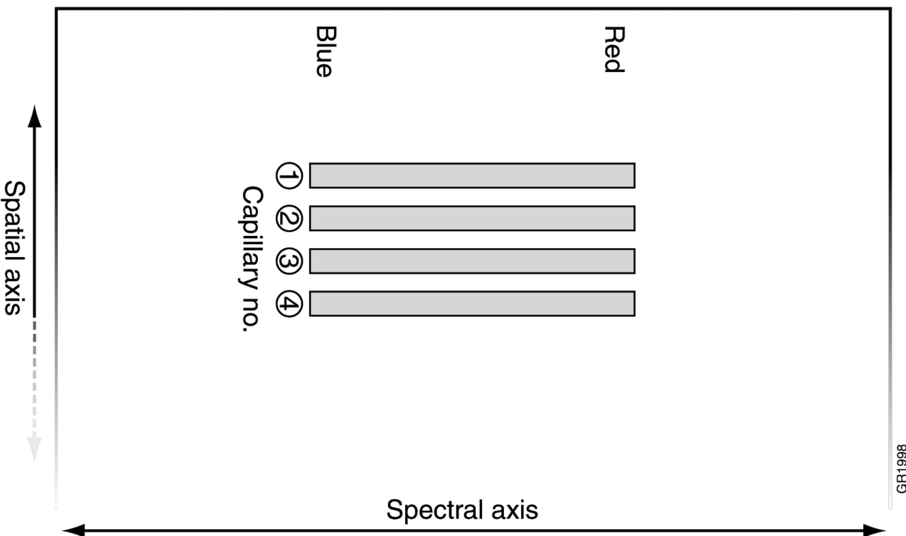


Figure 3-1 CCD camera with axes labeled

Section: Spatial Calibration

In This Section

The following topics describe how to perform a spatial calibration:

Topic	See Page
About Spatial Calibration	3-4
Performing a Spatial Calibration	3-5
Displaying a Spatial Calibration Profile	3-9
Overriding the Current Spatial Calibration Map	3-10
Overriding the Current Spatial Calibration Map	3-10

About Spatial Calibration

What is a Spatial Calibration?

The spatial calibration maps the position of each capillary detected on the CCD.

Spatial calibration runs are 2 min long (6 min for capillary fill and spatial calibration run). During this time, multiple frames of data are collected and summed. The collected data is analyzed and saved as a spatial map.

When to Calibrate

A spatial calibration must be performed whenever you install a new or used array on the instrument, or after you temporarily remove the capillary array from the detection block.

Spatial Maps

Only one spatial map is stored in the instrument database on drive E. The term “current” spatial map refers to that spatial map. You can replace (override) the “current” spatial map with a spatial map stored in a spatial calibration file. For the procedure, see page 3-10.

Spatial calibration maps are stored as text files (.scl extension) in:

- D:\AppliedBio\3100-Avant\DataCollection\SpatialCalLogs
- 3100 Calibration file
- Firmware

Calibration File Contents

Each spatial calibration file contains one spatial map from either the current or a previous calibration.

Calibration File Naming Convention

Spatial calibration files have the following file name format:

SpatialCal-*instrumentname*-Run*date-time*.scl

Spatial Calibration Log Files

A spatial calibration log file is created during a spatial calibration. It contains a summary of the data collected during the spatial calibration run, including the pixel positions assigned to each capillary.

The log file is a text file that can be opened and viewed in the Notepad accessory. It can be useful for troubleshooting spatial calibration problems.

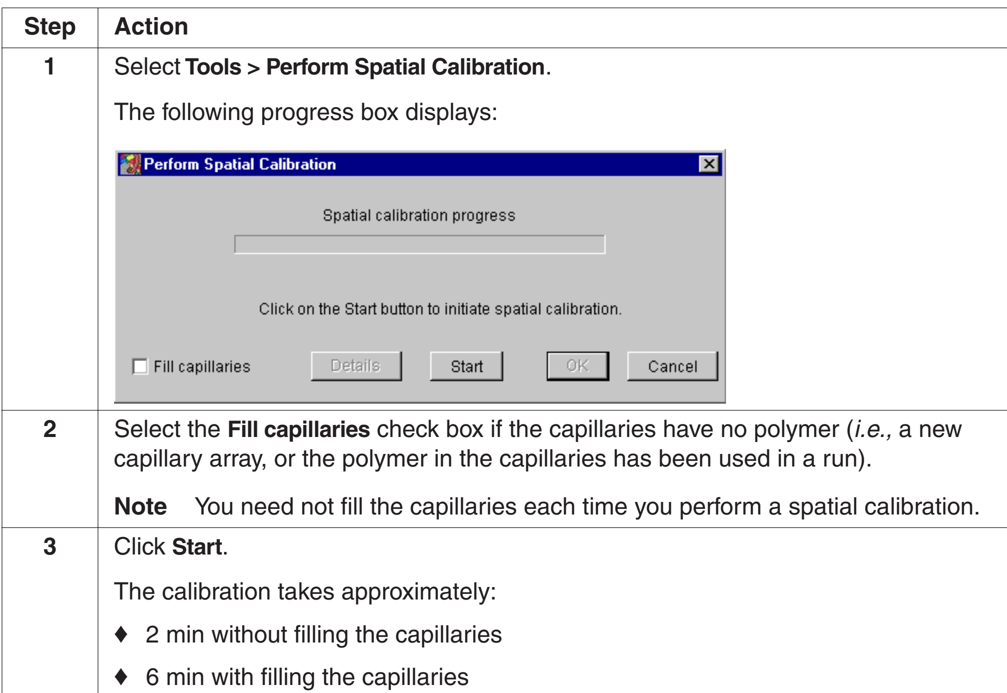
The log file is stored in the same directory as the spatial calibration files with the following file name format:

SpatialCal-*instrumentname*-Run*date-time*.log

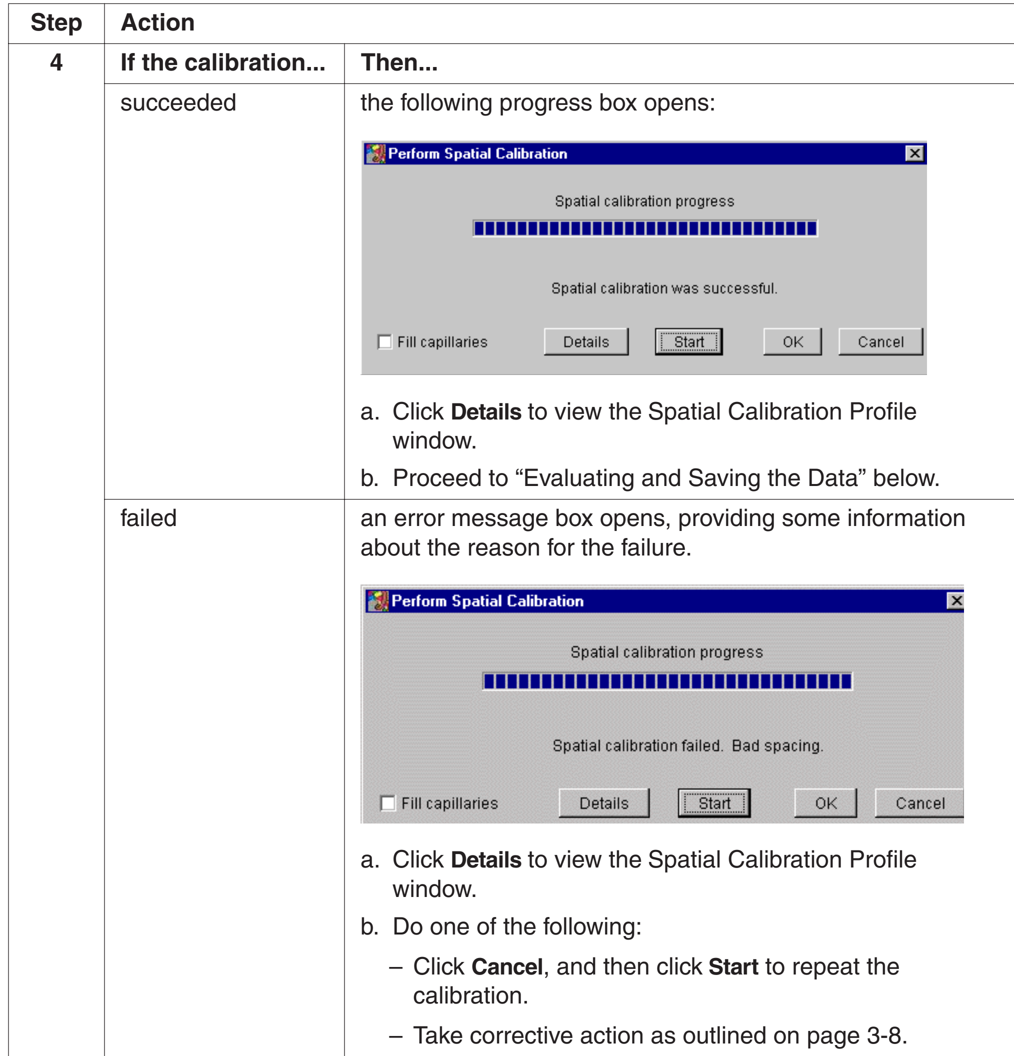
Performing a Spatial Calibration

Performing a Spatial Calibration

To perform a spatial calibration:

Step	Action
1	Select **Tools > Perform Spatial Calibration**. The following progress box displays: 
2	Select the **Fill capillaries** check box if the capillaries have no polymer (*i.e.,* a new capillary array, or the polymer in the capillaries has been used in a run). **Note** You need not 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 progress box opens: Perform Spatial Calibration Spatial calibration progress Spatial calibration was successful. Fill capillaries / Details / Start / OK / Cancel a. Click **Details** to view the Spatial Calibration Profile window. b. Proceed to “Evaluating and Saving the Data” below.
	failed	an error message box opens, providing some information about the reason for the failure. Perform Spatial Calibration Spatial calibration progress Spatial calibration failed. Bad spacing. Fill capillaries / Details / Start / OK / Cancel 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 3-8.

Evaluating and Saving the Data

To evaluate the spatial calibration results and save the data:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Evaluate the spatial calibration profile.

While viewing the calibration profile in the Details dialog box, use the following criteria to evaluate the data:

<table>
<tr><th>Peak Attribute</th><th>Criteria</th></tr>
<tr><td>Height</td><td>Similar heights for all peaks.</td></tr>
<tr><td>Red crosses</td><td>One red cross marking the top of every peak. No misplaced crosses.

To move a cross:
a. Change the value in a Capillary Position box.
b. Click outside of that box.
c. Click OK to accept the new value.</td></tr>
<tr><td>Shape</td><td>♦ Single sharp peak for each capillary.
♦ Small shoulders are acceptable.</td></tr>
<tr><td>Spacing</td><td>Position values are 13–16 higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.</td></tr>
</table>
</td></tr>
</table>

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>
<table>
<tr><th>If the spatial calibration profile is...</th><th>Then...</th></tr>
<tr><td>satisfactory</td><td>Continue on to step 3.</td></tr>
<tr><td>unsatisfactory</td><td>Perform one or more of the following:
♦ Click Cancel to close the Details box, and then click Start to repeat the calibration.
♦ 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.
♦ Override the data with data from a previous run (see page 3-10).
If the calibration continues to provide unsatisfactory results, see “If the Calibration Fails” on page 3-8.</td></tr>
</table>
</td></tr>
<tr><td>3</td><td>Click Yes to close the Perform Spatial Calibration window.
Question
Save spatial calibration data?
If you choose 'Yes', spatial calibration data will be saved in the database and sent to the instrument.
Yes No
The data becomes the current spatial calibration and the data is saved to the instrument and database.</td></tr>
</table>

If the Calibration Fails

If the calibration fails, 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 (see page 4-14).
- ♦ Reposition the array window in the detection cell, and then repeat the calibration.
- ♦ Try another capillary array.

Displaying a Spatial Calibration Profile

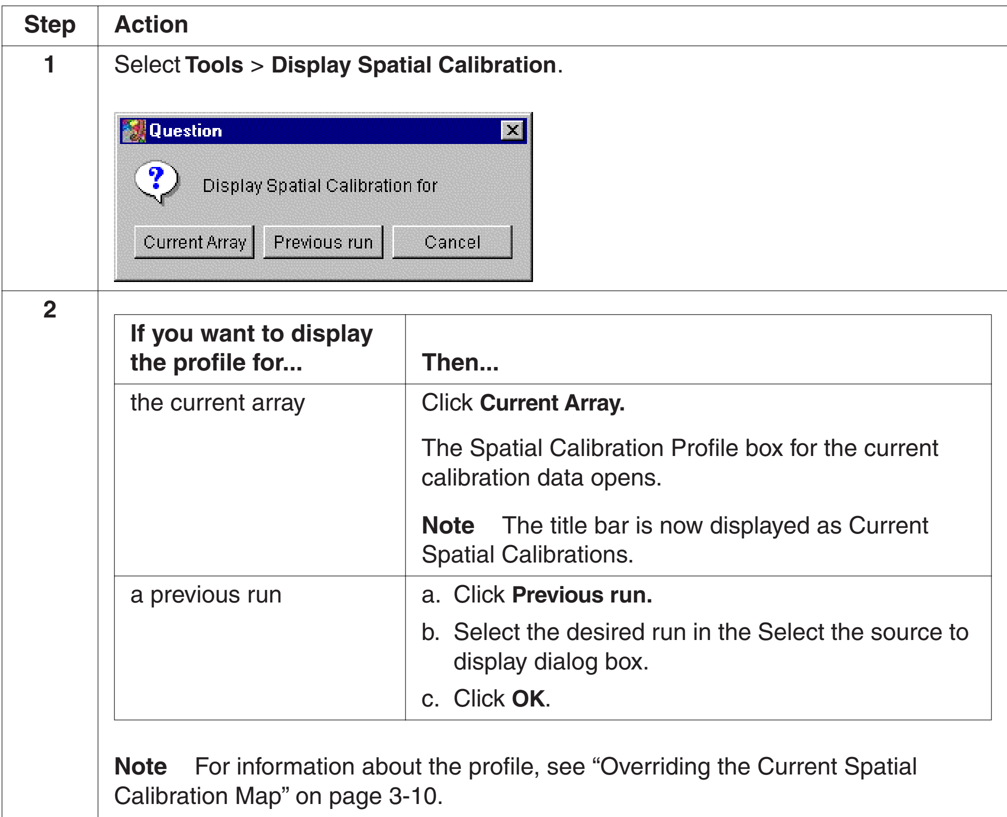
Introduction

By performing the procedure below, you can display the spatial calibration profile for the current capillary array or the profile that was used for a previous run.

Note With this procedure, you can view spatial calibration data, but you cannot change which data is set as the current map.

Displaying a Spatial Calibration Profile

To display a spatial calibration profile:



<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select Tools > Display Spatial Calibration.

Question
Display Spatial Calibration for
Current Array | Previous run | Cancel</td></tr>
<tr><td>2</td><td>
<table>
<tr><th>If you want to display the profile for...</th><th>Then...</th></tr>
<tr><td>the current array</td><td>Click Current Array.

The Spatial Calibration Profile box for the current calibration data opens.

Note The title bar is now displayed as Current Spatial Calibrations.</td></tr>
<tr><td>a previous run</td><td>a. Click Previous run.
b. Select the desired run in the Select the source to display dialog box.
c. Click OK.</td></tr>
</table>

Note For information about the profile, see “Overriding the Current Spatial Calibration Map” on page 3-10.</td></tr>
</table>

Overriding the Current Spatial Calibration Map

Introduction

Once the spatial calibration run has completed and you have accepted it, the new spatial calibration map is stored in the instrument database and sent to the instrument. This current map will be used to process sample run data.

