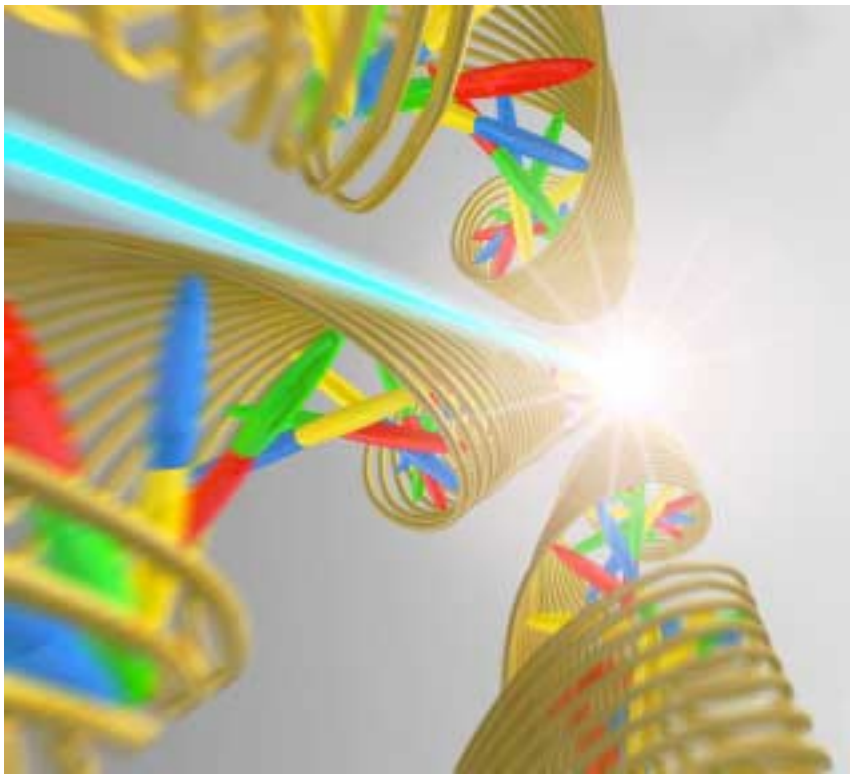

ABI PRISM® 3100 Genetic Analyzer and ABI PRISM® 3100-*Avant* Genetic Analyzer

User Reference Guide

Applied Biosystems | HITACHI

ABI PRISM® 3100 Genetic Analyzer and ABI PRISM® 3100-*Avant* Genetic Analyzer

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Part Number 4335393 Rev. A
07/2002

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

4 Working with Plate Records

5 System Management and Networking

6 Troubleshooting

1 Introduction and Safety

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

About the Instrument

System Components

The ABI PRISM® 3100 and 3100-Avant Genetic Analyzers are automated capillary electrophoresis systems that can separate, detect, and analyze fluorescent-labeled DNA fragments in one run.

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

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

Before You Begin

Important Safety Information

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

Audience

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

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

- Windows NT operating system
- General techniques for handling DNA samples and preparing them for electrophoresis. Networking, which is needed if you want to integrate the 3100 or 3100-Avant Genetic Analyzer into your existing laboratory data flow system

Documentation

List of User Documents

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

Title	Contents	P/N
	Instrument	
ABI PRISM 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer Site Preparation and Safety Guide	♦ Laboratory requirements for installation ♦ Instrument and chemical safety	4315835
ABI PRISM 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide	♦ Theory of operations ♦ System management ♦ Troubleshooting	4335393
ABI PRISM 3100 Genetic Analyzer User Guide	User procedures for using and maintaining the instrument	4334785
ABI PRISM 3100-Avant Genetic Analyzer User Guide	User procedures for using and maintaining the instrument	4333549
	Software	
ABI PRISM DNA Sequencing Analysis Software v. 3.7 NT User Guide	Detailed procedures for analyzing sequencing data	4308924
ABI PRISM GeneScan Analysis Software v. 3.7 NT User Guide	Detailed procedures for analyzing fragment analysis data	4308923
	Chemistry	
ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide	♦ Detailed chemistry procedures specific for the 3100 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

User Bulletins

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

Applied Biosystems will mail user bulletins related to the use of this instrument to you. We recommend storing the bulletins in this manual behind the tab labeled “User Bulletins.”

Safety

Documentation User Attention Words

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

Note Calls attention to useful information.

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

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

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

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

Chemical Hazard Warning

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

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

Chemical Waste Hazard Warning

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

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

Site Preparation and Safety Guide

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

About MSDSs

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

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

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

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

Ordering MSDSs

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

To order documents by automated telephone service:

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

To order documents by telephone:

In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	Dial **1.800.668.6913**, and press **1** for English or **2** for French.

To obtain documents through the Applied Biosystems Web site:

Step	Action
1	Go to **http://docs.appliedbiosystems.com/msdssearch.html**
2	In the **SEARCH** field, type in the chemical name, part number, or other information that will appear in the MSDS and click **SEARCH**. **Note** You may also select the language of your choice from the drop-down list.
3	When the **Search Results** page opens, find the document you want and click on it to open a PDF of the document.

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

Instrument Safety Labels

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

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

About Waste Disposal

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

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

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

Before Operating the Instrument

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

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

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

Computer Workstation Safety

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

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

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

Electric Shock

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

Lifting/Moving

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

System Overview 2

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

What the Instrument Does

Types of Analysis

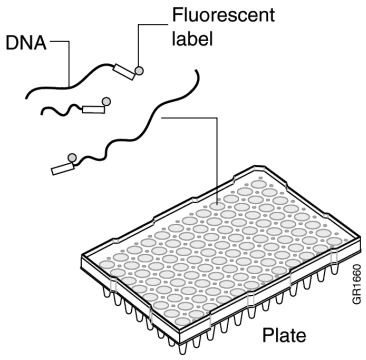
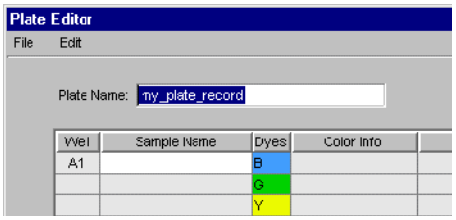
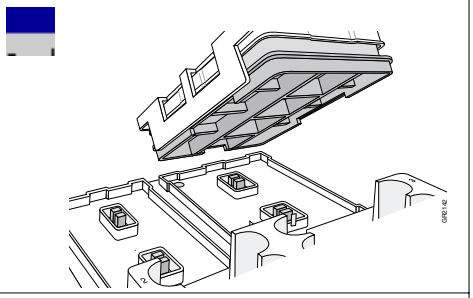
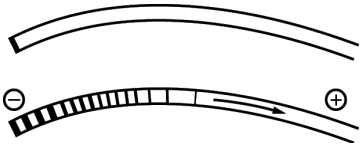
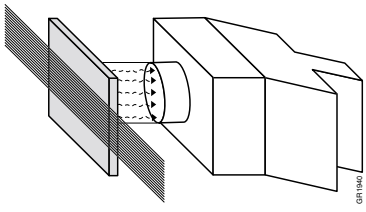
The ABI PRISM® 3100 and 3100-Avant Genetic Analyzer perform two kinds of analysis:

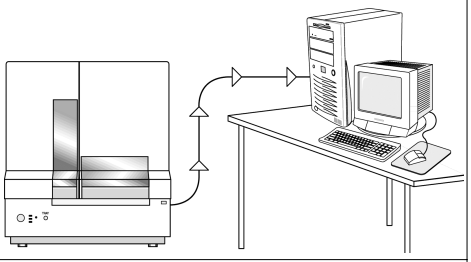
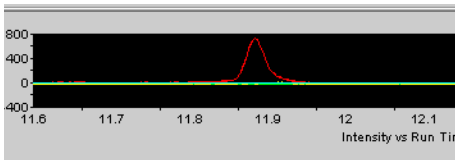
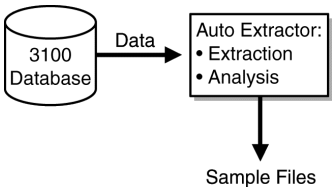
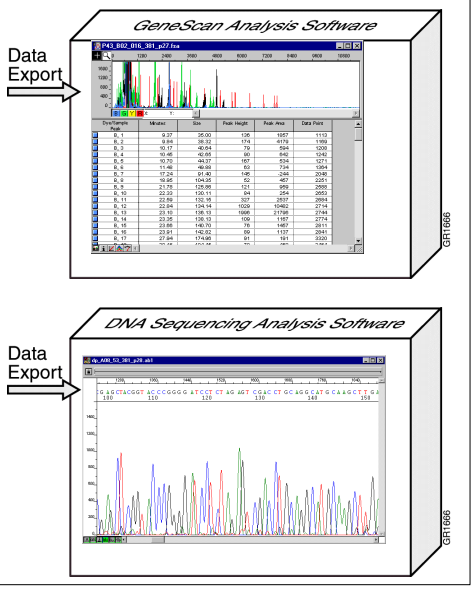
DNA Analysis	Purpose
Sequencing analysis	♦ Separates a mixture of DNA fragments according to their lengths ♦ Provides a profile of the separation ♦ Determines the order of the four deoxyribonucleotide bases
Fragment analysis	♦ Separates a mixture of DNA fragments according to their lengths ♦ Provides 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

Typical Run

The following table describes a typical run on the 3100 and 3100-Avant 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 3100 or 3100-Avant Genetic Analyzer 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 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 an excitation beam. The excitation beam causes the dye on the fragments to fluoresce. The fluorescence is captured by an optical detection device.	

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 and 3100-Avant 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 size.	
8	**Automatic Data Extraction and Data Analysis** The processed data is automatically extracted 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 ABI PRISM® DNA Sequencing Analysis software (for sequencing) or ABI PRISM® GeneScan® Analysis software (for fragment analysis). If necessary, the data is reanalyzed using different analysis parameters.	

Front View

Diagram and Description

The following diagram shows the front of the instrument:

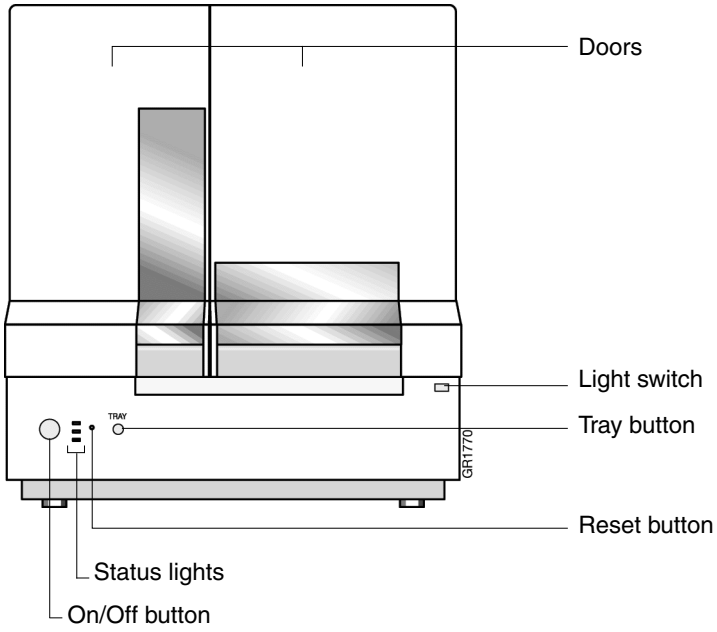


Table of Front View Components

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 the Maintenance section of the *ABI PRISM® 3100 Genetic Analyzer User Guide* or the *ABI PRISM® 3100-Avant Genetic Analyzer User Guide* for procedure.
Tray button	Brings the autosampler to the forward position **Note** This button works only when the instrument and oven doors are closed.

Table of Front View Components *(continued)*

<table>
<tr><th>Part</th><th>Function</th></tr>
<tr><td>Status lights</td><td>Indicates the status of the instrument as follows:
<table>
<tr><th>Light Appearance</th><th>Instrument Status</th></tr>
<tr><td>All off</td><td>Power off</td></tr>
<tr><td>Yellow solid</td><td>Loading firmware</td></tr>
<tr><td>Yellow blinking</td><td>♦ Loading calibration file
♦ Initializing subsystems</td></tr>
<tr><td>Green solid</td><td>Ready for use</td></tr>
<tr><td>Green blinking</td><td>Running</td></tr>
<tr><td>Red blinking</td><td>Error</td></tr>
</table></td></tr>
</table>

Front View with Doors Open

Diagram and Description

The following diagram shows inside the instrument's doors:

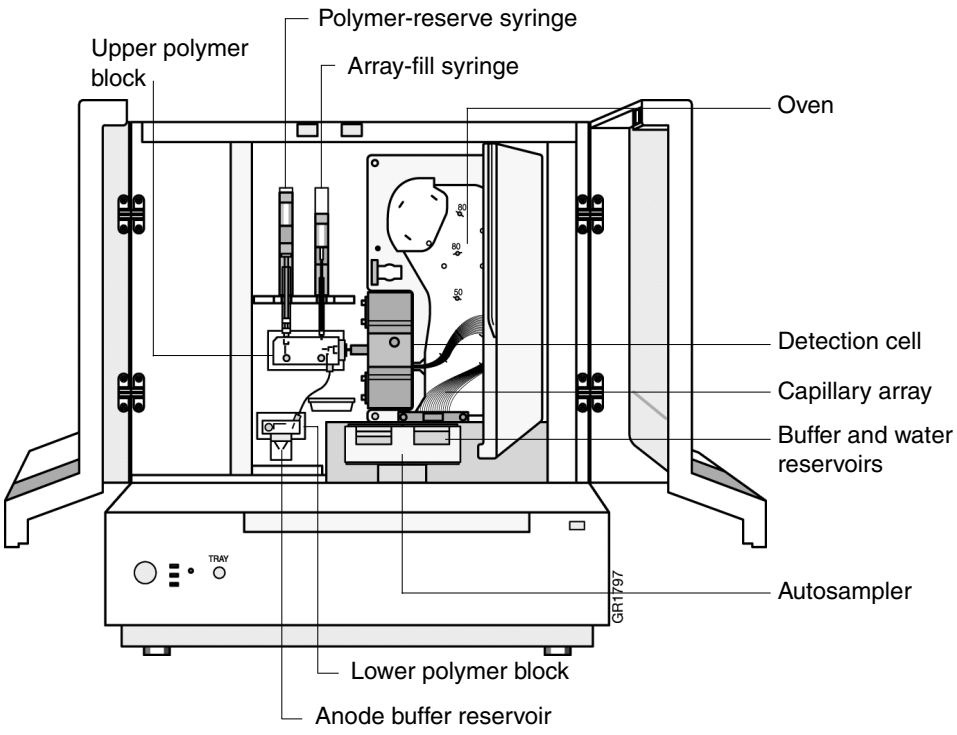


Table of Instrument Components

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	Enables the separation of the fluorescent-labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 4 or 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 and Description

The following diagram shows the back of the instrument:

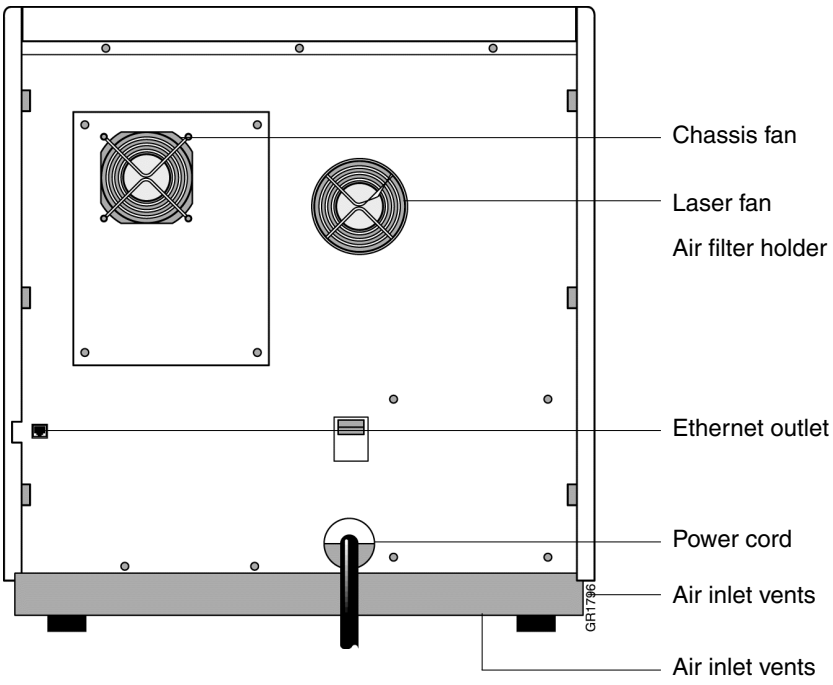


Table of Back View Components

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

Computer Workstation

Overview

The 3100 or 3100-Avant 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 and 3100-Avant 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 733 MHz

Hard Drive Partitions

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

Physical Hard Drive	Drive	Function
1	C	System operating files
	D	Reserved for the 3100/3100-Avant software and the analysis software
2	E	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 software installed on your computer, see “Software CD-ROMs” on page 3-2.

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, we recommend that you do not install third-party software onto the computer attached to the 3100 and 3100-Avant instrument.

Supported Dye Sets and Applications

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.

