User Guide

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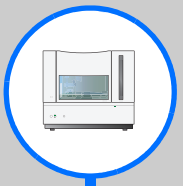
Applied Biosystems

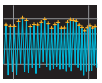
3730/3730xl

DNA Analyzers

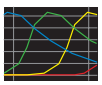
For use with Data Collection Software v2.0



Preparing the Instrument



Performing Spatial Calibration



Performing Spectral Calibration

Setting Up the Plate

Running the Instrument

Shutting Down the Instrument

Performing Instrument Maintenance

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

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NOTICE TO PURCHASER:

The purchase price of this [Capillary Sequencing Instrument Name] includes a grant of a limited, non-transferable license under U.S. Patent No. 5,567,292 and method claims of its foreign counterparts, and under U.S. Patent No. 6,358,385 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.

The Applied Biosystems 3730 and 3730*xl* DNA Analyzer includes patented technology licensed from Hitachi, Ltd. as part of a strategic partnership between Applied Biosystems and Hitachi, Ltd., as well as patented technology of Applied Biosystems.

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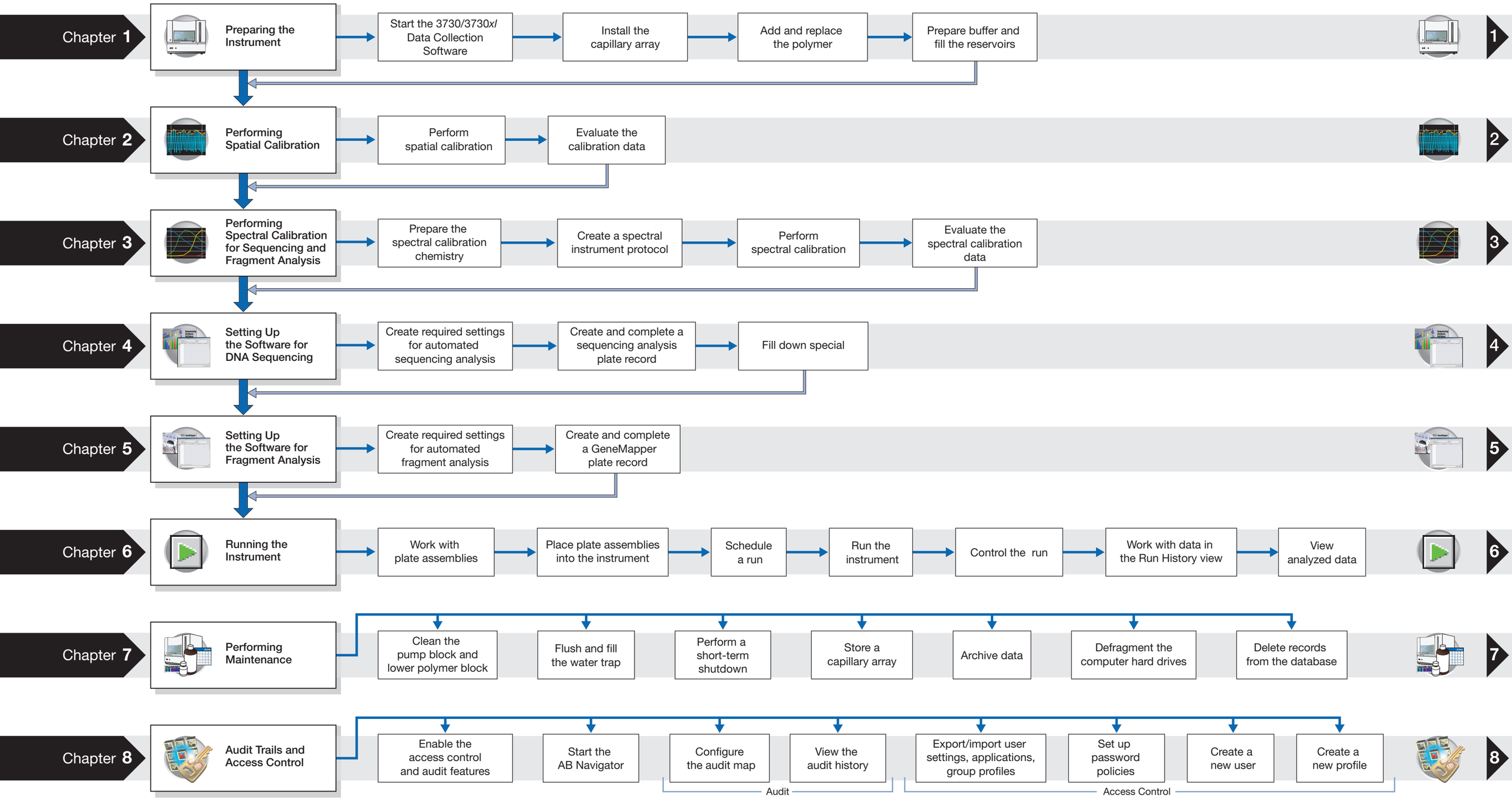
3730/3730*xl* Workflow



Contents

Preface

How to Use This Guide

Purpose of This Guide

This guide is written for the training of principle investigators and laboratory staff who operate and maintain the Applied Biosystems 3730/3730*xl* DNA Analyzers.

Assumptions

This guide assumes the following background:

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

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 xi

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

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

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, damage to an instrument, or loss of data.

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.

Sources of Safety Information

For System Operators

Operational safety information for the Applied Biosystems 3730/3730*xl* DNA Analyzer is provided in the following documents, which are included with each system.

Material Safety Data Sheets (MSDSs)

MSDSs provide information you need to store, handle, transport, and dispose of chemicals safely.

Symbols on Instruments

Electrical Symbols

The following electrical symbols 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 can receive or supply alternating current or voltage.

Symbol	Description
	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 or direct current or voltage.

Safety Symbols

The following safety *s*ymbols 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 xiii). 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.

Symbol	Description
	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 II laser radiation present when open and interlock defeated. Do not stare directly into the beam	**DANGER** de Class II rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class II laser radiation present when open. Do not stare directly into the beam.	**DANGER** de Class II rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

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

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

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.

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 actually 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).

Chemical Safety

Chemical Hazard Warnings

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

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.

CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

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 **E-mail** 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

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and 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, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, and/or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

CHEMICAL WASTE HAZARD. Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Safety Guidelines

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

Waste Profiles

A waste profile for the 3730/3730*xl* DNA analyzer is provided in the *3730/3730xl DNA 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

Shock Hazards

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the 3730/3730*xl* DNA 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.

Fuses

ELECTRICAL SHOCK HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the 3730/3730*xl* DNA analyzer, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power Supply

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 3730/3730*xl* DNA 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 3730/3730*xl* DNA Analyzer. Disconnect power before servicing the 3730/3730*xl* DNA Analyzer.

PHYSICAL INJURY HAZARD. Do not operate the 3730/3730*xl* DNA Analyzer without the arm shield in place. Keep hands out of the deck area when the 3730/3730*xl* instrument autosamplers are moving.

Solvents and Pressurized Fluids

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

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

- Be aware that Radel® 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 Radel® tubing that has been severely stressed or kinked.
- Do not use Radel® tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause Radel® tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Read and follow the guidelines published in:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4)
- Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030).

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.

Laser Safety

Laser Classification

The 3730/3730*xl* DNA Analyzer uses a laser. Under normal operating conditions, the instrument laser is categorized as a Class I 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 IIIb laser.

The 3730/3730*xl* DNA Analyzer laser has been tested to and complies with the "Radiation Control for Health and Safety Act of 1968 Performance Standard CFR 1040."

The 3730/3730*xl* DNA Analyzer laser 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 I 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.

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.

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.

Bar Code Scanner Laser Safety

Laser Classification

The bar code scanner included with the 3730/3730*xl* DNA Analyzer is categorized as a Class II laser.

Laser Safety Requirements

Class II lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

LASER HAZARD. Class II lasers can cause damage to eyes. Avoid looking into a Class II laser beam or pointing a Class II laser beam into another person's eyes.

Computer 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.

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

- Use equipment that comfortably supports 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

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.”

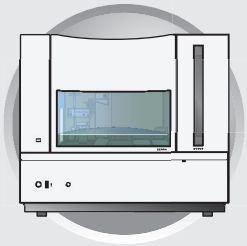
EMC

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 (Group 1, 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.”

Preparing the Instrument



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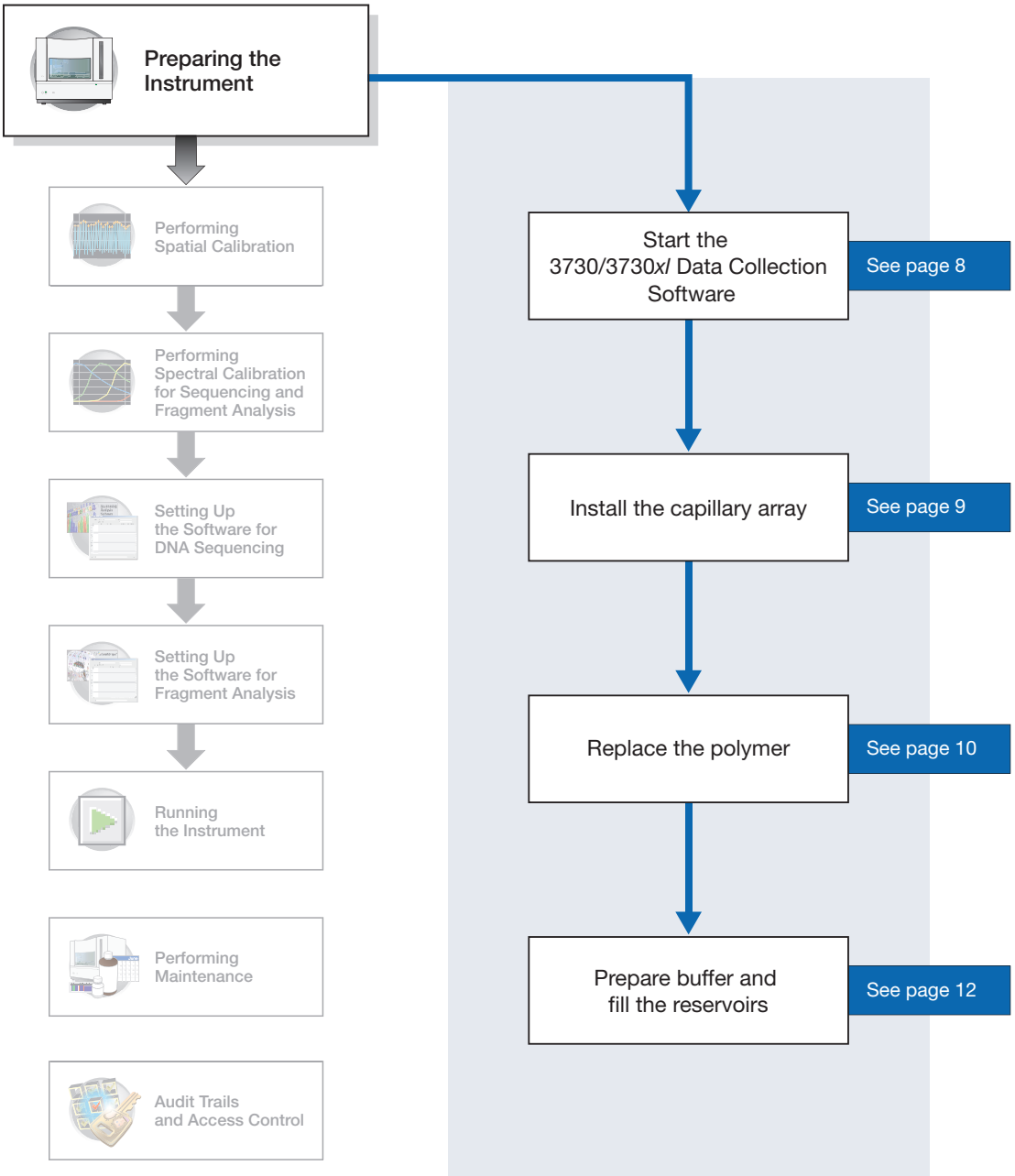
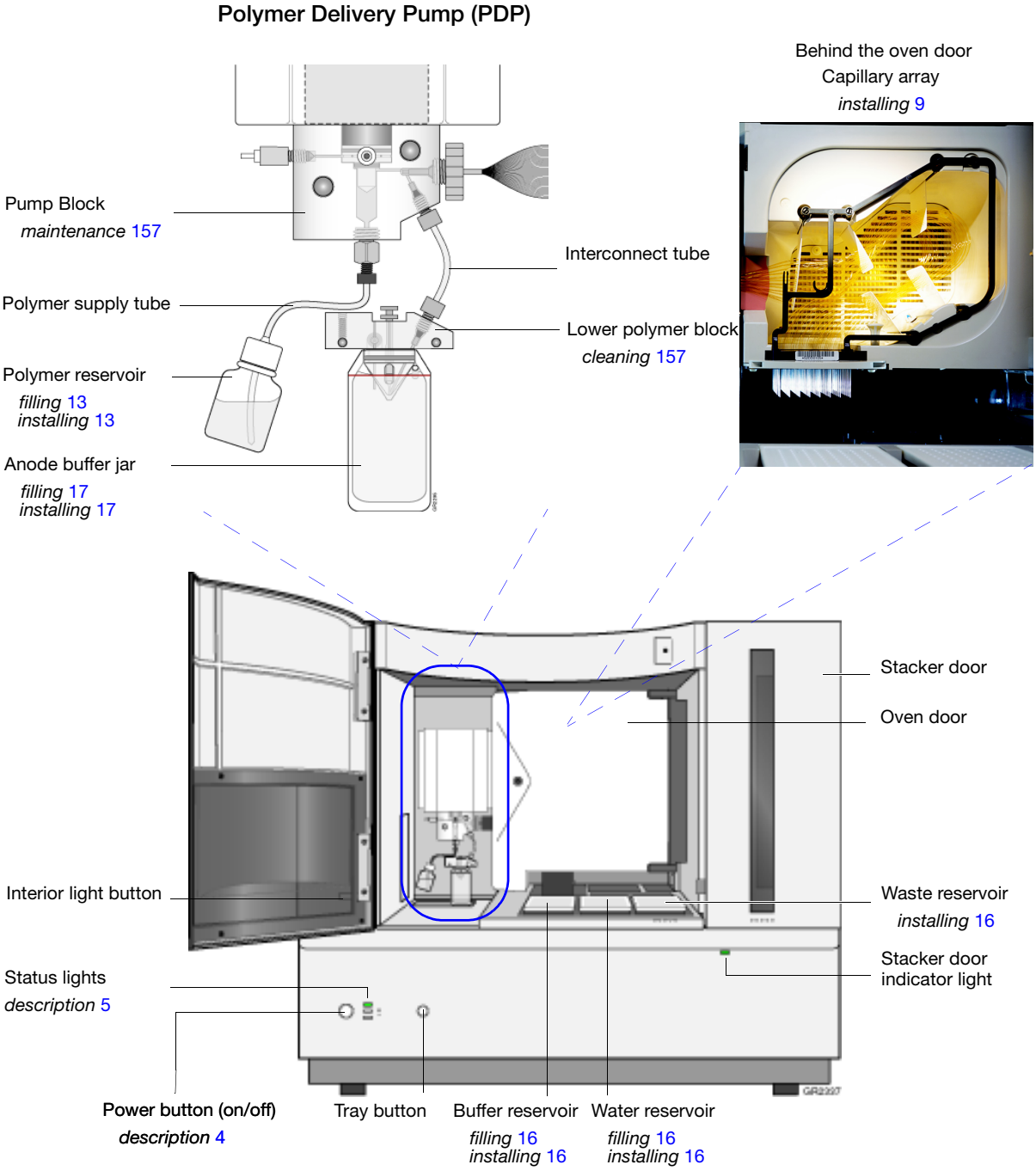
Workflow



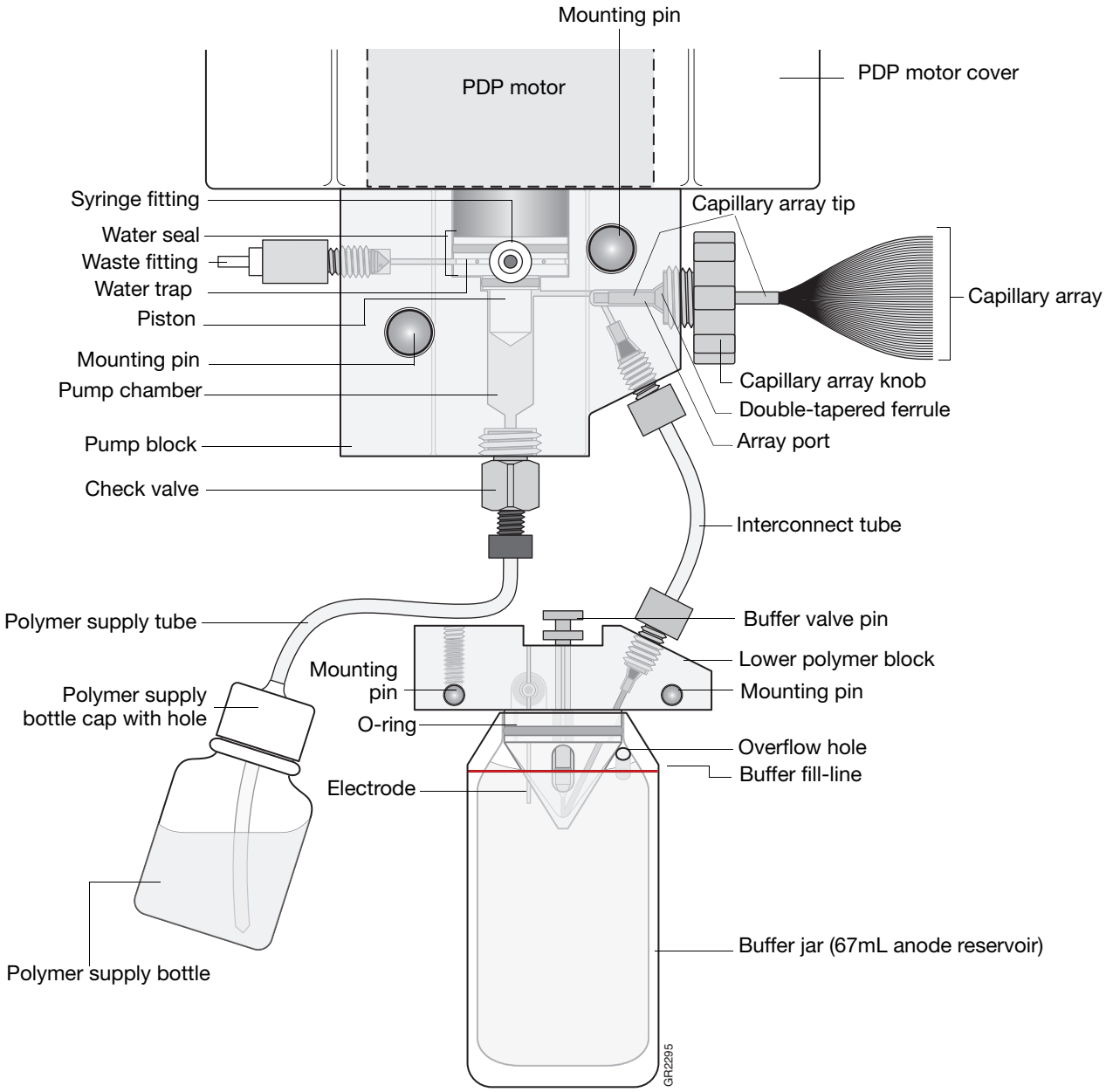
Notes

Instrument and Parts



Notes

Polymer Delivery Pump Detail



Notes

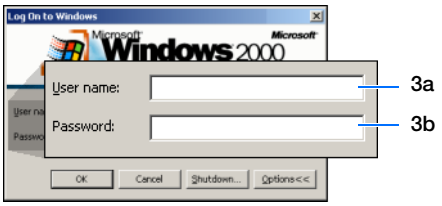
Overview

This chapter explains how to prepare the instrument for a run by installing the capillary array, buffer, and reservoirs.

Powering On the Computer and 3730/3730*xl* Instrument

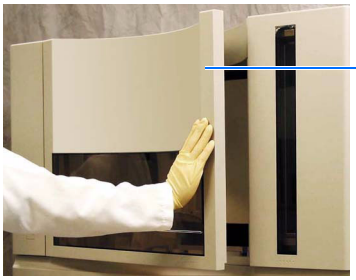
1. Press the power button on the monitor to turn it on.
2. Press the power button on the computer to turn it on.

3. In the **Log On to Windows** dialog box:
   a. In the **User Name** field, enter your user name.
   b. In the **Password** field, enter your password.
   c. Click OK.



4. Close the oven door.
5. Close the stacker drawer.

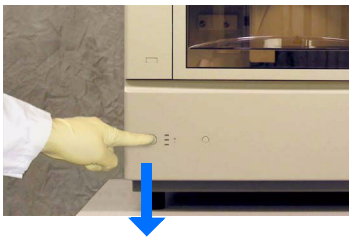
6. Close the instrument door.



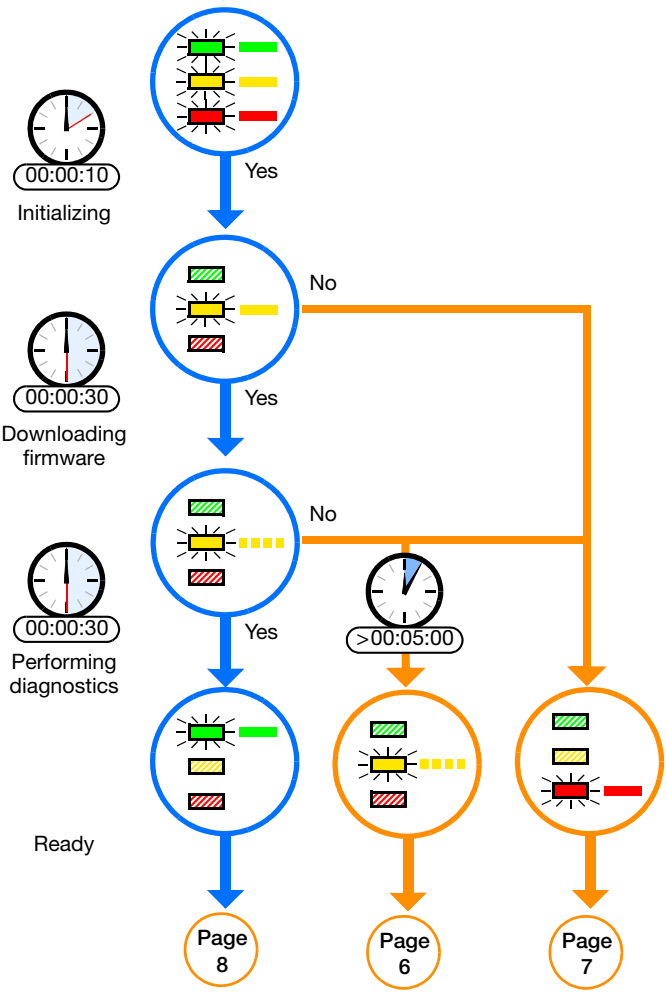
7. Wait until the monitor displays the desktop of the Windows® operating system.

Notes

8. Press the power button on the 3730/3730*xl* instrument to turn it on.



Status	Status Light	Action
• The instrument is ready • An automated wizard operation is in progress with the instrument door closed	solid green	Go to page 8.
• A run is in progress	flashing green	
• The instrument cannot communicate with the computer.	solid yellow	Go to page 7.
• The instrument is downloading firmware • The instrument is performing diagnostics • The oven door is open • The instrument door is open • The buffer reservoir is not installed • The capillary array is not installed • An automated wizard operation is in progress with the instrument door open	flashing yellow	Go to page 7.
• The instrument has detected a problem	solid red	Go to page 7.

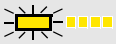


Notes

Troubleshooting

The instrument displays a flashing yellow light.

Determine the source of the problem as follows:



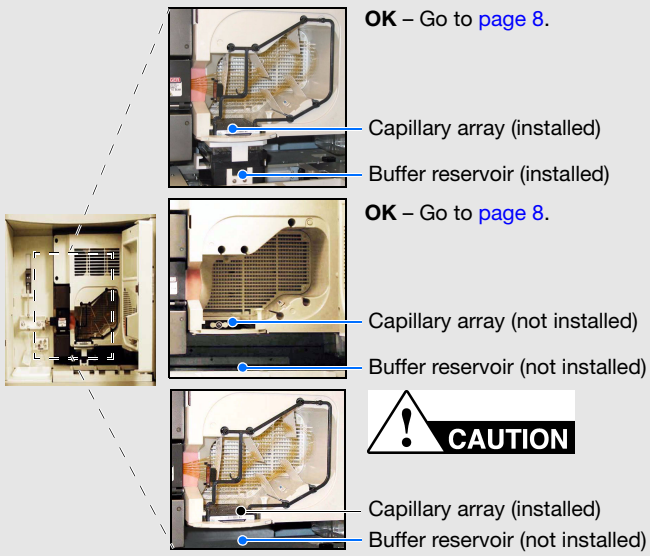
1. Press on the instrument door to ensure that it is closed.

   If the 3730/3730*xl* instrument displays the green status light, then the instrument door was open. Go to page 8

2. If the 3730/3730*xl* instrument continues to display the flashing yellow light:
   a. Open the instrument door.
   b. Press on the oven door to verify that it is closed.
   c. Close the instrument door.

   If the 3730/3730*xl* instrument displays the green status light, then the oven door was open. Go to page 8

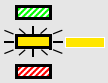
3. If the 3730/3730*xl* instrument continues to display the flashing yellow light:
   a. Open the instrument door.
   b. Open the oven door.
   c. Check that the buffer reservoir and capillary array are installed.
   d. Close the oven door.
   e. Close the instrument door.



Notes

Troubleshooting *(continued)*

The Instrument displays a solid yellow light.



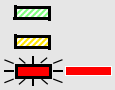
Determine the source of the problem as follows:

Verify that the ...

1. Monitor displays the desktop of the Windows operating system.
2. Ethernet cable is connected to the back of the 3730/3730*xl* instrument.
3. Other end of the Ethernet cable is connected to the computer.
4. Instrument door is closed.
5. Buffer, water, and waste reservoirs are in place.
6. 3730User account password is functional.

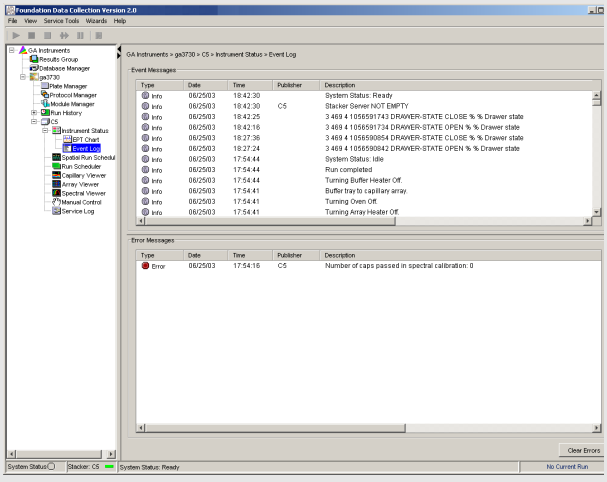
If the instrument continues to display the solid yellow light, contact Applied Biosystems technical support or your service representative for further assistance.

The Instrument displays a solid red light.



Determine the source of the problem as follows:

1. If the instrument continues to display the solid red light:
   a. Turn off the instrument.
   b. Wait for 30 seconds.
   c. Turn on the instrument.
2. If the instrument continues to display the solid red light:
   a. Start the 3730/3730*xl* Data Collection Software as explained on page 8.
   b. In the Tree pane of the Data Collection Software, double-click **GA Instruments** > **ga3730** > *instrument name* > **Instrument Status** > **Event Log**.
   c. In the Event Log view, find the last message in the log file.
   d. Using the error code, perform the required tasks to fix the problem.
3. If the instrument continues to display the solid red light, contact Applied Biosystems technical support or your service representative for further assistance.

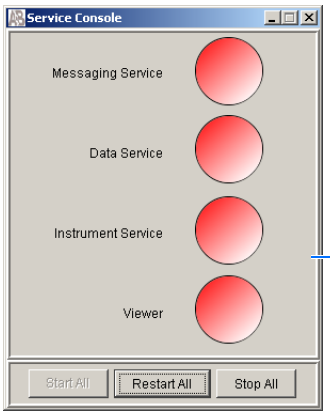


Notes

Starting the 3730/3730*x*l Data Collection Software

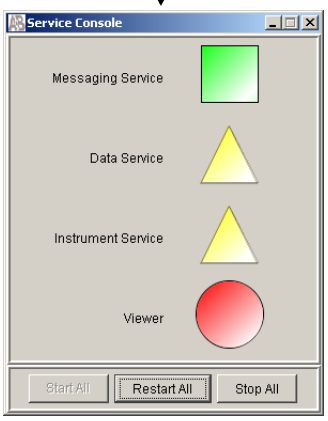
1. Select Start > **Programs** > **Applied Biosystems** > **Unified Data Collection** > **Run Unified Data Collection v2.0**.

   The data collection software opens the Service Console dialog box.

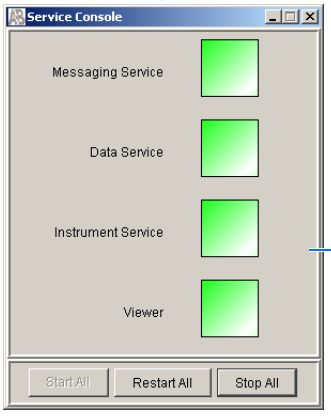


Red circles indicate that applications of the data collection software are not running.

Wait for the Service Console dialog box to open the applications of the data collection software.



When all applications are running (green squares), the Data Collection software opens the Data Collection Viewer.



Applications of the data collection software are running

Notes

Installing the Capillary Array

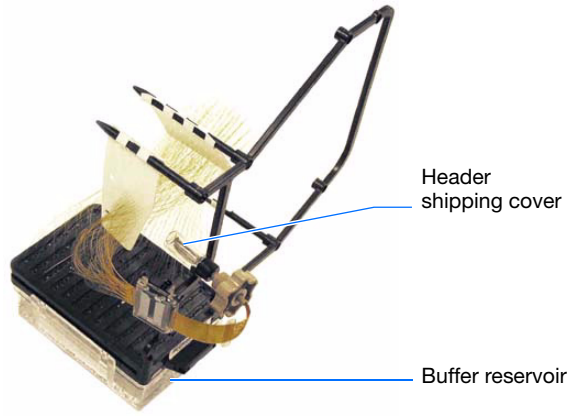
Required Materials

- Capillary array, 96- or 48-capillary
- Lab wipes, lint-free
- Gloves

WARNING **CHEMICAL HAZARD. POP-7 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.

Guidelines for Capillary Use

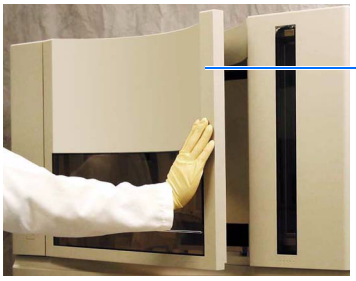
- Do not bend the capillaries
- Store capillary arrays using a buffer reservoir and the header shipping cover (for storing information see, page 168).



Installing a New or Used Capillary Array

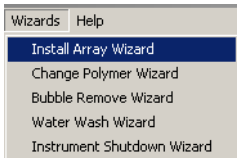
IMPORTANT! Wear gloves when you handle the capillary array.

1. Close the instrument door.



2. In the Data Collection software, select **GA Instructments** > **ga3730** > *instrument name* >.

3. On the toolbar, select **Wizards > Install Array Wizard**.



4. Install the array as instructed by the Array Wizard.

After Replacing the Capillary Array

- Perform a spatial calibration

Notes

Replacing the Polymer

Note: This section may be skipped if you have installed a capillary array using the Install Array wizard during the initial activation of the instrument.

Required Materials

- POP-7 polymer
- Wipes, lint-free
- Gloves

Guidelines for Polymer Use

- Check the polymer blocks and lines daily for bubbles.
- Ensure that you have enough polymer for operation:
  - A 96-capillary run uses approximately 250 µL of polymer
  - A 48-capillary run uses approximately 110 µL of polymer.

When to Replace the Polymer

Replace the polymer on the instrument:

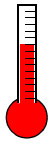
- Weekly (polymer lifetime is 7 days at 25 °C)
- If insufficient polymer remains for the planned run set

IMPORTANT! Failure to replace expired/old polymer may lead to loss of resolution and data quality.

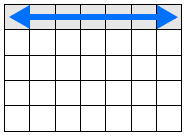
IMPORTANT! Wear gloves when you handle polymer.

WARNING **CHEMICAL HAZARD. POP-7 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.

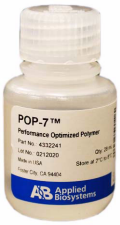
POP-7 Storage



2 °C to 8 °C

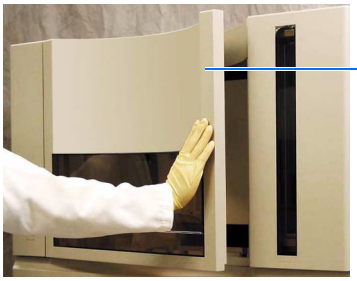


Until expiration date on the bottle

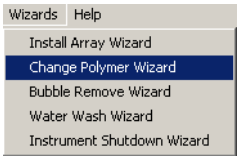


Notes

1. Close the instrument door.
2. In the Data Collection software, select **GA Instruments** > **ga3730** > *instrument name* >.



3. On the toolbar, select **Wizards** > **Change Polymer Wizard**.



4. Change the polymer as instructed by the Change Polymer wizard.

Notes

Preparing Buffer and Filling the Reservoirs

Required Materials

- Retainer, buffer/water/waste
- Septa
- Reservoir caps
- Reservoir, buffer/water/waste
- Plate base, water/waste
- Plate base, buffer
- Water, deionized, 180 mL plus, 160 mL for water and waste reservoirs
- 10X Genetic Analyzer Running Buffer with EDTA, 20 mL
- Graduated cylinder, 250-mL
- Gloves, silicone-free, powder-free

WARNING **CHEMICAL HAZARD. Running Buffer with EDTA**. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Guidelines for Buffer Use

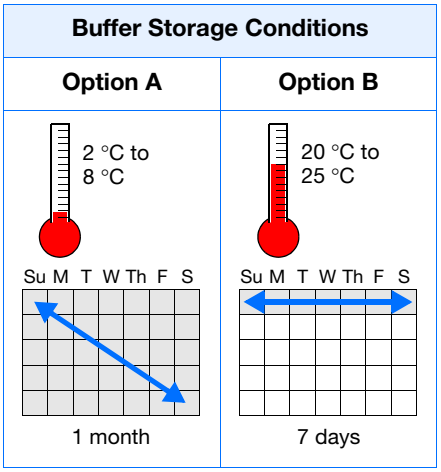
The 1X run buffer can be stored at:

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

When to Change the Buffer, Water, and Waste

Replace the buffer in the reservoirs every 48 hours, or before each batch of runs.

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

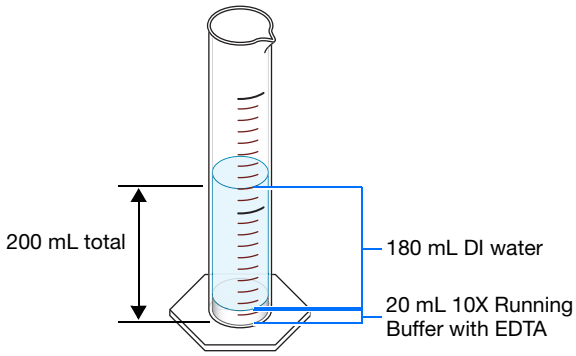


Notes

Preparing the 1X Run Buffer

IMPORTANT! Wear gloves when you handle running buffer with EDTA.

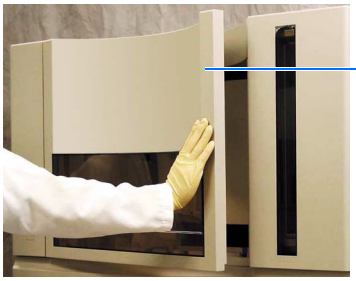
1. Add 20 mL 10X running buffer with EDTA into a graduated cylinder.
2. Add 180 mL (qs) deionized water to bring the total volume to 200 mL.
3. Mix well and set aside.



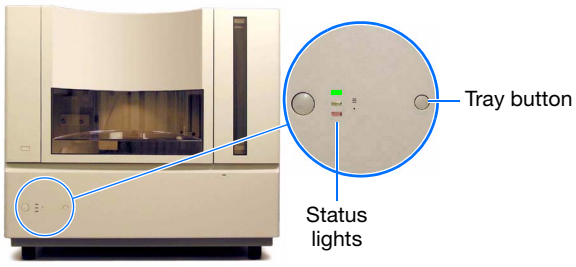
Filling the Water and Buffer Reservoirs

IMPORTANT! Wear gloves when you handle the reservoir.

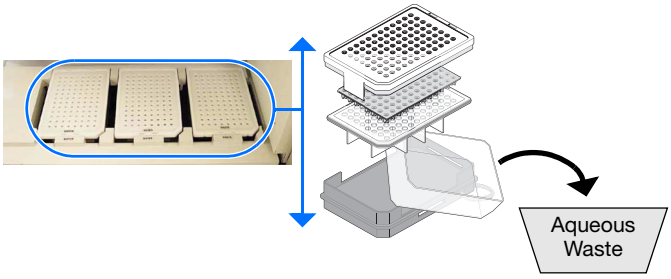
1. Close the instrument door.



2. Press the Tray button to bring the autosampler to the forward position.
3. Wait for the autosampler to stop moving and for the green status light to illuminate, before you open the instrument door.

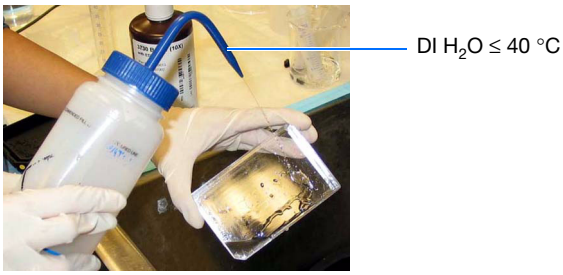


4. Unplug the buffer reservoir. Remove the buffer, water, and waste reservoir assemblies from the instrument.
5. Disassemble each reservoir assembly and empty the contents of the reservoirs into an aqueous waste container.



Notes

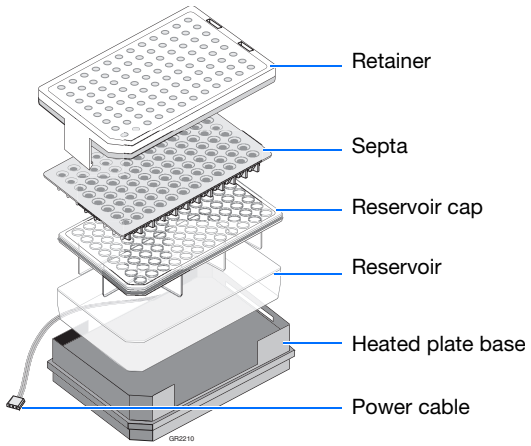
6. Rinse each reservoir using deionized water.
7. Dry the reservoirs using lint-free wipes.

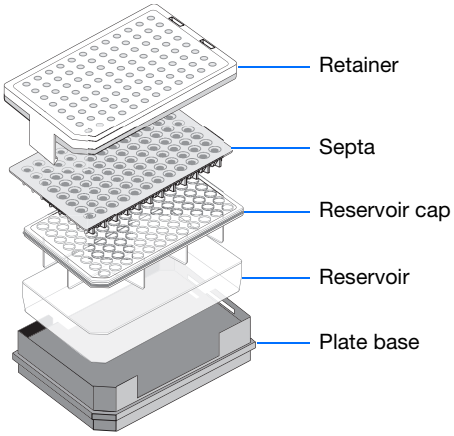


Notes

8. Fill and assemble the reservoirs.

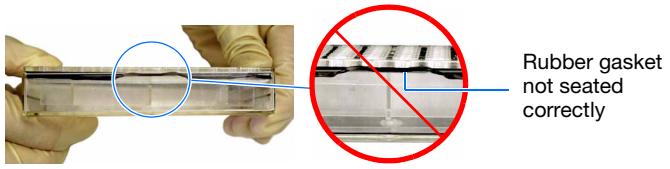
Buffer Reservoir Assembly	Water and Waste Reservoir Assemblies
1. Add 80 mL 1X run buffer to the Buffer reservoir. 2. Assemble the reservoir assembly as shown below:	1. Add 80 mL high-quality deionized water to each reservoir (Water and Waste). 2. Assemble each reservoir assembly as shown below:



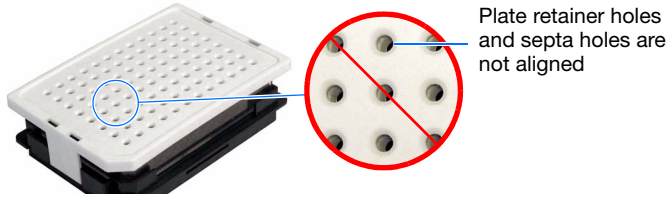


9. To prevent damage to the capillary array, inspect each reservoir assembly and verify that the:

- Septa fits snugly and flush on the reservoir cap
- Rubber gasket around the edge of the reservoir cap is seated correctly



- Holes of the plate retainer and the septa strip are aligned

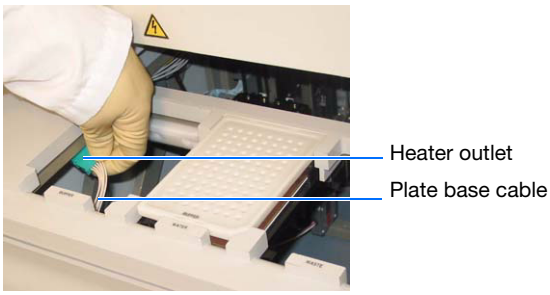


10. Dry the reservoirs using lint-free wipes.

Notes

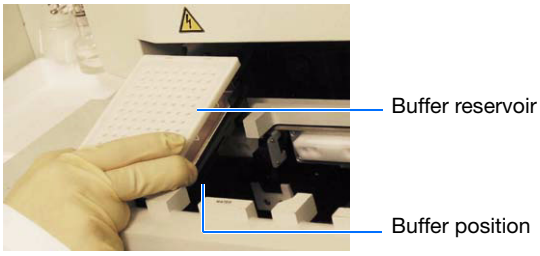
Placing Reservoirs into the Instrument

1. Connect the Buffer reservoir plate base cable into the heater outlet within the instrument.

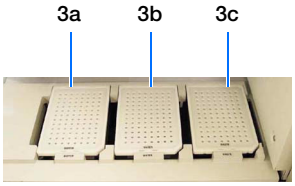


2. Move the buffer reservoir to the Buffer position (left).

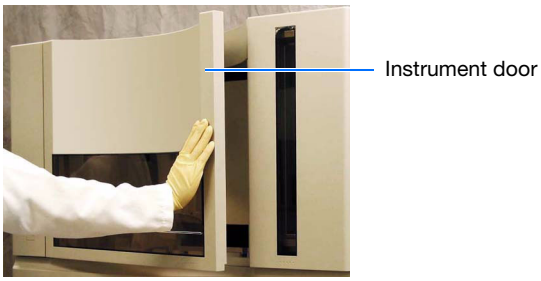
   IMPORTANT! After placing the buffer reservoir, make sure the cable is out of the way of the autosampler.



3. Place the Water and Waste reservoirs into the instrument. The reservoirs must be in the following order from left to right:
   a. Buffer reservoir
   b. Water reservoir
   c. Waste reservoir



4. Close the instrument door.



5. Press the tray button to return the autosampler to the array position.

Notes

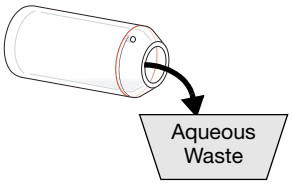
Filling the Anode Buffer Jar

Change the anode buffer:

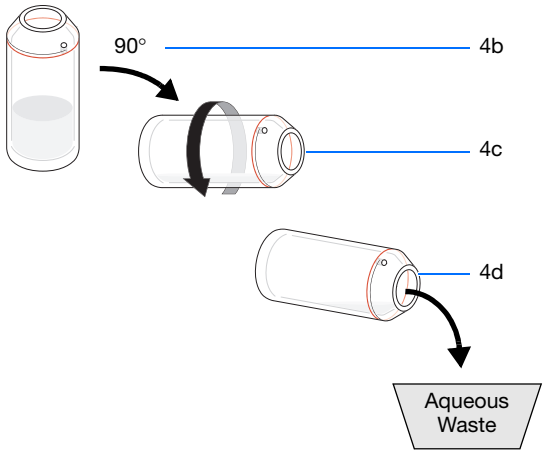
- Before each group of scheduled runs, or at least every 24 to 48 hours
- Every time you fill the polymer block with new polymer
- Every time you change the buffer reservoir

IMPORTANT! Wear gloves when you handle the anode buffer jar.

1. Remove the anode buffer jar by pulling down while twisting slowly.



2. Empty the anode buffer jar into an aqueous waste container.
3. Rinse the anode buffer jar using deionized water.
4. Rinse the anode buffer jar using 1X run buffer:
   a. Add 5 mL 1X run buffer to the anode buffer jar.
   b. Tilt the anode buffer jar 90°.
   c. Rotate the jar to rinse the interior with buffer.
   d. Empty the anode buffer jar into an aqueous waste container.



5. Add 67 mL 1X run buffer to the jar.

Notes

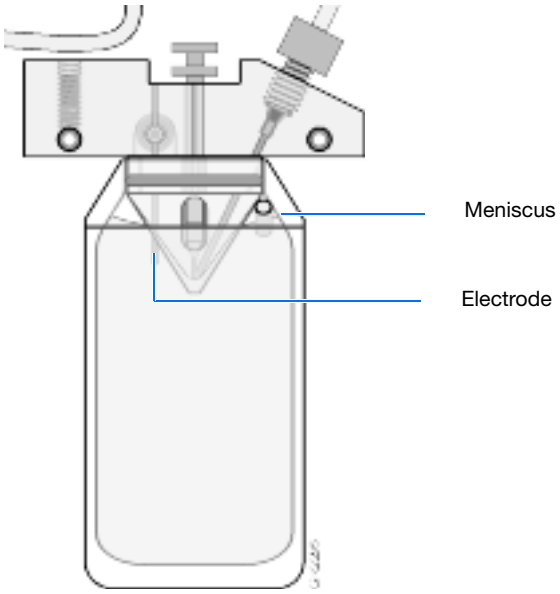
6. Put the anode buffer jar on the instrument with the overflow hole facing you.

   Note: The meniscus should line up just under the red fill line when installed on the instrument.

7. Verify that the electrode is immersed in the buffer.

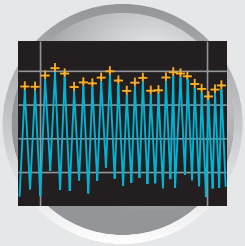
8. If the reservoir fills completely as polymer is added, perform steps 1 through 7 of this procedure to discard and replace the running buffer.

   Note: Replace buffer if excess polymer is expelled into the anode jar.

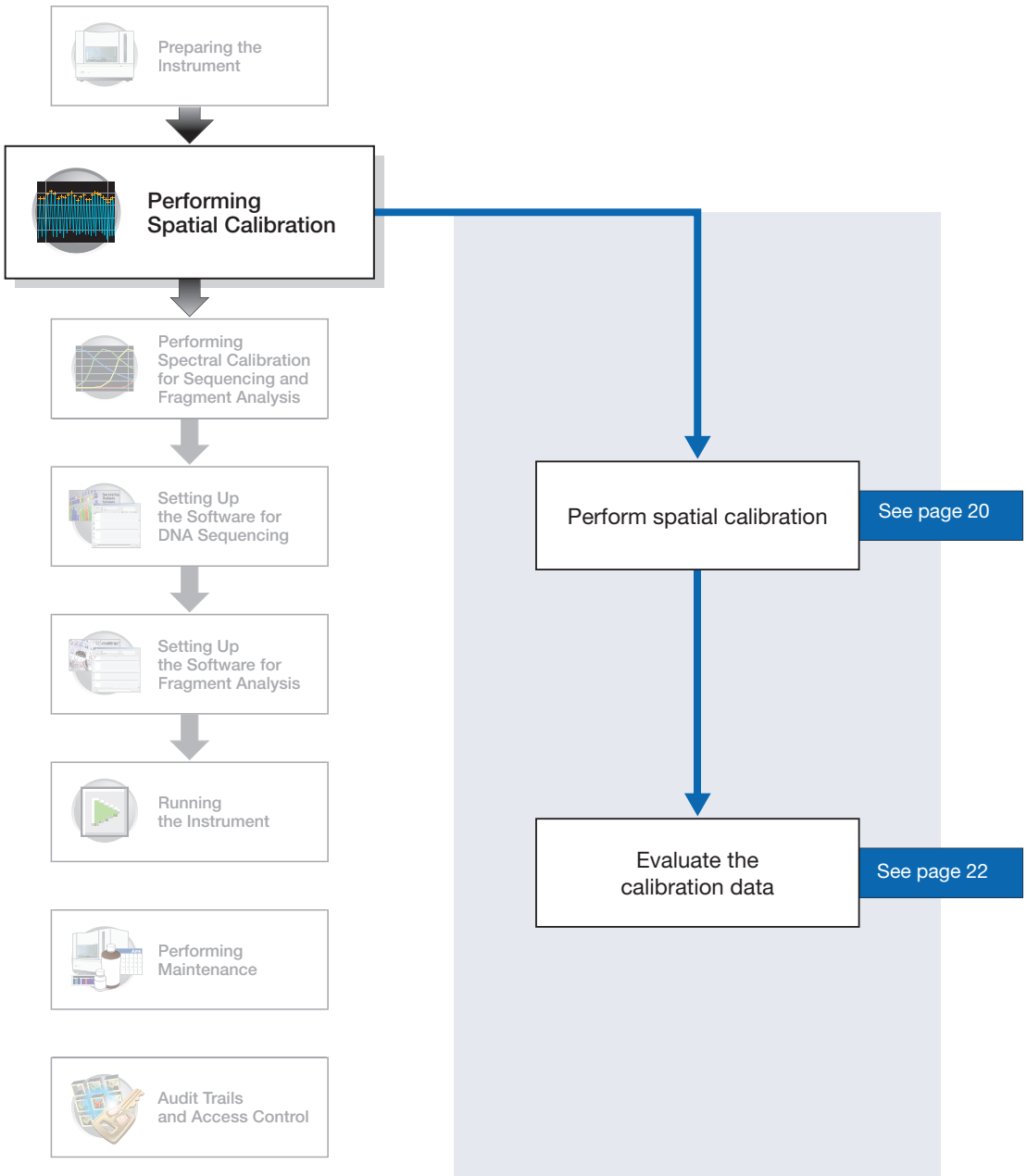


Notes

Performing Spatial Calibration



Workflow



Notes

Overview

What a Spatial Calibration Tells You

The 3730/3730*xl* Data Collection Software uses images collected during spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls and is detected by the CCD camera.

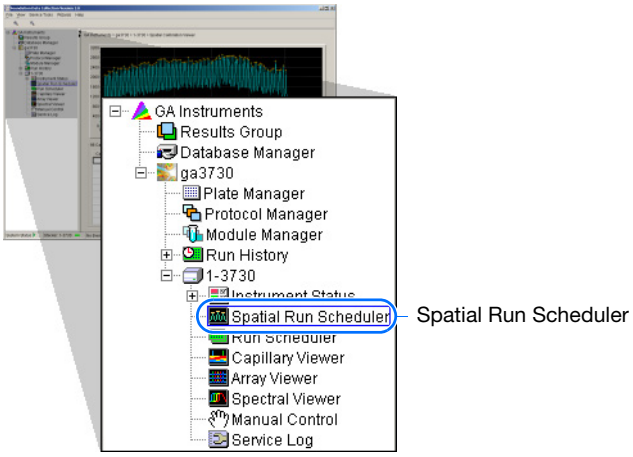
When to Perform the Spatial Calibration

Perform a spatial calibration after you:

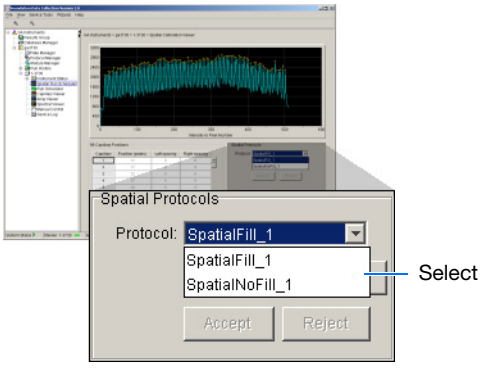
- Install a new or used capillary array
- Remove the capillary array from the detection cell block (even to adjust it)
- Move the instrument (even if the instrument was moved on a table with wheels)

Performing Spatial Calibration

1. In the Tree pane of the Data Collection Software, double-click **GA Instruments** > **ga3730** > *instrument name* > **Spatial Run Scheduler**.

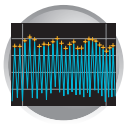


2. In the Spatial Run Scheduler view, do one of the following:
   - If the capillaries contain fresh polymer, select **Protocol** > **SpatialNoFill**.
   - Otherwise, select **Protocol** > **SpatialFill**.



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

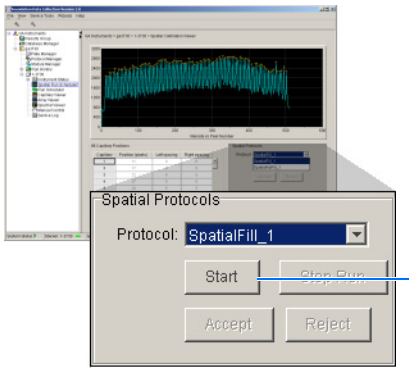
Notes



3. Click Start.

The approximate calibration run times are:

- 48-cap/36cm array with fill, 4 minutes.
- 96-cap/36cm array with fill, 3 minutes.
- No fill, 2 minutes.



4. Evaluate the calibration as explained on page 22.

Notes

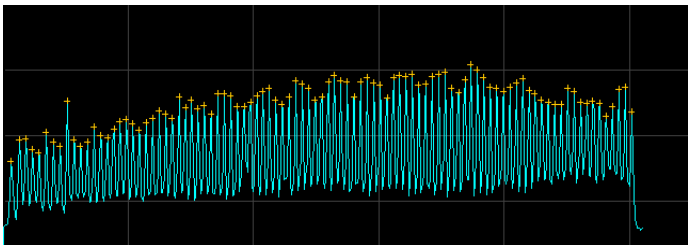
Evaluating the Calibration Data

Note: Examples of passing spatial calibration profiles start on page 27.

1. Verify that the peaks of the spatial are approximately the same height.

Are the peaks in the profile approximately the same height?

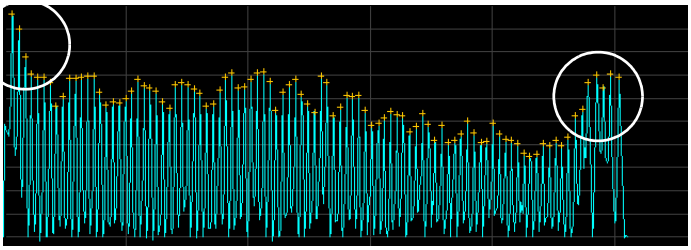
Yes – Go to step 2 on page 22.



No – How does the peak height vary?

- If the peak height increases at the beginning and the end of the spatial profile, then the variation in peak height is acceptable.

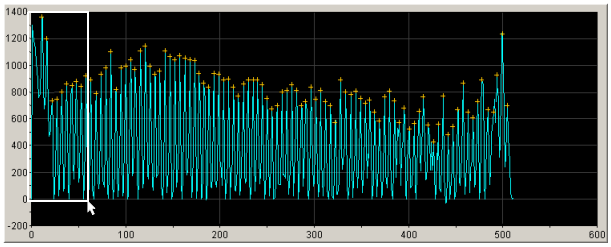
  Go to step 2 on page 22.



Irregular – If the peak heights are irregular, go to "If the Calibration Fails" on page 25.

Magnifying the Spatial Profile

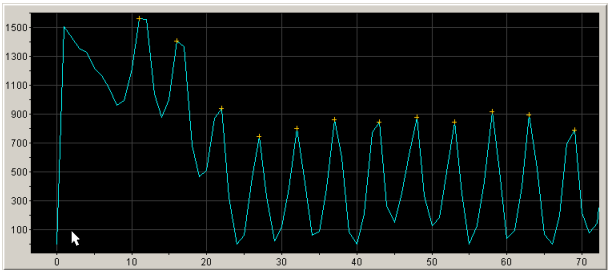
a. Click and drag the cursor to create a box around the area of interest.



b. Release the mouse button.

The data collection software displays the selected region.

c. Press **R** to reset the view.



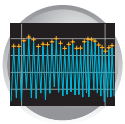
2. Verify that an orange cross appears at the top of each peak in the profile.

Does a cross appear at the top of each peak?

Yes – Go to step 3.

No – Where in the profile is the peak located?

Notes



- Left side of the profile

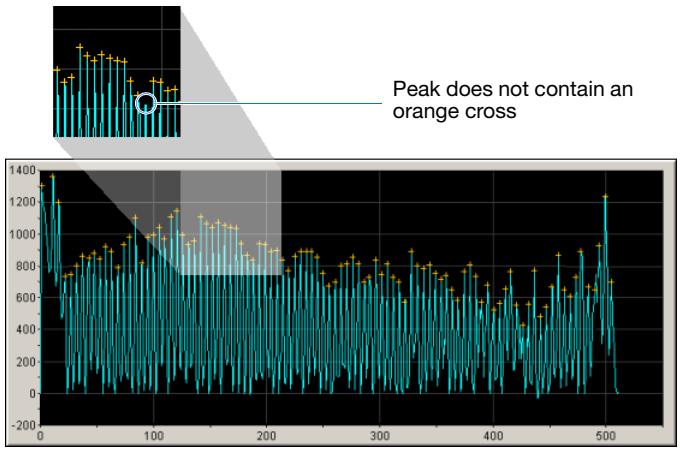
  If using a 96-capillary array, a small peak may appear in the left side of the profile.

  The peak is normal, go to step 3.

- After the first peak

  The data collection software did not locate the peak correctly.

  Move an orange cross to cover the peak. See, "To move an orange cross:" on page 24.