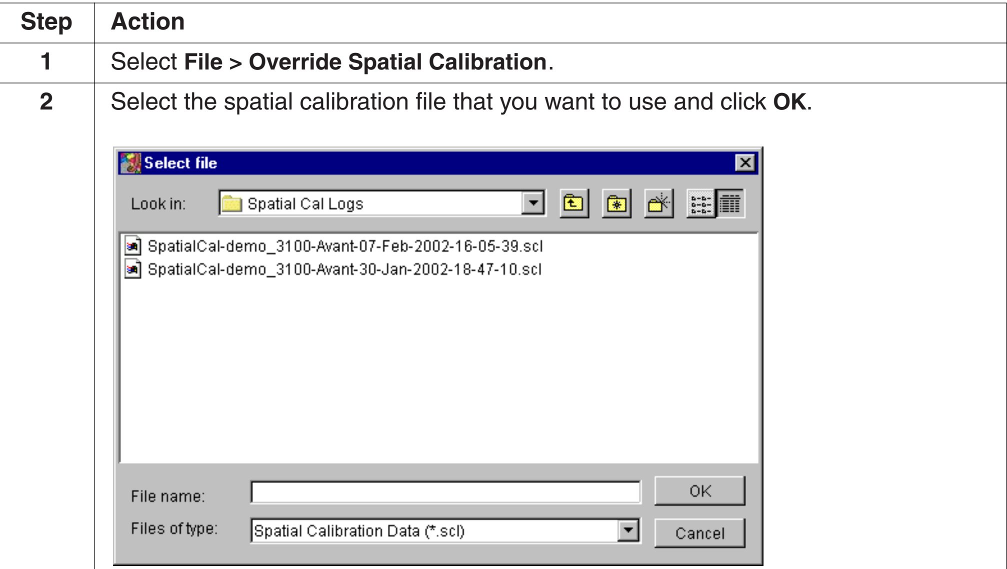
If the run data is bad when using the current map, you can override the calibration data with:

- Data collected during a previous run on the same capillary array if the detection cell has not been moved
- A spatial calibration map used to process any previous sample run still stored in the database

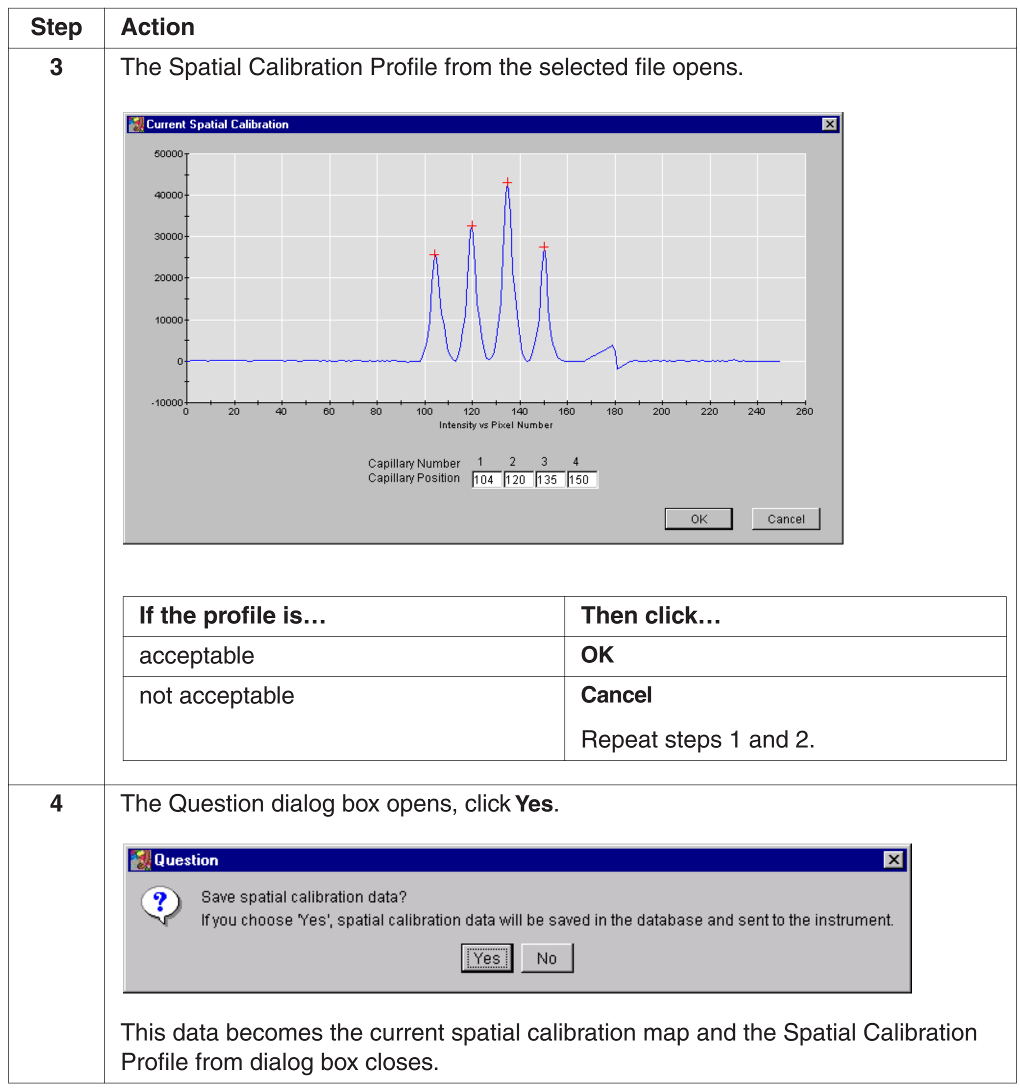
IMPORTANT Overriding calibration data is only allowed if the capillary array has not been removed and the detection cell has not been moved; Do not use calibration data collected from another capillary array.

Overriding the Current Spatial Calibration Profile

To override the current spatial calibration map:

Step	Action
1	Select **File > Override Spatial Calibration**.
2	Select the spatial calibration file that you want to use and click **OK**. 

To override the current spatial calibration map: *(continued)*



Step	Action
3	The Spatial Calibration Profile from the selected file opens.
4	The Question dialog box opens, click **Yes**. This data becomes the current spatial calibration map and the Spatial Calibration Profile from dialog box closes.

Step 3 decision table:

If the profile is…	Then click…
acceptable	**OK**
not acceptable	**Cancel** Repeat steps 1 and 2.

Section: Spectral Calibration

In This Section

The following topics are covered in this section:

Topic	See Page
About Spectral Calibrations	3-14
Preparing and Loading Spectral Calibration Standards	3-16
Performing a Spectral Calibration Using Default Processing Parameters	3-19
Displaying a Spectral Calibration Profile	3-27
Activating a Spectral Calibration for a Dye Set	3-31
Overriding a Spectral Calibration Profile	3-33

About Spectral Calibrations

Background

Although the dyes in a dye set fluoresce at different wavelengths, there is some overlap in the emission spectra of the dyes used. This spectral overlap must be eliminated for proper data analysis.

A spectral calibration is a run that produces a mathematical description of the overlap in the emission spectra of a given dye set. This mathematical description is called a matrix, and is required for each capillary. The process of applying a matrix to sample data is called multicomponenting.

A separate matrix is required for each dye set.

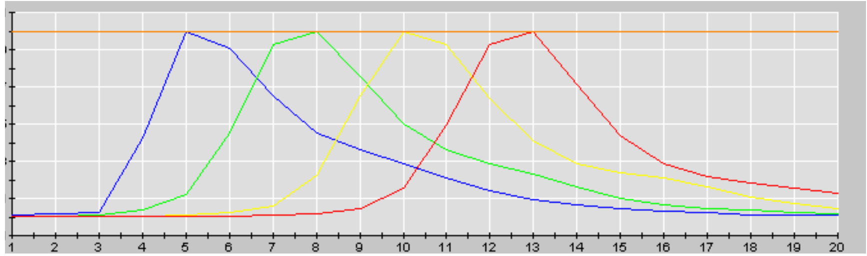


Figure 3-2 The spectral profile for dye set Z.

What Happens?

Spectral standards are run in all 16 capillaries. Then, the data collection software:

- Collects the data and stores it into 16 separate temporary files
- Analyzes the data and generates a mathematical description of the spectral overlap for each capillary
- Stores the data as a matrix

When to Calibrate

You must perform a spectral calibration:

- Whenever you use a new dye set on the instrument.
- Whenever a service engineer realigns/replaces the laser, optics, or CCD camera
- If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks).
- For fragment analysis only, if you change capillary array lengths (see below)

Changing Capillary Array Lengths

The impact of changing the capillary array length on spectral calibrations is different for sequencing and fragment analysis applications.

Analysis Application	Impact
Sequencing analysis	Typically, for each dye set, a single spectral calibration can be used for all capillary array lengths and polymer combinations.
Fragment analysis	For each dye set, a separate spectral calibration must be used for the 22- and 36-cm capillary arrays. Refer to “Activating a Spectral Calibration for a Dye Set” on page 3-31, for information on how to switch calibrations.

Procedure Overview

The procedures for performing a spectral calibration are basically the same for fragment analysis and DNA sequencing analysis applications.

Performing a spectral calibration is similar to performing a sample run, except that matrix calibration standards are run in place of samples, and a spectral calibration run module is used in place of a fragment or sequencing analysis run module.

Part	Description
Software setup	You will begin the procedure by preparing the instrument and calibration standards. Next, you will set up the run using the Plate View page of the data collection software. During the software setup, you will be prompted to select a specific: ♦ Spectral run module (determines the run conditions for each array type) ♦ Dye set (configures the software for the dye set you are using) ♦ Spectral parameter file (selects the type of algorithm you want to use to process the data: matrixStandard or sequenceStandard)
Standards calibration	During the calibration, dye-labeled DNA standards are electrophoresed, and the fluorescence data is collected and stored as temporary files. The matrix-making software analyzes this data and creates a spectral calibration matrix, which is used for sample data. Application of this matrix to the raw data is called multicomponenting.
Data analysis	After the calibration run, the software analyzes the matrices and assigns a capillary status value to each capillary. The matrix passes if it: ♦ Exhibits four or five distinct fluorescence emission maxima from dye sets with four or five dyes ♦ Meets the criteria specified in the selected spectral calibration parameter text file A passed matrix must be assigned to every capillary before a sample run can be performed. The software automatically replaces matrices for failed capillaries with matrices created from capillaries that passed. The replacements are made from the next nearest capillary, with the left side taking priority over the right side. Even though the algorithm has passed a calibration matrix from a capillary, it does not mean that the calibration data should necessarily be used for sample data analysis. We recommend that you examine all four calibration matrices before electing to save and use them for sample data processing. Ideally, each capillary has its own passed matrix. If you see a matrix that you do not want to use, you can use the Override Spectral Calibration command to replace the matrix with one from a neighboring capillary.

Preparing and Loading Spectral Calibration Standards

Determining the Correct Dye Set and Matrix Standard Set

Use the table below to determine the correct dye set and matrix standard set for the application you are using.

Application or Kit	Dye Set	Matrix Standard Set
ABI PRISM® BigDye® Terminator v3.0 chemistry	Z	ABI PRISM® BigDye® v3.0 Matrix Standard
		ABI PRISM® BigDye® v3.0 Terminator Sequencing Standard
ABI PRISM® BigDye® Terminator chemistry	E	DS-01
Custom oligos	D	DS-30
♦ ABI PRISM® Mouse Mapping Set v1.0 ♦ Custom oligos	D	DS-31 (DS-30 + VIC™ Matrix Standard)[1]
ABI PRISM® SNaPshot™ Multiplex System	E5	DS-02
♦ ABI PRISM® Linkage Mapping Set (LMS) v2.5 ♦ Custom Oligos	G5	DS-33

1. Replace the HEX™ matrix standard in DS-30 kit with the VIC matrix standard.

IMPORTANT Fragment Analysis: For optimal resolution, as in the case of fine mapping, Applied Biosystems recommends using the 36-cm capillary array. However, the 22-cm capillary array can be used to rapidly scan the genome when using markers less than 360 bp. Refer to *ABI PRISM® 3100 22-cm Capillary Array for High Throughput Microsatellite and SNP Genotyping User Bulletin* for more information. To select the appropriate capillary length for your application, refer to “Selecting a Capillary Array” on page 2-17.

DNA Sequencing: Preparing the Sequencing Standard

To prepare the matrix standard for Dye Set Z spectral calibration:

Step	Action
1	Resuspend a tube of ABI PRISM® BigDye® Terminator v3.0 Sequencing Standard with 170 µL of Hi-Di formamide. **⚠ WARNING** **CHEMICAL HAZARD. Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex thoroughly.
3	Spin the mixture briefly in a microcentrifuge.
4	Heat the standard tube at 95 °C for 5 min to denature the DNA.
5	Place the tubes immediately on ice for 2 min.

DNA Sequencing: Preparing the Matrix Standard

To prepare the matrix standard for Dye Set Z spectral calibration:

Step	Action
1	Mix thoroughly the ABI PRISM® BigDye® v3.0 Matrix Standard tube.
2	Spin the tube briefly in a microcentrifuge.
3	Prepare the BigDye v3.0 Matrix Standard for Dye Set Z by combining the following in a labeled 1.5-mL microcentrifuge tube: **Reagent** — **Volume (µL)** ABI PRISM BigDye v3.0 Matrix Standard — 10 Hi-Di™ Formamide (P/N 4311320) — 190 **Final Volume** — **200** **⚠ 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.
4	Vortex thoroughly.
5	Spin the mixture briefly in a microcentrifuge.
6	Heat the standard tube at 95 °C for 5 min to denature the DNA.
7	Place the tubes immediately on ice for 2 min.

Preparing Matrix Standard for Other Dye Sets

To prepare other dye sets, follow the product insert instructions included in the matrix standard box.

Fragment Analysis: Preparing the Matrix Standard

To prepare the matrix standards for Dye Set G5 spectral calibration:

Step	Action
1	Mix thoroughly a Matrix Standard Set DS-33 tube.
2	Spin the tube briefly in a microcentrifuge.
3	Prepare the Matrix Standard Set DS-33 for Dye Set G5 by combining the following in a labeled 1.5-mL microcentrifuge tube: **Reagent** — **Volume (µL)** Matrix Standard Set DS-33 — 5 Hi-Di Formamide (P/N 4311320) — 195 **Final Volume** — **200** **⚠ 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.
4	Vortex thoroughly.

To prepare the matrix standards for Dye Set G5 spectral calibration: *(continued)*

Step	Action
5	Spin the mixture briefly in a microcentrifuge.
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.

Preparing Matrix Standard for Other Dye Sets

To prepare other dye sets, follow the product insert instructions included in the matrix standard box.

Loading the Standards

To load the standards:

Step	Action
1	Dispense 10 μL of the denatured standard into the appropriate plate as shown below: ♦ 96-well plate, load wells A1 through D1 GR1315c ♦ 384-well plate, load wells A1, C1, E1, and G1 GR1316c

To load the standards: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Tap down or centrifuge the plate so that each standard is positioned at the bottom of its well. Your samples should:
<table>
<tr><th>Look like this...</th><th>Not look like this...</th><th>Not look like this...</th></tr>
<tr><td>GR1303
The sample is positioned correctly in the bottom of the well.</td><td>The sample lies on the side wall because the plate was not centrifuged.</td><td>An air bubble lies at the bottom of the well because the plate was not centrifuged with enough force or time.</td></tr>
</table>
</td></tr>
</table>

Performing a Spectral Calibration Using Default Processing Parameters

Introduction

Use the procedure below to perform a basic, “default” spectral calibration for DNA sequencing analysis or fragment analysis.

Note Dye Set Z or G5 are used for screen shot examples.

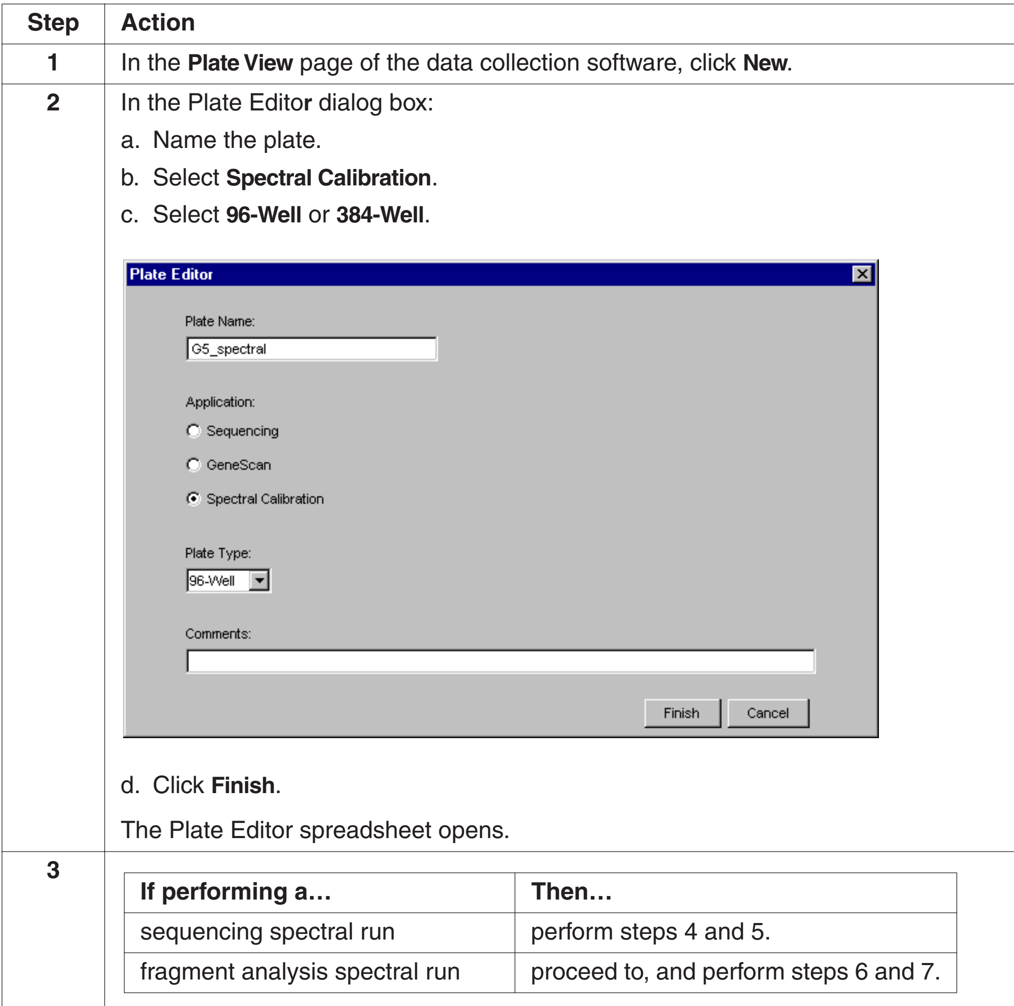
Preparing the Equipment

To prepare the equipment and supplies:

Step	Action
1	Power on the computer and the instrument.
2	Prepare the instrument for a run (see page 2-15).
3	Place the plate on the autosampler (see page 2-46).

Creating a Plate Record

To create a plate record for the denatured matrix standards:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>In the Plate View page of the data collection software, click New.</td></tr>
<tr><td>2</td><td>In the Plate Editor dialog box:
a. Name the plate.
b. Select Spectral Calibration.
c. Select 96-Well or 384-Well.


d. Click Finish.
The Plate Editor spreadsheet opens.</td></tr>
<tr><td>3</td><td><table><tr><th>If performing a…</th><th>Then…</th></tr><tr><td>sequencing spectral run</td><td>perform steps 4 and 5.</td></tr><tr><td>fragment analysis spectral run</td><td>proceed to, and perform steps 6 and 7.</td></tr></table></td></tr>
</table>

To create a plate record for the denatured matrix standards: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>For a sequencing spectral run, complete the Plate Editor spreadsheet for the wells you have loaded.

