ABI PRISM® 3100 Genetic Analyzer

User's Manual

Applied Biosystems | HITACHI

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER

This instrument, Serial No. _____, is Authorized for use in DNA sequencing and fragment analysis. This Authorization is included in the purchase price of this instrument and corresponds to the up-front fee component of a license under process claims of U.S. patents and under all process claims for DNA sequence and fragment analysis of U.S. patents now or hereafter owned or licensable by Applied Biosystems for which Authorization is required, and under corresponding process claims is foreign counterparts of the foregoing for which an Authorization is required. The running royalty component of licenses may be purchased from Applied Biosystems or obtained by using Authorized reagents purchased from Authorized supplier in accordance wit the label rights accompanying such reagents. Purchase of this instrument does not itself convey to the purchaser a complete license or right to perform the above processes. This instrument is also licensed under U.S. patents and apparatus and system claims in foreign counterparts thereof. No rights are granted expressly, by implication or by estoppel under composition claims or under other process or system claims owned or licensable by Applied Biosystems. For more information regarding licenses, please contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404.

The ABI PRISM® 3100 Genetic Analyzer includes patented technology licensed from Hitachi, Ltd. as part of a strategic partnership between Applied Biosystems and Hitachi, Ltd., as well as patented technology of Applied Biosystems.

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For international office locations, please call our local office or refer to our web site at www.appliedbiosystems.com.

Record information about your software below.

Software CD	Serial Number	Version Number	Registration Code
3100 Software			
Oracle® for NT			
GeneScan® Application			
Sequencing Analysis Application			

Contents

3 Performing a Run

4 Spatial and Spectral Calibrations

5 Software

6 Working with Plate Records

7 System Management and Networking

8 Maintenance

1 Introduction and Safety

Overview

In This Chapter

The following topics are covered in this chapter:

ABI PRISM 3100 Genetic Analyzer

Definition

The ABI PRISM® 3100 Genetic Analyzer is an automated capillary electrophoresis system that can separate, detect, and analyze up to 16 capillaries of fluorescently labeled DNA fragments in one run.

System Components

The 3100 Genetic Analyzer system includes the following components:

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

To Get Started Quickly

Important Safety Information

Before using the 3100 Genetic Analyzer, read the safety information starting on page 1-5 and in the *ABI PRISM 3100 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 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 Genetic Analyzer into your existing laboratory data flow system

Getting Started Quickly

The following table lists the sources of specific information to help you get started quickly:

For instruction on how to...	Refer to...
♦ prepare DNA templates ♦ perform cycle sequencing ♦ prepare extension products	*ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide* (P/N 4315831)
♦ prepare samples for sequencing analysis ♦ perform a sequencing analysis run and view run data	*ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Sequencing* (P/N 4315833)
♦ prepare samples for fragment analysis ♦ perform a fragment analysis run and view run data	*ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Fragment Analysis* (P/N 4315832)
calibrate this instrument	Chapter 4, "Spatial and Spectral Calibrations."
operate this instrument (detailed)	Chapter 3, "Performing a Run."

Additional Documentation

List of User Documents

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

Title	Contents	P/N
	Instrument	
ABI PRISM 3100 Genetic Analyzer Site Preparation and Safety Guide	♦ Laboratory requirements for installation ♦ Instrument and chemical safety	4315835
ABI PRISM 3100 Genetic Analyzer User's Manual	♦ User procedures ♦ Instrument maintenance ♦ Troubleshooting	4315834
ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Fragment Analysis	Abbreviated procedures for performing a fragment analysis run	4315832
ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Sequencing	Abbreviated procedures for performing a sequencing run	4315833
	Software	
ABI PRISM DNA Sequencing Analysis Software v. 3.6 NT User's Manual	Detailed procedures for analyzing sequencing data	4308924
ABI PRISM DNA Sequencing Analysis Software Release Notes		
ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual	Detailed procedures for analyzing fragment analysis data	4308923
ABI PRISM GeneScan Analysis Software Release Notes		
	Chemistry	
ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide	♦ Detailed chemistry procedures specific for the 3100 Genetic Analyzer ♦ Chemistry troubleshooting for the 3100 Genetic Analyzer	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 are the mechanism we use to inform our customers of technical information, product improvements, and related new products and laboratory techniques.

User bulletins related to the use of this instrument will be mailed 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.

⚠ 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 and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- 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 and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing).
- 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.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>a. Go to our Web site at www.appliedbiosystems.com/techsupp
b. Click MSDSs
<table><tr><th>If you have...</th><th>Then...</th></tr><tr><td>The MSDS document number or the Document on Demand index number</td><td>Enter one of these numbers in the appropriate field on this page.</td></tr><tr><td>The product part number</td><td rowspan="2">Select Click Here, then enter the part number or keyword(s) in the field on this page.</td></tr><tr><td>Keyword(s)</td></tr></table>c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or email.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Documents on Demand” under “Technical Support.”</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
<tr><td>By telephone from any other country</td><td>See the specific region under “To Contact Technical Support by Telephone or Fax” under “Technical Support.”</td></tr>
</table>

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

Instrument Safety Labels

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

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

About Waste Disposal

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

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

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

Before Operating the Instrument

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

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

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

Safe and Efficient Computer Use

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

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

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

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

  - Lumbar support should be provided to maintain the proper concave curve of the spine.
- Place the keyboard on a surface that provides:
  - The proper height to position the forearms horizontally and upper arms vertically.
  - Support for the forearms and hands to avoid muscle fatigue in the upper arms.
- Position the viewing screen to the height that allows normal body and head posture. This height depends upon the physical proportions of the user.
- Adjust vision factors to optimize comfort and efficiency by:
  - Adjusting screen variables, such as brightness, contrast, and color, to suit personal preferences and ambient lighting.
  - Positioning the screen to minimize reflections from ambient light sources.
  - Positioning the screen at a distance that takes into account user variables such as nearsightedness, farsightedness, astigmatism, and the effects of corrective lenses.
- When considering the user's distance from the screen, the following are useful guidelines:
  - The distance from the user's eyes to the viewing screen should be approximately the same as the distance from the user's eyes to the keyboard.
  - For most people, the reading distance that is the most comfortable is approximately 20 inches.
  - The workstation surface should have a minimum depth of 36 inches to accommodate distance adjustment.
  - Adjust the screen angle to minimize reflection and glare, and avoid highly reflective surfaces for the workstation.
- Use a well-designed copy holder, adjustable horizontally and vertically, that allows referenced hard-copy material to be placed at the same viewing distance as the screen and keyboard.
- Keep wires and cables out of the way of users and passersby.
- Choose a workstation that has a surface large enough for other tasks and that provides sufficient legroom for adequate movement.

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.

System Overview 2

Overview

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

Section: 3100 Instrument Overview

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

ABI PRISM 3100 System Components

Table of Components

The ABI PRISM® 3100 Genetic Analyzer system includes the following components:

Component	For detailed information, see...
ABI PRISM 3100 Genetic Analyzer	♦ "What the Instrument Does" on page 2-5. ♦ "How the Instrument Works" on page 2-6. ♦ "Instrument Hardware Overview" on page 2-9.
Computer workstation with Microsoft® Windows® NT operating system	♦ "Computer Workstation" on page 2-14. ♦ "Managing Hard Drive and Instrument Database Space" on page 7-3.
ABI PRISM 3100 Genetic Analyzer software	♦ "Software" on page 2-15. ♦ Chapter 5, "Software."
ABI PRISM® DNA Sequencing Analysis or GeneScan® Analysis software	♦ *ABI PRISM DNA Sequencing Analysis Software v. 3.6 NT User's Manual* (P/N 4308924). ♦ *ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual* (P/N 4308923).
Capillary Array	♦ "Capillary Array" on page 2-24. ♦ Appendix C, "Part Numbers."
Reagent consumables	♦ "Polymers" on page 2-20. ♦ "Injection Solution" on page 2-21. ♦ Appendix C, "Part Numbers."

What the Instrument Does

Types of Analysis

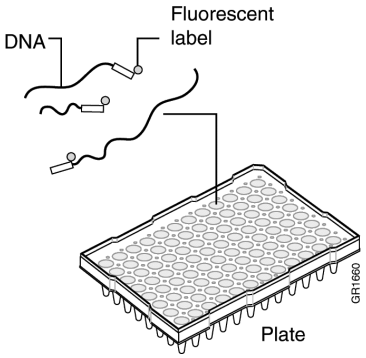
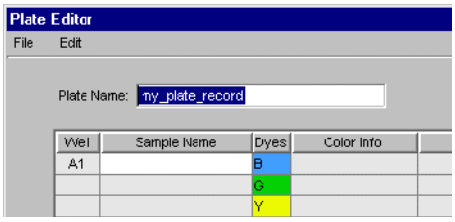
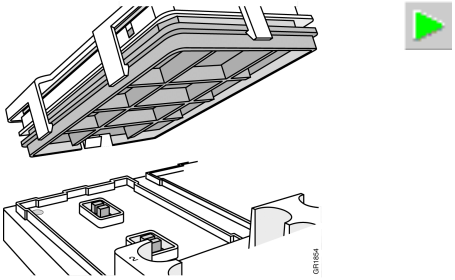
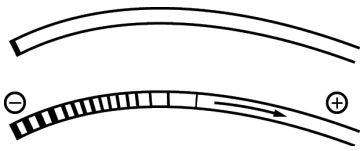
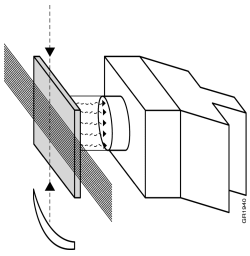
The 3100 Genetic Analyzer performs two kinds of analysis:

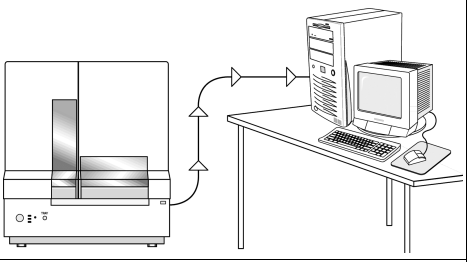
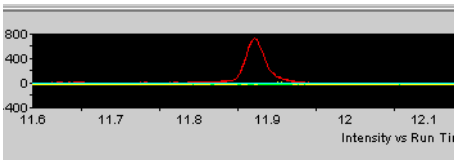
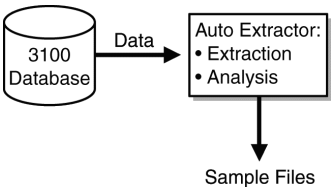
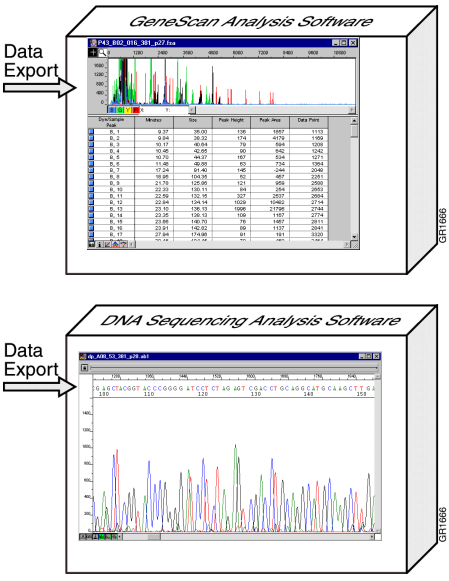
DNA Analysis	Purpose
Sequencing analysis	♦ Separates a mixture of DNA fragments according to their lengths ♦ Provides a profile of the separation ♦ Determines the order of the four deoxyribonucleotide bases
Fragment analysis	♦ Separates a mixture of DNA fragments according to their lengths ♦ Provides a profile of the separation ♦ Determines the length of each fragment (in basepairs) ♦ Estimates the relative concentration of each fragment in the sample

How the Instrument Works

Description of a Typical Run

The following table describes a typical run on the 3100 instrument:

Stage	Description	Diagram
1	**Sample Preparation** During sample preparation, the DNA fragments in a sample are chemically labeled with fluorescent dyes. The dyes facilitate the detection and identification of the DNA. Typically, each DNA molecule is labeled with one dye molecule, but up to five dyes can be used to label the DNA sample. Both the type of fluorescent labeling and the sample composition vary with the sample preparation method used. Samples are prepared in 96- or 384-well plates.	
2	**Software Setup** The operator creates a plate record and specifies the sample type and run module in the ABI PRISM® 3100 Data Collection software.	
3	**Beginning the Run** The operator places the plates on the instrument and starts the run. The autosampler automatically moves the sample plate into position to be sampled by the 16 capillaries.	
4	**Electrophoresis** Molecules from the samples are electrophoretically injected into thin, fused-silica capillaries that have been filled with polymer. Electrophoresis of all samples begins at the same time when a voltage is applied across all capillaries. The DNA fragments migrate towards the other end of the capillaries, with the shorter fragments moving faster than the longer fragments.	
5	**Excitation and Detection** As the fragments enter the detection cell, they move through the path of a laser beam. The laser light causes the dye on the fragments to fluoresce. The fluorescence is captured by a charge-coupled device (CCD) camera.	

Stage	Description	Diagram
6	**Data Collection** The CCD camera converts the fluorescence information into electronic information, which is then transferred to the computer workstation for processing by the 3100 Data Collection software.	
7	**Data Processing** After the data is processed, it is stored in the instrument database and displayed as an electropherogram. An electropherogram plots relative dye concentration (y-axis) against time (x-axis) for each of the dyes used to label the DNA fragments. Each peak in the electropherogram represents a single fragment.	
8	**Automatic Data Extraction and Data Analysis** The processed data is automatically extracted from the instrument database and analyzed. The positions and shapes of the electropherogram peaks are used to determine either the base sequence or fragment profile, depending on the type of run selected. The analyzed data is stored as sample files on the hard drive of the computer.	
9	**Viewing the Results** The analyzed data is viewed with either DNA Sequencing Analysis software (for sequencing) or GeneScan Analysis software (for fragment analysis). If necessary, the data is reanalyzed using different analysis parameters.	

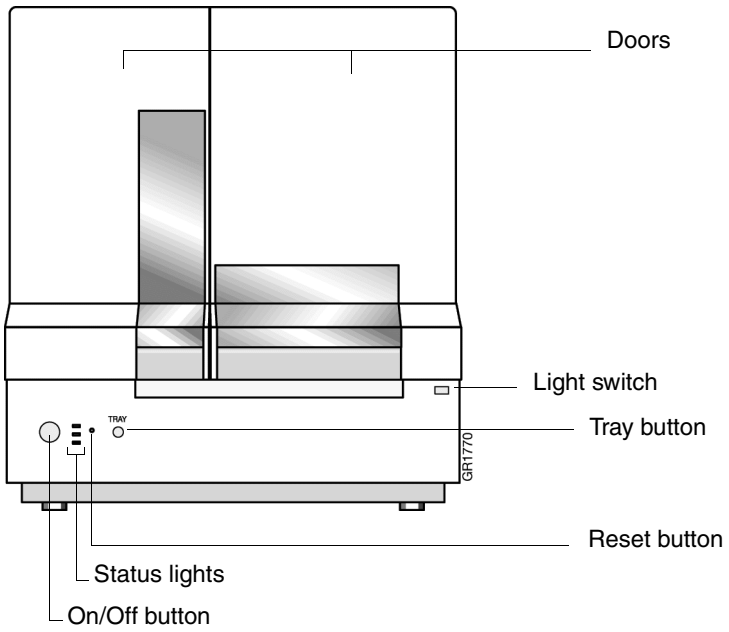
Section: Instrument Hardware Overview

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

Front View

Diagram

The following diagram shows the front of the instrument:



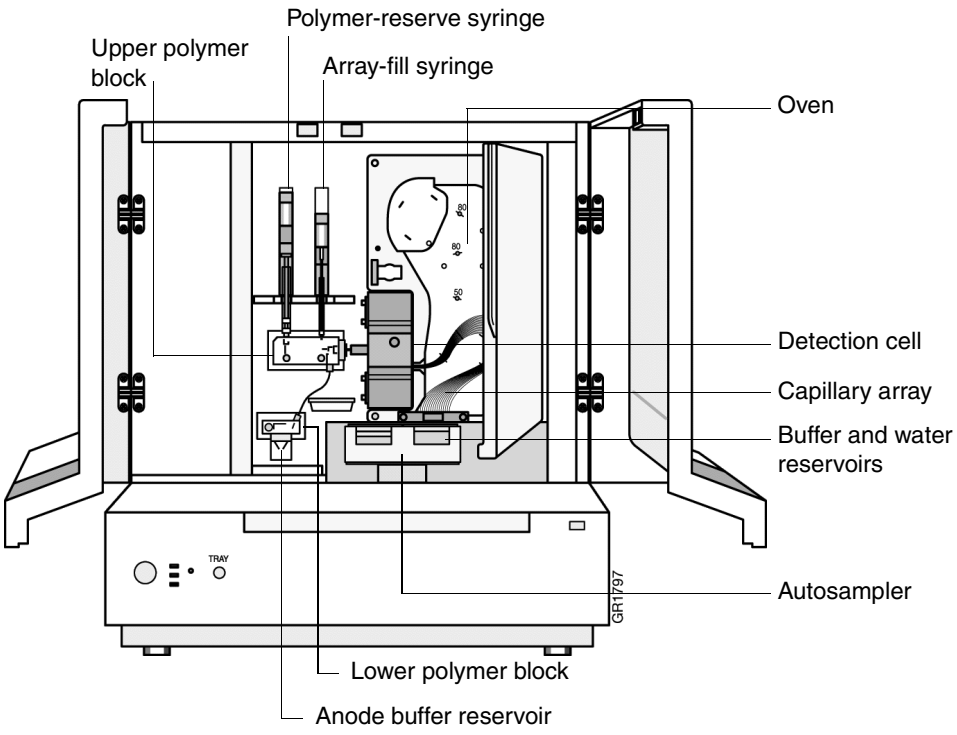
Description

Part	Function
Light switch	Switches on and off the interior lights
On/Off button	Switches on and off the instrument
Reset button	Resets all of the electronics on the instrument including the firmware and the calibration file **IMPORTANT** Use this button only as a last resort when the instrument is not responding. See page 8-7 for procedure.
Tray button	Brings the autosampler to the forward position **Note** This button works only when the instrument and oven doors are closed.
Status lights	Indicates the status of the instrument as follows:

Light Appearance	Instrument Status
All off	Power off
Yellow solid	Loading firmware
Yellow blinking	♦ Loading calibration file ♦ Initializing subsystems
Green solid	Ready for use
Green blinking	Running
Red blinking	Error

Front View with Doors Open

Diagram The following diagram shows inside the instrument's doors:



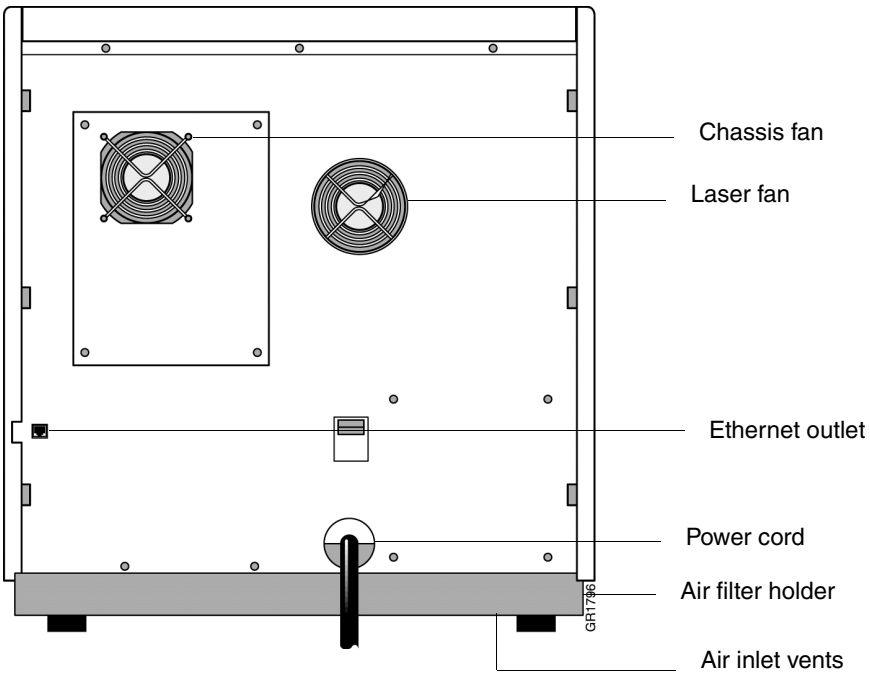
Description

Part	Function
Anode buffer reservoir	Contains 9-mL of 1X running buffer
Buffer and water reservoirs (four)	Contains 16-mL of 1X running buffer or water
Autosampler	Holds the sample plates and reservoirs and moves to align the samples, water, or buffer with the capillaries.
Capillary array	Enable the separation of the fluorescently labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 16 silica capillaries.
Detection cell	Holds the capillaries in place for laser detection
Lower polymer block	Contains the anode electrode. The anode buffer reservoir connects to this block.
Oven	Maintains uniform capillary array temperature
Polymer-reserve syringe	Contains and dispenses the polymer that fills the polymer blocks and the array-fill syringe. A 5-mL syringe.
Array-fill syringe	Contains and dispenses the polymer under high pressure to fill the capillaries. A 250-μL syringe.
Upper polymer block	Connects the two syringes and the detection end of the capillary array

Back View

Diagram

The following diagram shows the back of the instrument:



Description

Part	Function
Air filter holder	Holds the filter that cleans the air entering the instrument
Air inlet vents	Allows air into instrument **IMPORTANT** To ensure adequate air flow, do not place paper under the instrument.
Ethernet outlet	Provides a network connection to the computer workstation
Chassis fan	Pulls air out of the instrument
Laser fan	Cools the laser
Power cord	Supplies power to the instrument

Section: Computer and Software Overview

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

Computer Workstation

Overview

The 3100 Genetic Analyzer is shipped with a computer workstation running the Microsoft Windows NT operating system. An optional color printer is available.

This manual is written with the assumption that you know how to use a computer workstation running the Windows NT operating system. If you are not familiar with this computer, refer to the Windows NT workstation documentation shipped with this system for specific operating information.

Function

The computer workstation collects and analyzes data from the 3100 Genetic Analyzer.

System Requirements

The following table lists the minimum requirements for the computer workstation:

Item	Minimum Requirements
Hard drive storage	2 drives, 9 GB each
Memory	256 MB RAM
Monitor	17-in. SVGA
Operating system	Microsoft® Windows® NT v. 4.0 with Service Pack 5
Printer	Optional
Processor	Intel Pentium III 550 MHz

Hard Drive Partitions

During installation, the hard drives of your computer workstation were partitioned to create the following logical drives:

Physical Hard Disk	Drive	Size (GB)	Function
1	C	2	System operating files **Note** You may also install your own programs on this drive.
	D	7	Reserved for the 3100 software and the analysis software
2	E	9	Reserved for the instrument database

Software

Overview

The software installed on your computer workstation consists of:

- Data Collection software that controls, monitors, and collects data from the instrument
- An analysis application that either analyzes raw sequencing data or sizes and quantifies DNA fragments
- Software that automatically extracts and analyzes the data
- A database
- Utilities that enable you to manage the files in the database
- A toolkit that enables you to develop customized applications

For a complete list of the ABI PRISM 3100 software installed on your computer, see page 5-5.

Note Other programs are available from Applied Biosystems to align sequences, identify previously unsequenced regions, archive data, identify patterns of heredity, and perform other kinds of data manipulation. See your Applied Biosystems representative.

Note To avoid software conflicts, it is recommended that you do not install third-party software onto the computer attached to the 3100 instrument.

Section: Chemistry Overview

In This Section

The following topics are covered in this section:

Overview

This section provides an overview of the chemistry. For more detailed information, see the *ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide*.

Supported Dye Sets

Overview

DNA fragments are detected and identified by the fluorescent dyes with which they are chemically labeled. Dyes are purchased and used as dye sets, which are optimized for particular applications.

Table of Supported Dye Sets

Two dye sets are currently supported by Applied Biosystems for use with the 3100 Genetic Analyzer.

Note Other dye sets can also be used for sequencing with the 3100 Genetic Analyzer (see "dataType Parameter" on page 4-41).

Dye Set	Comprises...	Use for...
D	♦ 6FAM™ ♦ HEX™ ♦ NED™ ♦ ROX™	Fragment analysis
E	dRhodamine and ABI PRISM® BigDye™ versions of: ♦ dROX™ ♦ dTAMRA™ ♦ dR6G ♦ dR110	DNA sequencing
E[a]		SNP Detection Snapshot

a. The ABI PRISM® BigDye™ dye set has a similar spectral profile as Dye Set E. Customers have successfully used Dye Set E matrix standards for BigDye dyes. For best performance, however, we recommend that you create the matrix from Long-Read standards.

Labeling Chemistries

Supported Labeling Chemistries

The 3100 Genetic Analyzer is currently supported by Applied Biosystems for use with:

- DNA sequencing samples that are fluorescently labeled with:
  - ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit
  - ABI PRISM® BigDye™ Primer Cycle Sequencing Ready Reaction Kit
  - ABI PRISM™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit

Note These chemistries use the dyes in Dye Set E.

- Fragment analysis samples that are labeled with the fluorescent primers supplied with the ABI PRISM® Linkage Mapping Set-LD20, MD10, or HD5.

Note These chemistries use the dyes in Dye Set D.

Polymers

Overview

The ABI PRISM® 3100 Performance Optimized Polymer™ (POP) is used as a replaceable sieving medium that separates the DNA fragments by size during electrophoresis.

POP is shipped ready to use.

Supported Polymers

Two polymers are used with the 3100 Genetic Analyzer as follows:

Polymer Name	Use for...	Part Number
ABI PRISM® 3100 POP-4™ polymer	Fragment analysis	4316355
ABI PRISM® 3100 POP-6™ polymer	DNA sequencing	4316357

Chemical Hazard

⚠ 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.

Storage and Expiration

POP polymers are stable on the instrument for 7 days.

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.

Proper 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.

Injection Solution

Overview

The injection solution is a fluid that is used to:

- Denature (separate) the DNA strands.
- Resuspend DNA samples before starting a sample run.
- Resuspend calibration standards during the preparation of a calibration or sample run.
- Maintain the electrical connection between the polymer in the capillaries and the injection wells in the electrophoresis chamber by acting as an electrolyte (necessary for electrophoresis).

Hi-Di Formamide

The injection solution recommended for use with the 3100 Genetic Analyzer is Hi-Di™ Formamide (P/N 4311320) or formamide of equivalent 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.

Section: Electrophoresis Overview

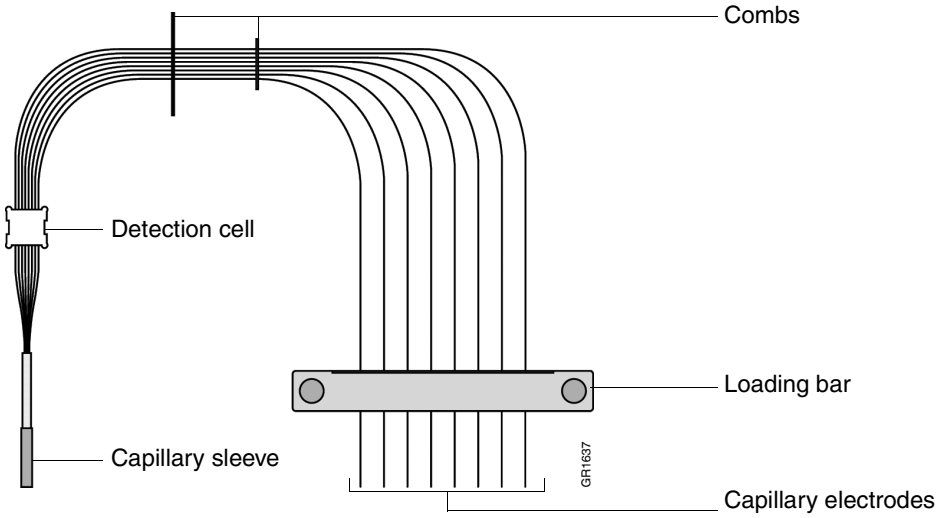
In This Section The following topics are covered in this section:

Capillary Array

Overview

The 3100 capillary array is a replaceable unit composed of 16 silica capillaries that, when filled with polymer, enable the separation of the fluorescently labeled DNA fragments by electrophoresis.

Diagram



Description

Part	Function
Capillary sleeve	Provides a seal, along with the ferrule and array ferrule knob, with the upper polymer block
Capillary electrodes	Hold the capillary ends in position
Combs	Separate the capillaries to maintain consistent positioning and heat distribution in the oven
Detection cell	Holds the capillaries in place for laser excitation
Loading bar	Supports the capillaries and provides a high-voltage connection to the capillary electrodes

Available Lengths

Length (cm)	Use for...
36	♦ Fragment analysis ♦ Rapid DNA sequencing
50	Standard DNA sequencing

For More Information

The following table lists capillary array topics covered elsewhere in this manual:

Topic	See Page
Changing a capillary array	8-15
Storing a capillary array	8-17
Part numbers for capillary arrays	C-1

Electrophoresis

Overview

Samples are electrophoretically separated as they travel through the polymer in the capillary array.

Temperature

Electrophoresis temperature is controlled by housing the capillary array in a sealed oven.

The following table lists the normal electrophoresis temperature for each type of run:

Type of Run	Temperature (°C)
Standard DNA sequencing	50
Rapid DNA sequencing	55
Standard fragment analysis	60

Electrophoresis Circuit

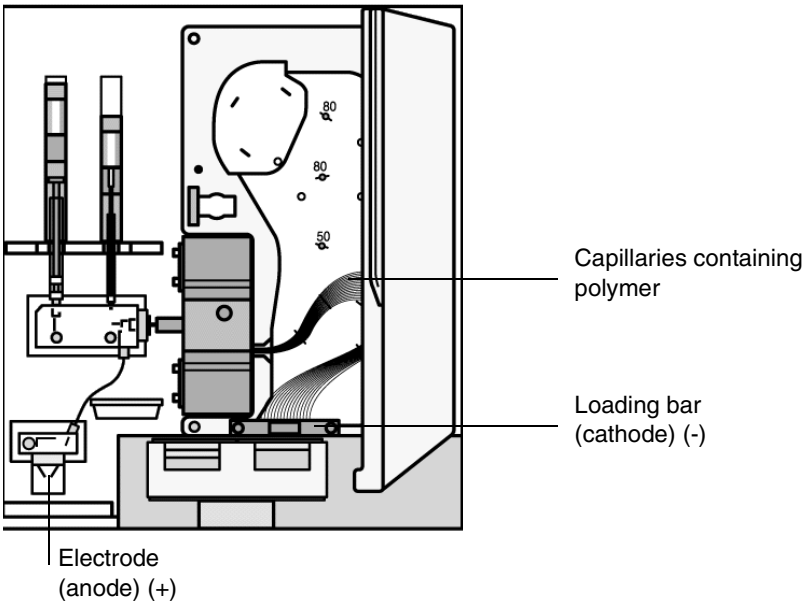
Overview

A high-voltage electrical circuit facilitates the electrophoresis of DNA fragments. The electrical charge is conducted through the circuit by:

- DNA and buffer ions in the polymer
- Buffer ions in the buffer
- Electrons in the electrical wires and electrodes

Diagram

The electrophoresis circuit is shown below.



Description

During electrophoresis, a high voltage is applied between the loading bar (cathode) and the electrode located on the lower polymer block (anode). The voltage drives the movement of negatively charged DNA fragments through the polymer in the capillaries towards the anode. From the anode, the current flows back in electrical wires through the power supply to the cathode to complete the circuit.

⚠ 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.

Section: Fluorescence Detection Overview

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

Introduction

Detection Overview

The dye-labeled DNA fragments are separated by electrophoresis within the capillary array. Once the fragments enter the detection cell, they pass through a laser beam. The light excites the attached dye labels causing them to fluoresce.

The detection components work together to collect the fluorescence and convert the information into electronic form. The electronic information is then processed and displayed by the 3100 Data Collection software.

Detection Components

The main components of the detection system and their function are listed in the following table.

Note The many lenses and mirrors integral to detection are not covered in this section.

Part	Function
Laser	Excites the attached dye labels as the DNA fragments pass through the detection cell
Transmission grating	Disperses the light by wavelength and a second set of lenses focuses the resulting light spectrum onto the CCD camera
CCD camera	Converts the incident fluorescence into digital information that is processed by the 3100 Data Collection software

Note More information on each of the components follows this section.

Laser

Overview

When a dye-labeled DNA fragment moves into the path of the laser beam, some electrons in the dye are excited to higher energy levels as the laser light is absorbed. Shortly afterwards, the electrons return to their ground states and emit fluorescence light energy. The light emitted from each dye has a different spectral profile (color).

Laser Type

The laser used to excite the dyes is an argon-ion laser.

Emission Wavelengths

The primary emission lines are at 488 nm and 514.5 nm.

Interlock

For your safety, an interlock switch shutters the laser and shuts off the electrophoresis power supply if the doors of the instrument are opened.

For more information on laser safety, refer to the *ABI PRISM 3100 Genetic Analyzer Site Preparation and Safety Guide* (P/N 4315835).

⚠ WARNING **LASER HAZARD**. Exposure to direct or reflected laser light at 40 mW for 0.1 seconds can burn the retina and leave permanent blind spots. Never look directly into the laser beam or allow a reflection of the beam to enter your eyes.

Transmission Grating

Overview

The transmission grating is a grooved disk that spectrally separates the fluorescence emitted (light) from the dye-labeled DNA fragments. After the light is spectrally separated, it is focused onto the charge-coupled device (CCD) camera.

CCD Camera

Overview

The CCD camera includes a rectangular silicon chip that converts the incident fluorescence light into digital information.

This digital information (data) will be processed by the 3100 Data Collection software. A description of the role of the CCD camera in data processing starts on page A-3.

Performing a Run 3

Overview

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

Section: Introduction

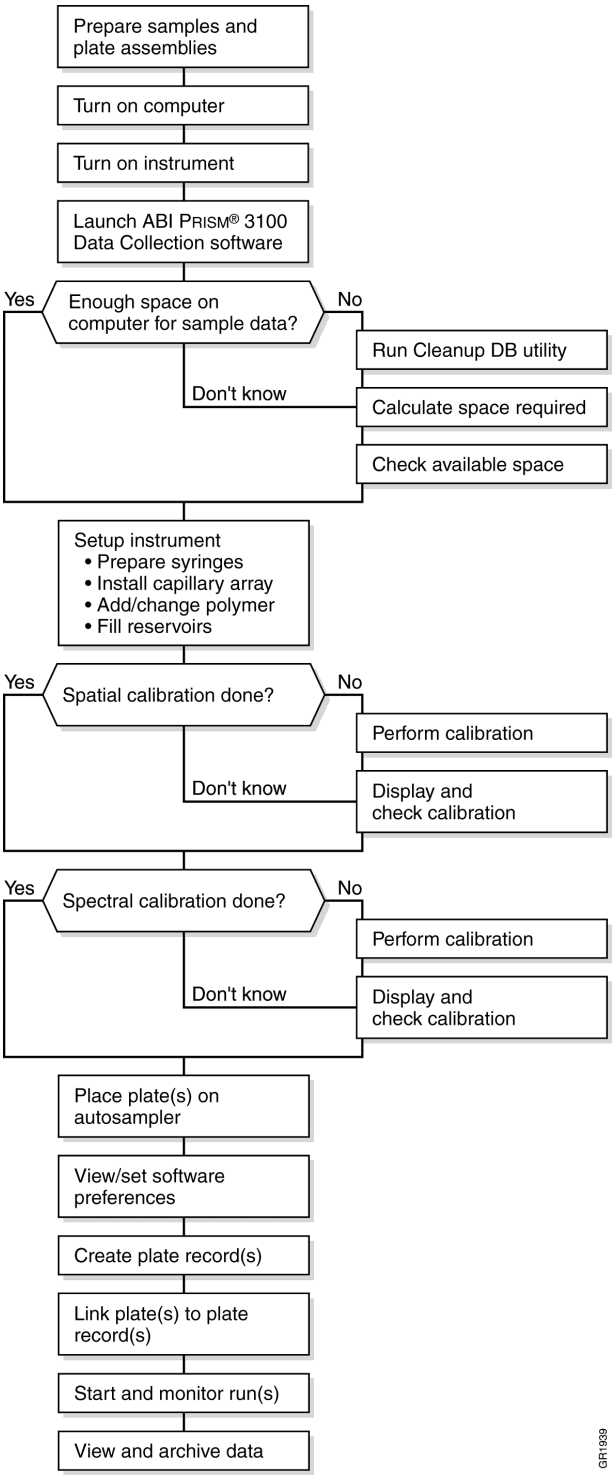
In This Section

The following topics are covered in this section:

Summary of Procedures

Flowchart of a Typical Run

This flowchart provides an overview of the steps required to perform a run on the ABI PRISM® 3100 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 sequencing or Rapid DNA Sequencing Analysis
Type of polymer	Either: ♦ ABI PRISM® 3100 POP-4™ for fragment analysis ♦ ABI PRISM® 3100 POP-6™ for DNA sequencing or rapid DNA sequencing
Length of capillary array	Either: ♦ 36-cm array for fragment analysis or rapid DNA sequencing ♦ 50-cm array for DNA 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.
Which analysis module to use	Either: ♦ Select one of the supplied analysis modules ♦ Create your own analysis module in the downstream application.
Which run module to use	Either: ♦ Select one of the supplied run modules ♦ Edit one of the supplied run modules to change the conditions used for a run
How many 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 identical run module columns and identical analysis module columns.
Whether to run the same sample again under different run conditions	Prepare two run module columns when creating the plate record, filling in the second with a different run module.
Whether to perform a single run or a batch run	Either: ♦ A single run that electrophoreses up to 16 samples ♦ A batch run that performs several sequential runs without needing operator attention

Decision Table *(continued)*

Decision	Comments
Whether to save data to a BioLIMS™ database (optional) or to ABIF sample files	If you do not enable BioLIMS in the Setting Preferences dialog box, the sample files are stored in the following directory: D:\AppliedBio\abi\3100\Data Extractor\Extracted Runs
Whether to perform a 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
Whether to perform a spectral calibration	A spectral calibration must be performed: ♦ Whenever you use a new dye set on the instrument ♦ Whenever you change the type of polymer used ♦ After the laser or CCD camera has been realigned by a service engineer ♦ If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks)

Section: Working with Samples and Plate Assemblies

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

Sample Preparation

References for Sample Preparation

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

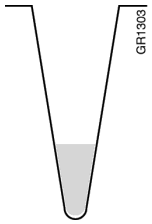
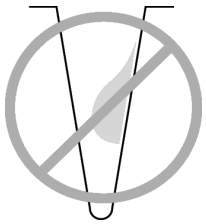
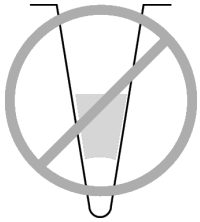
For...	Refer to the...
DNA sequencing samples	*ABI PRISM Automated DNA Sequencing Chemistry Guide* (P/N 4305080)
Fragment analysis samples	*ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Fragment Analysis* (P/N 4315832)

Checking the Plate

After centrifuging the plate of samples, ensure each sample is positioned at the bottom of its tube or well.

To check the plate of samples:

Step	Action
1	Hold the plate up to a light source. Your samples should:

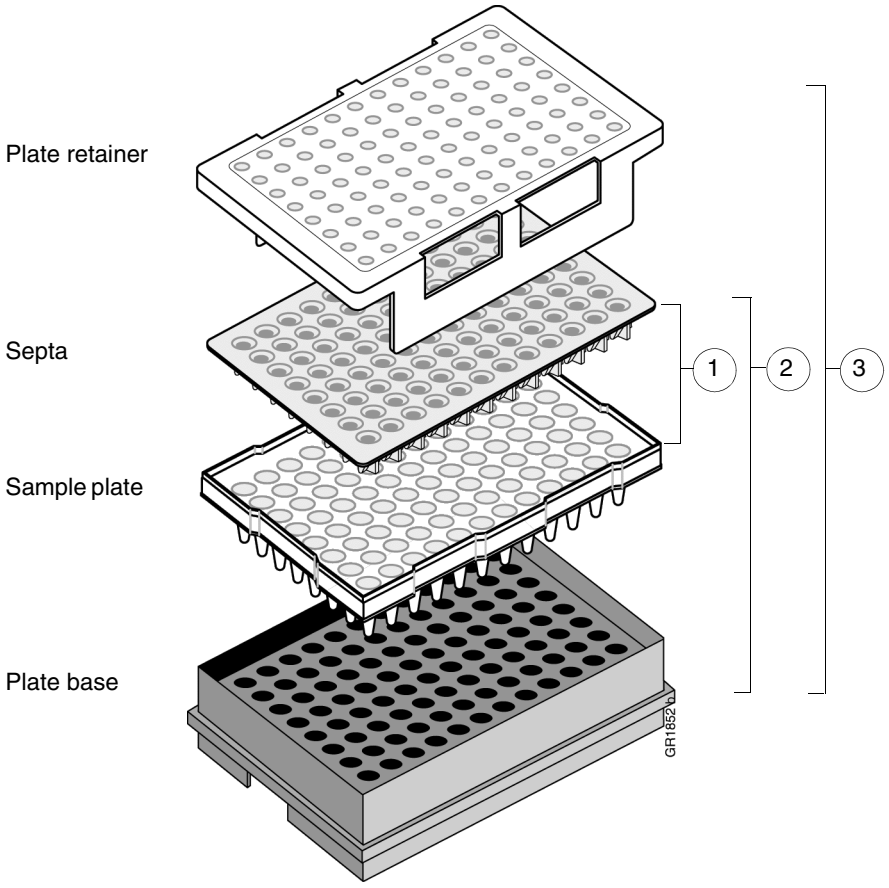
Look like this...	Not look like this...	Not look like this...
		
The sample is positioned correctly in the bottom of the well.	The sample lies on the side wall because the plate was not centrifuged.	An air bubble lies at the bottom of the well because the plate was not: ♦ Centrifuged with enough force,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

2	If any of the samples are not positioned at the bottom of their tube or well, recentrifuge the plate.

Working with Plate Assemblies

Plate Assembly Components

The plate assembly components are assembled as follows:

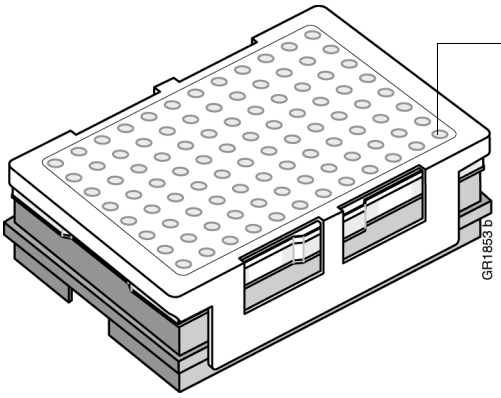


Preparing a Plate Assembly

To prepare a plate assembly:

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

To prepare a plate assembly: *(continued)*

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

Section: Starting the 3100 System

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

Starting the Computer

Starting the Computer Workstation

IMPORTANT You must start the computer workstation before starting the ABI PRISM® 3100 Genetic Analyzer.

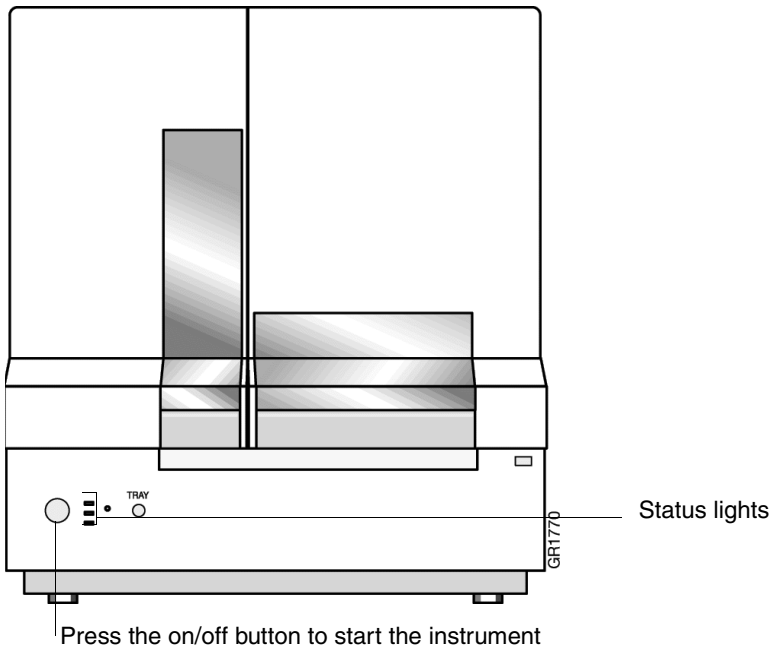
To start the computer workstation:

Step	Action
1	Turn on the monitor.
2	Power on the computer. The computer boots and then the **Begin Logon** dialog box appears.
3	Press **CTRL + ALT + DELETE** and enter the user name and password. **Note** ♦ The default user name for the workstation is **3100User**. 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 will launch automatically. If it does not launch, repeat steps 1–3.

Starting the Instrument

Starting the Instrument

To start the 3100 Genetic Analyzer:

Step	Action
1	Ensure that the: ♦ Oven door is closed and locked ♦ Instrument doors are closed **Note** If the doors are open during power-up, the red failure light will illuminate.
2	Ensure that the: ♦ Computer is powered on (see “Starting the Computer” on page 3-12) ♦ 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.  **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 ABI PRISM® 3100 Data Collection software and look at the event log. If this is not helpful, call a Technical Support representative. See Appendix B, “Technical Support.”