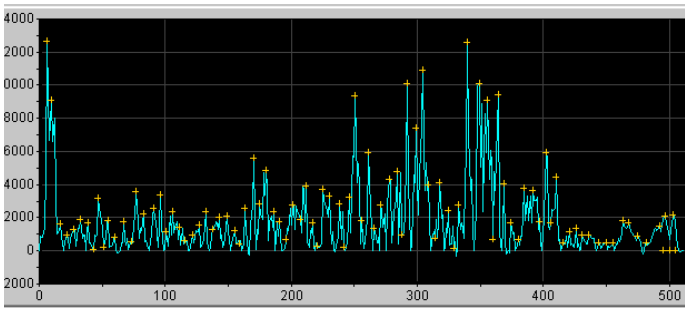
3. Check the profile for irregular peaks.

   Does the profile contain any irregular peaks?

   Yes – The calibration run has failed. Go to "If the Calibration Fails" on page 25.

   No – Go to step 4.

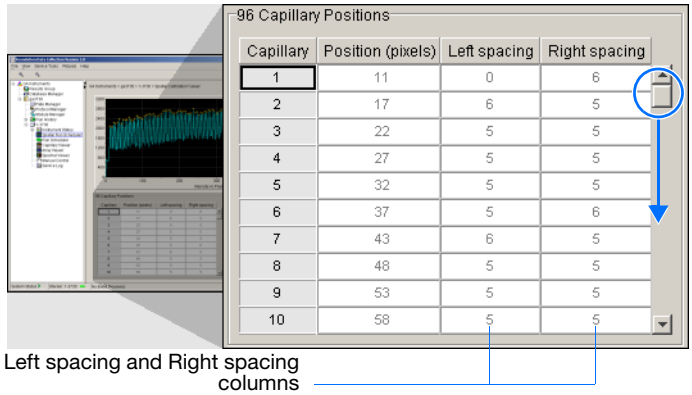
Elements of a poor spatial



4. Examine each row of the 96 Capillary Position table. Typical values for the **Left spacing** and **Right spacing** columns are:
   - 4 to 8 pixels for a 96-capillary array
   - 9 to 11 pixels for a 48-capillary array

   Note: Values greater than those stated above are acceptable if you are able to see a corresponding gap in the capillaries in the detection cell.

   Be sure to account for all capillaries (e.g., 96 capillary positions for 96 capillary array).
   - If *not*, verify that all peaks have crosses. If each peak does not each have a cross, see the Troubleshooting table below.
   - If *yes*, go to step 5.

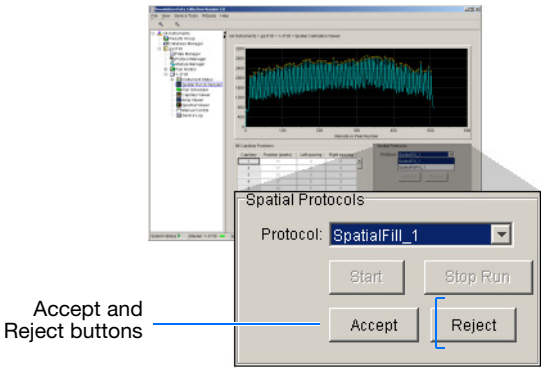


Notes

5. Accept or reject the spatial calibration as follows:

If the calibration:

- Passed, click Accept writes the calibration data to the database.
- Failed, click Reject, then go to "If the Calibration Fails" on page 25.



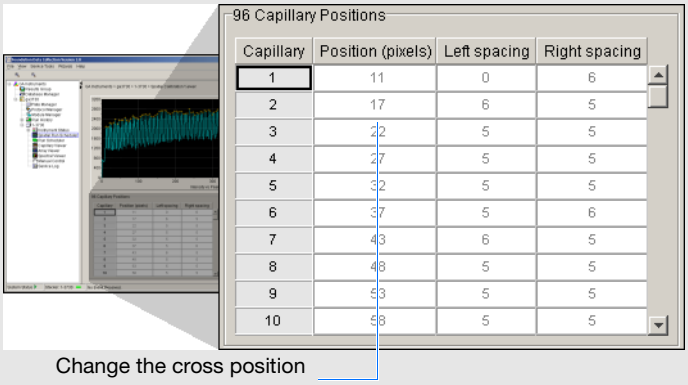
Troubleshooting

Peak does not contain an orange cross.

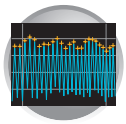
Note: The cross positions cannot be altered after you have accepted the calibration data.

To move an orange cross:

1. Magnify the view of the peak without a cross.
2. Determine the peak pixel position.
3. Change the value for the incorrectly positioned cross.
4. Click outside of that box.



Notes



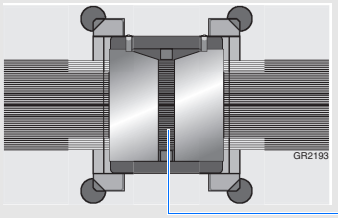
Troubleshooting

If the 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:

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

1. Go to step 1 on page 20 and repeat the spatial calibration.
2. If the calibration fails again:
   a. Follow the Bubble Remove wizard to remove bubbles and to fill the capillaries with polymer.
   b. Go to step 1 on page 20 to repeat the spatial calibration.
3. If the calibration fails again:
   a. Open the Instrument door.
   b. Open the oven door.
   c. Open the detection cell door and turn the cam knob 1/4 turn clockwise (pointer left).
   d. Pull the pump and lower polymer blocks forward until the detection cell comes out of the detection block.
   e. Remove from the pump block:
      – Tip of the capillary array
      – Array knob
      – Ferrule
   f. Add one drop of methanol to a sterile swab or lint-free wipe and apply to the front surface of the detection cell.
   g. Gently clean the front surface of the detection cell using the sterile swab or lint-free wipe.
   h. Replace the tip of the capillary array, array knob, and ferrule into the pump block.
   i. Push the pump and lower polymer blocks back against the pump panel, making sure that the buffer valve lever properly engages the buffer pin valve.
   j. Carefully place the detection cell into the detection block and secure it by rotating the cam knob 1/4 turn counterclockwise (pointer down).
   k. Close the detection cell door.
   l. Close the oven door.
   m. Close the Instrument door.
   n. Using the Bubble Remove wizard, remove all bubbles. Pay particular attention to the array port area.
   o. Go to step 1 on page 20 to repeat the calibration.

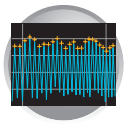


Notes

Troubleshooting

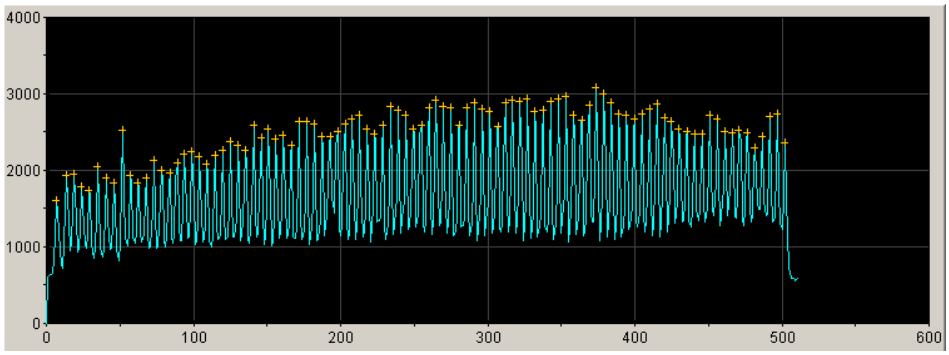
4. If the calibration fails again:
   a. Perform steps 3a through 3c.
   b. Reposition the capillary array window in the detection cell.
   c. Perform steps 3j through 3m.
   d. Go to step 1 on page 20 to repeat the calibration.
5. If calibration fails again, replace the capillary array as explained in "Installing the Capillary Array" on page 9.

Notes



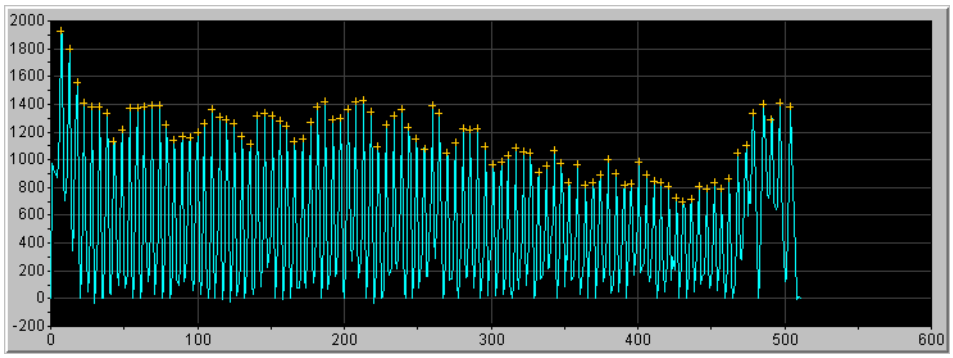
Examples of Passing Spatial Profiles

IMPORTANT! Improper peak identification may lead to sample mistracking on the instrument, and potential sample misnaming.

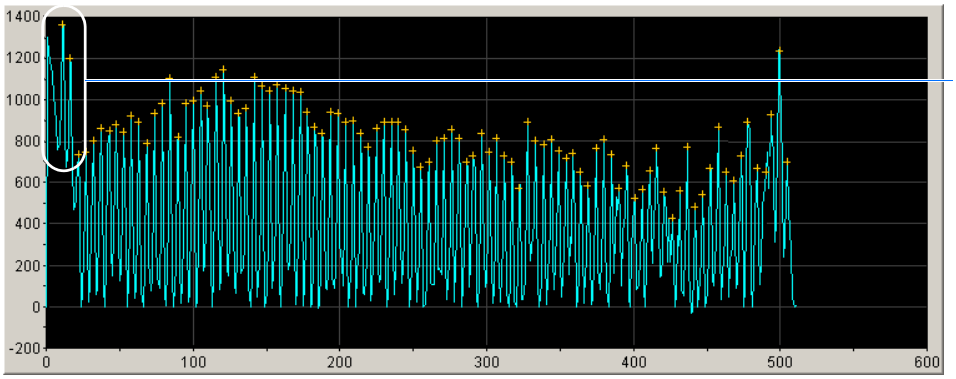


Passing Profile #1

This example shows a typical passing profile.



Passing Profile #2



Passing Profile #3

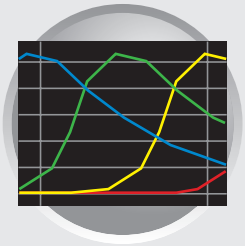
Background artifact

This example shows a passing profile with high artifactual background at the left margin.

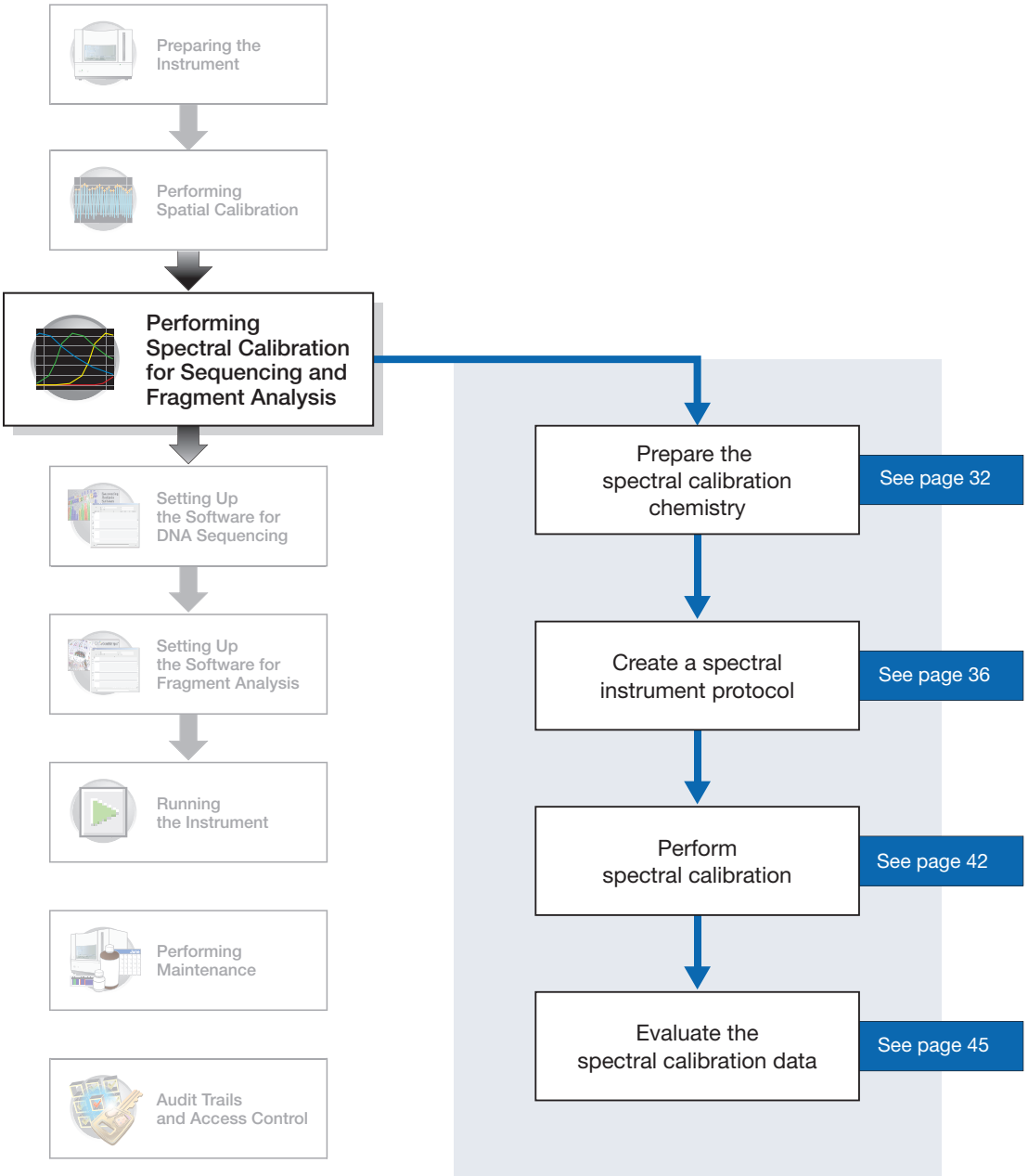
Notes

Notes

Performing Spectral Calibration For Sequencing and Fragment Analysis



Workflow



Notes

Overview

A spectral calibration creates a matrix that is used during a run to reduce raw data from the instrument to the 4- 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.

IMPORTANT! Do not run your computer operating systems' Internet Connection Wizard during a spectral calibration.

Note: A spectral calibration algorithm checks dye order. The error message if the algorithm determines that the dyes are in the incorrect order is, "failed calibration due to bad data: Bad dye order detected."

Spectral calibrations are performed with a specific combination of:

- Dye set (G5, G5-RCT, Any4Dye, Any5Dye, E or Z). For further information see, "Preparing the Spectral Calibration Chemistry" on page 32 and, Appendix B, G5, G5-RCT, Any4Dye, and Any5Dye Dye Sets.
- Array type (48-capillary or 96-capillary)
- Array length (36-cm or 50-cm)

IMPORTANT! Spectrals must be calibrated for dye set, array type, and array length.

When to Perform the Calibration

Perform a spectral calibration:

- Whenever you use a new dye set on the instrument
- After the laser or CCD camera has been realigned/replaced by a service engineer
- If you see a decrease in spectral separation (pull-up and/or pull-down peaks)
- If you alter any condition (dye set, array type, or array length)

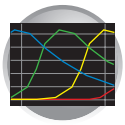
Changing Capillary Array Lengths

For each dye set, a single spectral calibration can not be used for all capillary array lengths.

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

Refer to page 53 for information on how to switch calibrations.

Notes



Required Materials

Part numbers are located in Appendix A.

Description
ABI PRISM® BigDye® Terminator v3.1 or v1.1 Sequencing Standard or, DS-33 Matrix Standard
ABI PRISM® 384- or 96-Well Reaction Plate w/ Barcode
Multichannel pipettor
Plate retainer
• Plate septum with black plate base or, • Heat-seal with gray plate base
Hi-Di™ formamide
Heated block or thermal cycler
Container with ice
Centrifuge with microplate adapter
Microcentrifuge
Vortex
Gloves

Two Types of Calibration Standards

Two types of calibration standards are used to create a matrix:

For Fragment Analysis:

- Matrix standards – four or five fragments of varying size are individually labeled with one of the four or five dyes of a set.

For Sequencing:

- Sequencing Standards – standard sequencing reaction fragments of varying size are individually labeled with one of the four dyes.

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

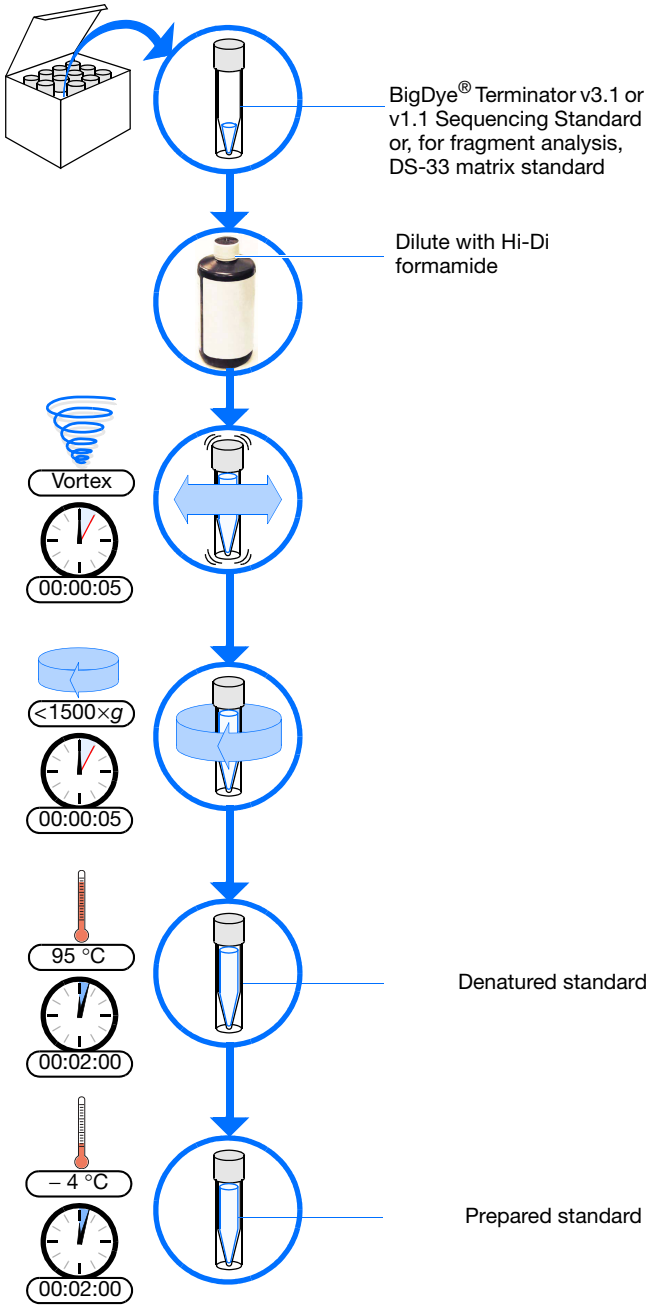
Sequencing Chemistry	Dye Set	Calibration Standards
ABI PRISM® BigDye® v3.1Terminator	Z_BigDyeV3	BigDye® v3.1 Terminator Sequencing Standard
ABI PRISM® BigDye® v1.1 Terminator	E_BigDyeV1	BigDye® v1.1 Terminator Sequencing Standard

Fragment Analysis Chemistry	Dye Set	Calibration Standards
ABI PRISM® Linkage Mapping Set v2.5/custom oligos	G5	DS-33
ABI PRISM® Linkage Mapping Set v2.5/custom oligos	G5-RCT	DS-33

Notes

Preparing the Spectral Calibration Chemistry

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.



1. Dilute spectral calibration standard with Hi-Di™ formamide according to the insert instructions.
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.
6. Vortex thoroughly and then briefly centrifuge the mixture.

Notes

Sealing and Preparing the Plate Assemblies

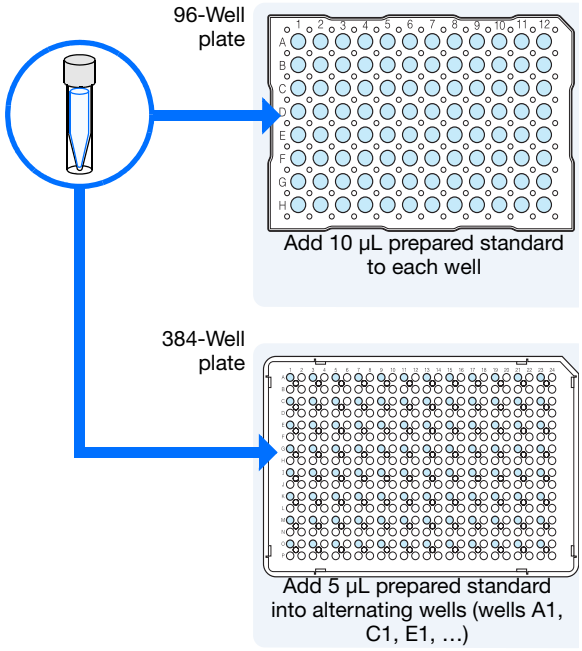
WARNING **WARNING** Do not use warped or damaged plates.

1. Add the denatured standard to the wells of a 384- or 96-well reaction plate:

   If using a:

   - **48-capillary, 96-well plate** – Add 10 µL of denatured standard to each well.
   - **384-well plate** – Add 5 µL of denatured standard into alternating wells of the plate.

   See **"Default Load Maps" on page 125**



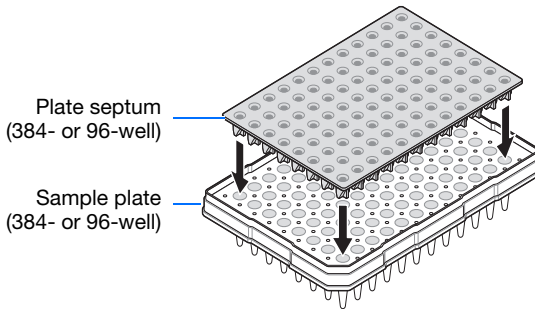
2. Seal the plate with septum or heat-seal:

   With septum:

   a. Place the plate on a clean, level surface.

   b. Lay the septum flat on the plate.

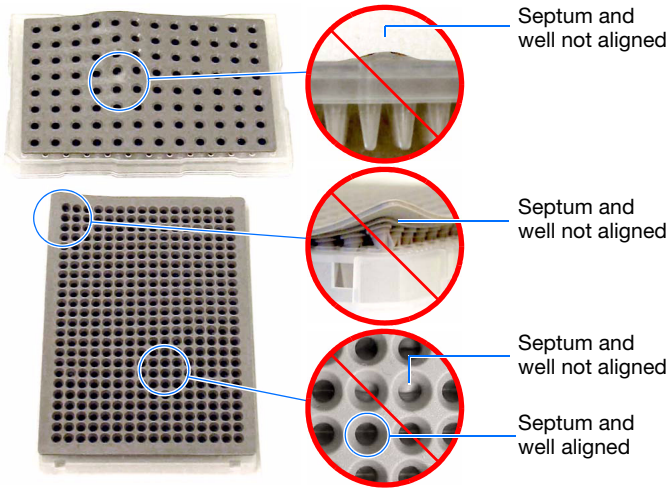
   c. Align the holes in the septum strip with the wells of the plate, then firmly press downward onto the plate.



Notes

d. Ensure that:

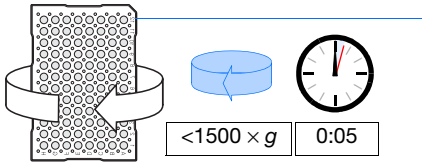
- The septa lie flat against the plate. You should not feel any lumps or raised edges.
- The septa are inserted straight into the wells. You should not see any bent or crooked duckbills when viewing the plate from above.



With heat-seal:

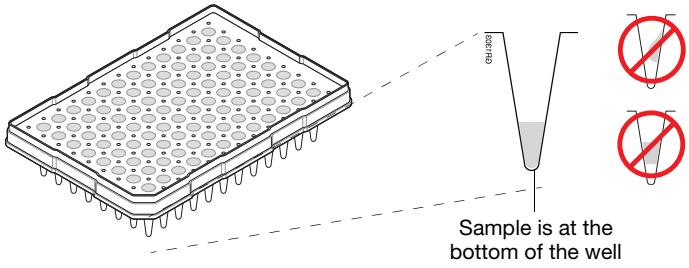
a. Follow your thermal sealer instruction instructions.

3. Briefly centrifuge the plate.

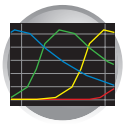


4. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.

If the reagents of any well contain bubbles or are not located at the bottom of the well, repeat steps 3 and 4.

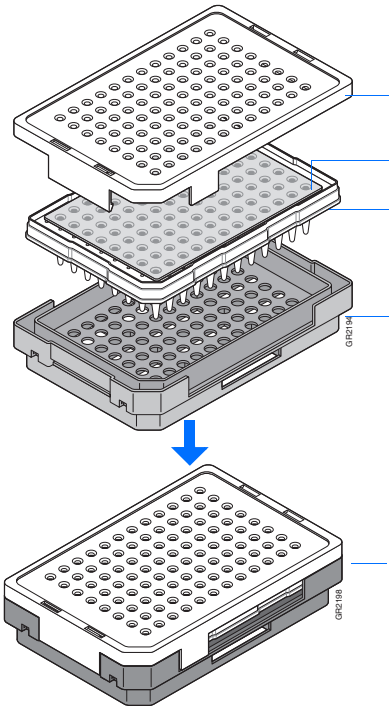


Notes



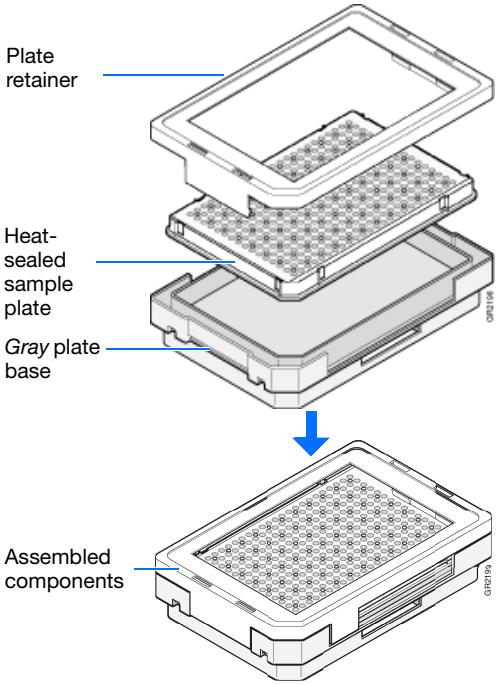
5. Assemble the plate assembly as shown below.

Septum Assembly



WARNING Use only ***black*** plate bases with septa-sealed plates.

Heat-sealed Assembly



WARNING Use only ***gray*** plate bases with heat-sealed plates.

6. Verify that the holes of the plate retainer and the septa are aligned.

IMPORTANT! The plate may damage the array if the retainer and the septum holes are not aligned.

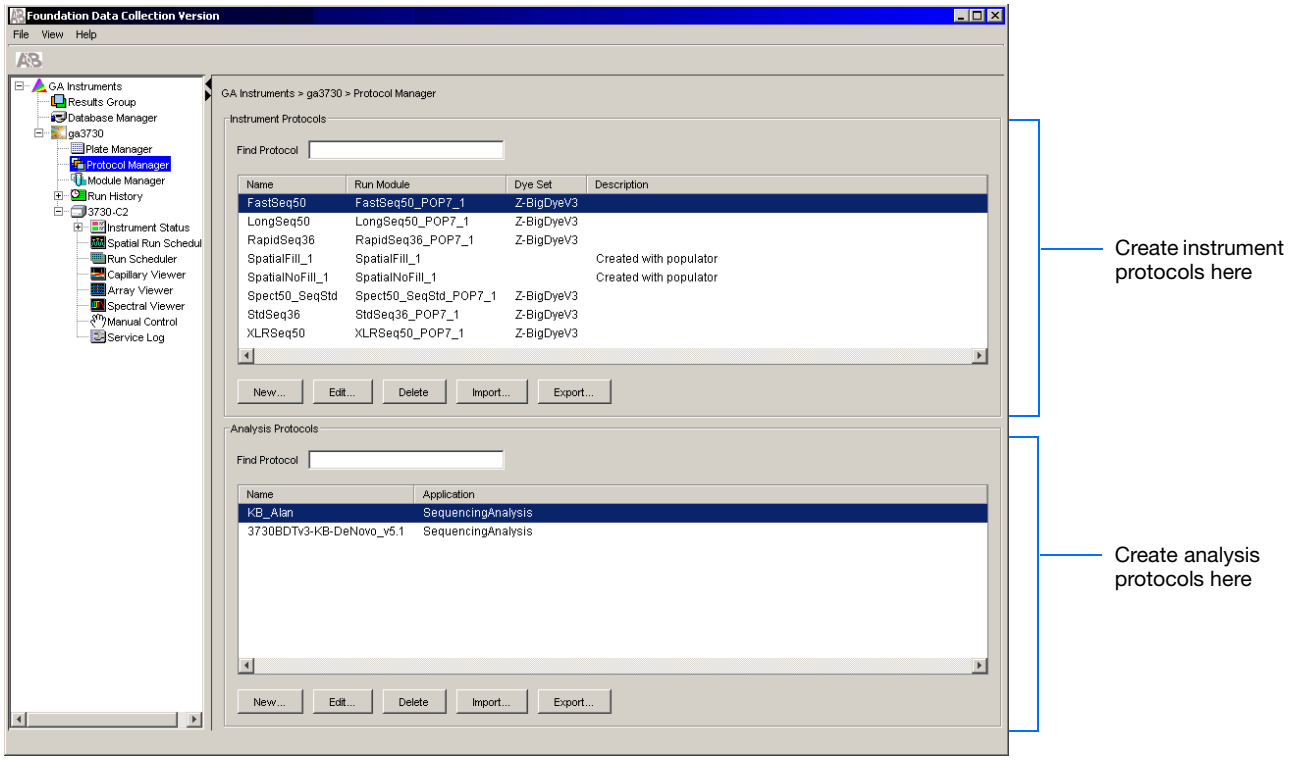
IMPORTANT! Heat Seal Recommendations

- Use 3-mil Applied Biosystems heat seal film (PN 4337570). This film is 3-mil before, and 1-mil after, heating.
- *Do not* use heat seal film thicker than 1-mil, after heating, on 3730/3730*xl* DNA Analyzer.
- Do *not* use heat-seal film containing adhesives or metals as these may damage the instrument's piercing needles.

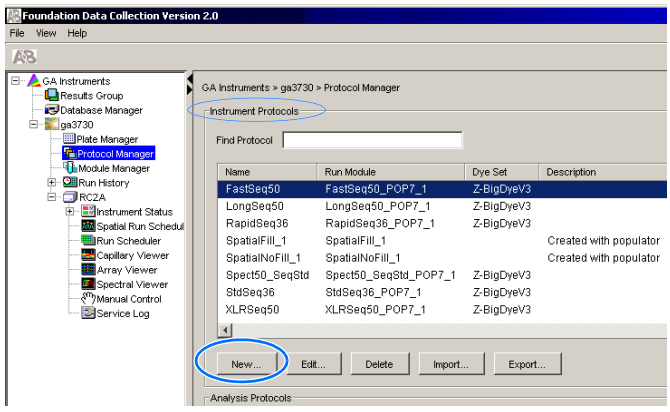
Notes

Creating a Spectral Instrument Protocol

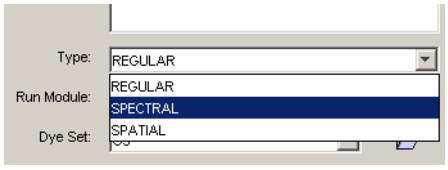
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.



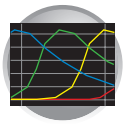
2. In the Instruments Protocols pane, click New... . The Protocol Editor dialog box opens.



3. Select **Spectral** from the Run Module drop-list.



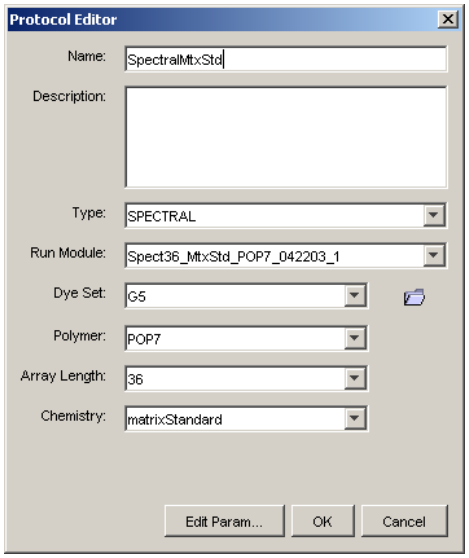
Notes



4. The Protocol Editor now displays additional drop-lists.Select from the following:

   If you are using a *matrix standard* for spectral calibration, choose:

   a. *Run Module*: **Spect36_MtxStd_1**
   b. *Array Length*: **36**
   c. *Chemistry:* **matrixStandard**



IMPORTANT! Select the appropriate capillary array length. The array length must match the array length information from the Install Array wizard.

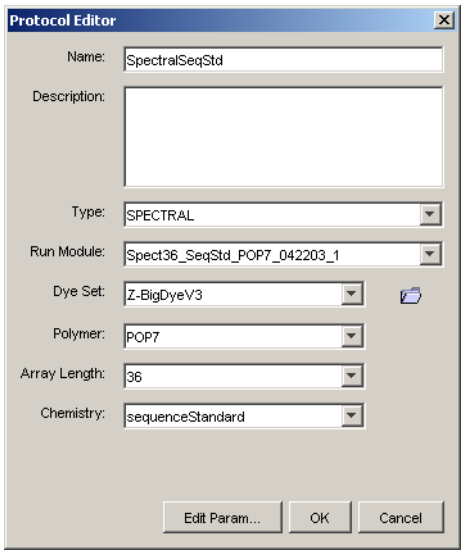
If you are using a *sequencing standard* for spectral calibration you may choose 36-cm or 50-cm array length:

36-cm capillary array:

   a. *Run module:* **Spect36_SeqStd_1**
   b. *Chemistry:* **sequenceStandard**

50-cm capillary array:

   a. *Run module:* **Spect50_SeqStd**
   b. *Chemistry:* **sequenceStandard**



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

IMPORTANT! Select the appropriate capillary array lengths. The array length must match the array length information from the Install Array wizard.

Dye Set	Standard Type	Chemistry File
Z_BigDyeV3	BigDye® v3.1 Terminator Sequencing Standard	Sequence Standard
E_BigDyeV1	BigDye® v1.1 Terminator Sequencing Standard	Sequence Standard

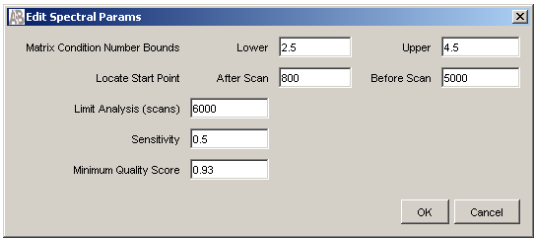
Notes

Dye Set	Matrix Standard Set	Chemistry File
G5	DS-33	Matrix Standard
G5-RCT	DS-33	Matrix Standard

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

5. (Optional) Click **Edit Param** to display the Spectral Params dialog box.

   Use this dialog box to edit the selection criteria for passing or failing spectral calibrations.



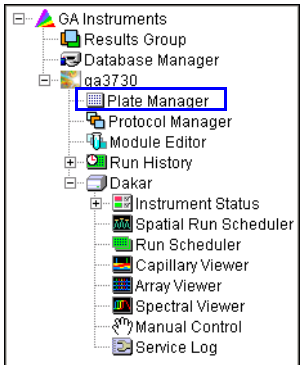
Parameters	Valid Data Ranges*
Matrix Condition Number Bounds	Lower: 1-10 Upper: 3-20
Locate Start Point	After Scan: 100-5000 Before Scan: 100-5000
Limit Analysis (scans)	400-20,000
Sensitivity	0-0.9
Minimum Quality Score	.80-.99
	*These ranges are dye-set independent

IMPORTANT! Default parameter values are optimized and are recommended for most situations

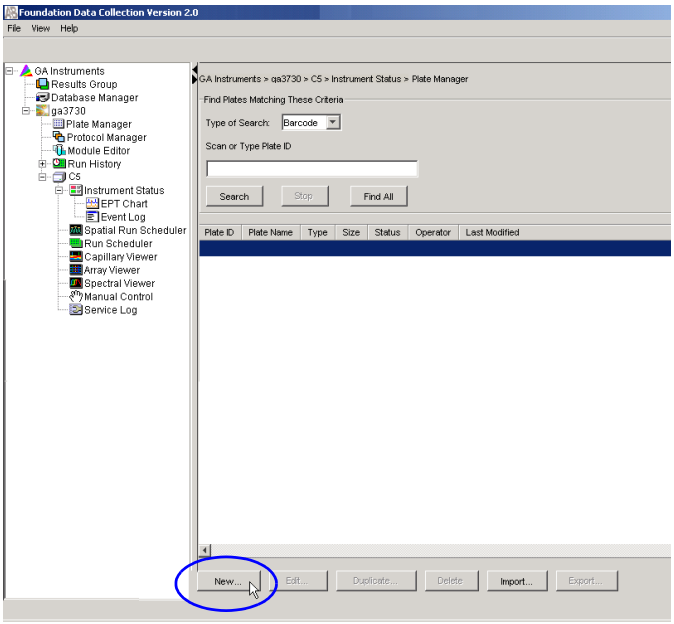
Notes

Creating a Spectral Calibration Plate Record

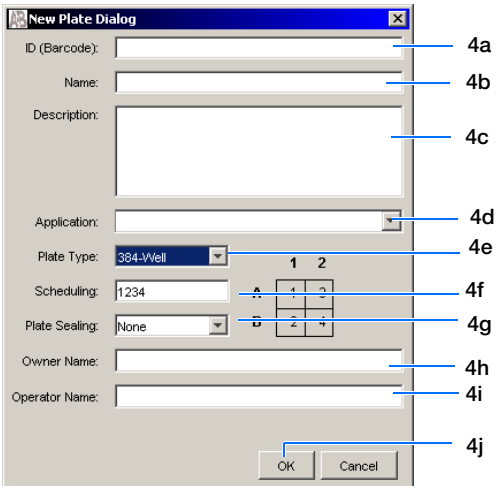
1. In the Tree pane of the Data Collection Software, double-click
   GA Instruments > **ga3730** > *instrument name* > **Plate Manager**.



2. Click **New** to create a new plate.



3. Complete the New Plate dialog box:
   a. Enter ID or Barcode number
   b. Enter a name for the plate.
   c. Optional: Enter a description for the plate record.
   d. In the Application drop-list, select **Spectral Calibration**.
   e. In the Plate Type drop-list, select **96-Well** or **384-Well**.
   f. Enter desired scheduling. For more information see, "Globally Modifying a Run Schedule" on page 122.



Notes

g. In the Plate Sealing drop-list, select **Septa** or **Heat Seal**.

h. Enter a name for the owner.

i. Enter a name for the operator.

j. Click OK.

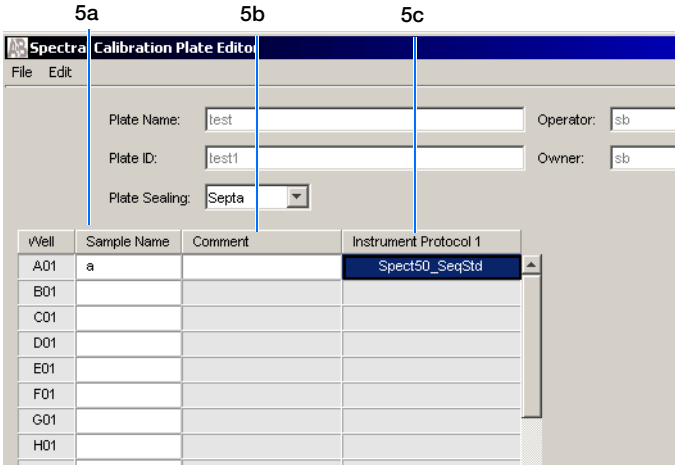
4. In the Spectral Calibration Plate Editor dialog box, enter the following information:

Note: This example assumes that you are loading the first quadrant.

a. In the Sample Name column of row A01, enter a sample name, then click the next cell.

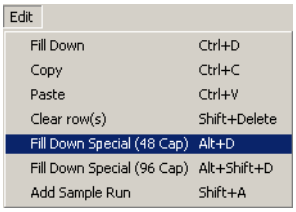
b. In the Comments column of row A01, enter any additional comments or notations for the sample at the corresponding position of the plate.

c. In the **Instrument Protocol 1** column of row A01, select a protocol from the drop-list.



5. Highlight the entire row.

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



Based on the plate type (96- or 384-well) and capillary array (48 or 96 capillaries) you are using, select the appropriate fill down option:

- 96 capillary/96-well plate: *Fill Down*
- 48 capillary/96-well plate: *Fill down Special (48 Cap)*
- 96 capillary/384-well plate: *Fill down Special (96 Cap)*
- 48 capillary/384-well plate: *Fill down Special (48 Cap)*

7. Click OK.

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

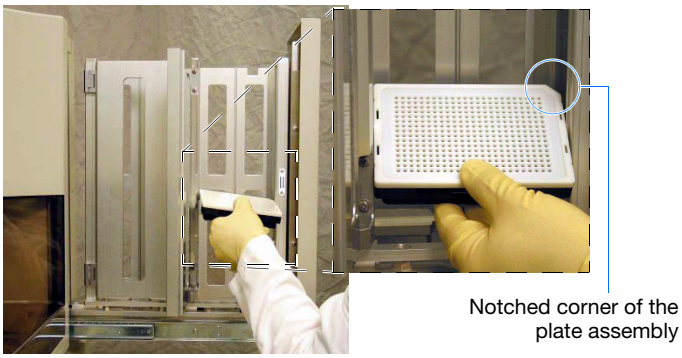
Notes

Loading the Plate into the Instrument

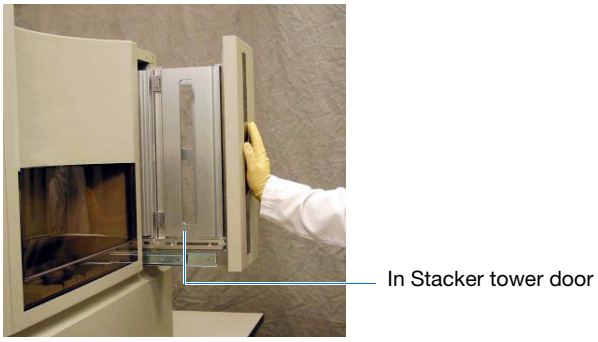
1. The name of the plate you just created is displayed in the Input Stack window of the Data Collection software, and is ready to run.
2. Open the stacker drawer.
3. Open the In Stack tower door.

4. Place the plate assembly into the stacker.

IMPORTANT! When placing the plate into the stacker, the plate must be oriented so that the notched corner of the plate assembly is located in the rear-right corner of the stacker.



5. Close the In Stack tower door.
6. Close the Stacker drawer.



Notes

Running the Spectral Calibration Plate

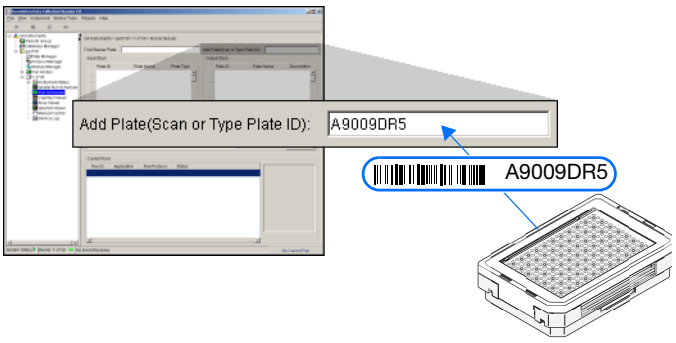
1. In the Tree pane of the Data Collection Software, double-click
   GA Instruments > **ga3730** > *instrument name* > **Run Scheduler**.

2. In the Run Scheduler view:

   a. In the Add Plate field, scan the barcode of a plate to add it to the input stack.

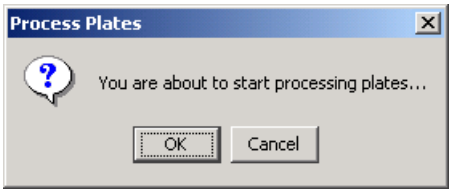
   Or,

   b. Type the plate ID and press **Enter** to add it to the input stack.



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

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



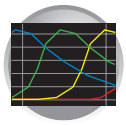
Note: The instrument may pause before running the plate to raise the oven temperature.

Application	Capillary Array Length (cm)	Approximate Spectral Run Time[a] (min)
Sequencing	50	120
Sequencing	36	60
Fragment Analysis	36	32

a. The data collection software may take up to 30 min to calculate the matrices after the run.

5. When the run is finished, remove the plate from the instrument.

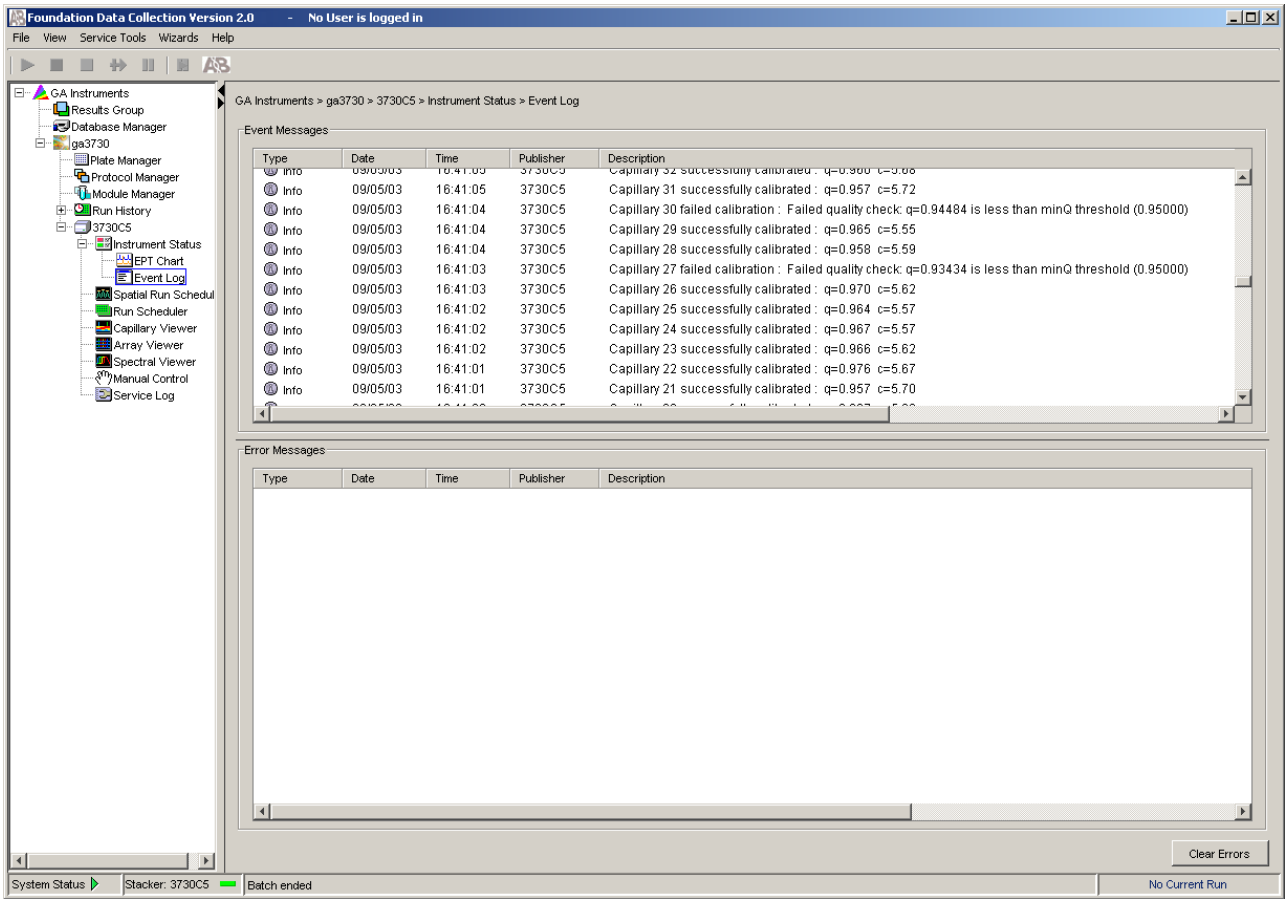
Notes



Viewing the Pass/Fail Status After the Run

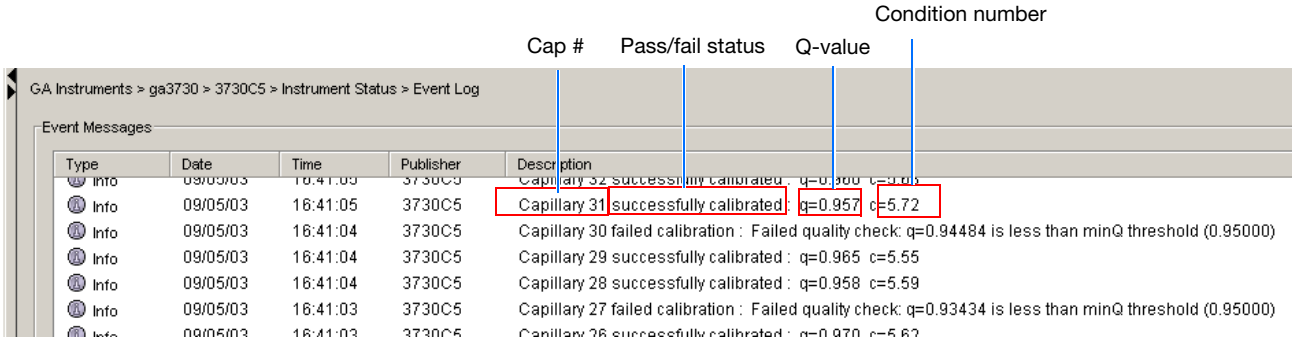
After the instrument completes the spectral calibration run, the pass or fail status of each capillary is recorded in the Events Messages section of the Instrument Status window.

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *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 for matrix standards
  - above 0.93 for sequence standards
- Condition number within range of:

Dye Set	Default Condition Number Range
Sequencing Analysis	
Z_BigDyeV3	2.5 to 4.5
E_BigDyeV1	3.0 to 5
Fragment Analysis	
G5	9.5 to 14.5
G5-RCT	9.5 to 14.5

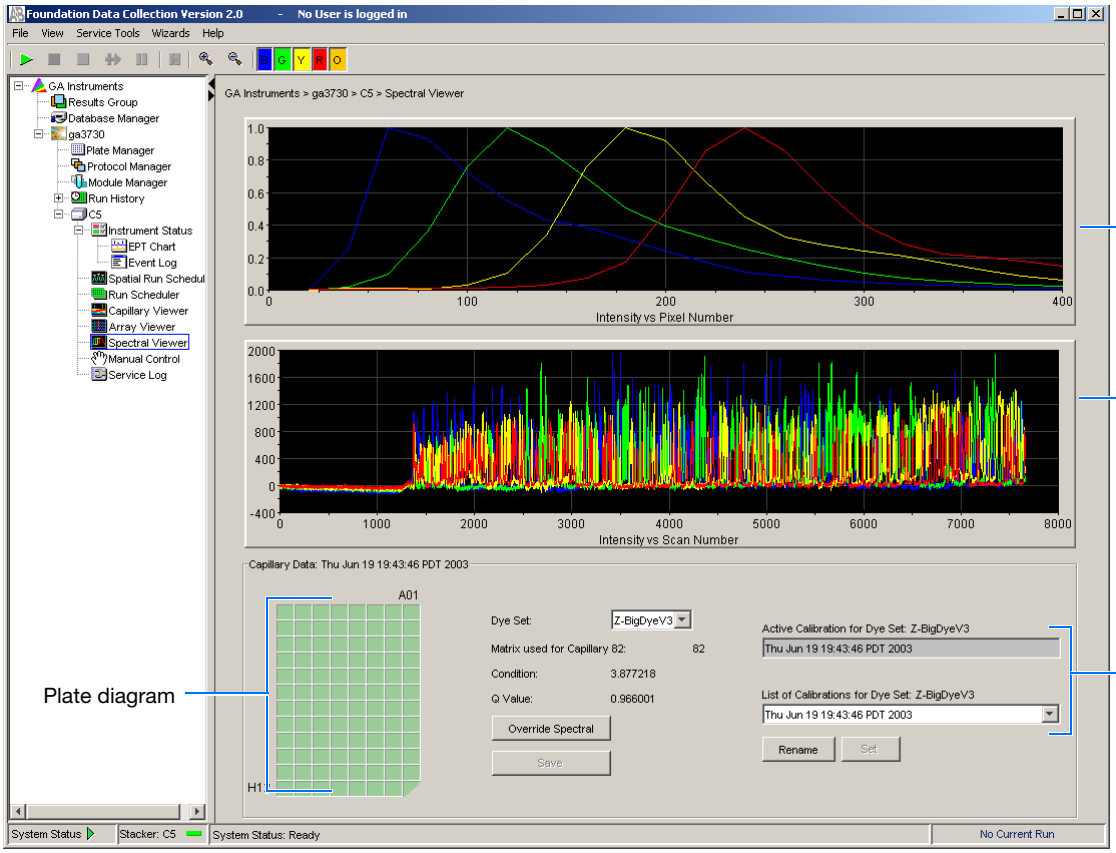
Notes

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.

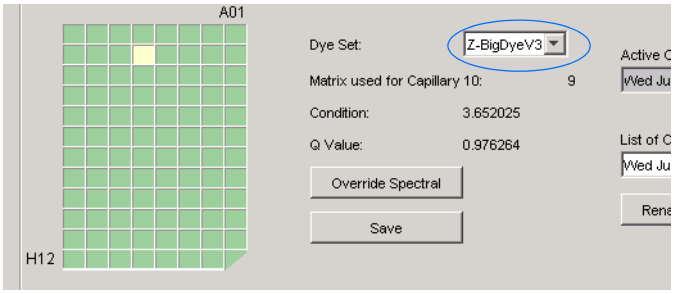
Note: Pages 49 and 50 contain examples of passing sequencing spectral calibration profiles, and page 51 contains an example of a passing fragment analysis spectral calibration profile.

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Spectral Viewer**.

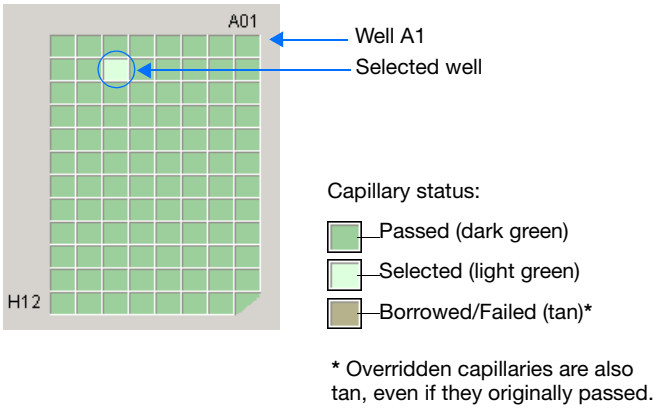


Notes

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



3. Select a well on the plate diagram to view the spectral results of associated capillary.



4. Evaluate the spectral calibration profile for the selected capillary:

a. Verify that the order of the peaks in the spectral profile from left to right are:

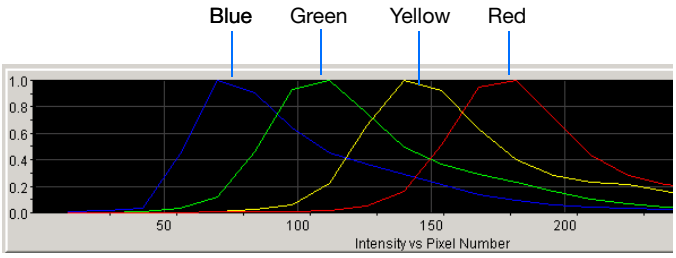
– 4-dye: blue-green-yellow-red

– 5-dye: blue-green-yellow-red-orange

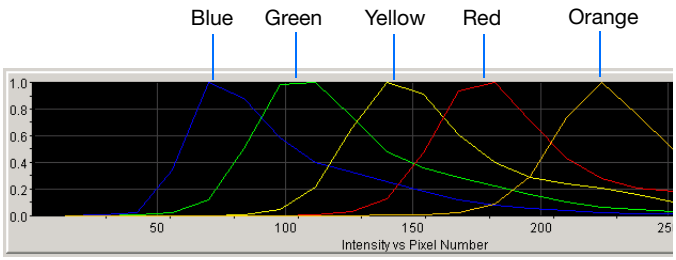
Do the peaks in the profile appear in the correct order?

Yes: Go to step c.

No: The calibration run has failed. Go to page 55.

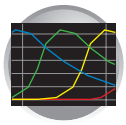


Example of a 4-dye spectral calibration profile



Example of a 5-dye spectral calibration profile

Notes

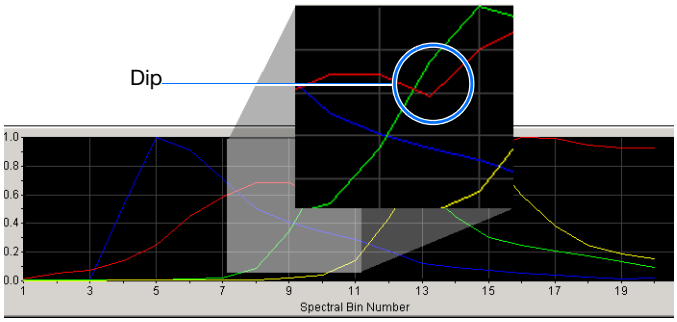


b. Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities (see "Tip: Magnifying the Spectral Profile" on page 48).

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 55.



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

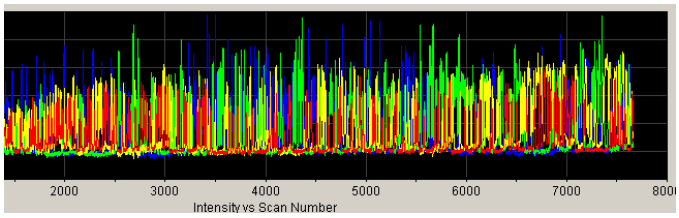
Fragment Analysis

– 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?

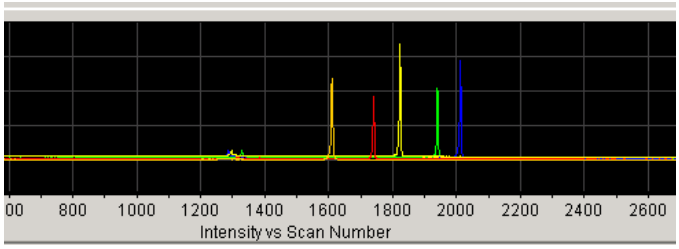
Yes: The calibration run has failed. Go to page 55.

