Applied Biosystems

ABI PRISM®

3100/3100-*Avant*

Genetic Analyzers

User Guide

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

NOTICE TO PURCHASER:

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

NOTICE TO PURCHASER

The purchase price of this ABI PRISM 3100/3100-Avant Genetic Analyzer includes a grant of a limited, non-transferable license under U.S. patent claims and method claims of its foreign counterparts, and under U.S. patent claims and element claims of its foreign counterparts, to use this particular instrument for electrophoresis methods employing fluorescence as a means of detection. No other licenses or rights are hereby conveyed either expressly, by implication, or estoppel including, but not limited to, any claims to a composition.

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Contents

Preface

How to Use This Guide

Audience

This manual is written for principle investigators and laboratory staff who are planning to operate and maintain the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers.

Assumptions

This guide assumes the following background:

- Familiarity with Microsoft® Windows® 2000 operating system.
- Knowledge of general techniques for handling DNA samples and preparing them for electrophoresis.
- A general understanding of hard drives and data storage, file transfers, and copying and pasting.

If you want to integrate the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers into your existing laboratory data flow system, you need networking experience.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File** > **Open** > **Spot Set**.

  Right-click the sample row, then select **View Filter** > **View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

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

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page xii.

How to Obtain More Information

Related Documentation

The following related document is shipped with the system:

ABI PRISM® 3100/3100-Avant Genetic Analyzers Quick Reference Card– Contains commonly used information and a flowchart of the ABI PRISM® 3100/3100-*Avant* Data Collection software.

Note: For additional documentation, see "Support" on the back cover.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

Safety and EMC Compliance Information

This section includes the following topics:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

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

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

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

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

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems instruments* (see “Safety Symbols” on page xiii).

Examples

The following examples show the use of safety alert words:

IMPORTANT! You must create a separate a Sample Entry Spreadsheet for each 96-well microtiter plate.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. **Formamide**. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xiv). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	**ATTENTION** Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING 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.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
DANGER Class 3B laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	**DANGER** Class 3B rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.
DANGER Class 3B laser radiation when open. Avoid direct exposure to laser beam.	**DANGER** Class 3B rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) laser radiation present when open and interlock defeated. Do not stare directly into the beam	**DANGER** de Class 2(II) rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) laser radiation present when open. Do not stare directly into the beam.	**DANGER** de Class 2(II) rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) LED when open and interlock defeated. Do not stare directly into the beam.	**DANGER** de Class 2(II) LED en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.

English	Francais
DANGER Class 2(II) LED when open. Do not stare directly into the beam.	**DANGER** de Class 2(II) LED en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page xvi.

Chemical Safety

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs” on page xvi.)

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, 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 Safety

Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

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

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- 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.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Profiles

A waste profile for the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer is provided in the *ABI PRISM® 3100 and 3100-Avant Genetic Analyzer Site Preparation Guide.*

Waste profiles show the percentage compositions of the reagents in the waste stream generated during installation and during a typical user application, even though the typical application may not be used in your laboratory.

The waste profiles help you plan for the handling and disposal of waste generated by operation of the instrument. Read the waste profiles and all applicable MSDSs before handling or disposing of chemical waste.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- 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, and/or national regulations.

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

Electrical Safety

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power

ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer system has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Moving Parts

PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

PHYSICAL INJURY HAZARD. Do not operate the instrument without the arm shield in place. Keep hands out of the deck area when the instrument is spotting.

Solvents and Pressurized Fluids

WARNING **PHYSICAL INJURY HAZARD.** Always wear eye protection when working with solvents or any pressurized fluids.

WARNING **PHYSICAL INJURY HAZARD.** To avoid hazards associated with high-pressure fluids in polymeric tubing:

- Be aware that PEEK™ tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.
- Always wear eye protection when in proximity to pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use PEEK tubing that has been severely stressed or kinked.

Laser Safety

Laser Classification

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer uses an argon laser. Under normal operating conditions, the instrument laser is categorized as a Class 3B laser. When safety interlocks are disabled during certain servicing procedures, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer complies with 21 CFR, 1040.10 and 1040.11, as applicable.

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer has been tested to and complies with the “Radiation Control for Health and Safety Act of 1968 Performance Standard CFR 1040.”

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzer has been tested to and complies with standard EN60825-1, “Radiation Safety of Laser Products, Equipment Classification, Requirements, and User’s Guide.”

Laser Safety Requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Applied Biosystems Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating (during service with safety interlocks disabled), you may be exposed to laser emissions in excess of the Class 3B rating.
- Do not remove safety labels or disable safety interlocks.

Additional Laser Safety Information

Refer to the user documentation provided with the laser for additional information on government and industry safety regulations.


WARNING **LASER HAZARD.** Lasers can burn the retina causing permanent blind spots. Never look directly into the laser beam. Remove jewelry and other items that can reflect the beam into your eyes. Do not remove the instrument top or front panels. Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the top or front panels are removed for service.


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

Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring 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.

To minimize musculoskeletal and repetitive motion risks:

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

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 3101-1, “Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements.”

This instrument has been tested to and complies with standard CSA 1010.1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements” and EN 61010-2-010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Class B), “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”

Laboratory Space Required

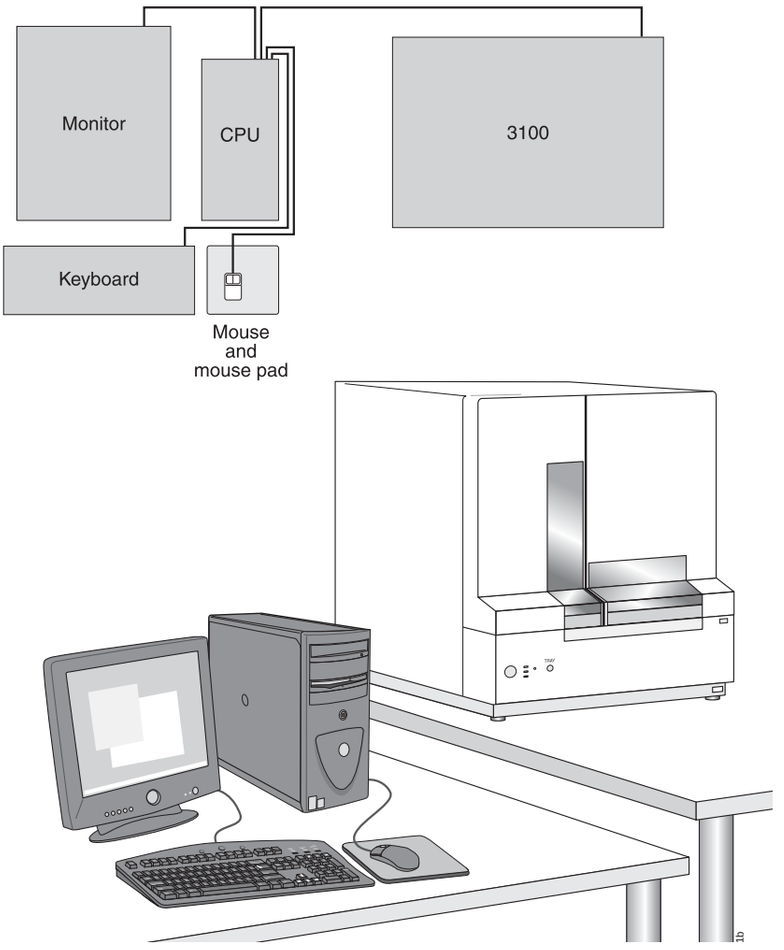
Dimensions and Weight

The ABI PRISM 3100 and 3100-*Avant* Genetic Analyzers and computer have the following dimensions:

Component	Width	Depth	Height	Weight
3100/3100-*Avant* Genetic Analyzer, crated	94 cm (37 in.)	70 cm (27.5 in.)	105 cm (41.5 in.)	142 kg (313 lb)
3100/3100-*Avant* Genetic Analyzer, uncrated	74 cm (29.2 in.)	54.8 cm (21.6 in.)	81 cm (32 in.)	120 kg (265 lb)
3100/3100-*Avant* Genetic Analyzer with all required clearances	148.6 cm (58.5 in.)	67 cm (26.4 in.)	111 cm (44 in.)	–
Computer CPU	21 cm (8.3 in.)	44 cm (17.4 in.)	44 cm (17.4 in.)	10 kg (25 lb)
Monitor	42 cm (16.6 in.)	53 cm (20.9 in.)	44 cm (17.4 in.)	18 kg (40 lb)

Typical Laboratory Layout

A typical laboratory layout is shown below.



Laboratory Environmental Requirements

Altitude

This instrument is for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

Temperature and Humidity

The laboratory temperature should be maintained between 15 to 30 °C (59 to 86 °F), and remain stable at the set temperature (within ± 2 ˚C). The instrument can tolerate between 20 to 80% relative humidity. Avoid placing the instrument adjacent to heaters or cooling ducts, or in direct sunlight.

Pollution Degree and Overvoltage Protection

The installation category (overvoltage category) for this instrument is II, and it is classified as portable equipment. The instrument has a pollution degree rating of 2 and may be installed in an environment that has nonconductive pollutants only.

Electrical Requirements

Power

The following table specifies the electrical operating range for various parts of the world:

IMPORTANT! In Japan, the unit must have a dedicated 200-volt outlet. The unit will not operate properly with a 100-volt outlet.

Location	Voltage (VAC)	Frequency	Maximum Current (A)
Japan	200 to 229 230 to 240	50/60 Hz ±1%	11.2
USA/Canada		60 Hz ±1%	
Europe		50 Hz ±1%	
Australia		50 Hz ±1%	

Power Line

The electrical receptacle must have a dedicated 2.5 kVA power line and ground.

Electrical Outlets

The instrument requires a 30 amp receptacle to match one of the two power cord configurations that ship with the system. See the “Power Cords” section below.

The electrical receptacle must be located within 3 m (10 ft) of the back of the instrument. Do not use extension cords.

IMPORTANT! Despite the apparent dissimilarity between the capacity requirements for the power line (2.5 KVA) and the receptacle (30 A), these are the system requirements.

Power Rating

This instrument is rated for a maximum input of 2500 VA.

Power Cords

In the USA, Canada, and Japan, the instrument is supplied with a detachable cord equipped with a Nema L6-30P plug.

In Europe and Australia, the instrument is supplied with a detachable electrical cord equipped with an IEC 309 plug.

The computer, CPU and monitor, can be plugged into any standard electrical receptacles after they have been configured for the proper voltage.

Grounding

Certain types of electrical noise are greatly exaggerated by poor or improper electrical ground connections. To prevent these problems, it is very important to have a dedicated ground line between the instrument and the building's main electrical service.

Power Line Regulator

In areas where the supplied power is subject to voltage fluctuations that exceed ±10% of the nominal voltage (see the section, "Power," above) a power line regulator may be required. High or low voltages can have adverse effects on the electronic components of the instrument.

Voltage Fluctuations

Main supply voltage fluctuations not to exceed +/- 10% of the nominal voltage.

Voltage Spikes

Short-duration, high-voltage spikes often cause random failures in microprocessor-controlled instrumentation. These spikes can be caused by other devices using the same power source (refrigerators, air conditioners, and centrifuges) or by outside influences such as lightning. A dedicated line and ground between the instrument and the building's main electrical service will prevent such problems.

If your environment contains devices that are electrically noisy, or you are in an area with frequent electrical storms, a line conditioner with a recommended capacity of 1.0 kVA will enhance the reliability of your system.

Power Outages

The instrument has not been designed to continue with a run after power failure. A run that has been interrupted by power failure must be restarted. For this reason, we recommend that you protect against power outage by installing an uninterruptible power supply (UPS) with an output capacity of 2.5 kVA (30 minutes at 2.4 kVA after full charge). We recommend the UPS from Franek Company. The order number for the appropriate UPS is FT1-B3100-DA. Franek's phone number is 1-800-326-6480, their fax number is 714-554-6957, their website is www.Franek-tech.com, and their E-mail address is sales@Franek.tech.com.

Preparing the Instrument

1

This chapter covers:

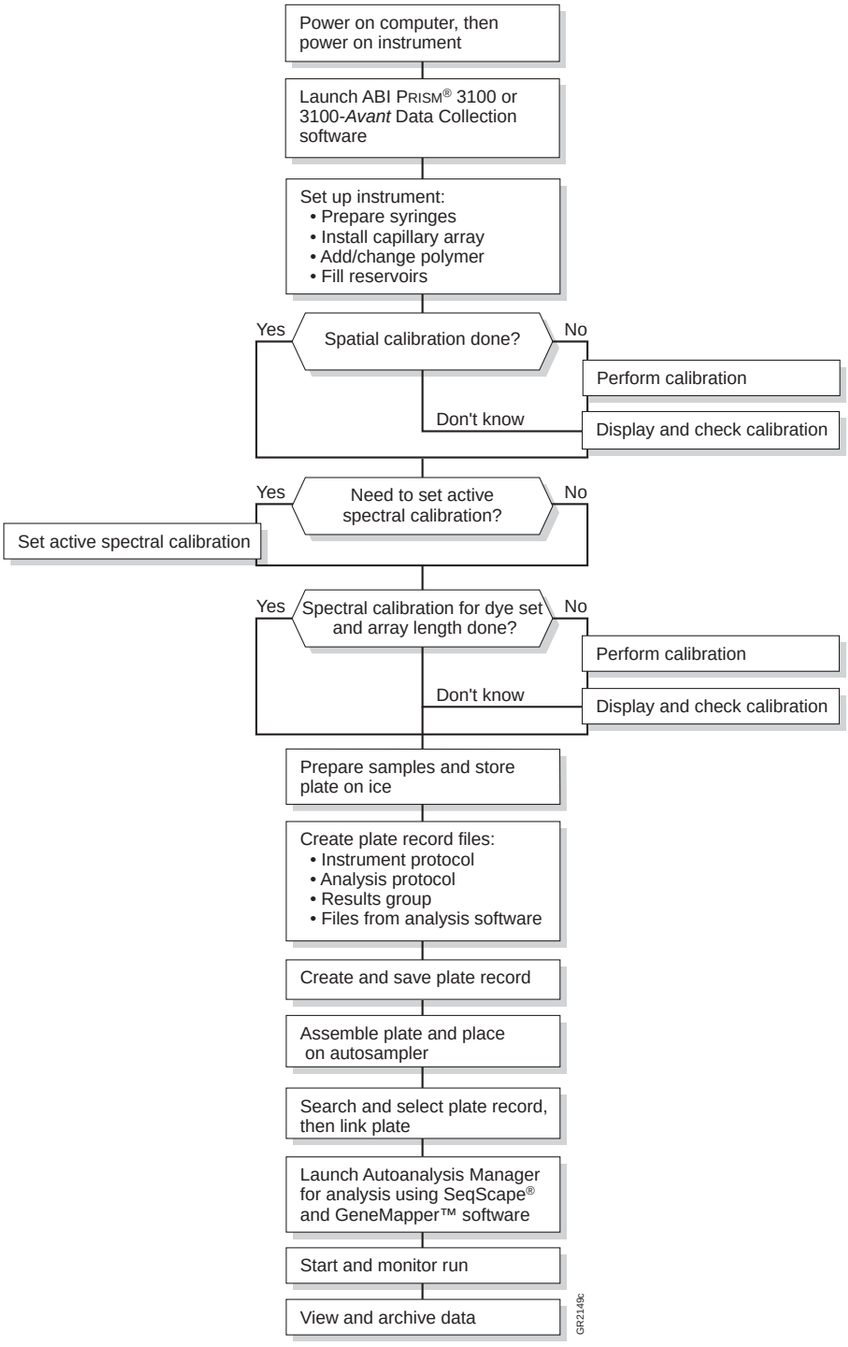
Notes

Notes

Introduction

A Typical Run

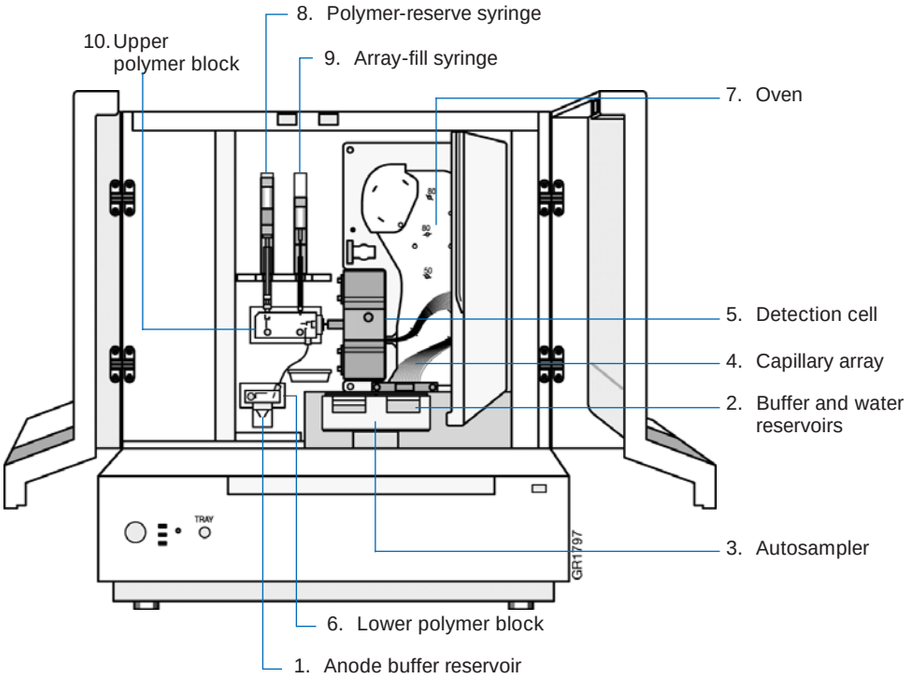
This flowchart provides an overview of the steps required to perform a run on the ABI PRISM® 3100/3100-*Avant* Genetic Analyzers.



Notes

Instrument Description

The following diagram shows inside the instrument's doors:



Instrument Components

Number and Part	Function
1. Anode buffer reservoir	Contains 9 mL of 1X running buffer.
2. Buffer and water reservoirs (four)	Contains 16 mL of 1X running buffer or water.
3. Autosampler	Holds the sample plates and reservoirs and moves to align the samples, water, or buffer with the capillaries.
4. 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.
5. Detection cell	Holds the capillaries in place for laser detection.
6. Lower polymer block	Contains the anode electrode. The anode buffer reservoir connects to this block.
7. Oven	Maintains uniform capillary array temperature.
8. Polymer-reserve syringe	Contains and dispenses the polymer that fills the polymer blocks and the array-fill syringe. A 5-mL syringe.
9. Array-fill syringe	Contains and dispenses the polymer under high pressure to fill the capillaries. A 250-µL syringe.
10. Upper polymer block	Connects the two syringes and the detection end of the capillary array.

Notes

Summary of Applications

Application	Polymer	Capillary Length	Run Time	Throughput (24 hrs)		Resolution	Performance
				3100-*Avant*	3100		
High throughput, small size fragment analysis	POP-4	22 cm	20 min	5,760* GT	23,040* GT	400 bp	0.50 SD†
Standard fragment analysis		36 cm	45 min	2,560* GT	10,240* GT	400 bp	0.15 SD
Ultra rapid sequencing			40 min	72,000 bp	288,000 bp	500 bp	98.5% base calling accuracy
Rapid sequencing	POP-6		60 min	48,000 bp	192,000 bp	500 bp	98.5% base calling accuracy
Standard sequencing	POP-4	50 cm	100 min	34,500 bp	138,000 bp	600 bp	98.5% base calling accuracy
Long fragment analysis			65 min	1,760* GT	7,040* GT	500 bp	0.15 SD
Standard sequencing	POP-6		2.5 hrs	23,400 bp	93,600 bp	650 bp	98.5% base calling accuracy
Long fragment analysis			90 min	1,200* GT	4,800* GT	500 bp	0.15 SD
Long read sequencing	POP-4	80 cm	3.5 hrs	22,800 bp	91,200 bp	950 bp	98.5% base calling accuracy

*20 GT/injection, †1bp resolution at 99.99% accuracy

Notes

Starting the 3100/3100-*Avant* System

Starting the Computer Workstation

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

1. Power on the monitor.

2. Power on the computer.

3. Press **Ctrl** + **Alt** + **Delete**.

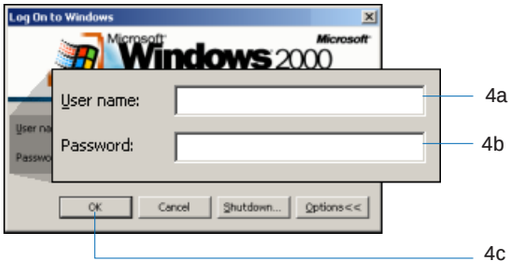
4. In the Log on to Windows dialog box:

   a. Enter the user name.

   b. If applicable, enter a password.

   Note: If the computer is connected to a network, you do not need to log on to the network before starting the instrument.

   c. Click OK.



Notes

Starting the 3100/3100-*Avant* Genetic Analyzer

1. On the instrument, ensure that the:
   - Oven door is closed and locked
   - Instrument doors are closed

   Note: If the doors are open during power on, the red failure light will illuminate.

2. On the computer, ensure that the:
   - Computer is powered on (see "Starting the Computer Workstation" on page 6)
   - Microsoft® Windows® 2000 operating system has loaded

   Note: The computer must be on and running the Windows 2000 operating system because the instrument must copy the firmware from the computer.

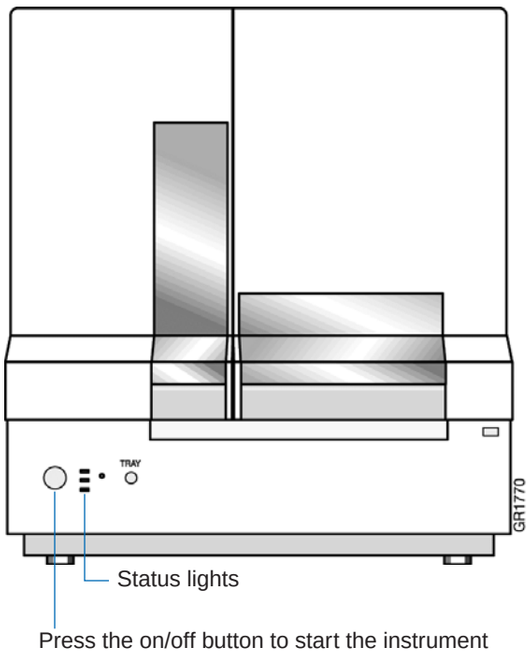
3. Turn on the instrument by pressing the on/off button on the front of the instrument.

   Note: While the instrument is booting up and performing self-checks, the yellow status light blinks.

4. Ensure the green status light is on and constant before proceeding.

   Note: If the green light does not come on, start the data collection software and look at the log. The pathway to the log is:

   E:\AppliedBiosystems\UDC\Data Collection\Log*Instrument Name*



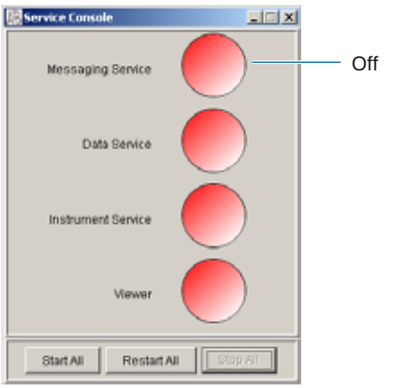
Notes

3100/3100-*Avant* Data Collection Software

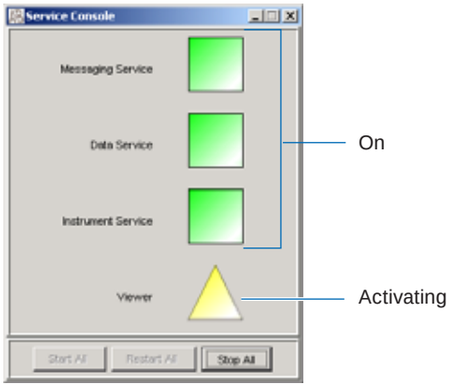
Starting the 3100/3100-*Avant* Data Collection Software

1. Select **Start** > **Programs** > **Applied Biosystems** > **Data Collection** > **Run Data Collection 3100 v2.0** or **Run Data Collection 3100-*Avant* v2.0**.

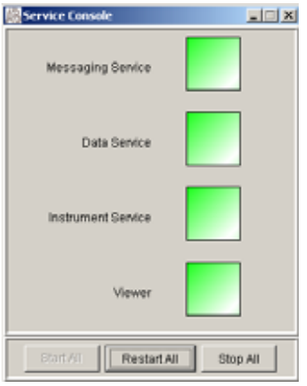
   The Service Console displays. By default, all applications are off as indicated by the red circles. However, they launch automatically with the 3100/3100-*Avant* Data Collection software.



   As each application automatically activates, the red circles (off) change to yellow triangles (activating), to green squares (on) when they are fully functional.

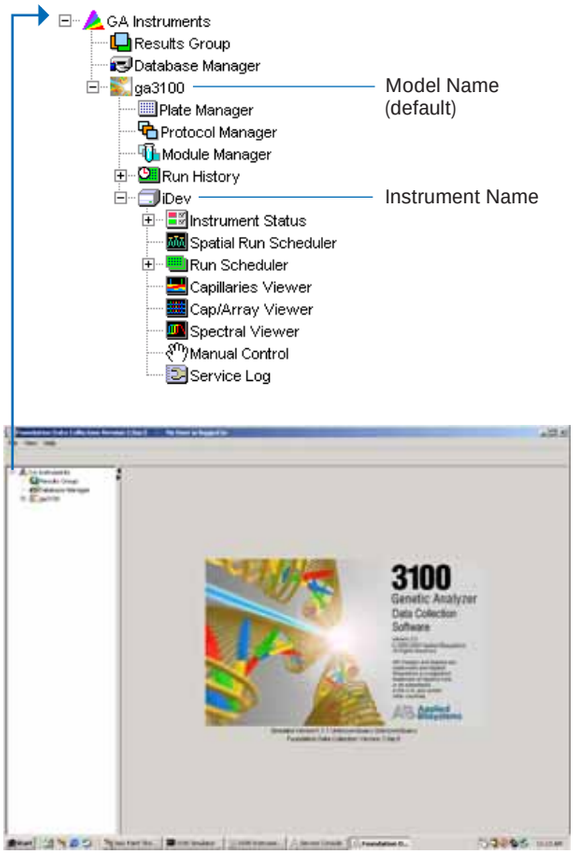


   When all the applications are running (all green squares–this could take several minutes), the Data Collection Viewer window displays.

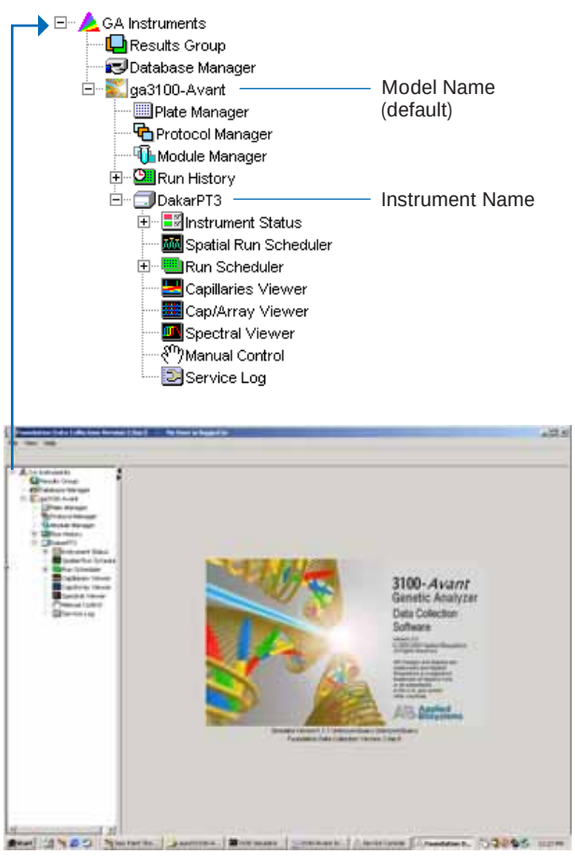


Notes

2. Click the + to expand subfolders in the left window pane. All application folders—except for Run History— are now visible and ready to access.



3100 Data Collection Viewer window



3100-*Avant* Data Collection Viewer window

Audit Trail and Access Control

The new Data Collection Software v2.0 incorporates Audit Trail and Access Control features to assist users with 21CFR Part 11 compliance. When the software is installed, the default configuration for these features are deactivated.

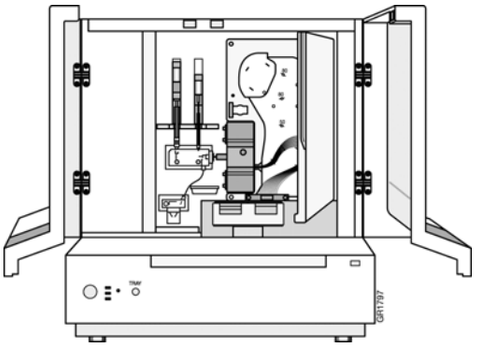
To activate these features and set up the software, refer to "Audit Trails and Access Control" on page 241.

Notes

Preparing the Instrument

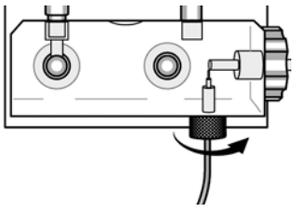
Attaching the Polymer Blocks to the Instrument

1. Open the instrument doors.



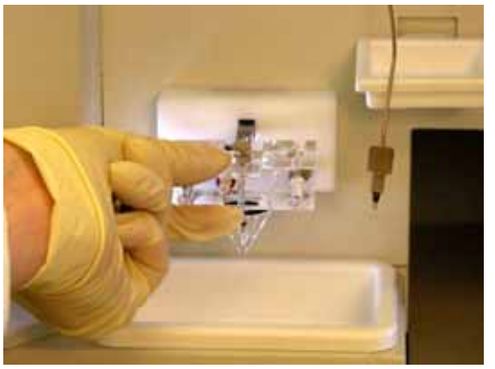
2. If necessary, clean the polymer blocks and the tubing as instructed on page 223.

3. Connect the polymer tubing to the upper polymer block by inserting one ferrule and nut into the upper polymer block and rotate counter clockwise until finger tight.



4. Push the upper polymer block onto the two guide pins on the instrument. Leave a 1-inch gap between the block and the back of the instrument.

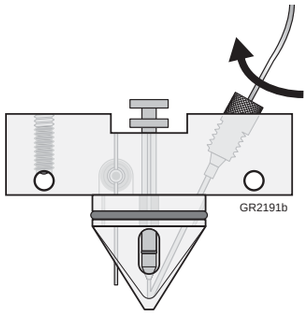
5. Install the lower polymer block. Ensure the block is pushed all the way against the instrument.



Notes

6. Connect the polymer tubing the lower polymer block by inserting the other ferrule and nut into the lower polymer block and rotate clockwise until finger tight.

   IMPORTANT! Do not overtighten.



7. Install clean drip trays, if they are not already on the instrument.

Selecting a Capillary Array

Use the tables below to select the correct capillary array length for your sequencing or fragment analysis application.

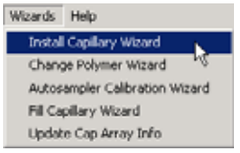
Sequencing Applications	Capillary Length (cm)
Ultra rapid sequencing	36
Rapid sequencing	36
Standard sequencing	50
Long read sequencing	80

Fragment Analysis Applications or Kits	Capillary Length (cm)
ABI PRISM® SNaPshot® Multiplex System	22
	36
• ABI PRISM® Linkage Mapping Set v2.5 • ABI PRISM® Mouse Mapping Set v1.0 • Custom oligos	22
	36
	50
• AmpFℓSTR® COfiler® Kit • AmpFℓSTR® Profiler® Plus Kit • AmpFℓSTR® Profiler Plus *ID*® Kit • AmpFℓSTR® SGM Plus® Kit • Other 4-Dye AmpFℓSTR Kits • AmpFℓSTR® Identifiler® Kit • AmpFℓSTR® SEfiler® Kit • Other 5-Dye AmpFℓSTR Kits	36

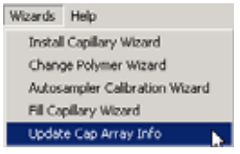
Notes

Installing or Replacing the Capillary Array

IMPORTANT! The capillary array length defined in either wizard must match the array length you are using for correct autoanalysis results.



If necessary, install a capillary array using the Install Capillary Array wizard. For instructions, see "Installing, Removing, or Replacing a Capillary Array" on page 211.



Alternatively, you can install the capillary array without using the wizard. Update the capillary array information using the Update Capillary Array Info utility. For instructions, see "Manually Installing a Capillary Array" on page 212.

IMPORTANT! If you installed or replaced an array that is a different length than the one you were using, you must reset the active spectral calibration (see page 59) or create a new spectral calibration for the dye set and array length combination (see Chapter 3, page 41).

Selecting the Polymer

There are two polymer types available for use on the 3100/3100-*Avant* system: ABI PRISM® POP-4™ and ABI PRISM® POP-6™. Use the following table to select the correct polymer for your application and capillary array length.

Polymer types:

Sequencing Applications

Sequencing Run Type	Capillary Length	Polymer
Ultra rapid sequencing	36	POP-4
Standard sequencing	50	
Long read sequencing	80	
Rapid sequencing	36	POP-6
Standard sequencing	50	

Fragment Analysis Applications

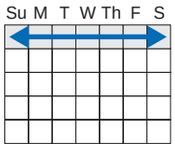
Fragment Analysis Kit	Capillary Length	Polymer
ABI PRISM SNaPshot Multiplex System	22	POP-4
	36	
• ABI PRISM® Linkage Mapping Set v2.5 • ABI PRISM® Mouse Mapping Set v1.0 • Custom oligos	22	
	36	
	50	
	50	POP-6

Notes

Fragment Analysis Applications *(continued)*

Fragment Analysis Kit	Capillary Length	Polymer
• AmpFℓSTR® COfiler® Kit • AmpFℓSTR® Profiler® Plus Kit • AmpFℓSTR® Profiler Plus *ID*® Kit • AmpFℓSTR® SGM Plus® Kit • Other 4-Dye AmpFℓSTR Kits • AmpFℓSTR® Identifiler® Kit • AmpFℓSTR® SEfiler® Kit • Other 5-Dye AmpFℓSTR Kits	36	POP-4

When to Add or Change Polymer



If syringes containing polymer are on the instrument, use the table below to determine whether to add or change the polymer before proceeding with instrument preparation.

IMPORTANT! Always replace polymer on the instrument that is older than 1 week.

If polymer on the instrument is ...	Then ...
less than 1 week old, and sufficient in quantity to complete your runs[a]	Ensure that there are no air bubbles, and then proceed with instrument preparation.
less than 1 week old, and insufficient in quantity to complete your runs	Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard (see page 207).
more than 1 week old wrong type (changing between POP-4 and POP-6 polymers)	1. Remove and clean the polymer blocks and syringes (see "Polymer Blocks" on page 223 and "Syringes" on page 216). 2. Fill the syringes and the upper polymer block with polymer by following the Change Polymer wizard (see page 207).

a. A 3100 run uses 50-80 µL of polymer and a 3100-*Avant* run uses ~20 µL of polymer.

Notes

Preparing and Installing the Syringes

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

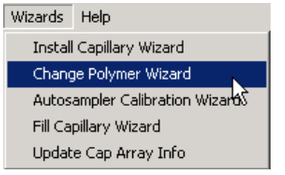
1. If necessary, clean and inspect the syringes as instructed on page 217.
2. Prime and fill the syringes as instructed on page 219.

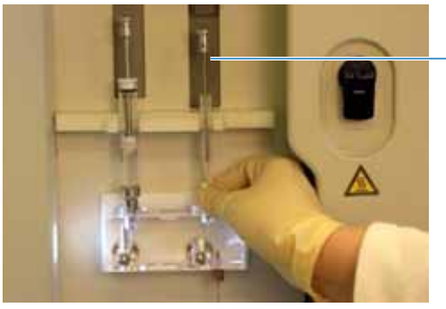
   IMPORTANT! The polymer type defined in the wizard must match the polymer you are using.

3. Install the syringes using the Change Polymer wizard. For instructions, see "Adding and Changing the Polymer" on page 207.

   IMPORTANT! Ensure that there are no air bubbles in the upper polymer block and polymer block tubing before proceeding. To remove any air bubbles, see page 228.

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.





Notes

Preparing Buffer and Filling the Reservoirs

Required Materials

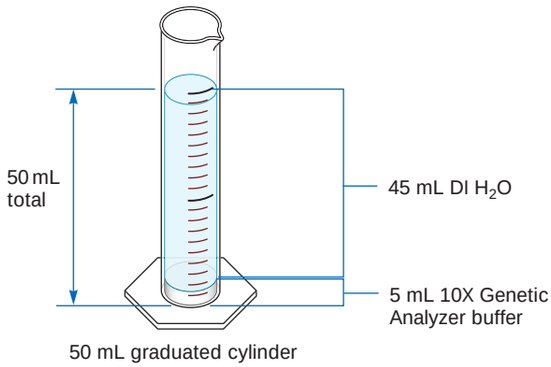
The following materials are required to prepare 1X running buffer:

- 10X Genetic Analyzer Buffer (P/N 402824)
- Quality deionized water
- 50 mL graduated cylinder

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.

Preparing Buffer for a Single Run

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

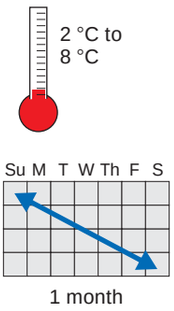
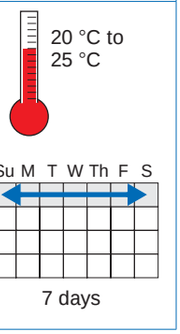


Storing the Buffer

The 1X running buffer can be stored at:

- 2 to 8 °C for up to 1 month
- Room temperature for 1 week

Buffer Storage Conditions

Option A	Option B
	

Notes

Replacing the Buffer

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

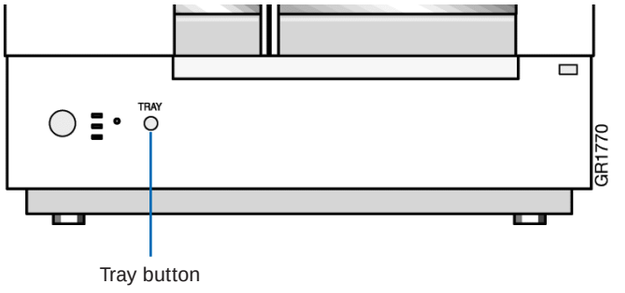
IMPORTANT! Failing to replace buffer may lead to loss of resolution and data quality.

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

Filling the Water and Cathode Buffer Reservoirs

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

1. Verify the oven and instrument doors are closed.
2. Press the Tray button on the outside of the instrument to bring the autosampler to the forward position.
3. Wait until the autosampler has stopped moving, then open the instrument doors.

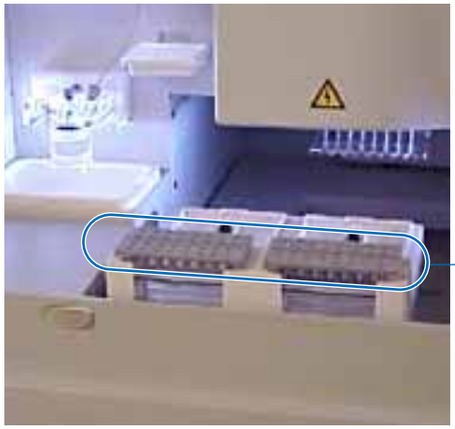


Notes

4. Remove the cathode buffer reservoir and water reservoirs from the instrument.

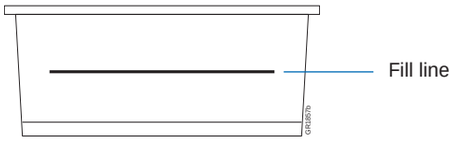
5. Dispose of remaining fluids and rinse out the reservoirs with deionized water.

   Note: The waste is very dilute; however, you should follow your company's waste disposal practices for appropriate disposal procedures.



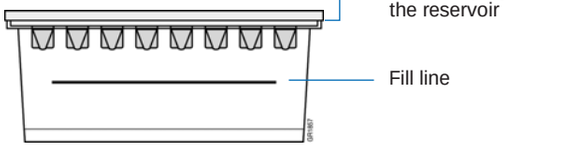
6. Rinse the cathode reservoir with 1X running buffer, and then fill to the line with 1X running buffer (about 16 mL).

7. Fill the three water reservoirs to the line with quality deionized water (about 16 mL).

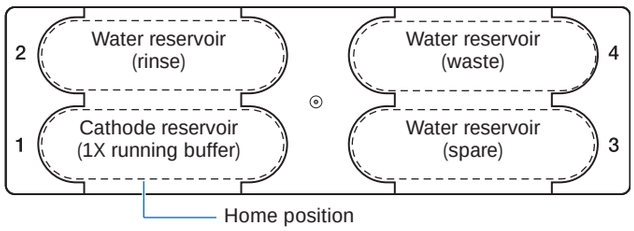


8. Place a clean reservoir septa on each reservoir, and dry the outside of the reservoirs using a lint-free wipe.

CAUTION Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips.



9. Place the reservoirs into position on the autosampler as shown below.



10. Close the instrument doors.

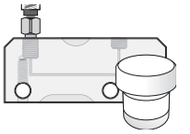
    Note: Closing the doors returns the autosampler to the last known position, placing the tips of the capillaries in water or buffer.

Notes

Filling the Anode Buffer Reservoir

Change the anode buffer:

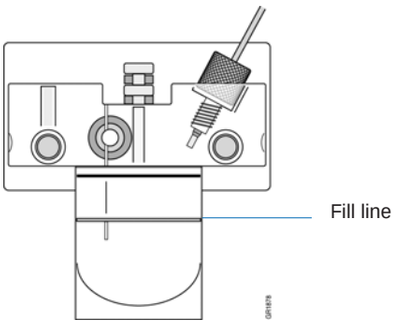
- Every 24 hours
- Before each run or batch of runs
- Every time you fill the polymer block with new polymer

Change the Anode Buffer		
 Every 24 hours	 Before each run or batch of runs	 Every time you add new polymer

1. Remove the anode buffer reservoir by firmly pulling down and twisting slowly.
2. Discard the used buffer appropriately.
3. Clean and rinse the reservoir with deionized water, and then rinse with buffer.

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

   Note: The meniscus should line up with the fill line.



5. Put the anode buffer reservoir on the instrument.
6. If the reservoir fills with fluid, repeat this procedure to discard and replace the running buffer.

   Note: The reservoir could fill during bubble clearing of the polymer blocks.

Notes

Plate Mapping

Introduction

Samples are scheduled for injection based on plate configuration to help arrange samples with high priority, run a plate that is partially filled and/or run a multiple application plate.

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

1. The order the plates are linked (3100 instrument only), see page 155 for linking plate information.
2. The priority value for samples in the plate record.

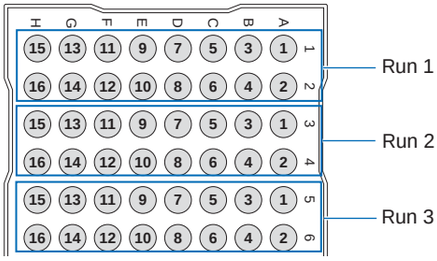
If all priorities are set to 100 (default), runs are scheduled as outlined below. Refer to "Run Priority Scheduling" on page 262 for information on how a change in the priority value changes run scheduling.

96-Well Plate Mapping

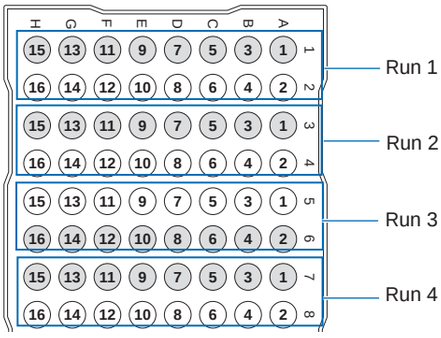
3100 Instrument

For a 96-well plate, injections are made from every well in two consecutive rows, starting with an odd row. A full 96-well plate requires six runs to inject all samples.

In the following example of a 96-well plate, the gray circles represent samples and the number in the well indicates capillary number. It takes three runs to inject 48 samples.



Below is an example of incorrect sample placement. To inject 32 samples using this configuration would require four runs.

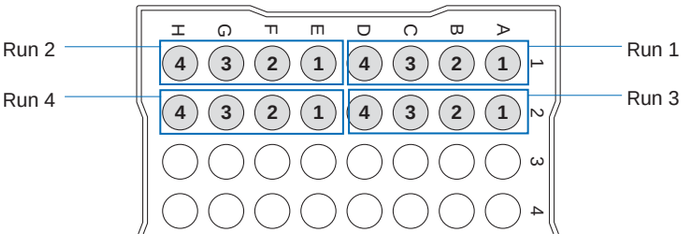


Notes

3100-*Avant* Instrument

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

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

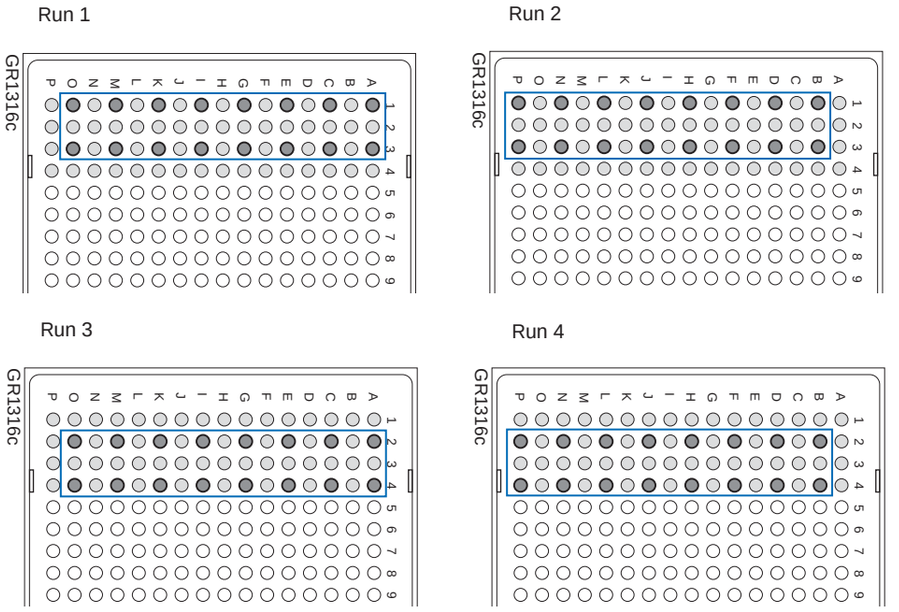


384-Well Plate Mapping

3100 Instrument

For a 384-well plate, injections are made from every other well and every other row. A full plate of 384 sample requires 24 runs to inject all samples once.

Below is an example of the injection pattern for the first four injections, starting with well A01. The light gray circles represent samples and the dark gray circles indicate the injection pattern.



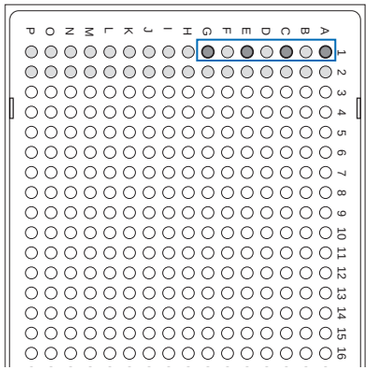
Notes

3100-*Avant* Instrument

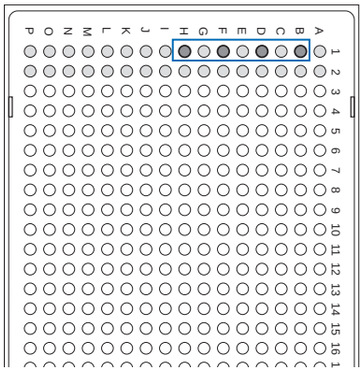
For a 384-well plate, injections are made from every other well. A full plate of 384 sample requires 96 runs to inject all samples once.

Below is an example of the injection pattern for the first six injections, starting with well A01. The light gray circles represent samples and the dark gray circles indicate the injection pattern.

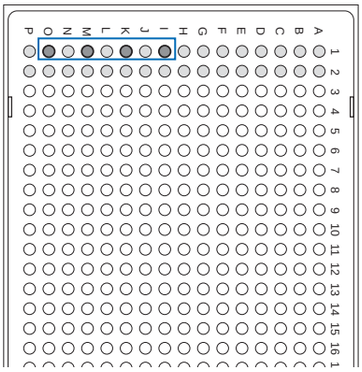
Run 1



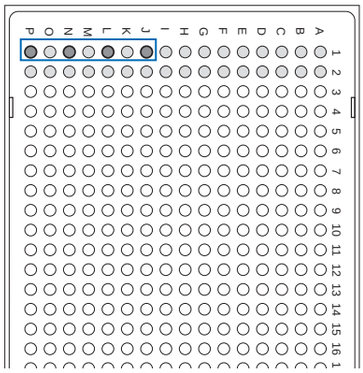
Run 2



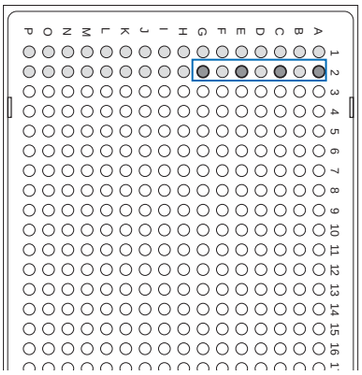
Run 3



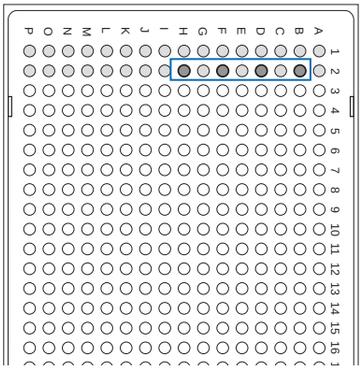
Run 4



Run 5



Run 6



Notes

Preparing and Loading Samples

References for Sample Preparation

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

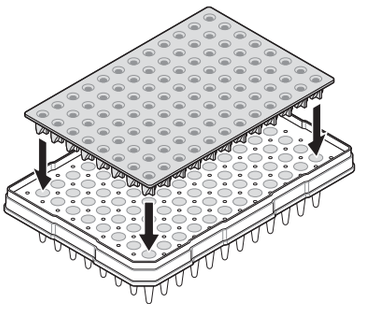
For samples of ...	Refer to the...
DNA sequencing	Individual kit protocols
Fragment analysis	

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

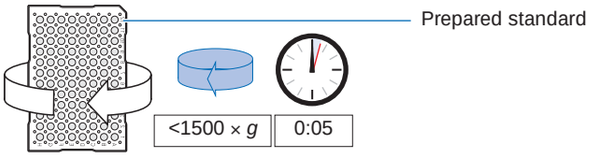
Loading the Samples

1. Dispense 10 to 30 µL of the denatured samples into the wells of a 96 well plate or 5 to 15 µL into the wells of a 384 well plate.
2. Seal the plate:
   a. Place the plate on a clean, level surface.
   b. Lay the septa flat on the plate.
   c. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.

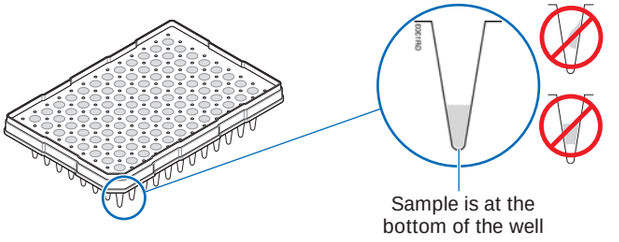
   IMPORTANT! Do not heat plates that are sealed with septa.



3. Briefly centrifuge the plate.



4. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.
5. If the reagents of any well contain bubbles or are not located at the bottom of the well, repeat steps 3 and 4.
6. Leave the plate on ice until you are ready to prepare the plate assembly and place the assembly on the autosampler.



Notes

Where to Go Next

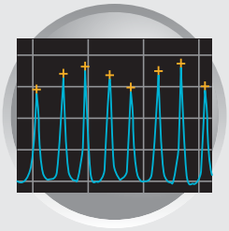
Use the table below to determine which chapter to proceed to next.

Do you need to...	Proceed to ...
Create a valid spatial calibration for the array you are using?	Chapter 2, page 26
Create a spectral calibration for the array length and dye set you are using?	Chapter 3, page 37
Set the active spectral calibration? You must set the active spectral, if you changed the array length. IMPORTANT! If you do not have a spectral for the dye set and array length you are using, perform a new spectral calibration.	Chapter 3, page 59
Set up the software for sequencing analysis runs?	Chapter 4, page 73
Set up the software for SeqScape analysis runs?	Chapter 4, page 99
Set up the software for fragment analysis runs?	Chapter 5, page 123
Perform maintenance, use wizards?	Chapter 7, page 195
Activate/modify the audit trail and access control features?	Chapter 8, page 241

Notes

Notes

Performing Spatial Calibration



This chapter covers:

Notes

Spatial Calibration

Overview

Spatial Calibration

A spatial calibration maps the pixel positions of the signal from each capillary in the spatial dimension of the CCD camera.

When to Perform the Calibration

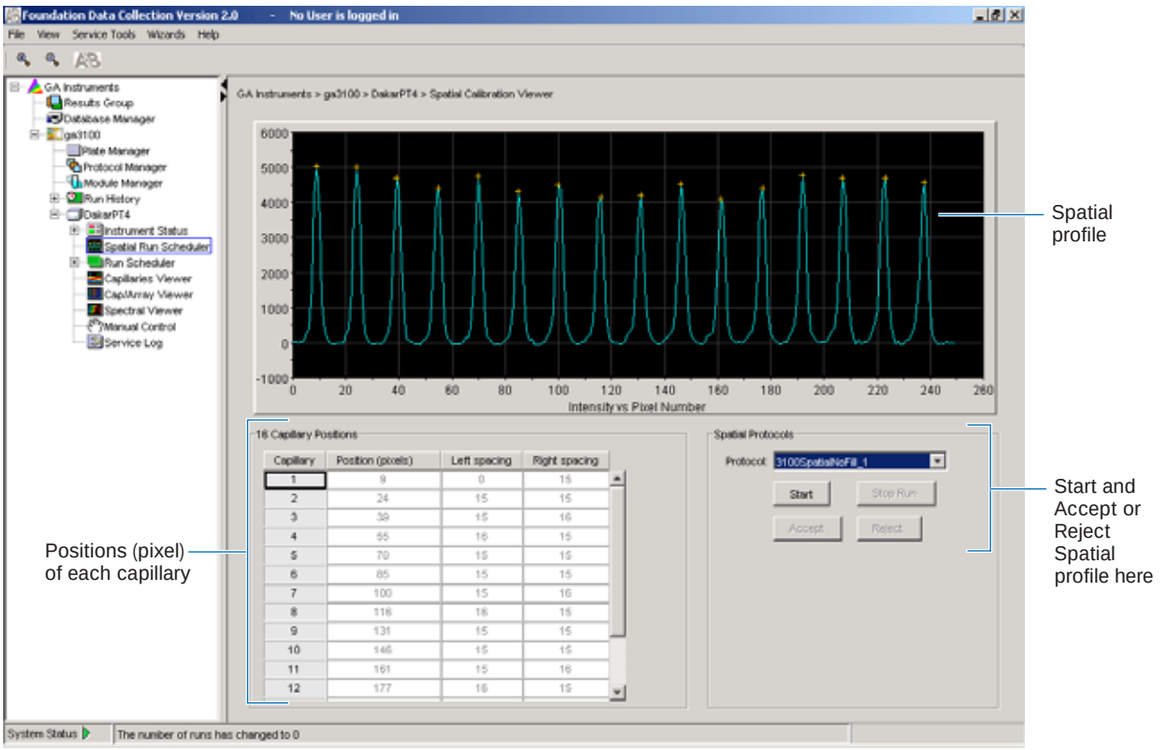
A spatial calibration must be performed after each time you:

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

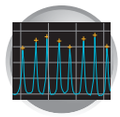
Performing and Evaluating a Spatial Calibration

Performing a Spatial Calibration

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Spatial Run Scheduler**.

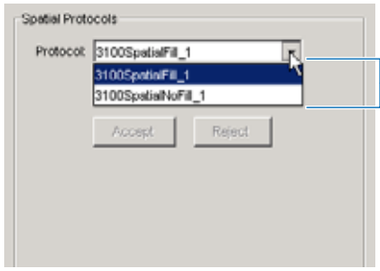


Notes



2. In the Spatial Protocols section, select one of the following:
   - If the capillaries contain fresh polymer, select **Protocol** > **SpatialNoFill_1**
   - Otherwise, select **Protocol** > **SpatialFill_1**

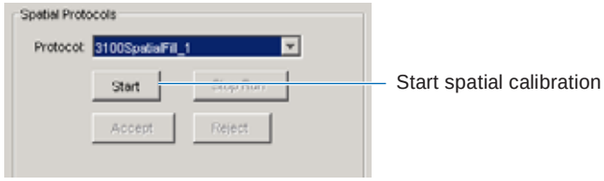
   Note: You do not need to fill the capillaries each time you perform a spatial calibration.



3. Click Start.

   The calibration run lasts approximately:
   - 2 min without filling the capillaries
   - 6 min with filling the capillaries

   Note: The spatial profile window turns black when you start a spatial calibration.



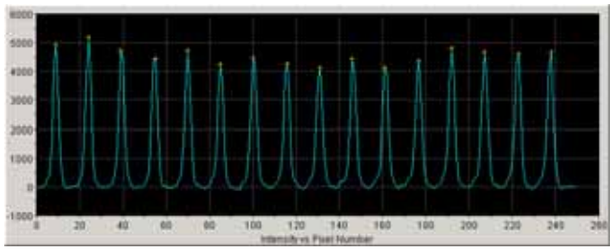
Evaluating a Spatial Calibration

1. Evaluate the spatial calibration profile.

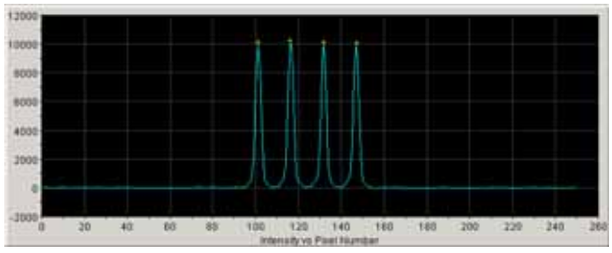
   While viewing the calibration profile, use the following criteria to evaluate the data:

Peak Attribute	Criteria
Height	Similar heights for all peaks.
Orange crosses	One orange cross marking the top of every peak. No misplaced crosses.
Shape	Single sharp peak for each capillary. Small shoulders are acceptable.
Spacing	Position values are 13–16 higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.

Spatial calibration profile for 3100 system



Spatial calibration profile for 3100-Avant system



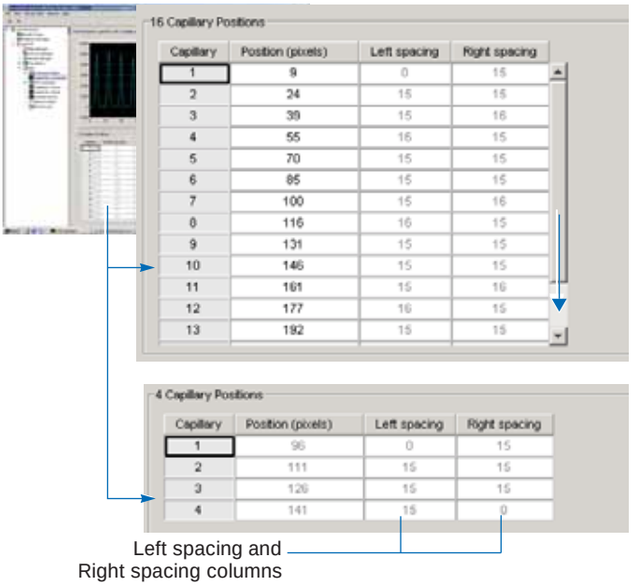
Notes

2. Examine each row in the 16 or 4 Capillary Positions table and verify that the values in the Left spacing and Right spacing columns are 13 to 16 pixels.

   To move a cross:

   a. Type a new value in the Positions (pixels) box for the capillary of interest.

   b. Click outside of that box or press **Enter**.

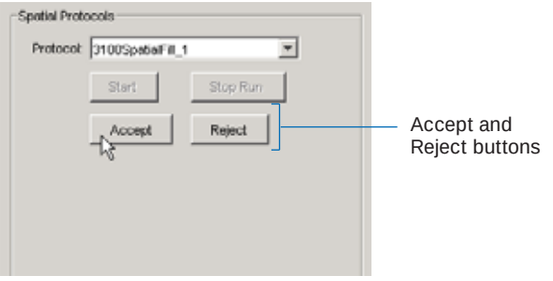


Accepting or Rejecting a Spatial Calibration

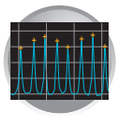
1. Accept or reject the spatial calibration as follows:

   If the calibration:

   - Passed, click Accept to write the calibration data to the database and .ini file.
   - Failed, click Reject, then go to "If the Spatial Calibration Fails" on page 29.



Notes



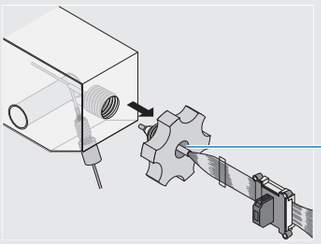
Troubleshooting

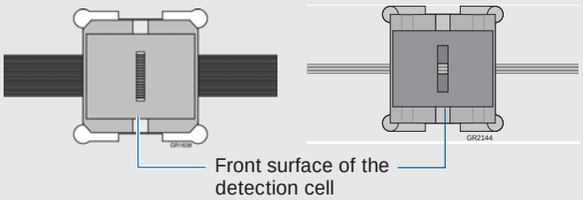
If the Spatial Calibration Fails

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

1. Click Reject, then go to step 1 on page 26 and repeat the spatial calibration.
2. If the calibration fails:
   a. Open the Instrument door.
   b. Open the oven door.
   c. Open the detection cell door.
   d. Remove the bundle tip of the capillary array from the upper polymer block.
   e. Add one drop of methanol to a sterile swab or lint-free wipe.
   f. Gently clean the front surface of the detection cell using the sterile swab or lint-free wipe.
   g. Replace the bundle tip of the capillary array into the upper polymer block.
   h. Close the detection cell door.
   i. Close the oven door.
   j. Close the Instrument door.
   k. Go to step 1 on page 26 to repeat the calibration.
3. If the calibration fails again:
   a. Perform "Adding and Changing the Polymer" on page 207 to fill the capillaries with polymer.
   b. Go to step 1 on page 26 to repeat the spatial calibration.
4. If the calibration fails again:
   a. Perform steps 2a through 2c.
   b. Reposition the capillary array window in the detection cell.
   c. Perform steps 2h through 2j.
   d. Go to step 1 on page 26 to repeat the calibration.
5. If the calibration fails again, replace the capillary array as explained on "Installing and Removing the Capillary Array" on page 210.

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.

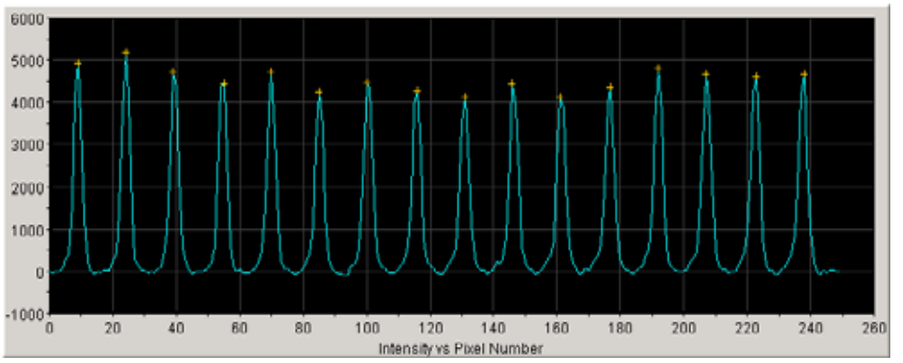




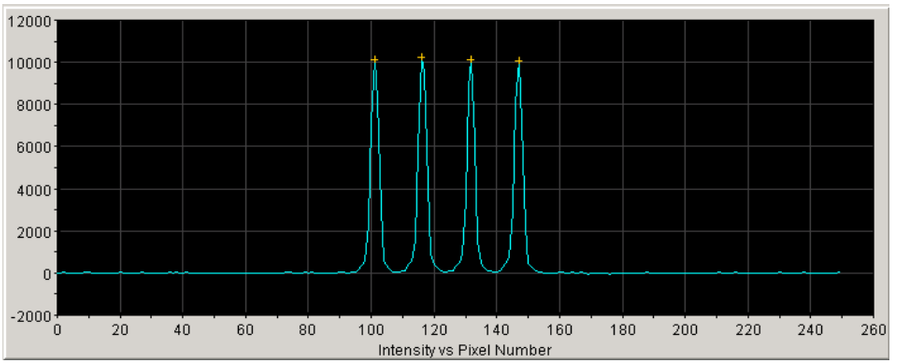
Notes

Examples of Spatial Profiles

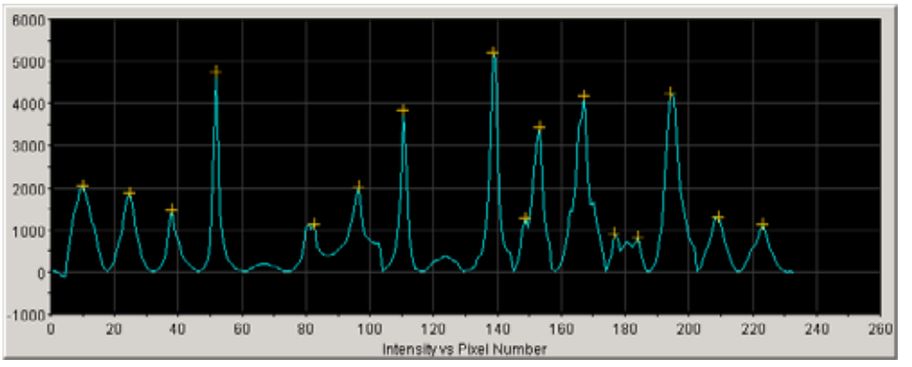
Passing Profile from a 3100 Instrument



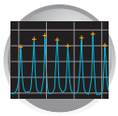
Passing Profile from a 3100-*Avant* Instrument



Failing Profile from a 3100 Instrument



Notes



Where to Go Next

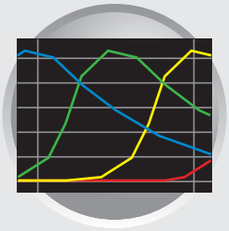
Use the table below to determine which chapter to proceed to next.

Do you need to...	Proceed to ...
Create a spectral calibration for the array length and dye set you are using?	Chapter 3, page 37
Set the active spectral calibration? You must set the active spectral, if you changed the array length. IMPORTANT! If you do not have a spectral for the dye set and array length you are using, perform a new spectral calibration.	Chapter 3, page 59
Set up the software for sequencing analysis runs?	Chapter 4, page 73
Set up the software for SeqScape runs?	Chapter 4, page 99
Set up the software for fragment analysis runs?	Chapter 5, page 123
Perform maintenance, use wizards?	Chapter 7, page 195
Activate/modify the audit trail and access control features?	Chapter 8, page 241
Learn more about troubleshooting?	Appendix C, page 279

Notes

Notes

Performing Spectral Calibration for Sequencing and Fragment Analysis



This chapter covers:

Notes

Overview

Spectral calibration creates a matrix. This matrix is used during a run to reduce raw data from the instrument to the 4-dye or 5 -dye data stored in the sample files. Performing a spectral calibration is similar to performing a sample run, except that calibration standards are run in place of samples, and a spectral calibration module is used in place of a run module.