3100 Dye Sets and Applications

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

Application or Kit	Dye Set	Matrix Standard Set
♦ ABI PRISM® BigDye® v3.0 Terminator chemistry ♦ ABI PRISM® BigDye® v3.0 Primer chemistry	Z	BigDye® v3.0 Matrix Standards
		BigDye® v3.0 Terminator Sequencing Standard
♦ ABI PRISM® BigDye® Terminator chemistry ♦ ABI PRISM® BigDye® Primer chemistry ♦ ABI PRISM® dRhodamine Terminator chemistry	E	DS-01
Custom oligos	D	DS-30
♦ ABI PRISM® Mouse Mapping Set v1.0 ♦ Custom oligos	D	DS-31 (DS-30 + VIC™ Matrix Standard)[a]
♦ AmpFℓSTR® COfiler® Kit ♦ AmpFℓSTR® Profiler Plus™ Kit ♦ AmpFℓSTR® SGM Plus® Kit ♦ Other 4-Dye AmpFℓSTR® Kits	F	DS-32
ABI PRISM® SNaPshot™ Multiplex System	E5	DS-02
♦ ABI PRISM® Linkage Mapping Set (LMS) v2.5 ♦ Custom Oligos ♦ AmpFℓSTR® Identifiler™ Kit ♦ Other 5-Dye AmpFℓSTR® Kits	G5	DS-33

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

3100-Avant Dye Sets and Applications

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

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

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

Polymers

Overview

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

POP polymer is shipped ready to use.

Supported Polymers

Two polymers used with the 3100 and 3100-Avant system are as follows:

Polymer Name	Use for...	Part Number
ABI PRISM® 3100 POP-4™ polymer	Fragment analysis Long read sequencing Ultra rapid sequencing	4316355
ABI PRISM® 3100 POP-6™ polymer	Standard sequencing Rapid read 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 and 3100-Avant 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.

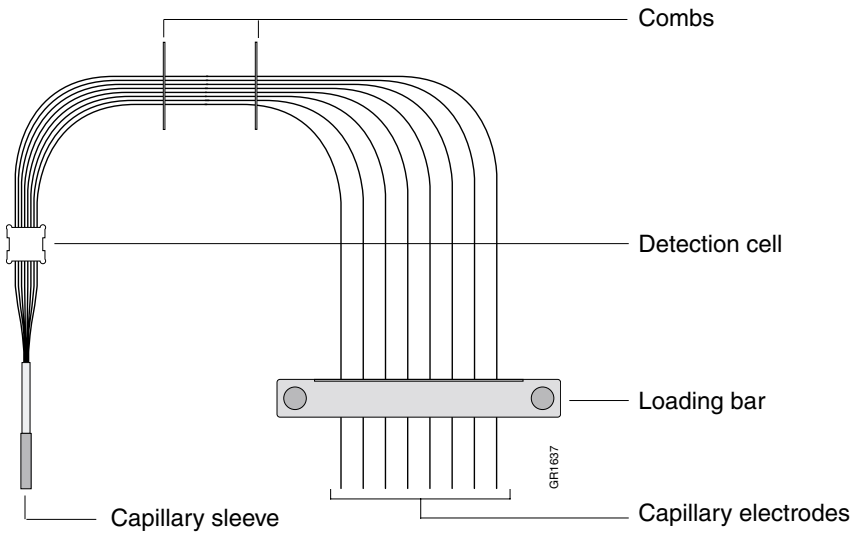
Capillary Array

Overview

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

Instrument	Capillary Number in an Array
3100	16
3100-Avant	4

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...
22	Rapid fragment analysis[a]
36	♦ Fragment analysis ♦ Ultra rapid DNA sequencing ♦ Rapid DNA sequencing
50	Standard DNA sequencing
80	Long read sequencing

a. Not supported for forensic applications.

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

Part Numbers

For capillary array part numbers, see page B-1.

Electrophoresis

Overview

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

Temperature

Housing the capillary array in a sealed oven controls electrophoresis temperature.

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

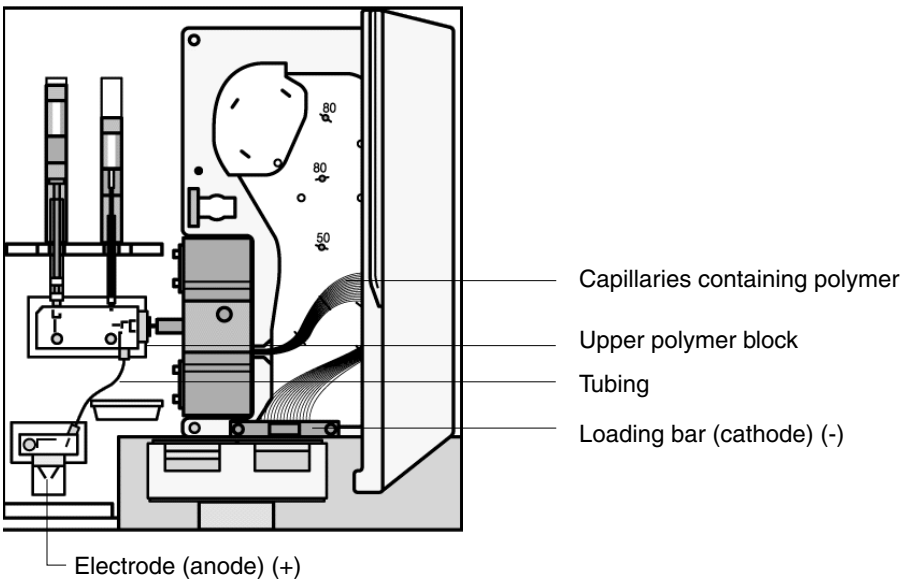
Type of Run	Temperature (°C)
Standard DNA sequencing	50
Rapid DNA sequencing	55
Standard fragment analysis	60
Long read DNA sequencing	50
Ultra rapid DNA sequencing	55

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 ions in the polymer
- 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.

Fluorescent Detection

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 laser 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 and 3100-Avant 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
Spectral dispersion device	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 and 3100-Avant 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 and 3100-Avant 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.

Spectral Dispersion Device

Overview The spectral dispersion device 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 and 3100-Avant Data Collection software.

Software

3

In This Chapter

The following topics are covered in this chapter:

Software CD-ROMs

Introduction

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

Contents of the CDs

This software is provided on a set of six CD-ROMs and their contents are listed below.

CD Title	Contents
3100 or 3100-Avant Software	♦ ABI PRISM® 3100 or 3100-Avant Firmware ♦ ABI PRISM® 3100 or 3100-Avant Data Collection software ♦ Auto Extractor ♦ Extractor utility ♦ Clean up database utility ♦ MethodImport utility ♦ Remove Run Modules utility ♦ Diskspace utility ♦ InitDB utility ♦ ABI Sample File Toolkit ♦ OrbixWeb™ v. 3.2 Professional Edition ♦ Orbix Desktop® v. 2.3 software ♦ Persistence Powertier® v. 4.321 ♦ Java Runtime Environment® v.1.1.7b ♦ Adobe Acrobat Reader® with Search v. 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® v. 8.0.5 database standard edition
Diagnostic software	This software consists of diagnostic utilities for use by Applied Biosystems service engineers only.
PowerQuest Drive Image 5 software	This software makes an image of the hard drive.

Determining the Software Versions on Your System

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

Note Both the software and the instrument have to be running.

Software Suite

Firmware

Firmware controls the most basic operations of the instrument, such as turning on the laser. 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.

The 3100 and 3100-Avant firmware resides on the computer workstation and is downloaded when the instrument is started. Therefore, the instrument and the computer workstation must be running to perform any functions.

Data Collection Software

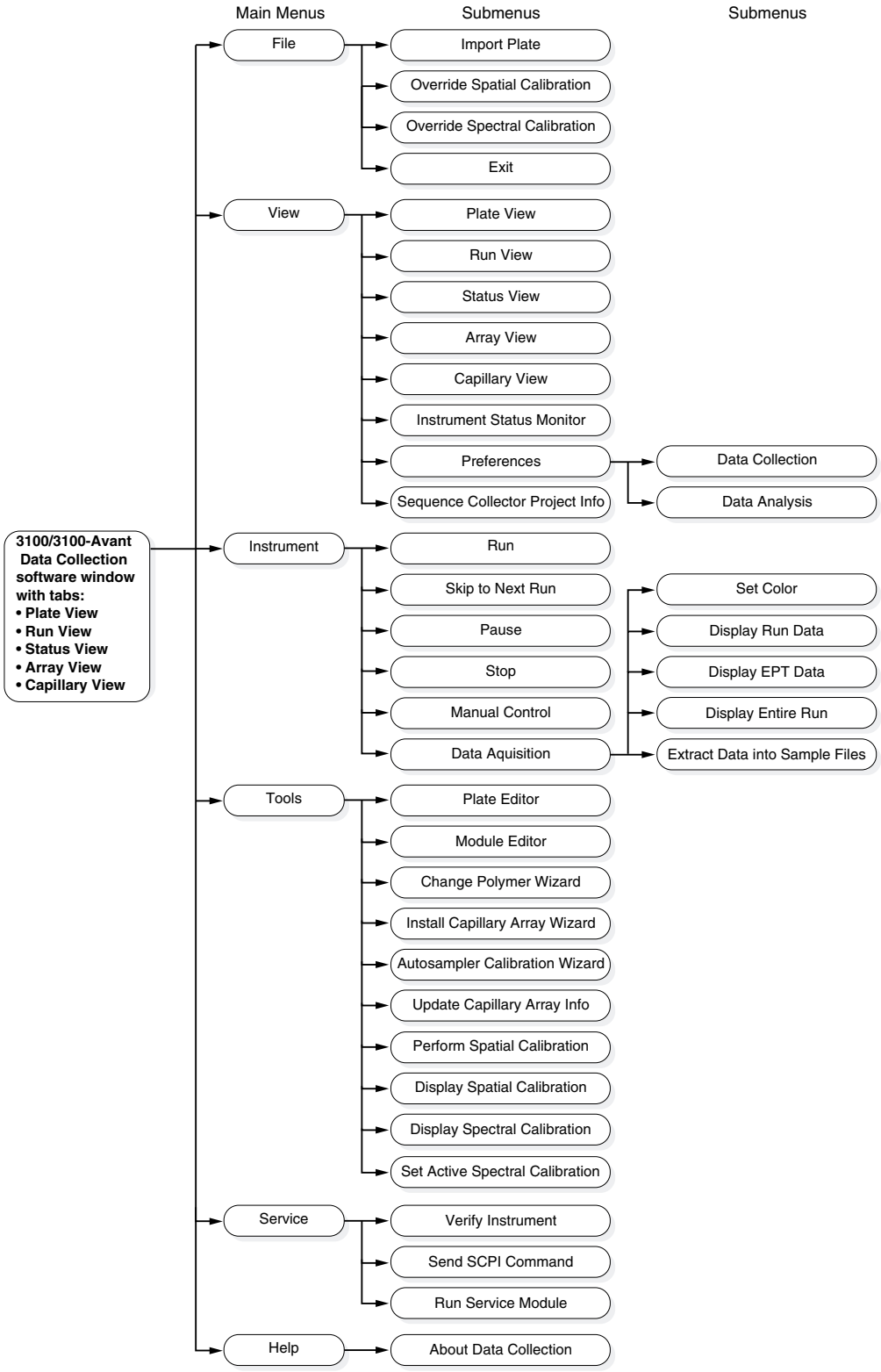
The 3100 and 3100-Avant Data Collection software performs the following functions:

- Works in conjunction with the 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 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 converts fluorescence emission data to digital data 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, that guide you through routine maintenance procedures
- Provides utilities, that when launched, automatically perform database maintenance

Additional Information

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

Data Collection Software Menu Flowchart



Applications in the Data Collection Software

Auto Extractor/AE Server	Auto Extractor is used to automatically extract and analyze the data after each run. The AE server is part of the Data Collection software and contains the Auto Extractor.
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.
Extractor Utility	The extraction utility (Extractor) uses the run data in the instrument database to create sample files. If an ABIF sample file becomes corrupt or if you accidently delete a file that you want, you can use the Extractor utility to re-extract the data into the sample file. Directions for using the Extractor utility start on page 5-2.
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 5-4.
Method Import Utility	The Method Import utility (MethodImport) imports the data contained in method files into the instrument database. Use this utility to install new versions of methods sent out by Applied Biosystems after your genetic analyzer is installed. Directions for running the Method Import utility start on page 5-6.
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 5-7.
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 5-8.
ABI Sample File Toolkit	Use ABI Sample File Toolkit to read ABIF sample files and to develop customized applications for the 3100 and 3100-Avant Genetic Analyzer.

Supporting Software

OrbixWeb

OrbixWeb v. 3.2 Professional Edition provides database management services between the 3100 or 3100-Avant Data Collection software, Extractor utility, and the Oracle database. OrbixWeb v. 3.2 Professional Edition has no user interface; however, it must always be running when the 3100 and 3100-Avant Data Collection software or Extractor utility is running.

Orbix Desktop

Orbix Desktop v. 2.3 software is middleware that is used by the 3100 and 3100-Avant Data Collection software and the Extractor utility.

Persistence Powertier

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

Java Runtime Environment

Java Runtime Environment v. 1.1.8 is software that enables the 3100 and 3100-Avant 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 modules
- EPT data

This manual describes how the database is used by the 3100 or 3100-Avant 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 data

Types and Locations of Files

Introduction

The 3100 and 3100-Avant 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 by the 3100 and 3100-Avant 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 for the 3100 system are listed below.

Note The 3100-Avant system will show 3100-Avant instead of 3100 in the directory.

Extension	File Type	Directory (if Applicable)
.ab1	ABIF sample file for sequencing analysis	D:\AppliedBio\3100\DataExtractor
.bat	Batch file initiates a series of software events (*e.g.*, 3100Collection.bat)	—
.bcp	Basecaller parameter file	D:\AppliedBio\Shared\Analysis\Basecaller\Params
.exe	Executable program	—
.fsa	ABIF sample file for fragment analysis	D:\AppliedBio\3100\DataExtractor
.fsf	Factura settings file	D:\AppliedBio\Shared\Analysis\Factura\Settings
.gsp	Analysis module for GeneScan	D:\AppliedBioi\Shared\Analysis\Sizecaller\Params
.ini	Initialization file	—
.log	Log file in text file format	—
.mcl	Spectral calibration file	D:\AppliedBio\3100\DataCollection\Spectral Cal Logs\Spectral Cal
.mob	Mobility file	D:\AppliedBio\Shared\Analysis\Basecaller\Mobility
.modexp	Exported run module file	—
.mtd	Method file	D:\AppliedBio\Support Files\Data Collection Support Files\Method Files
.par	Spectral calibration parameter files	D:\AppliedBio\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\Support Files\Data Collection Support Files\Plate Import Files
.saz	Analysis module for sequencing analysis	D:\AppliedBio\Shared\Analysis\Basecaller\Params
.scl	Spatial calibration file	D:\AppliedBio\3100\DataCollection\SpatialCalLogs
.scp	Sizecaller parameter file	D:\AppliedBioi\Shared\Analysis\Sizecaller\Params
.szs	Size standard file	D:\AppliedBio\Shared\Analysis\SizeCaller\SizeStandards
.tmp	Temporary run or calibration data file written in code	—
.txt	Text file that can be read by Notepad	—

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 can 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 “Set Color Dialog Box” on page 3-9.)

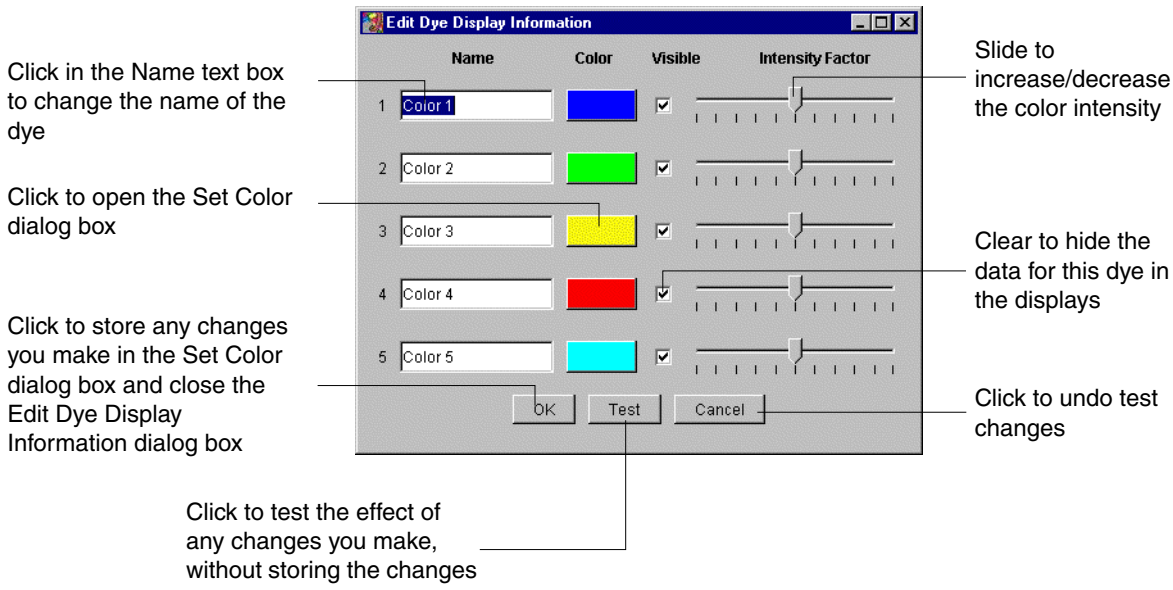
Opening the Dialog Box

To open the Edit Dye Display Information dialog box:

Step	Action
1	Select **Instrument > Data Acquisition**.
2	Select **Set Color**. This opens the Edit Dye Display Information dialog box as shown below.