No: Go to step 5.



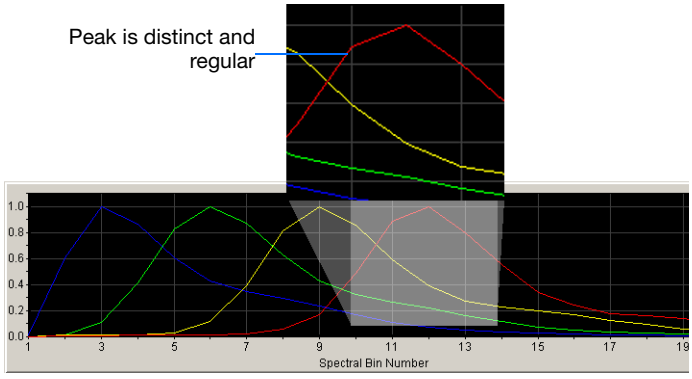
Example of a 4-dye sequencing raw data profile

Left to right: Orange, Red, Yellow, Green, Blue



Example of a 5-dye fragment analysis raw data profile

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



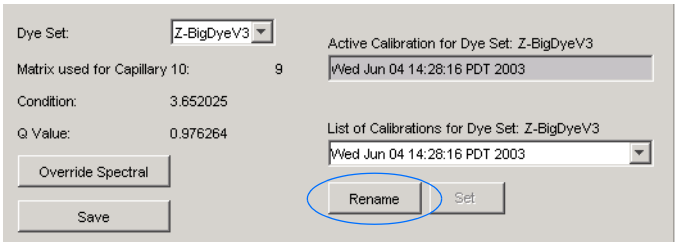
Notes

6. Rename the spectral run. The spectral file default name is the day, date and time of the run.

   a. Click Rename.

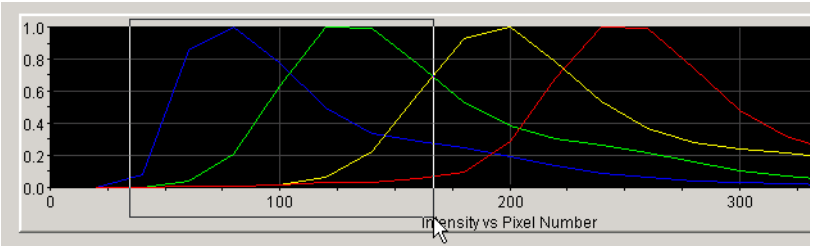
   b. In the Rename Calibration dialog box, enter a descriptive name for the spectral calibration including the dye set, array length and polymer type (optional).

   c. Click OK.



Tip: Magnifying the Spectral Profile

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Spectral Viewer**.
2. In the profile or raw data display, click - drag the cursor to create a box around the area of interest.

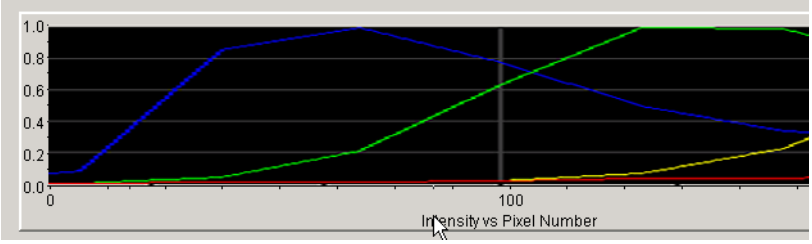


Selecting an area to magnify in a spectral profile

3. Release the mouse button.

   The data collection software displays the selected region.

4. Press **R** to reset the view.

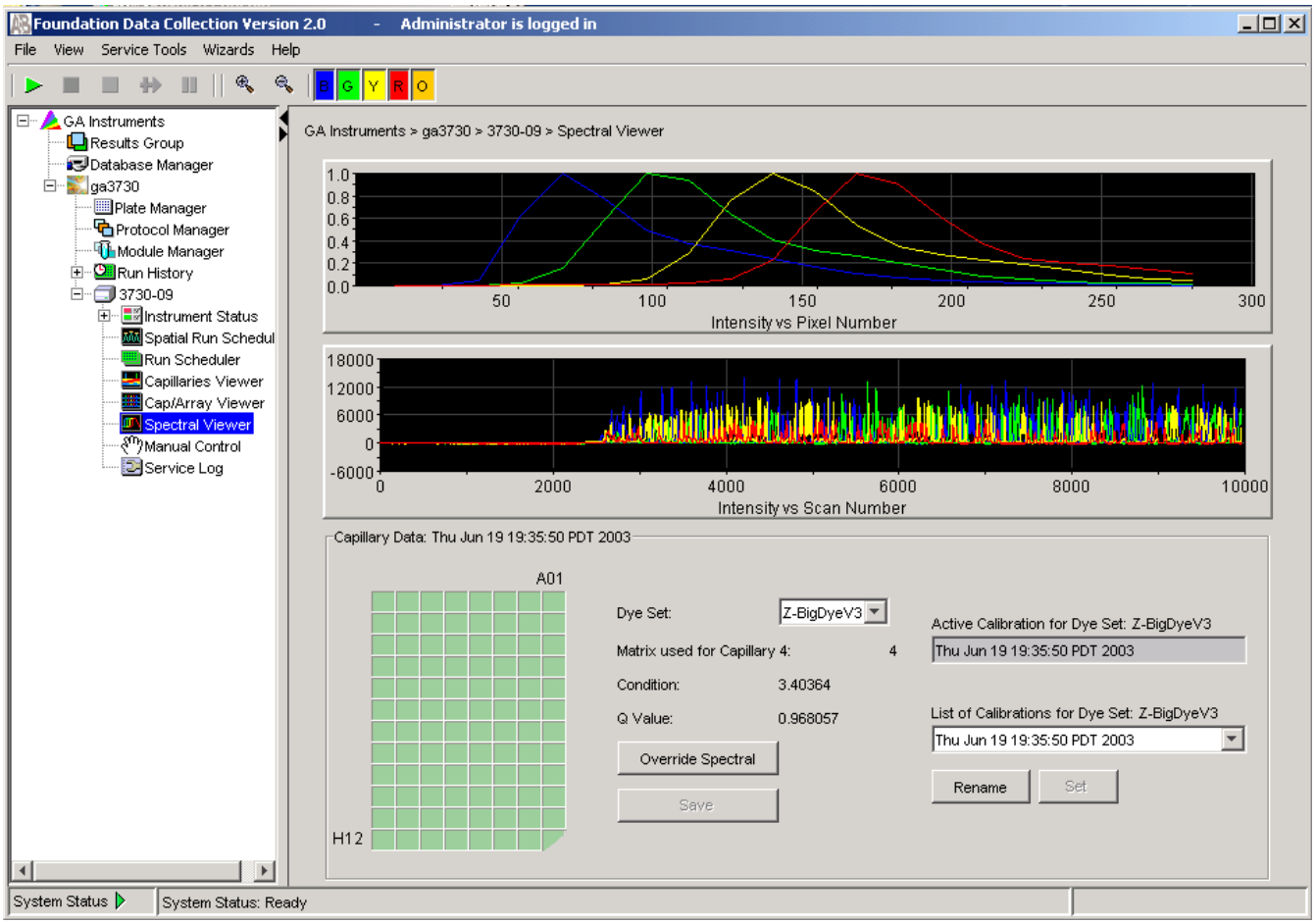


Magnified area of that spectral profile

Notes

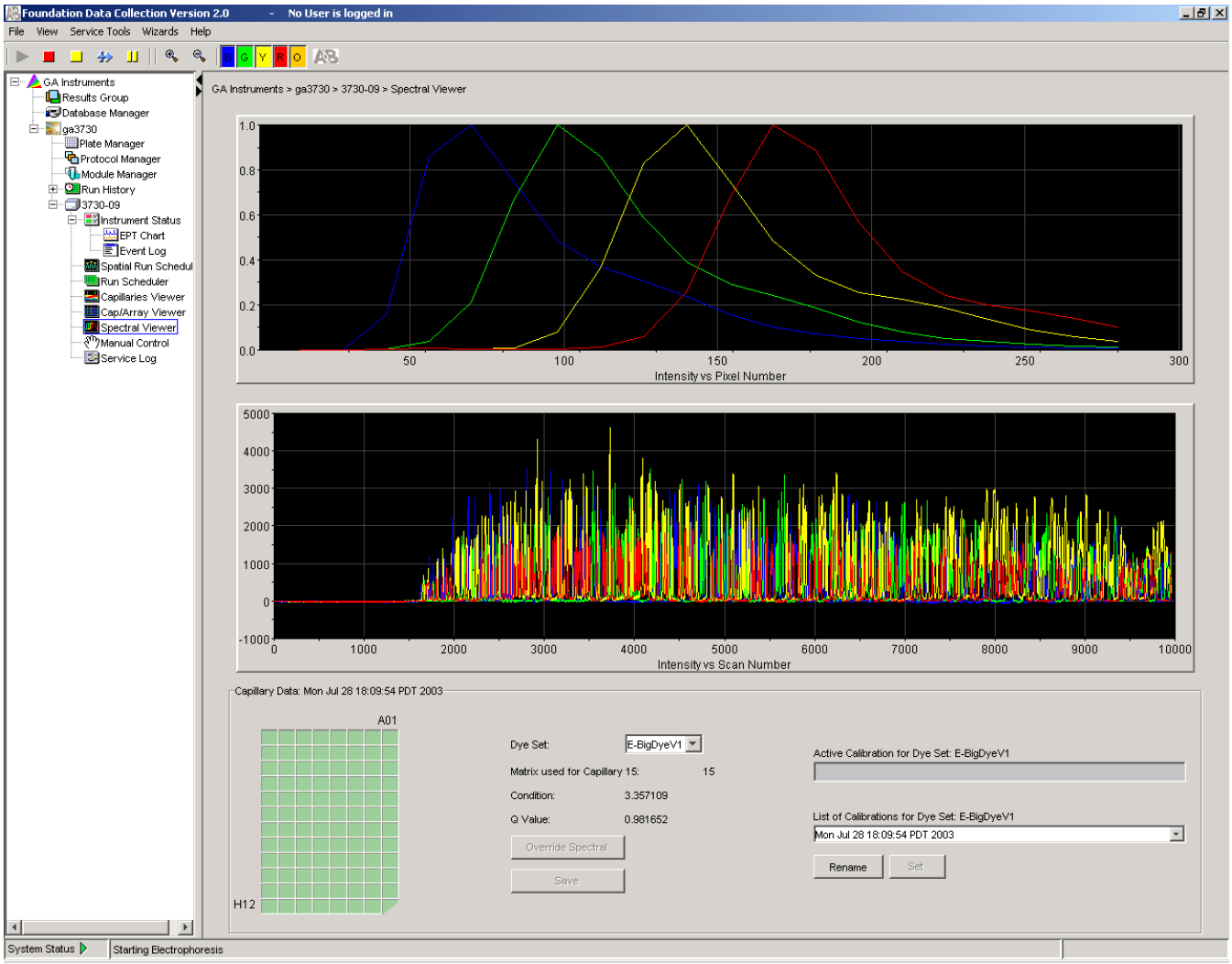
Examples of Passing Sequencing Spectral Calibrations

Dye Set Z Created from a Sequencing Standard



Notes

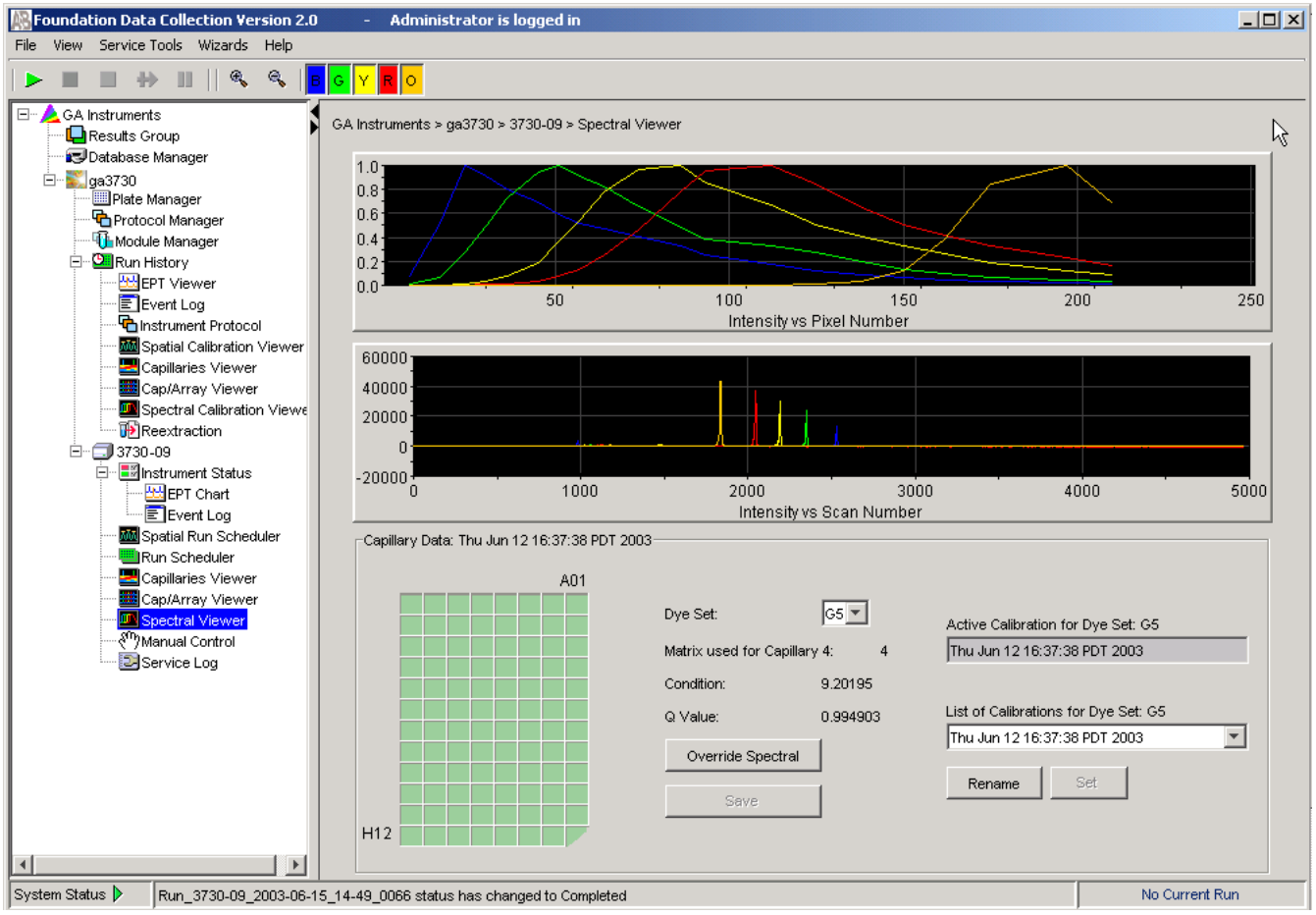
Dye Set E Created from a Sequencing Standard



Notes

Example of a Passing Fragment Analysis Spectral Calibration

Dye Set G5 Created from Matrix Standard Set DS-33



Notes

Spectral Viewer

Selecting Active Spectral Calibrations

For best quality data, we suggest that you perform spectral calibrations every time a new array is installed in the instrument. However, you may choose to reuse previous spectral calibrations to apply to new data that will be generated on the instrument. Once data is collected, you cannot reapply a different spectral calibration.

IMPORTANT! It is essential that you perform a spectral calibration any time the capillary array is moved or replaced when using DyeSetG5-RCT.

IMPORTANT! If you installed an array that is a different length or type (48 vs 96) than you were using previously and if a previous spectral calibration for the new array/new conditions exits, you *must* reset the active spectral calibration. if a previous spectral for those array conditions exist. Otherwise, you *must* run a new spectral calibration.

Poor quality data or failed analyses are results of using the wrong spectral calibration.

IMPORTANT! Spectrals must be calibrated for dye set, array type, and array length.

When a new *spatial* calibration is saved, the current spectral calibration for DyeSet G5-RCT is deactivated. Dye sets G5, E, and Z are not deactivated. If you wish to continue without a spectral recalibration, you may "Set" an active spectral using the instructions below.

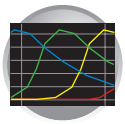
All calibrations for your current dye set are listed in the List of Calibrations drop-list. Therefore, you can choose a spectral to use from that list prior to the beginning of a new run.

Note: An asterisk * precedes failing calibrations.

Note: The most recent spectral for each dye set is automatically chosen as the active calibration.

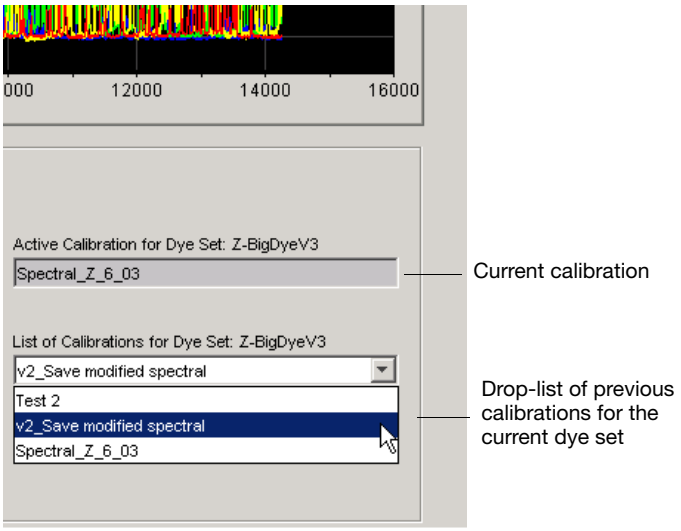
Each dye set can have its own active calibration. Thus, there is no need to manually set the active calibration if you are performing runs with various dye sets.

Notes

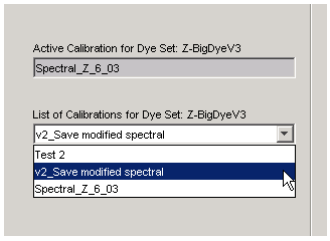


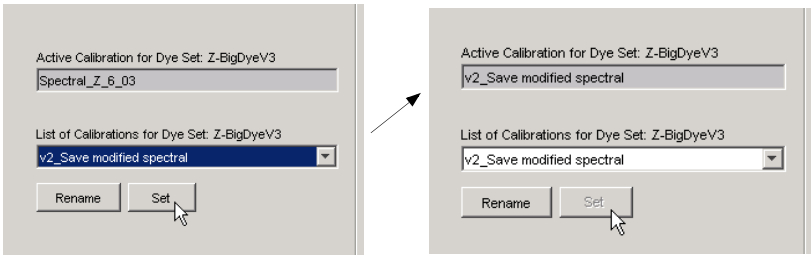
To select a previous spectral calibration:

1. Select the dye set of interest.
2. In the Spectral Viewer, click the List of Calibrations drop-menu in the lower, right pane.



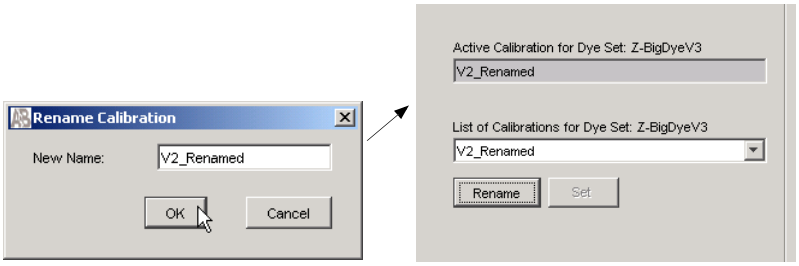
3. Select the spectral calibration you want to use for future runs.





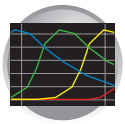
4. Click **Set** to display your chosen spectral calibration in the Active Calibration text box.

Notes



5. (Optional) Click **Rename** to display the Rename Calibration dialog box, enter a new name, and click **OK**.

Notes



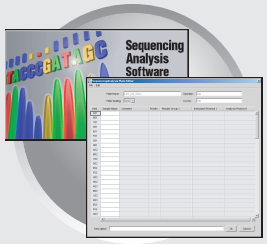
Troubleshooting

Troubleshooting spectral calibration		
Observation	**Possible Cause**	**Recommended Action**
No signal.	Incorrect sample preparation.	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.
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.
	Insufficient filling of array.	Check for broken capillaries and refill the capillary array.
	Expired spectral standards.	Check the expiration date and storage conditions of the spectral standards. If necessary, replace with a fresh lot.
Spikes in the data.	Expired polymer.	Replace the polymer with a fresh lot using the Change Polymer Wizard. **⚠ WARNING CHEMICAL HAZARD.** **POP-7 polymer** 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.	• Refill the capillaries using the Bubble Remove wizard. • Properly bring the polymer to room temperature. • Replace expired polymer.
	Possible contaminant in the polymer.	Replace the polymer using the Change Polymer wizard.

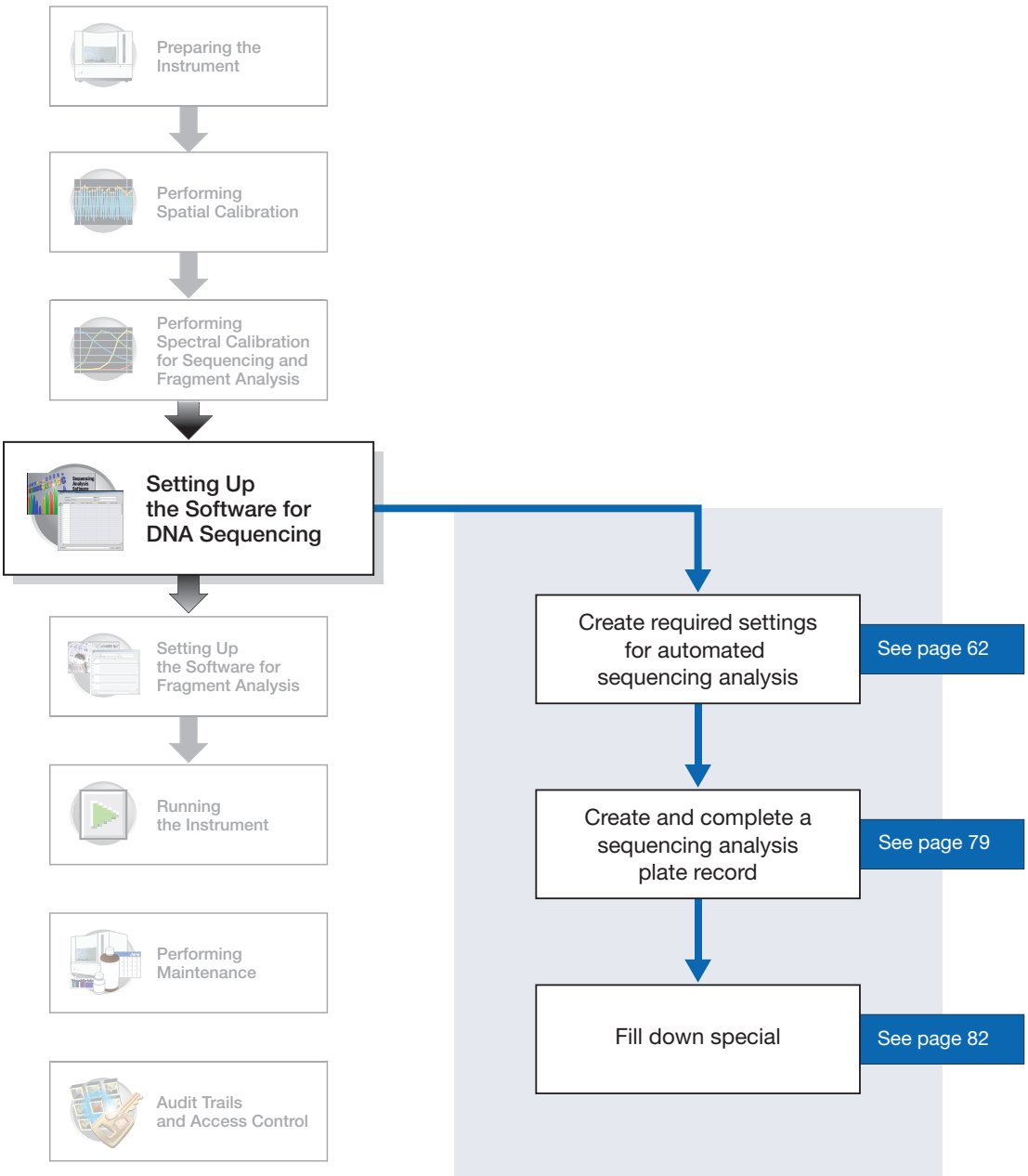
Notes

Notes

Setting Up the Software for DNA Sequencing



Workflow



Notes

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 Applied Biosystems instruments.

Important Notes

- A unique name must be assigned to the instrument computer before 3730/3730*xl* Data Collection software is installed.
- Do not rename the computer once 3730/3730xl Data Collection software has been installed. Doing so *will* cause the 3730/3730xl 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.

What Plate Records Contain

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 page 74
- Mobility file (in Analysis Protocol), see page page 66
- 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 62
- Name of the Analysis Protocol—Analysis Protocols specify how data is analyzed at the end of the run (in Analysis Protocol), see page page 66

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

Note: A plate record must be created in advance of the first run. Plate records can be created, and plates added to the stacker, while a run is in progress.

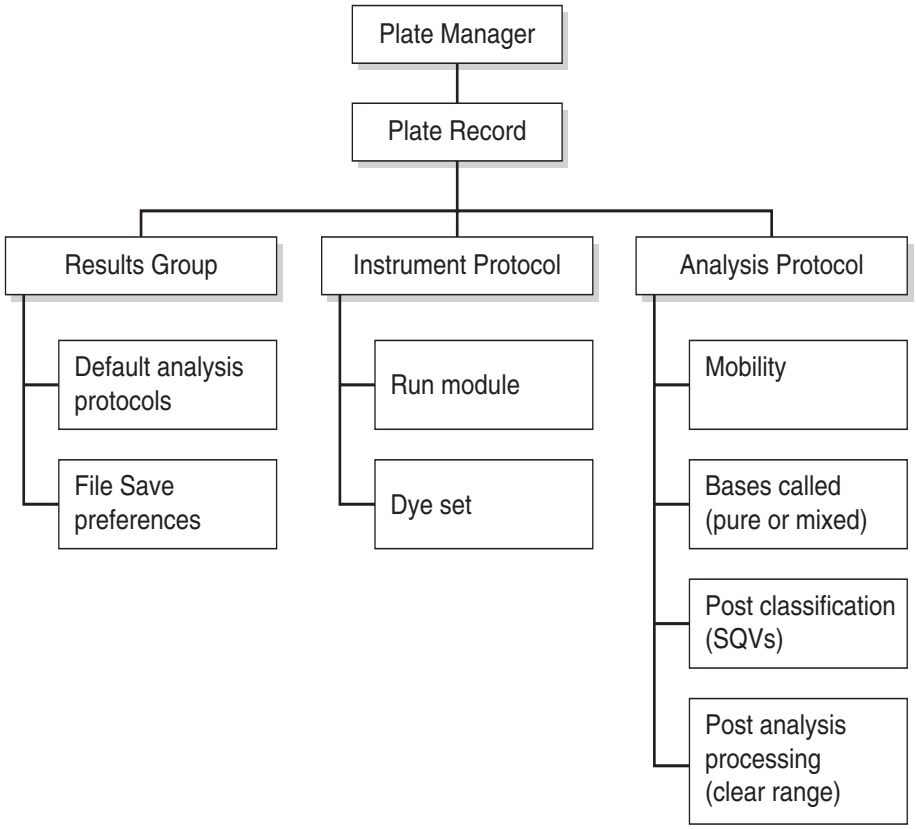
Notes

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 59 describes what each file specifies:

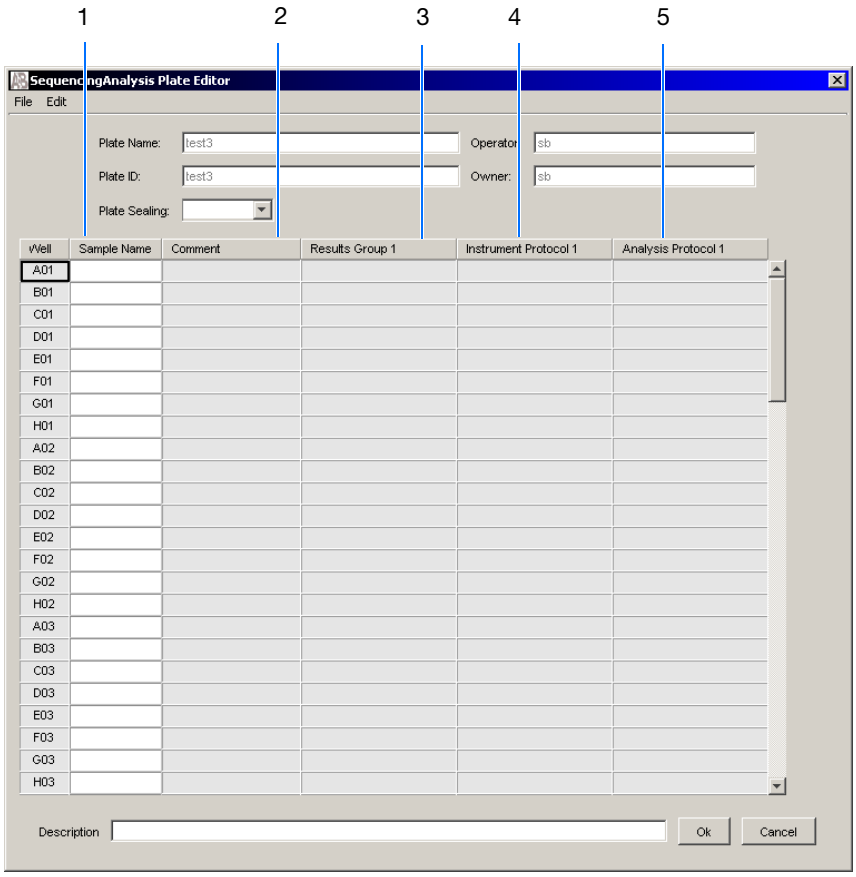
Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	62
Analysis Protocol	Contains everything needed to analyze sequencing data.	66
Results Group	Defines the file type, the file name, file save locations, analysis software and autoanalysis.	71



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



Default is one sample run. To add additional runs, see page 81.

Blank Sequencing Analysis plate record

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

Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. 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 Groups" on page 71.

Notes

Column	Description
4. 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 62.
5. 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 66.

Notes

Creating Required Settings for Automated Sequencing Analysis

If the Settings Already Exist

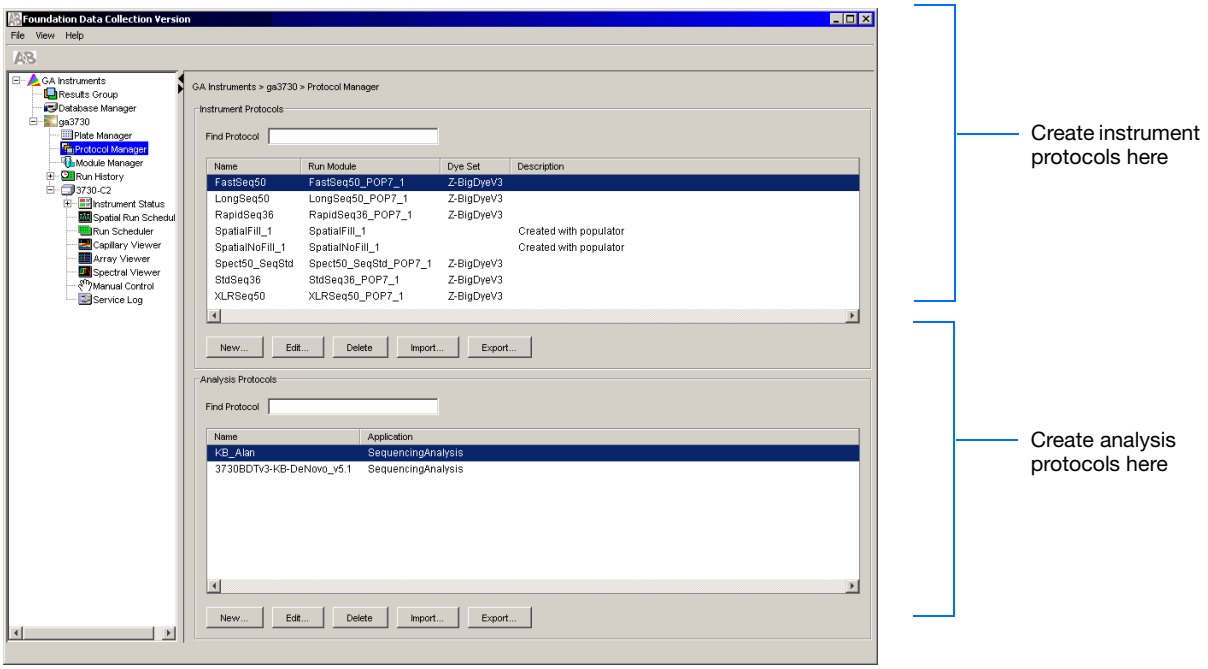
If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to "Creating and Completing a Sequencing Analysis Plate Record" on page 79.

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

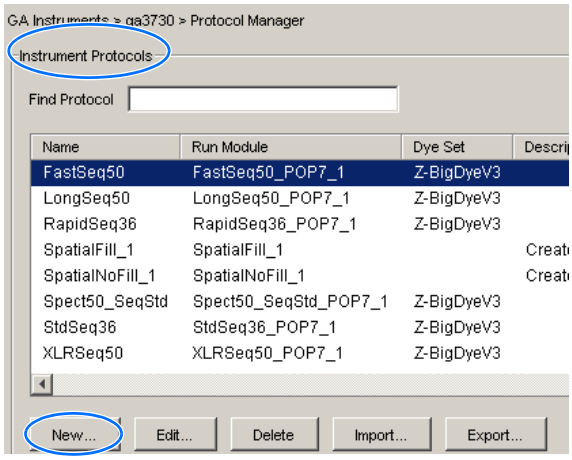
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.



Notes

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

   The Protocol Editor opens.

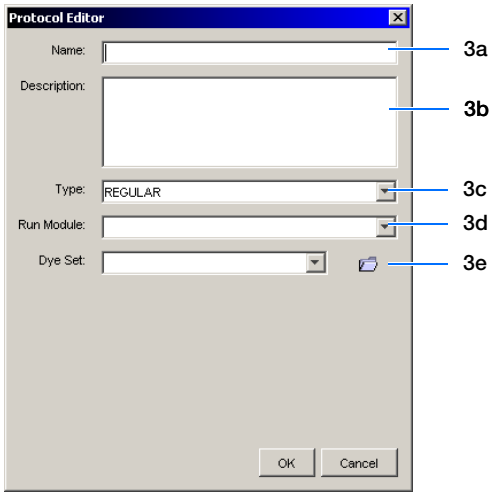


3. Complete the Protocol Editor:

   a. Type a name for the protocol.

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

   c. Select **Regular** in the Type drop-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 64.

Run Module	Capillary Array Length (cm)	Sequencing Run	Approximate Run Times* (min)
XLRSeq50_POP7	50	Extra long read	180
LongSeq50_POP7	50	Long read	120
FastSeq50_POP7	50	Fast read	60
StdSeq36_POP7	36	Standard read	60
RapidSeq36_POP7	36	Rapid read	35
* Approximate run times assume oven temperature has reached run temperature			

Notes

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

Dye Set	Chemistry
E_BigDyeV1	ABI PRISM® BigDye® v1.1 Terminator
Z_BigDyeV3	ABI PRISM® BigDye® v3.1 Terminator

f. Click OK.

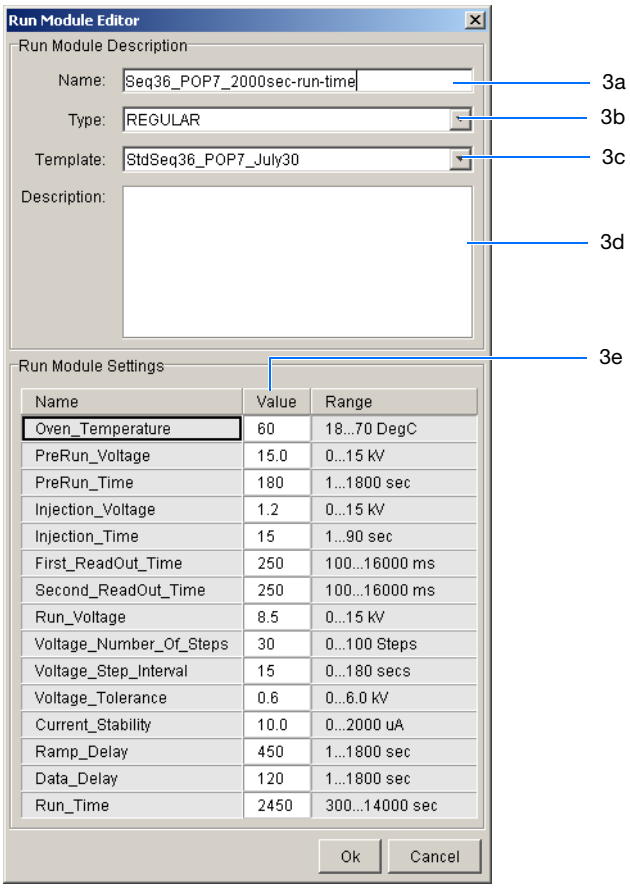
Tip: Customizing Run Modules

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

1. Click **GA Instruments >** **ga3730 >** *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-list, select the type of module (Regular, Spatial or Spectral).
   c. In the Template drop-list, select a template module as a basis for the new module.

Note: You cannot edit a default module installed with 3730/3730*xl* Data Collection software.

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



   e. Change to the desired module parameters using the range for the allowable parameters.
   f. Click **OK**.

Notes

Editable Run Module Parameters

Parameter Name	Range	Comment
Oven_Temperature	18-70 C	Temperature setting for main oven throughout run.
PreRun_Voltage	0-15 kV	Pre run voltage setting before sample injection.
PreRun Time	1-1800 sec	Prerun voltage time.
Injection_Voltage	0-15 kV	Injection voltage setting for sample injection.
Injection_Time	1-90 sec	Sample injection time.
First_ReadOut_time	100-16000 millisec	The interval of time for a data point to be produced. First_ReadOut_time should be equal to Second_ReadOut_time.
Second_ReadOut_Time	100-16000 millisec	The interval of time for a data point to be produced. Second_ReadOut_time should be equal to First_ReadOut_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	0-180 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.
Voltage_Tolerance	0.1-6 kV	Maximum allowed voltage variation. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel. If it goes beyond tolerance and shuts off, contact Applied Biosystems tech support.
Current_Stability	0-2000 microA	Maximum allowed electrophoresis current variation. Current fluctuations above this value will be attributed to air bubbles in system and the voltage automatically turned off. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Ramp_Delay	1-1800 sec	Delay During Voltage Ramp. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Data_Delay	1-1800 sec	Time from the start of separation to the start of sample data collection.
Run_Time	300-14000 sec	Duration data is collected after Ramp_Delay.

Notes

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 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.

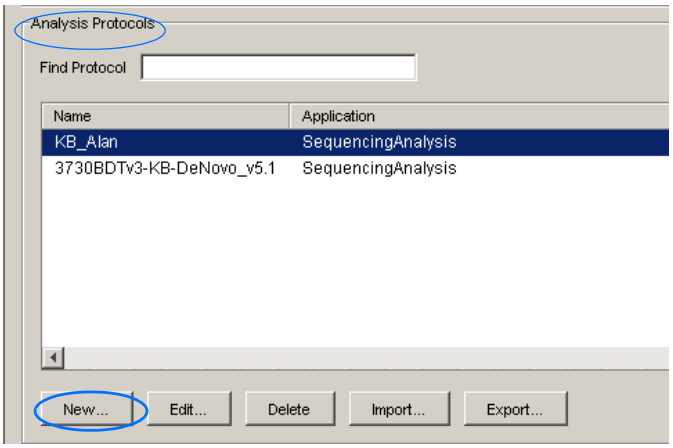
IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis will not be performed if you do so.

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....

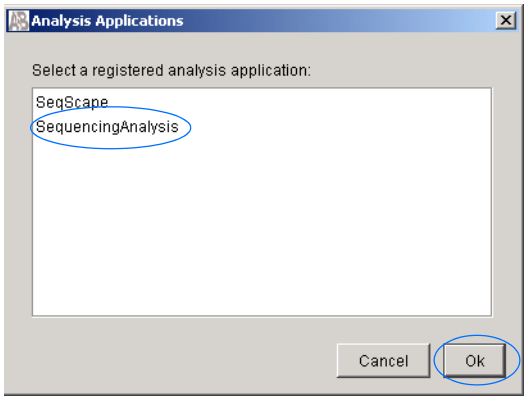
   If more than one analysis application is installed on the data collection computer, the Analysis Applications dialog box opens.



Notes

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

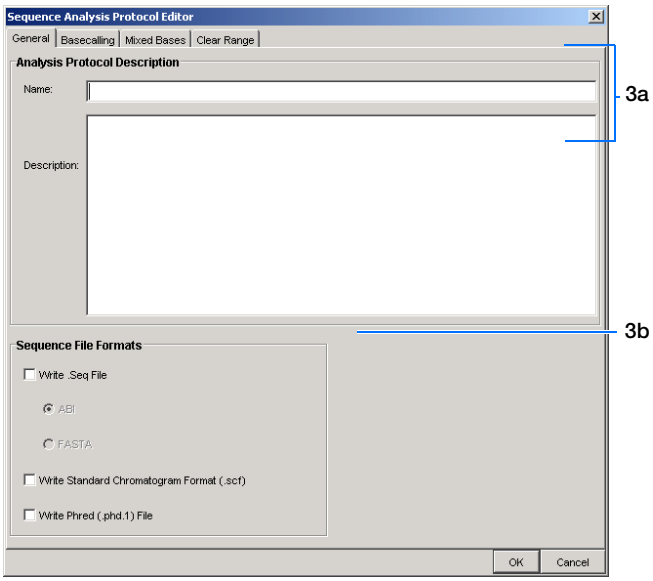
   The Analysis Protocol Editor dialog box opens.



3. In the **General** tab:

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

   b. Select the appropriate Sequence File formats settings.



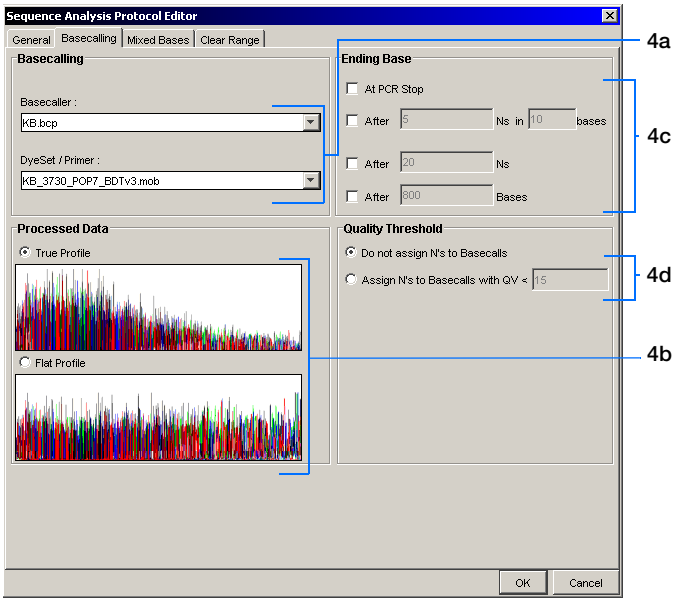
Option	If checked, the software creates…
Write .Seq File check box	a .seq file for printing the sequence as text file or for using the file in other software. • ABI format is used with Applied Biosystems software. • FASTA format is used with other software
Write Standard Chromatogram Format file (.scf)	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.

Notes

4. Select the **Basecalling** tab.

   a. Select the appropriate basecaller and DyeSet primer based on the chemistry and capillary array length you are using.

   Note: Sequencing Analysis Software v5.1 and 3730/3730*xl* Data Collection software filter .mob file choices to match the chosen .bcp file.



   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 option to 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 option to assign Ns to bases with QVs less than the set point. The QV will still be displayed.

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

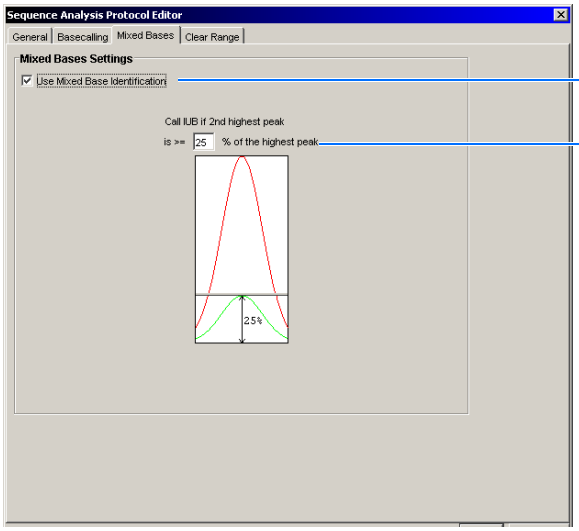
   Note: This function is active with the KB Basecaller only.

Notes

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.

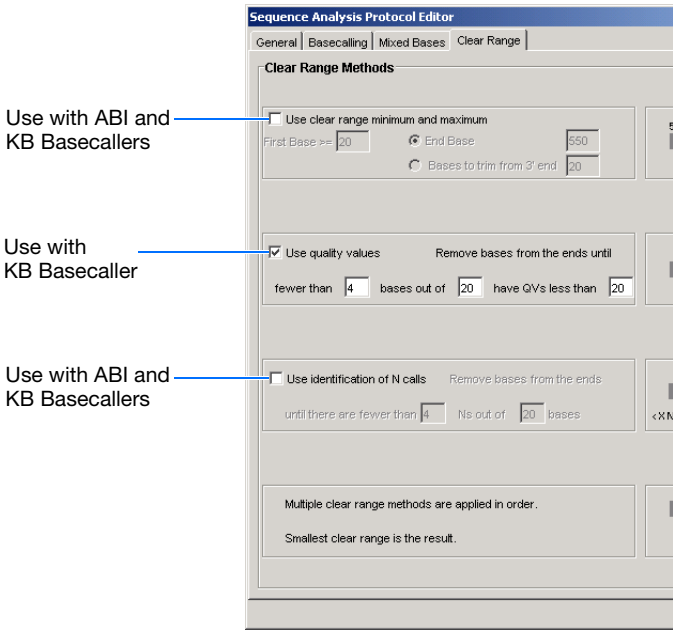


6. Select the **Clear Range** tab.

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.



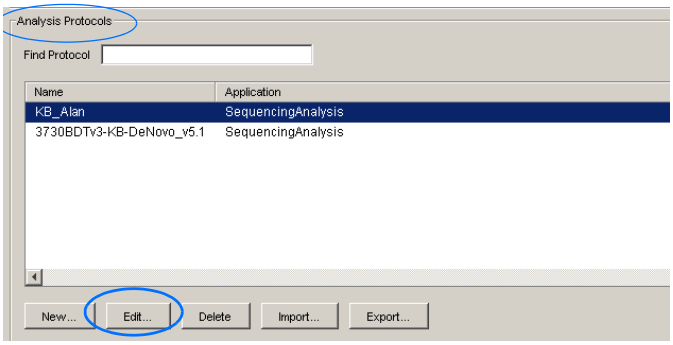
4

Notes

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.

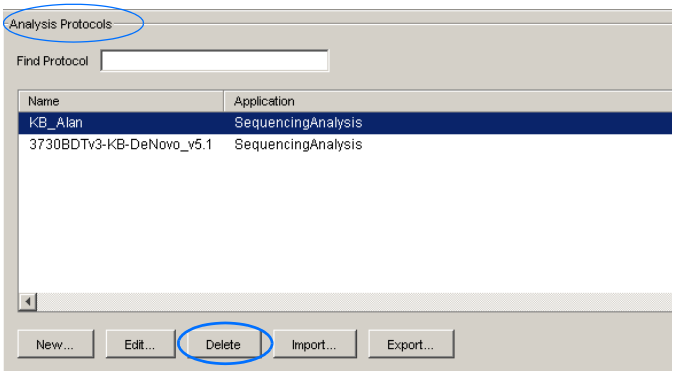


Deleting an Analysis Protocol

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 .



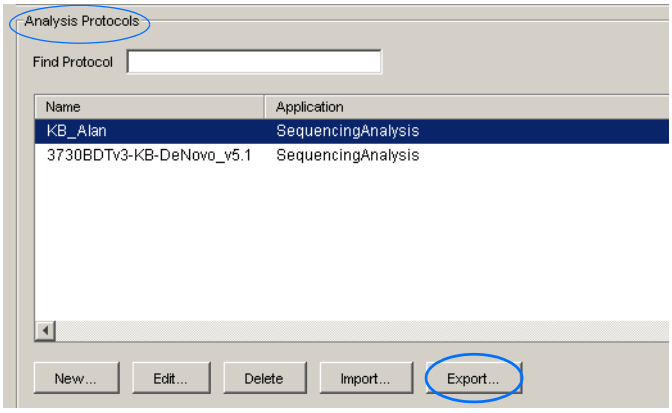
Notes

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.

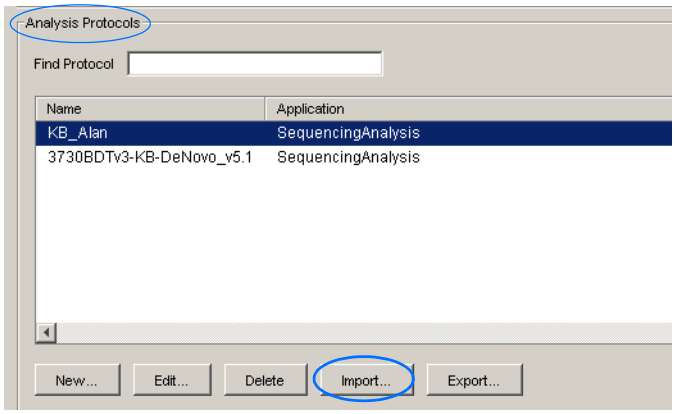
   The Export Confirmation dialog box displays.
3. Click **Save**.



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 Export Confirmation dialog box displays.
3. Click **Save**.



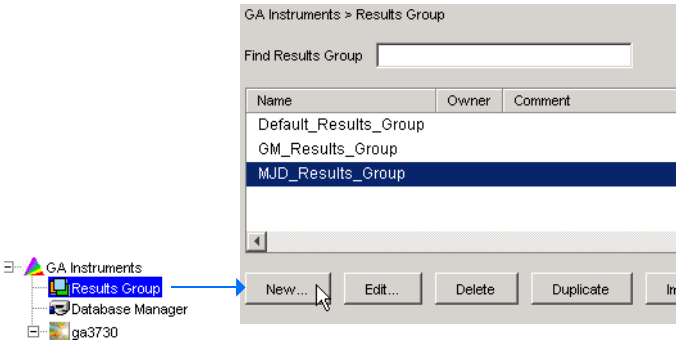
Results Groups

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

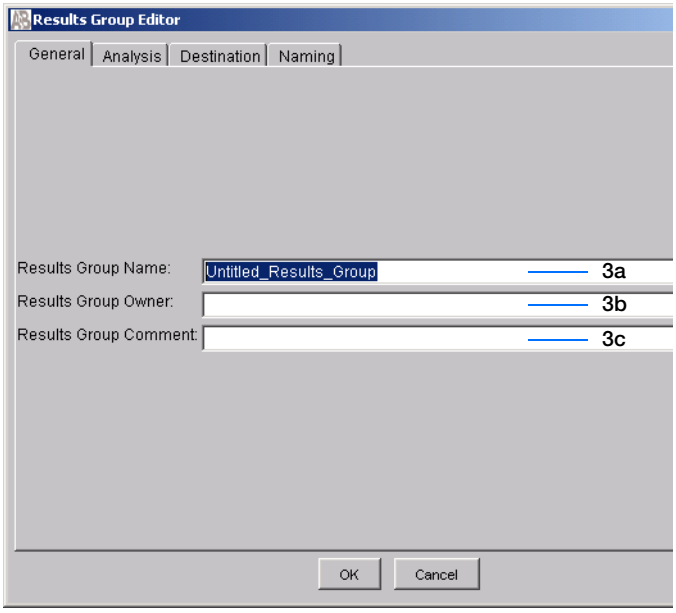
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click New....

   The Results Group Editor window displays.



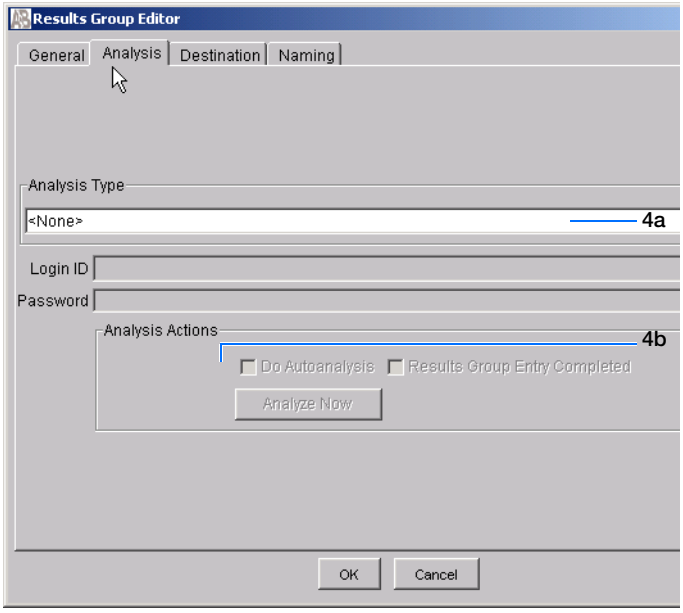
Notes

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).



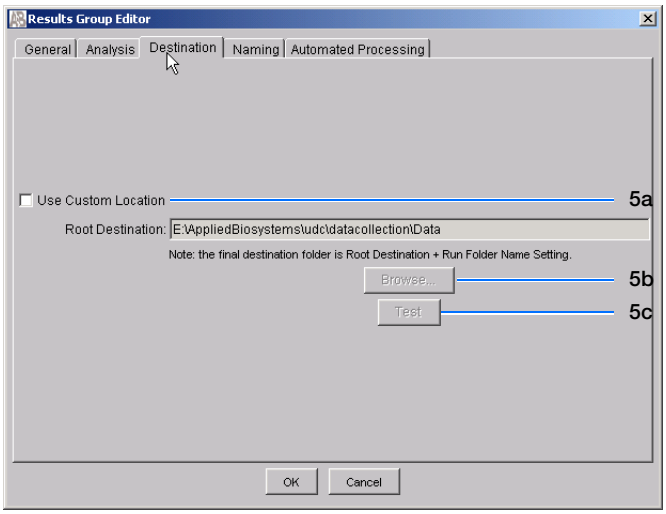
4. Select the **Analysis** tab, then:
   a. Select **Sequencing Analysis** from the Analysis Type drop-list.
   b. In the Analysis Actions section, select **Do Autoanalysis**, if you want your data automatically analyzed after a run.

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 step a and step b below

   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, this text displays "Path Name test successful."
      – If it fails, this text displays "Could not make the connection. Please check that the Path Name is correct." Click **Browse** and select a different location.

Sample File Destinations:

Locations where sample files are placed during extraction:

- Default Destination, default folder naming: *Data* / *instrument type* / *instrument name* / *run folder* (No ProcessedData 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.

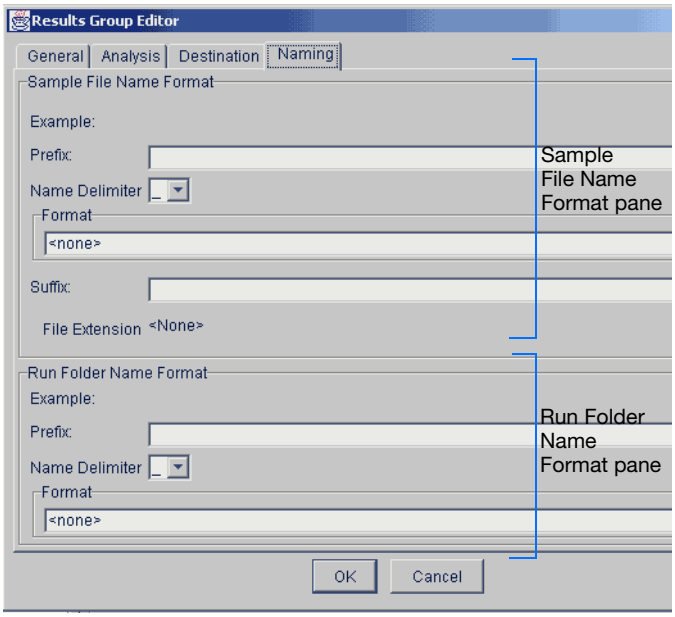
Notes

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 page 58 for accepted characters.

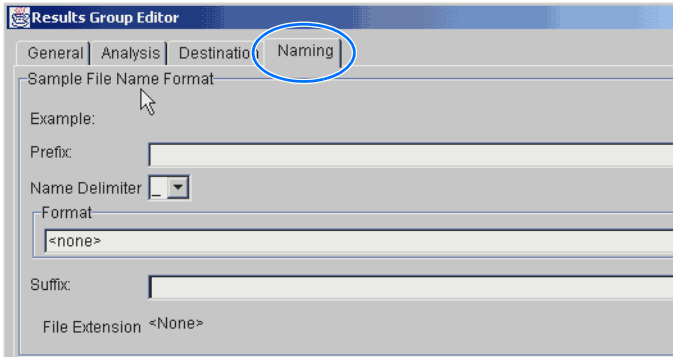
   The elements of the Naming tab are discussed in the following sections.



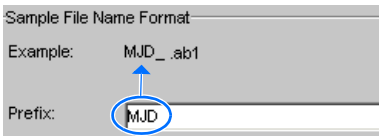
Sample File Name Format Pane

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

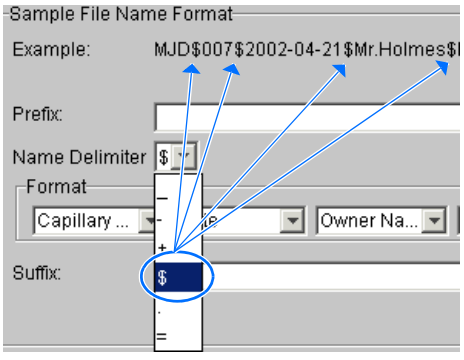
1. Select the **Naming** tab.