Starting the 3100 Data Collection Software

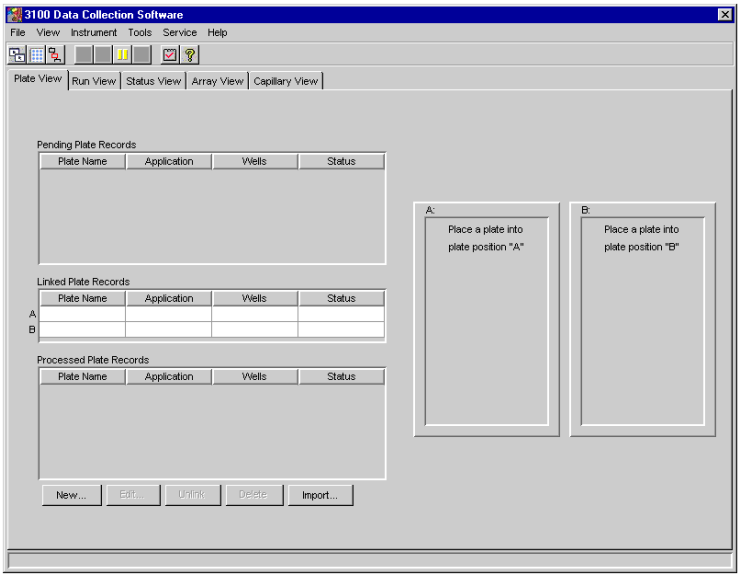
Before You Begin

Before starting the Data Collection software:

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

Starting the Data Collection Software

To start the Data Collection software:

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

Section: Checking the Available Space and Deleting Records

In This Section

The following topics are covered in this section:

Introduction

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

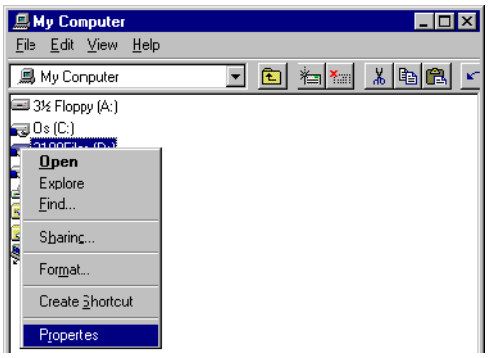
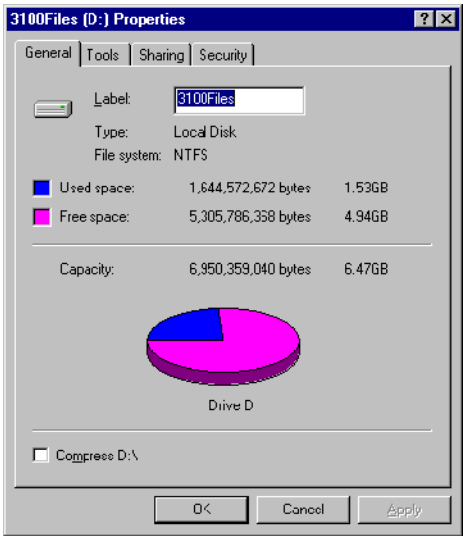
The sections that follow tell you:

- How to check the available hard drive 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
- Where to find the procedures for deleting database records

Checking the Available Hard Drive Space

Checking Hard Drive Space

To check the hard drive for space for sample files:

Step	Action
1	Double-click the **My Computer** icon on the desktop to view the drives.
2	Right-click on a the D drive and select **Properties**.  The **Properties** dialog box opens displaying the used and free space. 
3	Estimate how much free space you need by using the information provided below. **File Type** / **Approximate Space Required Per File (kB)[a]** Analyzed sample file for DNA sequencing — 250 Analyzed sample file for fragment analysis — 500 Unanalyzed sample file — 100 a. The values provided are estimates only. The actual file size depends on the run module selected.
4	If there is insufficient space: a. Archive the sample files to another volume. b. Delete the original files from the drive.

Checking the Available Database Space

Checking Database Space

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

To check the database space:

Step	Action
1	Run the Diskspace utility. For instructions, see “Checking Database Space: The Diskspace Utility” on page 7-5.
2	If the used space is more than 8 GB, purge the database of some or all data. See the procedure references below.

Deleting Records from the Database

Cleanup Database Utility Reference

See page 7-8 to run the Cleanup Database utility:

- Once per week, or
- When the used space is more than 8 GB, or
- Every 400–500 runs

Deleting an Individual Plate Record Reference

Delete individual plate records when you want to free database space without deleting all of the records (see page 6-39).

Section: Preparing the Instrument

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

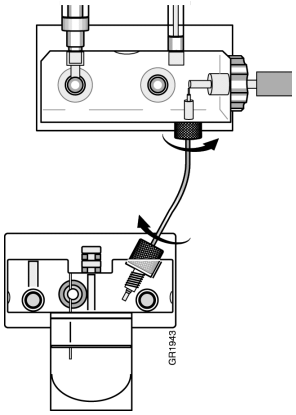
Instrument Setup

Using Manual Control

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. For a complete list of the commands, and instructions for using them, see “Controlling the Instrument Using Manual Control” on page 5-15.

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 8-27.
2	Push the upper polymer block onto the two guide pins on 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.

Preparing and Installing the Syringes

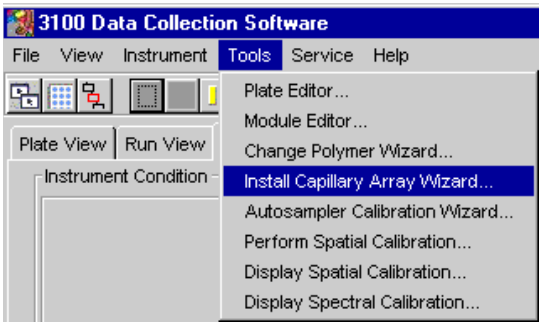
IMPORTANT Wear gloves while performing the following procedure, and any other time you handle the capillary array, glass syringes, septa, or buffer reservoirs.

To prepare and install the syringes:

Step	Action
1	Clean and inspect the syringes as instructed on page 8-21.
2	Prime and fill the syringes as instructed on page 8-22.
3	Install the syringes as instructed on page 8-23.

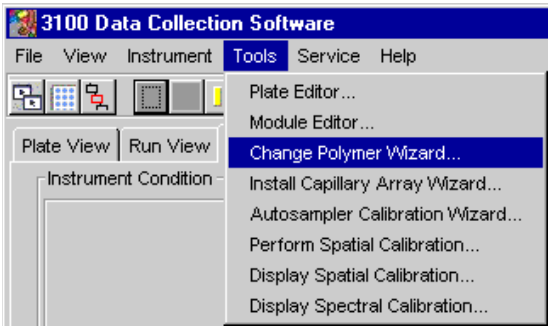
Installing or Replacing the Capillary Array

If necessary, install a capillary array using the Install Capillary Array wizard. For instructions, see “Installing and Removing the Capillary Array” on page 8-15.



Adding or Changing Polymer

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

If polymer on the instrument is...	Then...
less than 1 week old, and sufficient in quantity to complete your runs[a]	Ensure there are no air bubbles, and then proceed with instrument preparation. **Note** To remove any air bubbles, see page 8-28.
greater than 1 week old, or insufficient in quantity to complete your runs	Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard. For instructions, see page 8-10.  **⚠ 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.

a. A run uses 50–80 µL of polymer. This is equivalent to 60–100 runs from one 5-mL syringe. A minimum of 1 mL of polymer is required for the instrument to operate.

IMPORTANT Always replace polymer that is older than 1 week.

IMPORTANT Ensure there are no air bubbles in the upper polymer block before proceeding. To remove any air bubbles, see page 8-28.

Preparing Buffer and Filling the Reservoirs

Required Materials

The following materials are required to prepare 3100 1X running buffer:

- ABI PRISM® 3100 10X Running Buffer with EDTA (P/N 402824)
- Quality deionized water

Making Buffer for a Single Run

To prepare 50 mL of 1X running buffer:

Step	Action
1	Add 5.0 mL of 10X running 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 3100 1X running buffer can be stored at 2–8 °C for up to 1 month.

When to Replace the Buffer

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

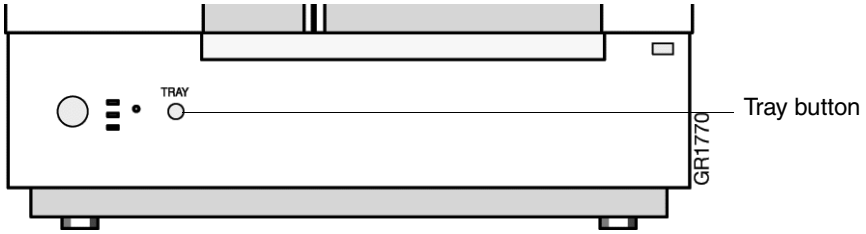
IMPORTANT Failing to replace buffer may lead to loss of resolution and data quality.

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.

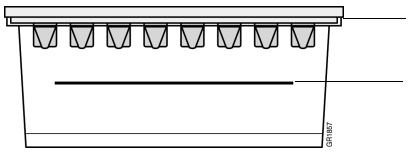
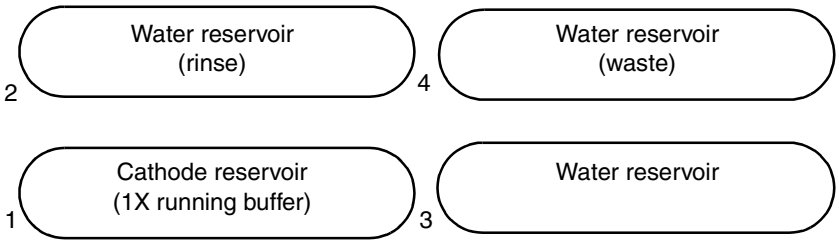
Filling the Water and Cathode Buffer Reservoirs

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

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

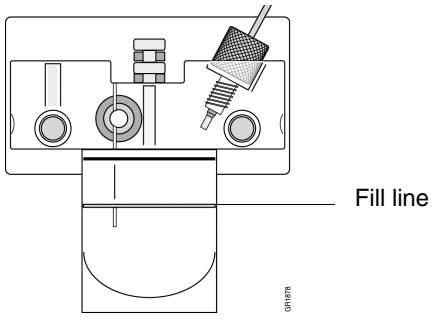
Step	Action
7	Fill the water reservoirs to the line with quality deionized water (about 16 mL).
8	Place a clean septa strip on each reservoir, and dry the outside of the reservoirs using a lint-free wipe. **⚠ CAUTION** Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips. 
9	Place the reservoirs into position on the autosampler as shown below. 

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.
4	Fill the reservoir to the fill line with fresh 1X running buffer (about 9 mL). 

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

Step	Action
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

When to Perform a 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
- Temporarily remove the capillary array from the detection block

For instructions, see “Spatial Calibration” on page 4-3.

When to Perform a Spectral Calibration

If necessary, perform a spectral calibration.

A spectral calibration must be performed:

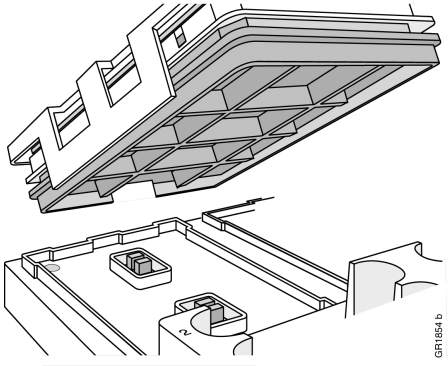
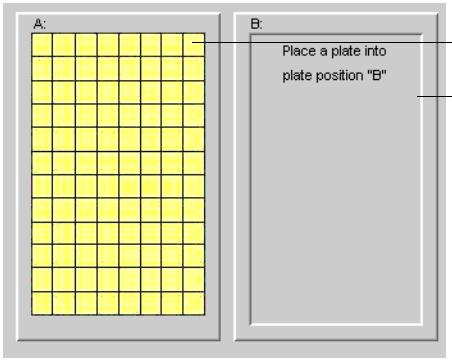
- Whenever you use a new dye set on the instrument
- After the laser has been realigned by a service engineer
- After the CCD camera has been realigned by a service engineer
- If you begin to see pull-up and/or pull-down peaks consistently

For instructions, see “Spectral Calibration” on page 4-15.

Placing the Plate onto the Autosampler

Placing the Plate onto the Autosampler

To place the plate onto the autosampler:

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

Section: Setting Up the Software

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

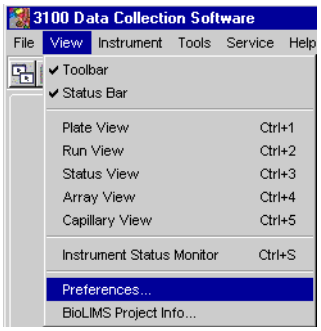
Setting Software Preferences

Introduction

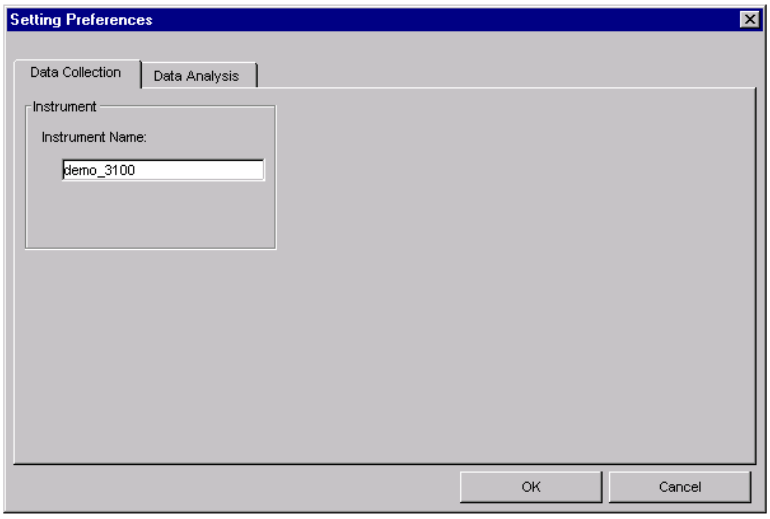
The Data Collection software preferences are set during instrument installation; however, you can view or change these preferences in the Setting Preferences dialog box.

Viewing the Setting Preferences Dialog Box

To view the Setting Preferences dialog box:

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

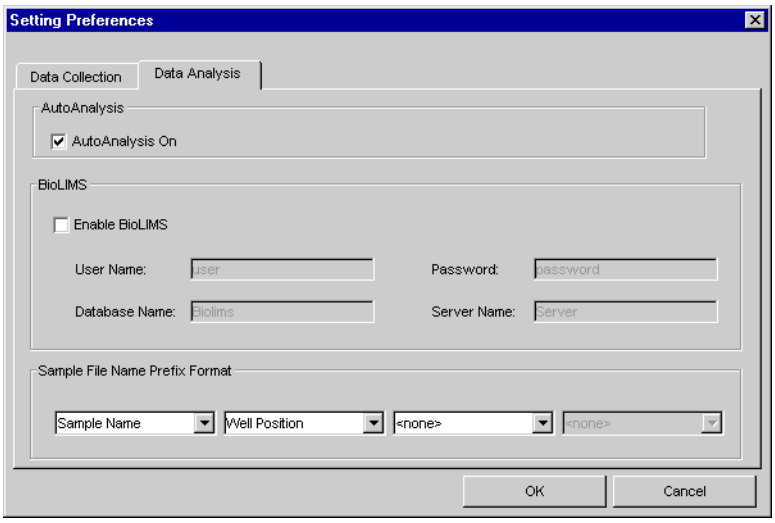
Data Collection Page



The following table describes the preferences that can be set within this page:

Preference	Description
Instrument Name	This field automatically populates with **demo_3100.** You can change it to any name (*e.g.*, the instrument's serial number).

Data Analysis Page



The following table describes the preferences that can be set within this page:

Preference	Description
AutoAnalysis On	Select AutoAnalysis On to have the samples automatically analyzed by the analysis software after the run. **Note** Selecting this option will not prevent you from reanalyzing your sample data.
BioLIMS	Use these settings to have data extracted to a BioLIMS database instead of to sample files on the hard drive.
Sample File Name Prefix Format	Specify the format for the sample file names by using the drop-down lists to reorder the identifiers. **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 wizard entry **Note** In addition to the four identifiers you set with the drop-down lists, all names are automatically appended with the capillary number and a file extension. Therefore, in the Data Analysis page example shown above, the sample name will be: *Sample Name_Well Position_Capillary Number.*fsa (or .abl for sequencing)

About Plate Records

Introduction

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, if present (GeneScan analysis only)
- Mobility file (DNA sequencing analysis only)
- Comments about the plate and about individual samples
- Dye set information
- BioLIMS project (this entry is mandatory, even when BioLIMS is not used)
- The name of the run module (run modules specify information about how samples are run)
- The 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 re-run. This will move the existing plate record from the Processed window to the Pending window.

Create plate records in advance of placing the plates on the instrument.

A plate record cannot be created while a run is in progress.

About the Procedure

The next two sections cover the most common method for creating a plate record. There is a separate procedure for each analysis application.

Note There are several other methods for creating a plate record. See Chapter 6, “Working with Plate Records.”

Plate Records for Spectral Runs

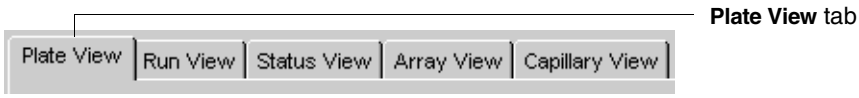
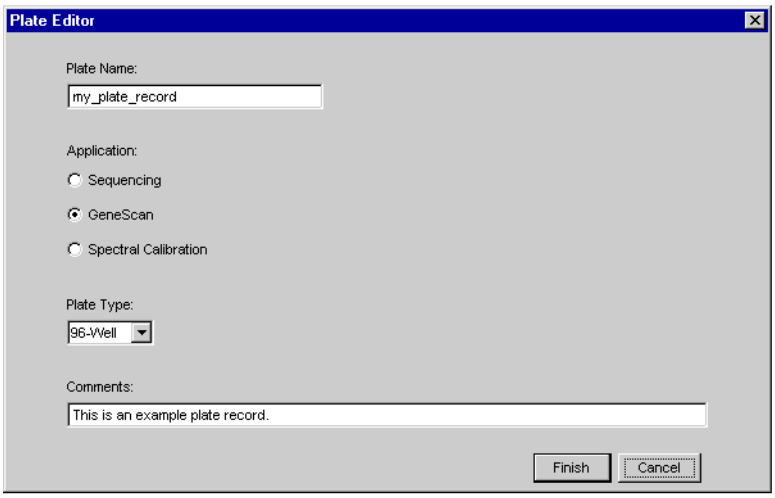
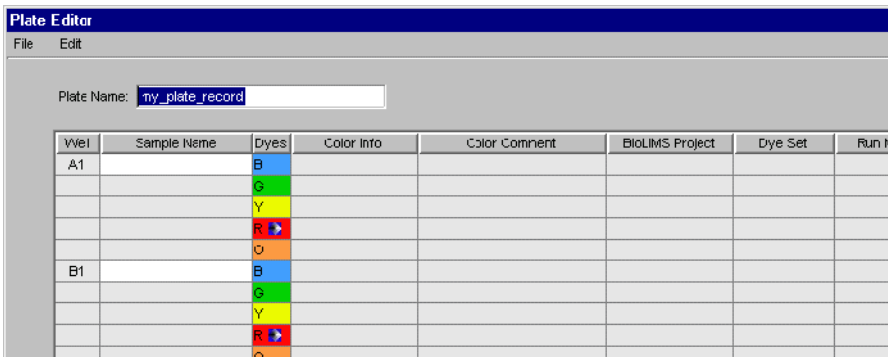
For information on creating plate records for spectral calibration runs, see “Creating a Plate Record” on page 4-21.

Creating a Plate Record for GeneScan Analysis

Entering Plate Record Information

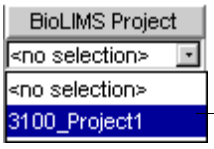
Note You cannot create a plate record while a run is in progress.

To enter plate record information:

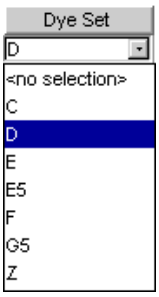
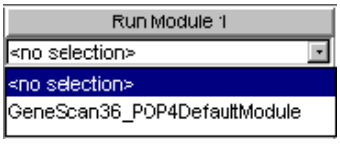
Step	Action
1	Click the **Plate View** tab on the **3100 Data Collection software** window to go to the **Plate View** page. 
2	On the **Plate View** page, click **New**. Or, click the Plate Editor button on the toolbar.  The **Plate Editor** dialog box appears. 
3	Use the **Plate Editor** dialog box to name your plate and to specify the application and plate type. Entering comments is optional. **IMPORTANT** When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces.
4	When done, click **Finish**. The **Plate Editor** spreadsheet 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. **Note** In the default naming convention, the sample name you type is incorporated into the sample file name. For example: **MySample_A01_01.fsa** Capillary position Well position Sample name you type The sample file naming convention used can be changed in the **Preferences** dialog box. See page 3-28 for details. **IMPORTANT** When naming the samples, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces. **IMPORTANT** Be sure that sample file names are not longer than 55 characters. An underscore separates each preference selected, so be sure to count the underscore in the number of characters. There is no automatic error checking for sample names that exceed this limit. Sample files with long names cannot be opened by the analysis software.
2	*Optional* For each sample, enter **Color Info** and **Color Comment** text.
3	Enter a BioLIMS project. **IMPORTANT** A BioLIMS project is required for every sample, even if a BioLIMS database is not used. a. Click in the **BioLIMS Project** cell for Well A1. b. Select a project name from the drop-down list.  **IMPORTANT** You must enter a BioLIMS **Note** To set up a BioLIMS project, see page 5-48. 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 whenever a field is the same for all samples in the plate record.

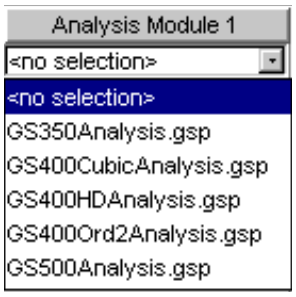
To enter sample information and save the plate record: *(continued)*

Step	Action
4	For each sample, select the appropriate **Dye Set** from the drop-down list. For GeneScan analysis, select Dye Set D.  **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.
5	For each sample, select the appropriate **Run Module** from the drop-down list.  The following table shows the run module to select based on your run type: **Analysis Type** | **Run Module** GeneScan | GeneScan36_POP4DefaultModule **Note** If you need to view or edit a run module file, see page 5-20. **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.

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

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

IMPORTANT The AutoAnalysis On preference must be selected if analysis is to take place automatically after the run (see page 3-29).

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

<table>
<tr><th>If using size standard...</th><th>Select this analysis module...</th></tr>
<tr><td>GS400HD</td><td>GS400HDAnalysis.gsp</td></tr>
<tr><td>GS350</td><td>GS350Analysis.gsp</td></tr>
<tr><td>GS500</td><td>GS500Analysis.gsp</td></tr>
<tr><td rowspan="2">GS500 (see footnote)</td><td>GS400CubicAnalysis.gsp[a]</td></tr>
<tr><td>GS400Ord2Analysis.gsp[a]</td></tr>
</table>
a. These modules are for advanced users with specific sizing needs. See the ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual.

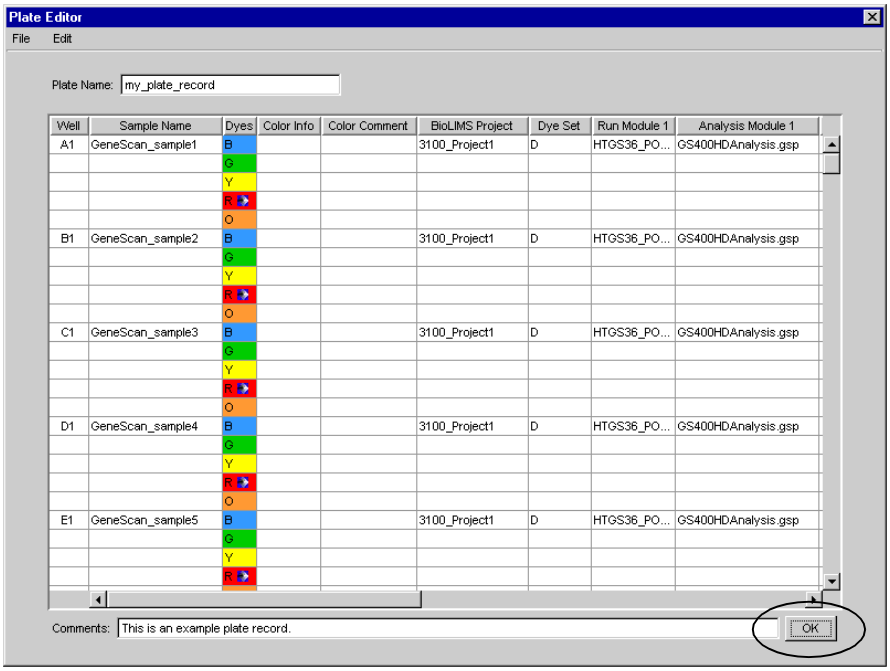
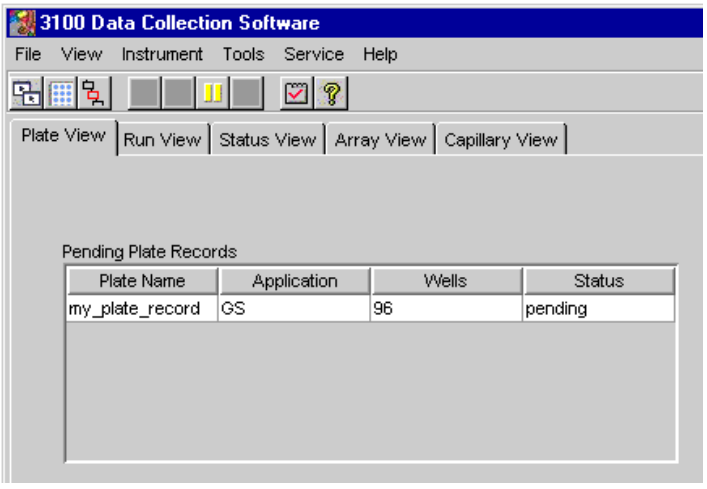
Note You can examine the settings for each of these files using GeneScan Analysis Software. The meanings of the settings are described in the ABI PRISM GeneScan Analysis Software v. 3.6 NT User's Manual.

Note For more information on module files, see Chapter 5, "Software."</td></tr>
<tr><td>7</td><td>If you want to run the same sample again, select a second run module and a second analysis module. You can run a sample in a linked plate up to five times.

Samples will be automatically grouped so that all samples with the same run module are run sequentially.</td></tr>
</table>

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

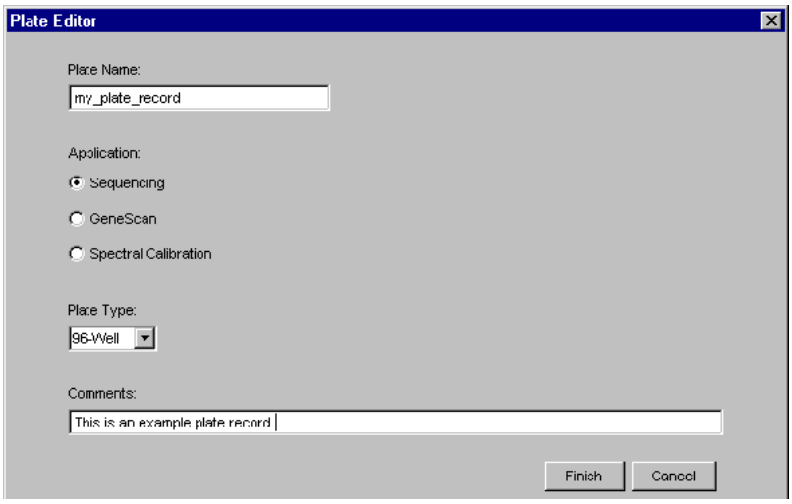
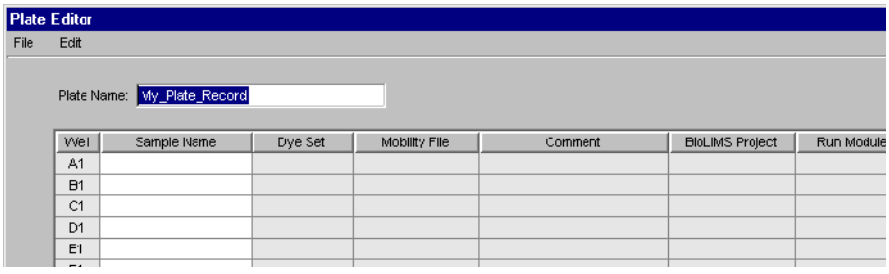
Step	Action
8	Make sure the plate record is correct, and then click **OK**.  **Note** It may take a while for the new plate record to be saved to the database and added to the **Pending Plate Records** table as shown below. 

Creating a Plate Record for DNA Sequencing Analysis

Entering Plate Record Information

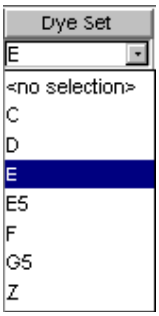
Note You cannot create a plate record while a run is in progress.

To enter plate record information:

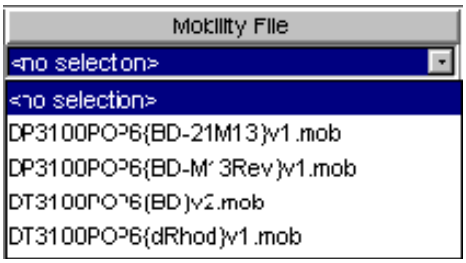
Step	Action
1	Click the **Plate View** tab on the **3100 Data Collection software** window to go to the **Plate View** page. 
2	In the **Plate View** page, click **New.** Or, double-click the Plate Editor button on the toolbar.  The **Plate Editor** dialog box appears. 
3	Use the **Plate Editor** dialog box to name your plate and to specify the application and plate type. Entering comments is optional. **IMPORTANT** When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces.
4	When done, click **Finish**. The **Plate Editor** spreadsheet opens. 

Entering Sample Information

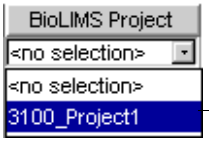
To enter sample information and save the plate record:

Step	Action
1	In the **Plate Editor** spreadsheet, type the names of all the samples in the **Sample Name** column. **Note** In the default naming convention, the sample name you type is incorporated into the sample file name. For example: **MySample_A01_01.ab1** Capillary position Well position Sample name you type The sample file naming convention used can be changed in the **Preferences** dialog box. See page 3-28 for details. **IMPORTANT** When naming the samples, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces. **IMPORTANT** Be sure that sample file names are not longer than 55 characters. An underscore separates each preference selected, so be sure to count the underscore in the number of characters. There is no automatic error checking for sample names that exceed this limit. Sample files with long names cannot be opened by the analysis software.
2	For each sample, select the appropriate **Dye Set** from the drop-down list. For DNA Sequencing analysis, select Dye Set E.  **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>3</td><td>For each sample, select the appropriate Mobility File from the drop-down list.
 

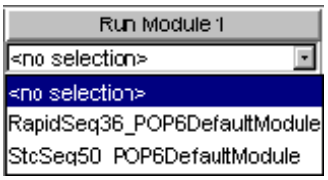
Note You may need to resize the column to see the whole file name. To do this, place the cursor between the column headers (it will become a double-headed arrow) and drag right.
 

The following table shows which mobility file to select based on your sequencing chemistry:
<table><tr><th>DNA Sequencing Chemistry</th><th>Mobility File</th></tr><tr><td>ABI PRISM® BigDye™ Primer chemistry; using the -21m13 primer</td><td>DP3100POP6{BD-21M13}v1.mob</td></tr><tr><td>BigDye Primer chemistry; using the reverse primer</td><td>DP3100POP6{BD-M13Rev}v1.mob</td></tr><tr><td>ABI PRISM® BigDye™ Terminator chemistry</td><td>DT3100POP6{BD}v2.mob</td></tr><tr><td>ABI PRISM™ dRhodamine Terminator chemistry</td><td>DT3100POP6{dRhod}v1.mob</td></tr></table></td></tr>
<tr><td>4</td><td>Enter a BioLIMS project.
IMPORTANT A BioLIMS project is required for every sample, even if a BioLIMS database is not used.
a. Click in the BioLIMS Project cell for Well A1.
b. Select a project name from the drop-down list.
 

IMPORTANT You must enter a BioLIMS project.
Note To set up a BioLIMS project, see page 5-48.
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.
– The Project Name for every sample in the plate record is now the same.
Note Press CTRL+D whenever a field is the same for all samples in the plate record.</td></tr>
</table>

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

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

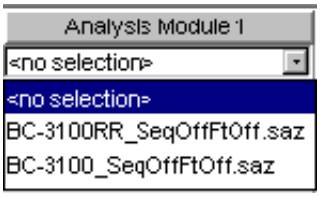
Note If you need to view or edit a run module file, see page 5-20.

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

<table>
<tr><th>Analysis Type</th><th>Run Module</th></tr>
<tr><td>Standard DNA sequencing</td><td>StdSeq50_POP6DefaultModule</td></tr>
<tr><td>Rapid DNA sequencing</td><td>RapidSeq36_POP6DefaultModule</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. To see the scheduled order of the runs, select the Run View tab.</td></tr>
<tr><td>6</td><td>For each sample, select the appropriate Analysis Module from the drop-down list.

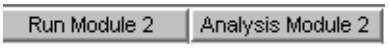
IMPORTANT The AutoAnalysis preference must be selected if analysis is to take place automatically after the run (see page 3-29).

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

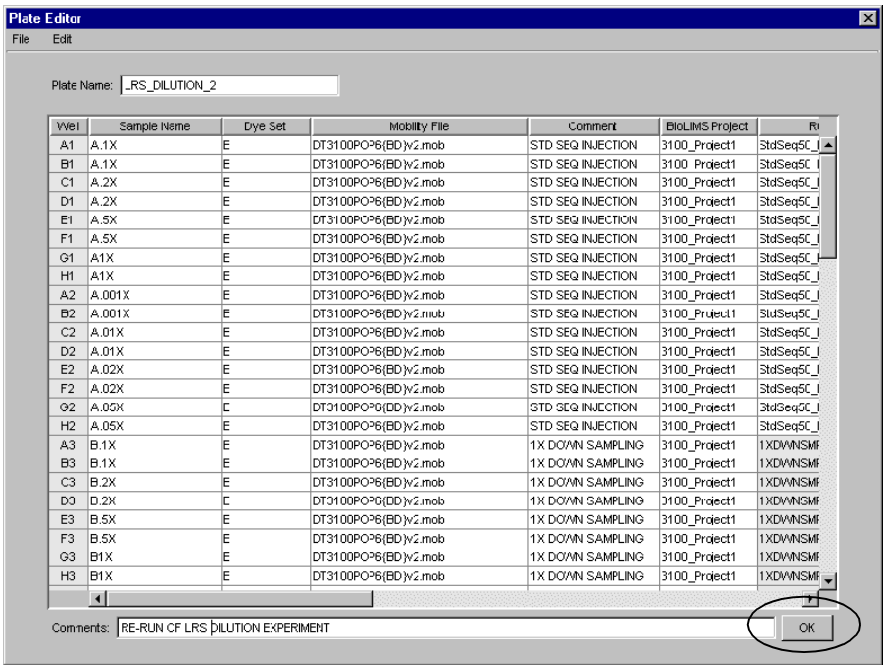
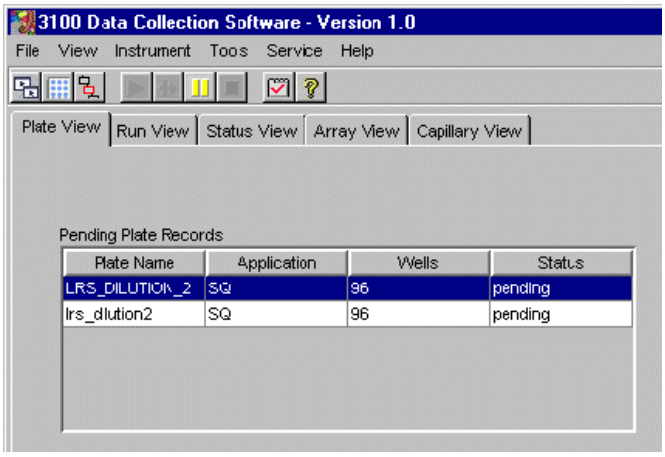
<table>
<tr><th>Run Type</th><th>Analysis Module</th></tr>
<tr><td>Standard DNA sequencing</td><td>BC-3100_SeqOffFtOff.saz</td></tr>
<tr><td>Rapid DNA sequencing</td><td>BC-3100RR_SeqOffFtOff.saz</td></tr>
</table>

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. 3.6 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.

Samples will be automatically grouped so that all samples with the same run module are run sequentially.</td></tr>
</table>

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

Step	Action
8	Make sure the plate record is correct, and then click **OK**.  **Note** It may take a while for the new plate record to be saved to the database and added to the **Pending Plate Records** table as shown below. 

Linking and Unlinking a Plate

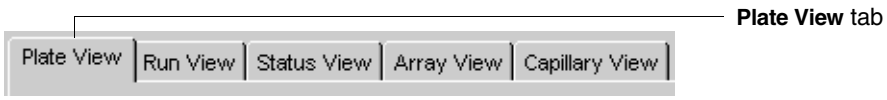
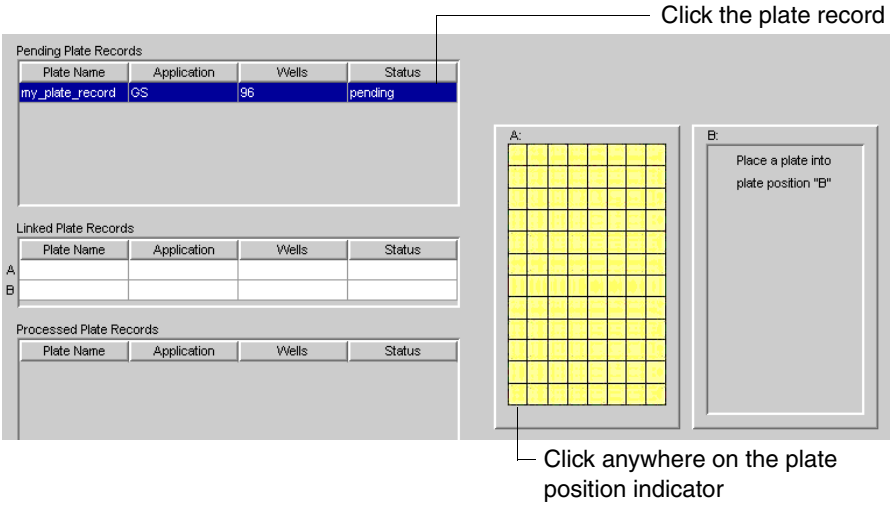
Introduction

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

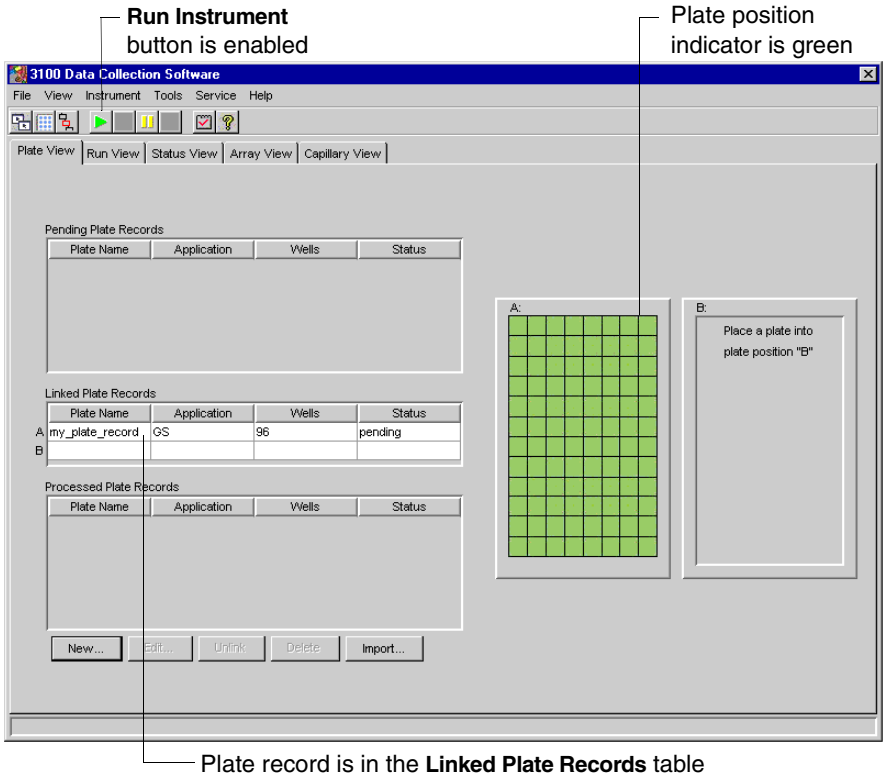
IMPORTANT A plate can be linked even if there are no run modules selected for its samples. In this case, there is no error message and runs for samples in the plate will not be scheduled.

Linking a Plate to a Plate Record

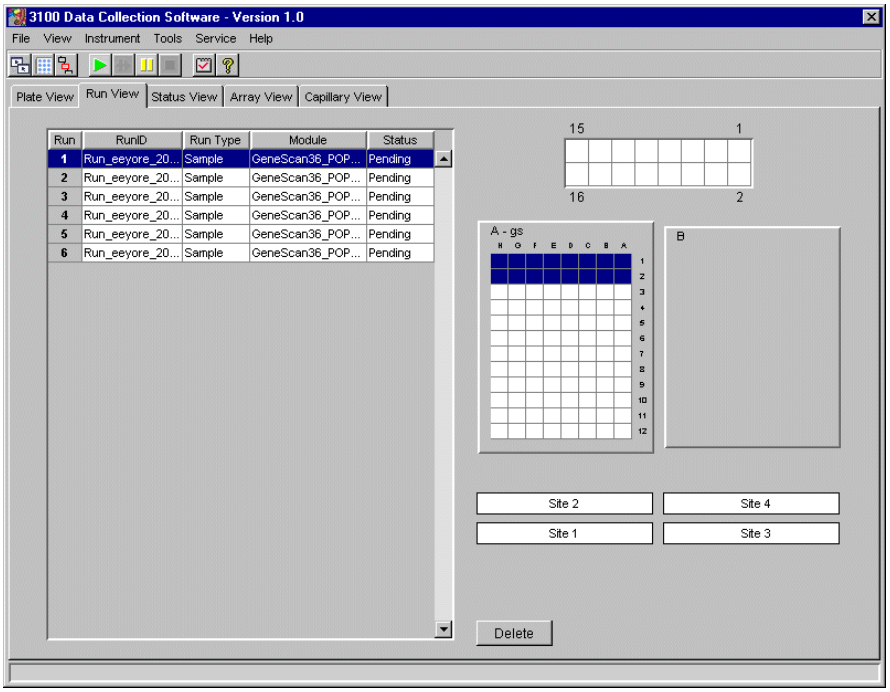
To link a plate to a plate record:

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

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

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

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

Step	Action
5	Click the **Run View** tab to view the run schedule. For more information about the **Run View** page, see page 3-50. **Note** Although individual runs can be deleted, the order in which the runs are scheduled cannot be altered. For more information on run scheduling, see page 3-46. 

Unlinking a Plate Record

To unlink a plate record:

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

Section: Running the Instrument

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

About Run Scheduling

Introduction To view the run schedule, click the Run View tab. The order in which the runs are scheduled cannot be altered. Run scheduling depends on the factors listed below.

Plate Run Order If two plates are being run, the order in which they are run is based on the following factors, in the order listed:

- If one plate is a spectral calibration run, it will be run first.
- For two plates of either fragment analysis or DNA sequencing, the plates will be run in the order in which the plates were linked.

Sample Run Order The order in which the samples are run is based on the following factors, in the order listed:

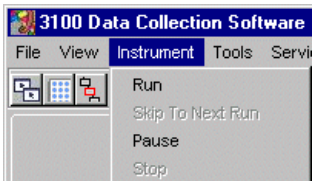
- First, samples will be sorted alphabetically by run module name.
  - Samples with module names beginning with capital letters come before those that begin with lower-case letters (*e.g.*, Z before a).
  - If samples have the same run module name, the samples on the plate that was linked first will be run first.
- Secondly, samples within a plate will be run in the order of their well designation (*i.e.*, A1, B1, C1, etc.).
- Lastly, samples with more than one run module specified will be run in the order that the run modules appear.

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

Controlling the Run

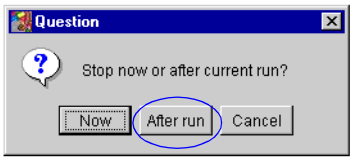
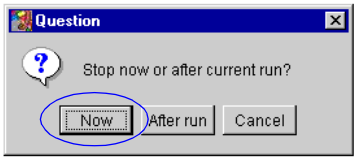
Controlling the Run Using the Instrument Menu

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 3100 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 for long periods may affect data quality.
♦ Complete the current run, and ♦ Stop the other scheduled runs	**a. Stop**  b. **After run** in the **Question** dialog box 	
♦ Stop the current run, and ♦ Stop the other scheduled runs	**a. Stop**  b. **Now** in the **Question** dialog box 	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 3-60.
♦ 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 3-60.

Run Times

DNA Sequencing Run Times

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

Type of Analysis	Run Module	Run Time
Standard DNA sequencing	StdSeq50_POP6DefaultModule	2 hr 30 min
Rapid DNA sequencing	RapidSeq36_POP6DefaultModule	1 hr

GeneScan Run Times

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

Type of Analysis	Run Module	Run Time
GeneScan	GeneScan36_POP4DefaultModule	45 min

Section: Monitoring a Run

In This Section

The following topics are covered in this section:

Introduction

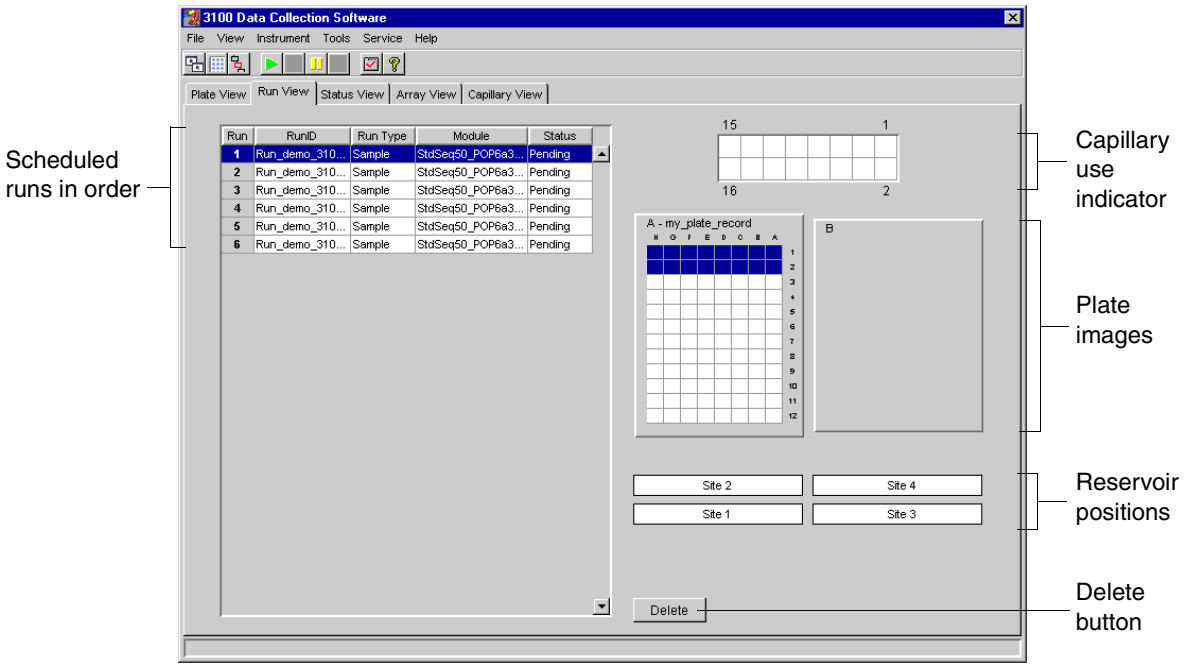
This section describes the functions and features of:

- The 3100 Data Collection software pages that are used to monitor a run, and
- The 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 individual runs can be deleted, the order in which the runs are scheduled cannot be altered. For more information on run scheduling, see page 3-46.