Dialog Box Operations

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



Set Color Dialog Box

Changing the Display Colors Using the RGB System

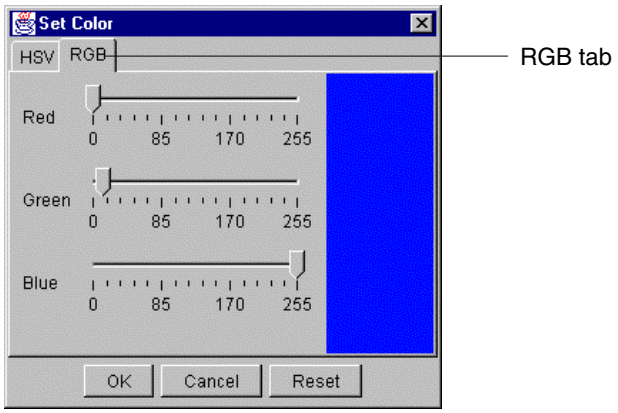
It is a good idea to change the colors used in the electropherogram and capillary displays if you find it hard to distinguish the default colors.

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

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

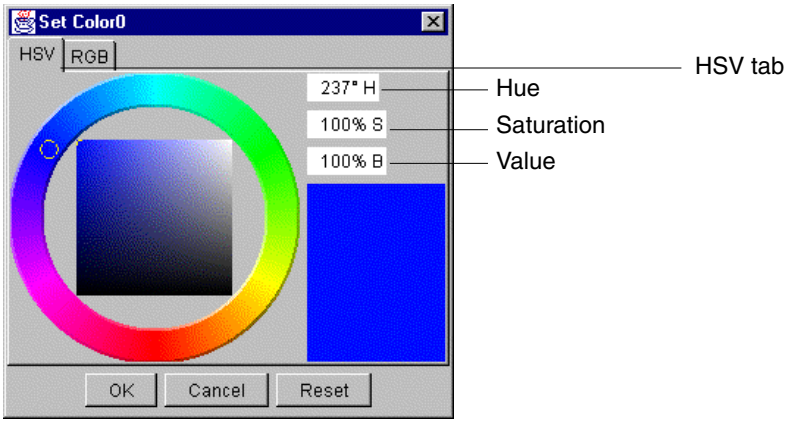
Step	Action
1	Select **Instrument > Data Acquisition > Set Color**.
2	a. Within the Edit Dye Display Information dialog box, click the **Color** box of the color you want to change. See “Dialog Box Operations” on page 3-8. b. Select the RGB tab. 
3	Move the sliders to mix the three colors until you produce 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	Select **Instrument > Data Acquisition > Set Color**.
2	a. Within the Edit Dye Display Information dialog box, click the **Color** box of the color you want to change. See “Dialog Box Operations” on page 3-8. b. Select the **HSV** tab if it is not already selected. 
3	Click in the circle and drag the cross-hair pointer around the circle to select the desired hue.
4	Click in the inner square and drag horizontally to select the desired saturation.
5	Click in the inner square and drag vertically to select the desired value.
6	<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>
7	Close the Edit Dye Display Information dialog box.

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

Manual Control Commands

Table of Commands

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

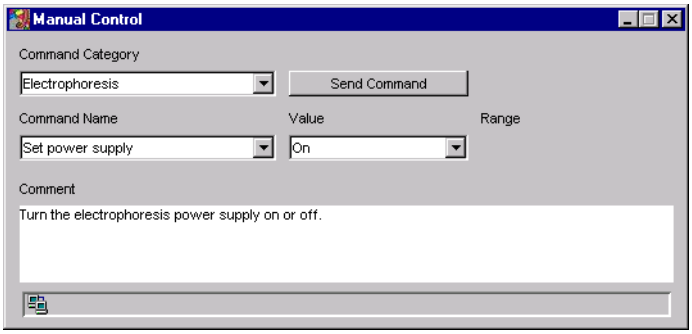
Command Category	Command Name	Value
Electrophoresis	Set Power Supply	♦ On ♦ Off
	Set Voltage	A number between 0 and 15 kV
Laser	Set State	♦ Idle ♦ On ♦ Off
	Set Power	A number between 0 and 25 mW
	Open/Close Shutter	♦ Open ♦ Closed
Oven	Set State	♦ On ♦ Off
	Set Temperature	A number between 18 and 65 °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 ♦ 22 cm/POP4 ♦ 80 cm/POP4

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	Select **Instrument > Manual Control**. 
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.

Run Modules

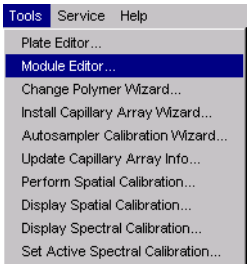
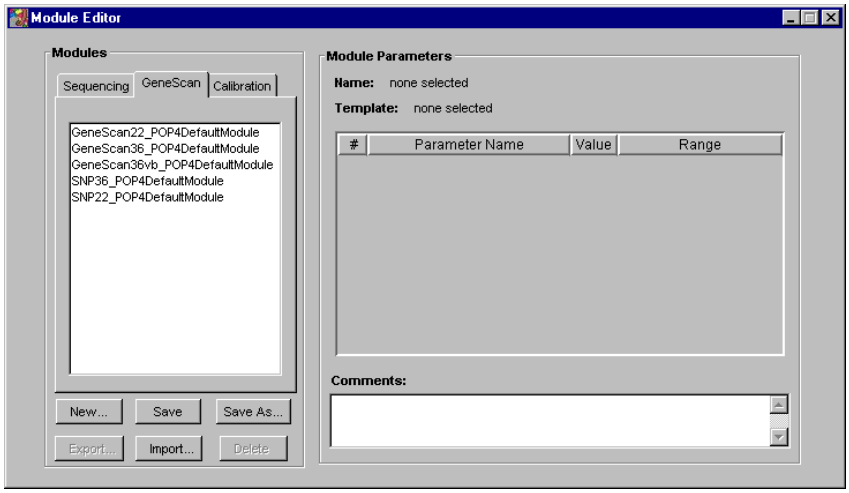
Introduction

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

- Duration of the run
- Run temperature
- Injection time

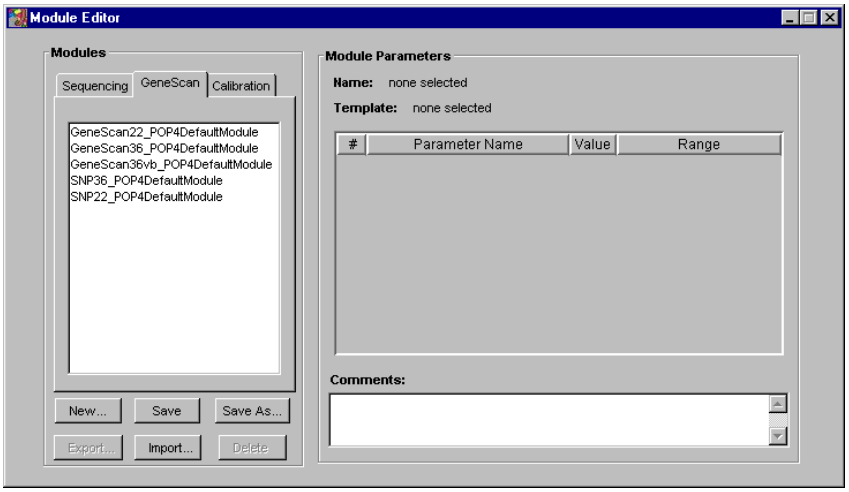
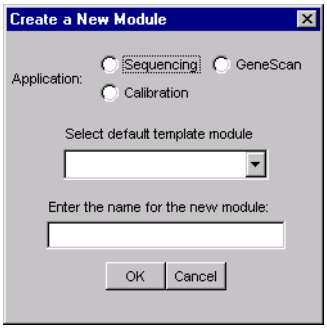
Viewing a Run Module

To view a run module:

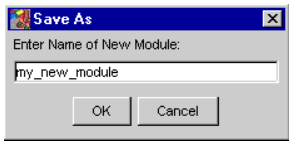
Step	Action
1	Select **Tools > Module Editor** or click the Module Editor button on the toolbar.   This opens the Module Editor dialog box.
2	a. In the Modules group box, select either the **Sequencing** or **GeneScan** tab, as appropriate.  **Note** The Calibration tab lists the spatial and spectral calibration modules. b. 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.

Creating a Run Module

To create a new run module:

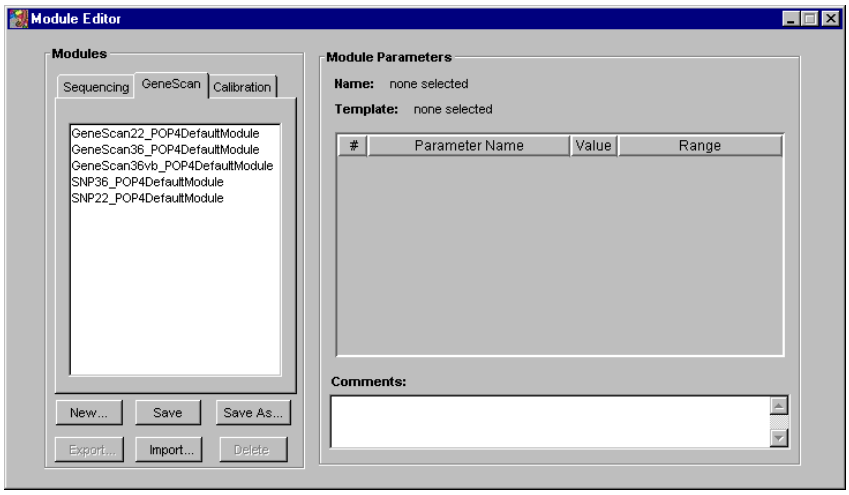
Step	Action
1	a. Click the Module Editor icon on the toolbar to open the Module Editor dialog box.  b. Click **New**. 
2	a. Select the: ♦ Application ♦ Template module ♦ Name for the new module  b. Click **OK**.
3	Edit the parameter values that you want to change. **IMPORTANT** Only whole numbers are accepted. **IMPORTANT** Be sure that all values are red. Values in black are not saved.

To create a new run module: *(continued)*

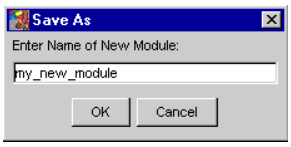
Step	Action
4	Click **Save**. Option: Click **Save As**. Enter a unique descriptive name and click **OK**.  **Note** Save cannot be applied to default run modules. Save the module under a different name.
5	When you are finished, click the Close button ( ) to exit the Module Editor.

Editing a Run Module

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

Step	Action
1	a. Click the Module Editor icon on the toolbar to open the Module Editor dialog box.  b. Select a module by double-clicking. 
2	Edit the parameter values that you want to change. **IMPORTANT** Enter whole numbers. **IMPORTANT** Be sure that all values are red. Values in black are not saved.

To edit an existing run module or to create a new run module: *(continued)*

Step	Action
3	Click **Save As**. Enter a unique descriptive name and click **OK**.  **Note** Save cannot be applied to default run modules. Save the module under a different name.
4	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 table below 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, GeneScan run method files, and calibration files.

Modifiable Run Module Parameters

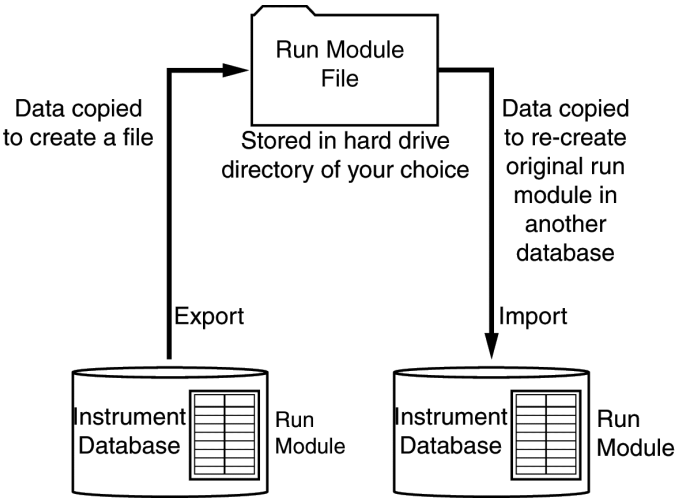
The following table lists the user-modifiable run module parameters:

Parameter	Comment
Run Temperature	The temperature of the oven 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 editable voltage applied across each capillary during a run. You may include a voltage ramp at the beginning of electrophoresis.
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.



Exporting a Module

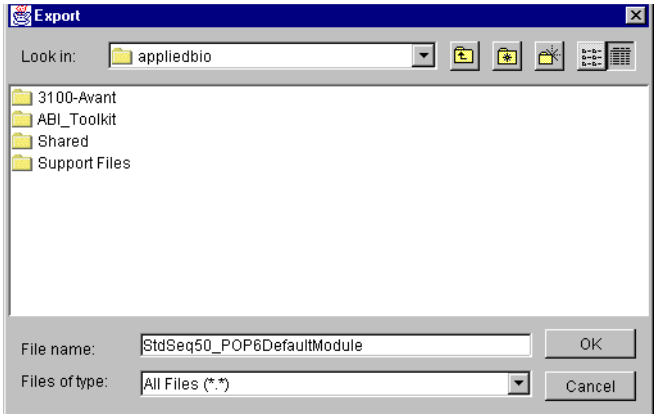
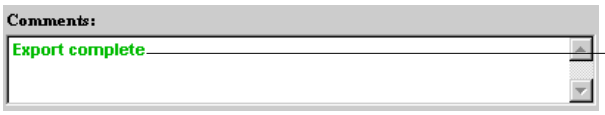
A run module cannot be transferred directly. The data in a run module must first be copied into a file that is created and stored on a hard drive. This is known as exporting the module because you are exporting it from the database.

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 icon on the toolbar to open the Module Editor dialog box. 
2	a. Select the appropriate application (GeneScan, Sequencing, or Calibration) tab. b. Select the module in the Modules group box that you want to export. c. In the Modules group box, click **Export**. This opens the Export browse dialog box as shown for the 3100-Avant instrument below. 
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. **Note** Keep the name to fewer than 32 characters. A file name longer than 32 characters will not import.
6	Click **OK**. This creates a run module in the specified folder.  This message confirms a successful export.

Importing a Module

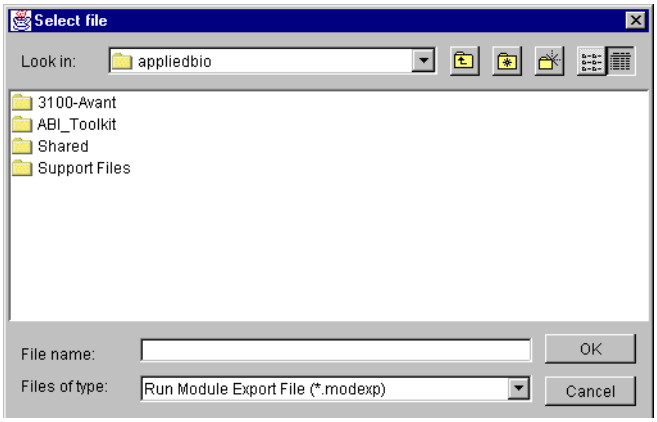
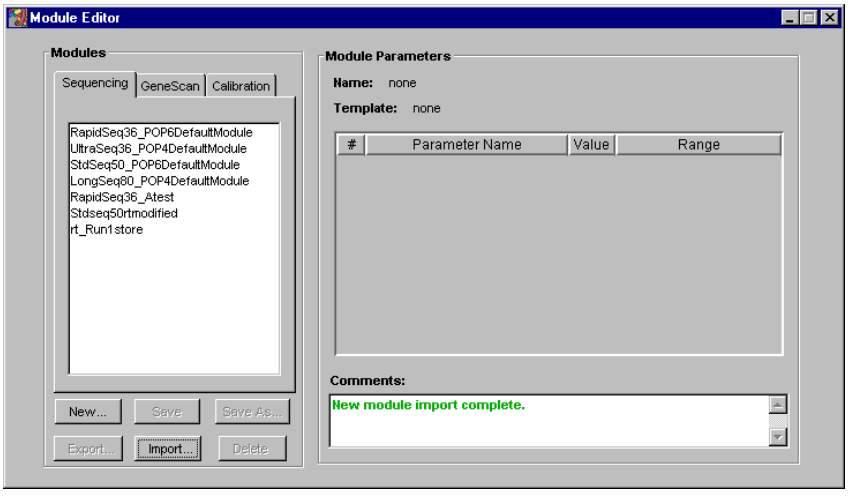
The data in the exported file is copied to the donor database to recreate the original run module. This is known as importing the module. The recreated run module has the same name as the original except for a unique number added by the software. The number is based on the date. This prevents conflicts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

Note A run module file name longer than 32 characters will not import. There is no error message.

