AB Applied Biosystems | **HITACHI**

Getting Started Guide

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Performing Spectral Calibration

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Autoanalysis and SeqScape Software

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Preface

How to Use This Guide

Purpose of This Guide

The *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* provides step-by-step instructions for preparing and analyzing a sample. It is designed to help you quickly learn how to use the Applied Biosystems 3130/3130*xl* Genetic Analyzers.

Audience

This manual is written for principle investigators and laboratory staff who are planning to operate and maintain the Applied Biosystems 3130/3130*xl* Genetic Analyzers.

Assumptions

This guide assumes you have the following background:

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

If you want to integrate the 3130/3130*xl* Genetic Analyzers into your existing laboratory data flow system, you need networking experience.

Text Conventions

This guide uses the following conventions:

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

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

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

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

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

User Attention Words

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

- **Note** – Provides information that may be of interest or help but is not critical to the use of the product.
- **IMPORTANT!** – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

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

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

Safety Alert Words

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

How to Obtain More Information

Related Documentation

The following related document is shipped with the system:

- *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide (*P/N 4352716) - Contains information on maintaining the instrument and computer system, troubleshooting tables to diagnose performance failures and advance reference content.
- *Applied Biosystems 3130/3130xl Genetic Analyzers Site Preparation Guide* (P/N 4352717) - Contains the space, environmental, and electrical requirements to support the 3130/3130xl Genetic Analyzer system.
- *Applied Biosystems 3730/3730xl DNA Analyzers and Applied Biosystems 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide* (P/N 4359472) - Contains information and procedures for the administrator maintaining the computer system and software files of the Applied Biosystems 3130/3130*xl* Genetic Analyzers.
- *Applied Biosystems 3130/3130xl Genetic Analyzers Quick Reference Card* (P/N 4362825) - Contains a flowchart on how to run your samples and instrument, a table of maintenance tasks, and a Data Collection software reference guide.

Note: For additional documentation, see "How to Obtain Support" 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

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

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

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

Safety

This safety section describes the following:

Safety Conventions Used in This Document

Safety Alert Words

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

Definitions

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

⚠ **CAUTION** – 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.

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

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

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

Examples

The following examples show the use of safety alert words:

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

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

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

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

Symbols on Instruments

Electrical Symbols on Instruments

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

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

Safety Symbols

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

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

Safety Labels on Instruments

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

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangeureux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	**ATTENTION** Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.

English	Francais
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
DANGER Class 3B laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	**DANGER** Class 3B rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.
DANGER Class 3B laser radiation when open. Avoid direct exposure to laser beam.	**DANGER** Class 3B rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) laser radiation present when open and interlock defeated. Do not stare directly into the beam	**DANGER** de Class 2(II) rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) laser radiation present when open. Do not stare directly into the beam.	**DANGER** de Class 2(II) rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) LED when open and interlock defeated. Do not stare directly into the beam.	**DANGER** de Class 2(II) LED en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2(II) LED when open. Do not stare directly into the beam.	**DANGER** de Class 2(II) LED en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

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

Moving and Lifting the Instrument

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

Moving and Lifting Stand-Alone Computers and Monitors

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

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

• Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.

• Make sure that the path from where the object is to where it is being moved is clear of obstructions.

• Do not lift an object and twist your torso at the same time.

• Keep your spine in a good neutral position while lifting with your legs.

• Participants should coordinate lift and move intentions with each other before lifting and carrying.

• Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

• Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.

• Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page xviii.

Chemical Safety

Chemical Hazard Warning

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

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

About MSDSs

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

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

Obtaining MSDSs

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

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**

2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.

3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.

5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

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

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.

- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

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

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

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste 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

DANGER **ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the Applied Biosystems 3130/3130*xl* Genetic Analyzers without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

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

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

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

Overvoltage Rating   The Applied Biosystems 3130/3130*xl* Genetic Analyzers have an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

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

Laser Safety

Laser Classification   The Applied Biosystems 3130/3130*xl* Genetic Analyzers use an Argon 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 3B laser.

The Applied Biosystems 3130/3130*xl* Genetic Analyzers has been tested to and complies with 21 CFR, 1040.10 and 1040.11, as applicable."

The Applied Biosystems 3130/3130*xl* Genetic Analyzers have been tested to and complies with standard EN60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide."

Laser Safety
Requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Applied Biosystems Technical Representative.

- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the laser is operating (during service with safety interlocks disabled), you may be exposed to laser emissions in excess of the Class 3B rating.

- Do not remove safety labels or disable safety interlocks.

Additional Laser
Safety
Information

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

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

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

Workstation Safety

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

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

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.

- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standard
- European Safety and EMC Standards
- Australian 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 (Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."
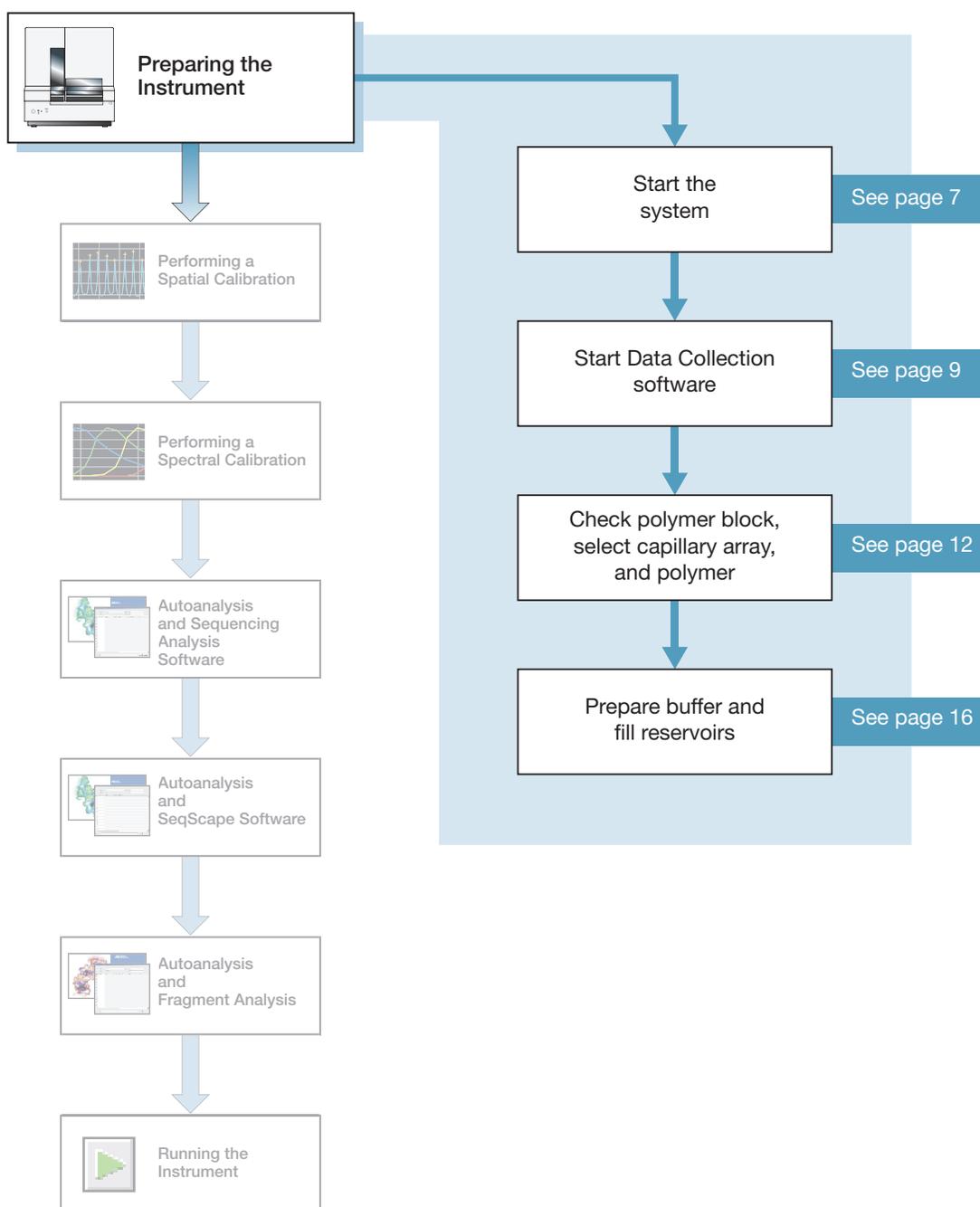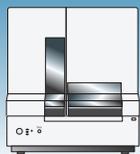
Australian EMC Standards

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

Preparing the
Instrument

Performing a
Spatial Calibration

Performing a
Spectral Calibration

Autoanalysis
and Sequencing
Analysis
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Autoanalysis
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Running the
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Start the
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Start Data Collection
software

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Check polymer block,
select capillary array,
and polymer

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Prepare buffer and
fill reservoirs

See page 16

Notes

Instrument Description

Instrument Layout   A typical laboratory layout is shown below.
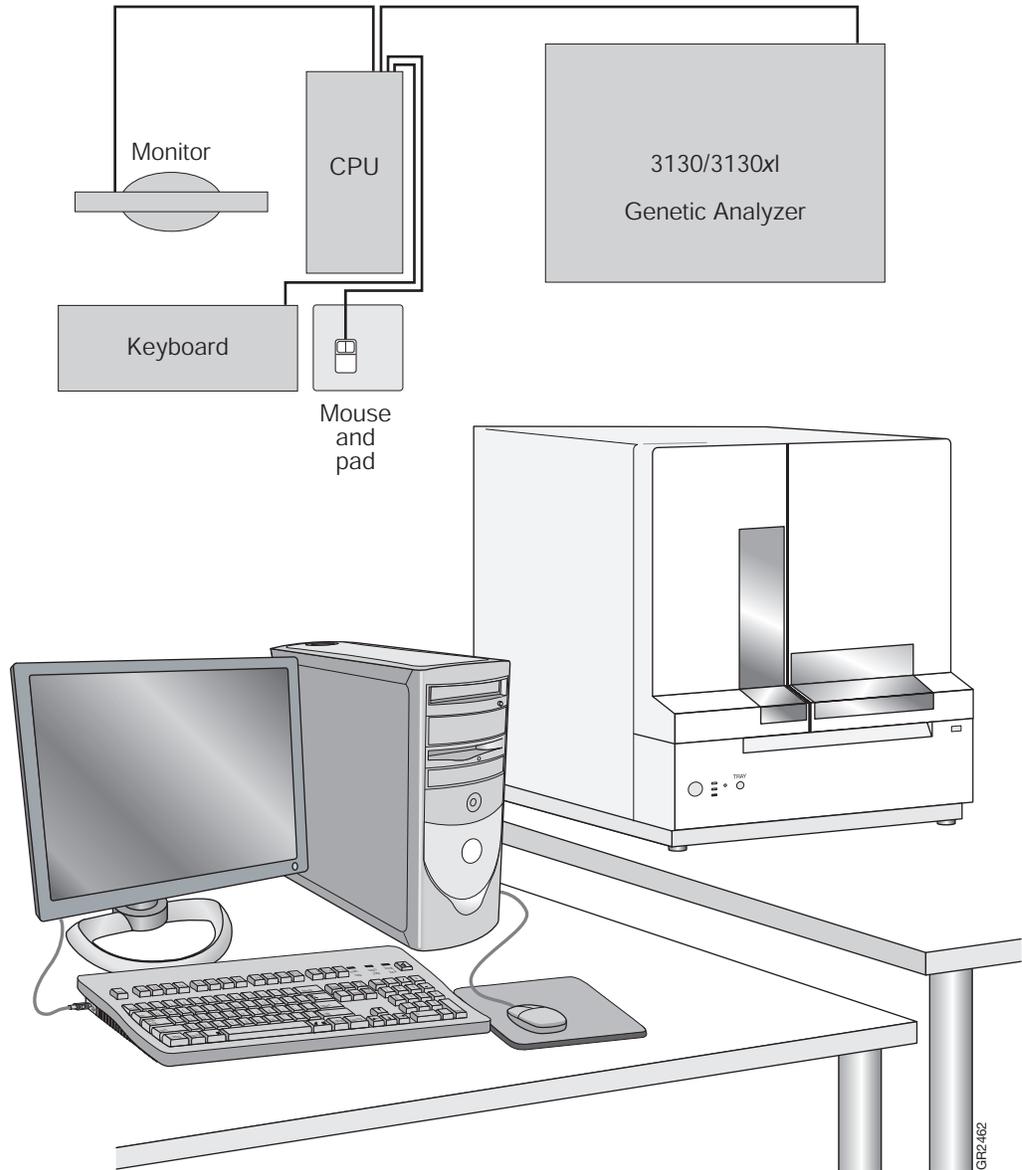
Notes
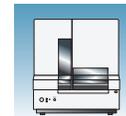
Application Summary Tables

Sequencing Resolution Performance and Specifications

Decide what combination of capillary array and polymer from the table below matches your resolution and performance specifications.

Type of Run	Capillary Length (cm)	Polymer Type	Module	Run Time (min)	24 hr Throughput (number of samples)		KB Basecaller QV$_{20}$ LOR[a] [b]
					3130 Genetic Analyzer	3130*xl* Genetic Analyzer	
Ultra rapid	36	POP-4™	UltraSeq36_POP4	40	144	576	400
		POP-7™	UltraSeq36_POP7	35	164	656	500
Rapid	36	POP-6™	RapidSeq36_POP6	60	96	384	500
		POP-7	RapidSeq36_POP7		96	384	600
Fast	50	POP-7	FastSeq50_POP7	60	96	384	700
Standard	50	POP-4	StdSeq50_POP4	100	56	224	600
		POP-6	StdSeq50_POP6	150	36	144	600
		POP-7	StdSeq50_POP7	120	48	192	850
Long read	80	POP-4	LongSeq80_POP4	210	24	96	700
		POP-7	LongSeq80_POP7	170	32	128	950

a Length of Read (LOR) is the usable range of high-quality or high-accuracy bases determined by Quality Values (QV) generated by KB™ Basecaller v1.2. The LOR is determined by using a sliding window of 20 bases, which has an average QV > 20.

b 98.5% basecalling accuracy, less than 2% Ns.

Notes _____

Fragment Analysis Resolution Performance and Specifications

Decide what combination of capillary array and polymer from the table below matches your resolution and performance specifications. See "Fragment Analysis Kits and Run Modules" on page 5 for information on capillary, polymer, and run module.

Run Modules	Capillary Length (cm)	Polymer Type	Run Time (min)	24 hr Throughput (GT[a])		Resolution (bp)	Specification (SD)[b]
				3130 Analyzer	3130*xl* Analyzer		
High Throughput, Small Size Fragment Analysis							
FragmentAnalysis22 _POP4	22	POP-4	20	5,760	23,040	250	0.15
SNP22_POP4	22		20	5,760	23,040	120	0.50
Standard Fragment Analysis							
FragmentAnalysis36 _POP4	36	POP-4	45	2,560	10,240	500	0.15
HIDFragmentAnalysis 36_POP4			45	2,560	10,240	500	0.15
SNP36_POP4			30	3,840	15,360	120	0.50
FragmentAnalysis36 _POP7		POP-7	35	3,290	13,170	500	0.15
FragmentAnalysis50 _POP4	50	POP-4	65	1,760	7,040	500	0.15
FragmentAnalysis50 _POP6		POP-6	90	1,200	4,800	500	0.15
FragmentAnalysis50 _POP7		POP-7	50	2,300	9,220	500	0.15

a 20 GT (Genotypes)/capillary/injection.
b 1bp resolution at 99.99% accuracy.

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Fragment Analysis Kits and Run Modules

The table below lists the Applied Biosystem kit types, with the available run module(s) and dye sets.

Application/Kit	SNP22_POP4	SNP36_POP4	HTSNP36_POP7	FragmentAnalysis22_POP4	FragmentAnalysis36_POP4	FragmentAnalysis36_POP7	FragmentAnalysis50_POP4	FragmentAnalysis50_POP6	FragmentAnalysis50_POP7	HIDFragmentAnalysis36_POP4
						Module				
SNaPshot® Multiplex System	E5	E5								
Custom oligos				D, F, G5	D, F, G5	D, F, G5	D, F, G5	D, F, G5	D, F, G5	
Linkage Mapping Set (human) v2.5				D, G5	D, G5	D, G5	D, G5	D, G5	D, G5	
ABI PRISM® Mouse Mapping Set v1.0				D, G5	D, G5	D, G5	D, G5	D, G5	D, G5	
4-Dye Stockmarks® kits (bovine and canine)					F					
5-Dye Stockmarks® kit (equine)					G5					
AFLP® kits					F					
4-Dye AmpFℓSTR® kits										F
5-Dye AmpFℓSTR kits										G5

AmpFℓSTR Kit Table

Kits	HIDFragmentAnalysis 36_POP4
AmpFℓSTR COfiler® Kit AmpFℓSTR Profiler Plus® Kit AmpFℓSTR Profiler Plus *ID* Kit AmpFℓSTR SGM Plus® Kit Other 4-Dye AmpFℓSTR Kits	F
AmpFℓSTR SEfiler™ Kit AmpFℓSTR Identifiler® Kit AmpFℓSTR Yfiler™ Kit Other 5-Dye AmpFℓSTR Kits	G5

Notes _____

Barcode Readers

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

External Barcode Readers

KEYENCE BL-80VE
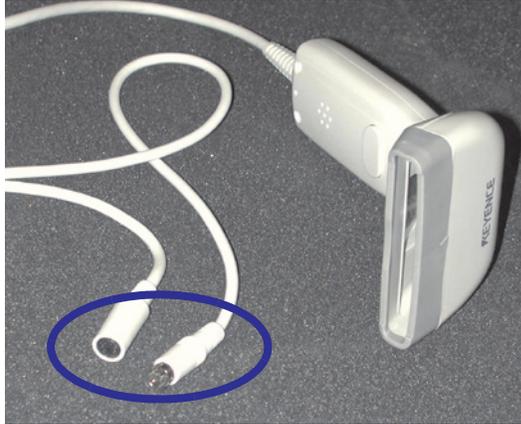
An external barcode reader can be used with the 3130/3130*xl* instrument. With the KEYENCE BL-80VE (see photo above), which connects to the instrument computer keyboard, you can scan barcodes into any text box in the Data Collection software. The KEYENCE BL-80VE barcode reader uses an LED as a light source.

Notes

Starting the 3130/3130*xl* Genetic Analyzer System

Starting the Computer Workstation

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

1. Power on the monitor.
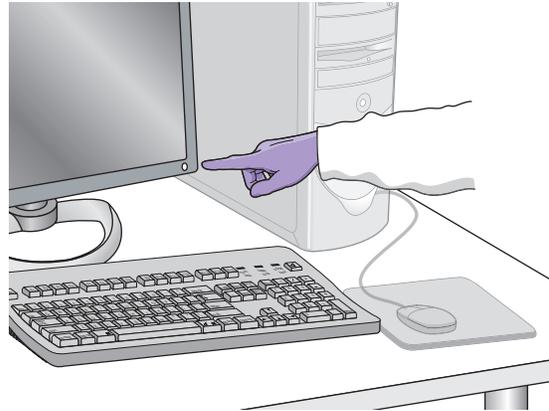
2. Power on the computer.
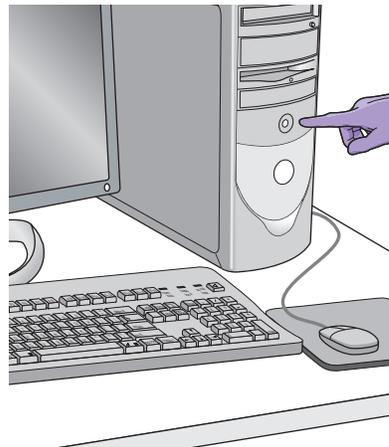
3. In the Log On to Windows dialog box:

    a. Enter the user name.

    b. If applicable, enter a password.

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

    c. Click ⬛ OK ⬛.

Notes _____

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Starting the 3130/3130*xl* Genetic Analyzer

1. Ensure that the:

- Oven door is closed and locked
- Instrument doors are closed

Note: If the doors are open during power on, the yellow light will continue to flash until you close the doors.

2. Ensure that the computer is powered on.

Note: The computer must be on and running to allow the instrument to copy the firmware from the computer.

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

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

4. Ensure the green status light is on and not flashing before proceeding.

Note: If the green status light does not come on, start the Data Collection software and view the log. The pathway to the log is: E:\AppliedBiosystems\UDC\DataCollection\Log*Instrument Name*

Status lights

Press the on/off button to start the instrument

Notes _____

1
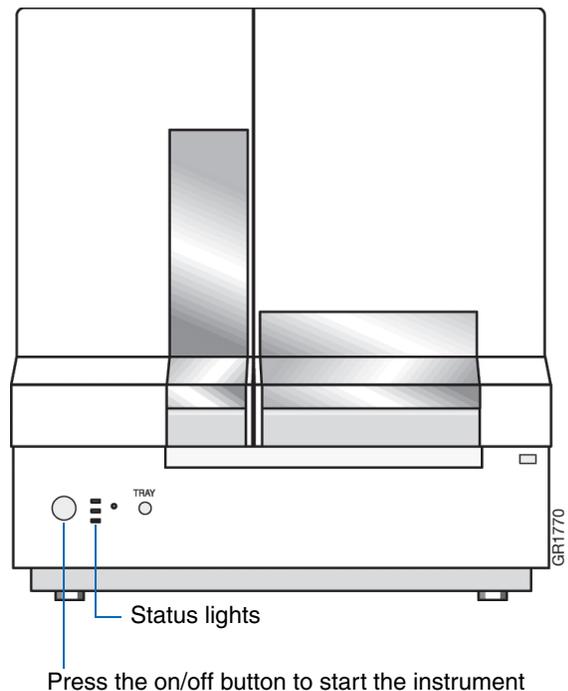
Data Collection Software

IMPORTANT! Do not rename the computer. The instrument computer was assigned a unique name before the 3130/3130*xl* genetic analyzer Data Collection software was installed. Do not rename the computer once the Data Collection software has been installed. Doing so may cause the Data Collection software to malfunction.

Starting the 3130/3130*xl* Data Collection Software

1. Select **Start > All Programs > Applied Biosystems > Data Collection > Run 3130 Data Collection v3.0** or **Run 3130***xl* **Data Collection v3.0** to display the Service Console. By default, all applications are off, indicated by the red circles. They launch automatically with the 3130/3130*xl* Data Collection software.
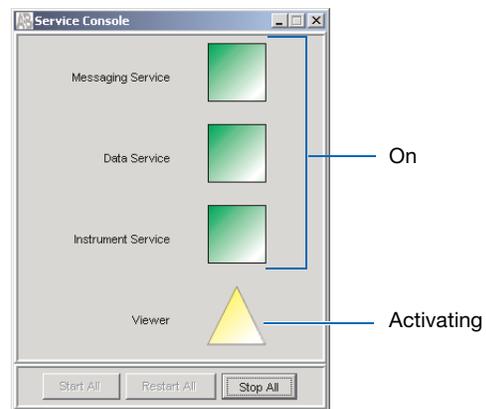
As each application activates, the red circles (off) change to yellow triangles (activating), and then to green squares (on) when they are fully functional.