When to Perform the Calibration

You must perform a spectral calibration:

- Whenever you use a new dye set on the instrument
- Change capillary array length (or polymer type for fragment analysis)
- After the laser or CCD camera has been realigned/replaced by a service engineer
- If you begin to see a decrease in spectral separation (pull-up and/or pull-down peaks) in the raw or analyzed data

What Happens?

Spectral standards are run in all 16 or 4 capillaries. Then, the data collection software:

- Collects the data and stores it into 16 or 4 separate temporary files
- Analyzes the data and generates a mathematical description of the spectral overlap for each capillary
- Stores the spectral calibration data for the dye set run

Changing Capillary Array Lengths and Polymer Type

For each dye set, a single spectral calibration *can not* be used for all capillary array lengths (and polymer type, for fragment analysis).

- For every sequencing dye set, you must create a separate spectral calibration for each capillary array length you use.
- For every fragment analysis dye set, you must create a separate spectral calibration for each capillary array length and polymer type combination you use.

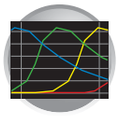
Refer to "Activating a Spectral Calibration" on page 59, for information on how to switch calibrations, once calibrations are performed for each dye set on each capillary length.

Supported Sequencing Chemistries

ABI PRISM® BigDye® Terminator v1.0, v2.0 and v3.0 chemistry kits have been discontinued. While these chemistries can be still run on the 3100 series instruments using supported basecaller, and mobility files, and run modules they are no longer fully supported.

Any new developments on the 3100series instruments in terms of the creation of new basecaller and mobility files, and run modules will be made solely in support of the ABI PRISM® BigDye® Terminator v1.1 and v3.1chemistry kits. For more information, please contact Applied Biosystems Technical Support.

Notes



Determining the Correct Dye Set and Calibration Standard

There are two types of spectral calibration standards:

- Matrix standards
  - A tube that contains a single labeled fragment for each of the four or five dyes
  - Four separate tubes, each containing fragments labeled with one of the four dyes
- BigDye® v3.1 or BigDye® v1.1 Terminator Sequencing Standard

  A tube the contains a standard chemistry reaction that contains multiple labeled fragments for each of the four dyes

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

Dye Sets and Calibration Standards for Sequencing Chemistry Using the ABI PRISM® 3100 Genetic Analyzer

Sequencing Chemistry	Dye Set	Spectral Calibration Standard
• ABI PRISM® BigDye® v3.1Terminator • ABI PRISM® BigDye® v3.1 Primer	Z_BigDyeV3	BigDye® v3.1 Matrix Standards
		BigDye® v3.1 Terminator Sequencing Standard
• ABI PRISM® BigDye® v1.1 Terminator • ABI PRISM® BigDye® v1.1 Primer	E_BigDyeV1	DS-01Matrix Standards
		BigDye® v1.1 Terminator Sequencing Standard
ABI PRISM® dRhodamine Terminator		dRhodamine Matrix Standards

Dye Sets and Calibration Standards for Sequencing Chemistry Using the ABI PRISM® 3100-*Avant* Genetic Analyzer

Sequencing Chemistry	Dye Set	Spectral Calibration Standard
ABI PRISM BigDye® v3.1Terminator	Z_BigDyeV3	BigDye v3.1 Matrix Standards
		BigDye v3.1 Terminator Sequencing Standard
ABI PRISM BigDye v1.1 Terminator	E_BigDyeV1	DS-01 Matrix Standards
		BigDye® v1.1 Terminator Sequencing Standard
ABI PRISM dRhodamine Terminator		dRhodamine Matrix Standards

Dye Sets and Calibration Standards for Fragment Analysis Chemistry using the 3100/3100-*Avant* Genetic Analyzers

Fragment Analysis Chemistry	Dye Set	Spectral Calibration Standard
Custom oligos	D	DS-30 Matrix Standards
• ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	D	DS-31 Matrix Standards
• AmpF*ℓ*STR® COfiler® Kit • AmpF*ℓ*STR® Profiler® Plus Kit • AmpF*ℓ*STR® Profiler Plus *ID*® Kit • AmpF*ℓ*STR® SGM Plus® Kit • Other 4-Dye AmpF*ℓ*STR Kits	F	DS-32 Matrix Standards

Notes

Dye Sets and Calibration Standards for Fragment Analysis Chemistry using the 3100/3100-*Avant* Genetic Analyzers

Fragment Analysis Chemistry	Dye Set	Spectral Calibration Standard
ABI PRISM® SNaPshot® Multiplex System	E5	DS-02 Matrix Standards
• ABI PRISM® Linkage Mapping Set v2.5 • Custom Oligos • AmpFℓSTR® Identifiler® Kit • AmpFℓSTR® SEfiler® Kit • Other 5-Dye AmpFℓSTR Kits	G5	DS-33 Matrix Standards

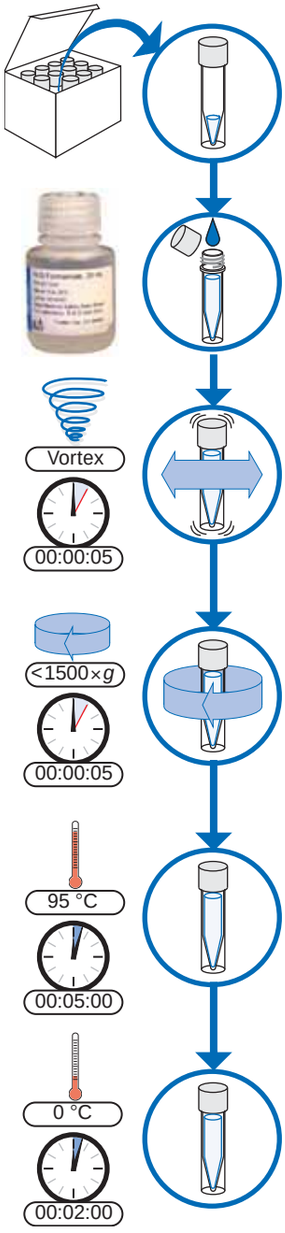
Notes

Preparing the Spectral Calibration Chemistry

Preparing the Calibration Standard

1. Prepare one of the following:
   - ABI PRISM BigDye Terminator v1.1 or v3.1 Sequencing Standard:
     – Remove a tube of the Sequencing Standard from the freezer.
     – Add 170 µL of Hi-Di™ formamide to resuspend the tube of BigDye Terminator v1.1 or v3.1 Sequencing Standard.
     – Skip to step 2.
   - Matrix standards:
     – Remove a tube of the matrix standard from the refrigerator.
     – Mix thoroughly, then spin briefly in a microcentrifuge.
     – Follow the matrix standard insert for matrix standard and Hi-Di formamide ratios.
2. Vortex thoroughly.
3. Briefly centrifuge the mixture.
4. Heat the standard tube at 95 °C for 5 minutes to denature the DNA.
5. Cool the tubes on ice for 2 minutes.

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.



Notes

Sealing and Preparing the Plate Assemblies

WARNING **WARNING** Do not use warped or damaged plates.

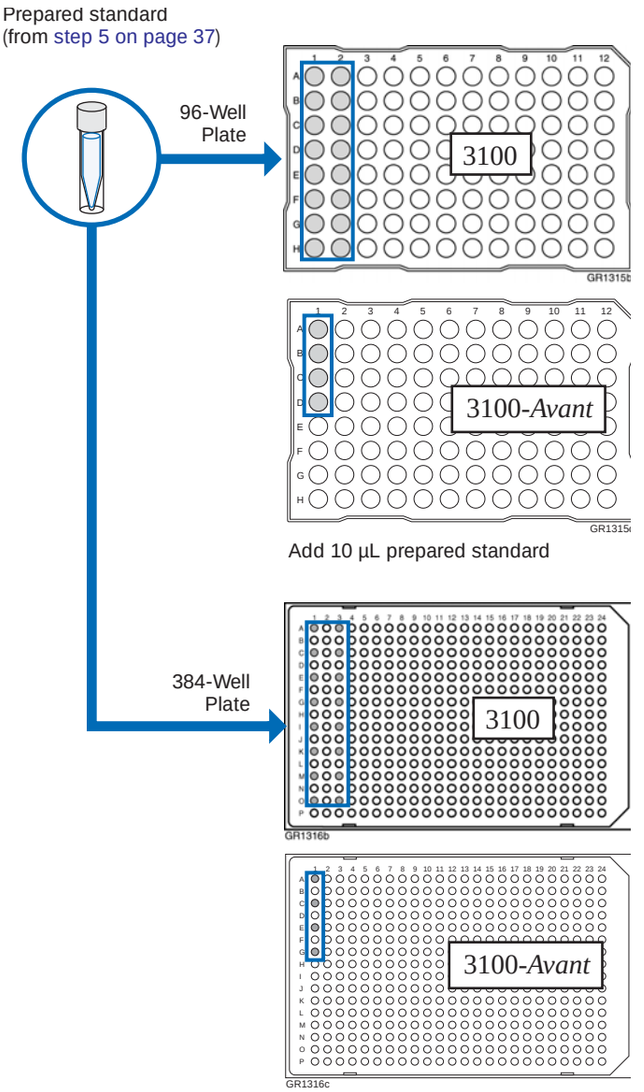
1. Add the denatured standard to the wells of a 96- or 384-well reaction plate:
   - If using 3100 instrument:
     - **96-well plate** – Add 10 µL of denatured standard to wells A1 through H2.
     - **384-well plate** – Add 5 µL of denatured standard into alternating wells of the plate:

       Row 1: A1, C1, E1, …K1, M1, O1

       Row 2: Empty

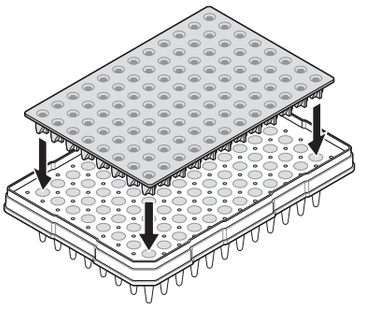
       Row 3: A3, C3, E3, …K3, M1, O1
   - If using 3100-*Avant* instrument:
     - **96-well plate** – Add 10 µL of denatured standard to wells A1, B1, C1 and D1.
     - **384-well plate** – Add 5 µL of denatured standard into alternating wells of the plate:

       Row 1: A1, C1, E1 and G1

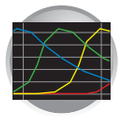


2. Seal the plate:
   a. Place the plate on a clean, level surface.
   b. Lay the septa flat on the plate.
   c. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.

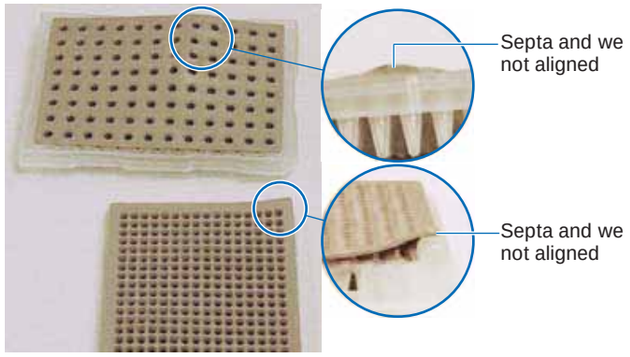
IMPORTANT! Do not heat plates that are sealed with septa.



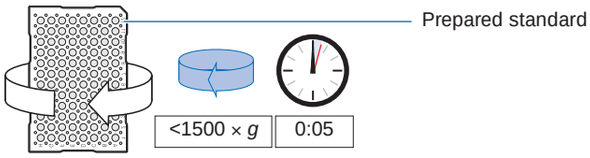
Notes



3. To prevent damage to the capillary array, inspect the plate and septa to verify the septa fits snugly and flush on the plate.

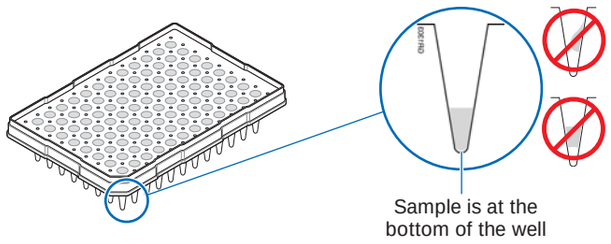


4. Briefly centrifuge the plate.

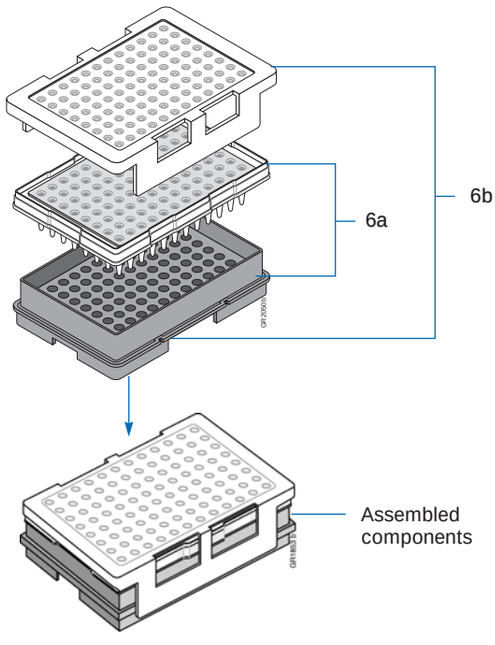


5. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.

   If the reagents of any well contain bubbles or are not located at the bottom of the well, repeat steps 4 and 5.



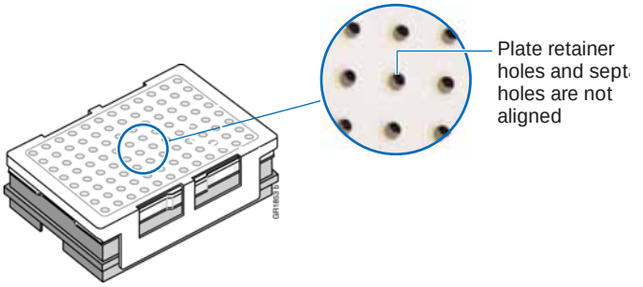
6. Assemble the plate assembly:
   a. Place the sample plate into the plate base.
   b. Snap the plate retainer onto the plate and plate base.



Notes

7. Verify that the holes of the plate retainer and the septa strip are aligned. If not, re-assemble the plate assembly (see step 6).

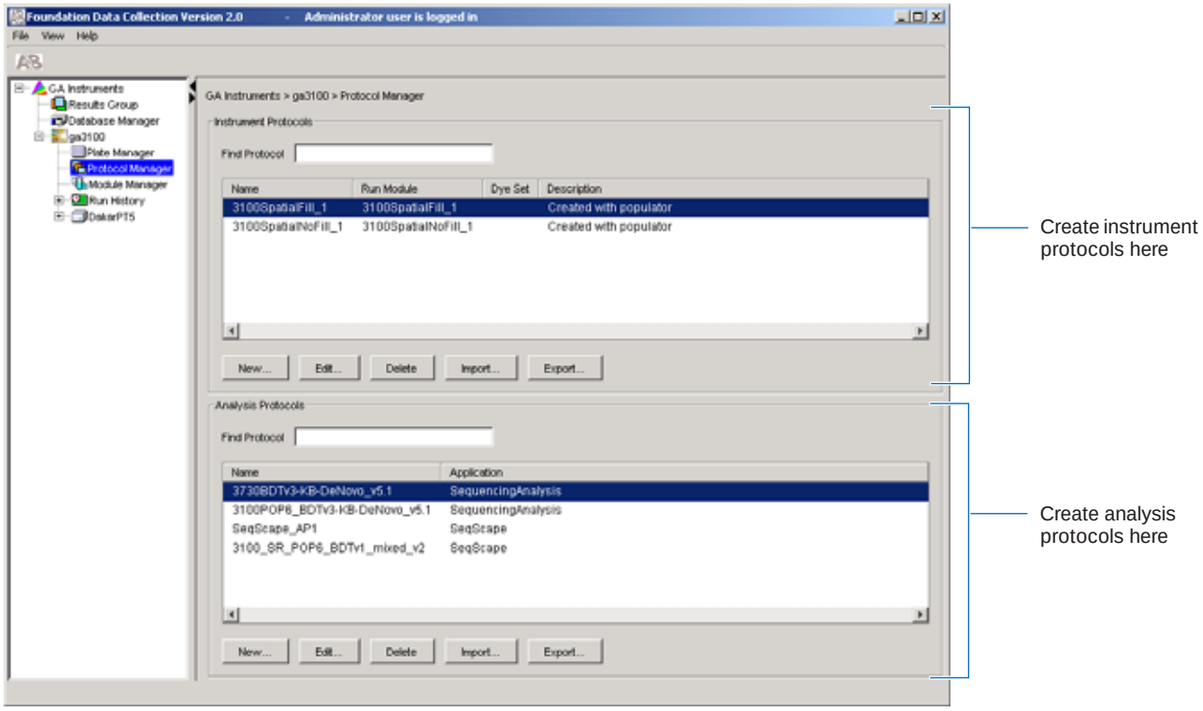
IMPORTANT! Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.



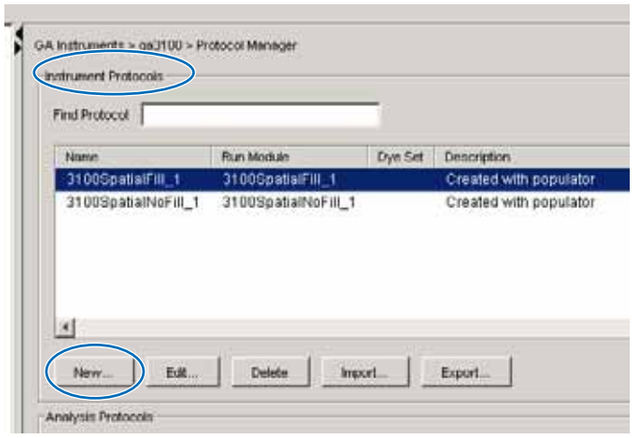
Notes

Creating a Spectral Instrument Protocol

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Protocol Manager**. This opens the Protocol Manager window.

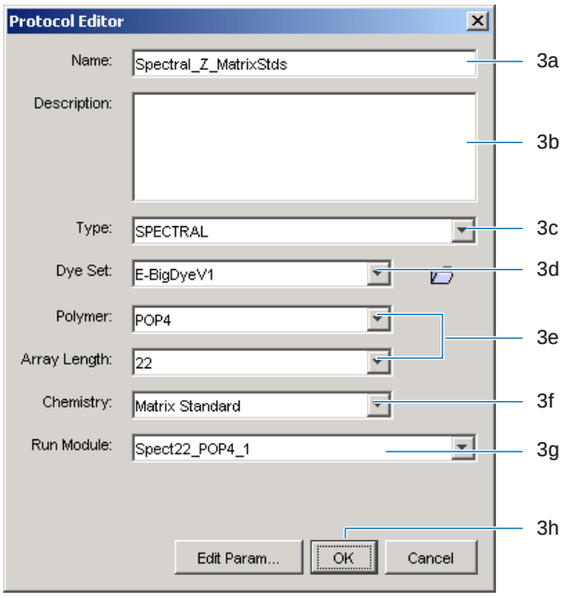


2. In the Instruments Protocols pane, click New... . The Protocol Editor dialog box opens.



Notes

3. Complete the Protocol Editor dialog box:
   a. Type a name for the protocol.
   b. Type a description for the protocol (optional).
   c. Select **Spectral** in the Type drop-down list.



   d. Using the information in the tables below, select the correct Dye Set for your run.

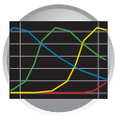
Dye Sets for Sequencing Chemistry

Instrument	Chemistry	Dye Set
3100 only	ABI PRISM® BigDye® v1.1 Primer	E_BigDyeV1
3100/3100-*Avant*	ABI PRISM® BigDye® v1.1 Terminator	
	ABI PRISM® dRhodamine Terminator	
	ABI PRISM® dGTP BigDye® Terminator	
3100 only	ABI PRISM® BigDye® v3.1 Primer	Z_BigDyeV3
3100/3100-*Avant*	ABI PRISM® BigDye® v3.1 Terminator	
	ABI PRISM® dGTP BigDye® v3.0 Terminator	

Dye Sets for Fragment Analysis Applications or Kits

Instrument	Application or Kit	Dye Set	Matrix Standard Set
3100 and 3100-*Avant*	Custom oligos	D	DS-30
	• ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	D	DS-31

Notes



Dye Sets for Fragment Analysis Applications or Kits *(continued)*

Instrument	Application or Kit	Dye Set	Matrix Standard Set
3100/3100-*Avant*	• AmpF*ℓ*STR® COfiler® Kit • AmpF*ℓ*STR® Profiler® Plus Kit • AmpF*ℓ*STR® Profiler Plus *ID*® Kit • AmpF*ℓ*STR® SGM Plus® Kit • Other 4-Dye AmpF*ℓ*STR Kits	F	DS-32
	ABI PRISM® SNaPshot® Multiplex System	E5	DS-02
	• ABI PRISM® Linkage Mapping Set v2.5 • Custom Oligos • AmpF*ℓ*STR® Identifiler® Kit • AmpF*ℓ*STR® SEfiler® Kit • Other 5-Dye AmpF*ℓ*STR Kits	G5	DS-33

e. Use the table below, to select the Polymer and Array Length you are using from the appropriate drop-down list.

Polymer Type	Array Length (cm)
POP4	22
	36
	50
	80
POP6	36
	50

f. In the Chemistry drop-down list, use the tables on page 44 select the correct chemistry file.

IMPORTANT! Failure to select the correct chemistry file for the spectral calibration samples the you are using results in a failing spectral run.

Note: The Chemistry file for fragment analysis dye sets automatically defaults to the Matrix Standard.

Notes

Chemistry Files for Sequencing Dye Sets

Dye Set	Standard Type	Chemistry File
Z_BigDyeV3	BigDye® v3.1 Matrix Standards	Matrix Standard
	BigDye® v3.1 Terminator Sequencing Standard	Sequence Standard
E_BigDyeV1	DS-01	Matrix Standard
	BigDye® v1.1 Terminator Sequencing Standard	Sequence Standard
	dRhodamine matrix standards	Matrix Standard

Chemistry Files for Fragment Analysis Dye Sets

Dye Set	Matrix Standard Set	Chemistry File
D	DS-30	Matrix Standard
D	DS-31	
F	DS-32	
E5	DS-02	
G5	DS-33	

g. In the Run Module drop-down list, select the run module for your run from the table.

Note: The modules list is filtered based on the polymer type, then the array length you selected in step e on page 43. You may have only one run module option available.

Polymer Type	Array Length (cm)	Run Module
POP4	22	Spect22_POP4_1
	36	Spect36_POP4_1
		SpectSQ36_POP4_1
	50	Spect50_POP4_1
	80	Spect80_POP4_1
POP6	36	Spect36_POP6_1
	50	Spect50_POP6_1

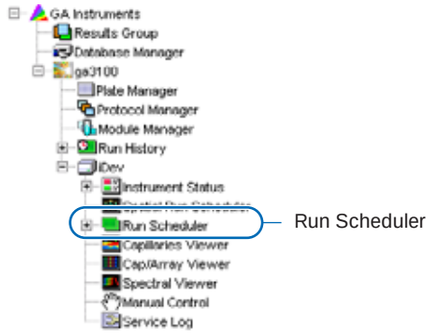
h. Click OK.

Notes

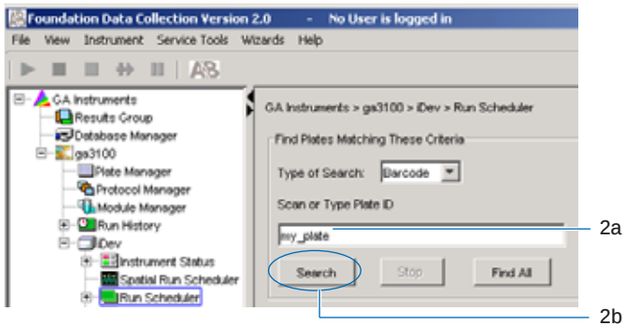
Performing a Spectral Calibration

Creating the Plate Record

1. In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Run Scheduler**.

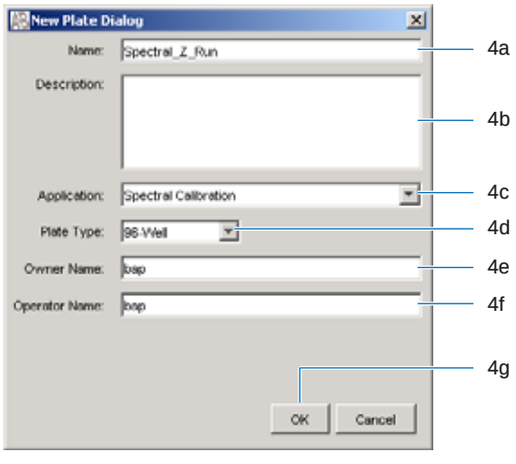


2. In the Run Scheduler view:
   a. In the Scan or Type Plate ID field enter a new plate name.
   b. Click Search.



3. In the Create new plate dialog box, click Yes.

4. Complete the New Plate dialog box:
   a. Enter a name for the plate.
   b. Optional: Enter a description for the plate record.
   c. In the Application drop-down list, select **Spectral Calibration**.
   d. In the Plate Type drop-down list, select **96-Well** or **384-Well**
   e. Enter a name for the owner.
   f. Enter a name for the operator.
   g. Click OK.



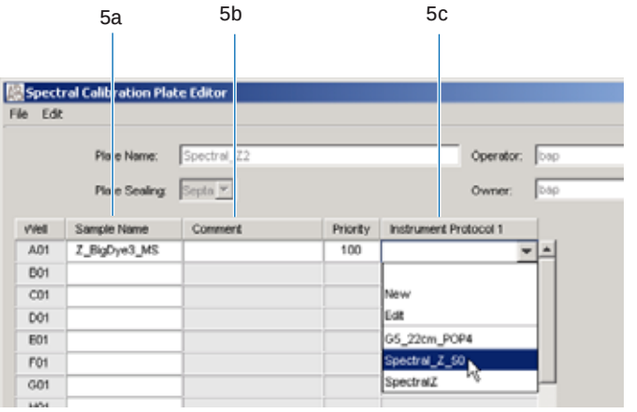
Notes

5. In the Spectral Calibration Plate Editor dialog box, enter the following information:

   a. In the Sample Name column of row A, enter a sample name, then click the next cell. The value 100 automatically display in the Priority column.

   b. In the Comments column of row A, enter any additional comments or notations for the sample at the corresponding position of the plate.

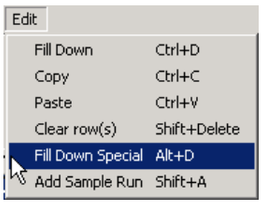
   c. In the **Instrument Protocol 1** column of row A, select a protocol from the list.



6. Highlight the entire row.

7. Select **Edit > Fill Down Special**.

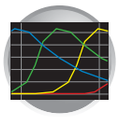
   Based on the plate type (96- or 384-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run (see page 265).



8. Click OK.

   You have successfully created the plate record for the spectral calibration plate.

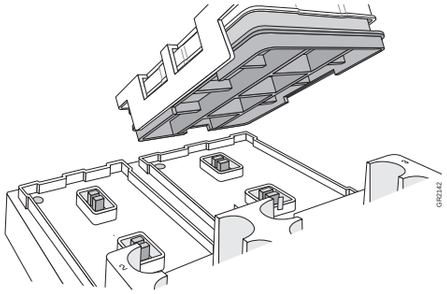
Notes



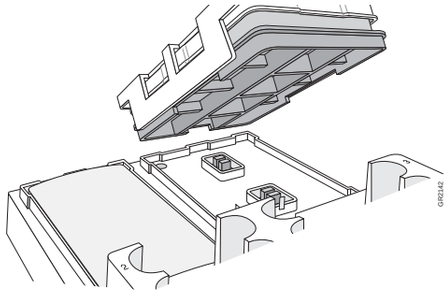
Placing the Plate Assembly into the Instrument

1. Verify the oven and front doors are closed.
2. Press the Tray button.
3. Open the front doors.
4. Place the plate assembly on the autosampler in position A or B for the 3100 instrument and position B for the 3100-*Avant* instrument.

   Note: There is only one orientation for the plate, with the notched end of the plate base away from you.



3100 instrument



3100-*Avant* instrument

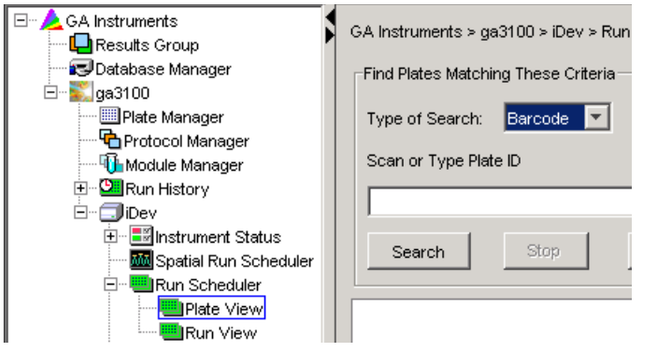
5. Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off of the autosampler.
6. Close the instrument doors.

   Note: Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in buffer.

Notes

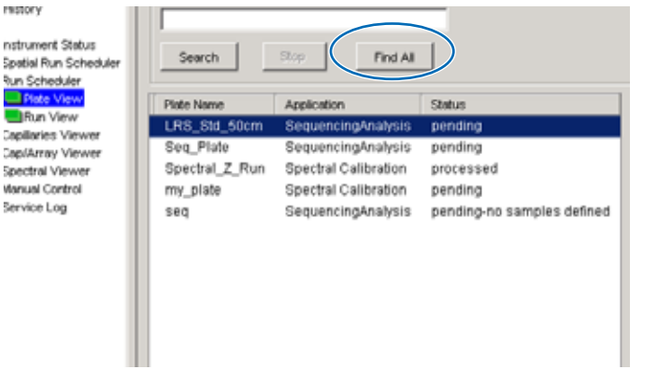
Running the Spectral Calibration Plate

1. In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Run Scheduler** > **Plate Viewer**.

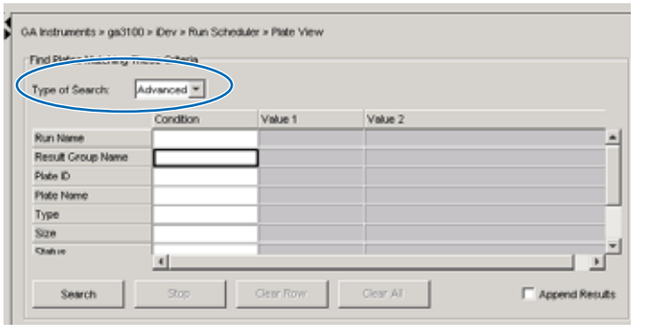


2. Search for your plate record. There are two options:

   - If you have a limited number of plates in the database, click Find All.



   - Perform an advanced search by selecting **Advanced** in the Type of Search drop-down list (see page 163).



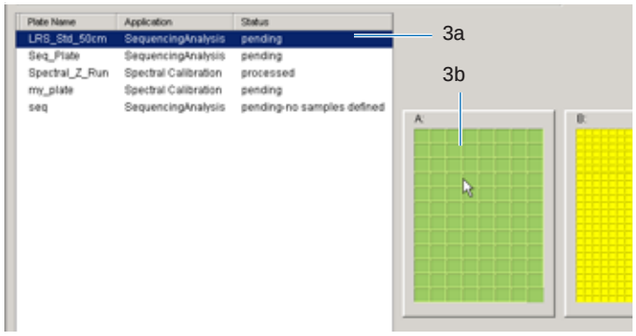
Notes

3. Link the plate.

   a. Select the plate record you want to run.

   b. Click the plate position indicator that corresponds to the plate you are link.

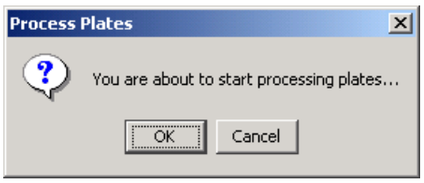
      The plate map color will change from yellow to green when it is successfully linked.



   > Note: The 3100-*Avant* instrument has only one plate position to link a plate record.

4. In the toolbar of the Data Collection Software window, click  to begin the run.

5. The Processing Plates dialog box opens, then click OK.



   > Note: The instrument may pause before running the plate to raise the oven temperature.

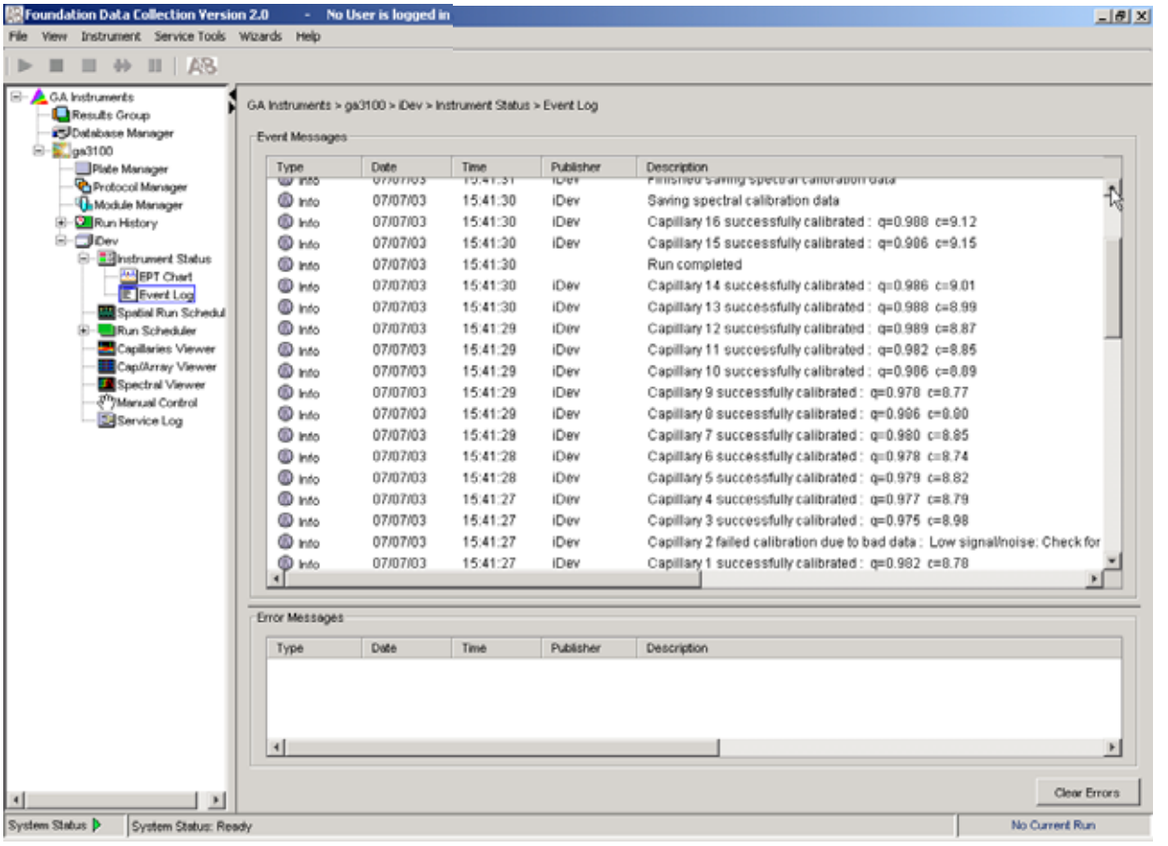
Run Type	Capillary Length (cm)	Approximate Run Time (min)
Spect22_POP4_1	22	20
Spect36_POP4_1	36	28
SpectSQ36_POP4_1		45
Spect36_POP6_1		50
Spect50_POP4_1	50	70
Spect50_POP6_1		95
Spect80_POP4_1	80	140

Notes

Viewing the Pass/Fail Status After the Run

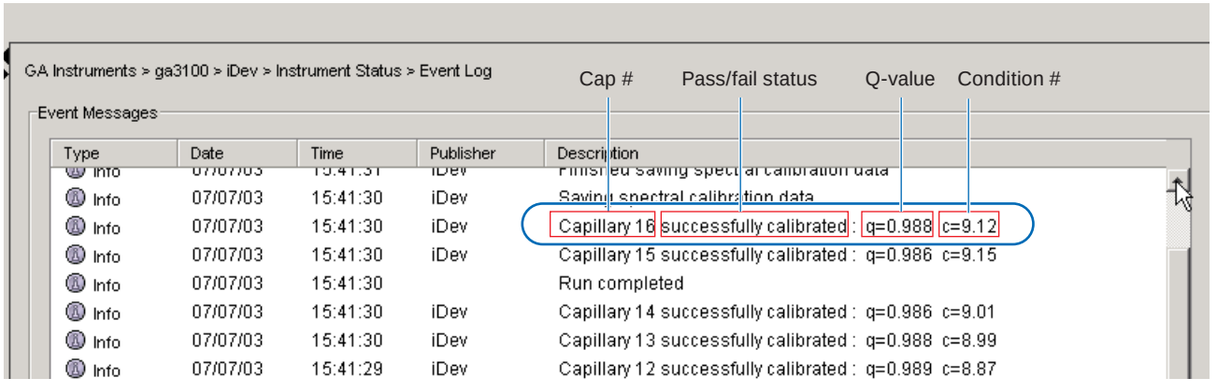
After the instrument completes the spectral calibration run, the pass or fail status of each capillary is recorded in the Events Messages section of the Instrument Status window.

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Instrument Status** > **Event Log**.



Notes

2. In the Events Messages section of the window, view the status of each capillary.



Dye set G5 status results

For a good-quality calibration, each capillary should have a:

- Q-value above 0.95
- Condition number within range of:

Dye Set	Condition Number Range
Sequencing Analysis	
Z_BigDyeV3	3 to 5
E_BigDyeV1	
Fragment Analysis	
D	4 to 8.5
F	6 to 12
E5	2.5 to 4
G5	7 to 12

3. If the entire spectral calibration failed, see "If All Capillaries Fail" on page 57.

Notes

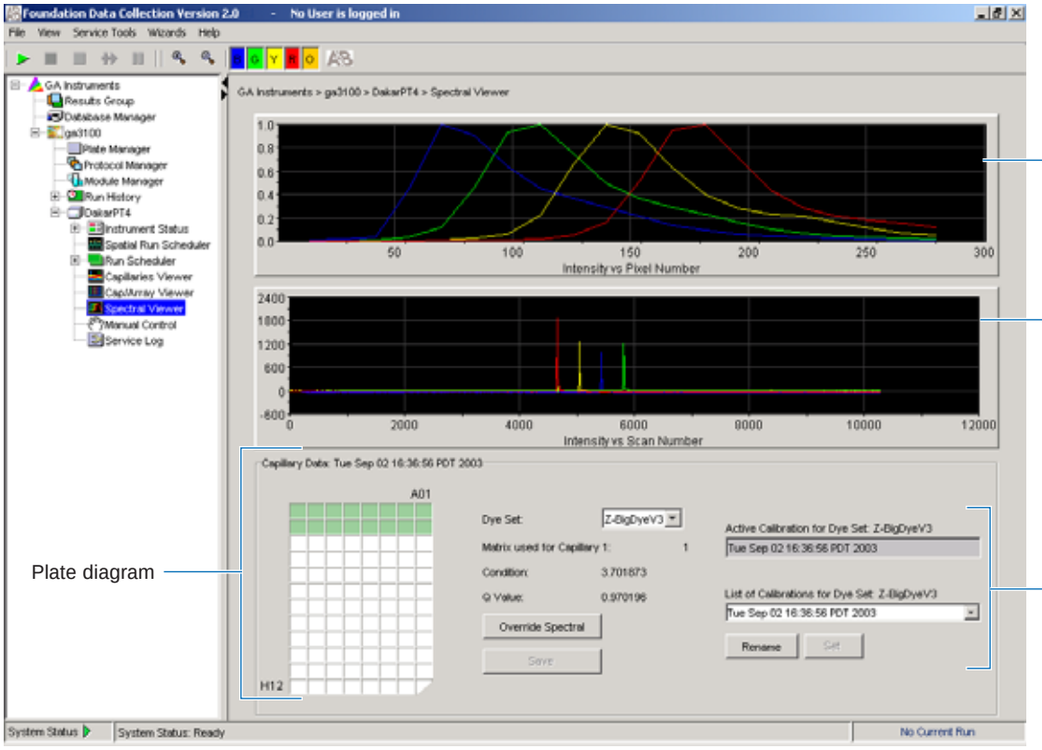
Evaluating the Spectral Calibration Data

IMPORTANT! Review and evaluate the spectral calibration profile for each capillary, even if the Spectral Calibration Results box indicated that they all passed.

Note: Pages 61 to 64 contain examples of passing sequencing spectral calibration profiles and pages 65 to 68 contain examples of passing fragment analysis spectral calibration profiles.

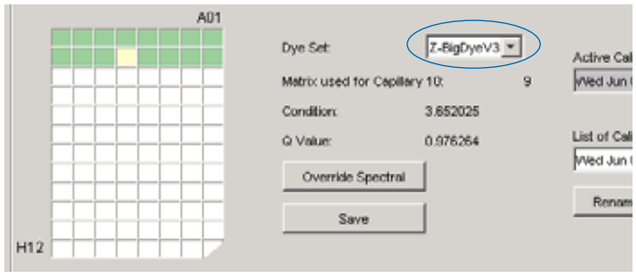
Evaluating the Spectral Profile and Raw Data

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Spectral Viewer**.



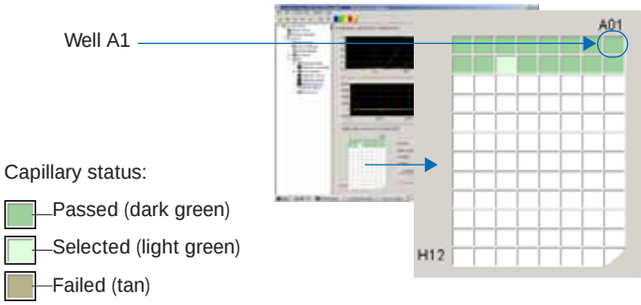
Notes

2. In the Dye Set drop-down list, select the dye set you just created.



> **Note:** If the spectral calibration failed (no spectral profiles created), see the troubleshooting table on page 57.

3. In the plate diagram, select a well on the plate diagram to view the capillary spectral results.



> **Note:** If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it is assigned the profile of the nearest passing capillary to the right.

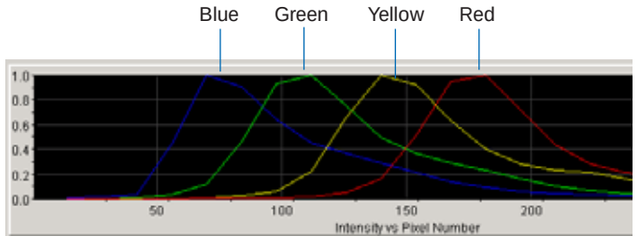
4. Evaluate the spectral profile and raw data for the selected capillary:

   a. Verify that the order of the peaks in the spectral profile from left to right are:
      - 4-dye: blue-green-yellow-red
      - 5-dye: blue-green-yellow-red-orange

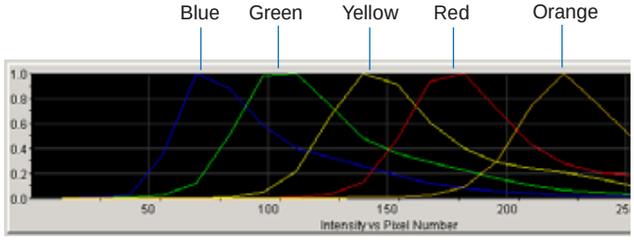
      Do the peaks in the profile appear in the correct order?

      Yes – Go to step b.

      No – The calibration run has failed. Go to page 56.



Example of a 4-dye spectral profile



Example of a 5-dye spectral calibration profile

Notes

b. Verify that the order of the peaks in the raw data profile from left to right are:

   Sequencing

   – 4-dye: red-yellow-blue-green

   Fragment Analysis

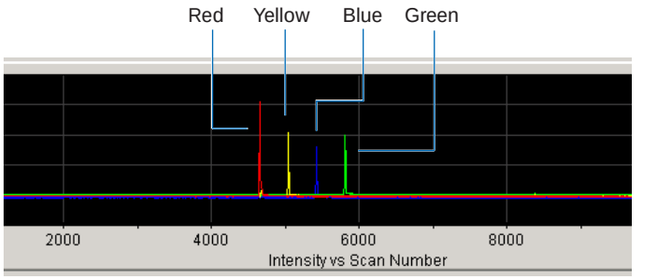
   – 4-dye: red-yellow-green-blue

   – 5-dye: orange-red-yellow-green-blue

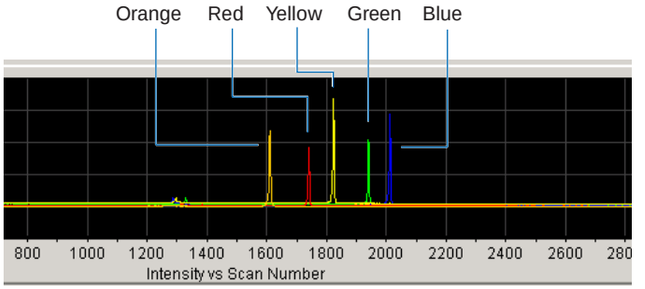
   Are the peaks in the wrong order or are there any extraneous peaks that adversely affect the spectral profile?

   Yes – The calibration run has failed. Go to page 56.

   No – Go to step c.



Example of a 4-dye sequencing raw data profile



Example of a 5-dye fragment analysis raw data profile

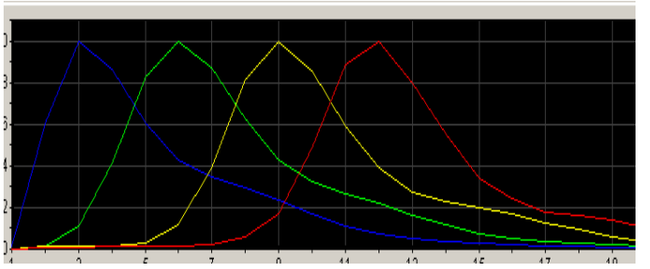
c. Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities (see "Tip: Magnifying the Spectral Profile or Raw Data" on page 55).

   Are the peaks in the spectral profile separate and distinct?

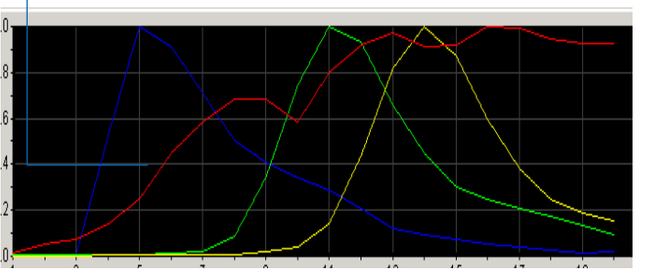
   Yes – The capillary has passed. Go to step 5.

   No – The calibration run has failed. Go to page 56.

Peaks are distinct, regular and in the proper order – pass

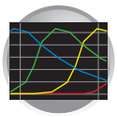


Red peak is not distinct, regular or in the proper order – fail

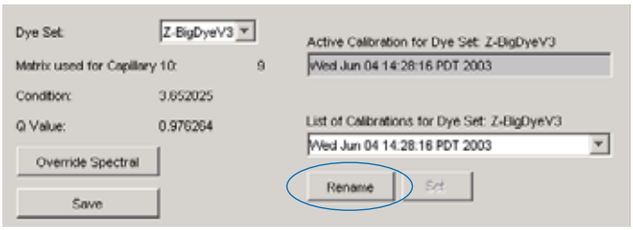


5. Repeat steps 3 and 4 for each capillary in the array.

Notes

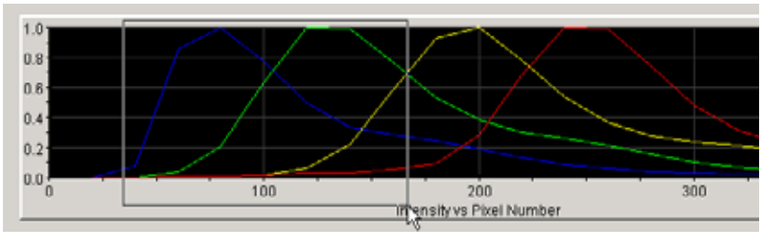


6. Rename the spectral run. The spectral file default name is the day, date and time of the run.
   a. Click Rename.
   b. In the Rename Calibration dialog box, enter a descriptive name for the spectral calibration including the dye set, array length and polymer type (optional).
   c. Click OK.



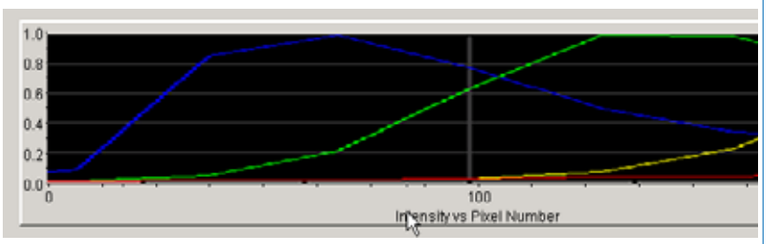
Tip: Magnifying the Spectral Profile or Raw Data

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Spectral Viewer**.
2. In the spectal profile or raw data display, click-drag the cursor to create a box around the area of interest.



Selecting an area to magnify in a spectral profile

3. Release the mouse button.
   The data collection software displays the selected region.
4. Press **R** to reset the view.



Magnified area of that spectral profile

Notes

Troubleshooting

If a Capillary Fails

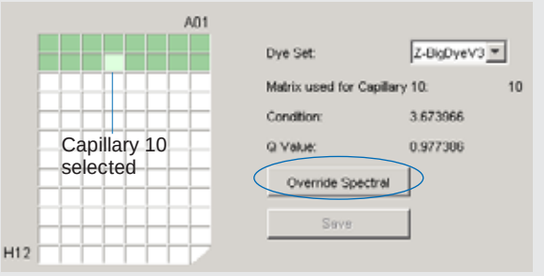
If a capillary fails, it is automatically assigned the spectral profile of its nearest passing capillary to the left. If there are no passing capillaries to the left, it is assigned the profile of the nearest passing capillary to the right.

For applications where pull-up and pull-down peaks cause critical errors, we recommend that you repeat the spectral calibration and use a unique spectral for each capillary.

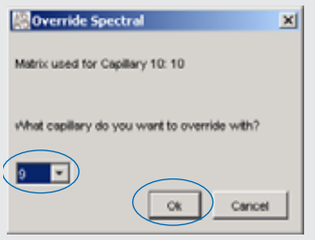
Manually Overriding a Spectral Profile

To override a spectral calibration profile:

1. Review the data.
2. In the plate diagram, select the capillary spectral profile you want to override.
3. Click **Override Spectral**. The Override Spectral dialog box opens.

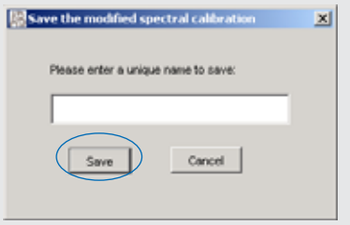


4. Select a new capillary value from the drop-down list.
5. Click **OK**.



6. Click **Save**.

7. In “Save the modified spectral calibration” dialog box, enter a new name, then click **Save**.

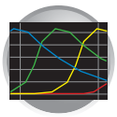


When a Calibration Fails

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

- Verify that the correct chemistry and run module were selected. If not, correct, and then repeat the run.
- Verify the freshness of the reagents used.

Notes



Troubleshooting *(continued)*

If All Capillaries Fail

If all capillaries fail, no spectral profiles are created. However, the raw data can still be viewed.

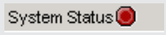
Viewing the Raw Data for a Failed Spectral Calibration

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Spectral Viewer**, then review the spectral data.

   You observe:

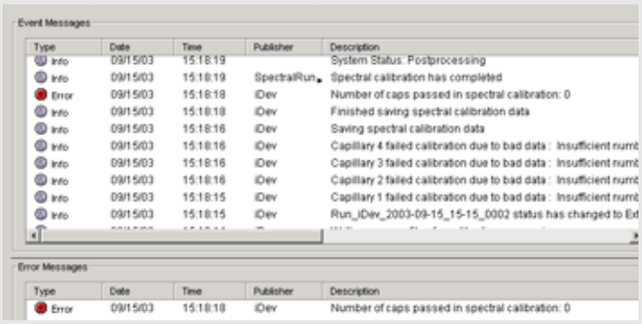
   – The window displays data from the previous passing spectral calibration.

   – This System Status is blinking red.



System failure, check Event Log

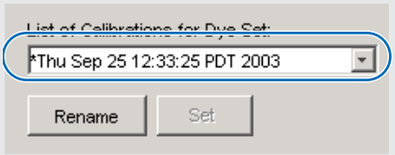
2. Click **Instrument Status > Event Log** to view the Event and Errors messages.



3. Click **Spectral Viewer**.

4. In the Dye Set drop-down list, select the dye set for the failed calibration.

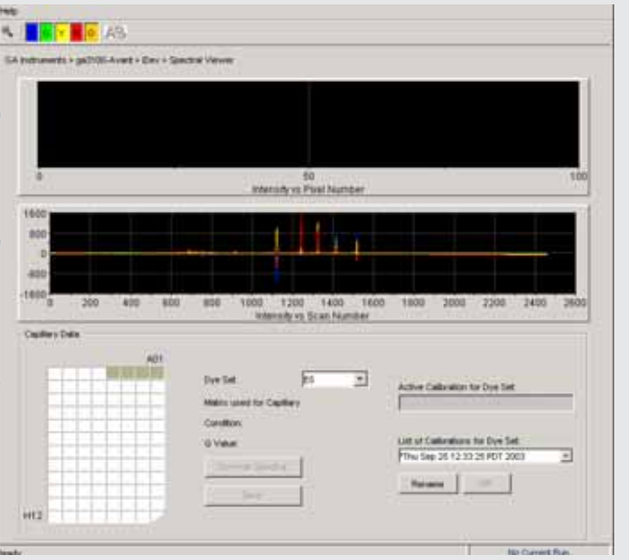
5. In the List of Calibrations drop-down list, select the failing run. The failing run has a asterisk (*) next to its name.



Notes

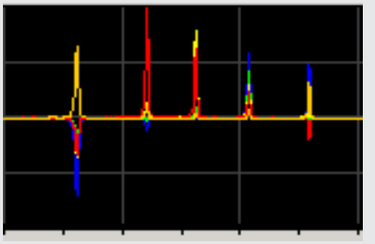
Troubleshooting *(continued)*

The window is updated with the information from the failed E5 spectral calibration.



6. Use the list of questions below when reviewing the raw data to determine the cause of the failure.
   - How many peaks?
   - Any extraneous peaks?
   - Peak order?
   - Peak shape and signal strength?
   - Negative peaks?
   - Additional peaks (positive or negative) under the main color peak?

Note: It is helpful to magnify the raw data for the review process (see "Tip: Magnifying the Spectral Profile or Raw Data" on page 55).



Raw data

Maginfied raw data

Notes

Activating a Spectral Calibration

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

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

IMPORTANT! If you installed or replaced an array that is a different length than you were using, you must set the active spectral calibration for that dye set and arrary length combination. If one does not exist, perform a new spatial calibration, then set the active spectral calibration.

A run will not start without the correct spectral calibration for a specific dye set and capillary array length.

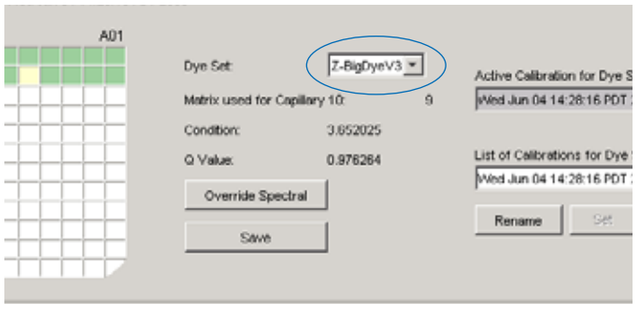
To set active spectral calibration

1. In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Spectral Viewer**.

   IMPORTANT! If the Spectral Viewer window is blank and deactivated, then either:

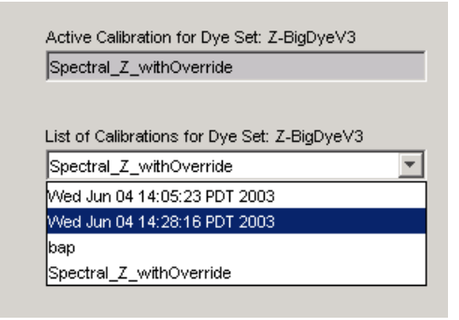
   - no spectral calibrations are in the database or,
   - you changed the arrary length and do not have a spectral for that dye set and array length combination. you will have to create one

2. In the Dye Set drop-down list, select a dye set**.**



Notes

3. In the List of Calibrations for Dye Set drop-down list, select the spectral calibration you want to use.

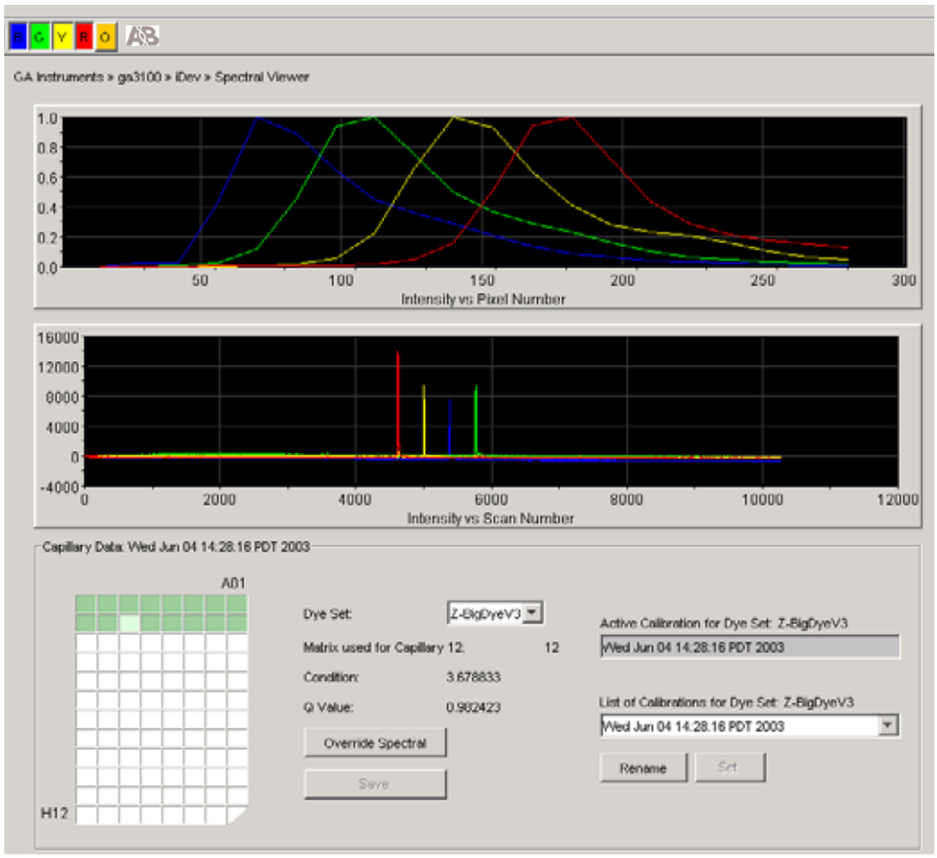


4. Click Set.

Notes

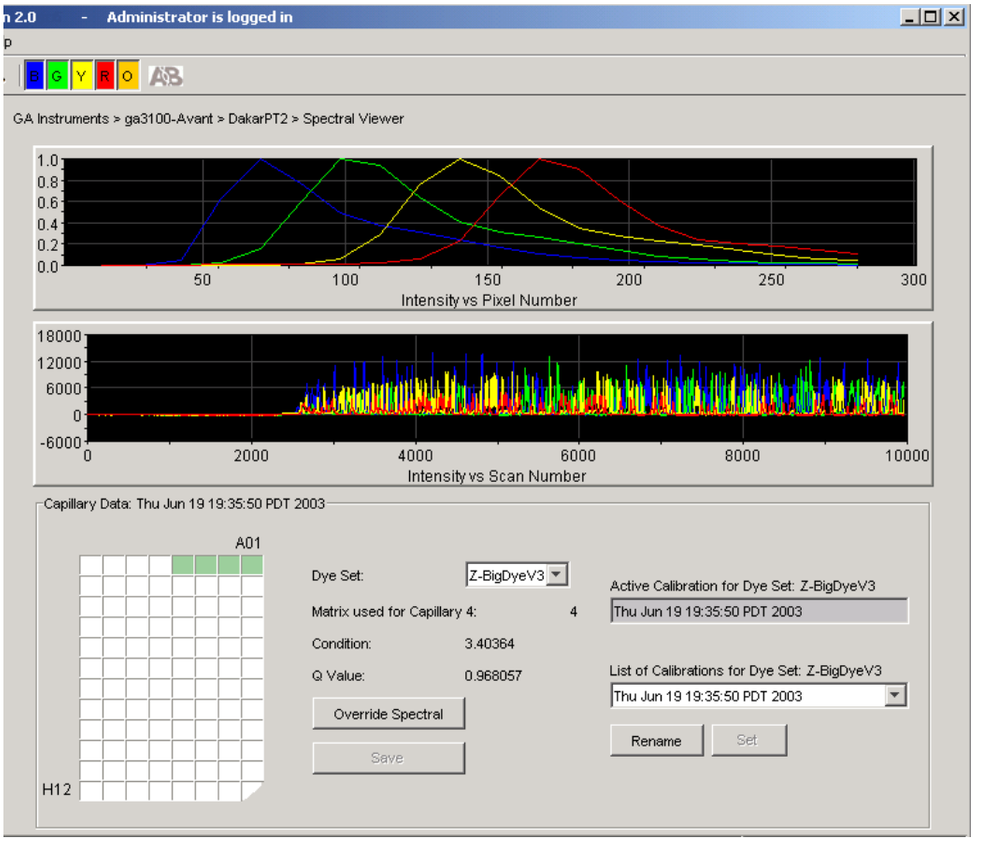
Examples of Passing Sequencing Spectral Calibrations

Dye Set Z Created from Matrix Standard



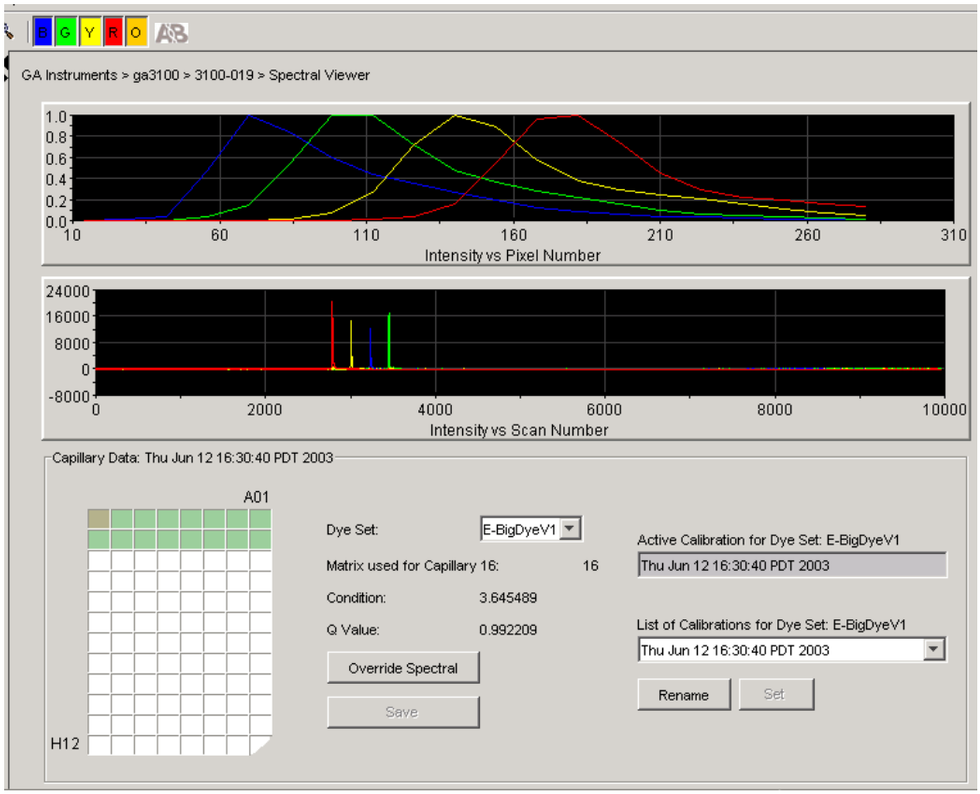
Notes

Dye Set Z Created from a Sequencing Standard



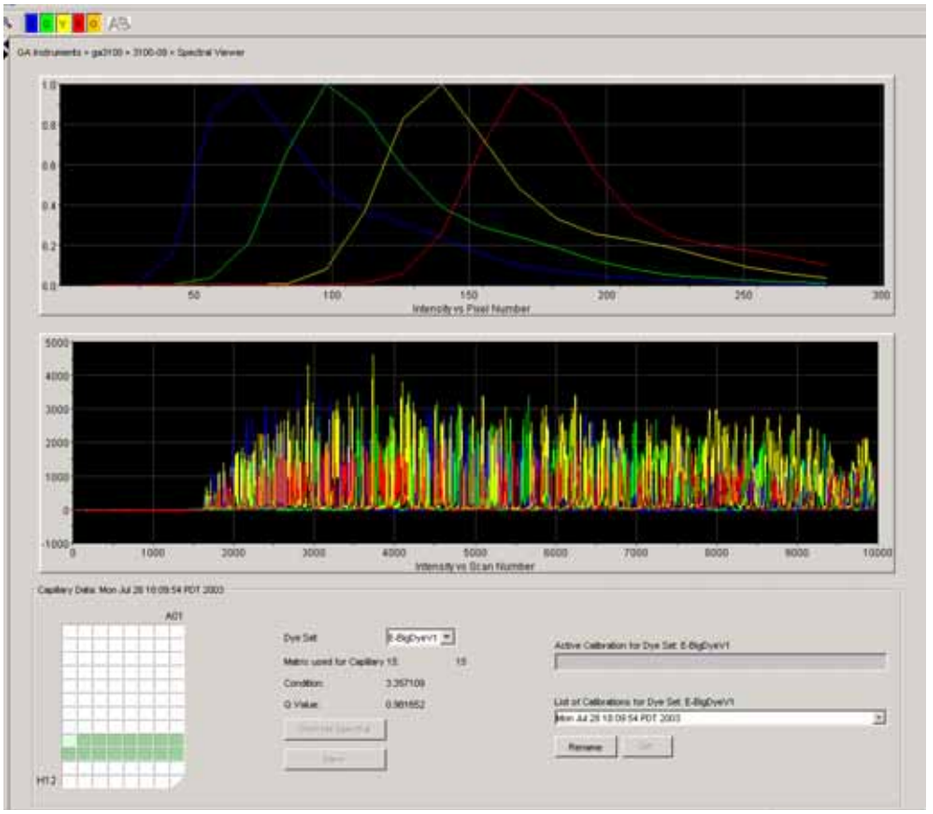
Notes

Dye Set E Created from Matrix Standard Set DS-01

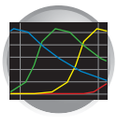


Notes

Dye Set E Created from a Sequencing Standard

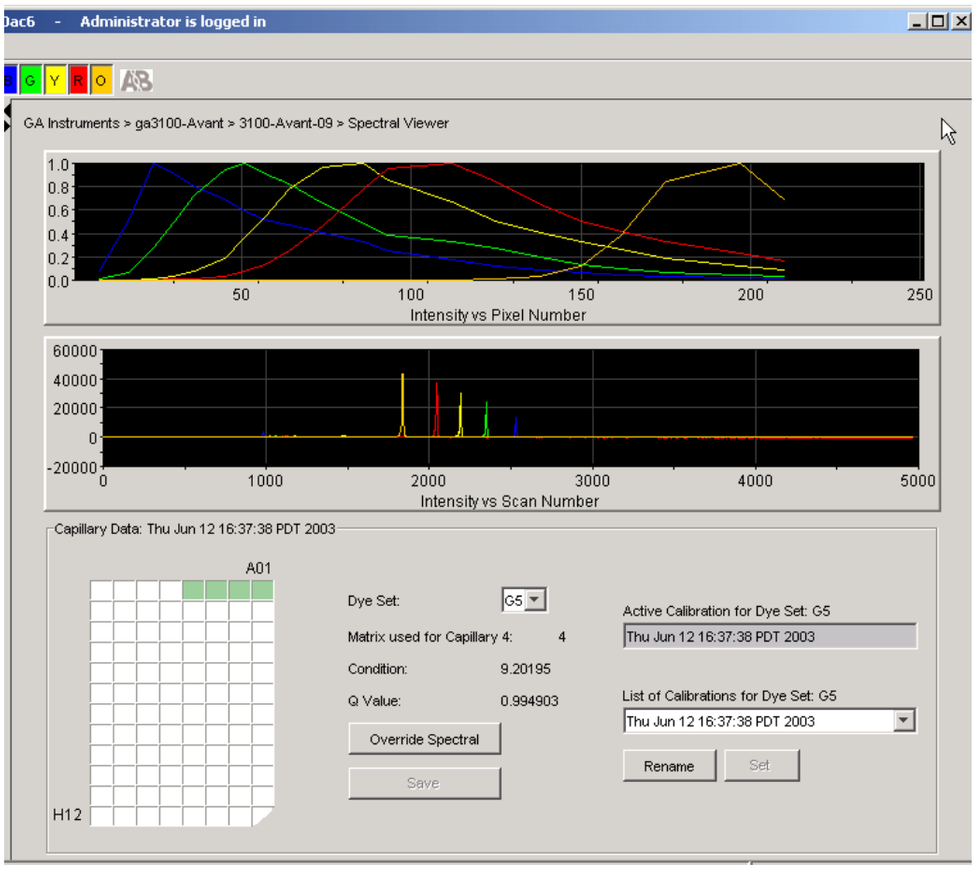


Notes



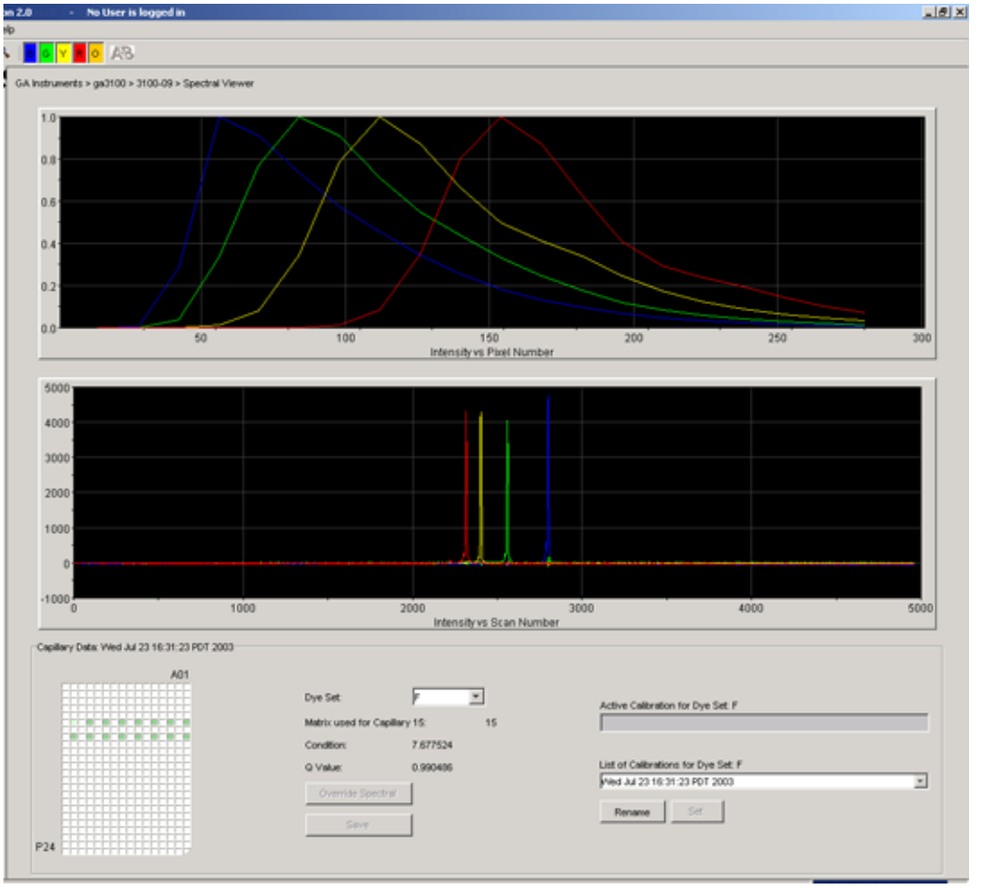
Examples of Passing Fragment Analysis Spectral Calibrations