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 icon on the toolbar to open the Module Editor dialog box. 
3	In the Modules group box, click **Import**. This opens a browser dialog box as shown for the 3100-Avant instrument below. 
4	Navigate to the folder in which you saved the run module file and select the file. **Note** Due to software limitations, you cannot select a folder on the desktop.
5	Click **OK** to import the file.  Your file shows up in the list. The Comments box confirms a successful import.

Sequencing Analysis Modules

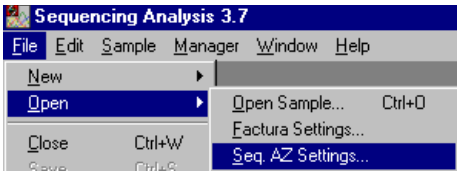
Introduction

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

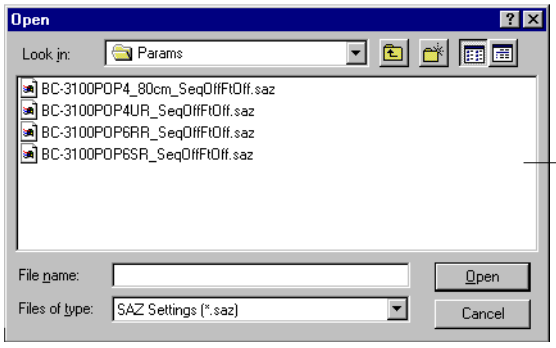
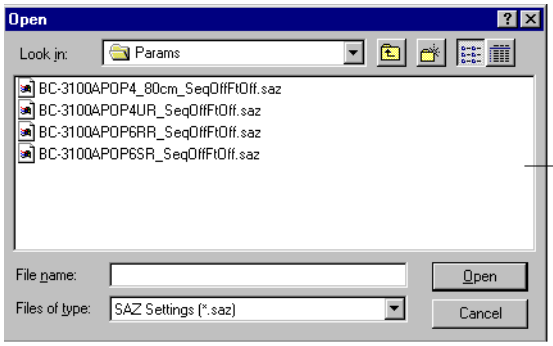
Some sequencing analysis modules are provided with the 3100 or 3100-Avant Data Collection software. In the DNA Sequencing Analysis software, the sequencing analysis module is called a sequencing analysis settings file.

Viewing and Editing Analysis Modules for DNA Sequencing

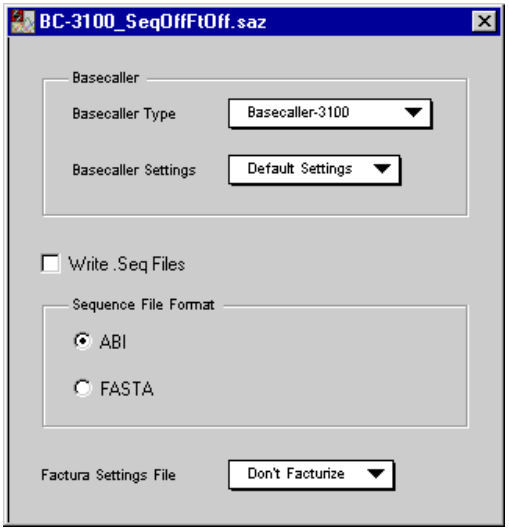
To view or edit a sequencing analysis module (.saz file):

Step	Action
1	Start the DNA Sequencing Analysis software. **Note** 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\SeqAnal\Bin
2	Select **File** > **Open** > **Seq. AZ Settings**. 

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

Step	Action
3	Select the analysis module that you want to view or edit. The analysis modules are stored in the following directory: D:\AppliedBio\Shared\Analysis\Basecaller\Params  3100 sequencing analysis modules  3100-Avant sequencing analysis modules This opens the sequencing analysis setting file (.saz).

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

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


You can edit the following settings:

♦ Basecaller Type can be Basecaller-3100 (for standard sequencing) or Basecaller-3100RR (for rapid-run sequencing). The files (.bcp) are located in the following directory:
D:\AppliedBio\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: Select File > Open > Factura Settings.

– The files are located in the following directory:
D:\AppliedBio\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

Overview

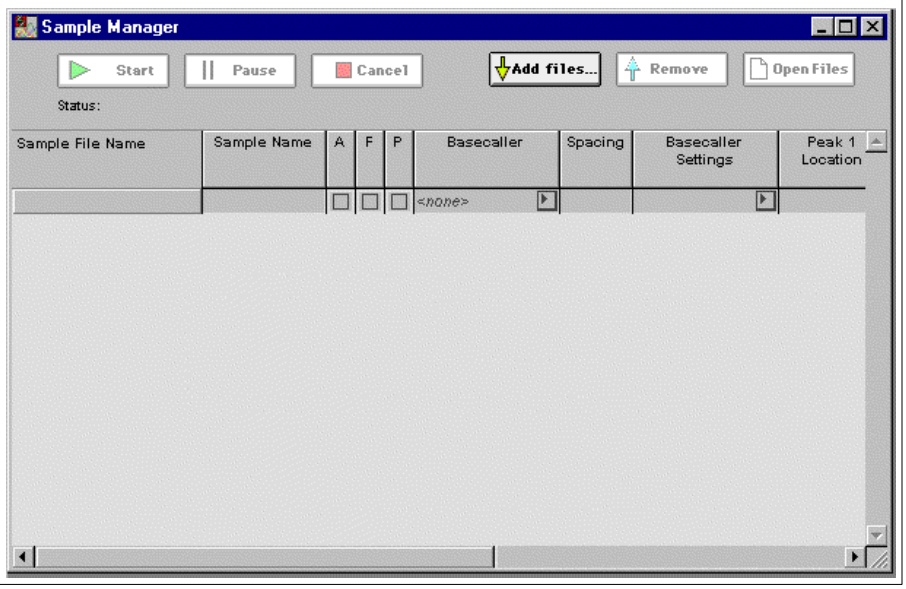
Creating a sequencing analysis module requires:

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

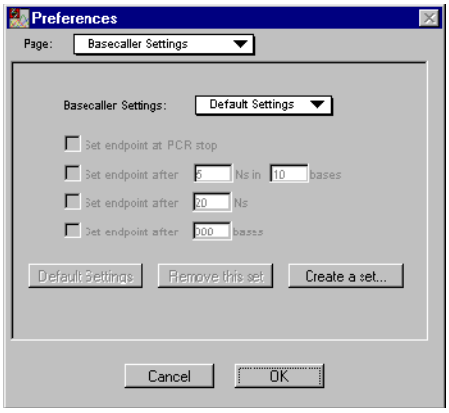
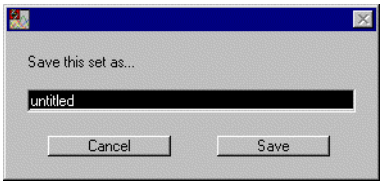
For detailed information about the topics covered in this section, see the *ABI PRISM® DNA Sequencing Analysis Software v. 3.7 NT User Guide* (P/N 4308924).

Creating a Basecaller Settings File

To create a basecaller settings file:

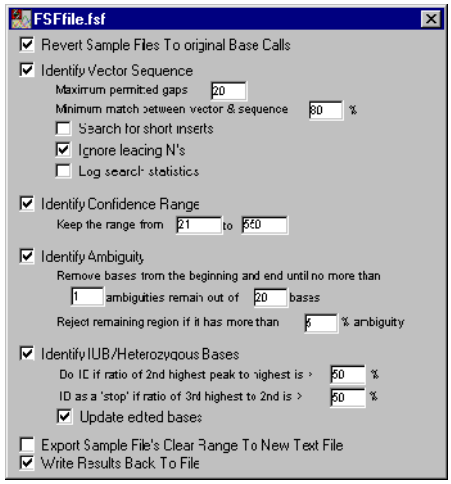
Step	Action
1	Quit the 3100 or 3100-Avant Data Collection software if it is running.
2	Start the DNA Sequencing Analysis software. The Sample Manager window opens inside the Sequencing Analysis window. 

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

Step	Action
3	To set a cutoff condition for the analysis, select **Edit > Preferences > Basecaller Settings**.  **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**.  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.

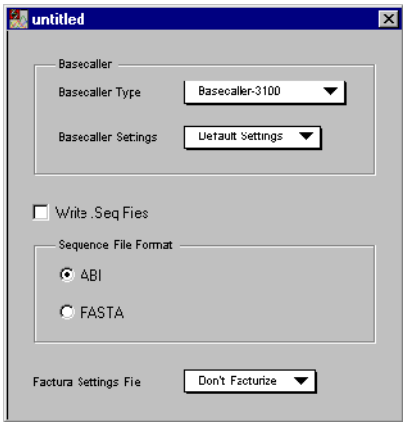
Creating a New Factura Settings File

To create a new Factura settings file:

Step	Action
1	Select **File > New > Factura Settings**. 
2	Select the required options, then click the Close button. A Sequencing Analysis alert box displays.
3	Click **Save**. The Save this document as dialog box displays.
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 use spaces.
5	Make sure that the file will be saved to the following directory: D:\AppliedBio\Shared\Analysis\Factura\Settings

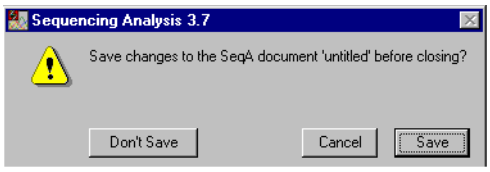
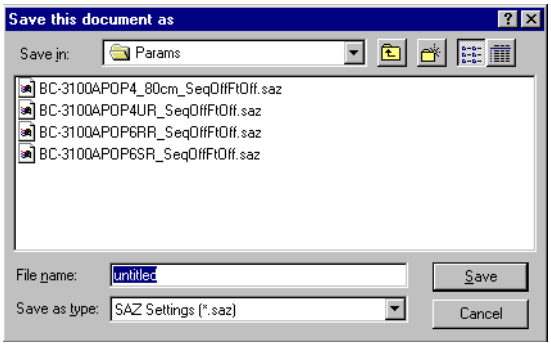
Creating a New Sequencing Analysis Module

To create a new sequencing analysis module:

Step	Action
1	Select **File > New > Seq.AZ Settings**. 
2	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
3	Select **Write .Seq Files** if you want a .Seq file created (this saves the sequence as a text file).
4	In the Sequence File Format group box, select either **ABI** or **FASTA**. **Note** Select **FASTA** only if you intend to export the data to a program that accepts FASTA files.
5	**If you do...** / **Then...** not want to use Factura software / leave Factura Settings File as Don't Facturize. want to use Factura software / select a Factura settings file from the drop-down list. 

Saving the Sequencing Analysis Module

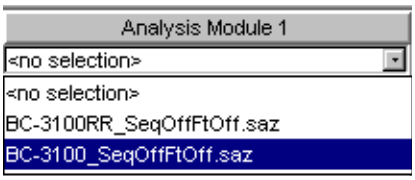
To save the newly created sequencing analysis module:

Step	Action
1	Click the Close button on the untitled dialog box.
2	Click **Save**. 
3	 3100-Avant analysis modules a. 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. b. Make sure that the file will be saved to the following directory: D:\AppliedBio\Shared\Analysis\Basecaller\Params c. 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 or 3100-Avant Data Collection software.
2	In the **Plate View** page, double-click a plate record to open 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.

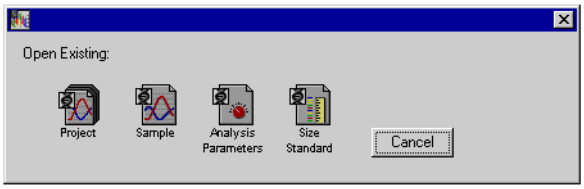
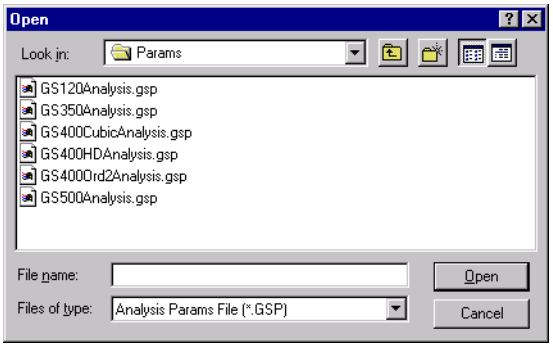
Analysis Modules for Fragment Analysis

Introduction

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

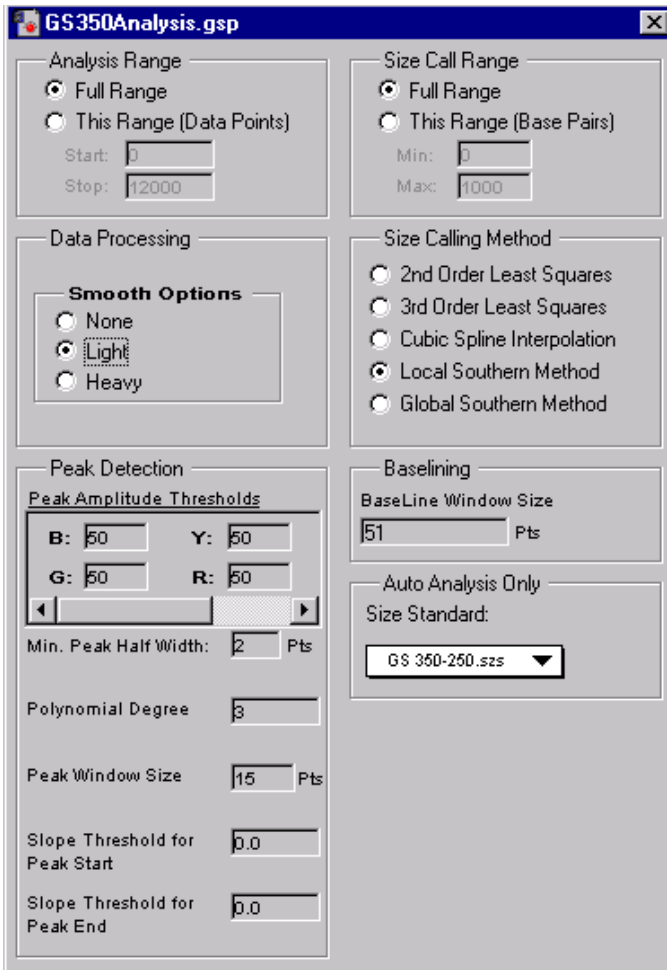
Viewing and Editing Analysis Modules

To view or edit an analysis module for fragment analysis (.gsp file):

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

To view or edit an analysis module for fragment analysis (.gsp file): *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</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. 3.7 NT User Guide (P/N 4308923).

 
</td></tr>
<tr><td>6</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>♦ Select File > Save As, assign a unique name, and then click OK, or
♦ Select File > Save to save the changes to the current analysis module.

IMPORTANT The analysis modules must be stored in the following folder: D:\AppliedBio\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>

Before Creating an Analysis Module

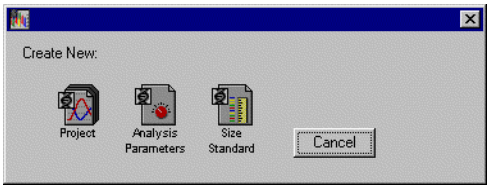
Before creating an analysis module for fragment analysis, you may need to create a custom size standard file.