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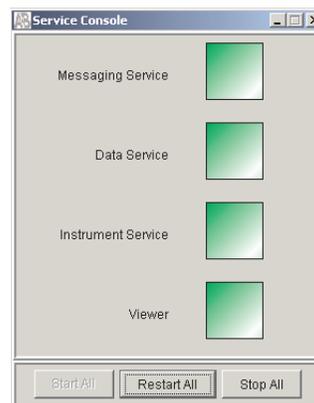
Notes

2. Click + to expand subfolders in the left tree pane. All application folders are now visible.

Select items from this tree pane to open manager, viewer, and other program windows.
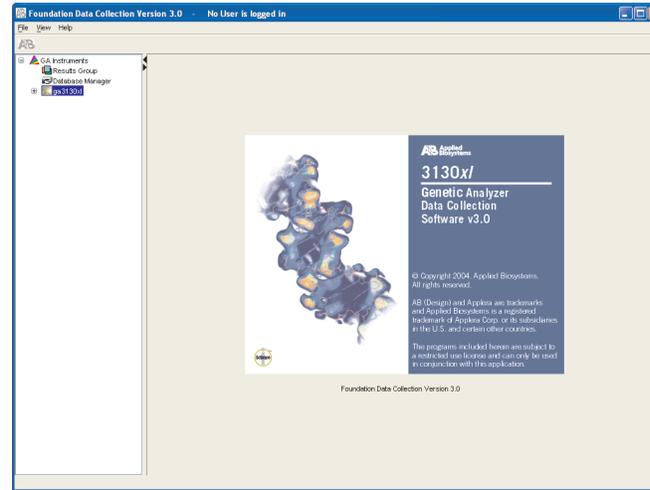
3. Click + to expand subfolders in the left tree pane. All application folders are now visible.

Select items from this tree pane to open manager, viewer, and other program windows.
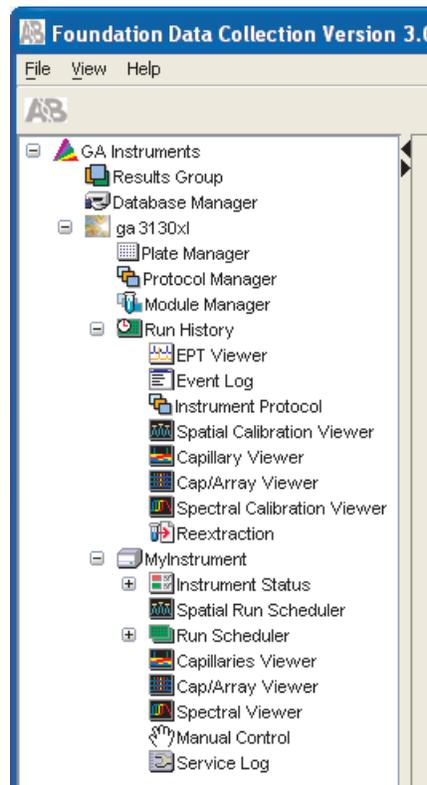
Preparing the Instrument

Instrument Doors and Interior
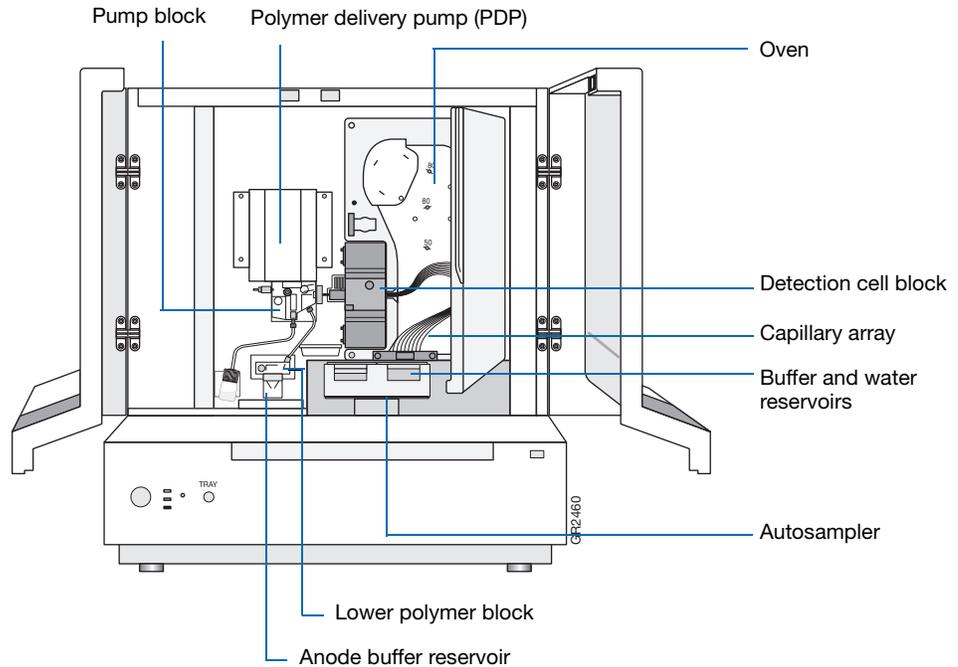
Part	Function
Anode buffer reservoir	Contains 16 mL of 1X running buffer.
Buffer and water reservoirs (four)	Each contain 16 mL of 1X running buffer or water.
Autosampler	Holds the sample plates and reservoirs and moves to align the samples, water, or buffer with the capillaries.
Capillary array	Enables the separation of the fluorescent-labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 4 or 16 silica capillaries.
Detection cell block and heater	Holds the capillaries in place for laser detection.
Lower polymer block	Contains the buffer valve, anode electrode, and anode buffer reservoir.
Oven	Maintains uniform capillary array temperature.
Polymer delivery pump (PDP)	Pumps polymer into the array and performs maintenance procedures.
Pump block	Includes the displacement pump chamber, piston water seal, array attachment point (array port), and connection to the lower polymer block through the interconnect tube.

Notes _____

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Inspecting the Instrument

1. Open the instrument doors.

2. Perform the daily maintenance tasks -see
   Chapter 1 in the *Applied Biosystems 3130/3130xl
   Genetic Analyzers Maintenance,
   Troubleshooting and Reference Guide*.

3. Install clean drip trays.

Polymer Delivery Pump
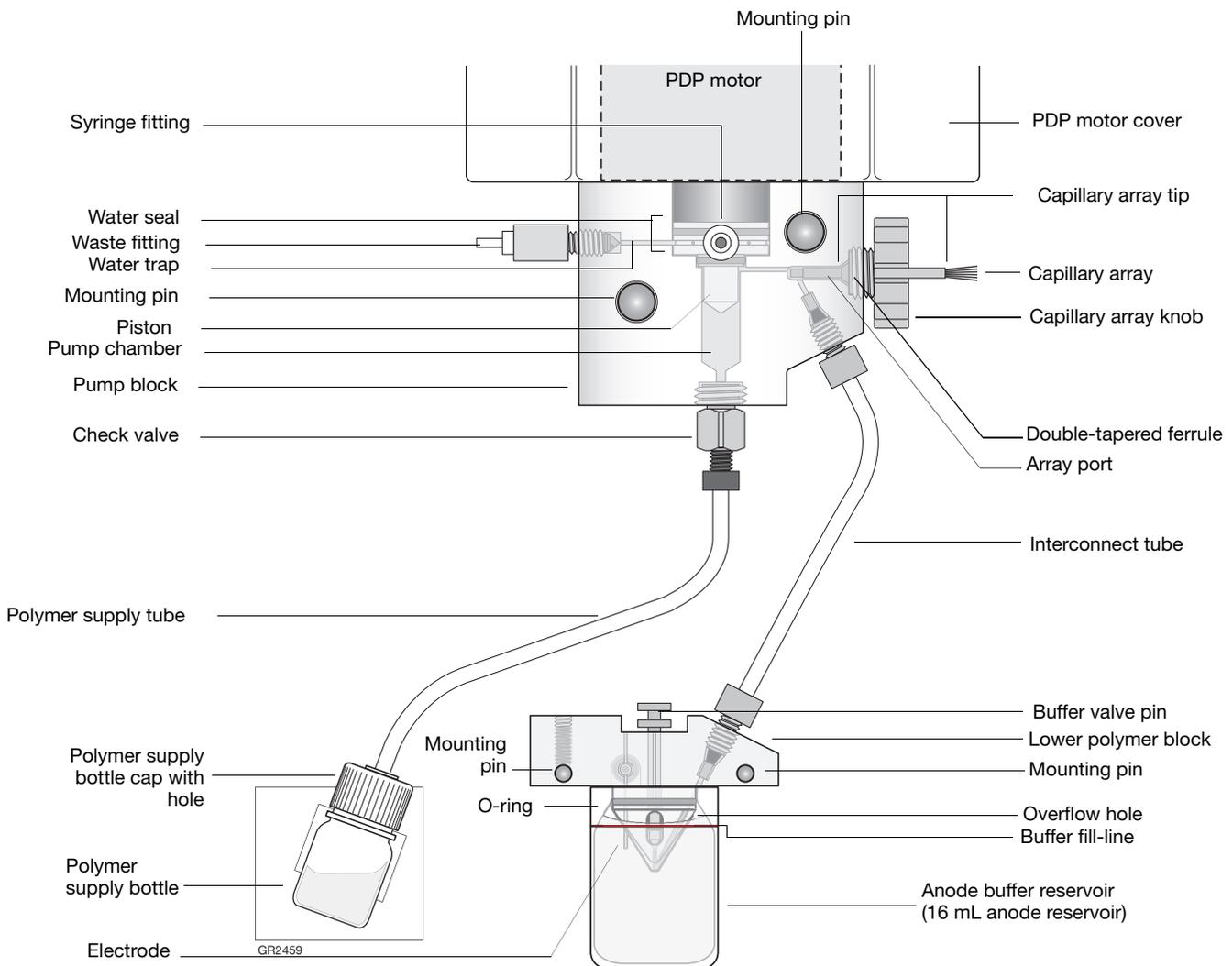
Inspecting the Instrument

1. Open the instrument doors.

2. Perform the daily maintenance tasks -see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide* for more detail on maintenance.

3. Install clean drip trays.

Installing or Replacing the Capillary Array

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

IMPORTANT! Wear gloves while handling polymer, the capillary array, septa, or buffer reservoirs.

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

1. Install a capillary array by clicking **<***instrument name***>,** selecting **Install Array Wizard** and following the prompts.

> **IMPORTANT!** To install or replace an array that is a different length than the one you were using, reset the active spectral calibration (see page 47) or create a new spectral calibration for your dye set and array length combination (see page 37).

Wizards	Help
Install Array Wizard	
Change Polymer Type Wizard	
Replenish Polymer Wizard	
Bubble Remove Wizard	
Water Wash Wizard	
Instrument Shutdown Wizard	
Autosampler Calibration Wizard	
Update Cap Array Info	

2. Optional: Select **Update Cap Array Info** wizard to correct any errors or update capillary array and serial number information.

Notes _____

When to Replenish or Change Polymer

IMPORTANT! Always replace polymer that has been on the instrument longer than one week.

Su M T W Th F S
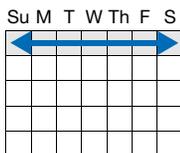
If polymer on the instrument...	Then ...
has been on less than one week and is in sufficient quantity to complete your runs[a]	Remove all bubbles, and then proceed with instrument preparation.
has been on less than one week, and insufficient in quantity to complete your runs	Add fresh polymer to the polymer supply by following the Replenish Polymer Wizard.
has been on longer than one week	
is the wrong type (a change between POP-4, POP-6, and/or POP-7 polymers is required)	Replace the installed polymer type with a different type by following the Change Polymer Type Wizard.

a A 3130*xl* genetic analyzer run uses 50 to 80 μL of polymer and a 3130 genetic analyzer run uses ~25 to 40μL of polymer.

Before Using the Polymer

1. Remove the polymer from 4 °C storage.

2. Loosen the cap and bring the polymer to room temperature.

3. To dissolve deposits, tighten the cap and gently swirl the polymer.

Replenishing the Polymer

IMPORTANT! Wear gloves while handling polymer, the capillary array, septa, or buffer reservoirs.

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

1. Click **< Instrument Name>** in the tree pane.

Notes

2. Select **Wizards > Replenish Polymer Wizard** to replenish polymer.

> **IMPORTANT!** The polymer type defined in the wizard must match the polymer type that you are using.
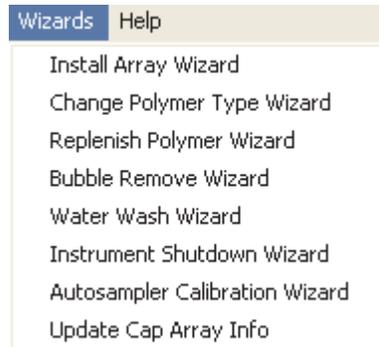
Changing Polymer Type

> **IMPORTANT!** Wear gloves while handling polymer, the capillary array, septa, or buffer reservoirs.

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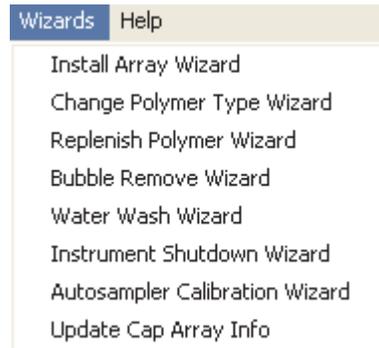
1. Click **< Instrument Name>** in the tree pane.

2. Select **Wizards > Change Polymer Type Wizard** to change to a different polymer.

Wizards Help
Install Array Wizard
Change Polymer Type Wizard
Replenish Polymer Wizard
Bubble Remove Wizard
Water Wash Wizard
Instrument Shutdown Wizard
Autosampler Calibration Wizard
Update Cap Array Info

Wizards Help
Install Array Wizard
Change Polymer Type Wizard
Replenish Polymer Wizard
Bubble Remove Wizard
Water Wash Wizard
Instrument Shutdown Wizard
Autosampler Calibration Wizard
Update Cap Array Info

Notes _____

Preparing Buffer and Filling Reservoirs

Required Materials

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

Required materials to prepare 1X running buffer:

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

Preparing Buffer for a Single Run

1. Add 5 mL of 10X Genetic Analyzer buffer into a graduated cylinder.

2. Add purified water to bring the total volume up to 50 mL.

3. Mix well.

50 mL total

45 mL DI H_2O

5 mL 10X Genetic Analyzer buffer

50 mL graduated cylinder
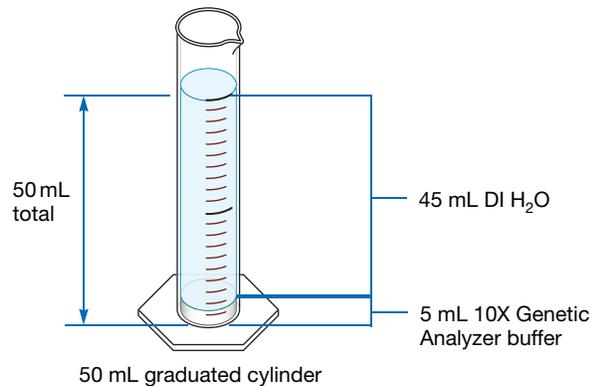
Storing Buffer

Store 1X running buffer at:

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

Buffer Storage Conditions	
Option A	**Option B**
2 °C to 8 °C	20 °C to 25 °C
Su M T W Th F S	Su M T W Th F S
1 month	7 days

1

Replacing Buffer and Water

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

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

IMPORTANT! To clean and refill the reservoirs, the autosampler is brought to the forward position, thereby leaving the capillary tips exposed to the air. Do not leave the autosampler in this position for an extended time because the capillaries can dry out and the array may be damaged.

Filling the Water and Cathode Buffer Reservoirs

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

⚠️ **CAUTION** **CHEMICAL HAZARD. 1✕ Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

1. Verify the oven and instrument doors are closed.

2. Press the Tray button on the outside of the instrument to bring the autosampler to the forward position

3. Wait until the autosampler stops at the forward position, then open the instrument door.

Tray button

Notes

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

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

   Note: Follow your company's waste disposal practices for appropriate disposal procedures.

Remove

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

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

   Fill line

   ⚠ **CAUTION** Be sure that the septa fit securely and flush on the tops of the reservoirs to prevent damaging the capillary tips.

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

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

| 2 | Water reservoir (waste) | Water reservoir (rinse) | 4 |
| 1 | Cathode reservoir (1X running buffer) | Water reservoir (unused) | 3 |

Home position

10. Close the instrument doors.

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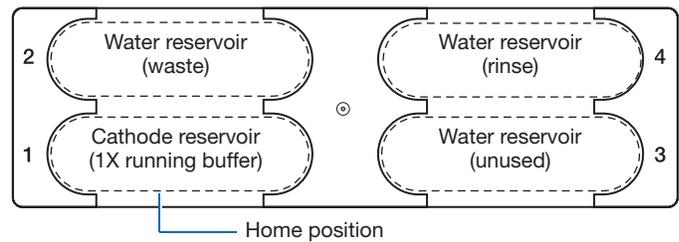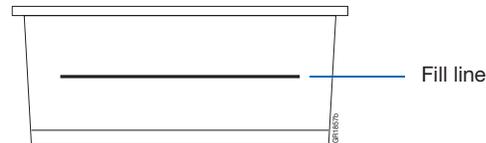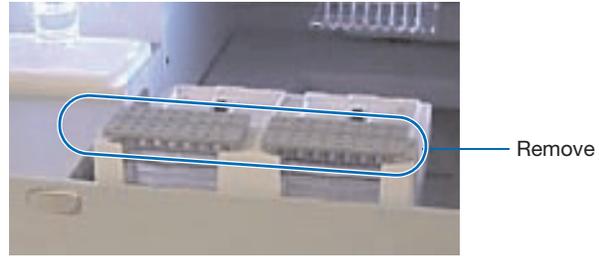
Notes _____

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Filling the Anode Buffer Reservoir

Change the anode buffer:

- Every 24 hours
- Before each run or batch of runs
- Every time you replenish the polymer or change polymer type

⚠ **CAUTION** **CHEMICAL HAZARD**. **1X Genetic Analyzer Buffer with EDTA** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Change the Anode Buffer		
Every 24 hours	Before each run or batch of runs	

1. Remove the anode buffer reservoir by firmly pulling down and twisting slowly.

2. Discard the used buffer appropriately.

3. Clean and rinse the reservoir with deionized water, and then rinse with buffer.

4. Fill the anode buffer reservoir to the fill line with fresh 1X running buffer (about 16 mL).

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

5. Put the anode buffer reservoir on the instrument.

6. If the reservoir fills with fluid, repeat this procedure to discard and replace the running buffer.

   Note: The reservoir could fill during bubble removal.
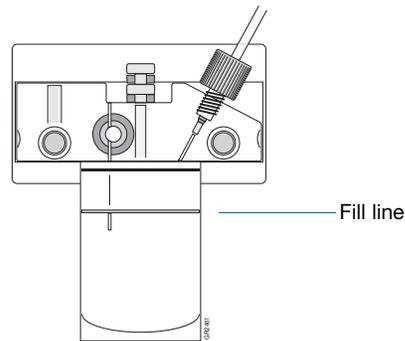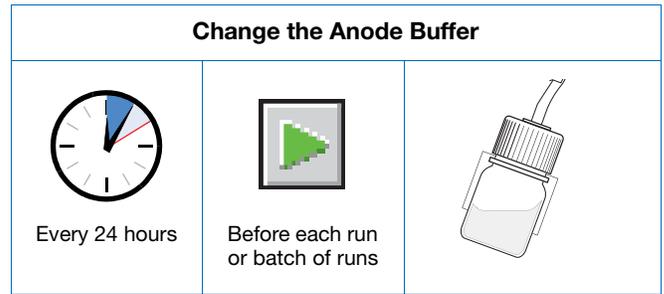
Fill line

Notes _____

Notes

Performing a Spatial Calibration

```
Preparing the
Instrument
        │
        ▼
Performing a
Spatial Calibration ──────────────┐
        │                         │
        ▼                         ▼
Performing a              ┌─────────────────────┐
Spectral Calibration      │ Start a spatial     │  See page 22
        │                 │ calibration run     │
        ▼                 └─────────────────────┘
Autoanalysis                      │
and Sequencing                    ▼
Analysis                  ┌─────────────────────┐
Software                  │ Evaluate spatial    │  See page 23
        │                 │ calibration profile │
        ▼                 └─────────────────────┘
Autoanalysis                      │
and                               ▼
SeqScape Software         ┌─────────────────────┐
        │                 │ Accept or reject    │  See page 24
        ▼                 │ spatial calibration │
Autoanalysis              └─────────────────────┘
and
Fragment Analysis
        │
        ▼
Running the
Instrument
```
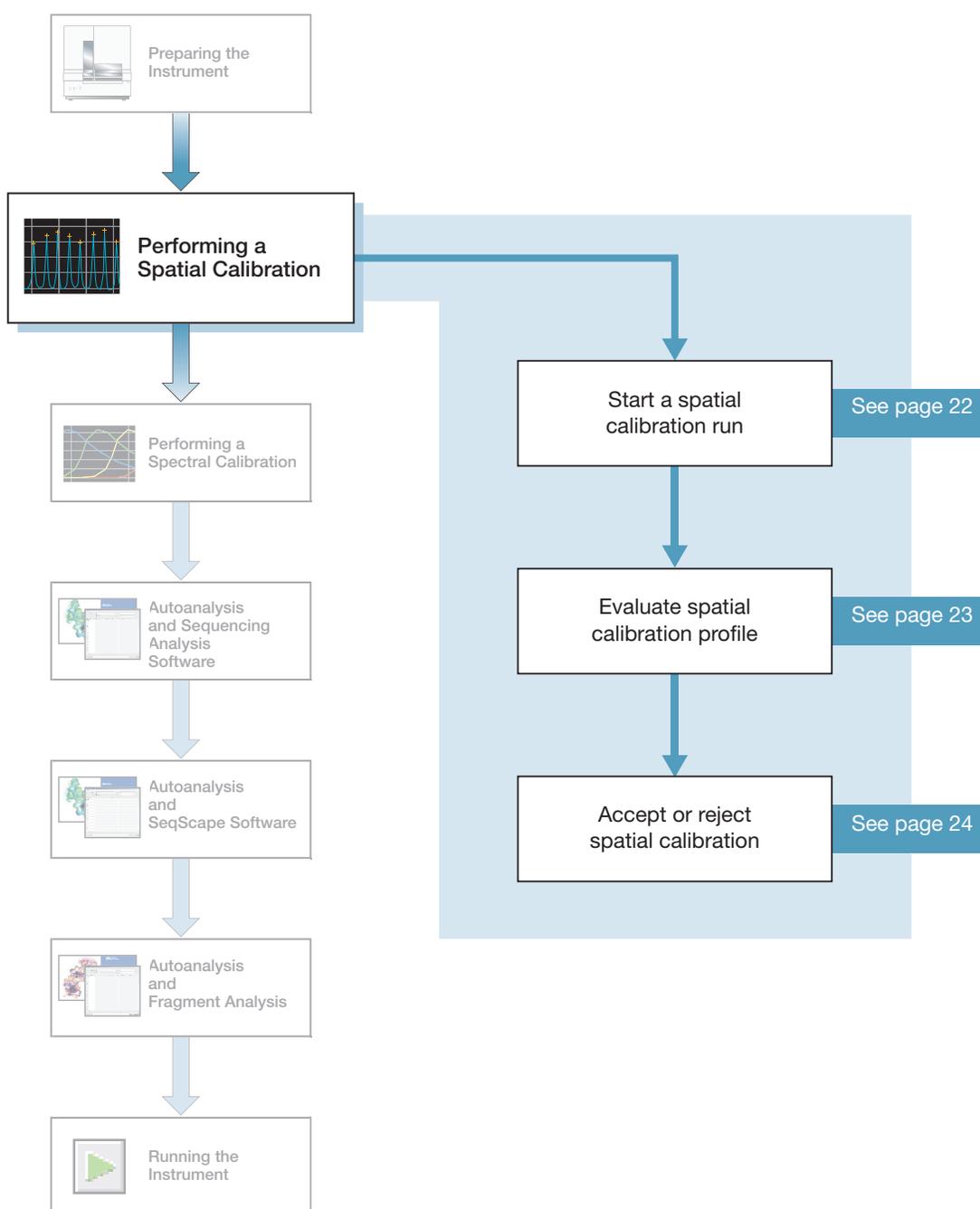
Notes _____

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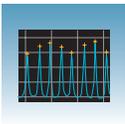
Spatial Calibration

The 3130/3130*xl* genetic analyzer Data Collection software uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera.