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. Placing the cursor over an individual cell will 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.

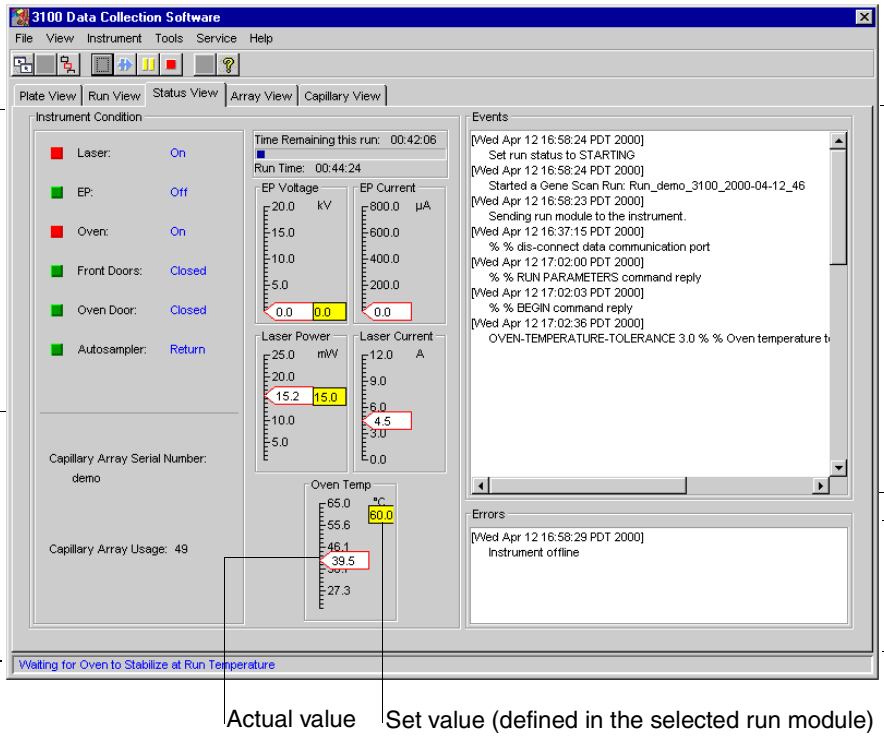
Status View Page

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

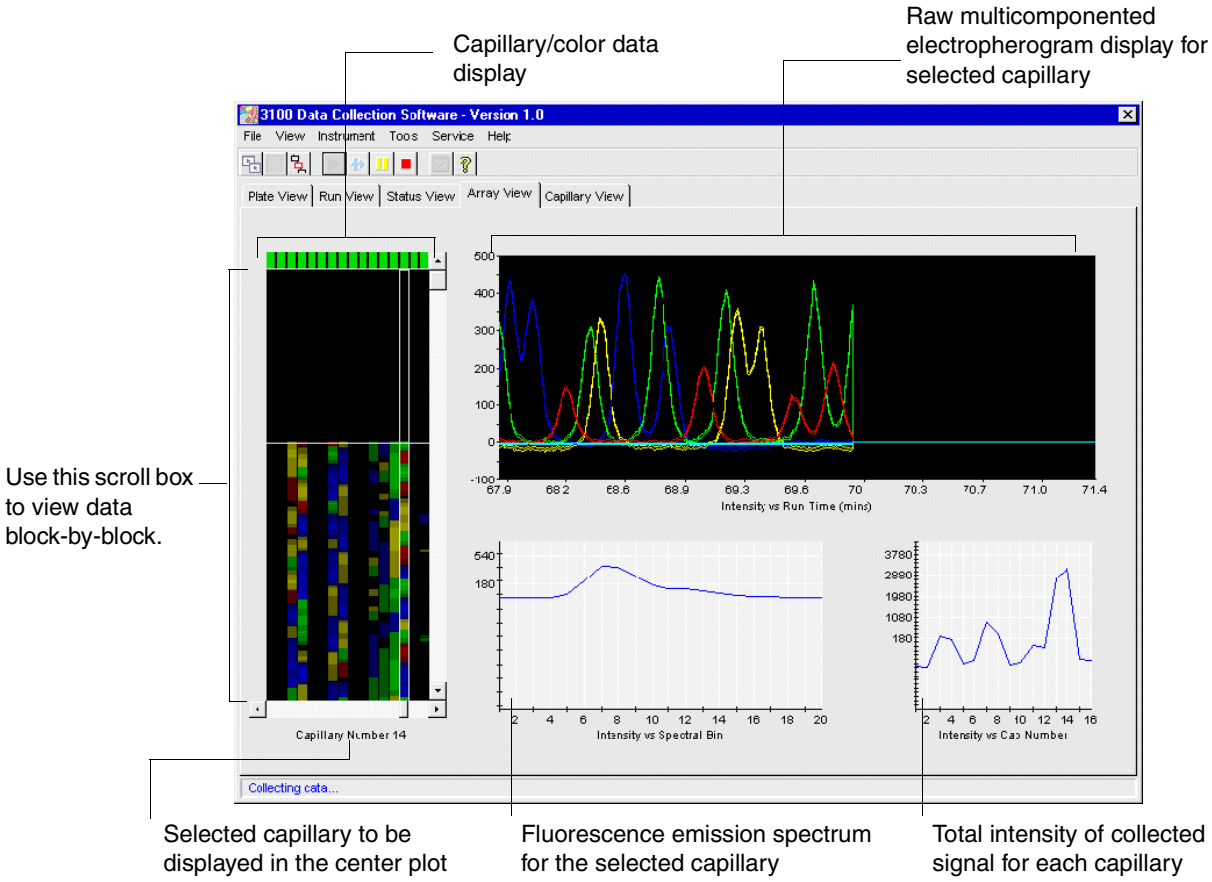
Function

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 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.

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 as described in the following table.

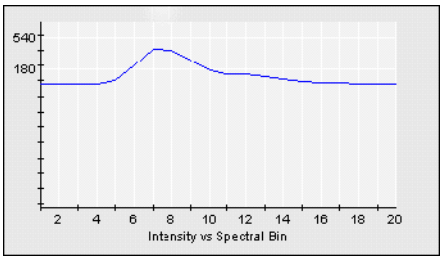
Cell Color	Status of the Capillary	Comment
Green	Operational	—
Yellow	Questionable	This capillary did not pass the spectral calibration and has been assigned the spectral profile of its nearest passing neighbor. There may be a problem with data collected from this capillary.
Red	Nonoperational	All capillaries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

Capillary Display

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.

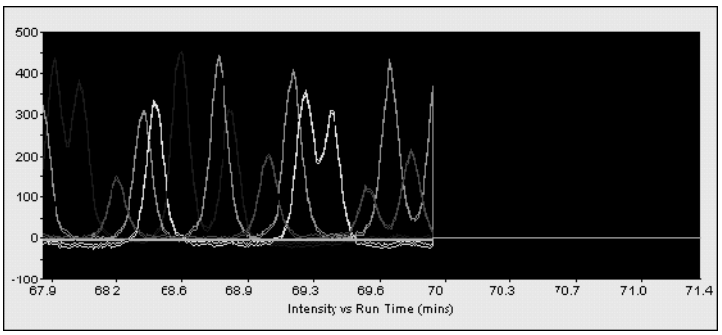


Note This window is updated each time you select a different capillary in the Capillary Display window during data collection.

Electropherogram Display

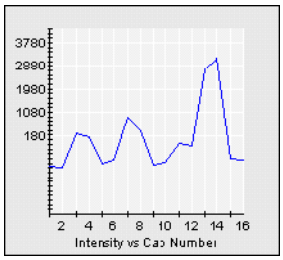
An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The data displayed has been multicomponented. The relative dye concentration is determined by applying chemometric algorithms to the collected fluorescence data.

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

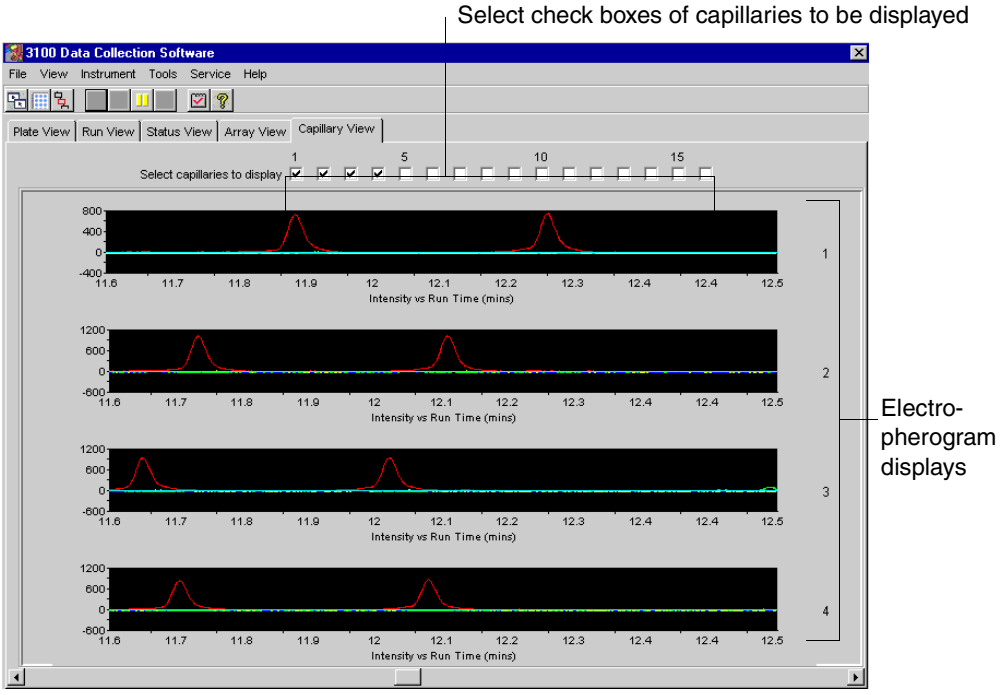
Function

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

IMPORTANT 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.

Features

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



Check Boxes

Select the check boxes of the capillaries for which you want electropherograms displayed.

Note Only four capillary electropherograms will fit on the screen at one time. If you select more than four, a scroll bar appears so that you can access the others.

Electropherogram Displays

An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The data displayed is 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.

Display Order

The capillaries are displayed in the order in which the boxes are checked. For example, to display capillary 1 under capillary 15: (a) Clear all check boxes. (b) Select check box 15. (c) Select check box 1.

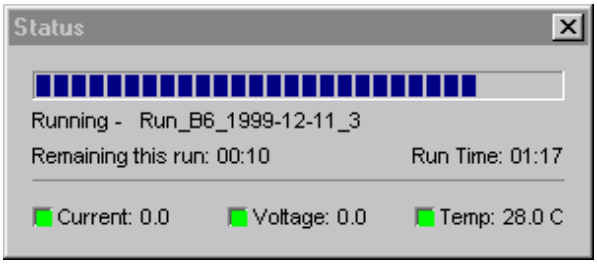
Instrument Status Monitor

Function

The Instrument Status Monitor displays the current run conditions.

Viewing the Instrument Status Monitor

To view the Instrument Status Monitor:

Step	Action
1	From the **View** menu, select **Instrument Status Monitor** or double-click the **Instrument Status Monitor** button on 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:

Recovering Data if Autoextraction Fails

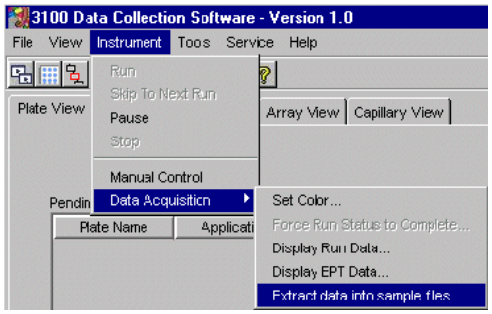
Introduction

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

The auto extractor should automatically extract data from stopped runs. If autoextraction fails, use the Extract data into sample files command as described below.

Recovering Data from a Stopped Run

To recover data from a stopped run:

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

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

Introduction

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

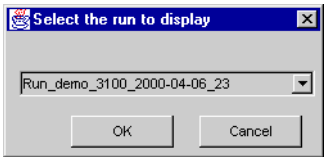
- In the Array View page (in much the same way that you might view the gel file output from an ABI PRISM® slab gel instrument)
- In the Capillary View page, capillary-by-capillary

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 3100 Data Collection software, click the **Array View** tab to display the **Array View** page.
2	From the **Instrument** menu, point to **Data Acquisition**, and choose **Display Run Data**. This opens the **Select the run to display** dialog box. 
3	From 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. **Note** It may take a few moments to retrieve the data.
4	Use the scroll features on the **Array View** page to view the data. **Note** For information on the **Array View** page, see page 3-53. **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.
5	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 3-56. **IMPORTANT** Always exit from the **Array View** and the **Capillary View** windows. During a run, do not leave these pages open for extended periods. This may cause unrecoverable screen update problems. Leave the **Status View** window open.

Viewing Analyzed GeneScan Data

Introduction

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

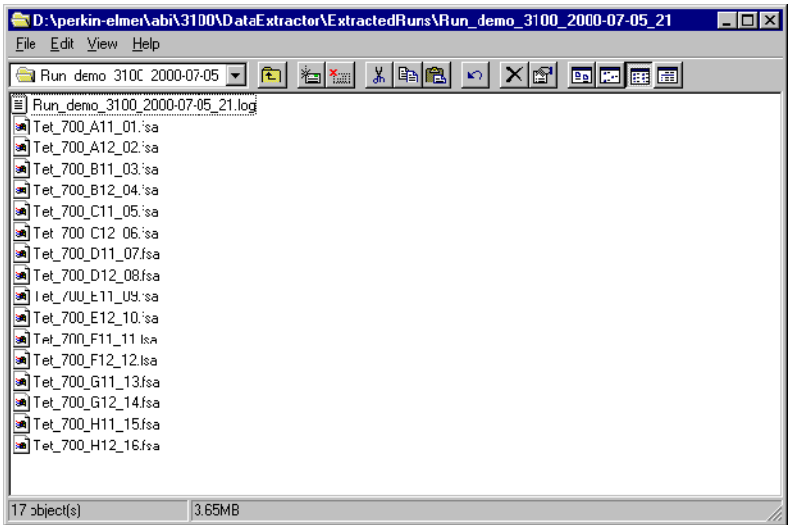
Refer to the *ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual* (P/N 4308923) for details on viewing and analyzing GeneScan data.

Locating Sample Files

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

D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns

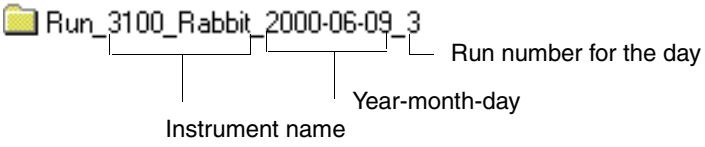
An example of the run folder and its contents is shown below.



Run Folder Default Name

The default name of the run folder is:
Run_<Instrument name>_<*date*>_<*runID*>

An example of a run folder name is shown below.



Viewing Analyzed DNA Sequencing Data

Introduction

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.

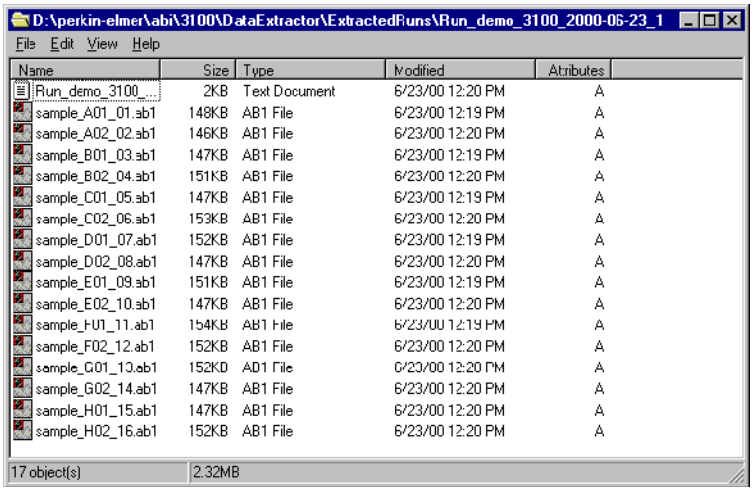
Refer to the *ABI PRISM DNA Sequencing Analysis Software v. 3.6 NT User's Manual* (P/N 4308924) for details on viewing and analyzing GeneScan data.

Locating Sample Files

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

D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns

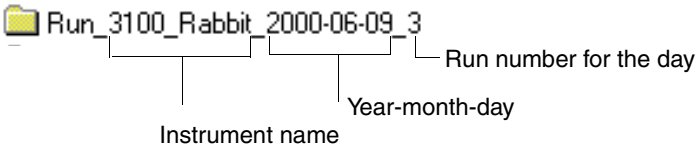
An example of the run folder and its contents is shown below.



Run Folder Default Name

The default name of the run folder is:
Run_<*Instrument name*>_<*date*>_<*runID*>

An example of a run folder name is shown below.



Archiving Data

Introduction

There are many options for archiving your data. You could, for example, copy the data to another networked computer and from there use any archiving system, such as an external SCSI storage device.

We do not recommend that you add a 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 Genetic Analyzer has been installed with the 3100 Software. Installing a SCSI device first will alter the drive letter assignments so that the instrument and software cannot be properly installed.

Installing a SCSI Storage Device

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. See page 2-14.

Spatial and Spectral Calibrations 4

Overview

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

Section: Spatial Calibration

In This Section The following topics describe how to perform a spatial calibration:

About Spatial Calibrations

When to Calibrate

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

What a Spatial Calibration Tells You

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

About Spatial Calibration Data

Spatial Maps

The spatial map is a simple numerical table that defines the number of pixels at the center of the fluorescence from each capillary in the spatial dimension of the CCD.

For information on the organization of the CCD and frame data, refer to Appendix A, “Data Flow.”

Only one spatial map at a time is stored in the database on drive E. The term “current” spatial map refers to the spatial map that is currently stored in the instrument database. You can replace (override) the “current” spatial map stored in the instrument database with a spatial map stored in a spatial calibration file. For the procedure, see page 4-12.

The maps are stored as text files in the SpatialCalLogs folder in the following directory:
D:\AppliedBio\abi\3100\DataCollection\SpatialCalLogs

Spatial maps are also saved to the 3100 Calibration file and to the firmware.

Spatial Calibration Files

Spatial calibration files are stored on drive D.

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

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.

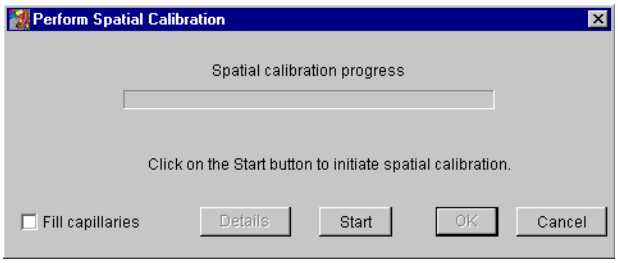
The log file is a text file that can be opened and viewed in the Notepad accessory. It can be useful for troubleshooting spatial calibration problems.

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

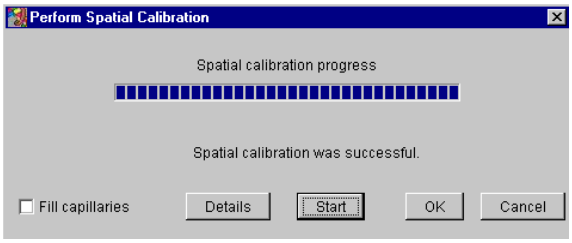
Performing a Spatial Calibration

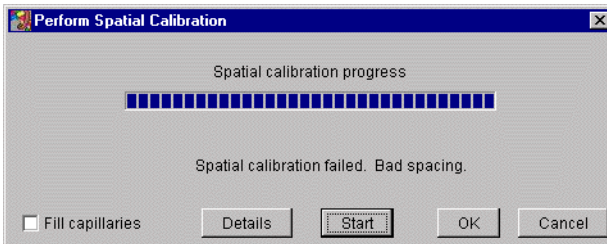
Performing a Spatial Calibration

To perform a spatial calibration:

Step	Action
1	From the **Tools** menu, select **Perform Spatial Calibration**. The following dialog box appears: 
2	Select the **Fill capillaries** check box if the: ♦ Capillaries have no polymer (*i.e.,* a new capillary array), or ♦ Polymer in the capillaries has been used in a run **Note** You 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)*

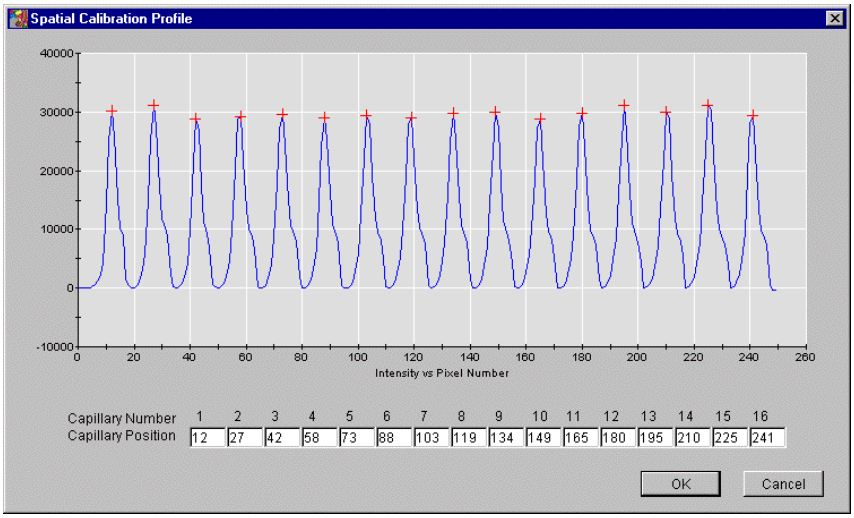
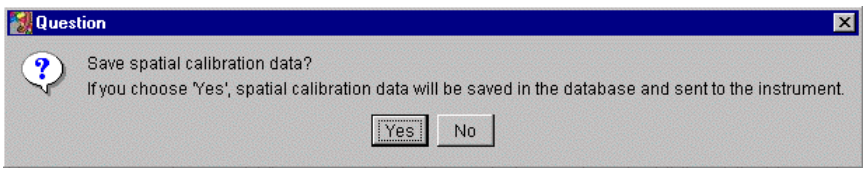
<table>
<tr><th>Step</th><th colspan="2">Action</th></tr>
<tr><td rowspan="3">4</td><td>If the calibration...</td><td>Then...</td></tr>
<tr><td>succeeded</td><td>the following dialog box appears:
 

a. Click Details to view the Spatial Calibration Profile window.
b. Continue on to “Viewing Successful Results and Saving the Data” below.</td></tr>
<tr><td>failed</td><td>an error message box appears, providing some information about the reason for the failure.
 

a. Click Details to view the Spatial Calibration Profile window.
b. Do one of the following:
– Click Cancel, and then click Start to repeat the calibration.
– Take corrective action as outlined on page 4-9.</td></tr>
</table>

Viewing Successful Results and Saving the Data

To view the spatial calibration results and save the data:

Step	Action
1	Evaluate the spatial calibration profile. **Note** For information about the profile, see “Evaluating a Spatial Calibration Profile” on page 4-11.  When you are finished, click **OK** to close the **Spatial Calibration Profile** box.
2	**If the spatial calibration profile is...** / **Then...** satisfactory: Continue on to step 3. unsatisfactory: a. Click **Cancel** to close the **Details** box, and then click **Start** to repeat the calibration, or b. Reposition one or more of the red crosses. To move a cross, change the value in the Capillary Position box, and then click outside of that box. c. Override the data with data from a previous run (see page 4-28.) If the calibration continues to provide unsatisfactory results, see “If the Calibration Fails” on page 4-9.
3	Click **OK** to close the **Perform Spatial Calibration** window. The **Question** dialog box opens. 

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

Step	Action
4	

To...	Then...
save this calibration data to the 3100 Data Collection Software database	Click **Yes.**
delete this data and use data from a previous run	a. Click **No.** b. Proceed to "Overriding the Current Spatial Calibration Map" on page 4-12.

If the Calibration Fails

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

- Repeat the calibration.
- Fill the capillaries with polymer, and then repeat the calibration.
- Clean the detection cell, and then repeat the calibration (see page 8-14).
- Reposition the array window in the detection cell, and then repeat the calibration.

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.

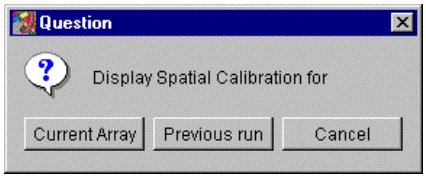
Note With this procedure, you can view spatial calibration data, but you cannot change which data is set as the current map.

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>From the Tools menu, select Display Spatial Calibration.

This opens the Question dialog box.

 
</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.

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

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 “Evaluating a Spatial Calibration Profile” on page 4-11.</td></tr>
</table>

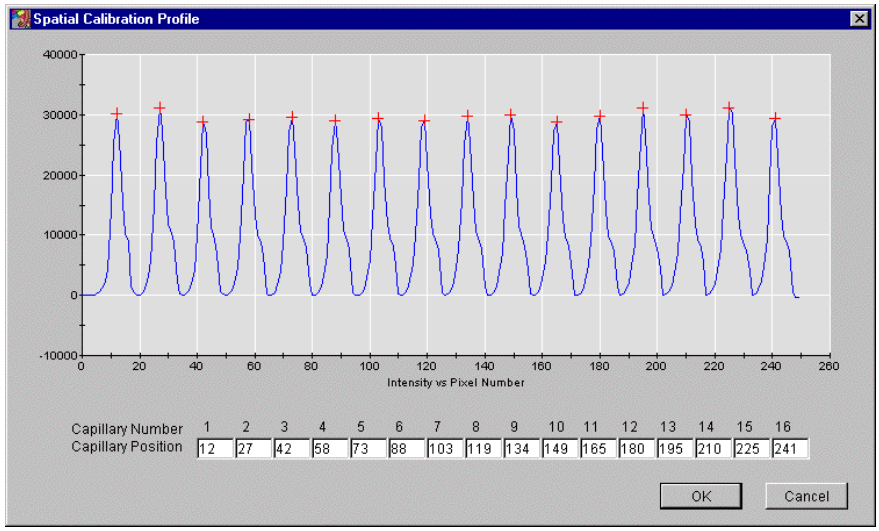
Evaluating a Spatial Calibration Profile

Evaluation Criteria

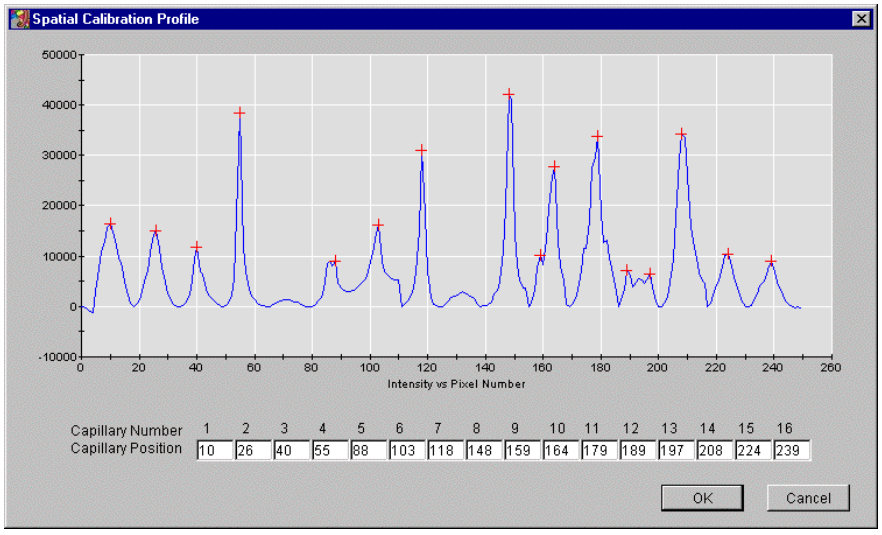
While viewing the calibration profile in the Details dialog box, use the following criteria to evaluate the data:

Peak Attribute	Criteria
Height	Similar heights for all peaks.
Red crosses	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.
Shape	♦ Single sharp peak for each capillary. ♦ Small shoulders are acceptable.
Spacing	Position values are 13–16 higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.

Example of Passing Profile



Example of Failed Profile



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 new map will be used to process sample run data.

If the current run did not provide good data, you can override the new 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

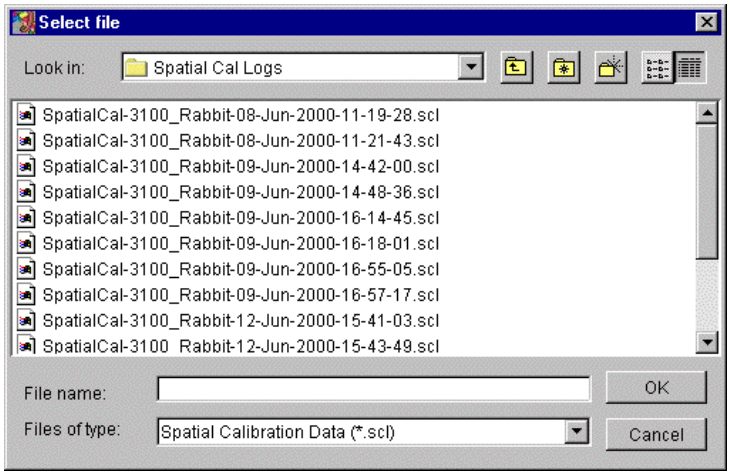
When overriding the data with data from a previous run, if possible, use data that was collected during a run performed:

- On the same capillary array
- Since the capillary array was last moved

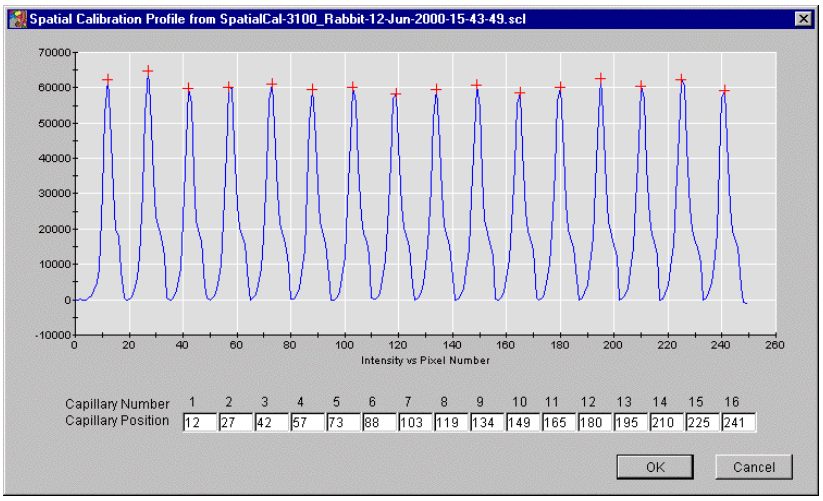
IMPORTANT Overriding calibration data is only allowed if the capillary array has not been removed and the detection cell has not been moved; do not use calibration data collected from another capillary array.

Overriding the Current Spatial Calibration Profile

To override the current spatial calibration map:

Step	Action
1	From the **File** menu, select **Override Spatial Calibration**. The **Select file** dialog box appears. 
2	Select the spatial calibration file that you want to use.

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

Step	Action
3	Click **OK**. This opens the **Spatial Calibration Profile from** box with the data for the selected file displayed. 
4	Click **OK**. This data is now the current spatial calibration map and the **Spatial Calibration Profile from** box closes.

Section: Spectral Calibration

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

About Spectral Calibrations

Introduction

A spectral calibration creates a matrix that corrects for the overlapping of fluorescence emission spectra of the dyes.

When to Calibrate

You must perform a spectral calibration:

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

Procedure Overview

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

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 method file is used in place of a run module.

Parts of the Spectral Calibration Procedure

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 3100 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 (see page A-6).

Parts of the Spectral Calibration Procedure *(continued)*

Part	Description
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 distinct fluorescence emission maxima ♦ 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 16 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.

Performing a Spectral Calibration Using Default Processing Parameters

Introduction

Use the procedure below to perform a basic, "default" spectral calibration for both DNA sequencing and fragment analysis.

Preparing the Equipment

To prepare the equipment and supplies:

Step	Action
1	Start the computer and the instrument.
2	Prepare the instrument for a run as described starting on page 3-19.
3	Prepare an ice bucket with wet ice.

DNA Sequencing: Sample Types for Spectral Calibration

There are two types of samples from which matrices can be made:

- Matrix standard
- BigDye sequencing sample

The procedures for preparing both sample types are covered in the tables below.

DNA Sequencing: Preparing the Matrix Standard for Dye Set E Matrices

To prepare the matrix standards for Dye Set E Matrices:

Step	Action
1	Thaw and mix thoroughly the DS-01 (P/N 4315974) matrix standard tube.
2	Spin the tube briefly in a microcentrifuge.
3	Prepare the Matrix Standard Set DS-01 for Dye Set E by combining the following in a labeled 1.5-mL microcentrifuge tube: **Reagent** / **Volume (μL)** Matrix Standard Set DS-01 (dROX, dTAMRA, dR6G, dR110) / 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.
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.

DNA Sequencing: Preparing BigDye Sequencing Sample for Dye Set E Matrices

The best samples to choose for making a matrix have approximately 25% each of A, C, G, and T. A good example of this is the BigDye Terminator Sequencing Standard, or pGem

To prepare the BigDye Terminator standard for Dye Set E Matrices:

Step	Action
1	Resuspend a tube of BigDye Terminator Sequencing Standard (P/N 4304154) 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	Immediately place the tubes on ice for 2 min.

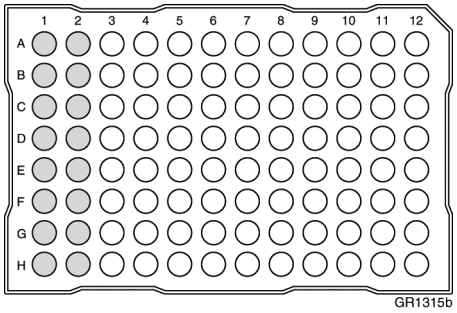
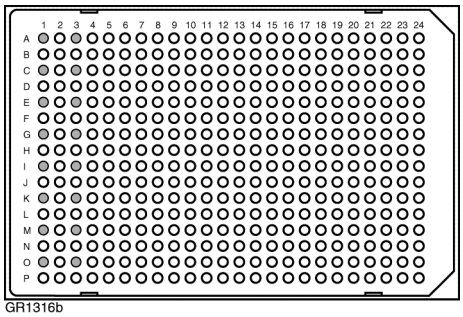
Fragment Analysis: Preparing the Matrix Standards for Dye Set D Matrices

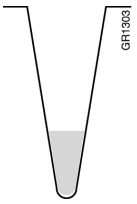
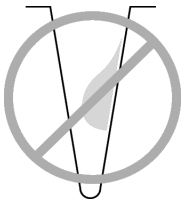
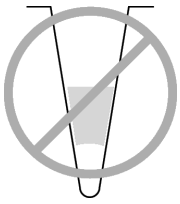
To prepare the Matrix Standards for Dye Set D Matrices:

Step	Action
1	Thaw and mix thoroughly the four DS-30 (P/N 4316100) matrix standard tubes.
2	Spin the tubes briefly in a microcentrifuge.
3	Prepare the Matrix Standard Set DS-30 for Dye Set D by combining the following in a labeled 1.5-mL microcentrifuge tube: **Reagent** / **Volume (µL)** 6FAM — 1.25 HEX — 1.25 NED — 1.25 ROX — 1.25 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.
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.

Loading the Standards

To load the standards:

Step	Action
1	Dispense 10 µL of the denatured matrix standard into a: ♦ 96-well plate, wells A1 through H2, as shown below.  GR1315b ♦ 384-well plate, wells A1, A3, C1, C3, E1, E3, etc. as shown below.  GR1316b
2	Centrifuge the plate so that each standard is positioned at the bottom of its well. Your samples should:

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

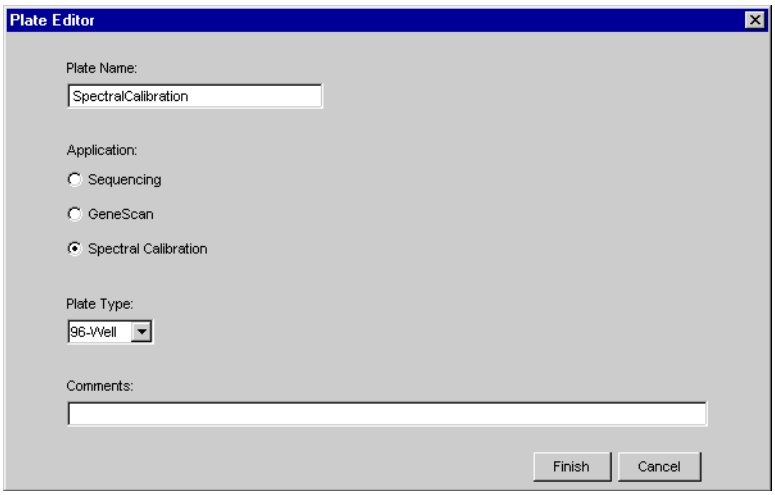
Preparing the Plate and Instrument

Follow the instructions in Chapter 3, “Performing a Run,” to:

- Assemble the plates (page 3-9).
- Check and refill the fluids on the instrument (page 3-22).
- Place the plate on the autosampler (page 3-25).

Creating a Plate Record

To create a plate record for the denatured matrix standards:

Step	Action
1	Within the **Plate View** page of the 3100 Data Collection software, click **New**. This opens the **Plate Editor** dialog box.
2	In the **Plate Editor** dialog box: a. Name the plate. b. Select **Spectral Calibration**. c. Make sure that the appropriate plate size is selected.  d. Click **Finish**. This opens the Plate Editor spreadsheet.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>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 E

Matrix standard</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: Spect36_POP6DefaultModule
– 50-cm: Spect50_POP6DefaultModule
d. Select the spectral parameter MtxStd{Sequencing-SetE}.par.
e. Click OK.</td></tr>
<tr><td>Dye Set E

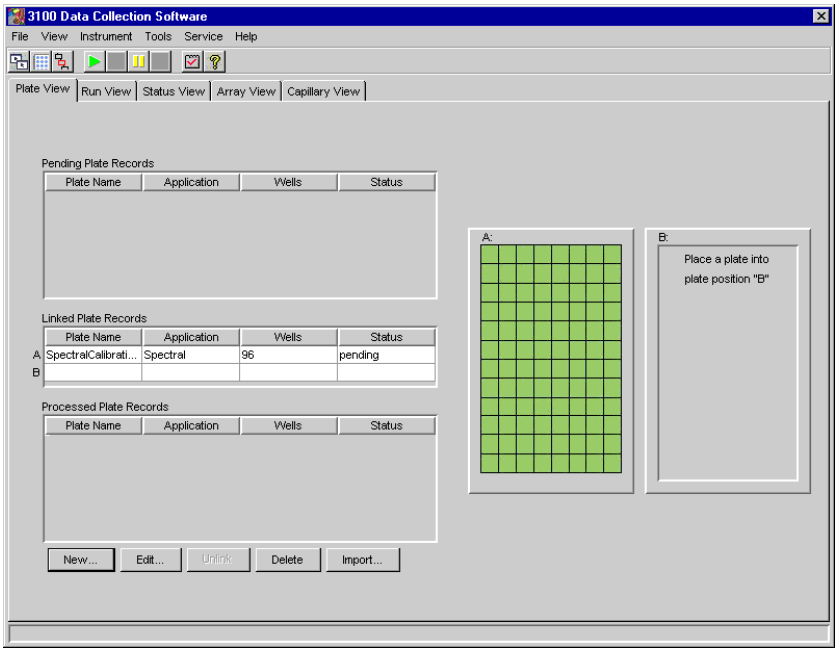
BigDye sample</td><td>a. Type a name for the samples.
b. Select Dye Set E.
c. Select the run module depending on your capillary array size:
– 36-cm: Spect36_POP6DefaultModule
– 50-cm: Spect50_POP6DefaultModule
d. Select the spectral parameter SeqStd{Sequencing-SetE}.par.
e. Click OK.</td></tr>
<tr><td>Dye Set D</td><td>a. Type a name for the samples.
b. Select Dye Set D.
c. Select the run module Spect36_POP4DefaultModule.
d. Select the spectral parameter MtxStd{GeneScan-SetD}.par.
e. Click OK.</td></tr>
</table>

IMPORTANT Make sure the correct spectral parameter file has been selected for the type of dyes you are running. Selecting the incorrect parameter file will cause the spectral calibration to fail.

This creates a plate record for the calibration run in the database. After a few seconds, the entry for the plate record appears in the Pending Plate Records table of the Plate Setup page.</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 on the toolbar is enabled, meaning that the instrument is ready to 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 on the toolbar to begin the run.

Run Times

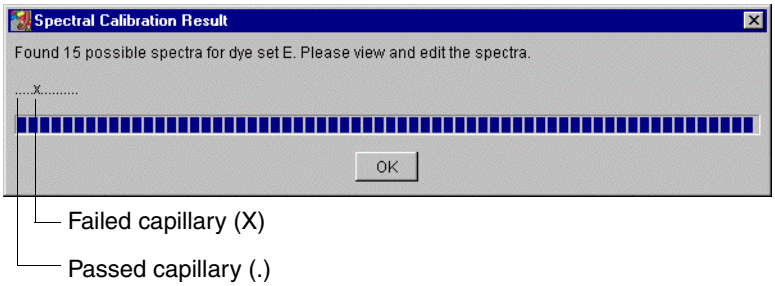
The following table lists the spectral calibration run times:

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

Spectral Calibration Result Box

At the end of the run, while the data is being analyzed, the Spectral Calibration Result dialog box opens to indicate which capillaries have passed and which have failed.

The example below for Dye Set E shows one failed capillary, which is represented by an “X”, and 15 passed capillaries, which are represented by a “.” dot.



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 4-25.

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 3-53).

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

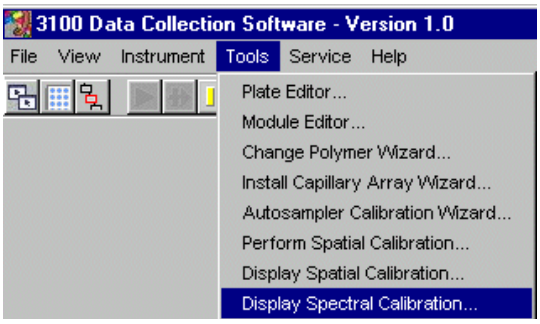
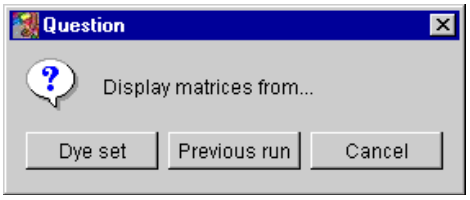
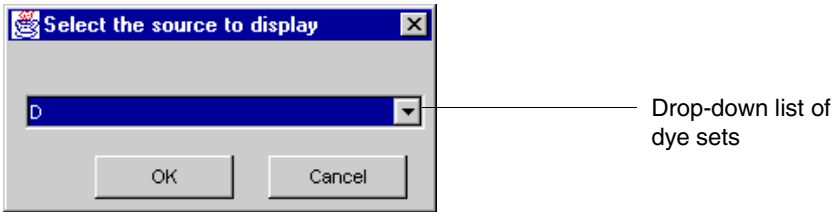
Introduction

At any time, you can display the:

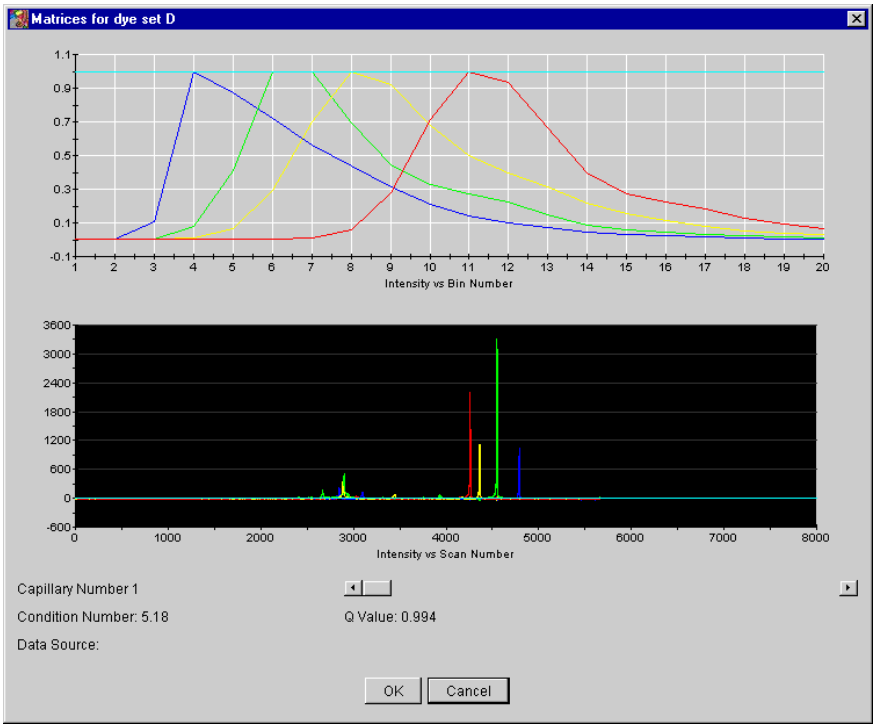
- Current spectral calibration profile for a specified dye set. The current profile is the one that was created when the last spectral calibration was performed and which is stored in the instrument database. The current profiles can be examined only if a spectral calibration has been performed for this 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:

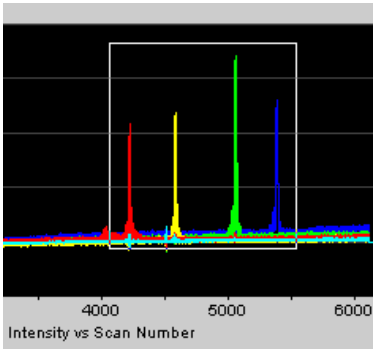
Step	Action
1	From the **Tools** menu, select **Display Spectral Calibration**.  The **Question** dialog box appears. 
2	Click **Dye set**. This opens the **Select the source to display** dialog box. 
3	From the drop-down list, select the dye set for the matrices that you want to examine.