Dye Set G5 Created from Matrix Standard Set DS-33

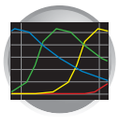


Notes

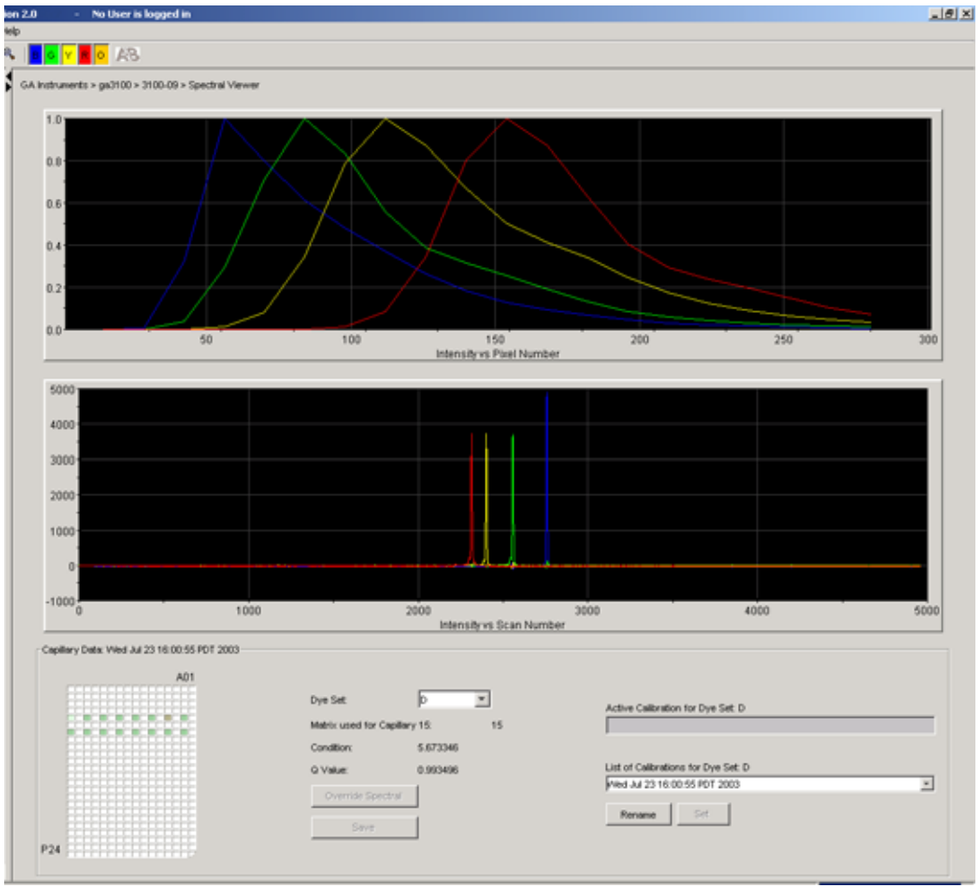
Dye Set F Created from Matrix Standard Set DS-32



Notes

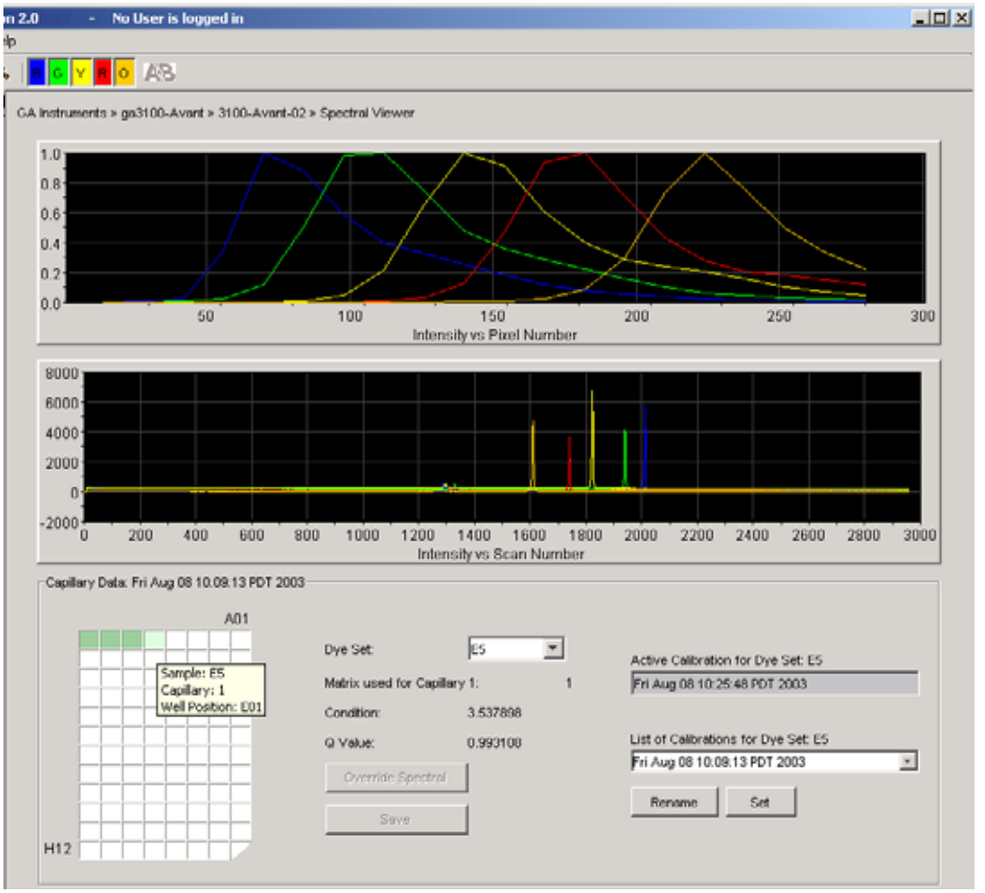


Dye Set D Created from Matrix Standard Set DS-30

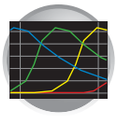


Notes

Dye Set E5 Created from Matrix Standard Set DS-02



Notes



Where to Go Next

Use the table below to determine which chapter to proceed to next.

Do you need to...	Proceed to ...
Set up the software for sequencing analysis runs?	Chapter 4, page 73
Set up the software for SeqScape analysis runs?	Chapter 4, page 99
Set up the software for fragment analysis runs?	Chapter 5, page 123
Perform maintenance, use wizards?	Chapter 7, page 195
Activate/modify the audit trail and access control features?	Chapter 8, page 241
Learn more about on plate record feature?	Appendix A, page 261
Learn more about troubleshooting?	Appendix C, page 279

Notes

Notes

3100/3100-*Avant* Data Collection Software and DNA Sequencing

This chapter covers:

Notes

Notes

3100/3100-*Avant* Data Collection Software and Sequencing Analysis Software v5.1

The Applied Biosystems Sequencing Analysis Software v5.1 must be installed and registered with the 3100/3100-*Avant* Genetic Analyzer Data Collection Software before you can create the files required for autoanalysis. Please refer to the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide* (P/N 4346366) for further information.

Important Notes

- A unique name must be assigned to the instrument computer before 3100/3100-*Avant* Data Collection software is installed. Do not rename the computer once 3100/3100-*Avant* Data Collection software has been installed. Doing so may cause the 3100/3100-*Avant* Data Collection software to malfunction.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. The invalid characters are below:

spaces

\ / : * ? " < > |

IMPORTANT! An error message is displayed if you use any of these characters. You must remove the invalid character to continue.

Using Sequencing Analysis Software

You may choose to perform autoanalysis of sequencing samples by utilizing features of the 3100/3100-*Avant* Data Collection and Applied Biosystems Sequencing Analysis Software v5.1.

Autoanalysis

Autoanalysis can only be performed on the same instrument computer that collected the sample files. Additionally, if you perform autoanalysis on samples, but wish to edit/review results on another computer, you must transfer the Analysis Protocol to the Sequencing Analysis Software v5.1 database. If you wish to analyze samples on another computer, you must transfer the files to that location.

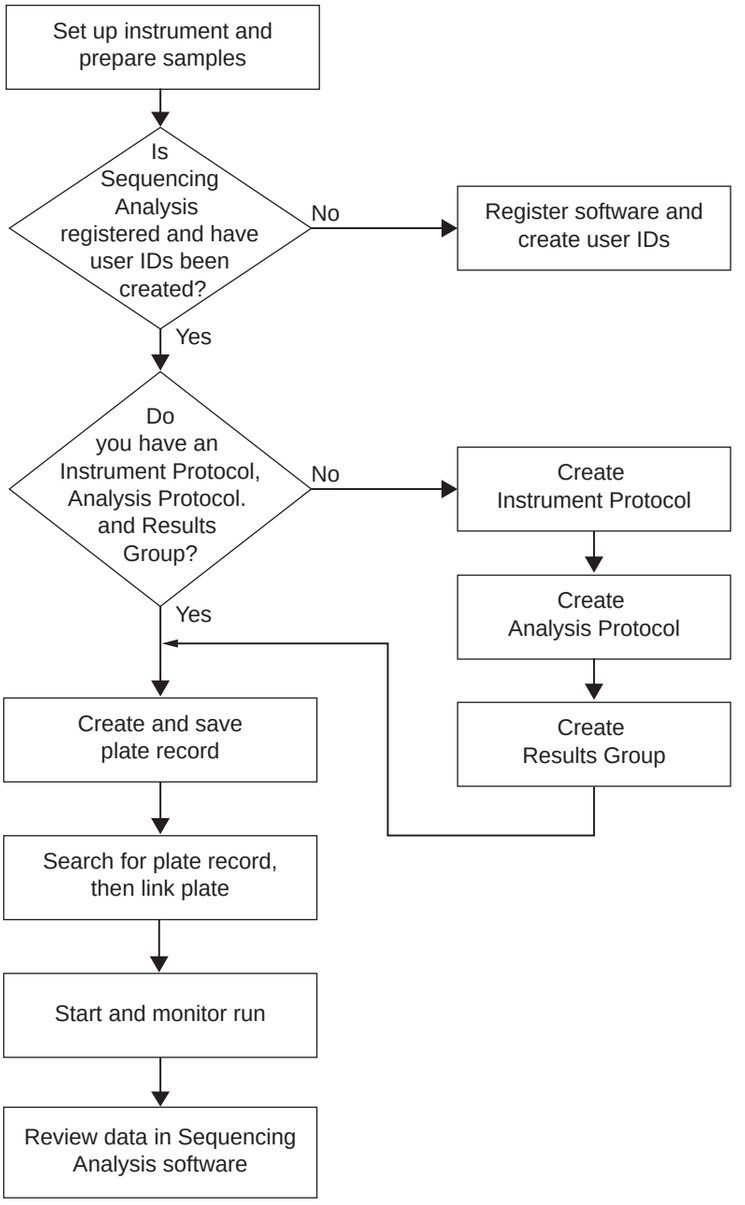
When completing the Plate Record, you need to fill in Instrument Protocol information for Data Collection to complete the run. Additionally, when creating a new Results Group for a set of samples to be autoanalyzed, you must check the “Do Autoanalysis” check box in the Analysis tab of the Results Group Editor, and choose an Analysis Protocol for each run.

Manual Analysis

If the run is not set up for autoanalysis, refer to the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide* (PN 4346366) for information on performing manual analysis.

Notes

Workflow for Autoanalysis Using Sequencing Analysis Software



Notes

About Plate Records and Sequencing Analysis

Overview

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

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

- Plate name, type, and owner
- Position of the sample on the plate (well number)
- Sample name, see page 91
- Mobility file (in Analysis Protocol), see page 84
- Comments about the plate and about individual samples
- Name of the run module and Dye set information (run modules specify information about how samples are run) (in Instrument Protocol), see page 79
- Name of the Analysis Protocol—Analysis Protocols specify how data is analyzed at the end of the run (in Analysis Protocol), see page 84

When to Create a Plate Record

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

- Spectral calibrations
- Sequencing analysis
- SeqScape analysis
- Fragment analysis
- Mixed (sequencing, SeqScape and/or fragment analysis samples) see "Multi-application (Mixed) Plate Record" on page 269

Additionally, Plate Records must be created in advance of placing the plates on the instrument. However, plate records, for subsequent plates can be created while a run is in progress.

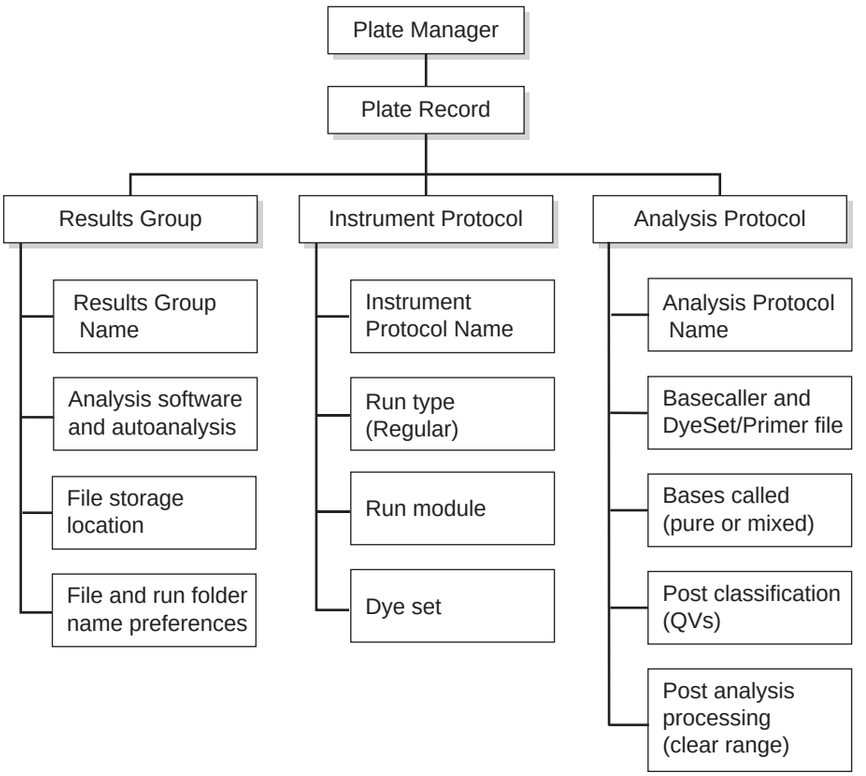
About Sequencing Analysis Plate Record

The Plate Editor displays an empty plate record for the selected application that is chosen in the New Plate dialog box. The data fields within a given plate record vary depending on the selected application. This section describes the data fields that are present in a sequencing analysis Plate Record.

The table below and the flow chart on page 76 describes what each file specifies:

Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	79
Analysis Protocol	Contains everything needed to analyze sequencing data.	83
Results Group	Defines the file type, the file name, file save locations, analysis software and autoanalysis.	89

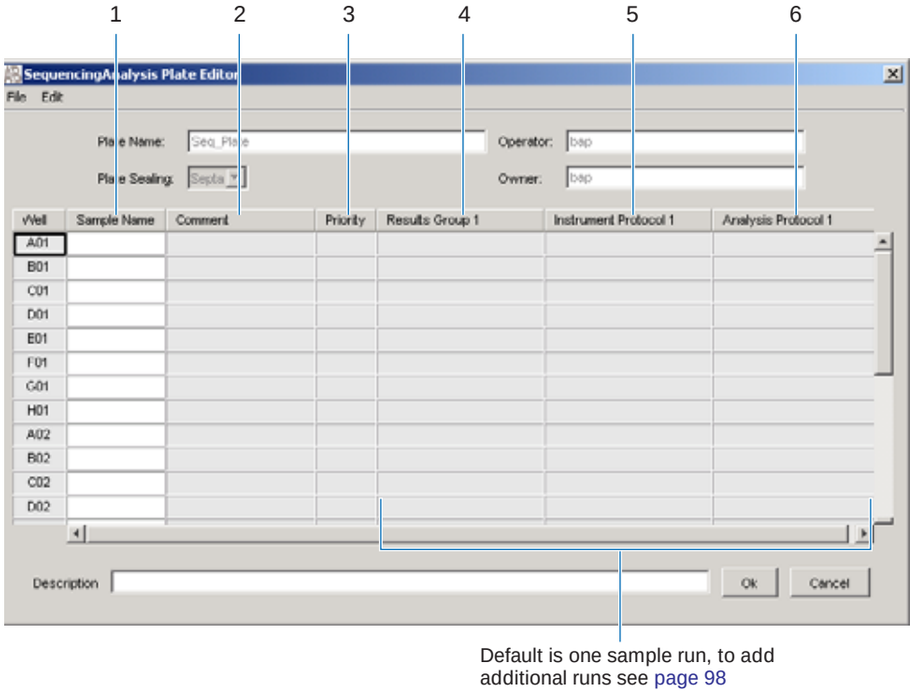
Notes



Elements of a Sequencing Analysis plate record

IMPORTANT! In order for data collection and autoanalysis to be successful, each run of samples must have an Instrument Protocol, an Analysis Protocol, and a Results Group assigned within a plate record.

Notes



Blank Sequencing Analysis plate record

The following table describes the columns inserted in a Plate Record for a sequencing analysis run.

Number and Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Priority	A default value of 100 to each sample. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list.
4. Results Group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the Results Group listed in the cell • None: Sets the cell to have no selected Results Group • Select one of the available Results groups from the list Note: You must have a Results Group selected for each sample entered in the Sample Name column. See, “Results Group for Sequencing Analysis” on page 89.

Notes

Number and Column	Description
5. Instrument Protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. Note: You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See, "Instrument Protocol for Sequencing Analysis" on page 79.
6. Analysis Protocol	• New: Opens the Analysis Protocol Editor dialog box. • Edit: Opens the Analysis Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Analysis Protocols: In alpha-numeric order Note: You must have an Analysis Protocol selected for each sample entered in the Sample Name column. See, "Analysis Protocol for Sequencing Analysis" on page 83.

Notes

Creating Required Files for Automated Sequencing Analysis

If the Files Already Exist in Data Collection

If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to "Creating and Completing a Sequencing Analysis Plate Record" on page 96.

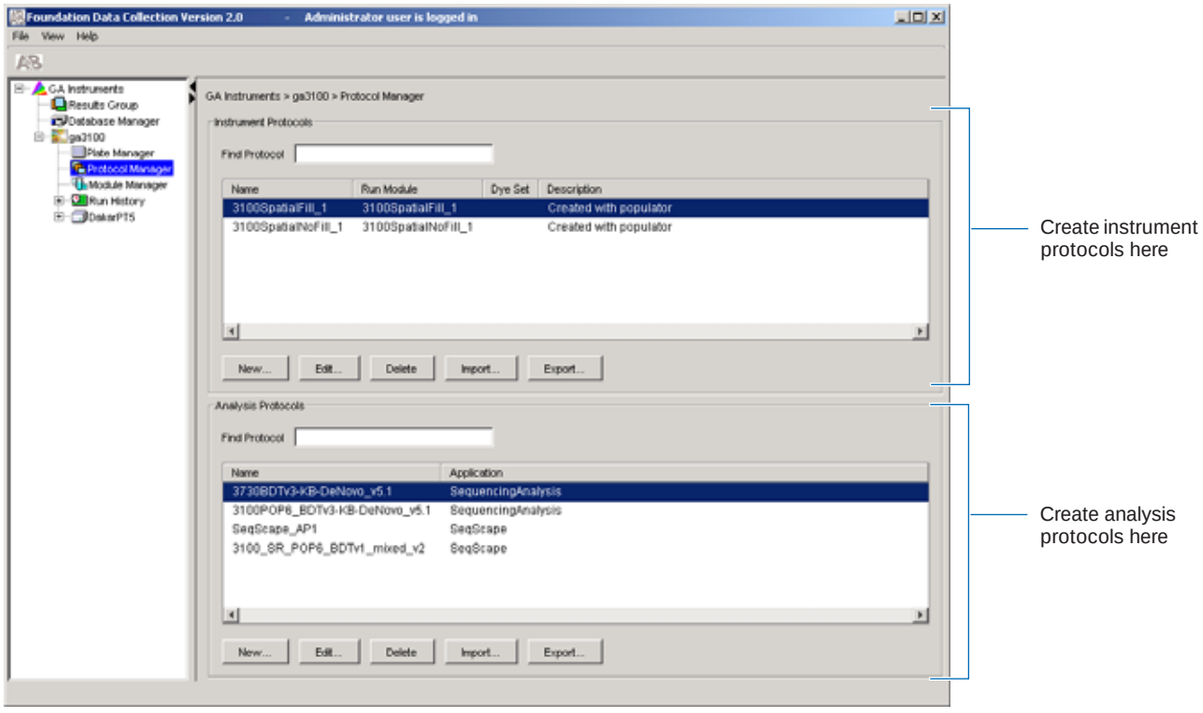
Instrument Protocol for Sequencing Analysis

About Instrument Protocols

An instrument protocol contains all the settings necessary to run the instrument. An instrument protocol contains the protocol name, type of run, run module, and dye set.

Creating an Instrument Protocol

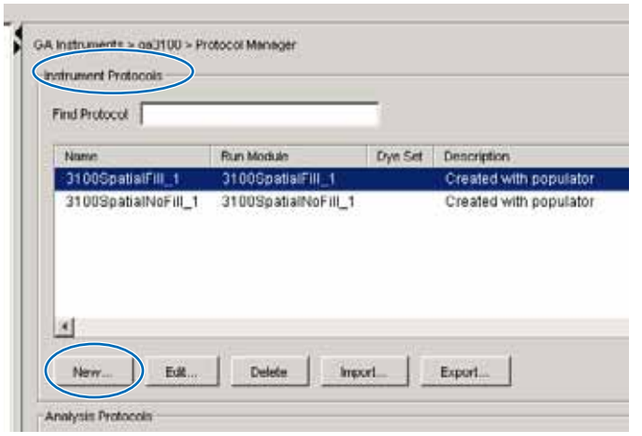
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Protocol Manager**.



Notes

2. In the Instruments Protocols section, click New... .

   The Protocol Editor opens.

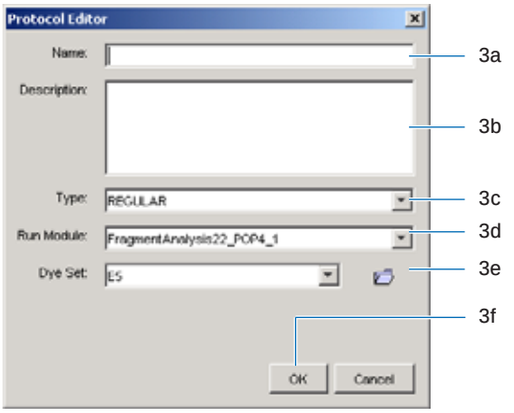


3. Complete the Protocol Editor:

   a. Type a name for the protocol.

   b. Type a description for the protocol (optional).

   c. Select **Regular** in the Type drop-down list.



   d. Using the information in the table below, select the correct run module for your run.

   Note: To customize a run module, see "Tip: Customizing Run Modules" on page 82.

Sequencing Run	Capillary Array Length (cm)	Run Module
Ultra rapid	36	UltraSeq36_POP4_1
Rapid	36	RapidSeq36_POP6_1
Standard	50	StdSeq50_POP4_1
		StdSeq50_POP6_1
Long read	80	LongSeq80_POP4_1

Notes

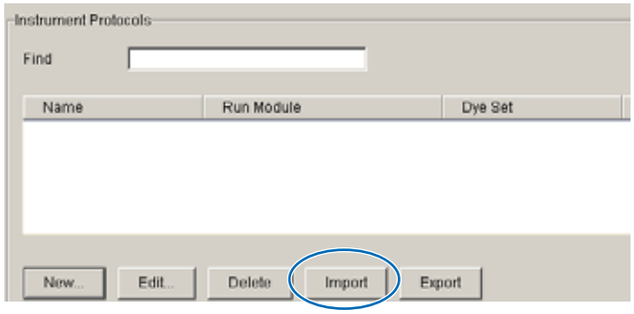
e. Using the information in the table below, select the correct Dye Set for your run.

Dye Set	Chemistry	Instrument
E_BigDyeV1	ABI PRISM® BigDye® v1.1 Primer	3100 only
	ABI PRISM® BigDye® v1.1 Terminator	3100/3100-*Avant*
	ABI PRISM® dRhodamine Terminator	
	ABI PRISM® dGTP BigDye® Terminator	
Z_BigDyeV3	ABI PRISM® BigDye® v3.1 Primer	3100 only
	ABI PRISM® BigDye® v3.1 Terminator	3100/3100-*Avant*
	ABI PRISM® dGTP BigDye® v3.0 Terminator	

f. Click OK.

Importing an Instrument Protocol

1. Click Import in the Instrument Protocols pane of the Protocol Editor window.

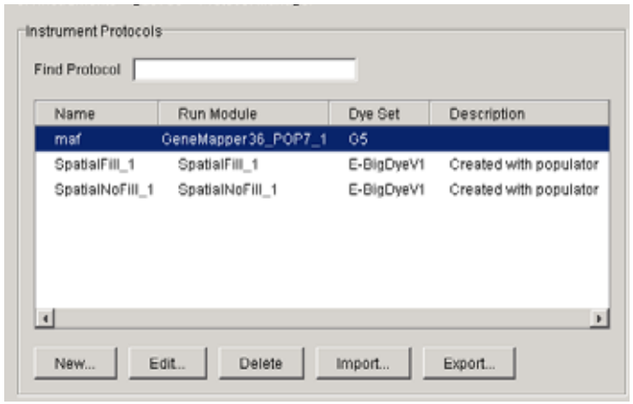


2. Navigate to the protocol you want to import.

   Note: Import file type is .txt (text).

3. Double-click the protocol to import it.

   The imported file is displayed as the top row in the Instrument Protocol pane.



Notes

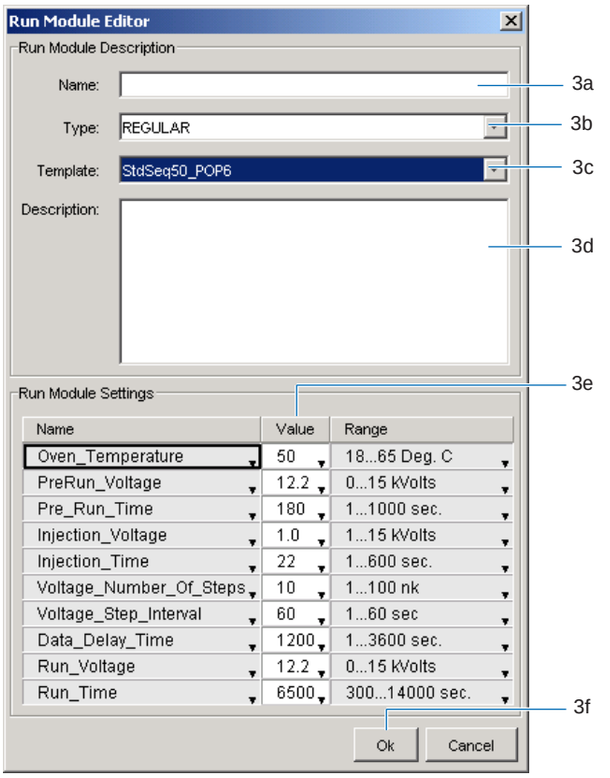
Tip: Customizing Run Modules

You can modify default run modules to suit your particular needs.

1. Click **GA Instruments >** **ga3100 or ga3100-*Avant* >** *instrument name* **> Module Manager.**
2. Click New... .
   The Run Module Editor dialog box opens.
3. Complete the Run Module Editor dialog box:
   a. Enter a name for your new module.
   b. In the Type drop-down list, select the type of module (Regular, Spatial or Spectral).
   c. In the Template drop-down list, select a template module as a basis for the new module.

      Note: You cannot edit a default module installed with 3100/3100-*Avant* Data Collection software.

   d. Optional: Enter a description of your new run module.



Notes

Tip: Customizing Run Modules *(continued)*

e. Change to the desired module parameters using the table below as a guide to the allowable parameters.

Name	Range	Comment
Oven_Temperature	18-65 C	Temperature setting for main oven throughout run.
PreRun_Voltage	0-15 kV	Pre run voltage setting before sample injection.
PreRun Time	1-1000 sec	Prerun voltage time.
Injection_Voltage	0-15 kV	Injection voltage setting for sample injection.
Injection_Time	1-600 sec	Sample injection time.
Run_Voltage	0-15 kV	Final run voltage.
Voltage_Number_Of_Steps	0-100 steps	Number of voltage ramp steps to reach Run_Voltage. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Voltage_Step_Interval	0-60 sec	Dwell time at each voltage ramp step. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Data_Delay_Time	1-3600 sec	Time from the start of separation to the start of data collection.
Run_Time	300-14000 sec	Duration data is collected after Data_Delay_Time.

f. Click **OK**.

Analysis Protocol for Sequencing Analysis

About Analysis Protocols

New to Data Collection is the implementation of analysis protocols. An analysis protocol contains all the settings necessary for analysis and post processing:

- Protocol name – The name, description of the analysis protocol, and the sequence file formats to be used
- Basecalling settings – The basecaller, DyeSet/Primer file, and analysis stop point to be used
- Mixed Bases – Option: to use mixed base identification, and if so, define the percent value of the second highest to the highest peak
- Clear Range – The clear range to be used based on base positions, sample quality values, and/or number of ambiguities (Ns) present

Note: If you created an appropriate analysis protocol in the Sequencing Analysis software, you can use it in data collection software.

IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis will not be performed if you do so.

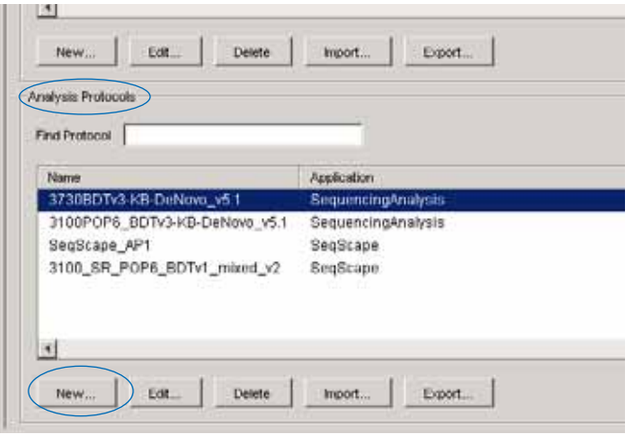
Notes

Creating an Analysis Protocol

Refer to the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide* (P/N 4346366), chapter 8 for more information regarding analysis protocols

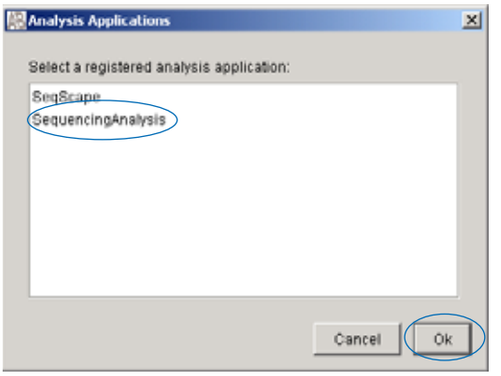
1. In the Analysis Protocol section of the Protocol Manager, click New....

   If more than one analysis application is installed on the data collection computer, the Analysis Applications dialog box opens.



2. Select **Sequencing Analysis**, then click OK.

   The Analysis Protocol Editor dialog box opens.

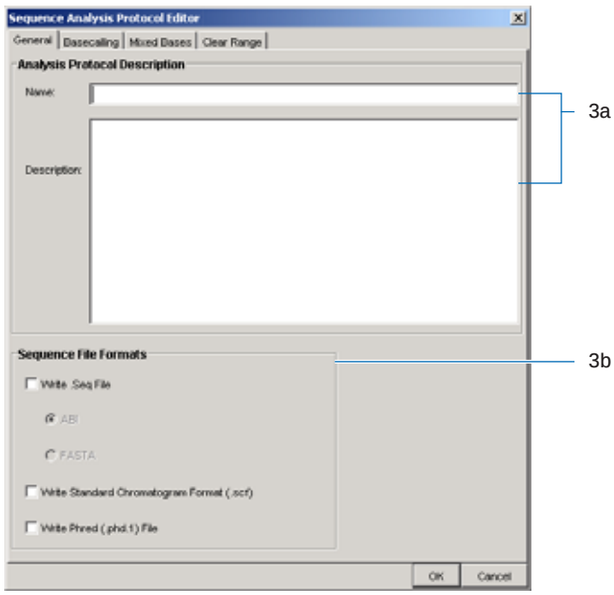


Notes

3. In the **General** tab:

   a. Enter a unique name and description for the new protocol.

   b. Select the appropriate Sequence File formats settings.

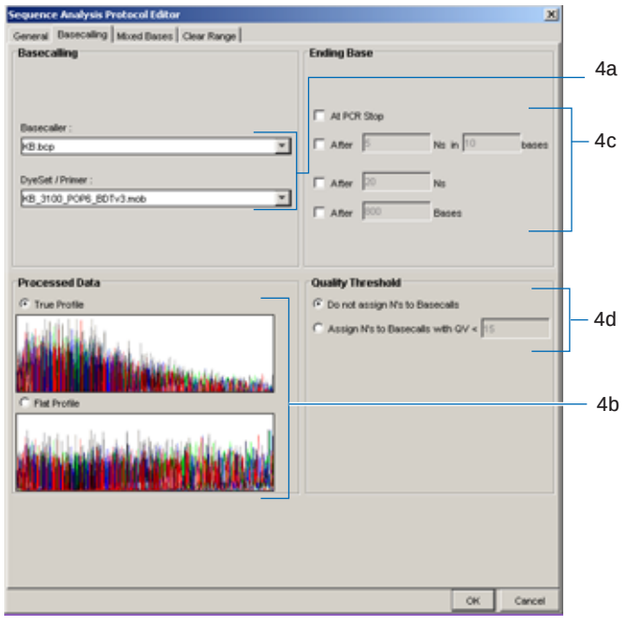


Option	If checked, the software creates…
Write .Seq File check box	a .seq file for printing the sequence as text file or for using the file in other software. • ABI format is used with Applied Biosystems software. • FASTA format is used with other software
Write Standard Chromatogram Format file (.scf)	When selected, the software creates a .scf file that can be used with other software. When created, the .scf extension is not appended to the file name.
Write Phred (.phd.1) File	When selected and the KB basecaller is used, the software creates a .phd.1 file that can be used with other software.

4. Select the **Basecalling** tab.

   a. Use Appendix B, "Basecallers and DyeSet/Primer Files," to select the appropriate basecaller and DyeSet primer based on the chemistry, capillary array length and polymer type you are using.

   Note: Sequencing Analysis Software v5.1 and 3100/3100-*Avant* Data Collection software filter .mob file choices to match the chosen .bcp file.



Notes

b. In the Processed data pane, select True or Flat profile.

Option	Function
True Profile	Used to display data as processed traces scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. The profile of the processed traces will be very similar to that of the raw traces.
Flat Profile	Used to display the data as processed traces scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces will be flat on an intermediate scale (> about 40 bases). Note: This option is applied to data that is analyzed with the KB basecaller only. If you use the ABI basecaller the profile option reverts to True Profile.

c. If desired, select one or more stop points for data analysis.

d. Select your Threshold Quality option.

Option	Function
Call all bases and assign QV	When using the KB basecaller, use this setting assign a base to every position, as well as the QV.
Assign 'N' for bases with QV < 15	When using the KB basecaller, use this setting assign Ns to base with QVs less than the set point. The QV will still be displayed.

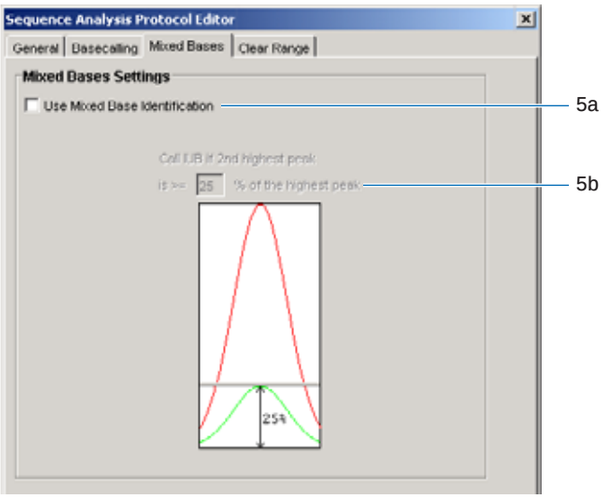
5. Select the **Mixed Bases** tab.

Note: This function is active with the KB Basecaller only.

a. For mixed bases only, select **Use Mixed Base Identification**.

b. Use the default setting of 25% or change the detection level by entering a new value or dragging the % line up or down.

Note: Do not use less than 15% as your detection limit.



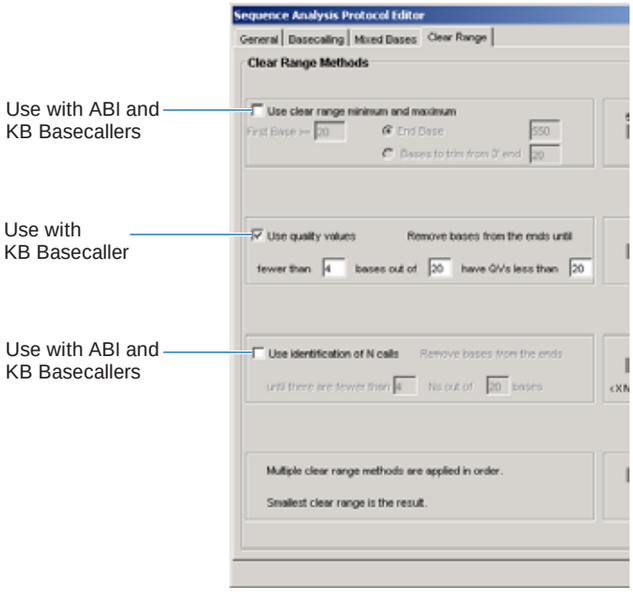
Notes

6. Select the **Clear Range** tab.

   Note: The clear range is the region of sequence that remains after excluding the low-quality or error prone sequence at both the 5´ and 3´ ends.

   Select one or more Clear Range methods. If you apply multiple methods, the smallest clear range results.

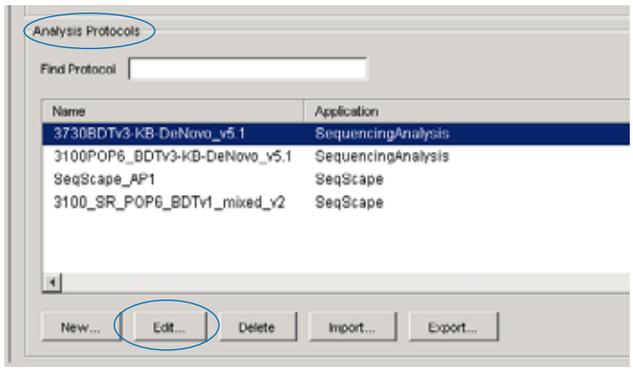
7. Click OK to save the protocol and close the Sequence Analysis Protocol Editor dialog box.



Editing and Deleting Analysis Protocols

Editing an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to edit.
2. Click Edit....
3. Make changes in the General, Basecalling, Mixed Bases and Clear Range tabs, as appropriate.
4. Click OK to save the protocol and close the Analysis Protocol Editor dialog box.

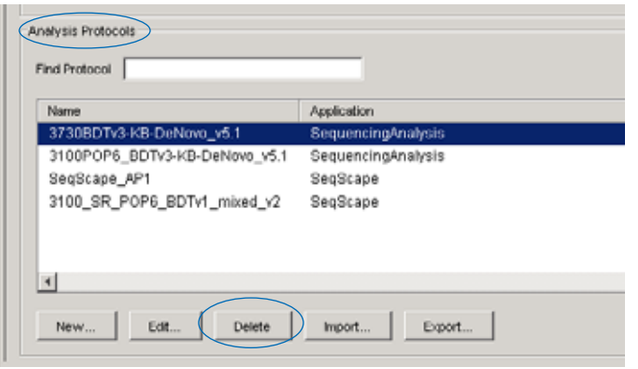


Notes

Deleting an Analysis Protocol

IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis will not be performed if you do so. Also, You must first delete any plate records using the Analysis Protocol before you can delete or modify the Analysis Protocol for these plate records.

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to delete.
2. Click Delete.
   The Deletion Confirmation dialog box displays.
3. Click Yes.

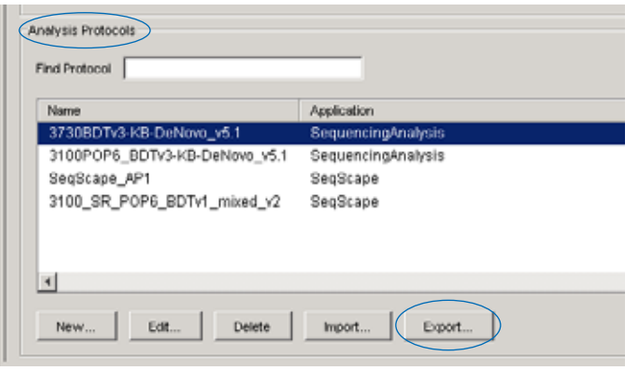


Note: It is better to delete the Analysis Protocol from the Sequencing Analysis Software v5.1.

Exporting and Importing Analysis Protocols

Exporting an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to export.
2. Click Export.
   The Export Confirmation dialog box displays.
3. Click Yes.

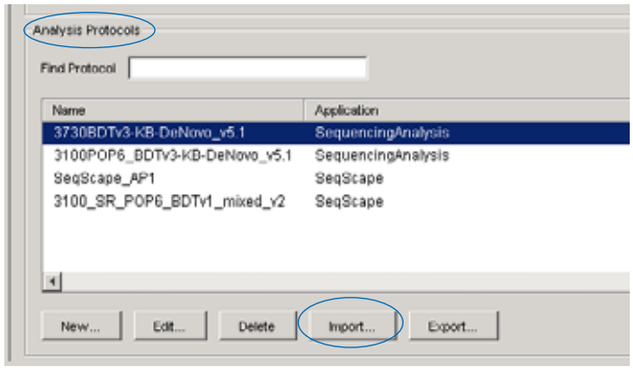


Notes

Importing an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to import.
2. Click Import.

   The Import Confirmation dialog box displays.
3. Click Yes.



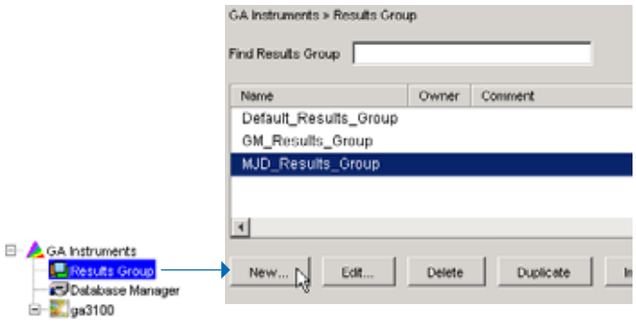
Results Group for Sequencing Analysis

A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. It is called a Results Group because it is used to analyze, name, sort, and deliver samples that result from a run.

Creating a Results Group

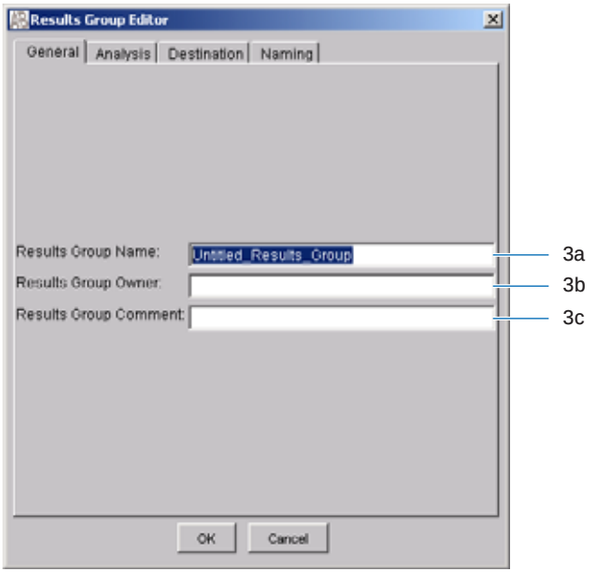
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click New....

   The Results Group Editor window displays.



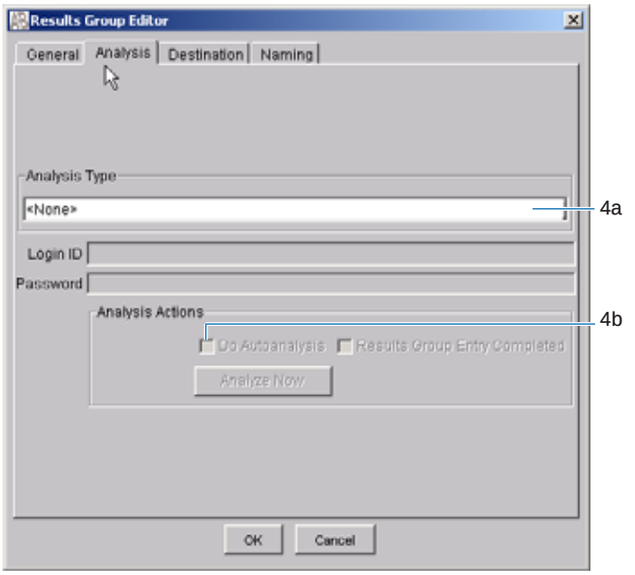
Notes

3. Complete the General tab:
   a. Type a Results Group Name. The name can be used in naming and sorting sample files. It must be unique (see page for a list of accepted characters).
   b. Type a Results Group Owner (optional). The owner name can be used in naming and sorting sample files.
   c. Type a Results Group Comment (optional).



4. Select the **Analysis** tab, then:
   a. Select **Sequencing Analysis** from the Analysis Type drop-down list.
   b. In the Analysis Actions section, select **Do Autoanalysis**, if you want your data automatically analyzed after a run.

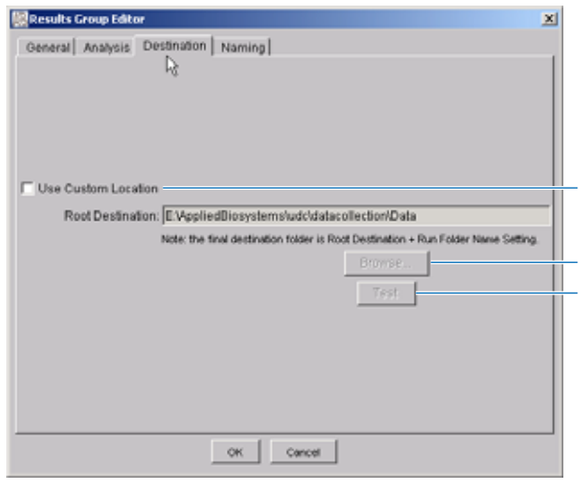
Note: Login ID and password are not required for Sequencing Analysis software.



Notes

5. Select the **Destination** tab, then use the default destination or define a new location for data storage.

To use …	Then …
default location	skip to step 6
custom location	complete steps a-c



   a. Click **Use Custom Location**, then click Browse... to navigate to a different save location.

   b. Click Test to test the Location path name connection:

      If it passes, a message box displays "Path Name test successful."

      If it fails, a message box displays "Could not make the connection. Please check that the Path Name is correct." Click and retry to establish a connection.

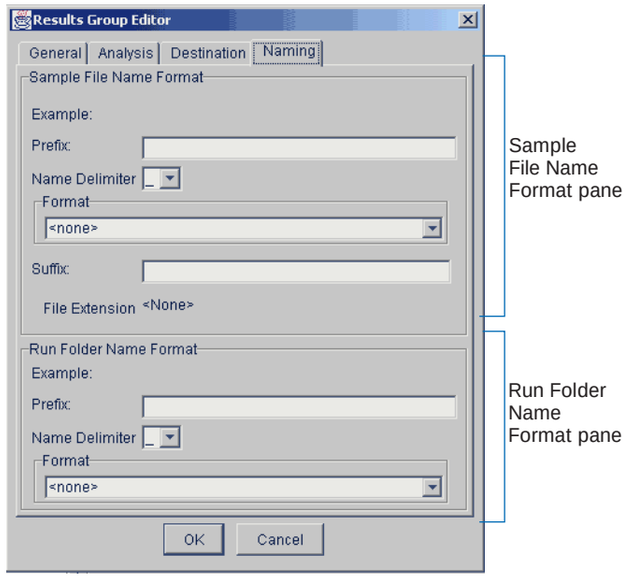
   c. Click OK.

6. Select the **Naming** tab.

   Use the Naming tab to customize sample file and run folder names.

   IMPORTANT! Sample name, run folder name, and path name, *combined*, can total no more than 250 characters. See page 73 for accepted characters.

   The elements of the Naming tab are discussed in the following sections.

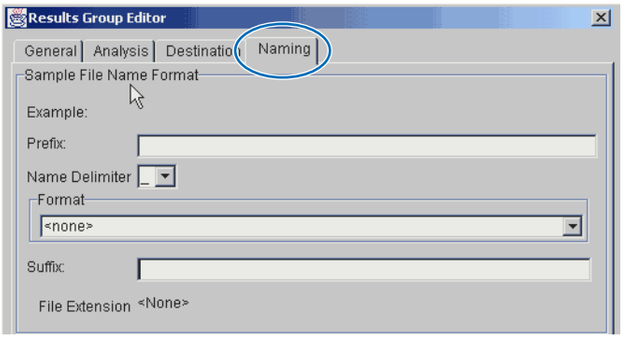


Sample File Name Format Pane

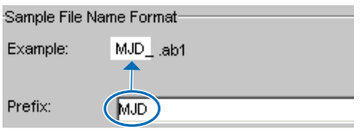
Follow the procedure below to complete the Sample File Name Format pane.

Notes

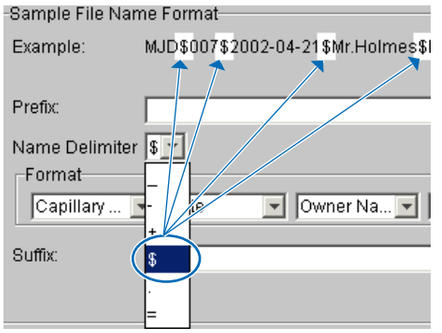
1. Select the **Naming** tab.



2. Click the **Prefix** box (optional) to type a prefix for the file name. Anything that you type here is shown in the Example line (see graphic below).



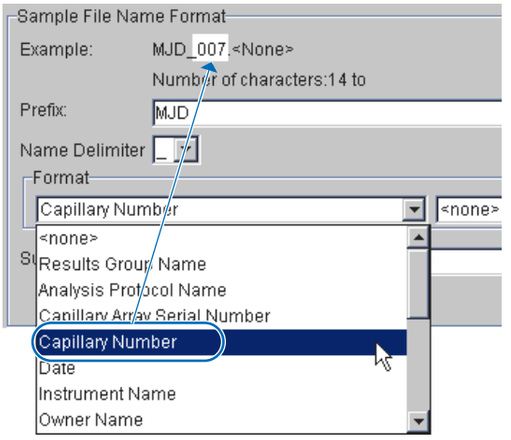
3. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3 below). Only one delimiter symbol may be chosen.



4. Click the Format list and then select the components that you want in the sample name.

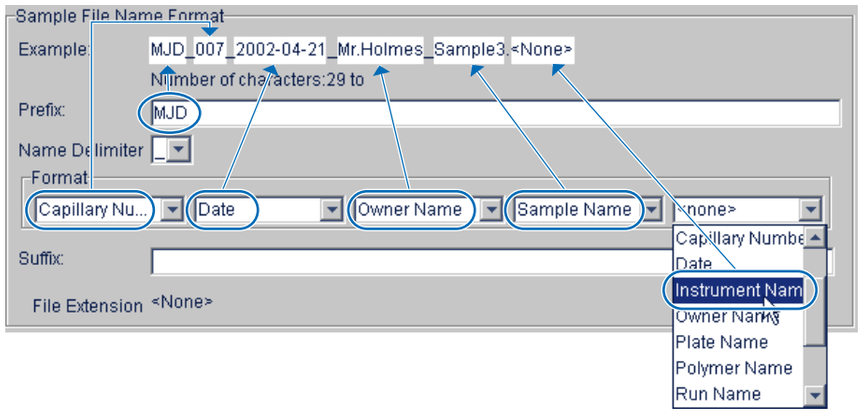
   Note: Generally, all the samples from a single run are placed in the same run or results folder, so the name of every sample from a single run should be different. Most of the Format options will not be different between samples, so you need to take care to select at least one of the options that make the sample names unique within a run.

   For example, if a unique identifier is not included in the name, a warning message displays. The Results Group **makes the** file name unique. As you select the elements for the file name, they are reflected in the Example line.

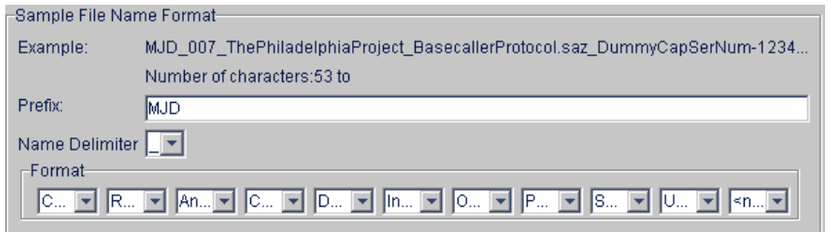


Notes

As you continue to select elements for the file name, additional elements display.

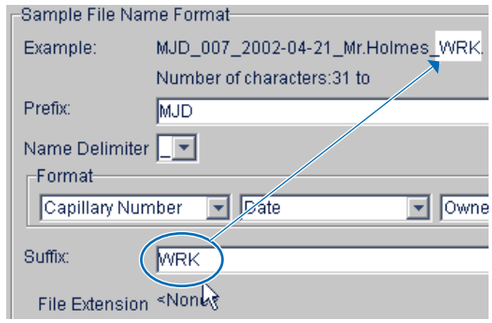


The names of the Format elements eventually truncate, but the Example field remains visible (up to 72 characters).



5. **Click the Suffix** box (optional) and type the suffix for the file name.

   The **File Extension** field displays the file extension generated from the Analysis Type specified on the **Analysis** tab (see page 90). For example, Sequencing Analysis and produces sample files with an .ab1 extension.



Notes

Run Folder/Sub-Folder Name Format Pane

Follow the same steps described above for the Sample File Name Format pane (see page 91) to change the sub-folder name within the run folder.

Saving a Results Group

Click OK from any tab once all the elements within the Results Group have been chosen.

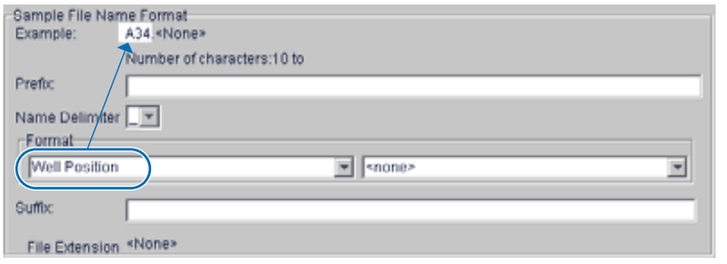
IMPORTANT! You must select at least one Format element for the Sample file and the Run folder names in order to proceed within the Results Group.

Note: Even if you create a custom run folder location, a separate default run folder is generated that contains the log file.

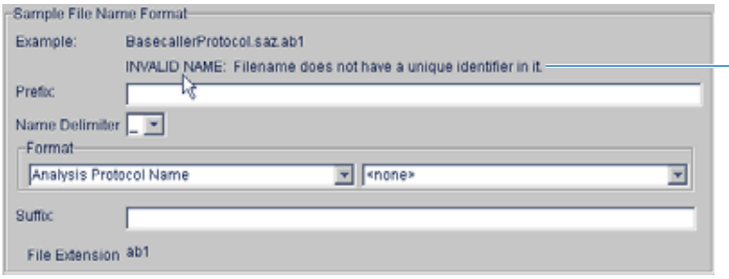
About Format Elements (Unique Identifiers)

While you may select a minimum of just one Format element for the Sample file and Run folder names in order to save a Results Group, selecting just the minimum may not provide enough information for you to identify the file or folder later.

For example, although acceptable, the 'A34' sample file name below (well position) may not be helpful when trying to locate and identify the file later.



If you choose elements from the Format lists that do not create unique Sample file or Run folder names, a warning message displays below the Example line (see figure 2-22).



To remove the warning message and proceed within the Results Group Editor window, simply select a Format element that distinguishes one file from another (for example, the capillary number is unique while the instrument name is not).

Notes

Importing and Exporting a Results Group

Results Groups can be imported from, or exported to, tab-delimited text files. This allows easy sharing of identical Results Groups between instruments.

Importing a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click Import.

   A standard File Import dialog box displays.
3. Navigate to the file you want to import.

   Note: Import file type is .txt (text).
4. Click Open.

   Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Exporting a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click the Results Group name to select it.
3. Click Export.

   A standard file export dialog box displays with the chosen Results Group name.
4. Navigate to the location where you want to save the exported file.
5. Click Save.

Note: If there is a name conflict with a Results Group that already exists at the save location, the Results groups can be duplicated in order to copy settings into a similar Results Group without the risk of user error when copying it manually (see procedure below).

Duplicating a Results Group

1. Click the Results Group to select it.
2. Click Duplicate.

Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Notes

Creating and Completing a Sequencing Analysis Plate Record

Creating a Sequencing Analysis Plate Record

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

3. Complete the information in the New Plate Dialog:

   a. Type a name for the plate.

   b. Type a description for the plate (optional).

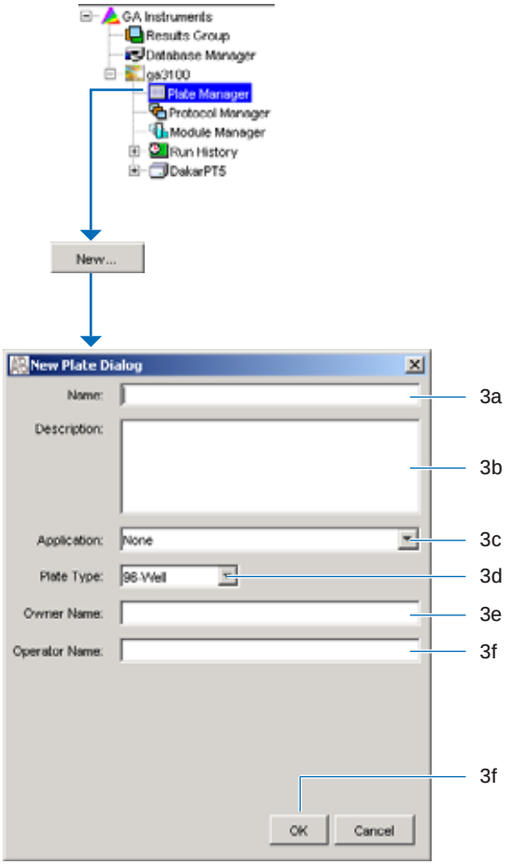
   c. Select your sequencing application in the Application drop-down list.

   d. Select **96-well** or **384-well** in the Plate Type drop-down list.

   e. Type a name for the owner and operator.

   f. Click OK.

   The Sequencing Analysis Plate Editor opens.



Notes

Completing a Sequencing Analysis Plate Record

1. In the **Sample Name** column of a row, enter a sample name, then click the next cell. The value 100 automatically display in the Priority column.

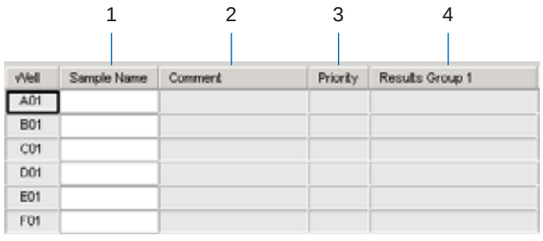
2. In the **Comments** column, enter any additional comments or notations for the sample.

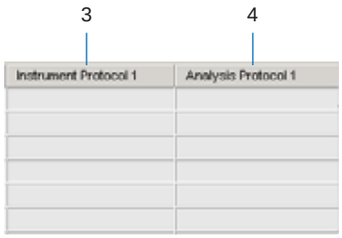
3. In the **Priority** column, change the priority value, if desired (see page 262).

4. In the **Results Group 1** column, select a group from the drop-down list (see page 89).

5. In the **Instrument Protocol 1** column, select a protocol from the drop-down list (see page 79).

6. In the **Analysis Protocol 1** column, select a protocol from the drop-down list (see page 84).

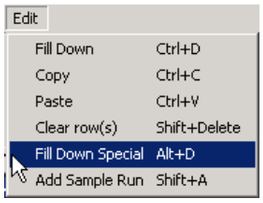




7. To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:

   - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down Special**.

     Based on the plate type (96- or 384-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run (see page 265).
   - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down**.
   - For the different samples and protocols – Complete the manually.

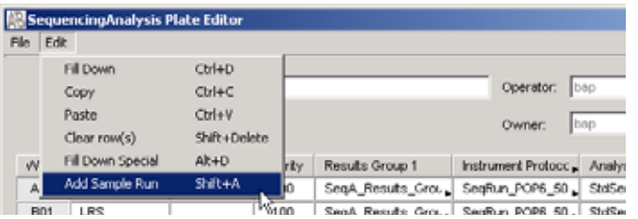


Notes

8. If you want to do more than one run, then select **Edit > Add Sample Run**.

   Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.

   You can add additional runs by selecting **Edit > Add Sample Run** again.



9. Complete the columns for the additional runs.

10. Click OK.

IMPORTANT! After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

Notes

3100/3100-*Avant* Data Collection Software and SeqScape Software v2.1

The ABI PRISM® SeqScape Software v2.1 must be installed and registered with the 3100/3100-*Avant* Genetic Analyzer Data Collection Software before you can create the files required for autoanalysis. Please refer to the *ABI PRISM® SeqScape Software v2.1 User Guide* (P/N 4346367) for further information.

Important Note

A unique name must be assigned to the instrument computer before 3100/3100-*Avant* Data Collection software is installed. Do not rename the computer once 3100/3100-*Avant* Data Collection software has been installed. Doing so may cause the 3100/3100-*Avant* Data Collection software to malfunction.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. The invalid characters are below:

spaces

\ / : * ? " < > |

IMPORTANT! An error message is displayed if you use any of these characters. You must remove the invalid character to continue.

Using SeqScape Software

Autoanalysis

Sequencing data that is generated on the ABI PRISM®3100/3100-*Avant* Genetic Analyzers can be automatically analyzed for use in the SeqScape Software v2.1. Autoanalysis can be performed only on the same instrument computer that collected the sample files. You can configure the software packages to perform data collection and then data analysis without requiring user interaction.