When to Perform the Calibration

You are required to perform a spatial calibration when you:

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

Creating a Spatial Calibration File

1. In the tree pane of the Data Collection software, click **GA Instruments** > **ga3130** or **ga3130***xl* > *instrument name* > **Spatial Run Scheduler**.

Spatial profile

Positions (pixel) of each capillary

Start, Accept or Reject Spatial profile

2. In the Spatial Protocols section, select one of the following:

- If the capillaries contain fresh polymer, select **Protocol** > **3130SpatialNoFill_1**
- Otherwise, select **Protocol** > **3130SpatialFill_1**

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

Select one

3. Click [Start].

The calibration run lasts approximately:

- 2 min. without filling the capillaries
- 6 min. when filling the capillaries

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

Start spatial calibration

Evaluating a Spatial Calibration File

1. Evaluate the spatial calibration profile using the following criteria:

Peak Attribute	Acceptable Criteria
Height	Similar heights for all peaks.
Orange crosses	One orange cross marking the top of every peak. No misplaced crosses.
Shape	Single sharp peak for each capillary. Small shoulders are acceptable.
Spacing	The difference between adjacent positions is 13 to 16 pixels. Theoretical spacing between capillaries is 15.

Spatial calibration profile for 3130*xl* system
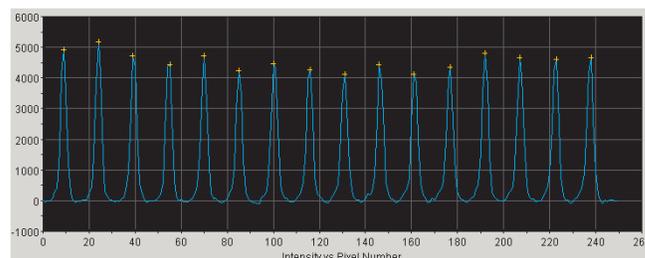
Spatial calibration profile for 3130 system
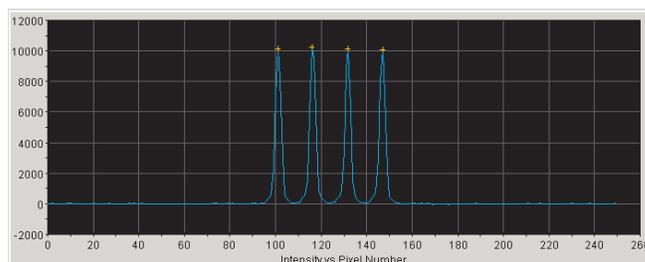
Notes
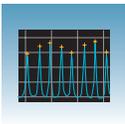
2. Examine each row in the 16 or 4 Capillary Positions table and verify that the values in both the Left spacing and Right spacing columns range between 13 to 16 pixels.

To move the cross:

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

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

16 Capillary Positions

Capillary	Position (pixels)	Left spacing	Right spacing
1	10	0	15
2	25	15	16
3	41	16	15
4	56	15	16
5	72	16	15
6	87	15	15
7	102	15	15
8	117	15	15
9	132	15	16
10	148	16	15
11	163	15	15
12	178	15	16
13	194	16	15

4 Capillary Positions

Capillary	Position (pixels)	Left spacing	Right spacing
1	96	0	15
2	111	15	15
3	126	15	15
4	141	15	0

Left spacing and Right spacing columns

Accepting or Rejecting a Spatial Calibration

If the calibration:

- Passed, click Accept to write the calibration data to the database and .ini file.
- Failed, click Reject , then see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide*.

Spatial Protocols

Protocol: 3100SpatialFill_1

Start   Stop Run

Accept   Reject

Accept and Reject buttons
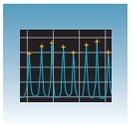
Notes

Examples of Spatial Profiles

Passing Profiles   3130 Genetic Analyzer
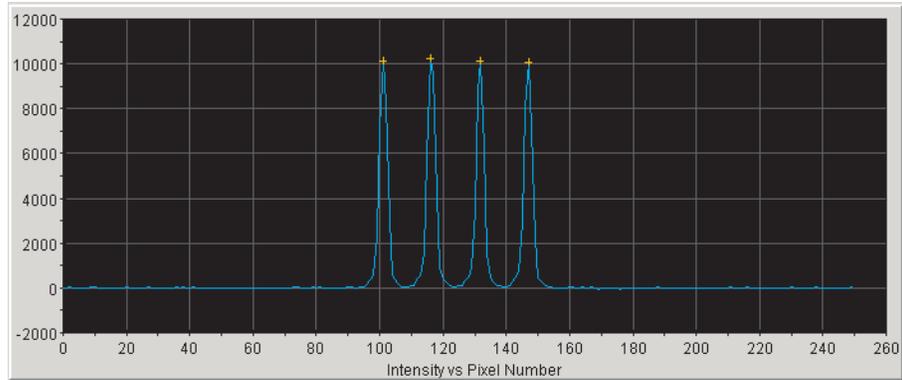
3130*xl* Genetic Analyzer
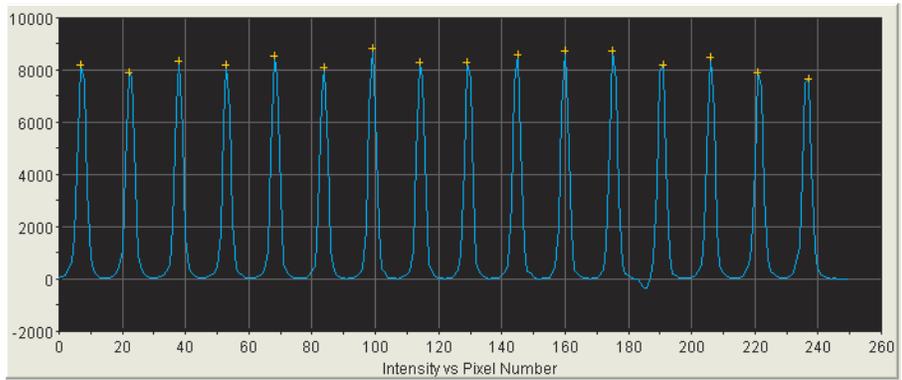
Failing Profile   3130*xl* Genetic Analyzer

Notes

Notes

Performing a Spectral Calibration

Preparing the Instrument	
Performing a Spatial Calibration	
Performing a Spectral Calibration	
Autoanalysis and Sequencing Analysis Software	
Autoanalysis and SeqScape Software	
Autoanalysis and Fragment Analysis	
Running the Instrument	

Prepare spectral calibration chemistry	See page 31
Create a spectral instrument protocol	See page 35
Perform a spectral calibration	See page 37
Evaluate the spectral calibration data	See page 43
Activate a spectral calibration	See page 47

Notes _____

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Spectral Calibration

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

When to Perform the Calibration

Perform a spectral calibration:

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

What Happens?

Run the spectral standards in all 16 or 4 capillaries. The Data Collection software then:

- Collects the data and stores it in 16 or 4 separate temporary files
- Analyzes the data and generates a matrix for each capillary
- Stores the spectral calibration data for the dye set run

Changing Capillary Array Lengths and Polymer Type

For each dye set, a unique spectral calibration is used for each different capillary array length.

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

See "Activating a Spectral Calibration" on page 47, on how to switch calibrations once calibrations are performed for each dye set on each capillary length.

Supported Sequencing Chemistries

New basecaller, mobility files, and run modules will be developed for the 3130/3130*xl* series instruments solely in support of the ABI PRISM® BigDye® Terminator v1.1 and v3.1chemistry kits. For more information, please contact Applied Biosystems Technical Support.

ABI PRISM® BigDye® Terminator v1.0, v2.0 and v3.0 chemistry kits have been discontinued.

Notes
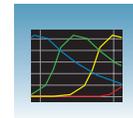
Types of Calibration Standards

There are two types of spectral calibration standards:

- **Matrix standards for fragment analysis or sequencing** - A tube that contains four or five fragments each labeled with a different single dye.

- BigDye® v3.1 or BigDye® v1.1 Terminator Sequencing Standard - A tube of a standard chemistry reaction that contains multiple labeled fragments in each of the four dyes

Dye Set Tables

Determine the correct dye set and matrix standard set for the application you are using by using the tables below.

Sequencing Chemistry Dye Sets, Calibration Standards, and Chemistry File

Sequencing Chemistry	Dye Set	Spectral Calibration Standard	Chemistry File
• BigDye® Terminator v3.1 Cycle Sequencing Kit • ABI PRISM® dGTP BigDye® Terminator v 3.0 Cycle Sequencing Ready Reaction Kit[a]	Z_BigDyeV3	BigDye® v3.1 Matrix Standards	Matrix Standard
		BigDye® v3.1 Terminator Sequencing Standard	Sequence Standard
• BigDye® Terminator v1.1 Cycle Sequencing Kit • ABI PRISM® BigDye® Primer Cycle Sequencing Kits • ABI PRISM® dGTP BigDye® Terminator Cycle Sequencing Ready Reaction Kit[a]	E_BigDyeV1	DS-01 Matrix Standards	Matrix Standard
		BigDye® v1.1 Terminator Sequencing Standard	Sequence Standard
ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit		dRhodamine Matrix Standards Kit	Matrix Standard

a   dGTP kits are not supported on capillary electrophoresis instruments due to compressions on certain sequence context regions; you can run the kits if compression is not an issue.

Notes _____
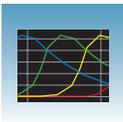
Fragment Analysis Dye Sets, Calibration Standards, and Chemistry File

Fragment Analysis Chemistry	Dye Set	Spectral Calibration Standard	Chemistry File
Custom oligos	D	DS-30 Matrix Standards	Matrix Standard
• ABI PRISM® Mouse Mapping Set v1.0 • Custom oligos	D	DS-31 Matrix Standards	
• AFLP® kits • Stockmarks® Kits 4-dye (bovine and canine) • AmpFℓSTR® COfiler® Kit • AmpFℓSTR® Profiler Plus® Kit • AmpFℓSTR® Profiler Plus® *ID* Kit • AmpFℓSTR® SGM Plus® Kit • Other 4-Dye AmpFℓSTR Kits	F	DS-32 Matrix Standards	
ABI PRISM® SNaPshot® Multiplex System	E5	DS-02 Matrix Standards	
• ABI PRISM® Linkage Mapping Set v2.5 • Stockmarks® Kit 5-dye (equine) • Custom Oligos • AmpFℓSTR® Identifiler® Kit • AmpFℓSTR® SEfiler™ Kit • AmpFℓSTR® Yfiler™ Kit • Other 5-Dye AmpFℓSTR Kits	G5	DS-33 Matrix Standards	

Notes
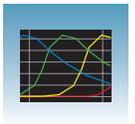
Preparing the Spectral Calibration Chemistry

Preparing the Calibration Standard

1. Prepare one of the following:

⚠️ **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.

- ABI PRISM BigDye Terminator v1.1 or v3.1 Sequencing Standard:
  – Remove a tube of the Sequencing Standard from the freezer.
  – Add 170 µL of Hi-Di™ formamide to resuspend the BigDye Terminator v1.1 or v3.1 Sequencing Standard.
- Sequencing or fragment analysis matrix standards:
  – Remove a tube of the matrix standard from the refrigerator.
  – Mix thoroughly, then spin briefly in a microcentrifuge.
  – Follow the matrix standard insert for matrix standard and Hi-Di formamide ratios.

2. Vortex thoroughly.
Briefly centrifuge the mixture.

3. Heat the standard tube at 95 °C for 5 minutes to denature the DNA.

4. Cool the tubes on ice for 2 minutes.
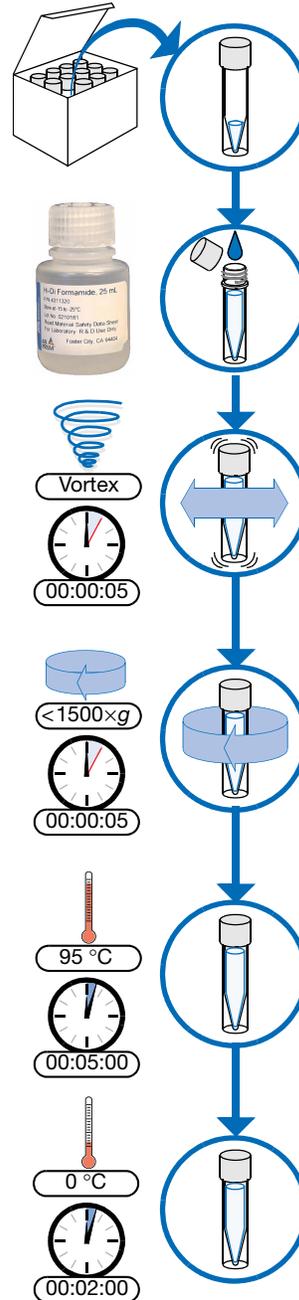
Notes

Loading Samples

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

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

Note: The efficient way to lay out samples in a plate is illustrated here. See Appendix A, "Plate Mapping," for the relationship between sample position and priority in scheduled injections.

To load samples:

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

   • If using a 3130*xl* genetic analyzer:

      – **96-well plate** – Add 10 μL of denatured standard to wells A1 through H2.

      – **384-well plate** – Add 5 μL of denatured standard into alternating wells of the plate:

      Row 1: A1, C1, E1, …K1, M1, O1

      Row 2: Empty

      Row 3: A3, C3, E3, …K3, M3, O3

   • If using 3130 genetic analyzer:

      – **96-well plate** – Add 10 μL of denatured standard to wells A1, B1, C1 and D1.

      – **384-well plate** – Add 5 μL of denatured standard into alternating wells of the plate:

      Row 1: A1, C1, E1 and G1

Prepared standard
(from step 4 on page 31)
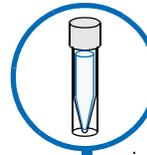
96-Well Plate

3130*xl* instrument layout
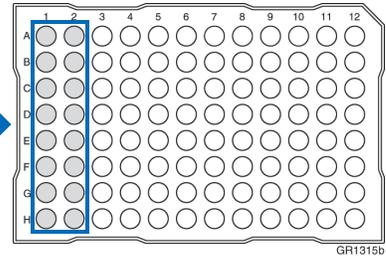
3130 instrument layout
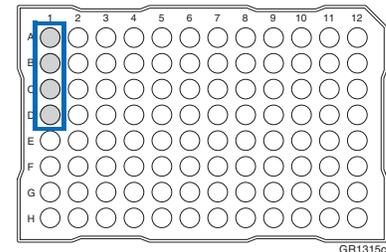
Add 10 µL prepared standard

384-Well Plate

3130*xl* instrument layout
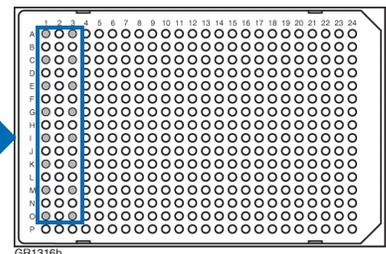
3130 instrument layout
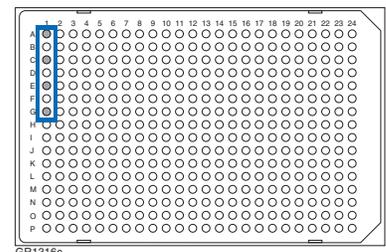
Add 5 µL prepared standard into alternating wells

Notes
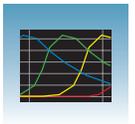
2. Seal the plate:

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

    b. Lay the septa flat on the plate.

    c. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
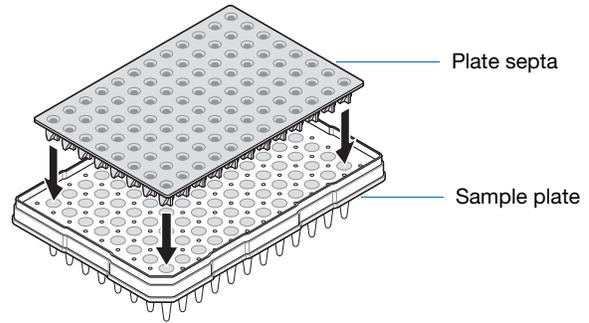
       IMPORTANT!: Do not heat plates that are sealed with septa.

Plate septa

Sample plate

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

Septa and well not aligned

Septa and well not aligned
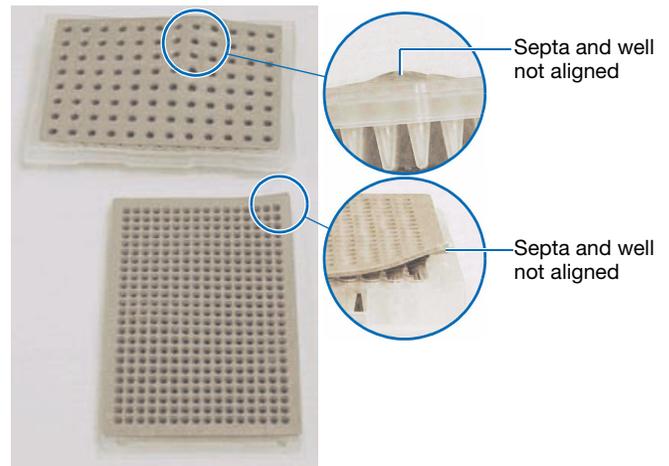
4. Briefly centrifuge the plate.

Prepared standard

$<1500 \times g$ 0:05
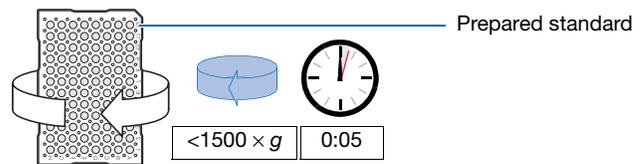
5. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.

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

Sample is at the bottom of the well
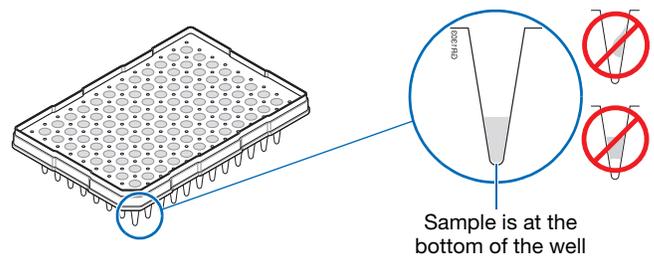
6. Leave the plate on ice until you are ready to prepare the plate assembly and place the assembly on the autosampler.

Notes _____

_____
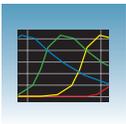
7. Assemble the plate assembly:

    a. Place the sample plate into the plate base.

    b. Snap the plate retainer onto the plate and plate base.

Plate retainer

6a

6b

Plate base

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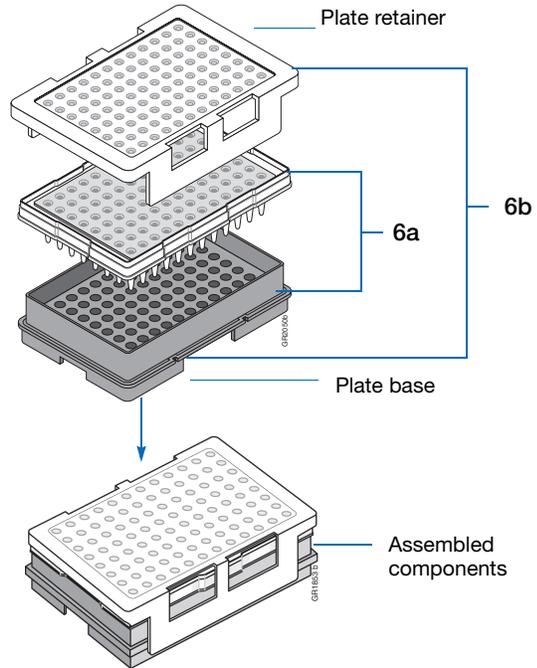
8. Verify that the holes of the plate retainer and the septa strip are aligned. If not, re-assemble the plate assembly (see step 7).