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

Step	Action
4	Click **OK**. This opens the **Matrices for dye set** box. 
5	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 (See “Q-Value” on page 4-42.) ♦ Condition number from 3–5 for sequencing, or 4–7 for fragment analysis. (See “Condition Number (C-Value)” on page 4-44.)
6	Click **Cancel** to close the dialog box.

For a Closer Look

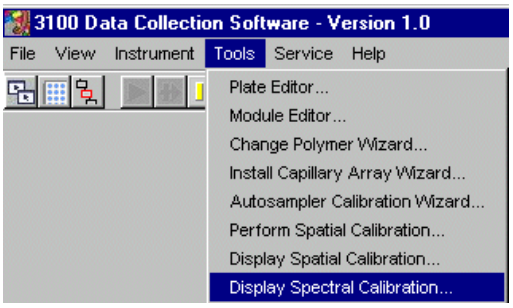
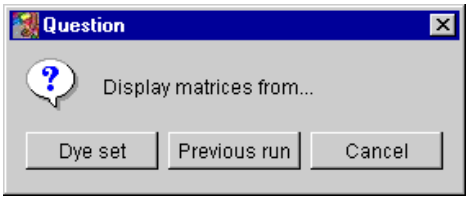
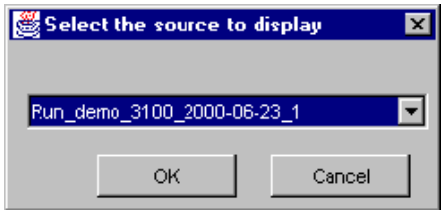
To zoom in on a portion of either graph, press SHIFT and drag the mouse.



To reset the view, press R.

Examining Profiles Used for Previous Runs

To examine the matrices used to process a previous run:

Step	Action
1	From the **Tools** menu, select **Display Spectral Calibration**.  The **Question** dialog box appears. 
2	Click **Previous Run**.
3	From the drop-down list, select the profile to be displayed, and then click **OK**. 

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 all 16 capillaries.

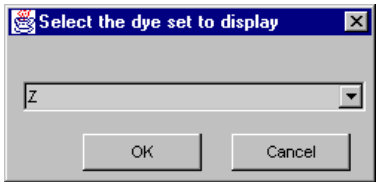
You can override a profile with a good-quality profile that was collected either:

- 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)

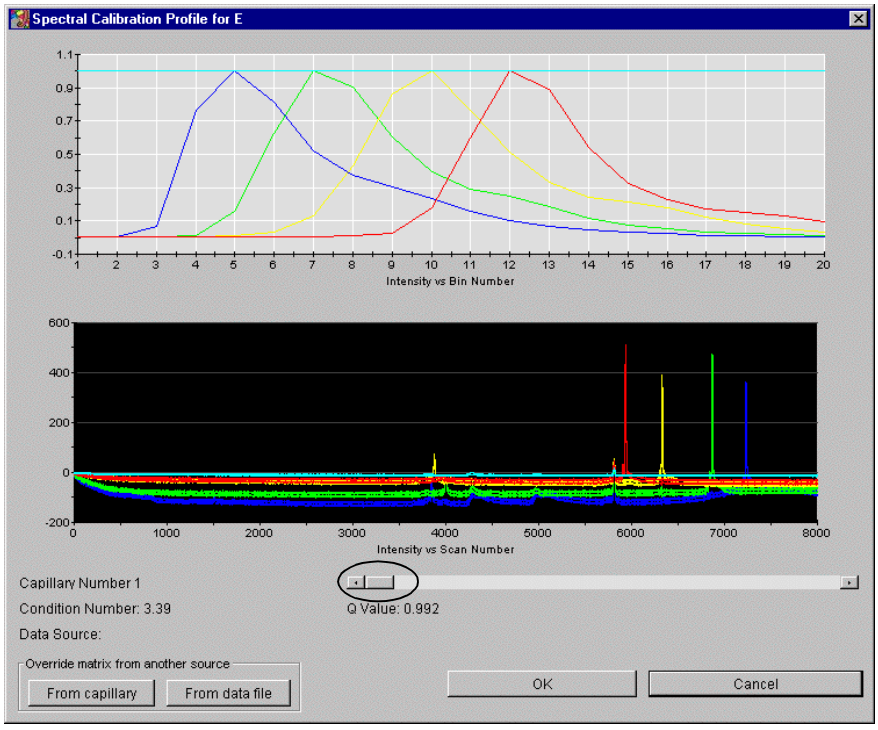
Overriding with Data from Another Capillary

Note To ensure the highest quality data, we recommend that you do not override capillary profiles.

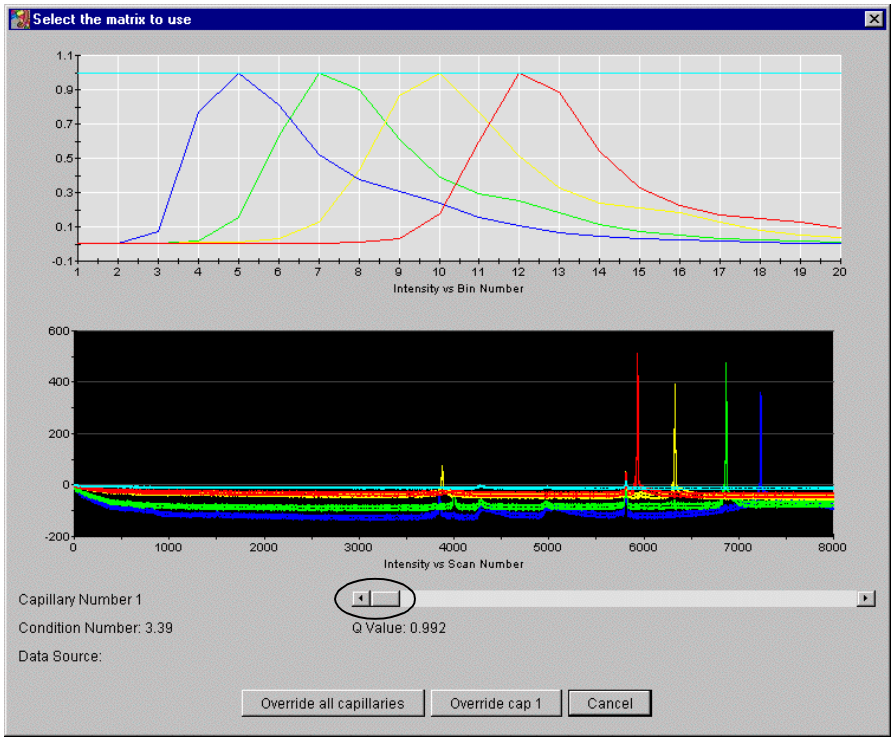
To override a spectral calibration profile with data from another capillary:

Step	Action
1	From the **File** menu, select **Override Spectral Calibration**. The **Select the dye set to display** dialog box appears. 

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

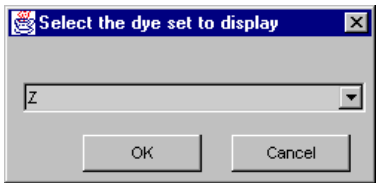
Step	Action
2	From the drop-down list, select the appropriate Dye Set, and then click **OK**. The current spectral profile is displayed. 
3	Use the slider bar to select the capillary to be overridden.
4	Click **From capillary**.

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

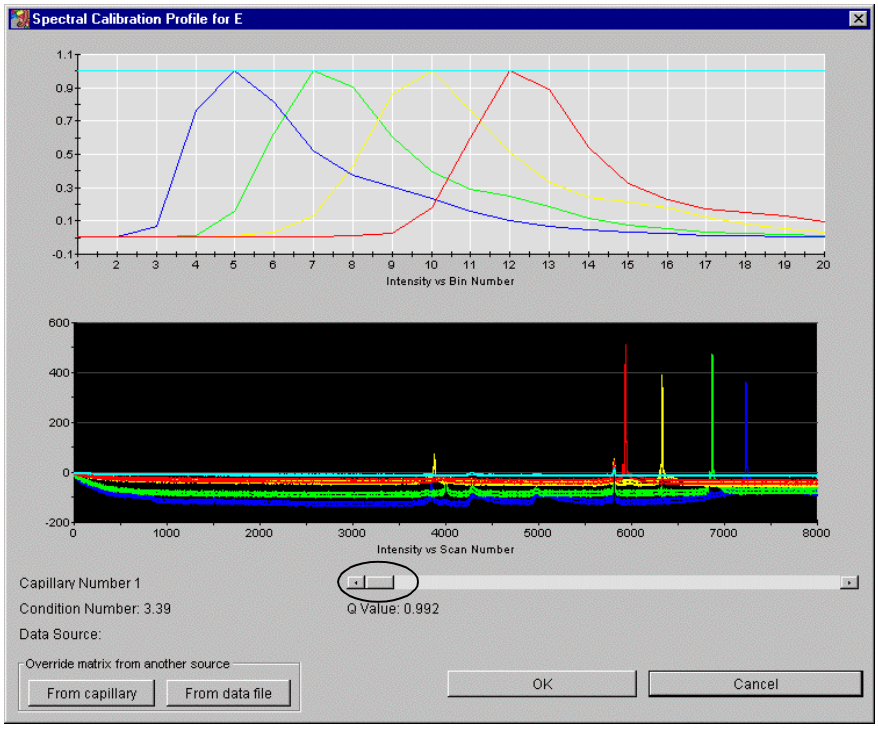
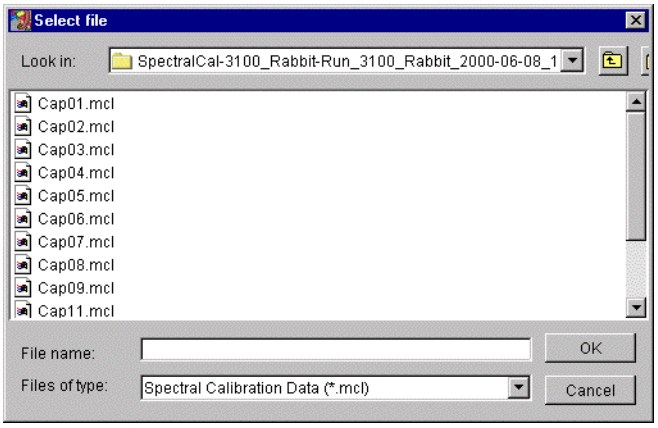
Step	Action
5	Use the slider bar to select the source capillary to override the unsatisfactory profile. 
6	Confirm that the correct capillary appears next to **Capillary Number**, and then click the appropriate button.

Overriding with Previously Collected Data

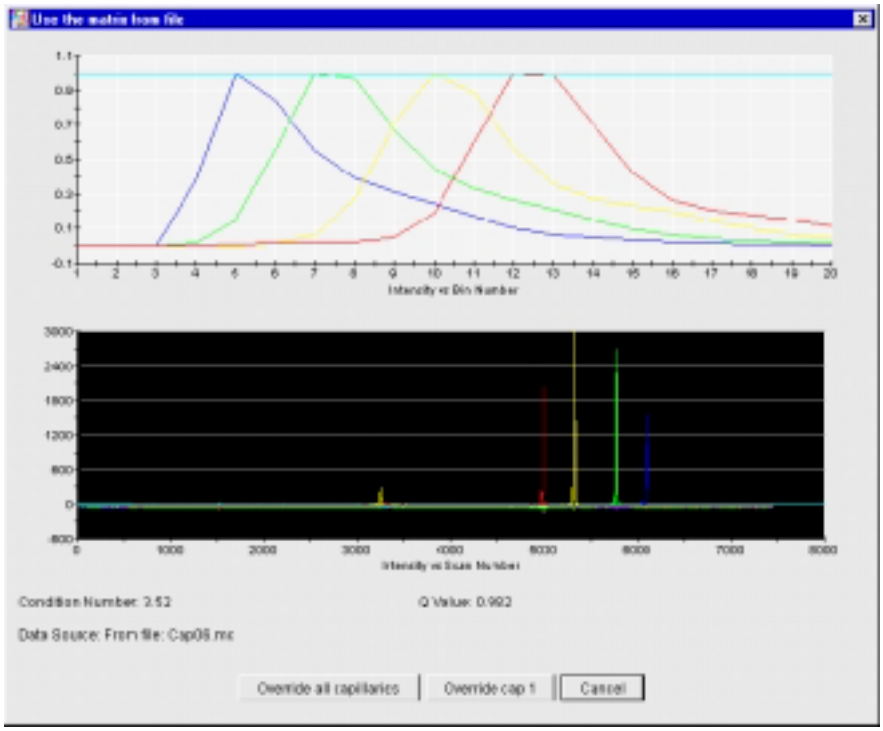
To override a spectral calibration profile with previously collected data:

Step	Action
1	From the **File** menu, select **Override Spectral Calibration**. The **Select the dye set to display** dialog box appears. 

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

Step	Action
2	From the drop-down lists, select the appropriate Dye Set, and then click **OK**. The current spectral profile is displayed. 
3	Use the slider bar to select the capillary with the profile to be overridden.
4	Click **From data file**.
5	Locate and select the spectral source file (.mcl) to override the unsatisfactory profile, and then click **OK**. 

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

Step	Action
6	In the **Use the matrix from file** dialog box, confirm that the correct file appears next to **Data Source: From file**, and then click the appropriate button. 
7	Click **OK**.

Section: Advanced Features of Spectral Calibration

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

Fine-Tuning a MatrixStandard Calibration

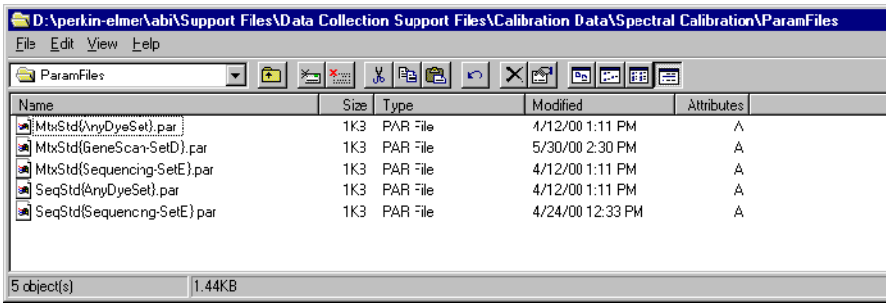
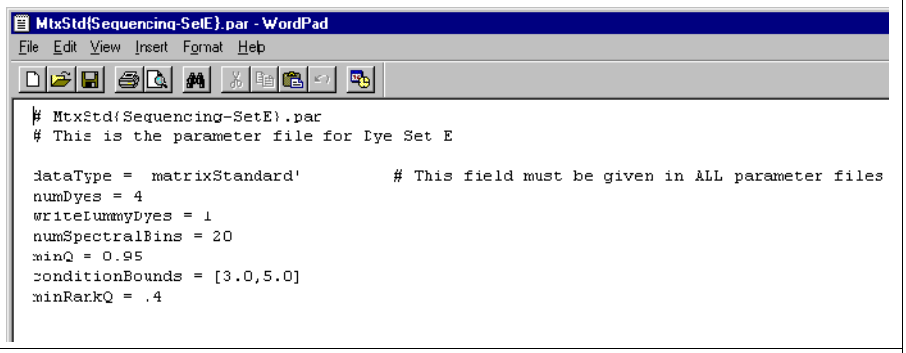
Introduction

Use the procedure below to fine-tune the parameter for a calibration run for:

- DNA sequencing with Dye Set E
- Fragment analysis with Dye Set D

Fine-Tuning a Calibration Run

To create a spectral calibration parameter file for matrixStandard dataType:

Step	Action
1	Navigate to the **Spectral Calibration** folder in the following directory: D:\AppliedBio\abi\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration
2	Double-click the **ParamFiles** folder. This opens the folder, displaying the stored parameter files. 
3	Double-click the supplied parameter file that is appropriate for the type of calibration run you are performing (see the explanation given in each parameter file). This opens the file. **Note** The file for the “matrixStandard” dataType and Dye Set E will be used in this example. 
4	Edit the values for **minQ** and/or **conditionBounds** as appropriate. **Note** Under normal circumstances, do not change the default value for the conditionBounds parameter. See page 4-44.
5	From the **File** menu, select **Save As**.
6	In the **File name** text box, type a name for the new spectral parameter file.

To create a spectral calibration parameter file for matrixStandard dataType: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>7</td><td>Click Save.

Note This saves the file in the ParamFiles folder.</td></tr>
<tr><td>8</td><td>
<table>
<tr><th>If you are performing a calibration run for...</th><th>Then follow the “Performing a Spectral Calibration Using Default Processing Parameters” on page 4-18, but...</th></tr>
<tr><td>DNA sequencing</td><td>select the parameter file you just created instead of the default parameter file MtxStd{Sequencing-SetE}.par.</td></tr>
<tr><td>Fragment analysis</td><td>select the parameter file you just created instead of the default parameter file MtxStd{GeneScan-SetD}.par.</td></tr>
</table>
</td></tr>
</table>

Spectral Calibration Matrices

Introduction

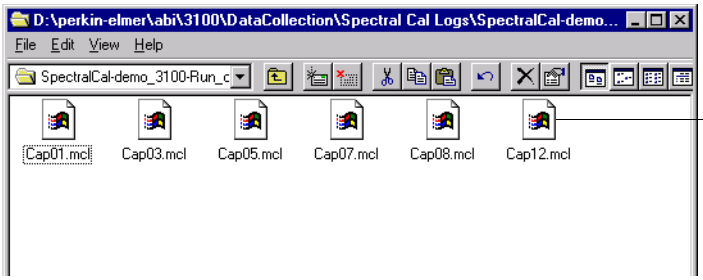
A spectral calibration matrix is a mathematical matrix that describes the fluorescence emission spectra of the four or five dyes being used.

For each capillary, only one spectral calibration matrix can be stored in the instrument database. At the end of a spectral calibration run, the spectral calibration matrix in the database for each capillary is overwritten.

Locating Matrix Files

For each matrix produced during the calibration run, a separate spectral calibration file is stored in a folder named Spectral Cal Logs in the following directory:

D:\AppliedBio\abi\3100\DataCollection\Spectral Cal Logs\SpectralCal



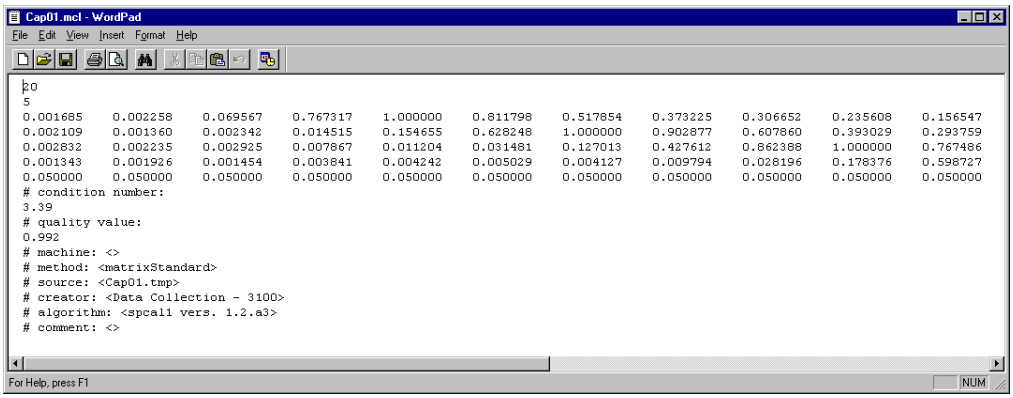
The naming conventions are given below.

Folder/File Type	Naming Format
Spectral calibration folder	SpectralCal-*instrumentname*-Run*year_date_time*
Spectral calibration file	Cap*capillarynumber*.mcl

Examining a Spectral Calibration Matrix

If you open a matrix file in an accessory application such as WordPad or Notepad, you can see that it is a matrix of numbers.

- The top two numbers define the dimensions of the matrix.
- Left to right, the columns represent the 20 spectral bins.
- Top to bottom, the numbers represent the relative fluorescence intensity of each of the dyes across a bin, in the following order: blue, green, yellow, red, fifth dye.



Spectral Calibration Log Files

Introduction

At the end of a spectral calibration run, a spectral calibration log file is automatically created. This file provides a list of the capillaries that passed and failed the calibration run.

Locating a Spectral Calibration Log Files

Spectral calibration log files are stored in the following directory:

D:\AppliedBio\abi\3100\DataCollection\Spectral Cal Logs

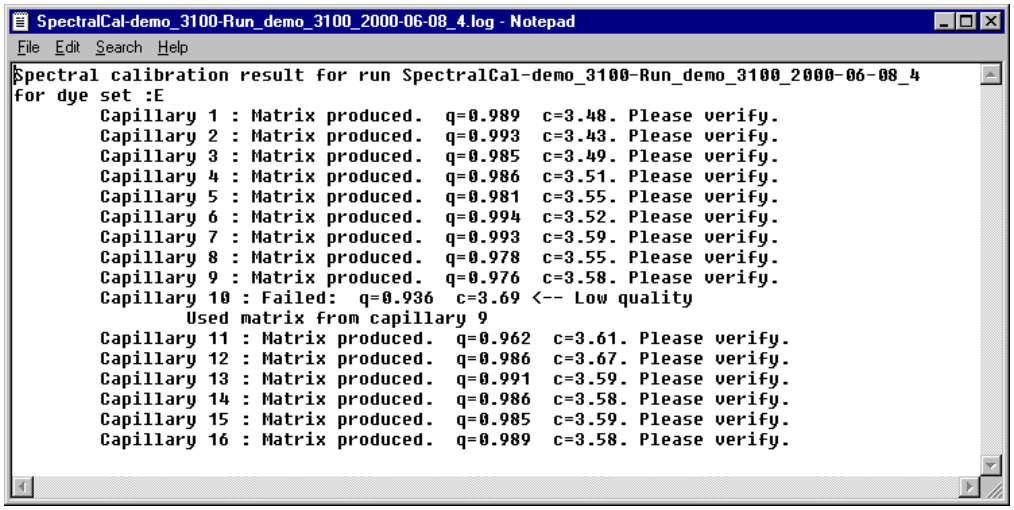
File Naming Convention

Spectral calibration log files have the following file name convention:

SpectralCal-*instrument name*-Run_*instrument name_year_date_run no. for day*.log

Example File Opened in Notepad

An example of a file opened in Notepad is shown below.



Spectral Calibration Parameter Files

Introduction

Spectral calibration parameter files are text files that contain the run parameters used for spectral calibrations. You can edit the parameters of an existing parameter file to create your own parameter file.

Locating Parameter Files

Spectral calibration parameters files are stored in the following directory:

D:\AppliedBio\abi\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\Param Files

List of Parameter Files Supplied

The spectral calibration parameter files included with the 3100 software are listed below:

Source of Dyes for Calibration	Parameter File Name	Application	With a conditionBounds Check?
Matrix standard for the desired dye set	MtxStd{AnyDyeSet}.par	Sequencing with any dye set	No
DS-30 matrix standard	MtxStd{GeneScan-SetD}.par	Fragment analysis with Dye Set D	Yes
DS-01 matrix standard	MtxStd{Sequencing-SetE}.par	Sequencing with Dye Set E	Yes
Sequencing sample from any dye set	SeqStd{AnyDyeSet}.par	Sequencing with any dye set	No
Sequencing sample from Dye Set E (BigDye and dRhodamine chemistry	SeqStd{Sequencing-SetE}.par	Sequencing with Dye Set E (preferred method)	Yes

Parameter Files Are Editable

You can use one of the spectral calibration parameter files supplied with the 3100 software or create your own using a supplied file as a template.

There are two reasons to set your own spectral calibration parameters:

- To fine-tune the conditions of a sequencing calibration run with Dye Set E or a fragment analysis calibration run with Dye Set D.
- To use a four-color dye set other than Dye Set D or Dye Set E. In other words, to use the sequenceStandard type of calibration rather than the matrixStandard type.

Editing a Spectral Calibration File

To edit a spectral calibration parameter file:

Step	Action
1	Locate the parameters folder in the following directory: D:\AppliedBio\abi\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\Param Files
2	Select the file to edit, and open it in WordPad or Notepad accessory application.

To edit a spectral calibration parameter file: *(continued)*

Step	Action
3	Edit the parameter values as desired. See “Spectral Calibration Parameters” on page 4-40 for the list of parameters and their acceptable values.
4	From the **File** menu, select **Save As**. Save the file with a unique name and a .par extension in the same directory. **IMPORTANT** Do not override original spectral calibration parameter files.

Spectral Calibration Parameters

About Spectral Calibration Parameters

Spectral calibration algorithms use variables known as spectral calibration parameters. Each parameter has one or more options that you can set.

How Parameters Are Stored

Spectral calibration parameters are stored in text files called spectral calibration parameters files. These text files have a .par extension and they are editable.

Parameters List

The spectral calibration parameters that have values you can define are listed below.

Parameter Name	Allowed Values	Default Value	Comments	See Page
dataType	♦ matrixStandard ♦ sequenceStandard	—	Selects the type of algorithm to use. Use the matrixStandard algorithm, except when using a sequencing sample to generate a matrix.	4-41
minQ	Any number from 0.0 to 1.0	0.95 for all parameter files except SeqStd{AnyDyeSet}.par, which is 0.92	Use with both dataType parameters.	4-42
conditionBounds	Any number from 1.0 for both [*minimum allowable c-value*, *maximum allowable c-value*] (see default as an example.)	♦ [3.0, 5.0] for sequencing analysis ♦ [4.0, 7.0] for fragment analysis	Use with both dataType parameters. No conditionBound numbers are used for the Any Dye Set.par files.	4-44
numDyes	Integers from 2 to 7	4	Indicates number of dyes used in sequencing or fragment analysis. Do not change this value.	4-46
writeDummyDyes	Integers from 0 to 9	0	Allows for the use of an additional dye. Do not change this value.	4-46
numSpectralBins	Integer from 2 to 50	20	Indicates the number of pixel bins on the CCD. Do not change this value.	4-46
startptOffset	Integer from 0 to 5000	200	Use only with the sequenceStandard dataType.	4-48
maxScansAnalyzed	Integers greater than or equal to 100	6000 for SeqStd{AnyDyeSet}.par	Use only with the sequenceStandard dataType.	4-49
startptRange	Integer for both the minimum and maximum: [*minimum*, *maximum*]	matrixStandard: none sequenceStandard: [800,500]	Use only with the sequenceStandard dataType.	4-49
minRankQ	Any number from 0.0 to 1.0	0.5	Indicates spectral data purity threshold. Use for matrixStandard only.	4-50

dataType Parameter

Types of Algorithm

There are two types of spectral calibration that correspond to two types of algorithm. They are:

- matrixStandard (assumes one peak detected per dye)
- sequenceStandard (assumes multiple peaks detected per dye)

Selecting the Type

By selecting the type of algorithm, you are deciding whether to perform a calibration run using one of the matrix standard sets, or using other dye sets about which the 3100 Data Collection software has no prior information.

The following table describes when to use each type of calibration:

When you are performing a calibration run with...	Use dataType...	See the procedure on page...
♦ Matrix Standard Set DS-30 for Dye Set D standard, or ♦ Matrix Standard Set DS-01 standard for Dye Set E	matrixStandard	4-34
A sequencing reaction that uses Dye Set E	sequenceStandard	4-34
A sequencing reaction that uses a four-color dye set other than Dye Set D or E	sequenceStandard	—
Matrix standards not using Dye Set D or E	matrixStandard	—

Pass/Fail Stringency Parameters

If you are using either dataType parameter, you can determine whether the calibration for a particular capillary will pass or fail by specifying the values for the parameters:

- minQ
- conditionBounds

If, for your particular samples, you want spectral calibration matrices that are very close to the perfect theoretical matrix, you can specify the parameter values so that only very high quality matrices will pass. Alternatively, you can select less stringent parameter values, which may give you calibrations that are reliable enough for your particular application and also result in more passing capillaries.

Note If you make the stringency very high, there will be more failed capillaries. If you use a failed capillary, the matrix may be overridden by a matrix from a distant capillary. The overriding matrix may give poorer results when applied to the new capillary. You must consider this when assigning the parameters, particularly for fragment analysis.

minQ Parameter

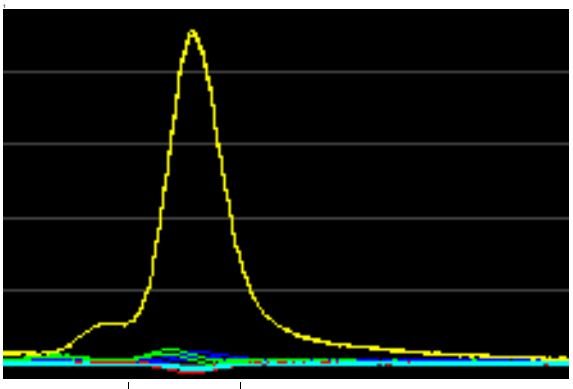
Introduction

The minQ parameter is used to set the tolerance for pull-up/pull-down peaks.

About Pull-Up and Pull-Down Peaks

A pull-up peak is a small peak of another color that appears under a main dye peak in an electropherogram.

A pull-down peak is the same, except it appears below the baseline under a main dye peak.



Pull-up/Pull-down peaks

Cause of Pull-Up and Pull-Down Peaks

Pull-up and pull-down peaks are caused by:

- Overloading the calibration standards.
- Differences in the shapes of the dye peaks recorded during a spectral calibration run compared to those in a theoretically perfect spectrum. The imperfections drag the intensity of the processed fluorescence data from a neighboring dye either up or down.

Q-Value

The 3100 Data Collection software calculates a value named “Q”, which is a measure of the consistency between the final matrix and the data from which it was computed. When the Q-value is 1.0 the fit is perfect, providing an ideal matrix with no detected pull-up/pull-down peaks.

The minQ value sets the minimum allowable Q-value for a passing capillary.

After a spectral calibration run, the software calculates the Q-value for each capillary used in the run. If this number is less than the minQ value set in the selected parameter file, the capillary will fail and the matrix will be automatically overridden by one from another capillary.

Note The methods for computing the Q-values for matrixStandard and known dye sets (Dye Sets D and E) sequenceStandard dataType parameters are slightly different. For this reason, the minQ values for the unknown dye sets sequenceStandard dataType should be set lower than the values for the known matrixStandard or sequencingStandard dataType.

High Q-Values

In rare cases, a high Q-value can be computed for a poor matrix. This can happen if the matrix standard is contaminated, leading to the creation of one or more extra peaks. The extra peak(s) causes the true dye peak to be missed by the algorithm. By chance, this can lead to a higher Q-value than would be computed with the correct peak. The best way to intercept this error is to visually inspect the spectral calibration profile for each capillary (see “Displaying a Spectral Calibration Profile” on page 4-25).

conditionBounds Parameter

Definition

The conditionBounds parameter value comprises two numbers that represent the lower and upper bounds of the matrix condition number, also called the c-value. The conditionBounds value format is [*lowest allowable c-value*, *highest allowable c-value*].

Condition Number (C-Value)

The condition number is a single number that indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set.

If there were no overlap in a dye set, the c-value would be 1.0, the lowest possible value. The condition number increases with increasing peak overlap. Because of slight variations in optics between instruments, a range is used to define the conditionBounds. As the expected range of condition numbers is different for different dye sets, the conditionBounds values are different for calibration runs that use different dye sets.

Using the Correct Values

Use the following table to select the correct conditionBound values.

<table>
<tr><th>If you are...</th><th>Then...</th></tr>
<tr><td>creating a parameter file and are intending to perform a spectral calibration for either dye set:
♦ D (with Matrix Standard Set DS-30)
♦ E (with Matrix Standard Set DS-01 or sequencing reaction)</td><td>you do not need to change the conditionBounds values. The conditionBounds values are listed below:
<table><tr><th>Parameter File</th><th>conditionBounds Value</th></tr><tr><td>MtxStd{Sequencing-SetE}.par</td><td>[3.0, 5.0]</td></tr><tr><td>SeqStd{Sequencing-SetE}.par</td><td>[3.0, 5.0]</td></tr><tr><td>MtxStd{GeneScan-SetD}.par</td><td>[4.0, 7.0]</td></tr></table></td></tr>
<tr><td>calibrating for a different dye set using the sequenceStandard dataType</td><td>determine the appropriate conditionBounds value range for that dye set.</td></tr>
<tr><td>you do not want to set the conditionBounds value (recommended the first time you use a new dye set other than D and E)</td><td>use the supplied parameter file SeqStd{AnyDyeSet}.par or MtxStd{AnyDyeSet}.par.</td></tr>
</table>

How the conditionBounds Value Is Used by the Software

After a spectral calibration run, the software computes the condition number of the spectral calibration matrix obtained for each capillary. If the condition number falls outside the conditionBounds range set in the selected parameter file, the capillary will fail and the matrix from a neighboring capillary will be used in its place.

In other words, the conditionBounds parameter allows you to discard spectral matrices that do not conform to the overlap pattern expected for the dye set used.

IMPORTANT If you select a parameter file that is prepared for a particular dye set and then use matrix standards for a different dye set, the capillaries will not pass the calibration. In addition, if you have a contaminant in your dyes that affects the spectra, the calibrations are likely to fail.

What the Condition Number Allows You to Compare

An ideal dye set has no spectral overlap. The condition number allows you to compare how close different dye sets are to the ideal, which is one. The lower the condition number, the smaller will be the overlap from neighboring dyes.

What the Condition Number Does Not Allow You to Compare

The condition number does not allow you to compare the quality of a matrix from one capillary with a matrix from another capillary collected during the same calibration run. This is because of slight differences in the optics from one side of a capillary array to the other. These differences may cause the condition numbers to be systematically higher on one side of the array than on the other.

IMPORTANT Do not manually override matrices that have slightly higher c-values on one side of the array because these matrices are still the most accurate for the capillaries they describe and will result in the smallest amount of pull-up/pull-down.

Determining Suitable conditionBounds Values for a Dye Set

To determine a suitable condition number range for a dye set:

Step	Action
1	Perform a spectral calibration without setting a conditionBounds value, such as with the SeqStd{AnyDyeSet}.par file or the MtxStd{AnyDyeSet}.par file.
2	Examine the spectral calibration profiles for each capillary.
3	Open the spectral calibration log file in the following directory: D:\AppliedBio\abi\3100\DataCollection\SpectralCalLogs
4	Record the computed condition numbers for each capillary.
5	Plot a frequency distribution histogram of the condition numbers. Do this by grouping the values into ranges and plotting the number of capillaries that fall within each range. The histogram will probably be a skewed normal distribution curve.
6	Use your judgment to determine minimum and maximum condition numbers. You are aiming to set numbers that are: ♦ Close enough to the mean to eliminate outliers ♦ Not so close to the mean that unnecessary failures on subsequent calibration runs are caused by: – Normal variation across the capillary array – Instrument-to-instrument variation The maximum and minimum numbers should typically be set generously out on the tails of the distribution curve.

numDyes and writeDummyDyes Parameters

Introduction The numDyes and writeDummyDyes parameters are used together to provide information about the number of dyes being used for a spectral calibration. The values of the two parameters must add up to five, which is the maximum number of dyes that can be used. With four dyes, the numDyes value is set to 4 and the number left over is the value assigned to writeDummyDyes, which is 1.

numSpectralBins Parameter

Introduction The numSpectralBins parameter defines the number of bins of data being collected in the spectral dimension of the CCD. The 3100 instrument has 20 bins.

This parameter exists because no header information is provided in the raw color data files (.tmp), so there is no mechanism for the algorithm to determine this value automatically.

Parameters Specific to sequenceStandard dataType

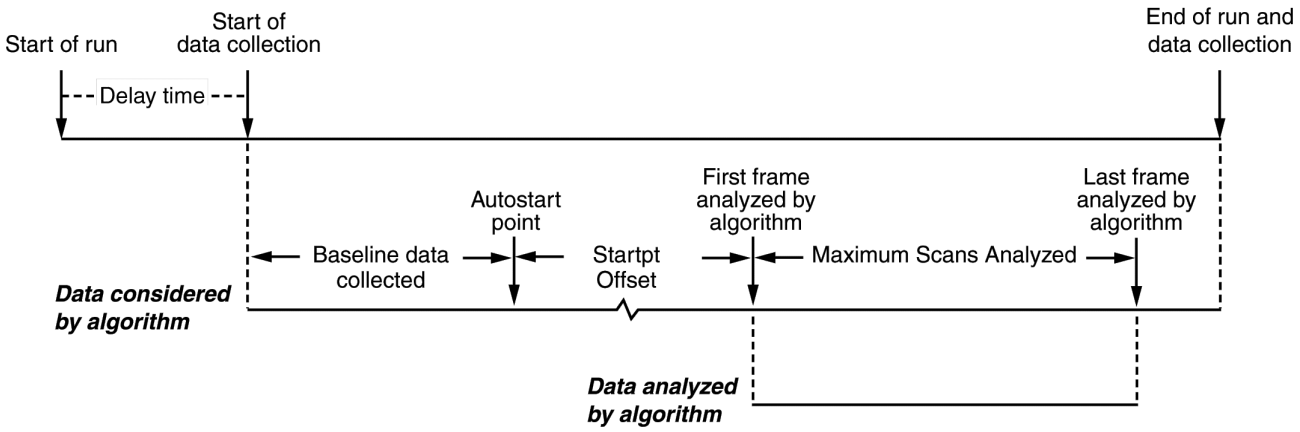
Parameters List

The following parameters are used only when the sequenceStandard dataType is selected:

- startptOffset
- maxScansAnalyzed
- startptRange

Summary Diagram

The following diagram summarizes the individual parameters that follow.



Note that there are two separate processes involved:

- Data collection, which happens during the run
- Data analysis, which occurs after the run, by the spectral calibration algorithm using the data collected and stored in the instrument database

startptOffset Parameter

Introduction If you use the sequenceStandard dataType parameter, you can select a value for the startptOffset (starting point offset) parameter.

Purpose The startptOffset parameter gives you control over which data frame the spectral calibration algorithm examines first.

Data Delay Time When you click the Start Run button to begin a run, time starts to be measured by the 3100 Data Collection software in data frames. During the first part of a run, there is no dye fluorescence because the DNA fragments have not yet migrated to the detection window. Typically, the software does not start to collect data immediately after sample injection to save instrument database space. The time between injection and the start of data collection is called the data delay time and is one of the parameters that you can set when you create a run module.

Collected Data All of the data frames from the start to the end of data collection are collected and stored in the database. After the run, this data is considered by the algorithm as it performs its analysis.

AutoStart Point The first data frames typically have no fluorescence data and are used by the algorithm to collect baseline information. The data frame at which the first fluorescence is detected, is called the Autostart point.

startptOffset Value When the startptOffset value is set to zero, the Autostart point is also the first frame used by the algorithm for analysis. When the startptOffset parameter is set to a higher value, the first analyzed frame is one that was collected later.

If you want to prevent early dye signals from being used by the algorithm, you can increase the startptOffset value. You might, for example, do this to prevent data for a primer peak from being used.

A typical starting point offset is 200 frames.

maxScansAnalyzed Parameter

Introduction If you use the sequenceStandard dataType parameter, you can select a value for the maximum number of scans analyzed (maxScansAnalyzed) parameter.

Purpose The maxScansAnalyzed parameter sets the number of data frames that are analyzed by the spectral calibration algorithm.

How to Use If the quality of the spectral data being collected is high, this value can be lowered to speed up the calibration procedure and conserve instrument database space. However, if you set this value too low, there may be insufficient good regions in the data to pass the calibration.A typical maximum number of scans analyzed value is 6000 frames.

startptRange Parameter

Introduction If you use the sequenceStandard dataType parameter, you can select the starting point range (startptRange) parameter.

Purpose The startptRange (not shown in the summary diagram) defines the minimum and maximum frame numbers that bound the start of data analysis.

The startptRange parameter gives you fine control over the first frame number that the algorithm uses for analysis. Although you can select the starting point offset, the Autostart point is selected by the algorithm, based on when the first dye is detected. By using just the startptOffset parameter, you do not control the actual point at which the algorithm starts its analysis. However, by specifying the starting point range, you can have this control.

The startptRange parameter forces the analysis starting frame to lie within the selected range. It is applied after the Autostart computation and startptOffset are applied. To set an exact start point, set the lower and upper bounds to be the same value (for example, [3000, 3000]).

Examples The following example shows the effect on the starting frame number using a startptRange value of [3000,4000].

Analysis Starting Frame Number Without Setting the startptRange	Analysis Starting Frame Number When startptRange Is Set	Comments
3300	3300	Frame number lies within range, so startptRange has no difference.
2000	3000	Frame number is less than lower bound of range, so lower bound is used.
4200	4000	Frame number is higher than lower bound of range, so upper bound is used.

minRankQ Parameter

Introduction

The minRankQ parameter is used as an internal check on spectral purity. Spectral purity is measured by a mathematical metric called “rank.” Pure peaks will have rankQ values close to 1, whereas peaks consisting of mixtures of two or more dyes will have lower rankQ values (closer to 0).

Peaks with rankQ values less than the value of minRankQ are rejected. For this instrument, minRankQ is set to 0.4.

5 Software

Overview

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

Section: About the 3100 Software

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

ABI PRISM 3100 Genetic Analyzer Software CD-ROMs

Introduction

The ABI PRISM® 3100 Genetic Analyzer software was installed on your computer by an Applied Biosystems service engineer.

This software is provided on a set of six compact discs.

Contents of the CDs

The software CD-ROMs and their contents are listed below.

CD Title	Contents
3100 Software	♦ ABI PRISM® 3100 Firmware ♦ ABI PRISM® 3100 Data Collection software ♦ Auto Extractor ♦ Re-extraction utility ♦ Clean up database utility ♦ NewMethodImport utility ♦ Remove Run Modules utility ♦ Diskspace utility ♦ InitDB utility ♦ ABI Sample File Toolkit ♦ OrbixWeb™ 3.2 Professional Edition ♦ Orbix Desktop® 2.3 software ♦ Persistence Powertier® 4.321 ♦ Java Runtime Environment® 1.1.7b ♦ Adobe Acrobat Reader® with Search 3.01
GeneScan Applications (optional)	ABI PRISM® GeneScan® Analysis software, including the GeneScan program and sizecaller
Sequencing Analysis Applications (optional)	ABI PRISM® DNA Sequencing Analysis software, including the Sequencing Analysis program, basecaller, and Factura™ Software
Oracle® Software	Oracle® 8.0.5 database standard edition
Microsoft® Windows® NT Image software	This software prepares the computer hard disks for installing the 3100 software.
Diagnostic software	This software consists of diagnostic utilities for use by Applied Biosystems service engineers only.

Determining the Software Versions on Your System

To determine the ABI PRISM 3100 firmware and the ABI PRISM 3100 Data Collection software versions installed on your system, click the About Data Collection button on the toolbar.

3100 Genetic Analyzer Software Suite

Introduction

This section contains overviews of the software provided on the ABI PRISM 3100 Genetic Analyzer software CD-ROMs.

Firmware

Introduction to Firmware

Firmware controls the most basic operations of the instrument, such as opening valves. The firmware is largely controlled by the commands sent from the computer workstation. It acts as the link between the software commands and hardware operations.

About the 3100 Firmware

Unlike the previous ABI PRISM® instruments for DNA analysis, the 3100 firmware resides on the computer workstation. The 3100 firmware is downloaded when the instrument is started. Therefore, the instrument and the computer workstation must be running to perform any functions.

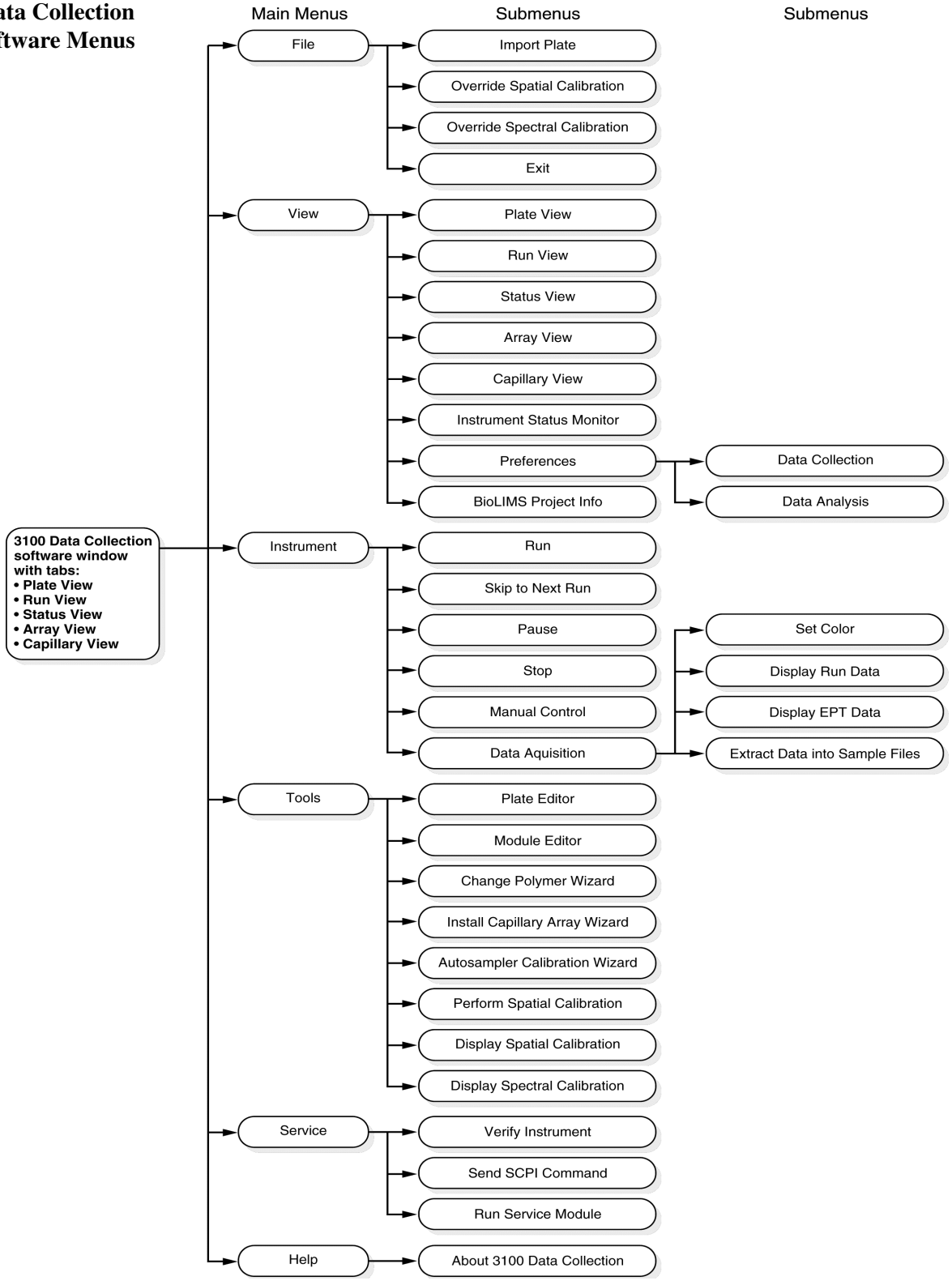
3100 Data Collection Software

Function

The 3100 Data Collection software performs the following functions:

- Works in conjunction with the 3100 firmware to control the mechanical operation of the instrument, such as moving the autosampler and switching on the oven
- Collects and stores plate record data and preference settings in the instrument database
- Automatically schedules samples to particular runs
- Monitors and displays the status of the instrument, and saves it to the instrument database as EPT data
- Collects and processes fluorescence emission data from the CCD camera during runs
- Stores the processed data in tables in the database and in temporary files on the hard drive
- Displays electropherograms for the current run or any previous run still stored in the instrument database
- Provides wizards, which guide you through routine maintenance procedures
- Provides utilities, which, when launched, automatically perform database maintenance

3100 Data Collection Software Menus



Auto Extractor

Auto Extractor is used by the Data Collection software to automatically extract and analyze the data after each run.