Autoanalysis requires three software packages:

- 3100 or 3100-*Avant* Data Collection software

  The data collection software is used to run the instrument and collect fluorescent data from samples. For autoanalysis to occur, the software must be set up properly to allow communication with downstream software.

  Data collection software uses a data service. Data used for data collection as well as that created in SeqScape software can be accessed through the data service in data collection software.

Notes

- Autoanalysis Manager

  The Autoanalysis Manager is software that is part of the integration between the data collection, SeqScape, and GeneMapper™ software. It can queue messages and track the status of their processing. Each message is considered a batch job, whether it contains a single sample, samples from a result group, or an entire run of samples.

  Autoanalysis Manager is installed by Seqscape or GeneMapper software when loaded on a system with data collection software.

- A version of SeqScape software with no user interface

  This version of SeqScape is identical to the regular version of the software except that no user interface exists. The Autoanalysis Manager opens and uses this version of software to analyze the data in the projects.

  The automated processing version and the standard version of SeqScape software are installed from the SeqScape Software installation CD.

IMPORTANT! When installing SeqScape software v2.1 on a computer that is connected to a 3100/3100-*Avant* Genetic Analyzer, the data collection software must be running. If data collection software is not running, the SeqScape software does not register with the Data Service. Refer to the *ABI PRISM® SeqScape Software v2.1 User Guide* (P/N 4346367), Chapter 2 for information on properly installing the software.

Manual Analysis

If the run is not set up for autoanalysis, refer to the *ABI PRISM® SeqScape Software v2.1 User Guide* (P/N 4346367) for information on performing manual analysis.

Notes

About Plate Records and Seqscape

Successful automatic analysis requires that the:

- SeqScape software is installed properly
- SeqScape software is registered and the appropriate user IDs have been created
- Autoanalysis Manager software is running
- The 3100/3100-*Avant* instrument is set up to run, and samples are prepared
- Files for a data collection software plate record are available

  For data collection and autoanalysis to be successful, each run of samples must have an instrument protocol, an analysis protocol, and a results group assigned within a plate record.

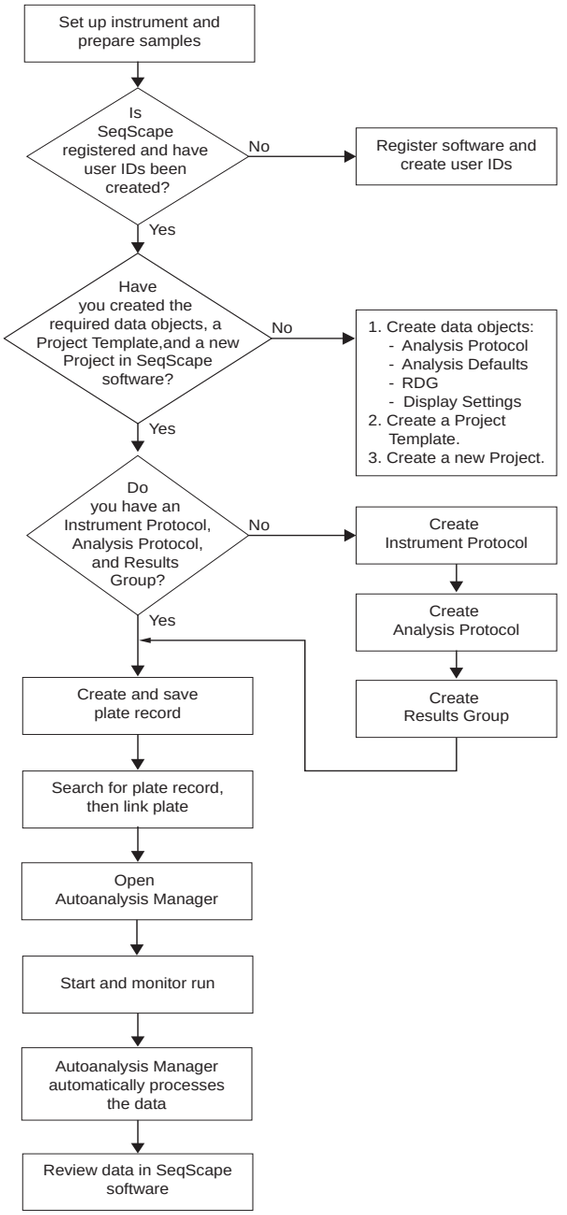
  The table below describes what each file specifies in the logical order of its use.

File Specifications

File	Description	Created in
Instrument Protocol	Contains everything needed to run the instrument.	Data collection software
Analysis Protocol	Contains everything needed to analyze sequencing data.	Data collection software or SeqScape software
Results Group	Defines the file type, the file name, file save locations, default analysis protocols linked to sample injections, and user name and password.	Data collection software

Notes

Work Flow for Autoanalysis Using SeqScape Software



About SeqScape Plate Record

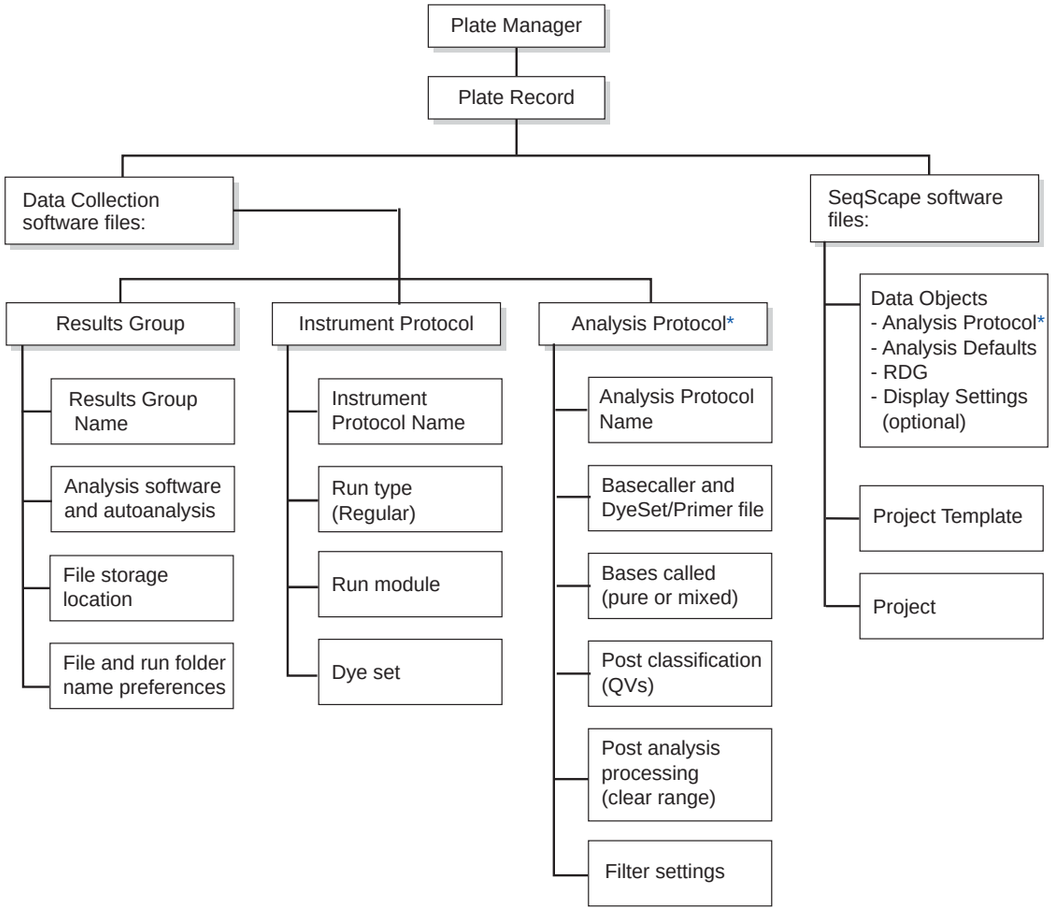
The Plate Editor displays an empty plate record for the selected application that is chosen in the New Plate dialog box. The data fields within a given plate record vary depending on the selected application. This section describes the data fields that are present in a sequencing analysis Plate Record.

For data collection and autoanalysis to be successful, each run of samples must have an instrument protocol, an analysis protocol, and a results group assigned within a plate record.

Notes

The table below and the flow chart on page 103 describes what each file specifies:

Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	106
Analysis Protocol	Contains everything needed to analyze sequencing data.	109
Results Group	Defines the file type, the file name, file save locations, analysis software, and autoanalysis.	115

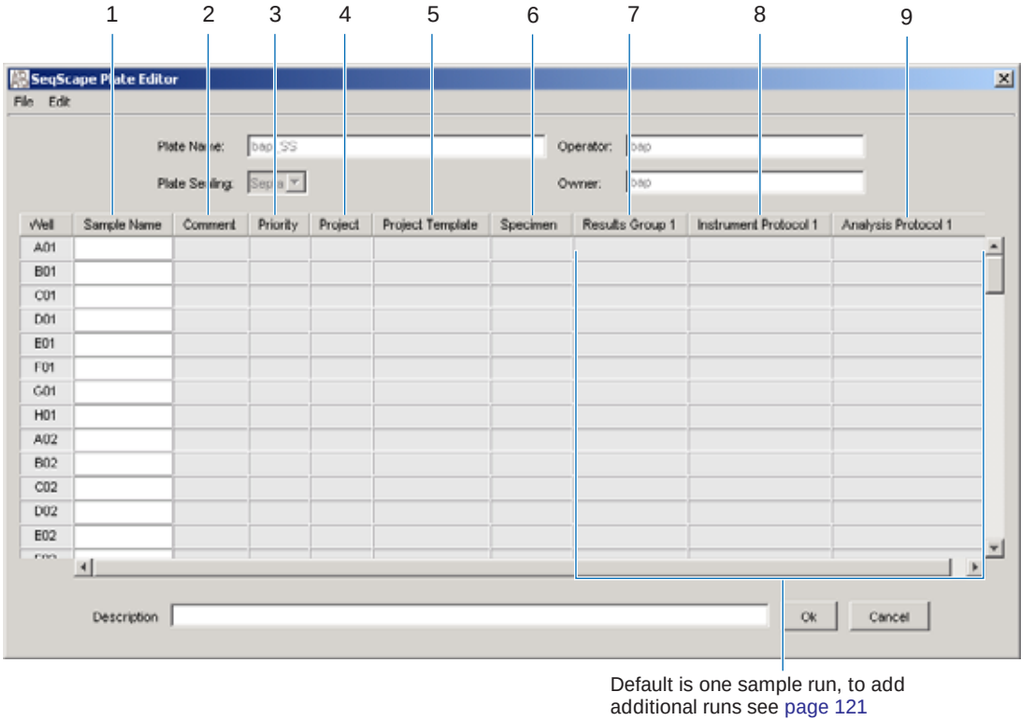


*You can create Analysis Protocols in either SeqScape or Data Colletion software

Elements of a SeqScape plate record

IMPORTANT! In order for data collection and autoanalysis to be successful, each run of samples must have an Instrument Protocol, an Analysis Protocol, and a Results Group assigned within a plate record.

Notes



Blank SeqScape plate record

The following table describes the columns inserted in a Plate Record for a SeqScape run.

Number and Column		Description
1.	Sample Name	Name of the sample
2.	Comment	Comments about the sample (optional)
3.	Priority	A default value of 100 to each sample. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list.
4.	Project	Select one of the available Project from the list, that was created in the SeqScape
5.	Project Template	Completed automatically based on the Project selected.
6.	Specimen	Select one of the available Specimen from the list, that was created in the SeqScape
7.	Results Group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the Results Group listed in the cell • None: Sets the cell to have no selected Results Group • Select one of the available Results groups from the list Note: You must have a Results Group selected for each sample entered in the Sample Name column. See, “Creating a Results Group” on page 115.

Notes

Number and Column	Description
8. Instrument Protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. Note: You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See, "Creating an Instrument Protocol" on page 106.
9. Analysis Protocol	• New: Opens the Analysis Protocol Editor dialog box. • Edit: Opens the Analysis Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Analysis Protocols: In alpha-numeric order Note: You must have an Analysis Protocol selected for each sample entered in the Sample Name column. See, "Creating an Analysis Protocol" on page 110.

Notes

Creating Required Files for Automated SeqScape Analysis

If the Files Already Exist

If the appropriate data collection and SeqScape files have been created, proceed to "Creating and Completing a SeqScape Plate Record" on page 119.

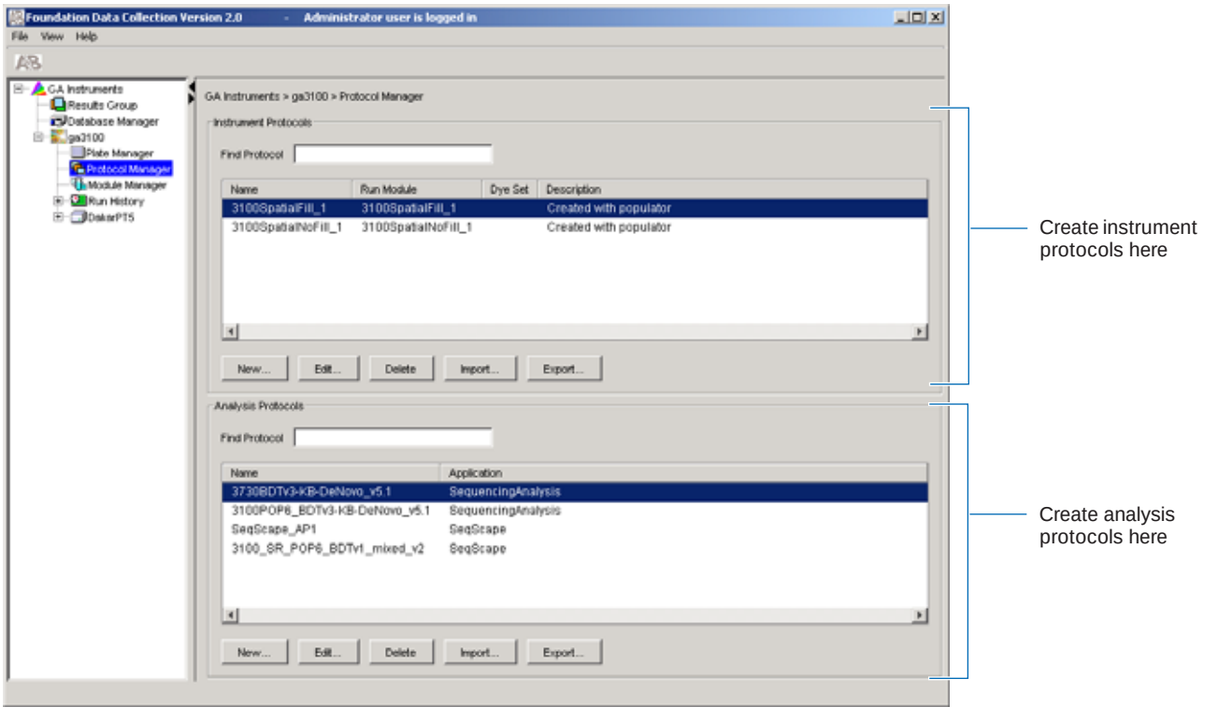
Instrument Protocol for SeqScape

About Instrument Protocols

An instrument protocol contains all the settings necessary to run the instrument. An instrument protocol contains the protocol name, type of run, run module, and dye set.

Creating an Instrument Protocol

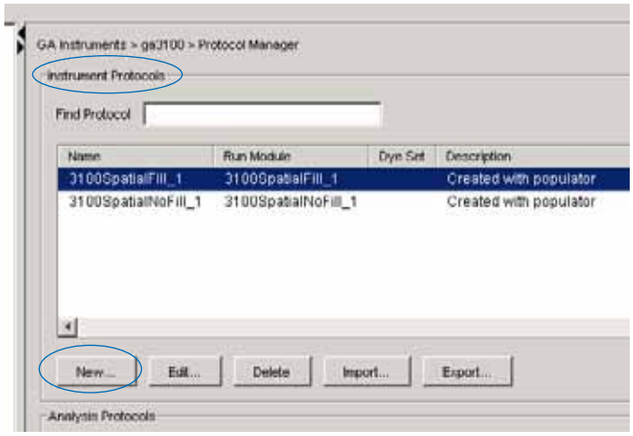
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Protocol Manager**.



Notes

2. In the Instruments Protocols section, click New....

   The Protocol Editor opens.

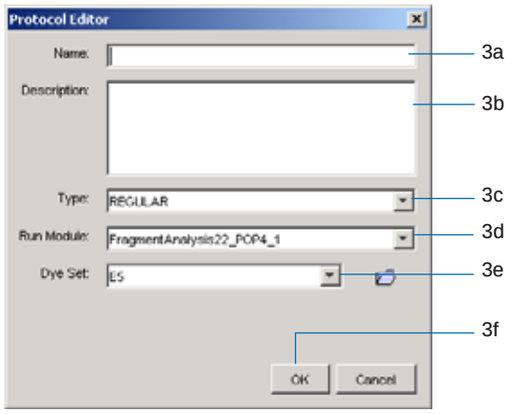


3. Complete the Protocol Editor:

   a. Type a name for the protocol.

   b. Type a description for the protocol (optional).

   c. Select **Regular** in the Type drop-down list.



   d. Using the information in the table below, select the correct run module for your run. (See "Tip: Customizing Run Modules" on page 82, if you want to modify a default module.)

Sequencing Run	Capillary Array Length (cm)	Run Module
Ultra rapid	36	UltraSeq36_POP4_1
Rapid	36	RapidSeq36_POP6_1
Standard	50	StdSeq50_POP4_1
		StdSeq50_POP6_1
Long read	80	LongSeq80_POP4_1

Notes

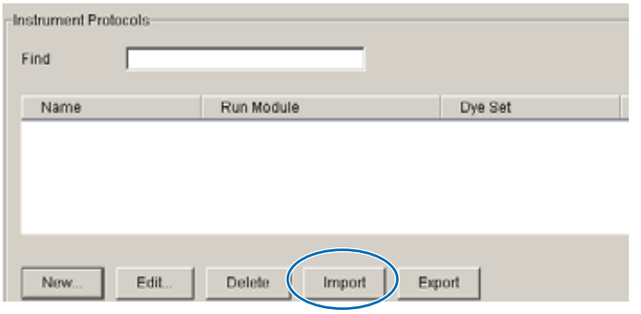
e. Using the information in the table below, select the correct Dye Set for your run.

Dye Set	Chemistry	Instrument
E_BigDyeV1	ABI PRISM® BigDye® v1.1 Primer	3100 only
	ABI PRISM® BigDye® v1.1 Terminator	3100/3100-*Avant*
	ABI PRISM® dRhodamine Terminator	
	ABI PRISM® dGTP BigDye® Terminator	
Z_BigDyeV3	ABI PRISM® BigDye® v3.1 Primer	3100 only
	ABI PRISM® BigDye® v3.1 Terminator	3100/3100-*Avant*
	ABI PRISM® dGTP BigDye® v3.0 Terminator	

f. Click OK.

Importing an Instrument Protocol

1. Select **Import** in the Instrument Protocols pane of the Protocol Editor window.

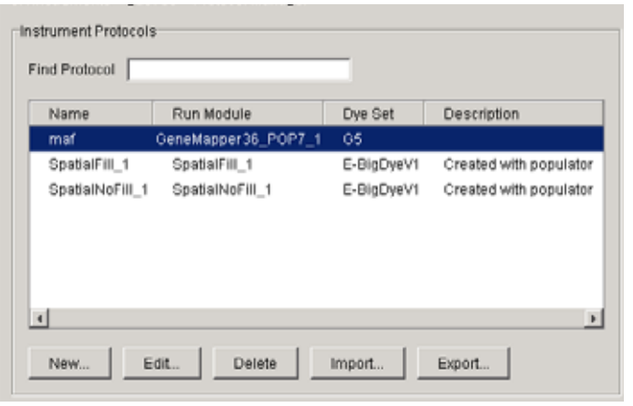


2. Navigate to the protocol you want to import.

Note: Import file type is .txt (text).

3. Double-click the protocol to import it.

The imported file is displayed as the top row in the Instrument Protocol pane.



Notes

Analysis Protocol for SeqScape

About Analysis Protocols

New to Data Collection is the implementation of analysis protocols. An analysis protocol contains all the settings necessary for analysis and post processing:

- Protocol name – The name, description of the analysis protocol, and the sequence file formats to be used
- Basecalling settings – The basecaller, DyeSet/Primer file, and analysis stop point to be used
- Mixed Bases – Option: to use mixed base identification, and if so, define the percent value of the second highest to the highest peak
- Clear Range – The clear range to be used based on base positions, sample quality values, and/or number of ambiguities (Ns) present
- Filter – The settings used to reject sequences that are not used in the assembly

Note: If you created an appropriate analysis protocol in the SeqScape software, you can use it in data collection software.

IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis will not be performed if you do so.

Notes

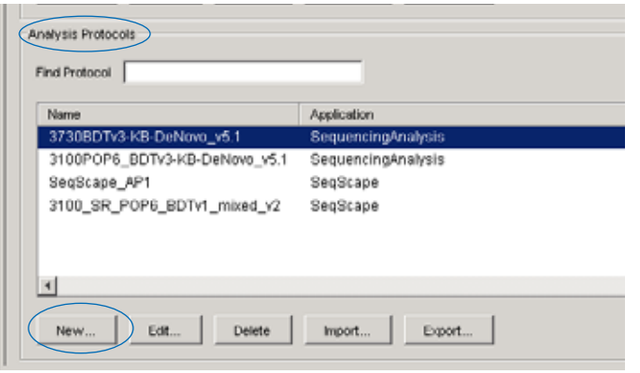
Creating an Analysis Protocol

IMPORTANT! If you created an appropriate analysis protocol in SeqScape software, you can use it in data collection software. You can also create an analysis protocol in the SeqScape software, if desired.

Note: Refer to the *ABI PRISM® SeqScape Software v2.1 User Guide* (P/N 4346367) for more information.

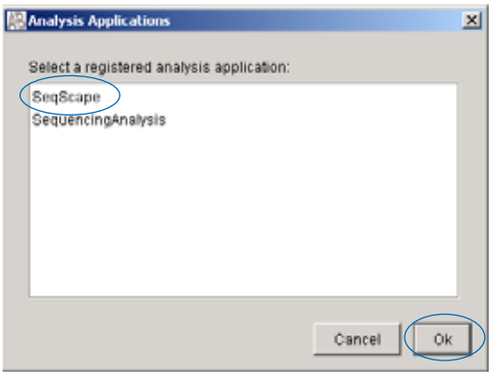
1. In the Analysis Protocol section of the Protocol Manager, click New... .

   If more than one analysis application is installed on the data collection computer, the Analysis Applications dialog box opens.



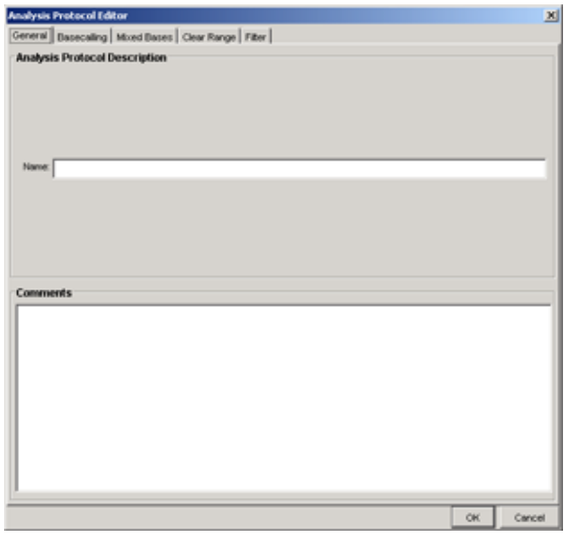
2. Select **SeqScape**, then click OK .

   The Analysis Protocol Editor dialog box opens.



Notes

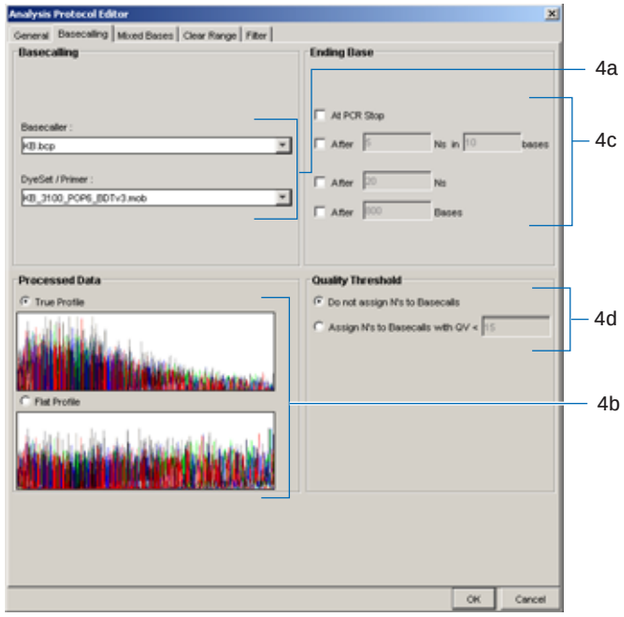
3. In the **General** tab, enter a unique name and description (optional) for the new protocol.



4. Select the **Basecalling** tab, then:

   a. Use Appendix B, "Basecallers and DyeSet/Primer Files," to select the appropriate basecaller and DyeSet primer based on the chemistry, capillary array length and polymer type you are using.

   Note: Sequencing Analysis Software v5.1 and 3100/3100-*Avant* Data Collection software filter .mob file choices to match the chosen .bcp file.



Notes

b. In the Processed data pane, select True or Flat profile.

Option	Function
True Profile	Used to display data as processed traces scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. The profile of the processed traces will be very similar to that of the raw traces.
Flat Profile	Used to display the data as processed traces scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces will be flat on an intermediate scale (> about 40 bases). Note: This option is applied to data that is analyzed with the KB basecaller only. If you use the ABI basecaller the profile option reverts to True Profile.

c. If desired, select one or more stop points for data analysis.

d. Select your Threshold Quality option.

Option	Function
Call all bases and assign QV	When using the KB basecaller, use this setting assign a base to every position, as well as the QV.
Assign 'N' for bases with QV < 15	When using the KB basecaller, use this setting assign Ns to base with QVs less than the set point. The QV will still be displayed.

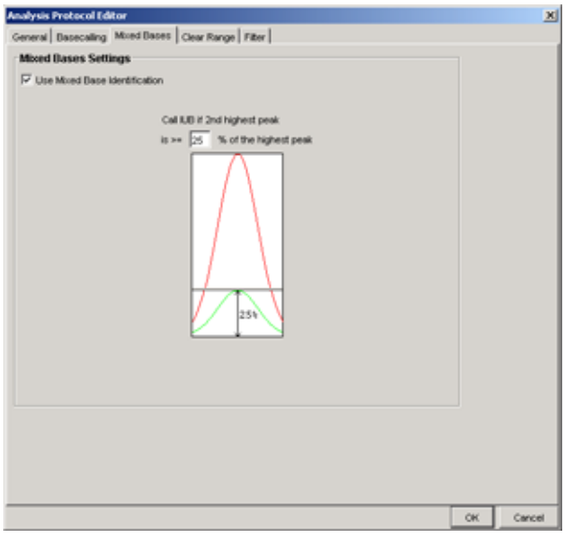
5. Select the **Mixed Bases** tab, then:

Note: This function is active with the KB basecaller only.

a. For mixed bases only, select **Use Mixed Base Identification**.

b. Use the default setting of 25% or change the detection level by entering a new value or dragging the % line up or down.

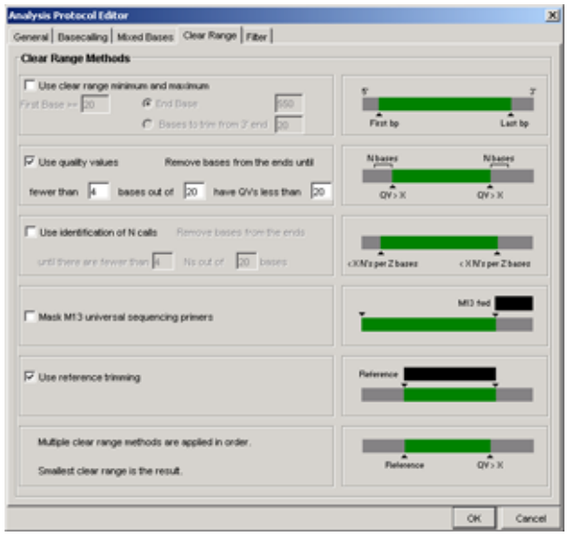
Note: Do not use less than 15% as your detection limit.



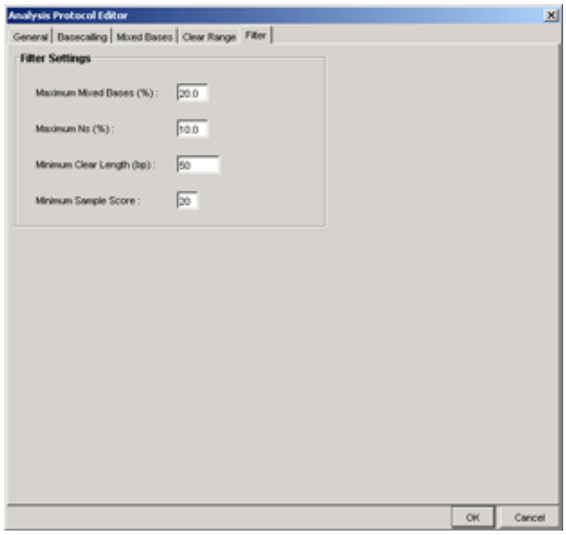
Notes

6. Select the **Clear Range** tab, then, if desired, select one or more stop points for data analysis.

   Note: The clear range is the region of the sequence that remains after excluding the low-quality or error-prone sequence at both the 5´ and 3´ ends.



7. Select the **Filter** tab, then, if desired, change one or more of the settings.

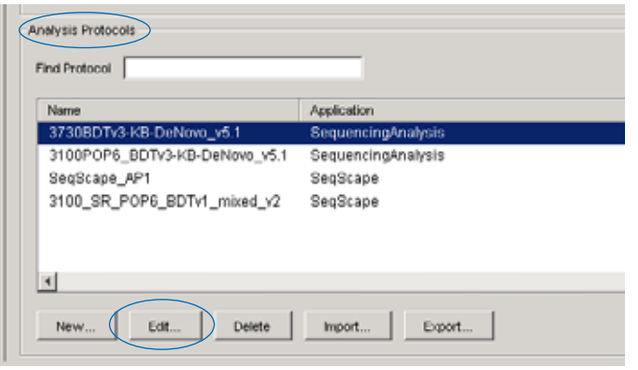


8. Click OK to save the protocol and close the Analysis Protocol Editor dialog box.

Notes

Editing an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to edit.
2. Click Edit... .
3. Make changes in the General, Basecalling, Mixed Bases and Clear Range tabs, as appropriate.
4. Click OK to save the protocol and close the Analysis Protocol Editor dialog box.



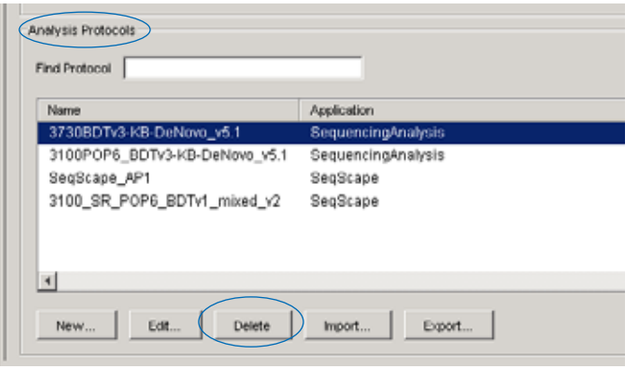
Deleting an Analysis Protocol

IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis will not be performed if you do so. Also, You must first delete any plate records using the Analysis Protocol before you can delete or modify the Analysis Protocol for these plate records.

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to delete.
2. Click Delete .

   The Deletion Confirmation dialog box displays.
3. Click Yes .

Note: It is better to delete the Analysis Protocol from the SeqScape Software v2.1.



Notes

Results Group for SeqScape

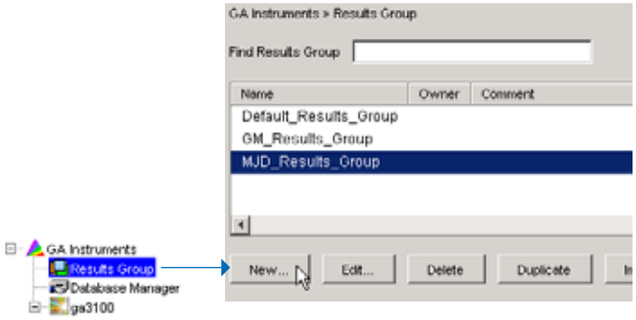
A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. It is called a Results Group because it is used to analyze, name, sort, and deliver samples that result from a run.

Creating a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.

2. Click New....

   The Results Group Editor window displays.

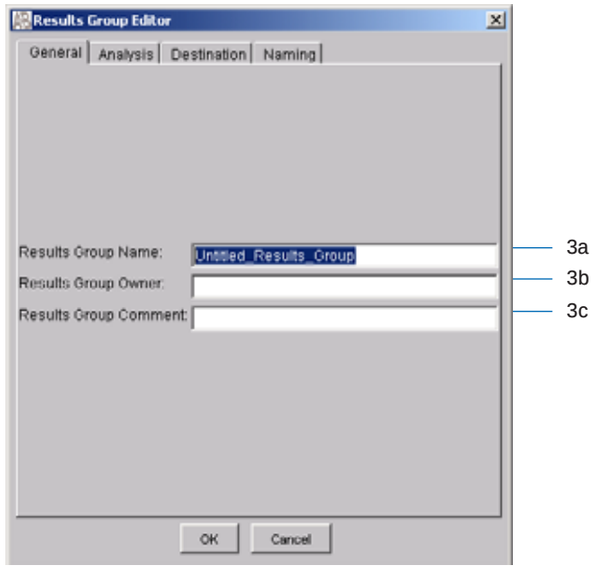


3. Complete the General tab:

   a. Type a Results Group Name. The name can be used in naming and sorting sample files. It must be unique (see page for a list of accepted characters).

   b. Type a Results Group Owner (optional). The owner name can be used in naming and sorting sample files.

   c. Type a Results Group Comment (optional).



Notes

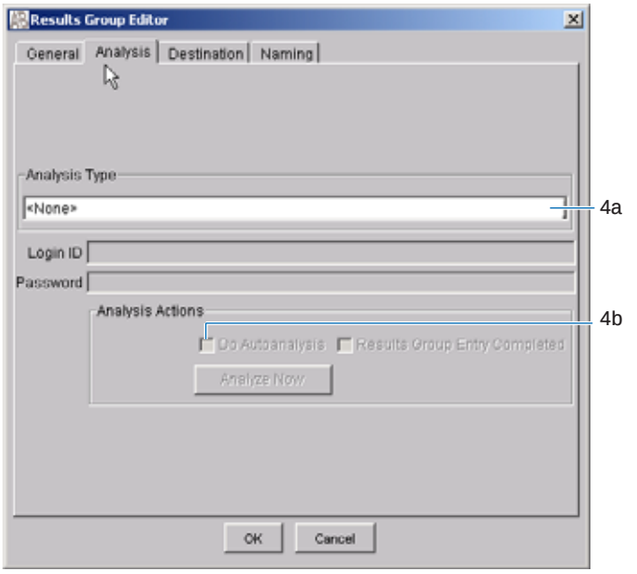
4. Select the **Analysis** tab, then:

   a. Select **SeqScape_** *computer name* in the Analysis Type drop-down list.

   b. Select **Do Autoanalysis** in the Analysis Actions section.

   c. Type a valid SeqScape Login ID and Password in the text boxes.

   IMPORTANT! Failure to use the proper login and password causes your samples not to be analyzed automatically.



5. Select the **Destination** tab, then use the default destination or define a new location for data storage.

To use ...	Then ...
default location	skip to step 6
custom location	complete steps a-c

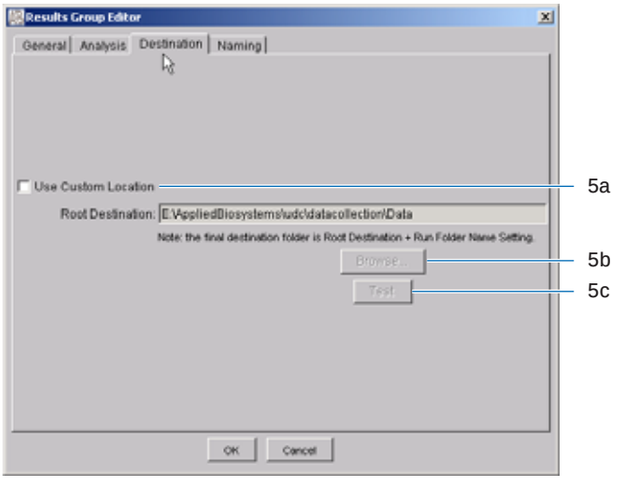
   a. Click **Use Custom Location**, then click Browse... to navigate to a different save location.

   b. Click Test to test the Location path name connection:

   If it passes, a message box displays “Path Name test successful.”

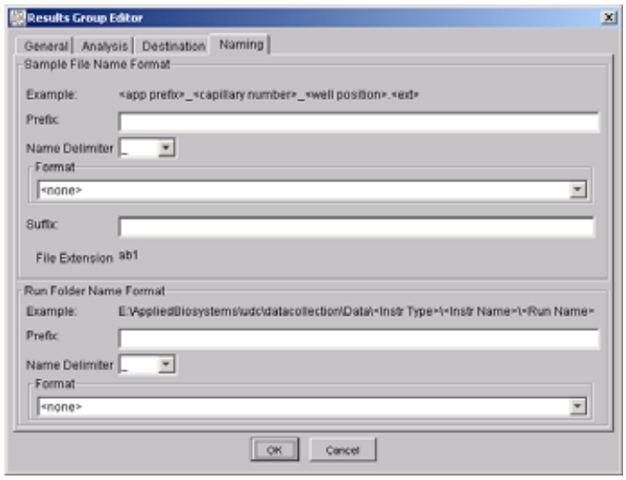
   If it fails, a message box displays “Could not make the connection. Please check that the Path Name is correct.” Click and retry to establish a connection.

   c. Click OK.



Notes

6. Select the **Naming** tab, then define custom names for sample file and run folder name.



7. Click OK to save and close the Results Group Editor.

4

Notes

Importing and Exporting a Results Group

Results Groups can be imported from, or exported to, tab-delimited text files. This allows easy sharing of identical Results Groups between instruments.

Importing a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click Import.

   A standard File Import dialog box displays.
3. Navigate to the file you want to import.

   Note: Import file type is .txt (text).
4. Click Open.

   Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Exporting a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click the Results Group name to select it.
3. Click Export.

   A standard file export dialog box displays with the chosen Results Group name.
4. Navigate to the location where you want to save the exported file.
5. Click Save.

Note: If there is a name conflict with a Results Group that already exists at the save location, the Results groups can be duplicated in order to copy settings into a similar Results Group without the risk of user error when copying it manually (see procedure below).

Duplicating a Results Group

1. Click the Results Group to select it.
2. Click Duplicate.

Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Notes

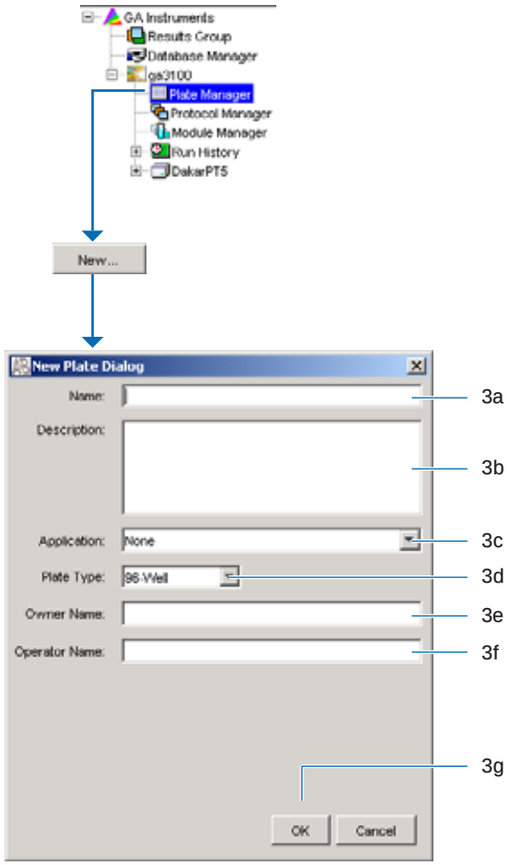
Creating and Completing a SeqScape Plate Record

Creating a SeqScape Plate Record

1. Click the **Plate Manager** icon in the navigation pane.
2. Click New....

   The New Plate Dialog dialog box opens.
3. In the New Plate dialog box:
   a. Type a name for the plate.
   b. Type a description for the plate (optional).
   c. Select **SeqScape_***computer name* in the Application drop-down list.
   d. Select **96-well** or **384-well** in the Plate Type drop-down list.
   e. Type a name for the owner.
   f. Type a name for the operator.
   g. Click OK.

      The SeqScape Plate Editor opens.

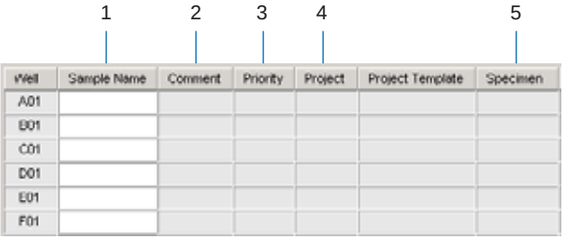


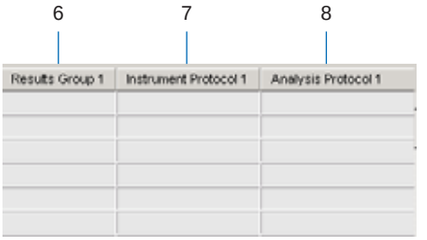
Notes

Completing a SeqScape Plate Record

1. In the **Sample Name** column of a row, enter a sample name, then click the next cell. The value 100 automatically display in the Priority column.
2. In the **Comments** column, enter any additional comments or notations for the sample.
3. In the **Priority** column, change the priority value, if desired (see page 262).
4. In the **Project** column, select a project from the drop-down list.

   Based on the Project you select, the project template is filled in automatically.
5. In the **Specimen** column, select a specimen.
6. In the **Results Group 1** column, select a group from the drop-down list (see page 115).
7. In the **Instrument Protocol 1** column of the row, select a protocol from the drop-down list (see page 106).
8. In the **Analysis Protocol 1** column of the row, select a protocol from the drop-down list (see page 110).





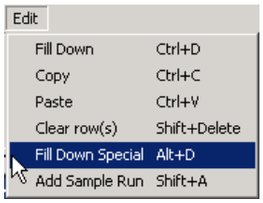
Notes

9. To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:

   - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down Special**.

     Based on the plate type (96- or 384-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run (see page 265).

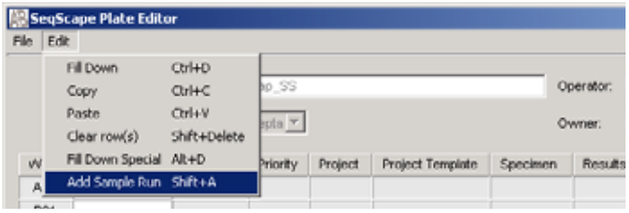
   - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down**.
   - For the different samples and protocols – Complete the manually.



10. If you want to do more than one run, then select **Edit** > **Add Sample Run**.

    Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.

    You can add additional runs by selecting **Edit** > **Add Sample Run** again.



11. Complete the columns for the additional runs.

12. Click OK to save, then close the plate record.

IMPORTANT! After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

Notes

Where to Go Next

Use the table below to determine which chapter to proceed to next.

Do you need to…	Proceed to …
Set up the software for fragment analysis runs?	Chapter 5, page 123
Start and monitor a run?	Chapter 6, page 149
Perform maintenance, use wizards?	Chapter 7, page 195
Activate/modify the audit trail and access control features?	Chapter 8, page 241
Learn more about on plate record feature?	Appendix A, page 261
Learn more about troubleshooting?	Appendix C, page 279

Notes

3100/3100-*Avant* Data Collection and Fragment Analysis

This chapter covers:

Notes

3100/3100-*Avant* Data Collection and GeneMapper Software

Note: This chapter is written for both GeneMapper™ Software v3.5 and GeneMapper™ *ID* Software v3.1. The graphics used in this chapter are of the GeneMapper software v3.5.

Important Notes

- A unique name must be assigned to the instrument computer before 3100/3100-*Avant* Data Collection software is installed.
- Do not rename the computer once 3100/3100-*Avant* Data Collection software has been installed. Doing so may cause the 3100/3100-*Avant* Data Collection software to malfunction.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. The invalid characters are below:

spaces

\ / : * ? " < > |

IMPORTANT! An error message is displayed if you use any of these characters. You must remove the invalid character to continue.

Autoanalysis

You may choose to perform autoanalysis of fragment analysis samples by utilizing features of the 3100/3100-*Avant* Data Collection and GeneMapper software.

GeneMapper Software v3.5

Autoanalysis can be performed on the same instrument that collected the sample files or on a remote computer.

GeneMapper *ID* Software v3.1

Autoanalysis can only be performed on the same instrument that collected the sample files. If you wish to analyze samples on another computer, you must transfer the files to that location.

If a user performs autoanalysis on samples, but wishes to edit/review results on another computer, they will need to transfer the GeneMapper software project, analysis methods, size standards, panel and bin set information to the other GeneMapper software database. There is no easy method for transferring all components of a project from one GeneMapper software database to another. All components need to be exported and imported individually.

When completing the Plate Record, you need to fill in Instrument Protocol information for Data Collection to complete the run. Additionally, when creating a new Results Group for a set of samples to be autoanalyzed, you must check the Do Autoanalysis check box (and for remote analysis, define a default location for sample file storage).

Notes

Manual Analysis

For information on manual analysis, refer to *GeneMapper Software Version 3.5 User Guide* (PN 4343790) or *GeneMapper ID Software Version 3.1 User Guide* (PN 4338775).

About Fragment Analysis and Data Collection

When GeneMapper software is installed on a computer that has 3100/3100-*Avant* Genetic Analyzer Data Collection Software, two applications are available through the Results Group Editor (see page 136):

- GeneMapper-Generic

and,

- GeneMapper-<Computer Name>

GeneMapper-Generic

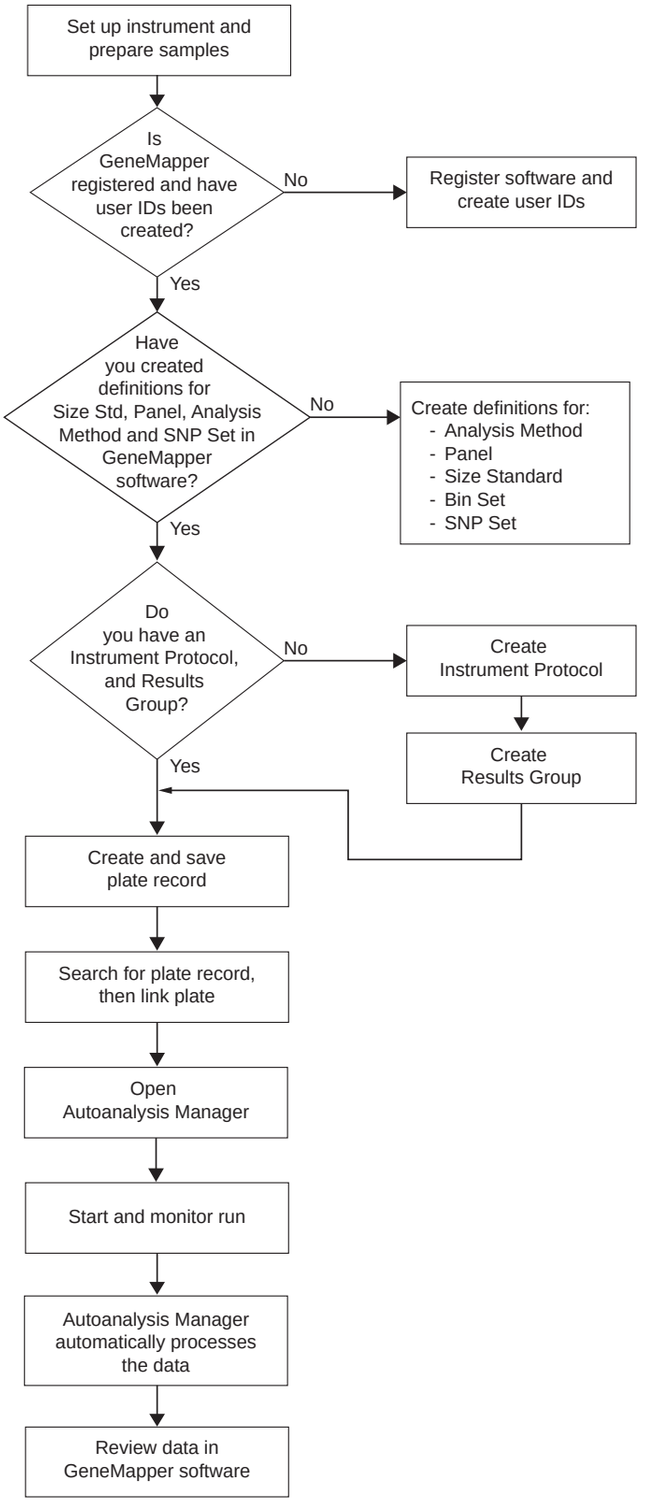
GeneMapper-Generic enables you to generate .fsa files, but not perform autoanalysis. When completing the Sample Sheet, you need to fill in basic information for Data Collection to complete the run; all other GeneMapper software related fields are text entries. This is useful if you are using other software applications for analysis. This is also useful if you choose to analyze your samples in GeneMapper software on another computer, but do not have the same entries in the GeneMapper software database stored on the Data Collection computer. For example, if you have a customized size standard definition on the other GeneMapper software computer, you can type in that size standard name in the size standard text field and it will populate that column in your GeneMapper software project.

GeneMapper-<Computer Name>

GeneMapper-<Computer Name> is for autoanalysis. The Size Standard, Analysis Method, and Panel columns in the Sample Sheet window read directly from the GeneMapper software database. These components must be created in GeneMapper software prior to setting up the plate record for a run. There is no way to create a new entry for these columns once inside the plate editor dialog box. If you create a new GeneMapper software component while the plate record dialog box is open, the columns will not update. The plate record must be closed and reopened to update the GeneMapper software components. For more information see, "Creating Required Files for Automated Fragment Analysis" on page 131.

Notes

Workflow for Autoanalysis Using GeneMapper Software



Notes

About GeneMapper Plate Records

Overview

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

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

- Plate name, type, and owner
- Position of the sample on the plate (well number)
- Comments about the plate and about individual samples
- Dye set information (in Instrument protocol)
- Name of the run module (run modules specify information about how samples are run) (in Instrument protocol)

When to Create a Plate Record

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

- Spectral calibrations
- Fragment analysis
- SeqScape analysis
- Sequencing analysis
- Mixed (sequencing, Seqscape, and fragment analysis samples) see "Multi-application (Mixed) Plate Record" on page 269

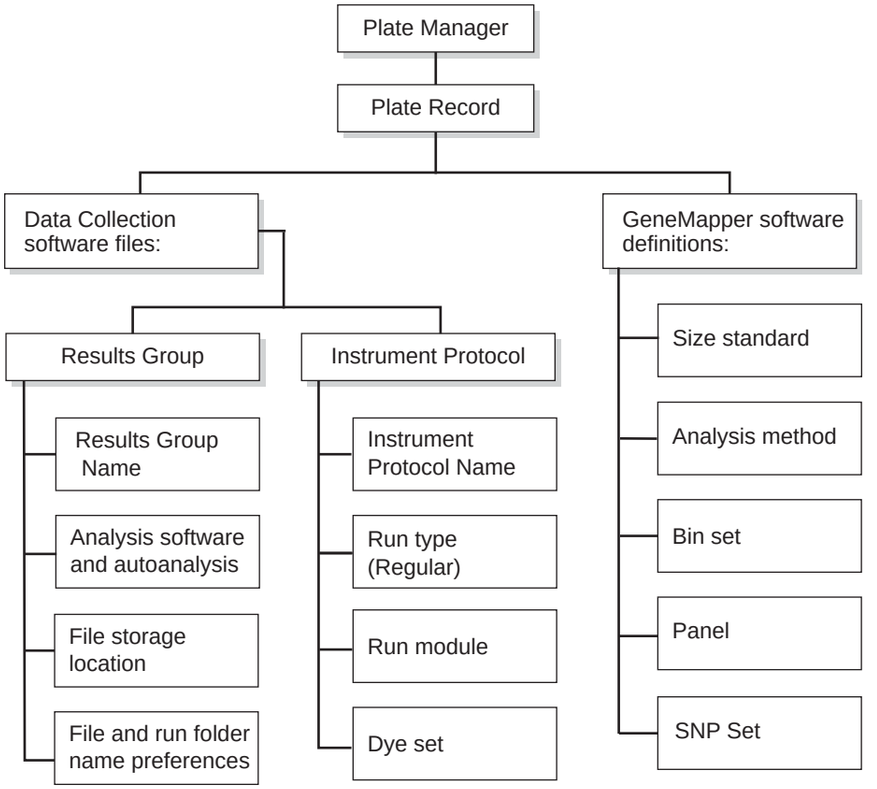
Additionally, Plate Records must be created in advance of placing the plates on the instrument. However, Plate Records can be created while a run is in progress.

The 3100/3100-*Avant* Data Collection Software contains several new features that are briefly described here and in more detail throughout this section.

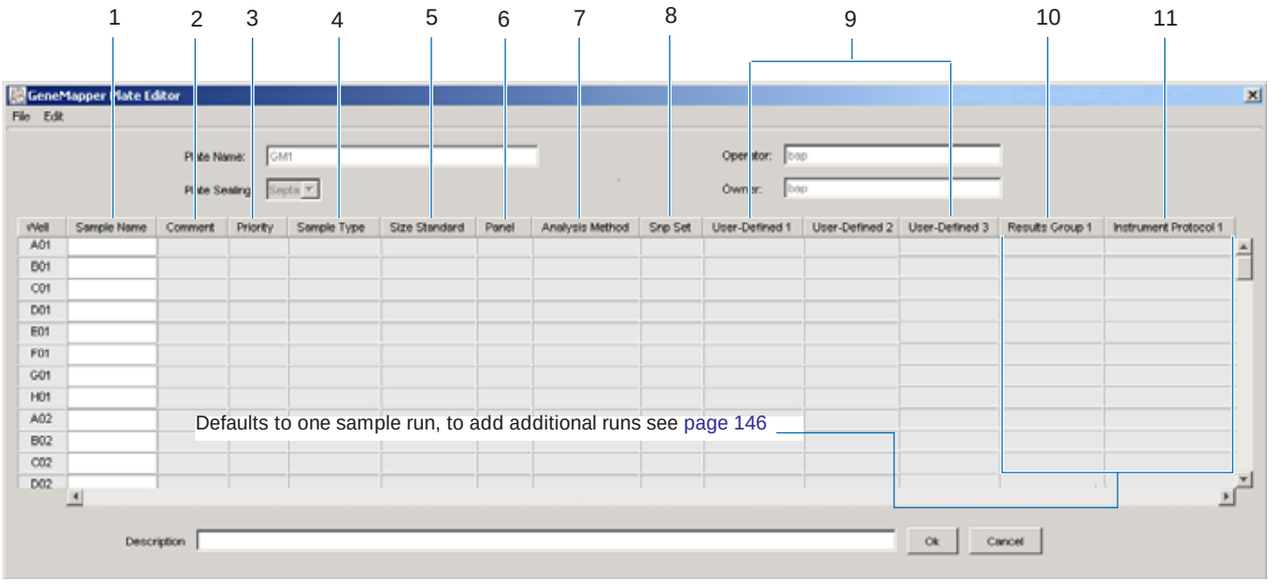
Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	131
Results Group	Defines the file type, the file name, autoanalysis, and file save locations that are linked to sample injections.	136

IMPORTANT! In order for data collection and auto-analysis to be successful, each run of samples must have an Instrument Protocol, a Results Group and files created in GeneMapper software assigned within a plate record.

Notes



Elements of a GeneMapper plate record



Blank GeneMapper plate record

Notes

The following table describes the columns inserted in a Plate Record for a fragment analysis run.

Number and Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Priority	A default value of 100 to each sample. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list.
4. Sample Type	Use to identify the sample as Sample, Positive Control, Allelic Ladder or Negative Control.
5. Size Standard IMPORTANT! For GeneMapper-<Computer Name> ONLY: Size Standard, Panel, and Analysis Method must be created in GeneMapper software before creating a new plate	• GeneMapper-Generic (optional): Manually enter size standards in the text field* • GeneMapper-<Computer Name>: Select a saved size standard from the drop-down list
6. Panel IMPORTANT! For GeneMapper-<Computer Name> ONLY: Size Standard, Panel, and Analysis Method must be created in GeneMapper software before creating a new plate	• GeneMapper-Generic (optional): Manually enter panels in the text field* • GeneMapper-<Computer Name>: Select a saved panel from the drop-down list
7. Analysis Method IMPORTANT! For GeneMapper <Computer Name> ONLY: Size Standard, Panel, and Analysis Method must be created in GeneMapper software before creating a new plate	• GeneMapper-Generic (optional): Manually enter analysis methods in the text field* • GeneMapper-<Computer Name>: Select a saved analysis method from the drop-down list
8. SNP Set IMPORTANT! For GeneMapper <Computer Name> ONLY: The SNP set is a file created in the GeneMapper software that links a SNP name to a marker name.	• GeneMapper-Generic (optional): Manually enter SNP set in the text field* • GeneMapper-<Computer Name>: Use for SNPlex chemistry, select a saved SNP set the drop-down list
9. 3 User-defined columns	Optional text entries
10. Results Group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the Results Group listed in the cell • None: Sets the cell to have no selected Results Group • Select one of the available Results groups from the list Note: You must have a Results Group selected for each sample entered in the Sample Name column. See, "Results Group for Fragment Analysis" on page 136.

Notes

Number and Column	Description
11. Instrument Protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. Note: You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See, "Results Group for Fragment Analysis" on page 136.

Notes

Creating Required Files for Automated Fragment Analysis

If the Files Already Exist

If the appropriate data collection and fragment analysis files have been created, proceed to "Creating and Completing a GeneMapper Plate Record" on page 144.

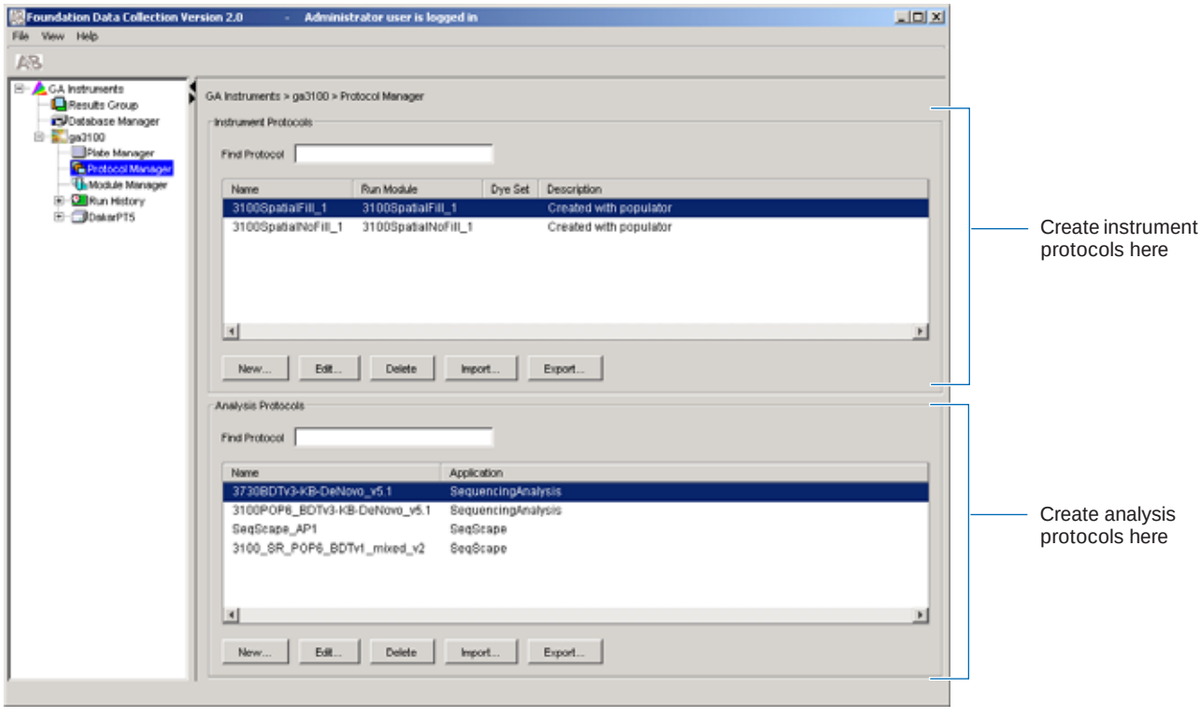
Instrument Protocol for Fragment Analysis

About Instrument Protocols

An instrument protocol contains all the settings necessary to run the instrument. An instrument protocol contains the protocol name, type of run, run module, and dye set.

Creating an Instrument Protocol

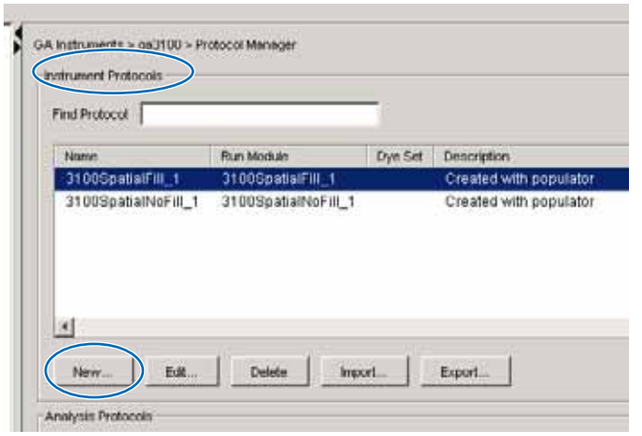
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Protocol Manager**.



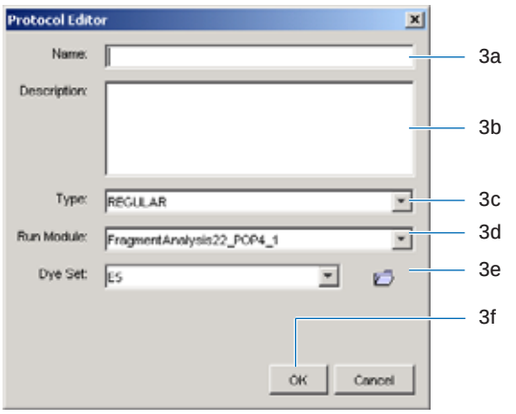
Notes

2. In the Instruments Protocols section, click New... .

   The Protocol Editor opens.



3. Complete the Protocol Editor:
   a. Type a name for the protocol.
   b. Type a description for the protocol (optional).
   c. Select **Regular** in the Type drop-down list.



Notes

d. Using the information in the table below, select the correct run module for your run. To customize a run module, see "Tip: Customizing Run Modules" on page 135.

Application or Kit	Capillary Array Length	Run Module
SNaPshot Multiplex System	22 cm	SNP22_POP4_1
	36 cm	SNP36_POP4_1
• LMS v2.5 • ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	22 cm	FragmentAnalysis22_POP4_1
	36 cm	FragmentAnalysis36_POP4_1
• AmpFℓSTR COfiler Kit • AmpFℓSTR Profiler Plus Kit • AmpFℓSTR SGM Plus Kit • AmpFℓSTR Profiler Plus *ID* Kit • AmpFℓSTR SEfiler Kit • Other 4-Dye AmpFℓSTR Kits • AmpFℓSTR Identifiler Kit • Other 5-Dye AmpFℓSTR Kits	36 cm	HIDFragmentAnalysis36_POP4_1
• LMS v2.5 • ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	50 cm	FragmentAnalysis50_POP4_1
• LMS v2.5 • ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	50 cm	FragmentAnalysis50_POP6_1

Notes

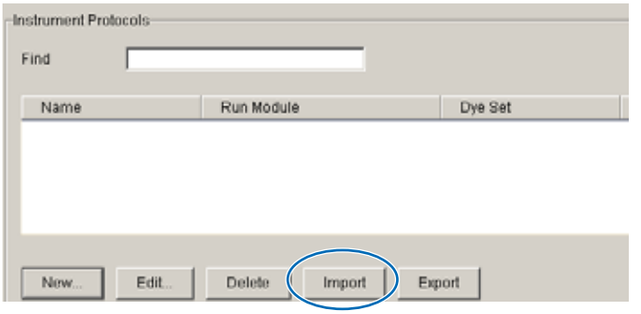
e. Using the information in the table below, select the correct Dye Set for your run.

Application or Kit	Dye Set	Matrix Standard Set
Custom oligos	D	DS-30
• ABI PRISM Mouse Mapping Set v1.0 • Custom oligos	D	DS-31
• AmpFℓSTR® COfiler® Kit • AmpFℓSTR® Profiler® Plus Kit • AmpFℓSTR® SGM Plus™ Kit • AmpFℓSTR® Profiler Plus *ID* Kit • Other 4-Dye AmpFℓSTR Kits	F	DS-32
ABI PRISM® SNaPshot® Multiplex System	E5	DS-02
• ABI PRISM® Linkage Mapping Set v2.5 • Custom Oligos • AmpFℓSTR® Identifiler™ Kit • AmpFℓSTR® SEfiler Kit • Other 5-Dye AmpFℓSTR Kits	G5	DS-33

f. Click OK.

Importing an Instrument Protocol

1. Click Import in the Instrument Protocols pane of the Protocol Editor window.



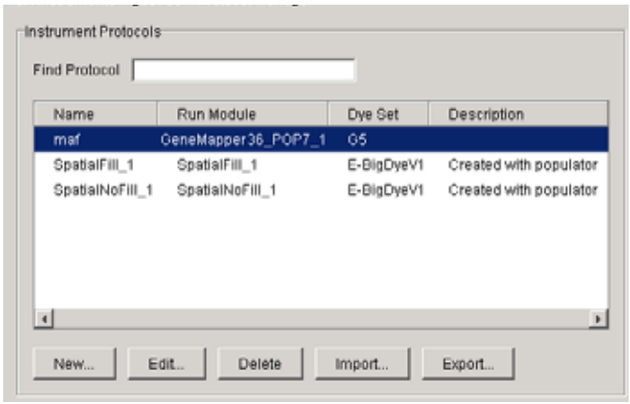
2. Navigate to the protocol you want to import.

Note: Import file type is .txt (text).

Notes

3. Double-click the protocol to import it.

   The imported file is displayed as the top row in the Instrument Protocol pane.