You will need to create a custom file for performing:

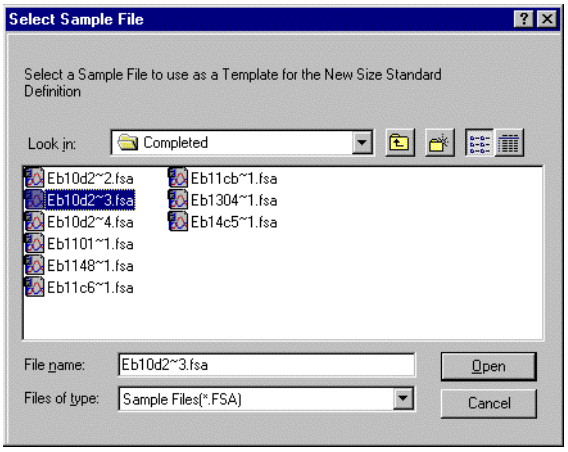
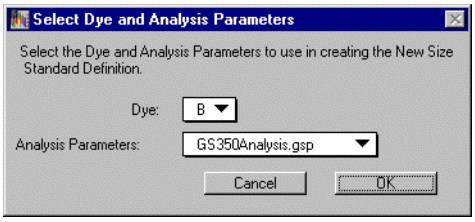
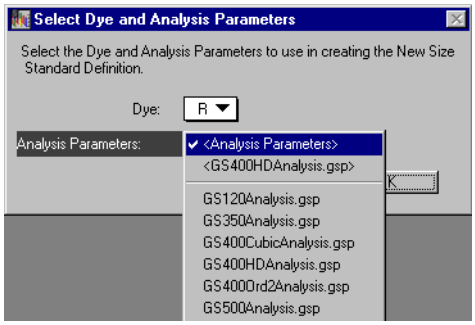
- Denaturing runs but not using GeneScan™-350 Size Standard, GeneScan™-400HD Size Standard, or GeneScan™-500 Size Standard
- Non-denaturing runs using applications such as SSCP
- Runs using one of the Applied Biosystems internal lane standards, but significantly altering collection time or analysis range
- Runs where the size standard data differs significantly (*i.e.*, extra or missing peaks)

Creating a Size Standard File

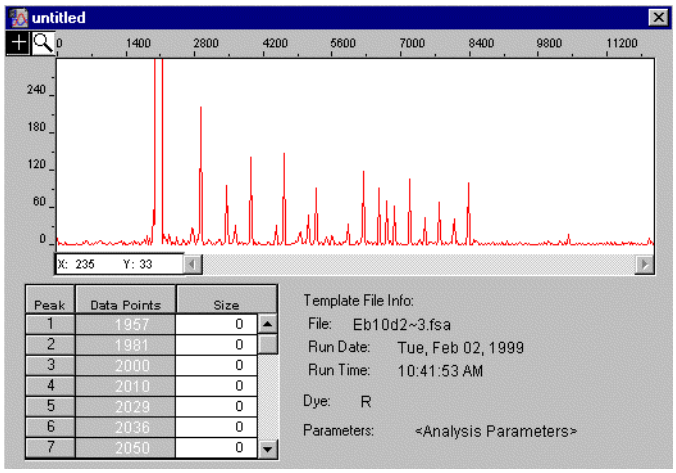
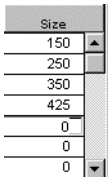
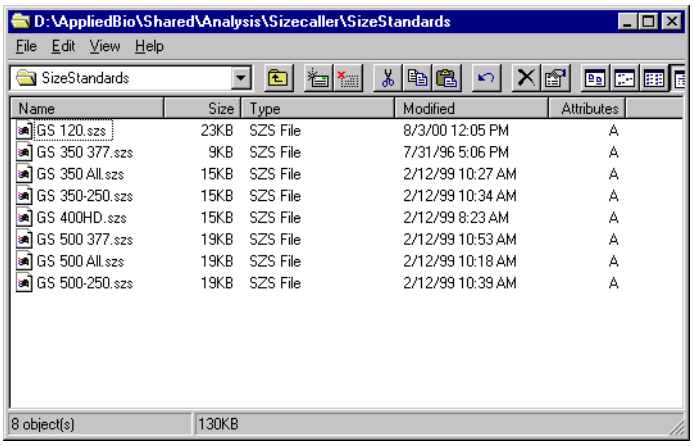
To create a size standard file:

Step	Action
1	Review the size standard data and optimize the analysis parameters.
2	Quit the 3100 or 3100-Avant 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\GeneScan\Bin 
4	Select **File > New** to open the **Create New** box. 
5	a. Click the **Size Standard** icon to open a browser dialog box. b. Navigate to the DataExtractor folder in the following directory: D:\AppliedBio\3100\DataExtractor or D:\AppliedBio\3100-Avant\DataExtractor

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

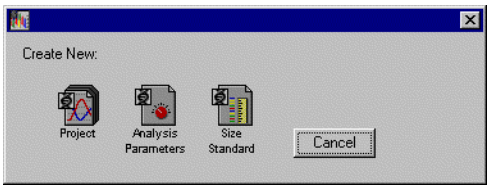
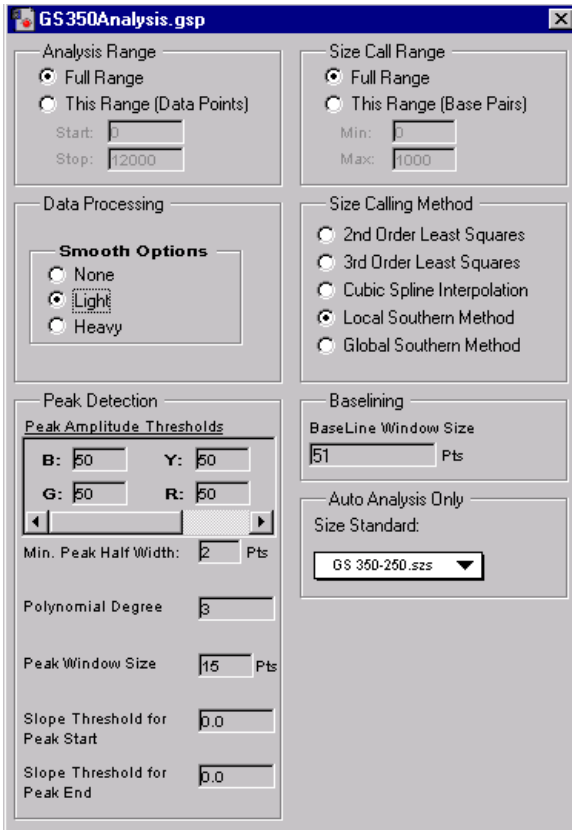
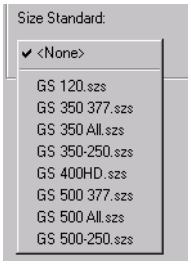
Step	Action
6	Select the GeneScan sample file (with the extension .fsa) that you want to use as a template. 
7	a. Click **Open**.  b. From the **Dye** drop-down list, select the dye-color that was used to label the size standard DNA fragments.
8	From the **Analysis Parameters** drop-down list, select <**Analysis Parameters**>. This references the current analysis parameter settings rather than an analysis parameter file. 

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

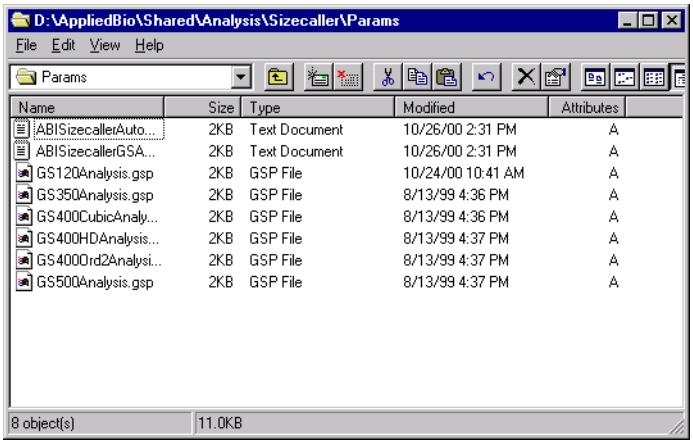
Step	Action
9	Click **OK**. 
10	In the Size column, enter the known sizes of the standard's peaks. 
11	Select **File** > **Save**.
12	Navigate to the **SizeStandards** folder located in the following directory: D:\AppliedBio\Shared\Analysis\SizeCaller\SizeStandards The folder contains a number of size standard (.szs) files. 
13	a. In the **File name** text box, type a file name for the size standard file. b. 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 an Analysis Module for Fragment Analysis

To create an analysis module for fragment analysis:

Step	Action
1	Select **File > New**. 
2	Click the **Analysis Parameters** icon.
3	Fill out the **untitled** dialog box according to the directions given in the *ABI PRISM® GeneScan Analysis Software v. 3.7 NT User Guide*.  In the AutoAnalysis Only group box, select the size standard file that you just created from the Size Standard drop-down list. 

To create an analysis module for fragment analysis: *(continued)*

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

Setting Up Sequence Collector Project Information

Introduction

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

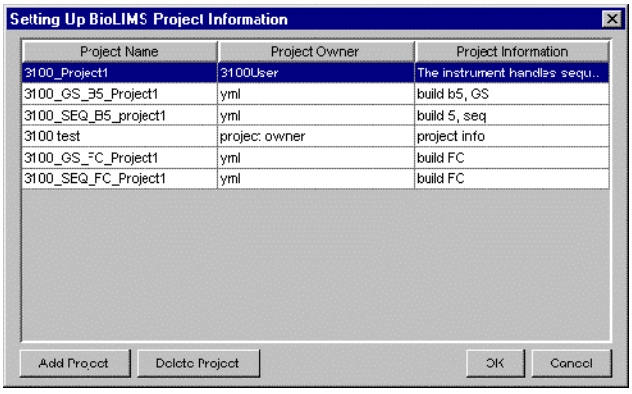
When samples are extracted into the Sequence Collector database, they are added to the specified Sequence Collector project.

Setting Up Sequence Collector Project Information

To set up the Sequence Collector project information:

Step 1

Select **View > Sequence Collector Project Info.**



Step 2

If you want to...	Then...
add a new project	a. Click **Add Project** and a blank row displays. 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.

Step 3

Enter the appropriate information in the text fields.

Text Field	Description/Constraints
Project Name	Type a descriptive name of your choice. **Note** The Project Name will be the Collection Name in the Sequence Collector database.
Project Owner	Type in your name. **Note** The Project Owner will be the Creator in the Sequence Collector database.
Project Information	Type in any comments, if desired. **Note** The Project Information will be the Comment in the Sequence Collector database.

To set up the Sequence Collector 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 **Seque**nce Collector Project column in the Plate Editor window.
5	Continue with “Preparing a Plate for Uploading to Sequence Collector” on page 3-39.

Preparing a Plate for Uploading to Sequence Collector

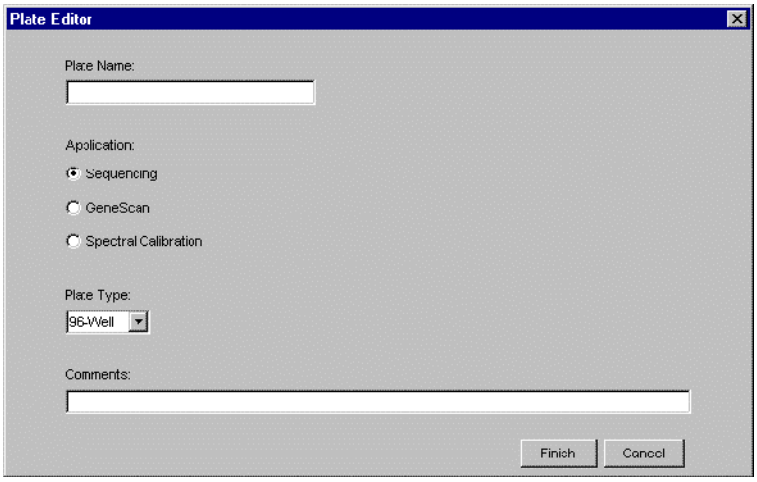
Introduction

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

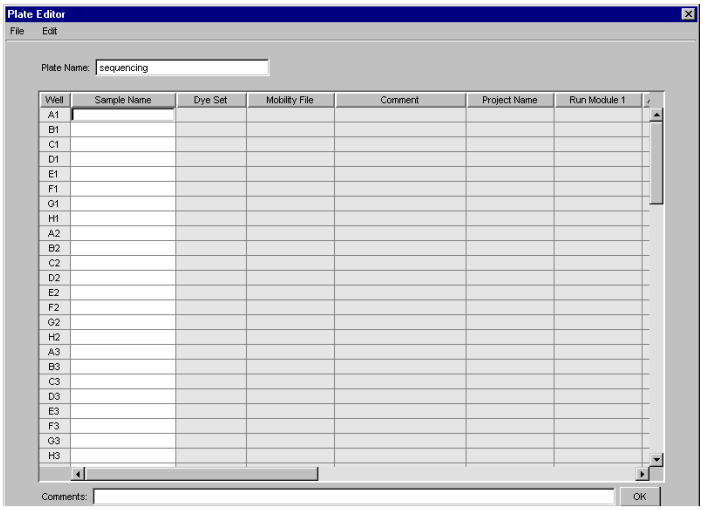
- Specifying a Sequence Collector project in the plate's sample sheet
- Setting Sequence Collector preferences for the plate

Specifying a Sequence Collector Project

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

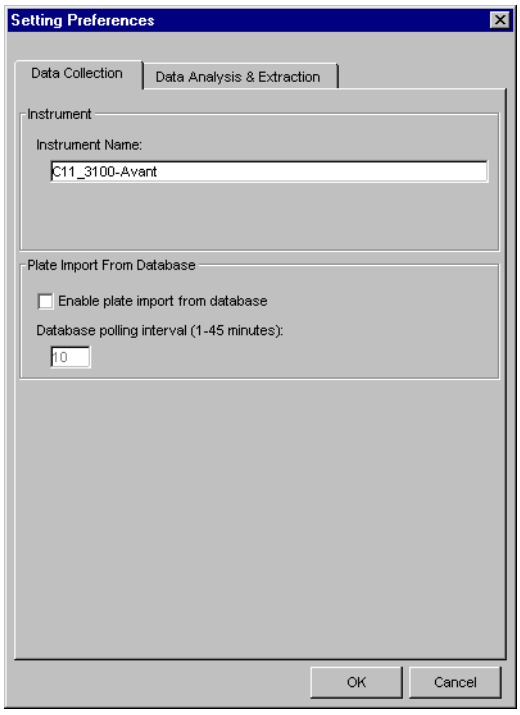
Step	Action
1	From the **Tools** menu, select **Plate Editor.** 
2	Fill in the window items as follows: **Item** / **Action** Plate Name — Type the plate name. Application — Click on the appropriate application. Plate Type — Choose the appropriate type from the drop-down list. Comments — Type comments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

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

Step	Action
3	Click **Finish**. The Plate Editor spreadsheet opens with the plate name you assigned in step 2. 
4	Fill in the spreadsheet, making sure to click on the **Project Name** column for each well and select a Sequence Collector project from the drop-down list. **IMPORTANT** All plates must have a Project Name.
5	Click **OK**. **Note** You will receive a Please wait message before the software returns to the Data Collection software window.
6	a. If it is not already selected, select the **Plate View** tab. b. Make sure your plate is listed under Pending Plate Records.
7	Highlight your plate and continue with "Setting Sequence Collector Preferences" on page 3-41.

Setting Sequence Collector Preferences

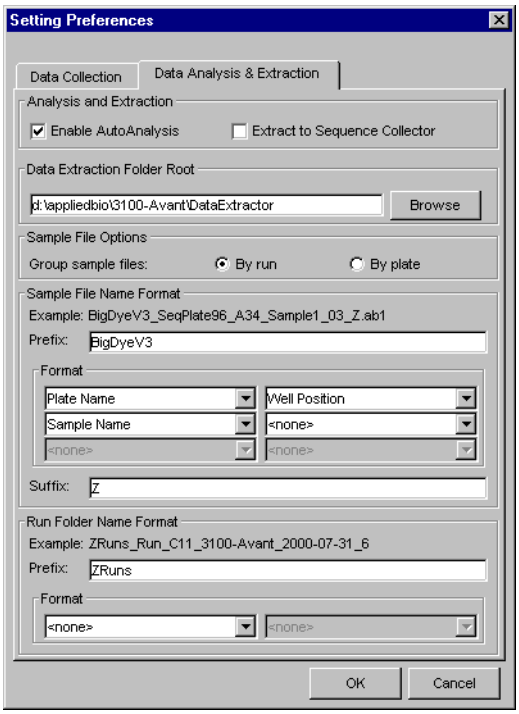
To set Sequence Collector preferences for the plate:

Step	Action
1	Select **View > Preferences.** 
2	In the Data Collection tab, set the following preferences: a. In the Instrument Name field, enter a name for the instrument. b. If you are importing plates from a database, then select the **Enable plate import from database** check box and enter a polling interval.

To set Sequence Collector preferences for the plate: *(continued)*

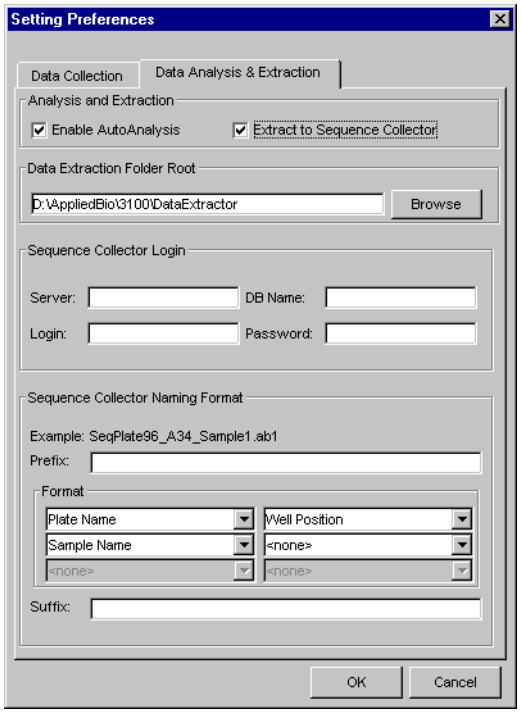
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3</td><td>Select the Data Analysis & Extraction tab.