Diskspace Utility

The Diskspace utility lists the amount of space that the database uses, the amount that is free for use, and the percent filled.

Directions for using the Diskspace utility start on page 7-5.

Re-extraction Utility

The Re-extraction utility (Reextractor) uses the run data in the instrument database to make a new file. If an ABIF sample file becomes corrupt or if you accidently delete a file that you want, you can use the Re-extraction utility to replace the sample file.

Directions for using the Re-extraction utility start on page 7-6.

Cleanup Database Utility

The Cleanup Database utility (CleanupDB) deletes some of the information stored in the instrument database to make room for new run data.

Directions for using the Cleanup Database utility start on page 7-8.

New Method Import Utility

The New Method Import utility (NewMethodImport) imports the data contained in method files into the instrument database. The utility is used to install new versions of methods sent out by Applied Biosystems after your 3100 Genetic Analyzer is installed.

Directions for running the New Method Import utility start on page 7-10.

Remove Run Modules Utility

The Remove Run Modules utility (RemoveRunModules) removes all modules and associated information from the instrument database. Use this utility to quickly delete all old modules before importing new ones.

Directions for running the Remove Run Modules utility start on page 7-11.

Initialize Database Utility

The Initialize Database utility (InitDB) completely erases and reinitializes the instrument database. Use this utility only when instructed to do so by an Applied Biosystems representative.

Directions for running the Initialize Database utility start on page 7-12.

ABI Sample File Toolkit

The ABI Sample File Toolkit is an option that can be used to read ABIF sample files and therefore develop customized applications for the ABI PRISM® 3100 Genetic Analyzer.

OrbixWeb

OrbixWeb 3.2 Professional Edition provides database management services between the 3100 Data Collection software, Auto Extractor, and the Oracle database.

OrbixWeb v. 3.2 Professional Edition has no user interface; however, it must always be running when the 3100 Data Collection software or Auto Extractor are running.

Orbix Desktop

Orbix Desktop 2.3 software is middleware that is used by the 3100 Data Collection software and Auto Extractor.

Persistence Powertier

Persistence Powertier 4.321 is an application server that allows the 3100 Data Collection software to interact with the instrument database.

Java Runtime Environment

Java Runtime Environment 1.1.7b is software that enables the 3100 Data Collection software to run.

Adobe Acrobat Reader

Adobe Acrobat Reader is a program that allows you to read electronic documents saved in the portable document format (PDF).

Oracle Database

The Oracle® instrument database stores the following types of information:

- Processed, but unanalyzed, fluorescence data, which is collected from the CCD
- Plate records, which contain information about plates and their samples
- Run schedules, which are lists of runs automatically assigned by the software
- Run log and error log data
- 3100 Data Collection software preference settings
- Electrophoresis modules (run modules and calibration modules)
- EPT data

This manual describes how the database is used by the 3100 software. Consult an Oracle database administrator for more information about administering the database.

GeneScan Analysis Software

If you purchased the GeneScan option, GeneScan Analysis software will be installed on the hard drive of your computer workstation. This software is used to:

- Review the fragment analysis profile and size data
- Reanalyze the data

DNA Sequencing Analysis Software

If you purchased the sequencing option, DNA Sequencing Analysis software will be installed on the hard drive of your computer workstation. This software is used to:

- Review basecalled sequences
- Reanalyze the basecalled sequence

Additional Information

Additional information about the ABI PRISM 3100 Genetic Analyzer software can be found in the readme files and release notes on the software CD-ROMs.

Types and Locations of Files

Introduction

The ABI PRISM® 3100 Genetic Analyzer software includes many different files and folders. Some of these are created to store run data and calibration data. Others are required to run the software.

IMPORTANT Never move or delete any file or folder unless specifically directed to do so by an Applied Biosystems representative or the 3100 Genetic Analyzer documentation. Doing this could render the software inoperable.

Filename Extensions

You can recognize certain file types by the three-letter extensions in their file names. The common file types and their extensions are listed below.

Extension	File Type	Directory (If Applicable)
.ab1	ABIF sample file for sequencing analysis	D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns
.bat	Batch file initiates a series of software events (*e.g.*, 3100Collection.bat)	—
.bcp	Basecaller parameter file	—
.exe	Executable program	—
.fsa	ABIF sample file for fragment analysis	D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns
.fsf	Factura settings file	D:\AppliedBio\abi\Shared\Analysis\Factura\Settings
.gsp	Analysis module for GeneScan	D:\AppliedBio\abi\Shared\Analysis\Sizecaller\Params
.ini	Initialization file	—
.log	Log file in text file format	—
.mcl	Spectral calibration file	D:\AppliedBio\abi\3100\DataCollection\Spectral Cal Logs\Spectral Cal
.mob	Mobility file	D:\AppliedBio\Abi\Shared\Analysis\Basecaller\Mobility
.mod	Run module	—
.modexp	Exported run module file	—
.mtd	Method file	D:\AppliedBio\abi\Support Files\Data Collection Support Files\Method Files
.par	Spectral calibration parameter files	D:\AppliedBio\abi\Support Files\Data Collection Support Files\Calibration Data\Spectral Calibration\Param Files
.pdf	Portable document format file that can be read by Adobe Acrobat Reader	—
.plt	Plate file (tab-delimited text file) for import into the instrument database to create a plate record	D:\AppliedBio\Abi\Support Files\Data Collection Support Files\Plate Import Files
.saz	Analysis module for sequencing analysis	D:\AppliedBio\abi\Shared\Analysis\Basecaller\Params
.scl	Spatial calibration file	D:\AppliedBio\abi\3100\DataCollection\SpatialCalLogs
.scp	Sizecaller parameter file	—
.szs	Size standard file	D:\AppliedBio\abi\Shared\Analysis\SizeCaller\SizeStandards
.tmp	Temporary run or calibration data file written in code	—
.txt	Text file that can be read by Notepad	—

Section: Setting the Format for the Displayed Dye Colors

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

Using the Edit Dye Display Information Dialog Box

Introduction

The formats for the dye colors shown in the electropherogram and capillary displays are set in the Edit Dye Display Information dialog box.

You may use the Edit Dye Display Information dialog box to:

- View the current settings for the displayed dye colors (*e.g.*, the blue plots may represent the base cytosine).
- Hide the data for particular dyes so that it does not appear in the displays.
- Change the names of the dye.
- Change the color intensity.
- Open the Set Color dialog box to change the colors shown. (See “Using the Set Color Dialog Box” on page 5-13.)

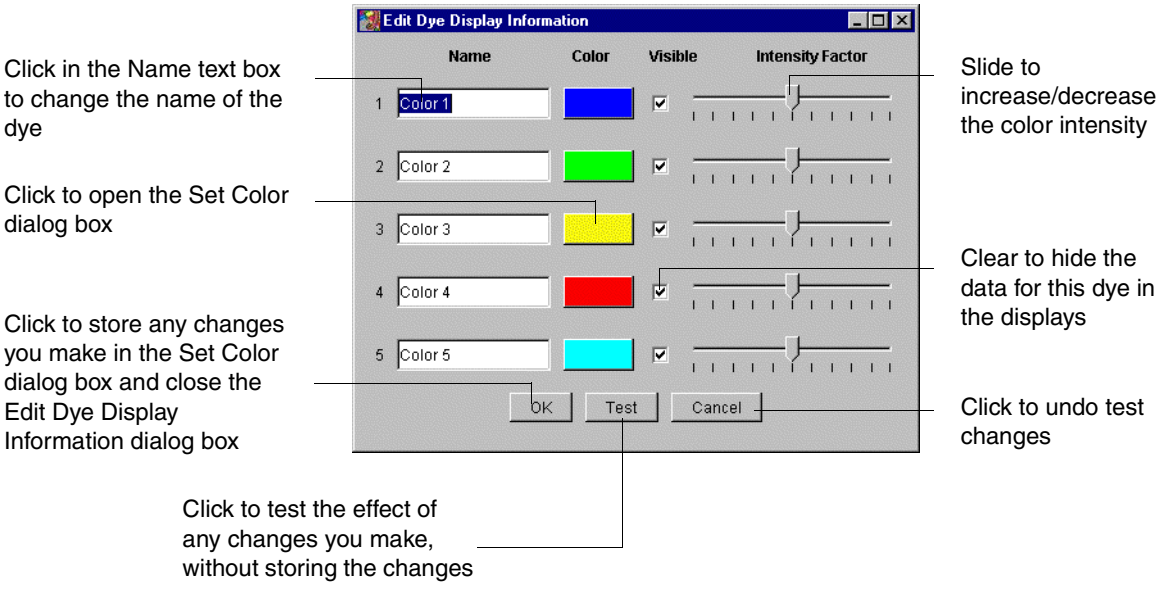
Opening the Edit Dye Display Information Dialog Box

To open the Edit Dye Display Information dialog box:

Step	Action
1	From the **Instrument** menu, point to **Data Acquisition**, and select **Set Color**. This opens the **Edit Dye Display Information** dialog box as shown below.

Using the Edit Dye Display Information Dialog Box

The operations of the Edit Dye Display Information dialog box are summarized in the diagram below.



Using the Set Color Dialog Box

Why Change the Display Colors?

It is a good idea to change the colors used in the electropherogram and capillary displays if you find it hard to distinguish the default colors.

Two Ways to Change the Display Colors

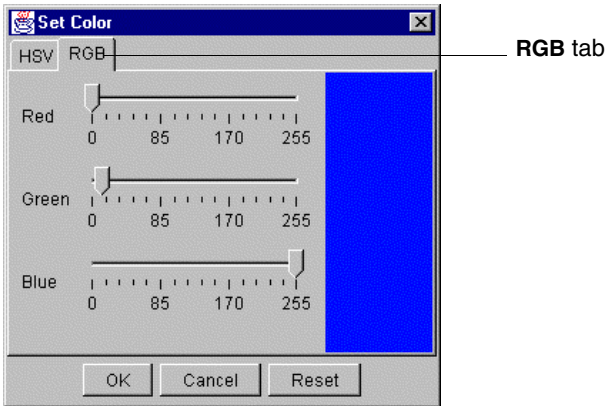
There are two ways to change the color used to represent the concentration of dye in the 3100 Data Collection software user interface:

- Using the red green blue (RGB) color system
- Using the hue saturation value (HSV) color system

Changing the Display Colors Using the RGB System

The RGB system uses the three primary colors (red, green, and blue) in various proportions to create the other colors.

To change the displayed dye color using the RGB system:

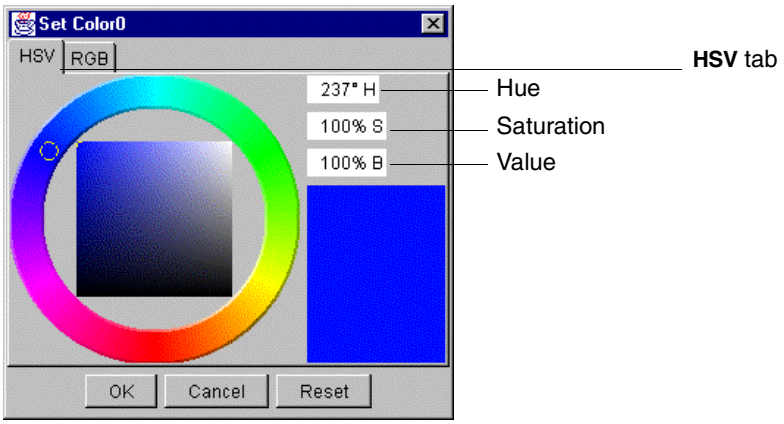
Step	Action
1	From the **Instrument** menu, point to **Data Acquisition**, and select **Set Color**.
2	Within the **Edit Dye Display Information** dialog box, click the **Color** box of the color you want to change. The **Set Color** dialog box is displayed. Click the **RGB** tab if it is not already selected. 
3	Move the sliders to mix the three colors until you produce the display color that you want.
4	<table><tr><th>To...</th><th>Click...</th></tr><tr><td>Incorporate the change</td><td>**OK**</td></tr><tr><td>Ignore the change</td><td>**Cancel**</td></tr><tr><td>Revert to the default colors</td><td>**Reset**</td></tr></table>
5	Close the **Edit Dye Display Information** dialog box.

Changing the Display Colors Using the HSV System

The Hue Saturation Value (HSV) system describes colors in terms of three properties[1]:

Property	Description
Hue	The wavelength composition of the color, *e.g.*, blue
Saturation (chroma)	The purity of the color in a scale from gray to the most vivid version of the color
Value (intensity)	The relative lightness or darkness of a color in a range from black to white; *e.g.*, light red, dark green, etc.

To change the displayed dye color using the HSV system:

Step	Action
1	Within the **Edit Dye Display Information** dialog box, click the **Color** box of the color you want to change. The **Set Color** dialog box is displayed. Click the **HSV** tab if it is not already selected. 
2	Click in the circle and drag the cross-hair pointer around the circle to select the desired hue.
3	Click in the inner square and drag horizontally to select the desired saturation.
4	Click in the inner square and drag vertically to select the desired value.
5	<table><tr><th>To...</th><th>Click...</th></tr><tr><td>Incorporate the change</td><td>**OK**</td></tr><tr><td>Ignore the change</td><td>**Cancel**</td></tr><tr><td>Revert to the default colors</td><td>**Reset**</td></tr></table>
6	Close the **Edit Dye Display Information** dialog box.

1. See the *Essential Guide to User Interface Design*, W. O. Galitz (1996), John Wiley & Sons.

Section: Controlling the Instrument Using Manual Control

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

Manual Control Commands

Table of Commands

The following table displays the manual control options as they are organized in the Data Collection software.

Command Category	Command Name	Value
Electrophoresis	Set Power Supply	♦ On ♦ Off
	Set Voltage	A number between 0 and 20 kV
Laser	Set State	♦ Idle ♦ On ♦ Off
	Set Power	A number between 0 and 25 mW
	Open/Close Shutter	♦ Open ♦ Closed
Oven	Set State	♦ On ♦ Off
	Set Temperature	A number between 18 and 65 °C
Autosampler	Move Forward	N/A
	Return	N/A
	Move Up/down	A number between –500 and 500 steps
	Move to Site	♦ Site 1 (left, front for 1X running buffer) ♦ Site 2 (left, rear for deionized water) ♦ Site 3 (right, front for deionized water) ♦ Site 4 (right, rear for deionized water)
Array-fill syringe	Move Home	N/A
	Move Up	A number between 1 and 1200 steps
	Move Down	A number between 1 and 1200 steps
Polymer-reserve syringe	Move Home	N/A
	Move Up	A number between 1 and 1200 steps
	Move Down	A number between 1 and 1200 steps
Pin-valve	Set Position	♦ Open ♦ Closed
Capillary	Fill	♦ 50 cm/POP6 ♦ 36 cm/POP4 ♦ 36 cm/POP6

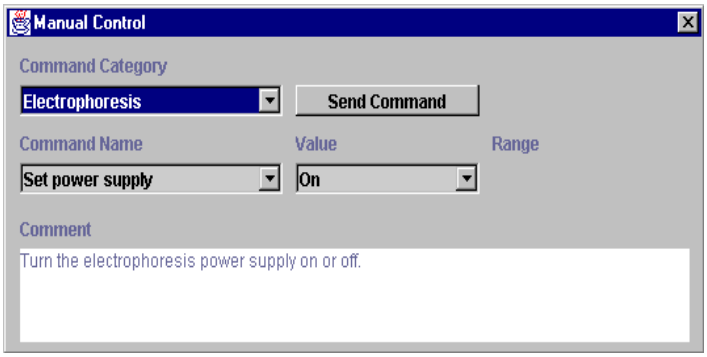
Using Manual Control Commands

Sending a Manual Control Command

IMPORTANT The oven and instrument doors must be closed for manual control commands to execute.

Note You cannot send a manual control command during a run.

To send a manual control command:

Step	Action
1	From the **Instrument** menu, select **Manual Control**. The **Manual Control** dialog box appears. 
2	Select a **Command Category** from the drop-down list.
3	Select a **Command Name**. **Note** To check a command's function, read the **Comment** box.
4	Enter or select a **Value**.
5	Click **Send Command**.

Note Some tasks require that you send more than one manual control command. For example, to heat the oven to 50 °C, you first send a command to turn on the oven, and then you send a command to set the temperature.

Section: Working with Run Modules

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

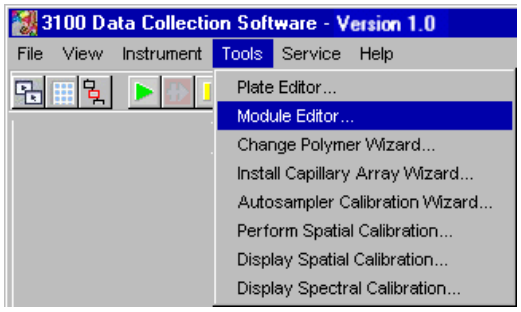
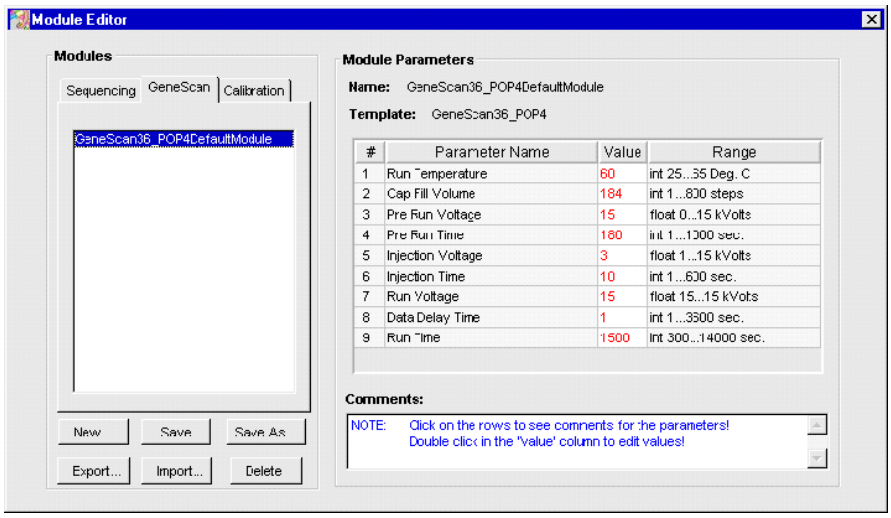
Introduction The run module specifies the conditions for how the sample is run. Examples include:

- Duration of the run
- Run temperature
- Injection time

Viewing a Run Module

Viewing a Run Module

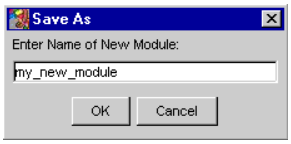
To view a run module:

Step	Action
1	From the **Tools** menu, select **Module Editor** or click the Module Editor button on the toolbar.   This opens the **Module Editor** dialog box.
2	In the **Modules** group box, click either the **Sequencing** or **GeneScan** tab, as appropriate.  **Note** The **Calibration** tab lists the spatial and spectral calibration modules.
3	To view the parameters for a particular module, select the name of the module from the list. All the parameters for the run module are displayed. **Note** The Run Voltage value is fixed. It cannot be edited.

Editing or Creating a Run Module

Editing or Creating a Run Module

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

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

Run Module Parameters

Introduction

You can change the module parameters listed below when creating run modules. The parameters are listed in the order in which they appear in the run module editor.

Note Not all parameters are visible in the run module editor for all supplied sequencing and GeneScan run method files.

Modifiable Run Module Parameters

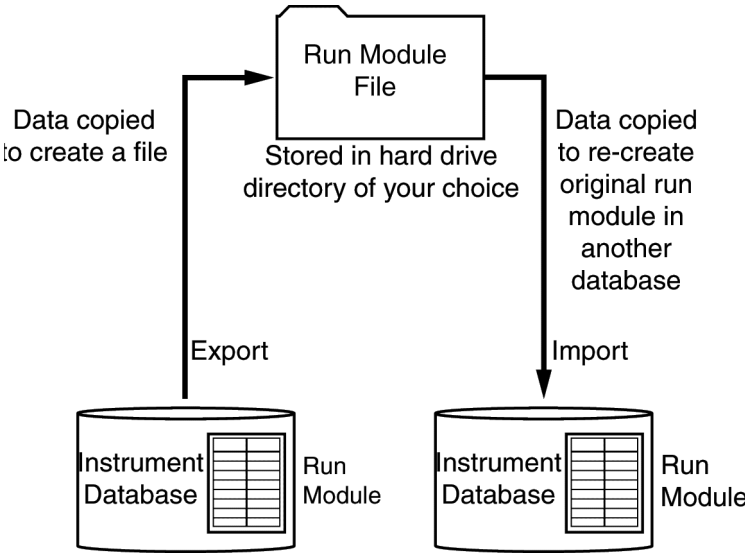
The following table lists the user-modifiable run module parameters:

Parameter	Comment
Run Temperature	The temperature of the electrophoresis chamber during the run. The speed of electrophoretic migration decreases as the electrophoresis temperature decreases.
Cap FillVolume	The time set for the array-fill syringe to pump polymer into the capillaries. **IMPORTANT** If this value is decreased from that in the supplied run module, the polymer used during the previous run may not be completely replaced. This could lead to an accumulation of residual, large DNA fragments in the capillaries over time, causing an increase in background signal.
Prerun Voltage	The voltage applied across the capillaries during the prerun period of electrophoresis. A prerun is performed to equilibrate the ionic strength across the capillary array before electrokinetic injection.
Prerun Time	The duration of the prerun period of electrophoresis.
Injection Voltage	The voltage applied across each capillary during electrokinetic injection. The injection voltage is directly proportional to the amount of DNA injected. This works in conjunction with the Injection Time to control the amount of DNA injected.
Injection Time	The duration of electrokinetic injection. This works in conjunction with the Injection Voltage to control the amount of DNA injected.
Run Voltage	The voltage applied across each capillary during a run.
Data Delay Time	The period of electrophoresis between the completion of electrokinetic injection and the time at which the software starts to collect data.
Run Time	The duration of electrophoresis, including the Data Delay Time. The maximum run time for DNA sequencing and fragment analysis runs is 16,000 seconds.

Transferring Run Modules Between Computers

Overview

The process of transferring run modules between two instrument databases on different computers is illustrated below.



About Exporting a Module

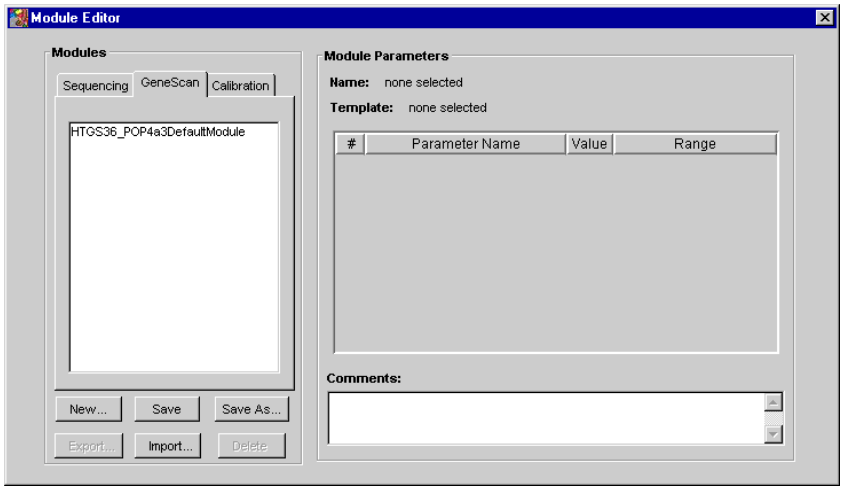
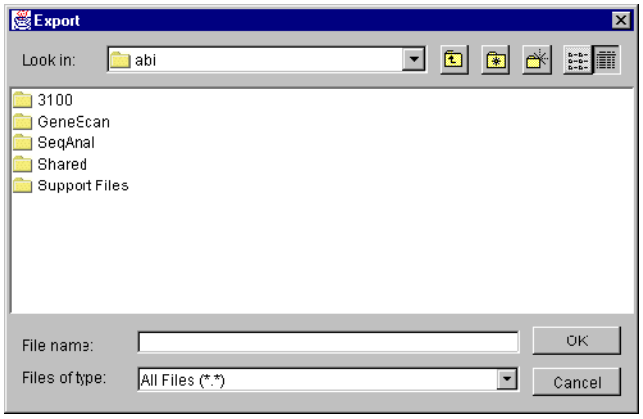
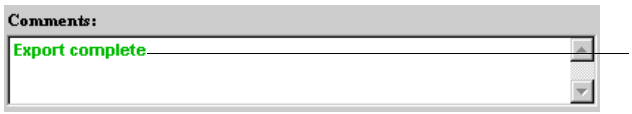
A run module cannot be transferred directly. The data in a run module must first be copied into a file that is created and stored on a hard drive. This is known as exporting the module because you are exporting it from the database.

The file created has the file name format: *filename*.modexp

The hard drive to which the run module file is saved could be the local drive of the donor or acceptor computer, or it could be a server that is accessible to both computers.

Exporting a Run Module

To export a run module:

Step	Action
1	Click the Module Editor button on the toolbar to open the **Module Editor** dialog box.  
2	Make sure that the module you want to export is selected in the **Modules** group box. In the **Modules** group box, click **Export**. This opens the **Export** browser dialog box. 
3	Navigate to the folder in which you want to save the run module file. **Note** Due to software limitations, you cannot select a folder on the desktop.
4	Double-click the destination folder so that its contents are displayed in the pane.
5	In the **File name** box, type a name for the file.
6	Click **OK**. This creates a run module in the specified folder.  This message confirms a successful export.

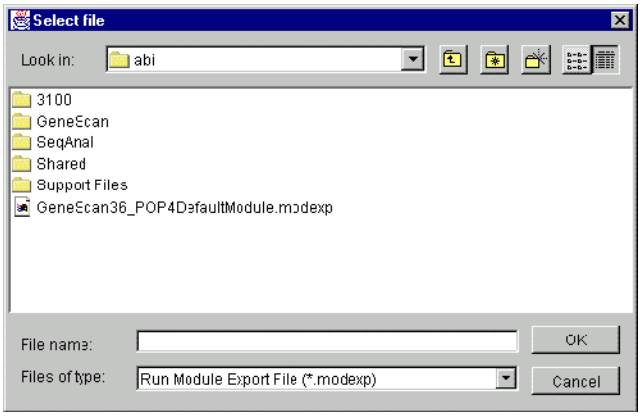
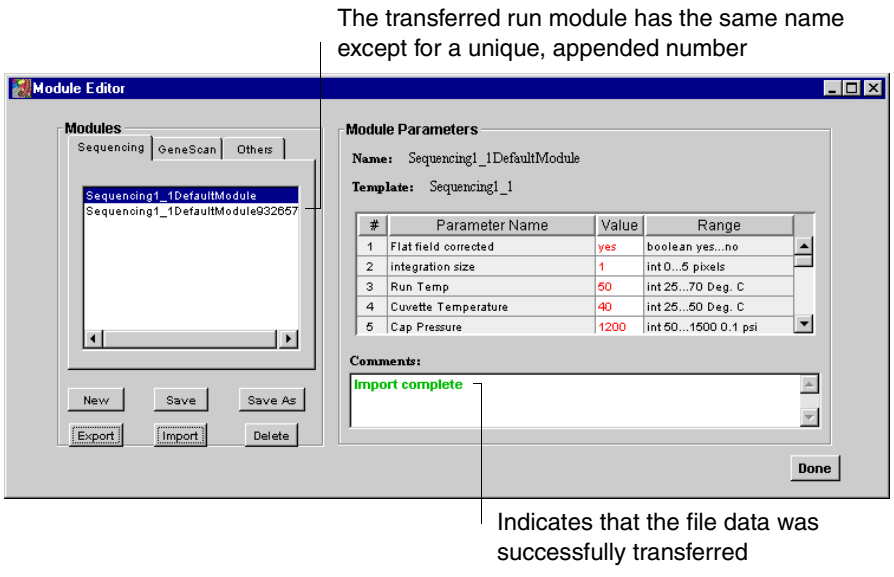
About Importing a Module

The data in the exported file is copied to the donor database to re-create the original run module. This is known as importing the module. The re-created run module has the same name as the original except for a unique number added by the software. The number is based on the date. This prevents conflicts with the original run module in the donor database.

Note You cannot read a run module file because it is written in code.

Importing a Run Module File

To import a run module file:

Step	Action
1	Go to the computer to which you want to transfer the run module.
2	Click the Module Editor button on the toolbar to open the **Module Editor** dialog box. 
3	In the **Modules** group box, click **Import**. This opens a browser dialog box. 
4	Navigate to the folder in which you saved the run module file. Select the file. **Note** Due to software limitations, you cannot select a folder on the desktop.
5	Click **OK** to import the file. 

Section: Working with Sequencing Analysis Modules

In This Section

The following topics are covered in this section:

Introduction

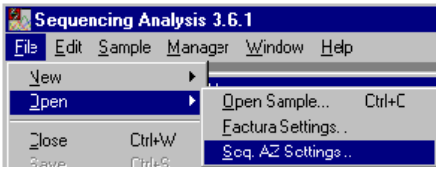
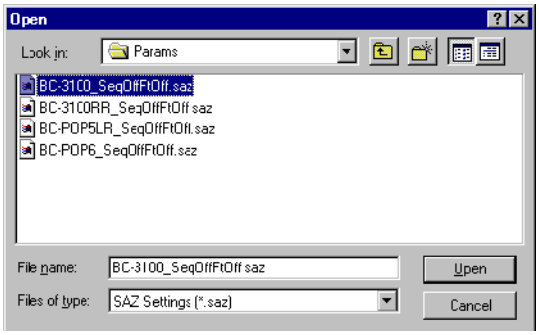
Sequencing analysis modules, created with DNA Sequencing Analysis software, provide the Auto Extractor with the parameters needed to analyze sequencing data.

Some sequencing analysis modules are provided with the 3100 Data Collection software. In the DNA Sequencing Analysis software, the sequencing analysis module is called a sequencing analysis settings file.

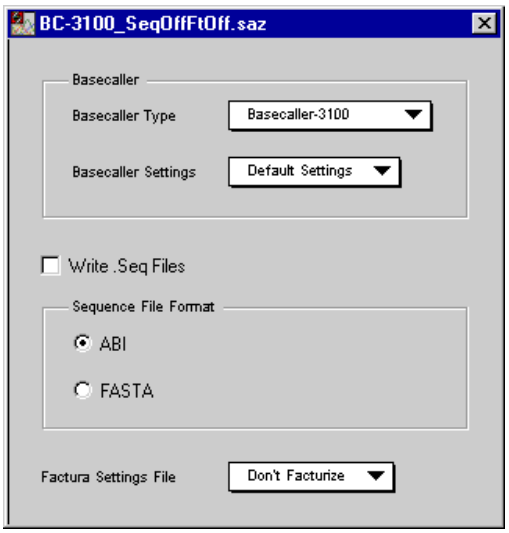
Viewing and Editing Analysis Modules for DNA Sequencing

Viewing and Editing Analysis Modules for DNA Sequencing

To view or edit an analysis module (.saz file):

Step	Action
1	Start the DNA Sequencing Analysis software. You may have an icon for the program on the **Start** menu. If not, you can find the DNA Sequencing Analysis software (SeqA.exe) in the following directory: D:\AppliedBio\abi\SeqAnal\Bin
2	From the **File** menu, point to **Open** and select **Seq. AZ Settings**. 
3	Select the analysis module that you want to view or edit. The analysis modules are stored in the following directory: D:\AppliedBio\abi\Shared\Analysis\Basecaller\Params  This opens the sequencing analysis setting file.

To view or edit an analysis module (.saz file): *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>4</td><td>


If you want, you can edit the settings:
♦ Basecaller Type can be Basecaller-3100 (for standard sequencing) or Basecaller-3100RR (for rapid-run sequencing). The files are located in the following directory:
D:\AppliedBio\abi\Shared\Analysis\Basecaller\Params
♦ Basecaller Settings are specified in the Preferences dialog box (accessed from the Edit menu).
♦ If the Write .Seq Files box is selected, text files of the basecalled sequence are written in either ABI or FASTA formats.
♦ If a Factura Settings File is selected, Factura processing will be applied during analysis.
– To view or edit a Factura settings file: From the File menu, point to Open, and select Factura Settings.
– The files are located in the following directory:
D:\AppliedBio\abi\Shared\Analysis\Factura
</td></tr>
<tr><td>5</td><td>
<table>
<tr><th>If you have made changes to the analysis module and you...</th><th>Then...</th></tr>
<tr><td>want to save the changes</td><td>click Save As to create a new analysis module. Enter a unique descriptive name and click OK.</td></tr>
<tr><td>don’t want to save the changes</td><td>click the Close button to close the window.</td></tr>
</table>
</td></tr>
</table>

Creating a Sequencing Analysis Module

Procedure Overview

Creating a sequencing analysis module requires:

- Creating a basecaller settings file
- Creating a Factura settings file
- Creating a new sequencing analysis module
- Saving the sequencing analysis module

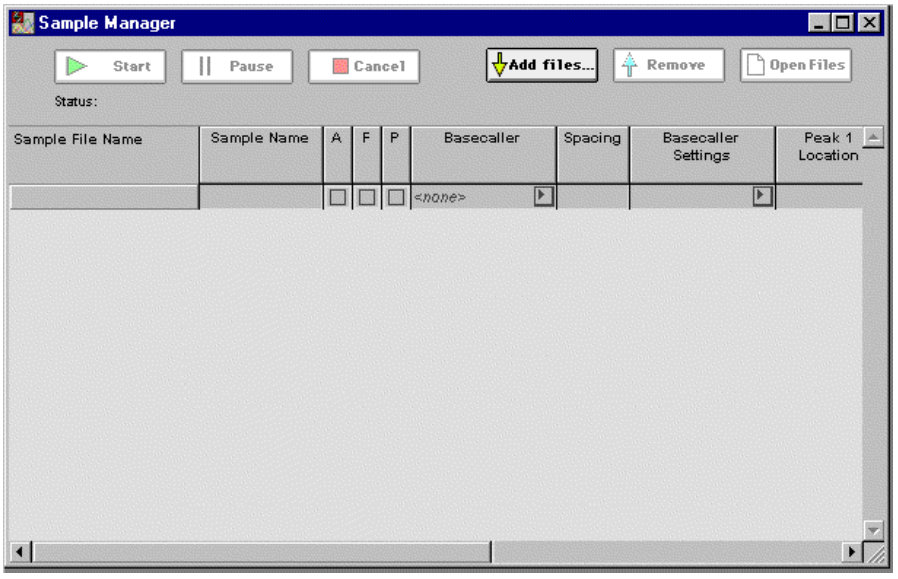
For More Information

For detailed information about the topics covered in this section, see the *ABI Prism DNA Sequencing Analysis Software v. 3.6 NT User's Manual.*

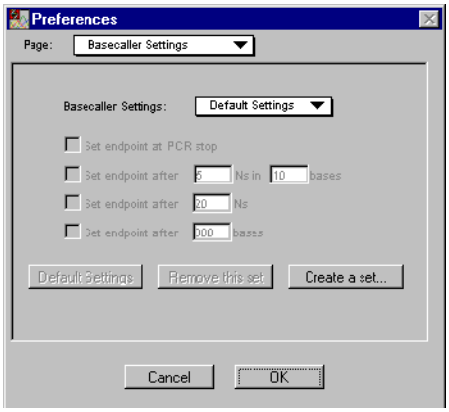
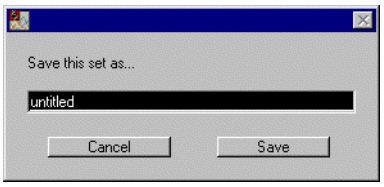
Creating a Basecaller Settings File

To create a basecaller settings file:

Step	Action
1	Quit the 3100 Data Collection software if it is running.
2	Start the DNA Sequencing Analysis software. The **Sample Manager** window opens inside the **Sequencing Analysis** window.

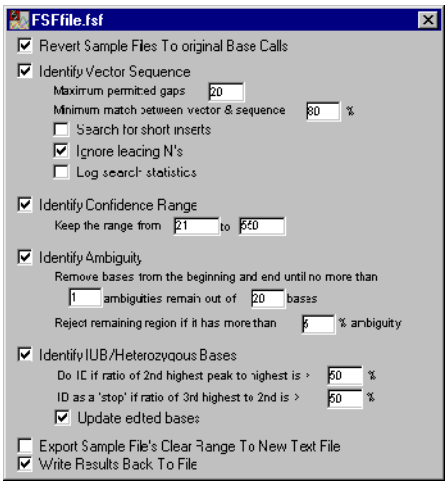
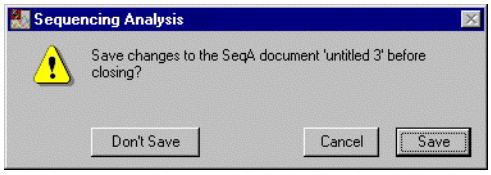
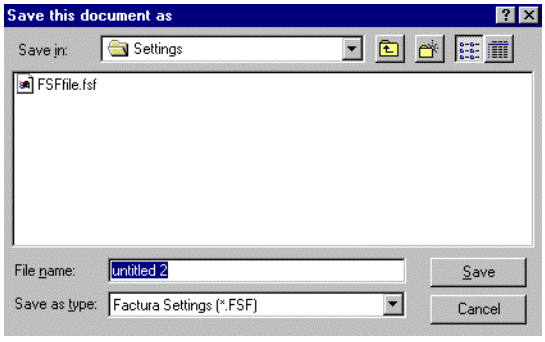


To create a basecaller settings file: *(continued)*

Step	Action
3	To set a cutoff condition for the analysis, from the **Edit** menu point to **Preferences**, and select **Basecaller Settings**. This opens the **Preferences** dialog box.  **Note** The default setting has the cutoff conditions disabled.
4	In the **Preferences** dialog box, click **Create a set**.
5	Check one or more of the **Set endpoint** check boxes as appropriate.
6	If you checked the second, third, or fourth check box, type the number(s) that you want to use into the text boxes. The **Create a set** button becomes **Save this set as.** **Note** "Ns" means bases that could not be assigned an identity.
7	Click **Save this set as**. This opens an unnamed dialog box.  a. Type a name for the basecaller settings file into the text box. b. Click **Save**.
8	In the **Preferences** dialog box, click **OK**. This saves the basecaller settings.

Creating a New Factura Settings File

To create a new Factura settings file:

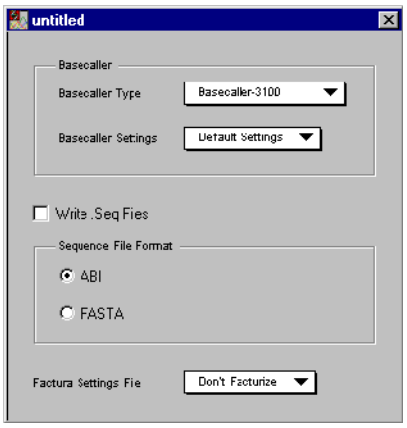
Step	Action
1	From the **File** menu, point to **New** and select **Factura Settings**. This opens the **FSFfile.fsf** dialog box. 
2	Select the required options, then click the Close button in the top-right corner of the dialog box. A **Sequencing Analysis** alert box appears. 
3	Click **Save**. The **Save this document as** dialog box appears. 
4	In the **File name** box, type the name you want to use for the Factura settings file. **Note** Do not use any of the following characters in the file name: * < > ? | / \ : ". Do not uses spaces.
5	Make sure that the file will be saved to the following directory: D:\AppliedBio\abi\Shared\Analysis\Factura\Settings

Creating a New Sequencing Analysis Module

To create a new sequencing analysis module:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>From the File menu, point to New and select Seq.AZ Settings.

This opens the untitled dialog box.

 
</td></tr>
<tr><td>2</td><td>From the Basecaller Type drop-down list, select a basecaller.

Either:

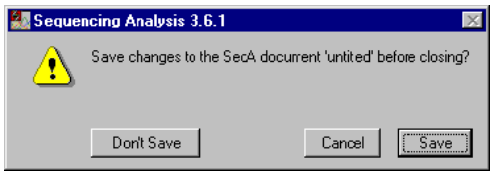
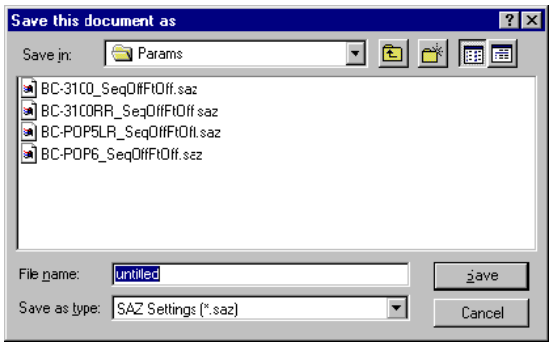
♦ Select the name of the basecaller settings file that you just created from the Basecaller Settings drop-down list, or

♦ Use the default settings</td></tr>
<tr><td>3</td><td>Select Write .Seq Files if you want a .Seq file created (this saves the sequence as a text file).</td></tr>
<tr><td>4</td><td>Select either ABI or FASTA in the Sequence File Format group box. Select FASTA only if you intend to export the data to a program that accepts FASTA files.</td></tr>
<tr><td>5</td><td>
<table>
<tr><th>If you do...</th><th>Then...</th></tr>
<tr><td>not want to use Factura software</td><td>leave Factura Settings File as Don't Facturize.</td></tr>
<tr><td>want to use Factura software</td><td>select a Factura settings file from the drop-down list.

 
</td></tr>
</table>
</td></tr>
</table>

Saving the Sequencing Analysis Module

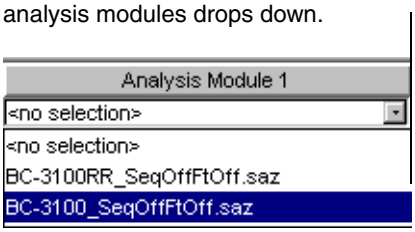
To save the sequencing analysis module:

Step	Action
1	Click the Close button. A **Sequencing Analysis** alert box appears. 
2	Click **Save**. The **Save this document as** dialog box opens. 
3	In the **File name** text box, type a name for the analysis module. **Note** Do not use any of the following characters in the file name: * < > ? | / \ : “. Do not uses spaces.
4	Make sure that the file will be saved to the following directory: D:\AppliedBio\abi\Shared\Analysis\Basecaller\Params
5	Click **Save**. This creates an analysis module with the format *file name*.saz.

Note You can check that the analysis module was saved by examining a plate record in the plate editor as described below.

Ensuring the Analysis Module Was Saved

To check that the analysis module was saved:

Step	Action
1	Open the 3100 Data Collection software.
2	In the **Plate View** page, double-click a plate record. This opens the plate editor. If the plate record is already open, close it, and then re-open it.
3	Scroll horizontally to the **Analysis Module 1** column.
4	Click in a cell that lists a sequencing analysis module. The list of sequencing analysis modules drops down. 
5	Make sure that the sequencing analysis module you just created is listed. **Note** If it is not listed, you may have saved the sequencing analysis module in the wrong folder.

Section: Working with GeneScan Analysis Modules

In This Section

The following topics are covered in this section:

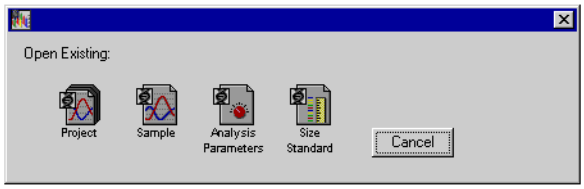
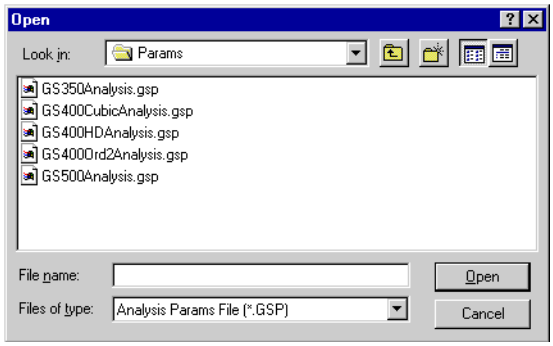
Introduction

GeneScan analysis modules provide Auto Extractor with the parameters needed for analyzing data from fragment analysis.

Viewing and Editing Analysis Modules for GeneScan

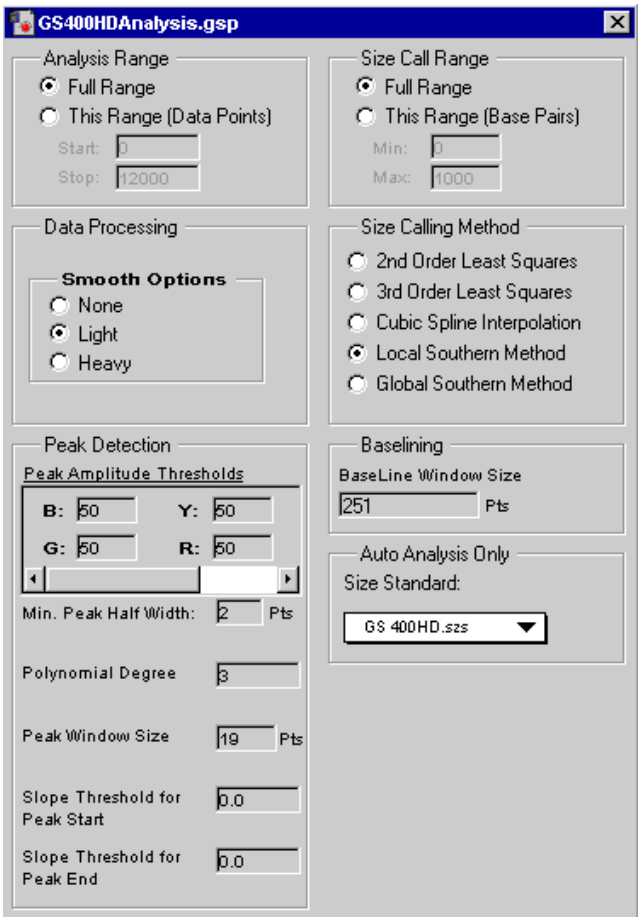
Viewing and Editing Analysis Modules

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

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

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>If you want, you can make changes to the analysis module. For more information about the parameters, see the ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual (P/N 4308923).