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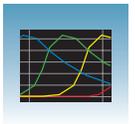
Plate retainer holes and septa holes are not aligned

Notes

Creating a Spectral Instrument Protocol

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▨ **ga3130***xl* or **ga3130** > ▤ **Protocol Manager** to open the Protocol Manager window.
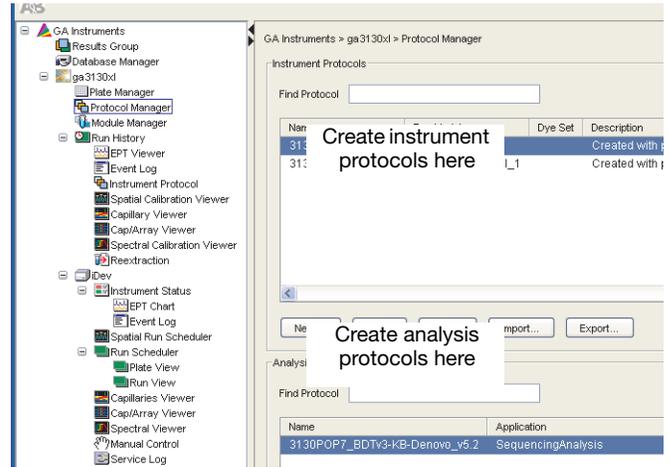
2. In the Instrument Protocols pane, click ⌑ New... ⌑. The Protocol Editor dialog box opens.
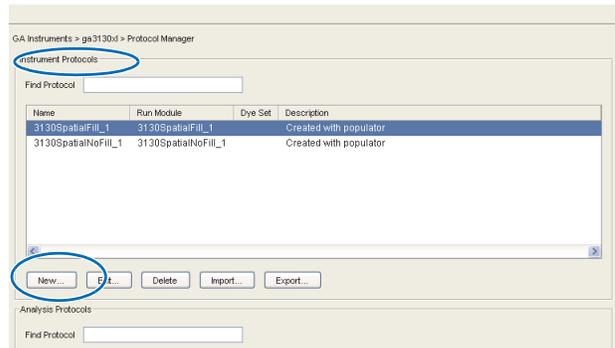
3. Complete the Protocol Editor dialog box.

    a. Type a name for the protocol.

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

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

    d. Select the correct dye set for your run. See "Dye Set Tables" on page 29.
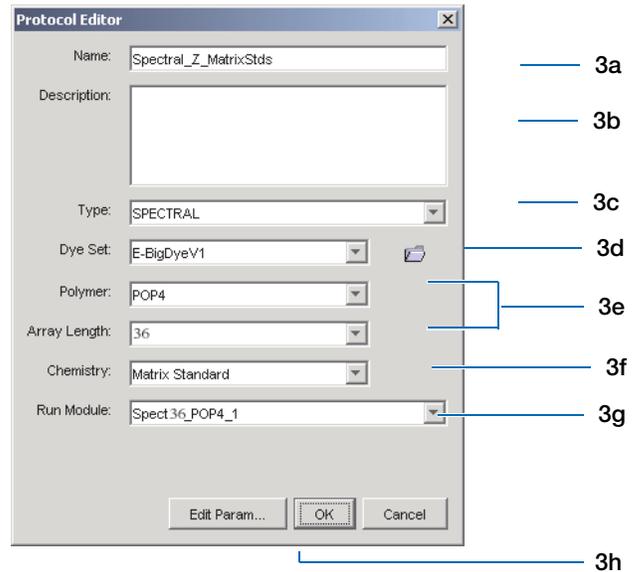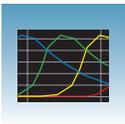
e. Select the Polymer and Array Length from the appropriate drop-down list. See table, "Polymer, Array Length and Spectral Run Modules."

f. Select your chemistry file in the Chemistry drop-down list. Failure to select the correct chemistry file for your spectral calibration samples results in a failing spectral run. See "Dye Set Tables" on page 29 for matching chemistry files.

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

---

g. In the Run Module drop-down list, select the run module. See the table "Polymer, Array Length and Spectral Run Modules."

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

---

h. Click ⌗ OK ⌗.

Polymer, Array Length and Spectral Run Modules

Polymer Type	Array Length (cm)	Run Module
POP-4	22	Spect22_POP4
	36	Spect36_POP4
		SpectSQ36_POP4
	50	Spect50_POP4
	80	Spect80_POP4
POP-6	36	Spect36_POP6
	50	Spect50_POP6
POP-7	36	Spect36_POP7
	50	Spect50_POP7
	80	Spect80_POP7
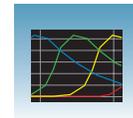
Performing a Spectral Calibration

Creating the Plate Record

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▨**ga3130***xl* or **3130** > ▭ *instrument name* > ▦ **Plate Manager.**

Plate Manager

2. Click [New...] to open the New Plate dialog box.

3. Complete the New Plate dialog box:

    a. Enter a name for the plate.

    b. Optional: Enter a description for the plate record.

    c. In the Application drop-down list, select **Spectral Calibration**.

    d. In the Plate Type drop-down list, select **96-Well** or **384-Well**

    e. Enter a name for the owner.

    f. Enter a name for the operator.

    g. Click [OK].

3a
3b
3c
3d
3e
3f
3g
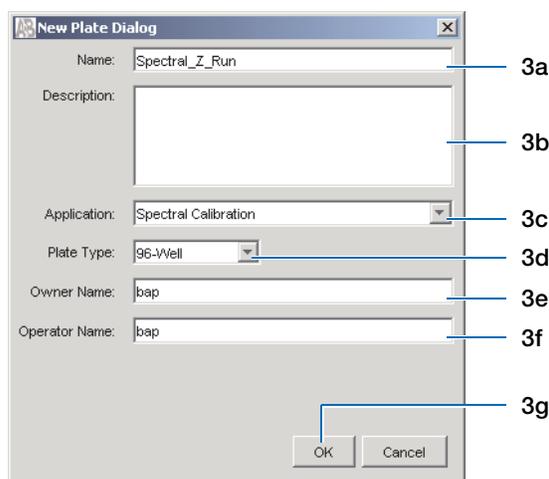
4. In the Spectral Calibration Plate Editor dialog box:

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

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

    c. In the **Instrument Protocol 1** column, select a protocol from the list or create a new protocol (see step 2 on page 35)

4a   4b   4c
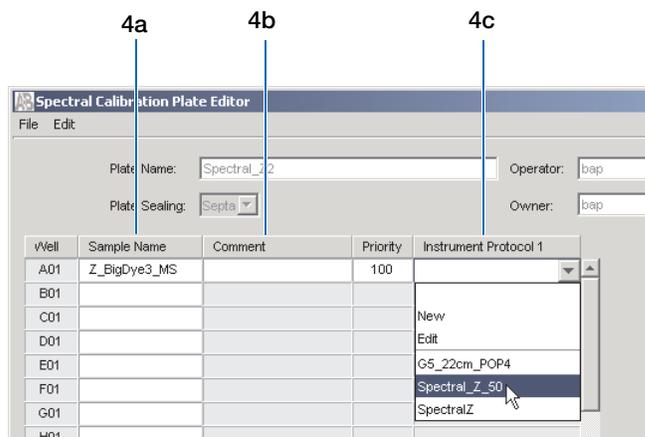
5. Highlight the entire row.

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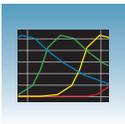
6. Select **Edit > Fill Down Special**.

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

7. Click OK.

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

Edit	
Fill Down	Ctrl+D
Copy	Ctrl+C
Paste	Ctrl+V
Clear row(s)	Shift+Delete
Fill Down Special	Alt+D
Add Sample Run	Alt+A

Notes
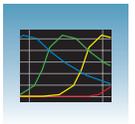
Placing the Plate Assembly into the Instrument

1. Verify the oven and front doors are closed.

2. Press the Tray button and wait for the autosampler to stop at the forward position.

3. Open the front doors.

4. Place the plate assembly on the autosampler in position A or B for the 3130*xl* genetic analyzer and position B for the 3130 genetic analyzer.

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

A   B

3130*xl* genetic analyzer

B
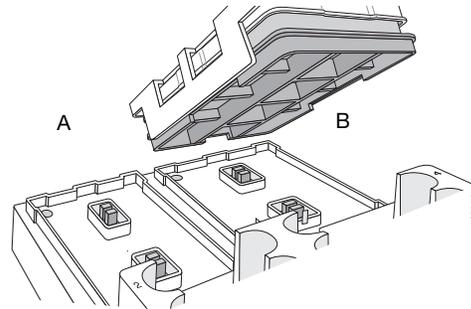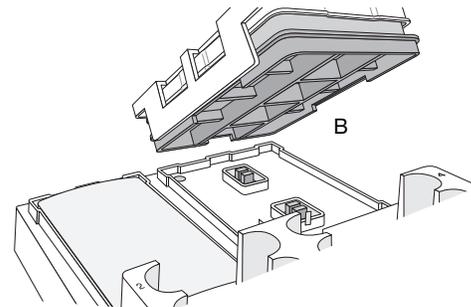
3130 genetic analyzer

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

6. Close the instrument doors.

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

Notes _____
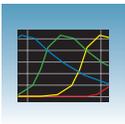
Running the Spectral Calibration Plate

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▨ **ga3130***xl* or **3130** > ▭ *instrument name* > ▤ **Run Scheduler** > ▤ **Plate View**.
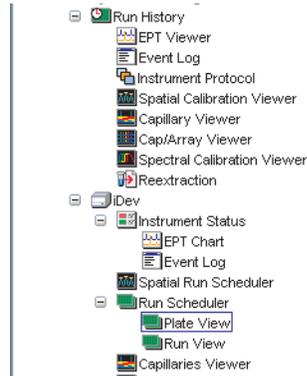
2. Search for your plate record. There are two options:

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

    – In the **Scan or Type Plate ID** text box, type in the plate name and click **Search** to find the plate.

    – Or if you have a limited number of plates in the database, click [Find All].
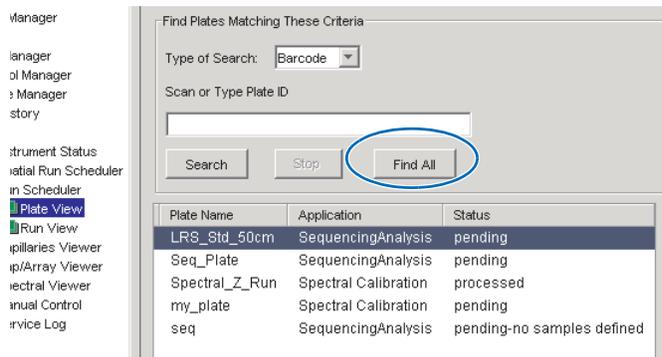
    All plates in the database display in plate record section.

- Perform an advanced search by selecting **Advanced** in the Type of Search drop-down list.

    – Use the drop-down list to define search conditions for a category or multiple categories (Run Name, Results Group Name, Plate Name, etc.)
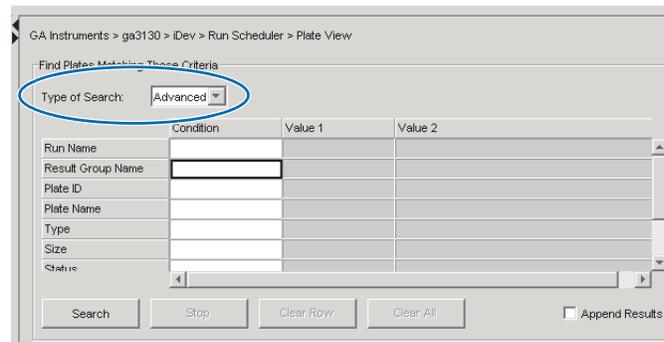
    Note: Your entry for Plate Name will also be set as the Plate ID.

    – For each category with a condition selected, type a value (primary search string) in the Value 1 column.

    – Click [Search]. All plates in the database that match the search criteria display in the plate record section.

Notes

Applied Biosystems 3130/3130*xl* Genetic Analyzers Getting Started Guide
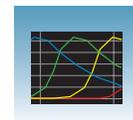
3. Link the plate.

    a. Select the plate record you want to run.

    b. Click the plate position indicator that matches the plate you want linked.

        The plate map color will change from yellow to green when it is successfully linked.
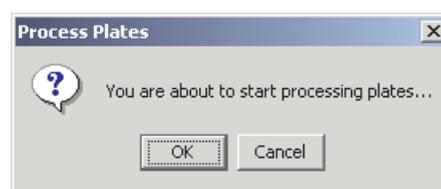
        Note: The 3130 genetic analyzer has only one plate position (Bay B) to link a plate record.
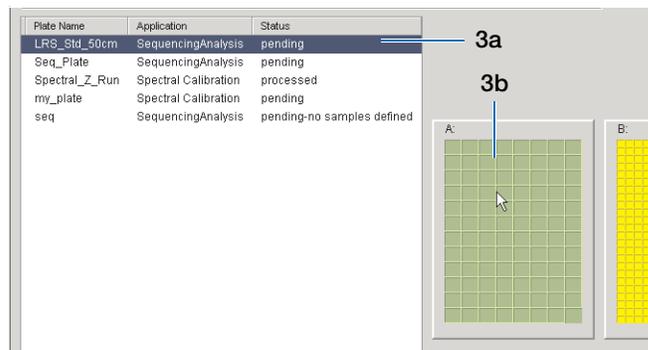
4. In the toolbar of the Data Collection software window, click ▶ to begin the run.

5. The Processing Plates dialog box opens. Click OK.

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

Spectral Calibration Approximate Run Times

Capillary Length (cm)	Run Type	Ramp Time (min)	Approximate Total Run Time (min)
22	Spec22_POP4	3	21
36	Spec36_POP4	10	35
	SpecSQ36_POP4	10	48
	Spec36_POP6	10	54
	Spec36_POP7	5	30
50	Spec50_POP4	7.5	110
	Spec50_POP6	10	95
	Spec50_POP7	7.5	52
80	Spec80_POP4	10	140
	Spec80_POP7	10	120

Notes
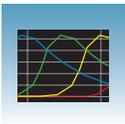
Viewing the Pass/Fail Status After the Run

After the spectral calibration run, the pass or fail status of each capillary is recorded in the Event Log section of the Instrument Status window.

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > ▱ *instrument name* > 🖼 **Instrument Status** > 📄 **Event Log**.

   ```
   Plate Manager
   Protocol Manager
   Module Manager
   Run History
   iDev
       Instrument Status
           EPT Chart
           Event Log
       Spatial Run Schedu
       Run Scheduler
       Capillaries Viewer
       Cap/Array Viewer
       Spectral Viewer
       Manual Control
       Service Log
   ```

2. In the Event Messages section of the window, view the status of each capillary.
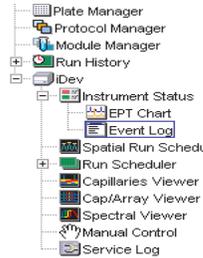
   Note: The window below shows the dye set G5 status results.

Cap #   Pass/fail status   Q-value   Condition Number

Type	Date	Time	Publisher	Description
Info	07/07/03	15:41:31	iDev	Finished saving spectral calibration data
Info	07/07/03	15:41:30	iDev	Saving spectral calibration data
Info	07/07/03	15:41:30	iDev	Capillary 16 successfully calibrated : q=0.988 c=9.12
Info	07/07/03	15:41:30	iDev	Capillary 15 successfully calibrated : q=0.986 c=9.15
Info	07/07/03	15:41:30		Run completed
Info	07/07/03	15:41:30	iDev	Capillary 14 successfully calibrated : q=0.986 c=9.01
Info	07/07/03	15:41:30	iDev	Capillary 13 successfully calibrated : q=0.988 c=8.99
Info	07/07/03	15:41:29	iDev	Capillary 12 successfully calibrated : q=0.989 c=8.87

Each capillary should have a Q-value above 0.95 and a Condition number range shown in the table.

Note: If the entire spectral calibration failed, see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide* for troubleshooting information.

Dye Set	Acceptable Condition Number Range	Q-Value
Sequencing Analysis		0.95
Z_BigDyeV3	3 to 5	
E_BigDyeV1		
Fragment Analysis		
D	4 to 8.5	
F	6 to 12	
E5	2.5 to 4	
G5	8.5 to 14.5	
Any 4- or 5-Dye set	1 to 20	0.80

Notes
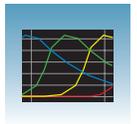
Evaluating the Spectral Calibration Data

IMPORTANT! Evaluate the spectral calibration profile for each capillary, even if the Spectral Calibration Results box indicates that they passed.

Note: Pages 49 to 50 for examples of passing sequencing spectral calibration profiles. See pages 51 to 52 for examples of passing fragment analysis spectral calibration profiles.

Evaluating the Spectral Profile and Raw Data

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > 🖥 *instrument name* > 🔳 **Spectral Viewer**.

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

Note: If the spectral calibration failed (no spectral profiles are created), see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide* for more information.

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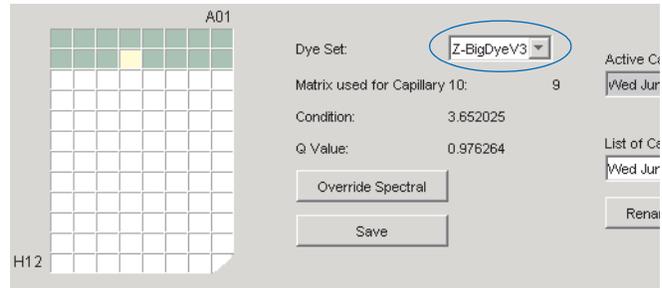
3. In the plate diagram, select a well on the plate diagram to view the capillary spectral results.

**Note:** A failing capillary is automatically assigned the spectral profile of its nearest passing capillary.

Capillary status:

Passed (dark green)

Passed well elected (light green)

Failed (tan)

Failed well selected (light tan)
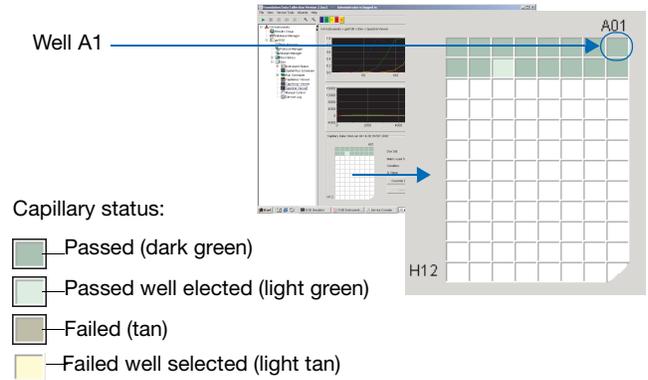
4. Evaluate the spectral profile and raw data for the selected capillary:

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

– 4-dye: blue-green-yellow-red

– 5-dye: blue-green-yellow-red-orange

Do the peaks in the profile appear in the correct order?	Then
Yes	Go to step b.
No	The calibration run has failed. See the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*.
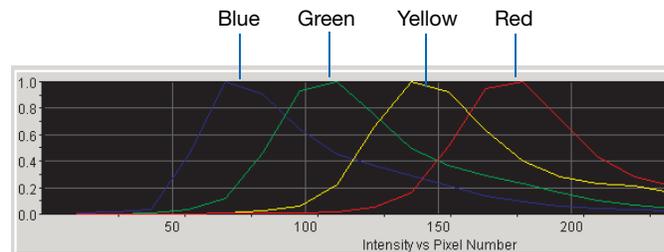
Example of a 4-dye spectral profile
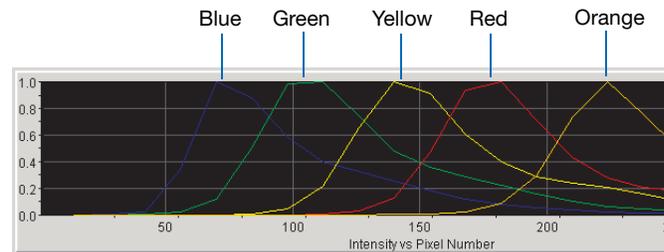
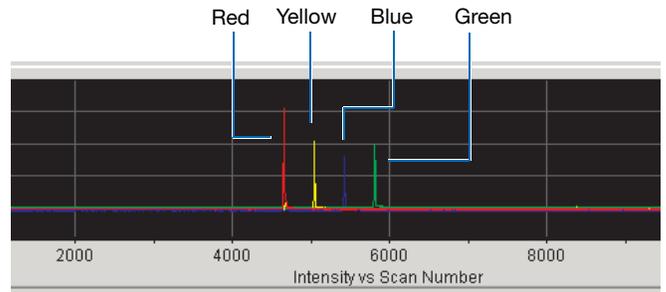**Example of a 5-dye spectral calibration profile**
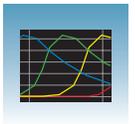
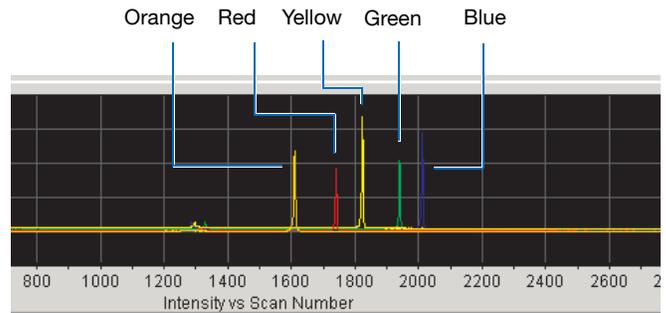**b.** Verify that the order of the peaks in the raw data profile from left to right are:

Sequencing

– 4-dye: red-yellow-blue-green

Fragment Analysis

– 4-dye: red-yellow-green-blue

– 5-dye: orange-red-yellow-green-blue

Are peaks in the wrong order or extraneous peaks adversely affecting the spectral profile?	Then
Yes	The calibration run has failed. See the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*.
No	Go to step c.

Example of a 4-dye sequencing raw data profile

Example of a 5-dye fragment analysis raw data profile

c. Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities (see "Magnifying the Spectral Profile or Raw Data" on page 46).

Are the peaks separate and distinct?	Then
Yes	The capillary has passed. Go to step 5.
No	The calibration run has failed. See the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*.

Peaks are distinct, regular and in the proper order – pass
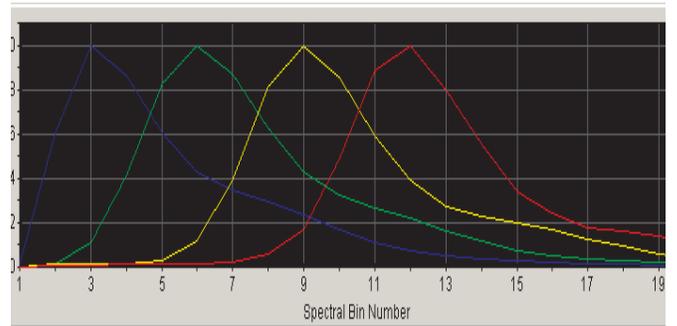
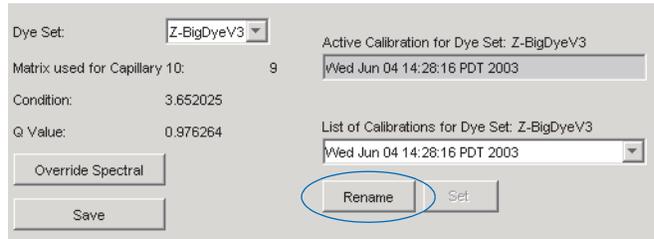Red peak is not distinct, regular or in the proper order – fail
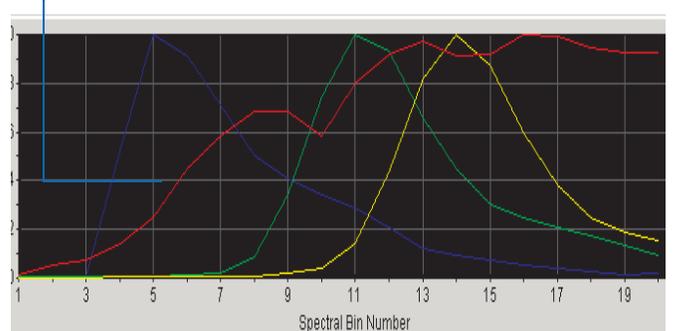
Notes _____

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

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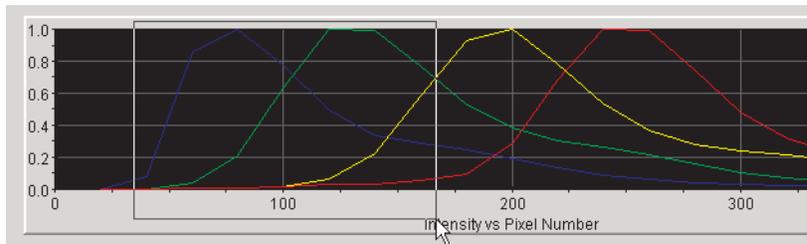
    a. Click Rename .