<table>
<tr><th>If you are…</th><th>Then…</th></tr>
<tr><td>generating sample files</td><td>complete step 4 and skip step 6.</td></tr>
<tr><td>uploading data to Sequence Collector</td><td>proceed to step 5.</td></tr>
</table>

Note When extracting to Sequence Collector the sample files are still created under D:\AppliedBio\3100\Data Extractor or D:\AppliedBio\ 3100-Avant\Data Extractor and they are uploaded to the Sequence Collector database.</td></tr>
<tr><td>4</td><td>a. In the Analysis and Extraction section, the Enable AutoAnalysis check box is selected as default. Clear the box if you do not want your samples autoanalyzed.
b. In the Data Extraction Folder Root section, use the default or click Browse to select a folder location for all generated data.
c. In Sample File Options, select how you want your sample files grouped. Select the By run button to group by individual run or select the By plate button to group by the entire plate.
d. In the Sample File Name Format section, use the drop-down lists to define the sample file name format. A prefix and/or suffix can be added as needed.
e. In the Run Folder Name Format section, use the drop-down lists to define the run folder name format. A prefix can be added as needed.
f. Click OK.</td></tr>
</table>

To set Sequence Collector preferences for the plate: *(continued)*

Step	Action
5	For database files, define the following: a. In the Analysis and Extraction section, select **Extract to Sequence Collector**. The Enable AutoAnalysis check box is selected as default. Clear the box if you do not want your samples autoanalyzed.  b. In the Data Extraction Folder Root section, use the default or click **Browse** to select a folder location for all generated data. c. In the Sequence Collector Login section, define the server, DB name, login, and password for the database you are using. **Note** The server name is in the tnsnames.ora file and is the tnsalias name. This name is on the left-hand side of the = sign in the tnsnames.ora file. d. In the Sequence Collector Naming Format section, use the drop-down lists to define the sample file name format. Add a prefix and/or suffix as needed.
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 Sequence Collector database automatically. **Note** View the xx_analysis.log or the xx_extraction .log file to see if the extraction completed successfully. Continue with “After Extracting to the Sequence Collector Database” on page 3-44.

After Extracting to the Sequence Collector Database

Introduction

After your samples have run, you must view the run log file to ensure the extraction to the Sequence Collector 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 Sequence Collector project information was entered incorrectly, etc.). The only way to check the status of the extraction is to view the *xx*_analysis.log or the *xx*_extraction.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 or 3100-Avant Data Collection software and navigate to the **ExtractedRuns** folder. In most cases, the path will be either: ♦ D:\AppliedBio\3100\DataExtractor\ExtracedRuns ♦ D:\AppliedBio\3100-Avant\DataExtractor\ExtracedRuns
2	Open the **ExtractedRuns** folder. A directory is created in this folder for each run you've performed on the instrument.
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.
4	Open either the *xx*_analysis.log or the *xx*_extraction.log to check:

If the extraction was...	Then...
completed successfully	The following message appears after each sample file listed "**Successfully uploaded to Sequence Collector**."
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 *Applied Biosystems Sequence Collection Software Version 3.0 User's Guide*, (P/N 4319527) for instructions.

Working with Plate Records

4

In This Chapter

The following topics are covered in this chapter:

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

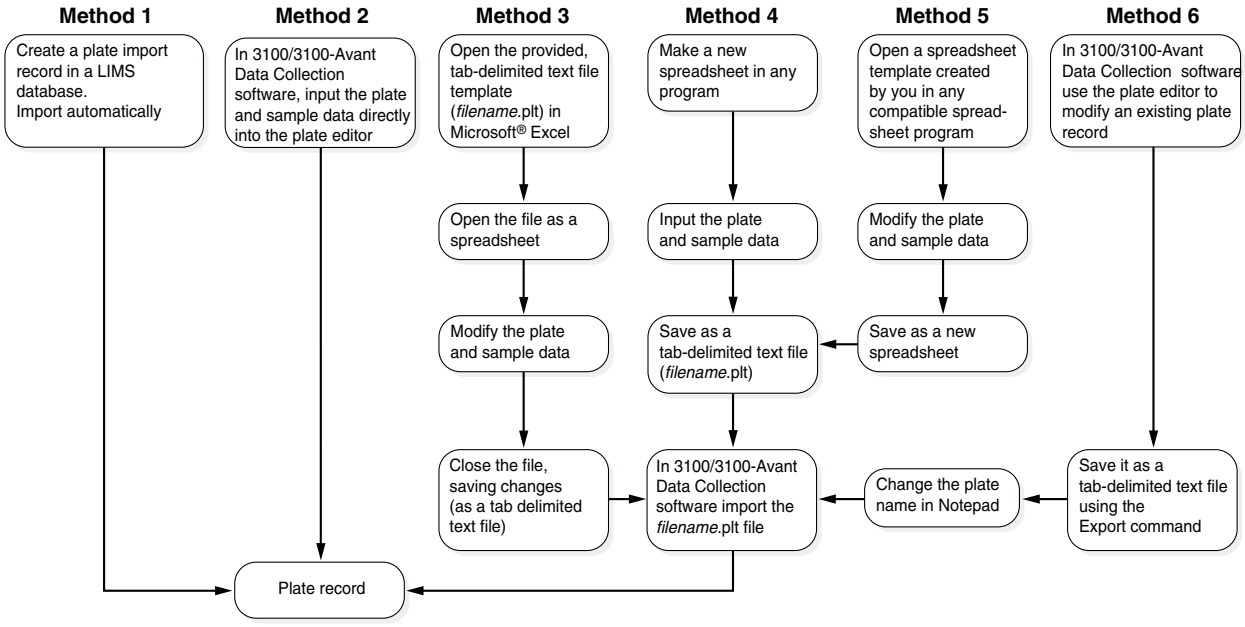
Create plate records in advance of placing the plates on the instrument or you can create a plate record while the instrument is running.

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.

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 and 3100-Avant 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	4-16	4	4-22
2	ABI PRISM 3100 or 3100-Avant User Guide	5	4-23
3	4-19	6	4-24

Plate Record Fields

Introduction

Plate record fields consist of the following:

- Dye sets
- Mobility files
- Size standards
- Run modules
- Analysis modules
- Sequence Collector

Dye Sets Provided

Select a dye set from among the following options:

For...	Select Dye Set
Fragment analysis	D
Fragment analysis - SNP	E5
Fragment analysis - Forensic applications	F
Fragment analysis - high throughput LMS and HID applications	G5
Fragment analysis - Molecular Diagnostic kits for the 3100 system only	C
DNA sequencing analysis	E
	Z

Note See “Supported Dye Sets and Applications” on page 2-11 for more details on dyes.

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 during the run.

About Mobility Files

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

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

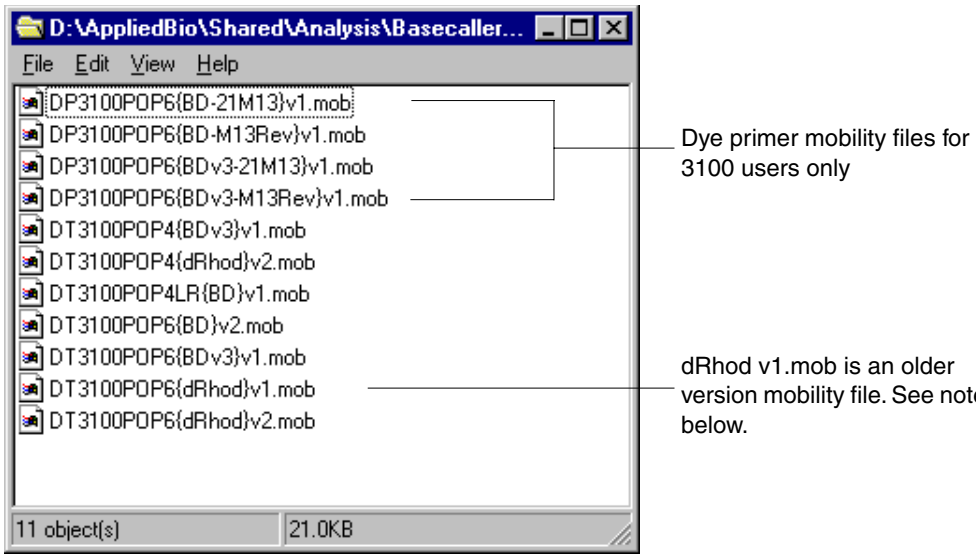
A mobility file contains the data that is used to compensate for differences in the electrophoretic mobilities of DNA fragments caused by labeling with different dye.

When a dye is bound to a DNA fragment, it changes the rate at which the fragment migrates during electrophoresis. Electrophoresing DNA fragments that are labeled with different dyes 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 Provided

The following mobility files are provided with the 3100 and 3100-Avant software and stored in the following directory and are described in the mobility file table:

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



Note Select the newer version mobility file DT3100POP6{dRhod}v2.mob instead of the older version DT3100POP6{dRhod}v1.mob file.

Note New versions of mobility files may become available from the Applied Biosystems Web site. Mobility files for dye sets other than the ABI PRISM® BigDye® sets must be provided by the manufacturer.

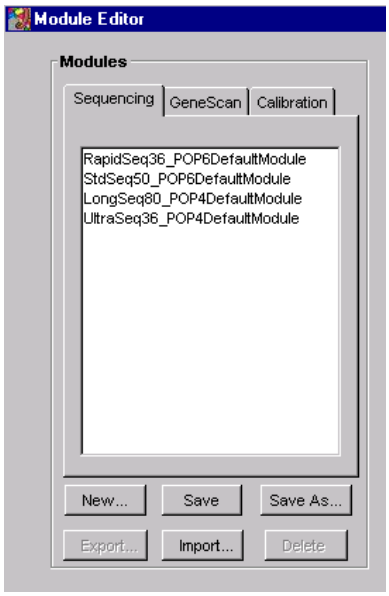
Run Modules

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 3-17.

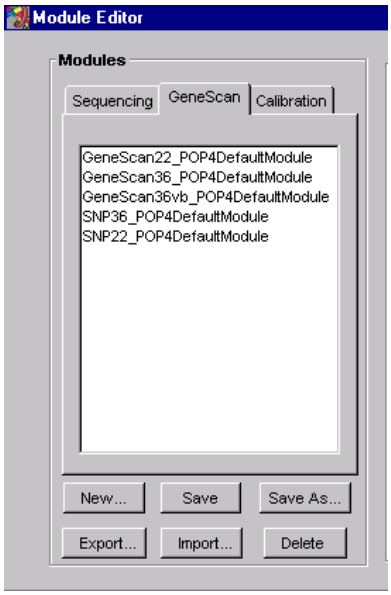
Run Modules Provided

The following run modules are provided with the 3100 and 3100-Avant software.

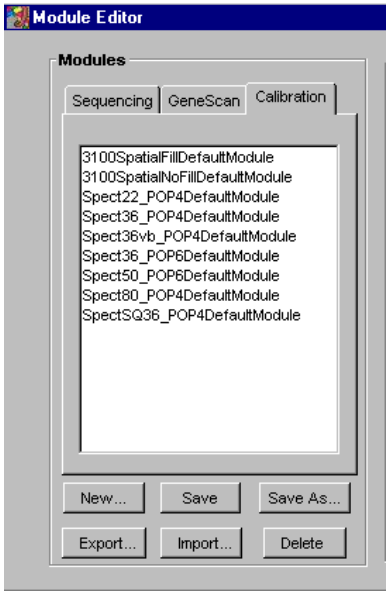
Sequencing modules



GeneScan modules



Calibration modules



Analysis Modules

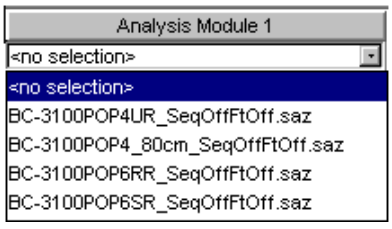
A module is a collection of routines that perform a task. Analysis modules tell the AutoAnalyzer which parameters to use for data analysis. You can use the analysis modules provided and/or create your own to define different analysis parameters.

Analysis Modules Provided

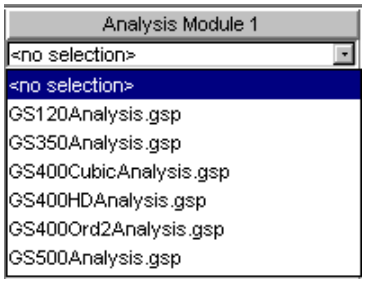
The following analysis modules are provided with the 3100 and 3100-Avant software. You can examine the settings for each of the files using the analysis software.

3100 Analysis Modules

Sequencing analysis modules

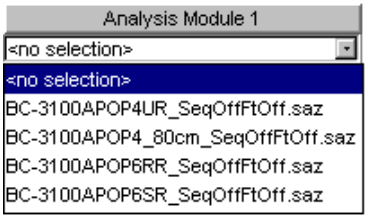


Fragment analysis modules

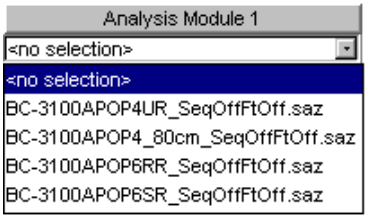


3100-Avant Analysis Modules

Sequencing analysis modules



Fragment analysis modules



Note Settings are described in the *ABI PRISM® DNA Sequencing Analysis Software v. 3.7 NT User Guide* and the *ABI PRISM® GeneScan® Analysis Software v. 3.7 NT User Guide.*

Analysis Module	**Run Type**
DNA Sequencing	
BC-3100POP4UR_SeqOffFtOff.saz	Ultra rapid DNA sequencing
BC-3100POP4_80cm_SeqOffFtOff.saz	Long read DNA sequencing
BC-3100POP6RR_SeqOffFtOff.saz	Rapid DNA sequencing
BC-3100POP6SR_SeqOffFtOff.saz	Standard DNA sequencing
Fragment Analysis	
GS120Analysis.gsp	GeneScan using size standard GS120
GS350Analysis.gsp	GeneScan using size standard GS350
GS400HDAnalysis.gsp	GeneScan using size standard 400HD
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. 3.7 NT User Guide.*

Tab-Delimited Text Files

Introduction

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.

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

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.

Creating Tab-Delimited Text Files

Introduction

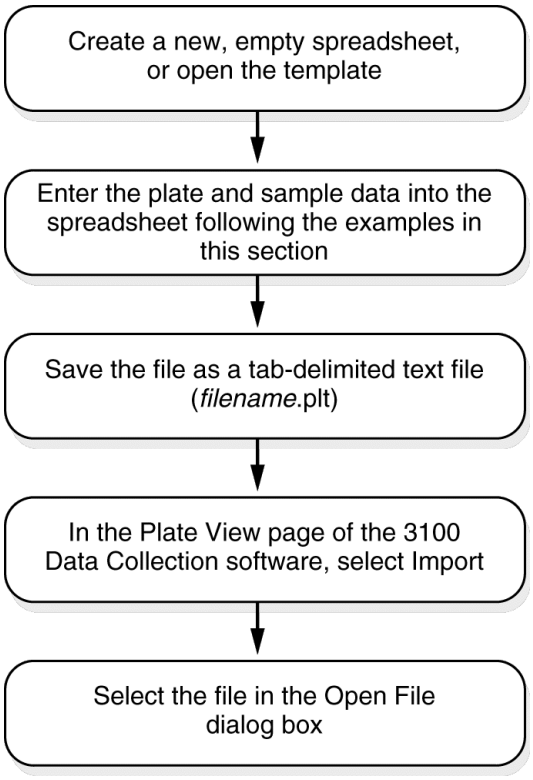
Although it is possible to create tab-delimited text files by typing the required information directly into a word-processing program, it is easier to first enter the data into a spreadsheet, and then save the spreadsheet as a tab-delimited text file. Spreadsheets are easier to work with because they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

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 4-16).

Overview of the Method

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



Using Spreadsheets to Create Tab-Delimited Text Files

Introduction

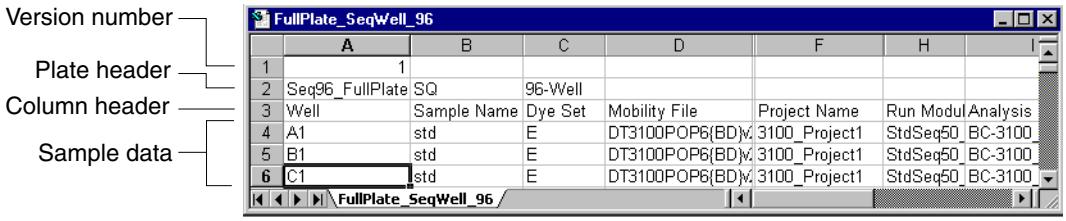
You can enter plate record data into any spreadsheet program that can save files as tab-delimited text files.

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. Examples are shown below.

Sequencing Spreadsheet

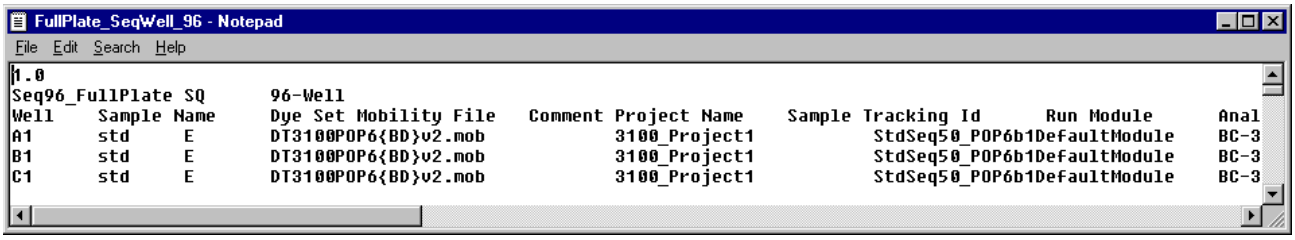
An example of a spreadsheet, prepared in Microsoft® Excel, for samples intended for DNA sequencing is shown below.

For an explanation of the labels, see page 4-11.