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).

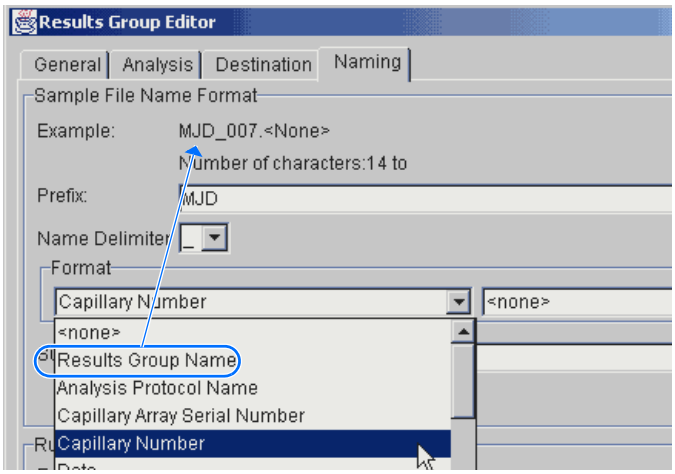


3. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3 below). Only one delimiter symbol may be chosen.



Notes

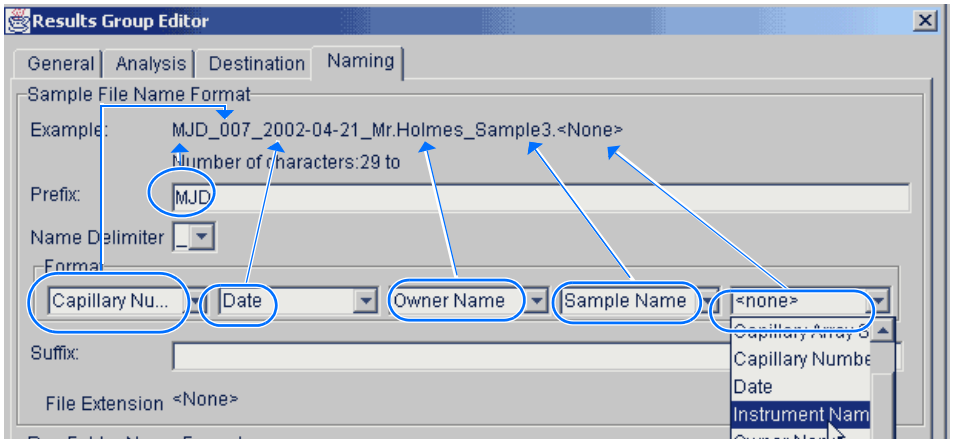
4. Click the Format list and then select the components that you want in the sample name.



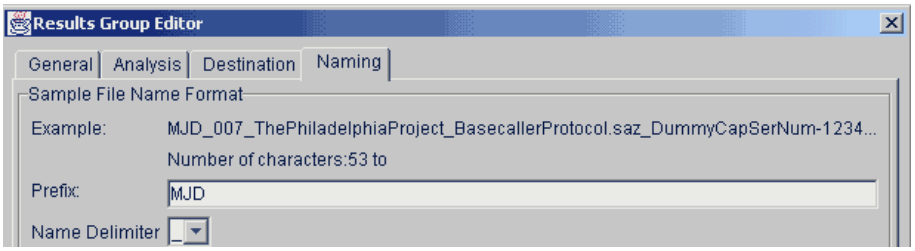
Note: Generally, all the samples from a single run are placed in the same run or results folder, so the name of every sample from a single run should be different. Most of the Format options will not be different between samples, so you need to take care to select at least one of the options that make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

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



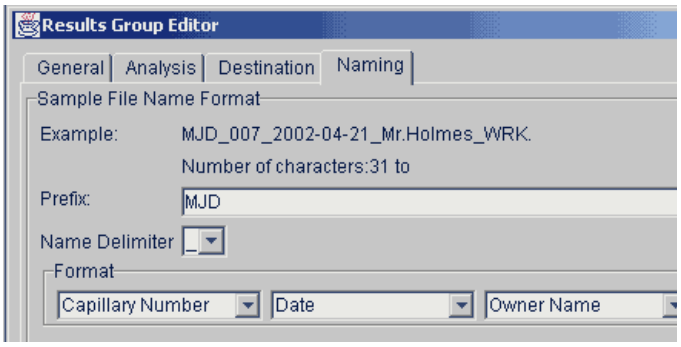
The names of the Format elements eventually truncate, but the Example field remains visible (up to 72 characters).



Notes

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 (page page 72). For example, Sequencing Analysis produces sample files with an .ab1 extension.



Saving a Results Group

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

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

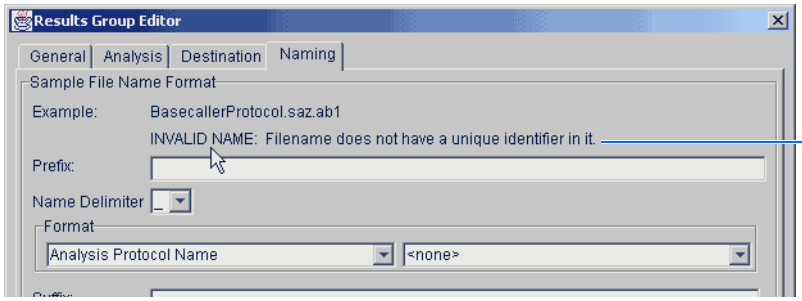
Format Elements (Unique Identifiers)

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.

Note: If you choose a non-unique file name, the software appends numbers (incrementally) before the file extension.

Notes

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 next figure).



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).

Run Folder/Sub-Folder Name Format Pane

Follow the same steps described above for the Sample File Name Format pane (page page 74) to specify the run folder name within the run folder.

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.

Notes

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

1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

3. Complete the information in the New Plate Dialog:

   a. Type a plate ID or barcode.

   b. Type a name for the plate.

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

   d. Select your sequencing application in the Application drop-list.

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

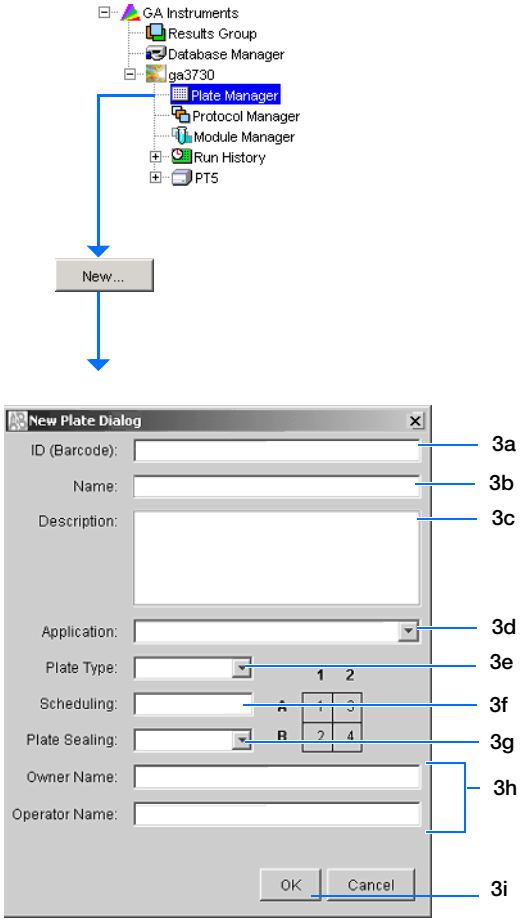
   f. Schedule the plate. For more information, see "Scheduling Runs" on page 121.

   g. Select **heat seal** or **septa**.

   h. Type a name for the owner and operator.

   i. Click OK.

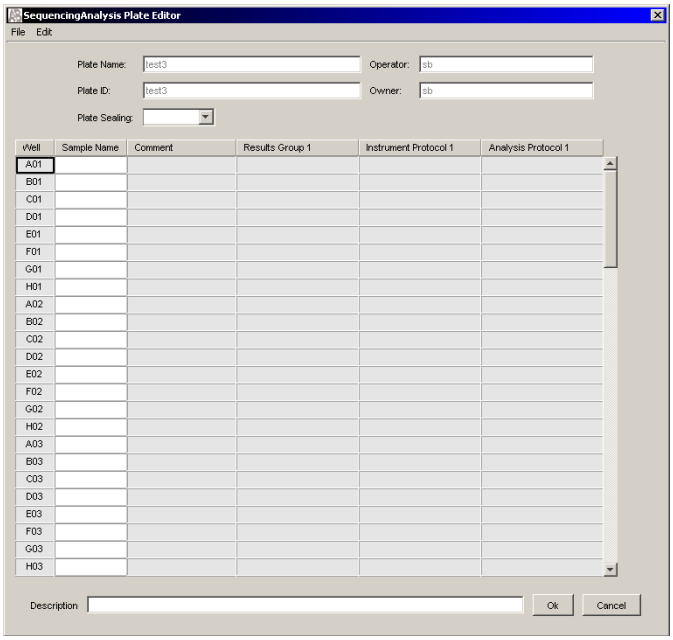
      The Sequencing Analysis Plate Editor opens.



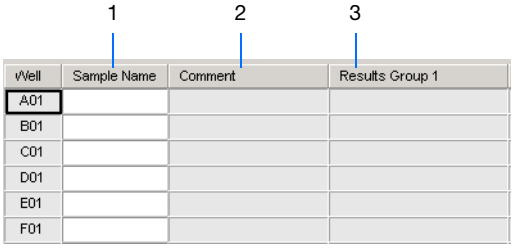
Notes

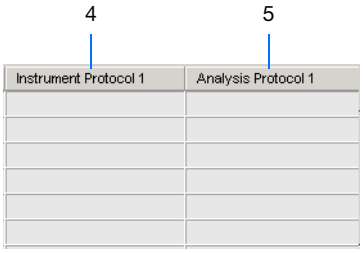
Completing a Sequencing Analysis Plate Record

Note: Plate records can be imported and exported as tab-delimited files (.txt).



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 **Results Group 1** column, select a group from the drop-list (see page 71).
4. In the **Instrument Protocol 1** column, select a protocol from the drop-list (see page 62).
5. In the **Analysis Protocol 1** column, select a protocol from the drop-list (see page 66).

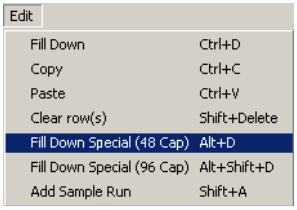




Notes

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

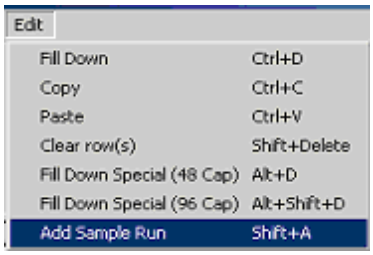
   - For the same samples and protocols – Highlight the entire row, then select **Edit > Fill Down Special** (see "Fill Down Special" on page 82)
   - Based on the plate type (96- or 384-well) and capillary array (48 or 96 capillaries) you are using, select the appropriate fill down option:
     - 96 capillary/96-well plate: *Fill Down.*
     - 48 capillary/96-well plate: *Fill down Special (48 Cap).*
     - 96 capillary/384-well plate: *Fill down Special (96 Cap).*
     - 48 capillary/384-well plate: *Fill down Special (48 Cap).*
   - For the same samples and protocols – Highlight the entire row, then select **Edit > Fill Down**.
   - For the different samples and protocols, complete the plate editor manually.



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

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

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



8. Complete the columns for the additional runs.

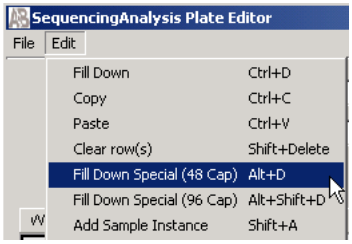
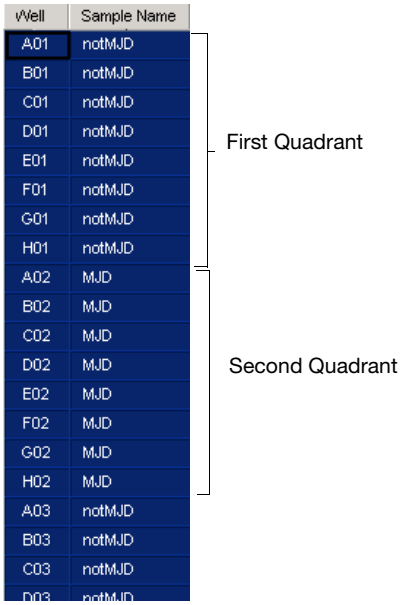
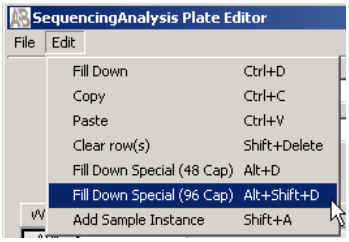
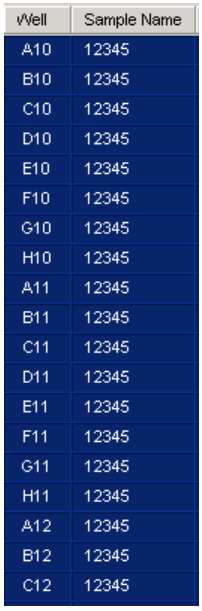
9. Click OK.

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

Fill Down Special

The following table illustrates the Fill Down Special feature.

If You Choose ...	Then ...
Fill Down Special (48 Cap)  	The fill down pattern matches the 48-capillary load pattern. 
Fill Down Special (96 Cap) *  * Especially useful for 384-well plates	The fill down pattern matches the 96-capillary load pattern. 

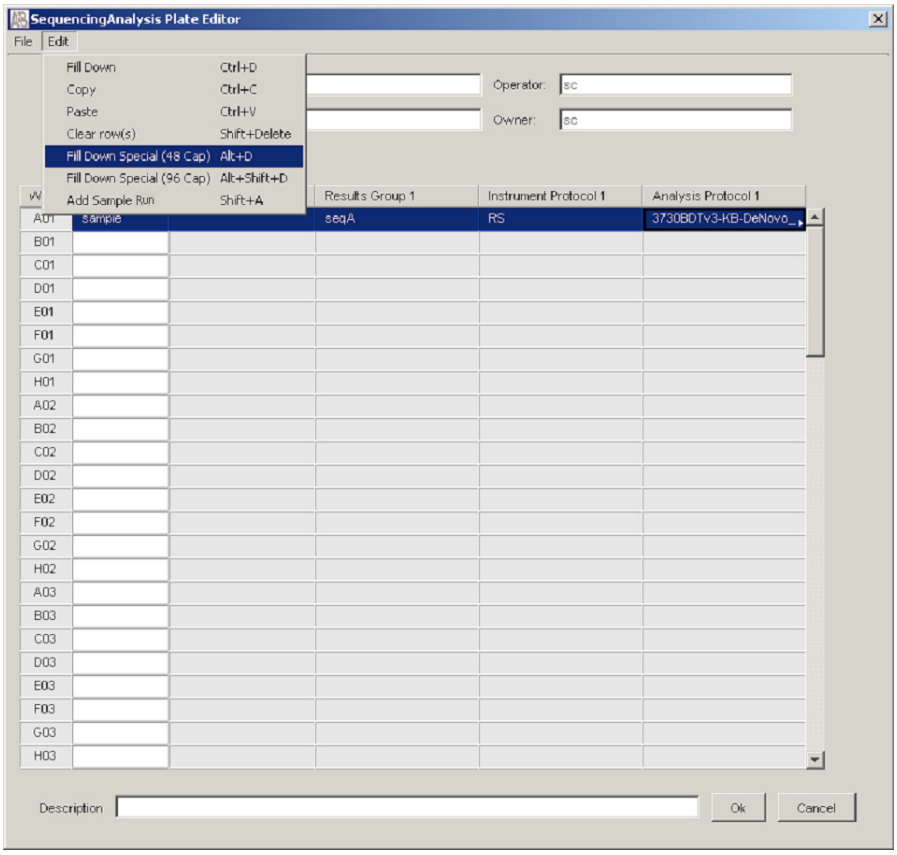
Notes

Fill Down Special for a 48 Cap/96-well Plate

The Fill Down Special feature allows you to fill the plate record based on the load pattern of the capillary array that you are using.

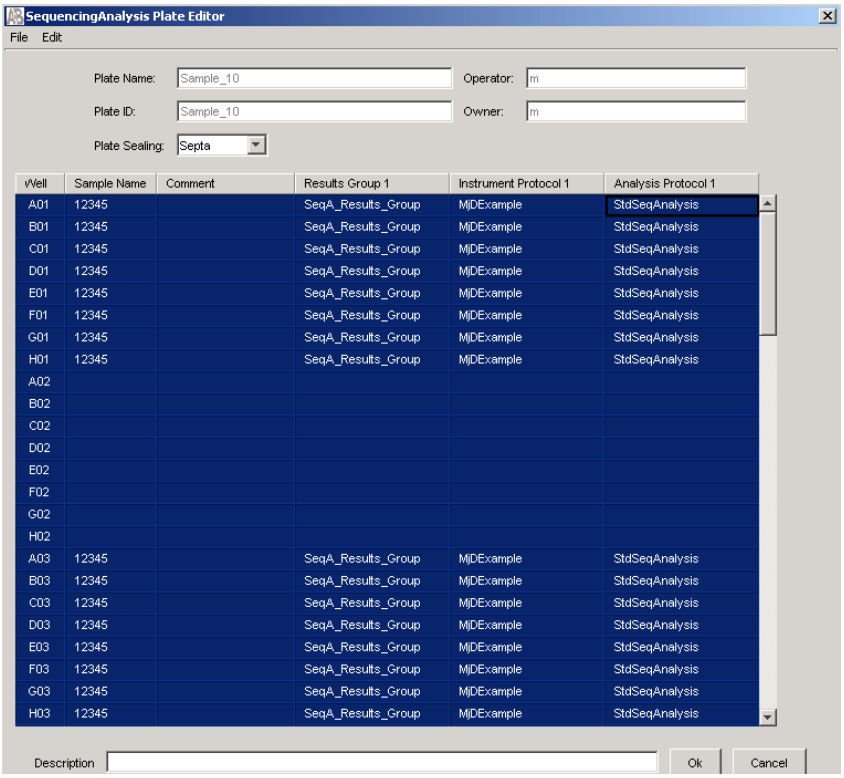
To use the fill down special function:

1. In the Plate Manager, double-click the plate of interest to display the Plate Editor.
2. Type the sample name and then double-click it to highlight the entire row.



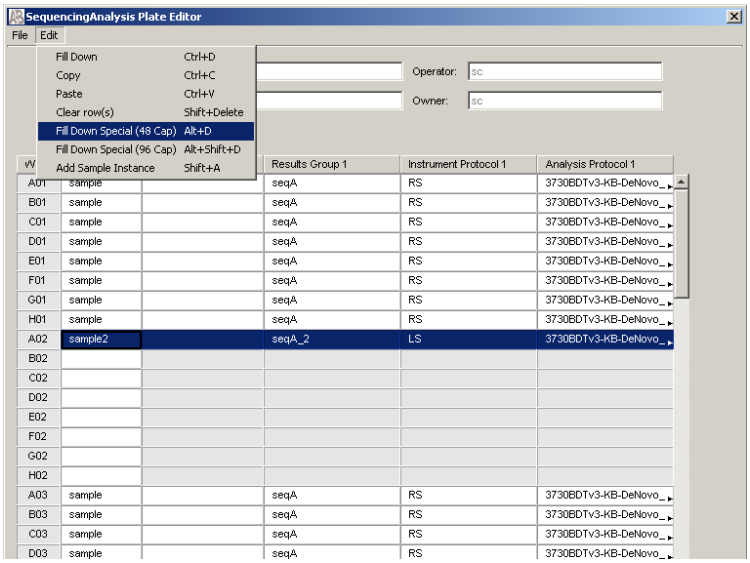
Notes

3. Select **Edit > Fill Down Special (48 Cap)** to fill the first quadrant.



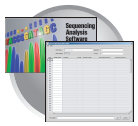
Well	Sample Name	Comment	Results Group 1	Instrument Protocol 1	Analysis Protocol 1
A01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
B01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
C01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
D01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
E01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
F01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
G01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
H01	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
A02					
B02					
C02					
D02					
E02					
F02					
G02					
H02					
A03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
B03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
C03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
D03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
E03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
F03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
G03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis
H03	12345		SeqA_Results_Group	MjDExample	StdSeqAnalysis

4. Click A02, type the name of sample 2 and highlight the entire row.

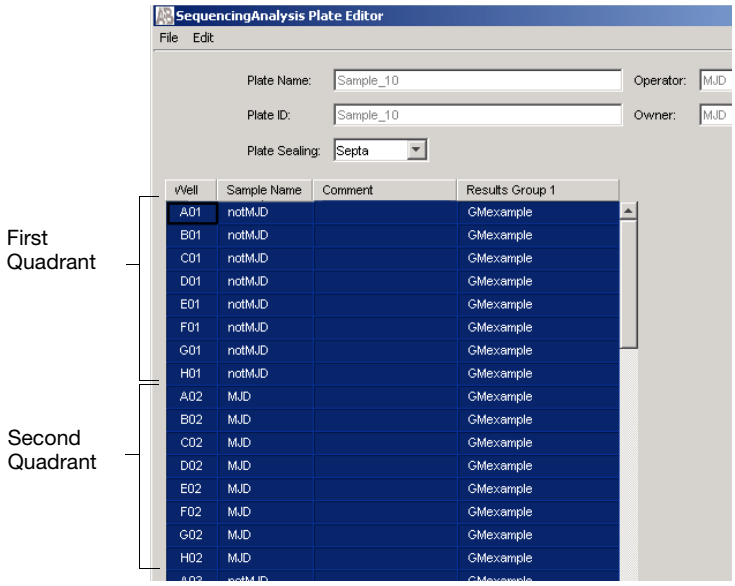


A01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
B01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
C01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
D01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
E01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
F01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
G01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
H01	sample		seqA	RS	3730BDTv3-KB-DeNovo_
A02	sample2		seqA_2	LS	3730BDTv3-KB-DeNovo_
B02					
C02					
D02					
E02					
F02					
G02					
H02					
A03	sample		seqA	RS	3730BDTv3-KB-DeNovo_
B03	sample		seqA	RS	3730BDTv3-KB-DeNovo_
C03	sample		seqA	RS	3730BDTv3-KB-DeNovo_
D03	sample		seqA	RS	3730BDTv3-KB-DeNovo_

Notes

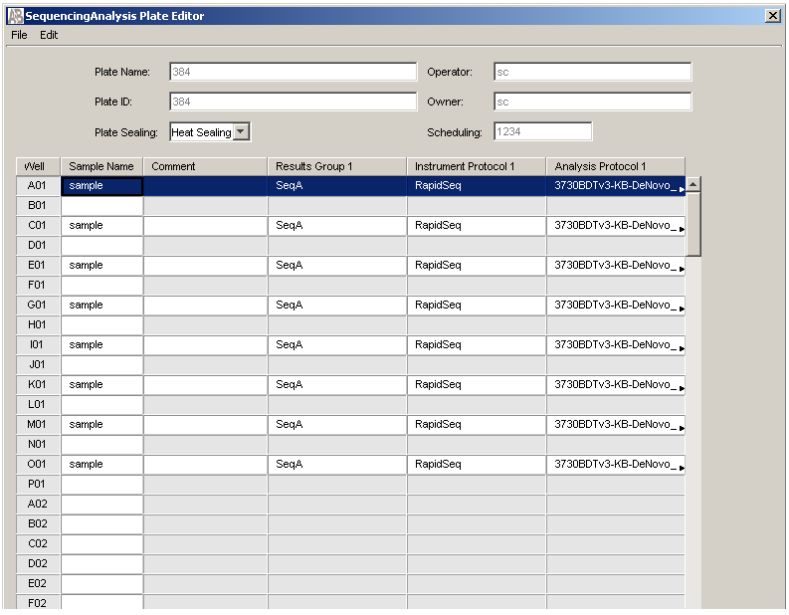


5. Select **Edit > Fill Down Special (48 Cap)** to fill the second quadrant.



Fill Down Special for a 96 Cap/384-well Plate

this is how the fill down pattern looks when you use the Fill Down Special (96 Cap) feature on a 384-well plate.



Notes

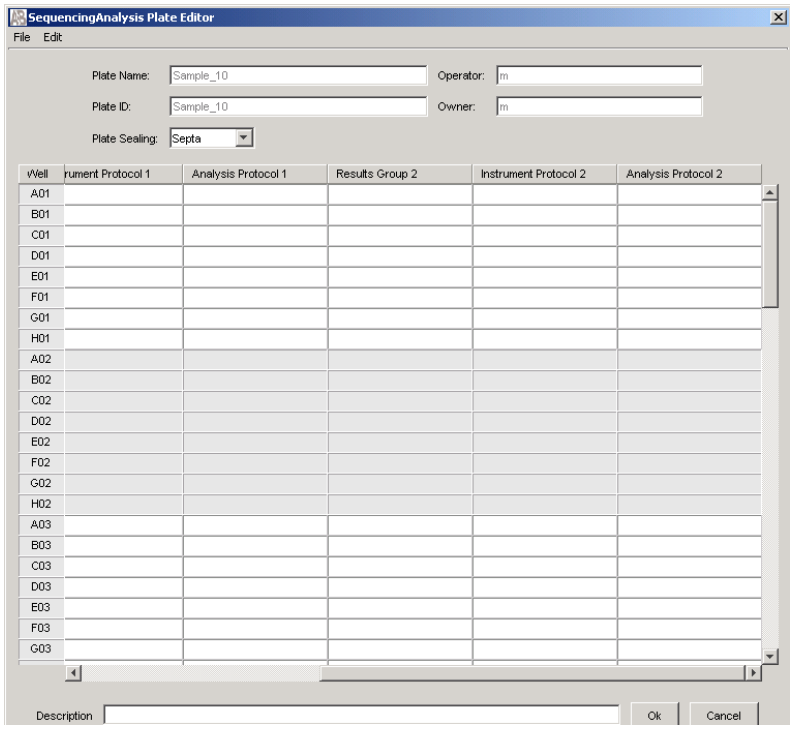
Adding a Sample Run

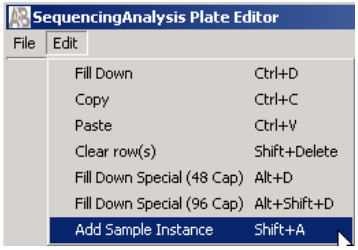
By adding additional sample runs, you can run samples with different variables (different run modules, for example).

Select **Edit > Add Sample Run**

Adding an instance opens an additional:

- Results Group
- Instrument Protocol
- Analysis Protocol (sequencing only)

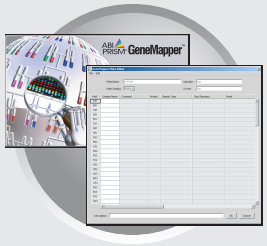




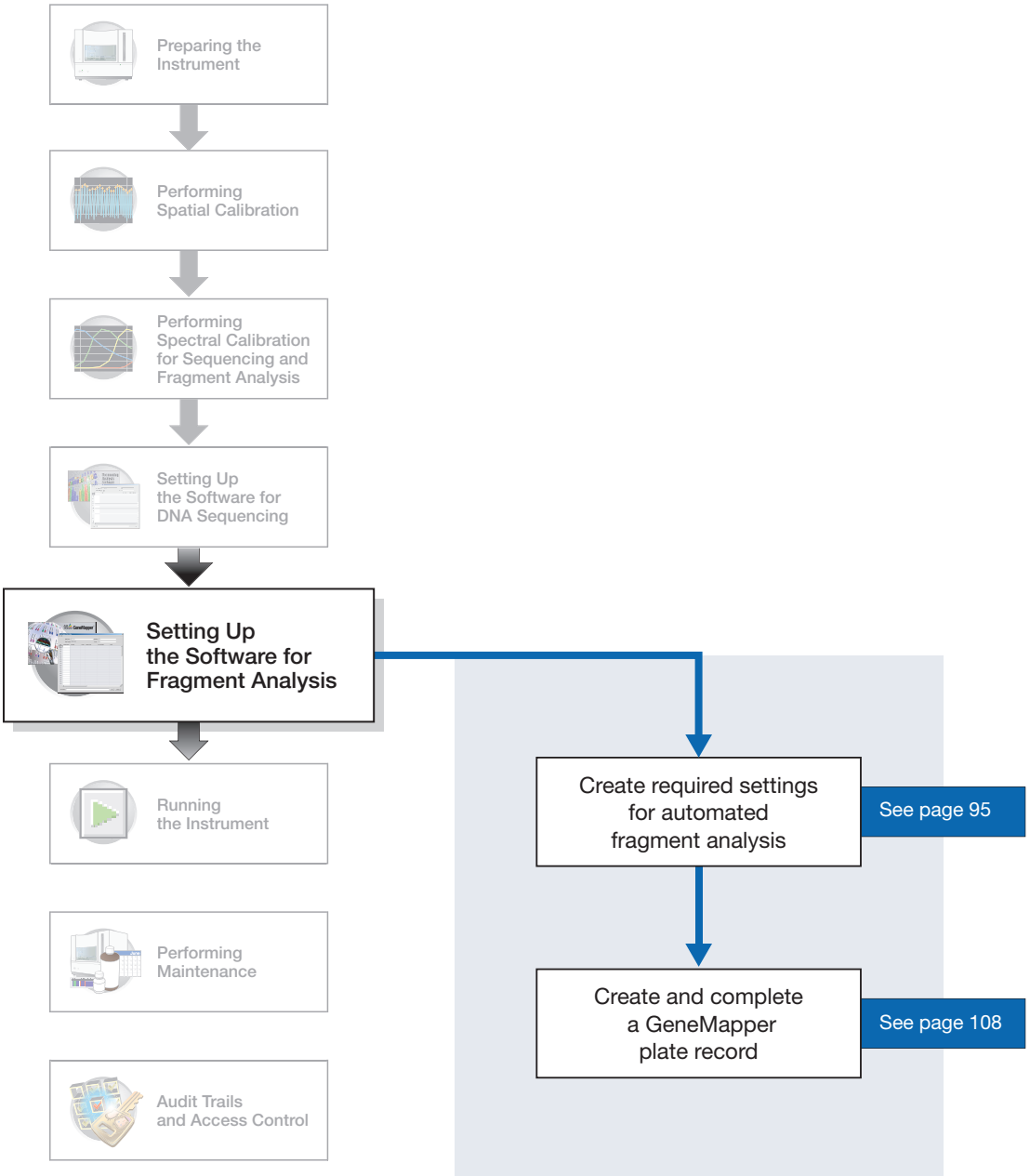
To run the plate(s), see "Running the Instrument" on page 115.

Notes

Setting Up the Software for Fragment Analysis



Workflow



Notes

3730/3730*xl* Data Collection and GeneMapper Software

Important Note

Do not rename the computer once 3730/3730xl Data Collection software has been installed. Doing so *will* cause the 3730/3730xl 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 3730/3730*xl* 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.

Manual Analysis

For information on manual analysis, refer to *GeneMapper Software Version 3.5 User Guide* (PN 4343790)

Fragment Analysis and Data Collection

When GeneMapper software is installed on a computer that has 3730/3730*xl* DNA Analyzer Data Collection Software, two applications are available through the Results Group Editor (see page 102):

- GeneMapper-Generic

and,

- GeneMapper-<Computer Name>

Notes

GeneMapper-Generic

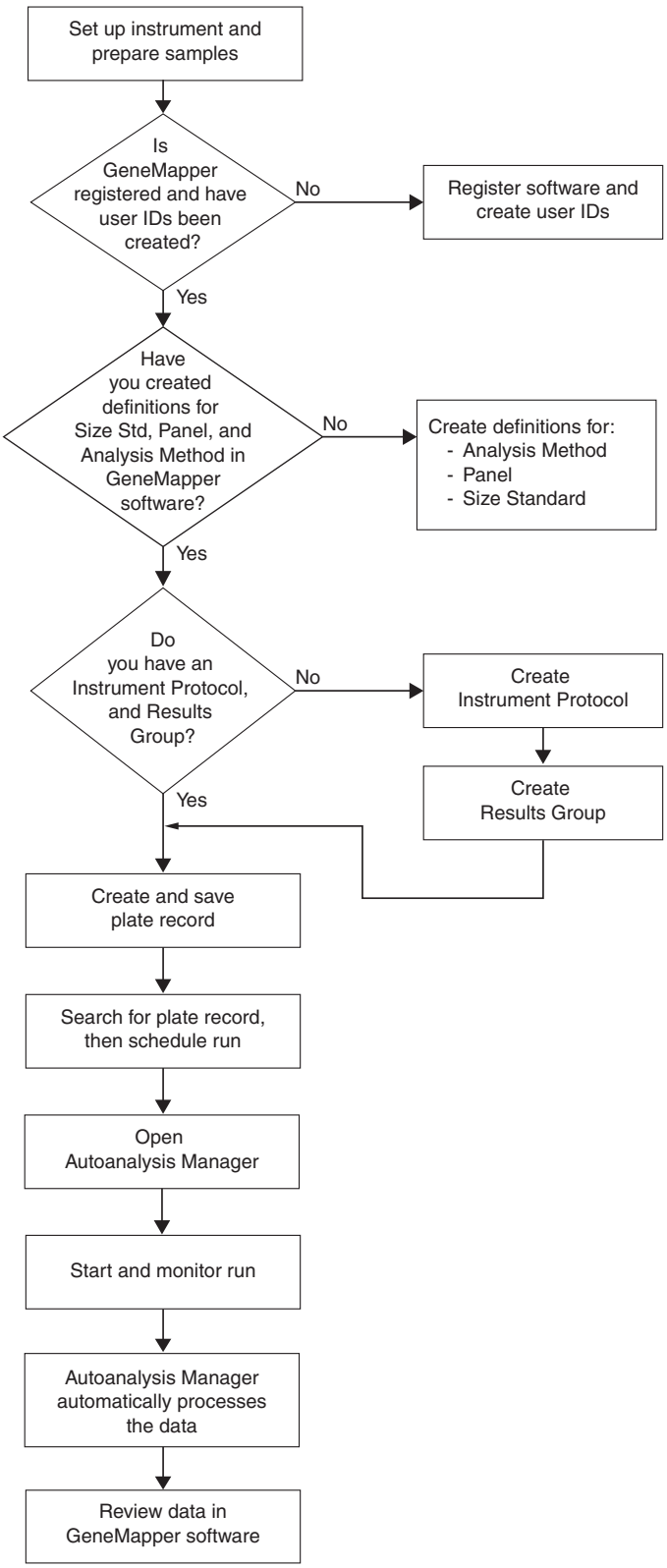
GeneMapper-Generic enables you to generate .fsa files, but not perform autoanalysis. When completing the Sample Sheet, you need to fill in basic information for Data Collection to complete the run; all other GeneMapper software related fields are text entries. This is useful if you are using other software 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, "Setting Up a Run for Autoanalysis" on page 136.

Notes

Workflow for Autoanalysis Using GeneMapper Software



Notes

GeneMapper Plate Records

Overview

A plate record is similar to a sample sheet or an injection list that you may have used with other Applied Biosystems 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

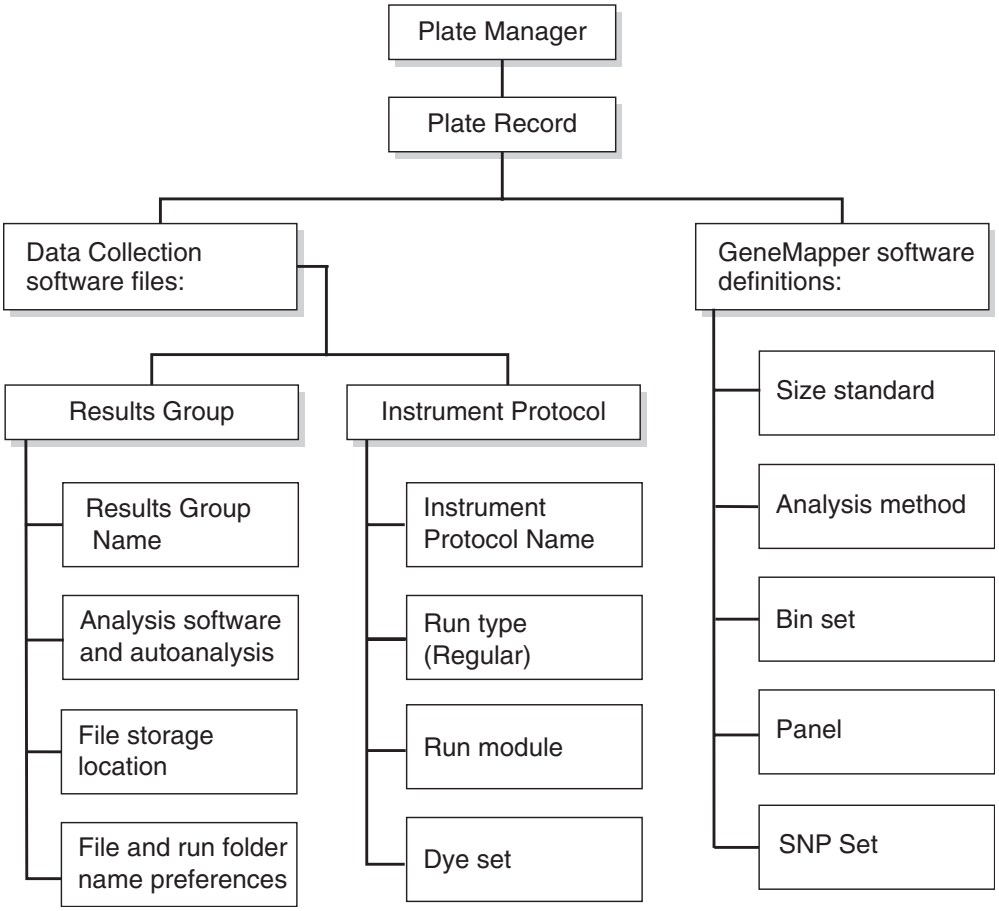
Note: A plate record must be created in advance of the first run. Plate records can be created, and plates added to the stacker, while a run is in progress.

Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	97
Results Group	Defines the file type, the file name, autoanalysis, and file save locations that are linked to sample injections.	102

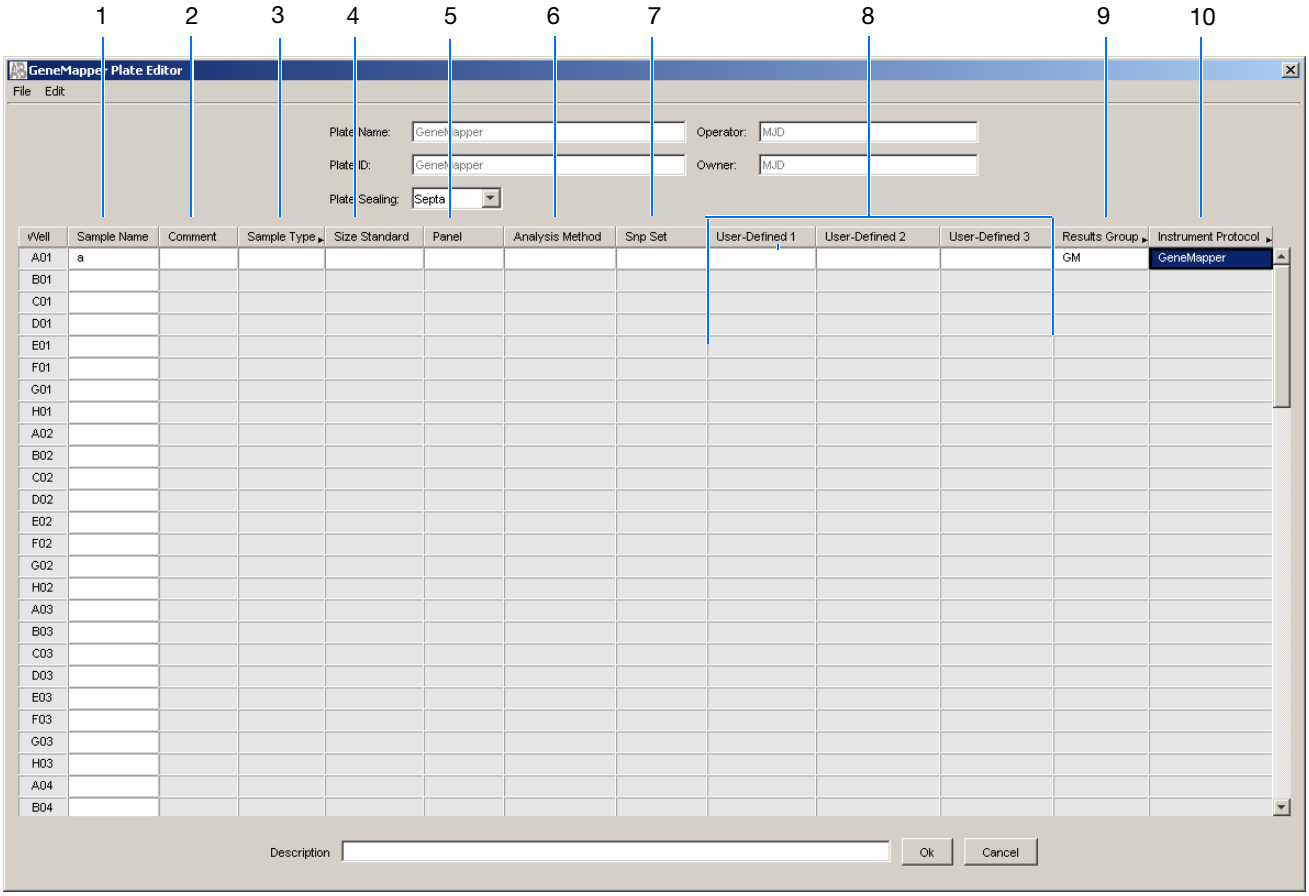
IMPORTANT! In order for data collection and auto-analysis to be successful, each run of samples must have an Instrument Protocol and a Results Group assigned within a plate record.

Notes

Elements of a GeneMapper Software Plate Record



Notes



Default is one sample run. To add additional runs, see page 112.

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

Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Sample Type	Use to identify the sample as Sample, Positive Control, Allelic Ladder or Negative Control.
4. 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-list

Notes

Column	Description
5. 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-list
6. 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-list
7. Snp **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>: Use for SNPlex chemistry; select a saved SNP set from the drop-list
8. 3 User-defined columns	Optional text entries
9. 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 Groups" on page 102.
10. 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 Protocols" on page 97.

Notes

Creating Required Settings for Automated Fragment Analysis

If the Settings Already Exist

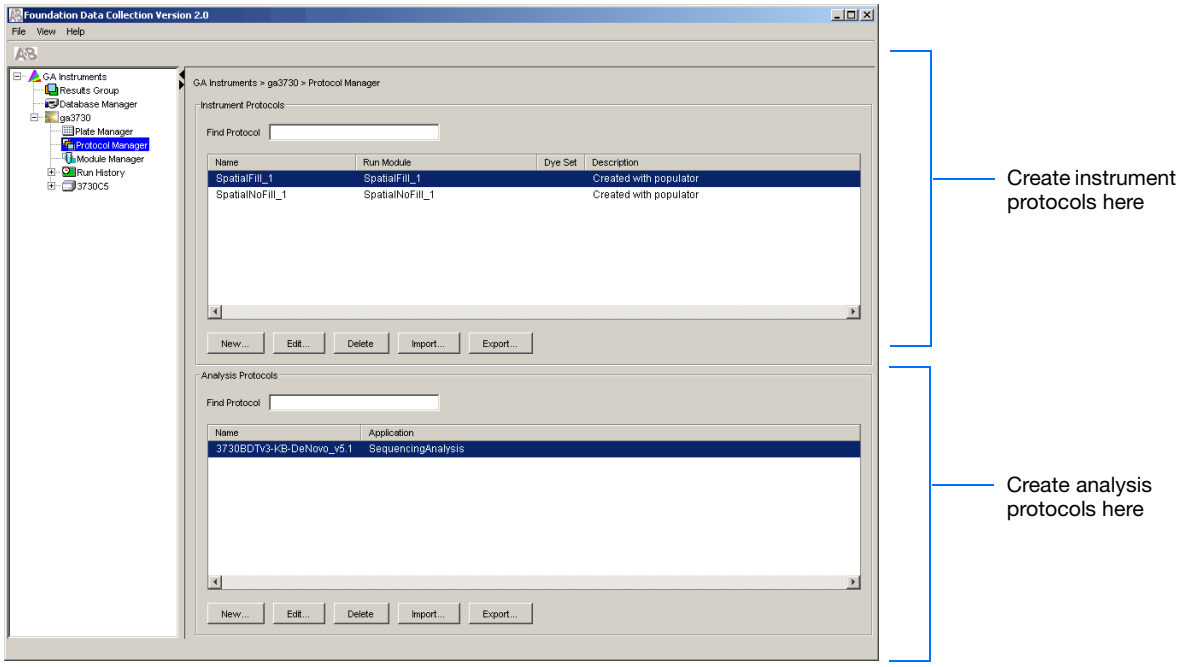
If the appropriate data collection and fragment analysis files have been created, proceed to "Creating and Completing a GeneMapper Plate Record" on page 110.

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

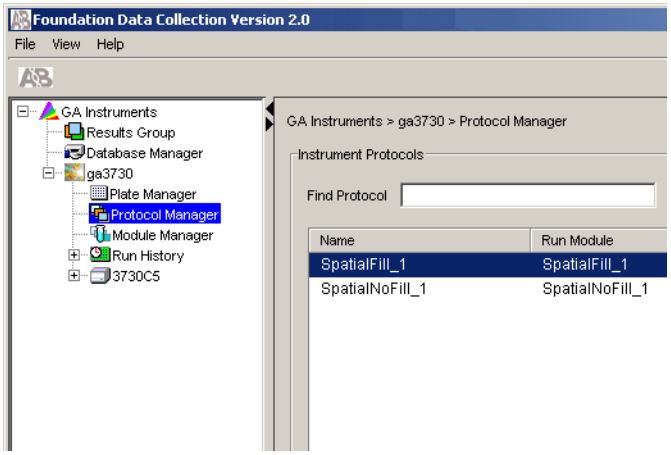
1. In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.



Notes

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

   The Protocol Editor opens.

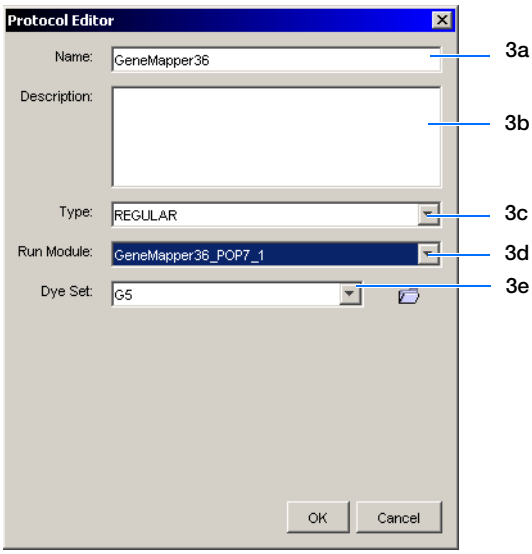


3. Complete the Protocol Editor:

   a. Type a name for the protocol.

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

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



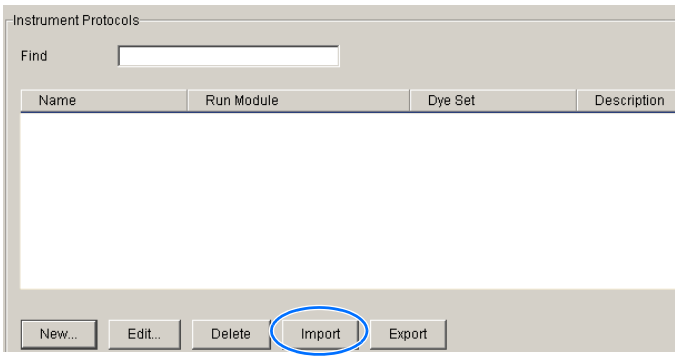
   d. Select **GeneMapper36_POP7**.

   e. Select **G5**.

   f. Click OK.

Importing an Instrument Protocol

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



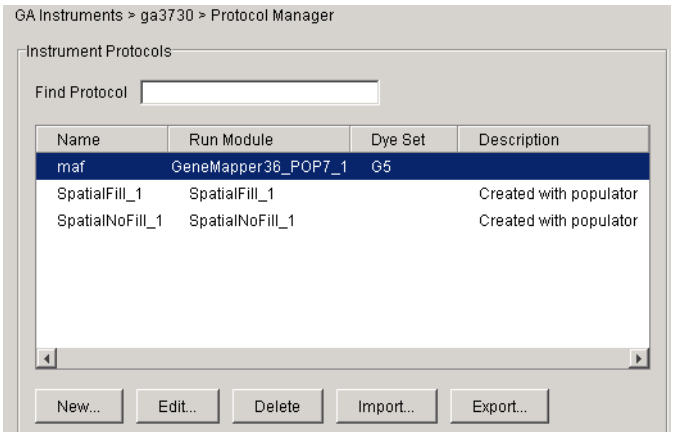
Notes

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 files are displayed alphabetically in the Instrument Protocol pane.

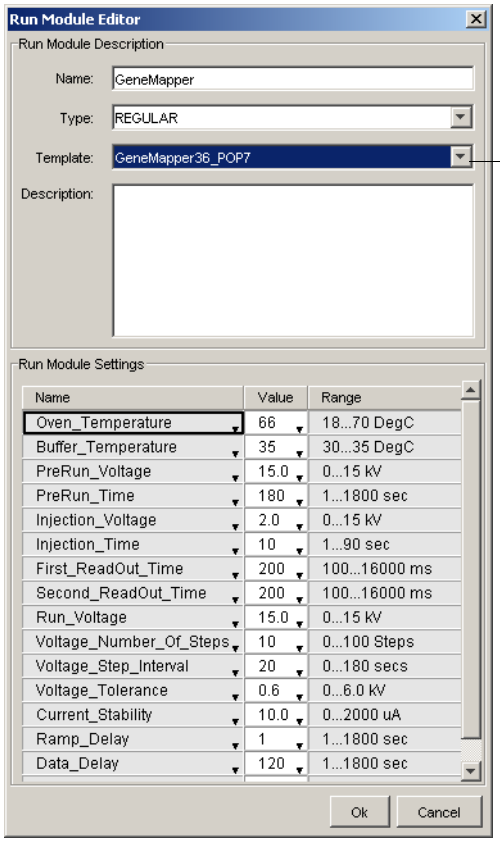


Notes

Customizing Run Modules

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

1. Click **GA Instruments** > **ga3730** > **Module Manager**.
2. Click New....
3. Select a template module as a basis for the new module.



Choose module template from the drop-down menu (step 3).

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

> **Note:** You cannot edit a default module installed with 3730/3730*xl* Data Collection.

Notes

The Run Module Parameters that you can edit:

Parameter Name	Range	Comment
Oven_Temperature	18-70 C	Temperature setting for main oven throughout run.
PreRun_Voltage	0-15 kV	Pre run voltage setting before sample injection.
PreRun Time	1-1800 sec	Prerun voltage time.
Injection_Voltage	0-15 kV	Injection voltage setting for sample injection.
Injection_Time	1-90 sec	Sample injection time.
First_ReadOut_time	100-16000 millisec	The interval of time for a data point to be produced. First_ReadOut_time should be equal to Second_ReadOut_time.
Second_ReadOut_Time	100-16000 millisec	The interval of time for a data point to be produced. Second_ReadOut_time should be equal to First_ReadOut_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	0-180 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.
Voltage_Tolerance	0.1-6 kV	Maximum allowed voltage variation. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel. If it goes beyond tolerance and shuts off, contact Applied Biosystems tech support.
Current_Stability	0-2000 microA	Maximum allowed electrophoresis current variation. Current fluctuations above this value will be attributed to air bubbles in system and the voltage automatically turned off. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Ramp_Delay	1-1800 sec	Delay During Voltage Ramp. We recommend that you do not change this value unless advised otherwise by Applied Biosystems support personnel.
Data_Delay	1-1800 sec	Time from the start of separation to the start of data collection.
Run_Time	300-14000 sec	Duration data is collected after Ramp_Delay.

Notes

Results Groups

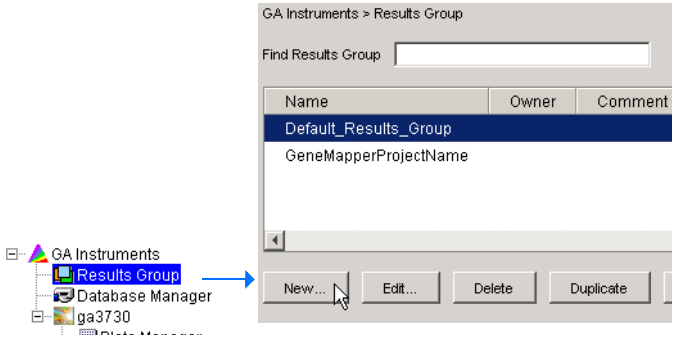
A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. A Results Group is used to prepare samples for analysis and, to name, sort, and deliver samples that result from a run.

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.

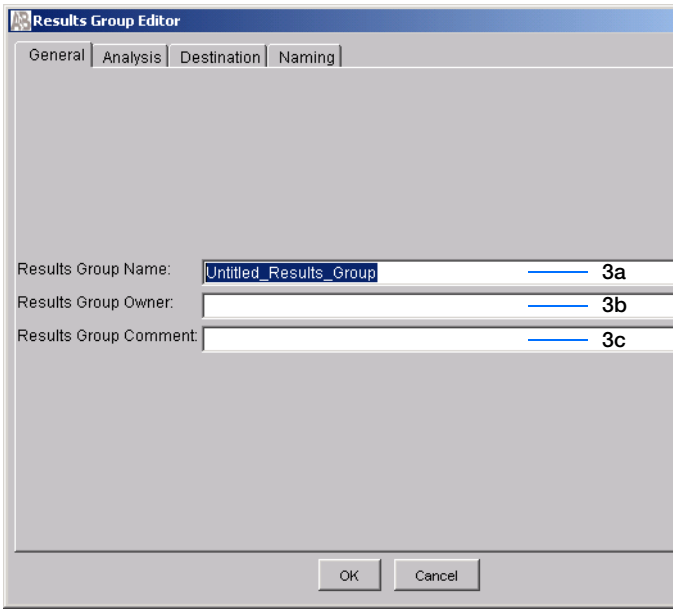


3. Complete the General tab:

   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).

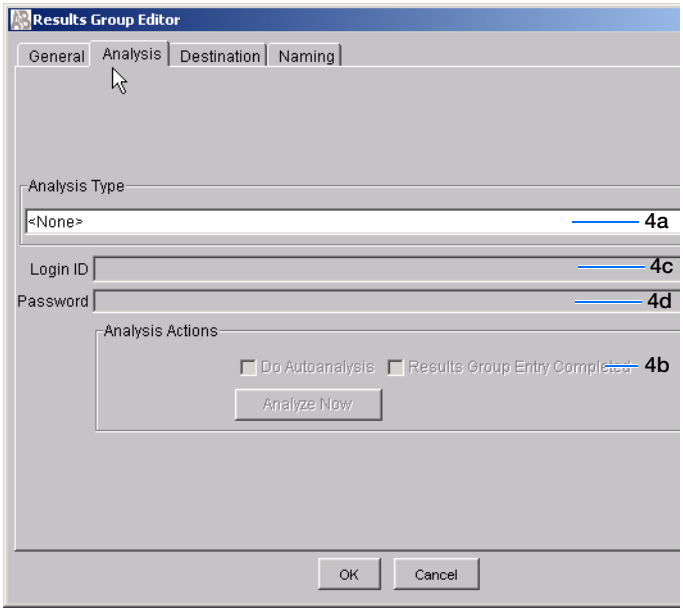
   b. Type a Results Group Owner (optional). The owner name can be used in naming and sorting sample files.

   c. Type a Results Group Comment (optional).



Notes

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



   a. 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 available and only .fsa files are generated
GeneMapper-<Computer Name>	• Autoanalysis of completed runs is available • Automated Processing tab is available 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 105)
Do Autoanalysis	Samples are analyzed after each run of 48 or 96 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**

   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.

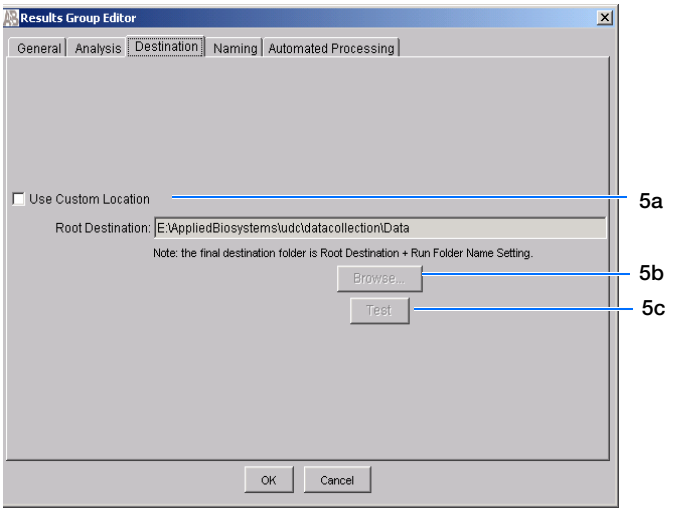
Notes

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 step a and step b below

   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, this text displays "Path Name test successful."
      – If it fails, this text displays "Could not make the connection. Please check that the Path Name is correct." Click **Browse** and select a different location.



Sample File Destinations:

Locations where sample files are placed during extraction:

- Default Destination, default folder naming: *Data* / *instrument type* / *instrument name* / *run folder* (No ProcessedData 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.

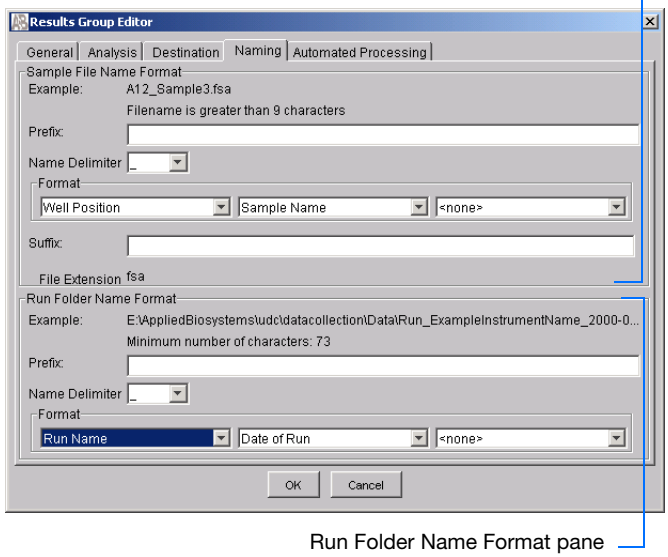
Notes

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 90 for accepted characters.