    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).
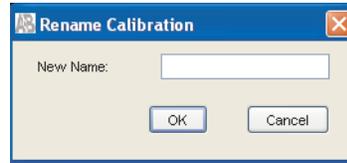
    c. Click OK .

Magnifying the Spectral Profile or Raw Data

1. In the tree pane of the Data Collection software, click
   GA Instruments > **ga3130xl** or **ga3130** > *instrument name* > **Spectral Viewer**.

2. In the spectral 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.
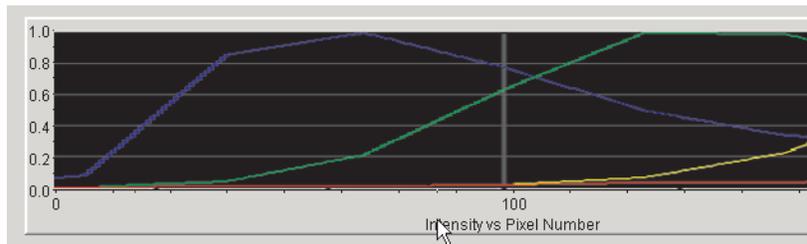
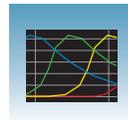**Magnified area of that spectral profile**

Activating a Spectral Calibration

IMPORTANT! A run cannot start unless a calibration file that matches the dye set and capillary array length combination to be used for the run, is active.

IMPORTANT! While the software does not force you to recalibrate spectrally after changing polymer types, it is strongly recommended that you perform a spectral calibration after a polymer type change.

IMPORTANT! Whenever you install or replace an array or switch dye sets, you must either set the active spectral calibration for that dye set and array length combination or perform a new spectral calibration and then activate it.

IMPORTANT! Every new spectral calibration is automatically the active one for that dye set.

You may activate a previously created spectral calibration for a run as long as the calibration matches the dye set and array length combination (and, for fragment analysis, the polymer type) that you wish to use. Use an active spectral calibration for:

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

Setting an Active Spectral Calibration

1. In the tree pane of the Data Collection software, click
   🔺 **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > 🖥 *instrument name* > 🟥 **Spectral Viewer**.

   IMPORTANT! If the Spectral Viewer window is blank and deactivated, then either:
   –The spectral calibration for that dye set is not in the database
   or,
   –You changed the array length and you do not have a spectral calibration file activated for that dye set and array length combination.

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

Notes _____
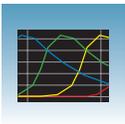
3. In the List of Calibrations for Dye Set drop-down list, select the spectral calibration you want to use. The spectral profile and raw data is displayed.
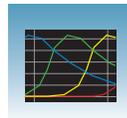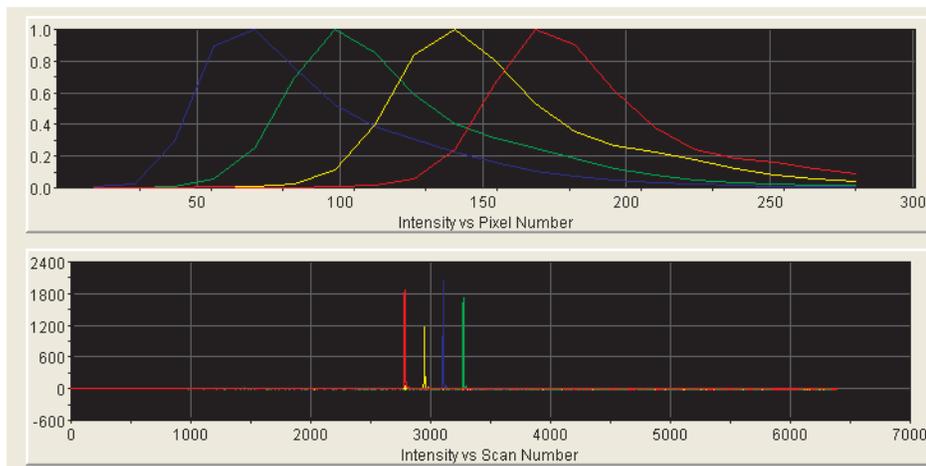

4. If the spectral calibration is acceptable, then click Set . Otherwise, run a new spectral calibration.
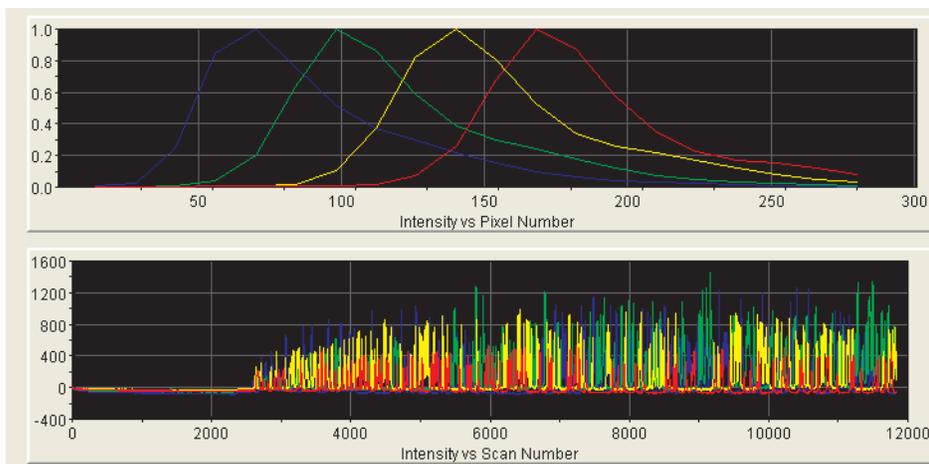
Notes

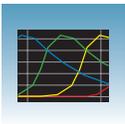# Examples of Passing Sequencing Spectral Calibrations

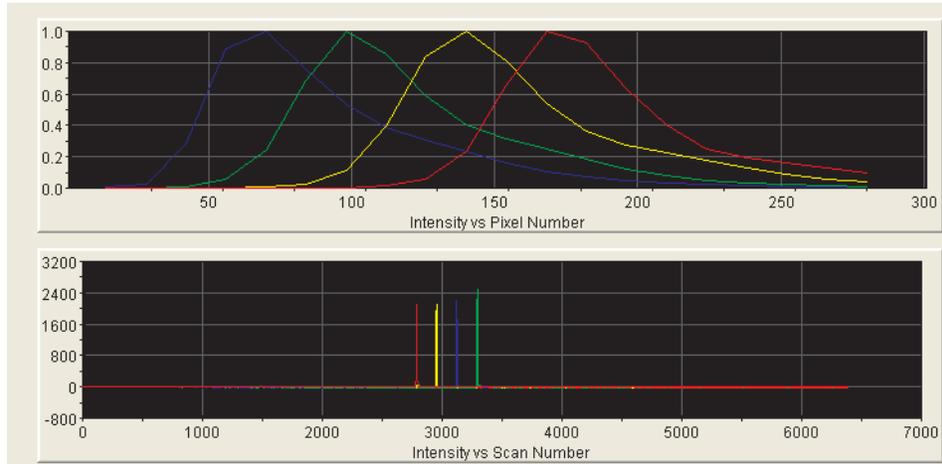**Dye Set Z Created from Matrix Standard**

Dye Set Z Created from a Sequencing Standard

Notes

Dye Set E Created from Matrix Standard Set DS-01

Dye Set E Created from a Sequencing Standard

Notes

Examples of Passing Fragment Analysis Spectral Calibrations

Dye Set G5 Created from Matrix Standard Set DS-33

Dye Set F Created from Matrix Standard Set DS-32
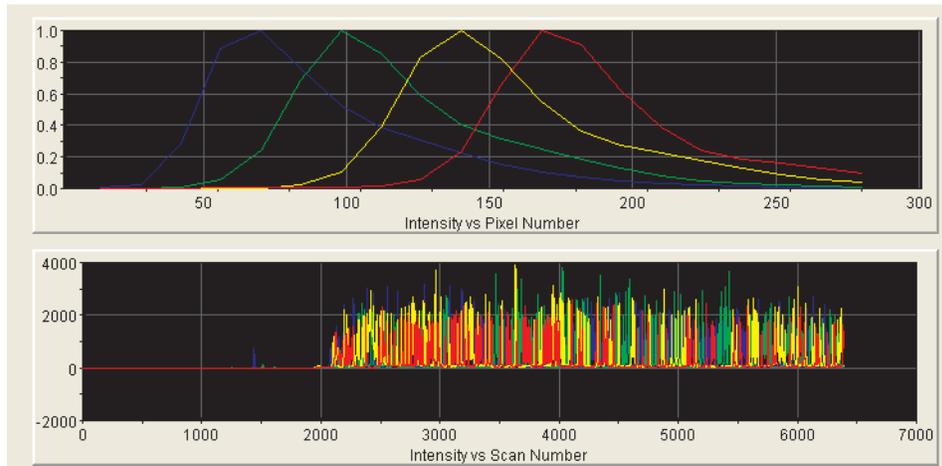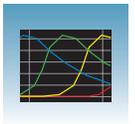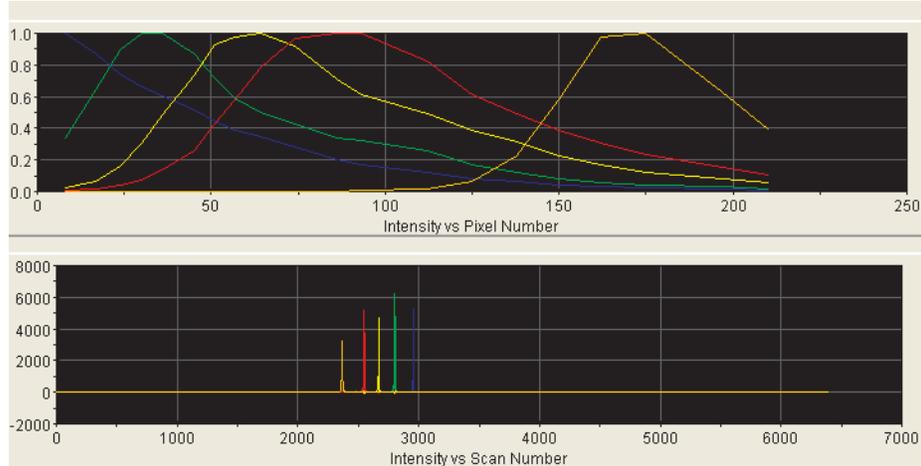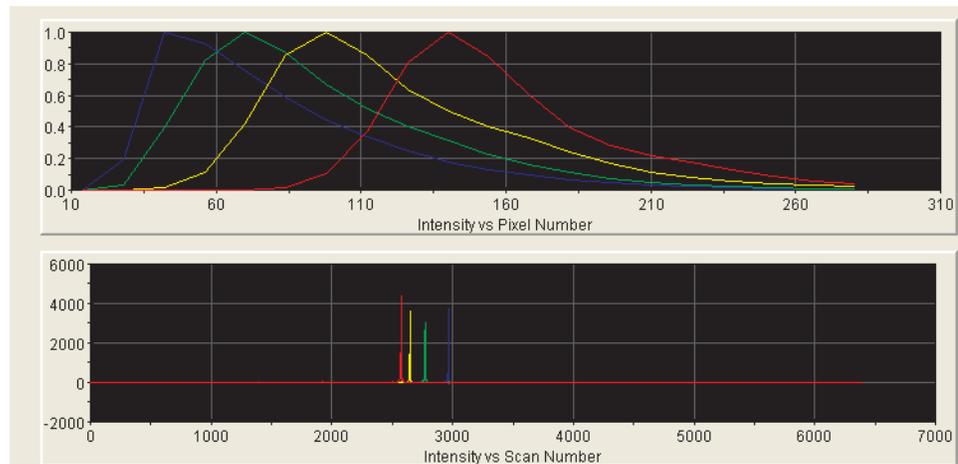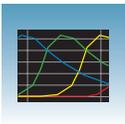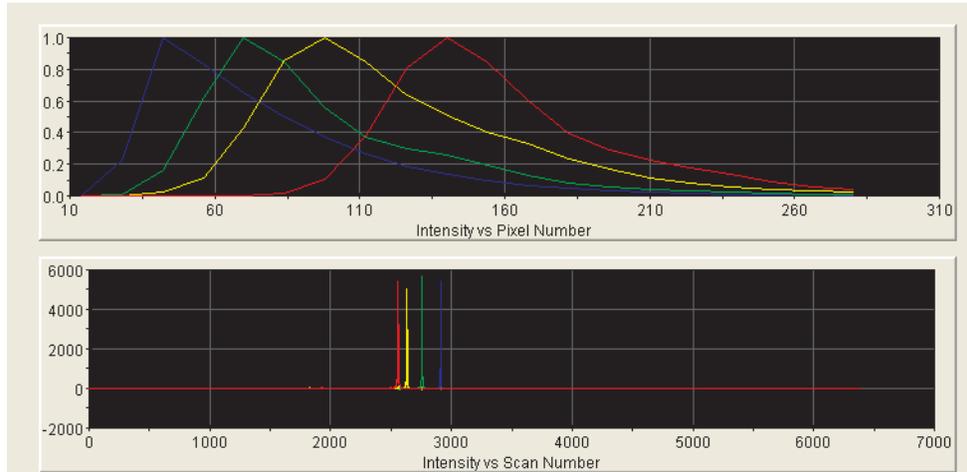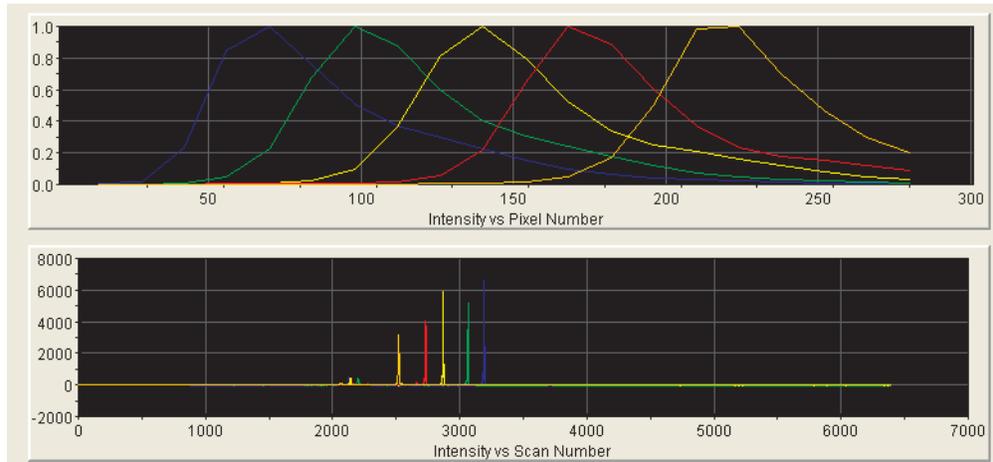

Notes _____

**Dye Set D
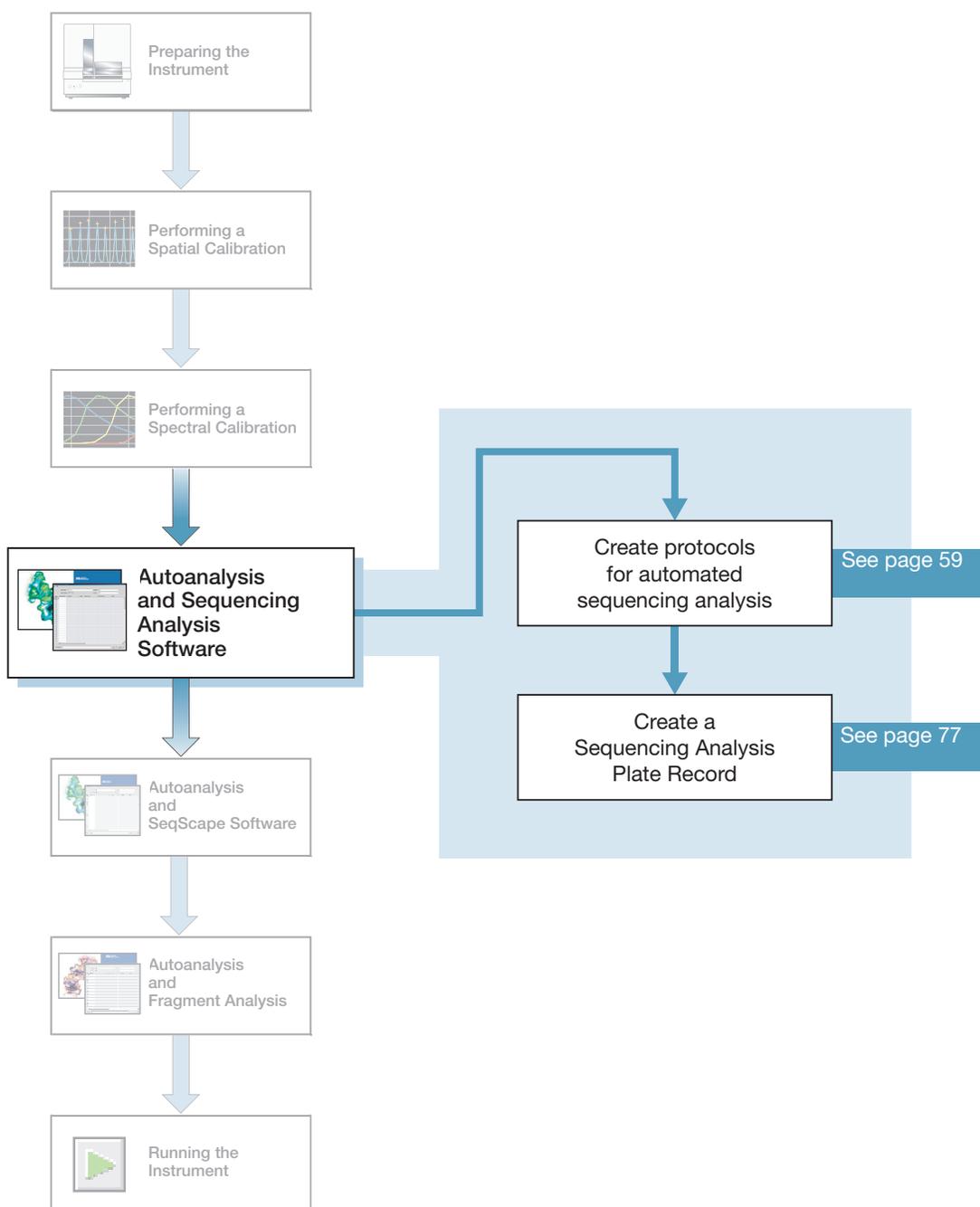Created from
Matrix Standard
Set DS-30**

**Dye Set E5
Created from
Matrix Standard
Set DS-02**

Autoanalysis and Sequencing Analysis Software

```
┌─────────────────────┐
│  Preparing the       │
│  Instrument          │
└─────────────────────┘
           │
           ▼
┌─────────────────────┐
│  Performing a        │
│  Spatial Calibration │
└─────────────────────┘
           │
           ▼
┌─────────────────────┐
│  Performing a        │
│  Spectral Calibration│
└─────────────────────┘
           │
           ▼
┌─────────────────────┐        ┌──────────────────────┐
│  Autoanalysis        │───────▶│  Create protocols    │  See page 59
│  and Sequencing      │        │  for automated       │
│  Analysis            │        │  sequencing analysis │
│  Software            │        └──────────────────────┘
└─────────────────────┘                   │
           │                              ▼
           │                   ┌──────────────────────┐
           ▼                   │  Create a            │  See page 77
┌─────────────────────┐        │  Sequencing Analysis │
│  Autoanalysis        │        │  Plate Record        │
│  and                 │        └──────────────────────┘
│  SeqScape Software   │
└─────────────────────┘
           │
           ▼
┌─────────────────────┐
│  Autoanalysis        │
│  and                 │
│  Fragment Analysis   │
└─────────────────────┘
           │
           ▼
┌─────────────────────┐
│  Running the         │
│  Instrument          │
└─────────────────────┘
```

4

Notes _____

_____

Sequencing Analysis Software

See the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide* (P/N 4346366) for details on sequencing analysis or the *Sequencing Analysis Software v5.2 User Bulletin* titled *New Features* (P/N 4358355) for an update on Sequencing Analysis Software v5.2.

Analyze your sequencing samples automatically using autoanalysis or manually.

Autoanalysis

Perform autoanalysis of sequencing samples using features of the 3130/3130*xl* genetic analyzer Data Collection software and Sequencing Analysis Software v5.2 or higher.

The Sequencing Analysis Software must be installed and registered with the 3130/3130*xl* genetic analyzer Data Collection software before you can create files required for autoanalysis.

Autoanalysis can only be performed on the same computer that collected the sample files. If you perform autoanalysis on samples, but wish to edit/review results on another computer, transfer the analysis protocol to the Sequencing Analysis Software database. To analyze samples on another computer, transfer the files to that location.

Manual Analysis

If the run is not set up for autoanalysis, see the *Applied Biosystems DNA Sequencing Analysis Software v5.1 User Guide* and the *Sequencing Analysis Software v5.2 User Bulletin - New Features*.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. Do not use the characters below:

spaces

\ / : * ? " < > |

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

Notes

About Plate Records and Sequencing Analysis

Overview
Plate records are data tables in the instrument database that contain the following information:

- Plate name, type, and owner
- Position of the sample on the plate (well number)
- Sample Name
- Mobility file (in Analysis Protocol)
- Comments about the plate and about individual samples
- Name of the run module and Dye set information (run modules specify the conditions to run the samples)
- Name of the Analysis Protocol

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

When to Create a Plate Record
Create a plate record for each plate of samples for the following types of runs:

- Spectral calibrations
- Sequencing Analysis Software
- SeqScape Software analysis
- GeneMapper® software
- Mixed (sequencing and fragment analysis samples - see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* for creating a mixed plate record)

For runs to begin, you must create a plate record for and link it to a plate loaded on the instrument. However, you can create plate records for new plates while a run is in progress.