 
</td></tr>
<tr><td>7</td><td>
<table>
<tr><th>If you have made changes to the analysis module and you...</th><th>Then...</th></tr>
<tr><td>want to save the changes</td><td>♦ From the File menu, select Save As, assign a unique name, and then click OK, or
♦ From the File menu, select Save to save the changes to the current analysis module.

IMPORTANT The analysis modules must be stored in the following folder:
D:\AppliedBio\abi\Shared\Analysis\Sizecaller\Params</td></tr>
<tr><td>do not want to save the changes</td><td>Click the Close button to close the window.</td></tr>
</table>
</td></tr>
</table>

Creating a GeneScan Analysis Module

Before Beginning

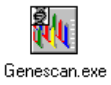
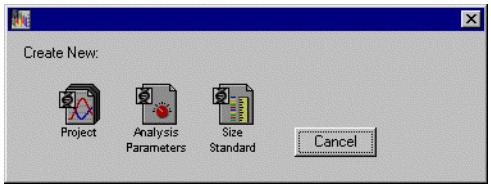
Before creating a GeneScan analysis module, you may need to create a custom size standard file.

You will need to create a custom file for performing:

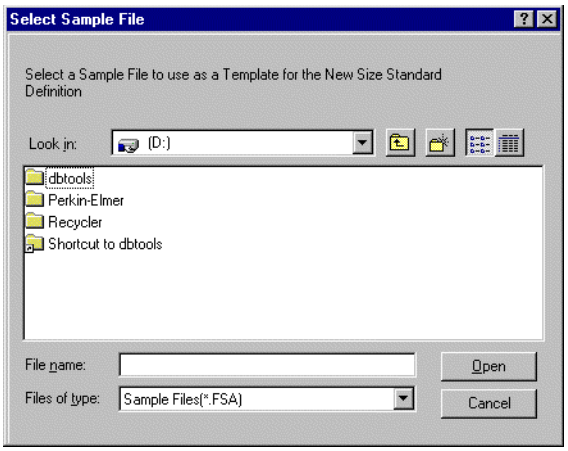
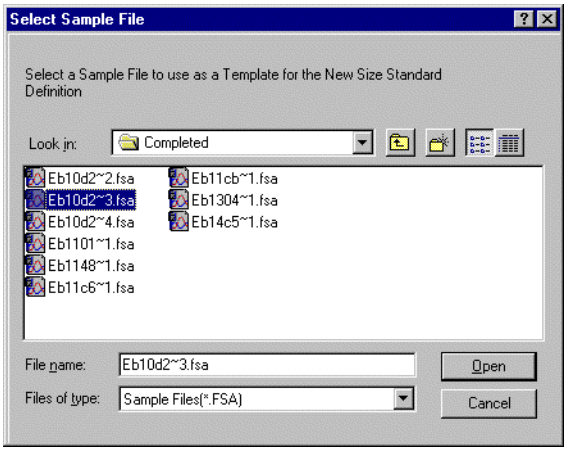
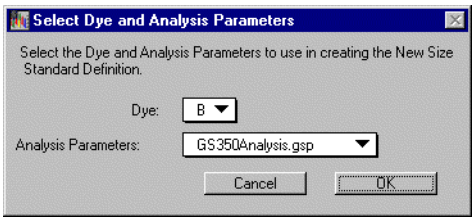
- Denaturing runs but not using GS350, 400HD, or GS500
- Non-denaturing runs using applications such as SSCP
- Runs using one of the Applied Biosystems internal lane standards, but significantly altering collection time or analysis range
- Runs where the red data differs significantly (*i.e.*, extra or missing peaks)

Creating a Size Standard File

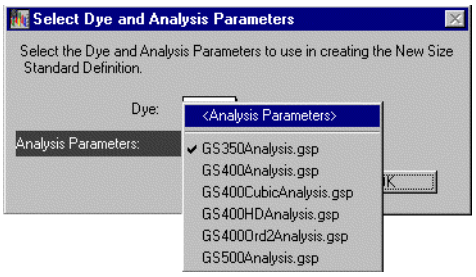
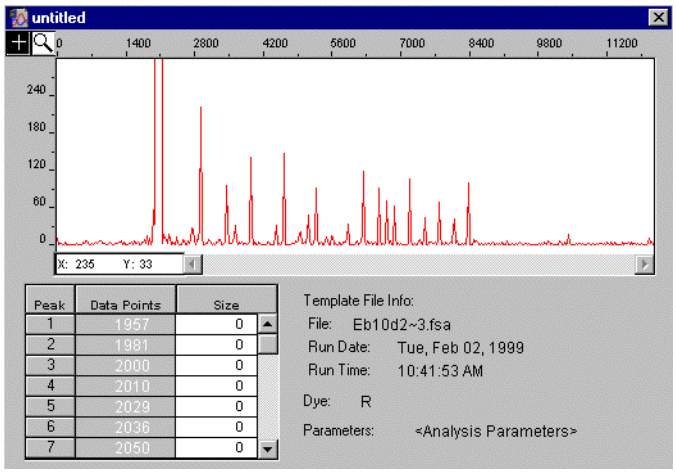
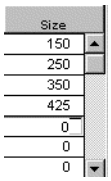
To create a size standard file:

Step	Action
1	Review the size standard data and optimize the analysis parameters.
2	Quit the 3100 Data Collection software if it is running.
3	Start GeneScan Analysis software. You may have a program icon for the GeneScan Analysis software on the **Start** menu or a shortcut icon on your desktop. If not, you can find the application (GeneScan.exe) in the following directory: D:\AppliedBio\abi\GeneScan\Bin 
4	From the **File** menu, select **New**. This opens the **Create New** box. 

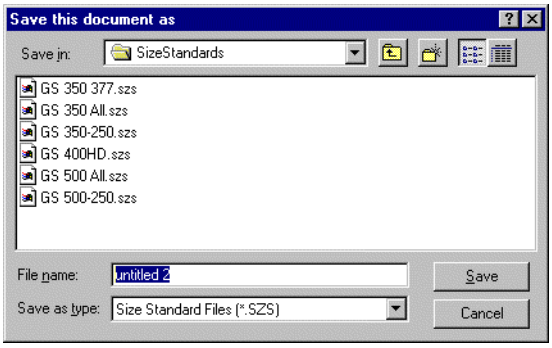
To create a size standard file: *(continued)*

Step	Action
5	Click the **Size Standard** icon. This opens a browser dialog box. 
6	Navigate to the Extracted Runs folder in the following directory: D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns **Note** This folder is created when Data Extractor is first used.
7	Select the GeneScan sample file (with the extension .fsa) that you want to use as a template. 
8	Click **Open**. This opens the **Select Dye and Analysis Parameters** dialog box.  From the **Dye** drop-down list, select the dye that was used to label the size standard DNA fragments.

To create a size standard file: *(continued)*

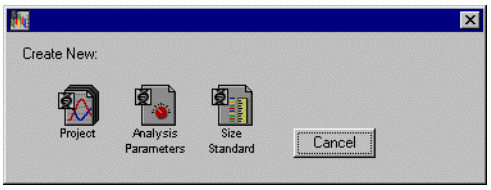
Step	Action
9	From the **Analysis Parameters** drop-down list, select <**Analysis Parameters**>. This references the current analysis parameter settings rather than an analysis parameter file. 
10	Click **OK**. This opens the **untitled** dialog box. 
11	In the **Size** column, enter the known sizes of the standard's peaks. 
12	From the **File** menu, select **Save**. This opens the **Save this document as** browser dialog box.

To create a size standard file: *(continued)*

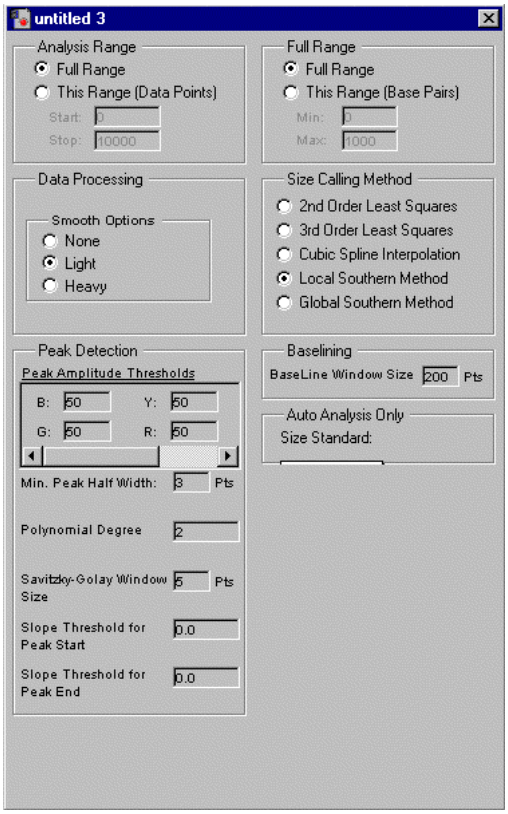
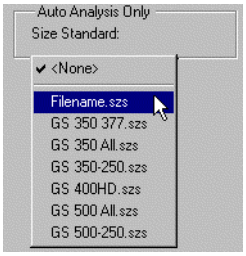
Step	Action
13	Navigate to the **SizeStandards** folder located in the following directory: D:\AppliedBio\abi\Shared\Analysis\SizeCaller\SizeStandards The folder contains a number of size standard (.szs) files. 
14	In the **File name** text box, type a file name for the size standard file.
15	Click **Save**. The browser dialog box closes and the file is saved to the correct directory location for Auto Extractor to read. In the newly created *Filename*.szs dialog box, click the Close button.

Creating a GeneScan Analysis Module

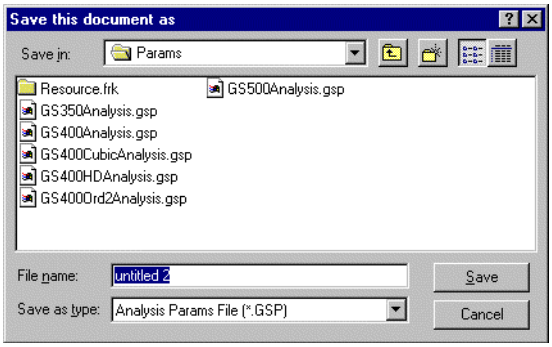
To create a GeneScan analysis module:

Step	Action
1	From the **File** menu, select **New**. This opens the **Create New** box. 

To create a GeneScan analysis module: *(continued)*

Step	Action
2	Click the **Analysis Parameters** icon. This opens the **untitled 3** dialog box. 
3	Fill out the **untitled** dialog box according to the directions given in the *ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual*. In the **AutoAnalysis Only** group box, select the size standard file that you just created from the **Size Standard** drop-down list. 

To create a GeneScan analysis module: *(continued)*

Step	Action
4	From the **File** menu, select **Save**. This opens a browser dialog box. Navigate to the **Params** folder in the following directory: D:\AppliedBio\abi\Shared\Analysis\Sizecaller\Params 
5	In the **File name** text box, type a file name for the analysis parameter file.
6	Click **Save**. The browser dialog box closes and the file is saved to the correct directory location for the Auto Extractor to read.
7	In the newly created *Filename*.szs dialog box, click the Close button.

Section: Working with BioLIMS

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

Setting Up BioLIMS Project Information

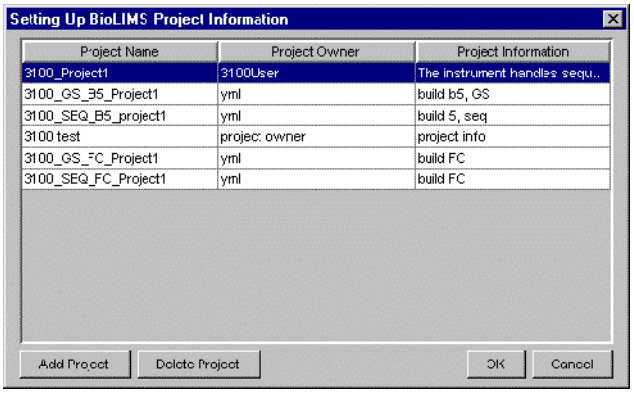
Introduction

In order to extract sample files into the BioLIMS™ database, you must first set up information for your BioLIMS project(s) in the 3100 Data Collection software. “BioLIMS projects” are the 3100 Data Collection software equivalent of “collections” in BioLIMS.

When samples are extracted into the BioLIMS database, they are added to the specified BioLIMS project.

Setting Up BioLIMS Project Information

To set up the BioLIMS project information:

Step	Action
1	From the **View** menu, select **BioLIMS Project Info.** The **Setting Up BioLIMS Project Information** window appears. 
2	**If you want to... / Then...** add a new project → a. Click **Add Project**. A blank row appears. b. Continue with step 3. delete an existing project → a. Highlight the project you want to delete. b. Click **Delete Project**. c. Skip to step 4.
3	Enter the appropriate information in the text fields. **Project Name** — Type a descriptive name of your choice. **Note** The **Project Name** will be the **Collection Name** in the BioLIMS database. **Project Owner** — Type in your name. **Note** The **Project Owner** will be the **Creator** in the BioLIMS database. **Project Information** — Type in any comments, if desired. **Note** The **Project Information** will be the **Comment** in the BioLIMS database.

To set up the BioLIMS project information: *(continued)*

Step	Action
4	Click **OK** to save your changes. The new project(s) will be listed in the drop-down list under the **BioLIMS Project** column in the **Plate Editor** window.
5	Continue with “Preparing a Plate for Extracting to BioLIMS” on page 5-50.

Preparing a Plate for Extracting to BioLIMS

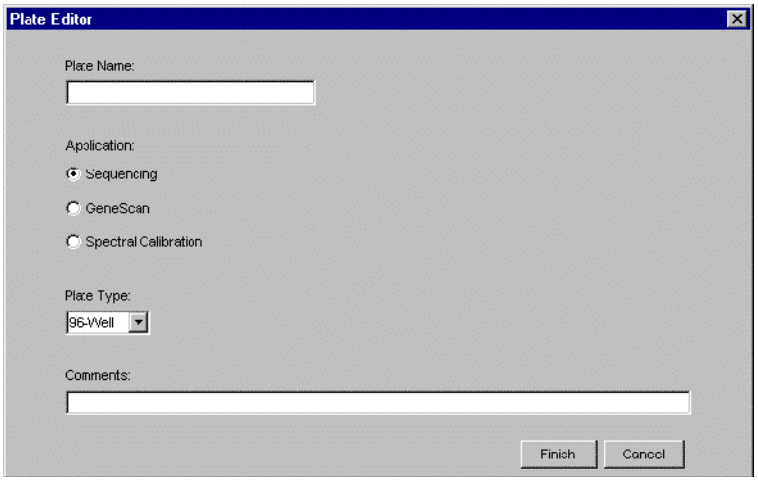
Introduction

After you have set up the BioLIMS project information, you must prepare a plate record in the 3100 Data Collection software for extraction to the BioLIMS database. This requires:

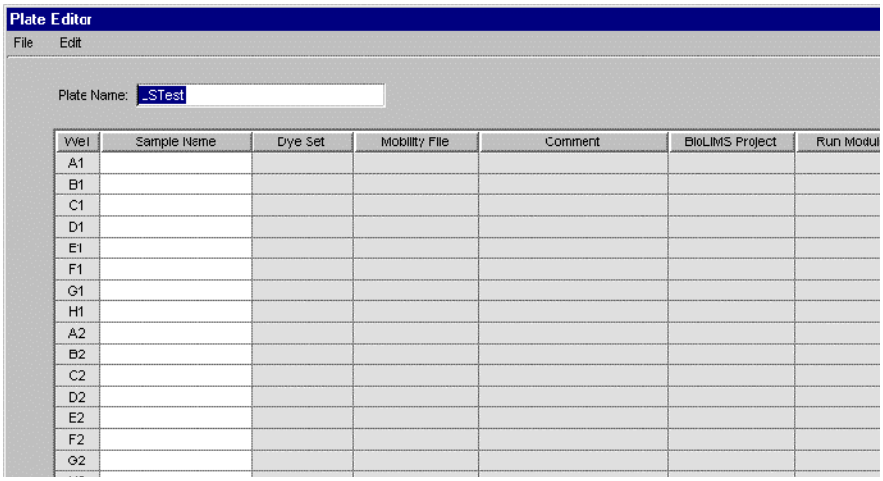
- Specifying a BioLIMS project in the plate's sample sheet
- Setting BioLIMS preferences for the plate

Specifying a BioLIMS Project

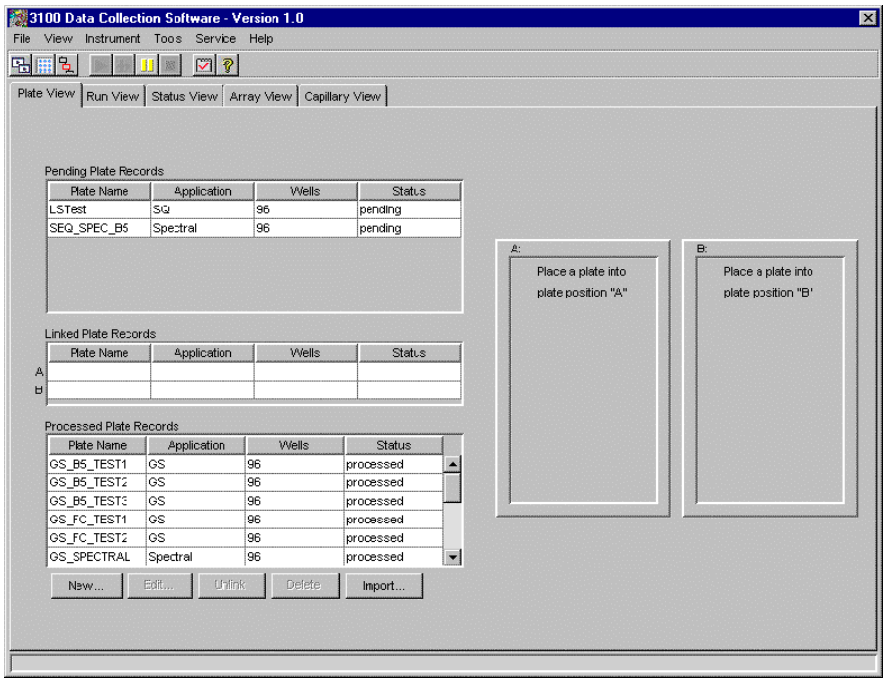
To specify a BioLIMS project in the plate's sample sheet:

Step	Action
1	From the **Tools** menu, select **Plate Editor.** The **Plate Editor** window appears. 
2	Fill in the window items as follows: **Item** / **Action** **Plate Name** — Type the plate name. **Application** — Click on the appropriate application. **Plate Type** — Choose the appropriate type from the drop-down list. **Comments** — Type comments if desired.

To specify a BioLIMS project in the plate's sample sheet: *(continued)*

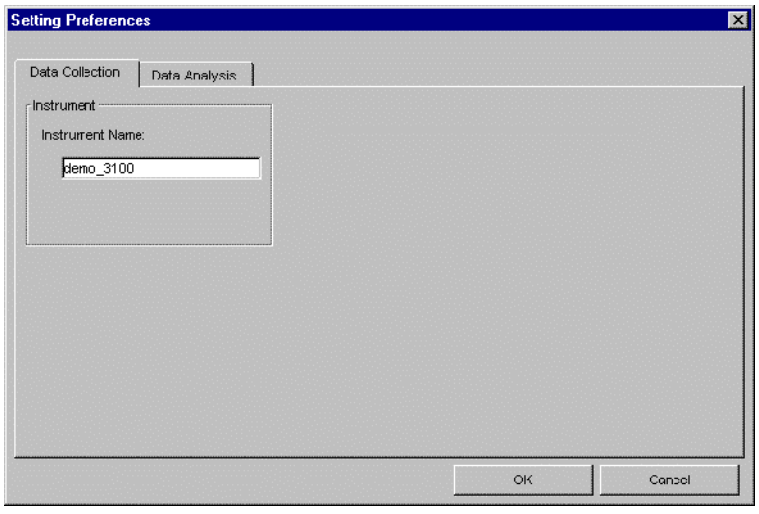
Step	Action
3	Click **Finish**. The **Plate Editor** spreadsheet appears with the plate name you assigned in step 2. 
4	Fill in the spreadsheet as appropriate, making sure to choose a BioLIMS project. To do this, click on the **BioLIMS Project** column for each well and choose a project from the drop-down list. **IMPORTANT** If you do not choose a BioLIMS project, your samples will not be extracted into the BioLIMS database successfully. **Note** If you need more information on filling out a spreadsheet, see page 3-32 for GeneScan analysis and page 3-37 for DNA sequencing.
5	Click **OK**. You will receive a **Please wait** message before the software returns to the **3100 Data Collection software** window. 

To specify a BioLIMS project in the plate's sample sheet: *(continued)*

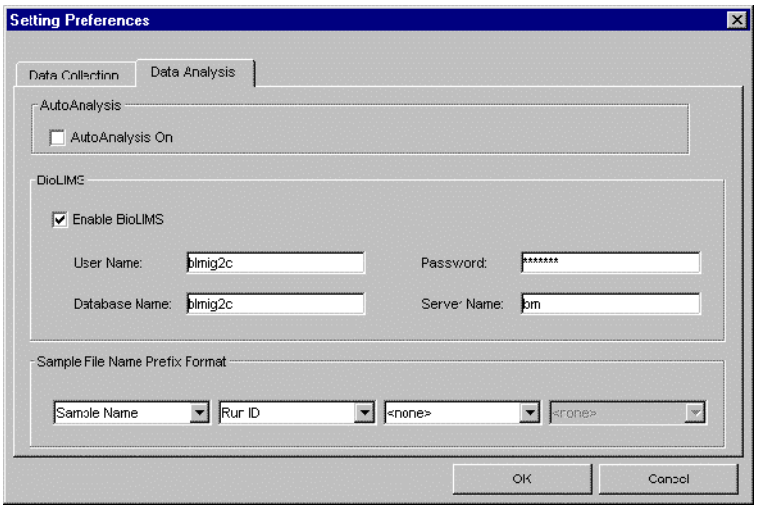
Step	Action
6	If it is not already selected, select the **Plate View** tab. 
7	Make sure your plate is listed under **Pending Plate Records**.
8	Highlight your plate and continue with "Setting BioLIMS Preferences" on page 5-52.

Setting BioLIMS Preferences

To set BioLIMS preferences for the plate:

Step	Action
1	From the **View** menu, select **Preferences.** The **Setting Preferences** window appears. 

To set BioLIMS preferences for the plate: *(continued)*

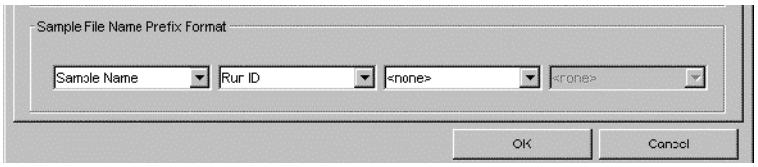
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Select the Data Analysis tab.
 
</td></tr>
<tr><td>3</td><td><table>
<tr><th>If you want to send...</th><th>Then...</th></tr>
<tr><td>raw data to the BioLIMS database</td><td>Leave the AutoAnalysis On check box blank.</td></tr>
<tr><td>analyzed data to the BioLIMS database</td><td>Check the AutoAnalysis On check box.</td></tr>
</table></td></tr>
<tr><td>4</td><td>In the BioLIMS portion of the window, check the Enables check box and type the appropriate information in the text fields.

Note The information below is used to connect to the BioLIMS database. It is assigned to your system when the Oracle software is installed. You may obtain the required information from the BioLIMS system administrator at your site.

<table>
<tr><th>Text Field</th><th>Description</th><th>Write your information here:</th></tr>
<tr><td>User Name</td><td>This is your account name on the server.</td><td></td></tr>
<tr><td>Database Name</td><td>This is the BioLIMS database/ schema name.</td><td></td></tr>
<tr><td>Password</td><td>This is the password for your server account.</td><td></td></tr>
<tr><td>Server Name</td><td>This is the server name of the BioLIMS database.

Note The server name is contained in the tnsnames.ora file.</td><td></td></tr>
</table></td></tr>
</table>

To set BioLIMS preferences for the plate: *(continued)*

Step	Action
5	In the **Sample File Name Prefix Format** portion of the window, assign a sample file name format for the BioLIMS project. To do this, choose from the drop-down list for each of the four identifiers. **IMPORTANT** You must select **Run ID** for one of the identifiers. In addition, you may want to limit the format to two identifiers only; if you choose more than two, the sample name will be truncated in the BioLIMS database. The recommended format is shown below. 
6	Click **OK**. The preferences will be applied to your highlighted plate.
7	Continue your setup and run your samples as usual. When the run has completed, the sample files will be extracted to the BioLIMS database automatically. However, you must view the debug.log file to see if the extraction completed successfully. Continue with “After Extracting to the BioLIMS Database” on page 5-55.

After Extracting to the BioLIMS Database

Introduction

After your samples have run, you must view the run's log file (debug.log) to ensure the extraction to the BioLIMS database was successful.

IMPORTANT You will not receive any error messages if the extraction was not completed successfully (*e.g.*, if the database connection was not established, if the BioLIMS project information was entered incorrectly, etc.). The only way to check the status of the extraction is to view the debug.log file.

Viewing a Run's Log File

To view a run's log file:

Step	Action
1	Open the directory that contains the 3100 Data Collection software and navigate to the **ExtractedRuns** folder. In most cases, the path will be: D:\AppliedBio\abi\3100\DataExtractor\ExtractedRuns
2	Open the **ExtractedRuns** folder. A directory is created in this folder for each run you've performed on the 3100 Genetic Analyzer.
3	Find the run for which you want to check the status and open its directory. All the data collected and extracted for this run is stored in this directory, along with a log file for the run (debug.log).
4	Open the run's debug.log file. **If the extraction was...** / **Then...** completed successfully: The following message appears after each sample file listed: **Successfully uploaded to BioLIMS**. not completed successfully: A message appears after each sample file listed, explaining why the extraction was not successfully completed. (For example: **Failed to open connection with database using specified credentials or database was not alive**.) You must manually upload the extracted data using Sample2DB. See the *Sample2DB User's Manual* for instructions.

Working with Plate Records 6

Overview

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

Section: Introduction

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

About Creating Plate Records

Introduction

The instrument database stores information about the plates and the samples they contain as data tables named plate records. Each plate placed on the instrument requires a plate record.

Note A plate record is analogous to the Sample Sheet used with the ABI PRISM® 377 DNA Sequencer and an Injection List used with the ABI PRISM® 310 Genetic Analyzer.

When to Create Plate Records

Create plate records in advance of placing the plates on the instrument.

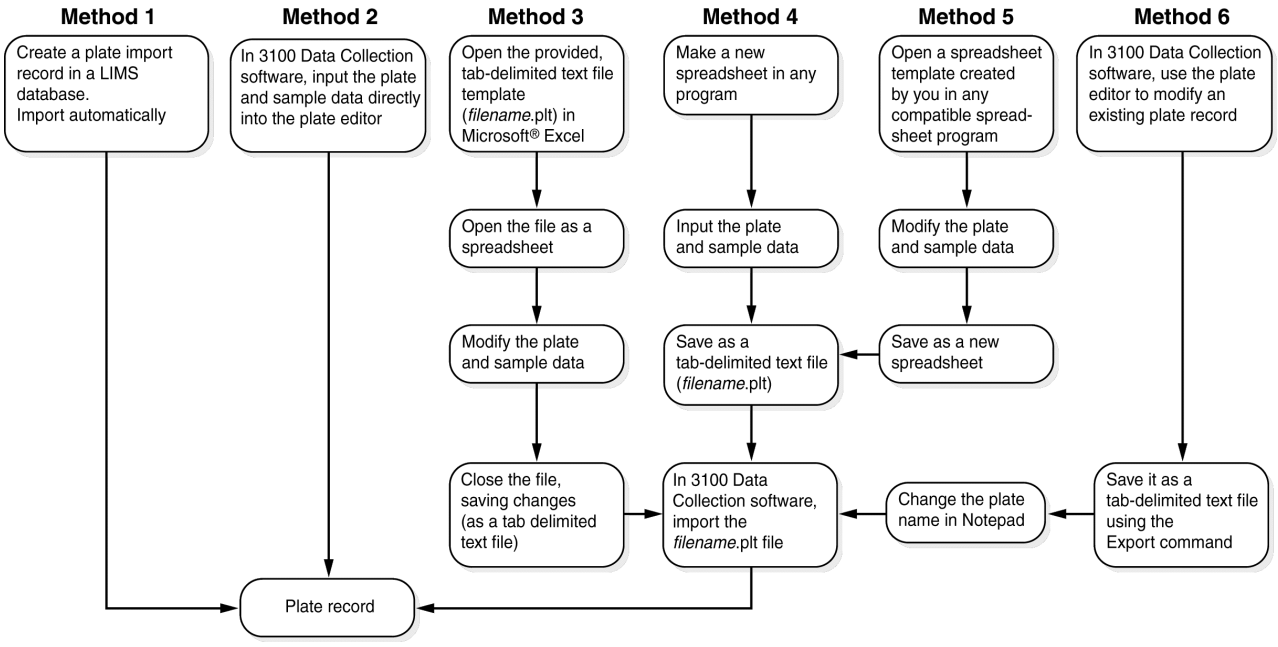
Data that will be imported for the creation of plate records can be prepared and stored on any networked computer or transferred from a computer on a disk.

Note Plate records cannot be created or linked while a run is in progress.

How to Create Plate Records

There are numerous methods used to create plate records. The most convenient method transfers data directly from a LIMS database. Once set up in the Preferences dialog box of the ABI PRISM® 3100 Data Collection software and the LIMS program, the transfer of data and creation of plate records is completely automatic, requiring no operator intervention.

Plate records can be created using the methods described in the following diagram:



For instructions for each method, see the pages listed in the following table:

Method	See Page...	Method	See Page...
1	6-19	4	6-28
2	3-31 and 3-36	5	6-29
3	6-24	6	6-30

About the Plate Record Fields

Introduction

Definitions and options for the following plate record fields are provided in this section:

- Dye sets
- Mobility files
- Run modules
- Analysis modules

Dye Sets

DNA fragments are detected and identified by the fluorescent dyes with which they are chemically labeled. Dyes are purchased and used as dye sets, which are optimized for particular applications.

When a dye is bound to a DNA fragment, it changes the rate at which the fragment migrates during electrophoresis. When DNA fragments that are labeled with different dyes are electrophoresed together, the fragments do not migrate with equal spacing because different dyes change the migration rate to different extents.

Without correction, this would lead to an uneven separation of peaks in the electropherogram. The data needed to perform mobility shift correction are contained in mobility files (see “Mobility Files” below).

Dye Sets Provided

Select a dye set from among the following options:

For...	Select Dye Set	Dyes in Set
DNA sequencing	E	dR6G, dR110, dTAMRA, dROX
Fragment analysis with ABI PRISM® Linkage Mapping Sets	D	6-FAM, HEX, NED, ROX

IMPORTANT If you select the wrong dye set you will have to re-run your samples. You cannot correct this after the run because multicomponenting is applied before run data storage.

Mobility Files

Note Mobility files are identical to the dye set/primer files used on other ABI PRISM genetic analyzers.

A mobility file is a software file containing the data that is used to compensate for differences in the electrophoretic mobilities of DNA fragments caused by labeling with different dyes (see “Dye Sets” above).

Mobility files are for DNA sequencing only. Mobility files are different for different dye sets and instrument types.

Mobility Files Provided

The following mobility files are provided with the 3100 software and stored in the following directory:

D:\AppliedBio\Abi\Shared\Analysis\Basecaller\Mobility

Mobility File	DNA Sequencing Chemistry
DP3100POP6{BD-21M13}v1.mob	BigDye Primer chemistry; using the -21m13 primer
DP3100POP6{BD-M13Rev}v1.mob	BigDye Primer chemistry; using the reverse primer
DT3100POP6{BD}v2.mob	BigDye Terminator chemistry
DT3100POP6{dRhod}v1.mob	dRhodamine Terminator chemistry

Note New versions of these mobility files may be available from our web site in the future. Mobility files for dye sets other than the ABI PRISM® BigDye™ sets must be provided by the manufacturer.

Run Modules

A module is a collection of routines that perform a task. Run modules define the run conditions for a sample. For a list of conditions you can set for running a sample, see “Modifiable Run Module Parameters” on page 5-22.

Run Modules Provided

The following run modules are provided with the 3100 software.

Run Type	Run Module
GeneScan	GeneScan36_POP4DefaultModule
Standard DNA sequencing	StdSeq50_POP6DefaultModule
Rapid DNA sequencing	RapidSeq36_POP6DefaultModule

Analysis Modules

A module is a collection of routines that perform a task. Analysis modules tell the AutoAnalyzer which parameters to use for data analysis. You can use the analysis modules provided and/or create your own to define different analysis parameters.

Analysis Modules Provided

The following analysis modules are provided with the 3100 software. You can examine the settings for each of the files using the analysis software.

Note The meanings of the settings are described in the *ABI PRISM DNA Sequencing Analysis Software v. 3.6 NT User's Manual* and the *ABI PRISM GeneScan Analysis Software v. 3.5 NT User's Manual.*

Analysis Module	Run Type
BC-3100_SeqOffFtOff.saz	Standard DNA sequencing
BC-3100RR_SeqOffFtOff.saz	Rapid DNA sequencing
GS400HDAnalysis.gsp	GeneScan using size standard 400HD
GS350Analysis.gsp	GeneScan using size standard GS350
GS500Analysis.gsp	GeneScan using size standard GS500
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. 3.5 NT User's Manual.*

Section: Tab-Delimited Text Files

In This Section

The following topics are covered in this section:

Introduction

The topics in this section explain tab-delimited text files, provide an overview of how to create them, and give examples of tab-delimited text files for DNA sequencing and fragment analysis.

Introduction to Tab-Delimited Text Files

Introduction

Tab-delimited text files can be imported directly into the instrument database to create plate records.

What They Are

Tab-delimited text files are text-only files that contain groups of information, called tokens, separated by tabs or end-of-line characters. Any text-only file (containing no graphics or tables) created in a word-processing program is a text file. Using tab stops to separate sections of text, and end-of-line characters to separate lines of text, makes a file a tab-delimited text file.

Examples

A tab-delimited text file created in Microsoft® Word is shown below. The symbols do not appear when the file is printed.

```
First·line-token·one    →    token·two  →  token·three  →  token·four·  →  token·five¶
Second·line-token·one   →    token·two  →  token·three  →  token·four  →  token·five¶
Third·line-token·one    →    token·two  →  token·three·  →  token·four  →  token·six¶
```

With the nonprinting symbols turned off, the file looks like this:

```
First line-token one      token two    token three    token four    token five
Second line-token one     token two    token three    token four    token five
Third line-token one      token two    token three    token four    token six
```

Word-Wrapped Example

As in word-processed documents, tab-delimited text files with long lines wrap around to the next line.

```
First line-token one          token two    token three    token four which is a long token
that wraps around to the next line         token five
Second line-token one         token two which is another long token          token three
          token four          token five
Third line-token one          token two    token three which is a further long token that
wraps around to the next line and makes the file difficult to read  token four    token six
```

Word wrapping does not affect the performance of a file, but it does make the information more difficult to comprehend.

Notepad

The Microsoft® Windows® NT operating system includes a simple text-only word processor called Notepad, located in the Accessories menu. Notepad will open any text-only file, even if the file was created by a program using the Macintosh® operating system. In this case, though, the end-of-line characters may need to be re-entered.

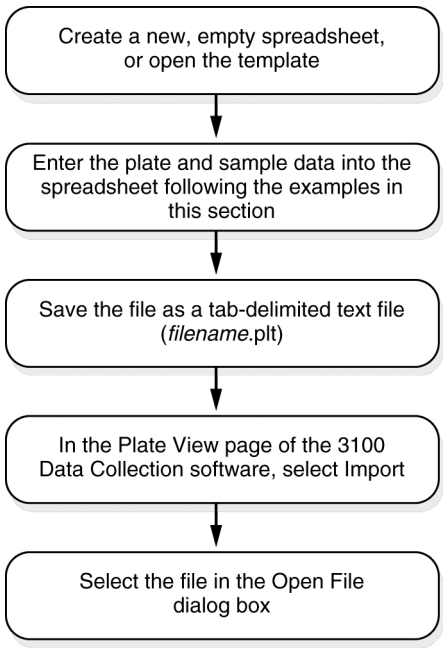
About Creating Tab-Delimited Text Files

Introduction

Although it is possible to create tab-delimited text files by typing the required information directly into a word-processing program, it is easier to first enter the data into a spreadsheet, and then save the spreadsheet as a tab-delimited text file. Spreadsheets are easier to work with because they organize data clearly in columns, and because repetitive typing can be reduced by using their fill-down function.

Overview of the Method

The typical method for importing information to create a plate record is outlined below.



Omitting Information

You do not need to include all of the information required for a run before importing a tab-delimited text file into the instrument database. Information can be added to the plate record using the plate editor after the tab-delimited text file has been imported.

Note If you are importing data from a LIMS database, there cannot be any errors in the data because there is no opportunity to review it and the import will fail (see page 6-19).

Using Spreadsheets to Create Tab-Delimited Text Files

Introduction

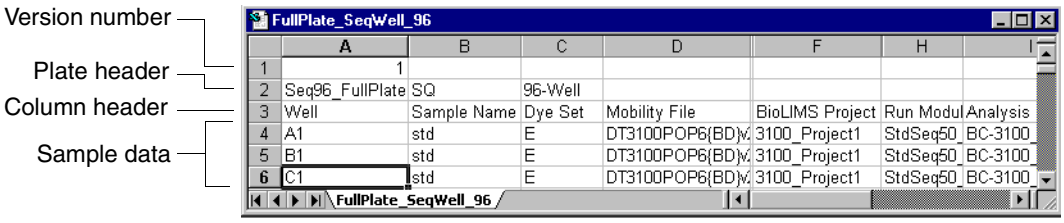
You can enter plate record data into any spreadsheet program that can save files as tab-delimited text files.

You can create spreadsheets in a program that uses the Macintosh operating system; however, you must then convert the files into Microsoft Windows format files.

Example of Sequencing Spreadsheet

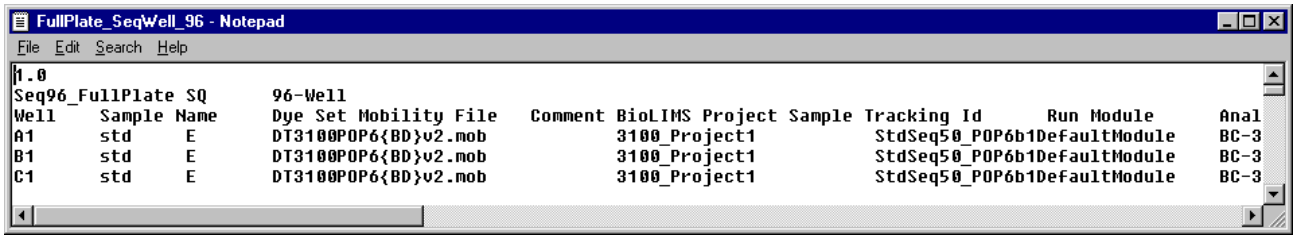
An example of a spreadsheet, prepared in Microsoft® Excel, for samples intended for DNA sequencing is shown below.

For an explanation of the labels, see page 6-14.



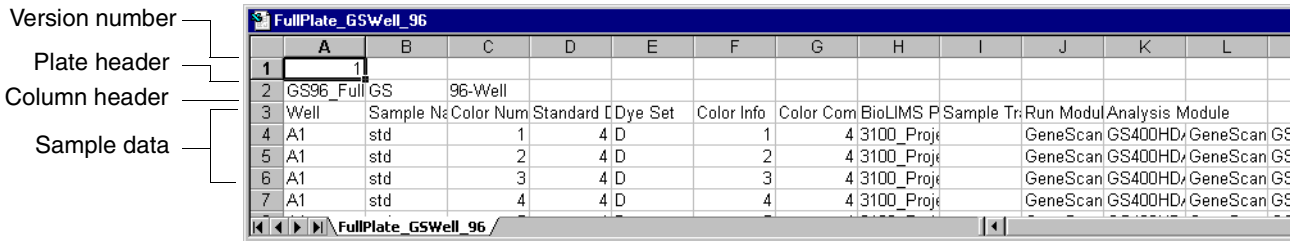
Example of Sequencing Tab-Delimited Text File

When the above spreadsheet is converted to a tab-delimited text file and opened with the Notepad program, it looks like the example below.



Example of Fragment Analysis Spreadsheet

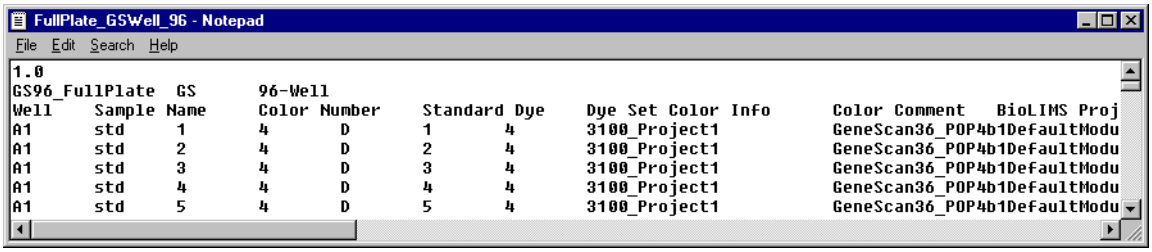
An example of a Microsoft Excel spreadsheet for samples intended for fragment analysis is shown below.



Example of Fragment Analysis Tab-Delimited Text File

When the above spreadsheet is converted to a tab-delimited text file and opened with the Notepad program, it looks like the example below.

For an explanation of the labels, see page 6-14.



FullPlate_GSWell_96 - Notepad

File Edit Search Help

```
1.0
GS96_FullPlate  GS      96-Well
Well    Sample Name     Color Number    Standard Dye    Dye Set Color Info      Color Comment   BioLIMS Proj
A1      std     1       4       D       1       4       3100_Project1           GeneScan36_POP4b1DefaultModu
A1      std     2       4       D       2       4       3100_Project1           GeneScan36_POP4b1DefaultModu
A1      std     3       4       D       3       4       3100_Project1           GeneScan36_POP4b1DefaultModu
A1      std     4       4       D       4       4       3100_Project1           GeneScan36_POP4b1DefaultModu
A1      std     5       4       D       5       4       3100_Project1           GeneScan36_POP4b1DefaultModu
```

Empty Cells or tokens

Tab-delimited text files may be imported with empty tokens. Missing data can be added using the plate editor.

IMPORTANT A space character (entered by pressing the space bar) must be entered between tab stops in a tab-delimited text file, as a place marker for missing information. A space character must be entered into each blank cell of a spreadsheet before converting it to a tab-delimited text file.

IMPORTANT Do not leave whole empty rows (with the exception of the Well Location row) in a spreadsheet or tab-delimited text file that is intended for import, as illustrated by the example below.

Do this:

	A	B
1	1.0	
2	PlateName0	SQ
3	Well	Sample Name
4	A1	cell_sample-01
5	B1	cell_sample-03
6	C1	cell_sample-04
7	F1	cell_sample-05

Do not do this:

	A	B
1	1.0	
2	PlateName0	SQ
3	Well	Sample Name
4	A1	cell_sample-01
5	B1	cell_sample-03
6	C1	cell_sample-04
7	D1	
8	E1	
9	F1	cell_sample-05

Typing Accuracy and Error Messages

It is extremely important to be accurate when typing information into a spreadsheet, tab-delimited text file, or LIMS database that will eventually be imported into the 3100 Data Collection software.

When the 3100 Data Collection software is importing data from a text file, it compares the relevant tokens with lists of run modules, analysis modules, etc., stored in the database or hard drive. The Data Collection software recognizes the data only if it can make a match. If an “illegal” value is typed into a cell in certain columns, the typed data will be deleted and the field will be blank in the imported plate record. If the sample name contains restricted characters, the entire plate will not be imported.

IMPORTANT When naming the plate, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces.

When importing data from a LIMS database, an error will be logged and no plate record will be created if the file contains a typing error.

Spreadsheet or Tab-Delimited Text File Information

Introduction

Four types of information are contained in a spreadsheet or tab-delimited text file intended for import into the 3100 Data Collection software:

- Version number
- Plate header
- Column header
- Sample data

See the spreadsheet examples in “Using Spreadsheets to Create Tab-Delimited Text Files” on page 6-12.

Version Number

The version number is the only cell or token on the first row of a spreadsheet or tab-delimited text file. It specifies the version of the formatting conventions used for importing plate records.

Currently, the version that must be entered into all spreadsheets is 1.0. If there are changes to the conventions, the version number will change, and you will be notified.

Plate Header

The plate header is a sequence of five cells or tokens separated by tabs. These cells or tokens must always be typed in the same order across the plate header.

Cell or Token	Function
Plate Name	Identifies a specific plate. The plate name you assign must not exceed 32 characters. **Note** This is the same as the Plate ID listed in the plate record tables of the Plate View page.
Application	Identifies a plate as containing samples for either GeneScan analysis (GS) or DNA sequencing (SQ). **IMPORTANT** Do not mix samples for sequencing analysis and fragment analysis in the same plate.
Plate Type	Defines the type of plate. The codes used for the two plate types are: ♦ 96-well plates: 96-Well ♦ 384-well plates: 384-Well
Owner	Identifies the name of the person who loaded the samples onto the plate and/or created the spreadsheet
Comment	Allows you to enter comments about the plate

Column Header for Sequencing Analysis

The column header for sequencing analysis contains up to eight cells or tokens that provide headings for the columns that will contain the sample data. The order in which the column cells or tokens are listed is important and is noted below.

Token	Function
Well Position	Identifies the well in which the sample is located, *e.g.*, A1, G6, O18, etc. For 96-well plates, the well positions are A–H and 1–12. For 384-well plates, the well positions are A–P and 1–24. **IMPORTANT** This cell or token must always be first (from left to right).
Sample Name	Identifies the sample. The sample name you assign must not exceed 63 characters. **IMPORTANT** When naming the sample, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces. **IMPORTANT** You must limit the sample name to 63 characters (59-character filename and 4-character extension). If you do, the name may be truncated when exported from the 3100 Data Collection software. **IMPORTANT** This cell or token must always be second (from left to right).
Dye Set	Specifies the dye set used to label the DNA. This name must match the names stored in the instrument database. **Note** If you select the wrong dye set you will have to re-run your samples. You cannot correct this problem after the run.
Mobility File	Specifies the dye mobility file used for processing the fluorescence data. **Note** This is identical to the dye set/primer file used with previous ABI PRISM® genetic analyzers.
Comment	Allows you to enter comments about the sample
Project Name	Designates the BioLIMS™ Genetic Information Management System Collection name into which this sample will be added.
Run Module	Specifies the run module used for the sample. **IMPORTANT** This cell or token must always be next to last (from left to right). **IMPORTANT** The name of the run module must be typed correctly. If the name is typed incorrectly, the plate will be imported but the run module will not be entered in the plate record.
Analysis Module	Specifies the analysis module used to run the sample. Sequencing analysis modules have the file format: *filename*.saz **IMPORTANT** This cell or token must always be last (from left to right). **IMPORTANT** You must always select an analysis module if you want the data to be extracted and analyzed. **IMPORTANT** The name of the analysis module must be typed correctly. If the name is typed incorrectly, the plate will be imported but the analysis module will not be entered in the plate record.