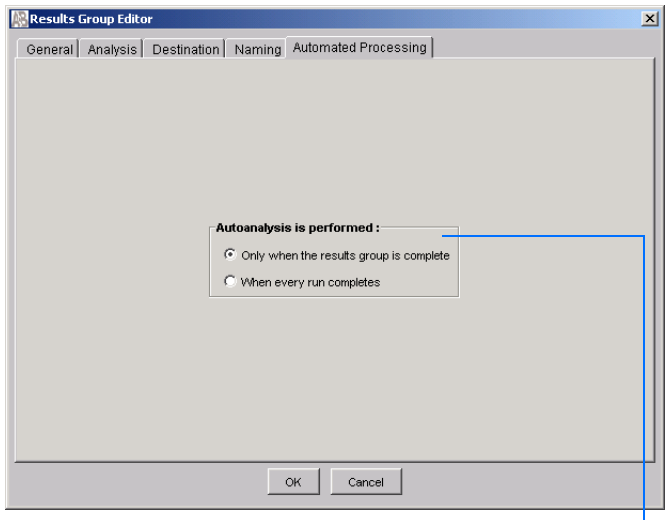
   The elements of the Naming tab are discussed in the following sections, see page 106.



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

   Note: The Automated Processing tab is available only if you selected GeneMapper-<Computer Name> in step 4 on page 101

   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 101)
Only when the result group is complete	Samples are analyzed after all samples using the same results group have been run.	• Do Autoanalysis **and** • Do Autoanalysis and Results Entry Group Complete
When every run completes	Samples are analyzed after each run of 48 or 96 samples.	Do Autoanalysis **only**

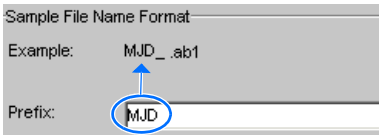
8. Click OK to save the Results Group.

Notes

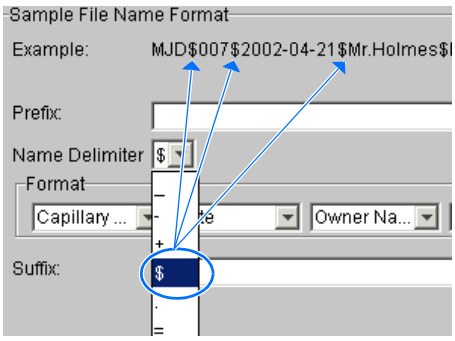
Sample File Name Format Pane

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).



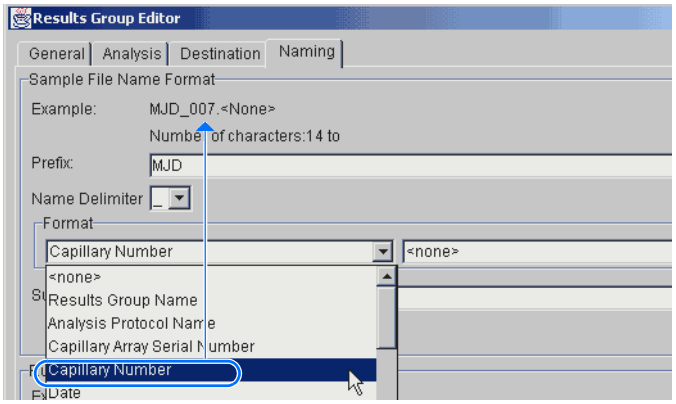
2. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3 below). Only one delimiter symbol may be chosen.



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.

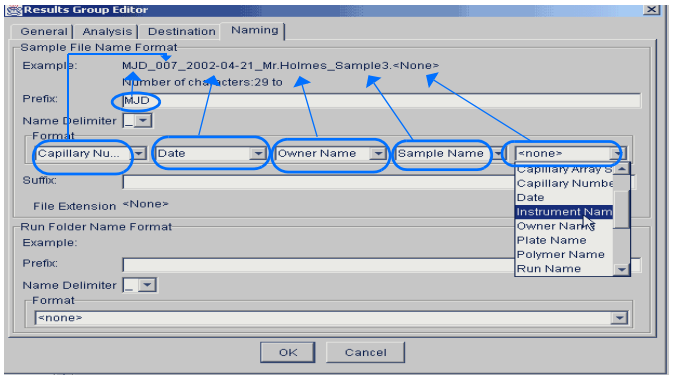
   For example, if a unique identifier is not included in the name, a warning message displays. The Results Group **makes the** file name unique. As you select the elements for the file name, they are reflected in the Example line.



Notes

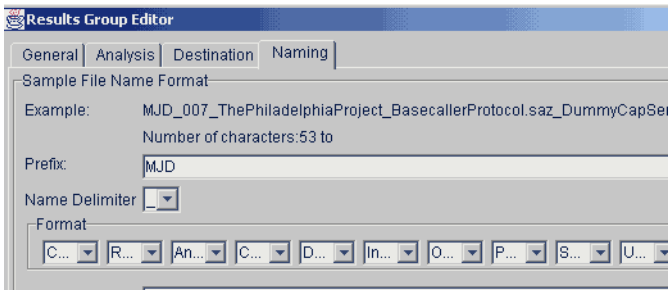
As you continue to select elements for the file name, additional elements display.

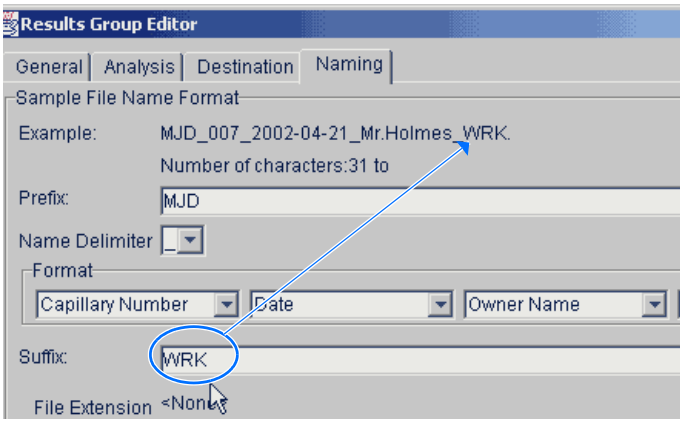
Note: An additional format list drop-menu displays after you select a format option.



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

Note: To view the truncated format elements, place the cursor on the edge of the window until it turns into a double-arrow. Drag the arrow to expand the window horizontally.





Notes

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 (page 101). For example, fragment analysis produces sample files with an .fsa extension.

Run Folder/Sub-Folder Name Format Pane

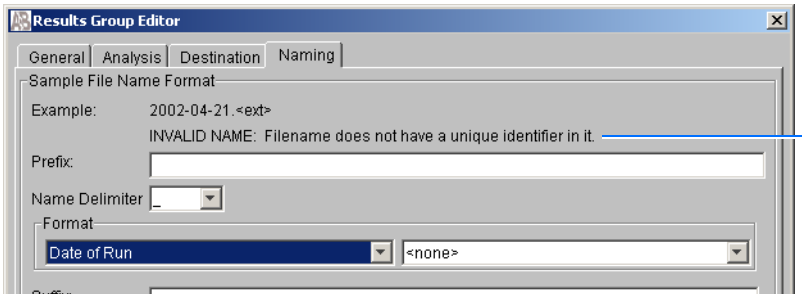
Follow the same steps described above for the Sample File Name Format pane (page 104) to change the sub-folder name within the run folder.

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.

Note: If you choose a non-unique file name, the software appends numbers (incrementally) before the file extension.

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 below).



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 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 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** > **ga3730** > **Plate Manager**.

2. Click New....

   The New Plate Dialog dialog box opens.

3. Complete the information in the New Plate Dialog:

   a. Type a plate ID.

   b. Type a name for the plate.

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

   d. Select your GeneMapper application in the Application drop-list.

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

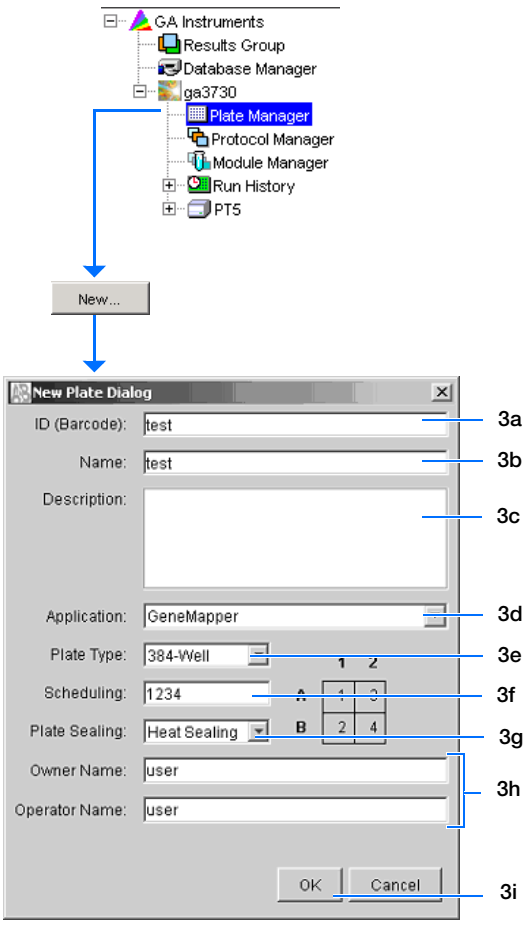
   f. Schedule the plate. For more information, see "Scheduling Runs" on page 121.

   g. Select **Heat Sealing** or **Septa**.

   h. Type a name for the owner and the operator.

   i. Click OK.

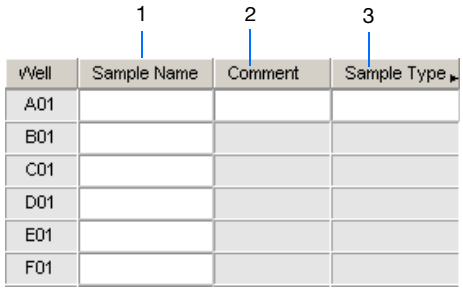
   The GeneMapper Plate Editor opens.

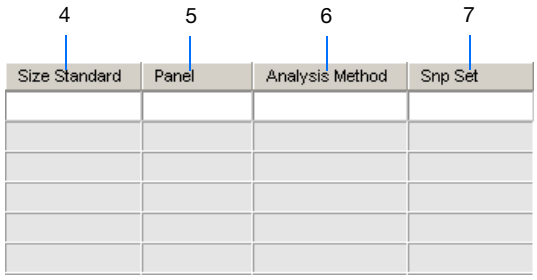


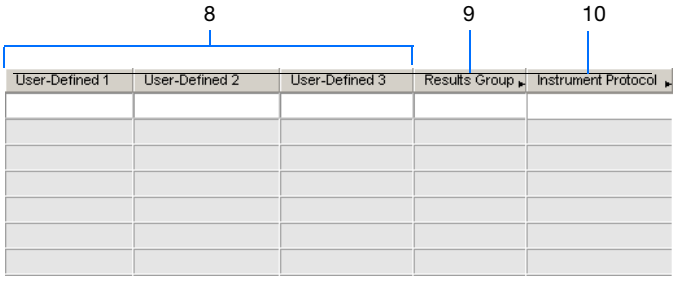
Notes

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.
2. In the **Comment** column, enter any additional comments or notations for the sample.
3. In the **Sample Type** column, select a sample type from the drop-list.
4. In the **Size Standard** column, select a size standard from the drop-list.
5. In the **Panel** column, select a panel from the drop-list.
6. In the **Analysis Method** column, select a method from the drop-list.
7. In the **Snp Set** column, select a SNP set from the drop-list.
8. Enter text for User-Defined columns 1 to 3.
9. In the **Results Group 1** column, select a group from the drop-list.
10. In the **Instrument Protocol 1** column, select a protocol from the drop-list.



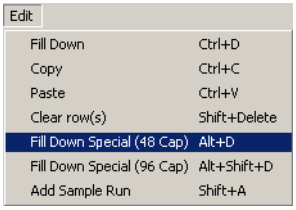




Notes

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

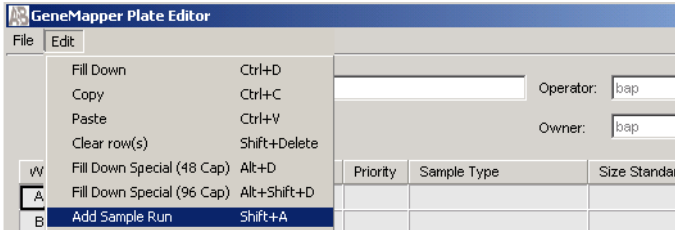
- For the same samples and protocols – Highlight the entire row, then select **Edit > Fill Down Special**. For more information see, "Fill Down Special" on page 111.
- Based on the plate type (96- or 384-well) and capillary array (48 or 96 capillaries) you are using, select the appropriate fill down option:
  - 96 capillary/96-well plate: *Fill Down*
  - 48 capillary/96-well plate: *Fill down Special (48 Cap)*
  - 96 capillary/384-well plate: *Fill down Special (96 Cap)*
  - 48 capillary/384-well plate: *Fill down Special (48 Cap)*
- For the different samples and protocols, complete the plate editor manually.



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

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

You can add additional runs by selecting **Edit > Add Sample Run** again (for more information see, "Adding a Sample Run" on page 113.



13. Complete the columns for the additional runs.

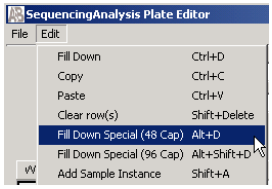
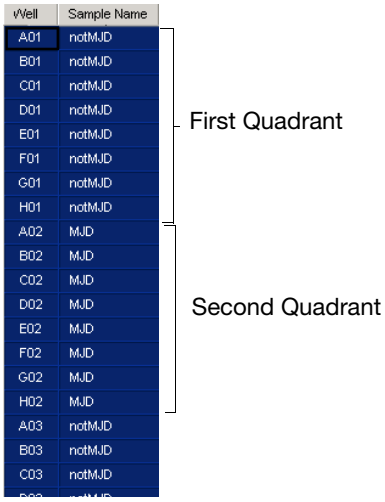
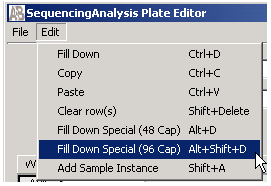
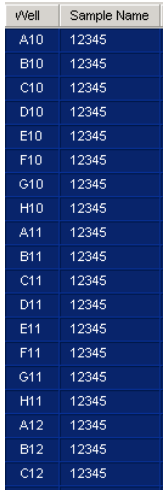
14. Click OK to save, then close the plate record.

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

Fill Down Special

The following table illustrates the Fill Down Special feature.

If You Choose ...	Then ...
Fill Down Special (48 Cap)  	The fill down pattern matches the 48-capillary load pattern. 
Fill Down Special (96 Cap) *  * Especially useful for 384-well plates	The fill down pattern matches the 96-capillary load pattern. 

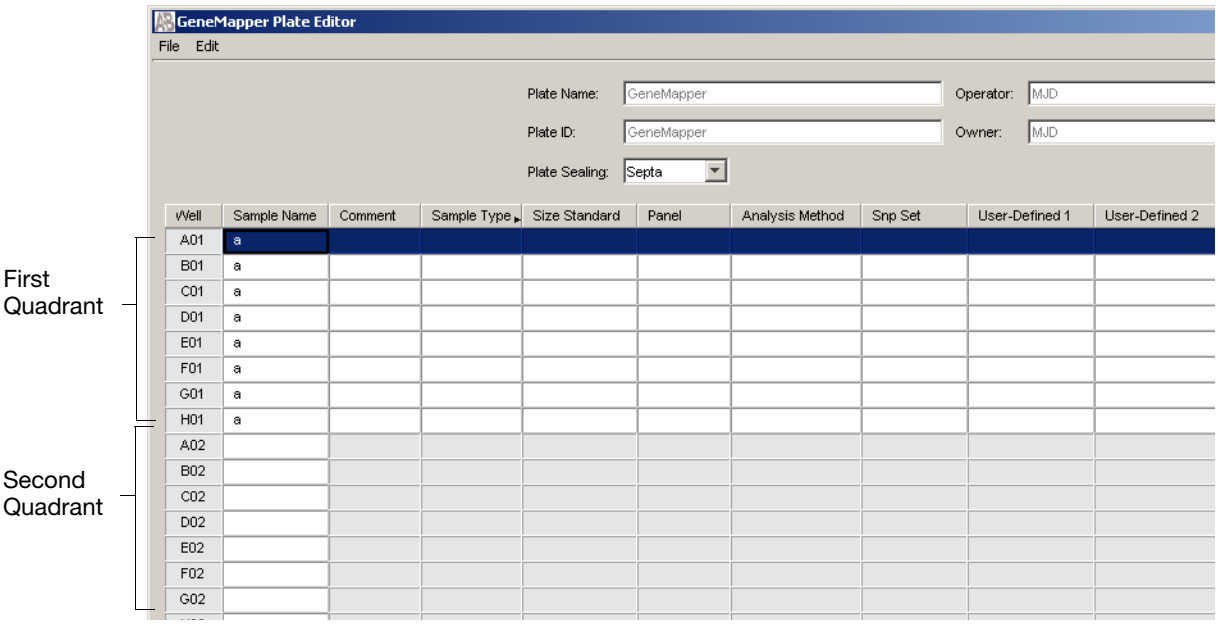
Fill Down Special for a 48 Cap/96-well Plate

The Fill Down Special feature allows you to fill the plate record based on the load pattern of the capillary array that you are using.

Notes

To use the fill down special function:

1. In the Plate Editor, complete the sample information in a row within the quadrant you want.
2. Highlight the entire row.
3. Select **Edit > Fill Down Special (48 Cap)** to fill the first quadrant.
4. Click A02, type the sample information, and highlight the entire row.

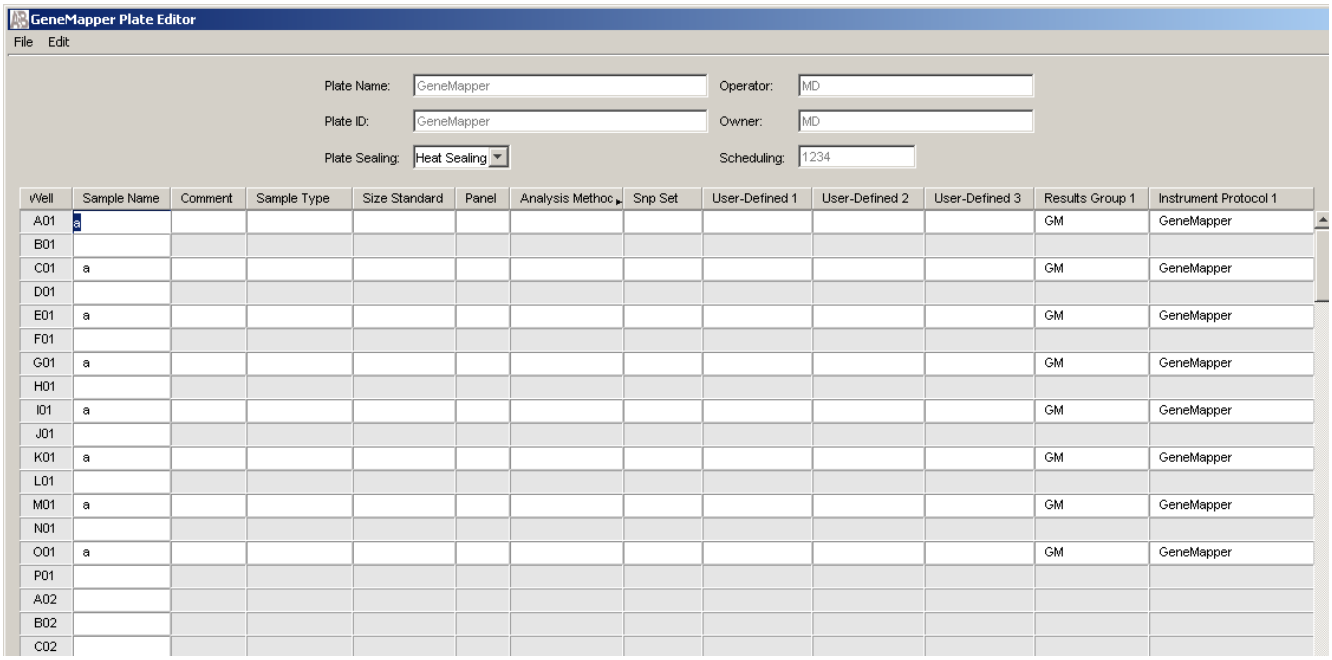


5. Select **Edit > Fill Down Special (48 Cap)** to fill the second quadrant.

Notes

Fill Down Special for a 96 Cap/384-well Plate

This is how the fill down pattern looks when you use the Fill Down Special (96 Cap) feature on a 384-well plate.



Adding a Sample Run

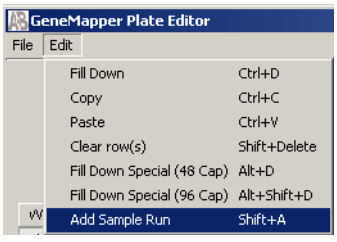
By adding additional sample runs, you can run samples with different variables (different run modules, for example).

Adding a sample run opens an additional:

- Results Group
- Instrument Protocol

1. Select **Edit > Add Sample Run**

To Run the plate(s), see "Running the Instrument" on page 115.

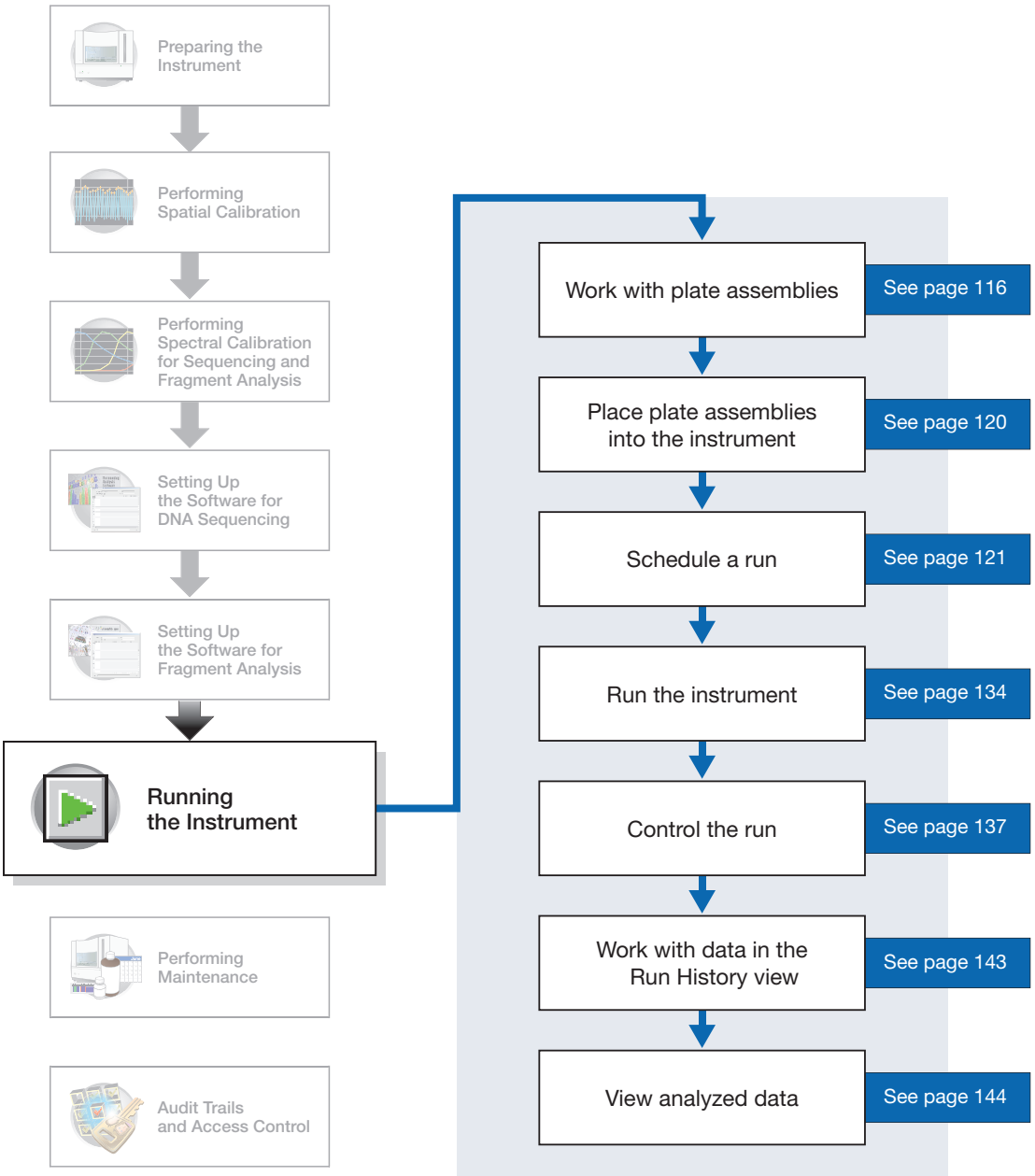


Notes

Notes

Running the Instrument

Workflow



Notes

Working with Plate Assemblies

Plate Assembly Components

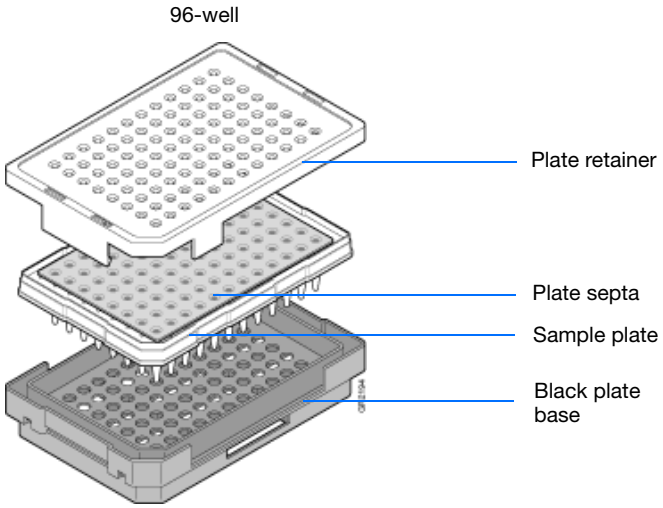
WARNING Do not use warped or damaged plates.

Materials Required for Septa Assemblies

Each 96- or 384-well septa assembly contains a:

- Plate retainer
- Plate septa
- Sample plate
- Base plate

WARNING Use only *black* plate bases with septa-sealed plates.



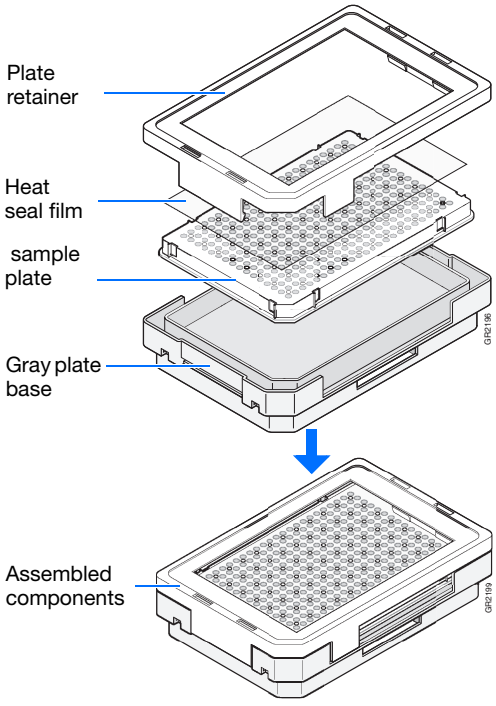
Notes

Materials Required for Heat-sealed Assemblies

Each 96- or 384-well heat-sealed assembly contains a:

- Plate retainer
- Heat seal film
- Sample plate
- Base plate

WARNING Use only *gray* plate bases with heat-sealed plates.



384-well plate

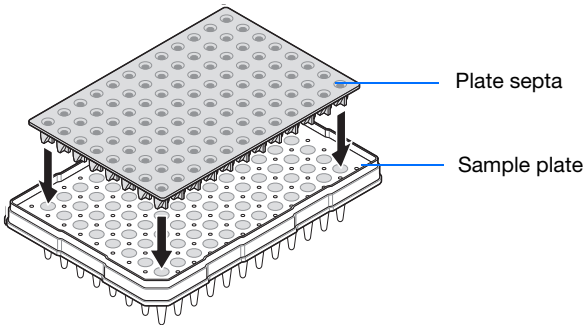
IMPORTANT! Heat Seal Recommendations

- Use 3-mil Applied Biosystems heat seal film (PN 4337570). This film is 3-mil before, and 1-mil after, heating.
- *Do not* use heat seal film thicker than 1-mil, after heating, on 3730/3730*xl* DNA Analyzer.
- Do *not* use heat-seal film containing adhesives or metals as these may damage the instrument's piercing needles

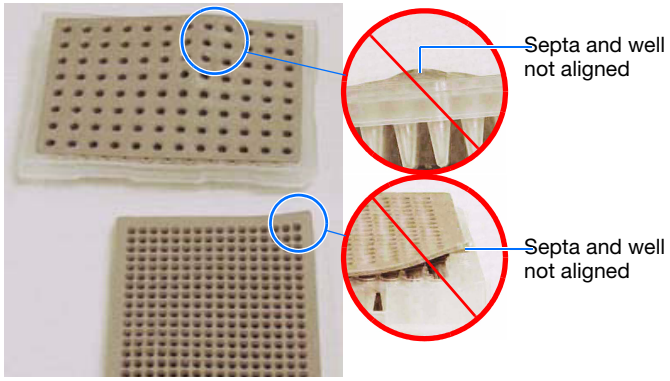
Notes

Preparing a Septa Plate Assembly

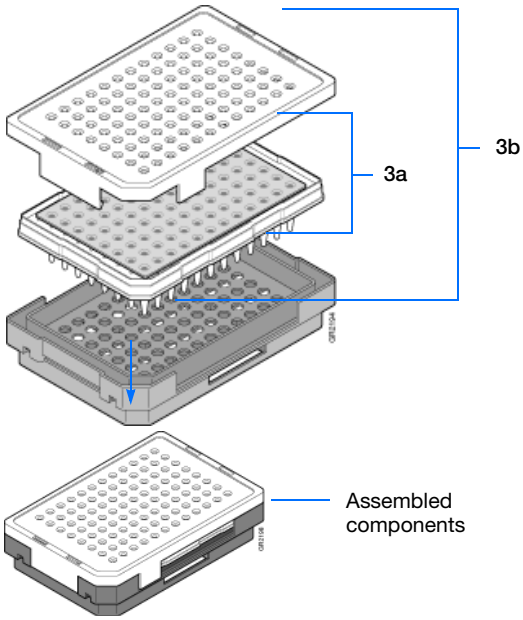
1. Seal the plate:
   a. Place the plate on a clean, level surface.
   b. Lay the septa flat on the plate.
   c. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.



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



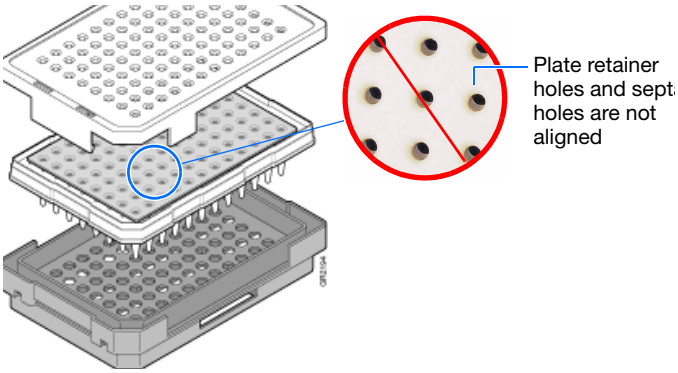
3. Assemble the plate assembly:
   a. Place the sample plate into the plate base.
   b. Snap the plate retainer onto the plate and plate base.



Notes

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

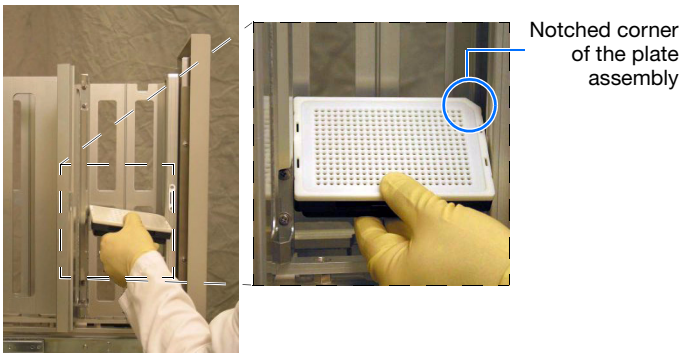
Placing Plate Assemblies into the Instrument

1. Open the stacker drawer.
2. Open the door of the In Stack tower.

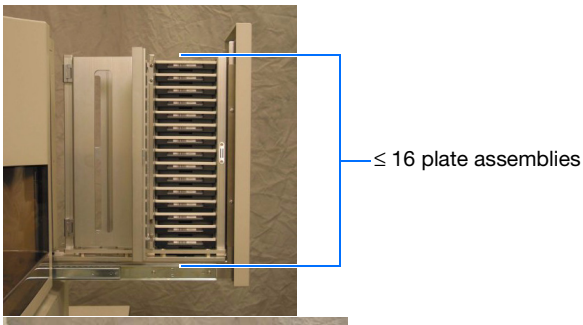
3. Place the plate assemblies into the stacker in any order.

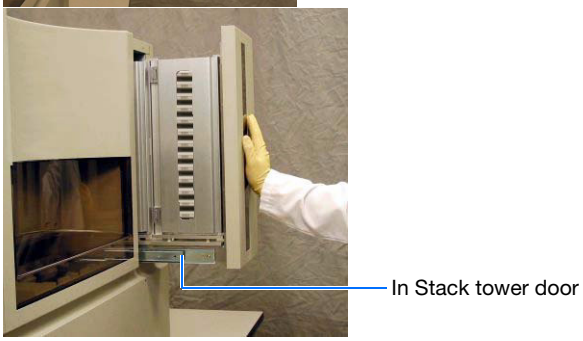
IMPORTANT! When placing the plate into the stacker, the plate must be oriented so that the notched corner of the plate assembly is located in the rear-right corner of the stacker.

IMPORTANT! Do not place more than 16 plates in the stacker.



4. Close the metal In Stack tower door.
5. Close the Stacker drawer.

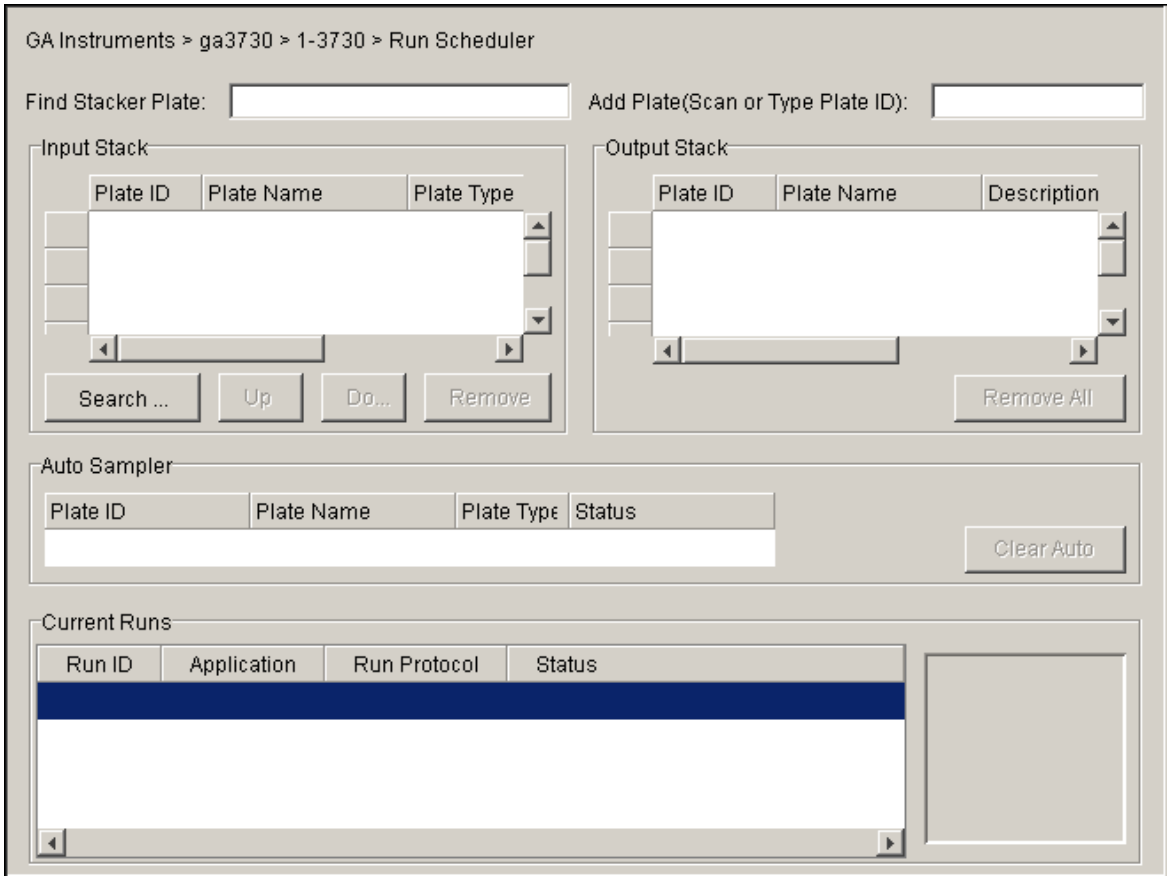




Notes

Scheduling Runs

In the Tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Run Scheduler**.



6

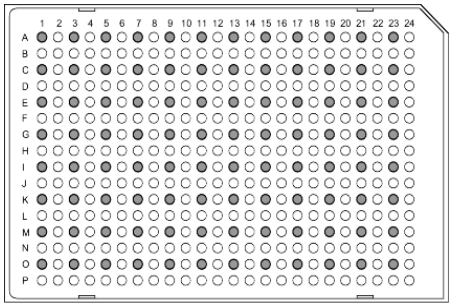
Notes

384-Well Plate Mapping and Default Run Scheduling

Samples within a plate run in the order of their well designation. For example, a default 384-well injection pattern looks like this:

- Plates that contain samples in a single quadrant and with more than one instrument protocol specified, run all the protocols in the order they appear in the plate record before the next quadrant is run.

Note: The analysis module of a sample plays no part in the order in which that sample quadrant runs.



Quadrant 1: wells A1, C1, E1, G1...
Quadrant 2: wells B1, D1, F1, H1...
Quadrant 3: wells A2, C2, E2, G2...
Quadrant 4: wells B2, D2, F2, H2...

For information on setting up a Plate record, page 58 for sequencing, and page 91 for fragment analysis.

The following table lists the default run priorities and load positions

Number of Capillaries	Plate Size	Run Priority	Quadrant	First Load Position
96	384-well	1	Q1	Well A1
		2	Q2	Well B1
		3	Q3	Well A2
		4	Q4	Well B2
48	96-well	1	Q1, load 1	Well A1
			Q1, load 2	Well A2
48	384-well	1	**Q1**, load 1	Well A1
			Q1, load 2	Well A3
		2	**Q2**, load 1	Well B1
			Q2, load 2	Well B3
		3	**Q3**, load 1	Well A2
			Q3, load 2	Well A4
		4	**Q4**, load 1	Well B2
			Q4, load 2	Well B4

Note: When using a 384-well plate and a 48-capillary array, you can change the run order of the main quadrant (**bold** numbers above) but not the load numbers.

Globally Modifying a Run Schedule

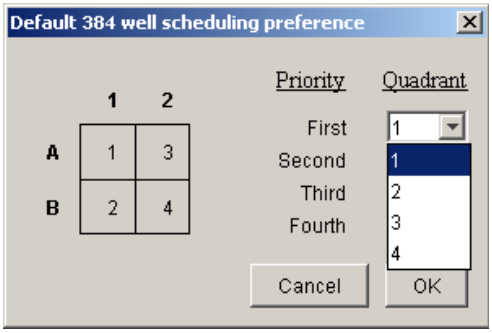
You can change the run order of quadrants and then apply it to all 384-well plates.

Notes

To modify the run order for all 384-well plates:

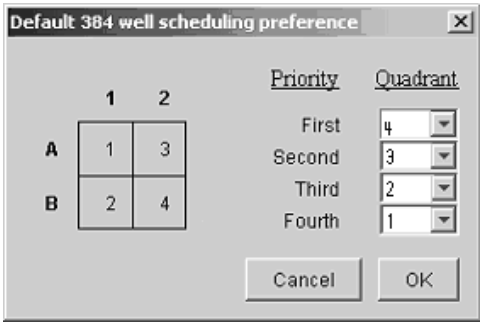
1. Click your instrument name in the left pane.
2. Select **Instrument > Scheduling Preference**.

   The Default 384 well scheduling preference dialog box displays.
3. Select the quadrant priority (run order) from the Quadrant list.



   You may select any run order. The example to the right shows a 4-3-2-1 quadrant priority (run order). With a 384-well and a 96-capillary array, the samples would run in this order:

   B2, A2, B1, A1...



Notes

Locally Modifying a Run Schedule

You can also change the run order of quadrants within a specific sample plate.

To locally modify the run order within a single 384-well plate:

1. In the Plate Manager, click **New Plate**.

   Note: For information about the Plate Manager, see page 79 for sequencing, and page 108 for fragment analysis.

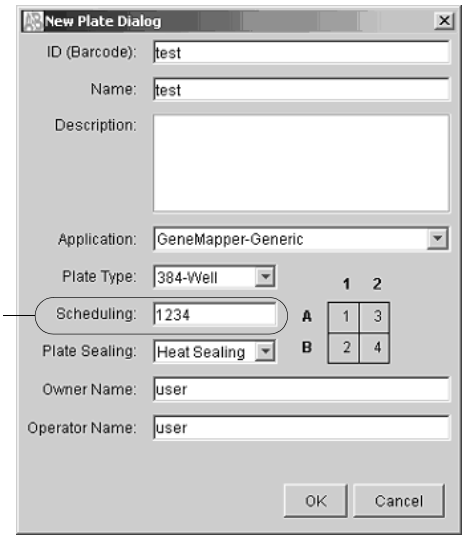
2. Select **384-Well** from the Plate Type list.

   The Scheduling box is activated.

3. Type the run priority in the Scheduling box.

4. Click **OK**.

Type run priorities here



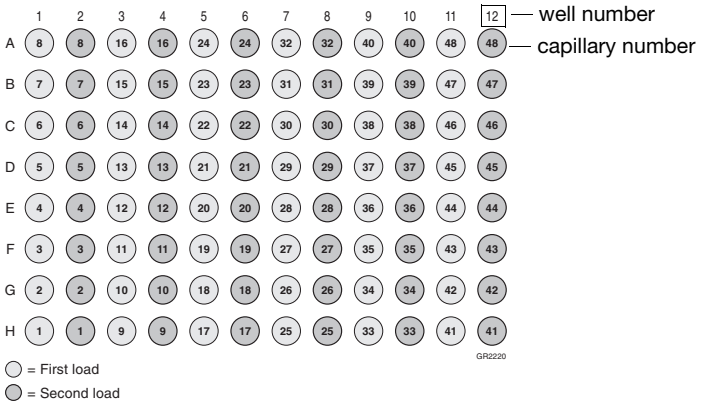
Notes

Default Load Maps

Refer to the following load maps for different sized arrays and sample plates.

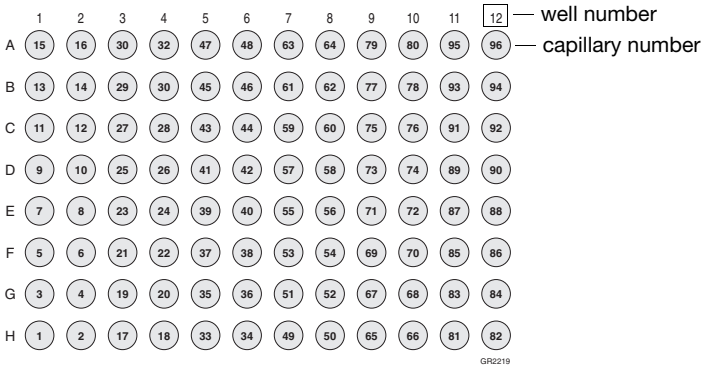
96-Well Plate, 48 Capillaries

Sample Plate: 96-well, Array: 48-capillary



96-Well Plate, 96 Capillaries

Sample Plate: 96-well, Array: 96-capillary

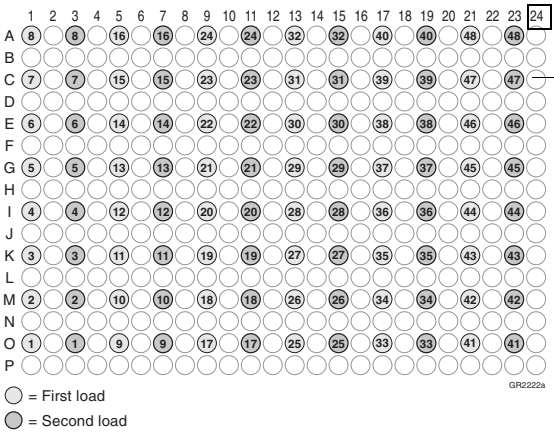


6

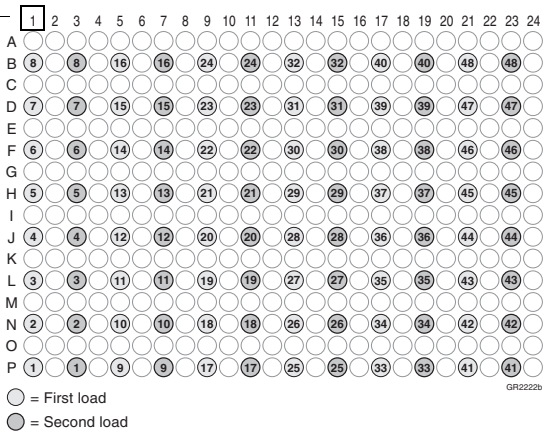
Notes

384-Well Plate, 48 Capillaries

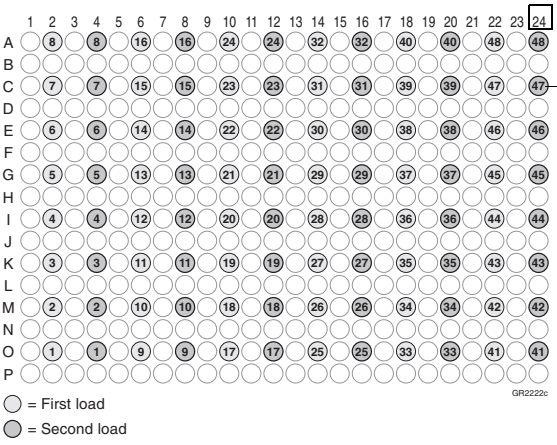
First quadrant pickup



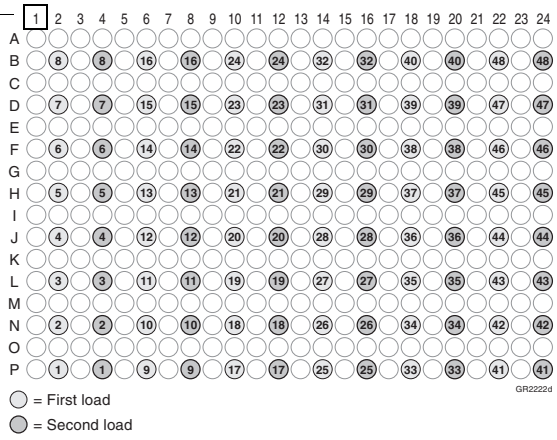
Second quadrant pickup



Third quadrant pickup



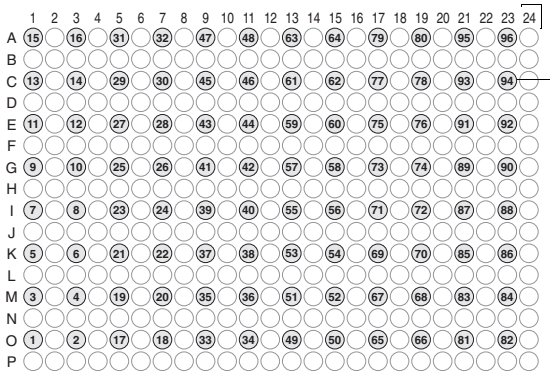
Fourth quadrant pickup



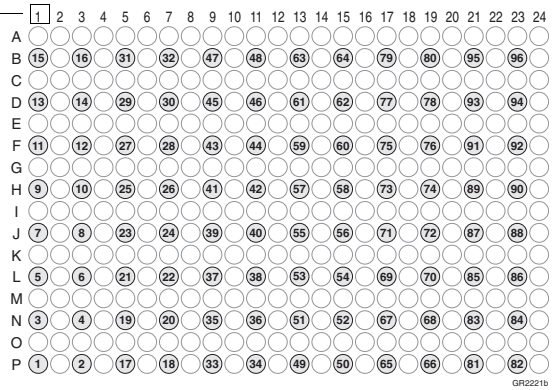
Notes

384-Well Plate, 96 Capillaries

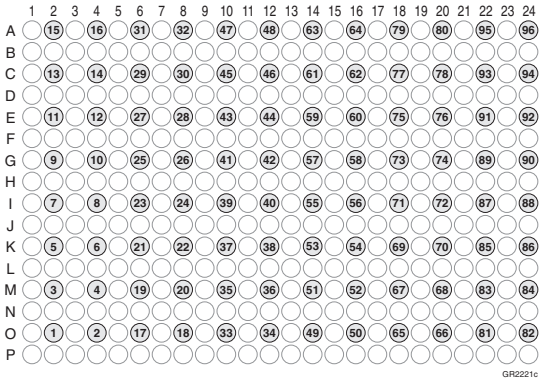
First quadrant pickup



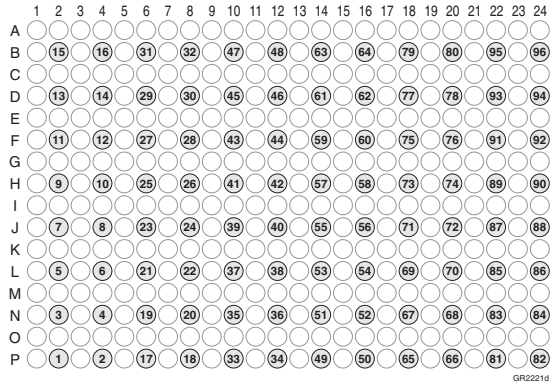
Second quadrant pickup



Third quadrant pickup



Fourth quadrant pickup



For a 384-well plate, injections are made from every other well and every other row. A full 384-well plate requires 4 runs for a 96-capillary array, and 8 runs for 48-capillary array, to inject all the samples once.

Notes

Barcode Readers

DANGER **ELECTRICAL HAZARD.** Power off the instrument and the computer before connecting an external barcode reader to the instrument.

Internal Barcode Reader

The 3730 & 3730*xl* internal barcode reader supports the following formats:

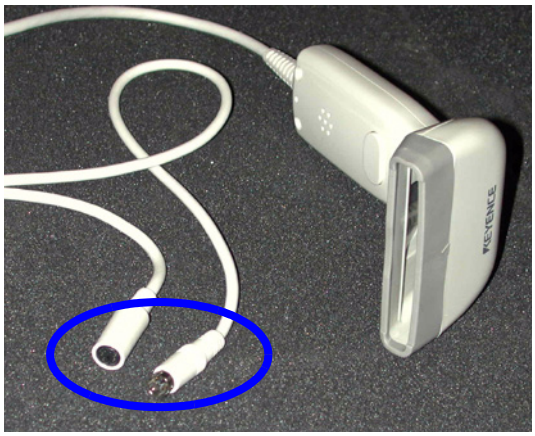
- Code 128
- Code 39
- Code 93
- LOGMARS
- EAN-8

Note: All Applied Biosystems barcoded plates for the 3730 and 3730xl instruments are code 128 format.

The barcode reader shares the default Windows illegal character list, which means the illegal characters for the reader are \ / : * ? " < > | and also having a space is illegal.

External Barcode Readers

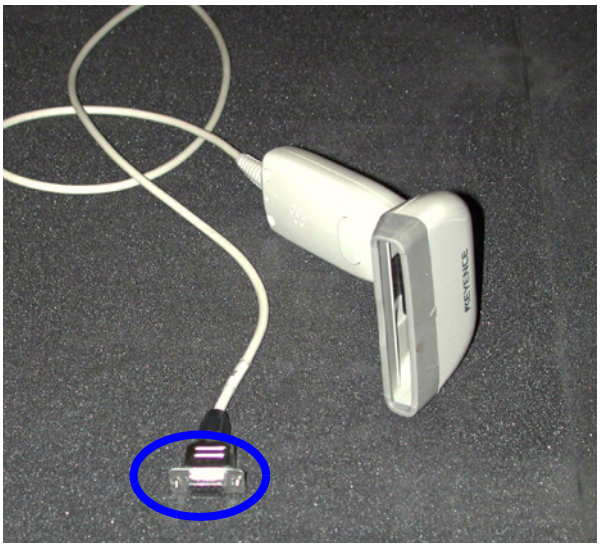
KEYENCE BL-80VE



An external barcode reader can also be used with the 3730 and 3730*xl* instruments. We have experience with the KEYENCE BL-80VE (see photo above), which connects to the instrument computer keyboard. With this reader, you can scan barcodes into any text box in the Data Collection software.

Notes

KEYENCE 80RKE



Another option is the KEYENCE 80RKE which you connect to the instrument serial port. With this reader, you can scan barcode information only into specific text boxes within the Data Collection software.

Note: The 80RE is not supported for either instrument.

Notes

Running the Instrument: Manual vs Auto Mode

Accessing Modes

You may schedule a run or runs using either manual mode or auto mode. Both modes are described below. Access either mode by selecting:

Run Scheduler >Instrument > Instrument Name > Run mode (Auto or Manual)

Note: You must be in the Run Scheduler view to see the instrument run mode menu.

Manual Mode Features

The benefits and features of using manual mode are:

- Plates can be added to the stacker individually and in order; runs are scheduled in the order the plates are in the stack.
- The internal reader is not necessary to link plates to plate records in the local database.
- Plates do not need to have a barcode.

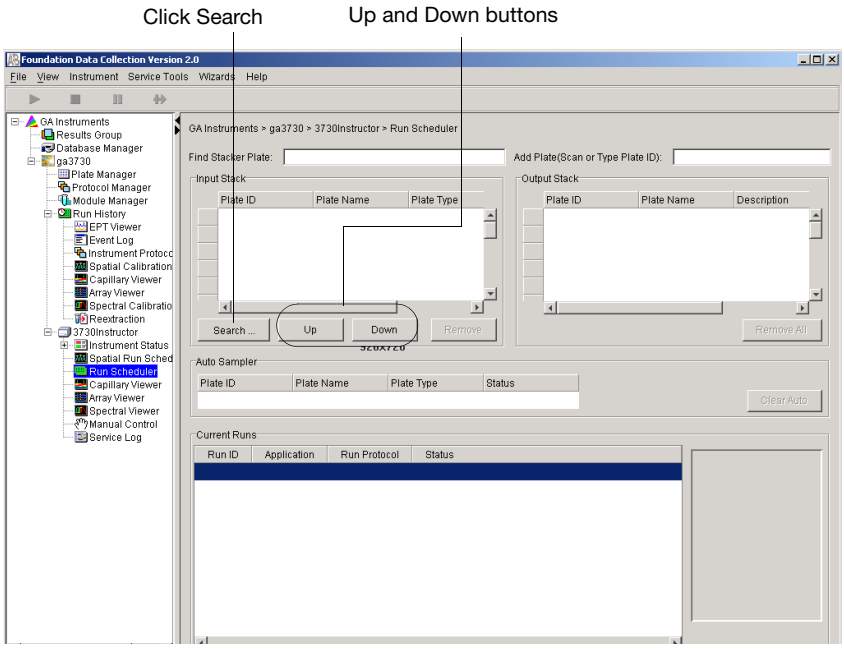
Scheduling Runs Using Manual Mode

To schedule runs using the manual mode (default):

1. Click the Run Scheduler icon.
2. Select **Instrument > Instrument Name > Manual mode**.

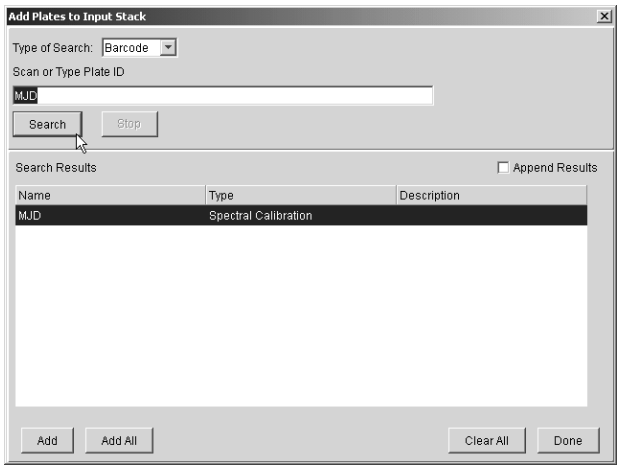
Notes

3. Click **Search** in the Run Scheduler to search for plate record(s).

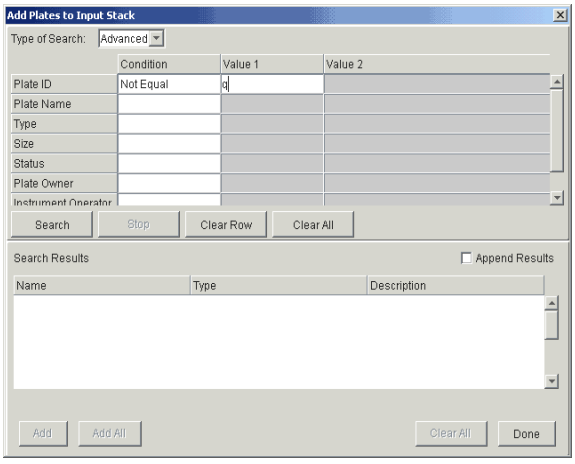


This opens the **Add Plates to In Stack** dialog box.

4. Type the name of the plate(s) or scan the plate ID and click **Search**.



Barcode search



Advanced search

Notes

5. Select run(s) to add and then click **Add** to add the plate record(s) to the Input Stack in the order in which you want them to run.

6. Click Done to close the Add Plates to In Stack dialog box.

7. Physically stack the plates in the In Stack in order. The bottom plate runs first.

   IMPORTANT! The order of the plate record must match the stack order of the plates in the In Stack. If the order does not match, processed runs will have the wrong plate record information.

   Note: You may assign more plates in the Run Scheduler than are actually available in the stacker.