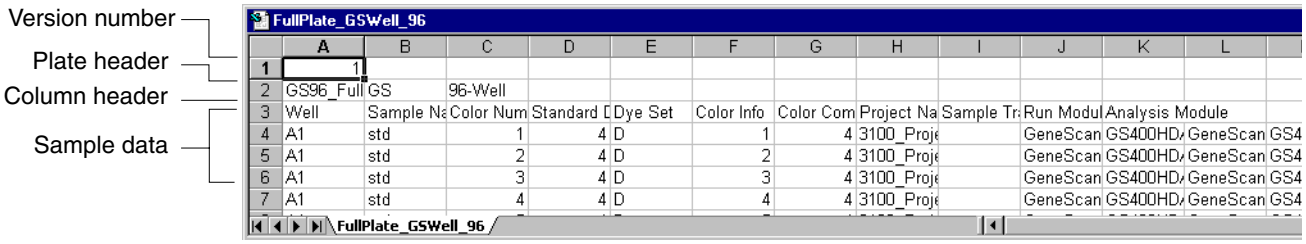
Sequencing Tab-Delimited Text File

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



Fragment Analysis Spreadsheet

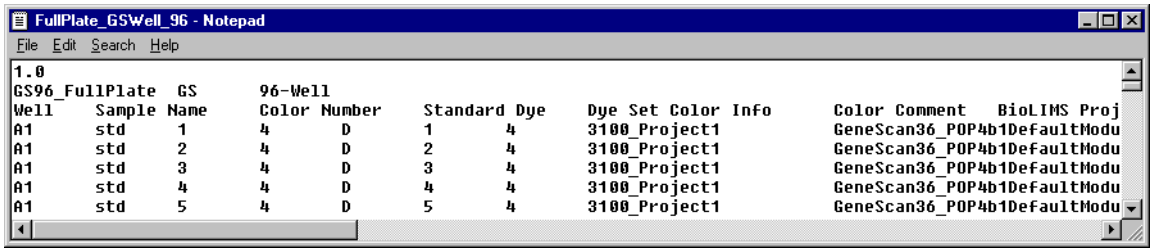
An example of a Microsoft Excel spreadsheet for samples intended for fragment analysis is shown below.



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 4-11.



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

Don't 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 or 3100-Avant Data Collection software.

When the 3100 or 3100-Avant 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 and 3100-Avant 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 4-9.

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 either: ♦ 96-Well ♦ 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.

Column Headings

Column Head	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 exceed 63 characters, the name may be truncated when exported from the 3100 and 3100-Avant Data Collection software. **IMPORTANT** This cell or token must always be second (from left to right).
Dye Set	Specifies the spectral information for the dyes 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 Sequence Collector™ Genetic Information Management System Collection name into which this sample will be added. **Note** Do not leave this cell blank.
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). You must always select an analysis module if you want the data to be extracted and analyzed. 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.

Column Header for Fragment Analysis

The column header for fragment analysis contains up to 10 cells or tokens that provide headings for the columns that will contain the sample data.

Column Headings

<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.
You must limit the sample name to 63 characters (59-character filename and 4-character extension). If you exceed 63 characters, the name may be truncated when exported from the 3100 and 3100-Avant 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 4-dye 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.

Use the number 5 for all 5-dye applications.</td></tr>
<tr><td>Dye Set</td><td>Specifies the spectral information for the dyes 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>(Optional) Enables you to customize the output for downstream analysis.</td></tr>
<tr><td>Project Name</td><td>Designates the Sequence Collector™ Genetic Information Management System Collection name into which this sample will be added.

Note Do not leave this cell blank.</td></tr>
</table>

Column Headings *(continued)*

Cell or Token	Function
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. Fragment 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. 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.

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 of runs you specify.

Setting 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

Below 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.
- 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

Below 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.
- Run modules and analysis modules are grouped in pairs, with the run module always placed to the left of its paired analysis module.

Creating a Plate Record by Importing LIMS Data

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.

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 Data Collection Software for LIMS Import

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

Sequence Collector is a type of LIMS database. For information about Sequence Collector, see page 3-37.

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.
Applied Biosystems recommends that you periodically delete data from the plate import table. It is best to do this when the 3100 and 3100-Avant Data Collection software is not running. To delete data from the plate import table, consult your Oracle database administrator.

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 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 also 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 or 3100-Avant Data Collection software
Bad	the transfer was unsuccessful	3100 or 3100-Avant 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.

Creating a Plate File Using a Provided Template

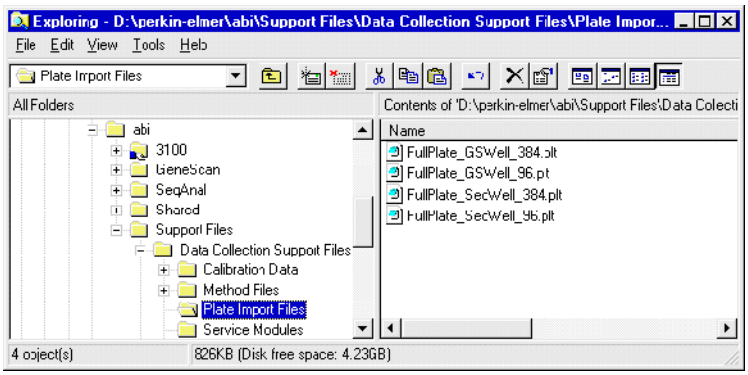
Locating the Templates

This method uses a tab-delimited text file template and Microsoft Excel to create a plate file. Templates are provided with the 3100 and 3100-Avant software and are listed below (See “Template File Names”). In Microsoft Excel, you are able to view a tab-delimited text file template in a spreadsheet format without saving it as a spreadsheet.

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

The templates provided with the 3100 and 3100-Avant Data Collection software are located in the following directory:

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



Template File Names

The templates provided with the 3100 and 3100-Avant Data Collection software are listed in the table.

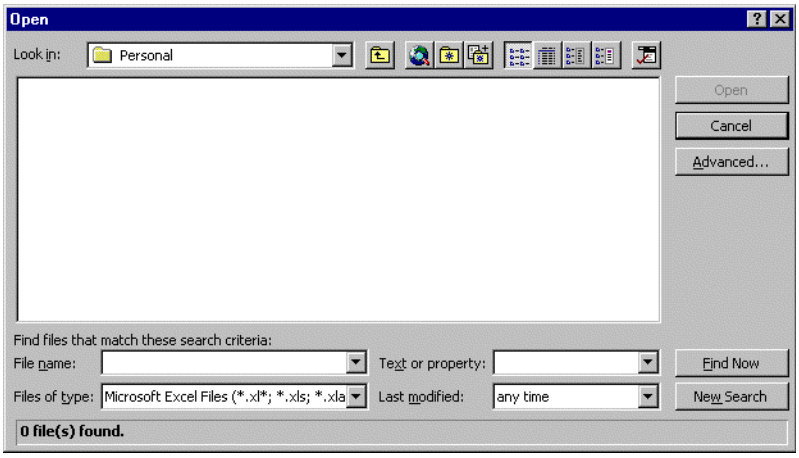
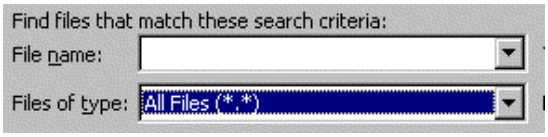
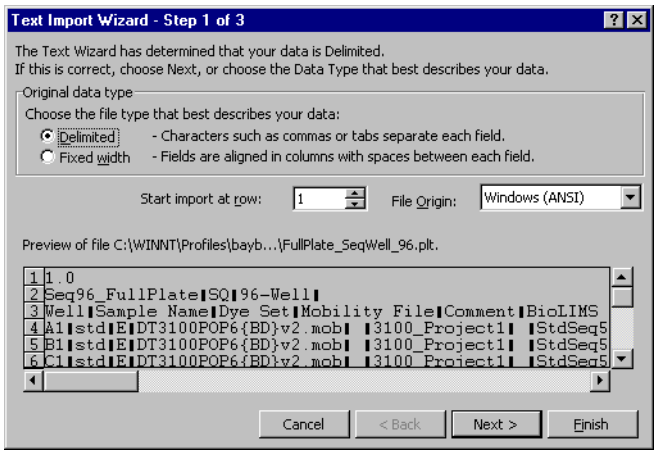
Template File Name	Type of Template
FullPlate_GSWell_384.plt	384-well for fragment analysis
FullPlate_GSWell_96.plt	96-well for fragment analysis
FullPlate_SeqWell_384.plt	384-well for sequencing analysis
FullPlate_SeqWell_96.plt	96-well for sequencing analysis

Creating a Plate Record Using a Template

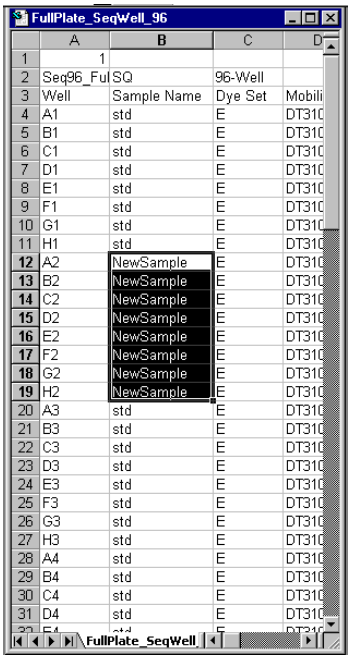
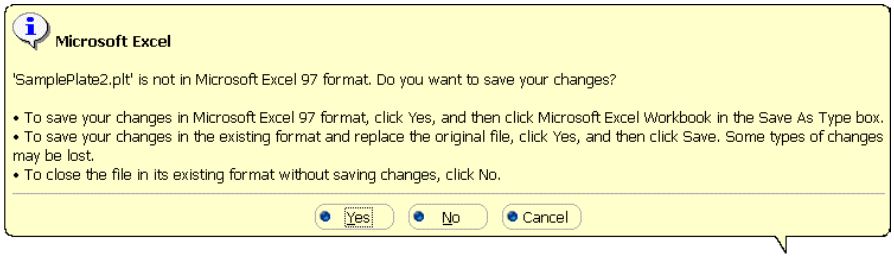
To create a plate record using a template:

Step	Action
1	Launch Microsoft Excel.

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

Step	Action
2	Select **File > Open**. 
3	Navigate to the **Plate Import Files** folder in the following directory: D:\AppliedBio\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. 
6	Click **Finish**. The file is displayed as a spreadsheet.

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

Step	Action
7	Modify any data in the cells by clicking the cell 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. 
8	Click the Close button. 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	a. In the **File name** drop-down list, 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. b. Click **Save**. This saves the edited file as a new file.
11	Follow the directions starting on page 4-25 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 4-11, type your information into the file.
3	Select **File > 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	a. Save the file with the following file name format: *filename*.plt. b. 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 4-25 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, that 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 4-19 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 modify or 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 to 4 to create other tab-delimited text files.
6	Follow the directions starting on page 4-25 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 or 3100-Avant Data Collection software.
2	a. In one of the plate record tables, double-click the plate record that you want to edit. b. 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\Support Files\Data Collection Support Files\Plate Import Files **Note** You cannot see Network Neighborhood directories from this browser dialog box.
5	a. In the **File name** dialog box, type a name for the file and add the extension .plt. b. 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 4-25 for importing a tab-delimited text file to create a plate record.

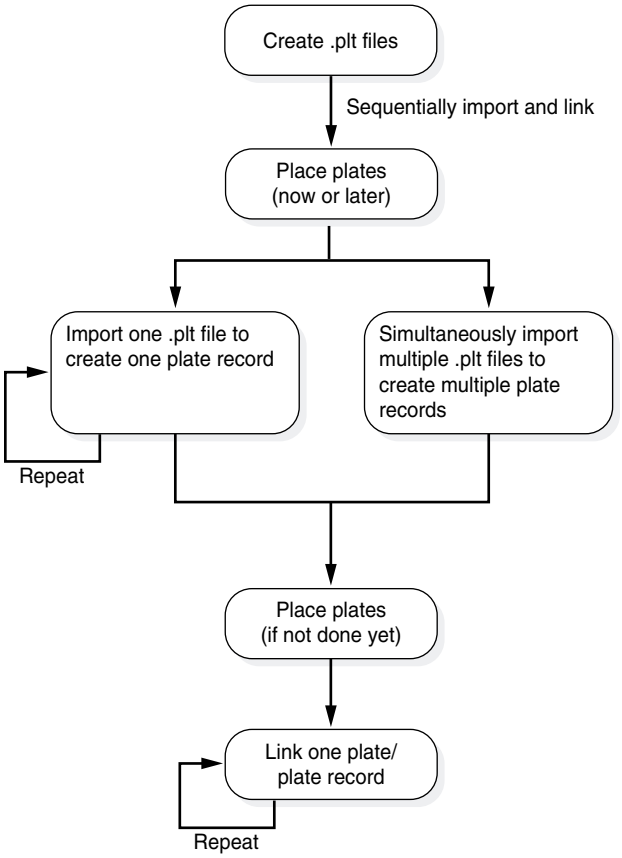
Importing Tab-Delimited Text Files and Linking Plate Records

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

In general, the steps for importing, placing, and linking are summarized in the diagram below.



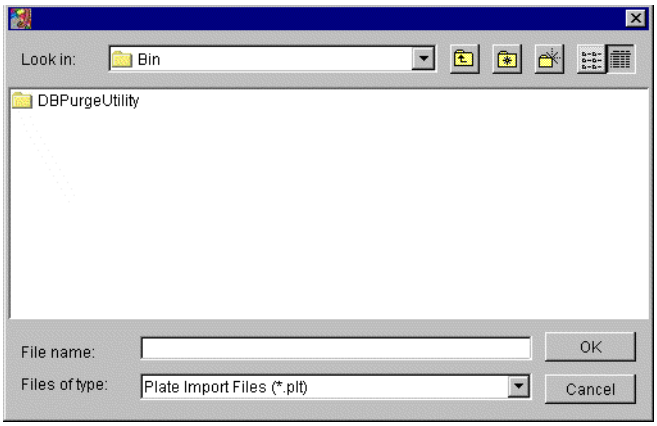
Sequentially Importing and Linking a Plate Record

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, you can import many tab-delimited text files at once.

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

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>In the Plate View page of the 3100 and 3100-Avant Data Collection software, click Import.

This opens an untitled browser dialog box.

 
</td></tr>
<tr><td>2</td><td>Navigate to the directory location of the plate file(s) (.plt) that you want to import and link.

<table>
<tr><th>If you want to create...</th><th>Then...</th></tr>
<tr><td>a single plate record</td><td>Select the .plt file.</td></tr>
<tr><td>more than one plate record</td><td>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.</td></tr>
</table></td></tr>
<tr><td>3</td><td>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.</td></tr>
<tr><td>4</td><td>Review the plate records in the plate editor.</td></tr>
<tr><td>5</td><td>Link the plate record to the plate.</td></tr>
</table>

Deleting Plate Records and Run Data

Introduction

Delete the plate records and run data when the used space on drive E is more than 8 GB.

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.

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: Cleanup Database Utility” on page 5-4.

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

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 D:Appliedbio\3100\DataExtractor or D:Appliedbio\3100-Avant\DataExtractor and verifying all sample files for all runs performed are there.

Deleting Individual Plate Records

To delete individual plate records:

Step	Action
1	Click the **Plate View** tab in the 3100 and 3100-Avant 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**. **Note** If you have created, linked or edited plates after runs have been deleted, the deleted runs will be rescheduled.

System Management and Networking 5

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

Storing Run Data

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	Approximate Data Storage Space
Processed frame data	In the instrument database E drive of the local computer workstation	100 MB for a 2.5-h run
ABIF sample file	On the local or networked hard drive D 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\3100\Data Extractor or D:\AppliedBio\3100-Avant\Data Extractor	♦ 250 KB per sample file for a ABI PRISM® 3100 POP-4™ polymer sequencing analysis run ♦ 210 KB per sample file for a ABI PRISM® 3100 POP-6™ polymer fragment analysis run ♦ 300 KB per sample file for a ABI PRISM® 3100 POP-4™ polymer fragment analysis run
Sequence Collector data	Sequence Collector database on another networked computer	–

Recovering Data: Extractor Utility

Function

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

Extractor Utility's re-extracted data will go to either:

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

Note Re-extracted data can also be stored to Sequence Collector if set up.

Note View the *xx*_analysis.log or the *xx*_extraction.log file to see if the extraction completed successfully.

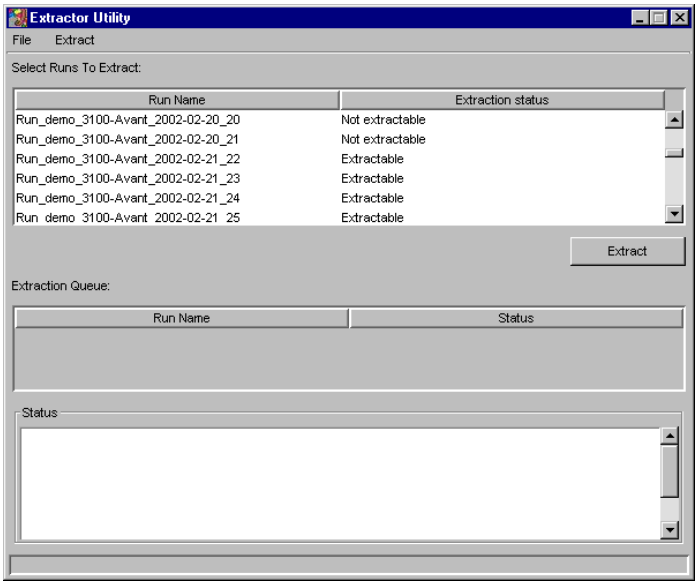
Selecting and Queuing Runs for Extraction

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

To select and queue runs for extraction:

Step	Action
1	Verify that the OrbixWeb™ Daemon and AEServer are running.
2	Quit the Data Collection software. **Note** The Extractor utility and Data Collection cannot run simultaneously.