<table>
<tr><th>For...</th><th>Perform the following...</th></tr>
<tr><td>Dye Set Z and

ABI PRISM BigDye v3.0 Sequencing Standard</td><td>a. Type a name for the samples.
b. Select Dye Set Z.
c. Select the run module depending on your capillary array size:
– 36-cm: SpectSQ36_POP4DefaultModule
– 36-cm: Spect36_POP6DefaultModule
– 50-cm: Spect50_POP6DefaultModule
– 80-cm: Spect80_POP4DefaultModule
d. Select the spectral parameter file SeqStd{Sequencing-SetZ}.par.
e. Click OK.</td></tr>
<tr><td>Dye Set Z and

ABI PRISM BigDye v3.0 Matrix Standard Set</td><td>a. Type a name for the samples.
b. Select Dye Set Z.
c. Select the run module depending on your capillary array size:
– 36-cm: SpectSQ36_POP4DefaultModule
– 36-cm: Spect36_POP6DefaultModule
– 50-cm: Spect50_POP6DefaultModule
– 80-cm: Spect80_POP4DefaultModule
d. Select the spectral parameter file MtxStd{Sequencing-SetZ}.par.
e. Click OK.</td></tr>
<tr><td>Dye Set E and

Matrix Standard Set DS-01</td><td>a. Type a name for the samples.
b. Select Dye Set E.
c. Select the run module depending on your capillary array size:
– 36-cm: SpectSQ36_POP4DefaultModule
– 36-cm: Spect36_POP6DefaultModule
– 50-cm: Spect50_POP6DefaultModule
– 80-cm: Spect80_POP4DefaultModule
d. Select the spectral parameter file MtxStd{Sequencing-SetE}.par.
e. Click OK.</td></tr>
</table>

IMPORTANT Verify that 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.</td></tr>
<tr><td>5</td><td>Proceed to “Linking the Plate” on page 3-24.</td></tr>
</table>

To create a plate record for the denatured matrix standards: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>IMPORTANT To select the appropriate capillary length for your application, refer to “Selecting a Capillary Array” on page 2-17.

For a fragment analysis spectral run, complete the Plate Editor spreadsheet for the wells you have loaded.

<table>
<tr><th>For...</th><th>Perform the following...</th></tr>
<tr><td>Dye Set D and
♦ Matrix Standard Set DS-30,
or
♦ Matrix Standard DS-31 (Matrix Standard Set DS-30 + VIC Matrix Standard)</td><td>a. Type a name for the samples.
b. Select Dye Set D.
c. Select the run module depending on your capillary array size:
– 22 cm: Spect22_POP4DefaultModule
– 36-cm: Spect36_POP4DefaultModule
d. Select the spectral parameter MtxStd{GeneScan-SetD}.par.
e. Click OK.</td></tr>
<tr><td>Dye Set E5 and
Matrix Standard Set DS-02</td><td>a. Type a name for the samples.
b. Select Dye Set E5.
c. Select the run module depending on your capillary array size:
– 22 cm: Spect22_POP4DefaultModule
– 36-cm: Spect36_POP4DefaultModule
d. Select the spectral parameter MtxStd{GeneScan-SetE5}.par.
e. Click OK.</td></tr>
</table>
</td></tr>
</table>

To create a plate record for the denatured matrix standards: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6
cont'd</td><td>
<table>
<tr><th>For...</th><th>Perform the following...</th></tr>
<tr><td>Dye Set G5

Matrix Standard Set DS-33</td><td>a. Type a name for the samples.
b. Select Dye Set G5.
c. Select the run module depending on your capillary array size:
– 22 cm: Spect22_POP4DefaultModule
– 36-cm: Spect36_POP4DefaultModule
– 36-cm: Spect36vb_POP4DefaultModule

Note To select the appropriate capillary length for your application, refer to “Selecting a Capillary Array” on page 2-17.

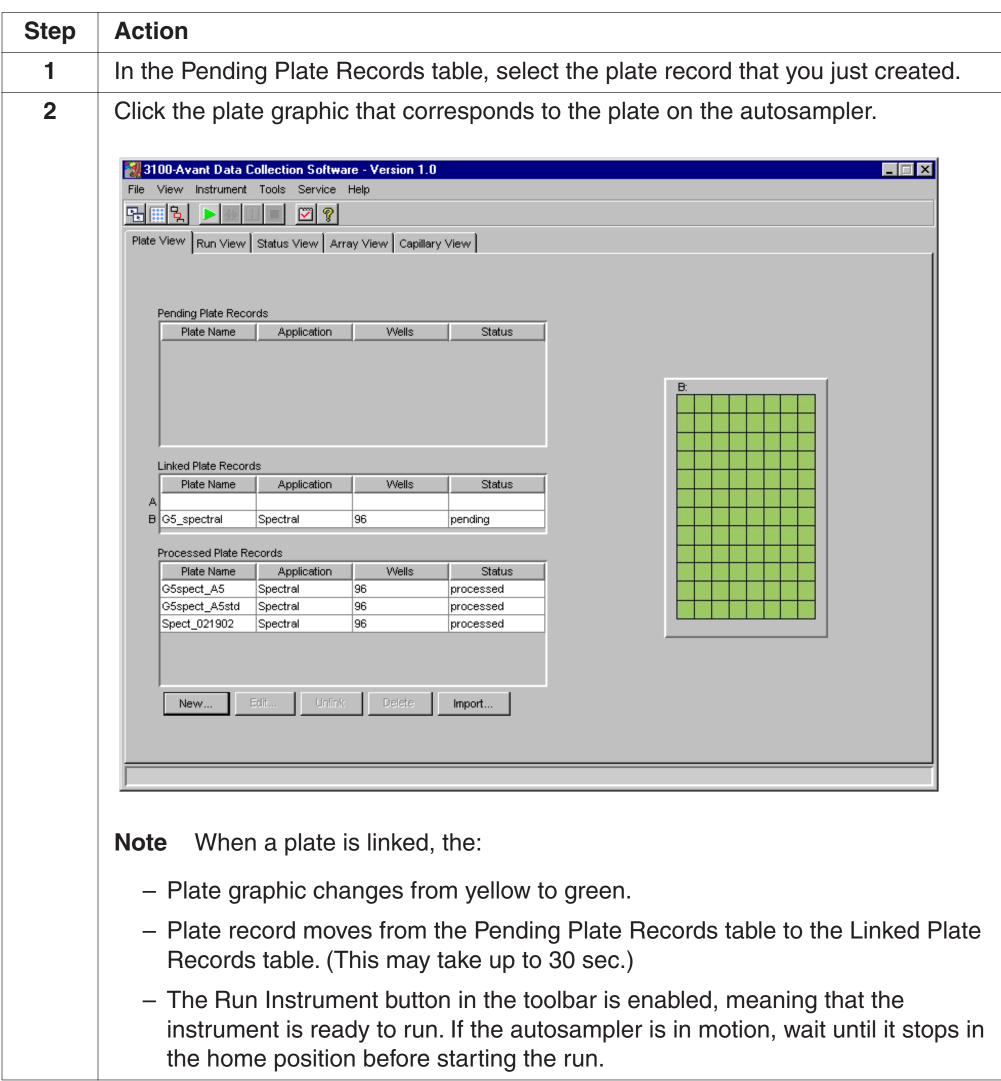
d. Select the spectral parameter MtxStd{GeneScan-SetG5}.par.
e. Click OK.</td></tr>
</table>

IMPORTANT Verify that 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 View page.</td></tr>
<tr><td>7</td><td>Proceed to “Linking the Plate” on page 3-24.</td></tr>
</table>

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 in the toolbar is enabled, meaning that the instrument is ready to run. If the autosampler is in motion, wait until it stops in the home position before starting the run.

Starting the Calibration

To start the calibration:

Step	Action
1	If you want to review the run schedule before beginning the run, click the **Run View** tab.
2	Click the **Run Instrument** button in the toolbar to begin the run.

Run Times

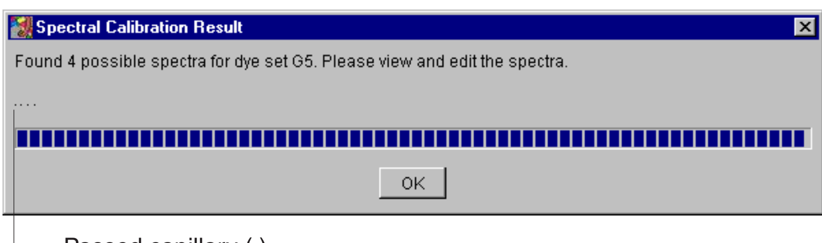
The following table lists the spectral calibration run times:

Application or Run Type	Capillary Array Length (cm)	Approximate Run Time (min)
SNP analysis	22	15
Microsatellite analysis	22	20
Microsatellite analysis	36	45
SNP analysis	36	30
Ultra rapid sequencing	36	40
Rapid sequencing	36	40
Standard sequencing	50	65
Long read sequencing	80	80

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 G5 shows four passing capillaries, which are represented by a "." dot. A failed capillary is represented by an "X".



To acknowledge the completed calibration run:

Step	Action
1	In the Spectral Calibration Result dialog 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 "Displaying a Spectral Calibration Profile" on page 3-27.

Error Message for Dye Set F or G5

The matrix-making algorithm has minimum peak requirements to create matrices for dye sets F and G5. The following message will display if peak amplitude falls below 750:

Data Error - One or more peaks fall below the minimum required amplitude of 750

If this error message occurs, rerun the spectral standards, and if necessary, increase the amount.

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.,* “Array View Page” on page 2-65).

For applications where pull-up and pull-down peaks 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.

Displaying a Spectral Calibration Profile

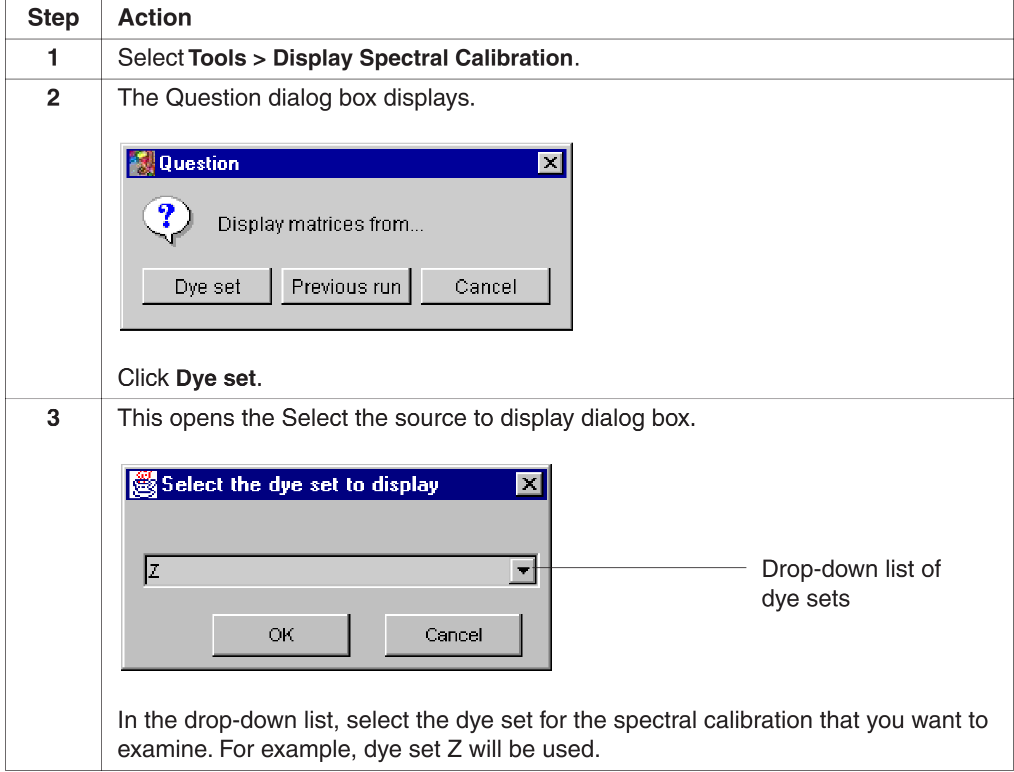
Introduction

At any time, you can display the:

- ♦ Current spectral calibration profile for a specified dye set. The current profile is the one that was created when the last spectral calibration was performed and which is stored in the instrument database. The current profiles can be examined only if a spectral calibration has been performed for this dye set.
- ♦ Spectral calibration profiles used to process any of the runs currently stored in the instrument database.

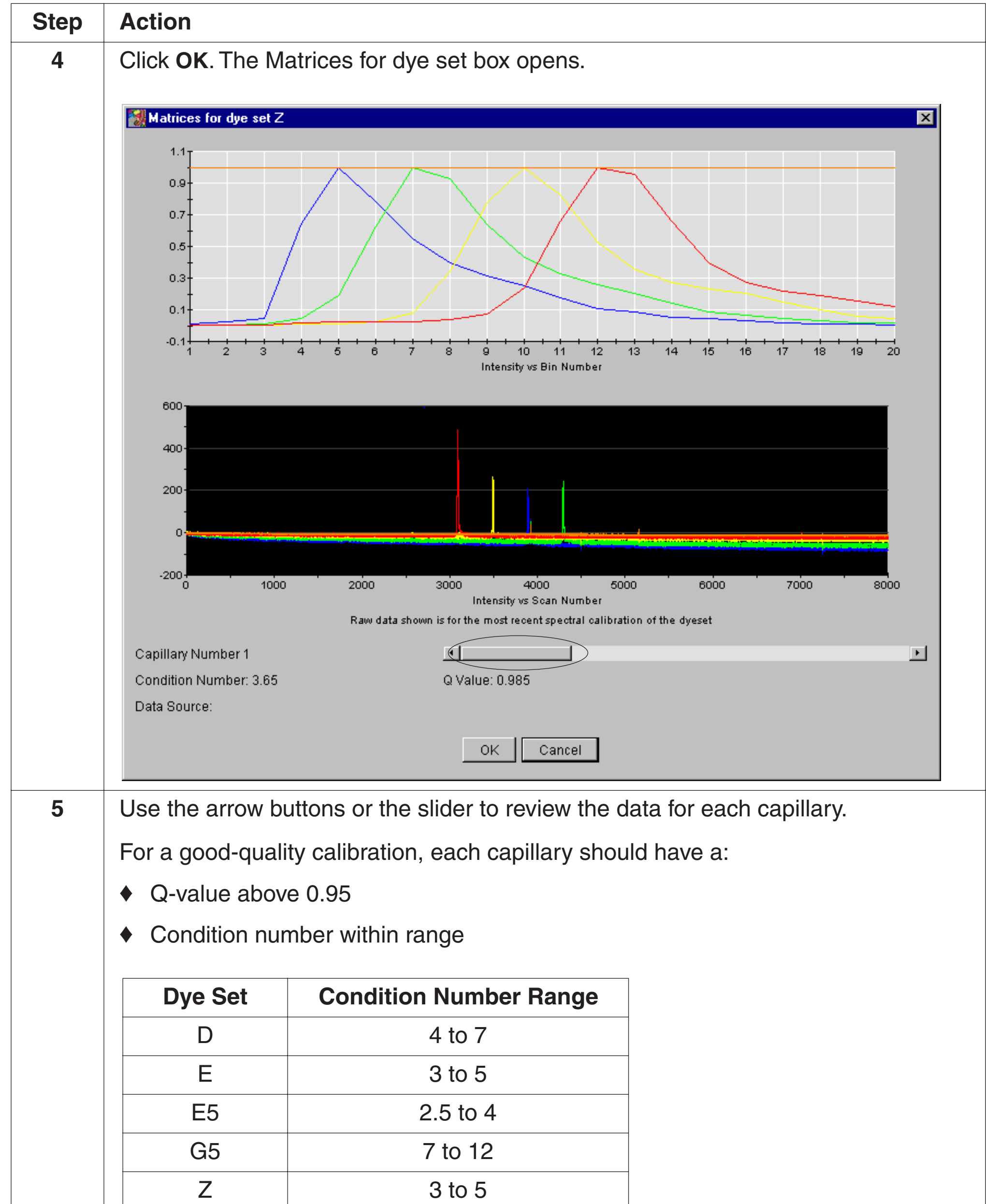
Examining a Spectral Calibration Profile for a Dye Set

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



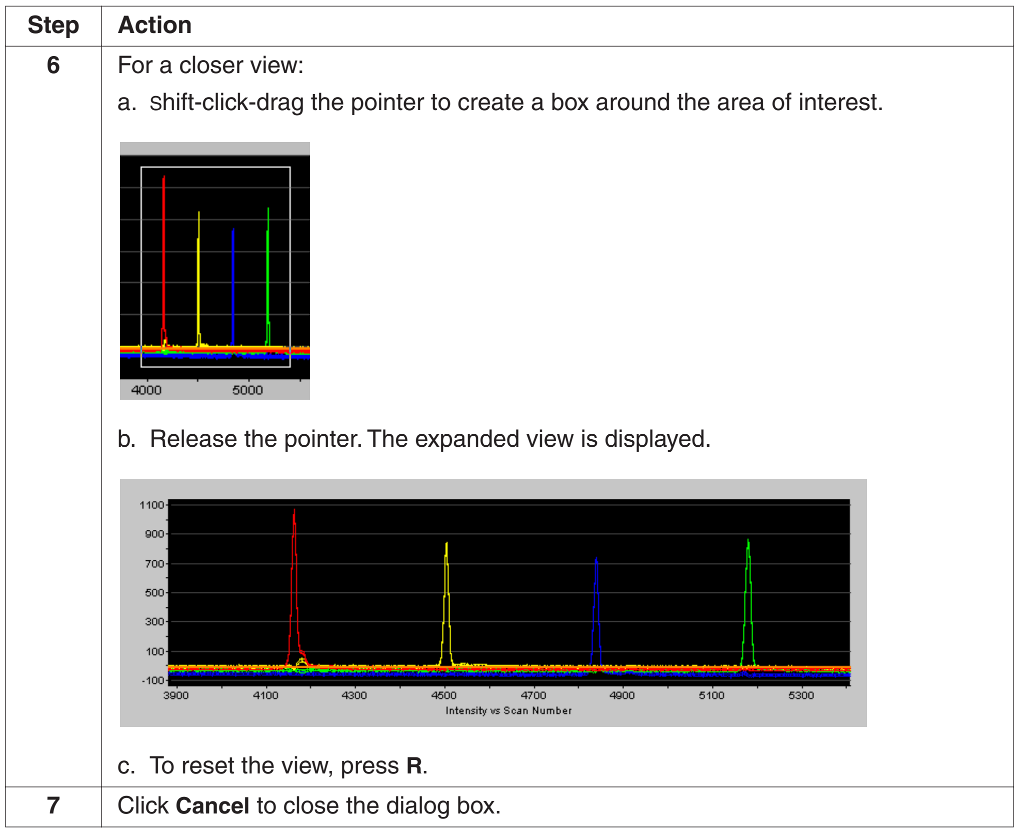
Step	Action
1	Select **Tools > Display Spectral Calibration**.
2	The Question dialog box displays. Click **Dye set**.
3	This opens the Select the source to display dialog box. In the drop-down list, select the dye set for the spectral calibration that you want to examine. For example, dye set Z will be used.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>Click OK. The Matrices for dye set box opens.