8. Click  (**Run).**

   As the plates are retrieved by the autosampler, they are run in the order they were placed in the In Stack.

Notes

Auto Mode Features

The features and benefits of the using Auto Mode are:

- Plates must have barcodes.
- Internal barcode reader is necessary in order to link plates to plate records in the local database.
- You can add plates to the In Stack in any order.
- Plates can be added or removed during instrument operation.

To schedule runs using the Auto mode:

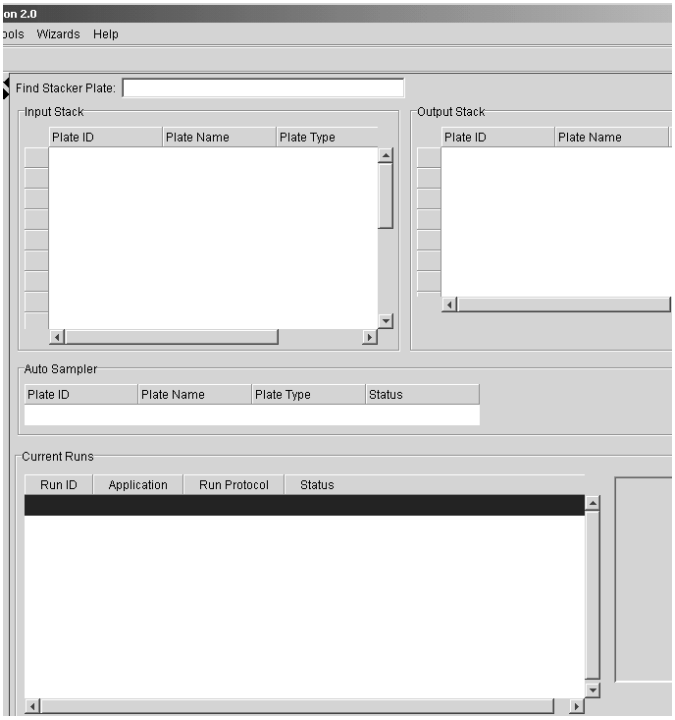
1. Select **Run Scheduler > Instrument Name > Auto mode**.

   Notice that the Search, Up, and Down buttons are no longer visible as they are in Manual mode. Also, you no longer have the Add Plate (Scan or Type Plate ID) option as you do in Manual mode.

2. Physically place plates in the In Stack in any order. Remember that the bottom plate runs first, the top plate runs last.

3. Click  (Run).

   As the plates are retrieved by the autosampler, plate barcodes are scanned and their plate records are associated with those stored in the local data collection database.



6

Notes

Running the Instrument: Launching the Run

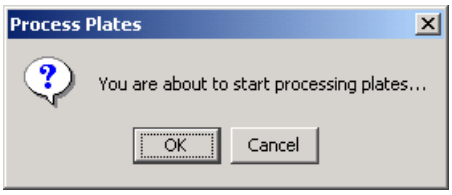
1. Verify the active spectral calibration matches your dye set and capillary array length.

2. If you want to review the run schedule before beginning the run, click
   GA Instruments > **ga3730** > *instrument name* > **Run Scheduler**

3. Click the green button in the toolbar.

   The Processing Plates dialog box opens.

4. Click **OK**.



5. The software automatically checks:
   - the capillary array length and polymer type in the Instrument Protocol column of the plate record against the capillary array length and polymer type
   - the available space in the database and drive E

If the database or drive E are ...	Then ...
full	A warning displays. Do the following: 1. Make more space by deleting unneeded files. See, "Working With Drives for Database and Sample Data Storage" on page 174 2. Click the green button to start the run.
not full	the run starts.

Notes

Basic Run Module Steps

When the run starts, the following basic steps are performed automatically by the instrument

Module Steps	Approximate Time
Turn Oven On	N/A
Wait for oven to equilibrate Initialize autosampler	1 min 40 sec (when oven is at set temperature)
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: ~38 minutes • Warm start ~10 minutes (oven is already at temperature)	

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

DNA Sequencing Run Times

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

Analysis	Capillary Array Length	Run Module	Approximate Run Time[a] (min)
Rapid read DNA sequencing	36-cm	RapidSeq36_POP7	35
Standard read DNA sequencing	36-cm	StdSeq36_POP7	60
Fast DNA sequencing	50-cm	FastSeq50_POP7	60
Long read DNA sequencing	50-cm	LongSeq50_POP7	120
Extra Long DNA sequencing	50-cm	XLRSeq50_POP7	180

a. Times assume oven is at temperature

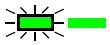
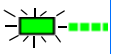
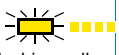
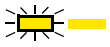
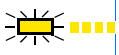
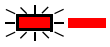
Notes

Fragment Analysis Run Times

The following table lists the approximate run time of a common fragment analysis run:

Capillary Array Length	Run Module	Approximate Run Time (min)
36-cm	GeneMapper36_POP7	32

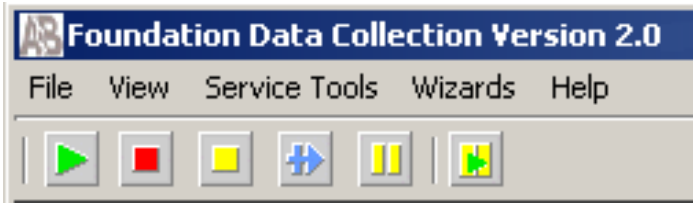
Instrument Status Lights

Status	Status Light	Action
• The instrument is ready. • An automated wizard operation is in progress with the instrument door closed.	 Solid green	Go to page 8.
• A run is in progess	 Flashing green	
• The instrument is downloading firmware.	 Flashing yellow	
• The instrument cannot communicate with the computer.	 Solid yellow	Go to page 7.
• The instrument is performing diagnostics. • The oven door is open. • The instrument door is open. • The buffer reservoir is not installed. • The capillary array is not installed. • An automated wizard operation is in progress with the instrument door open.	 Flashing yellow	Go to page 6.
• The instrument has detected a problem.	 Solid red	Go to page 7.

Notes

Controlling the Run

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

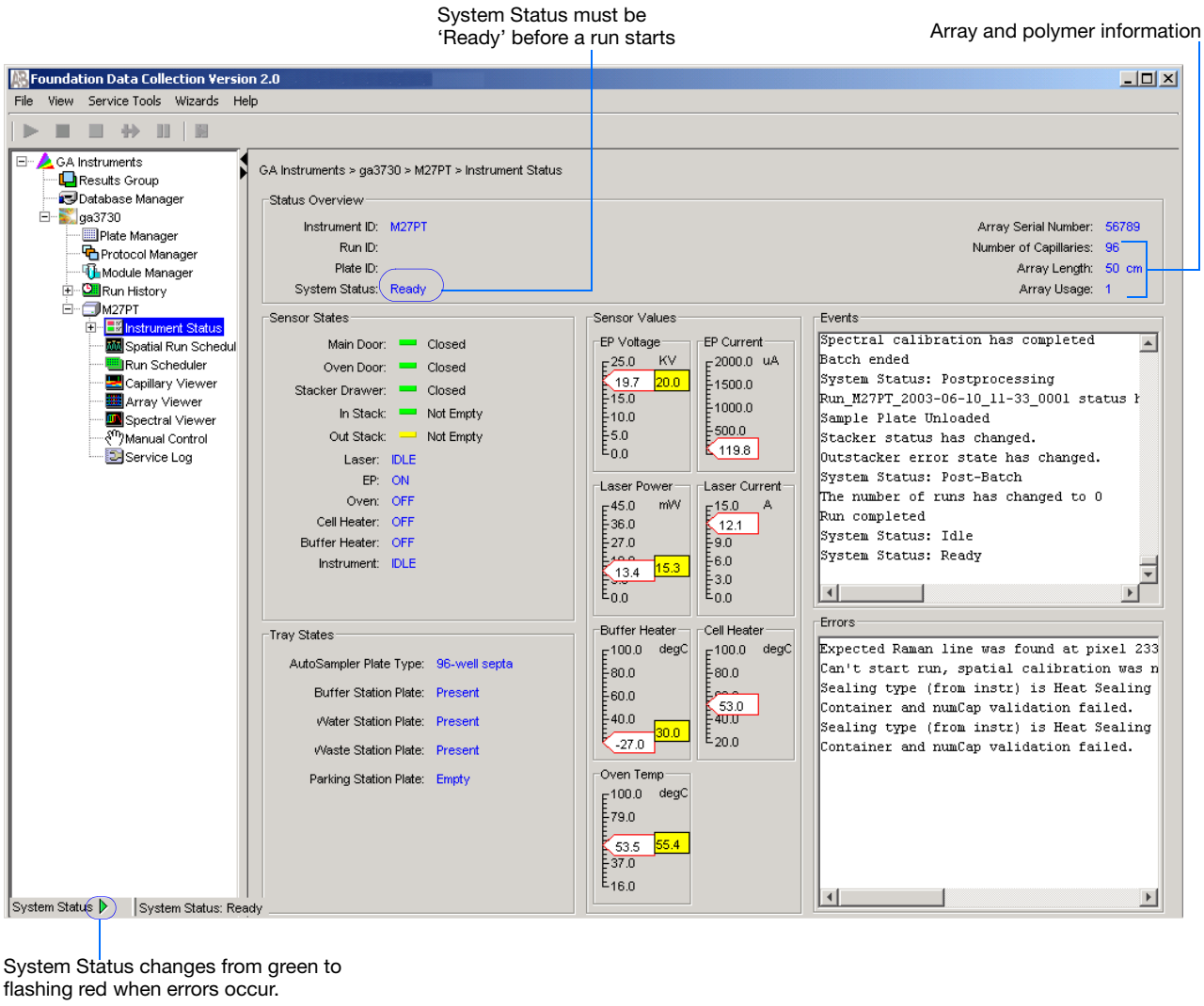


To ...	Click ...	Action
Start the run		Starts run(s).
Stop the current run		Stops the current run.
Stop after the current run		Finishes current run and then stops.
Skip to next run		Stops the current run and begins next scheduled run.
Pause after current run		Finishes current run and then waits for resume command to begin next scheduled run.
Resume after pause		Begin the next scheduled run after a pause.

Notes

Controlling the Run: Instrument Status

Click (Instrument Status) to monitor the status of the instrument or the current run.



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.

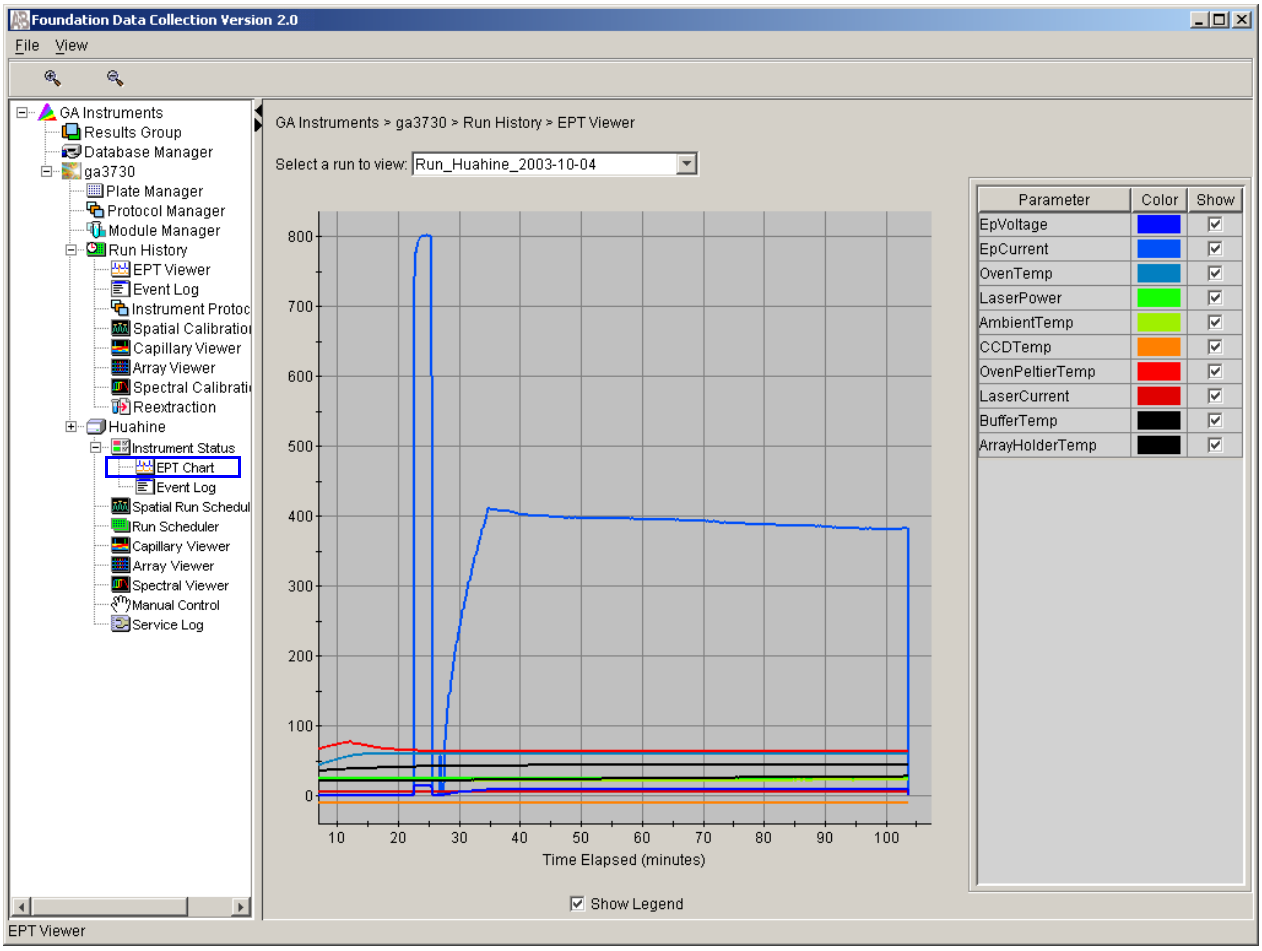
Some of the error messages provide information for service engineers. A "fatal" error usually requires that you restart the data collection software.

Notes

Controlling the Run: EPT Chart

The EPT Viewer displays real-time electrophoresis (EP) data during a run.

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Instrument Status** > **EPT Chart**.



Notes

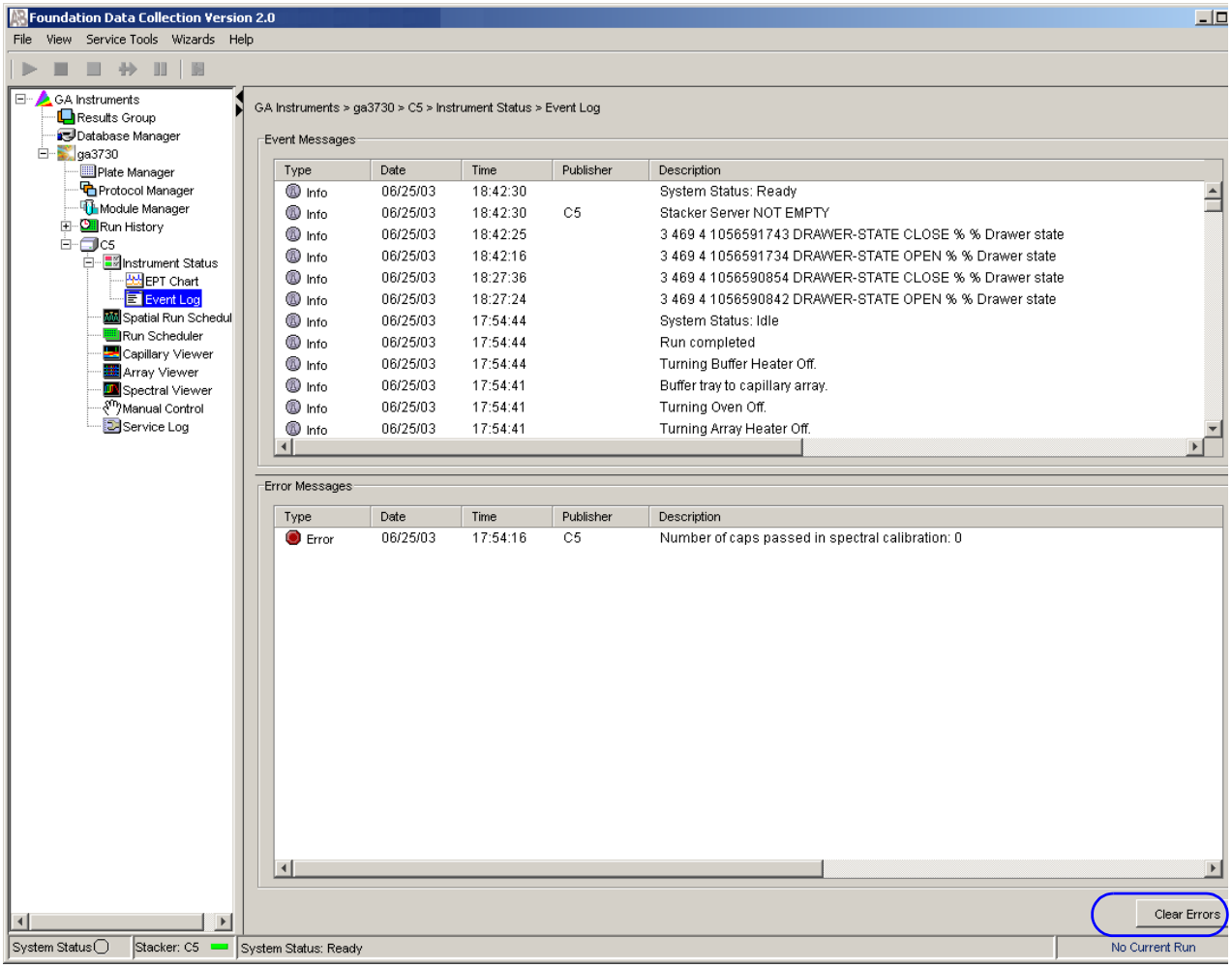
Controlling the Run: Event Log

The Event log graphically itemizes events such as errors and general information, as the graphic below illustrates.

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Instrument Status** > **Event Log**. This opens the event log window.

Clear error messages by clicking **Clear Errors**. The System Status light flashes red until all errors are cleared.

Note: This view can also be used to monitor a spectral calibration run in real time to verify the capillary-by-capillary processing status.



Note: If an error is generated while using manual control, re-boot the instrument and Data Collection software to recover from the error stage.

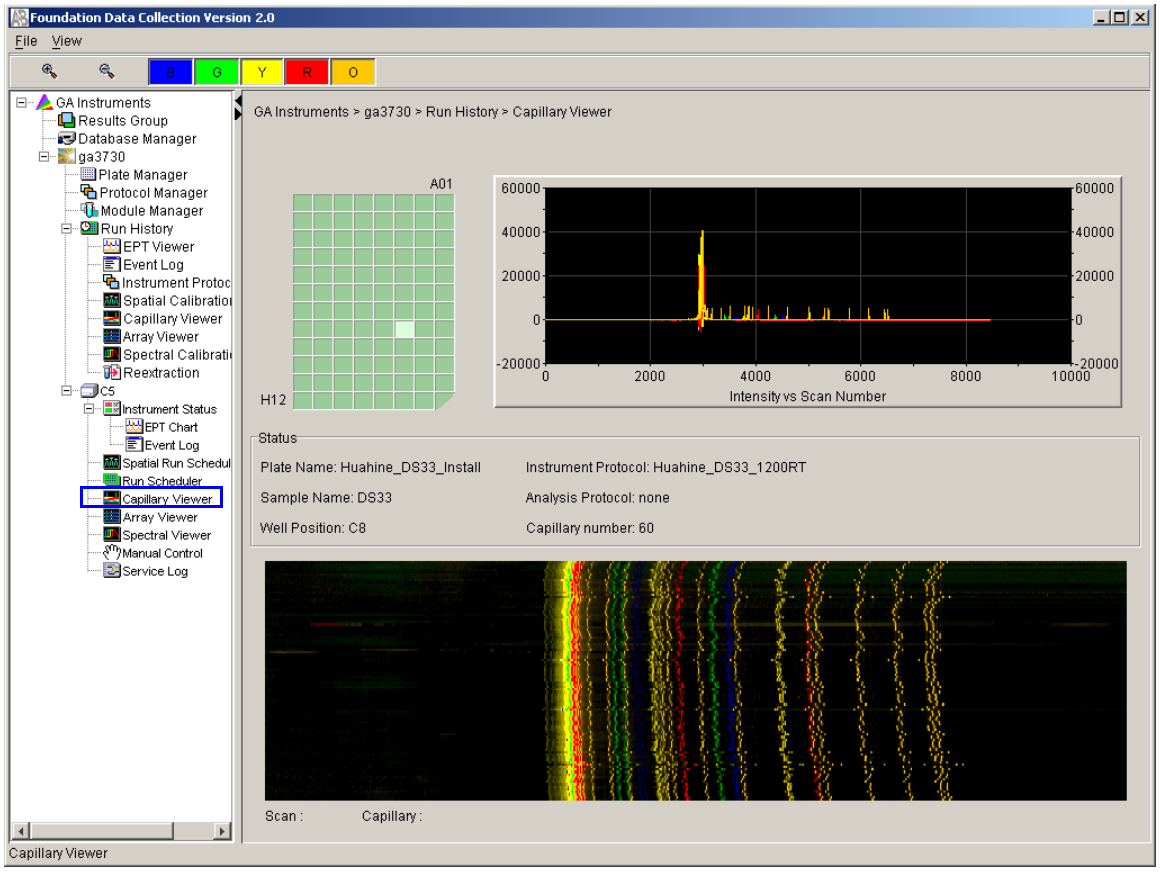
Notes

Controlling the Run: Capillary Viewer

Viewing Data in the Capillary Viewer

Use the Capillary Viewer to examine the quality of electropherogram data during a run for several capillaries at once.

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Instrument Status** > **Capillary Viewer**.



Electropherogram Displays

An electropherogram is a graph of relative dye concentration against time, plotted for each dye. The data displayed has been corrected for spectral overlap (multicomponented).

How to Zoom

To zoom:

1. Hold and drag the mouse over the area of interest.
2. Release the mouse, then click  to expand the view.
3. Click  to return to full view.

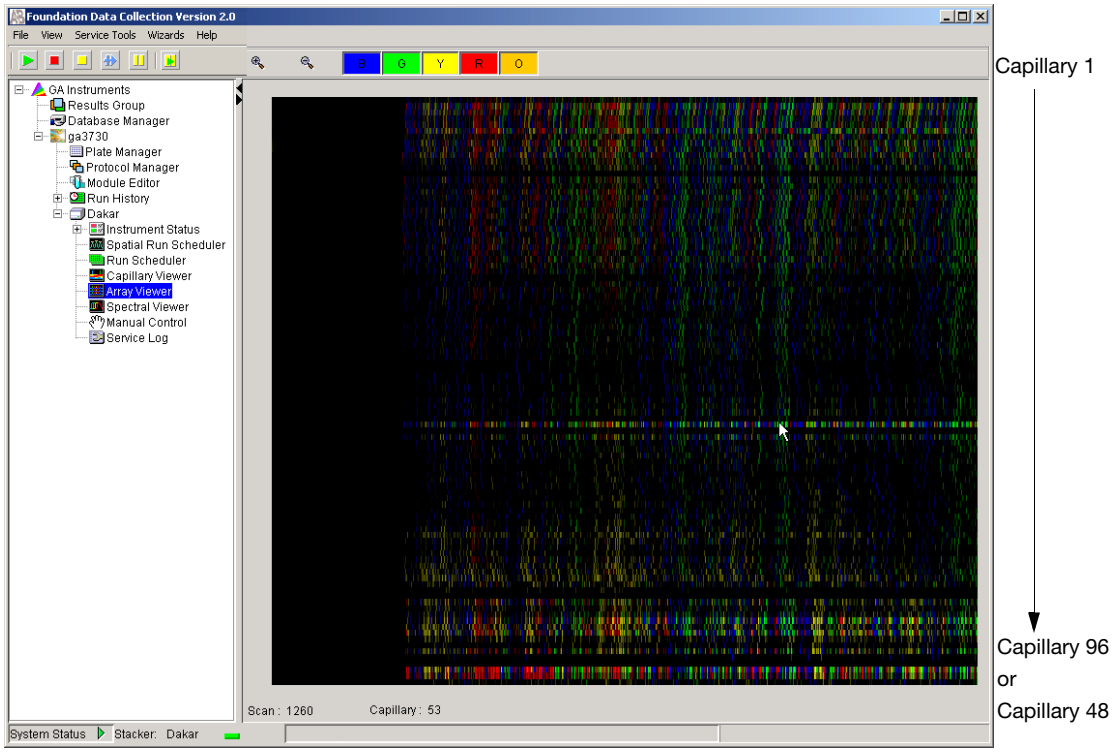
Click individual colors to view or hide them. 

Notes

Viewing Data in the Array Viewer

Use this during or after 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/data point (horizontal axis).

In the tree pane of the Data Collection Software, click **GA Instruments** > **ga3730** > *instrument name* > **Array Viewer**. This opens the Array Viewer window.



How to Zoom

1. To expand the view, hold and drag the mouse over the area of interest.
2. Click  to return to full view.

Color Bar

Click individual colors to view or hide them (same in Capillary Viewer).

Notes

Working with Data in The Run History View

Run History Components

The Run History utility can be used only with completed runs stored in the local 3730 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 Viewer **Note:** If Cleanup Database has been used, you cannot view processed data in Run History.	
Spatial Calibration Viewer	
Capillary Viewer **Note:** If Cleanup Database has been used, you cannot view processed data in Run History.	
Array Viewer **Note:** If Cleanup Database has been used, you cannot view processed data in Run History.	
Spectral Calibration 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

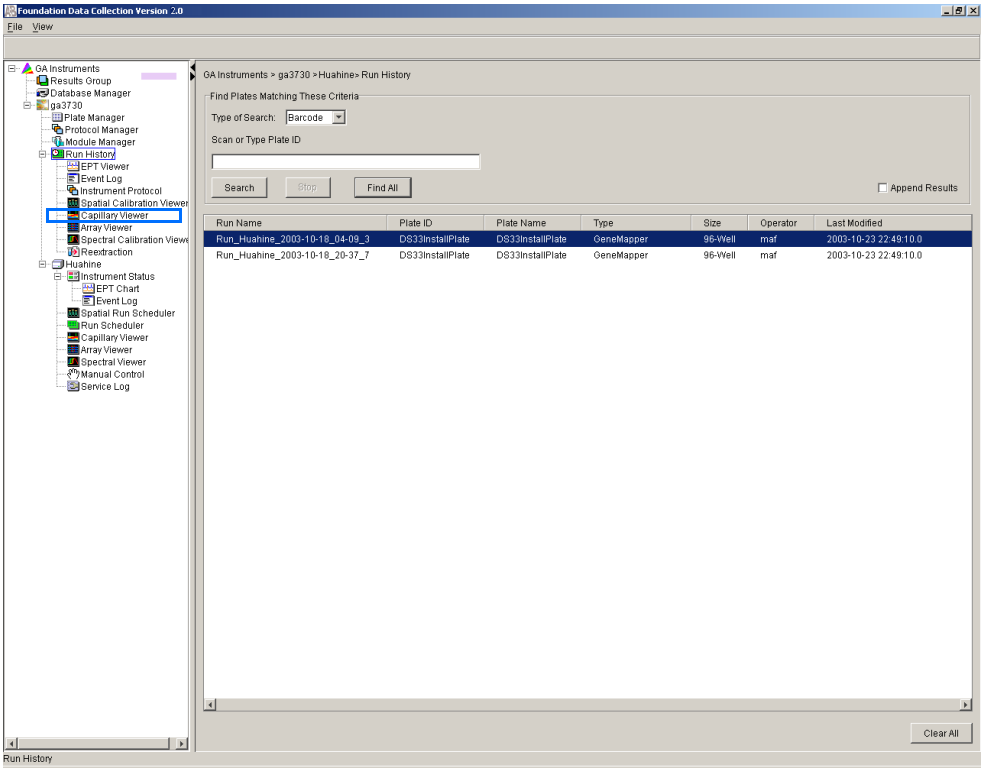
There are two formats for viewing data within the 3730 Data Collection Software under the Run History icon:

- In the Array Viewer window
- In the Capillary Viewer window, capillary-by-capillary

Notes

Viewing Data from a Completed Run

1. On the left tree pane in the 3730/3730*xl* Data Collection software, click (**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 Array Viewer or the Capillary Viewer from the left tree pane.

Notes

Viewing the Results of Autoextraction

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 several 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 files are created based on the your destination and folder naming selections.

Runs Stopped Before Complete Autoextraction

Runs that are stopped before completion display the status “Completed” in the Run Scheduler and the plate is moved to the Out Stack. In the Instrument View the status is changed to “Ready.” Successfully extracted and analyzed runs display the status “Processed” in the Run Scheduler.

The auto extractor component of the 3730/3730*xl* 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

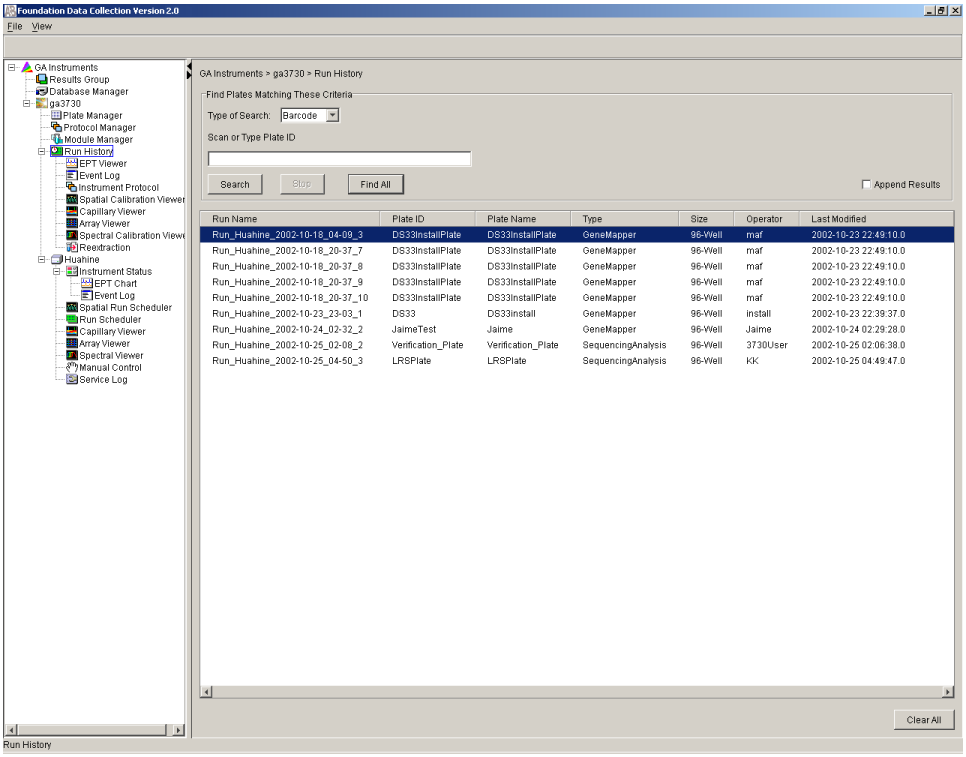
Changes made in the Reextraction Panel to a Sample name, Analysis protocol, etc., also change in the original plate record. The original plate information is overwritten.

Notes

Selecting and Queuing Samples for Reextraction

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 ID 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.
3. Select a run from the list.



4. Click (Reextraction) in the left tree pane.
   The Reextraction window displays
5. Click the checkboxes 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, unless you modify the results group settings.

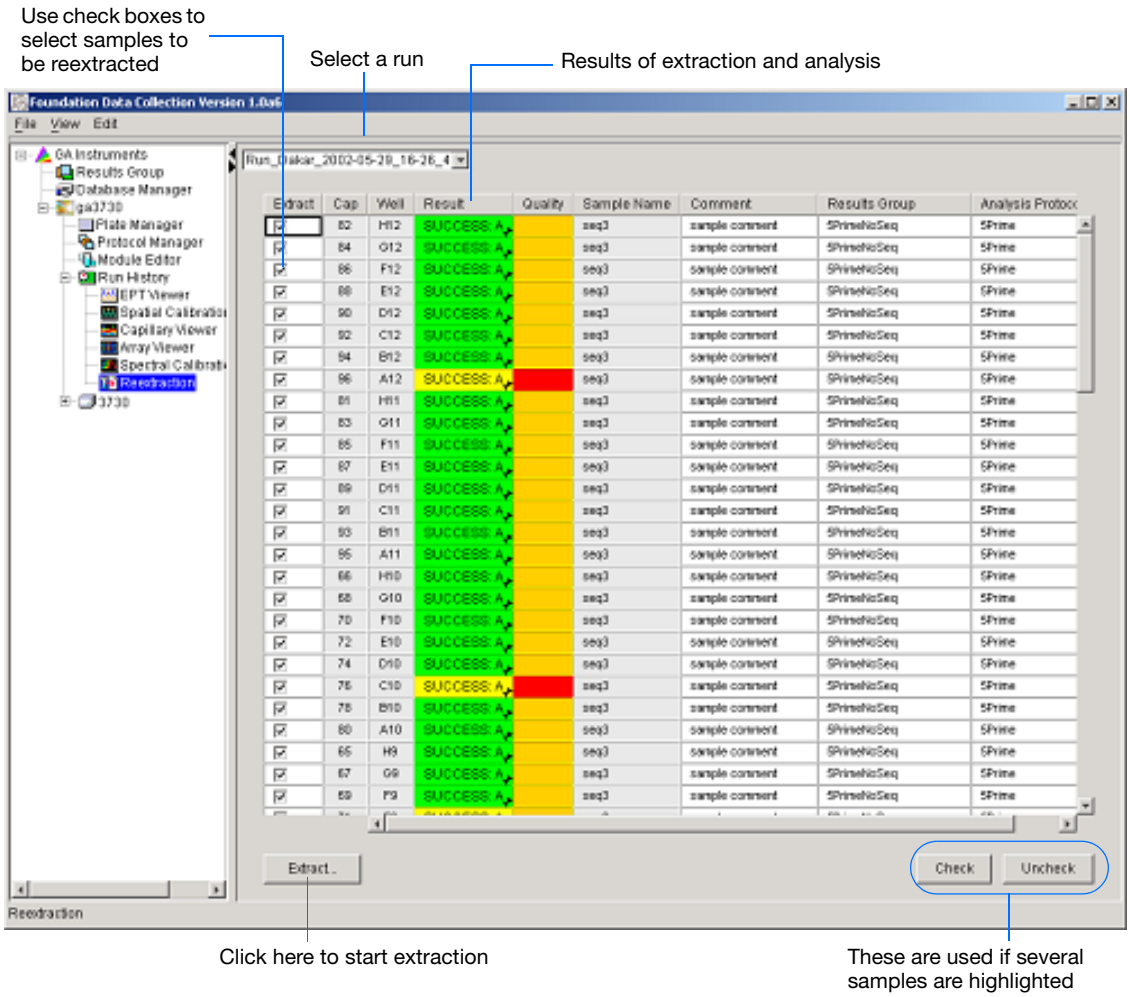
Notes

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.

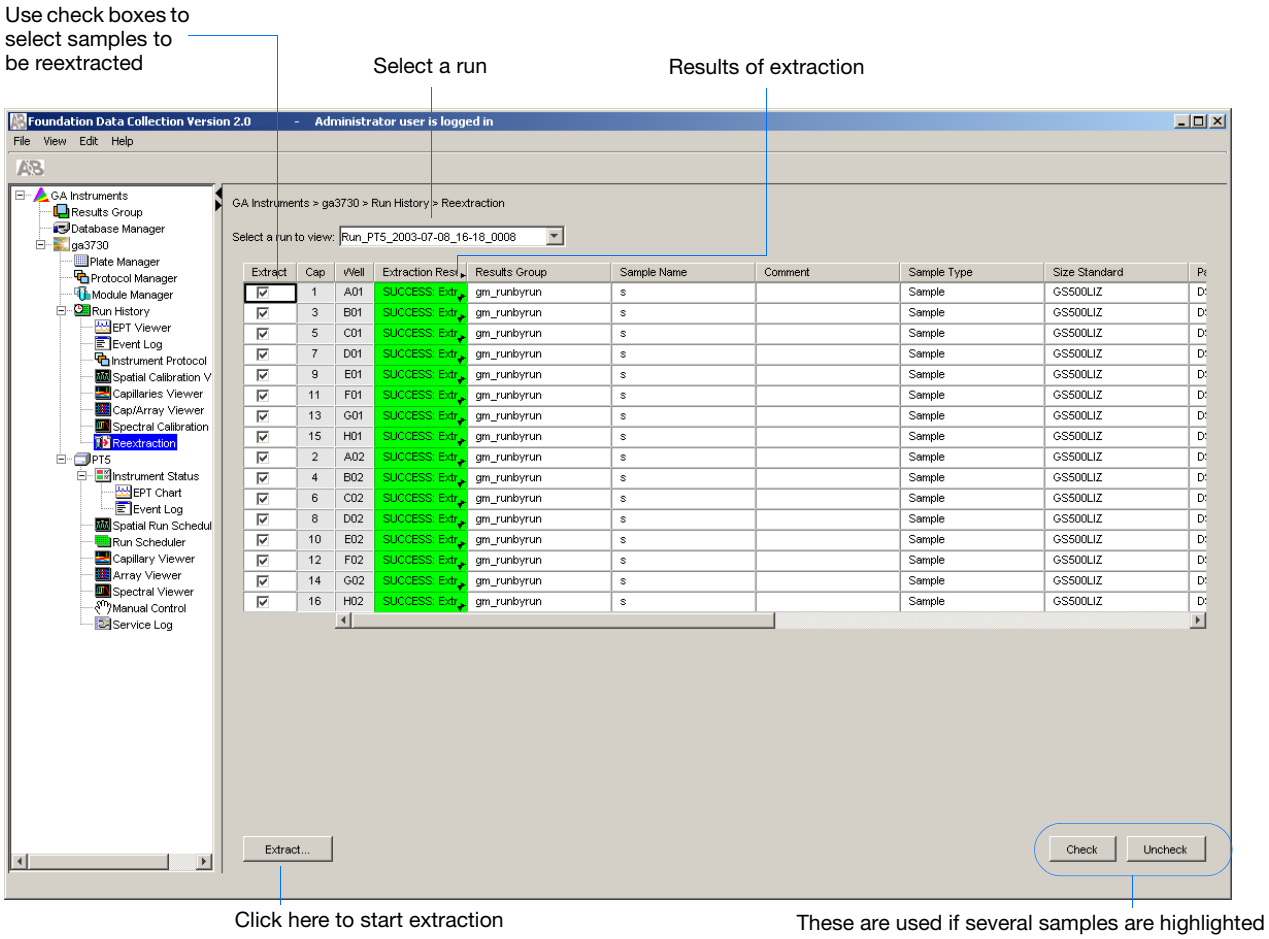
Reextraction Window for Sequencing Analysis



6

Notes

Reextraction Window for Fragment Analysis



Notes

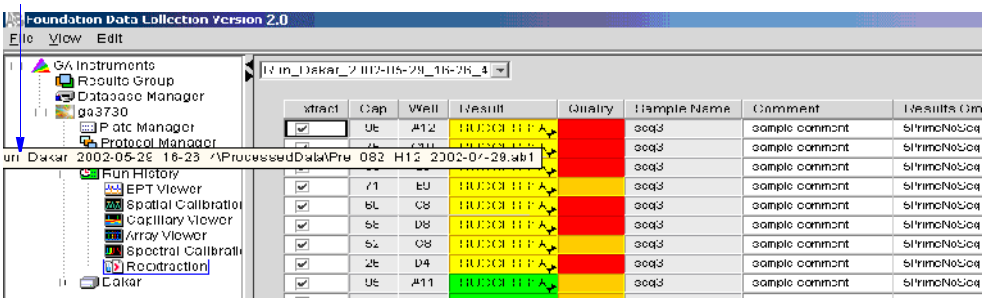
Results Column

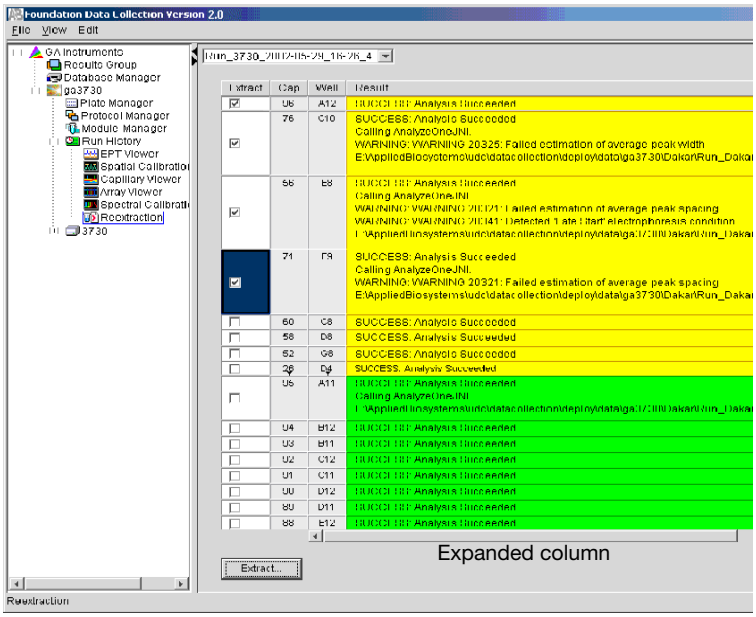
The results of extraction and analysis are color coded in the Results column. The following table lists the colors and their values.

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





Expanded column

Notes

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 checkboxes for each sample. Additionally, the fill-down command (Ctrl+D) works the same here as in the Plate Editor for easier information input.

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.

Notes

Reextracting 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.

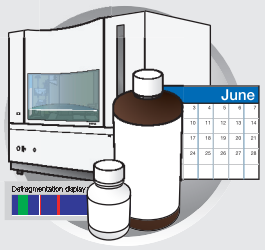
5. Click **Extract**.

   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.”

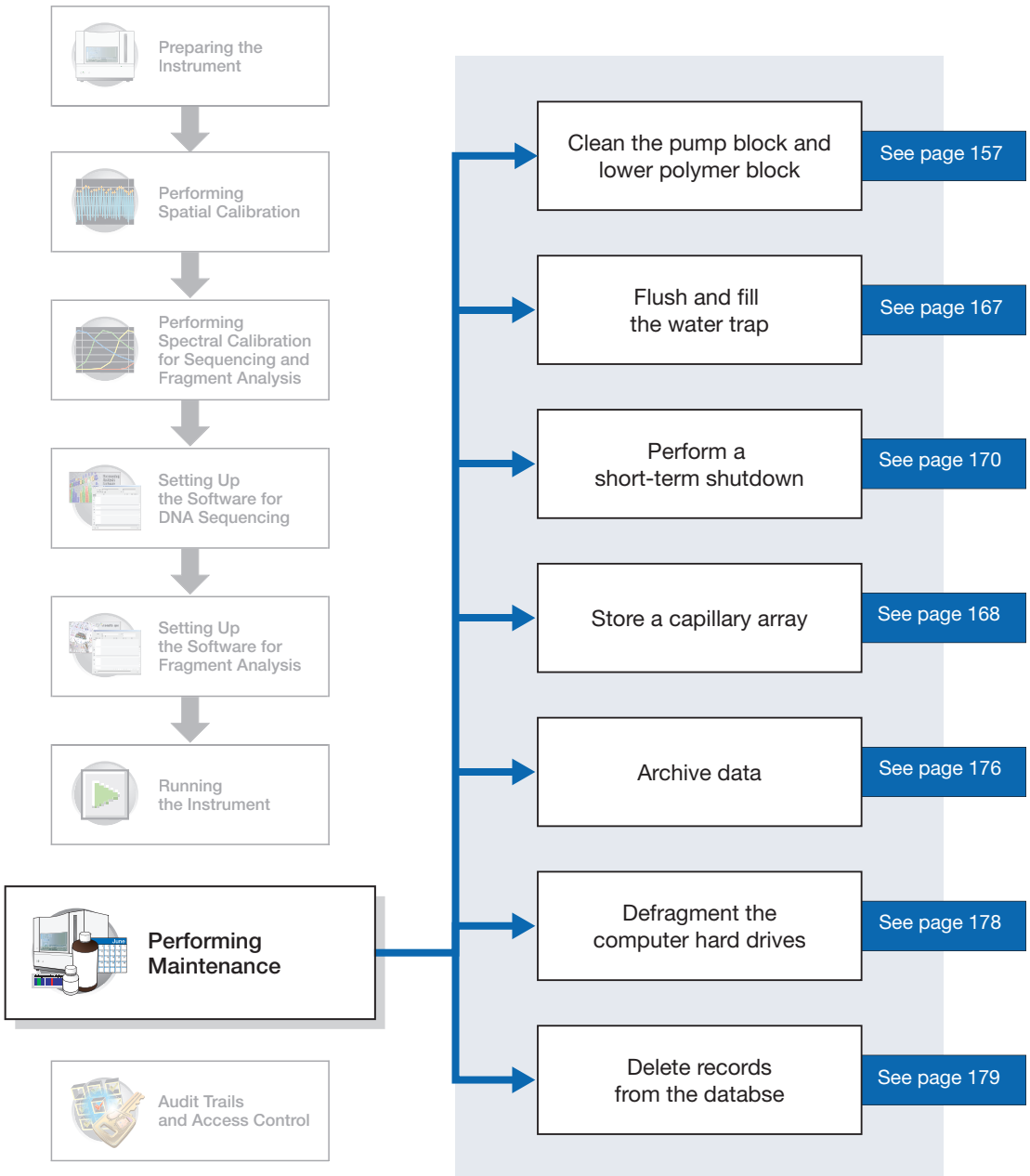
Notes

Notes

Performing Maintenance



Workflow



Notes

Performing Maintenance Tasks

Overview

This section lists common tasks required to maintain your Applied Biosystems 3730/3730*xl* DNA Analyzer in good working condition. The tasks are divided into tables based on how often you should perform each task.

WARNING Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids used on this instrument, or parts that may come into contact with these fluids.

Daily Tasks

Perform these tasks at least once per day.

Maintenance Task	Frequency
Ensure adequate levels of buffer and water in reservoirs.	Before each run
Ensure the plate assemblies are properly assembled. **IMPORTANT!** The holes in the plate retainer must align with the holes in the septa, or the capillary tips will be damaged.	Before each run
Ensure the plate assemblies are positioned on the plate deck properly. Plates should sit snugly on the deck. **IMPORTANT!** Never use warped plates.	Before each run
Check the level of buffer in the buffer jar and ensure that the overflow hole is not occluded and, that the overflow hole is facing toward the front of the instrument.	Before each run
Replace the water and 1X run buffer in the reservoirs on the instrument and, make sure that the outside of the assemblies are dry.	Every 48 hours
Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Remove all bubbles with the Bubble Remove wizard.	Daily or before each run
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 bottle to ensure sufficient volume for runs.	Daily or before each run
Check the pump block and the lower polymer block to ensure they fit securely on the instrument.	Daily
Clean the instrument surfaces.	Daily
Check for leaks around the array knob, interconnecting tube nuts, and check valve.	Daily

Notes

Weekly Tasks

Perform these tasks at least once per week.

Maintenance Task	Frequency
Replace the polymer using the Change Polymer Wizard.	Weekly or as needed
Check the storage conditions of the used arrays.	Weekly

Monthly Tasks

Perform these tasks at least once per month.

Maintenance Task	Frequency
Run the Water Wash Wizard. Flush the array port during this wizard, whether or not bubbles are present in the array port.	Monthly or as needed
Flush the water trap (see page 167).	Monthly or as needed

As-Needed Tasks

Perform these tasks as needed.

Maintenance Task	Frequency
Clean the drip tray.	As needed
Change the array.	As needed
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed

Notes

General Instrument Cleaning

To clean the instrument:

1. Ensure the oven door, the instrument door, and the stacker are closed.
2. Press the Tray button on the front of the instrument to move the autosampler to the forward position.
3. Wipe off any liquid on or around the autosampler using a lint-free tissue.
4. Clean out the drip tray with deionized water and lint-free tissue.
5. Clean off any polymer build-up (crystals) on the instrument including the capillary tips with deionized water and lint-free tissue.

   IMPORTANT! Never use organic solvents to clean the instrument or any of its components.

Notes

Guidelines for Pump Block and Lower Polymer Block Cleaning

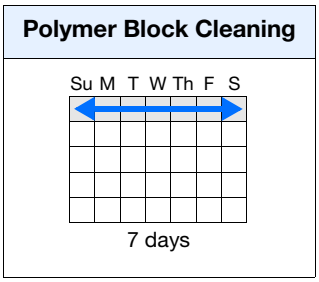
Cleaning Exterior Surfaces

- Do not expose the polymer blocks to organic solvents.
- Do not use sharp or pointed instruments to remove dried polymer from the polymer blocks.
- Do not use water > 50 °C to clean the polymer blocks.

WARNING **CHEMICAL HAZARD. POP-7 polymer** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

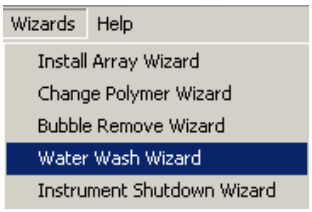
When to Clean the Pump Block and the Lower Polymer Block

- Clean the exterior every 7 days, when polymer is replenished.
- Clean the Polymer Delivery Pump (PDP) chamber, channels, and tubing once per month.



Cleaning the PDP Chamber, Channels and Tubing

1. Run Water Wash Wizard.
2. Inspect the channels of the Pump and Lower blocks for any possible contaminants. Repeat Water Wash Wizard until contaminants are removed.



7

Notes

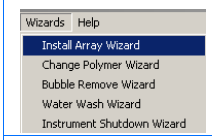
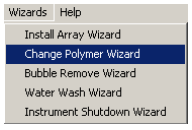
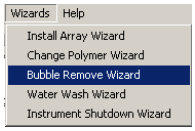
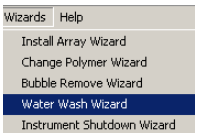
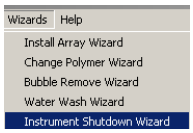
Wizards

Overview

The five wizards in the Data Collection Software v2.0 guide you through several maintenance procedures.

General Use Guidelines

The following table lists the wizards and when to use them.

Wizard	When to Use
Install Array 	• To install a capillary array: – On a new instrument – To reactivate an instrument that has been shut down • To replace an installed capillary array with another capillary array • To install an array without the wizard when the Data Collection software is reinstalled or upgraded
Change Polymer 	• To replenish the polymer supply • To replace the polymer in the PDP with polymer of the same or different lot • To enter polymer information when Data Collection software is installed or upgraded
Bubble Remove 	• To remove bubbles in the PDP chamber, channels, and tubing
Water Wash 	• To wash the PDP chamber, lower polymer block*, channels, and tubing with water: – As part of a monthly maintenance protocol – To remove any suspected contaminants in the PDP – To remove persistent bubbles (followed by the Bubble Remove Wizard, if needed) – To replace old polymer in the PDP * The lower polymer block should not be removed; clean on the instrument using this wizard.
Instrument Shutdown 	• To prepare the instrument for a period of disuse of greater than one week • To remove the array without the wizard

Using Wizards with the Instrument Door Open

IMPORTANT! Polymer Delivery Pump (PDP) operations with the door open are permitted only in wizards. If the door is opened at any other time during instrument operation, most functions, including PDP operations, are paused.

All wizards include instructions for manual operations and buttons that launch specific, automated operations of the Polymer Delivery Pump (PDP). You can use the 3730/3730xl version 2.0 to perform most automated operations of the wizards with the main instrument door open. With the door open, you can easily view the operations of the PDP, making possible close monitoring of bubble clearing and fluid changing. The wizards may also be performed more quickly, since the instrument door need not be closed (triggering a wait period for the green status light to illuminate) and opened repeatedly when the automated procedures are performed. Guidelines for using the capability to run automated wizard procedures with the door open are given in the guidelines table in the next section.

Guidelines for Wizard Use Depending on Instrument Door State

Follow the suggestions below to use the wizards effectively when the door is open or closed.

IMPORTANT! Whenever the door is closed (whether or not a wizard is open, or an automated procedure is in progress), the autosampler moves while determining position (initialization). Always wait for the autosampler to stop moving (finish initialization), and the green status light to illuminate before initiating any automated procedures. If you accidentally start an automated procedure while the autosampler is in motion, you may encounter irregularities in the instrument behavior. An error may be displayed in the Data Collection event window. However, you should be able to complete the Wizard. Restart the instrument and the Data Collection software.

If You...	With the Instrument Door ...	Then ...
IMPORTANT! Do not open or close the instrument door while an automated procedure is in progress. Leave the door in the starting state (whether open or closed) until the automated procedure is complete.		
Begin an automated procedure	Open	The procedure continues when the door is closed, and after the autosampler moves to initialize. If you open the door again, the procedure pauses until the door is closed.
Begin an automated procedure	Closed	The procedure pauses if the door is opened. Close the door again to resume the procedure.
Click *Fill Array*	Open	The procedure does not start; the door must be closed.
Perform an automated procedure	Closed	The green status light remains on (not flashing)
Perform an automated operation	Open	The yellow status light flashes.
Note: Regardless of whether or not automated procedures are in progress during wizard use: • If the instrument door is closed, then the green status light remains on (not flashing) • If the instrument door is open, then the yellow status light flashes		

Notes

Specific Use Guidelines

If ...	Then ...	Explanation
The polymer has been in the pump longer than 1 week	Use the Water Wash Wizard (instead of Change Polymer Wizard) to replace the polymer.	Using the Water Wash Wizard ensures that the system is well cleaned before fresh polymer is introduced. Urea decomposition can cause an increase in electrophoresis current in polymer that has been at room temperature for more than 1 week.
Bubbles move but are not completely cleared by the Bubble Remove Wizard	Use the Bubble Remove Wizard *repeatedly* until the bubbles are gone.	When clearing bubbles with repeated use of the wizard, note whether or not the target bubbles have moved during performance of the Wizard. Any bubbles that move but are not entirely cleared by running the wizard are likely to be cleared with a repeat of the Bubble Remove Wizard.
You want to clear *persistent* bubbles	Try one or both of the following: • Run the Water Wash Wizard followed, if necessary, by the Bubble Remove Wizard. (The Water Wash Wizard includes refilling the pump with polymer.) • Remove the polymer bottle and run the Bubble Remove Wizard (a large amount of air will be drawn into the pump chamber and other parts of the system). Reinstall the polymer bottle and repeat the Bubble Remove Wizard to remove all bubbles. **Note:** If the pump sits idle for a time, bubbles that previously did not move are often cleared by running the Bubble Remove Wizard.	
Many, or large bubbles are present in the pump chamber	The Water Wash Wizard may help to remove bubbles.	
No bubbles are present in the array port during the monthly water wash procedure	You should still perform the Flush Array Port procedure using the Water Wash Wizard as part of monthly maintenance, even if no bubbles are present.	Occasional flushing of the array port keeps this space filled with fresh solution.
You want to install a capillary array on an instrument without an array	Perform the Fill Array procedure at the end of the Install Array Wizard.	Filling the array helps to ensure complete changeover to polymer in this situation where the PDP has been extensively washed with water.
You remove or install a capillary array	Carefully follow the instructions in the appropriate wizard (Install Array or Instrument Shutdown wizards). Ensure that the instrument configuration and the database information agree.	A mismatch between the array configuration/identification and the database information may cause incorrect analysis parameters and result in reduced basecalling accuracy.
You select *Discard* during installation of an array using the Install Array Wizard	The information for that array cannot be entered again on the instrument.	
You plan to leave the instrument unused for more than 1 week	Use the Instrument Shutdown Wizard.	

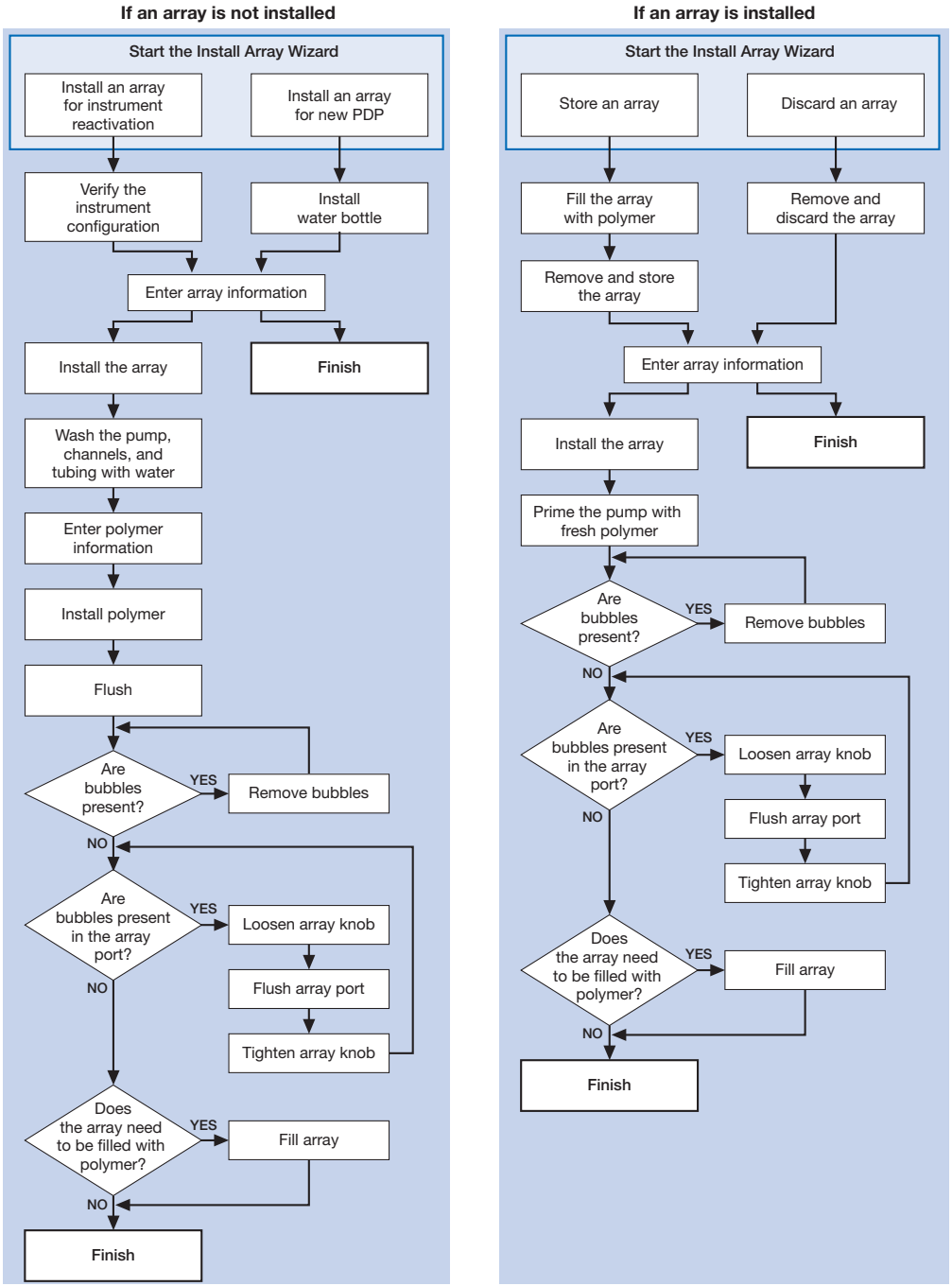
If ...	Then ...	Explanation
You are using the Install Array Wizard to reactivate the instrument	First turn on the instrument power to activate the wizard menu in Data Collection.	The instrument must be powered for the wizards to be available through Data Collection. If the instrument is turned off, the wizard names in the pull-down menu are grayed out.
You cancel a wizard during an automated procedure	The piston motion in progress will finish before the wizard is terminated.	The piston cannot stop immediately. During the period between cancellation and termination while the piston is in motion, a “Please wait...” dialog box displays.
You want to move the pump block on the mounting pins	Grasp the body of the pump, not the water seal fixtures.	Excessive force can damage the water seal fixtures.
You want to clean the array port knob, plug, or opening	Carefully clean the threads of these parts with moistened lab wipes.	Dried polymer deposits on the threads may cause poor sealing when the parts are rejoined.
You want to clean the PDP with the Water Wash wizard	Use only deionized water at ≤ 40 °C	Hot water may damage the PDP seals and joints. Do not use any solutions or fluids in the instrument other than water and polymer.