Note The Dye Set, Comment, and Project Name tokens can be presented in any order.

Column Header for GeneScan Analysis

The column header for GeneScan analysis contains up to 10 cells or tokens that provide headings for the columns that will contain the sample data. The order in which the column cells or tokens are listed is important and is noted below.

<table>
<tr><th>Cell or Token</th><th>Function</th></tr>
<tr><td>Well Position</td><td>Identifies the well in which the sample is located e.g., A1, G6, O18, etc.

For 96-well plates, the well positions are: A–H and 1–12. For 384-well plates, the well positions are A–P and 1–24.

IMPORTANT This cell or token must always be first (from left to right).</td></tr>
<tr><td>Sample Name</td><td>Identifies the sample. The sample name you assign must not exceed 63 characters.

IMPORTANT When naming the sample, you can use letters, numbers, and the following punctuation only: -_(){}#.+. Do not use spaces.

IMPORTANT You must limit the sample name to 63 characters (59-character filename and 4-character extension). If you do, the name may be truncated when exported from the 3100 Data Collection software.

IMPORTANT This cell or token must always be second (from left to right).</td></tr>
<tr><td>Color Number</td><td>Corresponds to a specific color button of the plate record Dye field.

<table>
<tr><th>Color Number</th><th>Color Button</th></tr>
<tr><td>1</td><td>B</td></tr>
<tr><td>2</td><td>G</td></tr>
<tr><td>3</td><td>Y</td></tr>
<tr><td>4</td><td>R</td></tr>
<tr><td>5</td><td>O</td></tr>
</table>
</td></tr>
<tr><td>Standard Dye</td><td>Represents the size-standard color. This should be the number 4 for all applications, which corresponds to the red dye. Selecting the number 4 in this field is equivalent to selecting the diamond in the “R” color box of the GeneScan Analysis software.</td></tr>
<tr><td>Dye Set</td><td>Specifies the dye set used to label the samples. It must match the names stored in the instrument database.</td></tr>
<tr><td>Color Info</td><td>Enables you to identify the sample in GeneScan analysis software when you are examining samples by color if you enter the sample name in this optional field.</td></tr>
<tr><td>Color Comment</td><td>Enables you to customize the output for downstream analysis. Optional.</td></tr>
<tr><td>Project Name</td><td>Designates the BioLIMS Genetic Information Management System Collection name into which this sample will be added.</td></tr>
<tr><td>Run Module</td><td>Specifies the run module used for the sample.

IMPORTANT This cell or token must always be next to last (from left to right).

IMPORTANT The name of the run module must be typed correctly. If the name is typed incorrectly, the plate will be imported but the run module will not be entered in the plate record.</td></tr>
</table>

Cell or Token	Function
Analysis Module	Specifies the analysis module used to run the sample. GeneScan analysis modules have the file format: *file name*.gsp **IMPORTANT** This cell or token must always be last (from left to right). **IMPORTANT** You must always select an analysis module if you want the data to be extracted and analyzed. **IMPORTANT** The name of the analysis module must be typed correctly. If the name is typed incorrectly, the plate will be imported but the analysis module will not be entered in the plate record.

Note The Dye Set, Comment, and Project Name tokens can be presented in any order.

Sample Data

The sample data begins on row 4 of a spreadsheet. A 96-well plate for sequencing analysis contains up to 96 rows of sample data (one row for each sample, and therefore each well). A 96-well plate for fragment analysis contains a multiple of 96 rows, since one well can contain several dye channels, each labeled with a differently colored dye.

Running the Same Sample with Different Conditions

Sample Run Options

You can run the same sample up to five times using different combinations of analysis modules and run modules as follows:

- Same run module, but different analysis module
- Same analysis module, but different run module
- Different run module and analysis module
- Same run module and analysis module (replicate run)

Note Make sure that you have enough sample for the number or runs you specify.

How to Set Up Multiple Runs

Multiple runs of the same sample are set up in the plate record or tab-delimited text files imported to create a plate record. To perform more than one run with the same sample, add additional pairs of run modules and analysis modules to the tab-delimited text file as shown in the examples below.

Example One: A Sample Running with More Than One Run Module

Here is part of a spreadsheet showing data for a sample that will be run with three different run modules with the same analysis module:

Run Module	Analysis Module				
runmod005-3	analmod35	runmod008-1	analmod35	runmod010-1	analmod35

- The Run Module and Analysis Module column headings are used only once.
- The run modules and analysis modules are grouped in pairs with the run module always placed to the left of its paired analysis module.

Example Two: A Sample Running with More Than One Analysis Module

Here is part of a spreadsheet showing data for a sample that will be run with three different analysis modules, but with the same run module:

Run Module	Analysis Module				
runmod005-3	analmod22	runmod005-3	analmod10	runmod005-3	analmod06

- The Run Module and Analysis Module column headings are used only once.
- The run modules and analysis modules are grouped in pairs, with the run module always placed to the left of its paired analysis module.

Section: Creating a Plate Record by Importing LIMS Data

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

Introduction This section provides an overview of transferring data from a laboratory information management system (LIMS) to the plate import table and a description of the format in which the LIMS data must be written.

This section does not describe the detailed procedure, which is beyond the scope of this manual.

Note To import LIMS data, you must know how to import binary data BLOBS into an Oracle® database.

Data Transfer

Advantages of Importing Data from a LIMS Database

Data transferred from a LIMS database creates plate records that are identical to plate records created from tab-delimited text files.

The advantages of using a LIMS database over tab-delimited text files are:

- The sample data is already entered into a LIMS database. Therefore, the data can be assembled quickly into the format required for import.
- Transferring data from a LIMS database is completely automatic.

Automatic Data Transfer

The data transfer process is automatic, it does not need to be initiated by a manual import command in the Data Collection software.

When the software is configured to import LIMS data, it:

- Periodically polls the plate import table (described below) for new data transferred into it by the LIMS database
- Automatically:
  - Creates plate records from the transferred data
  - Enters an event describing the import in the Events log
  - Registers the plate record in the Pending Plate Record table of the Plate View page

Configuring the 3100 Data Collection Software for LIMS Import

To use the automatic LIMS data-transfer feature, the 3100 Data Collection software must be configured to automatically poll the instrument database for plate import table entries.

BioLIMS is a type of LIMS database. For information about BioLIMS, see page 5-47.

Plate Import Table

Introduction

The instrument database contains a plate import table. It is the only part of the database that can be safely accessed by outside programs, as there are no links to other tables in the database.

Plate Import Table Capacity

The number of sets of plate data that can be accommodated in the plate import table is dependent on the amount of available space in the instrument database. Once the data in the table has been successfully imported into the main database, the data is stored as a plate record. As a result, there is little need to keep the imported data in the plate import table once the success of the import has been verified. We recommend that you periodically delete data from the plate import table. It is best to do this when the 3100 Data Collection software is not running. To delete data from the plate import table, consult your Oracle database administrator.

Plate Import Errors

If an error occurs while importing data from the plate import table, the error is registered in the following locations:

- Errors pane on the Status View page
- Run log table on the Run Log page
- Plate import table (status will be set to “Bad”)

Required Fields

A LIMS entry into the plate import table must contain the following five fields:

Field	Format
Plate ID	Up to 32 characters
Name	Up to 32 characters
Status	Up to 32 characters
Plate BLOB	BLOB
Plate BLOB version	Integer

Plate ID

The plate ID is a unique identifier or primary key for the plate. This ID should not be the same as the plate name. The plate ID must be unique. The instrument database will not allow entry of a plate ID if that value is already used by another row in the plate import table.

Name

The name is the name of the plate. This name should not be the same as the plate ID. The name is not a unique identifier for the plate in the plate import table and can be used more than once within the plate import table. However, once the data is used to create a plate record, the name becomes the database plate ID and must be unique among all existing plates.

Having the name field in addition to the plate ID field allows you to delete a plate record from the plate import table and then re-import it with the same name (but a different Plate ID).

The name must be the same as the plate name given to the header in the BLOB equivalent of the tab-delimited text file. It can be up to 32 characters and must not contain any restricted characters.

IMPORTANT Use only the following characters, which are a subset of the characters allowed by the Windows NT operating system: letters, numbers, and -_(){}#.+

Status

There are three status options:

Status	Assigned when...	Set by...
New	the data is ready for transfer	LIMS
Old	a plate table has been successfully imported	3100 Data Collection software
Bad	the transfer was unsuccessful	3100 Data Collection software

The status of any data set stored in the plate import table can be checked at any time through the LIMS software.

Plate BLOB Definition

The plate BLOB is an array of binary data that is equivalent (except in language) to a tab-delimited text file used for data import. The plate BLOB is written from a table in the LIMS database that contains data and formatting equivalent to a tab-delimited text file or spreadsheet used for data import.

The plate ID in the header of the binary BLOB must exactly match the plate name in the plate import table.

Converting the data into a plate BLOB format requires a knowledge of SQL and is a topic beyond the scope of this manual.

Plate BLOB Version Number

The plate BLOB takes its version number from the header of the table used to create the plate BLOB.

This number is 1.0 for the current release of the software, which is identical to the version in the tab-delimited text files prepared for import into the instrument database.

Section: Creating Plate Files

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

Definition A plate file is a tab-delimited text file saved with the file name extension .plt.

Creating a Plate File Using a Provided Template

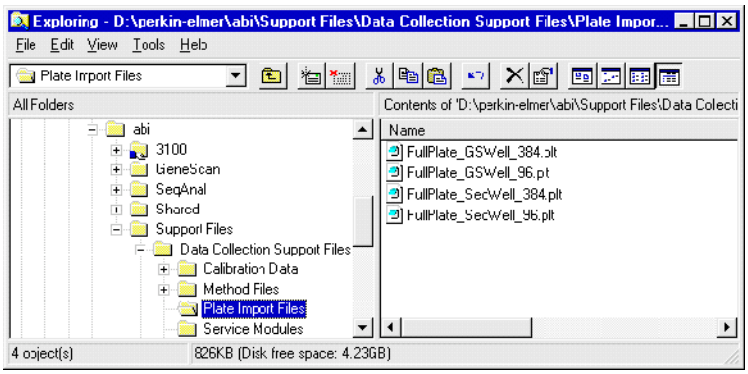
Introduction

This method uses a tab-delimited text file template and Microsoft Excel to create a plate file. Templates are provided with the 3100 software and are listed below. In Microsoft Excel, you are able to view a tab-delimited text file template in a spreadsheet format without actually saving it as a spreadsheet.

Locating the Templates

The templates provided with the 3100 software are located in the following directory:

D:\AppliedBio\Abi\Support Files\Data Collection Support Files\Plate Import Files



Template File Names

This table lists the file names and types of the templates.

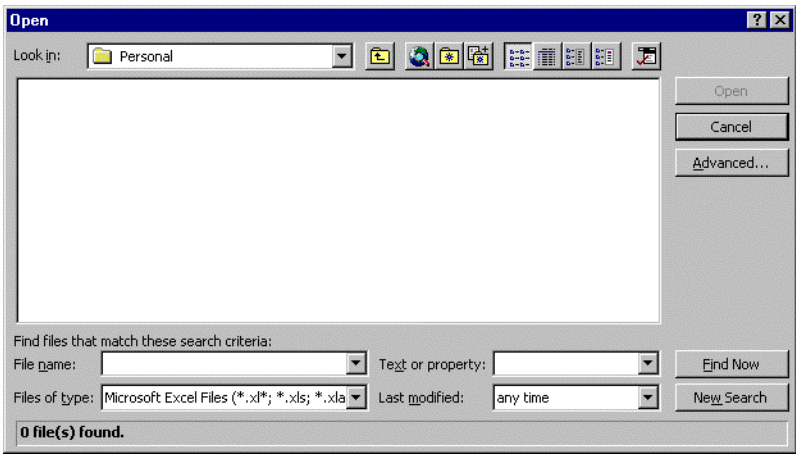
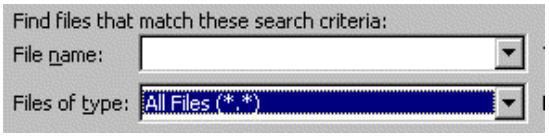
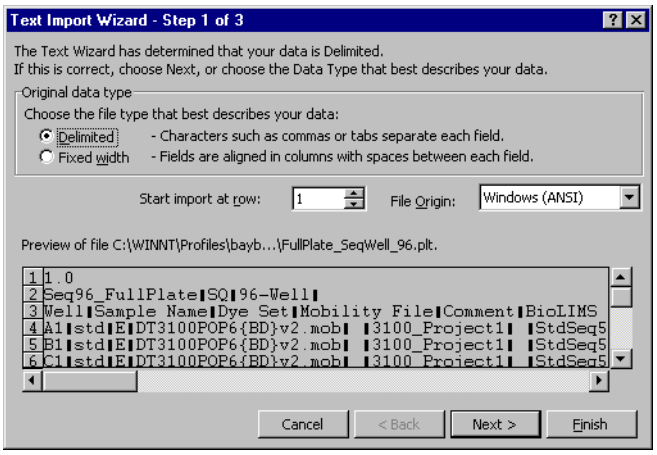
Template File name	Type of Template
FullPlate_GSWell_384.plt	384-well for fragment analysis
FullPlate_GSWell_96.plt	96-well for fragment analysis
FullPlate_SeqWell_384.plt	384-well for sequencing analysis
FullPlate_SeqWell_96.plt	96-well for sequencing analysis

Creating a Plate Record Using a Template

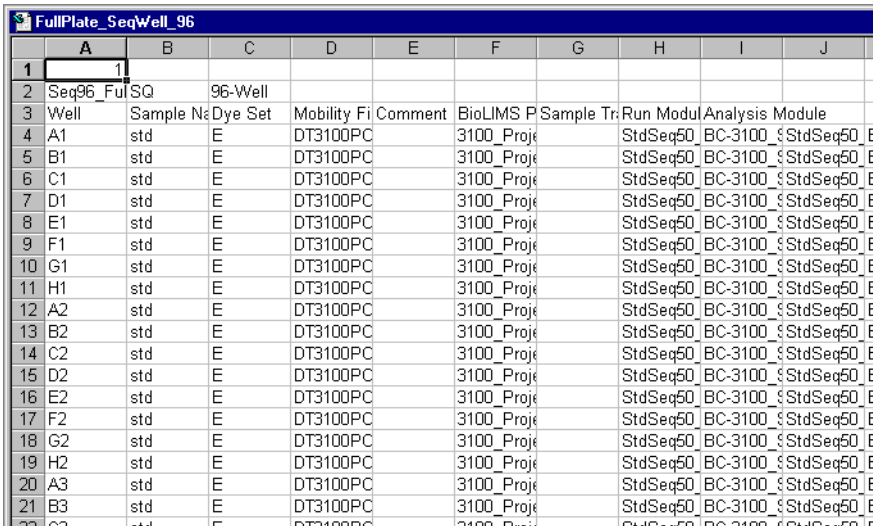
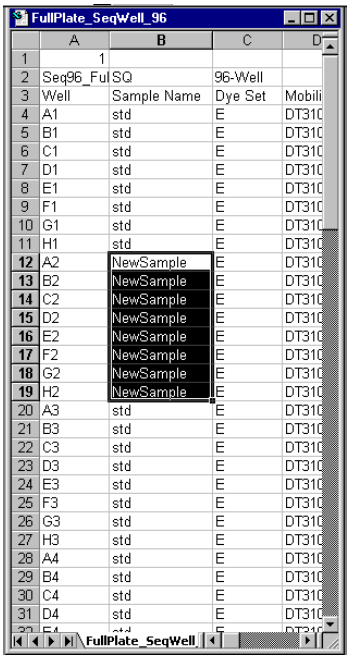
To create a plate record using a template:

Step	Action
1	Start Microsoft Excel.

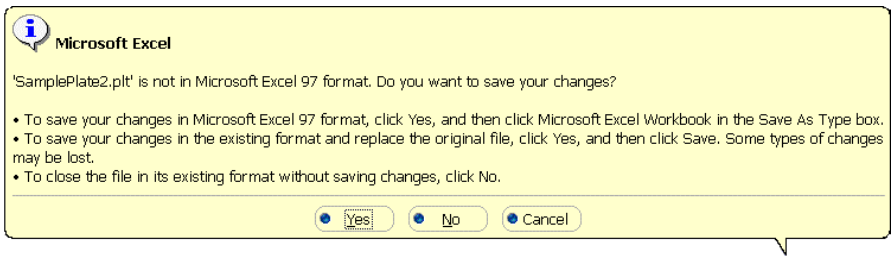
To create a plate record using a template: *(continued)*

Step	Action
2	From the **File** menu, select **Open**. This opens the **Open** dialog box. 
3	Navigate to the **Plate Import Files** folder in the following directory: D:\AppliedBio\Abi\Support Files\Data Collection Support Files\Plate Import Files Notice that no files are displayed. This is because there are no Microsoft Excel files in this folder.
4	In the **Files of type** list box, select **All Files**. 
5	Select the plate file (.plt file) template you want to use and click **Open**. The **Text Import Wizard** dialog box opens. 

To create a plate record using a template: *(continued)*

Step	Action
6	Click **Finish**. The file is displayed as a spreadsheet. 
7	Modify any data in the cells by clicking them and retyping. To save time, use the **Fill Down** command: ♦ Select the cell containing the information that you want to copy. ♦ From the **Edit** menu, select **Copy**. ♦ Drag the fill-down handle in the bottom-right corner of the cell to copy the information into adjacent cells. 

To create a plate record using a template: *(continued)*

Step	Action
8	Click the Close button in the upper right corner of the window. Either a standard Windows NT message box or an equivalent Office Assistant message box is displayed. 
9	Click **Yes**. This opens the **Save As** dialog box.
10	In the **File name** drop-down list of the **Save As** dialog box, delete the name of the file that you selected and type a new name for the edited file. Make sure that you add the .plt extension. Click **Save**. This saves the edited file as a new file.
11	Follow the directions starting on page 6-32 for importing a tab-delimited text file to create a plate record.

Creating a Plate File from a New Spreadsheet

Creating a Plate File from a New Spreadsheet

To create a plate file (.plt file) from a new spreadsheet:

Step	Action
1	On a computer using a Windows NT operating system, open a new spreadsheet file in a program that allows you to save a spreadsheet as a tab-delimited file.
2	Using the spreadsheet examples and the information about each token starting on page 6-14, type your information into the file.
3	From the **File** menu, select **Save As**. In most spreadsheet programs, the **Save As** dialog box will open.
4	Type in a name for the tab-delimited file that you are about to create. **IMPORTANT** Use only the following characters, which are a subset of the characters allowed by the Windows NT operating system: letters, numbers, and -_(){}#.+. Do not use spaces.
5	Save the file with the following file name format: *filename*.plt. In the **File Type** text box (or equivalent), select the **text file (tab delimited**) file type or equivalent. **Note** If you close Microsoft Excel before performing this step, the Office Assistant opens. Click **Yes**, and then **Save**.
6	Follow the directions starting on page 6-32 for importing a tab-delimited text file to create a plate record.

Creating a Plate File from a Custom Spreadsheet Template

Introduction

This method can be used to create a read-only spreadsheet template, which you can save as a different name and then modify to suit your needs.

If you are using similar samples and run conditions, this method allows you to type less each time you want to create a new plate record.

There are two parts to the procedure:

- Creating the template
- Modifying the template

Creating the Template

To create a custom spreadsheet template:

Step	Action
1	Use the directions starting on page 6-23 to create a plate file (.plt file) that contains the basic information that you need for a plate record.
2	Open the .plt file in a spreadsheet program.
3	Save the spreadsheet as a read-only file to ensure that it does not get overridden.

Modifying the Template

To create a plate record from a custom spreadsheet template:

Step	Action
1	Open the spreadsheet that you just created to use as a template.
2	Save the spreadsheet under a different name, making sure that it is not read-only as above.
3	Edit the plate and sample data in the spreadsheet according to the specific plate and samples you are using.
4	Save the spreadsheet as a tab-delimited text file, giving it the .plt extension.
5	If needed, repeat steps 1–4 to create other tab-delimited text files.
6	Follow the directions starting on page 6-32 for importing a tab-delimited text file to create a plate record.

Creating a Plate File from an Edited Plate Record

Introduction

To save time when preparing plate records, you can save the data entered into the plate editor table as a tab-delimited text file. After changing the plate name, the file can be re-imported. Alternatively, it can be saved as a read-only file and used as a template.

Creating a Plate File from an Edited Plate Record

To create a plate file from an edited plate record:

Step	Action
1	Open the **Plate View** page of the 3100 Data Collection software.
2	In one of the plate record tables, double-click the plate record that you want to edit. This opens the plate record in the plate editor. Edit the plate record as required.
3	From the **File** menu, select **Export**. This opens a browser dialog box.
4	Navigate to the folder in which you want to save the file. You may want to use the plate import files folder in the following directory: D:\AppliedBio\Abi\Support Files\Data Collection Support Files\Plate Import Files **Note** You cannot see Network Neighborhood directories from this browser dialog box.
5	In the **File name** dialog box, type a name for the file and add the extension .plt. Click **Save**. This saves the file as a tab-delimited text file to the specified directory.
6	If you want to use this file as a template, give the file a read-only status: a. Right-click the **Start** icon on the Windows NT taskbar, and from the pop-up menu select **Explore**. This opens Windows NT Explorer. b. Navigate to the file that you just created. c. Right-click the file and from the pop-up menu select **Properties**. d. From the **Attributes** group box, select **Read-only**. e. Click **OK**.
7	Follow the directions starting on page 6-32 for importing a tab-delimited text file to create a plate record.

Section: Importing Plate Files and Linking Plate Records

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

Introduction This section describes how to import tab-delimited text files to create plate records and how to link the plate records to their plates.

About Importing Tab-Delimited Text Files and Linking Plate Records

Introduction

To create and link a plate record by importing a plate file into the instrument database you must:

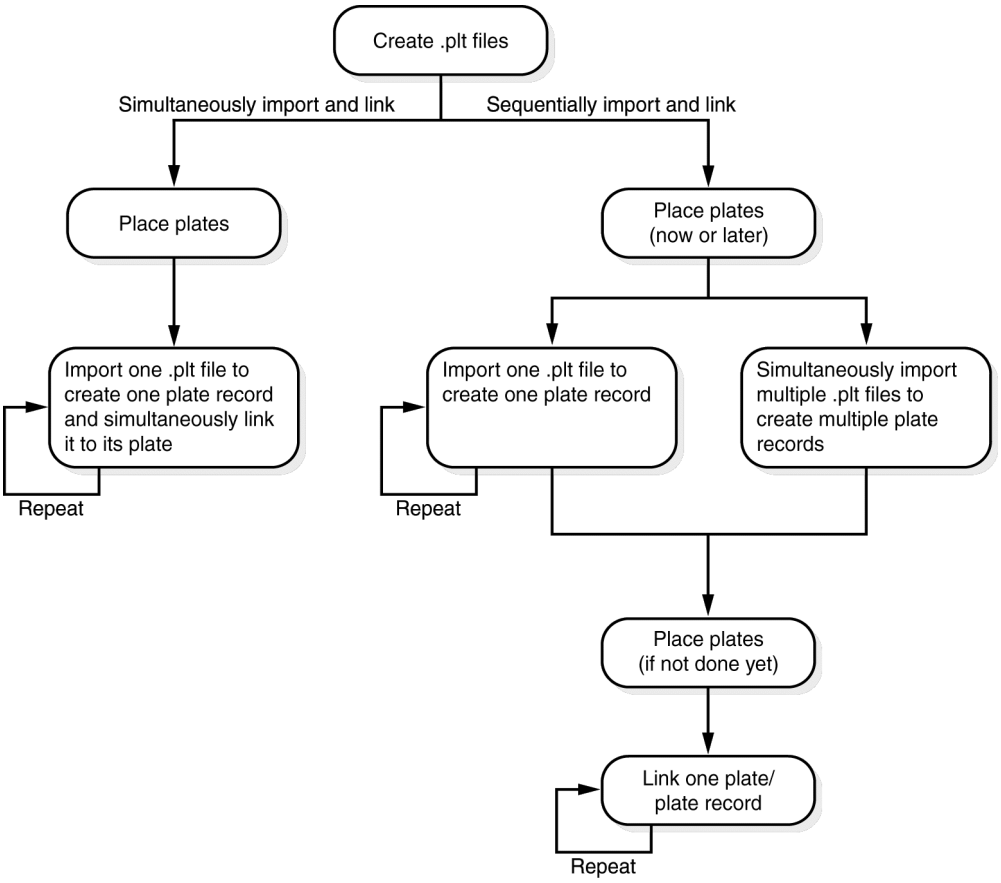
- Import the data
- Place the plates
- Link the plates

Options

In general, the options for importing, placing, and linking are as follows:

- Simultaneously import the file and link the plate record to its plate. This is the faster option and is highly recommended for importing data for 384-well plates, but you can only import and link one plate record at a time. Directions for this procedure start on page 6-33.
- Sequentially import one or more files, and then link the plate record(s) to their plates. Directions for these two procedures start on page 6-35.

The options are summarized in the diagram below:



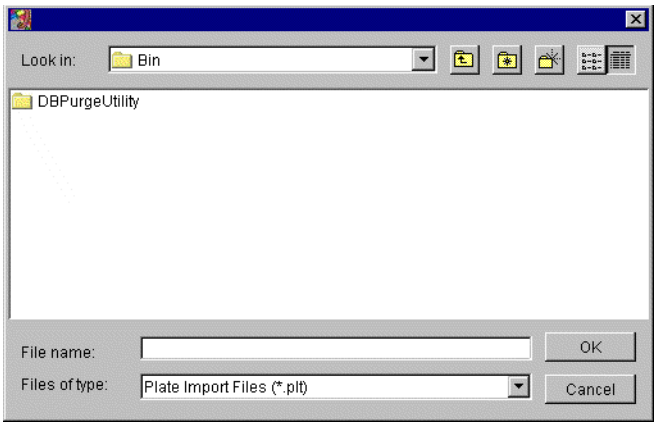
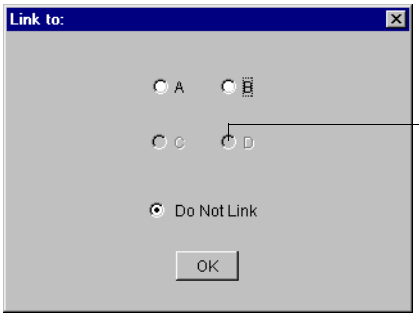
Simultaneously Importing and Linking a Plate Record

Introduction

You can speed up the process of importing a tab-delimited text file (.plt file) to create a plate record and link the plate record to its plate by performing the two procedures together. This decreases the number of read-write operations performed by the instrument database so it only takes about 2 min to import and link a 384-well plate. The disadvantage of using this procedure is that you can import and link only one plate record at a time.

Simultaneously Importing and Linking a Plate Record

To import a plate record and link it to its plate in a single procedure:

Step	Action
1	Place the plates that you want to link onto the plate deck.
2	In the **Plate Setup** page of the 3100 Data Collection software, click **Import**. This opens an unnamed browser dialog box. 
3	Navigate to the directory location of the plate file (.plt file) that you want to import and link, and then select the .plt file.
4	Click **OK**. This opens the **Link to** dialog box.  Disabled because no plate is currently in this position **Note** The **Do Not Link** button is currently enabled.
5	In the **Link to** dialog box, select the letter that corresponds to the plate position containing the plate that you want to link to. The **Do Not Link** option is cleared.

To import a plate record and link it to its plate in a single procedure: *(continued)*

Step	Action
6	Click **OK**. The .plt file is imported, and the plate record is created. **Note** If there is missing information in the file, you may be warned by an information box. This may happen, for example, if you make a typing error or list a module that no longer exists. Depending on the problem, the warning may accompany rejection of the entire plate record. However, in some circumstances, the data will be imported despite a warning. When this happens, the purpose of the warning is to prompt you to examine and correct the data in the plate editor.
7	Review the plate record in the plate editor.

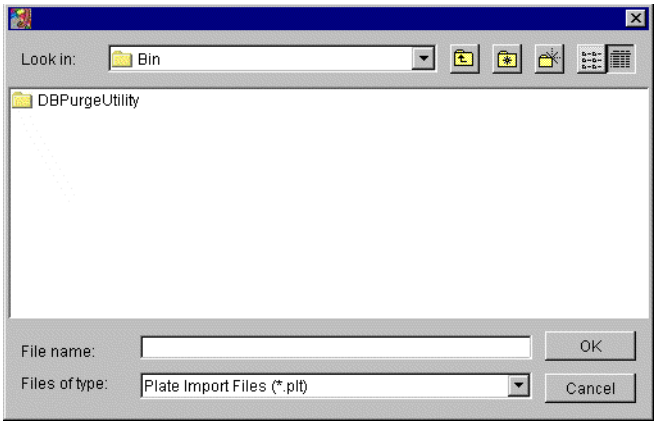
Sequentially Importing and Linking a Plate Record

Introduction

You can sequentially import a tab-delimited text file to create a plate record and then link it to its plate. It takes longer to perform these steps separately for a single plate record; however, but you can import many tab-delimited text files at once. This may make it faster than the procedure on page 6-33 if you have a lot of files to import.

Sequentially Importing and Linking a Plate Record

To import one or more tab-delimited text files to create plate records:

Step	Action
1	In the **Plate View** page of the 3100 Data Collection software, click **Import**. This opens an untitled browser dialog box. 
2	Navigate to the directory location of the plate file(s) (.plt) that you want to import and link. **If you want to create...** / **Then...** a single plate record: a. Select the .plt file. b. Click **OK**. This opens the **Link to** dialog box. c. Select the letter that corresponds to the plate position. more than one plate record: a. Click the **Up One Level** button.  b. Select a folder of .plt files. All .plt files are imported and appear in the pending plate records table ready to be linked.
3	Click **OK**. This imports the .plt file(s) and creates one or more plate records. **Note** If there is missing information in the file, you may be warned by an information box. This may happen, for example, if you make a typing error or list a module that no longer exists. Depending on the problem, the warning may accompany rejection of the entire plate record. However, in some circumstances, the data will be imported despite a warning. When this happens, the purpose of the warning is to prompt you to examine and correct the data in the plate editor.

To import one or more tab-delimited text files to create plate records: *(continued)*

Step	Action
4	Review the plate records in the plate editor.
5	Link the plate record to the plate.

Section: Deleting Plate Records and Run Data

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

Introduction

When to Delete Plate Records and Run Data

Delete the plate records and run data when the used space on drive E is more than 8 GB. See “Checking Database Space: The Diskspace Utility” on page 7-5.

Two Procedures

There are two ways to delete the processed frame data that is associated with plate records. You can:

- Use the Cleanup Database utility (CleanUpDB.bat)
- Delete individual plate records

Recommended Procedure

The Cleanup Database utility is the recommended way to delete plate records because:

- It is much faster to delete the processed frame data than to delete individual plate records.
- It prevents problems that result from incomplete deletion of data.

Cleanup Database Utility

Reference to the Cleanup Database Utility

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

To delete plate records and run data from the instrument database using the Cleanup Database utility, see “Deleting Processed Frame Data: The Cleanup Database Utility” on page 7-8.

Deleting Individual Plate Records

Introduction

When a plate record is deleted, the run data associated with samples in the plate is also deleted from the instrument database.

Note A new run cannot be started while a plate record is being deleted.

IMPORTANT You cannot delete a linked plate record, but plate records for unlinked, partially processed plates can be deleted. If the processed runs from unlinked partially processed plates have not yet been extracted, the run information will be deleted from the database. The pending plate record table is where unlinked partially processed plates are listed. Make sure that processed runs have been extracted by looking in the ExtractorEventLog.txt file.

When to Delete Individual Plate Records

Use this method if you want to delete only:

- Plate records that have no associated run data
- Certain plate records

Deleting Individual Plate Records

To delete individual plate records:

Step	Action
1	Click the **Plate View** tab in the 3100 Data Collection software. This opens the **Plate Setup** page.
2	In either the **Pending** or **Processed Plate Record** table, select the row that names the plate record you want to delete. **Note** You can select more than one row at a time by pressing CTRL while selecting additional rows.
3	Click **Delete**.

System Management and Networking 7

Overview

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

Section: Managing Hard Drive and Instrument Database Space

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

How Run Data Is Stored

Types of Run Data Storage

Run data is stored in different forms, depending on the configurations selected in the Preferences and Auto Extractor dialog boxes:

Data Storage Type	Where Stored	Approx. Data Storage Space
Processed frame data	In the instrument database of the local computer workstation	100 MB for a 2.5-hr run
ABIF sample file	On the local or networked hard drive at a directory location specified in the **Extraction Directory** dialog box of Auto Extractor The default setting is to store ABIF sample files in the following directory: D:\AppliedBio\abi\3100\Data Extractor\ExtractedRuns	♦ 250 KB per sample file for a ABI PRISM® 3100 POP-4™ sequencing analysis run ♦ 210 KB per sample file for a ABI PRISM® 3100 POP-6™ fragment analysis run ♦ 300 KB per sample file for a ABI PRISM® 3100 POP-4™ fragment analysis run
BioLIMS™ data	BioLIMS database on another networked computer	

Checking Database Space: The Diskspace Utility

Function

The Diskspace utility lists the amount of space that the database uses, the amount that is free for use, and the percent filled.

File Name and Directory

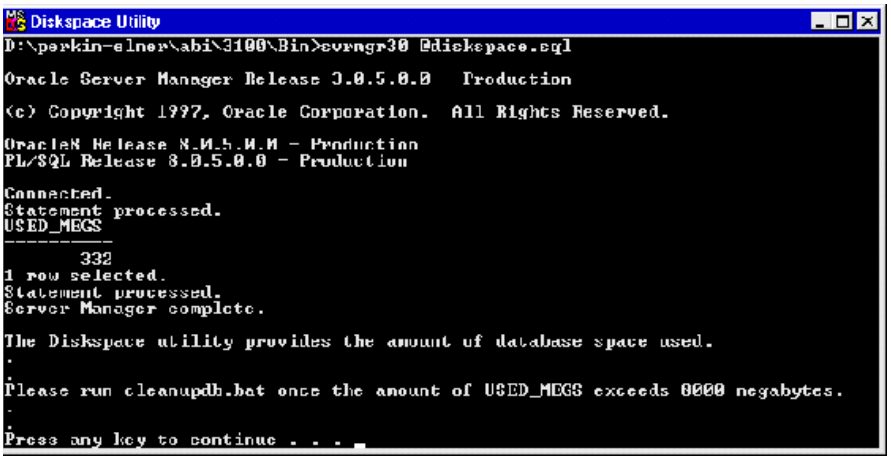
The Diskspace utility is named diskspace.bat and is located in the following directory: D:\AppliedBio\abi\3100\Bin

When to Perform

Run the Diskspace utility at least once per week to ensure you have adequate space for your sample files.

Checking the Space

To check the space using the utility:

Step	Action
1	Ensure OrbixWeb™ Daemon is running.
2	Quit the ABI PRISM® 3100 Data Collection software.
3	Using the Microsoft® Windows® NT Explorer, navigate to the following directory: D:\AppliedBio\abi\3100\Bin
4	Locate and double-click **diskspace.cmd**. 
5	If USED_MEGS is greater than 8000 MB, run the Cleanup Database utility. See page 7-8. **IMPORTANT** Never allow more than about 400 or 500 runs to reside in the database. This could lead to indiscriminate loss of data.

Re-Extracting Processed Frame Data: The Re-Extraction Utility

Function

The Re-extraction utility makes new ABIF sample files from run data stored in the database.

Note This utility cannot be used to replace a sample file once the run data in the instrument database has been deleted.

File Name and Directory

The Re-extraction utility file is named Reextractor.exe and is located in the following directory:

D:\AppliedBio\abi\3100\Bin

Used Only for Extracted Runs

The Re-extraction utility works only for runs that have the status Extracted.

You will not be able to re-extract failed runs, acquired runs, or runs that failed to be extracted.

Re-Extraction Into a BioLIMS Database

The Re-extraction utility cannot re-extract directly to a BioLIMS database. To re-extract into BioLIMS, use Reextractor.exe to extract from the instrument database to ABIF sample files. Then use the S2DB utility to upload the sample files into the BioLIMS database.

Re-Extracting On a Networked Computer

If you are re-extracting data on a networked computer, the computer must meet specific requirements and have certain software loaded.

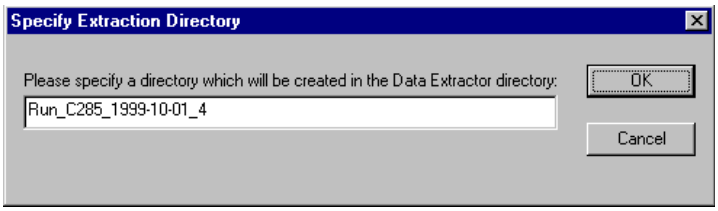
See “Requirements for a Networked Computer” on page 7-18.

Re-Extracting Processed Frame Data

To re-extract processed frame data:

Step	Action
1	Quit the 3100 Data Collection software.
2	Ensure OrbixWeb Daemon is running.
3	Right-click the **Start** button and select **Explore**. This opens Windows NT Explorer.
4	Navigate to the Bin folder in the following directory: D:\AppliedBio\abi\3100\Bin
5	Double-click the **Reextractor.exe** icon. This opens the **3100 Reextractor** box.
6	If the **3100 Reextractor** box was opened before one or more runs were completed, click **Refresh** to update the displayed runs.
7	In the **3100 Reextractor** box, select the run that you want to re-extract. The **Extract** button is now enabled. 

To re-extract processed frame data: *(continued)*

Step	Action
8	Click **Extract**. This opens the **Specify Extraction Directory** dialog box.  Type the name that you want to give the folder in which the re-extracted sample files will be saved. **Note** You cannot specify the location of this folder. It will be created in the existing Data Extractor folder in the following directory: D:\AppliedBio\abi\3100\Data Extractor
9	To analyze these sample files, open them in either ABI PRISM® DNA Sequencing Analysis software or ABI PRISM® GeneScan® Analysis software.

Deleting Processed Frame Data: The Cleanup Database Utility

Function

The Cleanup Database utility deletes the processed frame data and all associated run information stored in the 3100 Data Collection software database. This utility is used to make room for new run data.

The Cleanup Database utility deletes all of the:

- Processed frame data
- Plate records and run data

This utility does *not* delete the:

- Electrophoresis modules automatically imported from the supplied method files
- Run modules that you have created
- Spatial and spectral calibration data obtained from the last calibration runs performed
- Instrument-specific information such as the instrument name, serial number, user names, dye set information, etc.

File Name and Directory

The Cleanup Database utility is named CleanUpDB.bat and is located in the following directory:
D:\AppliedBio\abi\3100\Bin

When to Perform

Run the Cleanup Database utility at least once a week, and when the diskspace utility shows that the space used in association with diskspace.cmd exceeds 8000 MB.

IMPORTANT Never run the Cleanup Database utility more than once a day because previously extracted sample files may be overwritten. This can happen due to the format used for a run name.

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 Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\abi\3100\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 re-import the spatial, spectral, and run calibration methods or the calibration data obtained from the last calibration runs.

Another Method to Delete Processed Frame Data

Deleting the plate record for a plate of samples is another way to delete processed frame data stored in the instrument database.

Directions for deleting individual plate records start on page 6-39.

Importing a New Spatial or Spectral Calibration Method: The New Method Import Utility

About Method Files

Spatial and spectral method files contain the parameters that define the calibration run conditions (along with the SCPI commands that direct the operation of the instrument).

Note Spatial and spectral calibration method files are not the same as the spatial and spectral calibration files, which contain results of calibration runs.

Function

New methods provided by Applied Biosystems must be imported into the instrument database before they can be used. The New Method Import utility imports these methods.

File Name and Directory

The New Method Import utility is named NewMethodImportUtility.bat and is located in the following directory:
D:\AppliedBio\abi\3100\Bin

Importing a New Method

To import a method into the instrument database:

Step	Action
1	Ensure OrbixWeb is running.
2	Quit the 3100 Data Collection software.
3	Copy the new method file into the Method Files folder in the following directory: D:\AppliedBio\abi\Support Files\Data Collection Support Files\Method Files
4	Using Windows NT Explorer, navigate to the Bin folder in the following directory: D:\AppliedBio\abi\3100\Bin
5	Right-click **NewMethodImportUtility.bat**.
6	From the pop-up list, select **Edit**. This opens the utility's batch file in Notepad.
7	Look on the third line of the text for a string representing a file path to the method file. The end of that string states: **INSERTNAME.mtd**
8	Replace the INSERTNAME text with the actual name of the new method file, *e.g.*, **SeqXR101**. **Note** Do not replace or delete the .mtd file extension.
9	From the **File** menu, select **Save**. This opens the **Save As** dialog box.
10	In the **Save As** dialog box, type a name for the method and click **Save**.
11	From the **File** menu, select **Exit**. This closes Notepad.
12	Double-click the file that you just created to run the utility and import the new method.

Removing Run Modules from the Instrument Database: The Remove Run Modules Utility

Function

The Remove Run Modules utility removes all modules and associated information from the instrument database. This utility is used to quickly delete all old modules before you import new ones.

File Name and Directory

The Remove Run Modules utility is named RemoveRunModules.bat and is located in the following directory:
D:\AppliedBio\abi\3100\Bin

Removing Run Modules

To remove run modules using the utility:

Step	Action
1	Ensure OrbixWeb Daemon is running.
2	Quit the 3100 Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\abi\3100\Bin
4	Locate and double-click **RemoveRunModules.bat.**

Reinitializing the Instrument Database: The Initialize Database Utility

Function

The Initialize Database utility completely erases and reinitializes the instrument database.

File Name and Directory

The Initialize Database utility is named InitDB.bat and is located in the following directory:
D:\AppliedBio\abi\3100\Bin

Erasing and Reinitializing the Instrument Database

IMPORTANT Do not run this utility unless instructed to do so by a Applied Biosystems representative.

⚠ CAUTION The Initialize Database utility completely erases the instrument database. All raw data, plate records, customized run modules, spatial and spectral calibrations, and instrument-specific information such as polymer and capillary array information will be deleted.

To remove, erase, and reinitialize the instrument database using the utility:

Step	Action
1	Ensure OrbixWeb Daemon is running.
2	Quit the 3100 Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\abi\3100\Bin
4	Locate and double-click **InitDB.bat.**
5	Locate and double-click **CreateIndex.bat.**

Section: Networking

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

Networking Options

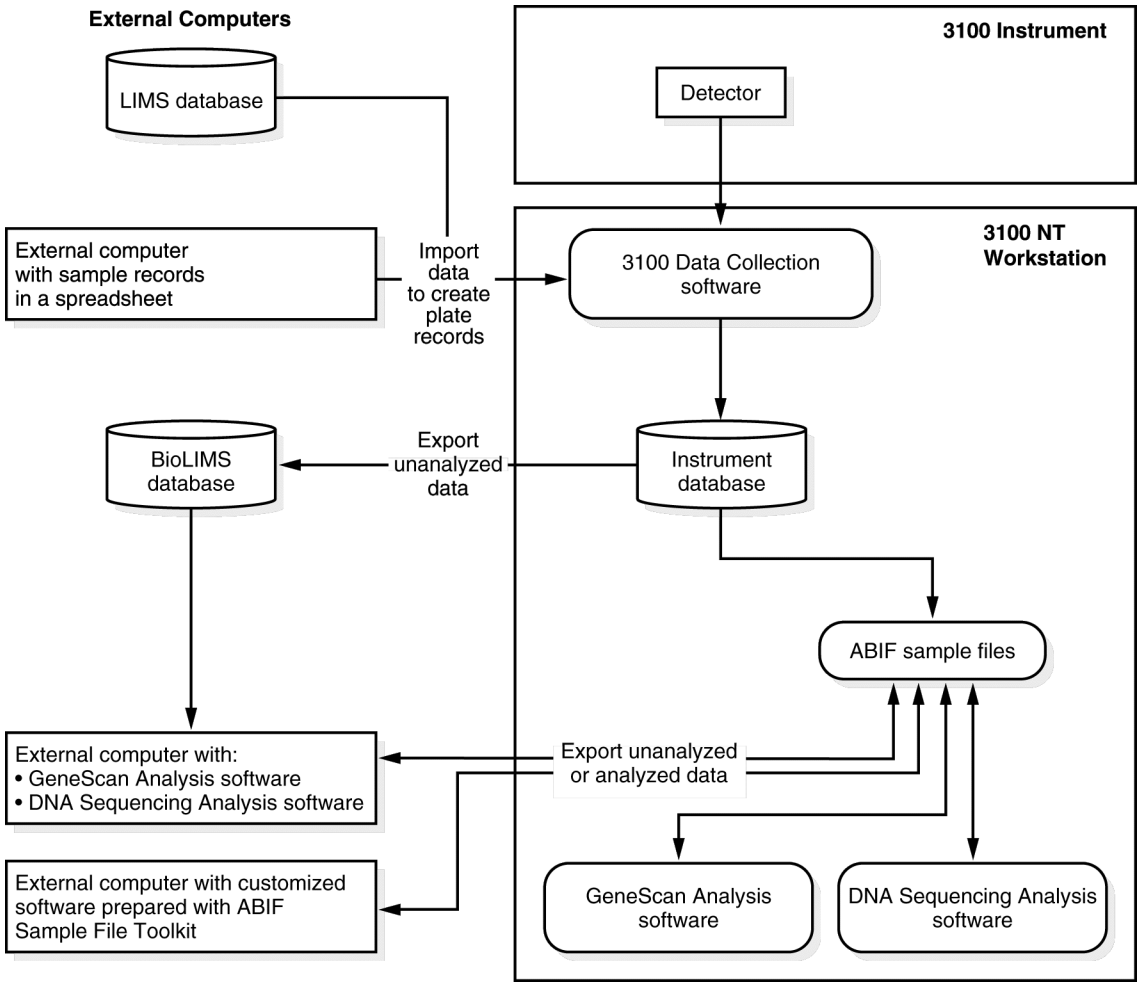
Introduction

You have the option of using the ABI PRISM® 3100 Genetic Analyzer as a stand-alone system. However, you will achieve optimal performance by integrating the 3100 Genetic Analyzer into your existing laboratory data flow system. The 3100 Genetic Analyzer has flexible import and export capabilities that can be tailored to meet your needs. Other computers can, for example, be used for preparing plate records, providing more comprehensive analysis, and storing data.

The networking options are configured in the 3100 Data Collection software.