</td></tr>
<tr><td>5</td><td>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 within range
<table>
<tr><th>Dye Set</th><th>Condition Number Range</th></tr>
<tr><td>D</td><td>4 to 7</td></tr>
<tr><td>E</td><td>3 to 5</td></tr>
<tr><td>E5</td><td>2.5 to 4</td></tr>
<tr><td>G5</td><td>7 to 12</td></tr>
<tr><td>Z</td><td>3 to 5</td></tr>
</table>
</td></tr>
</table>

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

Step	Action
6	For a closer view: a. shift-click-drag the pointer to create a box around the area of interest.  b. Release the pointer. The expanded view is displayed. c. To reset the view, press **R**.
7	Click **Cancel** to close the dialog box.

G5 Profiles Using the G5vb Module

The SpectG5vb_POP4Default Module supports specific fragment analysis applications using G5 chemistry. The spectral profile is significantly different than all other dye sets. The profile contains multiple peaks per dye color, this is normal.

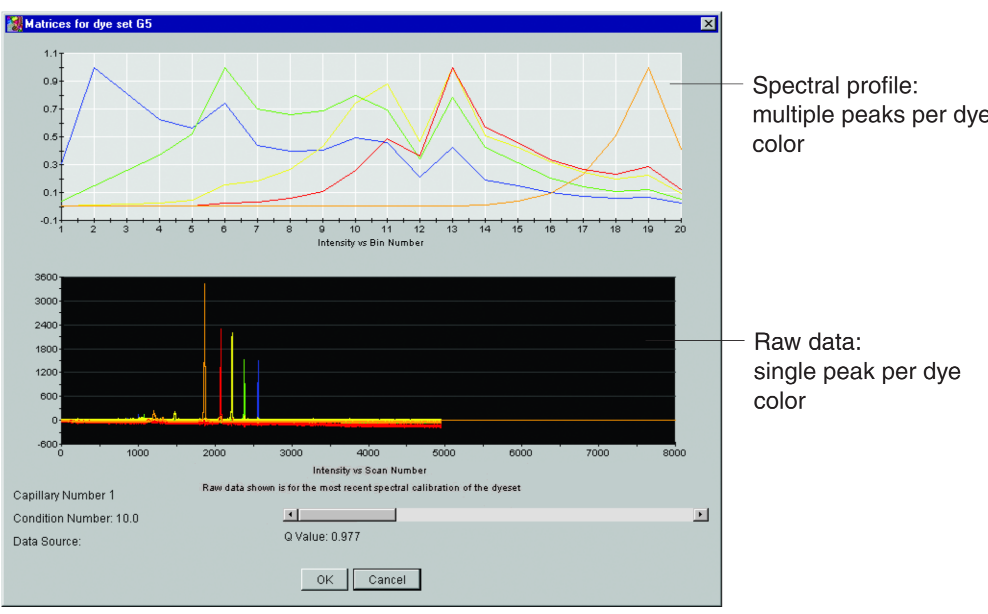
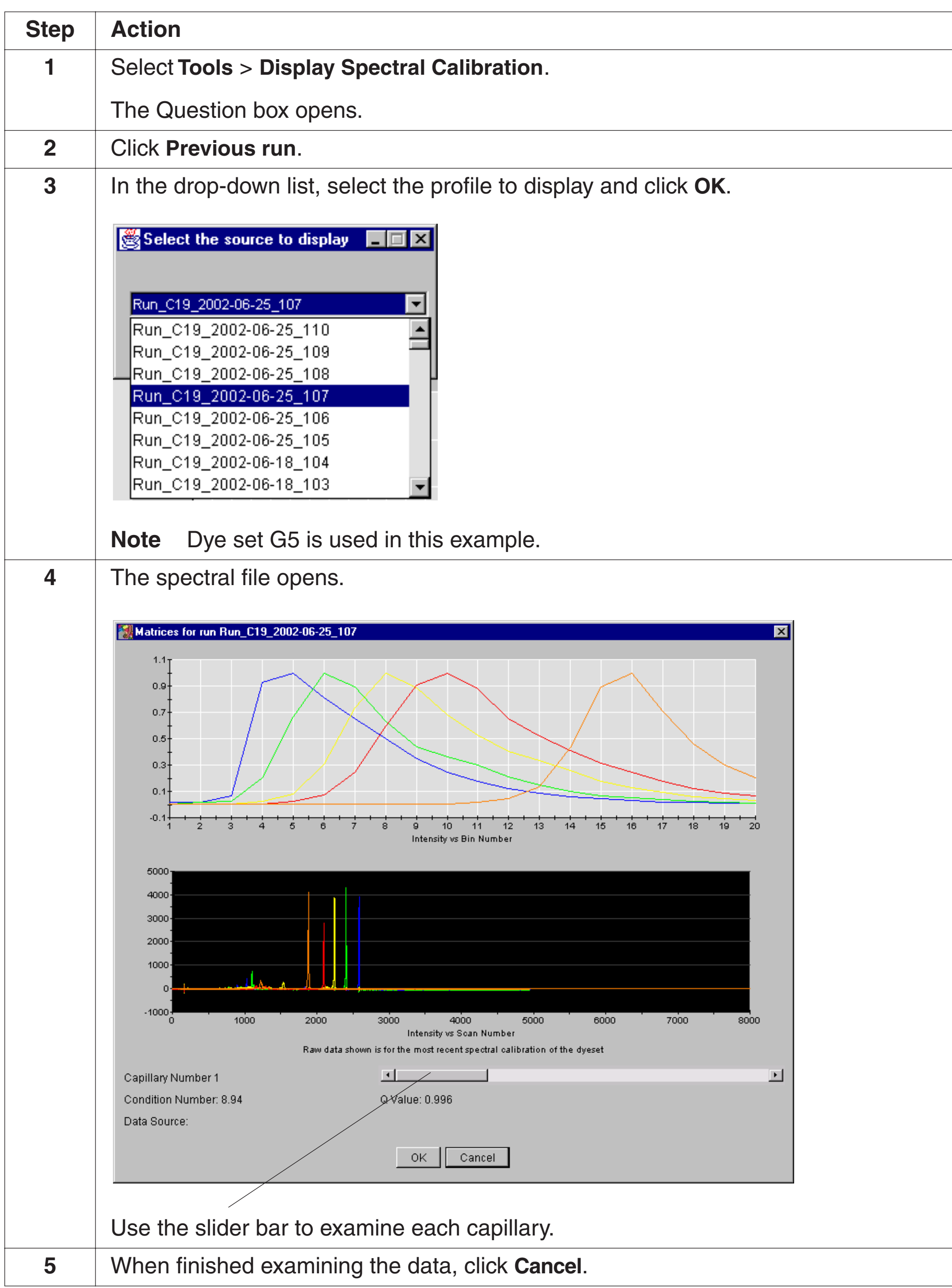


Figure 3-3 Spectral profile and raw data for a G5vb spectral calibration

Examining Profiles Used for Previous Runs

To examine the matrices used to process a previous run:



Step	Action
1	Select **Tools** > **Display Spectral Calibration**. The Question box opens.
2	Click **Previous run**.
3	In the drop-down list, select the profile to display and click **OK**. **Note** Dye set G5 is used in this example.
4	The spectral file opens. Use the slider bar to examine each capillary.
5	When finished examining the data, click **Cancel**.

Activating a Spectral Calibration for a Dye Set

Introduction

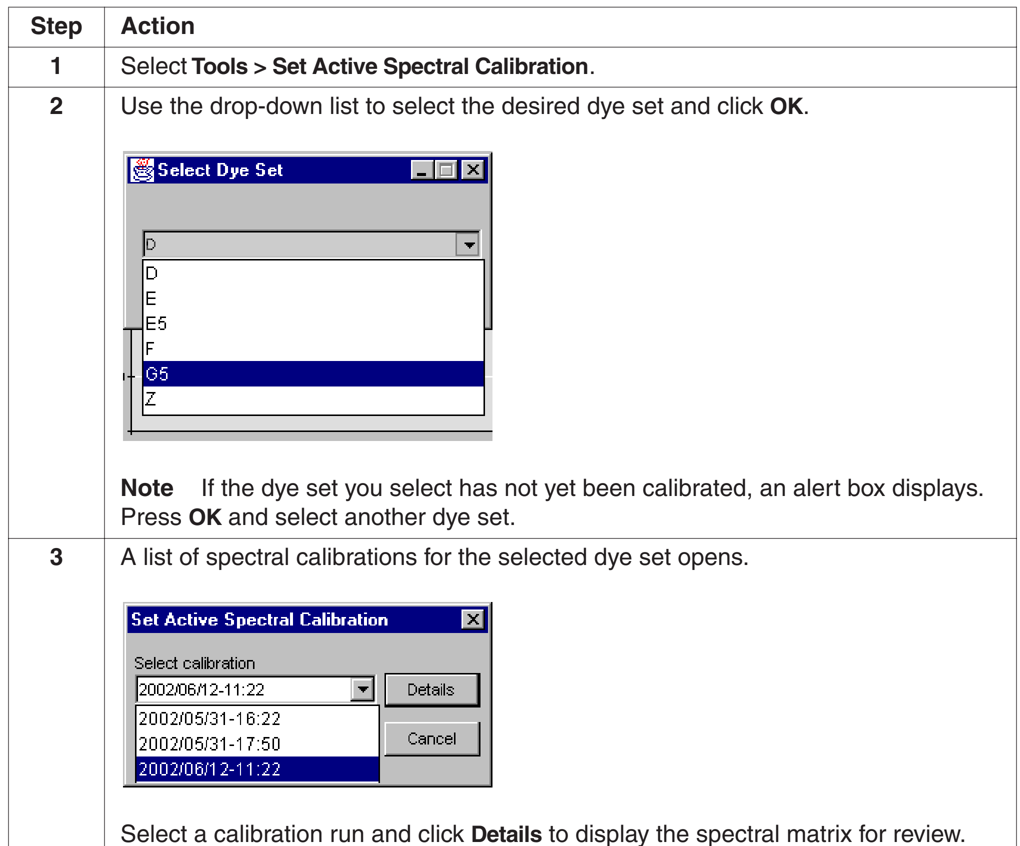
If you want to use a different spectral calibration for a specific dye set and/or capillary array length, you can select the active spectral calibration for a dye set from any previous spectral calibration runs.

This is useful for:

- ♦ Fragment analysis applications that require a separate spectral calibration (for the same dye set) for different capillary array lengths
- ♦ Applications that require different spectral calibrations for the same dye sets
- ♦ Repeat spectral calibrations where the original calibration is better than the second one

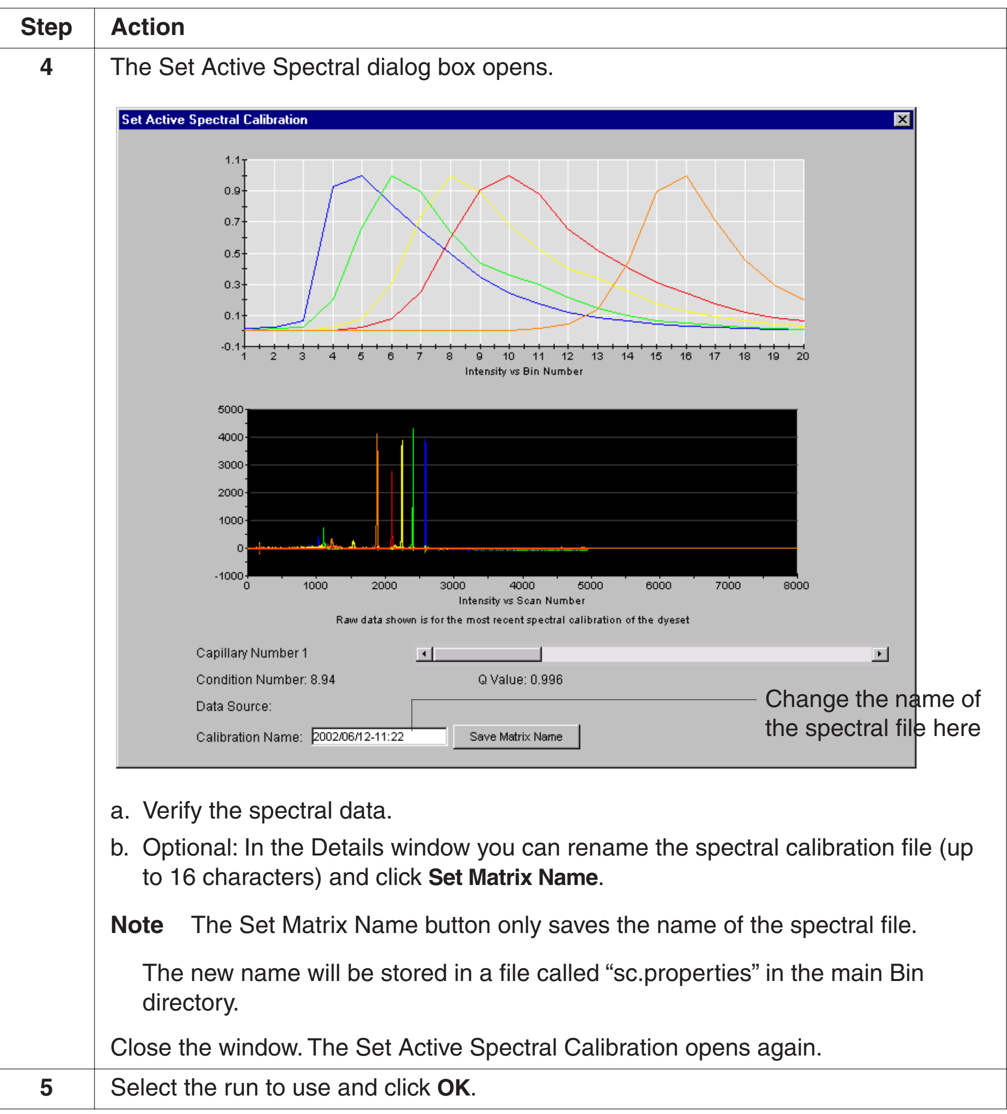
Activating a Previous Calibration

To activate a specific spectral calibration:



Step	Action
1	Select **Tools > Set Active Spectral Calibration**.
2	Use the drop-down list to select the desired dye set and click **OK**. **Note** If the dye set you select has not yet been calibrated, an alert box displays. Press **OK** and select another dye set.
3	A list of spectral calibrations for the selected dye set opens. Select a calibration run and click **Details** to display the spectral matrix for review.

To activate a specific spectral calibration: *(continued)*

Step	Action
4	The Set Active Spectral dialog box opens.  a. Verify the spectral data. b. Optional: In the Details window you can rename the spectral calibration file (up to 16 characters) and click **Set Matrix Name**. **Note** The Set Matrix Name button only saves the name of the spectral file. The new name will be stored in a file called “sc.properties” in the main Bin directory. Close the window. The Set Active Spectral Calibration opens again.
5	Select the run to use and click **OK**.

About the Raw Data Display

The data collection software stores the raw spectral data from a spectral calibration in temporary files. The temporary files are separated by dye set, and stored in the following location:

D:\AppliedBio\3100-Avant\DataCollection\SpectralCalLogs\CapRawData

There is only one copy of raw data per dye set. When a new spectral calibration is performed for an existing dye set, the current raw data is automatically replaced by the new raw data.

When viewing previous spectral calibrations, the spectral profile displayed is from the run you selected.The raw data view is from the most recent calibration (original data was replaced). The correct matrix is applied to the data.

Overriding a Spectral Calibration Profile

Introduction

You can override unsatisfactory spectral calibration profiles in the data collection software. The profiles can be overridden for individual capillaries (one at a time) or for all capillaries at once. However, we do not recommend applying a matrix from a single capillary to capillaries more than two positions over.