Notes

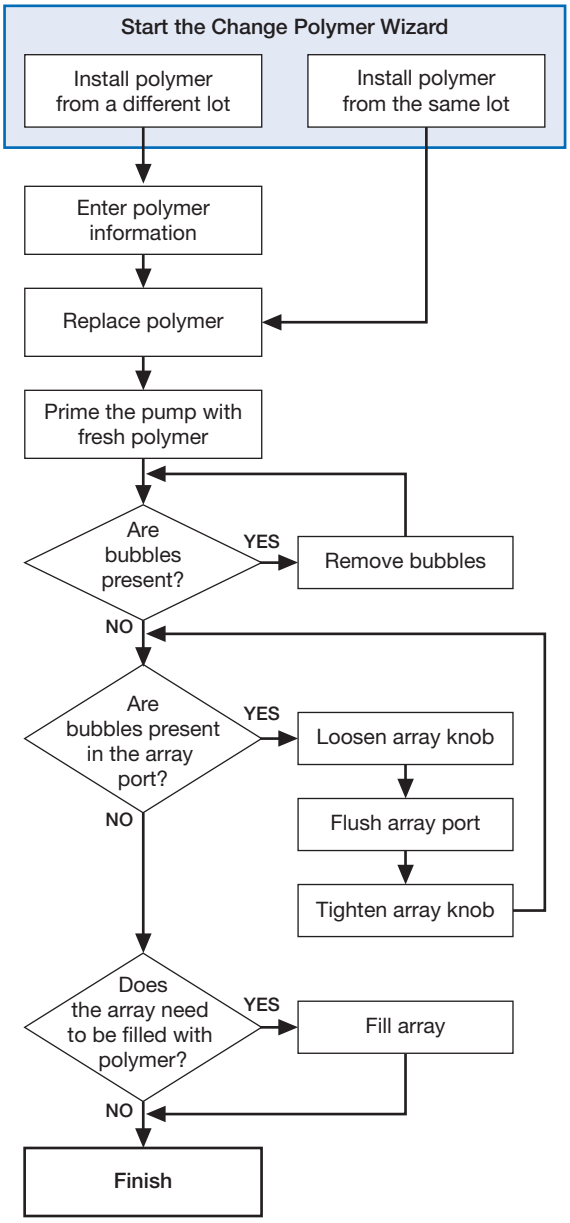
Wizard Flowcharts

The five wizards are depicted below as flowcharts. Each flowchart shows decision branches and alternative pathways.

Install Array Wizard

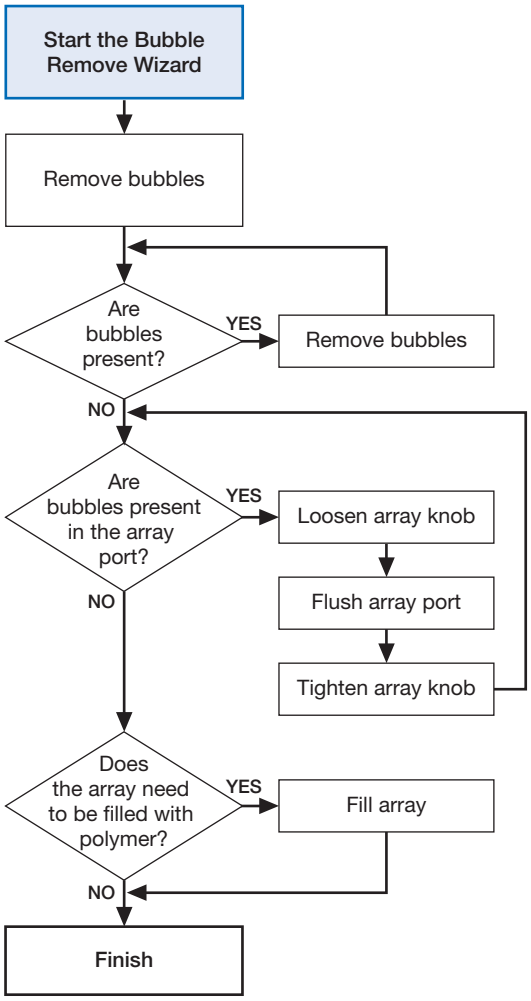


Change Polymer Wizard

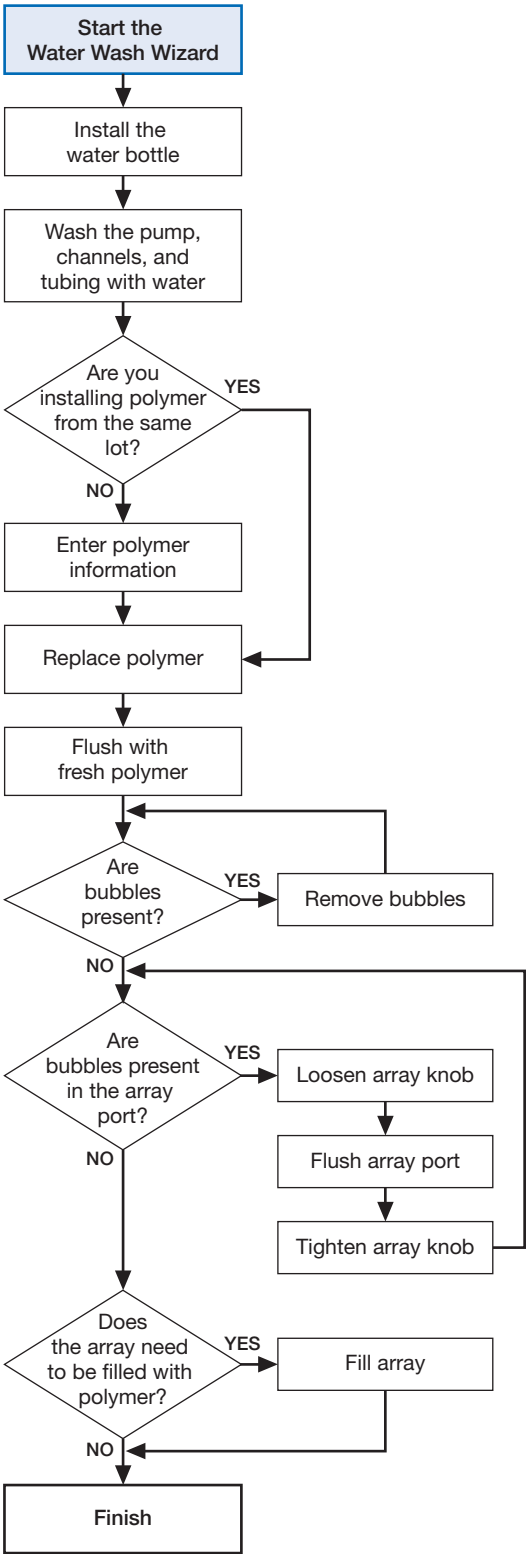


Notes

Bubble Remove Wizard

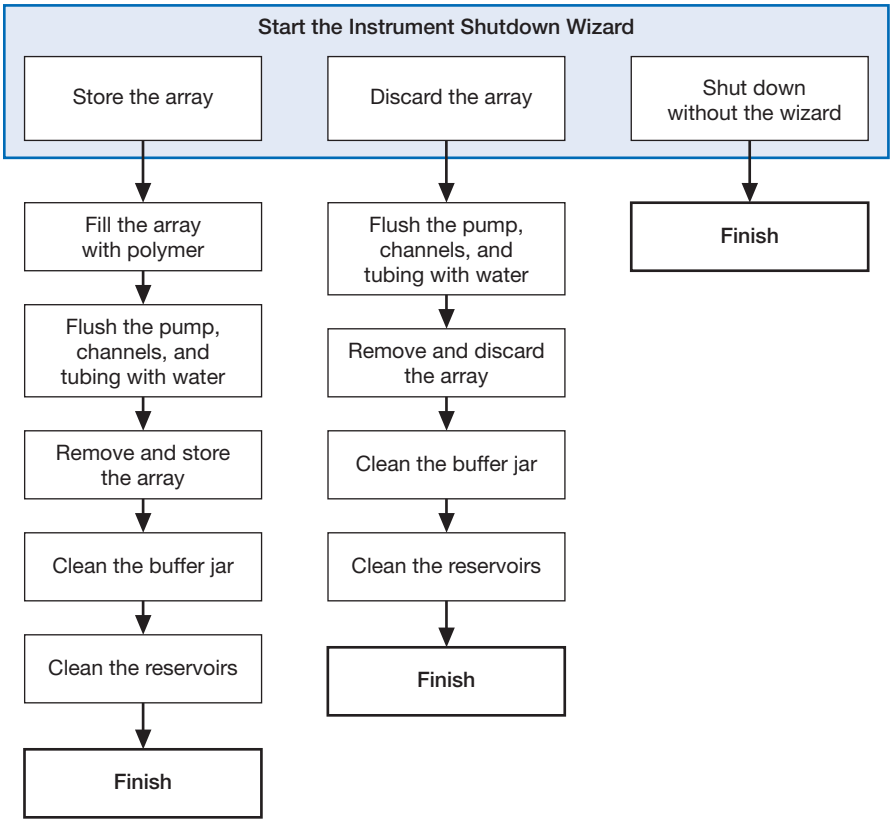


Water Wash Wizard



Notes

Instrument Shutdown Wizard



Flushing and Filling the Water Trap

Overview

The PDP water trap should be flushed with either distilled or deionized water at least once per month to wash out any diluted polymer, and to clear bubbles. Leave the trap filled with either distilled or deionized water.

To flush the water seal trap:

1. Fill the supplied 20 mL, all-plastic Luer lock syringe (PN 4324463) with distilled or deionized water. Expel any bubbles from the syringe.

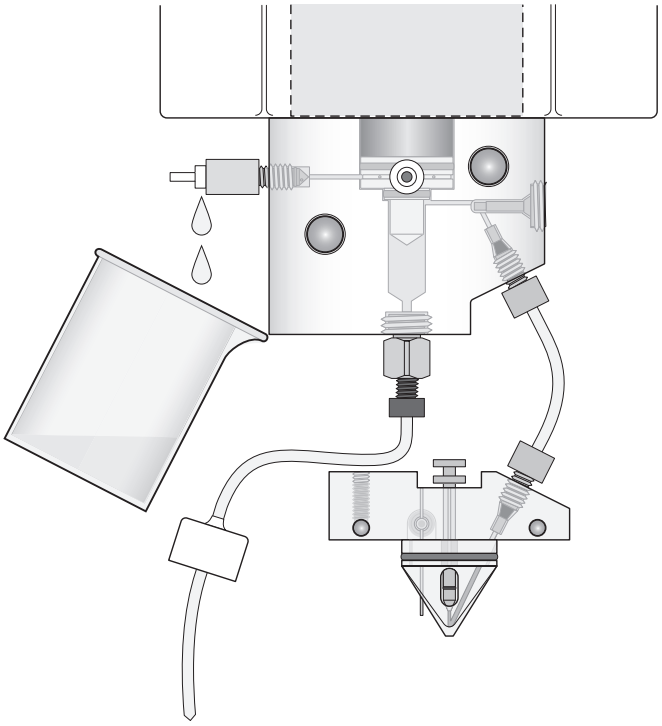
   Note: Do not use a syringe smaller than 20 mL. Doing so may generate excessive pressure within the trap.

2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

3. Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.

4. Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.

5. Hold an empty tube or beaker under the exit fitting to receive approximately 5 mL of waste. Flush the trap by pushing steadily on the syringe plunger.



IMPORTANT! DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals. Take approximately 30 seconds to flush 5 mL of either distilled or deionized water through the trap.

Notes

Note: Because the water trap volume is approximately 325 µL, a relatively small volume of water is adequate for complete flushing. However, a larger volume only improves flushing, as long as force and flow rate are kept within the limits given above

6. Close the fittings in this order by turning each clockwise until the fittings seal against the block:
   a. Luer fitting, first
   b. Exit fitting, second

IMPORTANT! Do not over-tighten the fittings. Very little pressure develops within the trap during pump operation, so the fittings require only enough tightening to prevent water leaks. Excessive tightening can damage the fittings.

7. Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

Storing a Capillary Array

IMPORTANT! Wear appropriate protection including gloves, laboratory goggles and coat whenever you work with the fluids used on this instrument or parts that may have come in contact with these fluids.

1. Remove the capillary array from the instrument using the Install Array wizard.

If You ...	Then ...
Follow the Install Array Wizard instructions	The capillaries are filled with fresh polymer and some of the steps described below will be complete when the array is removed.
Remove the array without using the Array Wizard instructions	The capillaries should first be filled with fresh polymer.

CAUTION To maintain serviceability during storage, keep both ends of the capillary array immersed in 1X run buffer. Failure to do so may result in array damage.

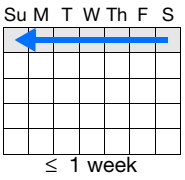
2. Put 80 mL of 1X run buffer in the capillary array header shipping cover.
3. Lower the capillary tips of the array header into the shipping cover and lock the header onto the cover. The tips of the capillaries should be immersed in buffer.
4. Clip the detection cell window cover onto the detection cell.
5. Attach the detection cell with its cover to the storage post on the array frame.
6. If the array knob and double-tapered ferrule are on the array tip:
   a. Remove them and rinse them with deionized water.
   b. Dry the parts with a lab wipe.
   c. If they are not to be used immediately, store them in a safe place.
7. Clean the array tip carefully with a lab wipe moistened with deionized water.
8. Attach the array tip shipping vial filled with 1X run buffer to the array tip. Loosen the vial cap slightly, insert the tip and then tighten the cap.
9. Clip the vial with the array tip onto the array frame.
10. Store the capillary array upright in a safe area.

IMPORTANT! Check the 1X run buffer levels in the shipping cover and vial at least once a week; replenish the buffer as necessary to keep the both ends of the capillaries immersed in buffer.

Notes

Performing a Short-Term Shutdown

Perform the short-term shutdown procedure if you will use the instrument again in 7 days or less.

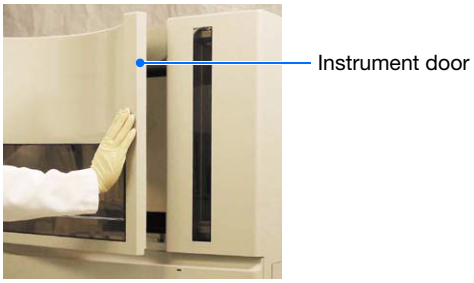


Materials Required

- 1X Run Buffer
- POP-7 polymer
- Deionized water
- Lab wipes
- Gloves

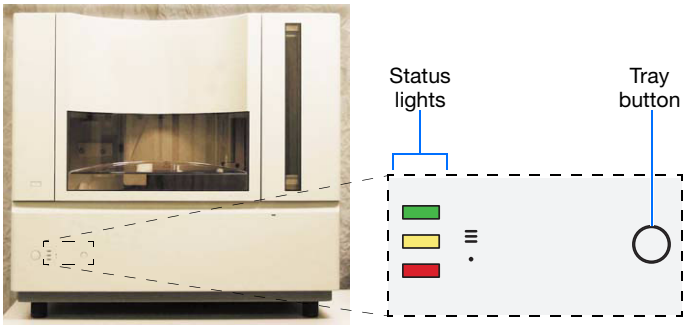
Performing a Short-Term Shutdown

1. Close the instrument door.

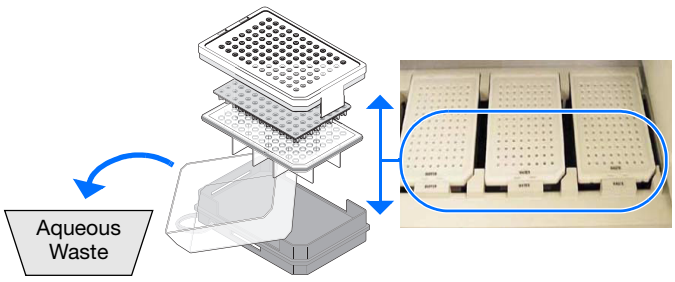


2. Press the tray button to bring the autosampler to the forward position.
3. Wait for the:
   - Autosampler to stop moving
   - Green status light to illuminate

   then open the instrument door.



4. Remove the buffer, water, and waste reservoir assemblies from the instrument.
5. Disassemble each reservoir assembly and empty the contents of the reservoirs into an aqueous waste container.

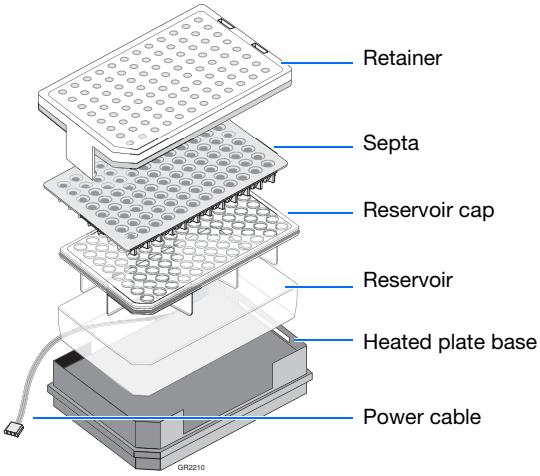


6. Rinse each reservoir using deionized water.
7. Dry the reservoirs using lint-free wipes.

8. Fill and assemble the reservoirs.

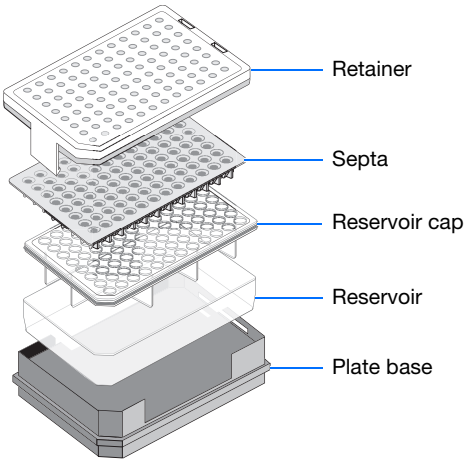
Buffer Reservoir Assembly

1. Add 80 mL 1X running buffer to the Buffer reservoir.
2. Assemble the reservoir assembly as shown below:

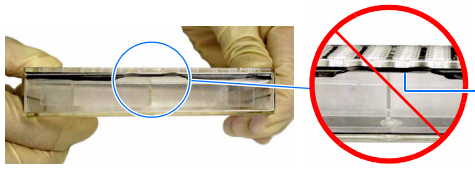


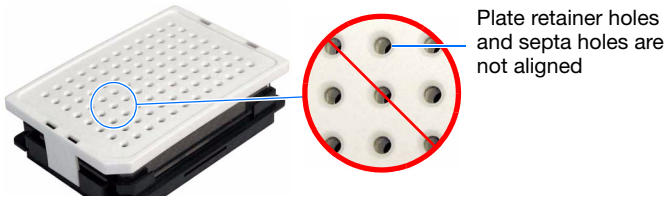
Water and Waste Reservoir Assemblies

1. Add 80 mL high-quality deionized water to each reservoir (Water and Waste).
2. Assemble each reservoir assembly as shown below:



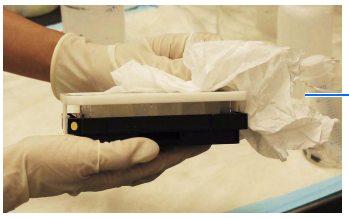
9. To prevent damage to the capillary array, inspect each reservoir assembly and verify that the:
   - Septa fit snugly and flush on the reservoir
   - Rubber gasket around the edge of the reservoir cap is seated
   - Plate retainer holes and the septa strip are aligned





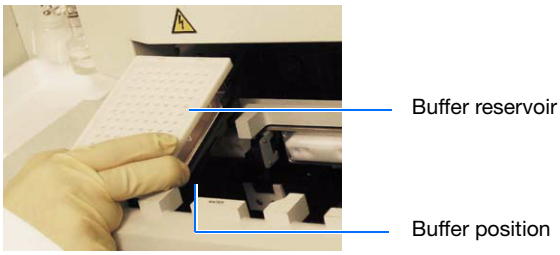
Notes

10. Dry the reservoirs using lint-free wipes.



11. Connect the Buffer reservoir plate base cable into the heater outlet within the instrument.

IMPORTANT! After placing the buffer reservoir, make sure the cable is out of the way of the autosampler.

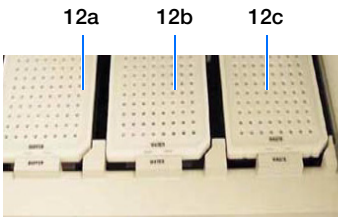


12. Place the Water and Waste reservoirs into the instrument. All three reservoirs will be in the following order:

a. Buffer reservoir

b. Water reservoir

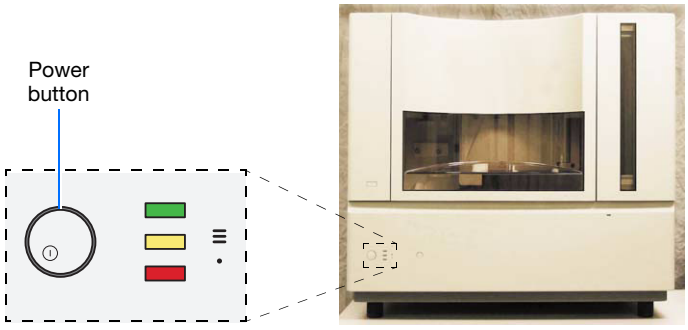
c. Waste reservoir



13. Close the instrument door.

14. Press the tray button.

15. Press the instrument power button.

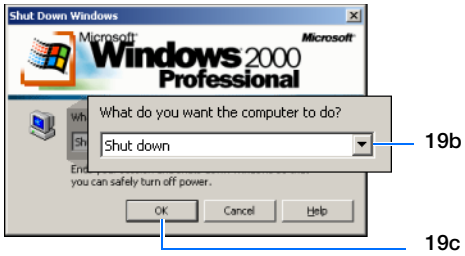


16. Shut down the computer:

a. Select Start > **Shutdown**.

b. In the Shut Down Windows dialog box, select **Shut down** from the drop-list.

c. Click OK.



17. Press the power button on the monitor.

7

Notes

Working With Drives for Database and Sample Data Storage

Checking Available Space on Drives D, E, and F

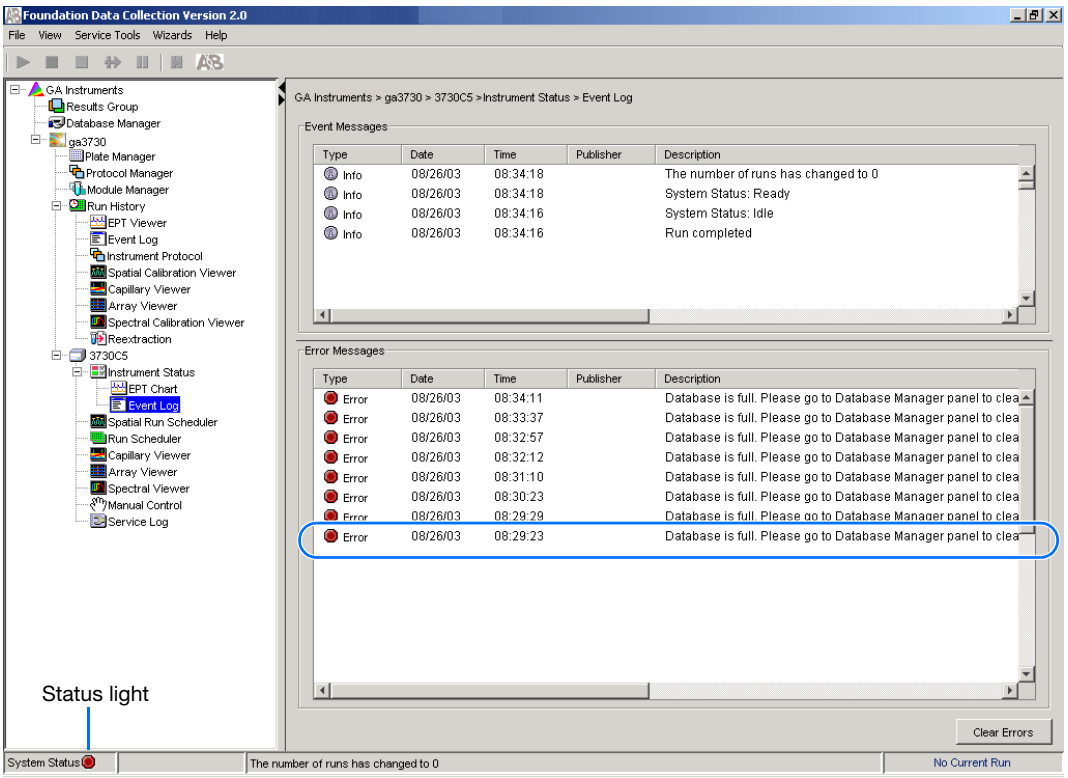
Before a run or batch of runs, the Data Collection software automatically checks the available space to ensure sufficient space to store the database and sample file data you create.

The Data Collection software sends a warning message to remove data when 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** > **ga3730** > *instrument name* > **Instrument Status**> **Event Log**.

IMPORTANT! 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
- Emptying the recyle bin
- Deleting unneeded files
- Defragmenting the drives

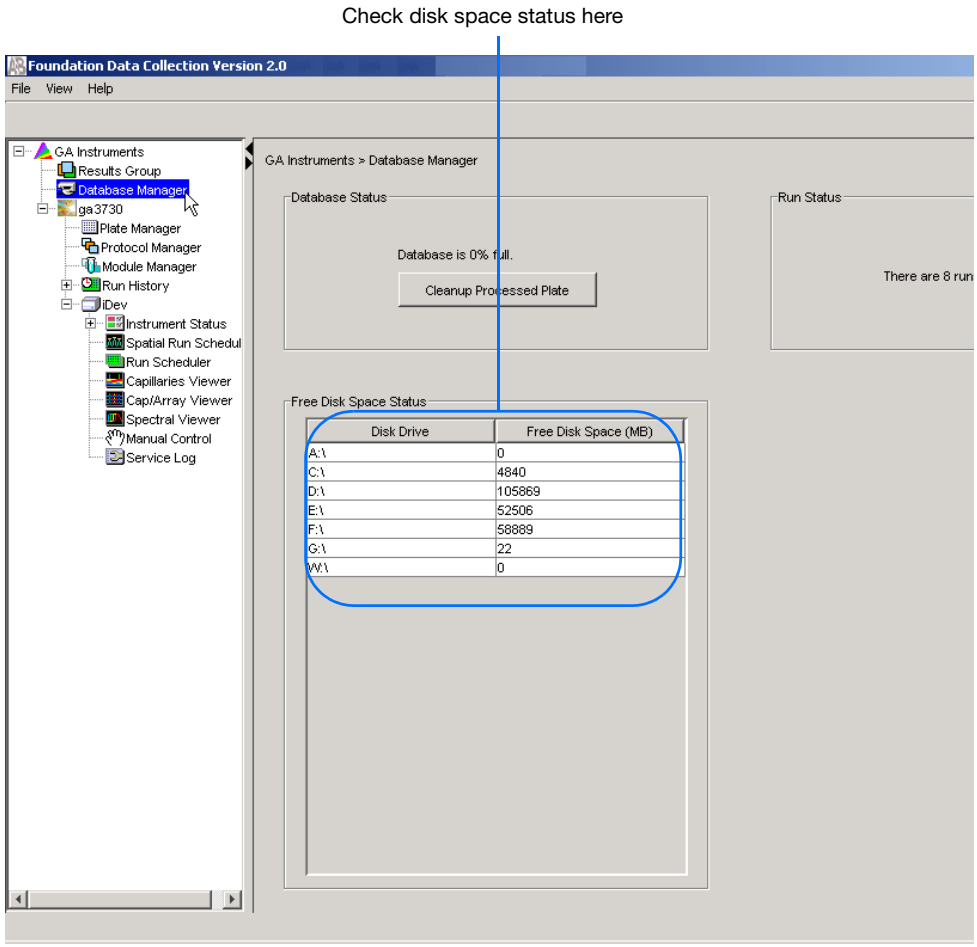
Notes

Hard Disk Status

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.



Checking available drive E space

2. If there is insufficient space:
   a. Archive the sample files to a CD-RW (see page 176) or another volume.
   b. Delete the sample file data from the drive E and empty the contents of the Recycle Bin.

Notes

Archiving Data

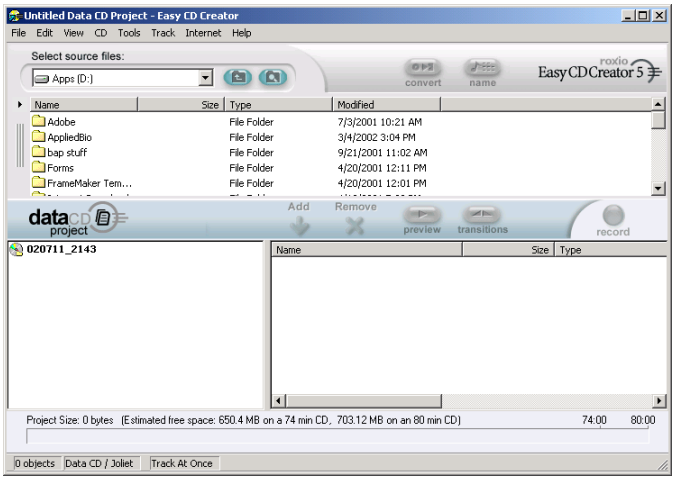
Creating a Data CD

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**.

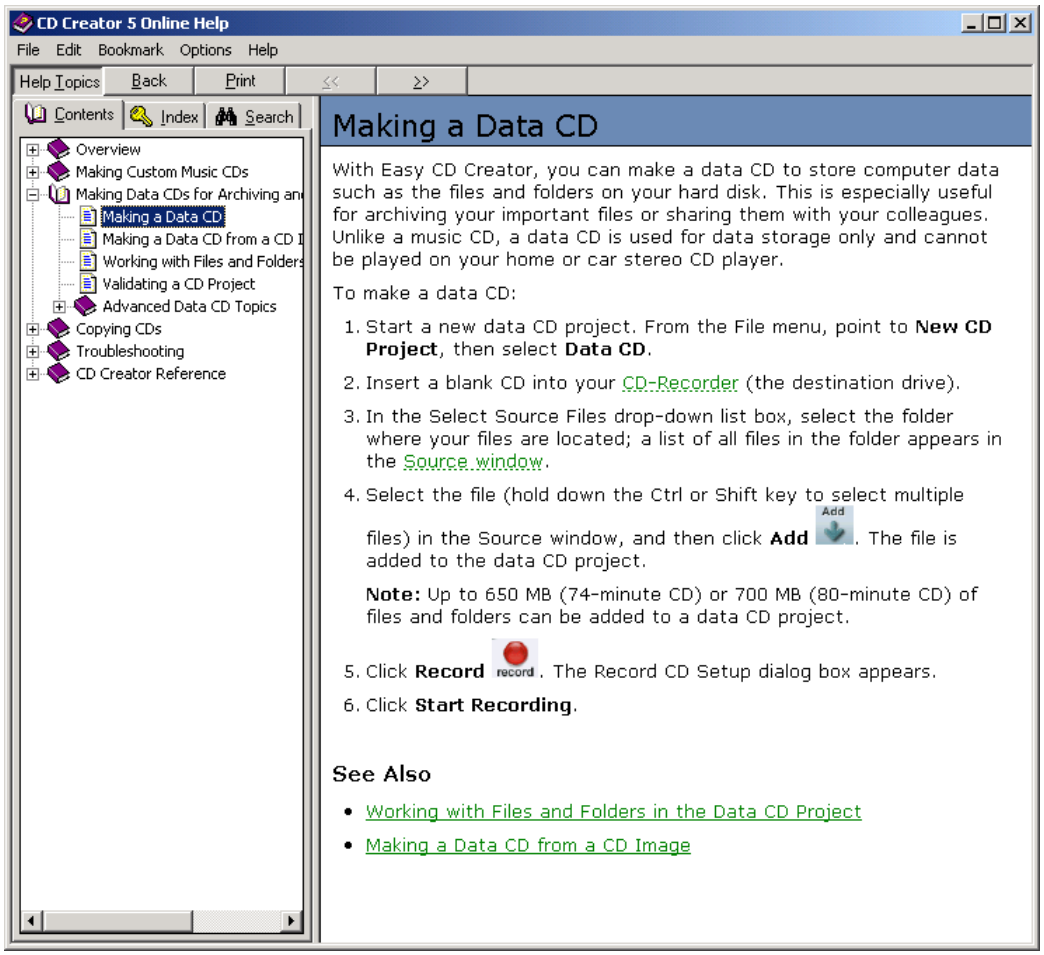
   The Untitled - Easy CD Creator dialog box opens.



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 instructions to create the CD.



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.

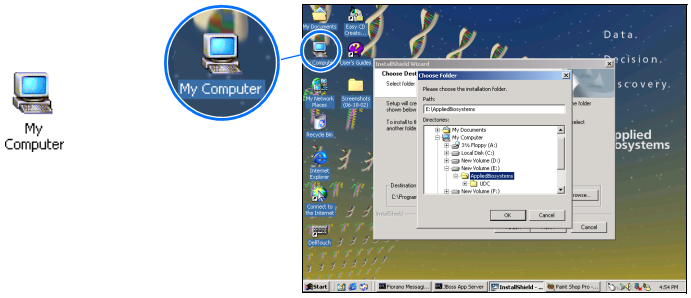
When to Defragment the Computer Hard Drive

Defragment the computer hard drive:

- at least once every month.
- before fragmentation reaches 10%.

Defragmenting the Hard Drive

1. In the Windows desktop, right-click **My Computer** (), then select **Manage**.

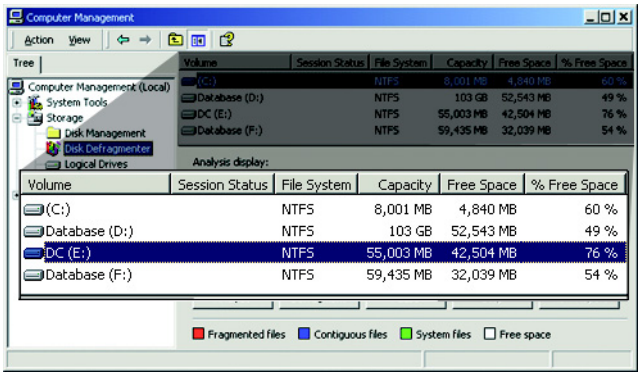


2. In the Tree tab of the Computer Management dialog box, click **Computer Management (Local)** > **Disk Fragmenter**.

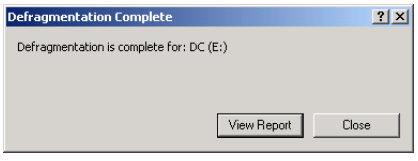
3. Select the **E** drive.

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 ×.

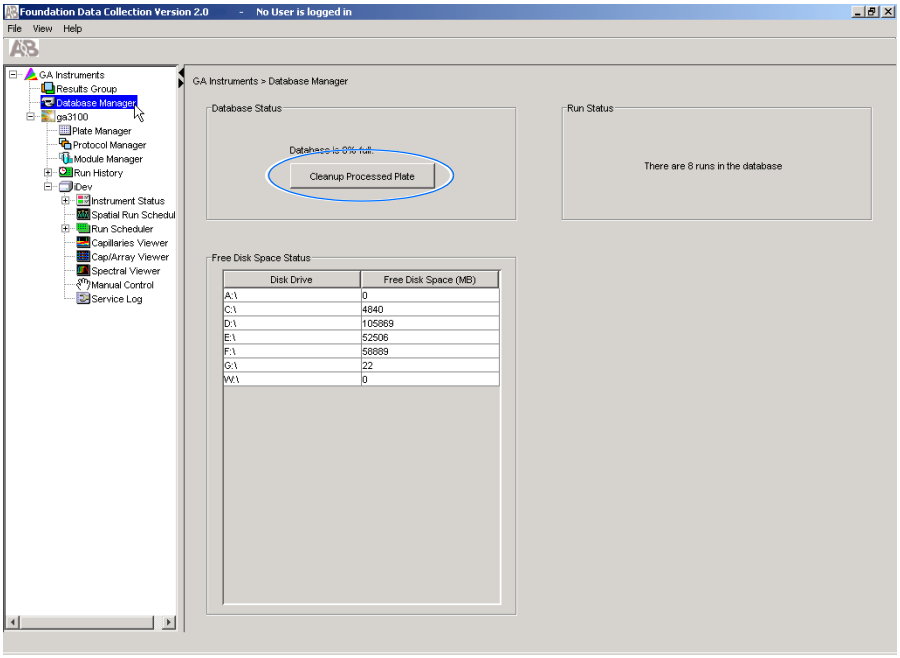
Deleting Records from the Database

Deleting Processed Frame Data

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

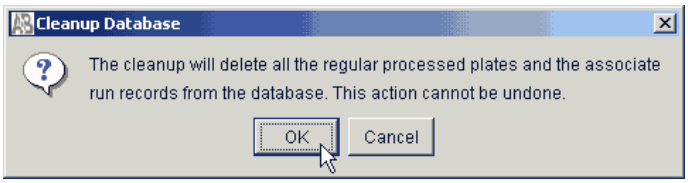
   The Database Manager view opens.

IMPORTANT! 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.



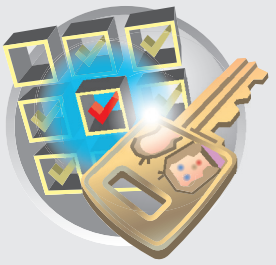
3. Click OK.

Note: There is no need to re-import the spatial and spectral calibrations or the custom run modules.

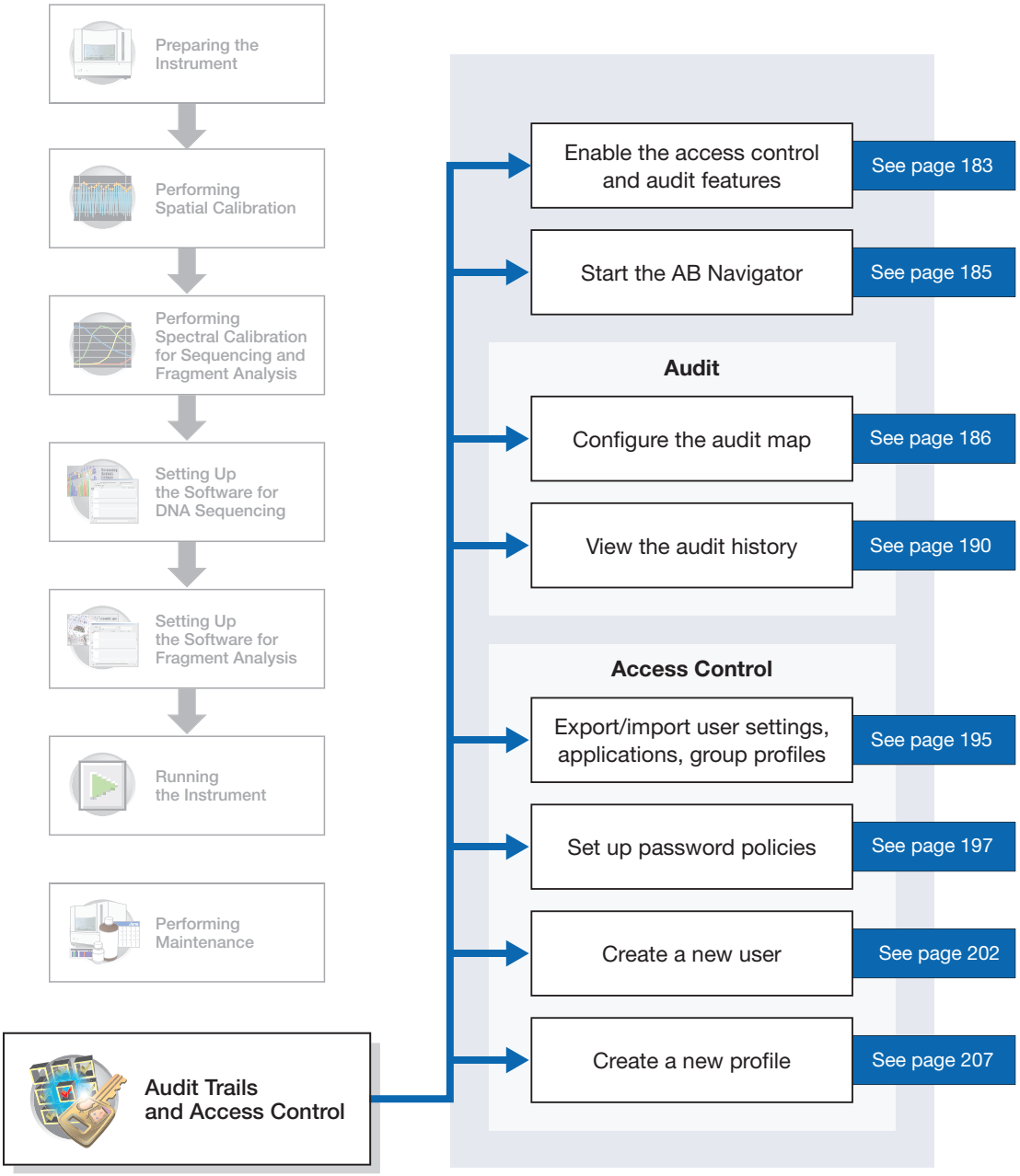
Note: It may take several minutes to clean up the database if it is full or contains a lot of data.

Notes

Audit Trails and Access Control



Workflow



Notes

Audit

Audit trails maintain a history of data changes made by the user.

Data Changes that Generate Audit Records in Data Collection Software

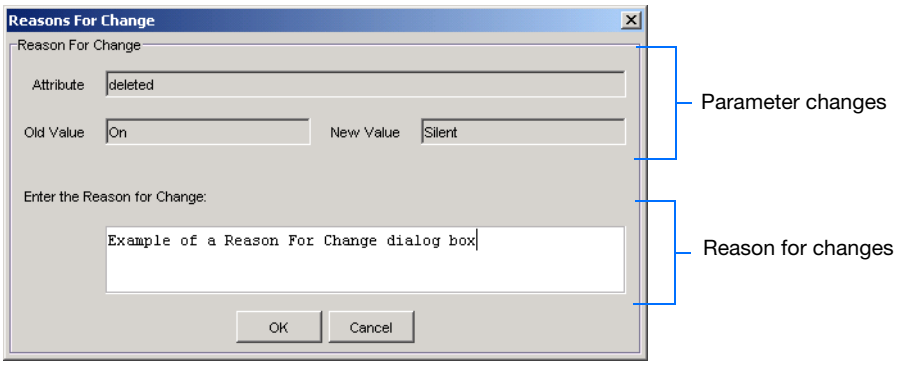
An audit record is generated when data are changed. The following table lists the three general categories and the events within them that generate an audit record in Data Collection software.

	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, edit, or import 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 created or changed.
- 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.

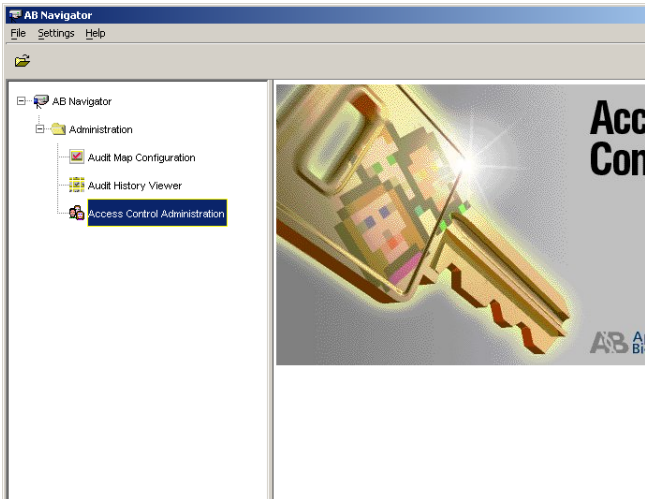


Notes

Enabling The Access Control and Audit Features

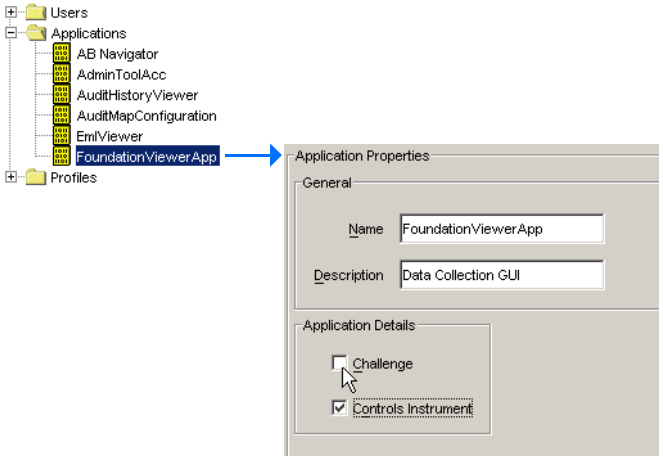
Enabling Access Control (Security)

1. Start the following:
   - Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3730 v2.0**.
   - Administrator application: **Start > Programs > Applied Biosystems > Administrator**.
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. In the left pane tree double-click **Access Control Administration.**



4. Select **Applications > FoundationViewerApp**.
5. Select the Challenge checkbox to activate it.

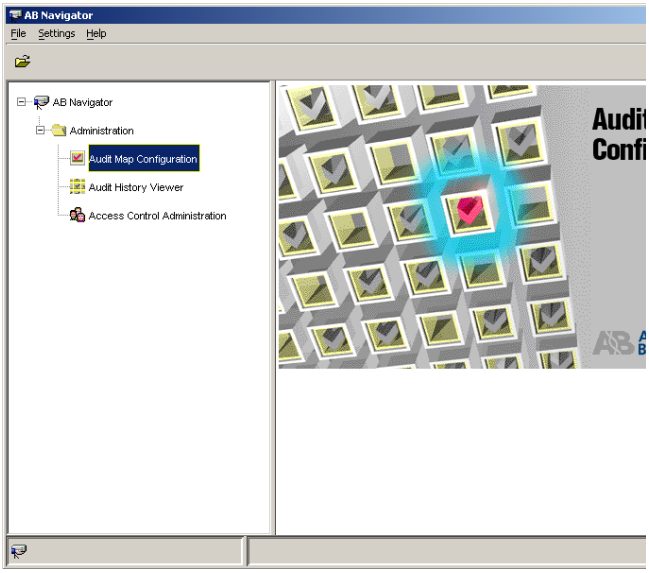
   The Login and Password dialog box is now enabled.
6. Select **File > Save**.
7. Exit Access Control Administration



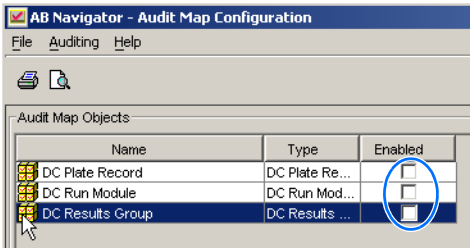
Notes

Enabling Audit

1. In the Navigator left pane tree, double-click Audit Map Configuration.



2. Select the Enabled checkbox for each audit map to activate it:
   - DC Plate Record
   - DC Run Module
   - DC Results Group



3. Exit Audit Map Configuration.

Notes

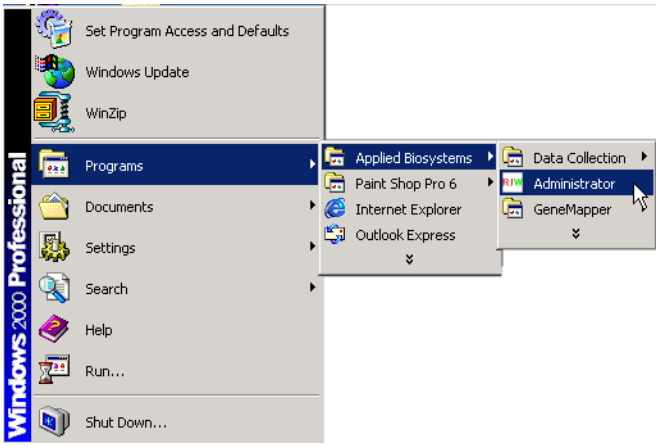
Starting AB Navigator

AB Navigator is the access point for these applications:

- Audit Map Configuration
- Audit Map History Viewer
- Access Control Administration

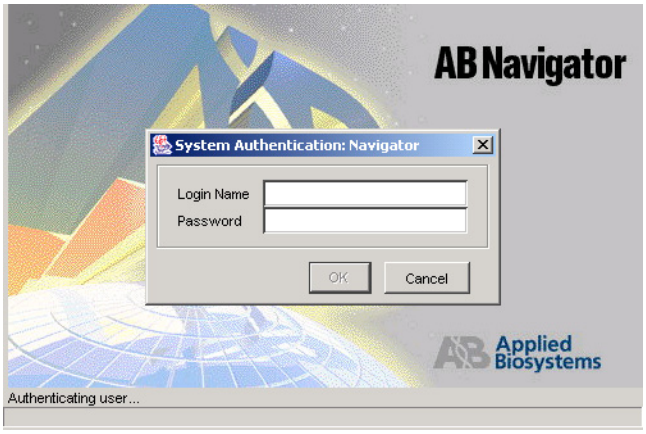
IMPORTANT! You must start Data Collection services in order for AB Navigator to function properly.

1. Start the Data Collection Services, then start the Administrator application:
   a. Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3730 v2.0**.
   b. Administrator application: **Start > Programs > Applied Biosystems > Administrator**.



The System Authentication dialog box displays.

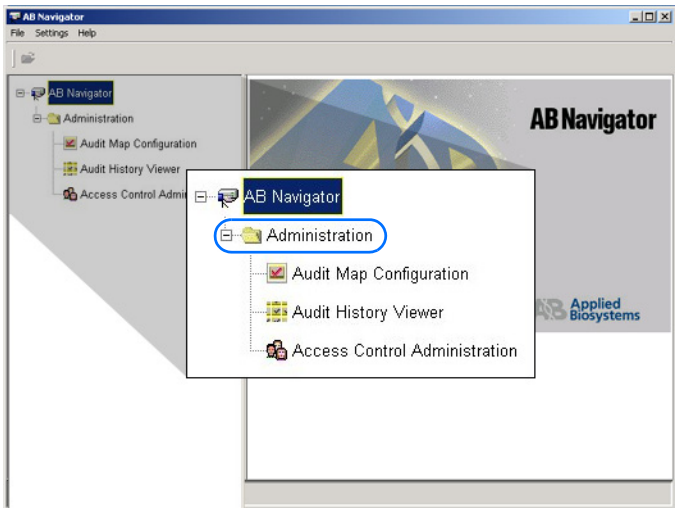
2. Enter login name and password and click **OK**.
   Default login name: “Administrator”
   Default password: “Administrator”



Note: To change your password, see step 4 on page 203

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 functional area.

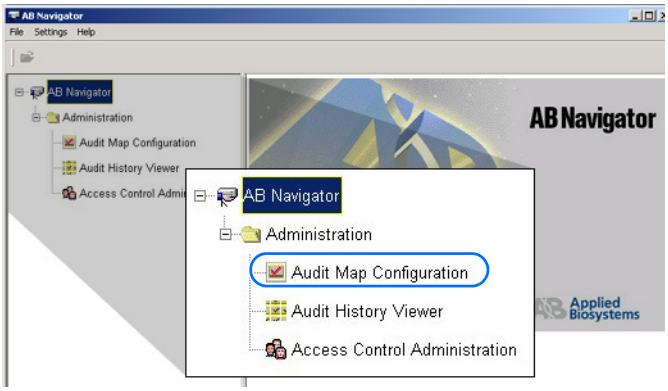
Some features of the Audit Map Configuration Tool:

- 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. Double-click the Audit Map Configuration icon in the left pane tree.

   The System Authentication dialog box displays.

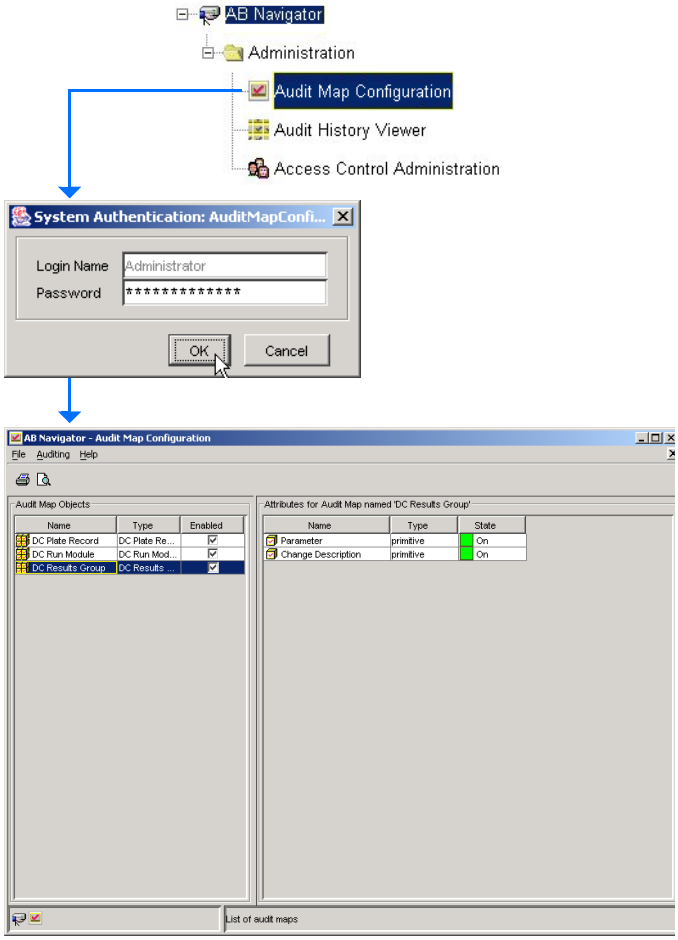


Notes

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**.

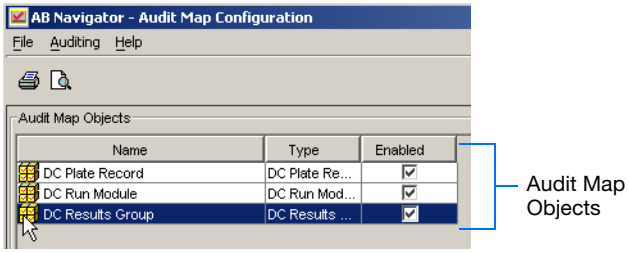
3. Click **OK**.

   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. 
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.

<table>
<tr><th>Toolbar Menu</th><th>Command</th><th>Function</th></tr>
<tr><td rowspan="5">File</td><td>Go To</td><td>Displays a list of applications that are currently running; select an application to go to that application</td></tr>
<tr><td>Visual Print</td><td>Displays Print Dialog</td></tr>
<tr><td>Visual Print Preview</td><td>Displays Print Preview</td></tr>
<tr><td>Exit Application</td><td>Exits the Audit Map Configuration application</td></tr>
<tr><td>Exit AB Navigator</td><td>Exits the AB Navigator application</td></tr>
<tr><td rowspan="3">Auditing</td><td>On</td><td>Select auditing to be turned on for the Audit Map Configuration.

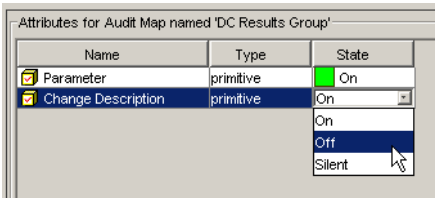
When a change is made to an Audit Map’s enabled state or when a change is made to the state of an attribute, auditing occurs, and A Reason For Change (RFC) dialog displays.

<table><tr><th>When RFC Dialog Displays and You...</th><th>Then ...</th></tr><tr><td>Click OK</td><td>The map or attribute state changes and an Audit Record is created.</td></tr><tr><td>Click Cancel</td><td>The map or attribute state does not change.</td></tr></table></td></tr>
<tr><td>Silent</td><td>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.</td></tr>
<tr><td colspan="2">Note: Changes to the auditing state from this toolbar menu item (Auditing) applies only to the Audit Map Configuration tool. To change the auditing states of Data Collection software functional areas, see the section below.</td></tr>
</table>

Notes

Attribute States

When you click an Audit Map Object, the Attributes Pane displays.



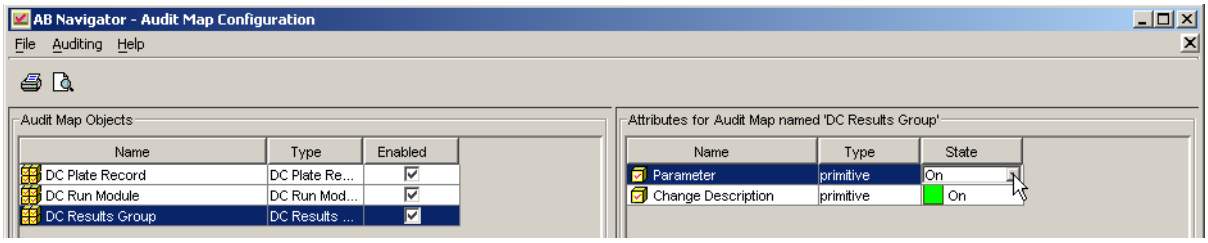
Change Description

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 graphic above—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 182).