To select and queue runs for extraction: *(continued)*

Step	Action
3	a. Click the **Start** menu. b. Point to **Applied Biosystems > 3100 Utilities > Extractor Utility** or **Applied Biosystems > 3100-Avant Utilities > Extractor Utility** 
4	Select a run or runs to extract. **Note** Do not select runs with Not extractable status.
5	Click **Extract**. The data will be extracted to the location defined in your preferences or the default location. For the 3100 system: D:\AppliedBio\3100\Data Extractor\Extracted Runs For the 3100-Avant system: D:\AppliedBio\3100-Avant\Data Extractor\Extracted Runs

Preferences

You may set the same preferences as in the data collection software by going to Extract > Preferences in the Extractor Utility.

Deleting Processed Frame Data: Cleanup Database Utility

Function

The Cleanup Database utility deletes the processed frame data and all associated run information stored in the 3100 or 3100-Avant 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.

Note The utility defragments the E partition.

File Name and Directory

The Cleanup Database utility is named CleanUpDB.bat and is located in either following directory:

- D:\AppliedBio\3100\Bin
- D:\AppliedBio\3100-Avant\Bin

When to Perform

You will be prompted by the software to run the Cleanup Database utility when the database is approximately 75% full.

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 that OrbixWeb Daemon and AE server are running.
2	Quit the 3100 or 3100-Avant Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\3100\Bin or D:\AppliedBio\3100-Avant\Bin

To delete processed frame data using the Cleanup Database utility: *(continued)*

Step	Action
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.

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 4-28.

Importing: Method Import Utility

Function

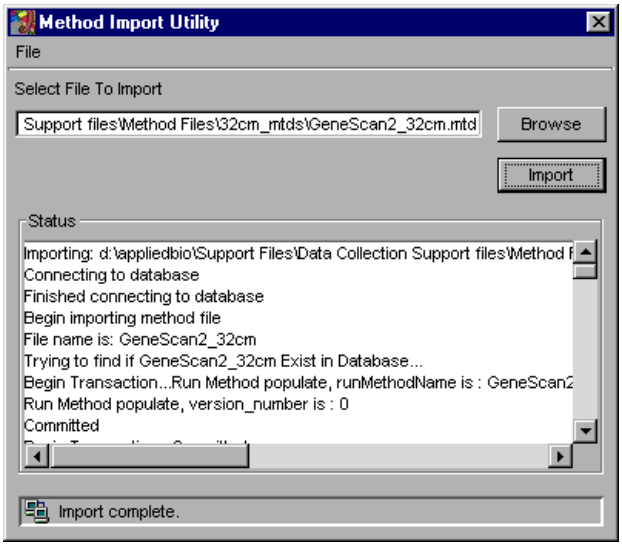
Method files contain the parameters that define the run conditions (along with the SCPI commands that direct the operation of the instrument).

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

Importing a Method

An application replaces editing and running the MethodImportUtility.bat batch file.

To import a method:

Step	Action
1	Ensure OrbixWeb Daemon is running.
2	Quit the data collection software. **Note** The method import utility and data collection software cannot run simultaneously.
3	Navigate to the following location: D:\AppliedBio\3100\Bin or D:\AppliedBio\3100-Avant\Bin
4	Open the **MethodImportUtility.bat** file. 
5	Click **Browse** and locate the method file you want to import into the database. **Note** All method files have an .mtd extension.
6	Click **Import**.
7	View the results in the Status section.

Removing Run Modules from the Instrument Database: 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\3100\Bin or D:\AppliedBio\3100-Avant\Bin

Removing Run Modules

To remove run modules using the utility:

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

Reinitializing the Instrument Database: 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\3100\Bin or D:\AppliedBio\3100-Avant\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 and AE are running.
2	Quit the 3100 or 3100-Avant Data Collection software.
3	Using Windows NT Explorer, navigate to the following directory: D:\AppliedBio\3100\Bin or D:\AppliedBio\3100-Avant\Bin
4	Locate and double-click **InitDB.bat.**
5	Locate and double-click **CreateIndex.bat.**
6	Restart the computer.

Networking Options

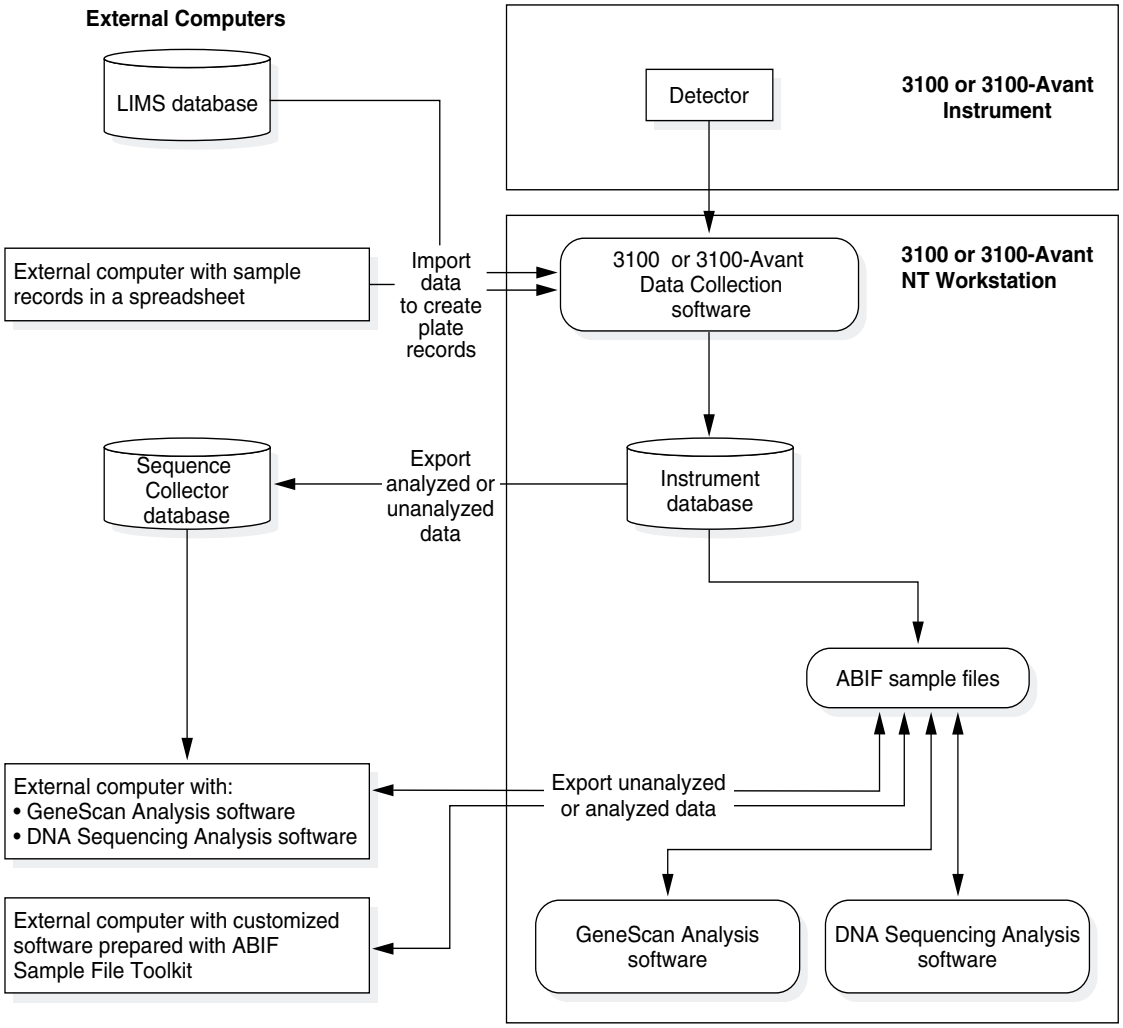
Introduction

You have the option of using the ABI PRISM® 3100 or 3100-Avant Genetic Analyzer as a stand-alone system. However, you will achieve optimal performance by integrating the 3100 or 3100-Avant Genetic Analyzer into your existing laboratory data flow system. The 3100 and 3100-Avant systems have 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 and 3100-Avant Data Collection software.

Overview Diagram

The diagram below 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 and 3100-Avant 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 LIMS database becomes automatic. Those plate records can then be exported from LIMS and imported into the 3100 or 3100-Avant Data Collection software.

Sequence Collector Option

With the Sequence Collector database system, data is collected on the computer workstation and written to a Sequence Collector database on a networked server using Auto Extractor. The data can later be 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 Sequence Collector 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 Sequence Collector 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 and 3100-Avant Genetic Analyzer fully support 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 or 3100-AvantUser 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 software, the person logged in as 3100User or 3100-AvantUser must be a member of the Administrators group.

Network Interface Cards

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

- On the motherboard, which is connected to the instrument
- 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 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" or "3100-AvantUser." This will break the connection with the 3100 or 3100-Avant Data Collection software and make the software inoperable.

To view 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 3100 or 3100-Avant instrument serial number.

To see the computer name and network domain:

Step	Action
1	Select **Start > Settings > Control Panel**.
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

Minimum Requirements

The minimum requirements for running 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.

Troubleshooting 6

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

Instrument Startup

Troubleshooting 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. Select **Start** > **Programs** > **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.
Instrument does not respond to commands immediately after closing the doors.	Autosampler reinitializes its location.	Wait for the autosampler to home before continuing.

Troubleshooting Instrument Startup *(continued)*

Observation	Possible Cause	Recommended Action
Auto analysis did not occur.	The AE server was not launched first.	Launch AE server.
	Auto analysis was not set in Preferences.	Select Auto analysis.

Spatial Calibration

Troubleshooting Spatial Calibrations

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. **⚠ WARNING CHEMICAL HAZARD.** **Methanol** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, and central nervous system depression and blindness. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
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

Troubleshooting Spectral Calibrations

Observation	Possible Cause	Recommended Action
No signal.	Incorrect preparation of sample.	Replace samples with fresh samples prepared with fresh Hi-Di™ formamide. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	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.
Data Error - One or more peaks fall below the minimum required amplitude of 750.	One or more peaks fall below the minimum required amplitude of 750.	Rerun the spectral standards, and if necessary, increase the amount of spectral standard added.
Spikes in the data.	Expired polymer.	Replace the polymer with a fresh lot using the Change Polymer Wizard. **⚠ WARNING CHEMICAL HAZARD.** **POP-4 polymer and POP-6** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	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

Troubleshooting 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. 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. **⚠ WARNING CHEMICAL HAZARD. POP-4 polymer and POP-6** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Possible contaminant in the polymer path.	Wash the polymer block with hot water. Pay 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. **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. **⚠ WARNING CHEMICAL HAZARD. POP-4 polymer and POP-6** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Troubleshooting Run Performance *(continued)*

Observation	Possible Cause	Recommended Action
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 Hi-Di formamide. **⚠ WARNING CHEMICAL HAZARD.** **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Pipetting error; not enough sample.	Increase the amount of DNA added.
		Recalibrate the pipets.
	Sample has high salt concentration.	Dilute in high-quality water.
		Desalt using a column purification method.
	Insufficient mixing.	Vortex the sample thoroughly, and then centrifuge the tube to condense the sample to the bottom of the tube.
	Autosampler out of calibration.	Check the injection with 20-µL samples. If the injection is OK, recalibrate the autosampler using the Autosampler Calibration Wizard. Pay particular attention to the Z-axis.
	Weak amplification of DNA.	Reamplify the DNA.
		Check DNA quality.

Troubleshooting Run Performance *(continued)*

Observation	Possible Cause	Recommended Action
Elevated baseline.	Possible contaminant in the polymer path.	Wash the polymer block with hot water. Pay 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. **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. **⚠ WARNING CHEMICAL HAZARD. POP-4 polymer and POP-6** cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	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. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Poor quality or breakdown of polymer.	Use a fresh lot of polymer.
	Capillary array used for more than 100 injections.	Replace with new capillary array.
	Degraded formamide.	Prepare fresh Hi-Di formamide and re-prepare samples. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	High salt concentration in samples.	Use a recommended protocol for salt removal. Dilute salts with water.
Poor resolution in some capillaries.	Insufficient filling of capillary array.	Refill the capillary 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.

Troubleshooting Run Performance *(continued)*

Observation	Possible Cause	Recommended Action
No current.	Poor quality water.	Use only high-quality ultra-pure water.
	Water placed in buffer reservoir position 1.	Replace with fresh 3100 1X running buffer.
	Not enough buffer in anode reservoir.	Add buffer up to the fill line.
	Buffer too dilute.	Prepare 1X running buffer. Add 3 mL 10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	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.
Elevated current.	Decomposed polymer.	Open fresh lot of polymer and store at 4 °C.
	Incorrect buffer dilution.	Prepare 1X running buffer. Add 3 mL 10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Arcing in the gel block.	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.

Troubleshooting Run Performance *(continued)*

Observation	Possible Cause	Recommended Action
Fluctuating current.	Bubble in polymer block.	Pause the run, check the polymer path for bubbles, and remove them if present.
	A slow leak may be present in the system.	Check polymer blocks and syringes for leaks. Tighten all fittings.
	Incorrect buffer concentration.	Prepare 1X running buffer. Add 3 mL 10X Genetic Analyzer Buffer with EDTA to 27 mL deionized water. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Not enough buffer in anode reservoir.	Add buffer up to the fill line.
	Clogged capillary.	Refill capillary array and check for clog.
	Arcing	Check for moisture in and around the septa, the reservoirs, the oven, and the autosampler.
Poor performance of capillary array used for fewer than 100 runs.	Poor quality samples, possible cleanup problems.	Desalt samples using a recommended purification protocol.
	Poor quality formamide.	Prepare fresh Hi-Di formamide and re-prepare samples. **⚠ WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Incorrect buffer.	Use 10X Genetic Analyzer Buffer with EDTA to prepare 1X running buffer. **⚠ CAUTION CHEMICAL HAZARD. 10X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
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.

Troubleshooting Run Performance *(continued)*

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

Software

Troubleshooting Software

Observation	Possible Cause	Recommended Action
An imported run module file does not import. There is no error message.	The file name is longer than 32 characters.	Rename the run module file using less than 32 characters.

Technical Support

A

Services and Support

Applied Biosystems Web Site

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

http://www.appliedbiosystems.com

and click the link for services and support.

At the services and support page, you can:

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

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

Part Numbers

B

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 computer workstation	3100-01
ABI PRISM® 3100-Avant Genetic Analyzer with computer workstation	3100-AVANT
Printers (sold only with ABI PRISM instruments)	
HP Deskjet 990CXI	4328881

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

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

DNA Sequencing Reagents and Consumables

Description	Part Number
ABI PRISM® 3100 POP-6™ polymer	4316357
ABI PRISM® 3100 POP-4™ polymer	4316355
ABI PRISM® 3100 capillary array, 50-cm	4315930
ABI PRISM® 3100 capillary array, 36-cm	4315931
ABI PRISM® 3100 capillary array, 80-cm	4319899
ABI PRISM® 3100-Avant capillary array, 50-cm	4333466
ABI PRISM® 3100-Avant capillary array, 36-cm	4333464
ABI PRISM® 3100-Avant capillary array, 80-cm	4333465
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® 3100 capillary array, 22-cm	4319898
ABI PRISM® 3100-Avant capillary array, 36-cm	4333464
ABI PRISM® 3100-Avant capillary array, 22-cm	4333463
Genetic Analyzer Buffer with EDTA (10X)	402824
Matrix Standard Set DS-02 (dR110, dR6G, dTAMRA™, dROX™, LIZ™)	4323014
Matrix Standard Set DS-30 (6FAM™, HEX, NED, ROX™)	4316100
Matrix Standard Set DS-32 (5-FAM, JOE, NED, ROX)	4323018
Matrix Standard Set DS-33 (6FAM, VIC, NED, PET™, LIZ™)	4323016
ABI PRISM® 3100 GeneScan™ Installation Standard DS-30	4316144
Hi-Di™ Formamide, 25-mL bottle	4311320

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

Description	Part Number
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 and ABI PRISM® 3100-Avant Genetic Analyzer User Reference Guide	4335393
ABI PRISM® 3100 Genetic Analyzer Sequencing Chemistry Guide v. 3.7	4315831
ABI PRISM® GeneScan® Analysis v. 3.7 NT User Guide	4308923
ABI PRISM® 3100 Genetic Analyzer User Guide	4334785
ABI PRISM® Sequencing Analysis Software v. 3.7 NT User Guide	4308924
ABI PRISM® 3100-Avant Genetic Analyzer User Guide	4333549
ABI Prism® 3100 Genetic Analyzer Operator Training CD	432559

Index

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