You can override a profile with a good-quality profile that was collected either:

- ♦ From another capillary during the same calibration run (stored as .tmp files), or
- ♦ From previously collected data, after the capillary array was last moved or replaced (stored in the spectral calibration folder as .mcl files)

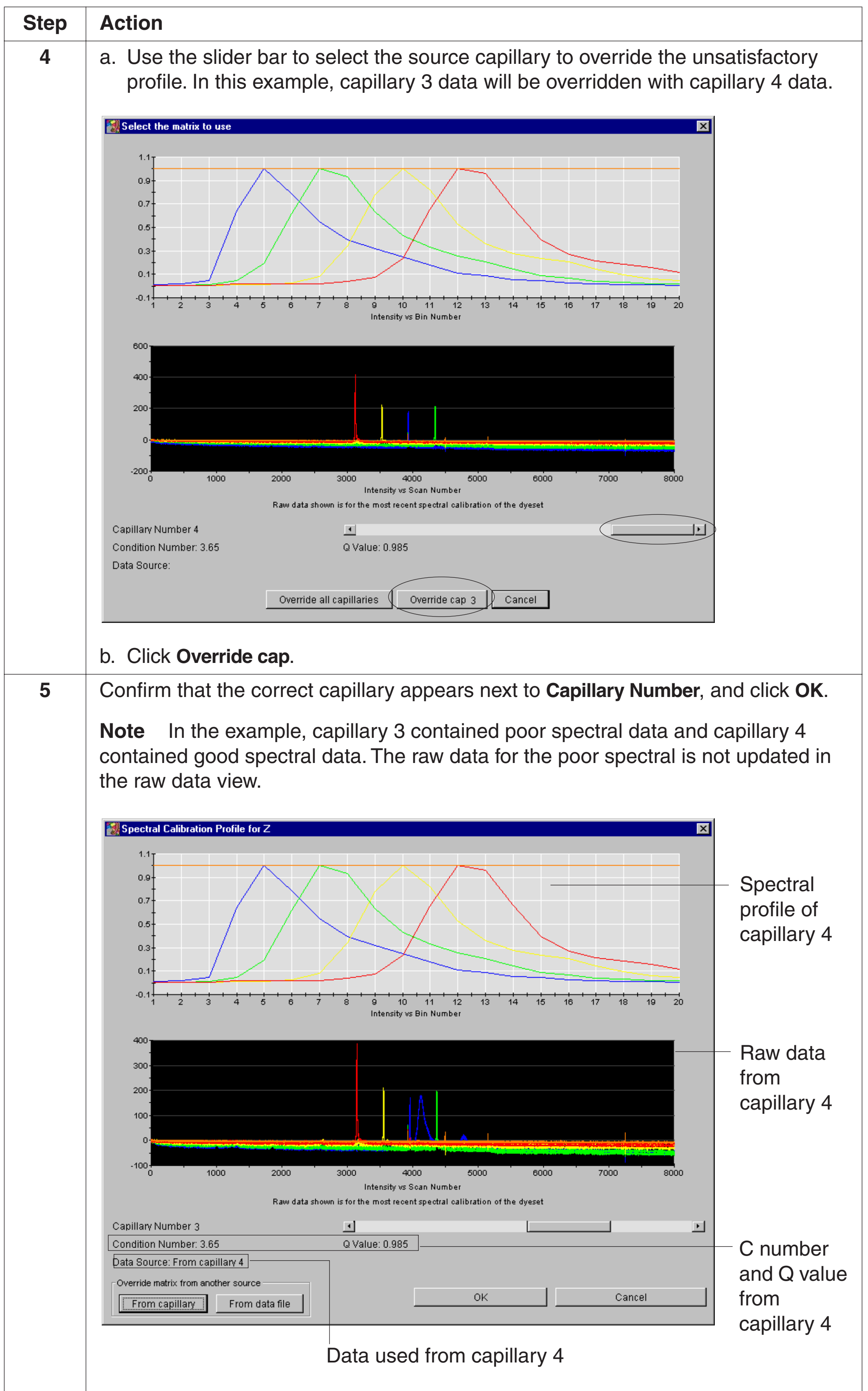
Overriding with Data from Another Capillary

Note To ensure the highest quality data, Applied Biosystems recommends that you do not override capillary profiles.

To override a spectral calibration profile with data from another capillary:

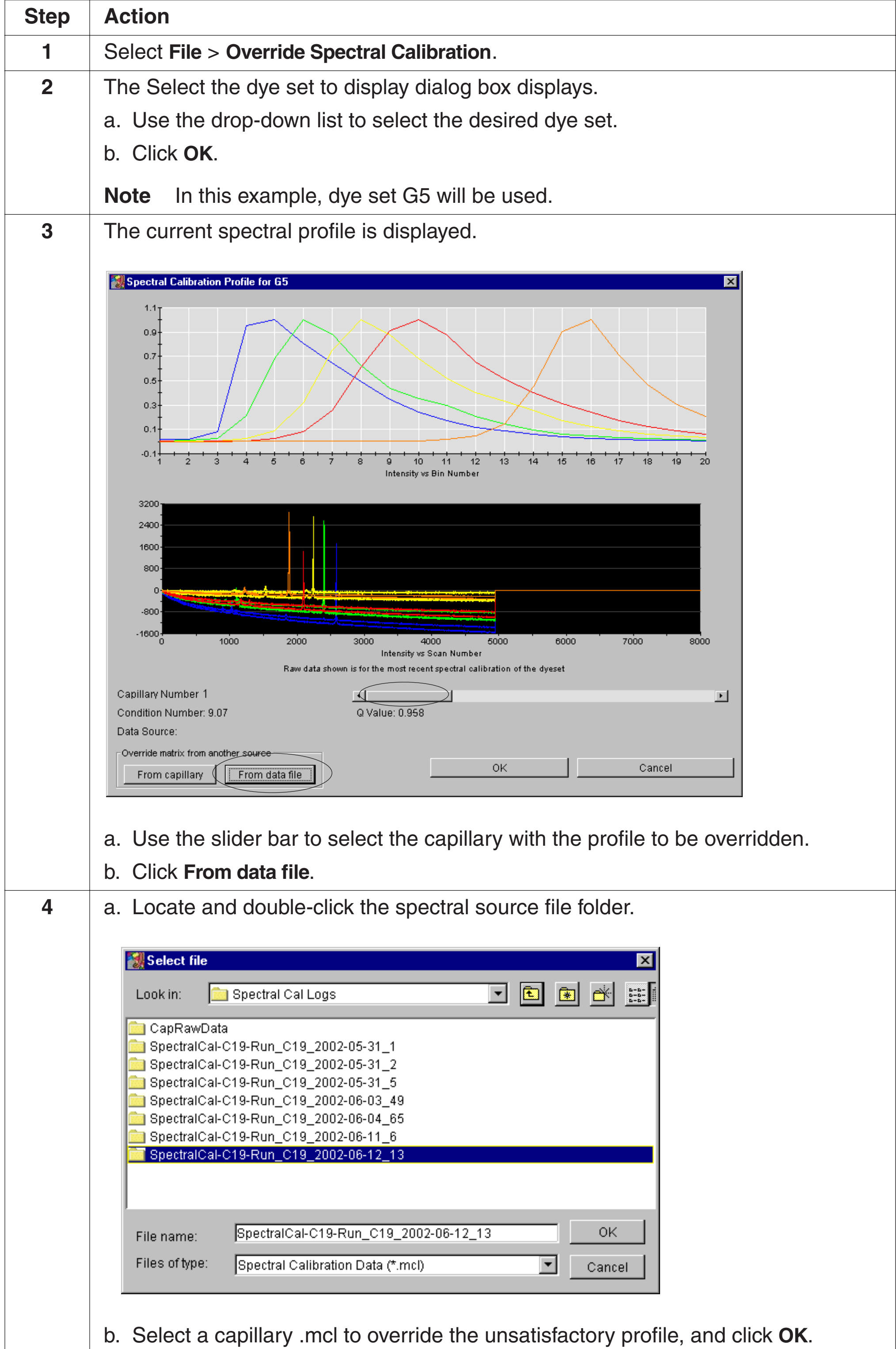
Step	Action
1	Select **File** > **Override Spectral Calibration**.
2	The Select the dye set to display dialog box displays. a. Use the drop-down list to select the desired dye set. b. Click **OK**. **Note** This example uses Dye Set Z.
3	The current spectral profile opens. a. Use the slider bar to select the capillary to be overridden. (Capillary 3 is selected for this example.) b. Click **From capillary**.

To override a spectral calibration profile with data from another capillary: *(continued)*

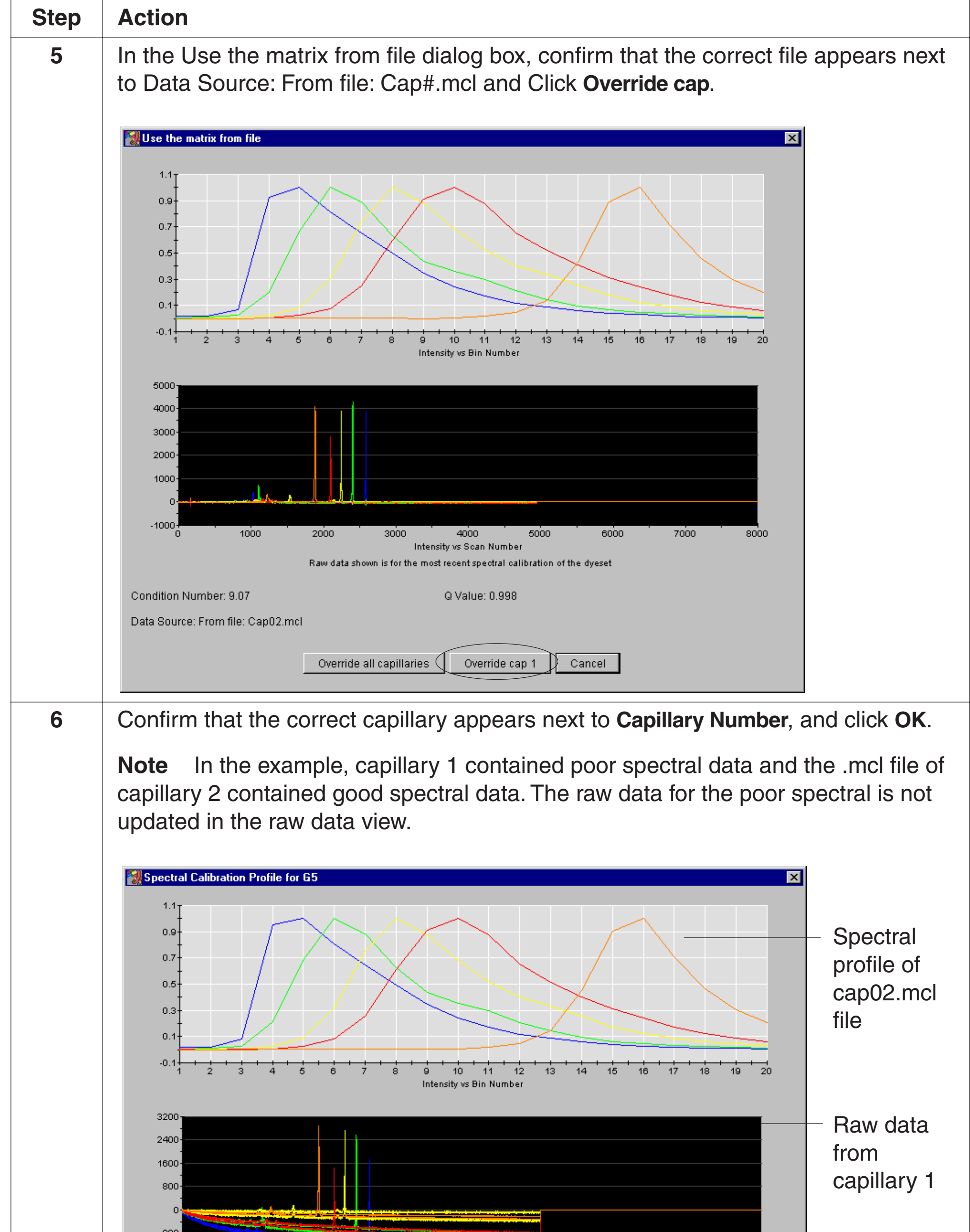
Step	Action
4	a. Use the slider bar to select the source capillary to override the unsatisfactory profile. In this example, capillary 3 data will be overridden with capillary 4 data.  b. Click **Override cap**.
5	Confirm that the correct capillary appears next to **Capillary Number**, and click **OK**. **Note** In the example, capillary 3 contained poor spectral data and capillary 4 contained good spectral data. The raw data for the poor spectral is not updated in the raw data view.

Overriding with Previously Collected Data

To override a spectral calibration profile with previously collected data:

Step	Action
1	Select **File** > **Override Spectral Calibration**.
2	The Select the dye set to display dialog box displays. a. Use the drop-down list to select the desired dye set. b. Click **OK**. **Note** In this example, dye set G5 will be used.
3	The current spectral profile is displayed.  a. Use the slider bar to select the capillary with the profile to be overridden. b. Click **From data file**.
4	a. Locate and double-click the spectral source file folder. b. Select a capillary .mcl to override the unsatisfactory profile, and click **OK**.

To override a spectral calibration profile with previously collected data: *(continued)*

Step	Action
5	In the Use the matrix from file dialog box, confirm that the correct file appears next to Data Source: From file: Cap#.mcl and Click **Override cap**. 
6	Confirm that the correct capillary appears next to **Capillary Number**, and click **OK**. **Note** In the example, capillary 1 contained poor spectral data and the .mcl file of capillary 2 contained good spectral data. The raw data for the poor spectral is not updated in the raw data view.

Maintenance 4

In This Chapter

The following topics are covered in this chapter:

Topic	See Page
Section: Instrument Maintenance	4-3
Maintenance Task Lists	4-4
Routine Cleaning	4-5
Moving and Leveling the Instrument	4-6
Resetting the Instrument	4-7
Shutting Down the Instrument	4-8
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Polymer	4-10
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Section: Instrument Maintenance

In This Section

The following topics are covered in this section:

Topic	See Page
Maintenance Task Lists	4-4
Routine Cleaning	4-5
Moving and Leveling the Instrument	4-6
Resetting the Instrument	4-7
Shutting Down the Instrument	4-8

Maintenance Task Lists

Overview

This section lists common tasks required to maintain your ABI PRISM® 3100-Avant Genetic Analyzer in good working condition. The tasks are divided into tables based on how often you should perform each task.

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

Daily Tasks

Perform these tasks at least once per day.

Maintenance Task	Frequency	See Page
Ensure that the reservoir septa are firmly seated and flat.	Before each run	—
Ensure that the plate assembly 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	2-45
Ensure that the plate assembly is positioned on the plate deck properly. Plate should sit snugly on the deck. **IMPORTANT** Never use warped plates.	Before each run	—
Replenish the water and 1X running buffer reservoirs on the instrument.	Daily or before each run	2-19
Check for bubbles in the polymer block and polymer block channels and remove.	Daily or before each run	4-32
Check the loading-end header to ensure the capillary tips are not crushed or damaged.	Daily or before each run	—
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	4-5
Check for dried polymer around the polymer block and clean as necessary.	Daily	—
Check for leaks around the syringes and screw nut.	Daily	—
Check data base space. Delete plate records from the instrument database and archive sample files.	Daily	4-35

Weekly Tasks

Perform these tasks at least once per week.

Maintenance Task	Frequency	See Page
Clean the syringes.	Weekly or as needed	4-22
Clean the water and buffer reservoirs with warm water.	Weekly	—
Clean the upper and lower polymer blocks.	Weekly	4-29
Replace the polymer in the syringes, upper polymer block, and capillary array.	Weekly or as needed	4-10
Check the storage conditions of the used arrays.	Weekly	—

As-Needed Tasks

Perform these tasks as needed.

Maintenance Task	Frequency	See Page
Clean the drip trays.	As needed	—
Change the array.	As needed	4-15
Replace syringes	3 months	—
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed	—
Calibrate the autosampler	Very rarely	4-33

Routine Cleaning

General Cleaning

To clean the instrument:

Step	Action
1	Press the **Tray** button on the front of the instrument to move the autosampler to the forward position.
2	Wipe off any liquid on or around the autosampler using a lint-free tissue.
3	Clean out the drip trays with deionized water and lint-free tissue.
4	Clean off any polymer build-up (crystals) on the instrument including the capillary tips and the stripper plate with deionized water and lint-free tissue. **IMPORTANT** Never use organic solvents to clean the instrument.

Moving and Leveling the Instrument

Before Moving the Instrument

⚠ WARNING **PHYSICAL INJURY HAZARD**. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Two or three people are required to lift the instrument, depending upon instrument weight.

To prepare for moving the instrument:

Step	Action
1	Remove the following components from the instrument: ♦ Any plate assemblies from the autosampler. ♦ Water and buffer reservoirs from the autosampler. ♦ Capillary array. For instruction see page 4-15. ♦ Syringes from the upper polymer block. For instruction see page 4-25. ♦ Upper polymer block. For instruction see page 4-28. ♦ Anode buffer reservoir. ♦ Lower polymer block. For instruction see page 4-28.
2	Switch off the breaker on the back of the instrument.
3	Disconnect the power cord and the Ethernet cable.
4	While moving the instrument, avoid any shock or vibration.

Leveling the Instrument

To level the instrument:

Step	Action
1	Place the bubble level on the autosampler deck.
2	Turn the instrument legs to level the instrument.

To move the instrument corner...	Turn the leg...
up	right (clockwise)
down	left (counterclockwise)

Resetting the Instrument

Overview

Reset the instrument when:

- There is a fatal error as indicated by the red status light
- The instrument does not respond to the ABI PRISM® 3100-Avant Data Collection software

There are two ways to reset the instrument:

- Press the Reset button on the front of the instrument to dump and reload the firmware and to reset the electronics. Try this method first.
- Shut down and restart the computer and the instrument.