About a Sequencing Analysis Plate Record
The Plate Editor displays an empty plate record for the application selected in the New Plate dialog box. The data fields within a given plate record vary depending on the selected application. This section describes the sequencing analysis plate record's data fields.
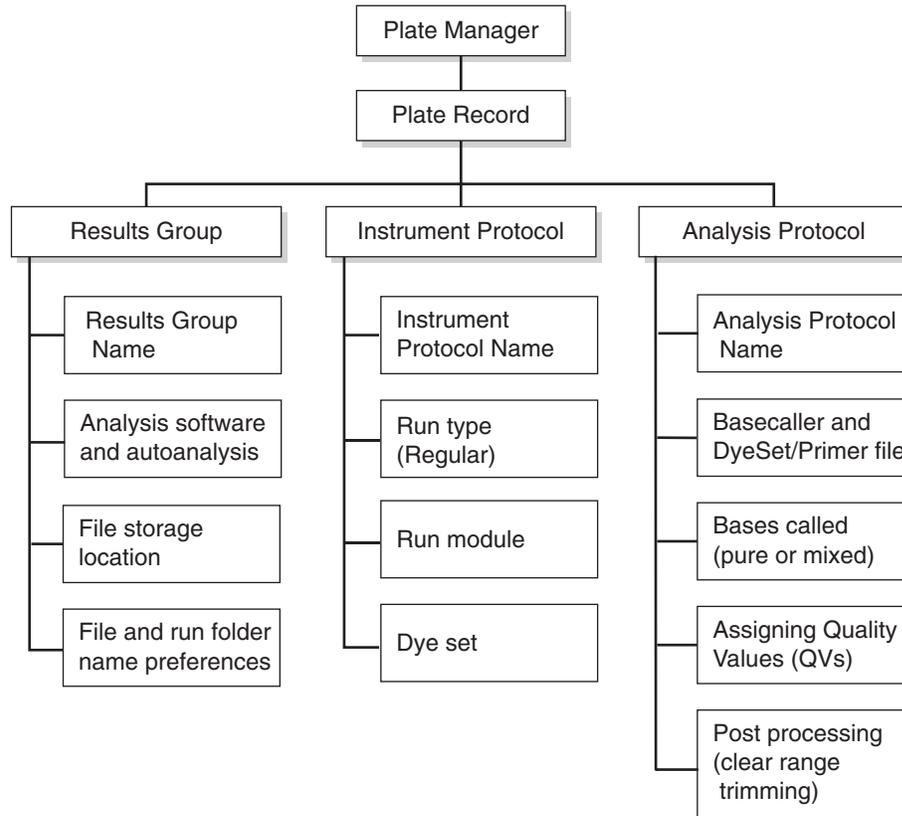
The table below describes required plate editor fields:

Parameters	Description	See Page
Instrument Protocol	Contains the run module and dye set needed to run the instrument.	59
Analysis Protocol	Contains everything needed to analyze sequencing data.	62
Results Group	Defines the file type, the file name, file save locations, analysis software and autoanalysis.	70

Notes _____

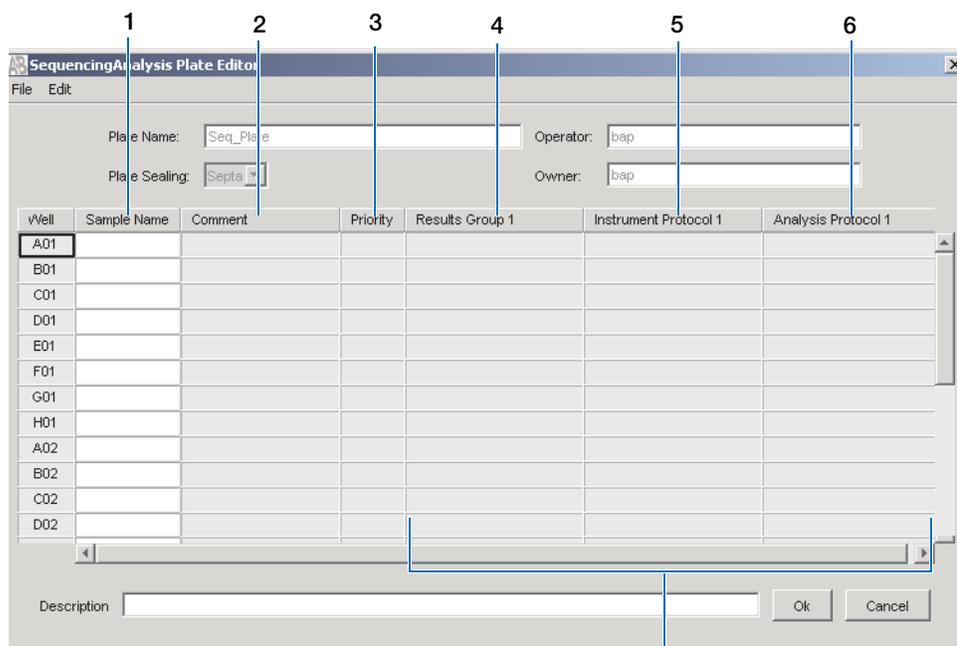### Elements of a Sequencing Analysis Plate Record

```
                          ┌──────────────────┐
                          │  Plate Manager   │
                          └──────────────────┘
                                   │
                          ┌──────────────────┐
                          │  Plate Record    │
                          └──────────────────┘
                                   │
        ┌──────────────────────────┼──────────────────────────┐
┌──────────────────┐      ┌──────────────────┐      ┌──────────────────┐
│  Results Group   │      │Instrument Protocol│     │ Analysis Protocol│
└──────────────────┘      └──────────────────┘      └──────────────────┘
```

Results Group	Instrument Protocol	Analysis Protocol
Results Group Name	Instrument Protocol Name	Analysis Protocol Name
Analysis software and autoanalysis	Run type (Regular)	Basecaller and DyeSet/Primer file
File storage location	Run module	Bases called (pure or mixed)
File and run folder name preferences	Dye set	Assigning Quality Values (QVs)
		Post processing (clear range trimming)

IMPORTANT! For data collection and autoanalysis to succeed, each run of samples must have an Instrument Protocol, an Analysis Protocol, and a Results Group assigned within a plate record.

**A Blank
Sequencing
Analysis Plate
Record**

Default is one sample run, to add
additional runs see page 79

Columns inserted in a plate record for a sequencing analysis run

Number and Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Priority	A default value of 100 to each sample. Changing the value to a smaller number increases the priority of that set of 16 or 4 samples to run before the others in the injection list.
4. Results Group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the Results Group listed in the cell • None: Sets the cell to have no selected Results Group • Select one of the available Results groups from the list **Note:** You must have a Results Group selected for each sample entered in the Sample Name column. See "Results Group for Sequencing Analysis" on page 70.

Notes

Number and Column	Description
5. Instrument Protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. **Note:** You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See "Instrument Protocol for Sequencing Analysis" on page 59.
6. Analysis Protocol	• New: Opens the Analysis Protocol Editor dialog box. • Edit: Opens the Analysis Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Analysis Protocols: In alpha-numeric order **Note:** You must have an Analysis Protocol selected for each sample entered in the Sample Name column. See "Analysis Protocol for Sequencing Analysis" on page 62.

Creating Protocols for Automated Sequencing Analysis

If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to "Filling Out a Sequencing Analysis Plate Record" on page 77.

Instrument Protocol for Sequencing Analysis

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

⚠️ **WARNING** Do not edit the instrument protocol currently being used, while the instrument is running.

Creating an Instrument Protocol

1. In the tree pane of the Data Collection software, click 🔺 **GA Instruments** > 🔳 **ga3130***xl* or **ga3130** > 🔲 **Protocol Manager**.

4

Create instrument protocols here

Create analysis protocols here
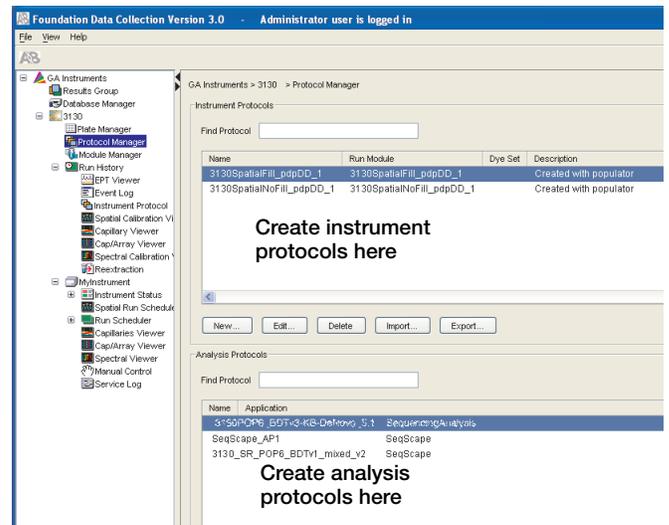
Notes

2. In the Instrument Protocol section, click
New... .

The Protocol Editor opens.
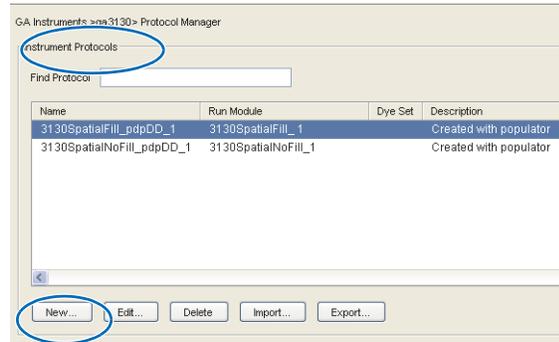
3. Complete the Protocol Editor:

    a. Type a name for the protocol.

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

    c. Select **Regular** in the Type drop-down list.
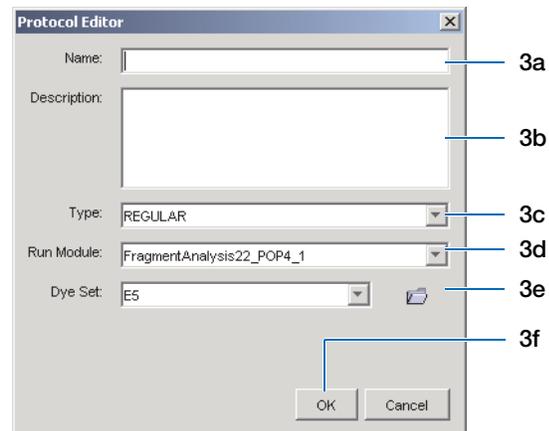
    d. Select the correct run module for your run. See "Sequencing Resolution Performance and Specifications" on page 3.

    Note: To customize a run module, see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide*.

Notes

e. Select the correct Dye Set for your run by using the table below.

Chemistry	Dye Set	POP-4 Polymer			POP-6 Polymer		POP-7 Polymer				
		UltraSeq36	StdSeq50	LongSeq80	RapidSeq36	StdSeq50	UltraSeq36	RapidSeq36	FastSeq50	StdSeq50	LongSeq80
BigDye® Terminator v3.1 Cycle Sequencing Kit	Z_BigDye V3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ABI PRISM® dGTP BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit		—	—	—	—	—	—	—	—	—	—
BigDye® Terminator v1.1 Cycle Sequencing Kit	E_BigDye V1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ABI PRISM® dGTP BigDye® Terminator Cycle Sequencing Kit*		—	—	—	—	—	—	—	—	—	—
ABI PRISM® dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit		✓	✓	✓	✓	✓	—	—	—	—	—
ABI PRISM® BigDye® Primer Cycle Sequencing Kits		—	—	—	✓	✓	—	—	—	—	—

*dGTP kits are not supported on capillary electrophoresis instruments due to compressions on certain sequence context regions; you can run the kits if you do not care about the compression issues.

f. Click OK .

Importing an Instrument Protocol

1. Click Import in the Instrument Protocols pane of the Protocol Editor window to display the standard File Import dialog box.

2. Navigate to the location of the .xml file you want to import.

3. Select the .xml file and click **Open**.
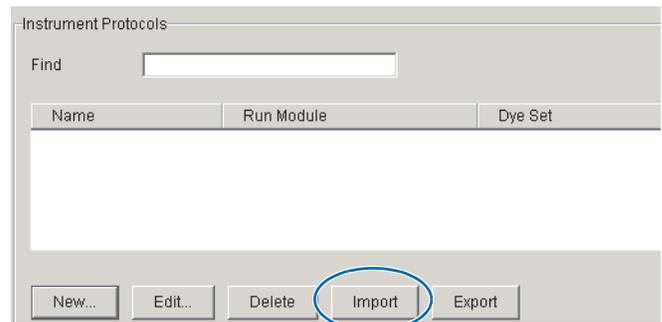
Exporting an Instrument Protocol

1. In the Instrument Protocols pane, highlight the protocol you want to export.

2. Click Export to display the standard File Export dialog box.

3. Browse to the desired folder location.

4. Click Save .

Notes

Analysis Protocol for Sequencing Analysis

An analysis protocol contains all the settings
necessary for analysis and post processing:

- **Protocol name** – The name, description of the
  analysis protocol, and the sequence file formats
  to be used
- **Basecalling settings** – The basecaller,
  DyeSet/Primer file, and analysis stop point to be
  used
- **Mixed Bases** (Optional) – When two bases are
  found at the same position. Define the percent
  value of the second highest to the highest peak
- **Clear Range** – The high quality sequence
  remaining after trimming low quality sequences
  typically found at the beginning and ends of the
  sequence. The clear range can be 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

See the *Applied Biosystems DNA Sequencing Analysis
Software v5.2 User Guide* (P/N 4346366) for more
information on 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.

    Note: When you name your analysis protocol,
    assign a version name to help you distinguish
    your analysis protocols.
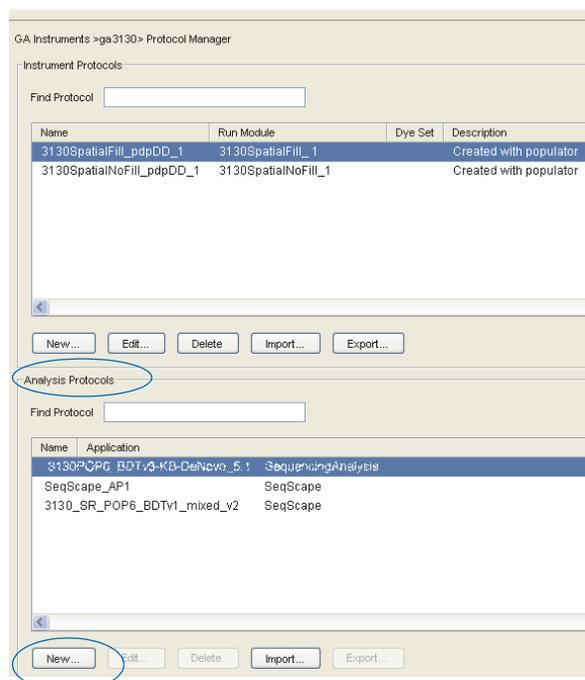
2. Select **Sequencing Analysis**, then click `OK` to open the Analysis Protocol Editor dialog box.
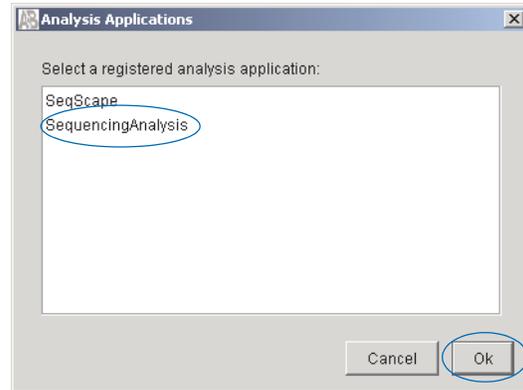
3. In the **General** tab:

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

    b. Select the appropriate Sequence File formats settings.

Option	Creates…
Write .Seq File check box	A .seq file for printing the sequence as text file or for use in other software. • Use ABI format with Applied Biosystems software. • Use FASTA format with other software.
Write Standard Chromatogram Format file (.scf)	A .scf file to use with other software. When created, the .scf extension is not appended to the file name.
Write Phred (.phd.1) File	A .phd.1 file to use with other software if you used the KB™ basecaller.
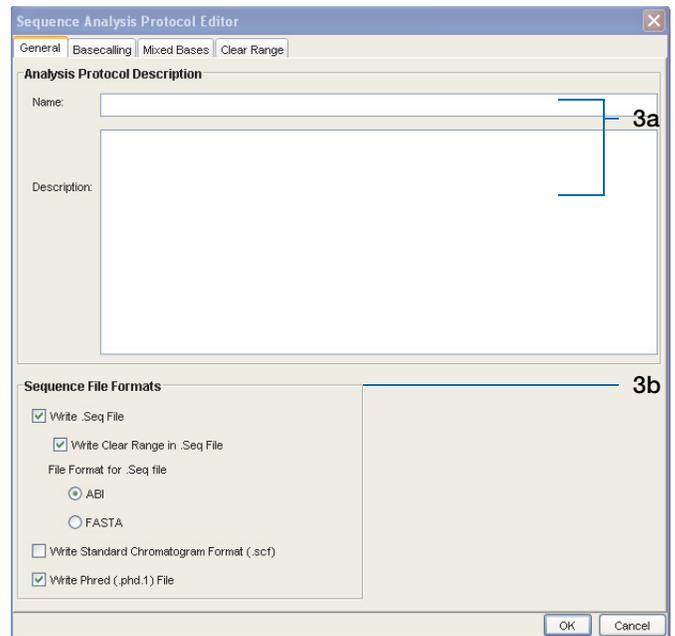
Notes

4. Select the **Basecalling** tab.

   a. See "3130/3130xl Genetic Analyzer Basecaller and DyeSet/Primer Files" on page 66 to select your basecaller and DyeSet primer file.

   Note: Sequencing Analysis Software and 3130/3130*xl* genetic analyzer Data Collection software filter .mob file choices to match the chosen .bcp file.
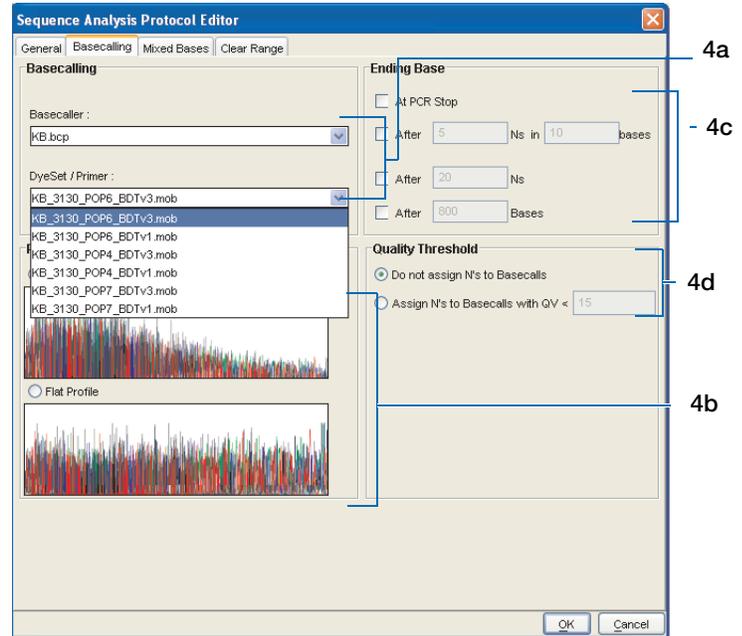
b. In the Processed data pane, select True or Flat Profile.

Option	Used to display data as processed traces scaled...
True Profile	Uniformly - 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	Semi-locally - 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	With the KB™ basecaller, use this setting to assign a base to every position, as well as the QV.
Assign 'N' for bases with QV < 15	With the KB basecaller, use this setting to assign Ns to base with QVs less than the set point. The QV is still displayed.

Notes

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

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

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

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

   **Note:** Do not use less than 15% as your detection limit.
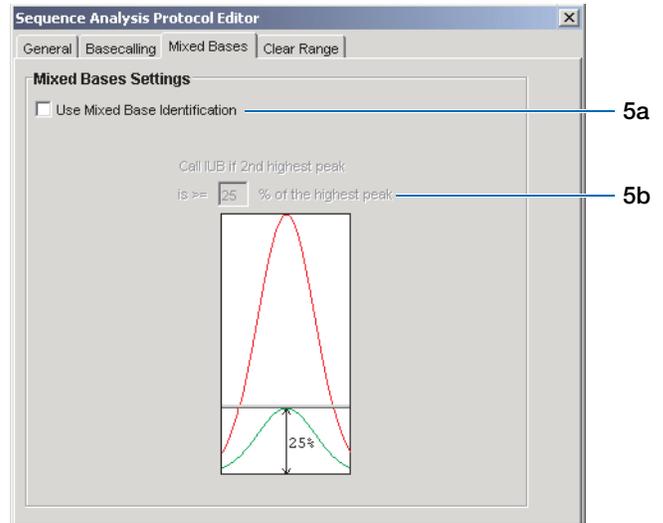
6. Select the **Clear Range** tab.