On, Off, and Silent

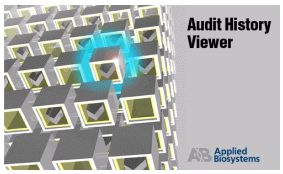
The following table describes the On, Off, and Silent states for audit map attributes, Change Description and Parameter.

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.

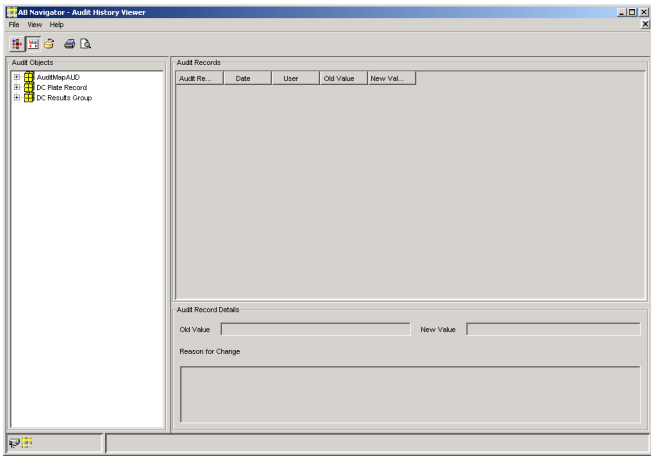
Starting the Audit History Viewer

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**.

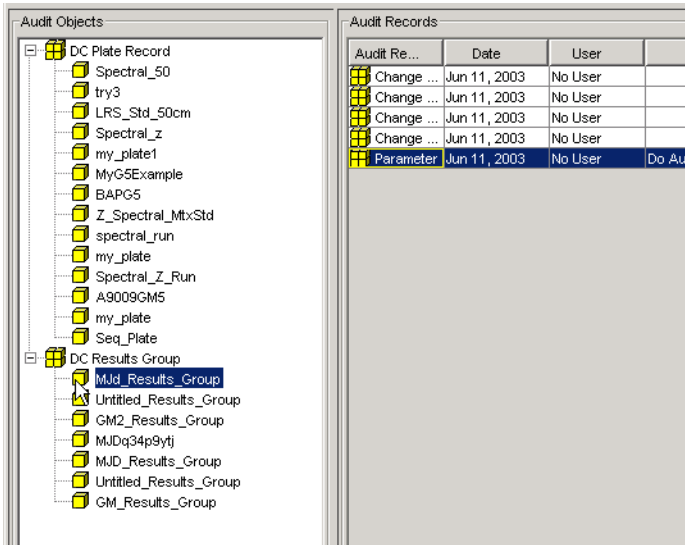
   The Audit History Viewer displays.



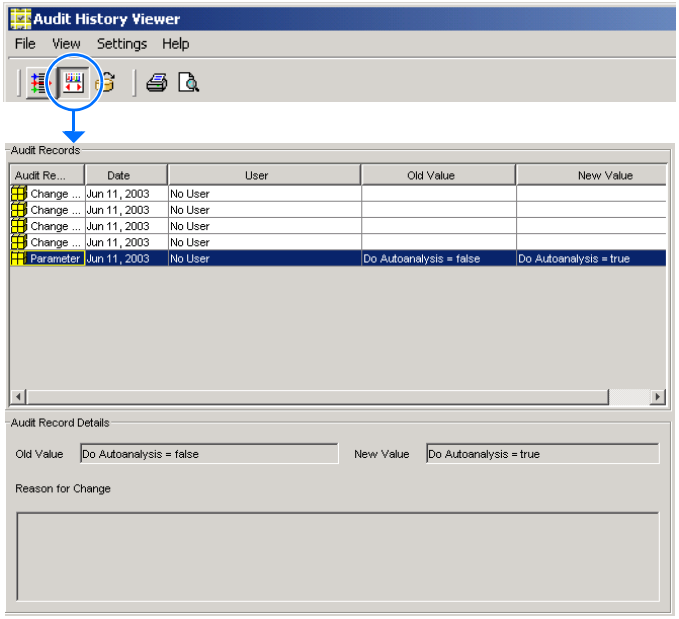
Notes

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).

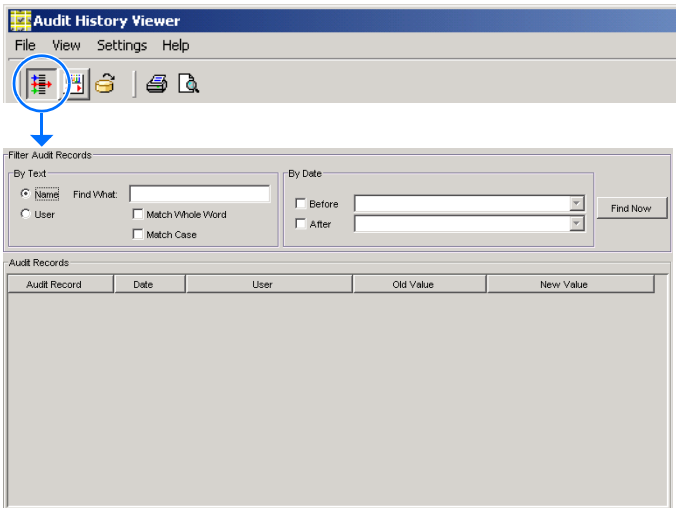
   The Filter Audit Records pane displays.

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

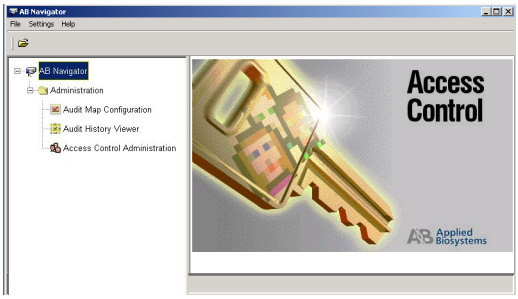
Also, Access Control allows an administrator to restrict or grant users access to features and functions of the software.

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

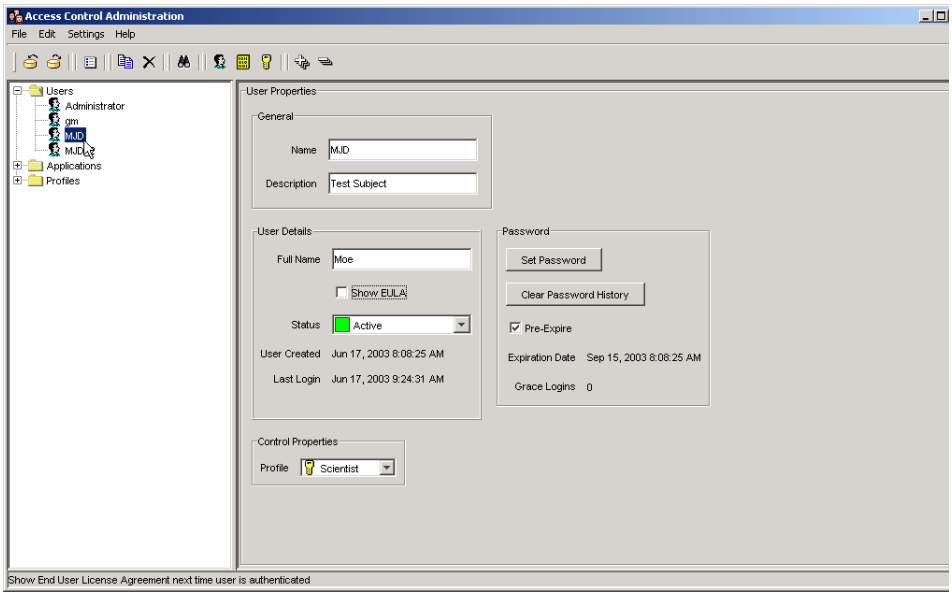
IMPORTANT! You must start Data Collection services in order for AB Navigator to function properly.

1. Start the following:
   - Data Collection services: **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3730 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**.



Notes

4. Click **OK** to display Access Control Administration



Access Control Administration Tool Bar

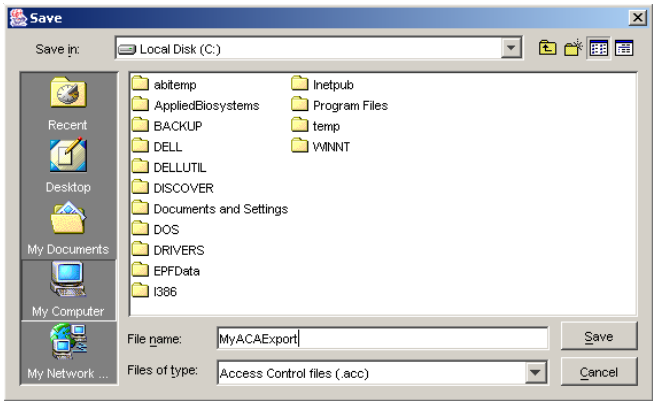
Icon	Command	Function
	Save	Saves changes
	Reload	Refreshes the Access Control Administration window with the latest changes
	Report	Customize and print a report of the selected Access Control Indentifiers
	Duplicate	Duplicates the selected entity
	Delete	Deletes the selected entity
	Find	Finds a specific identifier
	New User	Opens a new user window
	New Application	Create a new application (not needed for Data Collection software)
	New Profile	Opens a new profile window
	Expand	Expands all tree nodes making all items visible
	Collapse	Collapses all tree nodes making only the root tree items visible

Notes

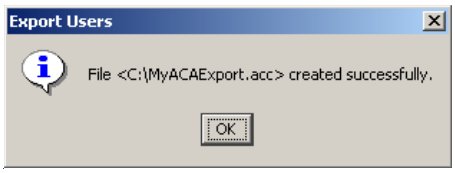
Exporting User Settings, Applications, and Group Profiles

Exporting and Importing the Access Control Administration settings can be useful if the same users and group profiles will be shared amongst many computers. This procedure can minimize the time needed to re-create the access control settings on the other computers. The Administrator will only need to create the User, Application, and Group profiles settings on one computer, and then import these settings to the other computers.

1. Select **File > Export Database**.
2. Choose a local or network location and type in a file name for the export file, and click **Save**.



3. The Export Users dialog box displays after a successful export.

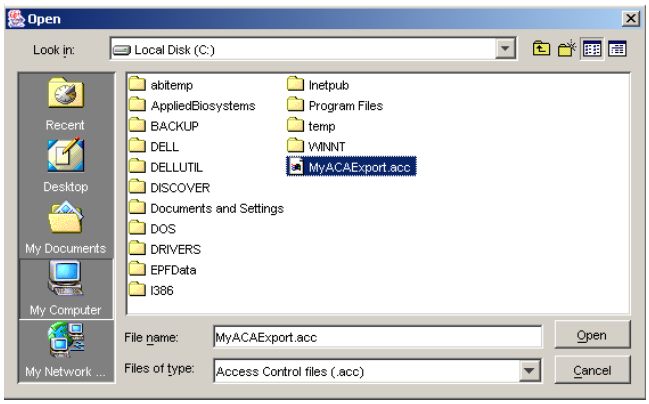


Notes

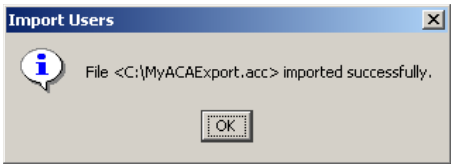
Importing User Settings, Applications, and Group Profiles

Importing the Access Control Administration settings will over-write any existing User, Application, and Profile settings. The Audit Map Configuration and Audit History Viewer will not be affected.

1. On the computer that you want to import the Access Control Administration database, enter the Access Control Administration.
2. Select **File > Import Database**.
3. Locate the file that was exported, and click **Open**.



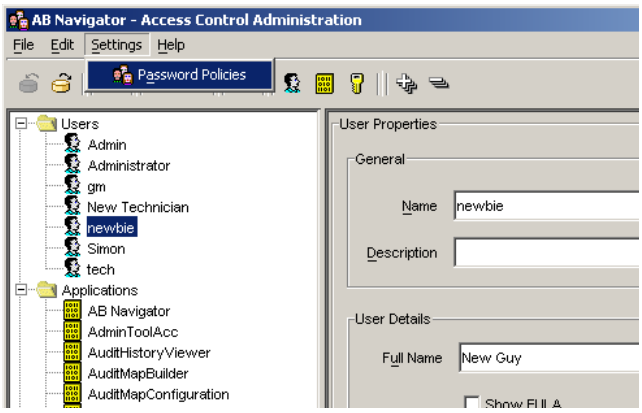
4. The Import Users dialog box displays after a successful import.



Notes

Password Policies

1. Customize passwords by accessing **Settings > Password Policies**.

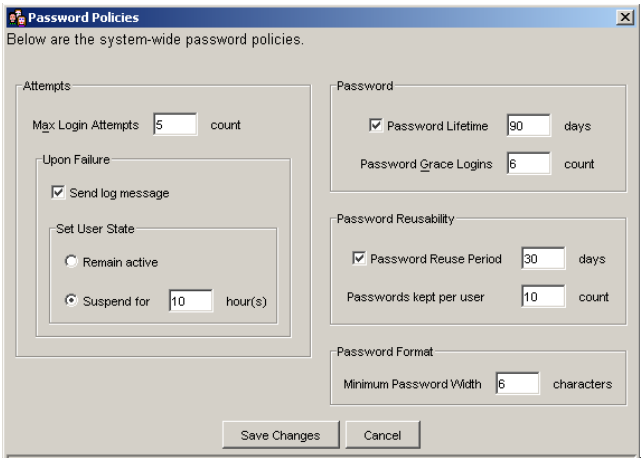


The following fields are customizable:

- **Max Login Attempts**:

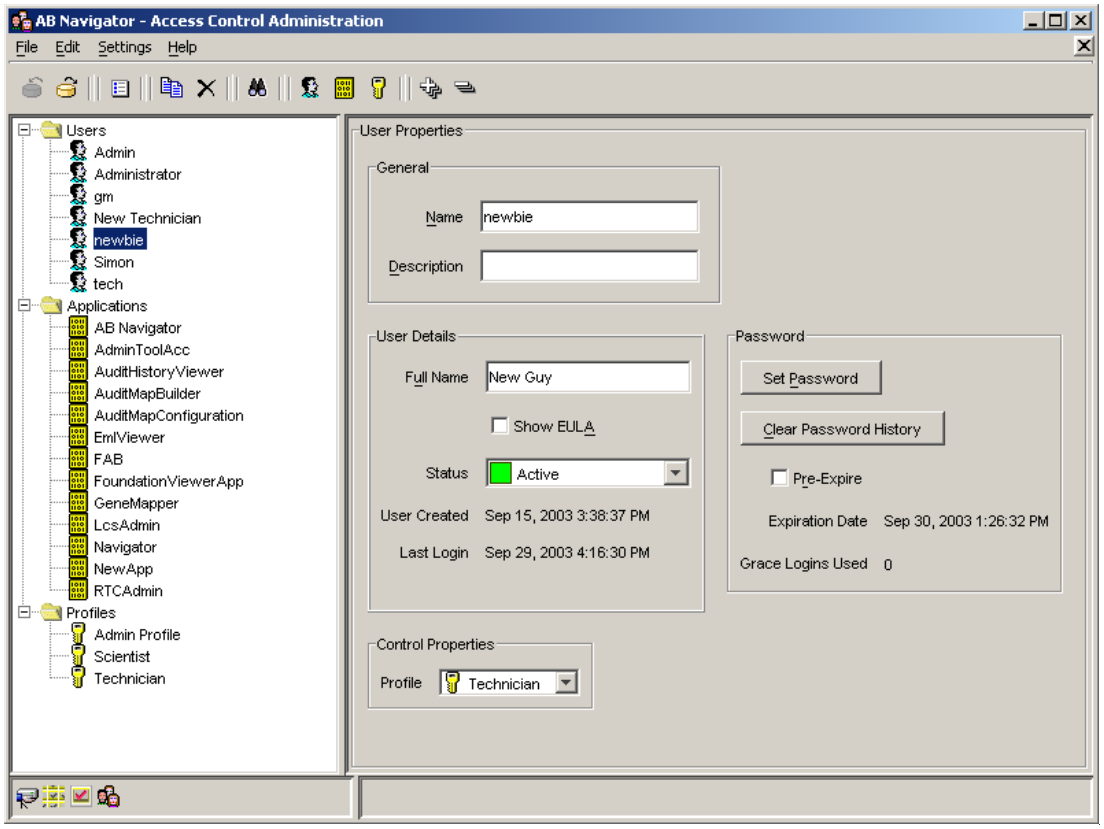
  Sets the maximum number of failed login attempts allowed before the user account is suspended. The User will not be able to log in again until the suspension time period is over, or the Administrator re-sets the user status to Active.

- **Set User State**:
  - *Remain active* - Users are never suspended, even though they exceed the maximum login attempts.
  - *Suspend for x hour(s)* - Users are suspended for the set number of hours if they exceed the maximum login attempts. The user can not login to the Data Collection viewer until the end of the suspension, at which time the User's "Suspended" status reverts to "Active."
- **Password**:
  - *Password Lifetime* - At the end of the set period, users will be asked to change their password.
  - *Password Grace Logins* - Sets the number of times that users are able to login with their old password before they are forced to change their password.



Notes

- **Password Reusability**:
  - *Password Reuse Period* - Sets the number of days that users are prevented from re-using their old password(s). For more information see, "User Password Change" on page 199.
  - *Passwords kept per user* - Sets the number of the latest passwords chosen by each user that will be remembered.



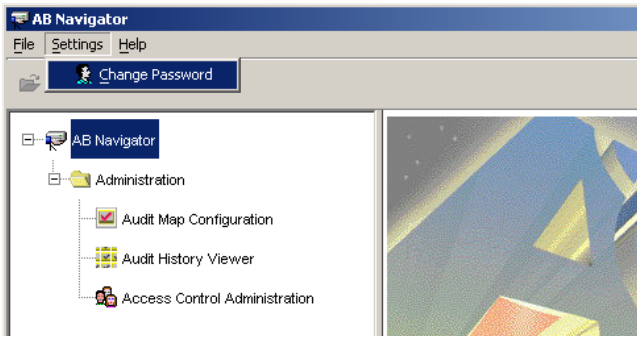
Note: The password history can be cleared by the Administrator by Clicking Clear Password History for a particular user. If the user forgets his password the Administrator can reset his password at any time without being required to provide the old password.

Notes

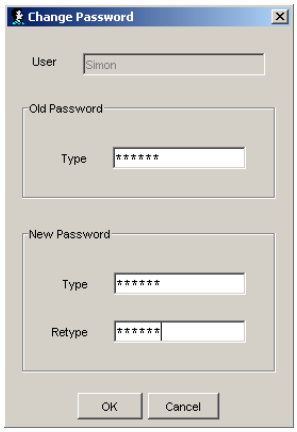
User Password Change

Users can change their passwords by going to the Administrator tool.

1. Select **Settings > Change Password**.



2. Users must type in their old password, and then their new password twice in the Change Password dialog box.

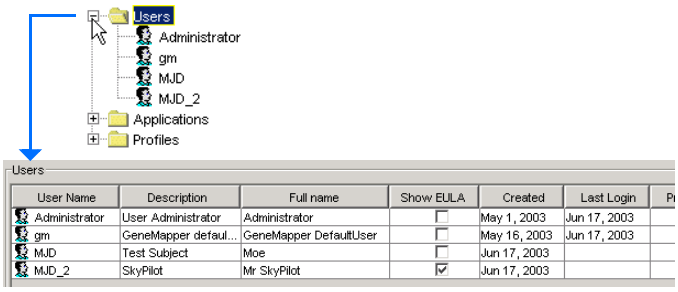


Notes

Type Selection

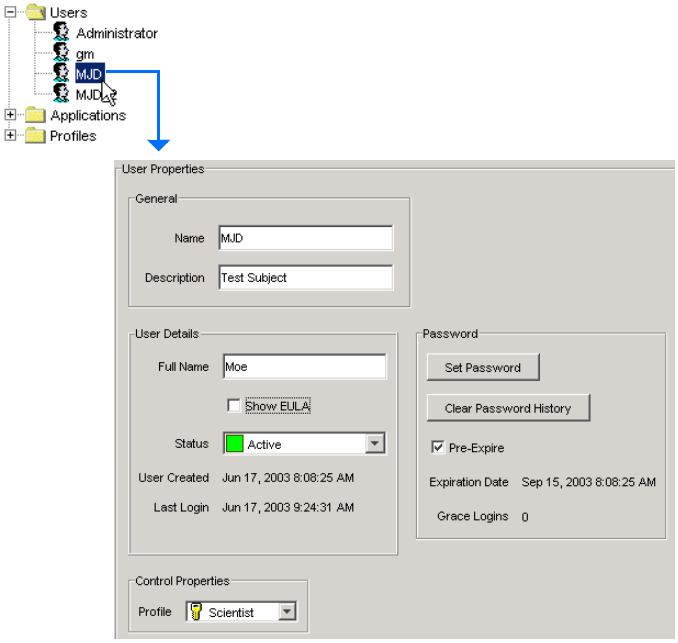
In the left pane tree, Users and Applications are types. When you select a type, the List of Users pane displays a list of identifiers of the type selected.

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.



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.

Three default profiles and their functional access levels:

- *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

The broad functional access levels are further described below:

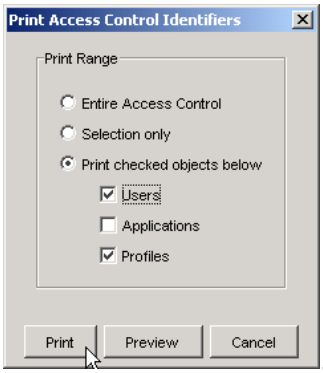
- *Instrument Protocols* include: Run Module Operations, Results Group Operations, Analysis Protocol Operations, Instrument Protocol Operations and Reextraction.
- *Instrument Operation* include: Plate Operations, Event Log, and Instrument Control Operations.
- *Instrument Maintenance*: Spatial Calibration Operations, Manual Instrument Control, Service Tools, and Wizards.

Notes

Commands

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*: Commits changes in the Admin Tool to data store and is accessible from the menu bar, keyboard shortcut, or toolbar.
- *Exit*: 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

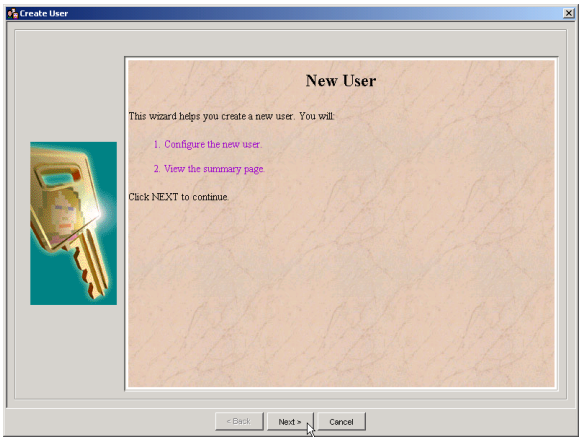
Creating a New User

IMPORTANT! You must set a default password for each new user.

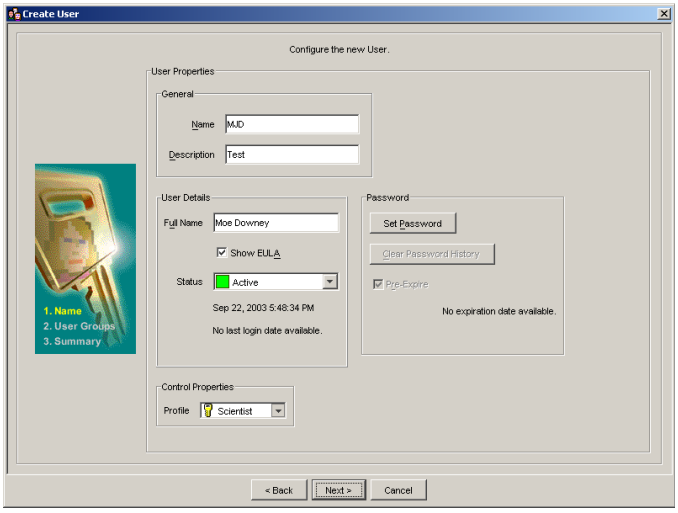
1. Click the New User icon .

   The New User dialog displays.

2. Click **Next**.

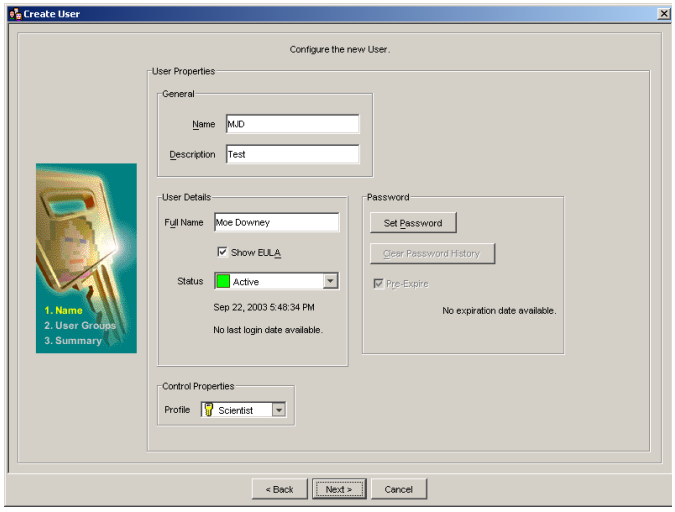


   The Configure pane displays.



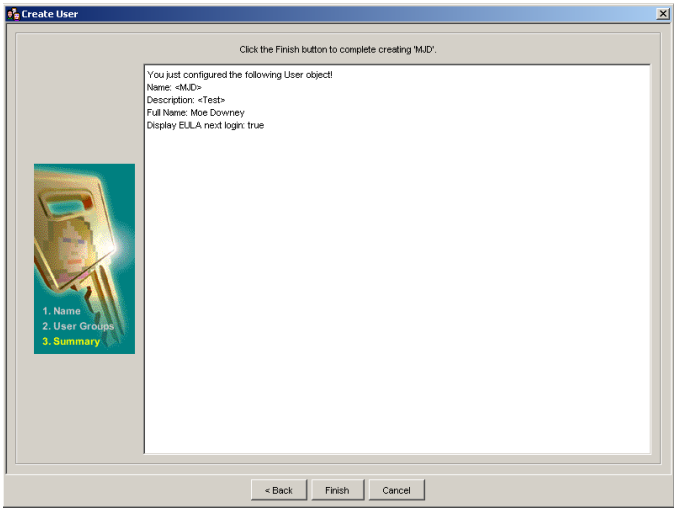
Notes

3. Complete the information in the window.
4. Click **Set Password**.



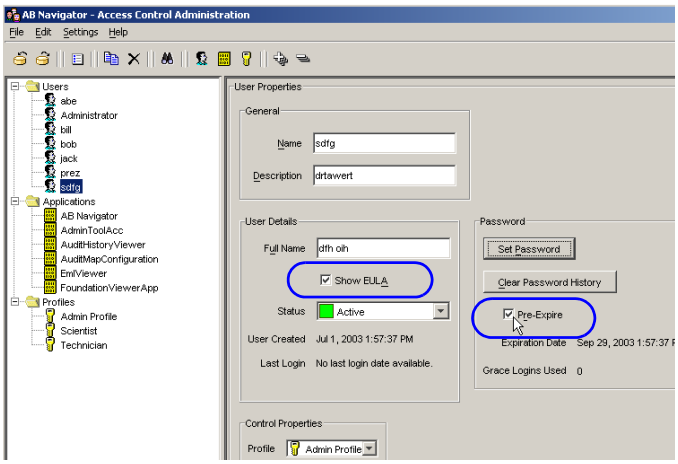
The Change Password dialog box displays.

5. Complete the new password and click **OK**.
6. Click **Finish** to complete the creation of a new user.
7. Click (Save).
8. Click **Next**.



Notes

9. *(Optional)* The Show EULA and Pre-Expire checkboxes are selected by default.



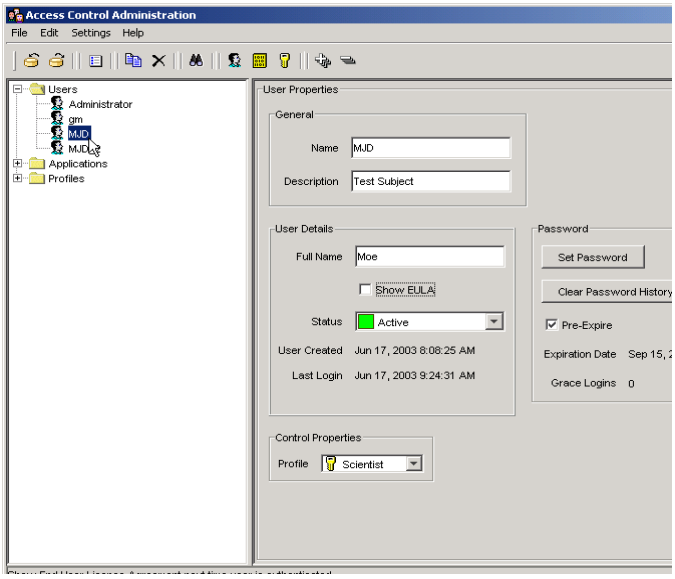
- If Show EULA checkbox is enabled, the first time the user logs into the 3730/3730*xl* Data Collection Software, the End User License Agreement displays. Uncheck this option if you do not want to force users to view the EULA.
- If Pre-Expire checkbox is enabled, users are forced to personalize their passwords when they log into the 3730/3730*xl* Data Collection Software with the default password for the first time. Uncheck this option if you do not want to give the user the ability to change the default password.

10. Click (Save).

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.



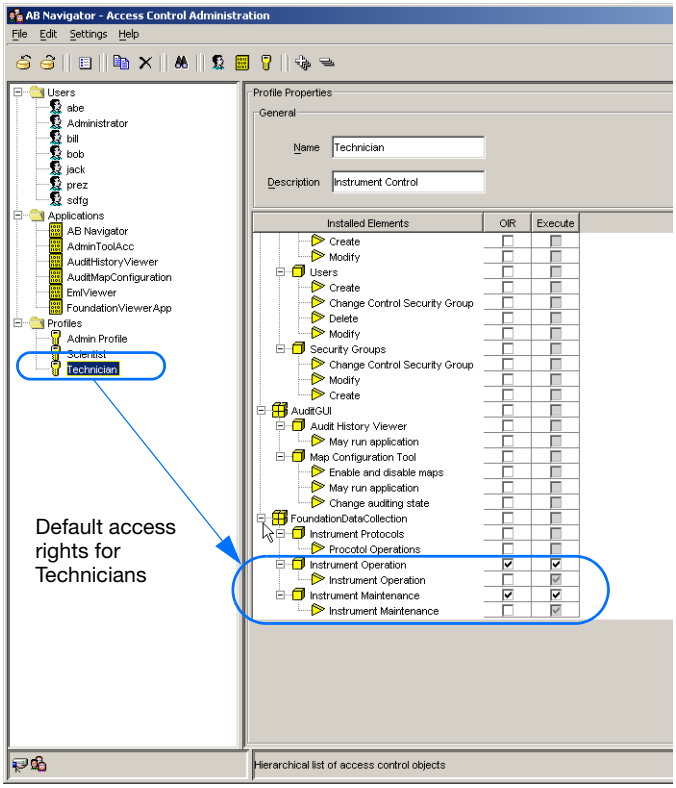
Notes

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

The broad functional access levels are further described below:

- *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.

Notes

Overriding Inherited Rights

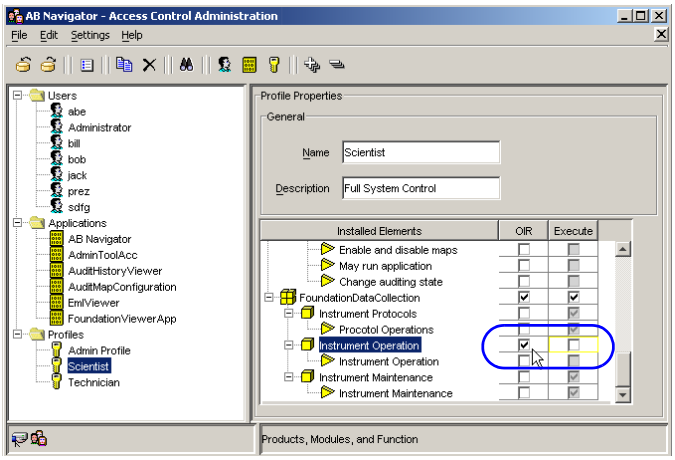
Selecting the OIR check box, allows the Administrator to change the default rights inherited from a parent node. The Scientist Group, for example, is allowed Full System Control, because they have the ability to execute any function contained under the FoundationDataCollection node. In another words, all the sub nodes under FoundationDataCollection is checked because the Executable rights are “inherited” from the FoundationDataCollection node.

To override the inherited rights of a User Group:

1. Deselect the OIR check box next to the function you want to change.
2. Select the Execute check box to allow access to the feature or deselect the Execute check box to deny access to the feature.

To override the inherited rights of a group:

1. Deselect the OIR check box next to the function you want to deny. In the example to the right, the Scientist group is denied access to Instrument Operation.

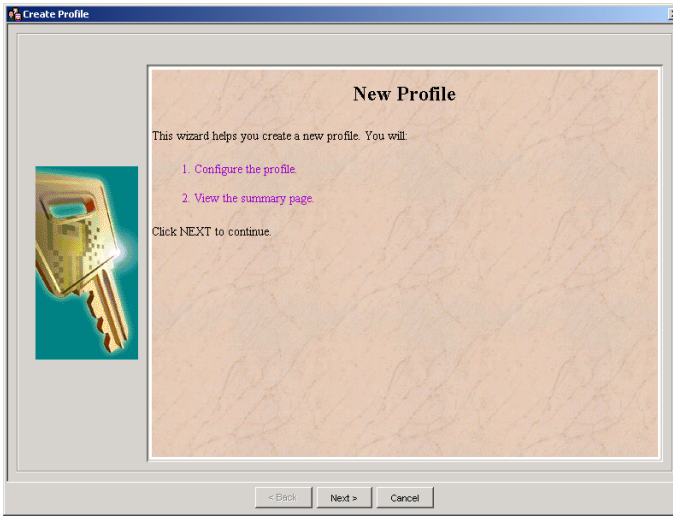


Notes

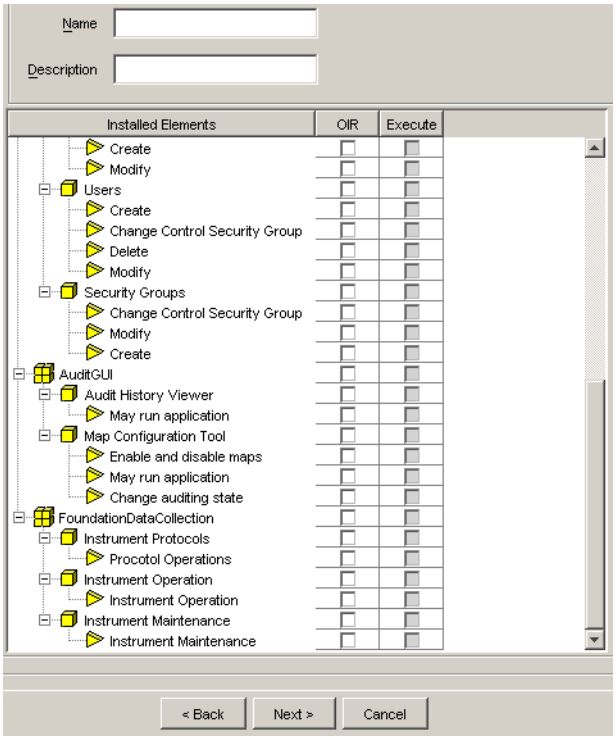
Creating a New Profile

1. Click the New Profile icon 
   The New Profile dialog displays.

2. Click **Next**.
   The Configure pane displays.

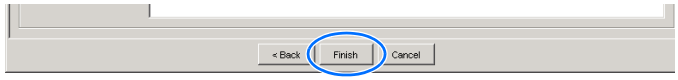


3. Complete:
   a. Profile properties.
   b. Select OIR and/or Execute.
      Execute: Select this to give access to the function to any user assigned to this Profile.
      OIR: Select this to override inherited rights. Any lower level in the hierarchy inherits the access rights of the node above it.
      To override inherited defaults, check the OIR check box. This allow the administrator to grant or deny a group's 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

Notes

Parts List A

Description	Part No
3730 36-cm capillary array	4331247
3730 50-cm capillary array	4331250
3730xl 36-cm capillary array	4331244
3730xl 50-cm capillary array	4331246
3700/3730 BigDye Terminator v3.1 Sequencing Std	4336943
3700/3730 BigDye Terminator v1.1 Sequencing Std	4336799
Matrix Standard Set DS-33	4318254
HiDi formamide - 25 mL	4311320
POP-7 (5 bottles of 25ml)	4335615
Buffer (10X) with EDTA - 500 mL	4335613
Buffer (10X) with EDTA - 4L	4318976
96-well Sample Plates w/barcode	4306737
96-well Sample Plates, no bar code	N801-0560
96-well plate septa	4315933
96-well Plate Base (septa sealed)	4334873
96-well Plate Base (heat sealed)	4334875
96-well Plate Retainer (Septa sealed)	4334869
96-well and 384-well Plate Retainer (heat sealed)	4334865
384-well Sample plates with barcode	4309849
384-well Plate septa	4315934
384-well Plate Base (Septa-Sealed)	4334874
384-well Plate Base (Heat-Sealed)	4334877
384-well Plate retainer (Septa-Sealed)	4334868
Heat Seal film - 3-mil	4337570

Notes

Notes

G5, G5-RCT, Any4Dye, and Any5Dye Dye Sets

B

Dye Sets G5 and G5-RCT For Fragment Analysis

Overview

Even small levels of crosstalk could be a concern for users of the 3730/3730*xl* instruments who perform fragment analysis as well as for applications with a high dynamic range. In fragment analysis applications that have few sample peaks and varying peak intensities, a crosstalk peak may appear as a real sample peak and be incorrectly identified as an allele. Crosstalk is not a concern with sequencing applications as there is a constant stream of peaks electrophoresing past the detector.

Dye Set G5-RCT

To reduce crosstalk for fragment analysis applications, a new dye set has been created for Data Collection Software v2.0, called dye set G5-RCT (reduced crosstalk). G5-RCT uses the same chemistry as dye set G5 (6-FAM™, VIC® NED™, PET®, LIZ® dyes). This dye set reduces signal, but reduces potential crosstalk to a greater degree, so the reduction in signal-to-noise ratio is less pronounced than the reduction in signal overall. Higher concentration peaks can be used without going offscale – this results in a higher dynamic range for the G5-RCT dye set.

Recommendations for Using GS or G5-RCT

Dye set G5-RCT may be particularly helpful for users performing fragment analysis with a 96 capillary array, as well as users interested in applications with a high dynamic range (large peaks much higher than small peaks). Most other users will prefer the G5 dye set.

Applied Biosystems supports:

- Fragment analysis on the 96-capillary array using G5-RCT only
- G5 and G5-RCT on the 48-capillary array.

Notes

Please see the chart that follows for more information about the advantages and issues to consider for each dye set.

Dye Set	Advantages
G5-RCT	**Advantages**: • Potential crosstalk is reduced, which can improve allele calling accuracy for 96 capillary array or high dynamic range applications • Higher dynamic range – higher concentration peaks can be used without peaks going off scale **Issues**: • Signals are reduced compared to signals generated with G5 dye set • It is essential to run a spectral calibration *each time* the capillary array is replaced or moved in the detection cell heater
G5	**Advantages**: • Higher sample signals compared to the G5-RCT dye set, as more light is collected from the CCD • While we recommend spectral recalibration when the capillary array is replaced or moved in the detection cell heater, *spectral recalibration is needed less often* with G5 than with G5-RCT • Optimized for the highest signal-to-noise ratio **Issues**: • You may observe more crosstalk with this dye set compared to the G5-RCT dye set, particularly on the 96 capillary array or in high dynamic range applications

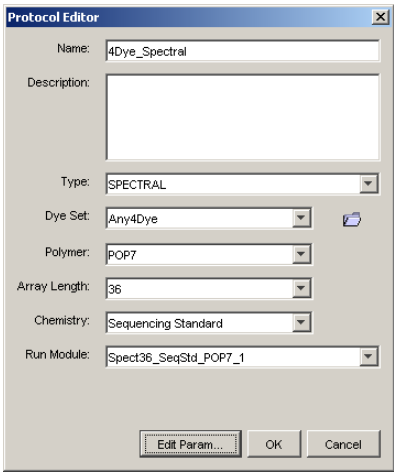
Notes

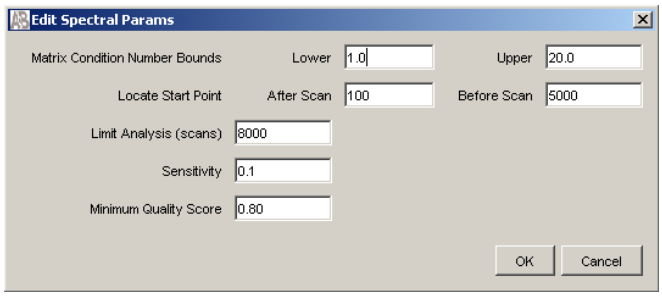
Creating a Spectral Calibration for the Any4Dye or Any5Dye Dye Sets

The steps to creating and running a customized 4- or 5- DyeSet is similar to running a supported dye set.

The following example highlights the use of Any4Dye dye set; it works the same for Any5Dye dye set.

1. Create a spectral protocol for the 4-Dye dye set, specifying the appropriate protocol parameters.
2. Click **OK** to save the spectral protocol.



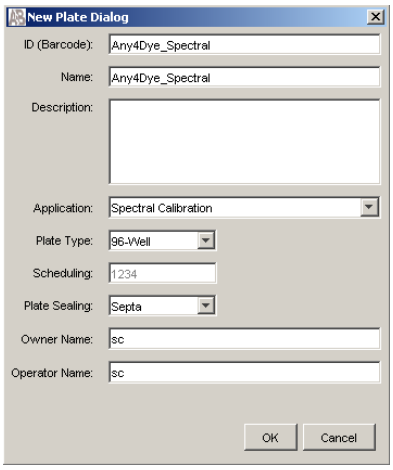


Note: Customize the Spectral parameters as needed. For more information see, step 5 on page 38.

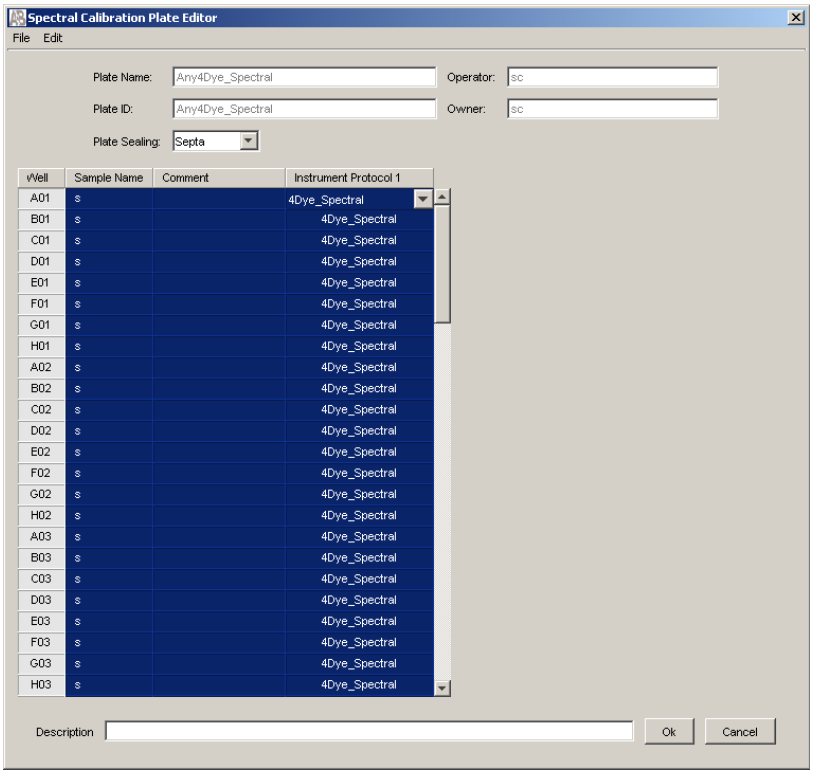
Notes

3. Click New in the plate manager to display the New Plate Dialog box.

4. Create a spectral plate for the Any4Dye dye set by completing the New Plate Dialog box.

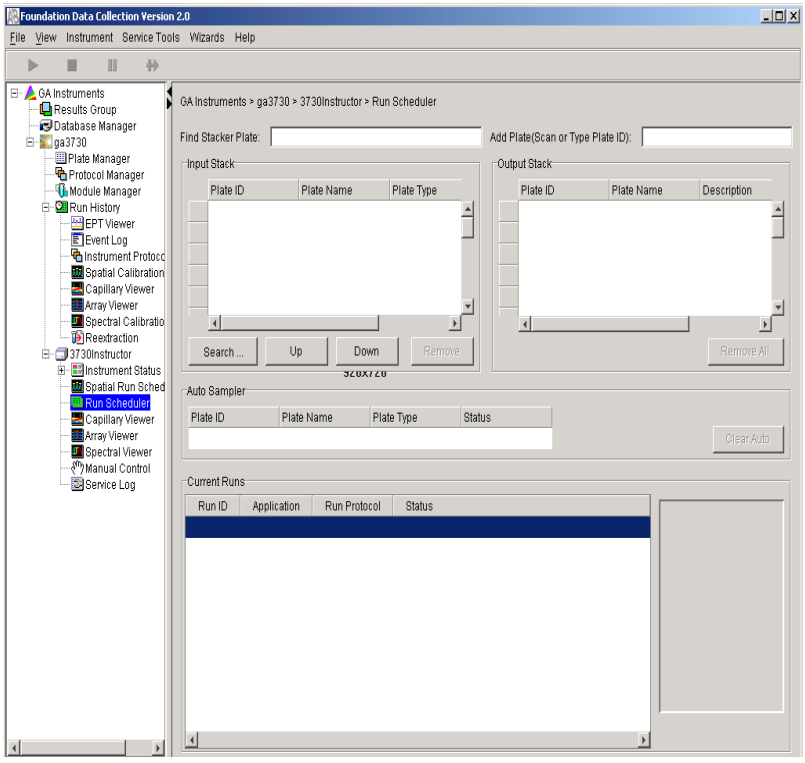


5. In the Plate Editor, select the Instrument Protocol that you just created in the previous steps and click **OK** to save the plate.



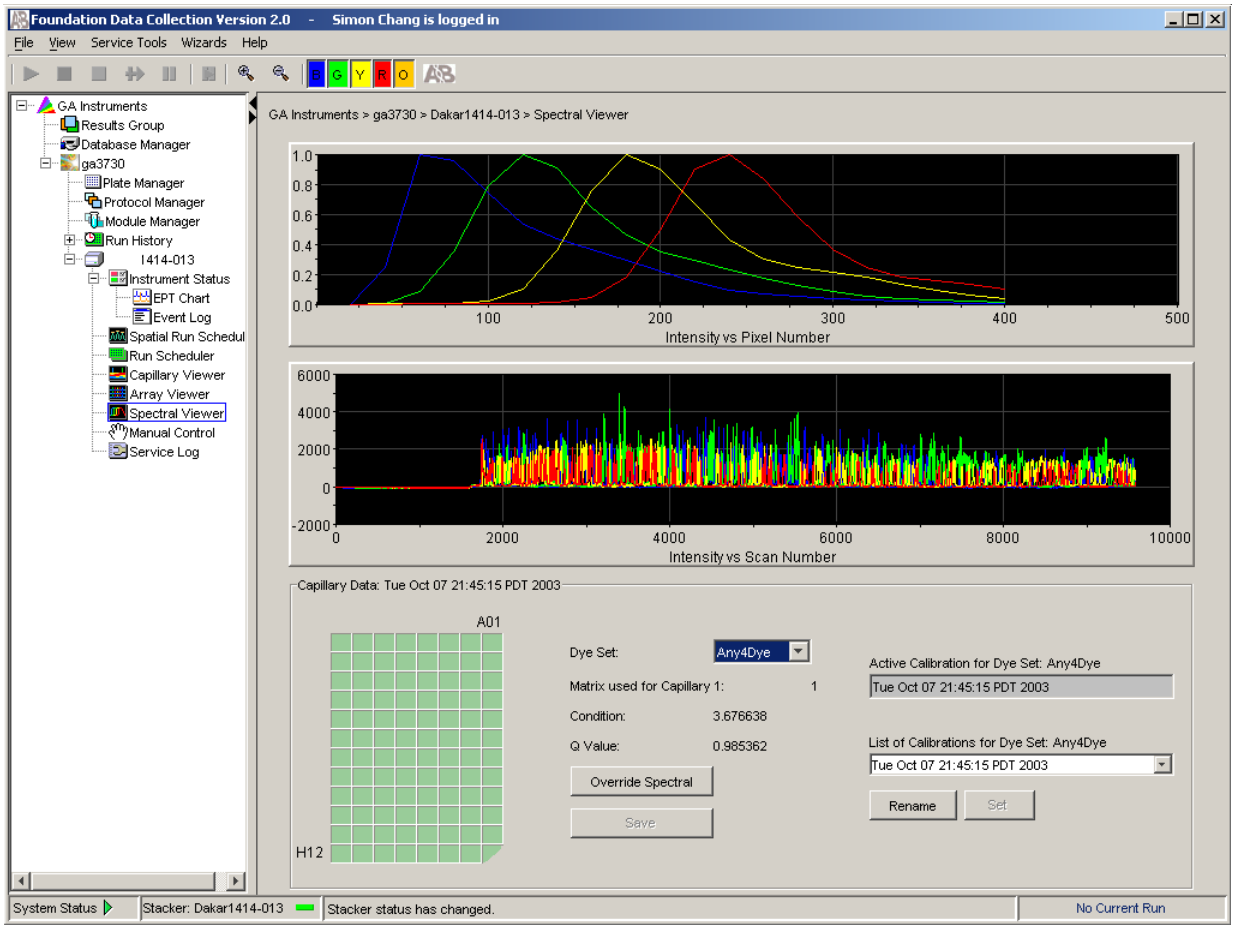
Notes

6. From the Run Scheduler, add this spectral plate to the Input Stack and run it.



Notes

7. Confirm that spectral matrices for all capillaries pass. Override individual capillaries and rename calibration as needed.

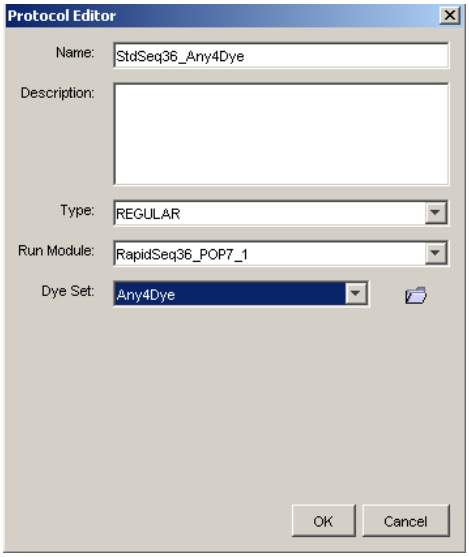


Notes

Regular Runs Using Any4Dye or Any5Dye Dye Sets

The following example highlights the use of Any4Dye dye set. This process works the same for Any5Dye set.

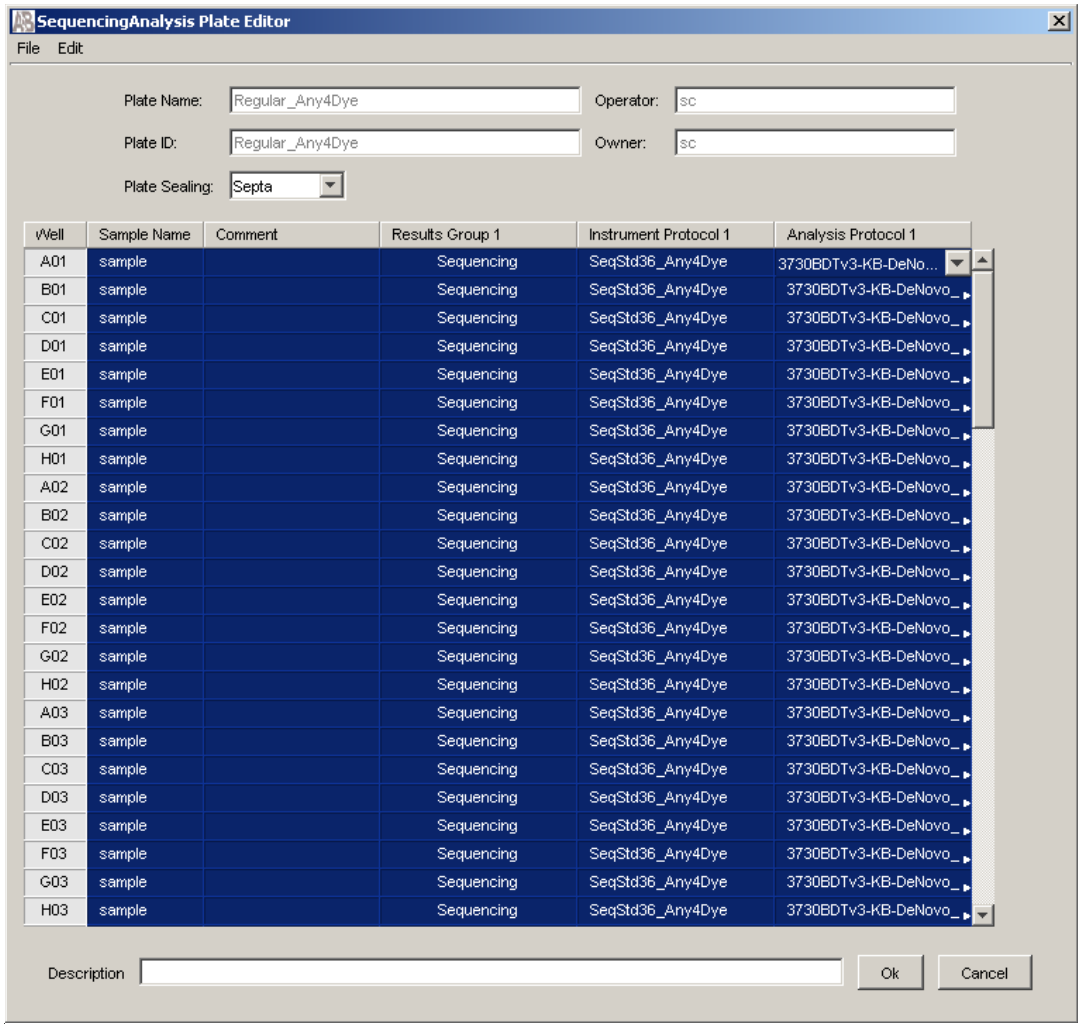
1. In the Protocol Editor, create a regular instrument run protocol for the Any4Dye dye set, and choose the appropriate default run module template. (You can create a customized run module in the module editor if desired).



2. In the Plate Manager, create a regular plate, selecting the Any4Dye instrument protocol you created in step 1.

Notes

3. In the Plate Editor, select the Instrument Protocol that you just created in step 1, and click **OK** to save the plate.



Well	Sample Name	Comment	Results Group 1	Instrument Protocol 1	Analysis Protocol 1
A01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNo...
B01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
C01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
D01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
E01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
F01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
G01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
H01	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
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C02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
D02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
E02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
F02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
G02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
H02	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
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C03	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
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E03	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
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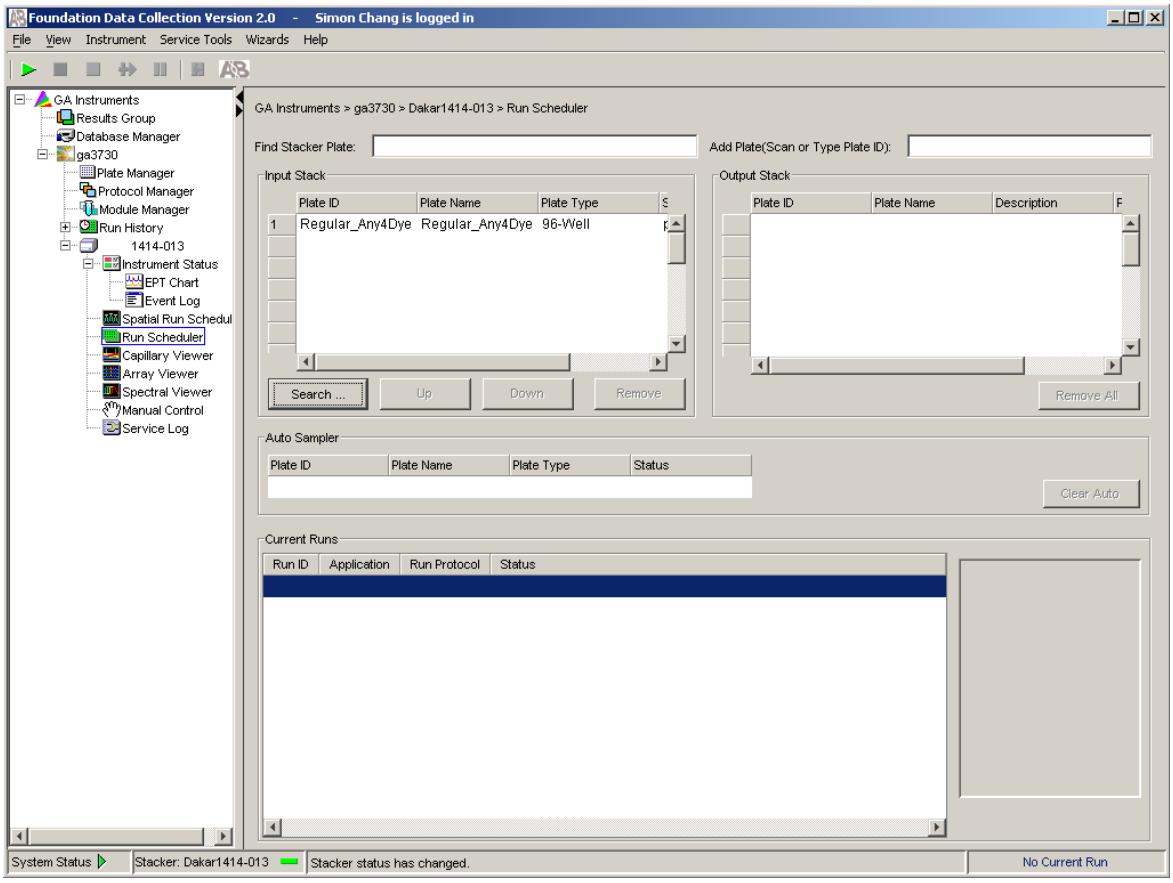
G03	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_
H03	sample		Sequencing	SeqStd36_Any4Dye	3730BDTv3-KB-DeNovo_

Notes

4. From the Run Scheduler, add this plate into the Input Stack and run it.



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