Resetting With the Reset Button

To reset the instrument:

Step	Action
1	Close the instrument doors.
2	Using a long narrow implement, such as a straightened paper clip, press the Reset button on the front of the instrument. TRAY Reset button GR1770

Resetting by Powering Down

To reset the instrument:

Step	Action
1	Close the instrument doors.
2	Turn off the instrument by pressing the On/Off button on the front of the instrument.
3	Restart the computer. a. Select **Start > Shutdown**. b. In the Shutdown Windows dialog box, select **Restart** and click **OK**. **IMPORTANT** Wait until the computer has completely restarted before proceeding.
4	Turn on the instrument and wait for the solid green light. **Note** When the instrument is shut down, the firmware is not saved. Upon restart, the instrument will reload a copy of the firmware and the calibration file from the computer.
5	Open the data collection software.

Shutting Down the Instrument

Short- and Long-Term Shutdowns

Perform the appropriate shutdown procedure as follows:

If the instrument will be unattended for...	Perform this shutdown 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 in 1X running 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. For instructions, see page 4-18.
2	Push the **Tray** button to move the autosampler forward.
3	Fill the buffer reservoir with 1X running buffer to just below the top of the reservoir.
4	Fill other reservoirs with fresh deionized water.
5	Secure a septa onto the reservoir and place the reservoir in position 1 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 4-19 to remove and store the capillary array off the instrument.
2	Remove from the instrument: ♦ Syringes from the upper polymer block. For instructions see page 4-25. ♦ Upper polymer block. For instructions see page 4-28. ♦ Lower polymer block. For instructions see page 4-28.
3	Remove from the autosampler: ♦ Plate assembly ♦ 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.
7	Wash the syringes, polymer blocks, and reservoirs with warm water. Rinse with deionized water. **IMPORTANT** Make sure all parts are completely dry before long-term storage.

Section: Fluids and Waste

In This Section

The following topics are covered in this section:

Topic	See Page
Buffer	4-10
Polymer	4-10
Instrument Waste	4-11

Buffer

When to Change the Buffer

We recommend that you change the buffer before each run or at **least every 24 hours**.

Making Buffer for a Single Run

⚠ CAUTION **CHEMICAL HAZARD**. **10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare 50 mL of 1X running buffer:

Step	Action
1	Add 5 mL of 10X Genetic Analysis Buffer into a graduated cylinder.
2	Add deionized water to bring the total volume up to 50 mL.
3	Mix well.

Storing Buffer

The 1X running buffer can be stored at 2 to 8 °C for up to 1 month.

Polymer

Storing Polymer

Store any remaining ABI PRISM® 3100 POP™ polymer at 2 to 8 °C until the expiration date printed on the jar.

Note Excessively hot environments may shorten the working life of the polymer.

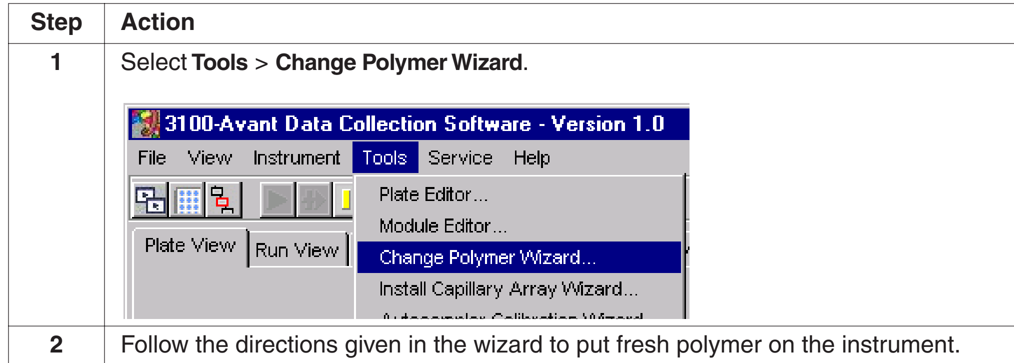
When to Change the Polymer

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

Adding and Changing the Polymer

⚠ CAUTION **CHEMICAL HAZARD**. **POP polymers** may cause eye, skin, and respiratory tract irritation. Read the MSDS for the polymer you are using, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

To put fresh polymer on the instrument:

Step	Action
1	Select **Tools** > **Change Polymer Wizard**. 
2	Follow the directions given in the wizard to put fresh polymer on the instrument.

Instrument Waste

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.
- ♦ 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.
- ♦ Handle chemical wastes in a fume hood.
- ♦ 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.

Section: Capillary Array

In This Section The following topics are covered in this section:

Topic	See Page
Before Installing a Previously Used Capillary Array	4-14
Installing and Removing the Capillary Array	4-15
Capillary Array Maintenance	4-17
Storing a Capillary Array on the Instrument	4-18
Storing a Capillary Array off the Instrument	4-19

Before Installing a Previously Used Capillary Array

Introduction

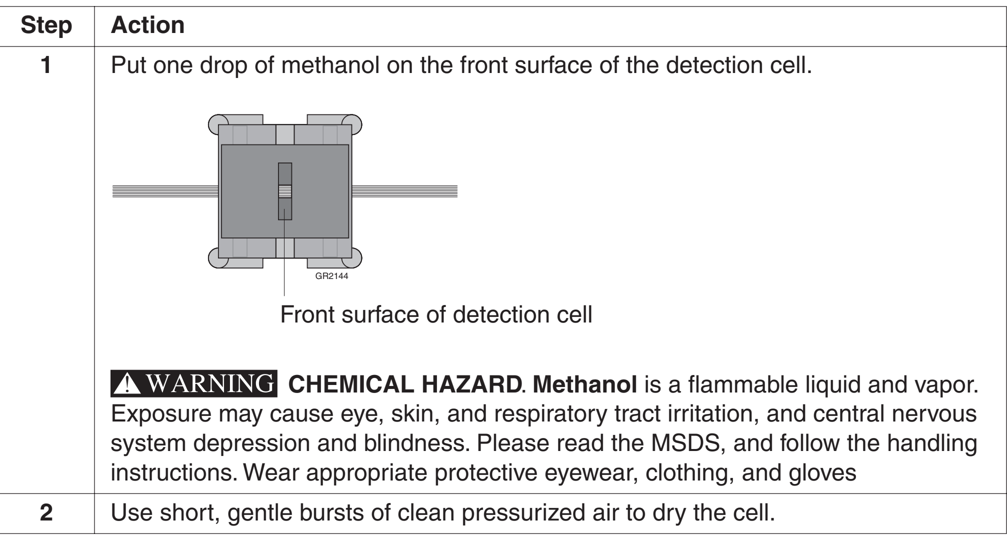
Before you reinstall a capillary array, it is recommended that you:

- ♦ 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.

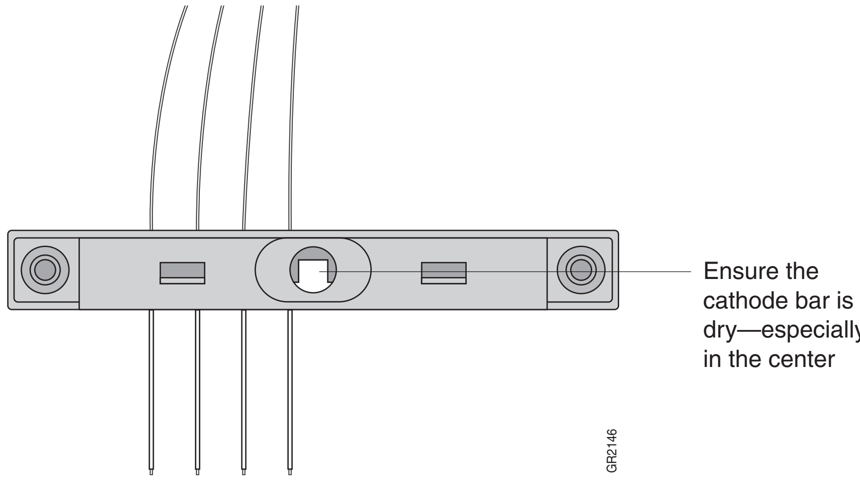
To clean the detection cell:

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
2	Use short, gentle bursts of clean pressurized air to dry the cell.

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:

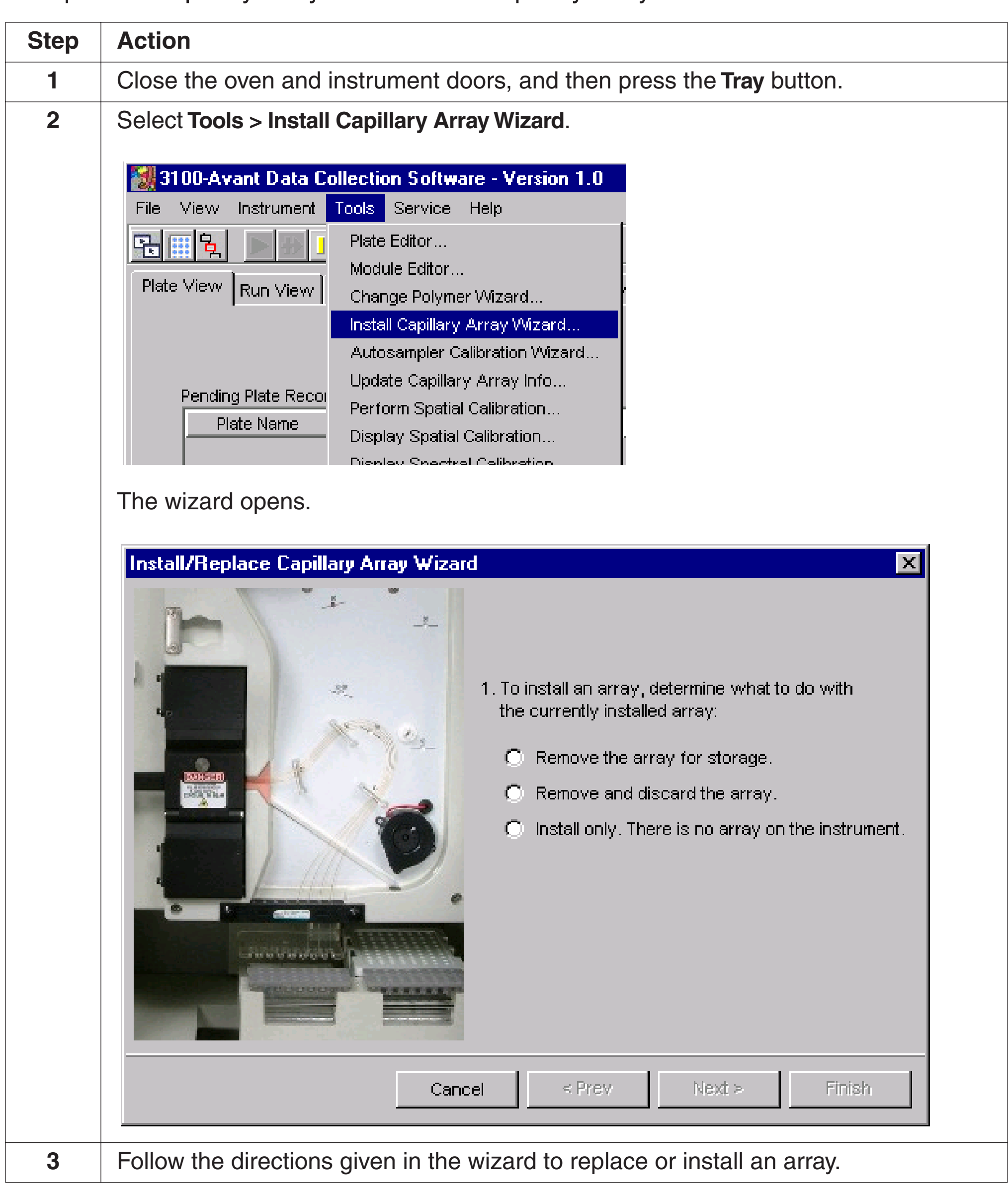
- ♦ Poor sizing precision or allele calling
- ♦ Poor resolution and/or decreased signal intensity

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 polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, 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:

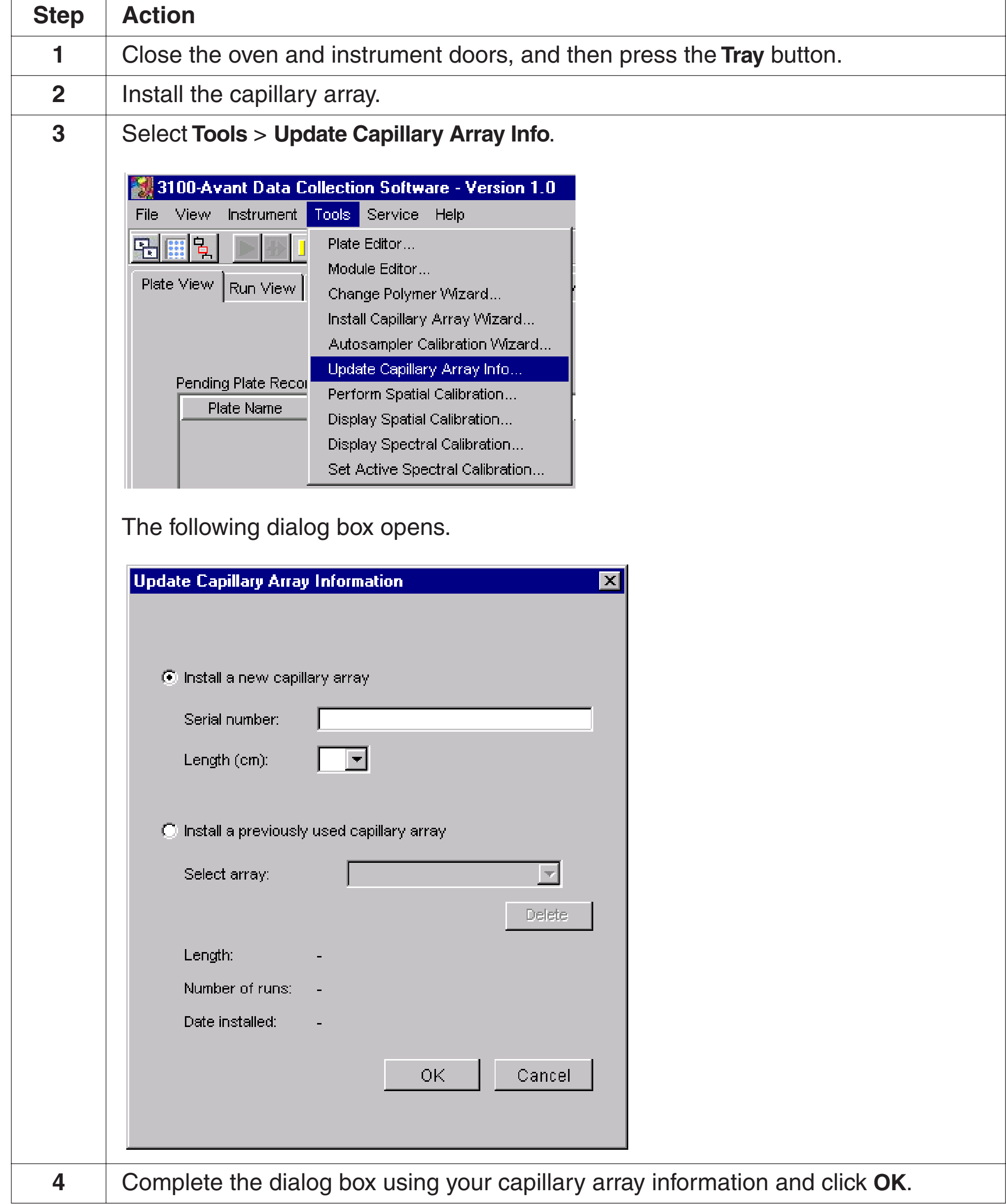
Step	Action
1	Close the oven and instrument doors, and then press the **Tray** button.
2	Select **Tools > Install Capillary Array Wizard**.  The wizard opens.
3	Follow the directions given in the wizard to replace or install an array.

Installing the Capillary Array Without Using the Install 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 polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

To install a capillary array on an instrument:

Step	Action
1	Close the oven and instrument doors, and then press the **Tray** button.
2	Install the capillary array.
3	Select **Tools** > **Update Capillary Array Info**.  The following dialog box opens.
4	Complete the dialog box using your capillary array information and click **OK**.

Capillary Array Maintenance

Caring for the Capillary Array

Follow these guidelines to properly care for the capillary array:

- ♦ Wear gloves and handle the capillary array gently.
- ♦ Do not touch the detection cell. If it is dirty, see “Cleaning the Detection Cell” on page 4-14.
- ♦ Keep the ends of the capillary array wet at all times.
- ♦ Always loosen the capillary array nut before pulling out the upper polymer block.
- ♦ Do not overtighten the capillary array nut.

Cleaning the Capillary Array

⚠ CAUTION **CHEMICAL HAZARD**. POP polymer may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

To clean the capillary array:

Step	Action
1	Flush the capillary array with fresh polymer as instructed in the “Installing and Removing the Capillary Array” on page 4-15.
2	Clean off any polymer buildup (crystals) on the instrument, including the capillary electrodes and the stripper plate, with deionized water and lint-free tissue. **Note** When cleaning the capillary electrodes, be careful not to bend them out of position. If the electrodes do get bent, follow the procedure “Verifying Capillary Alignment Using the Capillary Ruler” below. **IMPORTANT** Never use organic solvents to clean the instrument.
3	Clean the detection cell as instructed on page 4-14.

Filling the Capillary Array with Polymer Using Manual Control

To fill the capillary array with polymer using manual control commands:

Step	Action
1	Select **Instrument** > **Manual Control**.
2	In the Command Category drop-down list, select **Capillary**.
3	In the Command Name drop-down list, select **Fill**.
4	In the Value drop-down list, select the appropriate array length and polymer.
5	Click **Send Command**. Wait until you see the message Command complete before continuing.