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.
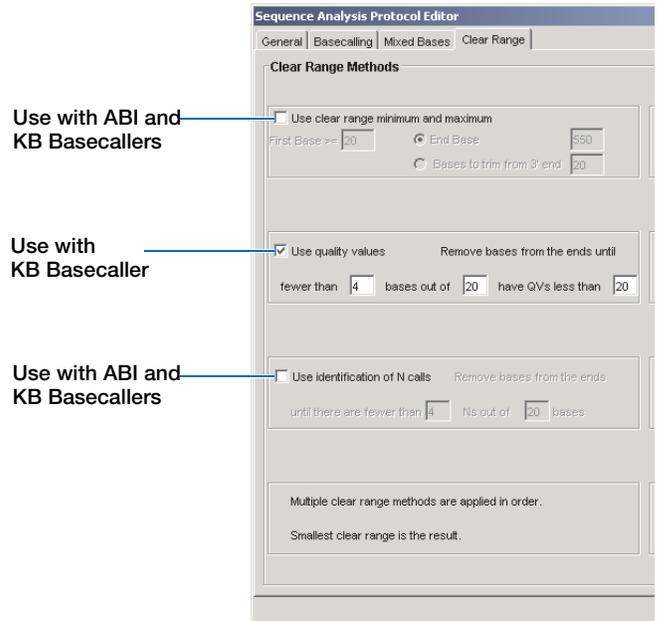
Notes _____

3130/3130xl Genetic Analyzer Basecaller and DyeSet/Primer Files

Basecaller and DyeSet/Primer Files Used with BigDye® Terminator Chemistry and KB Basecalling

DNA Sequencing Chemistry	Polymer	KB Basecalling Run Module	DyeSet/Primer	Basecaller
BigDye® Terminator v1.1 Cycle Sequencing Kit	POP-4™	UltraSeq36_POP4	KB_3130_POP4_BDTv1.mob	KB.bcp
		StdSeq50_POP4		
		LongSeq80_POP4		
	POP-6™	RapidSeq36_POP6	KB_3130_POP6_BDTv1.mob	
		StdSeq50_POP6		
	POP-7™	UltraSeq36_POP7	KB_3130_POP7_BDTv1.mob	
		RapidSeq36_POP7		
		FastSeq50_POP7		
		StdSeq50_POP7		
		LongSeq80_POP7		
BigDye® Terminator v3.1 Cycle Sequencing Kit	POP-4	UltraSeq36_POP4	KB_3130_POP4_BDTv3_.mob	
		StdSeq50_POP4		
		LongSeq80_POP4		
	POP-6	RapidSeq36_POP6	KB_3130_POP6_BDTv3.mob	
		StdSeq50_POP6		
	POP-7	UltraSeq36_POP7	KB_3130_POP7_BDTv3.mob	
		RapidSeq36_POP7		
		FastSeq50_POP7		
		StdSeq50_POP7		
		LongSeq80_POP7		

Basecaller and DyeSet/Primer Files Used with BigDye® Terminator Chemistry and ABI Basecalling

DNA Sequencing Chemistry	Polymer	ABI Basecalling Run Module	Basecaller	DyeSet/Primer
BigDye® Terminator v1.1 Kit	POP-4™	UltraSeq36_POP4	Basecaller-3130POP4UR.bcp	DT3130POP4LR{BD}v1.mob
		LongSeq80_POP4	Basecaller-3130POP4_80cmv3.bcp	
	POP-6™	RapidSeq36_POP6	Basecaller-3130POP6RRv2.bcp	DT3130POP6{BD}v2.mob
		StdSeq50_POP6	Basecaller-3130POP6SR.bcp	

Notes

Basecaller and DyeSet/Primer Files Used with BigDye® Terminator Chemistry and ABI Basecalling *(continued)*

BigDye Terminator v3.1 Cycle Sequencing Kit	POP-4	UltraSeq36_POP4	Basecaller-3130POP4UR.bcp	DT3130POP4{BDv3}v1.mob
		LongSeq80_POP4	Basecaller-3130POP4_80cmv3.bcp	
	POP-6	RapidSeq36_POP6	Basecaller-3130POP6RRv2.bcp	DT3130POP6{BDv3}v1.mob
		StdSeq50_POP6	Basecaller-3130POP6SRv2.bcp	
ABI PRISM® dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit	POP-4	UltraSeq36_POP4	Basecaller-3130APOP4UR.bcp	DT3130POP4{dRhod}v2.mob
		LongSeq80_POP4	Basecaller-3130POP4_80cmv3.bcp	
	POP-6	RapidSeq36_POP6	Basecaller-3130POP6RRv2.bcp	DT3130POP6{dRhod}v2.mob
		StdSeq50_POP6	Basecaller-3130POP6SR.bcp	

Dye Primer Chemistry

Basecaller and DyeSet/Primer Files Used for Dye Primer Chemistry

DNA Sequencing Chemistry	Polymer	ABI Basecalling Run Module	Basecaller	DyeSet/Primer
ABI PRISM® BigDye® Primer Cycle Sequencing Kit	POP-6	RapidSeq36_POP6	Basecaller-3130POP6RRv2.bcp	DP3130POP6{BD-21M13}v1.mob
		StdSeq50_POP6	Basecaller-3130POP6SR.bcp	DP3130POP6{BD-21M13Rev}v1.mob

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, if appropriate.

4. Click **OK** to save the protocol and close the Analysis Protocol Editor dialog box.
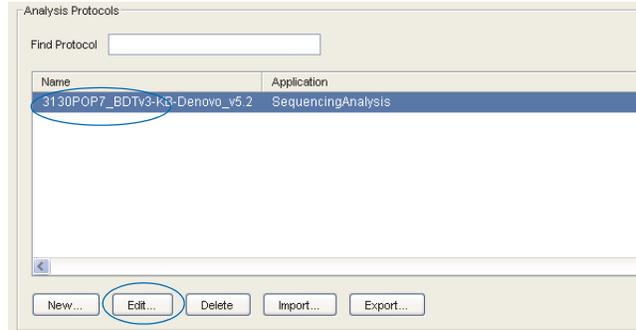
Deleting an Analysis Protocol

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. 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** to display the Deletion Confirmation dialog box.

3. Click **Yes** .
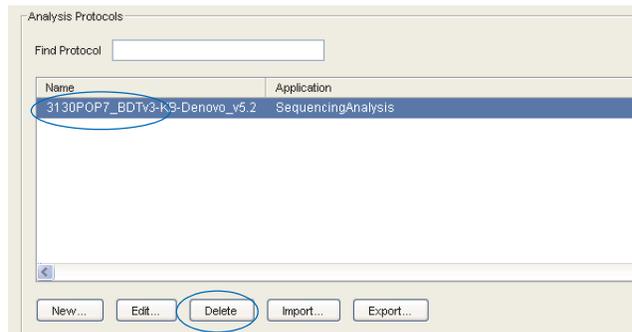
Exporting an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to export.

2. Click Export to display the standard File Export dialog box.

3. Browse to the desired folder location.

4. Click **Save**.
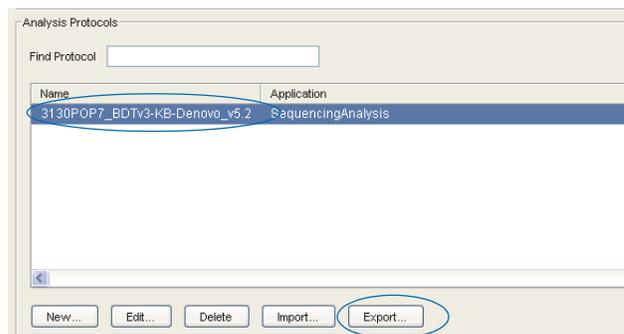
Importing an Analysis Protocol

1. Click Import to display the standard File Import dialog box.

2. Browse for the .xml file to import and click **Open**.

> **Note:** For multiple applications, select the appropriate application to associate with the analysis protocol.
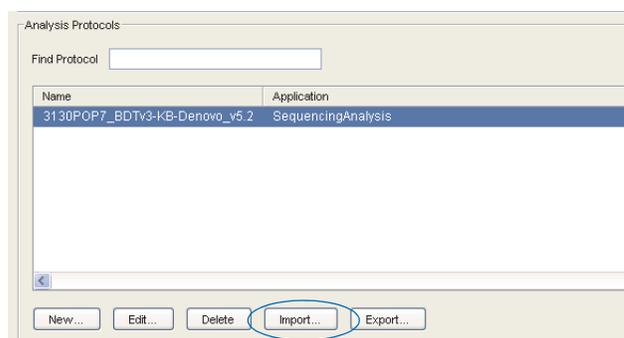
Results Group for Sequencing Analysis

A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. It is called a Results Group because it is used to 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... to display the Results Group Editor window.
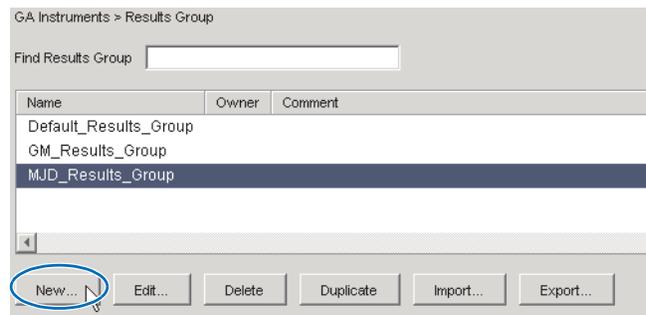
3. Complete the General tab:

    a. Type a unique Results Group Name. The name can be used in grouping sample files.

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

    c. Type a Results Group Comment (optional).
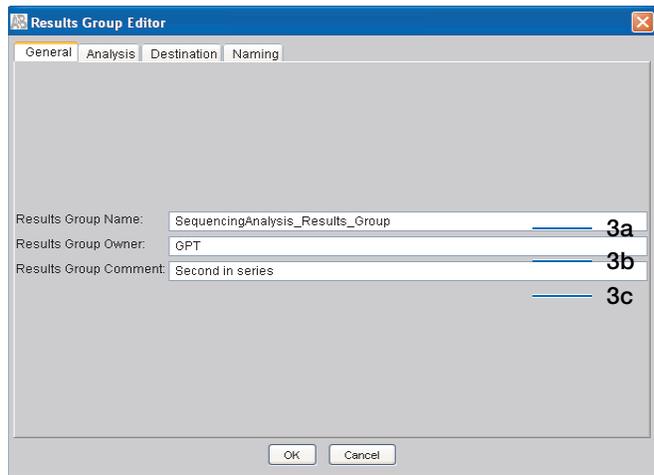
4. Select the **Analysis** tab, then:

   a. Select **Sequencing Analysis** from the Analysis Type drop-down list.

   b. In the Analysis Actions section, select **Do Autoanalysis**, if you want your data automatically analyzed after a run.

   Note: Login ID and password are not required for Sequencing Analysis software.
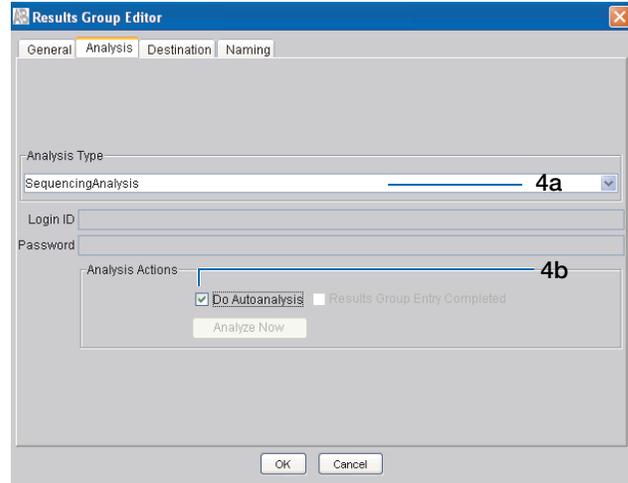
5. Select the **Destination** tab, then use the default destination or define a new location for data storage.

To use …	Then …
default location*	skip to step 6.
custom location	complete steps a-b.

   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…	Then a message box displays …
Passes	Test succeeded: <"path">.
Fails	Test failed:<"path">.

---
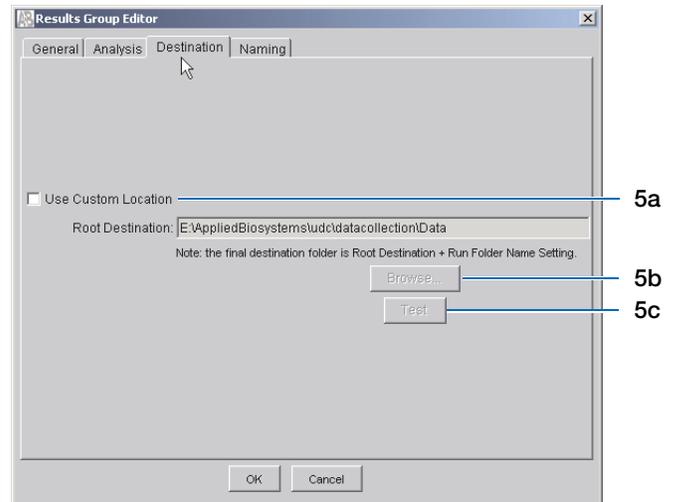
*Sample File Destinations

Locations where sample files are placed during extraction:

- Default Destination, default folder naming: E:\AppliedBiosystemsUDC\DataCollection\data*<instrument type>*\ *<instrument name>*\run folder
- Default Destination, custom folder naming: E:\AppliedBiosystemsUDC\DataCollection\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 customer folder\subfolders, etc.

Notes

6. Select the **Naming** tab.

Use the Naming tab to customize sample file and run folder names. You have two options:

- Use the default names for the folder and sample file.
- Use custom names for the folder and sample file. See "Optional: Completing Sample File Name Format Pane" on page 73 for elements of the Naming tab.

Note: Sample name, run folder name, and path name, *combined*, can total no more than 250 characters.

Sample File Name Format pane

Run Folder Name Format pane
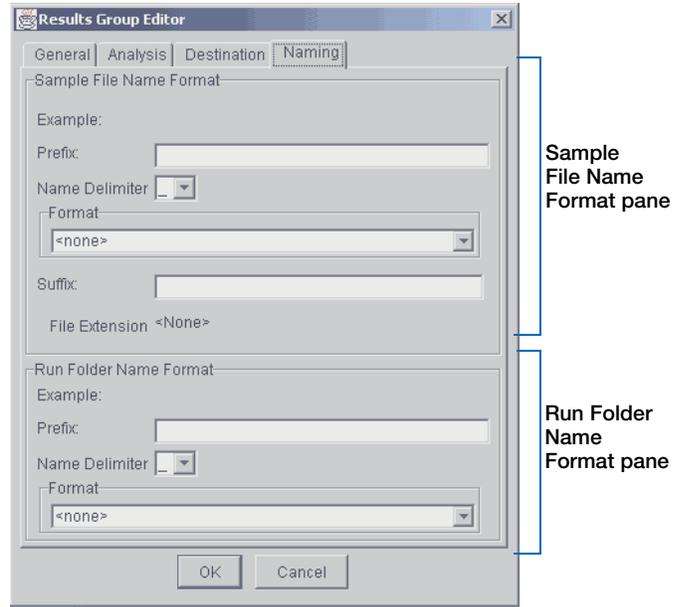
Optional: Completing Sample File Name Format Pane

Note: The default sample file naming for sequencing analysis is:
seq_<capillary number>_<wellposition>.ab1

1. Select the **Naming** tab.
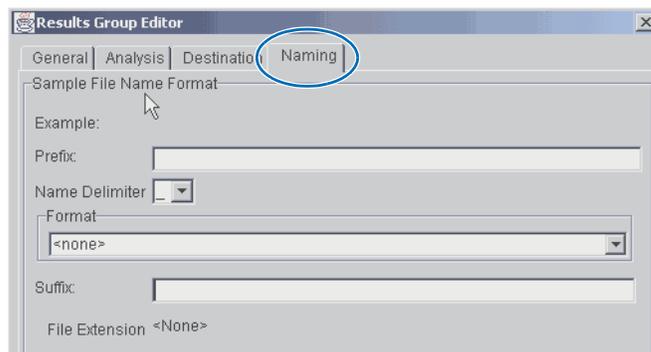
2. Type a prefix for the file name in the Prefix box. Your entry is shown in the Example line.
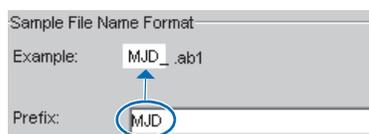
3. Select the symbol that separates the Format elements in the file name from the **Name Delimiter** drop-down list. Only one delimiter symbol may be chosen.
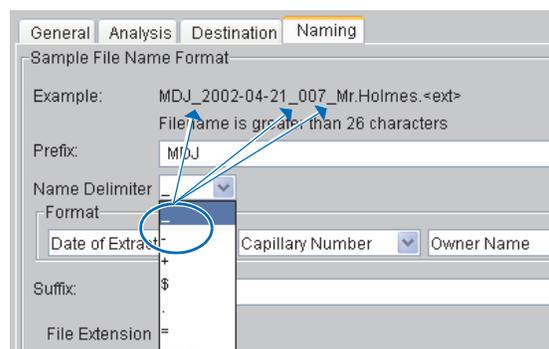
4. Click the Format list and then select the components that you want in the sample name.
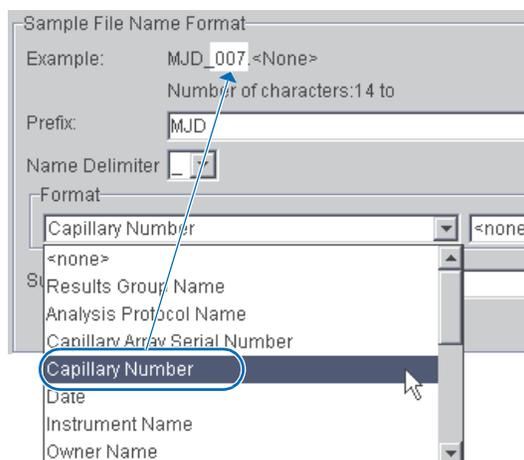
Note: All the samples from a single run can be placed in the same run or results folder, so the name of every sample from a single run should be different. Most of the Format options are not different between samples, so select at least one of the options that make the sample names unique within a run.

Notes

If a unique identifier is not included in the name, a warning message displays. Select from the elements to make a unique sample file name. Examples are:

– Well position

– Capillary number

– Run sequence number

– Plate Quadrant

Warning message

Note: some identifiers, such as well position, capillary number and plate quadrant are not unique in multiple sample instances. Plate Quadrant is not a unique identifier for a 96-well plate.

As you select the elements for the file name, they are placed in the Example line. An additional element drop-down menu displays allowing you the option of selecting an additional element.
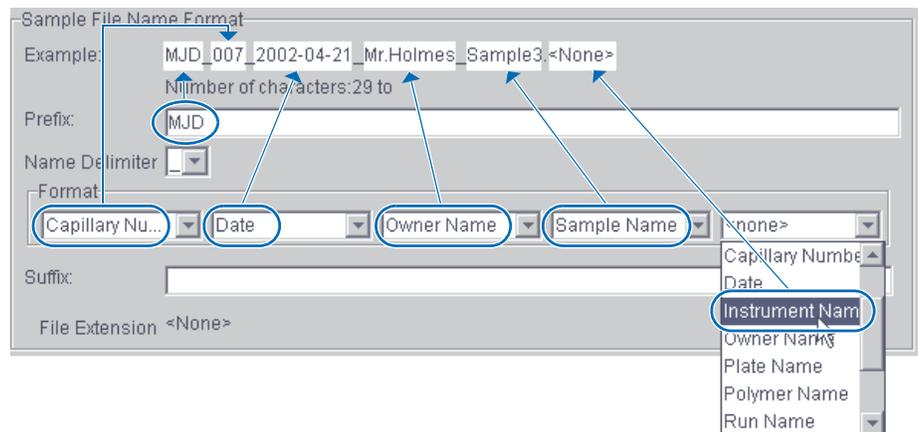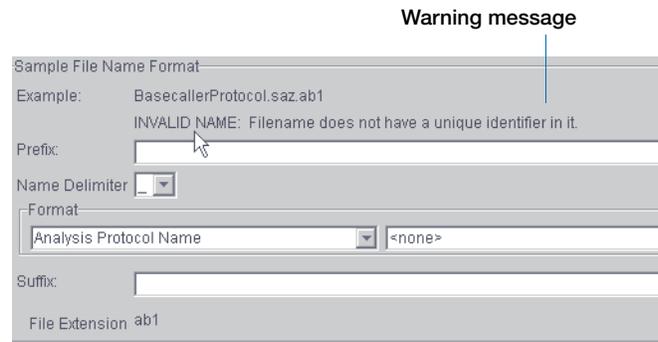
The names of the Format elements eventually truncate, but the Example field remains visible (up to 72 characters).

Note: Resize the Results Group window horizontally to view the element options.
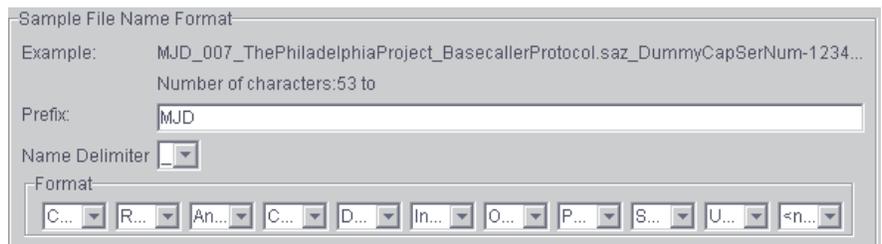
5. Type the suffix for the file name in the Suffix box.

> **Note:** The File Extension field displays the file extension generated from the Analysis Type specified on the Analysis tab (see page 71). For example, Sequencing Analysis produces sample files with an .ab1 extension.
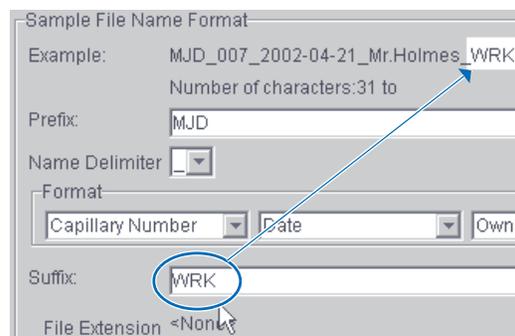
Optional: Run Folder/Sub-Folder Name Format Pane

> **Note:** The default run folder naming for sequencing analysis is Run_<Instrumentname>_<Dateand TimeofRun>_<RunSeq#>.

Follow the same steps described for the Sample File Name Format pane (see page 73) to change the sub-folder name within the run folder. Make the sub-folder name unique, such as using the Run Folder name.

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, the system generates a separate default run folder that contains the log file.

Importing and Exporting a Results Group

Results Groups can be imported from, or exported to, XML files allowing 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] to display the standard File Import dialog box.

Notes

3. Navigate to the file you want to import.

   Note: Import file type is .xml (XML file).

4. Click [Open] .

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] .
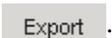
   Note: If there is a name conflict with a Results Group that already exists at the save location, then duplicate the Results group. Duplication copies the settings into a similar Results Group without the risk of user error when copying it manually (see procedure below).

Duplicating a Results Group

1. Click the Results Group name.

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

Filling Out a Sequencing Analysis Plate Record

Creating a Sequencing Analysis Plate Record

1. In the tree pane of the Data Collection software, click ⛰ **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > ⌗ **Plate Manager**.

2. Click ⌷New...⌷ to display the New Plate Dialog dialog box.

3. Complete the information in the New Plate Dialog:

    a. Type a name for the plate.