Overview Diagram

The following diagram summarizes the relationships among the different elements of the software and the options for networking with external computers:



Using an Additional Networked Computer

Using an additional networked computer makes more efficient use of the 3100 Genetic Analyzer. While the instrument is performing a run, you cannot create plate records, review data from past runs, or reanalyze data. By using another computer to perform these functions, you can perform more runs in a day.

The networked computer can run with a Microsoft Windows NT or Macintosh® operating system; however, if Macintosh versions of analysis applications are used, you can only view and edit the data. To reanalyze the data, you must use the Windows NT versions of analysis applications.

LIMS Database Option

An external LIMS database can be used to assemble all of the data needed to create plate records. Once a LIMS database has been set up correctly and the data has been entered into the LIMS database, the creation of plate records in the database becomes completely automatic.

BioLIMS Option

With the BioLIMS database system, data is collected on the computer workstation and written to a BioLIMS database on a networked server. The data can later be analyzed using Auto Extractor, and viewed and reanalyzed using DNA Sequencing Analysis software or GeneScan Analysis software. These programs can either be on the computer workstation, which is used to collect the data, or on a different computer that has access to the BioLIMS database. The data can also be viewed and edited (but not analyzed) using DNA Sequencing Analysis software or GeneScan Analysis software on a Macintosh computer with access to the BioLIMS database.

Stand-Alone Option

With the stand-alone option, all operations, including the creation of plate records, collection of data, and review of data with GeneScan Analysis software or DNA Sequencing Analysis software, are carried out on the computer workstation.

Networking the Computer Workstation

Introduction

The 3100 Genetic Analyzer fully supports connections to local area networks (LANs). Your network system must be planned and set up by a systems administrator who is familiar with the Windows NT operating system.

If you plan to add the computer workstation to a LAN, you should be aware of the following:

- The person logged in as 3100User must have system administration rights on the computer workstation.
- The computer workstation has two network interface cards.

Administrator Privileges

For installation and upgrades to the 3100 software, the person logged in as 3100User must be a member of the Administrators group.

Network Interface Cards

The computer workstation has two network interface cards. These are:

- The card on the motherboard, which is connected to the instrument
- The card installed in an expansion slot in the system unit, which can be used to connect to the network. (This card requires that drivers be installed.)

IMPORTANT Use only the network interface card in the expansion slot to connect to the LAN. The network interface card on the motherboard is reserved for the Ethernet connection to the instrument.

IP Address

Your network's system administrator must provide you with an IP address for networking to the LAN. This is not the same as the Internet Protocol (IP) address already being used to connect the computer workstation to the instrument.

IMPORTANT Do not modify the given IP address.

Windows NT User Name

IMPORTANT Do not change the default Windows NT logon user name from "3100User." This will break the connection with the 3100 Data Collection software and make the software inoperable.

To see the Windows NT logon user name:

Step	Action
1	Press CTRL+ALT+DELETE. This opens the **Windows NT Security** dialog box. The user name is displayed in the **Logon Information** group box in the following message: *Name* is logged on as *name-instrument serial number*

Viewing the Computer Name

The computer name is set during installation using the following format:

3100-*instrument serial number*

To see the computer name and network domain:

Step	Action
1	From the **Start** menu, point to **Settings** and select **Control Panel**. This opens the **Control Panel** window.
2	In the **Control Panel** window, double-click **Network**. This opens the **Network** property sheet. The **Identification** tabbed page displays the computer name and domain.

Requirements for a Networked Computer

Introduction

This section describes:

- Hardware requirements for a networked computer on which you intend to perform:
  - Remote data extraction and analysis
  - Additional data analysis using DNA Sequencing or GeneScan Analysis software
- Components of the 3100 software that must be installed on the computer

Minimum Requirements

The minimum requirements for running Auto Extractor and either DNA Sequencing Analysis or GeneScan Analysis software are:

- Intel Pentium processor, 400 MHz or faster
- Microsoft® Windows® NT 4.0 operating system with Service Pack 5
- 256-color display adapter card
- CD-ROM drive

For...	RAM (MB)	Hard Disk Space (MB)
Extraction only	64	80
Extraction and analysis	256	120

Hard Disk Space

Ensure that the networked computer has sufficient hard disk space to hold as many sample files as desired. One analyzed sample file is about 250 KB.

Software Required for Remote Extraction

The following table lists the software that must be installed on the networked computer for remote extraction to work:

If...	Install...
data extraction is to be performed on this computer	Auto Extractor
re-extraction is to be performed on this computer	Reextractor.exe
Auto Extractor or 3100DBUtils.exe are used	♦ All supporting shared files ♦ OrbixWeb™ Professional Edition ♦ Orbix Desktop® 2.3 software ♦ Persistence Powertier® 4.321 ♦ Oracle® Client database (not distributed with the 3100 software)

Note For directions on performing remote extraction, see page 7-6.

8 Maintenance

Overview

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

Section: Instrument Maintenance

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

Maintenance Task Lists

Overview

This section lists common tasks required to maintain your 3100 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 the reservoir septa are firmly seated and flat.	Before each run	—
Ensure the plate assemblies were put together properly. **IMPORTANT** The holes in the plate retainer must align with the holes in the septa or the capillary tips will be damaged.	Before each run	3-9
Ensure the plate assemblies are positioned on the plate deck properly. Plates should sit snugly on the deck. **IMPORTANT** Never use warped plates.	Before each run	—
Replenish the water and 1X running buffer reservoirs on the instrument.	Daily or before each run	3-22
Check for bubbles in the polymer block and polymer block channels and remove.	Daily or before each run	8-28
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	—
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	7-5

Weekly Tasks

Perform these tasks at least once per week.

Maintenance Task	Frequency	See Page...
Clean the syringes.	Weekly or as needed	8-21
Clean the water and buffer reservoirs with warm water.	Weekly	—
Clean the upper and lower polymer blocks.	Weekly	8-27
Replace the polymer in the syringes, upper polymer block, and capillary array.	Weekly or as needed	8-10
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	8-15
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed	—
Calibrate the autosampler.	Very rarely	8-29

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 8-15. ♦ Syringes from the upper polymer block. For instruction see page 8-23. ♦ Upper polymer block. For instruction see page 8-26. ♦ Anode buffer reservoir. ♦ Lower polymer block. For instruction see page 8-26.
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

Introduction

Reset the instrument when:

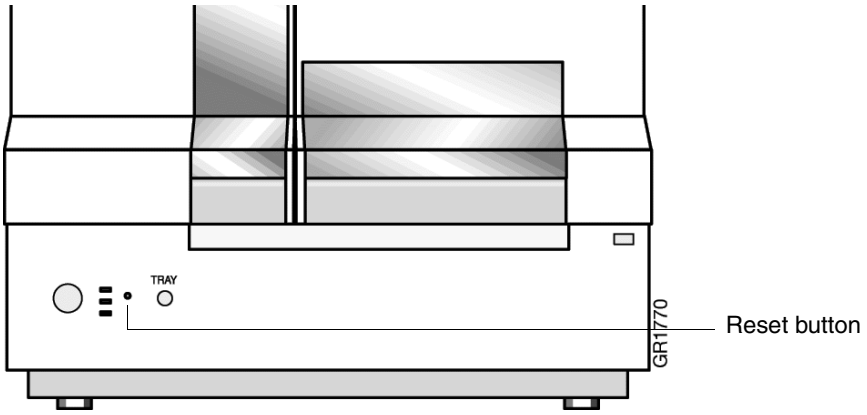
- There is a fatal error as indicated by the red status light
- The instrument does not respond to the ABI PRISM® Data Collection software

There are two ways to reset the ABI PRISM® 3100 Genetic Analyzer:

- 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 Using 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. 

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. From the **Start** menu, select **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. **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

When to Perform Each Shutdown Procedure

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 8-16.
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 8-17 to remove and store the capillary array off of the instrument.
2	Remove from the instrument: ♦ Syringes from the upper polymer block. For instructions see page 8-23. ♦ Upper polymer block. For instructions see page 8-26. ♦ Lower polymer block. For instructions see page 8-26.
3	Remove from the autosampler: ♦ Plate assemblies ♦ Reservoirs
4	Wipe the autosampler and drip trays with lint-free tissue dampened with water.
5	Close the instrument doors.
6	Shut down the computer and turn off the instrument.
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:

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

To prepare 50 mL of 1X 3100 running buffer:

Step	Action
1	Add 5.0 mL of 10X 3100 running 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 3100 running buffer can be stored at 2–8 °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.

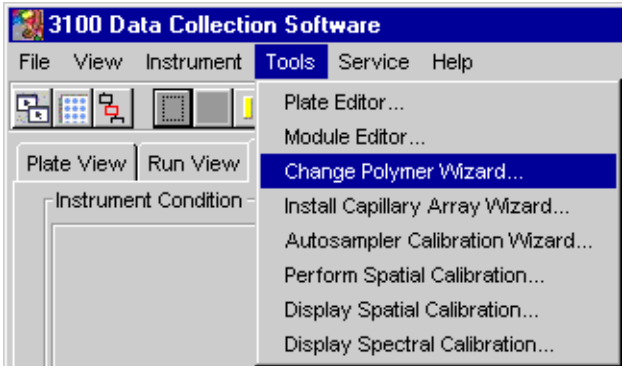
When to Change the Polymer

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** 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 put fresh polymer on the instrument:

Step	Action
1	From the **Tools** menu, select **Change Polymer Wizard**. 

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

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

Handling Instrument Waste

3100 Genetic Analyzer Waste

The waste produced by the ABI PRISM® 3100 Genetic Analyzer is not classified as hazardous; however, local regulatory personnel should be contacted before dispensing the waste into a sanitary sewer system.

Waste should be disposed of in accordance with all local, state, and federal regulations.

See also the *ABI PRISM 3100 Genetic Analyzer Site Preparation and Safety Guide*.

Composition

The 3100 Genetic Analyzer generates nonhazardous waste composed of polymer, buffer, and water.

Pure, Formulated 3100 POP Waste

Pure, formulated ABI PRISM 3100 POP polymer should not be dispensed into the sewer system because the concentration of its component reagents means that it is classified as hazardous waste. If you have old polymer that you want to dispose of, arrange to have it removed to an appropriate waste treatment facility by a local waste hauler. Tests conducted in accordance with the guidelines of the Environmental Protection Agency show that 3100 POP polymer is not acutely toxic. For more information, consult the MSDS for POP polymer.

Section: Capillary Array

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

Before Installing a Previously Used Capillary Array

Introduction

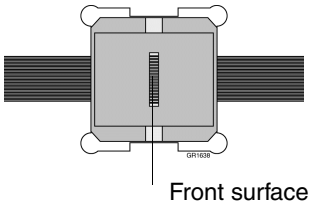
Before you reinstall a capillary array, it is recommended that you:

- 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.

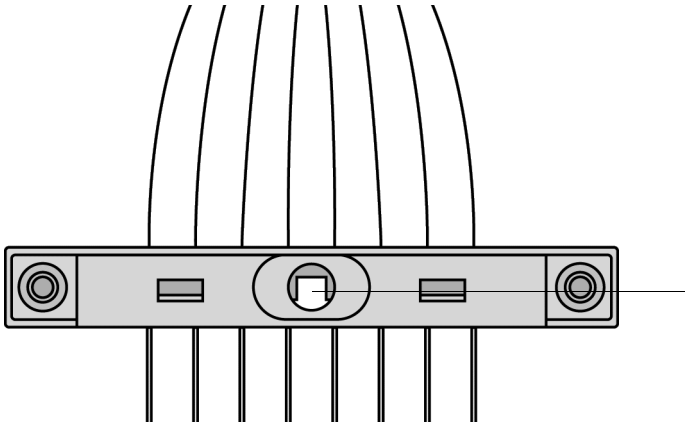
To clean the detection cell:

Step	Action
1	Put one drop of methanol on the front surface of the detection cell.  Front surface of 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	Blow dry the cell using clean pressurized air.

Checking the Cathode Bar

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

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



Installing and Removing the Capillary Array

When to Change a Capillary Array

A capillary array should last approximately 100 runs.

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

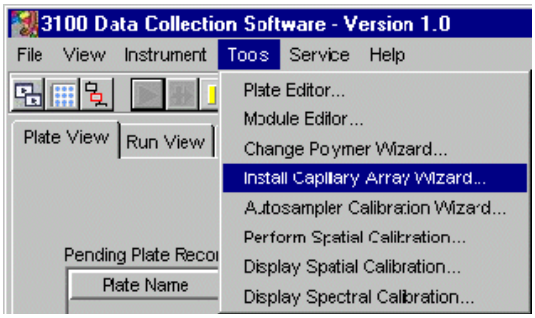
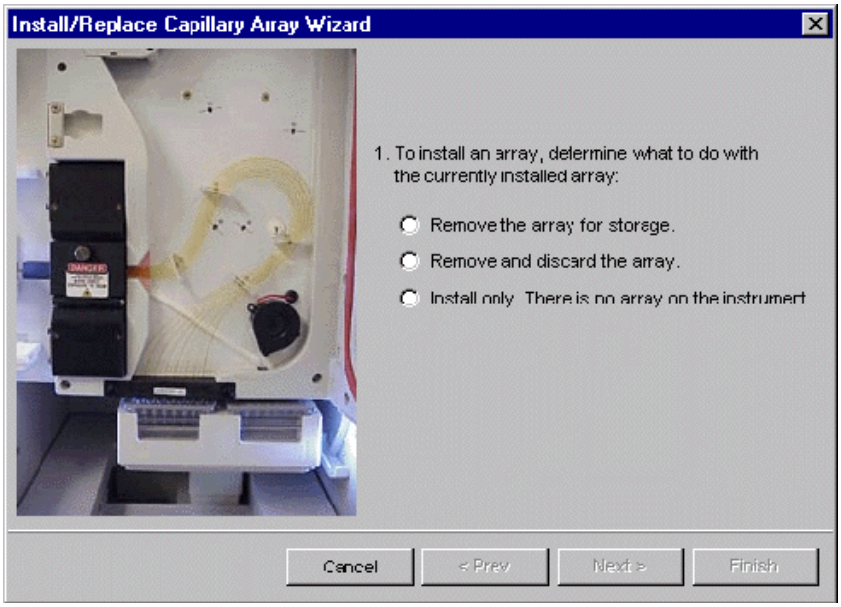
- Poor sizing precision or allele calling
- Poor resolution and/or decreased signal intensity

Installing or Removing the Capillary Array Using the Wizard

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

⚠ CAUTION CHEMICAL HAZARD. POP 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	From the **Tools** menu, select **Install Capillary Array Wizard**.  This opens the wizard. 
3	Follow the directions given in the wizard to replace or install an array.

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 8-14.
- 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

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 8-15.
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 “Checking Capillary Alignment Using the Capillary Ruler” on page 8-16. **IMPORTANT** Never use organic solvents to clean the instrument.
3	Clean the detection cell as instructed on page 8-14.

Filling the Capillary Array with Polymer Using Manual Control

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

Step	Action
1	From the **Instrument** menu, select **Manual Control**.
2	From the **Command Category** drop-down list, select **Capillary**.
3	From the **Command Name** drop-down list, select **Fill**.
4	From the **Value** drop-down list, select the appropriate array length and polymer.
5	Click **Send Command**.

Checking 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	Check the capillaries to 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	Check 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

When to Store on the Instrument

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 8-8.

Storing a Capillary Array off the Instrument

When to Store off the Instrument

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

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

Storing the Capillary Array off the Instrument

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

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

To store the capillary array off 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 8-15.
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 a 1.5-mL conical tube with deionized water and insert the detection end of the capillary array.
9	Wrap the tube with laboratory film (such as Parafilm) to prevent evaporation.
10	Store the capillary array upright.
11	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:

Syringe Maintenance

Syringe Types

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

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

Caring for the 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.

When to Replace the Syringes

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

Cleaning and Inspecting Syringes

When to Clean Syringes

Thoroughly clean the syringes:

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

Cleaning Syringes

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

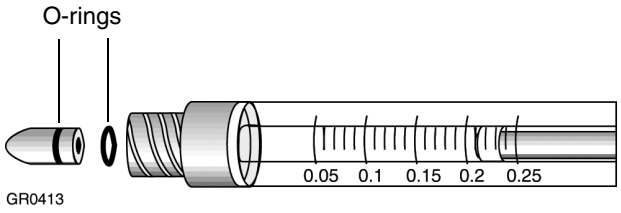
To clean a syringe:

Step	Action
1	Remove the syringe guard.
2	Remove the syringes as described on page 8-23.
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

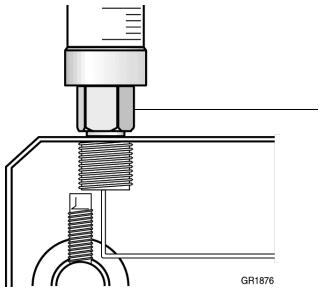
Installing Syringes

To install the syringes:

Step	Action
1	Follow the procedures to remove, clean, and dry the upper polymer block starting on page 8-26.
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.  **IMPORTANT** Be careful not to remove the fitting. There are several rings and check valves that could come out if this fitting is removed.
3	Grasp the array-fill syringe and rotate the syringe counterclockwise.
4	Properly dispose of any remaining polymer.
5	Proceed to “Cleaning and Inspecting Syringes” on page 8-21.

Section: Polymer Blocks

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

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 8-21.
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 8-17.
4	Disconnect the lower polymer block by unscrewing the polymer block tube fitting on the upper polymer block's under right side.
5	Grasp the upper polymer block with two hands and pull it straight out.
6	The upper polymer block rides on two steel shafts and slides out easily after a spring moves past a check point.

Removing the Lower Polymer Block

To remove the lower polymer block:

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

Cleaning the Polymer Blocks

When to Clean the Polymer Blocks

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 Polymer Blocks

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

To wash the upper and lower polymer blocks:

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

Cleaning the Anode Reservoir and Polymer Tubing

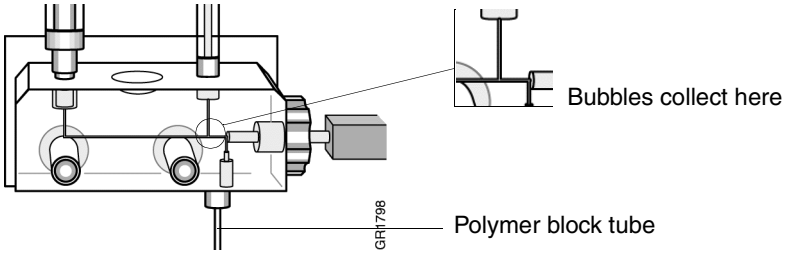
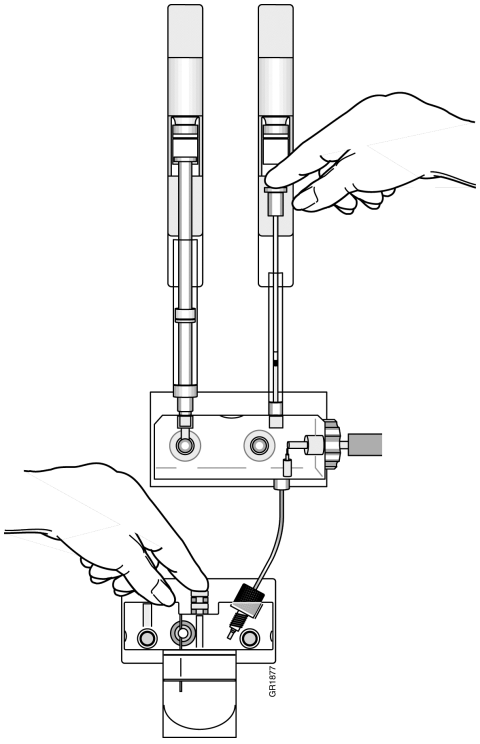
To wash the anode reservoir and polymer tubing:

Step	Action
1	Use a squirt bottle to flush deionized water into the polymer block tubing.
2	Wash the anode reservoir with warm water, and then rinse it with deionized water.
3	Dry both components with compressed air.

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** Make sure all air bubbles are pushed out of the tubing assembly into the lower buffer reservoir before proceeding. There should be no bubbles in the tubing or channel of the lower polymer block.

Section: Autosampler Calibration

When to Calibrate the Autosampler

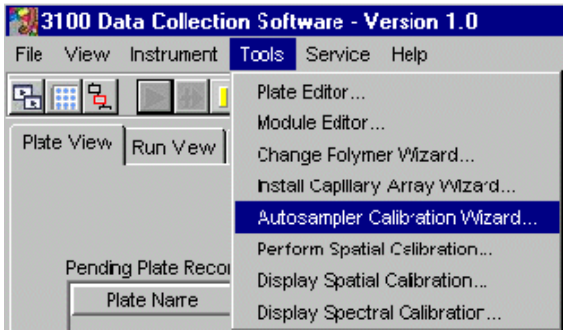
Calibrate the autosampler only as needed.

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	From the **Tools** menu, select **Calibrate Autosampler**. 
2	Follow the directions given in the wizard to calibrate the autosampler.

9 Troubleshooting

Overview

In This Chapter

The following troubleshooting topics are covered in this chapter:

Instrument Startup

Observation	Possible Cause	Recommended Action
No communication between the instrument and the computer. The event viewer is blank.	Incorrect Ethernet configuration.	Check the configuration of the IP address. a. From the **Start** menu, point to **Programs**, and select **Command Prompt**. b. At the **C:** prompt, type **IPconfig/all** c. Press Enter. The command prompt window displays information on the network. d. Ensure the IP address for Ethernet adapter 1 is set for the machine (*i.e.*, the motherboard Ethernet connection). The correct IP address is: **192.168.0.1** **Note** The local IT group should use Adapter 2 for networking.
Red light is blinking.	Incorrect start up procedure.	Start up in the following sequence: a. Log out of the computer. b. Turn off the instrument. c. Boot up the computer. d. After the computer has booted completely, turn the instrument on. Wait for the green status light to come on. e. Launch the Data Collection software.
Data Collection software will not launch.	Did not launch Orbixweb Daemon first.	Relaunch application following Orbixweb Daemon.
Computer screen is frozen.	Communication error. This may be due to leaving the user interface in the Capillary View or Array View window.	There will be no loss of data. However, if the instrument is in the middle of a run, wait for the run to stop. Then, exit the Data Collection software and restart as described above.
Autosampler does not move to the forward position.	Possible communication error.	Restart the system, and then press the Tray button.
	Oven or instrument door is not closed.	a. Close and lock the oven door. b. Close the instrument doors. c. Press the Tray button.

Spatial Calibration

Observation	Possible Cause	Recommended Action
Unusual peaks or a flat line for the spatial calibration.	The instrument may need more time to reach stability. An unstable instrument can cause a flat line with no peaks in the spatial view.	Check or repeat spatial calibration.
	Improper installation of the detection window.	Reinstall the detection window and make sure it fits in the proper position.
	Broken capillary resulting in a bad polymer fill.	Check for a broken capillary, particularly in the detection window area. If necessary, replace the capillary array using the Install Array Wizard.
	Dirty detection window.	Place a drop of methanol onto the detection window, and dry with compressed air. Use only light air force.
Persistently bad spatial calibration results.	Bad capillary array.	Replace the capillary array, and then repeat the calibration. Call Technical Support if the results do not improve.

Spectral Calibration

Observation	Possible Cause	Recommended Action
No signal.	Incorrect preparation of sample.	Replace samples with fresh samples prepared with fresh HiDi Formamide.
	Air bubbles in sample tray.	Centrifuge samples to remove air bubbles.
	Autosampler not correctly aligned. The capillary tips may be hitting the bottom of the wells, or they may not be touching the samples.	Check the autosampler calibration. If necessary, recalibrate the autosampler using the Autosampler Calibration Wizard.
If the spectral calibration fails, or if a message displays “No candidate spectral files found.”	Clogged capillary.	Refill the capillaries using manual control. Look for clogged capillaries during capillary fill on the cathode side.
	Incorrect parameter files and/or run modules selected.	Correct the files and rerun the calibration.
	Insufficient filling of array.	Check for broken capillaries and refill the capillary array.
	Expired matrix standards.	Check the expiration date and storage conditions of the matrix standards. If necessary, replace with a fresh lot.
Spikes in the data.	Expired polymer.	Replace the polymer with a fresh lot using the Change Polymer Wizard.
	Air bubbles, especially in the polymer block tubing assembly.	Refill the capillaries using manual control.
	Possible contaminant or crystal deposits in the polymer.	Properly bring the polymer to room temperature; do not heat to thaw rapidly. Swirl to dissolve any solids. Replace the polymer if it has expired.

Run Performance

Observation	Possible Cause	Recommended Action
No data in all capillaries.	♦ Bubbles in the system. ♦ No sample injection.	Visually inspect the polymer block and the syringes for bubbles. Remove any bubbles using the Change Polymer Wizard. Or, follow the procedure on page 8-28 for manual bubble removal. If bubbles still persist, perform the following: a. Remove the capillary array. b. Clean out the polymer block and syringes. c. Replace polymer with fresh polymer. Make sure to draw the polymer into the syringe very slowly.
No signal.	Autosampler calibration is not optimal.	Check the injection with 20-µL samples. If the injection is OK, recalibrate the autosampler using the Autosampler Calibration Wizard. Pay particular attention to the Z axis. If the injection is not OK, perform the procedures below.
	Dead space at bottom of sample tube.	Centrifuge the sample tubes.
	Bent capillary array.	Replace the capillary array and recalibrate the autosampler using the Calibrate Autosampler Wizard.
	Failed reaction.	Repeat reaction.
	Cracked or broken capillary	Visually inspect the capillary array, including the detector window area for signs of breakage.
Signal too high.	Sample concentration is too high.	Dilute the sample.
		Decrease the injection time.
	Too much DNA added to the reaction, resulting in uneven signal distribution.	Optimize chemistry.
Low signal strength.	Poor quality formamide.	Use a fresh lot of HiDi Formamide.
	Pipetting error; not enough sample.	Increase the amount of DNA added.
		Recalibrate the pipets.
	Sample has high salt concentration.	Dilute in high-quality water.
		Desalt using a column purification method.
	Insufficient mixing.	Vortex the sample thoroughly, and then centrifuge the tube to condense the sample to the bottom of the tube.
	Autosampler out of calibration.	Check the injection with 20-µL samples. If the injection is OK, recalibrate the autosampler using the Autosampler Calibration Wizard. Pay particular attention to the Z axis.
	Weak amplification of DNA.	Re-amplify the DNA.
		Check DNA quality.

Observation	Possible Cause	Recommended Action
Elevated baseline.	Possible contaminant in the polymer path.	Wash the polymer block with hot water. Pay particular attention to the upper polymer block, the ferrule, the ferrule screw, and the peek tubing. Dry the parts with compressed air before replacing them onto the instrument. See “Routine Cleaning” on page 8-5. **IMPORTANT** Do *not* wash syringes in hot water because the Teflon plungers will get damaged.
	Possible contaminant or crystal deposits in the polymer.	Bring the polymer to room temperature, swirl to dissolve any deposits. Replace the polymer if it has expired.
	Poor spectral calibration.	Perform new spectral calibration.
	Detection cell is dirty.	Place a drop of methanol onto the detection window and dry with compressed air. Use only light air force.
Loss of resolution.	Too much sample injected.	Dilute the sample and re-inject.
	Poor quality water.	Use high-quality, ultra-pure water.
	Poor quality or dilute running buffer.	Prepare fresh running buffer from 10X 3100 buffer with EDTA.
	Poor quality or breakdown of polymer.	Use a fresh lot of polymer.
	Capillary array used for more than 100 injections.	Replace with new capillary array.
	Degraded formamide.	Use fresh HiDi and ensure correct storage conditions.
	High salt concentration in samples.	Use a recommended protocol for salt removal. Dilute salts with water.
Poor resolution in some capillaries.	Insufficient filling of array	Refill array and look for cracked or broken capillaries. If problem persists contact Technical Support.
		Re-inject the same samples.
	Poor quality samples.	Check the sample preparation.
No current.	Poor quality water.	Use only high-quality ultra-pure water.
	Water placed in buffer reservoir position 1.	Replace with fresh 3100 1X running buffer.
	Not enough buffer in anode reservoir.	Add buffer up to the fill line.
	Buffer too dilute.	Prepare 3100 1X running buffer. Add 3 mL 3100 10X buffer with EDTA to 27 mL deionized water.
	Bubble(s) present in the polymer block and/or the capillary and/or PEEK tubing.	Pause run and inspect for the instrument for bubbles. They may be hidden in the PEEK tubing. Remove any bubbles according to the remove bubble procedure in the Replace Polymer Wizard.

Observation	Possible Cause	Recommended Action
Elevated current.	Decomposed polymer.	Open fresh lot of polymer and store at 4 °C.
	Incorrect buffer dilution.	Prepare 3100 1X running buffer. Add 3 mL 3100 10X buffer with EDTA to 27 mL deionized water.
	Arcing in the gel block.	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.
Fluctuating current.	Bubble in polymer block.	Pause the run, check the polymer path for bubbles, and remove them if present.
	A slow leak may be present in the system.	Check polymer blocks and syringes for leaks. Tighten all fittings.
	Incorrect buffer concentration.	Prepare 3100 1X running buffer. Add 3 mL 3100 10X buffer with EDTA to 27 mL deionized water.
	Not enough buffer in anode reservoir.	Add buffer up to the fill line.
	Clogged capillary.	Refill capillary array and check for clog.
	Arcing	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.
Poor performance of capillary array used for fewer than 100 runs.	Poor quality samples, possible cleanup problems.	Desalt samples using a recommended purification protocol
	Poor quality formamide.	Prepare fresh HiDi formamide and re prep samples
	Incorrect buffer.	Use 3100 10X running buffer with EDTA to prepare 3100 1X running buffer.
Migration time becomes progressively slower	Leak in system.	Tighten all ferrules, screws, and check valves. Replace any faulty parts.
	Improper filling of polymer block.	Check polymer pump force. If the force needs to be adjusted, call a service representative.
	Expired polymer.	Check expiration of polymer. If necessary, change the lot.
Migration time becomes progressively faster.	Water in syringe resulting in diluted polymer.	Clean the syringe and dry it with compressed air.
Extra peaks in the electropherogram.	Data off scale.	Dilute the sample and re-inject the sample.
	Possible contaminant in sample.	Re-amplify the DNA.
	Sample renaturation.	Heat-denature the sample in good-quality formamide and immediately place on ice.
Peaks exhibit a shoulder effect in GeneScan applications	Sample renaturation.	Heat-denature the sample in good-quality formamide and immediately place on ice.
Purging of polymer from the polymer reserve syringe.	Arcing in the anode gel block.	Replace the lower polymer block.
	Bubbles in syringes.	Remove bubbles.
Leaking polymer at the top of either syringe.	Insufficient seal around the Teflon tip of the plunger.	Make sure to wet the Teflon before filling the syringe with polymer. If the leaking persists, replace the syringe. **Note** Do not mix and match barrels and plungers

Observation	Possible Cause	Recommended Action
Leaking polymer at the bottom of the polymer-reserve syringe.	Improper tightening of the array ferrule knob to the syringe or/and to the polymer block.	Ensure the array ferrule knob is tightened.
Error message, “Leak detected” appears. The run aborts.	Air bubbles in the polymer path.	Check for bubbles and remove if present. Then, look for leaks.
Buffer jar fills very quickly with polymer.	Air bubbles in the polymer path.	Check for bubbles and remove if present. Bubbles can cause polymer to fill the jar.
Detection window pops out while replacing the capillary array. Replacing the window in the correct orientation is difficult.	Tightening of the array ferrule knob at the gel block causes high tension.	Loosen the array ferrule knob to allow the secure placement of the window. Retighten and close the detection door.
Detection window stuck. It is difficult to remove when changing the capillary array.		To loosen the detection window: a. Undo the array ferrule knob and pull the polymer block towards you to first notch. b. Remove the capillary comb from the holder in oven. c. Hold both sides of the capillary array around the detection window area, and apply gentle pressure equally on both sides. d. Release.

Data Flow

A

Overview

In This Appendix

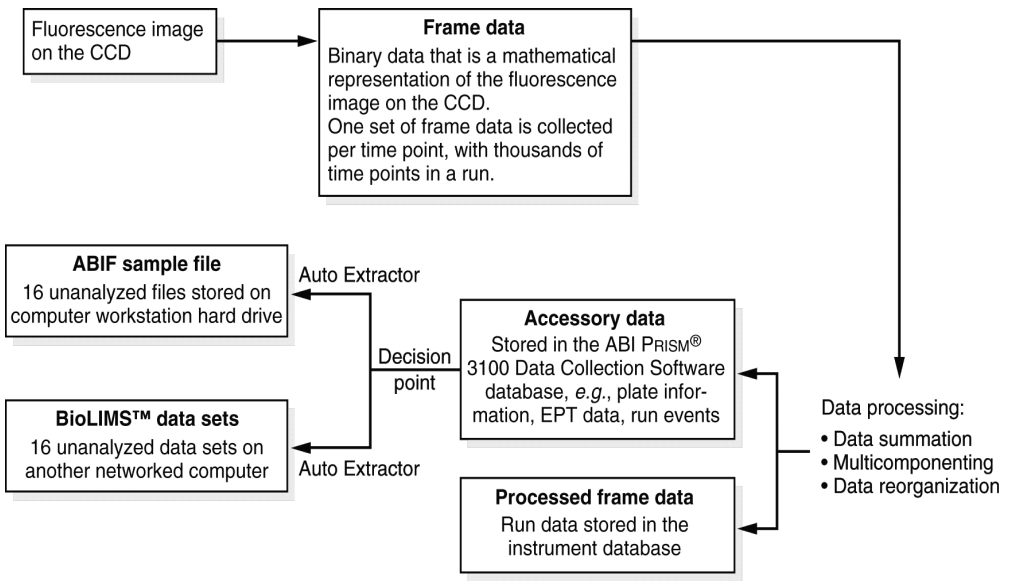
The following topics are covered in this appendix:

About Data Flow

Introduction

To successfully operate and troubleshoot the ABI PRISM® 3100 Genetic Analyzer, it helps to have a basic understanding of how data is collected and processed prior to analysis. A summary of the data flow is shown below.

Data Collection Summary



Organization of the CCD

Pixelated Array

As the dye-labeled DNA fragments pass through the laser beam, the fluorescence emitted is focused onto the CCD by the spectrograph. The CCD is a silicon chip that is divided into a two-dimensional array composed of thousands of electrically insulated pixels. Each pixel stores an amount of electrical charge proportional to the intensity of light striking it.

Spatial and Spectral Dimensions

The spatial and spectral dimensions of the array are as follows:

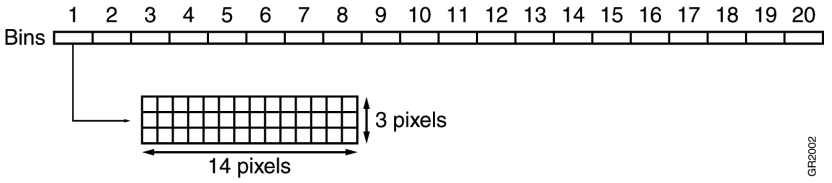
Axis	Dimension
X-axis	Spatial
Y-axis	Spectral

Bins

When the fluorescence data is read from the CCD, the charges from 3 pixels in the spatial dimension and 14 pixels in the spectral dimension are combined to form a bin. This process is called binning. Binning increases the signal without adding noise.

For each capillary, 20 adjoining spectral bins are read creating a full spectrum profile for the dye. The 20-bin data can be viewed in the spectral calibration profiles (see page 4-25) and the spectral calibration matrix files (see page 4-36).

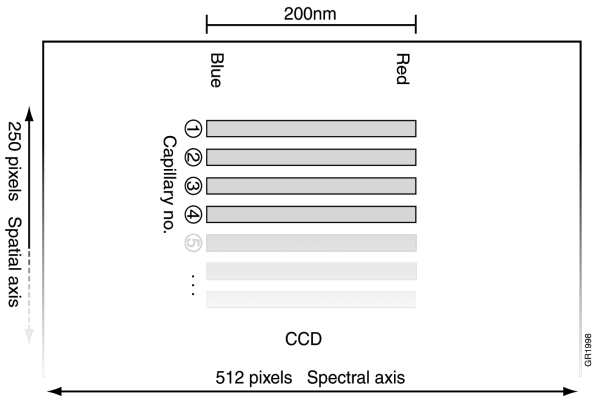
The 20-bin data is converted using the spectral matrix into intensity values for the four or five dyes. This process is called multicomponenting. Using full spectrum data reduces the amount of noise in the multicomponented data.



Incident Fluorescence

Fluorescence Pattern

The fluorescence from the dye-labeled DNA molecules passing through the detection window of all 16 capillaries simultaneously falls onto the CCD in the pattern illustrated below:



The collected emission spectrum ranges from 500 nm (blue) to 700 nm (red).

Note Dyes that emit green fluorescence, which is towards the shorter-wavelength end of this spectrum, are referred to as blue dyes because they emit on the blue side of the red dyes, which emit true red fluorescence.

Capillary Mapping

The position of the fluorescence onto the CCD is not fixed. The fluorescence will fall in a slightly different place in the spectral axis if you move the detection-end header of the capillary array. This means that you must run a new spatial calibration each time you re-install or replace the capillary array.

Note A new spectral calibration is not required when you change the capillary array because the spectral position is a function of the laser position, which does not change when the capillary array is replaced.

For the fluorescence data collected from DNA samples to be meaningful, each bin must be mapped to the fluorescence pattern falling onto the CCD. This mapping is achieved by performing a spatial calibration (see page 4-3) followed by a spectral calibration (see page 4-15). The calibrations generate a spatial map and a spectral matrix, which are used in the processing of sample data.

Frame Data

About Frame Data

Frame data is a two-dimensional (spectral dimension vs. spatial dimension) matrix that represents a single frame of data captured by the CCD camera.

How It Works

When light falls on the CCD camera, pixels that are struck by the light become electrically charged in proportion to the intensity of the light. The information carried by the fluorescence is therefore converted into electronic information.

The 3100 Data Collection software records which charges originated from which bins, allowing the software to reconstruct the charge pattern into a digital format.

Data Summation

At uniform time intervals, the charges on the pixels within each bin are summed. By summing data from bins that are adjacent in the spatial dimension of the CCD camera, the number of data points is reduced. The data matrix for each time point becomes 16 data points in the spatial dimension (three pixels for each capillary) by 20 data points in the spectral dimension (14 pixels per spectral bin). This data matrix is named 16X20 data.

Note For more detailed information on the CCD camera, pixels, bins, and the spatial and spectral dimensions, see “Organization of the CCD” on page A-3.

Raw Frame Data Sets

The 3100 Data Collection software organizes the electronic information into sets of binary frame data. At this point, these are unprocessed, or raw, frame data sets. This is still 16X20 data (*i.e.*, each raw frame data set is 16 capillaries by 20 bins). One raw frame data set is produced for every time point of data collected, which can generate thousands of raw frame data sets during a single run. Due to the large numbers produced, the raw frame data sets are transient and never stored.

Processed Frame Data Sets

The raw frame data sets are processed to remove data from pixels outside the fluorescence area. In the 3100 Data Collection software, “processing” means multicomponenting. This converts the data from 16 capillaries by 20 bins to16 capillaries by 4 dyes (or 5 dyes).

Note For more information on multicomponenting, see “Multicomponenting” on page A-6.

The processed frame data sets are stored in the instrument database.

Re-Extracting Processed Frame Data

If a sample file created from the processed frame data is deleted or damaged, the processed frame data stored in the instrument database can be re-extracted to make a replacement sample file. For more information, see “Re-Extracting Processed Frame Data: The Re-Extraction Utility” on page 7-6.

Deleting Processed Frame Data

For information on deleting processed frame data, see “Deleting Processed Frame Data: The Cleanup Database Utility” on page 7-8.

Multicomponenting

Background

Each dye in a dye set has a unique fluorescence emission, but the emission spectra are sufficiently broad for there to be overlap between them. This spectral overlap is corrected for during the chemometric processing described below.

Definition

Multicomponenting is the process of using a spectral calibration matrix to correct for the overlapping fluorescence emission spectra of the dyes in a dye set. It is carried out by the 3100 Data Collection software.

Function

Multicomponenting reduces the data to four or five data points, one per dye, in the spectral dimension.

During a spectral calibration, all dyes in a dye set are run in each capillary and their fluorescence emission profiles are collected. A mathematical description of the spectral overlap for each capillary is generated and stored as a matrix. This data is used to correct the sample run fluorescence data during the chemometric processing.

Chemometric Method

The mathematical method used to perform multicomponenting on the 3100 Genetic Analyzer is called the chemometric method. As the data is collected for each capillary, a comparison is made between the dye set matrix collected during the spectral calibration and the sample data being collected. Using this comparison at each spectral bin, the portion of the fluorescence emitted from a neighboring dye in the dye set is subtracted from the data, thus compensating for the spectral overlap.

Confidence Limits

The confidence limits associated with the measured fluorescence intensity are also calculated during multicomponenting. The confidence limits do not indicate systematic errors that may be introduced during multicomponenting, such as pull-up error, but rather indicate a random error associated with noise in the detection system.

This error is displayed as confidence bands in the real-time electropherogram views of the Array View.

Two traces are displayed for each dye. Each pair represent the upper and lower limits of detection for that dye. The greater the separation between two plots of color, the less confidence we have in the detection of the dye.

Separation of confidence bands will be normally higher at the end of a run and at the peak baseline.

Configuring Data Flow

Data Flow Options

After the data has been processed, the data is:

- Stored in the instrument database as processed frame data
- Converted to either ABIF sample files or BioLIMS data sets (depending on the Data Analysis preferences settings)

Processed Frame Data

After multicomponenting, the data is stored as groups of binary streams in the instrument database. This data is called processed frame data to distinguish it from other data stored in the database, such as plate records and EPT data.

Mobility Shift Correction for DNA Sequencing

Introduction

Mobility shift correction is an additional data processing step that occurs when performing DNA sequencing. The correction is carried out during autoanalysis when the basecaller software is assigning bases to the collected and processed data.

Background

When a dye is bound to a DNA fragment, it changes the rate at which the fragment migrates during electrophoresis. When DNA fragments that are labeled with different dyes are electrophoresed together, the fragments do not migrate with equal spacing because different dyes change the migration rate to different extents. Without correction, this would lead to an uneven separation of peaks in the electropherogram.

Mobility Files

The data needed to perform mobility shift correction are contained in mobility files. Mobility files are different for different dye sets and instrument types.

]When creating a plate record, you select the mobility file that you want to use for processing each sample. For the names of the mobility files provided with the ABI PRISM® 3100 Genetic Analyzer Software and for which file to use when, see page 6-6.

Mobility files have the general format *filename*.mob. They must never be moved from the Mobility folder located in the following directory:

D:\AppliedBio\Abi\Shared\Analysis\Basecaller\Mobility

Technical Support

B

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section "To Obtain Documents on Demand" following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844**, then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS™, BioMerge™, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**
Voyager™ MALDI-TOF Biospectrometry and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and Poros® Perfusion Chromatography Products)	**1-800-899-5858,** then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858**, then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**
Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 22358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 331400**	**31 (0)180 331409**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**

Region	Telephone Dial...	Fax Dial...
Latin America		
Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the **Troubleshooting** heading, click **Support Request Forms,** then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
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Part Numbers

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Applied Biosystems Part Numbers

Introduction

Part numbers for many consumables are given in this appendix. Refer to these part numbers when ordering from Applied Biosystems.

More information about Applied Biosystems kits and consumables is available from your sales representative or on the web at http://www.appliedbiosystems.com

Instrument Hardware

Description	Part Number
ABI PRISM® 3100 Genetic Analyzer with Dell Workstation	3100-01
Printers (sold only with ABI PRISM instruments)	
Epson Stylus 900 Color Printer (International)	4311692
Epson Stylus 900 Color Printer (for use at 100–120 volts)	4311623

Plate Assembly Kits

Description	Part Number
96-well plate kit	4316471
384-well plate kit	4316472

Software Kits

Description	Part Number
ABI PRISM® 3100 GeneScan® Analysis Software Module Kit	4317379
ABI PRISM® 3100 DNA Sequencing Analysis Software Module Kit	4317380

DNA Sequencing Reagents and Consumables

Description	Part Number
ABI PRISM® 3100 POP-6™ polymer	4316357
ABI PRISM® 3100 capillary array, 50-cm	4315930
ABI PRISM® 3100 capillary array, 36-cm	4315931
Genetic Analyzer Buffer with EDTA (10X)	402824
Matrix Standard Set DS-01 (dROX, dTAMRA, dR6G, dR110)	4315974
ABI PRISM® BigDye™ Terminator Sequencing Standards Kit	4304154
Hi-Di™ Formamide, 25-mL bottle	4311320

GeneScan Reagents and Consumables

Description	Part Number
ABI PRISM® 3100 POP-4™ polymer	4316355
ABI PRISM® 3100 capillary array, 36-cm	4315931
ABI PRISM® 10X buffer with EDTA	402824
Matrix Standard Set DS-30 (6FAM™, HEX, NED™, ROX™)	4316100
ABI PRISM® 3100 GeneScan™ Installation Standard DS-30	4316144
Hi-Di™ Formamide, 25-mL bottle	4311320

Instrument Consumables

Description	Part Number
96-well plate septa	4315933
MicroAmp® Optical 96-well Reaction Plates	N801-0560
384-well plate septa	4315934
MicroAmp® 384-well Reaction Plates	4305505
Reservoir septa	4315932

Instrument Spare Parts

Description	Part Number
96-well plate retainer	4317241
96-well plate base (AB)	4317237
384-well plate retainer	4317240
384-well plate base	4317236
Reservoirs (for buffer, water, and waste)	628-0163
Glass syringe, 5.0-mL polymer-reserve	628-3731
Glass syringe, 250-µL array-fill	4304470
Syringe O-rings	221102
Syringe ferrule	005401
Anode buffer reservoir jar	005402
Upper polymer block drip tray	628-3720
Lower polymer block drip tray	628-3088
Autosampler drip tray	628-3059
Polymer block tubing assembly	628-3732
Array calibration ruler	628-3214
Array comb holders	628-3403
Array ferrule sleeves	628-0165
Array ferrule knob	628-3730

Reference Materials

Description	Part Number
ABI PRISM 3100 Genetic Analyzer User's Manual	4315834
ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide v.3.6	4315831
ABI PRISM GeneScan Analysis v. 3.6 NT User's Manual	4308923
ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Fragment Analysis	4315832
ABI PRISM Sequencing Analysis Software v. 3.6 NT User's Manual (with v.3.6.1 update)	4308924
ABI PRISM 3100 Genetic Analyzer Quick Start Guide for Sequencing	4315833
ABI PRISM 3100 Genetic Analyzer Operator Training CD	432559

Limited Warranty Statement

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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 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 Genetic Analyzer Specification Sheet (the “Specifications”) publication number 106SP02-01.

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 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's Manual. This Warranty does not cover the customer-installable accessories or customer-installable consumable parts for the Instrument that are listed in the Instrument User's Manual. 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.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the Instrument that is not contained in Applied Biosystems printed product literature or this Warranty Statement. Any such affirmation, representation, or warranty made by any agent, employee, or representative of Applied Biosystems will not be binding on Applied Biosystems.

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