Verifying Capillary Alignment Using the Capillary Ruler

To check capillary alignment using the capillary ruler:

Step	Action
1	Place the ruler beside the capillaries and detach a side of the ruler to the bottom of the holder.
2	Verify that the capillaries match the lines of the ruler.
3	Check both sides of the capillaries.
4	Place the capillary array holder on the flat surface and stand the ruler up at the end of capillaries.
5	Verify that the cross points of line on the ruler to match the end of capillaries. If some of capillaries are bent, adjust each capillary carefully.

Storing a Capillary Array on the Instrument

Overview

Store the capillary array on the instrument when the capillary array will be **unused for less than 1 week**.

Storing the Array on the Instrument

To store the capillary array on the instrument, follow the instructions to perform a short-term shutdown on page 4-8.

Storing a Capillary Array off the Instrument

Overview

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

⚠ CAUTION **CHEMICAL HAZARD**. **POP polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

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 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 4-15.
6	Replace the cover over the detection cell.
7	Fill a buffer reservoir with fresh 1X running buffer and cover with a septa strip. Insert the capillary tips into the buffer.
8	Fill the shipping vial with fresh 1X running buffer and insert the detection end of the capillary array.
9	Store the capillary array upright.
10	Check the 1X running buffer level in the reservoir and tube weekly.

Section: Syringes

In This Section The following topics are covered in this section:

Topic	See Page
Syringe Maintenance	4-22
Priming and Filling Syringes	4-24
Installing and Removing Syringes	4-25

Syringe Maintenance

Syringe Types

The following table lists the name, volume, and function of the two syringes:

Name	Volume	Function
Array-fill syringe	250 *µ*L	High pressure syringe that displaces polymer into the capillary array
Polymer-reserve syringe	5 mL	Stores polymer for multiple sequential runs

Caring for Syringes

IMPORTANT To extend the lifetime of the syringe plunger's Teflon fitting, do not insert a dry plunger into the barrel of the syringe. Place a small drop of deionized water on the plunger's end before inserting it into the syringe. Pump the plunger slowly.

IMPORTANT Do not mix the barrels and plungers from different syringes. Mixing and matching is a common cause of leaks.

IMPORTANT Wear gloves while handling the glass syringes.

Replacing the Syringes

To maintain optimal performance, we recommend that you replace syringes about every 3 months.

Cleaning Syringes

Clean the syringes thoroughly:

- ♦ 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

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	Remove the syringe guard.
2	Remove the syringes as described on page 4-25.
3	Clean the syringe thoroughly by rinsing the inside and outside of the syringe barrel and the syringe tip with warm water. **IMPORTANT** Be sure there is no dried polymer left in the syringes.
4	Rinse the syringe barrel and tip with deionized water.
5	Blow dry with compressed air.
6	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.

Priming and Filling Syringes

Priming and Filling the Polymer-Reserve Syringe

Follow this procedure after cleaning the polymer-reserve syringe or before the polymer in the syringe is 1 week old.

⚠ CAUTION **CHEMICAL HAZARD. POP polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

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

To prepare the polymer-reserve syringe for use:

Step	Action
1	Draw approximately 0.3 mL of room-temperature polymer into a clean polymer-reserve syringe.
2	Pull up the plunger to the 5-mL mark.
3	Invert the syringe about six times to coat the walls with polymer. Discard this polymer into aqueous waste. **Note** Priming the syringe ensures that the running polymer is at the intended concentration and not diluted by residual water.
4	Fill the polymer-reserve syringe with a maximum of 4.5 mL of polymer. **IMPORTANT** Avoid introducing air bubbles into the polymer by keeping the syringe tip just submerged in the polymer while aspirating gently.
5	Remove any air bubbles by inverting the syringe and pushing a small amount of polymer out of the tip. **Note** Do not return the unused portion of the polymer to the bottle.

Priming and Filling the Array-Fill Syringe

⚠ CAUTION **CHEMICAL HAZARD. POP polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

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

To prepare the array-fill syringe for use:

Step	Action
1	Draw a small volume of room-temperature polymer into a clean array-fill syringe.
2	Pull up the plunger to the 250-μL mark.
3	Invert the syringe about six times to coat the walls with polymer. Discard this polymer into aqueous waste. **Note** Priming the syringe ensures that the running polymer is at the intended concentration and not diluted by residual water.
4	To prevent air bubbles, gently and slowly aspirate the polymer into the syringe until the desired volume has been reached.
5	Point the syringe up and slightly press the plunger to purge any air.

Installing and Removing Syringes

Installing Syringes

To install the syringes:

Step	Action
1	Follow the procedures to remove, clean, and dry the upper polymer block starting on page 4-28.
2	Place the polymer-reserve syringe tip in the left port on the top of the upper polymer block and screw the syringe tip clockwise into the polymer block. **IMPORTANT** Always hold the syringe by the metal sleeve—not the glass—when screwing the syringe into the block. The syringe should be finger tight in the block.
3	Place the array-fill syringe tip in the right port on the top of the upper polymer block and screw the syringe tip clockwise into the polymer block. **IMPORTANT** Always hold the syringe by the metal sleeve—not the glass—when screwing the syringe into the block. The syringe should be finger tight in the block.
4	Push the polymer block all the way against the instrument.
5	Replace the syringe guard.

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. Do not loosen this fitting while removing the syringe. GR1876 **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	Dispose of any remaining polymer properly.

Section: Polymer Blocks

In This Section

The following topics are covered in this section:

Topic	See Page
Removing the Polymer Blocks	4-28
Cleaning the Polymer Blocks	4-29
Removing Air Bubbles from the Upper Polymer Block	4-32

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 4-25.
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 4-18.
4	Disconnect the polymer block tube from the lower polymer.
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 dispose of the buffer properly.
2	Grasp the lower polymer block and pull it straight out.

Cleaning the Polymer Blocks

Frequency

Clean the upper and lower polymer blocks:

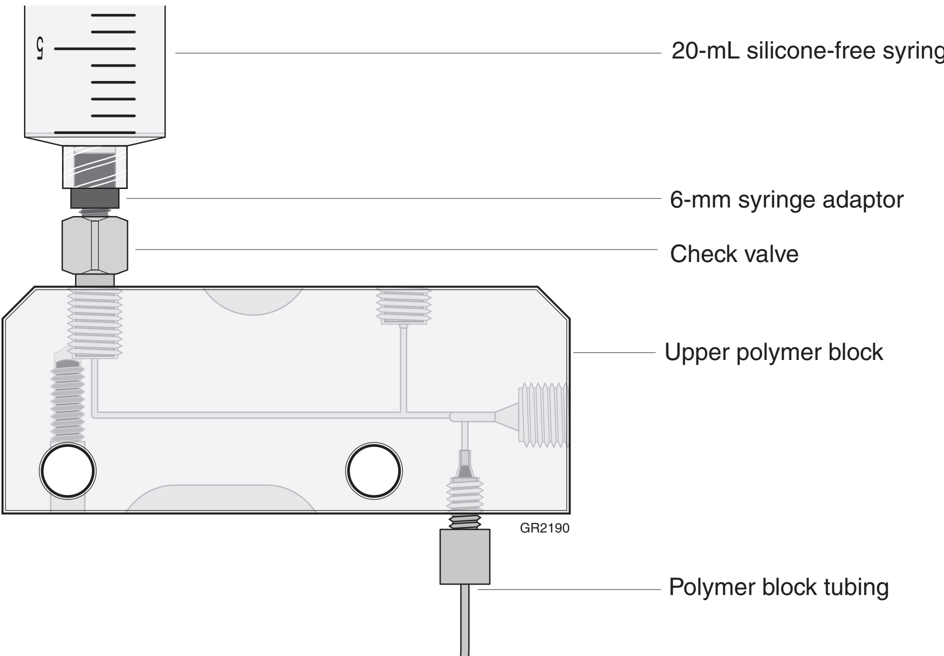
- ♦ Before replacing the polymer on the instrument
- ♦ When the polymer has been on the instrument for longer than 1 week

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

Cleaning the Upper Polymer Block

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

Below is a picture of the upper polymer block with the syringe and syringe adaptor attached.



To clean the upper polymer block:

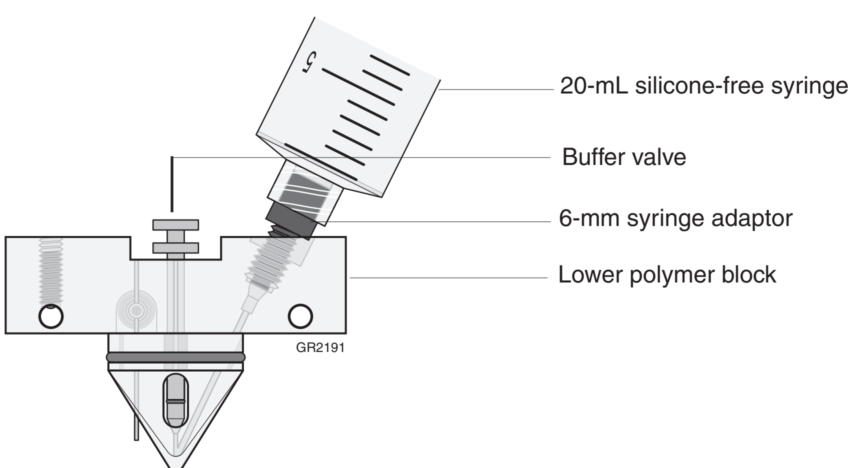
Step	Action
1	Rinse all the fittings with hot deionized water. Soak any fittings that are covered with polymer. **IMPORTANT** Do not use boiling water to rinse the fittings or the polymer block.
2	Hold the upper polymer block under hot deionized water.
3	Fit the 6-mm syringe adaptor (P/N 4322928) onto the 20-mL silicone-free syringe (P/N 4324463).
4	Thread the 6-mm syringe adaptor into the stainless-steel check valve.
5	Force several syringe loads of hot deionized water through each channel in turn by sealing their openings with your fingers. **Note** Force deionized water through polymer block tubing also.

To clean the upper polymer block: *(continued)*

Step	Action
6	Inspect the channels visually for dried polymer, which is white residue. Wash partially occluded channels with hot deionized water until the dried polymer is gone. **IMPORTANT** It may take a long time for the hot water to clear the obstruction. Do not use a sharp pointed instrument to clear the channel, even if the channel is completely occluded with dried polymer.
7	Rinse the upper polymer block and all the fittings thoroughly using deionized water.
8	a. Remove any residual water from the upper polymer block and fittings to ensure that fresh polymer is not diluted. This can be done by forcing air through the channels using the silicone-free syringe or by using canned compressed air. b. Force the air through the channels until the channels are dry. **IMPORTANT** When using canned compressed air, be careful not to blow propellent from the can into the block. Do not shake or invert the can. Some propellants may increase background fluorescence. **IMPORTANT** Do not use the 5.0-mL glass syringe to force air through the channels. This will damage the syringe's plunger and cause the syringe to leak.

Cleaning the Lower Polymer Blocks

Below is a picture of the lower polymer block with the syringe and syringe adaptor attached.



To clean the lower polymer block:

Step	Action
1	Verify that the buffer valve is open (in the up position).
2	Remove the polymer block tubing and fitting from the upper polymer block, if this was not done before.
3	Remove the lower polymer block from the instrument.
4	Rinse all the fittings with hot deionized water. Soak any fittings that are covered with polymer. **IMPORTANT** Do not use boiling water to rinse the fittings or the polymer block.

To clean the lower polymer block: *(continued)*

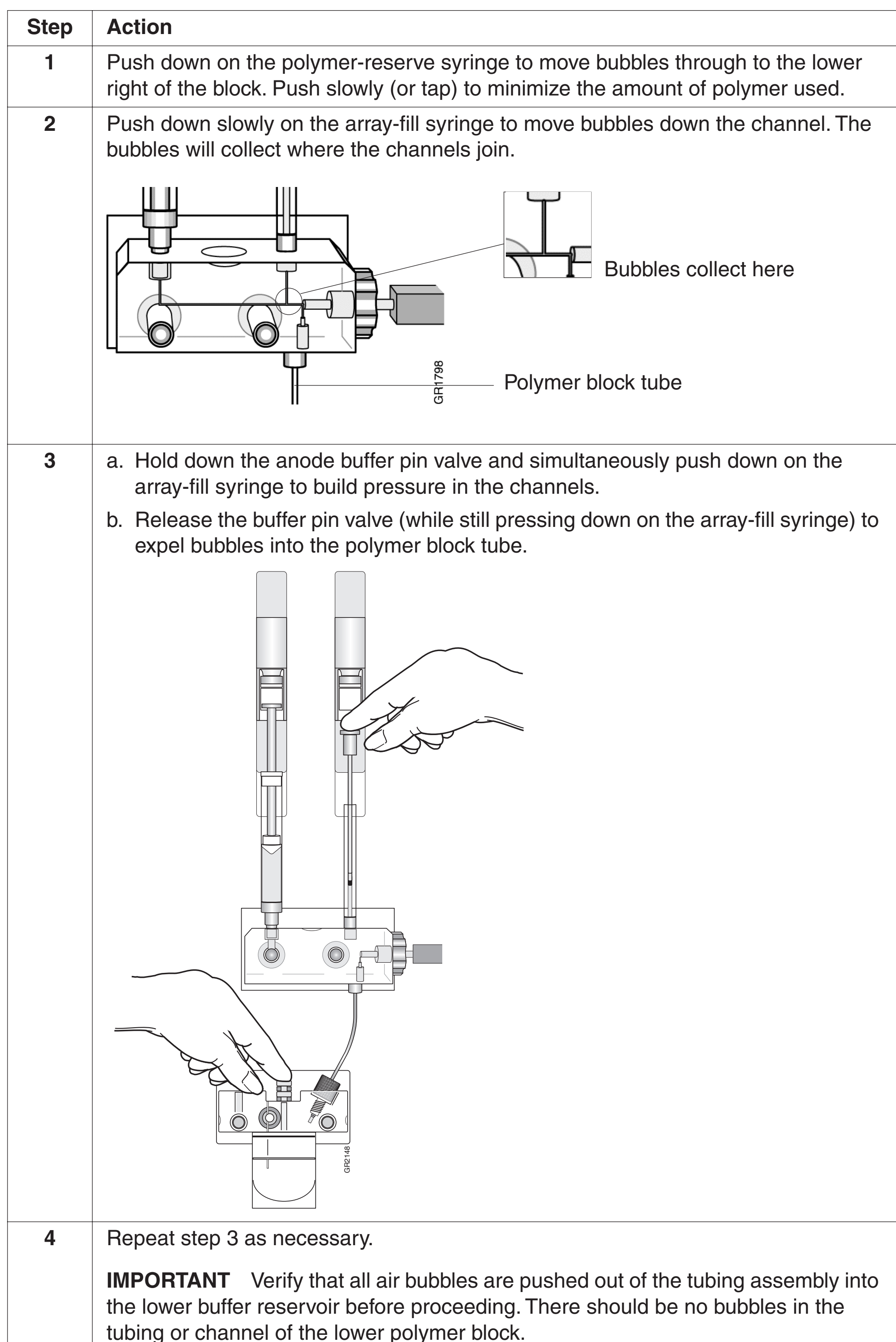
Step	Action
5	Hold the lower polymer block under hot deionized water. Using your fingers, move the buffer valve in and out to ensure any encrusted polymer is cleaned out of its guide channel. **IMPORTANT** Do not remove any of the components from the lower polymer block.
6	Fit the 6-mm syringe adaptor (P/N 4322928) onto the 20-mL silicone-free syringe (P/N 4324463).
7	Thread the 6-mm syringe adaptor into the polymer block where the polymer block tube fitting was originally located.
8	Force several syringe loads of hot deionized water through the channel.
9	Inspect the channels visually for dried polymer, which is white residue. Wash partially occluded channels with hot deionized water until the dried polymer is gone. **IMPORTANT** It may take a long time for the hot water to clear the obstruction. Do not use a sharp pointed instrument to clear the channel, even if the channel is completely occluded with dried polymer.
10	Rinse the lower polymer block and all the fittings thoroughly using deionized water.
11	a. Remove any residual water from the lower polymer block and fittings to ensure that fresh polymer is not diluted. This can be done by forcing air through the channels using the silicone-free syringe or by using canned compressed air. b. Force the air through the channels until the channels are dry. **IMPORTANT** When using canned compressed air, be careful not to blow propellent from the can into the block. Do not shake or invert the can. Some propellants may increase background fluorescence. **IMPORTANT** Do not use the 5.0-mL glass syringe to force air through the channels. This will damage the syringe's plunger and cause the syringe to leak.

Removing Air Bubbles from the Upper Polymer Block

Clearing Air Bubbles

To clear air bubbles from the upper polymer block:

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.
4	Repeat step 3 as necessary. **IMPORTANT** Verify that 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.