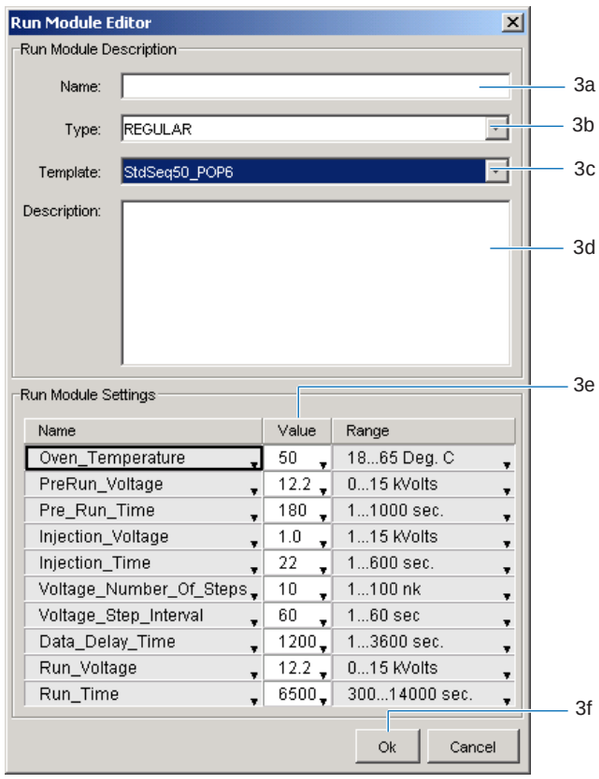
Tip: Customizing Run Modules

You can modify default run modules to suit your particular needs.

1. Click **GA Instruments >** **ga3100 or ga3100-*Avant* >** *instrument name* **> Module Manager**.
2. Click New....

   The Run Module Editor dialog box opens.
3. Complete the Run Module Editor dialog box:
   a. Enter a name for your new module.
   b. In the Type drop-down list, select the type of module (Regular, Spatial or Spectral).
   c. In the Template drop-down list, select a template module as a basis for the new module.

      Note: You cannot edit a default module installed with 3100/3100-*Avant* Data Collection software.
   d. Optional: Enter a description of your new run module.



Notes

Tip: Customizing Run Modules *(continued)*

e. Change to the desired module parameters using the table below as a guide to the allowable parameters.

Name	Range	Comment
Oven_Temperature	18-65 C	Temperature setting for main oven throughout run.
PreRun_Voltage	0-15 kV	Pre run voltage setting before sample injection.
PreRun Time	1-1000 sec	Prerun voltage time.
Injection_Voltage	0-15 kV	Injection voltage setting for sample injection.
Injection_Time	1-600 sec	Sample injection time.
Run_Voltage	0-15 kV	Final run voltage.
Voltage_Number_Of_Steps	0-100 steps	Number of voltage ramp steps to reach Run_Voltage. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Voltage_Step_Interval	0-60 sec	Dwell time at each voltage ramp step. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Data_Delay_Time	1-3600 sec	Time from the start of separation to the start of data collection.
Run_Time	300-14000 sec	Duration data is collected after Data_Delay_Time.

f. Click **OK**.

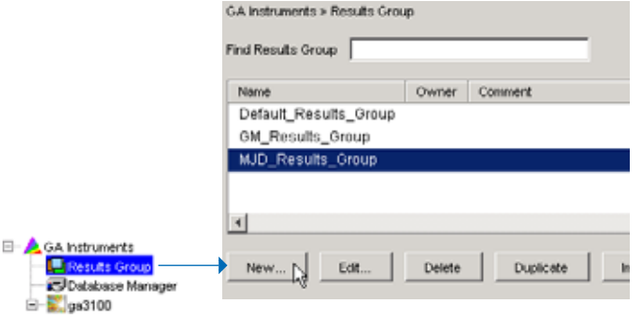
Results Group for Fragment Analysis

A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. It is called a Results Group because it is used to analyze, name, sort, and deliver samples that result from a run.

Creating a Results Group for Autoanalysis

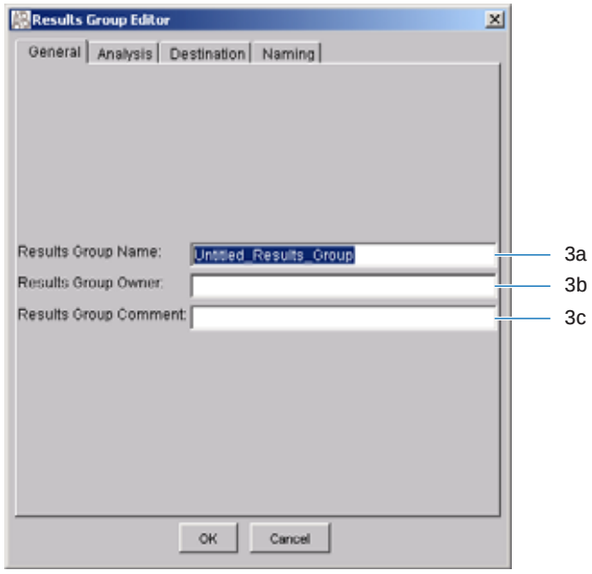
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click New....

   The Results Group Editor window displays.



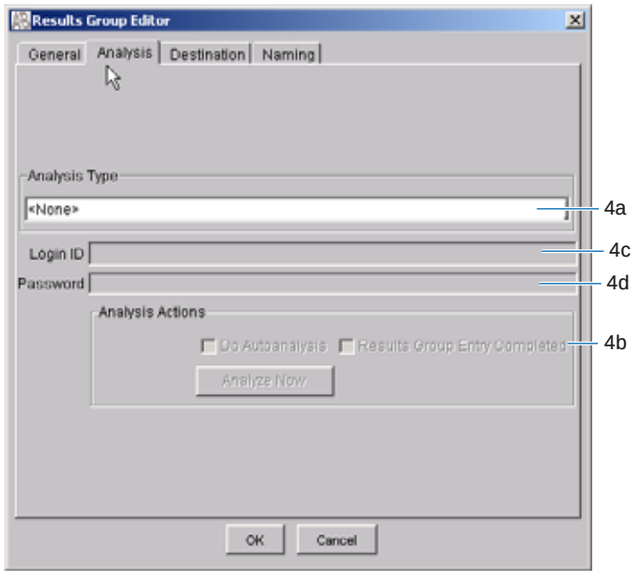
Notes

3. Complete the General tab:
   a. Type a Results Group Name. The name can be used in naming and sorting sample files. It must be unique (see page for a list of accepted characters).
   b. Type a Results Group Owner (optional). The owner name can be used in naming and sorting sample files.
   c. Type a Results Group Comment (optional).



4. Select the **Analysis** tab, then:
   a. Click the Analysis Type and then select one of the following:

If You Select ...	Then ...
None	Only raw data files are generated
GeneMapper-Generic	Autoanalysis is not enabled and only .fsa files are generated
GeneMapper-<Computer Name>	Autoanalysis of completed runs is enabled Steps b, c, and d below apply only to GeneMapper-<Computer Name> (not GeneMapper-Generic).



   b. In the Analysis Actions section, use the table below to select an option.

If You Select ...	Then ...	Use with Setting from Automated Processing Tab (page 139)
Do Autoanalysis	Samples are analyzed after each run of 16 or 4 samples.	**When every run completes**
Do Autoanalysis and Results Entry Group Complete	Samples are analyzed after all samples using the same results group have been run.	**Only when the result group is complete**

Notes

c. Type the Login ID.

d. Type the login password.

The login ID and password relate to the GeneMapper software UserName and Password. These items can only be created through the GeneMapper software Options Users tab.

5. Select the **Destination** tab, then use the default destination or define a new location for data storage.

To use a ...	Then ...
default location	skip to step 6
custom location *Use for remote analysis using GeneMapper v3.5*	complete steps a-c

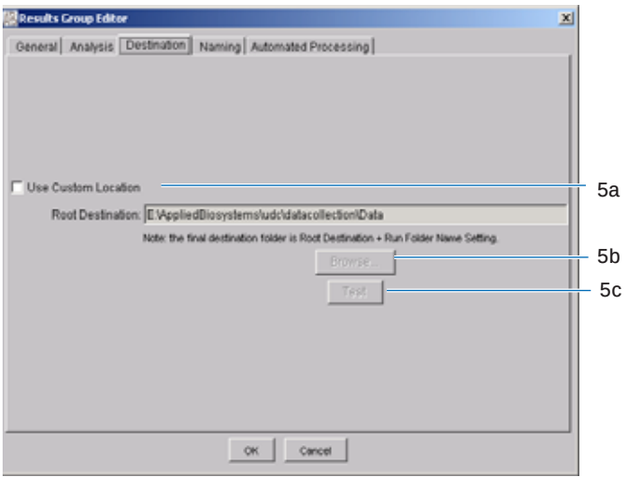
a. Click **Use Custom Location**, then click Browse... to navigate to a different save location.

b. Click Test to test the Location path name connection:

If it passes, a message box displays “Path Name test successful.”

If it fails, a message box displays “Could not make the connection. Please check that the Path Name is correct.” Click and retry to establish a connection.

c. Click OK.



Notes

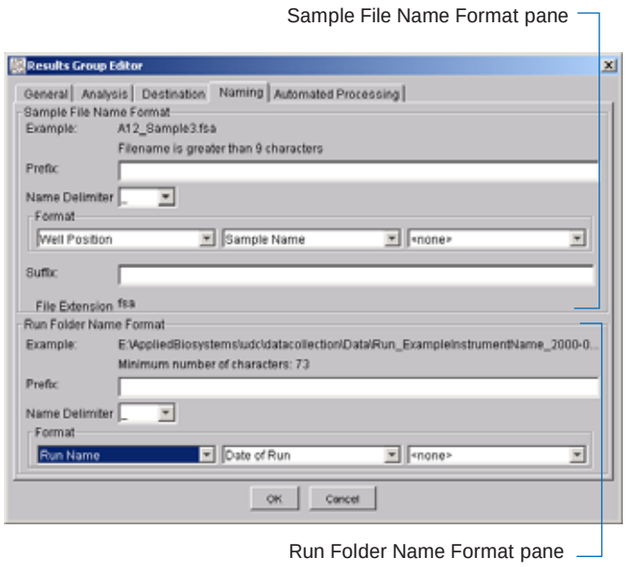
6. Select the **Naming** tab.

   Use the Naming tab to customize sample file and run folder names.

   IMPORTANT! Sample name, run folder name, and path name, *combined*, can total no more than 250 characters. See page 124 for accepted characters.

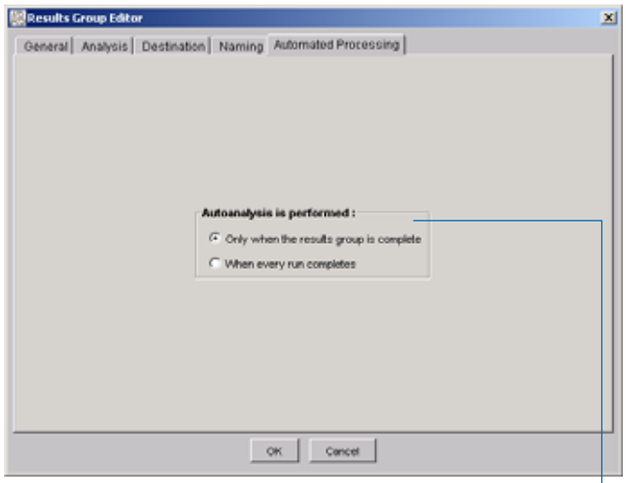
   IMPORTANT! You must select at least one Format element for the Sample file and the Run folder names in order to proceed within the Results Group.

   The elements of the Naming tab are discussed in the following sections, see page 140.



7. Select the **Automated Processing** tab.

   In the "Autoanalysis is performed" section, use the table below to select when you want your samples autoanalyzed.



If You **Select ...**	**Then ...**	**Use with Settings from Analysis Tab (page 137)**
Only when the result group is complete	Samples are analyzed after all samples using the same results group have been run.	**Do Autoanalysis and Results Entry Group Complete**
When every run completes	Samples are analyzed after each run of 16 or 4 samples.	**Do Autoanalysis**

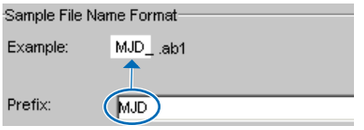
8. Click OK to save the Results Group.

Notes

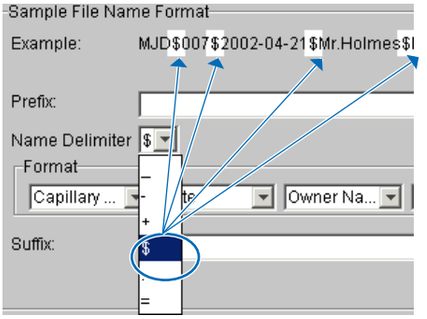
Sample File Name Format Pane

Follow the procedure below to complete the Sample File Name Format pane.

1. Click the **Prefix** box (optional) to type a prefix for the file name. Anything that you type here is shown in the Example line (see graphic below).



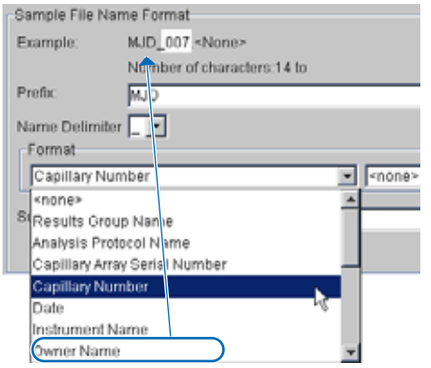
2. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3 below). Only one delimiter symbol may be chosen.



3. Click the Format list and then select the components that you want in the sample name.

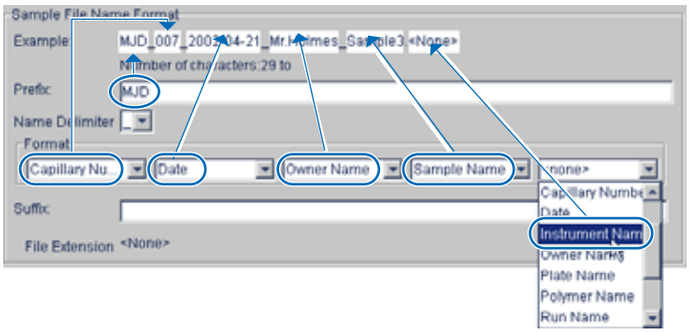
   Note: Generally, all the samples from a single run are placed in the same run or results folder, so the name of every sample from a single run should be different. Most of the Format options will not be different between samples, so you need to take care to select at least one of the options that make the sample names unique within a run.

   For example, if a unique identifier is not included in the name, a warning message displays. The Results Group **makes the** file name unique. As you select the elements for the file name, they are reflected in the Example line.

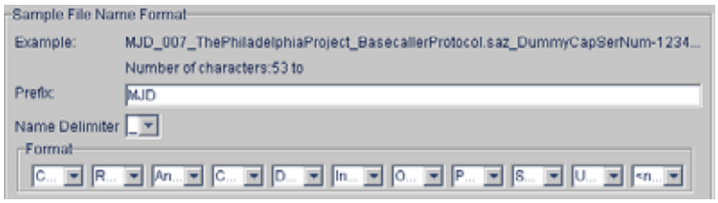


Notes

As you continue to select elements for the file name, additional elements display.

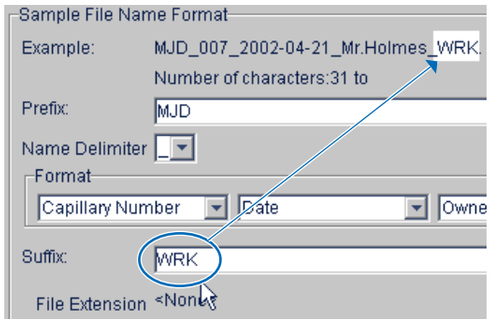


The names of the Format elements eventually truncate, but the Example field remains visible (up to 72 characters).



4. **Click the Suffix** box (optional) and type the suffix for the file name.

   The **File Extension** field displays the file extension generated from the Analysis Type specified on the **Analysis** tab (see page 137). For example, Sequencing Analysis and produces sample files with an .ab1 extension.



Run Folder/Sub-Folder Name Format Pane

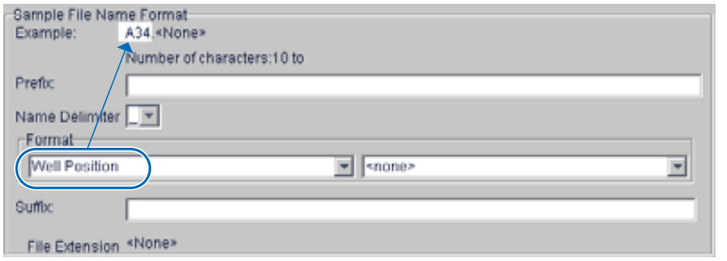
Follow the same steps described above for the Sample File Name Format pane (see page 140) to change the sub-folder name within the run folder.

About Format Elements (Unique Identifiers)

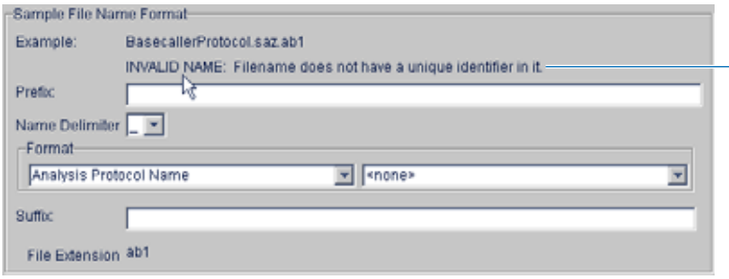
While you may select a minimum of just one Format element for the Sample file and Run folder names in order to save a Results Group, selecting just the minimum may not provide enough information for you to identify the file or folder later.

Notes

For example, although acceptable, the ‘A34’ sample file name below (well position) may not be helpful when trying to locate and identify the file later.



If you choose elements from the Format lists that do not create unique Sample file or Run folder names, a warning message displays below the Example line.



To remove the warning message and proceed within the Results Group Editor window, simply select a Format element that distinguishes one file from another (for example, the capillary number is unique while the instrument name is not).

Notes

Importing and Exporting a Results Group

Results Groups can be imported from, or exported to, tab-delimited text files. This allows easy sharing of identical Results Groups between instruments.

Importing a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click Import.

   A standard File Import dialog box displays.
3. Navigate to the file you want to import.

   Note: Import file type is .txt (text).
4. Click Open.

   Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Exporting a Results Group

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click the Results Group name to select it.
3. Click Export.

   A standard file export dialog box displays with the chosen Results Group name.
4. Navigate to the location where you want to save the exported file.
5. Click Save.

Note: If there is a name conflict with a Results Group that already exists at the save location, the Results groups can be duplicated in order to copy settings into a similar Results Group without the risk of user error when copying it manually (see procedure below).

Duplicating a Results Group

1. Click the Results Group to select it.
2. Click Duplicate.

Note: When you import or duplicate a Results Group, you are asked to type a name for the new Results Group and for the analysis application type.

Notes

Creating and Completing a GeneMapper Plate Record

Creating the GeneMapper Plate Record for Autoanalysis

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

3. Complete the information in the New Plate Dialog:

   a. Type a name for the plate.

   b. Type a description for the plate (optional).

   c. Select your GeneMapper application in the Application drop-down list.

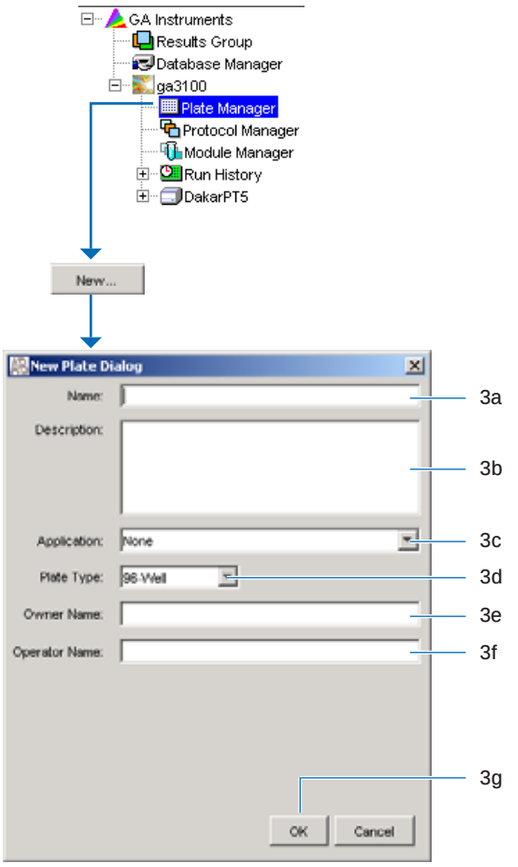
   d. Select **96-well** or **384-well** in the Plate Type drop-down list.

   e. Type a name for the owner.

   f. Type a name for the operator.

   g. Click OK.

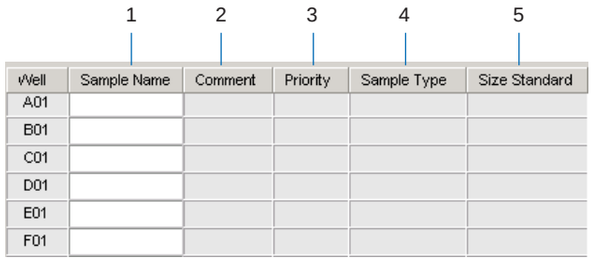
   The GeneMapper Plate Editor opens.

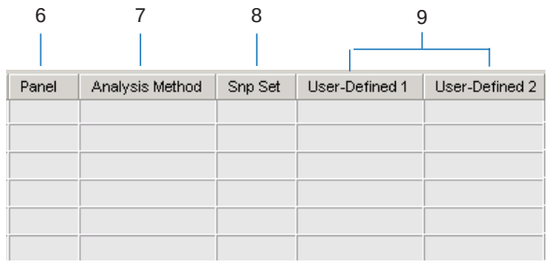


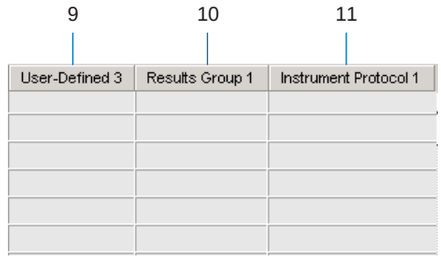
Notes

Completing a GeneMapper Plate Record for Autoanalysis

1. In the **Sample Name** column of a row, enter a sample name, then click the next cell. The value 100 automatically display in the Priority column.
2. In the **Comment** column, enter any additional comments or notations for the sample.
3. In the **Priority** column, change the priority value, if desired (see page 262).
4. In the **Sample Type** column, select a sample type from the drop-down list.
5. In the **Size Standard** column, select a size standard from the drop-down list.
6. In the **Panel** column, select a panel from the drop-down list.
7. In the **Analysis Method** column, select a method from the drop-down list.
8. In the **Snp Set** column, select a SNP set from the drop-down list.
9. Enter text for User-Defined columns 1 to 3.
10. In the **Results Group 1** column, select a group from the drop-down list (see page 136).
11. In the **Instrument Protocol 1** column, select a protocol from the drop-down list (see page 131).





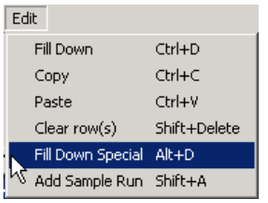


Notes

12. To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:

    - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down Special**.

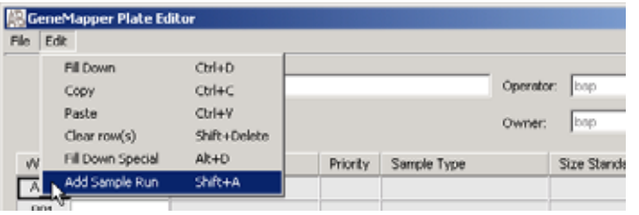
      Based on the plate type (96- or 384-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well numbers for a single run (see page 265).
    - For the same samples and protocols – Highlight the entire row, then select **Edit** > **Fill Down**.
    - For the different samples and protocols – Complete the manually.



13. If you want to do more than one run, then select **Edit** > **Add Sample Run**.

    Additional Results Group, Instrument Protocol and Analysis Protocol columns are added to the right end of the plate record.

    You can add additional runs by selecting **Edit** > **Add Sample Run** again.



14. Complete the columns for the additional runs.

15. Click OK to save, then close the plate record.

IMPORTANT! After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

Notes

Where to Go Next

Use the table below to determine which chapter to proceed to next.

Do you need to…	Proceed to …
Start and monitor a run?	Chapter 6, page 149
Perform maintenance, use wizards?	Chapter 7, page 195
Activate/modify the audit trail and access control features?	Chapter 8, page 241
Learn more about on plate record feature?	Appendix A, page 261
Learn more about troubleshooting?	Appendix C, page 279

Notes

Notes

Running the Instrument

This chapter covers:

Notes

Notes

Notes

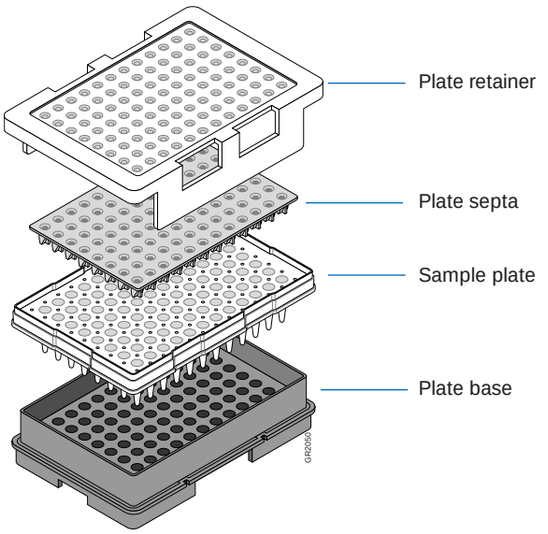
Working with Plate Assemblies

WARNING **WARNING** Do not use warped or damaged plates.

Plate Assembly Components

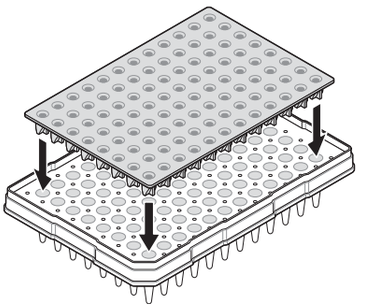
Each 96- or 384-well assembly contains a:

- Plate retainer
- Plate septa
- sample plate
- Base plate



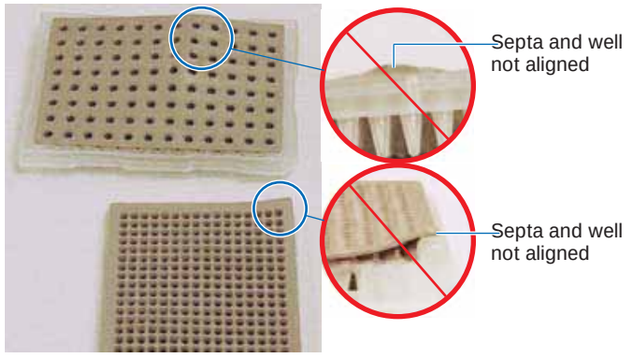
Preparing a Plate Assembly

1. Seal the plate:
   a. Place the plate on a clean, level surface.
   b. Lay the septa flat on the plate.
   c. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.

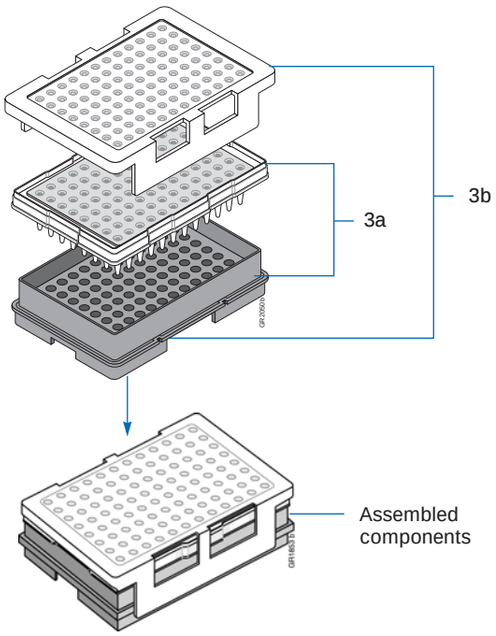


Notes

2. To prevent damage to the capillary array, inspect the plate and septa to verify the septa fits snugly and flush on the plate.

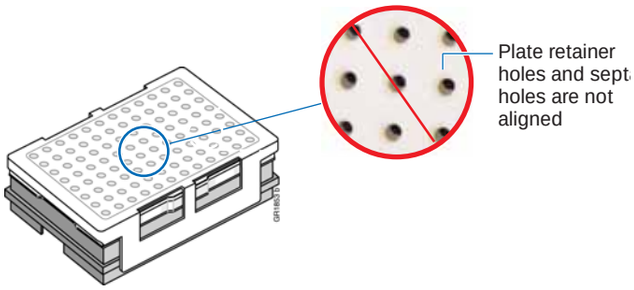


3. Assemble the plate assembly:
   a. Place the sample plate into the plate base.
   b. Snap the plate retainer onto the plate and plate base.



4. Verify that the holes of the plate retainer and the septa strip are aligned. If not, re-assemble the plate assembly (see step 3).

IMPORTANT! Damage to the array tips will occur if the plate retainer and septa strip holes do not align correctly.

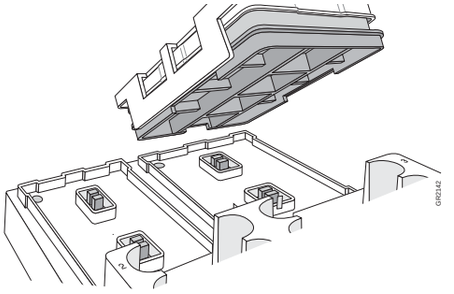


Notes

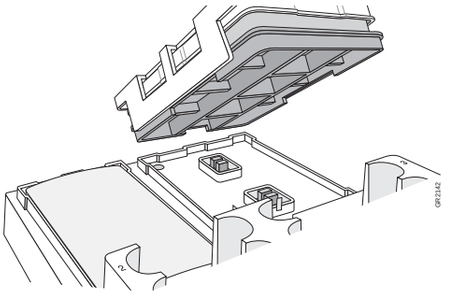
Placing the Plate onto the Autosampler

1. Close the oven and front doors.
2. Press the Tray button.
3. Open the front doors.
4. Place the plate assembly on the autosampler in position A or B for the 3100 instrument and position B for the 3100-*Avant* instrument.

   Note: There is only one orientation for the plate, with the notched end of the plate base away from you.



3100 instrument



3100-*Avant* instrument

5. Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off of the autosampler.
6. Close the instrument doors.

   Note: Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in buffer.

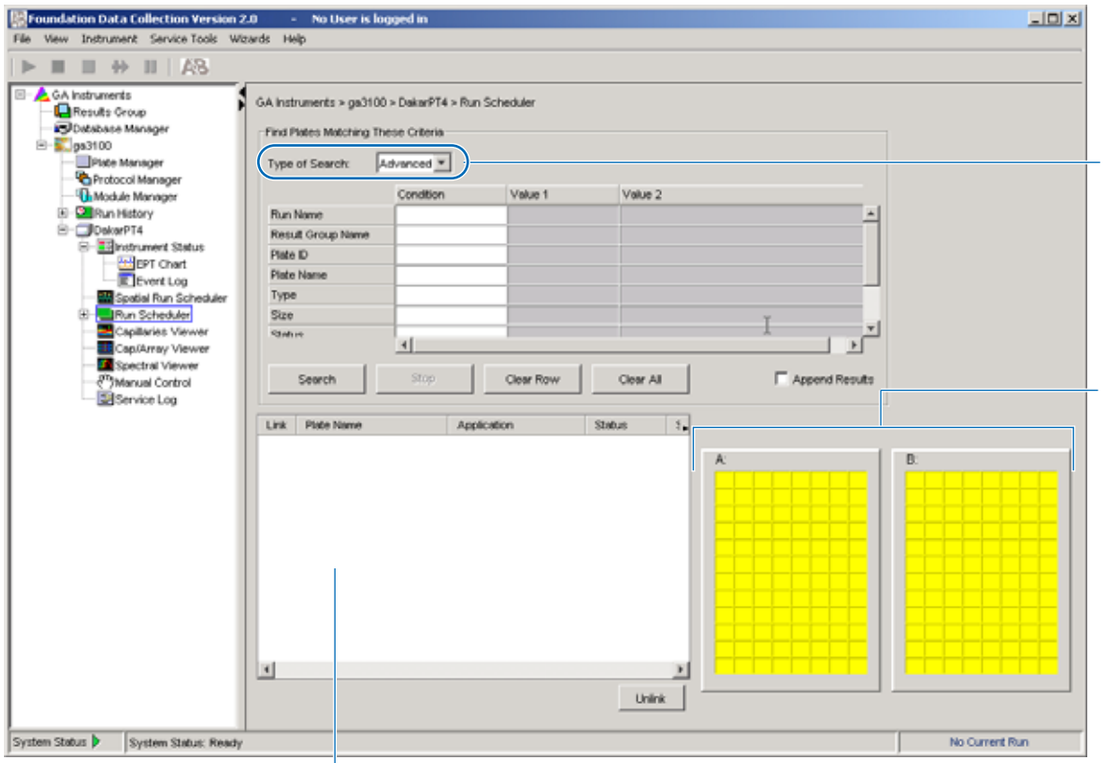
Notes

Linking and Unlinking a Plate

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

Searching for Plate Records

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Run Scheduler**.

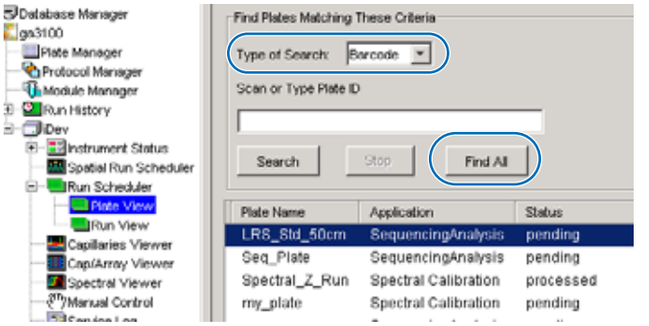


Notes

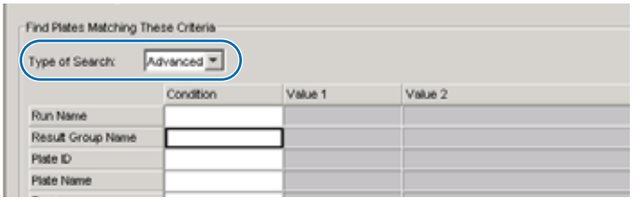
2. Search for your plate record. There are two search options, Find All and Advanced.

   - **Find All**
     - Select **Barcode** in the Type of Search drop-down list.
     - If you have a limited number of plates in the database, click Find All.

       All plates in the database display in plate record section.

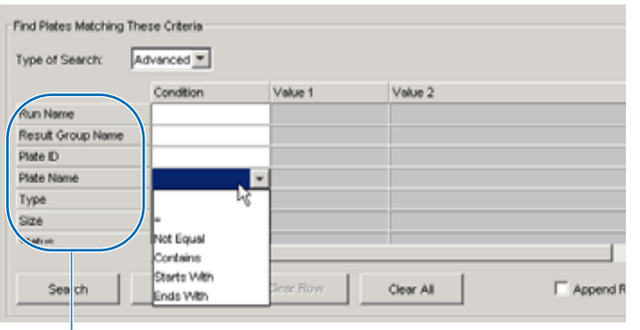


   - **Advanced**
     - Select **Advanced** in the Type of Search drop-down list.

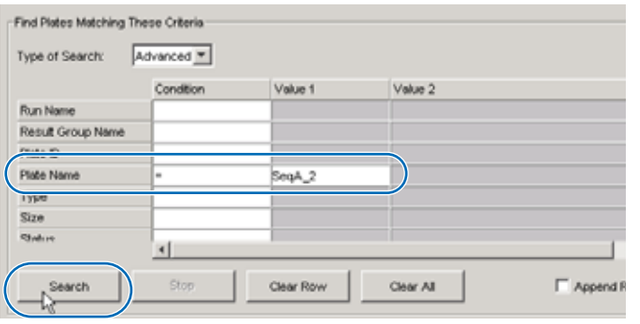


     - Use the drop-down list to define search conditions for a category or multiple categories (Run Name, Results Group Name, Plate Name, etc.).

       Note: Use the Plate Name for the Plate ID category.



     - For each category with a condition selected, type a value (primary search string) in the Value 1 column.
     - Click Search. All plates in the database that match the search criteria display the in plate record section.



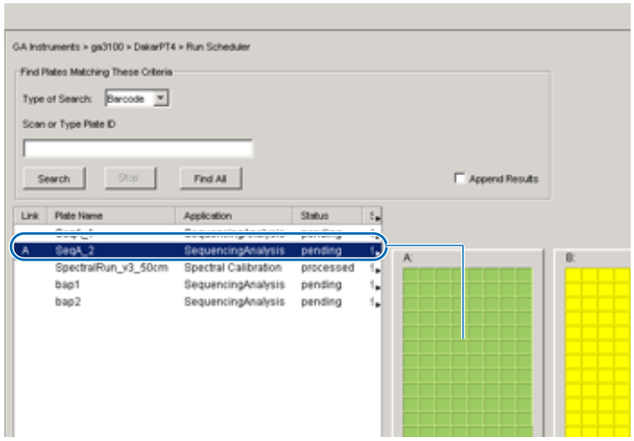
Notes

Linking a Plate

Select the plate record you want to run, then click the plate position indicator that corresponds to the plate you are linking.

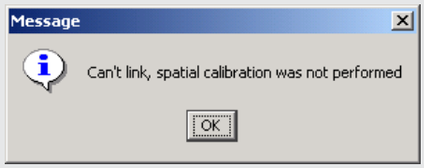
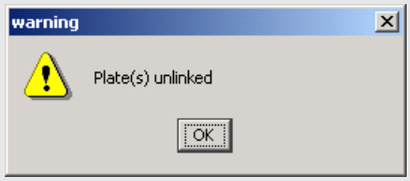
Note: The 3100-*Avant* instrument has only one plate position to link a plate record.

The plate position indicator changes from yellow to green when linked and the green run button is active.



Troubleshooting

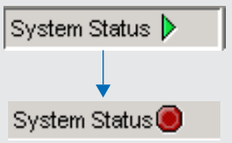
If a Plate Does Not Link

Observation/Problem	Cause	Corrective Action
Plate does not link.	Spatial calibration was not performed. 	1. Preform a spatial calibration. 2. Relink the plate(s) in the Run Scheduler.
The plates in the Run Scheduler were linked, but now are unlinked.	Used a wizard after linking a plate, but before starting a run. 	Relink the plate(s) in the Run Scheduler.

Notes

Troubleshooting *(continued)*

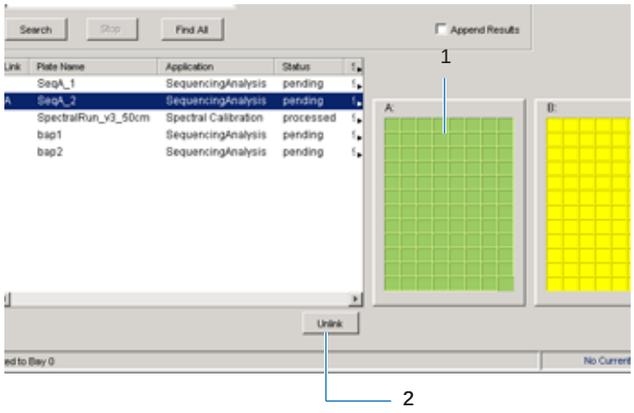
If a Plate Does Not Link

Observation/Problem	Cause	Corrective Action
The plate links, but System Status changes from green to red. 	A different length capillary array was installed, and the appropriate active spectral calibration was not selected or does not exists.	1. View the error messages in the Event Log. 1. In the Spectral Calibration Viewer, active the spectral calibration for the dye set and array length you are using (see page 59). 2. If one does not exist, create a new spectral calibration for the dye set and array length you are using, then set as the active spectral calibration (see page 41). 3. Relink the plate(s) in the Run Scheduler.
	The capillary array length and/or polymer type selected in the Instrument Protocol does not match capillary array length and/or polymer type stored in the database.	Correct the Instrument Protocol, or 1. Use the wizards to update the information in the database. 2. Set (see page 59) or create an active spectral calibration (see page 41). 3. Relink the plate.
	The database and/or drive E is full	1. View the error messages in the Event Log. 2. Proceed to "Working With Drives for Database and Sample Data Storage" on page 234. 3. Make more space. 4. Relink the plate(s) in the Run Scheduler.

Notes

Unlinking a Plate Record

1. Click the plate record that you want to unlink.
2. Click Unlink.



Notes

Run Scheduling

Sample Run Order

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

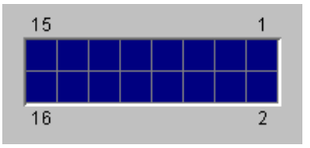
1. The order the plates are linked (3100 instrument only), see page 155 for linking plate information.
2. The priority value for samples in the plate record.

If all priorities are set to 100 (default), runs are scheduled as outlined below. Refer to "Run Priority Scheduling" on page 262 for information on how a change in the priority values changes run scheduling.

Capillary Array Map

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

Capillary numbers:

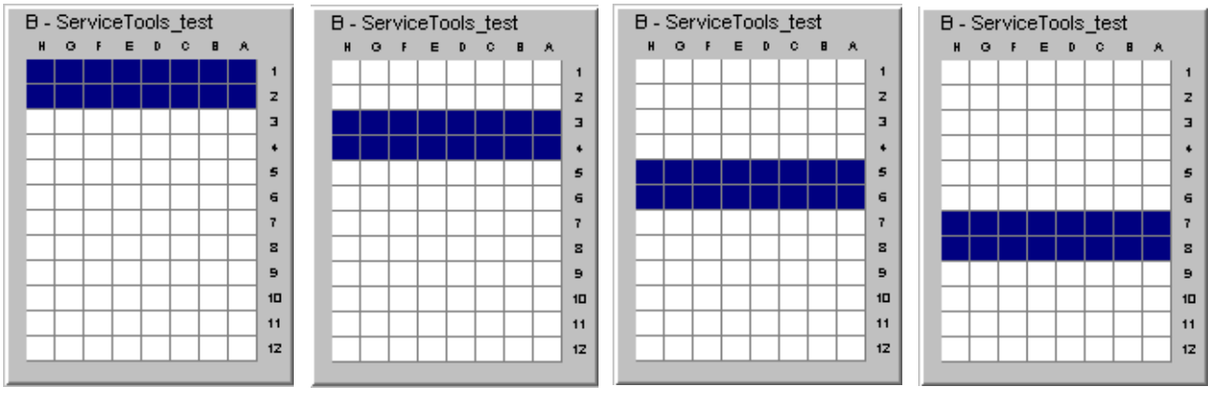


96-Well Plate Mapping

3100 Instrument

For a 96-well plate, injections are made from every well in two consecutive rows, starting with an odd row. A full 96-well plate requires six runs to inject all samples once.

Run 1 Run 2 Run 3 Run 4

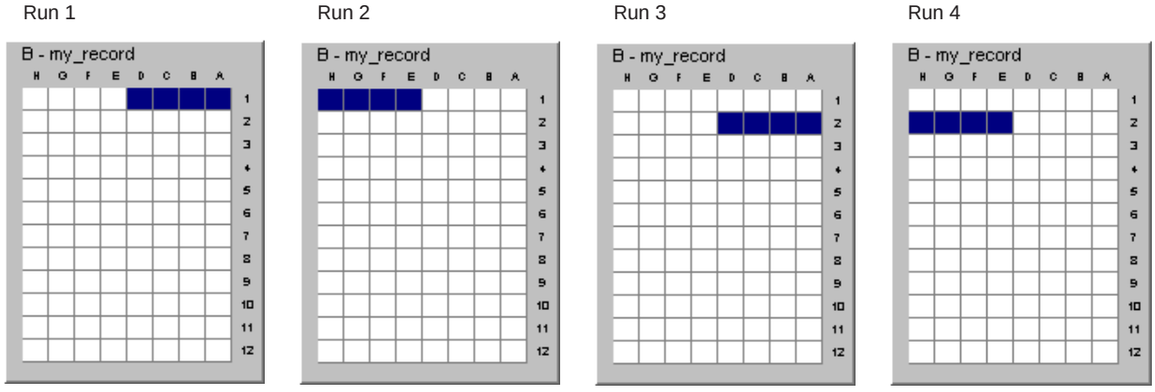


Note: Multiple injections (Instrument Protocol 2 - Instrument Protocol 5) are run before moving to the next set of wells.

Notes

3100-*Avant* Instrument

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

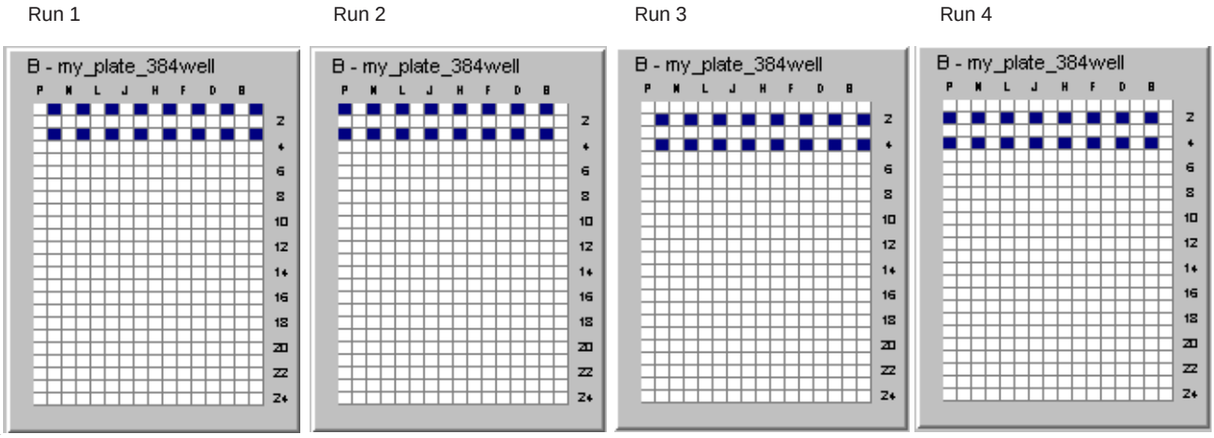


Note: Multiple injections (Instrument Protocol 2 - Instrument Protocol 5) are run before moving to the next set of wells.

384-Well Plate Mapping

3100 Instrument

For a 384-well plate, injections are made from every other well and ever other row. A full 384-well plate requires 24 runs to inject all the samples once.

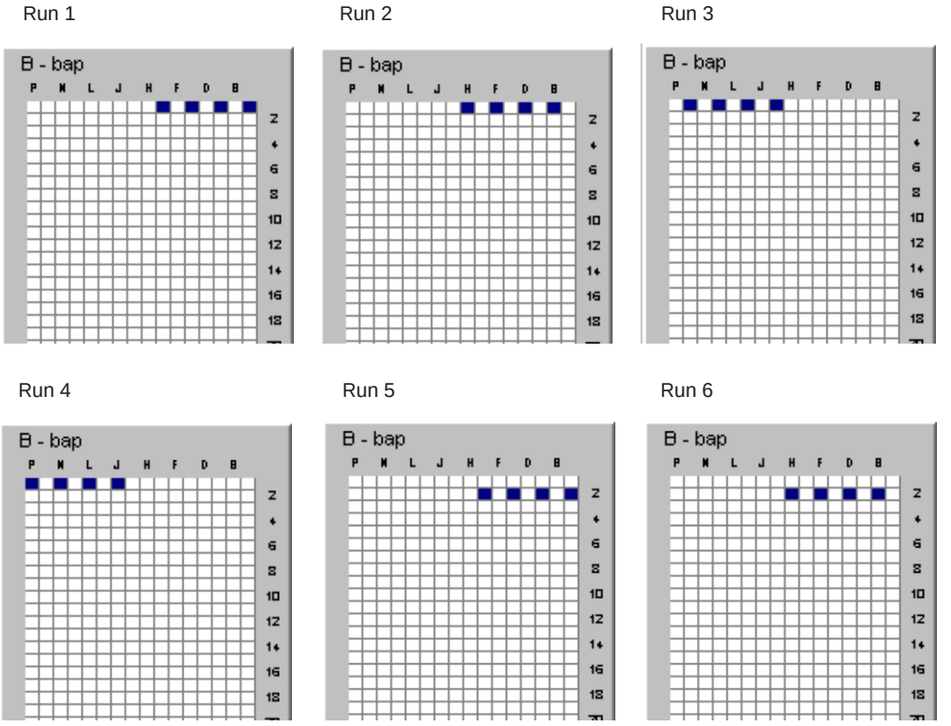


Note: Multiple injections (Instrument Protocol 2 to 5) from the same well are run before moving to the next set of wells.

Notes

3100-*Avant* Instrument

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



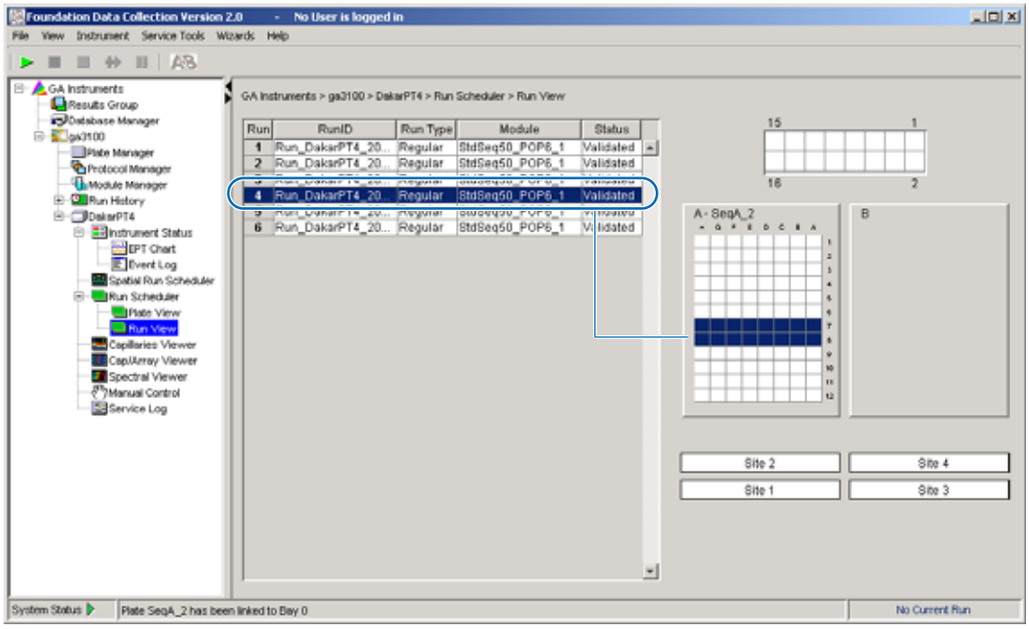
Note: Multiple injections (Instrument Protocol 2 to 5) from the same well are run before moving to the next set of wells.

Notes

Viewing the Run Schedule

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

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Run Scheduler** > **Run View**.



2. Select a row for any run. The corresponding wells to be injected for that run are highlighted in the plate diagram.

Notes

Running the Instrument

Launching the Run

Starting the Run

1. Verify the active spectral calibration matches your dye set and capillary array length.

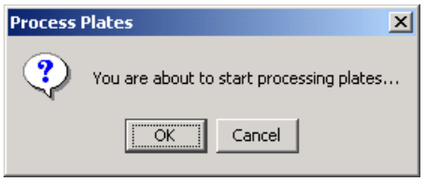
   To change the active spectral calibration, refer to "Activating a Spectral Calibration" on page 59.

   To create a new spectral calibration, refer to "Creating a Spectral Instrument Protocol" on page 41.

2. If you want to review the run schedule before beginning the run, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Run Scheduler** > **Run View**.

3. Click the green button in the toolbar.

4. The Processing Plates dialog box opens, then click OK.



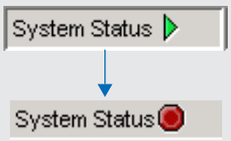
5. The software automatically performs a run validation:
   - if the validation passes, the run starts
   - if any of the validation test fails, the run does not start (use the troubleshooting table, "Run Validation" on page 165)

Notes

Troubleshooting

Run Validation

Below are the validation tests, all tests must pass before the run starts.

Test Checks	Look For	Corrective Action
The capillary array length and/or polymer type in the Instrument Protocol against the capillary array length and/or polymer type in the database	1. System Status changes from green to red.  2. View the error messages in the Event Log.	Correct the Instrument Protocol, or 1. Use the wizards to update the information in the database. 2. Set (see page 59) or create an active spectral calibration (see page 41). 3. Relink the plate, then click ▶.
The available space in the database and drive E		1. Proceed to "Working With Drives for Database and Sample Data Storage" on page 234. 2. Make more space. 3. Click ▶.
A different length capillary array was installed, and the appropriate active spectral calibration was not selected or does not exists.		1. In the Spectral Calibration Viewer, active the spectral calibration for the dye set and array length you are using (see page 59). 2. If one does not exist, create a new spectral calibration for the dye set and array length you are using, then set as the active spectral calibration (see page 41). 3. Click ▶.

Notes

Starting the Autoanalysis Manager for SeqScape and/or GeneMapper Software

The Autoanalysis Manager (AAM) software is used with SeqScape and/or GeneMapper software to automatically analyze the data.

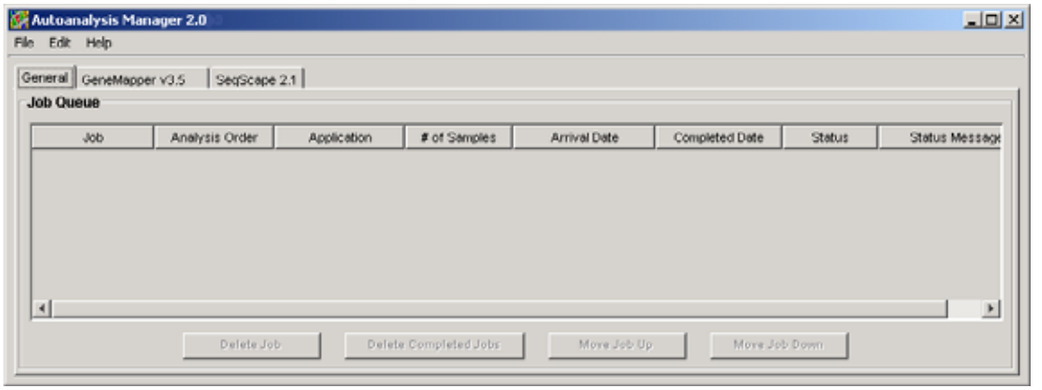
Note: The Data Collection Messaging Service must be running in order for analysis messages to be received by the Autoanalysis Manager.

To start the Autoanalysis Manager:

1. Select **Start** > **Programs** > **Applied Biosystems** > **Autoanalysis Manager** > **Autoanalysis Manager 2.0**.

   Note: Autoanalysis Manager does not start automatically. Autoanalysis Manager must be open to receive messages from 3100/3100-*Avant* Data Collection for autoanalysis in SeqScape and/or GeneMapper software.

   The Autoanalysis Manager window opens.



2. Quit the SeqScape and/or GeneMapper software.

   No other interaction with the AAM software is needed until the completion of the runs. See "Using the Autoanalysis Manager Software" on page 180 for information on how to use the Autoanalysis Manager.

Notes

Basic Run Module Steps

When the run starts, the following basic steps are performed automatically by the instrument. To customize a run module, see "Tip: Customizing Run Modules" on page 82.

Module Steps	Approximate Time
Turn Oven On	N/A
Wait for oven to equilibrate Initialize autosampler Fill syringes	1 min 40 sec
Fill Array	3-4 min
PreRun	3 min
Inject samples	30 sec
Start separation Ramp voltage	10 min
Collect Data	Variable
Run ends: Leave oven on Laser to idle	Until next run starts
Total time prior to separation: • Cold start: ~16.5 min • 2nd run: ~6.5 min	

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

Run Times

DNA Sequencing Run Times

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

Type of Run	Run Module	Run Time
Ultra rapid DNA sequencing	UltraSeq36_POP4_1	45 min
Rapid DNA sequencing	RapidSeq36_POP4_1	1 h
Standard DNA sequencing	StdSeq50_POP4_1	1 h 20min
	StdSeq50_POP6_1	2 h 30 min
Long read DNA sequencing	LongSeq80_POP4_1	3 h 40 min

Notes

Fragment Analysis Run Times

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

Type of Analysis	Run Module	Run Time
Fragment analysis	FragmentAnalysis22_POP4_1	20 min
Fragment analysis	FragmentAnalysis36_POP4_1	45 min
Fragment analysis	FragmentAnalysis50_POP4_1	65 min
Fragment analysis	FragmentAnalysis50_POP6_1	95 min
Fragment analysis	HIDFragmentAnalysis36_POP4_1	45 min
SNP analysis	SNP22_POP4_1	16 min
SNP analysis	SNP36_POP4_1	25 min

Controlling the Run

Controlling the Run Using the Toolbar

Use the toolbar at the top of the data collection software window to control the run.

Click ...	Description
 Start Run	Starts the run
 Stop	Stops the current run, and all other scheduled runs
 Stop After Current Run	Completes the current run, then stops all other scheduled runs
 Skip to Next Run	Stops the current run, then starts the other scheduled runs
 Pause Run	Pauses the current run[a]

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

Notes

Set Up for Continuous Operation

Running Continuously

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

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

Adding or Replacing a Plate During a Run

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

If the plate is in use, see "Replacing a Plate Currently in Use" on page 171.

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

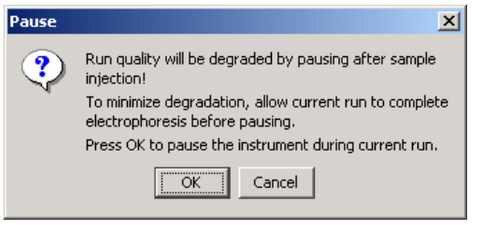
Notes

Replacing or Adding a Plate to a Run

Use this procedure to replace a completed plate or add a new plate to an unused plate bay (3100 instrument only).

1. Prepare your plate and create the plate record.
2. Click (Pause).

   In the Pause dialog box, read the pause warning, then click OK to pause the run.



   The following dialog box opens when the run is paused.

3. *Do not* click OK to resume the run. Temporarily ignore the dialog box.
4. Remove the old plate, if applicable.
   a. Press the Tray button to bring the autosampler forward.
   b. Open the instrument door.
   c. Remove the old plate.
5. Mount the new plate.
6. Close the door.

   The instrument resumes when the autosampler completes the initialization and returns to the home position.

   If the 3100 instrument does not resume automatically, open and close the door again.
7. Search for the plate record, then link the new plate.

   The new plate runs after the current plate completes all scheduled injections.

Notes

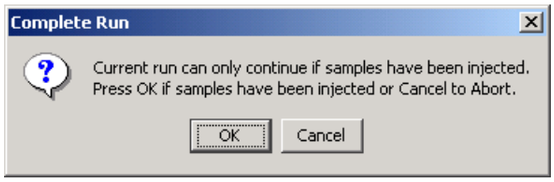
Replacing a Plate Currently in Use

Use this procedure to replace a plate in use. To avoid potential problems, it is best to allow the plate to complete the scheduled runs.

1. Follow steps 1 to 3 in the procedure "Adding or Replacing a Plate During a Run" on page 169.
2. Remove the plate.
   a. Press the Tray button to bring the autosampler forward.
   b. Open the 3100 instrument door.
   c. Remove the old plate.
3. Mount the new plate.
4. Close the door.

   The instrument resumes when the autosampler completes the initialization and returns to the home position.

   If the 3100 instrument does not resume automatically, open and close the door again.
5. In the Completed Run dialog box, click OK to continue if the samples have been injected, or click Cancel to abort the run and return the instrument to an idle state.



IMPORTANT! If you click OK, the instrument will continue running the current run regardless if the samples have actually been injected or not. If the samples have not been injected, the samples will be injected from the new plate.

6. Search for the plate record, then link the new plate.

Note: If you unmount the currently running plate prior to the first frame of data being collected but after sample injection (clicked OK to continue), the plate status changes to processed even though the run is actually continuing.

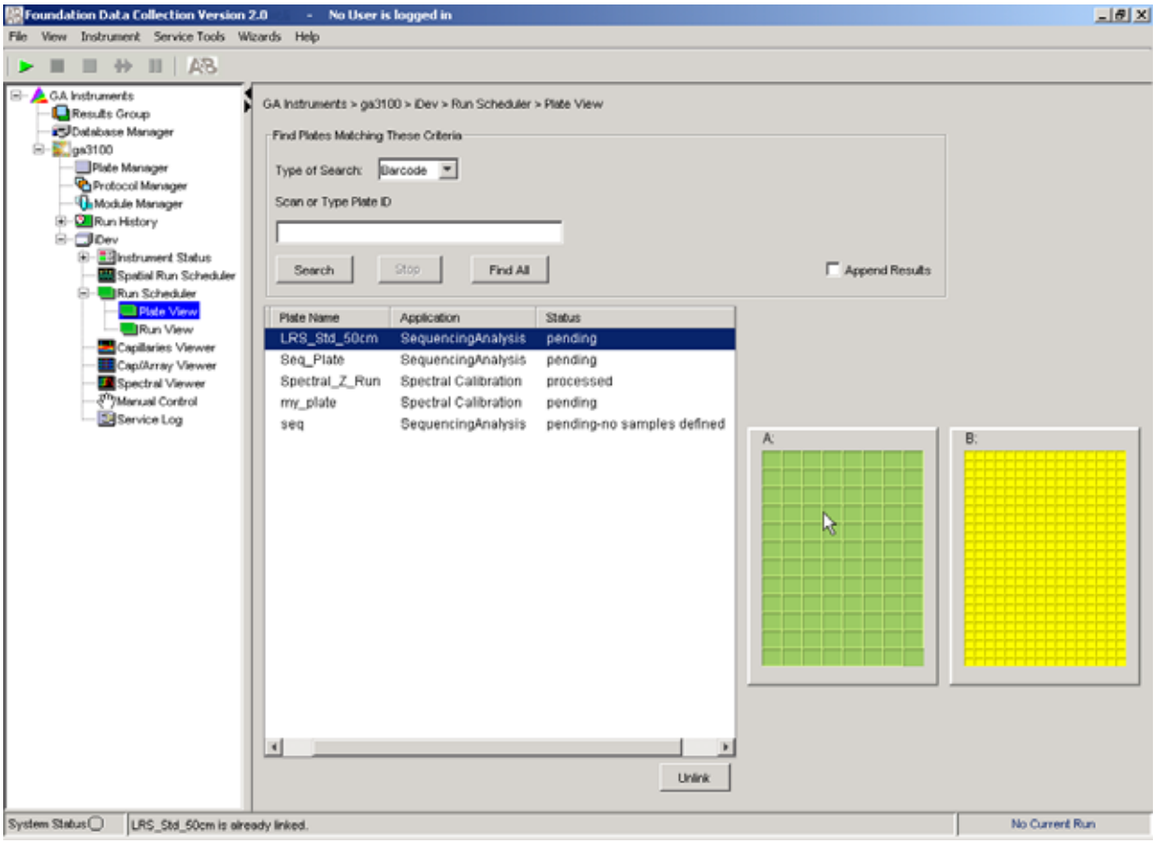
Notes

Viewing Data During a Run

Run Scheduler > Plate View

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Run Scheduler** > **Plate View**.

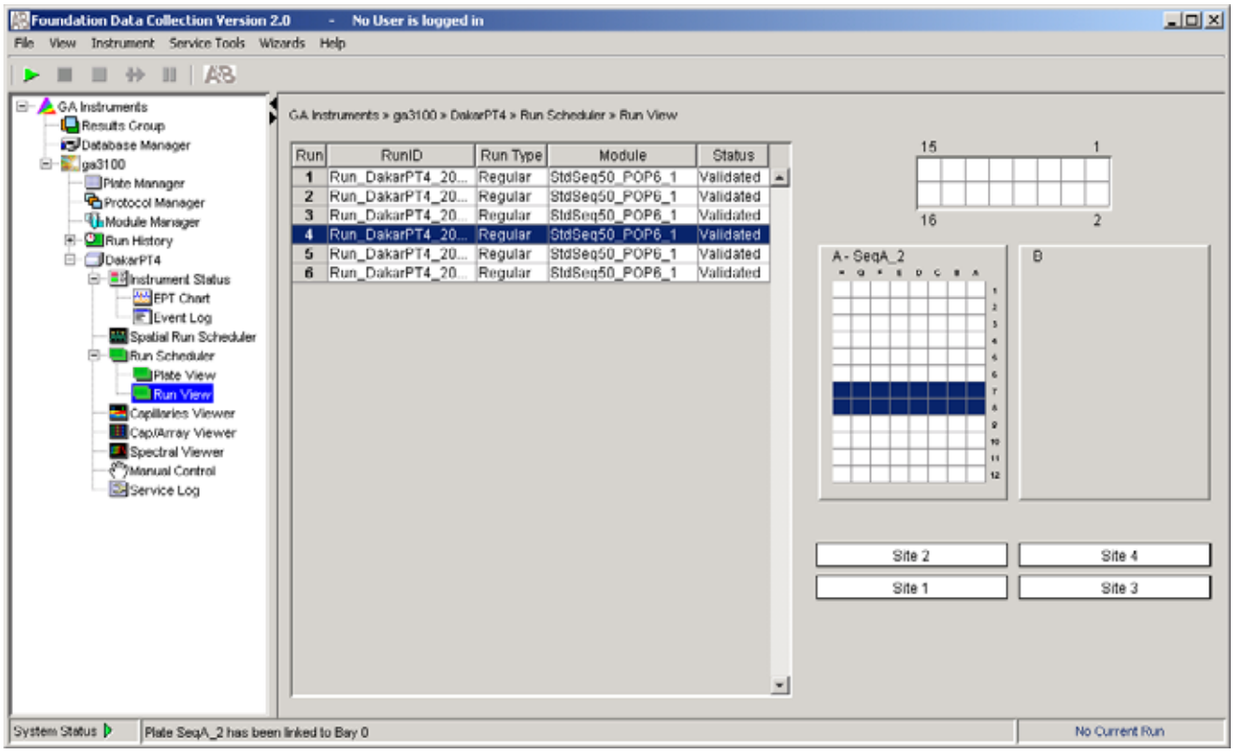
Note: The **Run Scheduler** and **Plate View** windows display the same information.



Notes

Run Scheduler > Run View

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Run Scheduler** > **Run View** to monitor the status of the scheduled runs.