Section: Autosampler Calibration

When to Calibrate the Autosampler

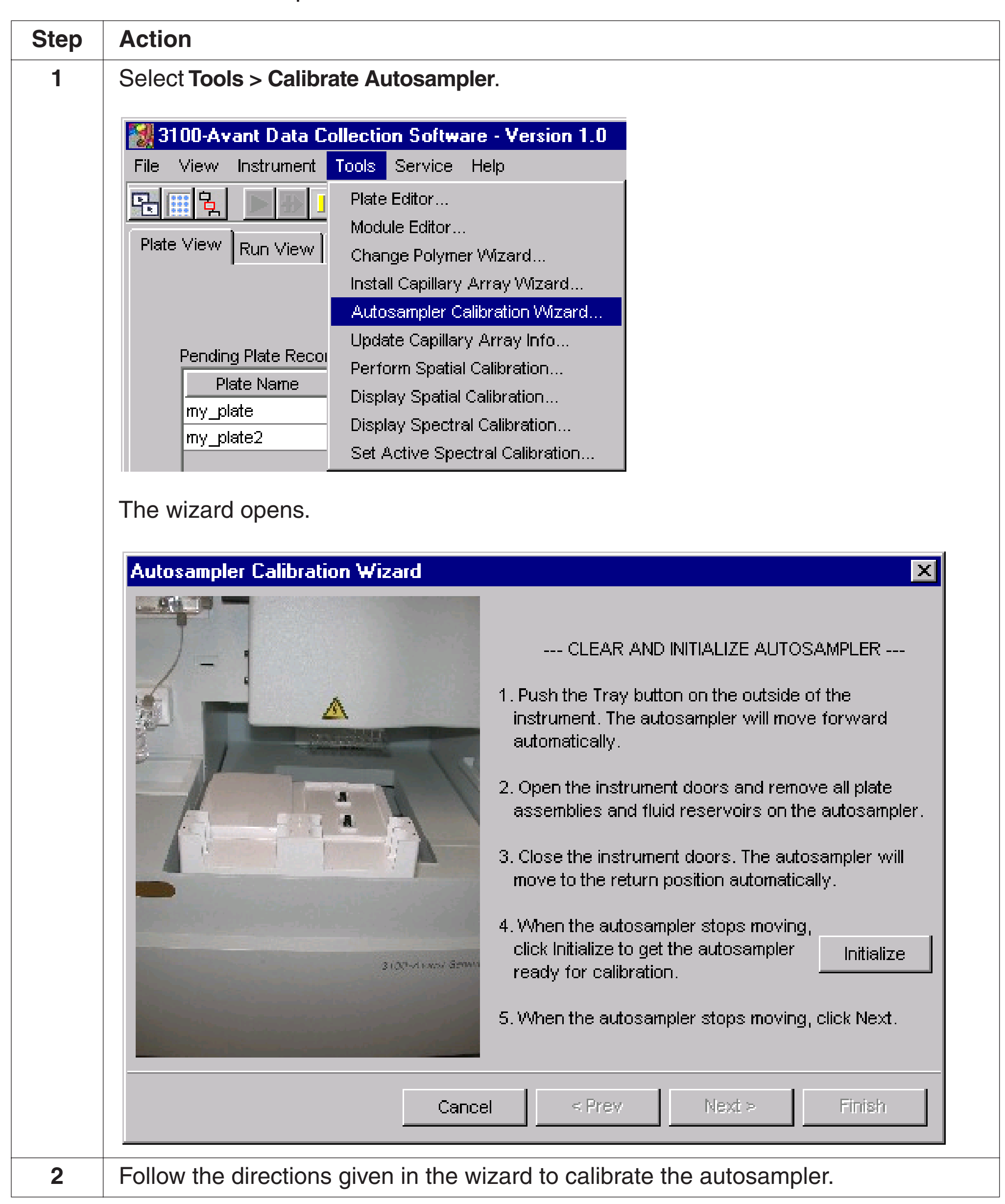
Calibrate the autosampler only as needed.

Symptoms of autosampler alignment problems may include:

- ♦ Poor injection for a small number of capillaries
- ♦ Low signal strength
- ♦ No evidence of sample

Calibrating the Autosampler

To calibrate the autosampler:



Step	Action
1	Select **Tools > Calibrate Autosampler**. The wizard opens.
2	Follow the directions given in the wizard to calibrate the autosampler.

Section: Checking the Available Space and Deleting Records

In This Section

The following topics are covered in this section:

Topic	See Page
Checking the Available Hard Drive Space	4-36
Archiving Data	4-37
Checking the Available Database Space	4-39
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Overview

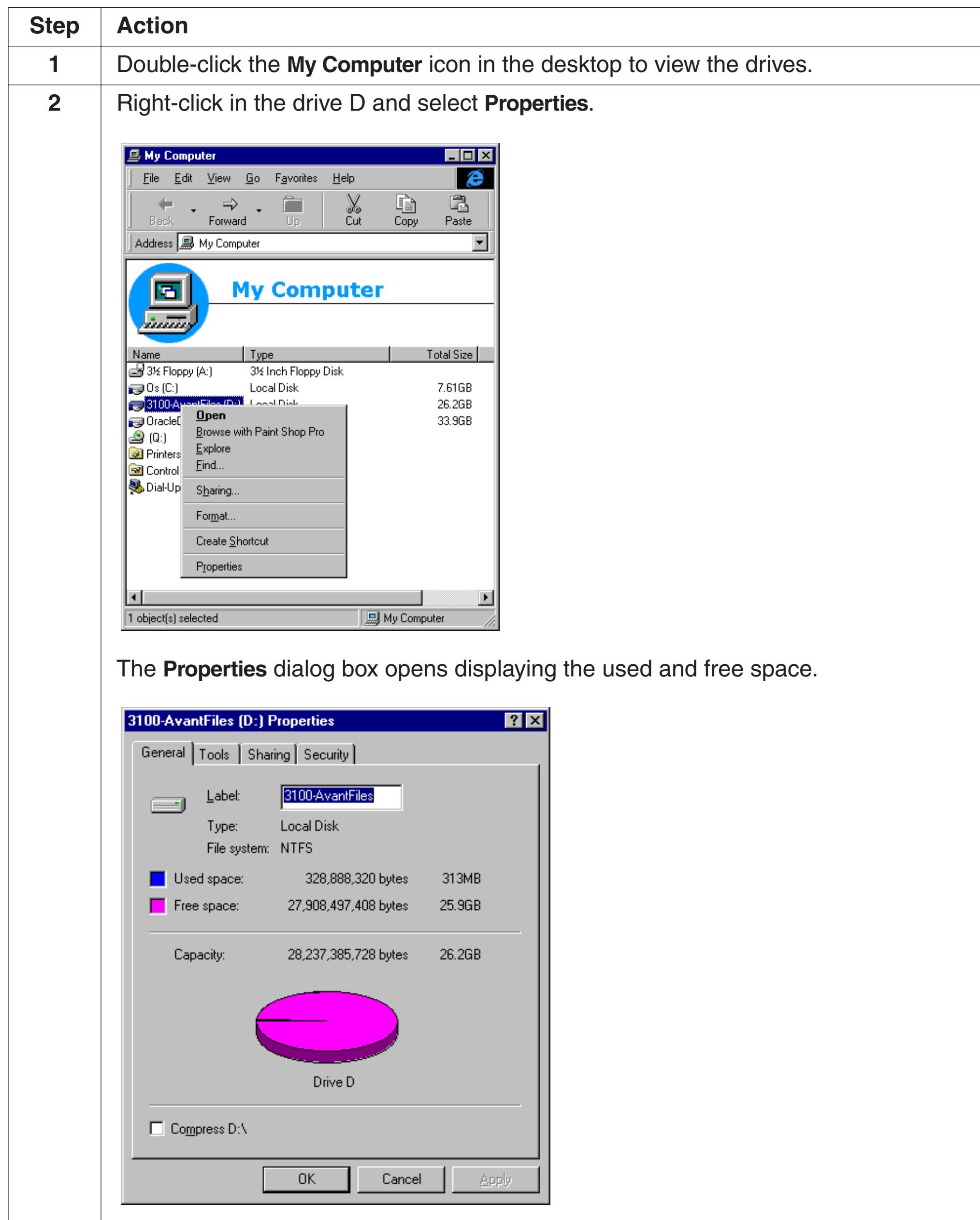
The sections that follow tell you:

- How to check the available hard disk space on drive D for the extracted sample files
- How to check the available space in the instrument database on drive E for the raw data

Checking the Available Hard Drive Space

Checking Hard Disk Space

To check the hard disk for space for sample files:

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

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

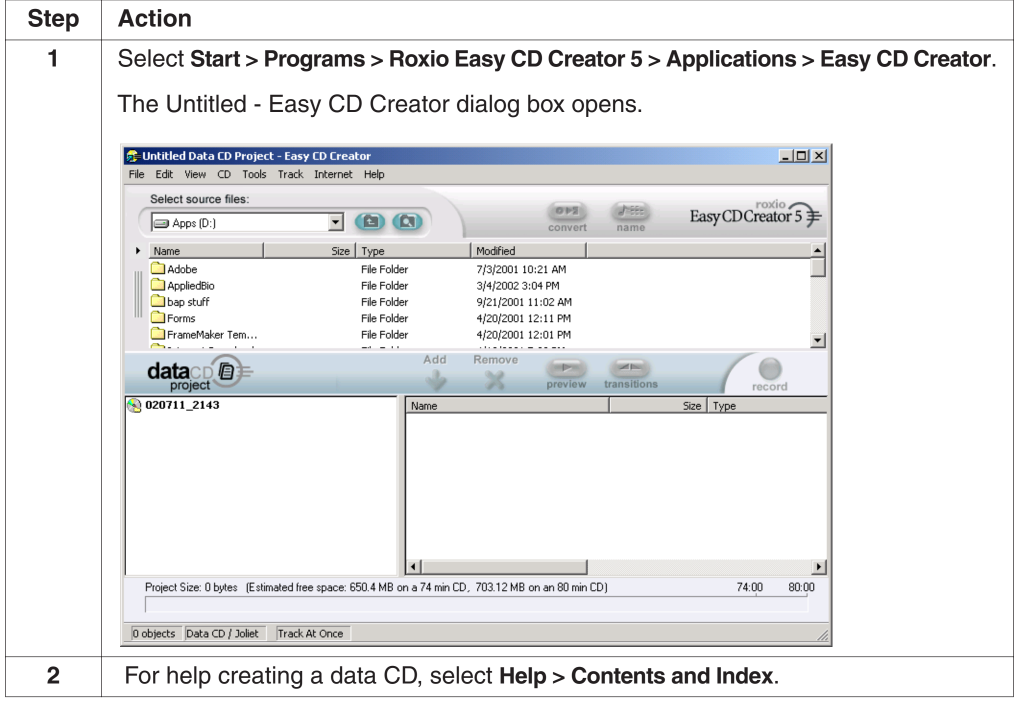
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Estimate how much free space you need by using the information provided below.
<table>
<tr><th>File Type</th><th>Approximate Space Required Per File (kB)[a]</th></tr>
<tr><td>Analyzed sample file for DNA sequencing</td><td>250</td></tr>
<tr><td>Analyzed sample file for fragment analysis</td><td>500</td></tr>
<tr><td>Unanalyzed sample file</td><td>100</td></tr>
</table>
a. The values provided are estimates only. The actual file size depends on the run module selected.</td></tr>
<tr><td>4</td><td>If there is insufficient space:
♦ Archive the sample files to a CD-RW or another volume.
♦ Delete the original files from the drive.</td></tr>
</table>

Archiving Data

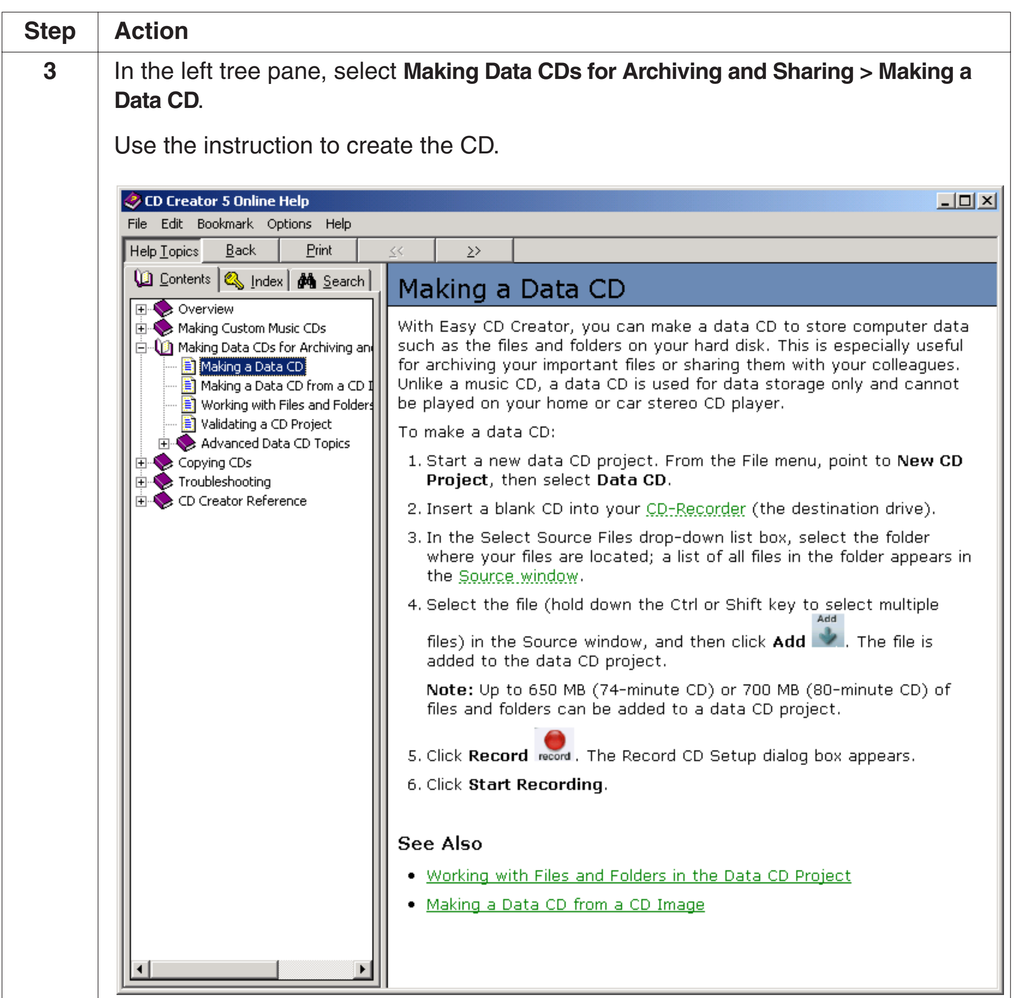
Creating a Data CD

A basic version of Roxio Easy CD Creator™ 5 software was loaded on your Dell™ computer. Use this software to archive data to a CD. The software is also part of the CD set you received with your Dell computer.

To archive data:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select Start > Programs > Roxio Easy CD Creator 5 > Applications > Easy CD Creator.
The Untitled - Easy CD Creator dialog box opens.

</td></tr>
<tr><td>2</td><td>For help creating a data CD, select Help > Contents and Index.</td></tr>
</table>

To archive data: *(continued)*

Step	Action
3	In the left tree pane, select **Making Data CDs for Archiving and Sharing > Making a Data CD**. Use the instruction to create the CD. 

Installing a SCSI Storage Device

We do not recommend that you add an SCSI storage device to the computer workstation. However, if you need to temporarily install one, follow the procedure below.

IMPORTANT Do not install a SCSI device on the computer workstation before the 3100-Avant Genetic Analyzer has been installed with the 3100-Avant Data Collection software. Installing a SCSI device first will alter the drive letter assignments so that the instrument and software cannot be properly installed.

To install a SCSI storage device:

Step	Action
1	Shut down the computer workstation.
2	Plug the device into the external SCSI port.
3	Turn the computer workstation back on.
4	Ensure the drive letter assignments have not changed.

Checking the Available Database Space

Automatic Checking of the Database

Before a run or batch of runs, the data collection software will automatically check the available space to ensure sufficient space to store the data you will create.

If the database does not contain enough space (~75% full), then use the cleanup DB utility to remove data and plate records.

Deleting Records from the Database

Deleting Processed Frame Data

⚠ CAUTION The Cleanup Database utility deletes all run data and plate records in the database. Before running the utility, be sure that all runs have been extracted from the database.

To delete processed frame data using the Cleanup Database utility:

Step	Action
1	Ensure OrbixWeb Daemon is running.
2	Quit the 3100-Avant Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\3100-Avant\Bin
4	Locate and double-click **CleanUpDB.bat**. This runs the Cleanup Database utility, which takes a few seconds to complete.
5	Shut down and then relaunch OrbixWeb Daemon. **⚠ CAUTION** If you do not perform this step, any new run data will not be saved to the database.

Note There is no need to reimport the spatial, spectral, and run calibration methods or the calibration data obtained from the last calibration runs.

Technical Support A

Services and Support

Applied Biosystems Web Site

A services and support page is available on the Applied Biosystems Web site. To access this, go to:

http://www.appliedbiosystems.com

and click the link for services and support.

At the services and support page, you can:

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

In addition, the services and support page provides worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Limited Warranty Statement

Applied Biosystems Limited Warranty Statement

Applied Biosystems warrants to the customer that, for a period ending on the earlier of one year from the completion of installation or fifteen (15) months from the date of shipment to the customer (the "Warranty Period"), the ABI PRISM® 3100-Avant Genetic Analyzer purchased by the customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the published performance specifications contained in the 3100-Avant Genetic Analyzer Specification Sheet (the "Specifications") publication number 106SP04-01.

During the Warranty Period, if the Instrument's hardware becomes damaged or contaminated or if the Instrument otherwise fails to meet the Specifications, Applied Biosystems will repair or replace the Instrument so that it meets the Specifications, at Applied Biosystems expense. However, if the 3100-Avant Genetic Analyzer becomes damaged or contaminated, or if the chemical performance of the Instrument otherwise deteriorates due to solvents and/or reagents other than those supplied or expressly recommended by Applied Biosystems, Applied Biosystems will return the Instrument to Specification at the customer's request and at the customer's expense. After this service is performed, coverage of the parts repaired or replaced will be restored thereafter for the remainder of the original Warranty Period.

This Warranty does not extend to any Instrument or part which has been (a) the subject of an accident, misuse, or neglect (including but not limited to failure to follow the recommended maintenance procedures), (b) modified or repaired by a party other than Applied Biosystems, or (c) used in a manner not in accordance with the instructions contained in the Instrument User Guide. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User Reference Guide. Those items are covered by their own warranties.

Applied Biosystems obligation under this Warranty is limited to repairs or replacements that Applied Biosystems deems necessary to correct those failures of the Instrument to meet the Specifications of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty will be performed by Applied Biosystems on site at the Customer's location at Applied Biosystems sole expense.

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Printed in USA, 7/2002
Part Number 4333549 Rev. B

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