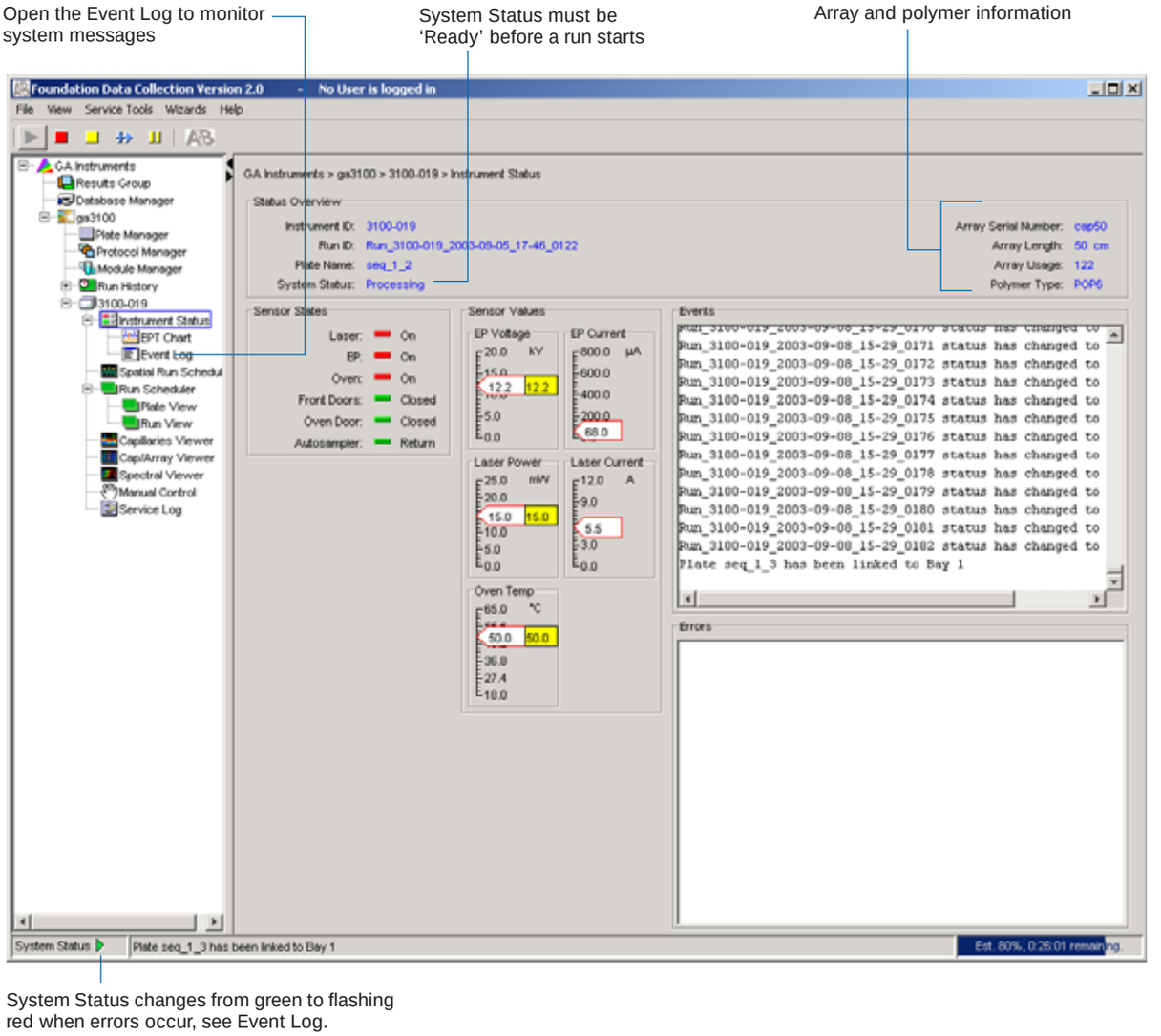


Note: For default load maps, see page 160.

Notes

Instrument Status

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Instrument Status** to monitor the status of the instrument or the current run.



Notes

Instrument Condition Group Box

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

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

Events Box

The Events box lists the:

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

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

Errors Box

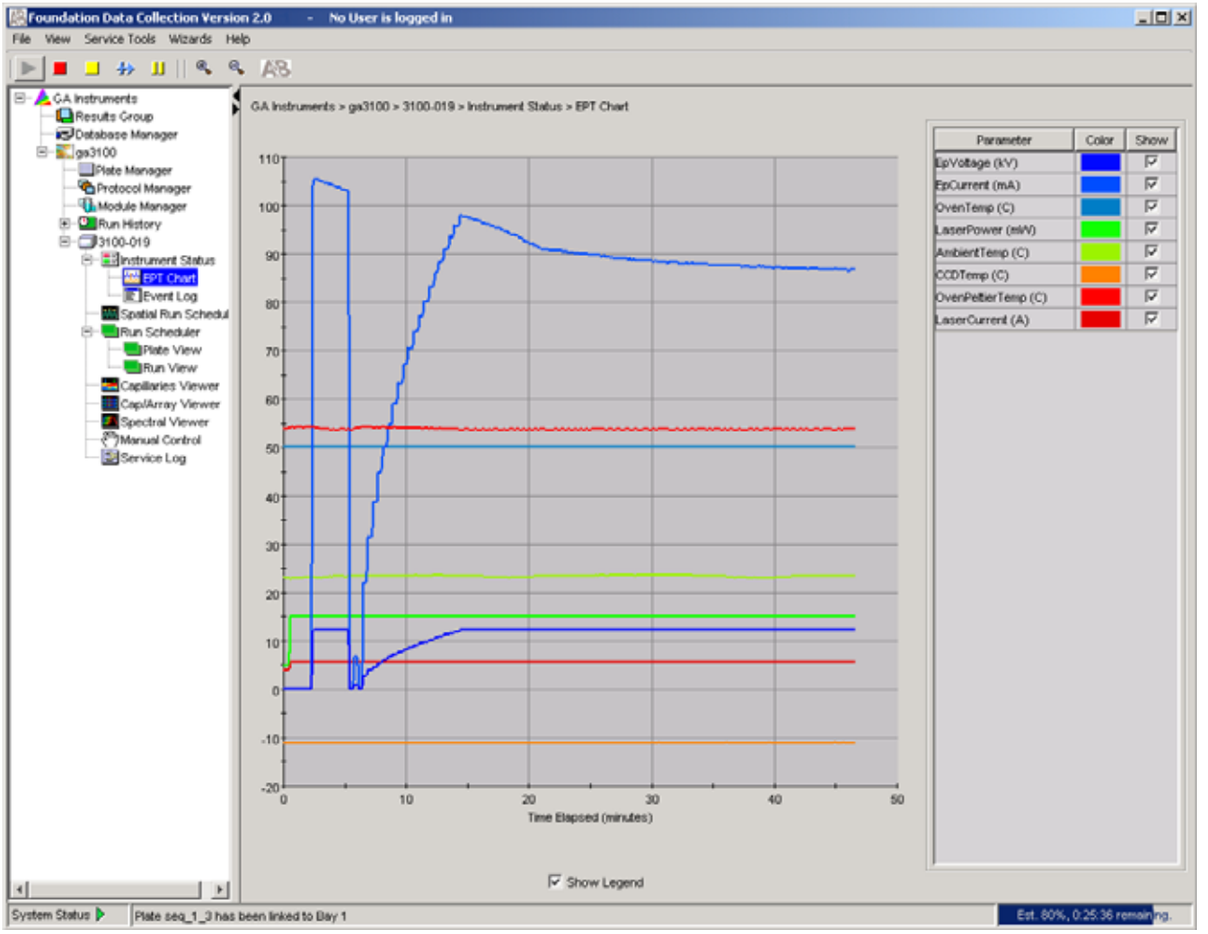
The Errors box lists errors that have occurred during the current run.

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

Notes

Instrument Status > EPT Chart

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Instrument Status** > **EPT Chart**. The EPT chart displays real-time electrophoresis (EP) data during a run.



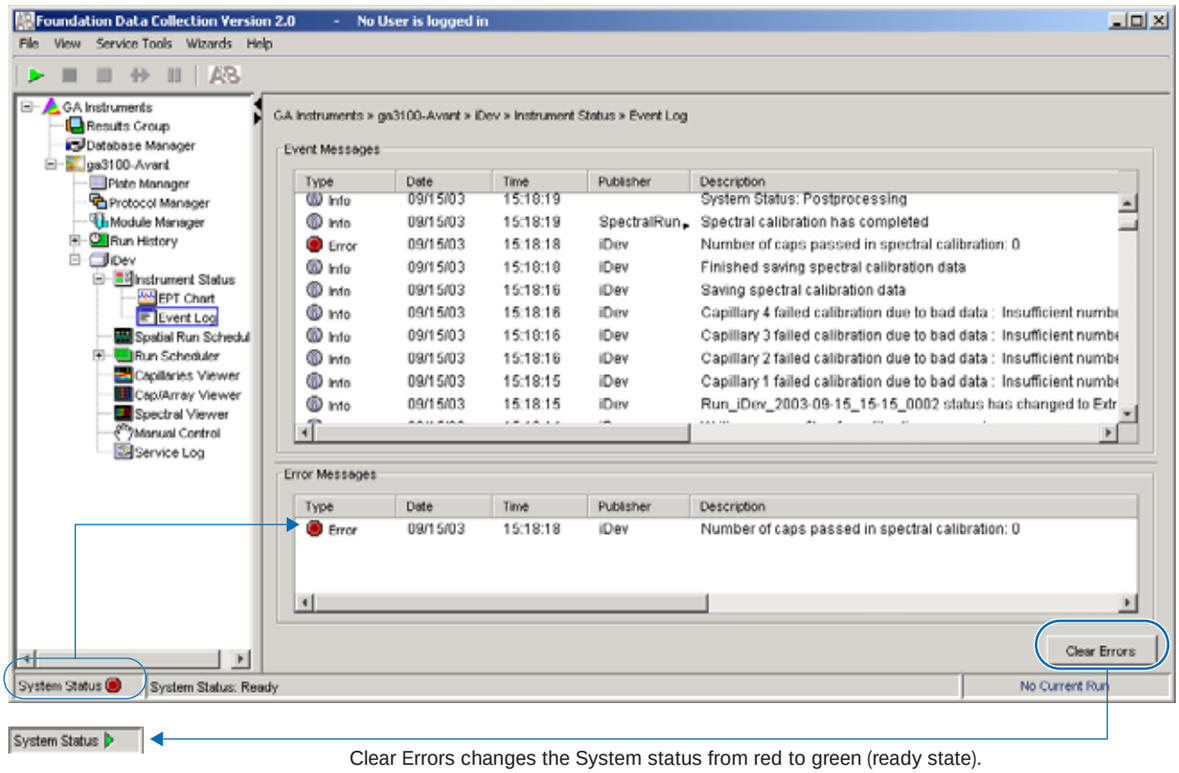
Notes

Instrument Status > Event Log

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Instrument Status** > **Event Log**. The Event log itemizes events such as errors and general information for all data collection steps.

Clear error messages by clicking **Clear Errors**. The System Status light flashes red until all errors are cleared. Take corrective action based on error message, then repeat the action that caused the error.

Note: This view can also be used to monitor spectral calibration results in real time to verify the capillary-by-capillary processing status.

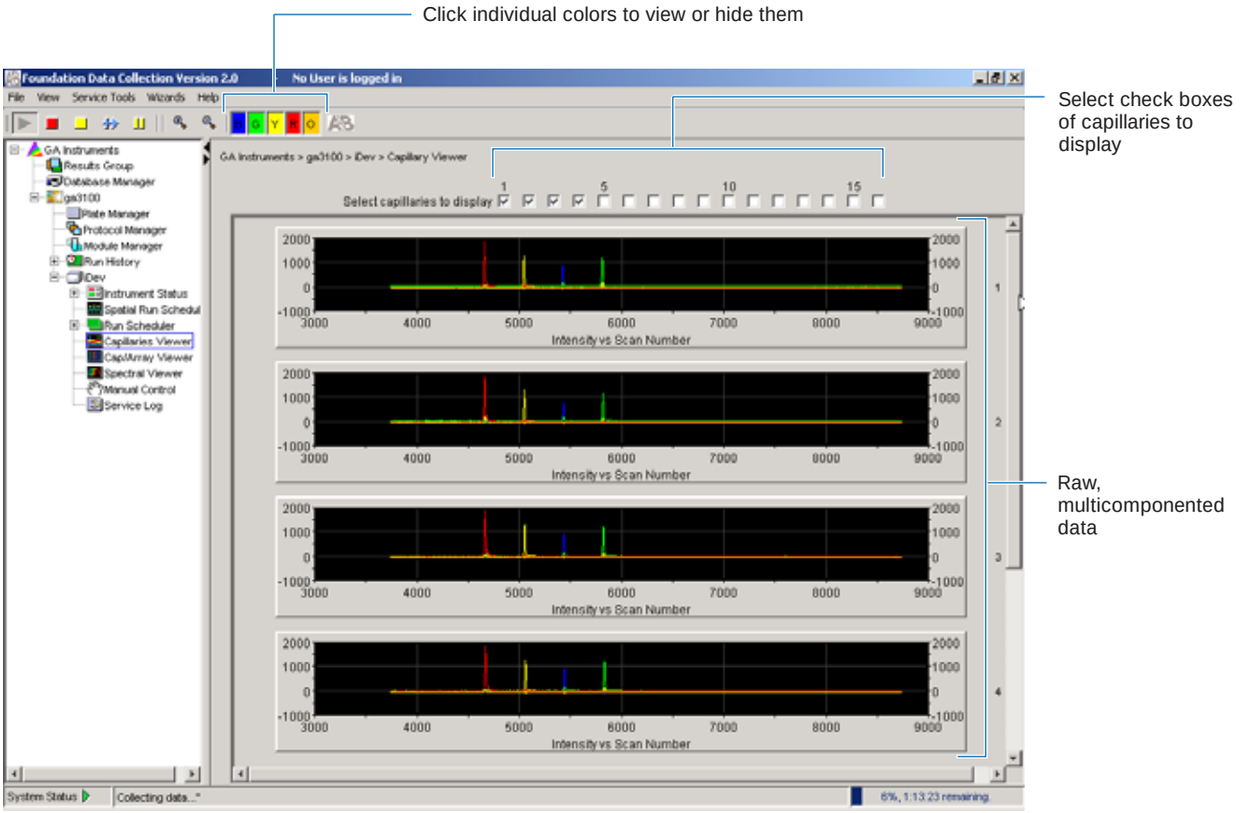


Clear Errors changes the System status from red to green (ready state).

Notes

Capillaries Viewer

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Capillaries Viewer**. Use the Capillary Viewer to examine the quality of the raw data during a run for several capillaries at once.



Check Boxes

Select the check boxes of the capillaries for which you want electropherograms displayed. The capillaries are displayed in the order in which the boxes are checked. The more boxes that are selected, the slower the refresh window rate.

Raw Data

An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The raw data displayed has been corrected for spectral overlap (multicomponented).

How to Zoom

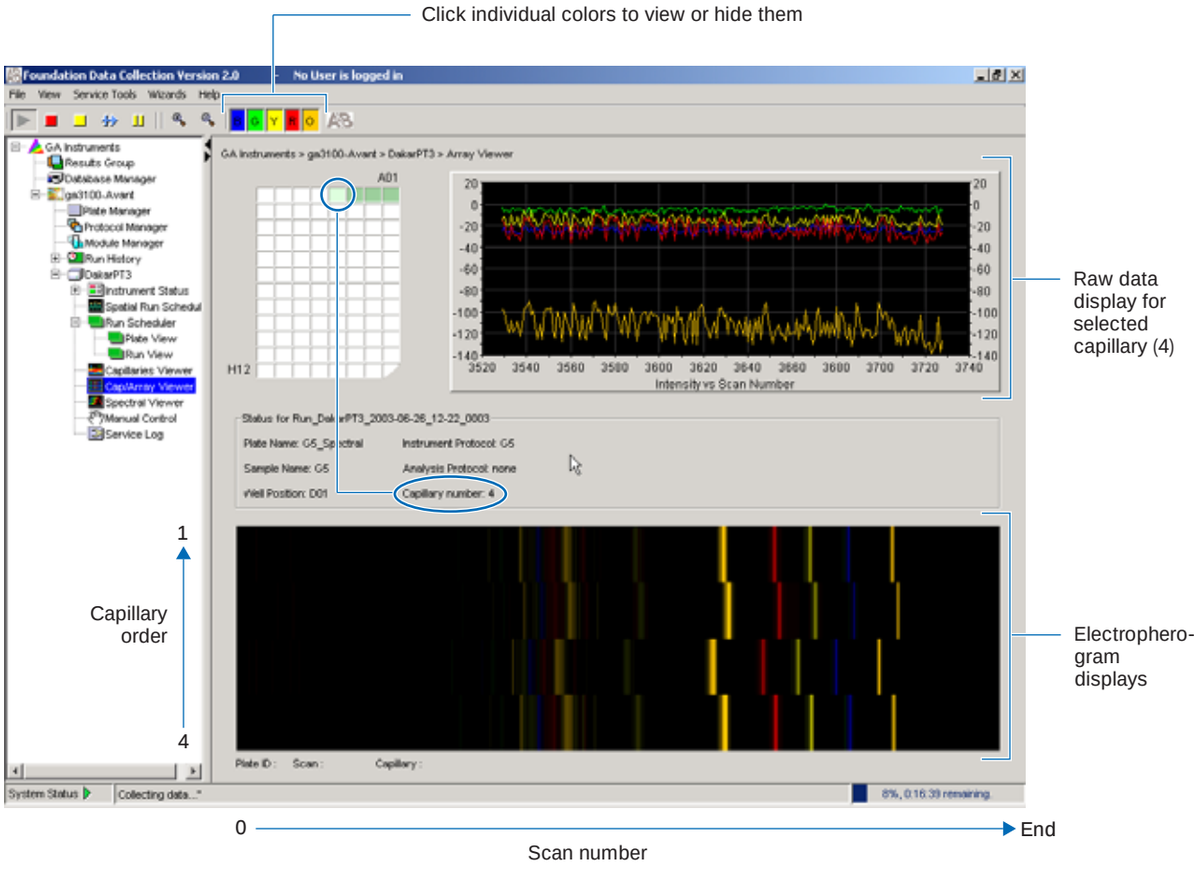
To zoom in and out:

1. Click , then place the pointer over the area of interest and click to expand the view.
2. Click to return to full view.

Notes

Cap/Array Viewer

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > *instrument name* > **Cap/Array Viewer**. Use the window during a run to examine the quality of your data, which is displayed as color data for the entire capillary array. You can view all the capillaries (vertical axis) as a function of time/scan numbers (horizontal axis).



How to Zoom

To zoom in and out:

1. Click , then place the pointer over the area of interest and click to expand the view.
2. Click to return to full view.

Notes

Using the Autoanalysis Manager Software

Overview

The Autoanalysis Manager software is used with SeqScape and/or GeneMapper software. The Autoanalysis Manager software is installed by the SeqScape or GeneMapper software installation CD.

Autoanalysis occurs in the following sequence:

- When data collection software finishes a run, the Message Service sends the message “Run Completed.”
- The Autoanalysis Manager receives the message, and the job is submitted. The job appears in the General tab.
- The Autoanalysis Manager polls for jobs every 2 minutes and opens the automated processing version of SeqScape and/or GeneMapper software to analyze the data.
- At the end of analysis, the status in the Autoanalysis Manager is updated.

Files Created

The data collection software stores the sample files in the location specified in the results group. The Autoanalysis Manager copies the files into the DataStore for SeqScape or GeneMapper processing.

To maintain sufficient storage space on your hard drive, delete the sample files created by data collection software that are no longer needed.

Autoanalyzing Samples

Once an internal message from the instrument is received by the Autoanalysis Manager, it opens the automated processing version of SeqScape and/or GeneMapper software to autoanalyze the samples. The standard user version of SeqScape and/or GeneMapper software must be closed in order for autoanalysis to begin.

If SeqScape and/or GeneMapper software is open, a dialog box message displays asking if you want to close the software in order to process the new runs. Do one of the following:

If You Select...	Then...
Yes	Any pending changes to the current project are saved, GeneMapper software closes and the AutoAnalysis Manager takes over.
No	The runs continue to collect and queue in the AutoAnalysis Manager until GeneMapper software is closed.

The message dialog box has a timer so that if you leave the SeqScape and/or GeneMapper software open but are not using it, once time expires, any pending changes to the current project are saved, SeqScape and/or GeneMapper software closes, and Autoanalysis Manager takes over.

Notes

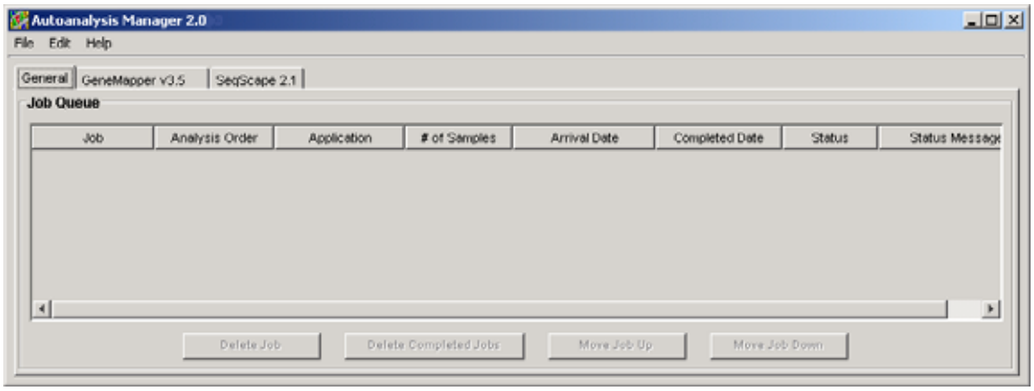
Components

The Autoanalysis Manager has two or three tabs:

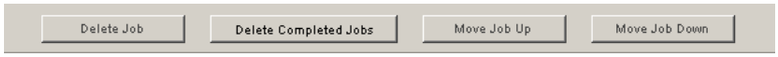
- General tab
- GeneMapper tab, if GeneMapper v3.5 or GeneMapper *ID* v3.1software is installed
- SeqScape tab, if SeqScape software is installed

General Tab

The General tab shows the jobs that have been submitted and their status.



Command Buttons in the General Tab



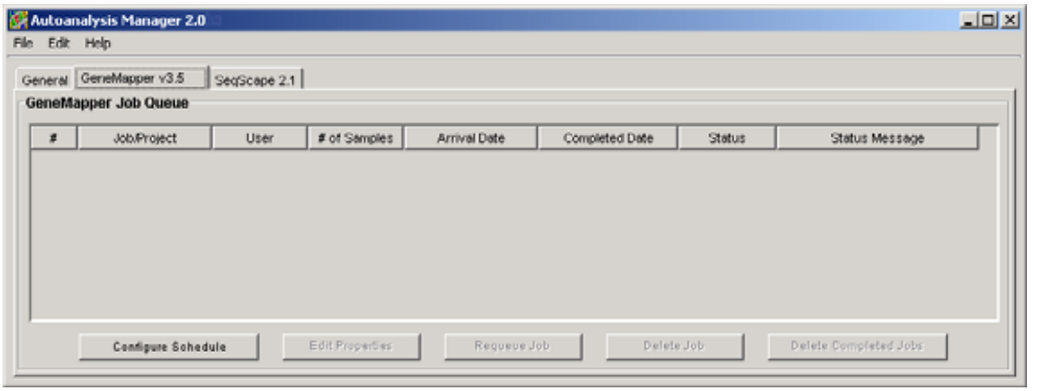
The table below describes the functions of the command buttons in the General tab

Button Name	Function
Delete Job	Deletes an individual job/project from the Autoanalysis Manager list. Does not delete sample files, SeqScape or GeneMapper software project.
Delete Completed Jobs	Deletes all completed jobs/projects from the Autoanalysis Manager list. Only successful jobs are deleted. Does not delete sample files, SeqScape or GeneMapper software projects.
Move Job Up	The active job/project is always given a queue number of 1. Once job 1 is finished analyzing, job 2 becomes job 1 and all other numbers are changed accordingly. Use the Move Up/Down buttons if you want to rearrange the analysis order.
Move Job Down	

Notes

GeneMapper Tab

The GeneMapper tab shows the jobs that have been submitted and their status.



Command Buttons in the GeneMapper Tab

The table below describes the functions of the command buttons in the GeneMapper tab.

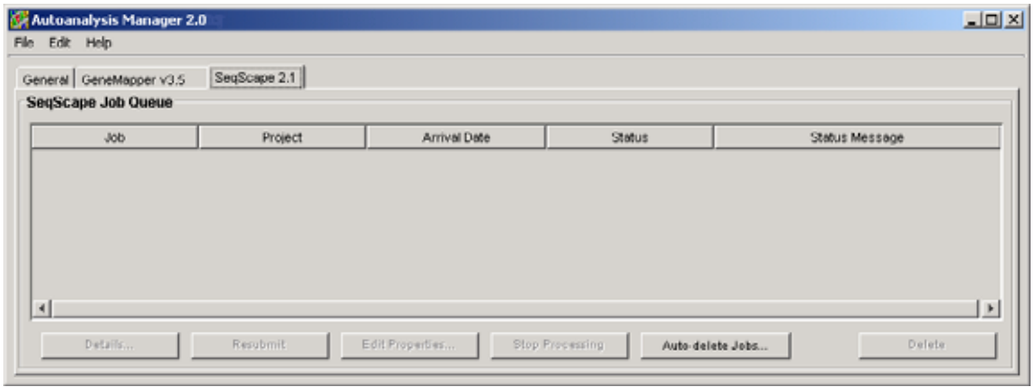
Button Name	Function
Configure Schedule	• Next Analysis Time: Enables you to set a start time for autoanalysis. Before this time arrives, no autoanalysis of projects will occur. • Periods restricting automated analysis: Enables you to set times during which autoanalysis will not occur. Useful if you know that you are going to be reviewing data during a certain time period and don't want to be bothered by the "Runs ready for processing" dialog box. Runs build up in the queue until the restricting time period is over, then runs will be autoanalyzed. • Automatic Deletion of Completed Jobs: Enables you to set the software to automatically delete successfully completed jobs. Jobs that failed or have not been analyzed will not be deleted. Only the Autoanalysis Manager job is deleted, sample files and GeneMapper software projects are not.
Edit Properties	Enables you to change the following settings: • Job/Project Name • UserName: GeneMapper software UserName • Password: Matching password for GeneMapper software UserName • Queue position: Enter a new queue position number for the project

Notes

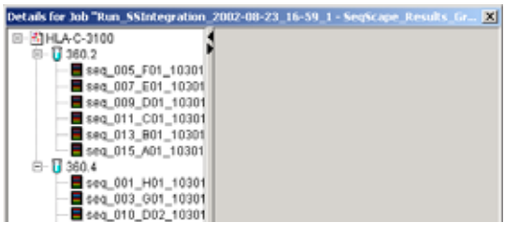
Button Name	Function
Requeue Job	• Samples that need to be autoanalyzed have queue numbers listed in the # column. • Samples that are already analyzed or failed, have a blank cell in the # column. To resubmit a job for autoanalysis, use the Requeue Job button to assign a queue number to that job.
Delete Job	• Deletes an individual job/project from the AutoAnalysis Manager list. Does not delete sample files or GeneMapper software project.
Delete Completed Jobs	• Deletes all completed jobs/projects from the AutoAnalysis Manager list. Only successful jobs are deleted. Does not delete sample files or GeneMapper software projects.

SeqScape 2.1 Tab

The SeqScape 2.1 tab shows the jobs, project, and status information.



The table below describes the functions of the command buttons in the SeqScape 2.1 tab:

Button Name	Function
Details	Displays the project in the navigation pane 
Resubmit	Submits a job for analysis
Edit Properties	Edits the name and password (active only if analysis failed)
Delete	Deletes a job from the Autoanalysis Manager

Notes

Working with Data in The Run History View

Run History Components

Elements of the Run History Utility

The Run History utility can be used only with completed runs stored in the local 3100/3100-*Avant* Data Collection database. It does not provide real-time viewing of collecting runs.

In the left tree pane, click the icon next to the function to launch it.

Elements Within the Run History Utility	Icon
EPT Chart Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Spatial Calibration Viewer	
Capillaries Viewer Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Cap/Array Viewer Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Spectral Viewer	
Reextraction Note: If Cleanup Database has been used, you cannot view processed data in Run History.	

Viewing Data from a Completed Run in the Data Collection Software

Overview

There are two formats for viewing data within the 3100/3100-*Avant* Data Collection Software under the Run History icon:

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

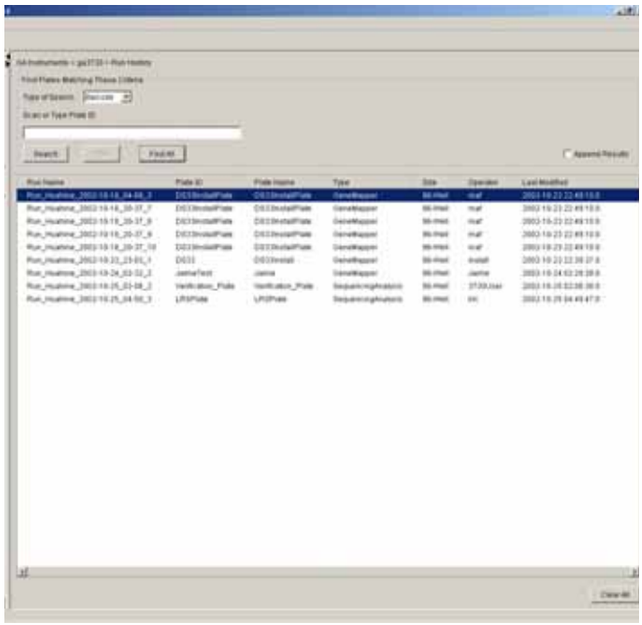
Notes

Viewing Data from a Completed Run

1. In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **3100-*Avant*** > **Run History** to select the run you want to view.

2. Search for your run by either Barcode or Advanced search.

3. After choosing the run, click the Cap/Array Viewer or the Capillary Viewer from the left tree pane.



Notes

Viewing the Results of Autoextraction

Overview

After a run is completed, extraction and analysis is performed automatically, according to the settings in the Plate Editor and the Results Group. The results of extraction and analysis can be viewed in the Reextraction Panel. Samples can be extracted again with the same settings, or with different Analysis Protocols or different Results Groups. This can be useful for many reasons:

- The destination location may not have been available during extraction.
- Some samples may have failed analysis and a different Analysis Protocol might be more successful.
- Samples might be saved in different locations, or with no analysis at all to save space.

Sample File Destinations

Locations where sample files are placed during extraction:

- Default Destination, and default folder naming: Data / instrument type / instrument name / run folder
- Default Destination, custom folder naming: Data/top custom folder/subfolders, etc.
- Custom Destination, default folder naming: Destination/instrument type/instrument name/run folder
- Custom Destination, custom folder naming: Destination/top custom folder/subfolders, etc.

Runs Stopped Before Complete Autoextraction

Runs that are stopped before completion display the status “Completed” in the Run Scheduler. In the Instrument Status the status is changed to “Ready.” Successfully extracted and analyzed runs display the status processed in the same Run View page.

The auto extractor component of the 3100/3100-*Avant* Data Collection automatically extracts data from stopped runs. If autoextraction fails, click the **Reextraction** icon  to extract data.

Effects of Changes Made in the Reextraction Panel

Changes made in the Reextraction Panel to a Results Group, Analysis protocol, Comments, etc., also change in the original plate record. The original plate information is overwritten.

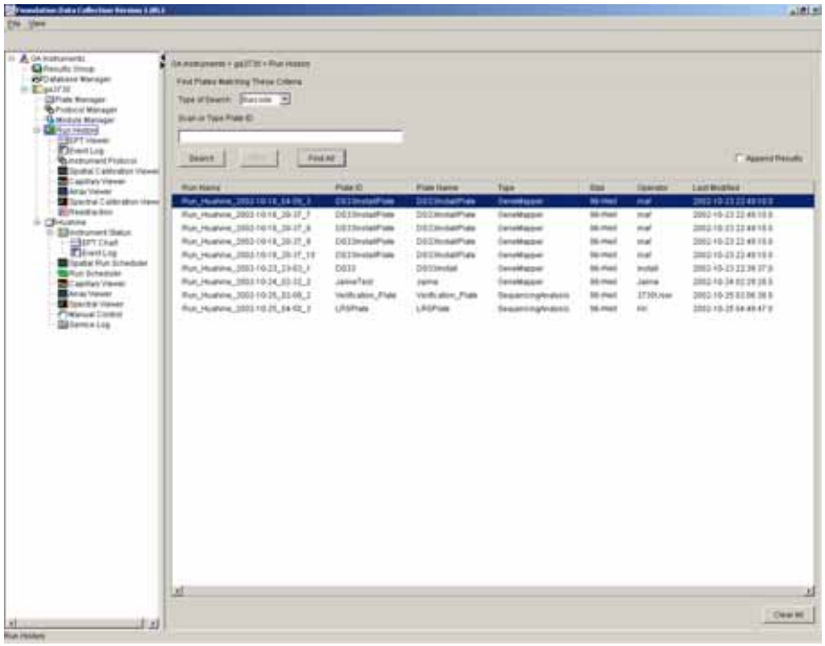
Selecting and Queuing Samples for Extraction

You can queue individual samples for reextraction. This is especially useful for experimenting with different Analysis Protocols for samples that have failed initial extraction.

1. Click  (Run History).
2. Enter the plate name for a plate that has been completed, or click **Search**. Plates that have runs still pending cannot be reextracted. All the runs from that plate appear in the window.

Notes

3. Select a run from the list.



4. Click (Reextraction) in the left tree pane.
   The Reextraction window displays

5. Click the check boxes in the Extract column to select the samples to be reextracted.

6. Click **Extract** to start the reextraction.

> Note: Reextracted sample files are saved in the original folder that data was extracted to.

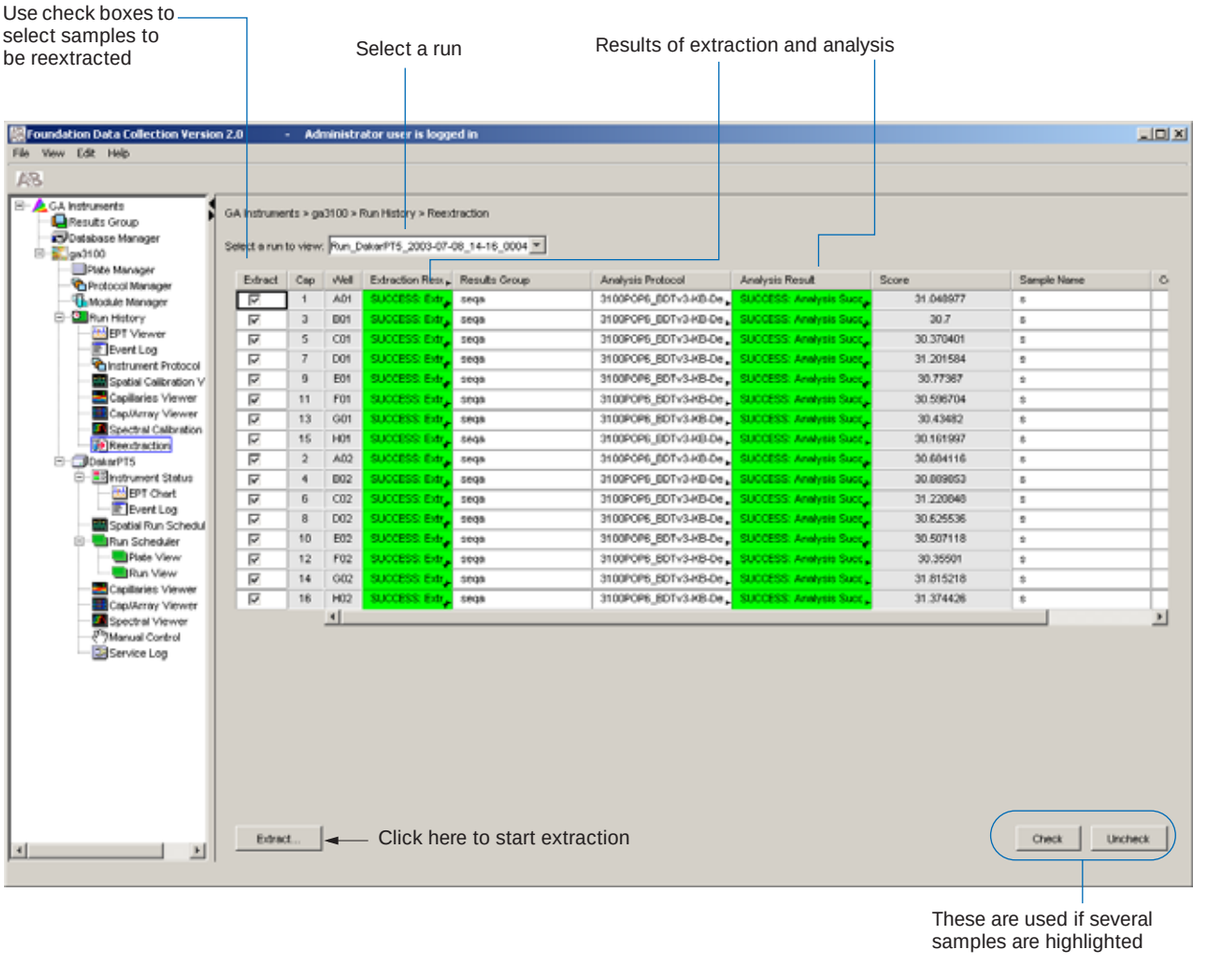
Notes

Elements of the Reextraction Window

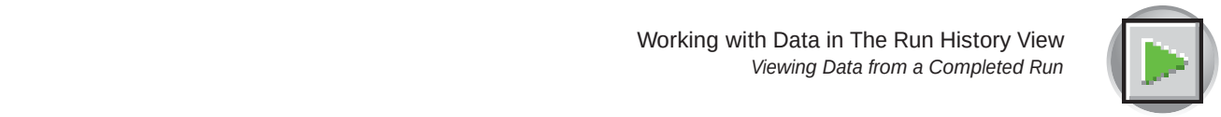
All the samples are displayed with the results of extraction and analysis.

Note: Sort the columns of the re-extraction panel by holding the shift key and then clicking on a column header.

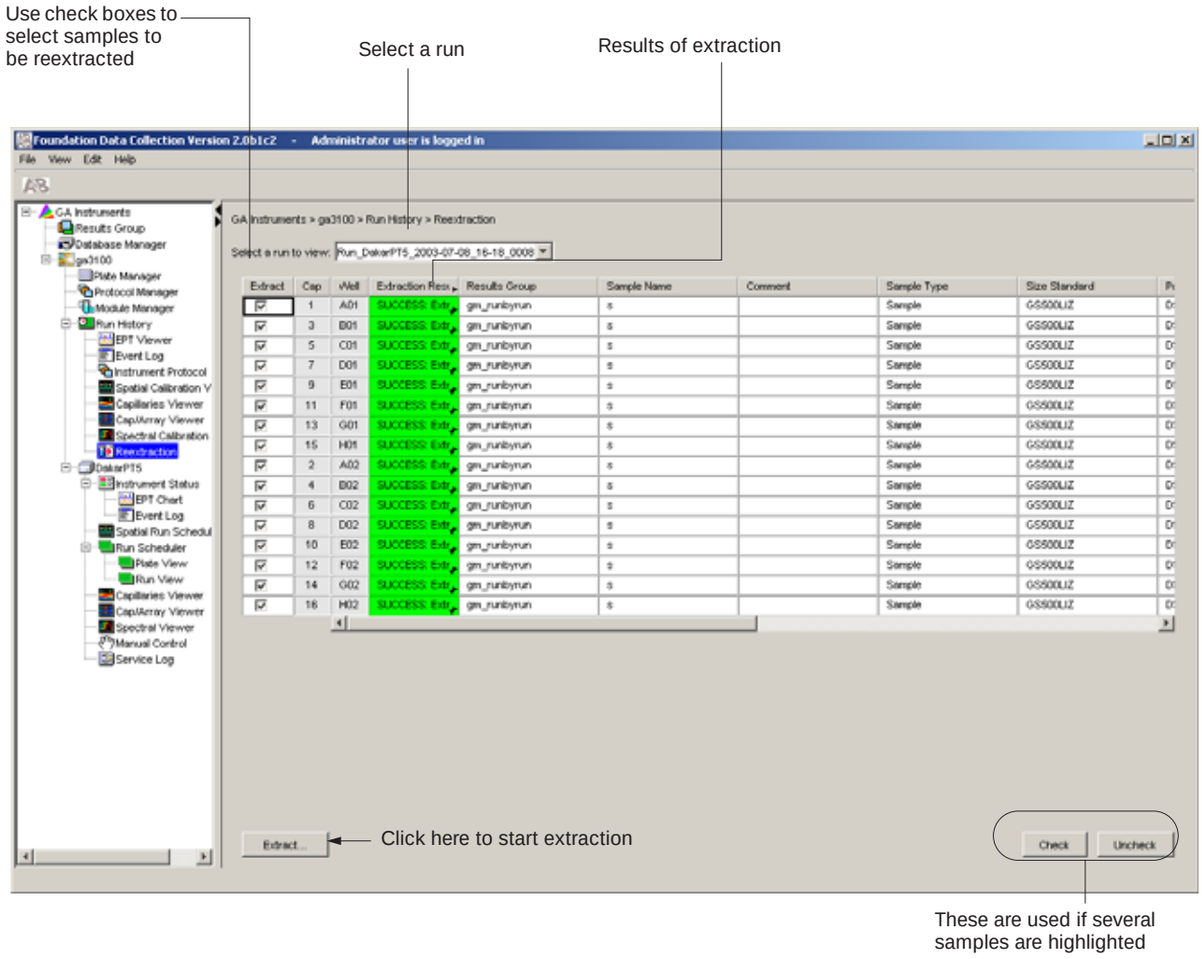
Reextraction Window for Sequencing Analysis



Notes



Reextraction Window for Fragment Analysis



Notes

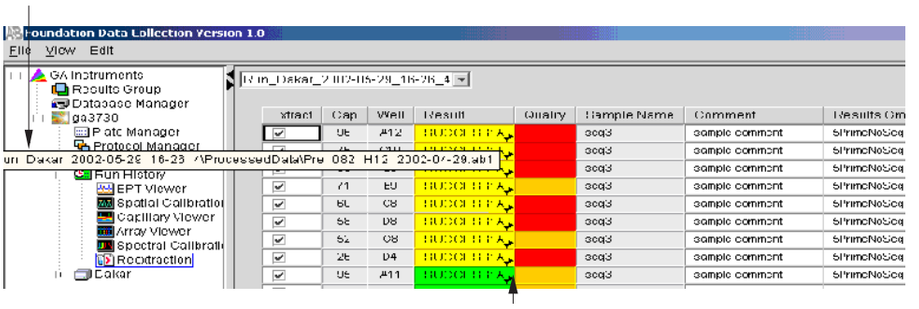
Results Column

The results of extraction and analysis are color coded in the Results column. The following table lists the colors and their values for Sequencing Analysis.

Color	Value	Notes
Red	Extraction or analysis failed	Descriptive messages can be viewed by resizing the Results column to view all text (click on the arrow)
Yellow *	Warnings for extraction or analysis	
Green	Successful extraction (with no analysis intended), or successful extraction and analysis.	
* Note: The text message for samples that produce yellow is: “FAILURE: Analysis Failed Bad Data; Error Number=nnnnn WARNING...		

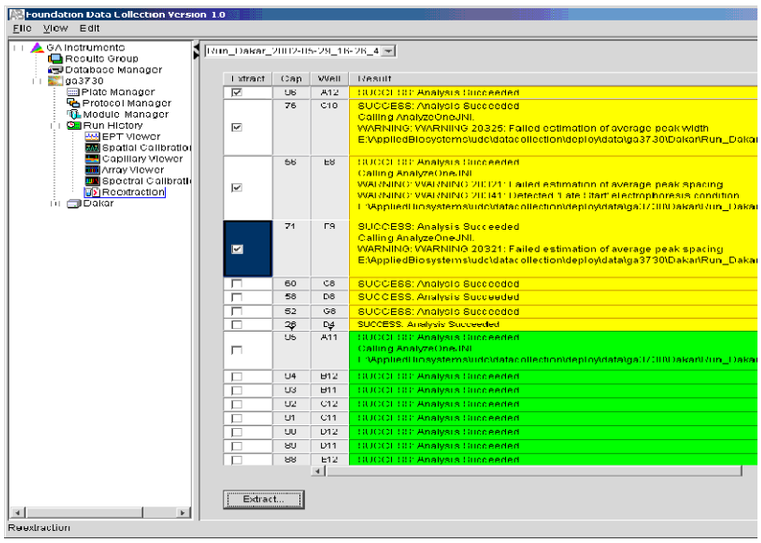
The Results column, by default, shows only the beginning of any processing message. The entire message returned from extraction and autoanalysis is inside the cell and can be viewed by expanding the cell. The location of the stored sample is also found there. In addition, there is a tooltip view for each sample results message.

Tooltip view. Access by placing the cursor over the sample of interest



Drag the cell’s edge to expand the column

Notes



Expanded column

Quality Column

The Quality column represents the quality values for an entire sequence. Quality Values are only assigned to analyzed samples when using the KB Basecaller. The following table lists the displayed colors and their associated value range.

Color	Quality Value Range
Red	< 15
Orange	≥ 15 and < 20
Yellow	≥ 20 and < 30
Green	> 30

Note: For more information on KB Basecaller and Quality Values, see the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide*, PN 4346366.

The column is empty (white) if:

- Analysis was not performed
- Analysis failed
- ABI Basecaller was used for analysis. This basecaller does not assign Quality Values.

Results Group and Analysis Protocol Columns

The Results Group and the Analysis Protocol (Analysis Method in the GeneMapper™ software) can be edited and the changes used for reextraction.

Note: Select an entire column in the Reextraction window by clicking on the column header. For example, clicking on the Extract column header selects all samples. Clicking the Uncheck or Check buttons at the bottom of the window, enables or disables the check boxes for each sample. Additionally, the fill-down command (**Ctrl**+**D**) works the same here as in the Plate Editor for easier information input.

Notes

Sorting The Samples

The samples can be sorted according to any of the column properties by holding down the shift key while clicking on the column header. Shift-clicking again sorts them in the reverse order. This is most useful for sorting by capillary number, by well position, by results, by quality, and by the Extract column. For example, it is often useful to bring all of the samples that failed analysis or extraction to the top of the column where they can be examined without having to scroll down to each sample individually.

Reextracting Selected Samples

To reextract selected samples:

1. Expand the Results column cells for any yellow or red results, to see a description of the warning or failure.
2. If desired, select a new Results Group, or edit the current one. This allows you to turn off autoanalysis, change the samples and folder naming options, the location where they are placed, the owner of the Results Group, etc.
3. If desired, change the Analysis Protocol to experiment with different ways of analyzing the sample, using a different basecaller for example.
4. Check the check box in the Extract column for the samples you wish to extract again.
5. Click **Extract**.

   IMPORTANT! Reextraction creates an entirely new sample file and does not replace the previously saved sample file. The presence of a previous sample file has no effect on the creation of a new sample file. If the same naming options that are used for reextraction are identical to those used previously, a number is appended to the filename. For example, if the first sample is, “sample 01.ab1” then the second sample would be, “sample 01 (1).ab1.”

Notes

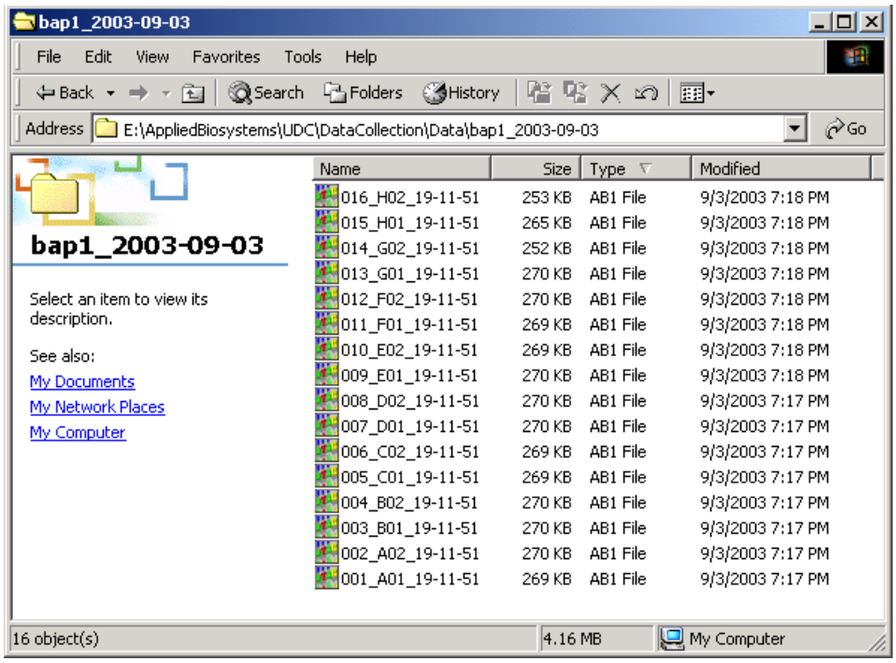
Viewing Analyzed Data

Locating Sample Files

When a run is finished, the analyzed sample files are extracted into a run folder, to a location defined in the Destination tab and the name of your run folder defined in the Name tab of your Results Group.

The default location is:

E:\AppliedBiosystems\UDC\Data Collection\Data\ *destination location+run folder name*



Locating Sample Files

If the data has been re-extracted, the data is in the location defined by the applied Results Group or the default destination location:

Viewing Sample Files

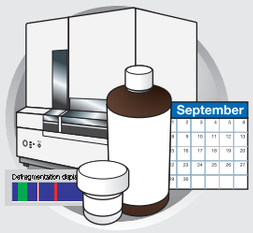
After a run has been extracted to sample files, you can use the Sequencing Analysis Software v5.1, SeqScape, or the GeneMapper Software to view the electropherogram data, both raw and analyzed. All sequencing sample files contain the .ab1 extension, and all fragment analysis sample files contain the .fsa extension.

IMPORTANT! If the run is not set up for autoanalysis, refer to the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide*, *SeqScape® Software v2.1 User Guide*, or *GeneMapper™ Software v3.5 User Guide* for information on manual analysis.

Notes

Notes

Performing Instrument Maintenance



This chapter covers:

Notes

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Notes

Instrument Maintenance

Maintenance Task Lists

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

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

Daily Tasks

Perform these tasks at least once per day.

Maintenance Task	Frequency	See Page
Ensure that the reservoir septa are firmly seated and flat.	Before each run	—
Ensure that the plate assembly were put together properly. The holes in the plate retainer must align with the holes in the septa or the capillary tips will be damaged.	Before each run	152
Ensure that the plate assembly is positioned on the plate deck properly. Plate should sit snugly on the deck. Never use warped plates.	Before each run	—
Replenish the water and 1X running buffer reservoirs on the instrument.	Daily or before each run	15
Check for bubbles in the polymer block and polymer block channels and remove.	Daily or before each run	228
Check the loading-end header to ensure the capillary tips are not crushed or damaged.	Daily or before each run	—
Check the level of polymer in the polymer-reserve syringe to ensure there is enough for all your runs.	Daily or before each run	—
Check the polymer block to ensure it fits securely on the instrument.	Daily	—
Clean the instrument surfaces.	Daily	—
Check for dried polymer around the polymer block and clean as necessary.	Daily	—
Check for leaks around the syringes and screw nut.	Daily	—

Notes

Weekly Tasks

Perform these tasks at least once per week.

Maintenance Task	Frequency	See Page
Clean the syringes.	Weekly or as needed	216
Clean the water and buffer reservoirs with warm water.	Weekly	—
Clean the upper and lower polymer blocks.	Weekly	224
Replace the polymer in the syringes, upper polymer block, and capillary array.	Weekly or as needed	207
Check the storage conditions of the used arrays.	Weekly	—
Check data base space. Delete plate records from the instrument database and archive sample files.	Weekly	234

As-Needed Tasks

Perform these tasks as needed.

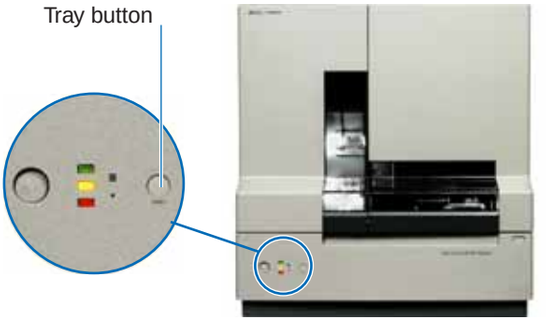
Maintenance Task	Frequency	See Page
Clean the drip trays.	As needed	—
Change the array.	As needed	209
Replace syringes	3 months	—
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed	—
Calibrate the autosampler	Very rarely	231

Notes

Routine Cleaning

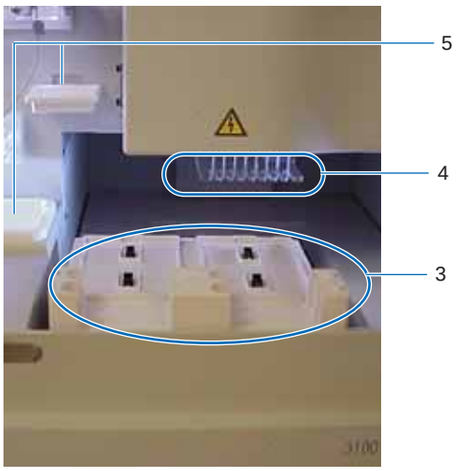
General Cleaning

1. Ensure the oven and instrument doors are closed.
2. Press the Tray button on the front of the instrument to move the autosampler to the forward position.



IMPORTANT! Never use organic solvents to clean the instrument.

3. Wipe off any liquid on or around the autosampler using a lint-free tissue.
4. Clean off any polymer build-up (crystals) on the instrument including the capillary tips and the stripper plate with deionized water and lint-free tissue.
5. Clean out the drip trays with deionized water and lint-free tissue.



Moving and Leveling the Instrument

Before Moving the Instrument

CAUTION **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.

1. Remove the following components from the instrument:
   - Any plate assemblies from the autosampler.
   - Water and buffer reservoirs from the autosampler.
   - Capillary array. For instruction see page 211.
   - Syringes from the upper polymer block. For instruction see page 222.
   - Upper polymer block. For instruction see page 223.

Notes

- Anode buffer reservoir.
- Lower polymer block. For instruction see page 223.

2. Switch off the breaker on the back of the instrument.

3. Disconnect the power cord and the Ethernet cable.

IMPORTANT! While moving the instrument, avoid any shock or vibration.

Leveling the Instrument

1. Place the bubble level on the autosampler deck.

2. Turn the instrument legs to level the instrument.

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

Notes

Resetting the Instrument

Reset the instrument when:

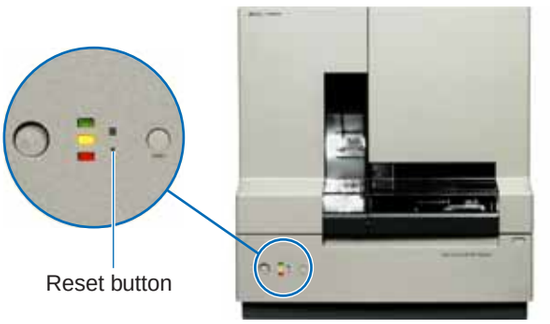
- There is a fatal error as indicated by the red status light
- The instrument does not respond to the ABI PRISM® 3100/3100-*Avant* Data Collection software

There are two ways to reset the 3100/3100-*Avant* Genetic Analyzer:

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

Resetting With the Reset Button

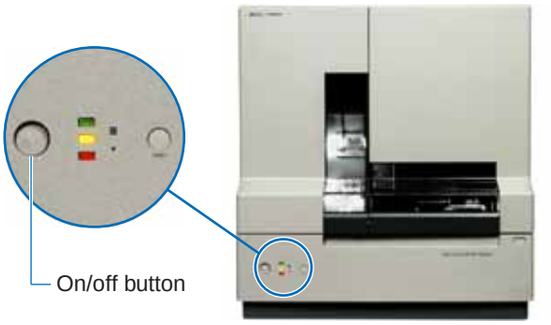
1. Close the instrument doors.
2. Using a long narrow implement, such as a straightened paper clip, press the Reset button on the front of the instrument.



Resetting by Powering Down

1. Close the instrument doors.
2. Power off the instrument by pressing the on/off button on the front of the instrument.
3. Restart the computer.
   a. Select **Start** > **Shutdown**.
   b. In the Shutdown Windows dialog box, select **Restart**, then click **OK**.

IMPORTANT! Wait until the computer has completely restarted before proceeding.



Notes

4. Turn on the instrument, then wait for the solid green light.

   Note: When the instrument is shut down, the firmware is not saved. Upon restart, the instrument reloads a copy of the firmware and the calibration file from the computer.

5. Launch the data collection software (all applications in the Service Console start automatically).

Shutting Down the Instrument

Short- and Long-Term Shutdowns

Perform the appropriate shutdown procedure based on the information in the following table:

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

Performing a Short-Term Shutdown

1. Ensure the oven and instrument doors are closed.
2. Fill the capillaries with fresh polymer. For instructions, see page 214.
3. Push the Tray button to move the autosampler forward.
4. Open the doors, then remove the plates and reservoirs
5. Remove the cathode buffer reservoir and water reservoirs from the instrument.

Notes

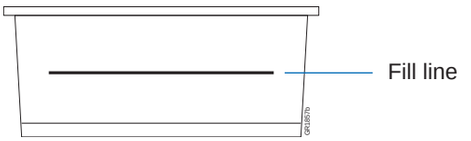
6. Dispose of remaining fluids and rinse out the reservoirs with deionized water.

   Note: The waste is very dilute; however, you should follow your company's waste disposal practices for appropriate disposal procedures.

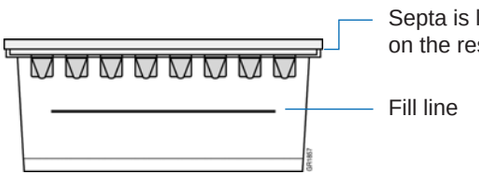
7. Rinse the cathode reservoir with 1X running buffer, and then fill to the line with 1X running buffer (about 16 mL).

8. Fill the three water reservoirs to the line with quality deionized water (about 16 mL).

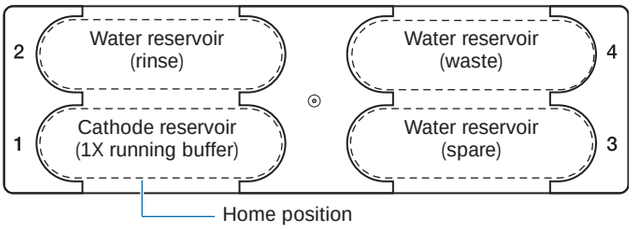
9. Place a clean reservoir septa on each reservoir, and dry the outside of the reservoirs using a lint-free wipe.



CAUTION Be sure that the septa fit snugly and flush on the tops of the reservoirs in order to prevent damaging the capillary tips.



10. Place the reservoirs into position on the autosampler as shown below.



11. Close the instrument doors.

    Note: Closing the doors returns the autosampler to the home position, placing the tips of the capillaries in buffer.

12. Shut down the computer and turn off the instrument.

Notes

Performing a Long-Term Shutdown

1. Follow the procedure on page 215 to remove and store the capillary array off the instrument.

2. Remove from the instrument:
   - Syringes from the upper polymer block. For instructions see page 222.
   - Upper polymer block. For instructions see page 223.
   - Lower polymer block. For instructions see page 223.

3. Remove the plate assembly and reservoirs from the autosampler:

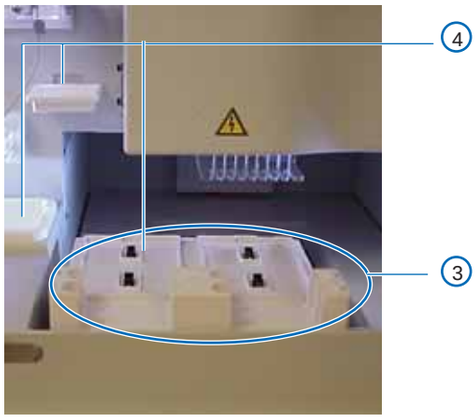
4. Wipe the autosampler and drip trays with lint-free tissue dampened with water.

5. Close the instrument doors.

6. Shut down the computer and power off the instrument.

7. Wash the syringes, polymer blocks, and reservoirs with warm water. Rinse with deionized water.

IMPORTANT! Make sure all parts are completely dry before long-term storage.



Notes

Fluids and Waste

Buffer

When to Change the Buffer

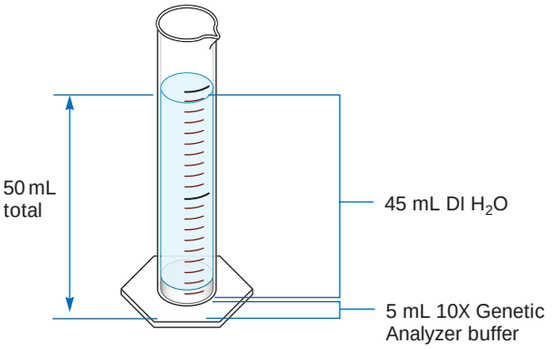
We recommend that you **change the buffer before each batch of runs or at least every 24 hours**.

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

Making Buffer for a Single Run

To prepare 50 mL of 1X running buffer:

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

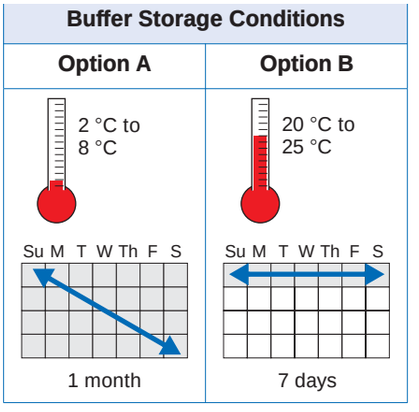


50 mL graduated cylinder

Storing the Buffer

The 1X running buffer can be stored at:

- 2 to 8 °C for up to 1 month
- Room temperature for 1 week



Notes

Polymer

Storing Polymer

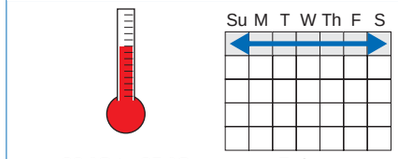
Store any remaining POP™ polymer at 2 to 8 °C until the expiration date printed on the jar.

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

When to Change the Polymer

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

POP-4 or POP-6 Storage Conditions



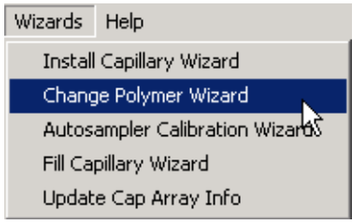
Adding and Changing the Polymer

IMPORTANT! Wear gloves when you handle the polymer.

WARNING **CHEMICAL HAZARD. POP Polymers** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

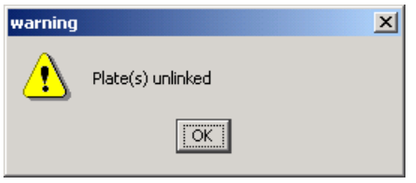
To put fresh polymer on the instrument:

1. Click **Wizards** > **Change Polymer Wizard**.

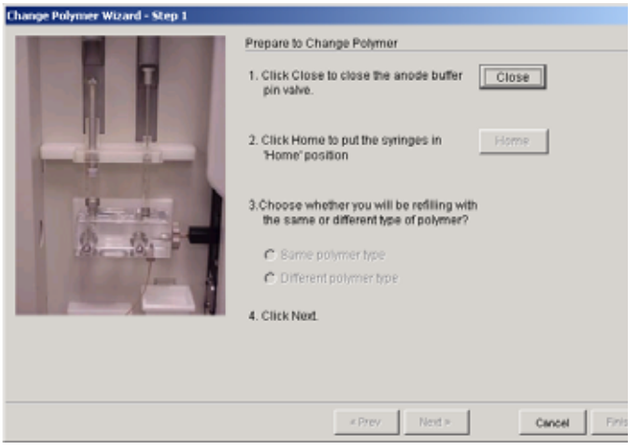


Notes

2. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.



3. Follow the directions given in the wizard to put fresh polymer on the instrument.



4. Relink plate(s), if applicable.

Notes

Capillary Array

Before Installing a Previously Used Capillary Array

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

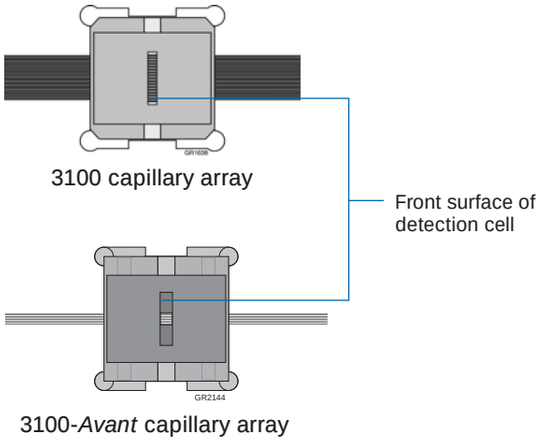
- Clean the front of the detection cell
- Check that the cathode bar is dry

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

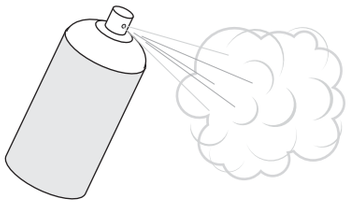
Cleaning the Detection Cell

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

1. Put one drop of methanol on the front surface of the detection cell.



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

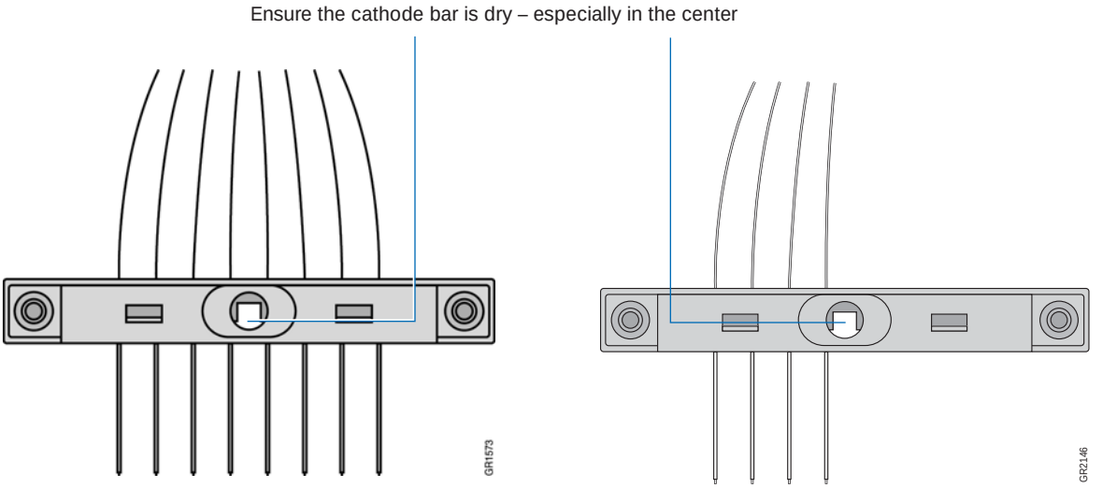


Notes

Checking the Cathode Bar

When putting a used array back on the instrument, be sure that the cathode bar is dry (see page 210). A wet bar could lead to arcing.

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



3100 capillary array

3100-*Avant* capillary array

Installing and Removing the Capillary Array

When to Change a Capillary Array

A capillary array should last approximately 100 runs.

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

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

Notes

Installing, Removing, or Replacing a Capillary Array

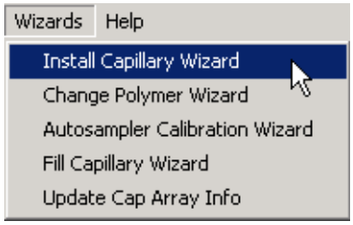
Follow the procedures in the Install Capillary Wizard to install, remove, or replace an array.

IMPORTANT! Wear gloves when you handle the polymer blocks.

IMPORTANT! The capillary array length defined in the wizard must match the array length you are using.

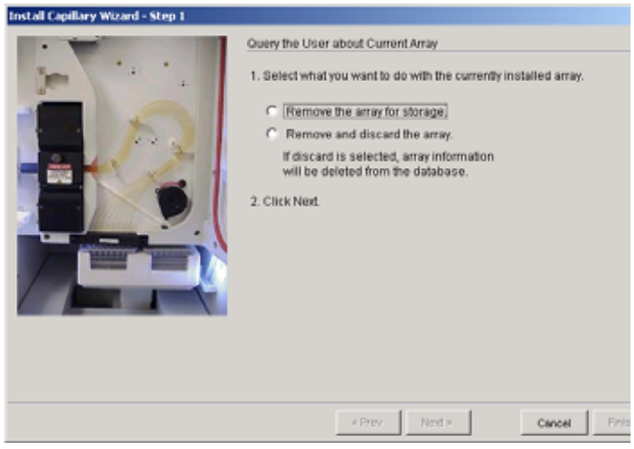
WARNING **CHEMICAL HAZARD. POP polymer** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Close the oven and instrument doors, then press the Tray button.
2. Select **Wizards** > **Install Capillary Wizard**.



3. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.

4. Open instrument and oven doors.
5. Follow the directions given in the wizard to install or replace an array.



6. Click Finish when done.
7. Close and lock the oven door, then close the instrument doors.

   IMPORTANT! If you installed or replaced an array that is a different length than the one you were using, you must reset the active spectral calibration (see Chapter 3, page 59) or create a new spectral calibration for the dye set and array length combination (see Chapter 3, page 37).

8. Relink plate(s), if applicable.

Notes

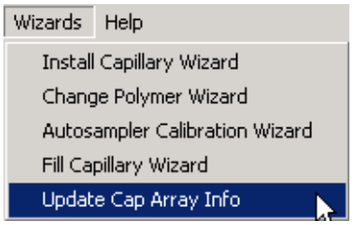
Manually Installing a Capillary Array

You can manually install a capillary array, then use the Update Cap Array Info wizard to enter the capillary array length and serial number into the database.

IMPORTANT! The capillary array length defined in the wizard must match the array length you are using.

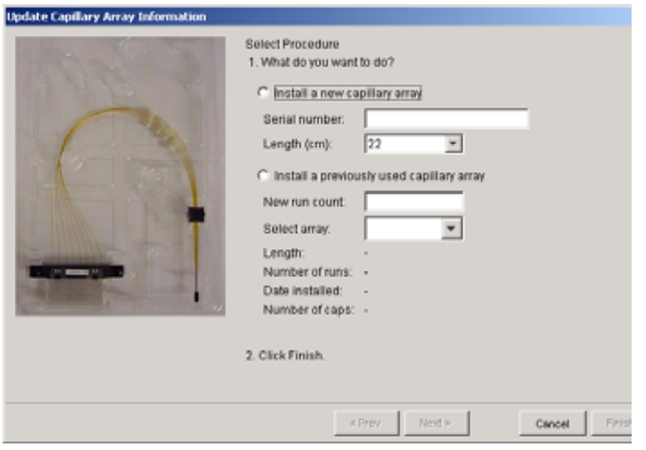
WARNING **CHEMICAL HAZARD. POP polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

1. Close the oven and instrument doors, then press the Tray button.
2. Open the instrument door and oven doors.
3. Install the capillary array.
4. Close and lock the oven door, then close the instrument doors.
5. Select **Wizards** > **Update Cap Array Info**.



6. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.

7. Complete the dialog box using your capillary array information, then click Finish.



IMPORTANT! If you installed or replaced an array that is a different length than the one you were using, you must reset the active spectral calibration (see Chapter 3, page 59) or create a new spectral calibration for the dye set and array length combination (see Chapter 3, page 33).

8. Relink plate(s), if applicable.

Notes

Capillary Array Maintenance

Caring for the Capillary Array

Follow these guidelines to properly care for the capillary array:

- Wear gloves and handle the capillary array gently.
- Do not touch the detection cell. If it is dirty, see "Cleaning the Detection Cell" on page 209.
- Keep the ends of the capillary array wet at all times.
- Always loosen the capillary array nut before pulling out the upper polymer block.
- Do not overtighten the capillary array nut.

Cleaning the Capillary Array

1. Flush the capillary array with fresh polymer as instructed in the "Installing and Removing the Capillary Array" on page 210.

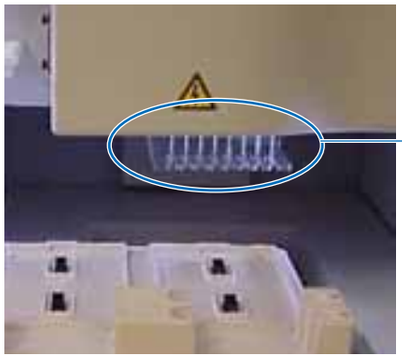
2. Clean off any polymer buildup (crystals) on the instrument, including the capillary electrodes and the stripper plate, with deionized water and lint-free tissue.

   Note: When cleaning the capillary electrodes, be careful not to bend them out of position. If the electrodes do get bent, follow the procedure "Verifying Capillary Alignment Using the Capillary Ruler" below.

   IMPORTANT! Never use organic solvents to clean the instrument.

3. Clean the detection cell as instructed on page 209.

WARNING CHEMICAL HAZARD. POP polymer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



Notes

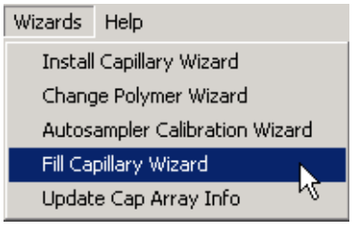
Filling the Capillary Array with Polymer Using the Fill Capillary Wizard

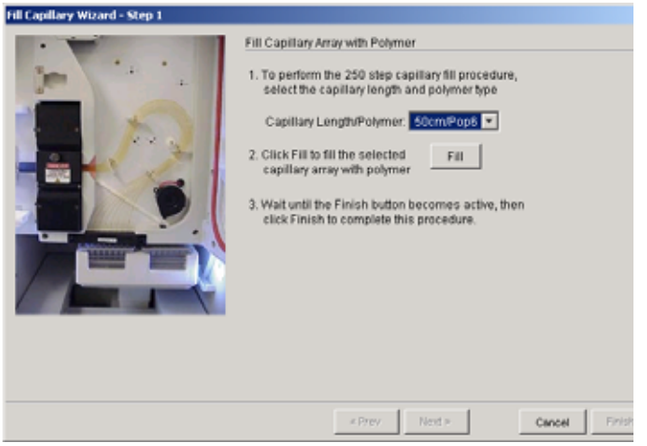
1. Select **Wizard** > **Fill Capillary Wizard**.

   The wizard opens.

2. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.

3. Follow the directions in the wizard, then click Finish.





Verifying Capillary Alignment Using the Capillary Ruler

1. Place the ruler beside the capillaries and detach a side of the ruler to the bottom of the holder.

2. Verify that all the capillaries match the lines of the ruler.

3. Place the capillary array holder on the flat surface and stand the ruler up at the end of capillaries.

4. Verify that the cross points of line on the ruler to match the end of capillaries. If some of capillaries are bent, adjust each capillary carefully.

Notes

Storing a Capillary Array on the Instrument

When to Use

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

Storing the Capillary Array on the Instrument

To store the capillary array on the instrument, follow the instructions to perform a short-term shutdown on page 203.

Storing a Capillary Array off the Instrument

When to Use

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

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

Storing the Capillary Array off the Instrument

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

WARNING **CHEMICAL HAZARD**. **POP polymer** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Fill the capillary array with fresh polymer using the Fill Capillary Array wizard or manual control commands.
2. Remove the syringe guard.
3. Remove both syringes from the upper polymer block and properly dispose of any remaining polymer.
4. Wash the syringes.
5. Remove the capillary array from the instrument using the Install/Replace Capillary Array wizard. For instructions see, "Installing and Removing the Capillary Array" on page 210.
6. Replace the cover over the detection cell.
7. Fill a buffer reservoir with fresh 1X running buffer and cover with a septa strip. Insert the capillary tips into the buffer.

Notes

8. Fill the shipping vial with fresh 1X running buffer and insert the detection end of the capillary array.
9. Store the capillary array upright.
10. Check the 1X running buffer level in the reservoir and tube weekly.

Syringes

Required Materials

- Polymer-reserve syringe, 5-mL
- Array-fill syringe, 250-µL
- Syringe, 20-mL, silicone-free
- Squeeze bottle, 1-L containing deionized water
- POP-4 or POP-6 polymer
- Lab wipes, lint-free
- Gloves

When to Clean the Syringe

Clean the syringe:

- When a syringe is removed from the instrument, or at least once per week
- When replacing polymer, including when switching to a new type or lot of polymer

Guidelines for Syringe Use

- Do not move the plunger when the barrel is dry.
- Do not combine the barrels and plungers from different syringes.
- Do not draw or expel a full volume of fluid from the syringe faster than 5 sec.

Syringe Maintenance

Syringe Types

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

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

Replacing the Syringes

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

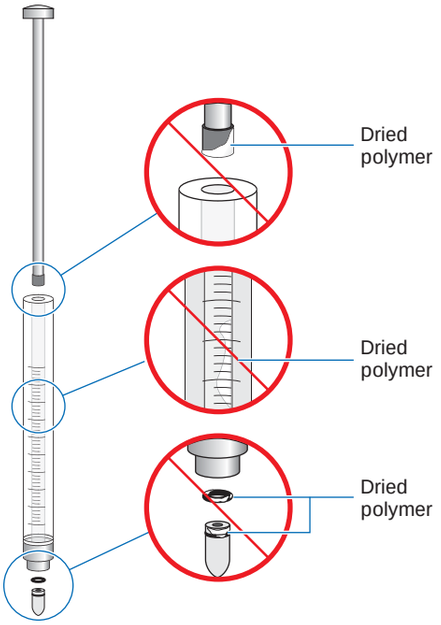
Notes

Cleaning Syringes

IMPORTANT! Be sure there is no dried polymer left in the syringes.

1. Remove the syringe guard.
2. Remove the syringes as described on page 222.
3. Clean the syringe thoroughly by rinsing the inside and outside of the syringe barrel and the syringe tip with warm water.
4. Inspect the components of the syringe for dried polymer (white residue).

   If the syringe contains dried polymer, repeat step 3.



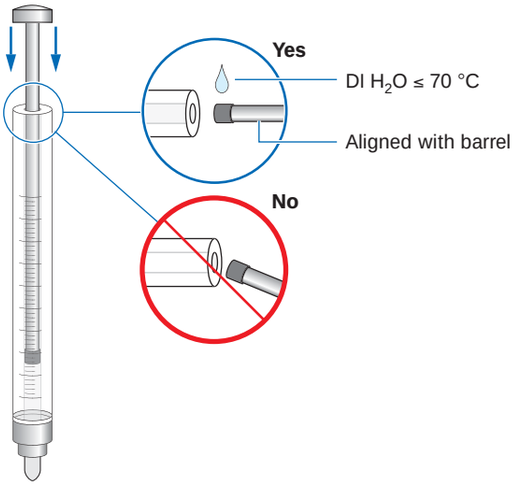
5. Rinse the syringe barrel and tip with deionized water.

Notes

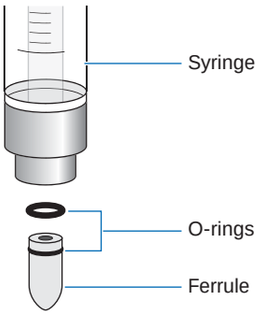
6. Assemble the syringe.

IMPORTANT! Add a drop of deionized water to the tip of the plunger before inserting the plunger.

IMPORTANT! Align the tip of the plunger with the barrel of the syringe before inserting the plunger.



7. Confirm that two O-rings (one behind the ferrule and one around the ferrule) are attached correctly.
8. Confirm that the ferrule is firmly attached to the end of the syringe.
9. Use a lint-free wipe to dry the syringe.

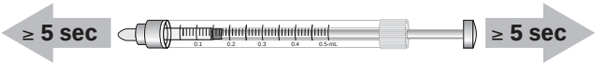


Priming and Filling Syringes

IMPORTANT! Wear gloves when you handle the glass syringe.

WARNING CHEMICAL HAZARD. POP polymer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Do not draw or expel a full volume of fluid from the syringe faster than 5 sec.

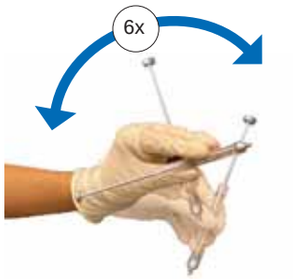


Notes

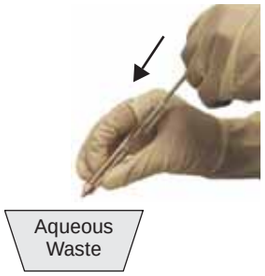
Priming and Filling the Polymer-Reserve Syringe

1. Draw approximately 0.5 mL of room-temperature polymer into a clean polymer-reserve syringe

2. Draw the plunger to the 5 mL mark to draw a volume of air into the syringe.
3. Invert the syringe six times to coat the walls with polymer.



4. Slowly expel the polymer into an aqueous waste container.



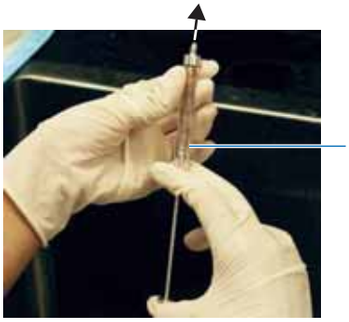
Note: The syringe is now primed for use on the instrument. Priming ensures that residual water from the wash does not dilute the concentration of the polymer.

5. Draw 5 mL of room-temperature polymer into the syringe.

IMPORTANT! Submerge the syringe tip in the polymer while filling to avoid creating air bubbles in the syringe.

Notes

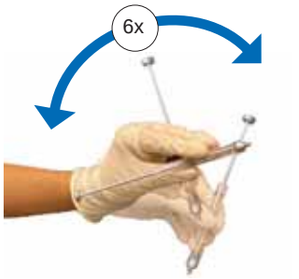
6. Invert the syringe and slowly expel a small amount of polymer out of the tip to remove any air bubbles.



Note: Do not return the unused portion of the polymer to the polymer jar.

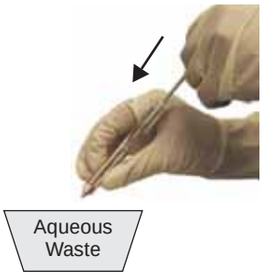
Priming and Filling the Array-Fill Syringe

1. Draw approximately 100 µL of room-temperature polymer into a clean array-fill syringe.
2. Draw the plunger to the 250 µL mark to draw a volume of air into the syringe.
3. Invert the syringe six times to coat the walls with polymer.



4. Slowly expel the polymer into an aqueous waste container.

Note: The syringe is now primed for use on the instrument. Priming ensures that residual water from the wash does not dilute the concentration of the polymer.



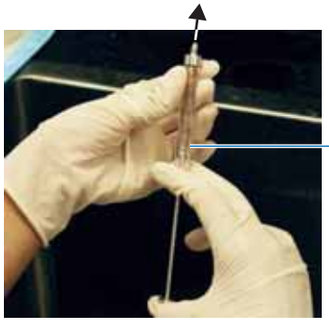
5. Draw 250 µL of room-temperature polymer into the syringe.

IMPORTANT! Submerge the syringe tip in the polymer while filling to avoid creating air bubbles in the syringe.

Notes

6. Invert the syringe and slowly expel a small amount of polymer out of the tip to remove any air bubbles.

   Note: Do not return the unused portion of the polymer to the polymer jar.



Installing and Removing Syringes

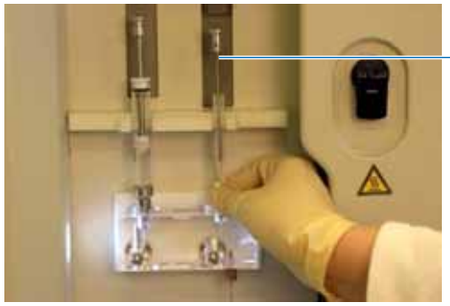
Installing Syringes

1. Follow the procedures to remove, clean, dry, and replace the upper polymer block starting on page 223.
2. Place the polymer-reserve syringe tip in the left port on the top of the upper polymer block and screw the syringe tip clockwise into the polymer block.

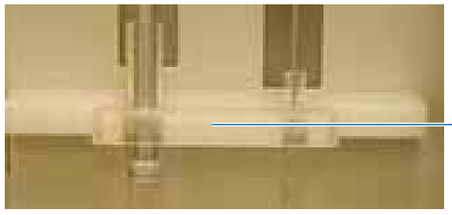
   IMPORTANT! Always hold the syringe by the metal sleeve – not the glass – when screwing the syringe into the block.

3. The syringe should be finger tight in the block.
4. Place the array-fill syringe tip in the right port on the top of the upper polymer block and screw the syringe tip clockwise into the polymer block.

   IMPORTANT! Always hold the syringe by the metal sleeve—not the glass—when screwing the syringe into the block.



5. The syringe should be finger tight in the block.
6. Replace the syringe guard.



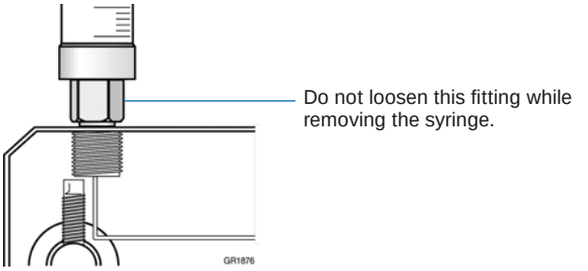
Notes

Removing Syringes

1. Remove the syringe guard.
2. Grasp the polymer-reserve syringe just above the fitting or at the base (not the glass barrel) and rotate the syringe counterclockwise.

   IMPORTANT! Be careful not to remove the fitting. There are several rings and check valves that could come out if this fitting is removed.

3. Grasp the array-fill syringe and rotate the syringe counterclockwise.
4. Dispose of any remaining polymer properly.



Notes

Polymer Blocks

Removing the Polymer Blocks

Removing the Upper Polymer Block

1. Verify the oven and instrument doors are closed, then press the Tray button.
2. Remove the syringe guard.
3. Remove the syringes as described on page 221.
4. Disconnect the capillary array from the polymer block:
   a. Open the oven, and detection block doors.
   b. Loosen the capillary array nut.
   c. Pull out the upper polymer block part way.
   d. Remove the detection cell from the detection block.
   e. Remove the capillary array sleeve from the polymer block.
   f. If the capillary array is to be reused, store it as described on page 215.
5. Disconnect the polymer block tube from the lower polymer.
6. Grasp the upper polymer block with two hands and pull it straight out.

   Note: The upper polymer block rides on two steel shafts and slides out easily after a spring moves past a check point.

Removing the Lower Polymer Block

1. Remove the anode reservoir and dispose of the buffer properly.
2. Grasp the lower polymer block and pull it straight out.

Notes

Cleaning the Polymer Blocks

When to Clean

Clean the upper and lower polymer blocks:

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

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

Cleaning the Upper Polymer Block

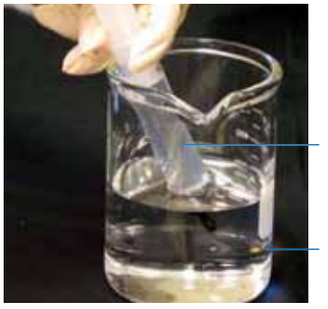
IMPORTANT! Do not expose the polymer blocks to any organic solvents.

1. Rinse all the fittings with hot water. Soak any fittings that are covered with polymer.

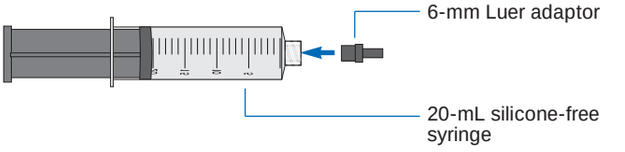
   IMPORTANT! Do not use boiling water to rinse the fittings or the polymer block.

2. Rinse the upper polymer block under hot water.

3. Fill the 20-mL silicone-free syringe with warm deionized water (≤70 °C).

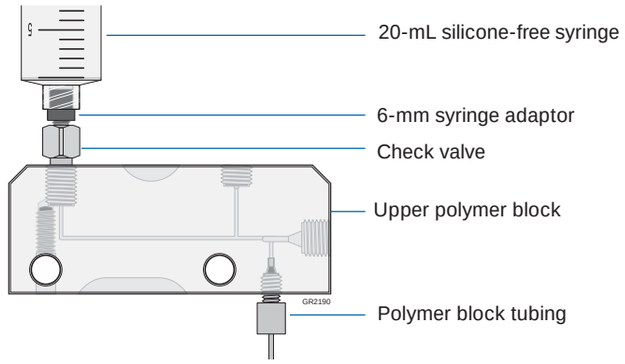


4. Fit the 6-mm syringe adaptor (P/N 4322928) onto the 20-mL silicone-free syringe (P/N 4324463).



Notes

5. Thread the 6-mm syringe adaptor into the stainless-steel check valve.

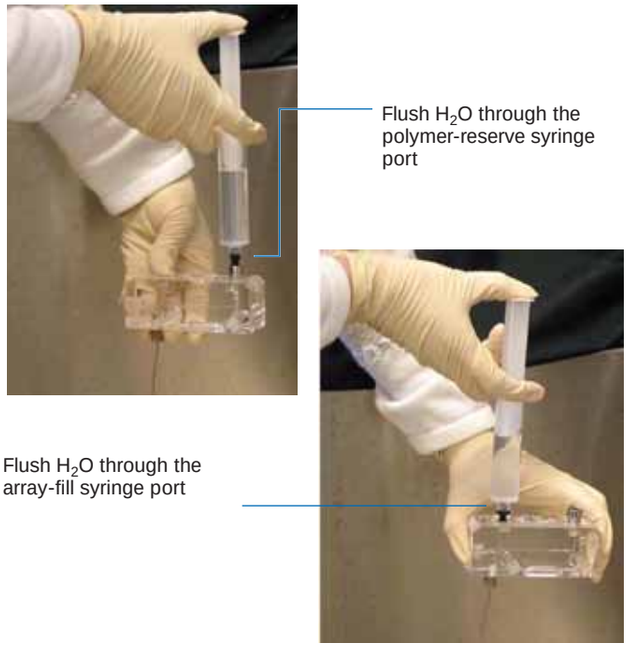


6. Force several syringe loads of hot water through each channel in turn by sealing their openings with your fingers.

   Note: Force deionized water through polymer block tubing also.

7. Remove the syringe from the polymer-reserve syringe port and attach it to the array-fill syringe port.

8. Force several syringe loads of hot water through each channel in turn by sealing their openings with your fingers.

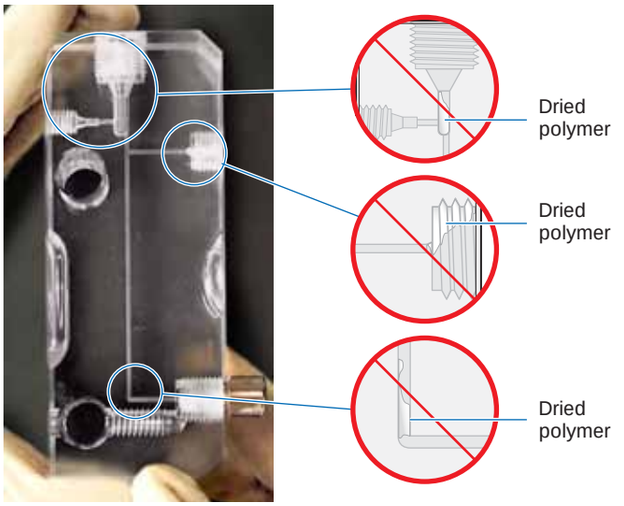


Notes

9. Inspect the channels visually for dried polymer, which is white residue. Wash partially occluded channels with hot water until the dried polymer is gone.

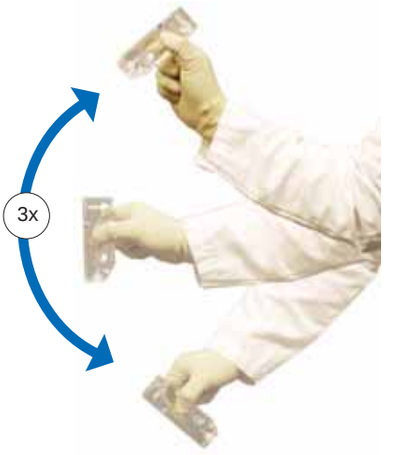
   IMPORTANT! It may take a long time for the hot water to clear the obstruction. Do not use a sharp pointed instrument to clear the channel, even if the channel is completely occluded with dried polymer.

10. Rinse the upper polymer block and all the fittings thoroughly using deionized water.



11. Remove any residual water from the upper polymer block by forcing air through the channels using the silicone-free syringe or shaking the polymer block.

    IMPORTANT! Do not use the 5.0-mL glass syringe to force air through the channels. This will damage the syringe's plunger and cause the syringe to leak.



Cleaning the Lower Polymer Blocks

To clean the lower polymer block:

1. Verify that the buffer valve is open (in the up position).

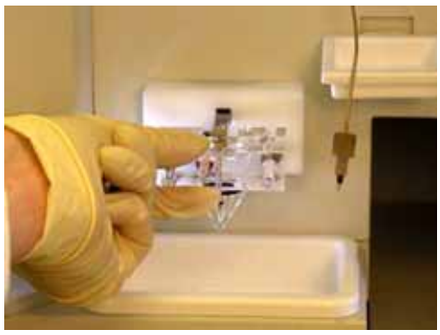
2. Remove the polymer block tubing and fitting from the upper polymer block, if this was not done before.

Notes

3. Remove the lower polymer block from the instrument.

4. Rinse all the fittings with hot water. Soak any fittings that are covered with polymer.

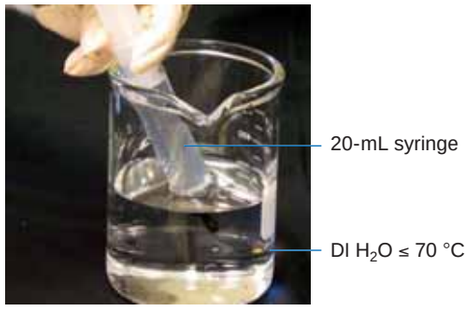
   IMPORTANT! Do not use boiling water to rinse the fittings or the polymer block.



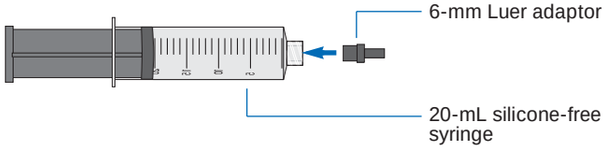
5. Hold the lower polymer block under hot water. Using your fingers, move the buffer valve in and out to ensure any encrusted polymer is cleaned out of its guide channel.

   IMPORTANT! Do not remove any of the components from the lower polymer block.

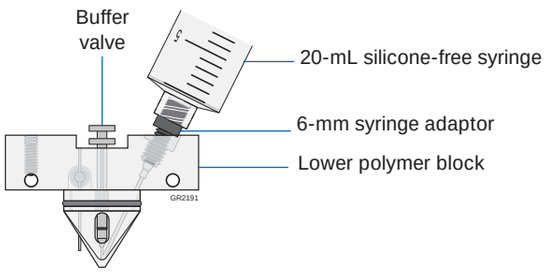
6. Fill the 20-mL silicone-free syringe with deionized water (≤70 °C).



7. Fit the 6-mm syringe adaptor (P/N 4322928) onto the 20-mL silicone-free syringe (P/N 4324463).



8. Thread the 6-mm syringe adaptor into the polymer block where the polymer block tube fitting was originally located.

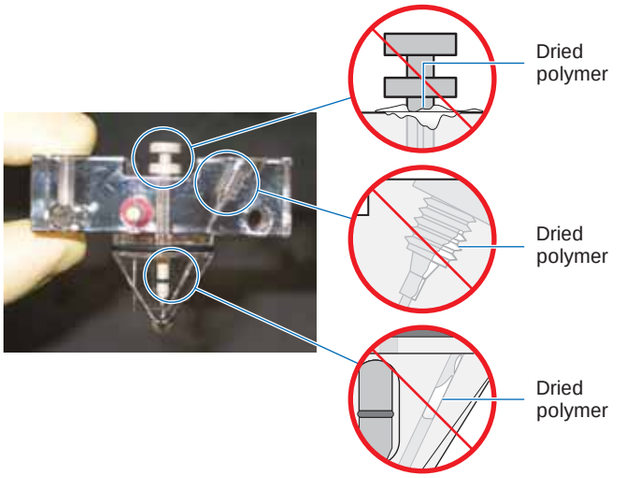


Notes

9. Force several syringe loads of hot water through the channel.

10. Inspect the channels visually for dried polymer, which is white residue. Wash partially occluded channels with hot deionized water until the dried polymer is gone.

IMPORTANT! It may take a long time for the hot water to clear the obstruction. Do not use a sharp pointed instrument to clear the channel, even if the channel is completely occluded with dried polymer.



11. Rinse the lower polymer block and all the fittings thoroughly using deionized water.

12. Remove any residual water from the lower polymer by forcing air through the channels until the channels are dry using the silicone-free syringe or shaking the polymer block.

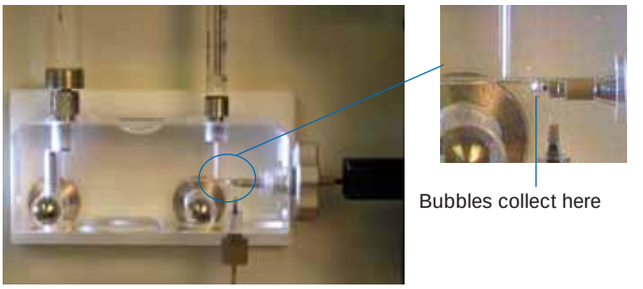
IMPORTANT! Do not use the 5.0-mL glass syringe to force air through the channels. This will damage the syringe's plunger and cause the syringe to leak.

Removing Air Bubbles from the Polymer Blocks

Clearing Air Bubbles

In the upper polymer block, bubbles tend to collect where the channels join after changing polymer, installing syringes and/or installing a capillary array.

The bubbles must be removed from the upper polymer block channel, polymer tubing and lower polymer block channel.

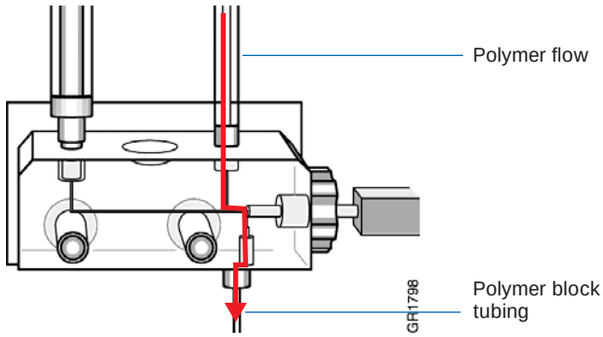


Notes

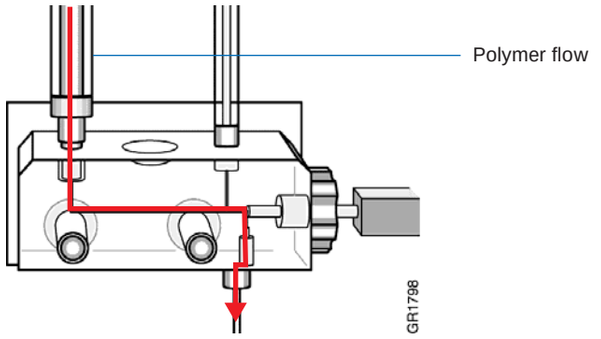
To clear air bubbles from the upper polymer block:

1. Push down slowly on the array-fill syringe to move bubbles down the channel and into the polymer block tubing.

   Note: Push slowly (or tap) to minimize the amount of polymer used.

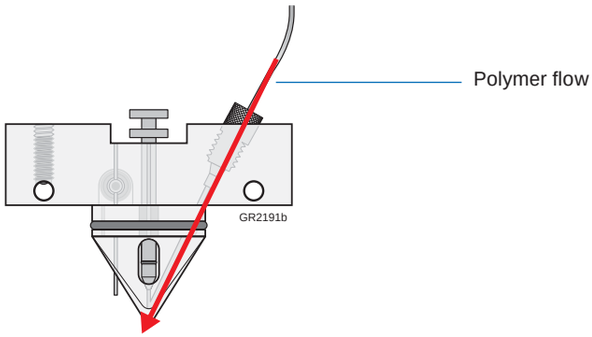


2. Push down slowly on the polymer-reserve syringe to move bubbles down the channel and into the polymer block tubing.



3. Continue to push down slowly on the polymer-reserve syringe to move the bubbles through polymer tubing and out the channel of the lower polymer block.

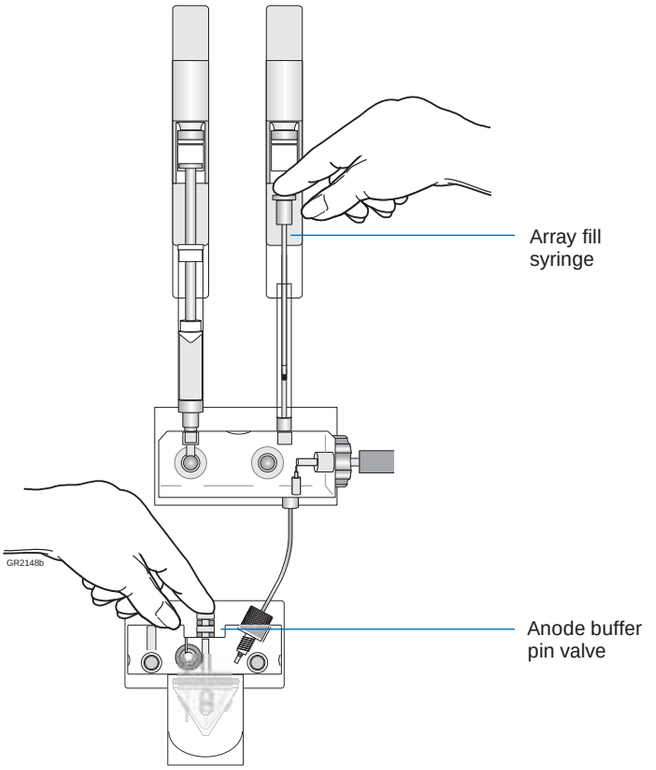
   IMPORTANT! Verify that all air bubbles are pushed out of the tubing assembly into the lower buffer reservoir before proceeding. There should be no bubbles in the tubing or channel of the lower polymer block.



4. If the buffer jar is attached, replace the buffer if excess polymer is expelled into the anode buffer jar.

Notes

5. If air bubbles are still present in the polymer block, expel the bubbles as follows:
   a. Hold down the anode buffer pin valve and simultaneously push down on the array-fill syringe to build pressure in the channels.
   b. Release the anode buffer pin valve (while still pressing down on the array-fill syringe) to expel bubbles into the polymer block tube.
6. Repeat step 5 as necessary.



Notes

Autosampler Calibration

When to Calibrate the Autosampler

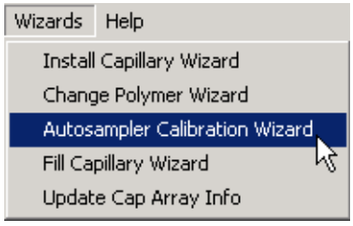
Calibrate the autosampler only as needed.

Symptoms of autosampler alignment problems may include:

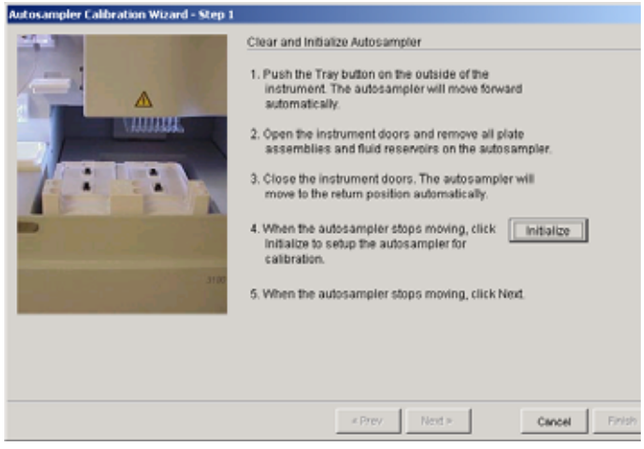
- Poor injection for a small number of capillaries
- Low signal strength
- No evidence of sample

Calibrating the Autosampler

1. Close the oven and instrument doors.
2. Select **Wizards** > **Autosampler Calibration Wizard**.



3. If plates are linked in the Run Scheduler, the plates automatically are unlinked. In the Warning dialog box, click OK.
4. Follow the directions given in the wizard to calibrate the autosampler.



5. Click Finish.

> IMPORTANT! The new X, Y, and Z positions are saved in the .ini file, which is store on the computer. The new values take effect only when the instrument power is cycled, and the values are uploaded to the instrument from the .ini file on the computer.

6. Cycle the instrument power off and on.

   The new X, Y, and Z positions from the .ini file are uploaded to the instrument software.

Notes

Manual Control

Manual control is active only if the oven and instrument doors are closed.

Table of Commands

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

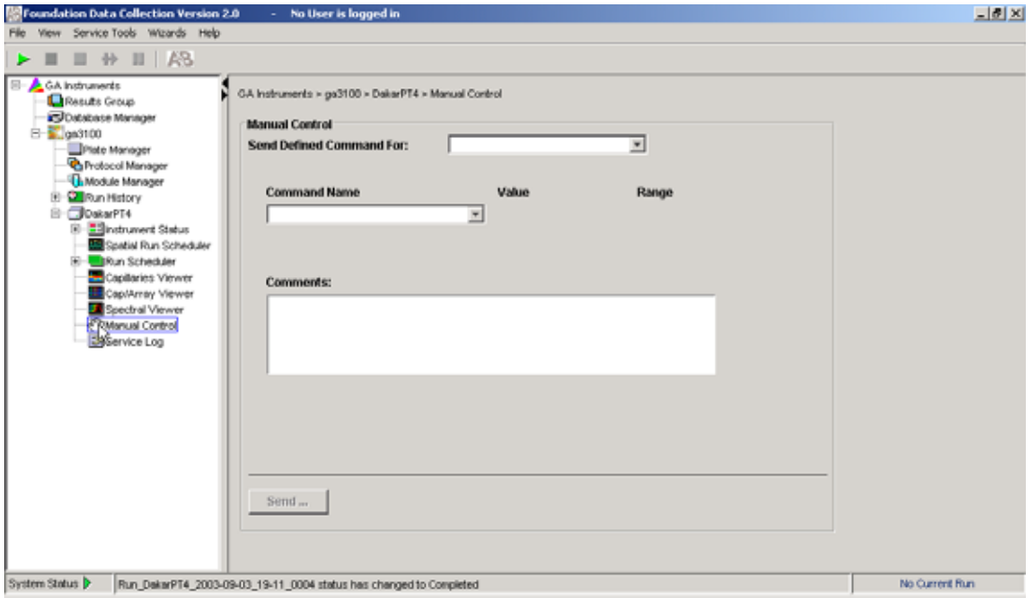
Command Function	Command Options	Value
Electrophoresis	Set power supply	• On • Off
	Set voltage	A number between 0 and 15 kV
Laser	Set state	• Idle • On • Off
	Set power	A number between 0 and 25 mW
	Open/Close shutter	• Open • Closed
Oven	Set state	• On • Off
	Set temperature	A number between 18 and 65 °C
Autosampler	Move forward	N/A
	Return	N/A
	Move up/down	A number between –500 and 500 steps
	Move to site	• Buffer (left, front for 1X running buffer), home position • Water1 (left, rear for deionized water) • Water2 (right, front for deionized water) • Waste (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

Notes

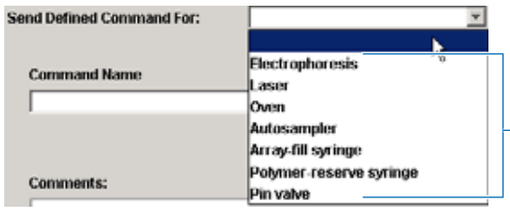
Using Manual Control

Manual control functions cannot be use during a run.

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name*> **Manual Control**.

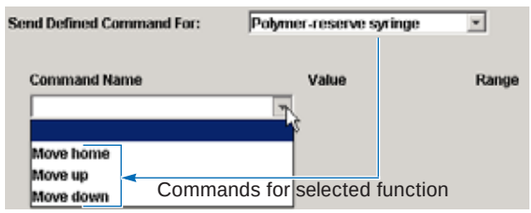


2. In the Send Defined Command For drop-down list, select a function.



3. In the Command Name drop-down list, select a command and enter a value, if required.

   Note: The command names are filtered based the function selected in step 2.



4. Click **Send ...**.

Notes

Working With Drives for Database and Sample Data Storage

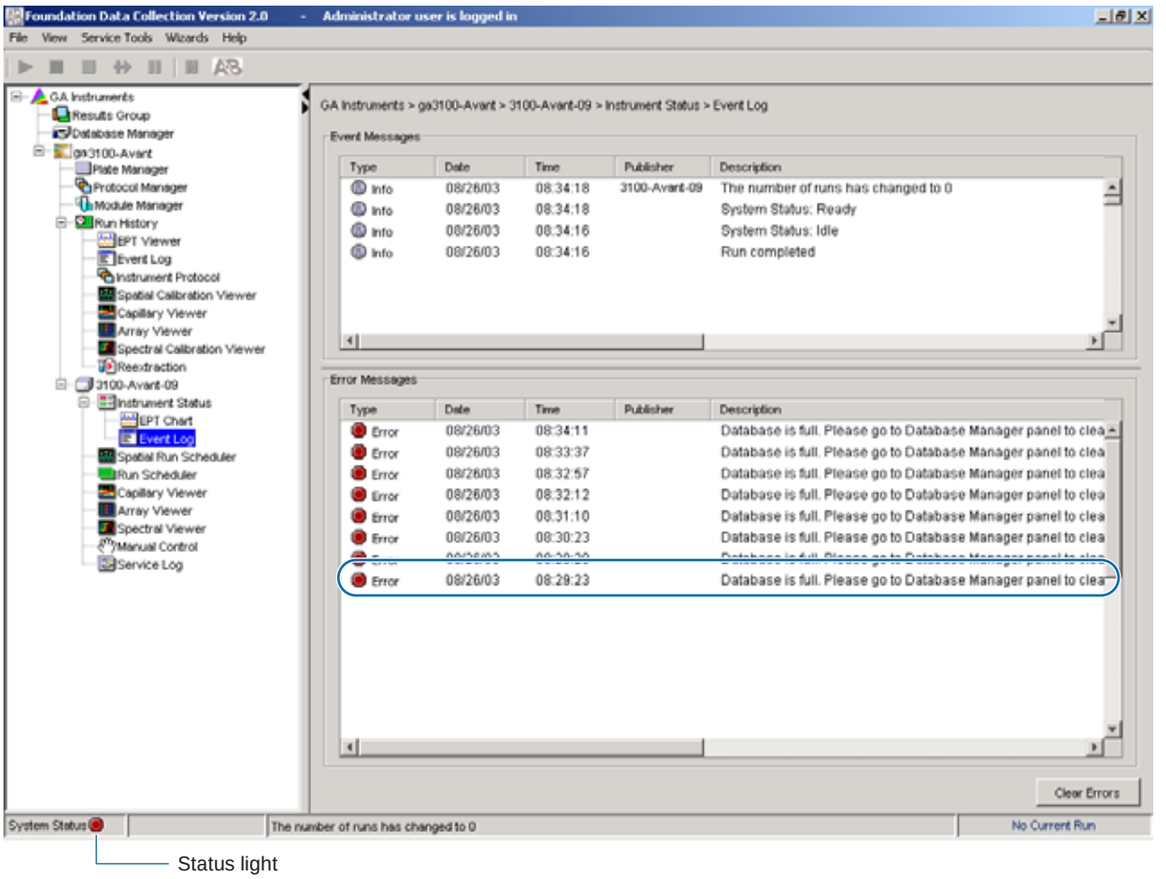
Checking Available Space on Drives D, E, and F

Before a run or batch of runs, the Data Collection software automatically checks the available space on drives C, D, and F to ensure sufficient space to store the database and sample file data you create.

The Data Collection software send a warning message to remove data the drive is getting full and/or clean up the database when the database is getting full (~80% of capacity). An error is generated and displayed in the Instrument Status window in the Errors pane and in the Event Log window in the Errors pane. Also, the status light in the bottom left-hand corner of the data collection window flashes red.

Full Database Error

To view the error messages, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Instrument Status**> **Event Log**.

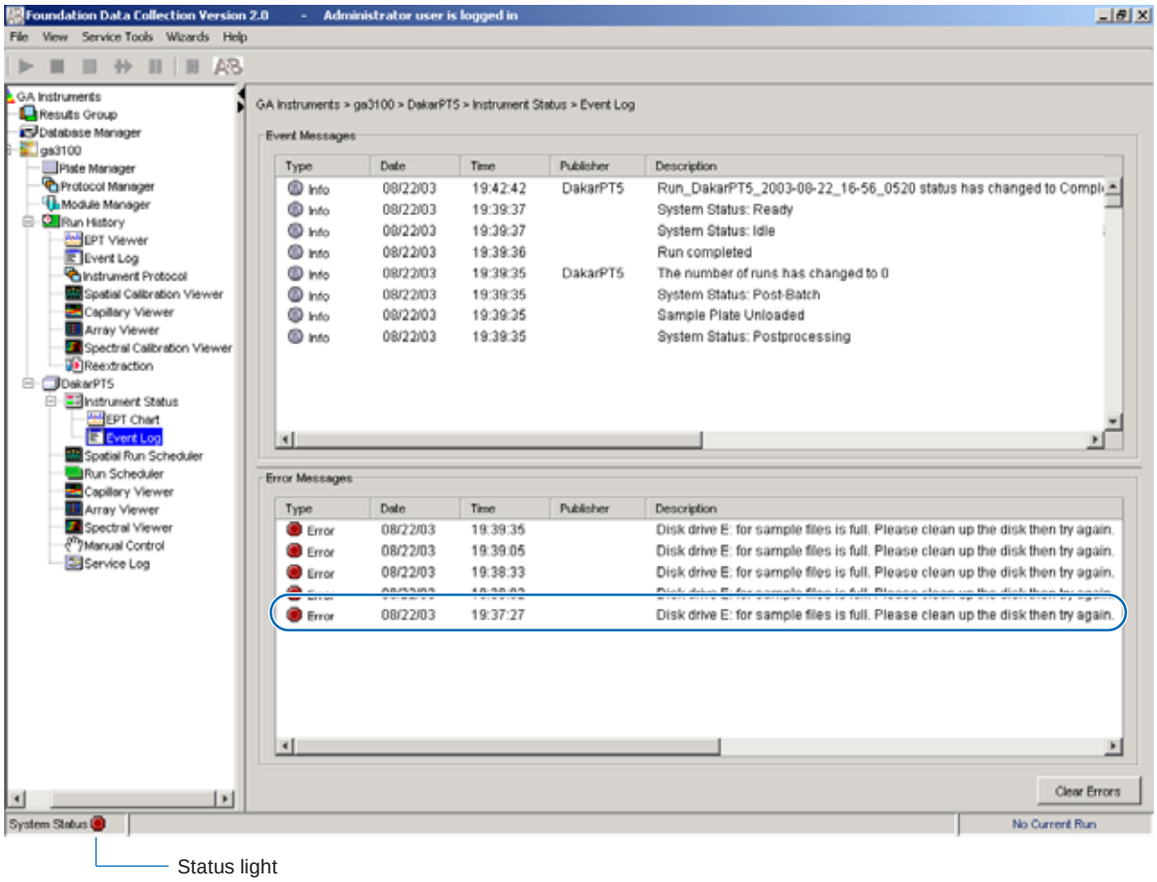


Database full error message

Notes

Disk Drive Full Error

To view the error messages, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > *instrument name* > **Instrument Status** > **Event Log**.



Disk drive full error message

Runs can not be started until the data is removed from the drive and/or database is cleaned up.

Cleaning Drives

Ensure that you have sufficient drive space by regularly:

- Archiving data
- Deleting unneeded files
- Emptying the trash
- Defragmenting the drives

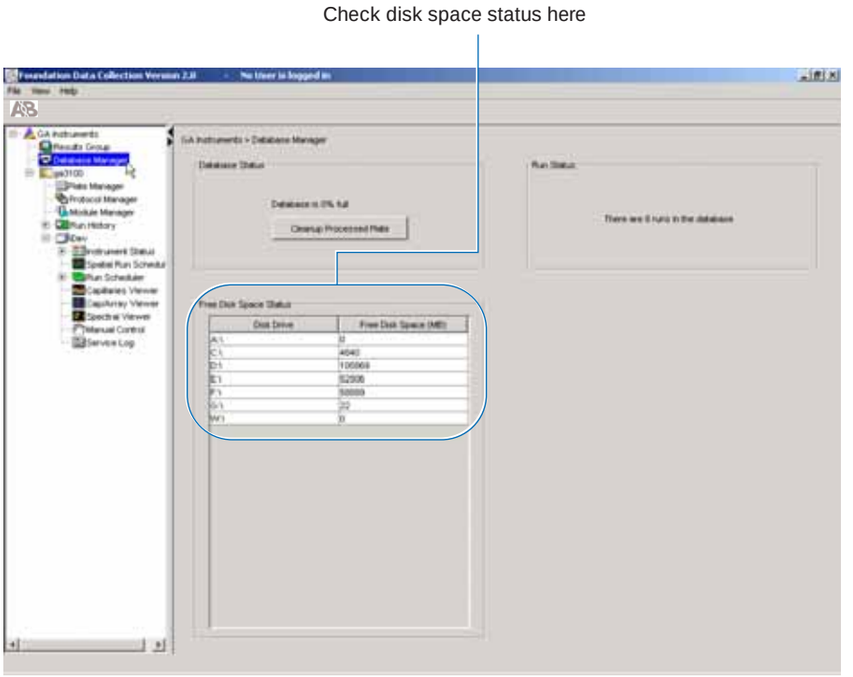
Notes

Hard Disk Status

Manually Checking Available Disk Space on Drive E

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Database Manager**.

   The Database Manager view opens.



2. If there is insufficient space:
   a. Archive the sample files to a CD-RW (see page 237) or another volume.
   b. Delete the sample file data from the drive E and empty the contents of the Recycle Bin.

Notes

Archiving Data

Creating a Data CD

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

To archive data:

1. Select **Start** > **Programs** > **Roxio Easy CD Creator 5** > **Applications** > **Easy CD Creator**.

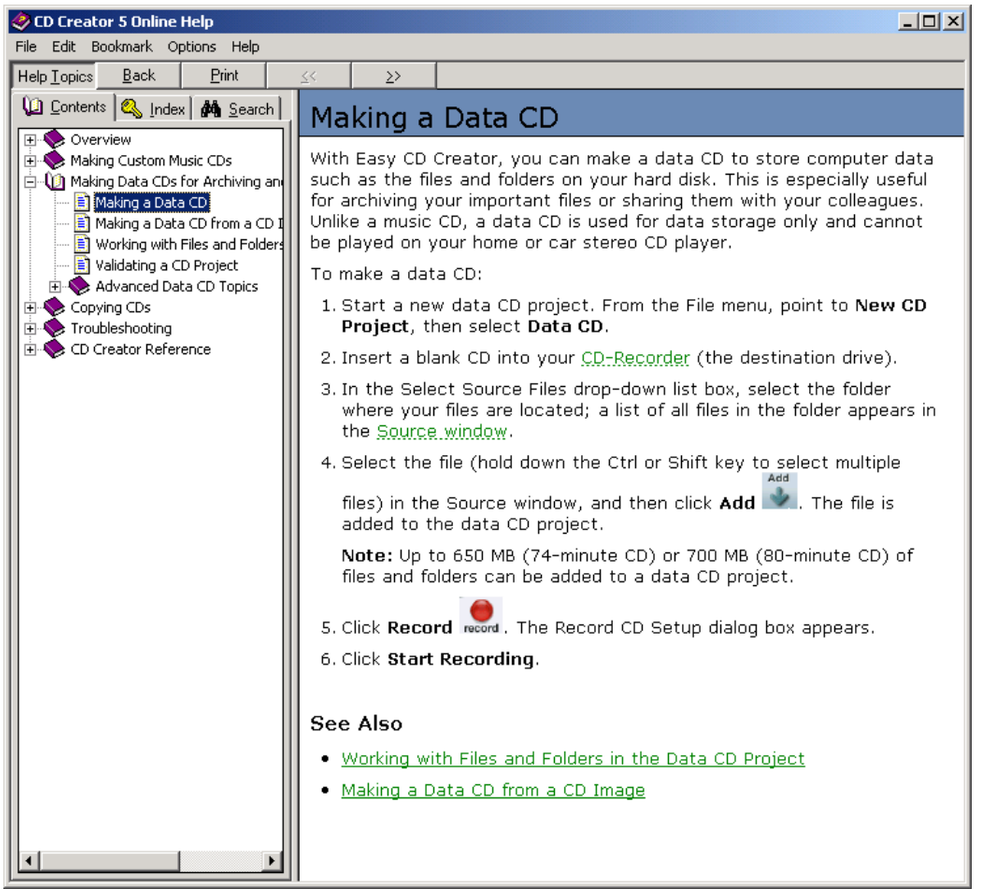
   The Untitled - Easy CD Creator dialog box opens.

2. For help creating a data CD, select **Help** > **Contents and Index**.

3. In the left tree pane, select **Making Data CDs for Archiving and Sharing** > **Making a Data CD**.

   Use the instruction to create the CD.

Notes



Instructions for creating a data CD

Notes

Defragmenting the Computer Hard Drives

The fragmentation of files decreases the performance of both the data collection software and the computer operating system. As the hard drive becomes fragmented, programs take greater time to access files because they must perform multiple seek operations to access the fragments.

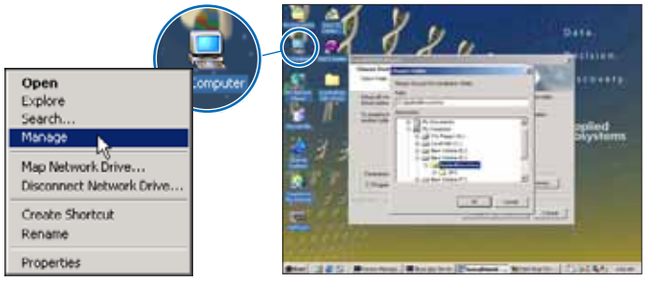
When to Defragment the Computer Hard Drive

Defragment the computer hard drive:

- at least once every month.
- before fragmentation reaches 10%.

Defragmenting the Drives

1. In the Windows desktop, right-click **My Computer** (), then select **Manage**.

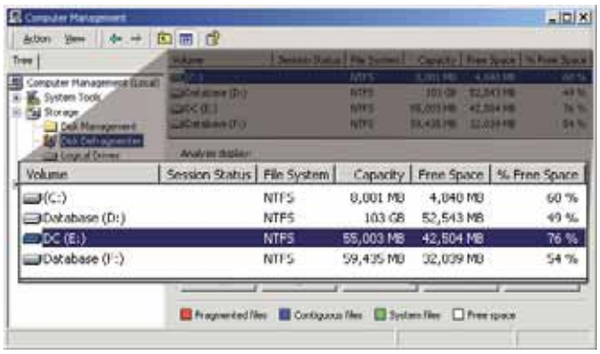


2. In the Tree tab of the Computer Management dialog box, click **Computer Management (Local)** > **Disk Fragmenter**.

3. Select the **E** drive.

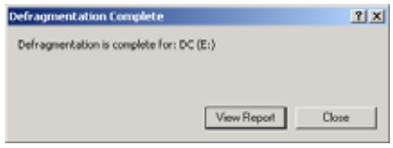
4. Click Defragment.

   The computer displays the Defragmentation Complete dialog box upon completion of the defragmentation of the drive.



5. In the Defragmentation Complete dialog box, click Close.

6. In the Computer Management dialog box, click ×.



Notes

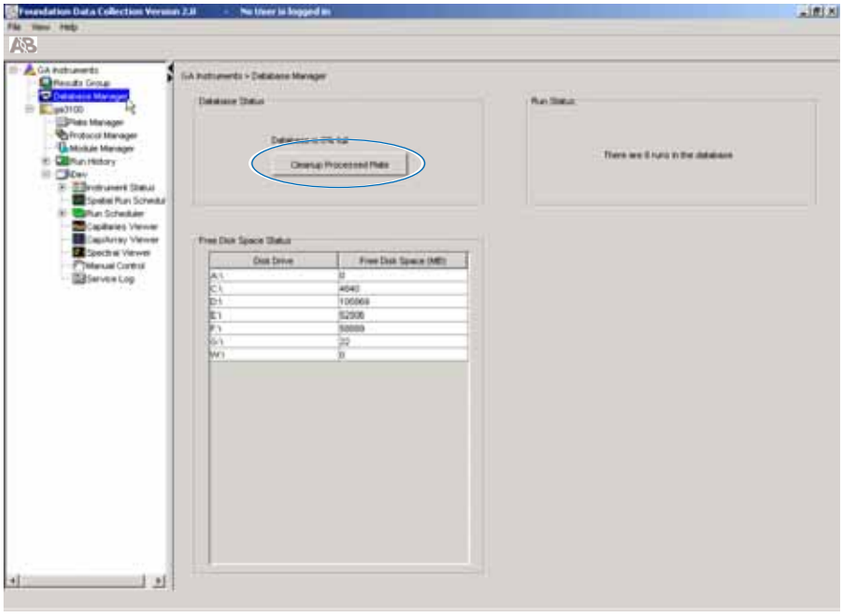
Deleting Records from the Database

Deleting Processed Frame Data

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Database Manager**.

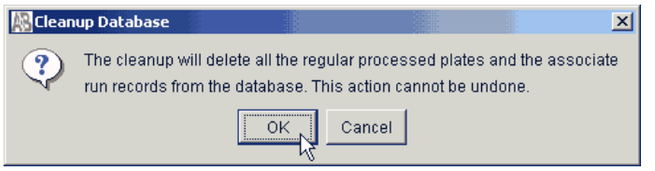
   The Database Manager view opens.

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.



2. Click **Cleanup Processed Plates**.

   The following dialog box opens.



3. Click OK.

Note: There is no need to re-import the spatial and spectral calibrations or the custom run modules.

Note: It may take several minutes to clean up the database if it is full or contains a lot of data.

Notes

Audit Trails and Access Control

This chapter covers:

Notes

Audit

Audit trails maintain a history of data changes made by the user.

Data Changes that Generate Audit Records in Data Collection Software

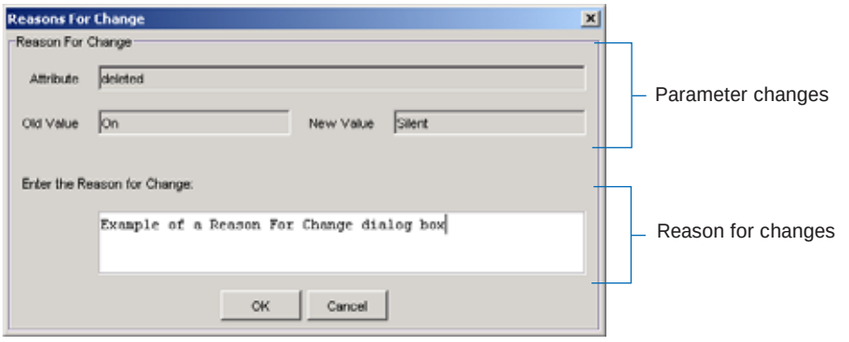
An audit record is generated when data are changed. The following table lists the three general categories and the events within them that generate an audit record in Data Collection software.

	Plate Record	Run Module	Results Group
An audit record is generated in Data Collection software when you ...	• Create, edit, or import a plate record	• Create a run module • Edit the parameters of a run module	• Create, edit, or import a results group

Reason For Change

When a change occurs and auditing is required, the Reasons For Change dialog displays and contains:

- The attribute that was changed, created, or deleted.
- The old and new values, if applicable, in the top half of the dialog box.
- A Text box to enter the reason for the change.
  - When you click OK, changes to the attribute and the audit data are saved.
  - When you click Cancel, no changes are saved and you return to the previous window.



Notes

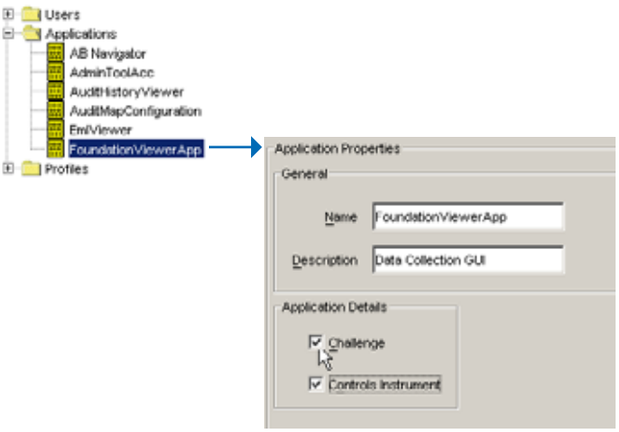
Enabling The Access Control and Audit Features

Enabling Access Control (Security)

1. Start the following:
   - Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3100 v2.0** or **Run Data Collection 3100-*Avant* v2.0**.
   - Administrator application: **Start > Programs > Applied Biosystems > Administrator**.
2. In the left pane tree double-click **Access Control Administration.**

3. In the System Authentication dialog box, type **Administrator** for the login name and type your password if you have changed it; if not, type **Administrator**.
4. Select **Applications > FoundationViewerApp**.
5. Select the Challenge check box to activate it.

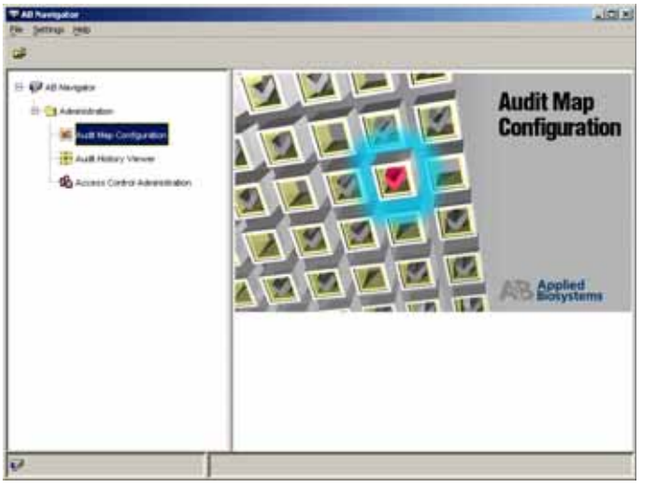
   The Login and Password dialog box is now enabled.
6. Select **File > Save**.
7. Exit Access Control Administration.



Notes

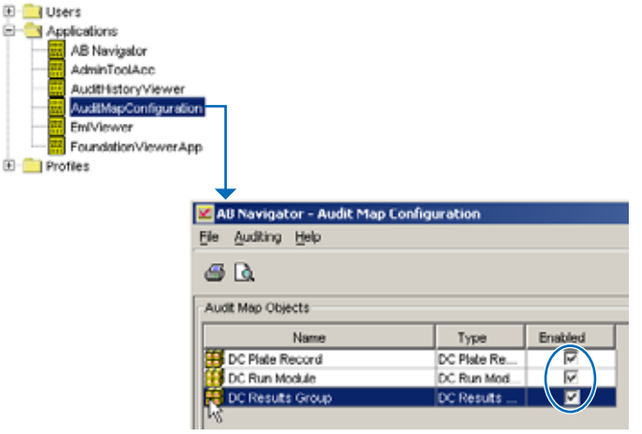
Enabling Audit

1. In the Navigator left pane tree, double- click Audit Map Configuration.



2. Select Enabled audit map object to activate it:
   - DC Plate Record
   - DC Run Module
   - DC Results Group

3. Exit Audit Map Configuration.



Notes

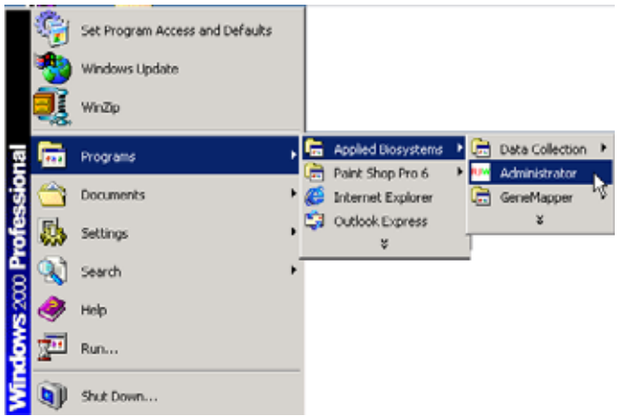
Starting AB Navigator

AB Navigator is the access point for these applications:

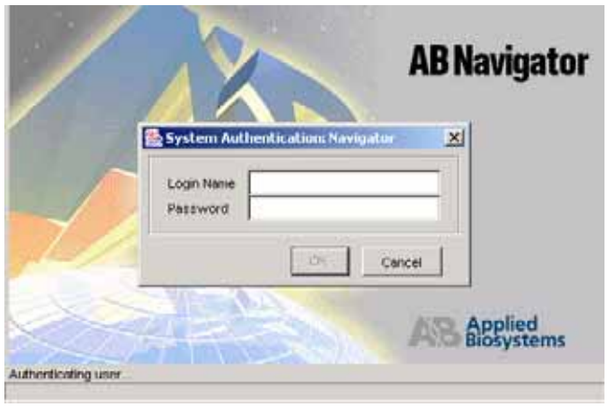
- Audit Map Configuration
- Audit Map History Viewer
- Access Control Administration r

IMPORTANT! You must start Data Collection services in order for AB Navigator to function properly.

1. Start the Data Collection Services, then start the Administrator application:
   a. Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3100 v2.0** or **Run Data Collection 3100-*Avant* v2.0**.
   b. Administrator application: **Start > Programs > Applied Biosystems > Administrator**.



The System Authentification dialog box displays.



2. Enter login name and password, then click **OK**.

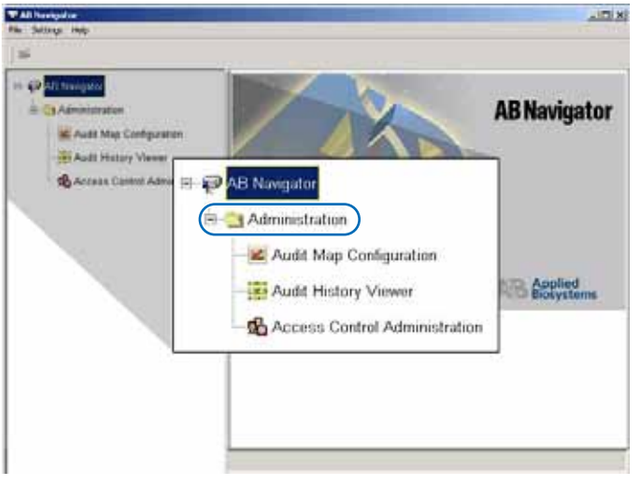
   Default login name: “Administrator”

   Default password: “Administrator”

   Note: To change your password, see step 4 on page 257

Notes

3. In the left pane tree, click Administration to expand the options.



Audit Map Configuration

The Audit Map Configuration Tool is used to manage Audit Maps. Audit Maps are used to control how auditing is done for a given data type.

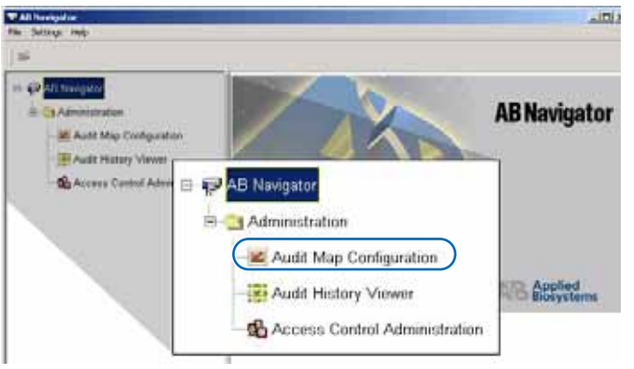
Some features of the Audit Map Configuration Tool:

- You can set the audit states of an audit map to On, Off, or Silent.
- There is no SAVE command. All changes to audit maps are saved automatically.

Starting the Audit Map Configuration Tool

1. Click the Audit Map Configuration icon in the left pane tree.

   The System Authentification dialog box displays.

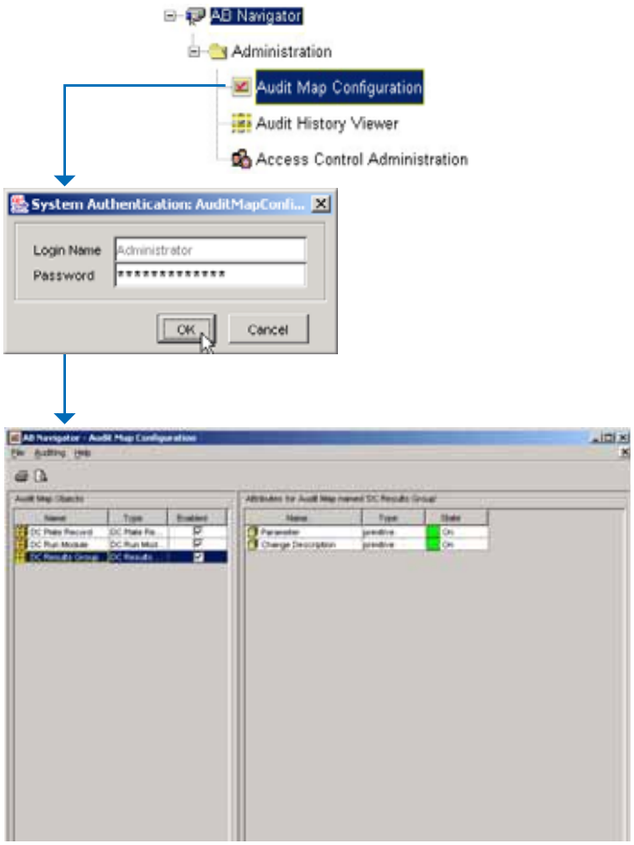


Notes

2. In the System Authentication dialog box, type **Administrator** for the login name and type your password if you have changed it; if not, type **Administrator**.

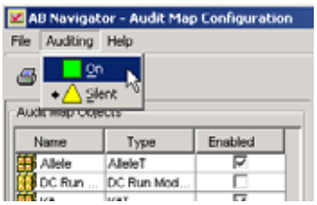
   Click **OK**.

   The Audit Map Configuration window displays.



Audit Map Configuration Functions

Audit Map Configuration Functions:

If you want to ...	Then ...
Enable or disable all the attributes in an audit map	Select or deselect a cell in the Enabled column in the Audit Map Objects pane.
Change the audit state of an attribute in this window only.	Select a different audit state in a cell under the State column in the Attributes pane. Audit states are: On or Silent. 
Sort a row	Click on a column header.

Note: Disabled Audit Maps (Enabled column) display their attribute list in italics.

Notes

Commands

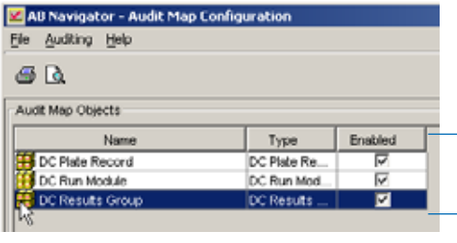
The following table lists the commands you can perform in the Audit Map Configuration Tool.

Toolbar Menu	Command	Function
File	Go To	Displays a list of applications that are currently running; select an application to go to that application
	Visual Print	Displays Print Dialog
	Visual Print Preview	Displays Print Preview
	Exit Application	Exits the Audit Map Configuration application
	Exit AB Navigator	Exits the AB Navigator application
Auditing	On	Select auditing to be turned on for the Audit Map Configuration. When a change is made to an Audit Map's enabled state or when a change is made to the state of an attribute, auditing occurs, and A Reason For Change (RFC) dialog displays.
	Silent	When a change is made to an Audit Map's enabled state or when a change is made to the state of an attribute, auditing occurs. Although the RFC Dialog does *not* display, a 'silent' Audit Record is created.

When RFC Dialog Displays and You...	Then ...
Click OK	The map or attribute state changes and an Audit Record is created.
Click Cancel	The map or attribute state does not change.

Attribute States

When you click an Audit Map Object, the Attributes Pane (right) displays.

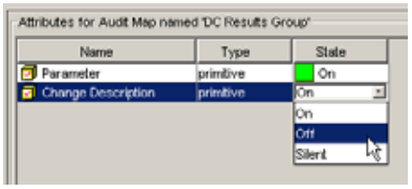


Notes

Change Description

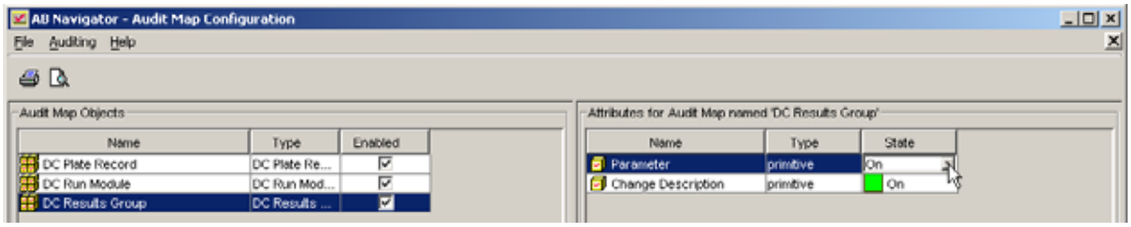
This function controls the Reason for Change dialog box. When it is on, any changes to the enabled Audit Map Object forces the user to type a reason for the change.

To disable this feature for an enabled object—The DC Results Group in the example below—change the state to Off.



Parameter Change

This function records old and new values that are displayed in the upper half of the Reason for Change dialog box (see "Reason For Change" on page 242).



On, Off, and Silent

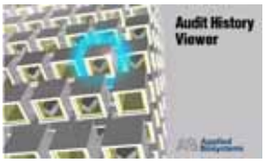
The following table describes the On, Off, and Silent states for audit map attributes, Change Description and Parameter.

	Audit Map Attributes	
State	**Change Description**	**Parameter**
On	Reason for change required	Records old and new values
Off	Reason for Change dialog box does not display	Does not record old or new value changes
Silent	Reason for Change dialog box does not display	Records old and new values

Notes

Audit History Viewer

The Audit History Viewer is used to view historical audit data. This tool is used as a read-only viewer for audit records. The tool provides data filtering so that audit records can be viewed in different formats.



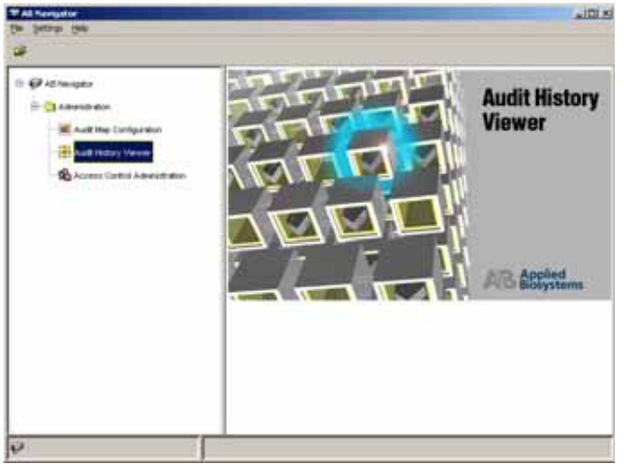
Audit records that you can view with the Audit History Viewer are:

- Date and time the audit record was created.
- The user who triggered the audit event.
- The attribute that was changed.
- The old and the new values.
- The reason for the change.

Note: The audit records are stored in a permanent data store.

Starting the Audit History Viewer

1. Double-click the Audit History Viewer icon in the left pane tree.



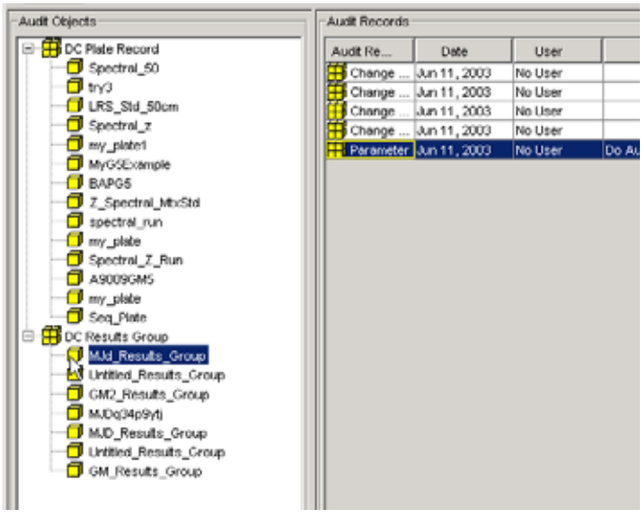
2. In the System Authentication dialog box, type **Administrator** for the login name and type your password if you have changed it. If not, type **Administrator**.
3. Click **OK**.

The Audit History Viewer displays.

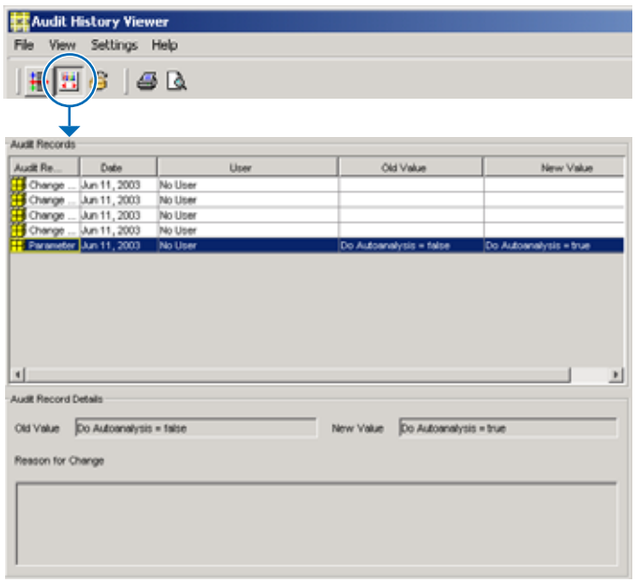
Notes

Viewing an Audit History

1. In the Audit Objects pane, expand the objects tree until the object of interest displays.



2. Highlight an object and then click (Detail Panel) to display audit record details.



> **Note:** Click the column headers to sort the read-only records columns.

8

Notes

Filter Command

The filter allows you to categorize audit history records.

1. Click (Filter).

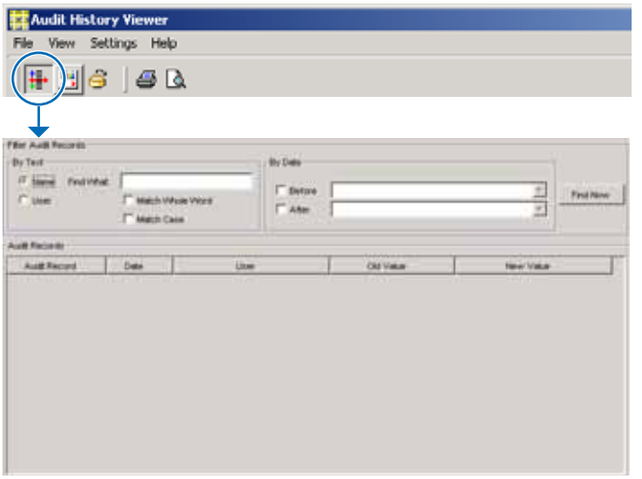
   The Filter Audit Records pane displays.

2. Enter search criteria in the applicable text boxes.

3. Click **Find Now**.

   You can filter audit records by:

   - Name
   - Date (and, before or after a date or between two dates)
   - User name
   - Matching whole words
   - Case sensitivity



Commands

Toolbar Menu	Command	Function
File	Reload	Refreshes the Audit History Viewer with the latest changes
	Report	Customize and then print a report of the selected Audit History Record
	Print Preview	Customize and then preview a report of the selected Audit History Record
	Page Setup	Customize the page setup of the Report printout
	Go To	Displays a list of applications that are currently running; select an application to go to that application
	Visual Print	Displays Print Dialog
	Visual Print Preview	Displays Print Preview
	Exit Application	Exits the Audit Map Configuration application
	Exit AB Navigator	Exits the AB Navigator application
View	Filter	Displays the filter pane on the top of the frame when selected. It allows the user to specify criteria that limits the amount of audit records in the Audit Record table.

Notes

Access Control Administration

The Access Control Administration tool allows an administrator to manage the creation and deletion of:

- Users
- Profiles

Also, Access Control allows an administrator to restrict or grant users access to features and functions of the software.

An administration user is always associated with the Administration User Group and cannot be deleted. And, only one administrator is allowed to modify Access Control data at one time.

Starting the Access Control Administration Tool

IMPORTANT! You must start Data Collection services in order for AB Navigator to function properly.

1. Start the following:
   – Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3100 v2.0** or **Run Data Collection 3100-*Avant* v2.0**.
   – Administrator application: **Start > Programs > Applied Biosystems > Administrator**.
2. Double-click the Access Control Administration icon in the left pane tree.
3. In the System Authentication dialog box, type **Administrator** for the login name and type your password if you have changed it. If not, type **Administrator**.
4. Click **OK**.

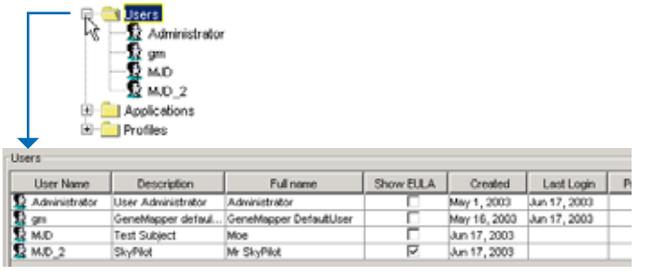
Notes

The Access Control Administration tool displays.

Type Selection

In the left pane tree, Users and Applications are types. When you select a type, the List of Users pane displays a list of identifiers of the type selected.

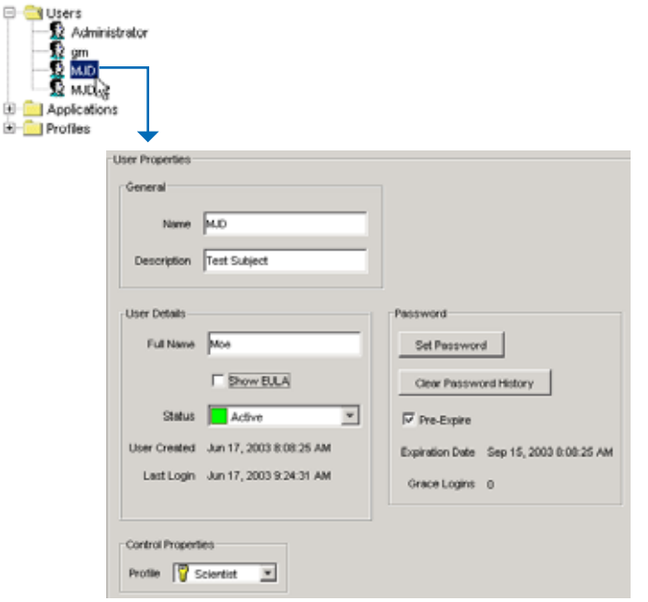
IMPORTANT! Do not remove any applications from the default list in the left pane tree.



Name Selection

When you highlight a name, properties of that name display in the User Properties pane.

Note: If you select the EULA (End User License Agreement) check box, the license agreement displays the next time the user is authenticated.



Properties Panes

Access control identifiers have an additional drop-list labeled “Control Properties”. This defines the access level an individual is allowed in the Data Collection software.

The identifiers under access control are:

- User
- Profile

Notes

Inherited Rights

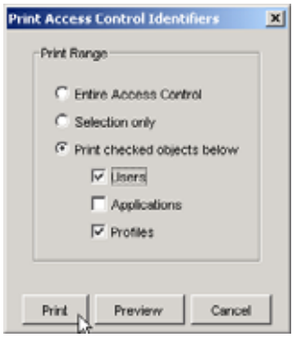
Each default user group has certain inherited rights related to their group profile.The group profile definitions are:

- *Instrument Protocols* include: Run Module Operations, Results Group Operations, Analysis Protocol Operations, Instrument Protocol Operations and Reextraction.
- *Instrument Operation include: Plate Operations, Event Log, and Instrument Control Operations.*
- *Instrument Maintenance*: Spatial Calibration Operations, Manual Instrument Control, and Miscellaneous Operations.

Commands

Commonly used commands:

- **Toolbar.** Frequently used commands appear in the application toolbar. Tool tip help text appears when you place the cursor over a button in the toolbar.
  - *Save*:  Save commits changes in the Admin Tool to data store and is accessible from the menu bar, keyboard shortcut, or toolbar.
  - *Exit*: Exit is invoked by the standard upper-right-corner control or by the Files/Exit menu selection. If you have updated memory but have not yet committed changes to data store, the application asks, “Information has been modified, Save changes?” The message box provides buttons for Yes, No, and Cancel.
  - *Duplicate:*  Duplicates the selected indentifier. Duplicate is accessible from the menu bar and toolbar.
  - *Find*:  locates the name specified in the text field in the navigator tree
  - *Print:* Prints all or some identifiers in various formats selected from the dialog shown below. Go to **File > Report**  to display the Print Options dialog box.



Notes

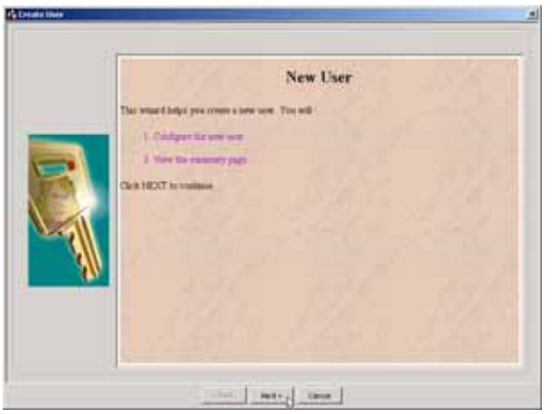
Creating a New User

IMPORTANT! You must set a default password for each new user.

1. Click the New User icon 

   The New User dialog displays.

2. Click **Next**.



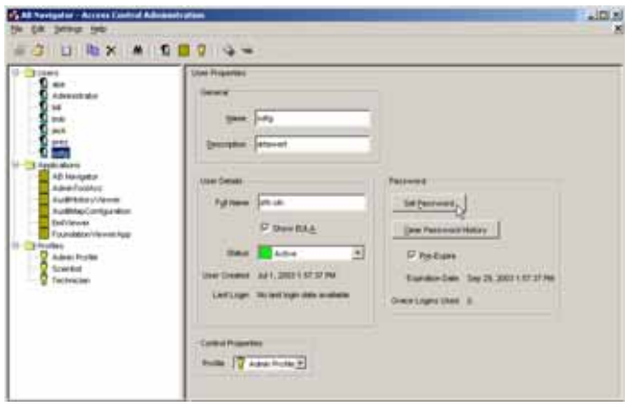
   The Configure pane displays.

Notes

3. Complete the information in the window.
4. Click Set Password.

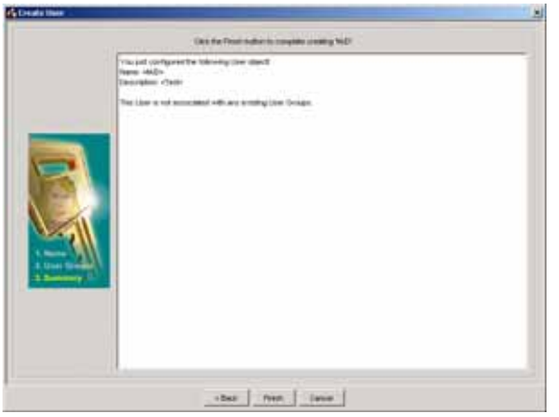
The Change Password dialog box displays.

5. Complete the new password, then click **OK**.
6. Click **Finish** to complete the creation of a new user.
7. Click (Save).
8. Click **Next**.

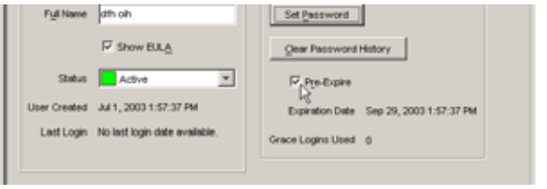


Notes

The Summary pane displays the new user profile data.



9. *(Optional)* To force the user to create a password when they login for the first time, enable the Pre-Expire check box. If the Pre-Expire check box is not enabled, first time users use the default login password.



User Properties

A user must be assigned to a profile, which allows the administrator to grant or deny a user the right to execute functions defined by applications

When one user is selected in the left navigator tree, the user profile displays in the User Properties pane and the User Details pane.

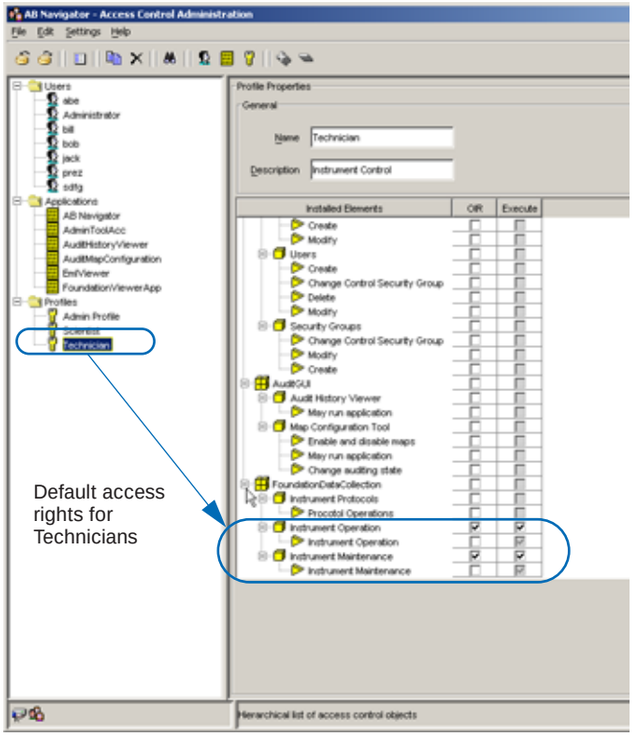
Notes

Default Profiles

User Groups

Default profiles show the access each user group has. The default user groups and their default profiles are:

- *Administrator*: Complete access to Instrument Protocols, Instrument Operation, Instrument Maintenance
- *Scientist*: Complete access to Instrument Protocols, Instrument Operation, Instrument Maintenance
- *Technician*: Access to Instrument Operation and Maintenance



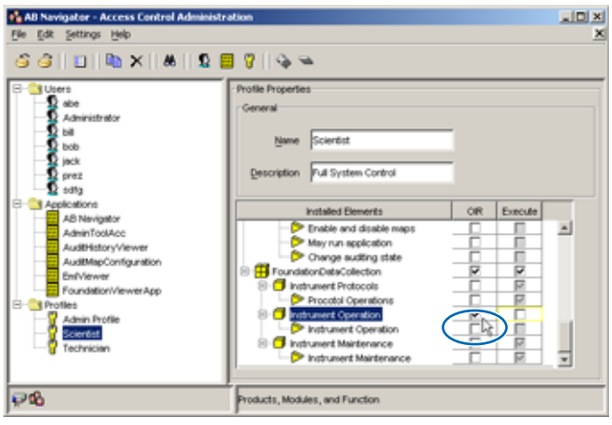
Inherited Rights

Each default user group has certain inherited rights related to their group profile.The group profile definitions are:

- *Instrument Protocols* include: Run Module Operations, Results Group Operations, Analysis Protocol Operations, Instrument Protocol Operations and Reextraction.
- *Instrument Operation include: Plate Operations, Event Log, and Instrument Control Operations.*
- *Instrument Maintenance*: Spatial Calibration Operations, Manual Instrument Control, and Miscellaneous Operations.

Overriding Inherited Rights

To override the inherited rights of a group, simply deselect the OIR check box next to the function you want to deny. In the graphic below, the scientist group is denied access to Instrument Operation.



Notes

Creating a New Profile

1. Click the New Profile icon 

   The New Profile dialog displays.

2. Click **Next**.

   The Configure pane displays.

3. Complete:

   a. Profile properties

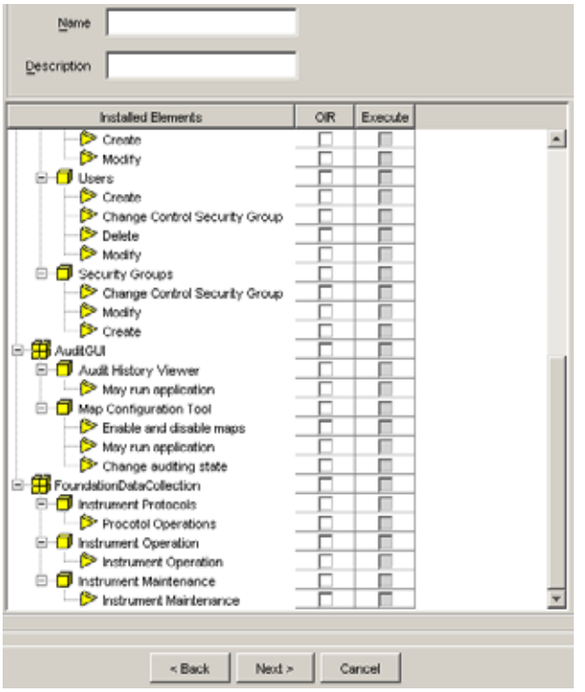
   b. From the drop list, select the control security group with which the new profile is to be associated.

   c. Select OIR and/or Execute

   Execute: Select this to give access to the function to any user assigned to this Profile.

   OIR: Select this to override inherited rights. Any lower level in the hierarchy inherits the access rights of the node above it.

   To override the inherited defaults, check the OIR check box. This allow the administrator to grant or deny the groups' ability to execute a specific function on a lower level of the hierarchy tree.



4. Click **Next**.

   The Summary pane displays the properties and associations of the new profile name.

5. Click **Finish** to complete the creation of a new User Profile Name.

Notes

Additional Information About Plate Records

A

This appendix covers:

Notes

Run Priority Scheduling

Priority Values

The user-definable run priority scheduling function allows for scheduling of runs in custom order, thereby providing more flexibility when scheduling runs.

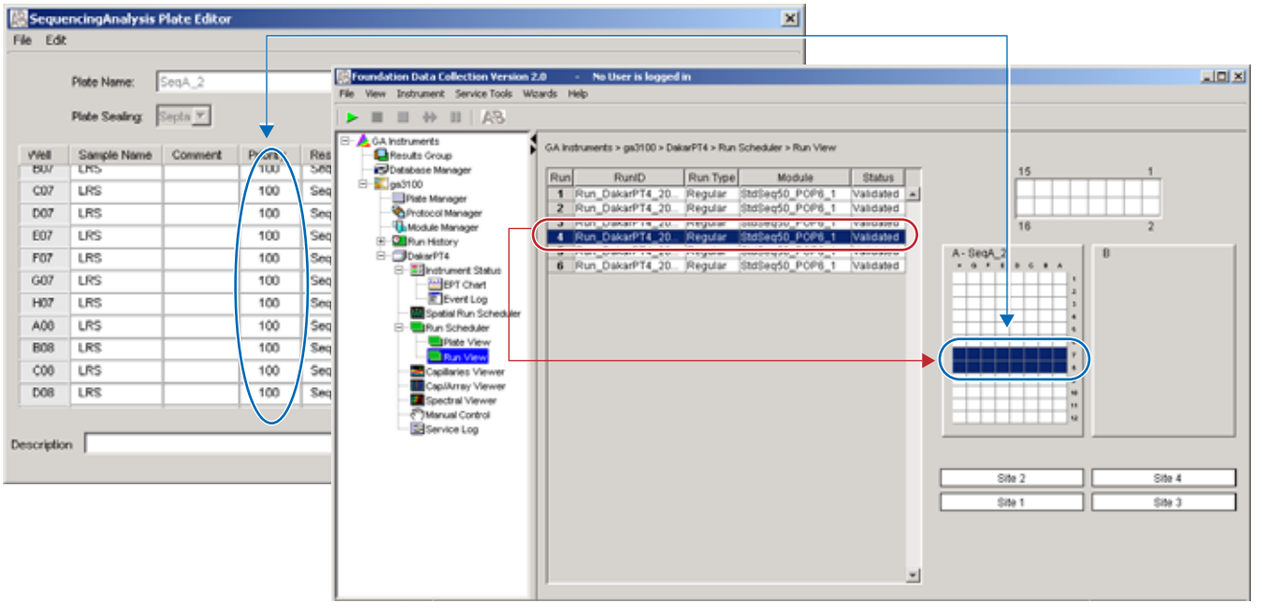
A default value of 100 is assigned to each sample in the plate record. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list. See "Run Scheduling" on page 160 for the default run schedule for 96- and 384-well plates for the both 16 and 4 capillary arrays.

Scheduling Examples Using a 96-well Plate and 16 Capillary Arrary

Default Run Scheduling

In this example, 100 is the priority value for all samples in the plate record. The default run priority schedule is used (see table below). Samples B07–D08 called out on the plate record, correspond to Run 4 as displayed in the **Run Scheduler > Run View** window.

Well Numbers	Run Number Priority
A01–H02	1
A03–H04	2
A05–H06	3
A07–H08	4
A09–H10	5
A11–H12	6



Default run priority schedule, samples in wells A07–H08 are scheduled as Run 4

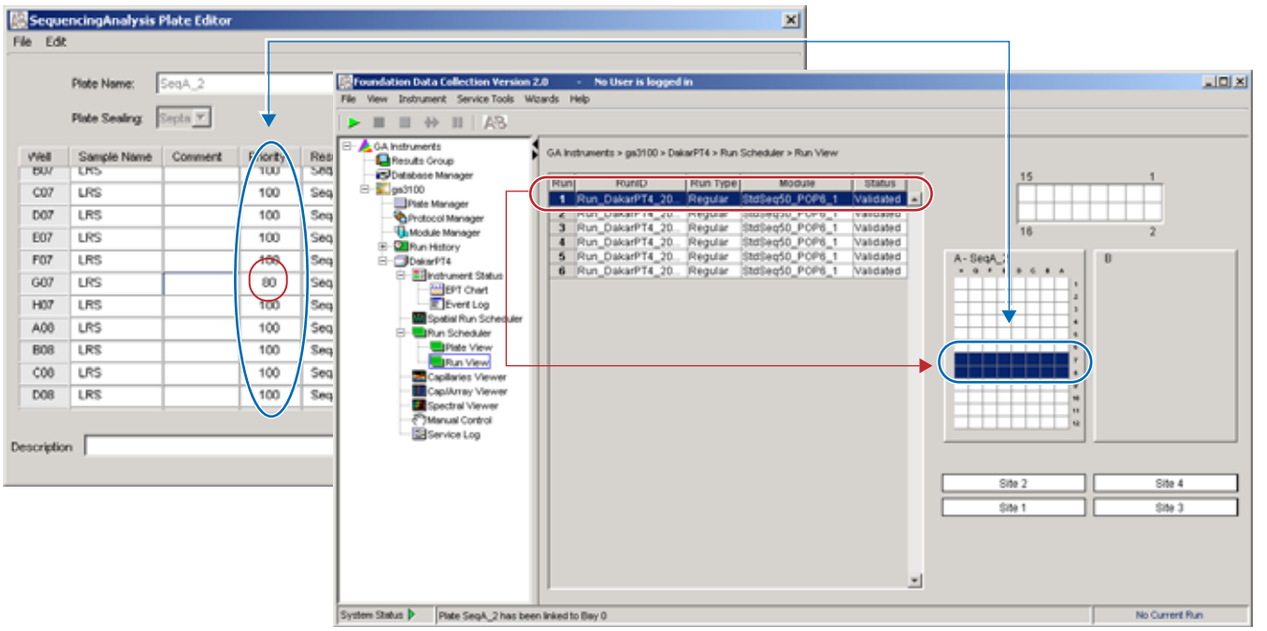
Notes

User-definable Run Priority Scheduling

In this example, the priority value for sample G07 is 80 and all other samples remain 100. Sample well G07 is contained in the A07–H08 injection set. All 16 samples now correspond to Run 1, as displayed in the **Run Scheduler** > **Run View** window.

The table below shows the change in the run priority schedule.

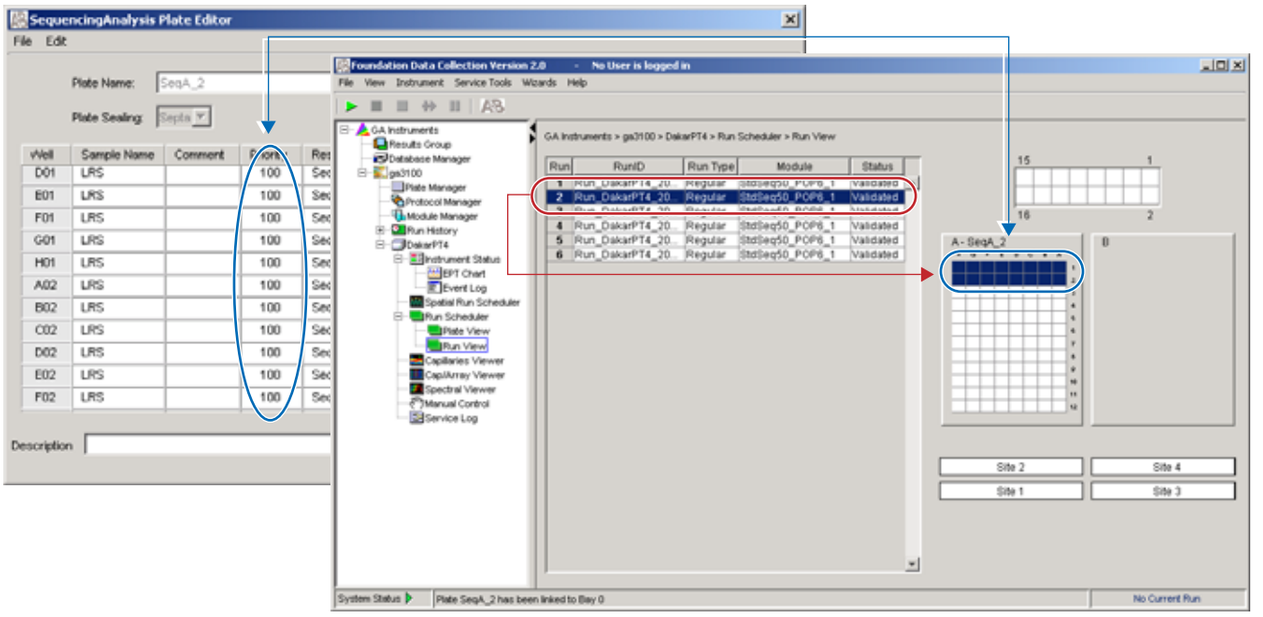
Well Numbers	Run Number Priority
A07–H08	1
A01–H02	2
A03–H04	3
A05–H06	4
A09–H10	5
A11–H12	6



User defined run priority schedule, samples in wells A07–H08 are scheduled as Run 1

Notes

The rest of the samples are run after the samples in wells A07–H08. Samples in wells A01–H02 are now scheduled as Run 2.



User defined run priority schedule, samples in wells A01–H02 are now scheduled as Run 2

Notes

Edit > Fill Down Special Option for Plate Records

Using Fill Down Special Option

Based on the plate type (96- or 384-well) and capillary array (16 or 4 capillaries) you are using, the software automatically fills in the appropriate well positions for a single run.

The Fill Down Special option works with all plate records (Spectral, Sequencing Analysis, SeqScape, GeneMapper and Mixed plates).

Creating and Completing the Plate Record

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

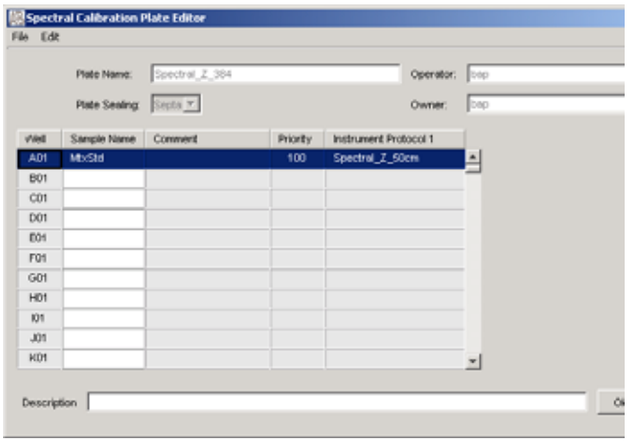
3. Complete the information in the New Plate Dialog box, then click OK.

   The Plate Editor opens.

4. Complete the columns for a single well position.

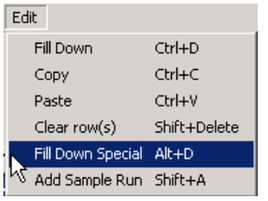
   > Note: You can start at any well position, the software automatically fills up or down based on the default run scheduling patterns (see Chapter 6, page 160).

5. Highlight the entire row.



Notes

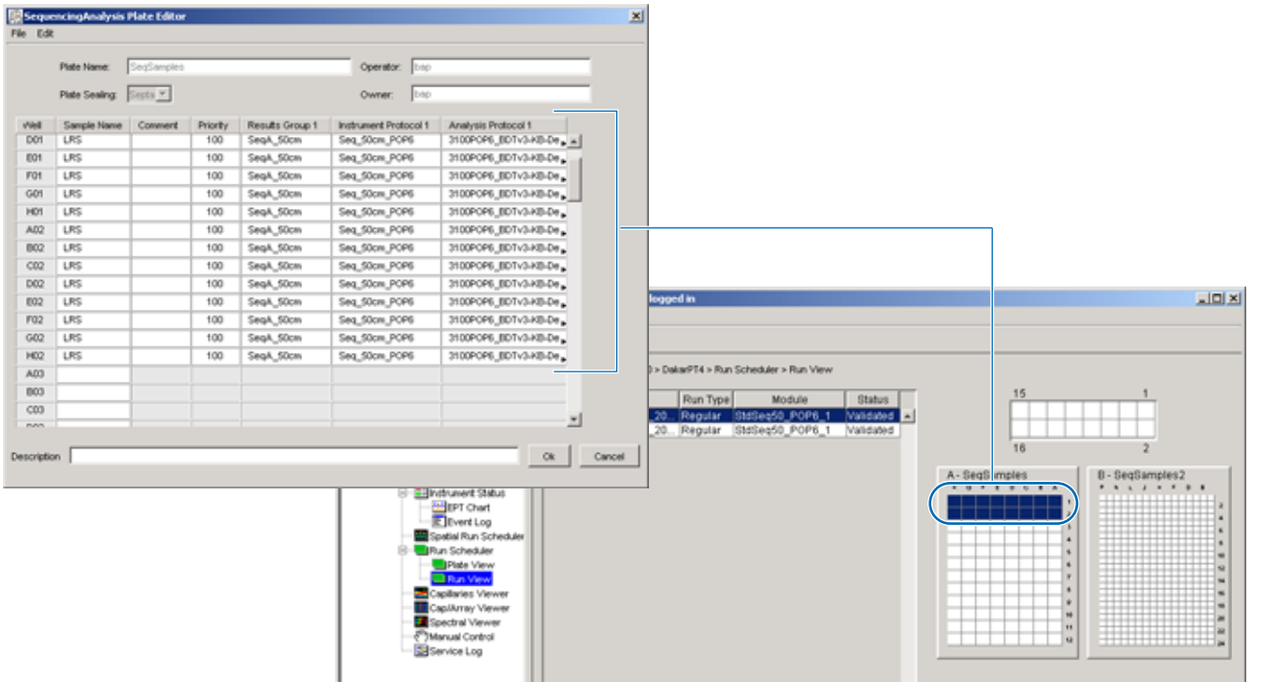
6. Select **Edit > Fill Down Special**.



7. Click OK to save the plate record.

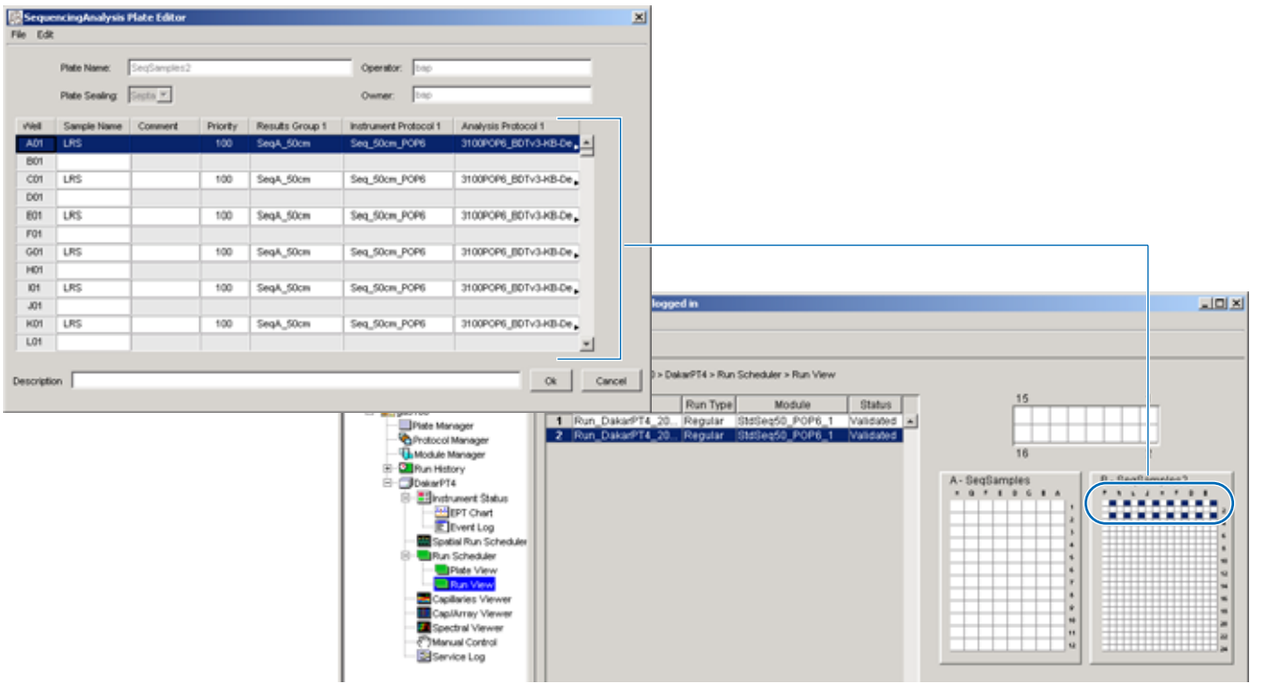
Examples of Fill Down Special

Examples of completed plate records and run scheduling for the 3100 and 3100-*Avant* instruments, and 96- and 384-well plates are shown below.

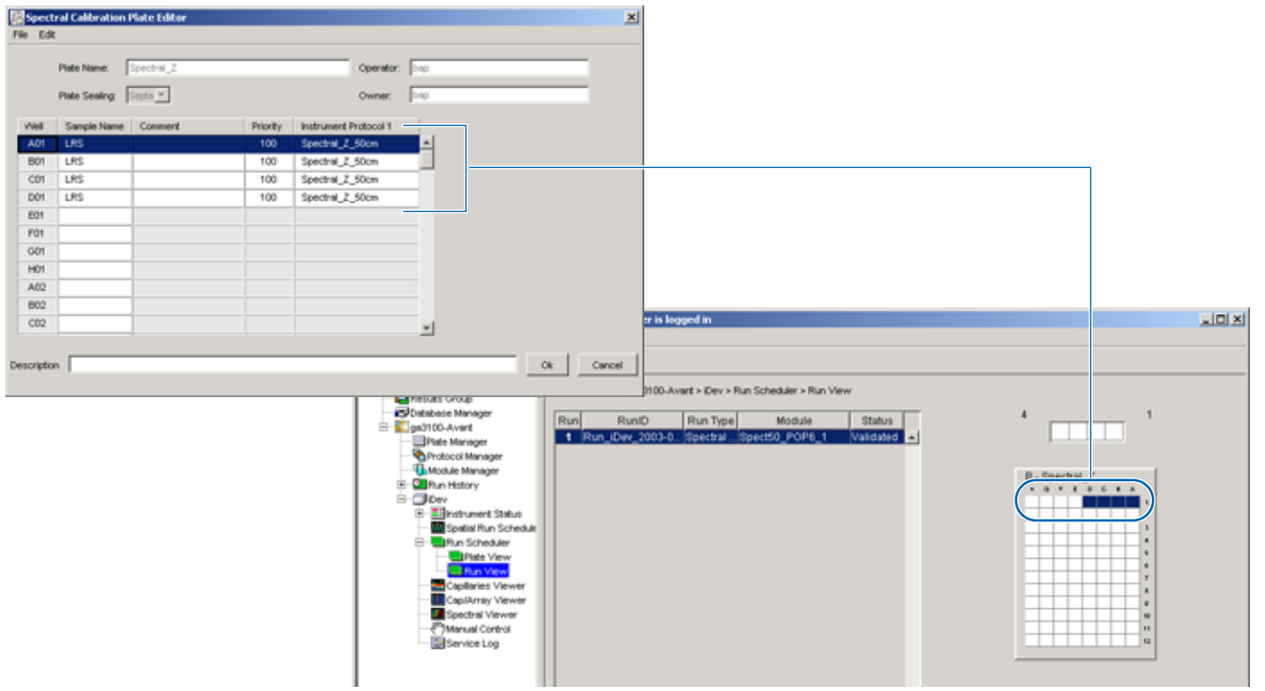


96-well plate on a 3100 instrument

Notes

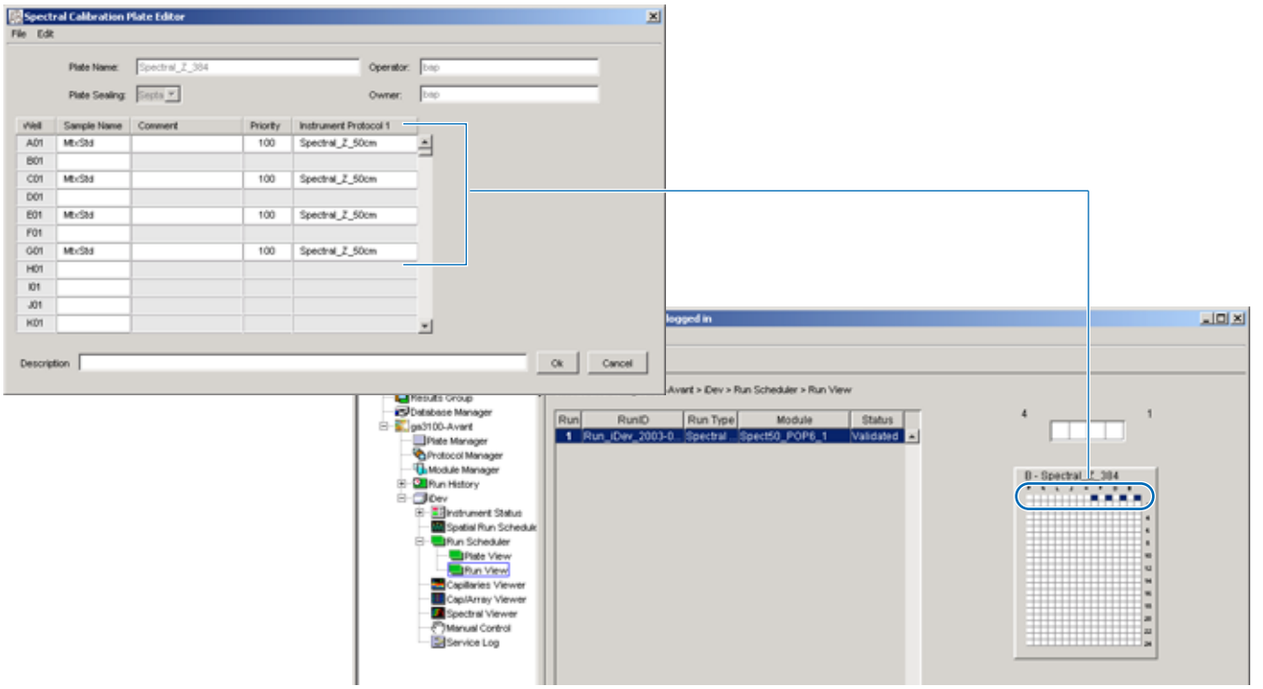


384-well plate on a 3100 instrument



96-well plate on a 3100-*Avant* instrument

Notes



384-well plate on a 3100-*Avant* instrument

Notes

Multi-application (Mixed) Plate Record

Required Files for a Mixed Plate Record

To run a mixed plate with sequencing, SeqScape and/or fragment analysis samples, the following files are required:

- Sequencing analysis (see Chapter 4, page 75)
  - Results Group
  - Instrument Protocol
  - Analysis Protocol
- SeqScape analysis (see Chapter 4, page 101)
  - Results Group
  - Instrument Protocol
  - Analysis Protocol
  - Files created in SeqScape software
- Fragment analysis (see Chapter 5, page 127)
  - Results Group
  - Instrument Protocol
  - Files created in GeneMapper software

Spectral Calibrations

Creating Spectral Calibrations

For every dye set and capillary array length combination you use, a separate spectral calibration *must be* created. Refer to "Performing a Spectral Calibration" on page 45 for more information.

Setting the Active Spectral Calibration

If you changed the capillary arrary length to run multi-application samples, you must set the active spectral calibration for each dye set used. Refer to "Activating a Spectral Calibration" on page 59, for information on how to set the active calibrations, once calibrations are performed for each dye set on each capillary length.

Notes

Creating and Completing a Mixed Plate Record

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3100** or **ga3100-*Avant*** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

3. Complete the information in the New Plate Dialog:

   a. Type a name for the plate.

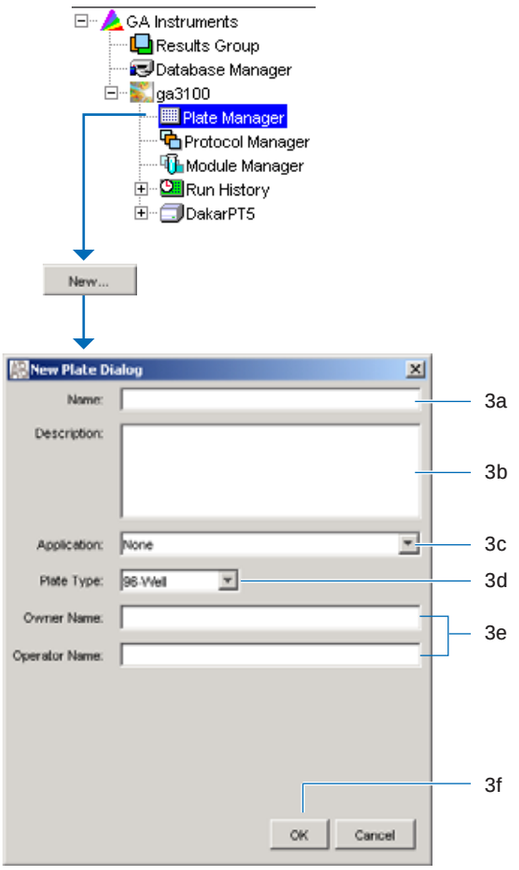
   b. Type a description for the plate (optional).

   c. Select **Mixed** in the Application drop-down list.

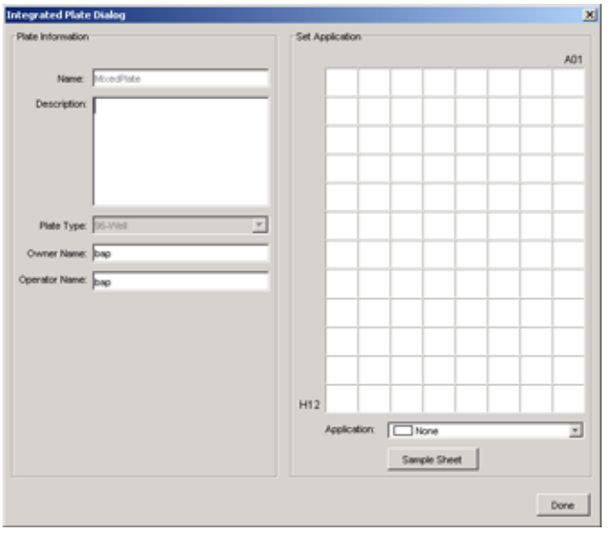
   d. Select **96-well** or **384-well** in the Plate Type drop-down list.

   e. Type a name for the owner and operator.

   f. Click OK.

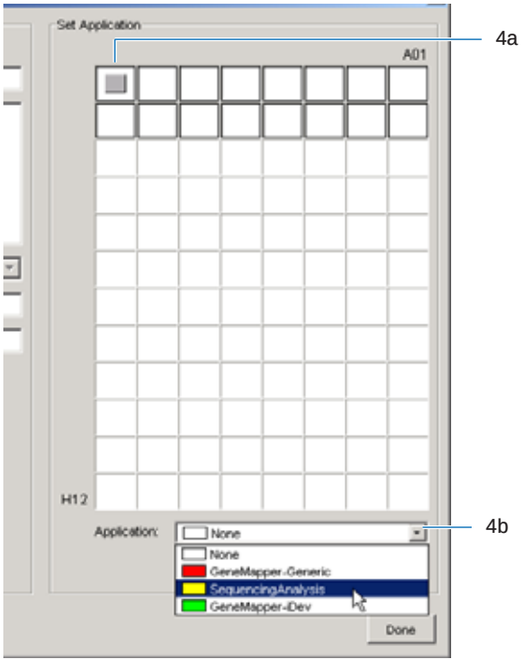


The Integrated Plate Dialog box opens.

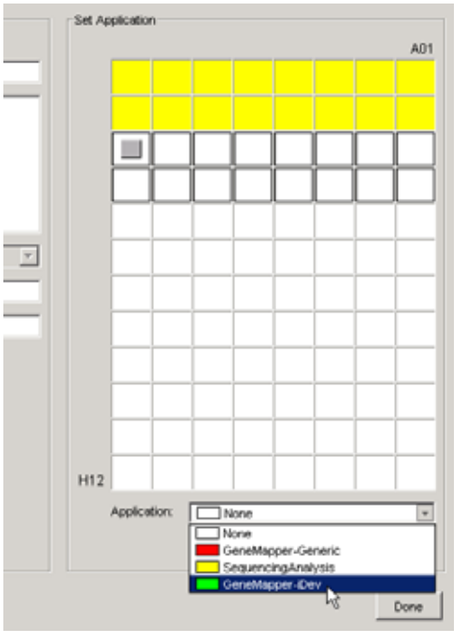


Notes

4. In the Set Application pane:
   a. On the plate map, click a well position. The run of 16 or 4 capillaries is outlined.
   b. In the Application drop-down list select the appropriate application.



   c. Repeat the process for additional samples and applications.

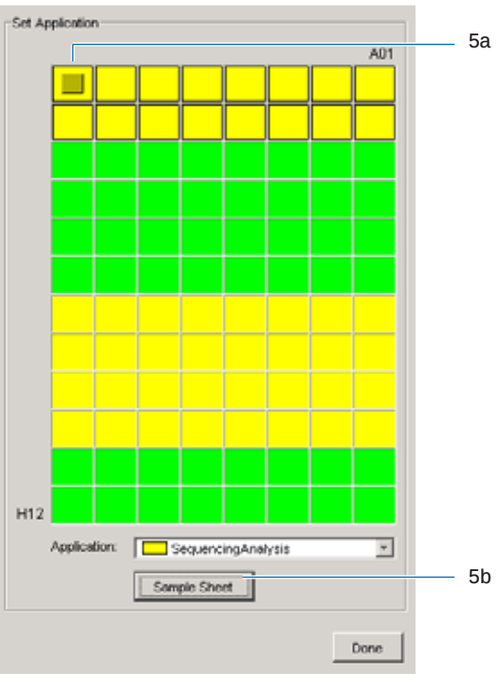


Notes

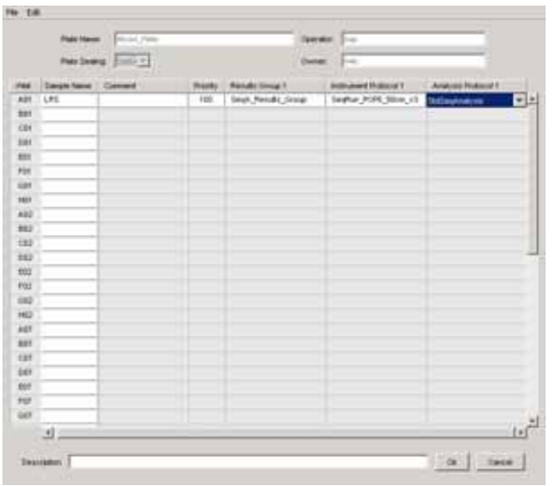
5. Create the Sequencing sample sheets (plate record).

   a. On the plate map, click a well position that represents a sequencing sample.

   b. Click **Sample Sheet**.



   The Sequencing Analysis Plate editor opens.



   c. Complete the plate record.

   Note: The well column contains only those wells that were designated as sequencing samples on the plate map.

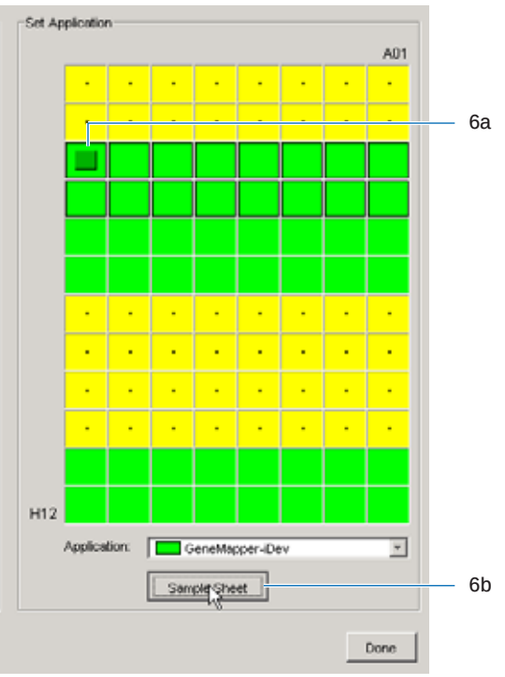
   d. Click OK. You are automatically returned to the Integrated Plate dialog box.

Notes

6. Create the GeneMapper sample sheet (plate record).

   a. On the plate map, click a well position that represents a GeneMapper sample.

   b. Click **Sample Sheet**.

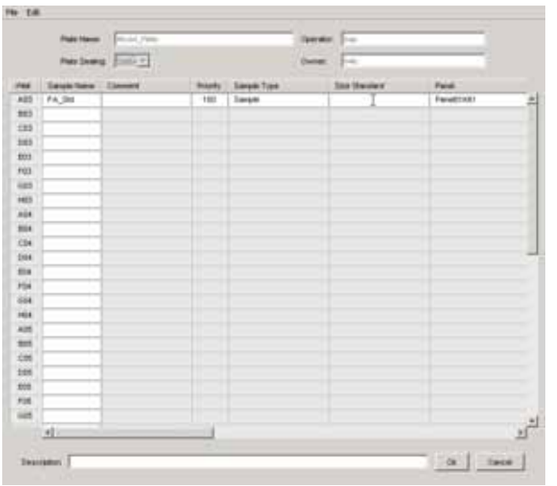


   The GeneMapper Plate editor opens.

   c. Complete the plate record.

   Note: The well column contains only those wells that were designated as fragment analysis samples on the plate map.

   d. Click OK. You are automatically returned to the Integrated Plate dialog box.



7. In the Integrated Plate dialog box, click Done.

Notes

Notes

Basecallers and DyeSet/Primer Files B

This appendix covers:

Notes

ABI PRISM® 3100 Genetic Analyzer Files

3100 Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
KB Basecalling			
ABI PRISM® BigDye® Terminator v1.1	36: ultra rapid	KB.bcp	KB_3100_POP4_BDTv1.mob
	50: std read		
	80: long read		
	36: rapid read		KB_3100_POP6_BDTv1.mob
	50: std read		
ABI PRISM® BigDye® Terminator v3.1	36: ultra rapid		KB_3100_POP4_BDTv3_.mob
	50: std read		
	80: long read		
	36: rapid read		KB_3100_POP6_BDTv3.mob
	50: std read		
ABI Basecalling			
• ABI PRISM BigDye Terminator v1.1	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4LR{BD}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
• ABI PRISM® dGTP BigDye® Terminator	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{BD}v2.mob
	50: std read	Basecaller-3100POP6SR.bcp	
• ABI PRISM BigDye Terminator v3.1	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
• ABI PRISM dGTP BigDye v3.0 Terminator	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{BDv3}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
ABI PRISM® dRhodamine Terminator	36: ultra rapid	Basecaller-3100POP4UR.bcp	DT3100POP4{dRhod}v2.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100POP6RRv2.bcp	DT3100POP6{dRhod}v2.mob
	50: std read	Basecaller-3100POP6SR.bcp	

Notes

3100 Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
ABI Basecalling			
ABI PRISM® BigDye® Primer v1.1	36: rapid read	Basecaller-3100POP6RRv2.bcp	DP3100POP6{BD-21M13}v1.mob DP3100POP6{BD-M13Rev}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
ABI PRISM® BigDye® Primer v3.1	36: rapid read	Basecaller-3100POP6RRv2.bcp	DP3100POP6{BDv3-21M13}v1.mob DP3100POP6{BDv3-M13Rev}v1.mob
	50: std read	Basecaller-3100POP6SR.bcp	
ABI PRISM® BigDye® Primer (All primers)	36: ultra rapid	Basecaller-3100POP4UR.bcp	DP3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100POP4_80cmv3.bcp	

Notes

ABI PRISM® 3100-*Avant* Genetic Analyzer Files

3100-*Avant* Basecaller and DyeSet/Primer Files Used for Dye Terminator Chemistry

DNA Sequencing Chemistry	Capillary Array Length (cm)	Basecaller	DyeSet/Primer
KB Basecalling			
ABI PRISM BigDye Terminator v1.1	36: ultra rapid	KB.bcp	KB_3100_POP4_BDTv1.mob
	50: std read		
	80: long read		
	36: rapid read		KB_3100_POP6_BDTv1.mob
	50: std read		
ABI PRISM BigDye Terminator v3.1	36: ultra rapid		KB_3100_POP4_BDTv3_.mob
	50: std read		
	80: long read		
	36: rapid read		KB_3100_POP6_BDTv3.mob
	50: std read		
ABI Basecalling			
ABI PRISM BigDye Terminator v1.1	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4LR{BD}v1.mob
	80: long read	Basecaller-3100APOP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{BD}v2.mob
	50: std run	Basecaller-3100APOP6SR.bcp	
ABI PRISM BigDye Terminator v3.1	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4{BDv3}v1.mob
	80: long read	Basecaller-3100APOP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{BDv3}v1.mob
ABI PRISM dRhodamine Terminator	36: ultra rapid	Basecaller-3100APOP4UR.bcp	DT3100POP4{dRhod}v2.mob
	80: long read	Basecaller-3100APOP4_80cmv3.bcp	
	36: rapid read	Basecaller-3100APOP6RRv2.bcp	DT3100POP6{dRhod}v2.mob
	50: std run	Basecaller-3100APOP6SR.bcp	

Notes

Troubleshooting C

This appendix covers:

Notes

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. 1. Select **Start** > **Programs** > **Command Prompt**. 2. At the **C:** prompt, type **IPconfig /all**. 3. Press **Enter**. The command prompt window displays information on the network. 4. 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.
Instrument red light is blinking.	Incorrect start up procedure.	Start up in the following sequence: 1. Log out of the computer. 2. Turn off the instrument. 3. Boot up the computer. 4. After the computer has booted completely, turn the instrument on. Wait for the green status light to come on. 5. Launch the Data Collection software.
Data Collection software will not launch.	Applications in the Service Console did not start properly. (It takes several minutes before data collection software opens.)	Restart the computer.
Computer screen is frozen.	Communication error.	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.	1. Close and lock the oven door. 2. Close the instrument doors. 3. 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.

Notes

Spatial Calibration

Troubleshooting spatial calibration		
Observation	**Possible Cause**	**Recommended Action**
Unusual peaks or a flat line for the spatial calibration.	The instrument may need more time to reach stability. An unstable instrument can cause a flat line with no peaks in the spatial view.	Check or repeat spatial calibration.
	Improper installation of the detection window.	Reinstall the detection window and make sure it fits in the proper position.
	Broken capillary resulting in a bad polymer fill.	Check for a broken capillary, particularly in the detection window area. If necessary, replace the capillary array using the Install Array Wizard.
	Dirty detection window.	Place a drop of methanol onto the detection window, and dry with compressed air. Use only light air force. **⚠ 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.

Notes

Spectral Calibration

Troubleshooting spectral calibration		
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 chemistry file, dye set, and/or run module 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.

Notes

Troubleshooting spectral calibration *(continued)*		
Observation	**Possible Cause**	**Recommended Action**
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.

Notes

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: 1. Remove the capillary array. 2. Clean out the polymer block and syringes. 3. 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.

Notes

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. IMPORTANT! You must cycle the power on the instrument to use the new values. • 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 reaction conditions.

Notes

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

Notes

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 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.

Notes

Troubleshooting run performance *(continued)*		
Observation	**Possible Cause**	**Recommended Action**
Loss of resolution.	Too much sample injected.	Dilute the sample and re-inject.
	Poor quality water.	Use high-quality, ultra-pure water.
	Poor quality or dilute running buffer.	Prepare fresh running buffer from 10X 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.

Notes

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.

Notes

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.

Notes

Troubleshooting run performance *(continued)*		
Observation	**Possible Cause**	**Recommended Action**
Migration time becomes progressively slower.	Leak in system.	Tighten all ferrules, screws, and check valves. Replace any faulty parts.
	Improper filling of polymer block.	Polymer pump force may need 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.
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 GeneMapper 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.

Notes

Troubleshooting run performance *(continued)*		
Observation	**Possible Cause**	**Recommended Action**
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: 1. Undo the array ferrule knob and pull the polymer block towards you to first notch. 2. Remove the capillary comb from the holder in oven. 3. Hold both sides of the capillary array around the detection window area, and apply gentle pressure equally on both sides. 4. Release.

Software

Troubleshooting software		
Observation	**Possible Cause**	**Recommended Action**
Autoanalysis does not occur.	Do Autoanalysis was not selected in the Results Group.	Select Do Autoanalysis in the Analysis tab of the Results Group.
Autoanalysis using SeqScape and GeneMapper software does not occur, and Do Autoanalysis is selected in the Results Group.	For SeqScape and GeneMapper software, the log in name and password are incorrect or missing.	Enter the proper log in name and password in the Analysis tab of the Results Group.
Linked plates become unlinked and unlink plate message occurs.	Used a wizard. All linked plated are automatically unlinked when any of the wizards are used.	Relink the plates after completing all wizards.
Cannot set active spectral calibration.	Instrument is running a module.	Set active spectral before starting a run.
Error message occurs while using manual control.		Restart the computer and instrument to recover.

Notes

Troubleshooting software *(continued)*		
Observation	**Possible Cause**	**Recommended Action**
Cannot link a plate.	Wrong array length installed for the Instrument protocol selected.	The software validates the array length in the database against the array length selected in the instrument protocol. Either change the array or edit the Instrument protocol using the correct length. Set or verify the active spectral calibration before starting the run.
Cannot import my plate record created in Microsoft® Excel.	Plate record was saved as an Excel document (.xl s extension).	Save the plate record as a tab deliminated text document (.txt extension), then import the plate record into data collection.
Run will not start, and System Status is blinking red.	Check the error messages in the Event Log: • Database is full	1. Extract any unextracted data. 2. In the Database Manager, click Cleanup Processed Plates, then click OK to confirm. 3. Start run.
	• Drive E is full	1. Archive sample files. 2. Delete files and empty the recycle bin. 3. Start run.
System Status is blinking red.	Check error messages in the Event Log.	Take corrective action based on error messages.

Notes

Notes

Instrument Warranty Information D

This appendix covers:

Notes

Computer Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation.
Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty

Applied Biosystems warrants that all standard components of its ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its ABI Prism 3100 and 3100-*Avant* Genetic Analyzers , such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Notes

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

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Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, Claims, and Returns

Damages

If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns

Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

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