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

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

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

    e. Type a name for the owner and operator.

    f. Click ⌷ OK ⌷ to open the Sequencing Analysis Plate Editor.
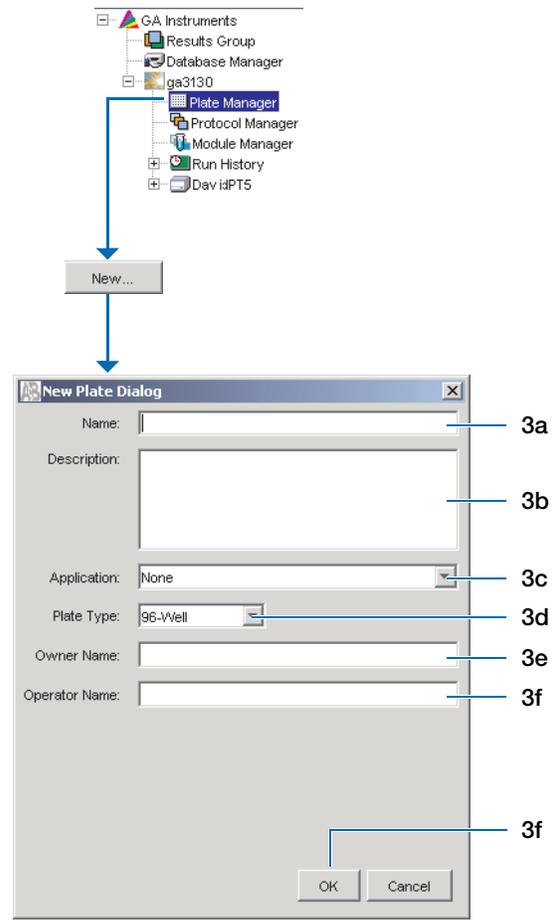
Notes

Completing a Sequencing Analysis Plate Record

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

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

3. In the **Priority** column, change the priority value, if desired. A lower number for each 4 or 16 set, receives a higher run priority.

4. In the **Results Group 1** column, select a group from the drop-down list (see page 70) or create a new Results Group.

5. In the **Instrument Protocol 1** column, select a protocol from the drop-down list (see page 59) or create a new instrument protocol.

6. In the **Analysis Protocol 1** column, select a protocol from the drop-down list (see page 62) or create a new analysis protocol.

7. Complete the plate record based on the samples loaded in your plate:

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

     Based on your plate type (96- or 384-well) and capillary array (16 or 4 capillaries), the software automatically fills in the appropriate well numbers for a single run

     or

   - For entire plates that use the same samples and protocols – Highlight the entire row, then select **Edit > Fill Down** to fill down the entire plate editor.

   - For plates with different samples and protocols – complete the entries manually.

Well	Sample Name	Comment	Priority	Results Group 1
A01				
B01				
C01				
D01				
E01				
F01				

Instrument Protocol 1	Analysis Protocol 1

Edit	
Fill Down	Ctrl+D
Copy	Ctrl+C
Paste	Ctrl+V
Clear row(s)	Shift+Delete
Fill Down Special	Alt+D
Add Sample Run	Alt+A

Notes

8. To do more than one injection of the same sample, select **Edit > Add Sample Run**.

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

Edit	
Fill Down	Ctrl+D
Copy	Ctrl+C
Paste	Ctrl+V
Clear row(s)	Shift+Delete
Fill Down Special	Alt+D
Add Sample Run	Alt+A

9. Complete the columns for the additional runs.

10. Click OK .

Note: After clicking OK in the Plate Editor, the completed plate record is stored in the Plate Manager database. The plate record can be searched for, edited, duplicated, exported, or deleted in the Plate Manager.

4

Notes

Notes

Autoanalysis and
SeqScape Software

Preparing the
Instrument

Performing a
Spatial Calibration

Performing a
Spectral Calibration

Autoanalysis
and Sequencing
Analysis
Software

**Autoanalysis
and
SeqScape Software**

Create protocols
for automated
SeqScape Software Analysis

Create a
SeqScape Software
Plate Record

Autoanalysis
and
Fragment Analysis

Running the
Instrument
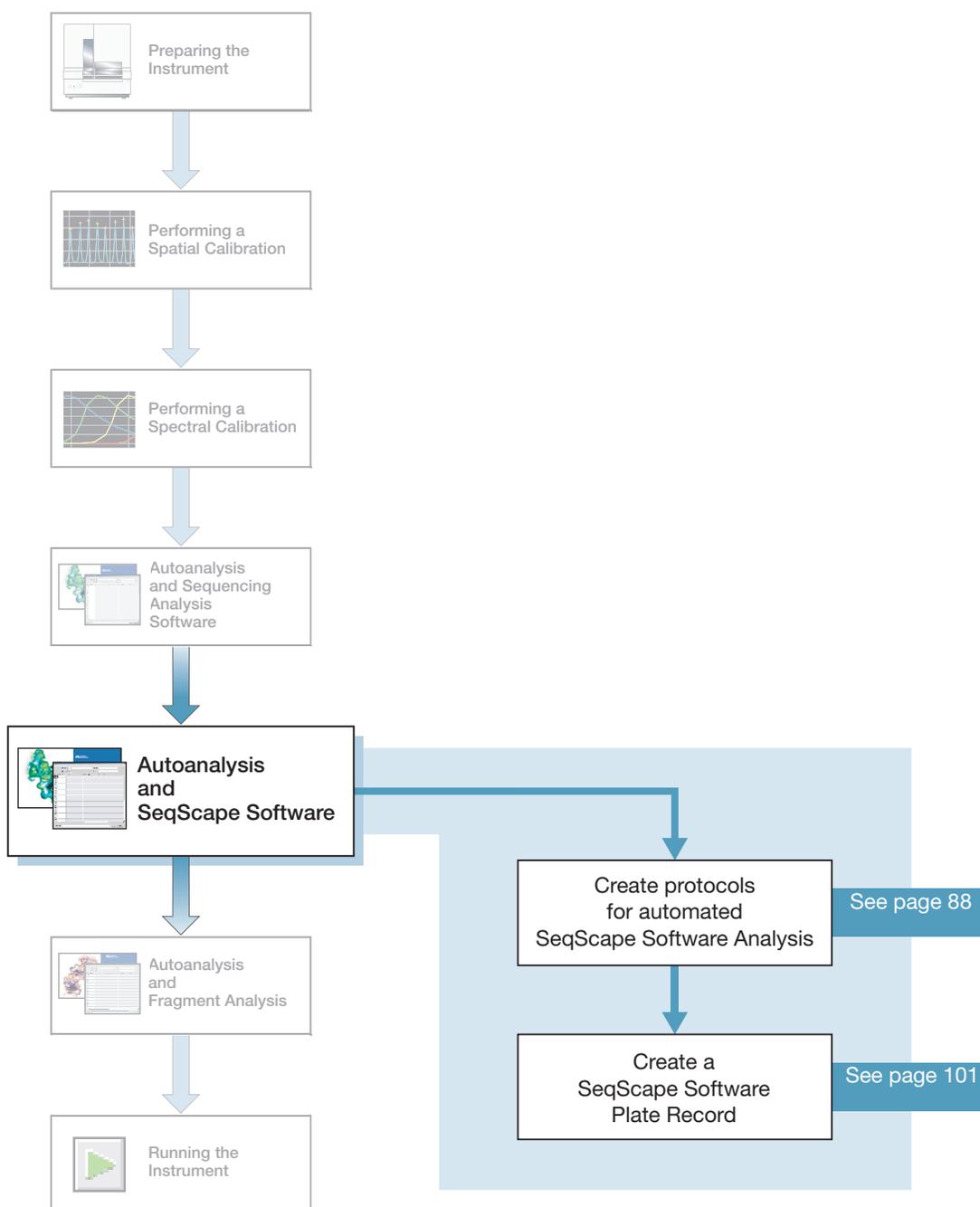
Notes

SeqScape Software

Analyzing your sequencing samples may be done by autoanalysis or manual analysis.

Autoanalysis Use the ABI PRISM® SeqScape® software to automatically analyze Applied Biosystems 3130/3130*xl* Genetic Analyzers sequencing data. Perform autoanalysis only on the same instrument computer that collected the sample files. You can configure the software packages to perform data collection and then data analysis without requiring user interaction.

Install and register the SeqScape software with the 3130/3130*xl* genetic analyzer Data Collection software before creating required files for autoanalysis.

See Appendix B in the *SeqScape Software User Guide v2.5* (P/N 4359442), for more details on setting up autoanalysis.

Required Software Packages Autoanalysis requires three software packages:

- 3130/3130*xl* genetic analyzer Data Collection software

  The Data Collection software runs the instrument and collects fluorescent data from samples. For autoanalysis to occur, the software must communicate with downstream software.

  Analysis parameters used for data collection as well as that created in SeqScape software are accessible in Data Collection software.

- Autoanalysis Manager

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

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

- A version of SeqScape software without a user interface

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

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

IMPORTANT! When installing SeqScape software on a computer that is connected to a 3130/3130*xl* genetic analyzer, the Data Collection software must be running. Otherwise, the SeqScape software does not register with the Data Service. See Chapter 2 in the *SeqScape Software User Guide v2.5*, for information on installing the software.

Notes _____

Importing Reports and Projects After Autoanalysis

In the SeqScape Software v2.5, select **Tools** > **Options** tab. You can set the options to automatically import:

- Reports after autoanalysis
- Projects after autoanalysis

See the *SeqScape Software User Guide v2.5* (P/N 4359442) for more details.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. Do not use the invalid characters below:

spaces

\ / : * ? " < > |

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

Manual Analysis

See the *SeqScape Software User Guide v2.5* (P/N 4359442) for information on performing manual analysis.

About Plate Records and Seqscape Software

Requirements

Successful automatic analysis requires that:

- SeqScape software is installed properly
- SeqScape software is registered and the appropriate user IDs have been created
- Autoanalysis Manager software is running
- The 3130/3130*xl* genetic analyzer is set up to run, and samples are prepared
- Files for a Data Collection software plate record are available:
  - An instrument protocol
  - An analysis protocol
  - A results group assigned within a plate record

When to Create a Plate Record

Create a plate record for each plate of samples for the following types of runs:

- Spectral calibrations
- Sequencing Analysis Software
- SeqScape Software analysis
- GeneMapper® software
- Mixed (sequencing and fragment analysis samples - see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide* for creating a mixed plate record)

Notes _____

For runs to begin, you must create a plate record for and link it to a plate loaded on the instrument. However, you can create plate records for new plates while a run is in progress.

SeqScape Software Plate Record Overview

The Plate Editor displays an empty plate record for the application selected in the New Plate dialog box. The data fields within a given plate record vary depending on the application. This section describes the SeqScape Plate Record's data fields.

IMPORTANT! For data collection and SeqScape software autoanalysis to succeed, each run of samples must have an instrument protocol, an analysis protocol, and a results group assigned within a plate record. You are also required to have a project, project template, and specimen information.

The table below describes required plate editor fields.

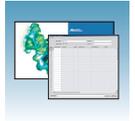| Parameters	Description	See Page
Instrument Protocol	Contains the run module and dye set needed to run the instrument. Created in Data Collection software.	88
Analysis Protocol	Contains everything needed to analyze sequencing data. Created in Data Collection software or SeqScape software. If you prefer to use analysis protocols created in SeqScape software, then select "Always use this protocol" - in the SeqScape software analysis defaults settings.	92
Results Group	Defines the file type, the file name, file save locations, default analysis protocols linked to sample injections, and user name and password. Created in Data Collection software.	98
Project	A group of related sequences that share the same reference or for which there is no explicit reference.	See the *SeqScape Software User Guide v2.5* for more information
Project Template	Contains a reference data group (RDG), analysis defaults, and display settings.	
Specimen	The container that holds all the sample data as assembled contigs from a biological source or PCR product	

Notes
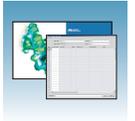
Elements of a SeqScape Plate Record

```
                    Plate Manager
                         |
                    Plate Record
                         |
        ┌────────────────┴───────────────────────────────────────┐
 Data Collection                                          SeqScape software
 software files:                                          files:
        |                                                         |
   ┌────┼──────────────┬──────────────┐              ┌────────────┼──────────┐
Results Group   Instrument Protocol  Analysis Protocol*   Project Template
                                                          - Analysis Protocol*
  Results Group   Instrument          Analysis Protocol   - Analysis Defaults
  Name            Protocol Name       Name                - RDG
                                                          - Display Settings
  Analysis software Run type          Basecaller and
  and autoanalysis  (Regular)         DyeSet/Primer file     Project
  File storage     Run module         Bases called
  location                            (pure or mixed)        Specimen
  File and run folder Dye set         Assigning Quality
  name preferences                    Values (QVs)
                                      Post processing
                                      (clear range
                                        trimming)
                                      Filter settings
```

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

Notes

SeqScape Software Plate Record

A Seqscape software plate record displaying the columns and descriptions is shown below.
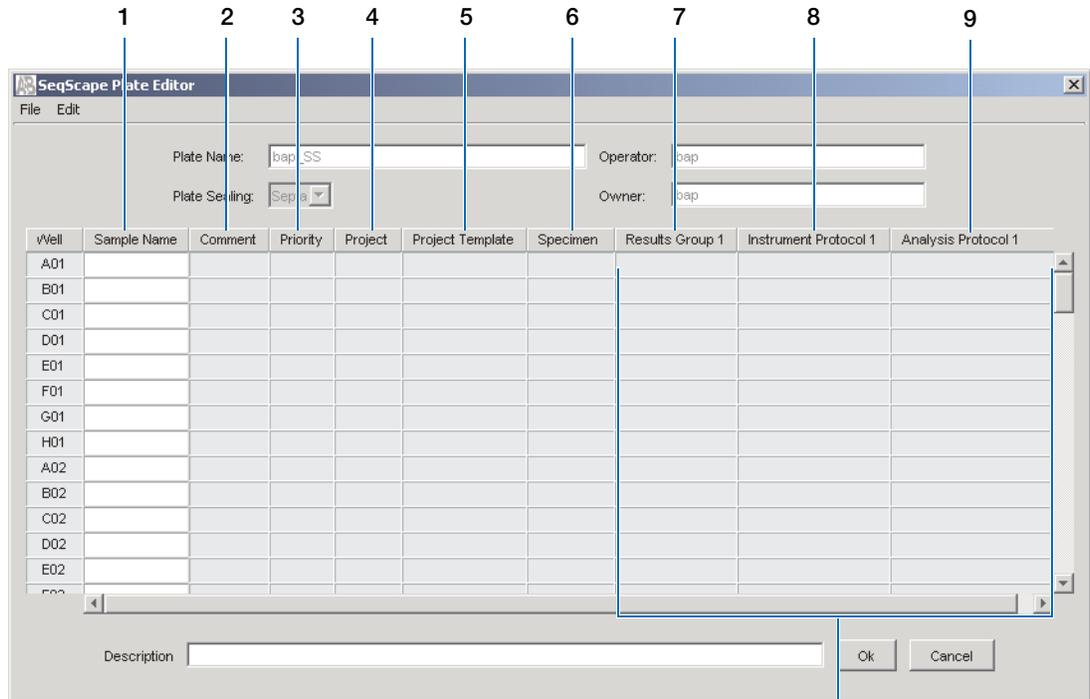
Default is one sample run, to add runs see

Number and Column		Description
1.	Sample Name	Name of the sample.
2.	Comment	Comments about the sample (optional).
3.	Priority	A default value of 100 to each sample. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list.
4.	Project	Select one of the available Project from the list that was created in Data Collection or SeqScape software.
5.	Project Template	Completed automatically based on the Project selected.
6.	Specimen	Select one of the available Specimen from the list that was created in Data Collection or SeqScape software.

Number and Column	Description
7. Results Group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the Results Group listed in the cell • None: Sets the cell to have no selected Results Group • Select one of the available Results groups from the list **Note:** You must have a Results Group selected for each sample entered in the Sample Name column. See "Creating a Results Group" on page 98.
8. Instrument Protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. **Note:** You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See "Creating an Instrument Protocol" on page 88.
9. Analysis Protocol	• New: Opens the Analysis Protocol Editor dialog box. • Edit: Opens the Analysis Protocol Editor dialog box for the Instrument Protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Analysis Protocols: In alpha-numeric order **Note:** You must have an Analysis Protocol selected for each sample entered in the Sample Name column. See "Creating an Analysis Protocol" on page 93.

5

Notes

Creating Protocols for Automated SeqScape Software Analysis

If the appropriate SeqScape software protocols and results group have been created, proceed to "Filling Out a SeqScape Software Plate Record" on page 101.

Instrument Protocol for SeqScape Software

An instrument protocol contains all the necessary settings to run the instrument:

- Protocol name
- Type of run
- Run module
- Dye set

Creating an Instrument Protocol

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▦ **ga3130** or **ga3130***xl* > ▦ **Protocol Manager**.
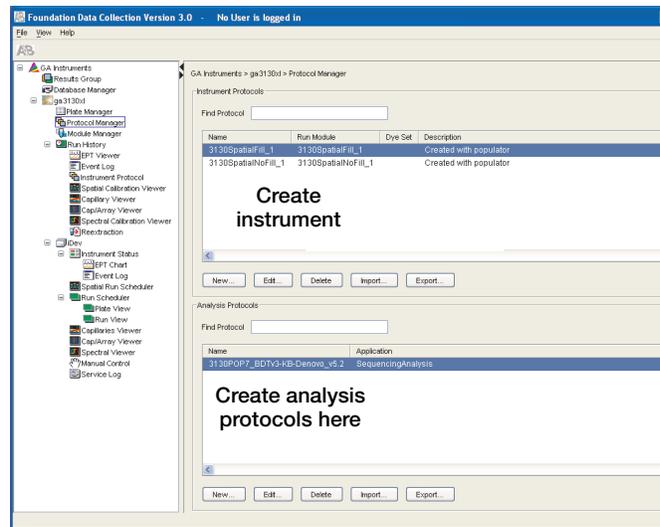
2. In the Instrument Protocols section, click **New...** to open the Protocol Editor.

3. Complete the Protocol Editor:

    a. Type a name for the protocol.

    b. Type a description for the protocol (optional).
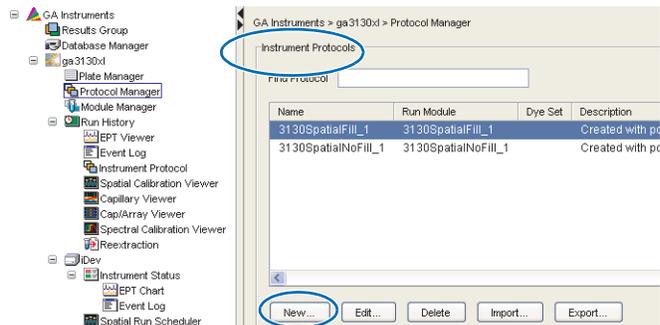
    c. Select **Regular** in the Type drop-down list.

Protocol Editor	✕
Name:	———— 3a
Description:	———— 3b
Type: REGULAR	———— 3c
Run Module: FragmentAnalysis22_POP4_1	———— 3d
Dye Set: E5	———— 3e
	———— 3f
OK Cancel	
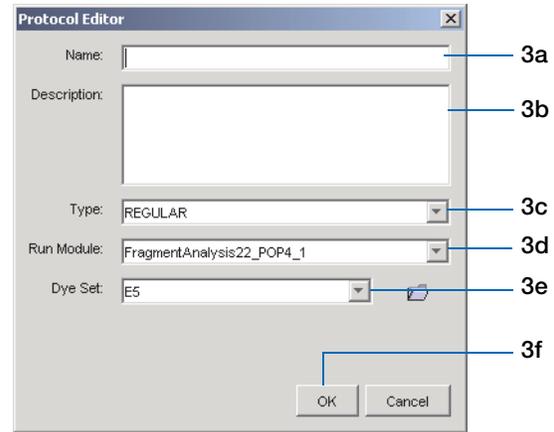
    d. Select the correct run module for your run. See "Sequencing Resolution Performance and Specifications" on page 3 to see a list of modules or the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide* to modify a default module.

Notes

e. Select the correct Dye Set for your run using the table below.

Chemistry	Dye Set	POP-4 Polymer			POP-6 Polymer		POP-7 Polymer				
		UltraSeq36	StdSeq50	LongSeq80	RapidSeq36	StdSeq50	UltraSeq36	RapidSeq36	FastSeq50	StdSeq50	LongSeq80
BigDye® Terminator v3.1 Cycle Sequencing Kit	Z_BigDye V3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ABI PRISM® dGTP BigDye® Terminator v3.0 Cycle Sequencing Ready Reaction Kit		—	—	—	—	—	—	—	—	—	—
BigDye® Terminator v1.1 Cycle Sequencing Kit	E_BigDye V1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ABI PRISM® dGTP BigDye® Terminator Cycle Sequencing Kit*		—	—	—	—	—	—	—	—	—	—
ABI PRISM® dRhodamine Dye Terminator Cycle Sequencing Ready Reaction Kit		✓	✓	✓	✓	✓	—	—	—	—	—
ABI PRISM® BigDye® Primer Cycle Sequencing Kits		—	—	—	✓	✓	—	—	—	—	—

*dGTP kits are not supported on capillary electrophoresis instruments due to compressions on certain sequence context regions; you can run the kits if you do not care about the compression issues.

f. Click   OK .

Notes

Importing an Instrument Protocol

1. Click [Import] in the Instrument Protocols pane of the Protocol Editor window to display the standard File Import dialog box.

2. Navigate to the location of the .xml file you want to import.

3. Select the .xml file and click **Open**.

Exporting an Instrument Protocol

1. In the Instrument Protocols pane, highlight the protocol you want to export.

2. Click [Export] to display the standard File Export dialog box.

3. Browse to the desired folder location.

4. Click [Save].

Notes

Analysis Protocol for SeqScape Software

About Analysis Protocols

An analysis protocol contains all the settings necessary for analysis and post processing:

- **Protocol name** – The name, description of the analysis protocol, and the sequence file formats to be used
- **Basecalling settings** – The basecaller, DyeSet/Primer file, and analysis stop point to be used
- **Mixed Bases** – (Optional) – When two bases are found at the same position. Define the percent value of the second highest to the highest peak
- **Clear Range** – The high quality sequence remaining after trimming low quality sequences typically found at the beginning and ends of the sequence. The clear range can be based on base positions, sample quality values, and/or number of ambiguities (Ns) present
- **Filter** – The settings that define which samples will be used in the assembly

Note: If you created an appropriate analysis protocol in the SeqScape software, you can use it in the Data Collection software. See Appendix B in the *SeqScape Software User Guide v2.5* (P/N 4359442) for more information on autoanalysis and analysis protocols.

IMPORTANT! Do not delete the current analysis protocol being used, during a run. Autoanalysis will not be performed if you do so.

IMPORTANT! You are required to select the Always use this Analysis Protocol in Analysis Defaults > Analysis Protocol in the Seqscape software if you want to use it for SeqScape analysis. See the *SeqScape Software User Guide v2.5* (P/N 4359442) for more information.

Creating an Analysis Protocol

IMPORTANT! If you created an appropriate analysis protocol in SeqScape software, you can use it in the Data Collection software. You can also create an analysis protocol in the SeqScape software.

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

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

2. Select **SeqScape**, then click OK to open the Analysis Protocol Editor dialog box.

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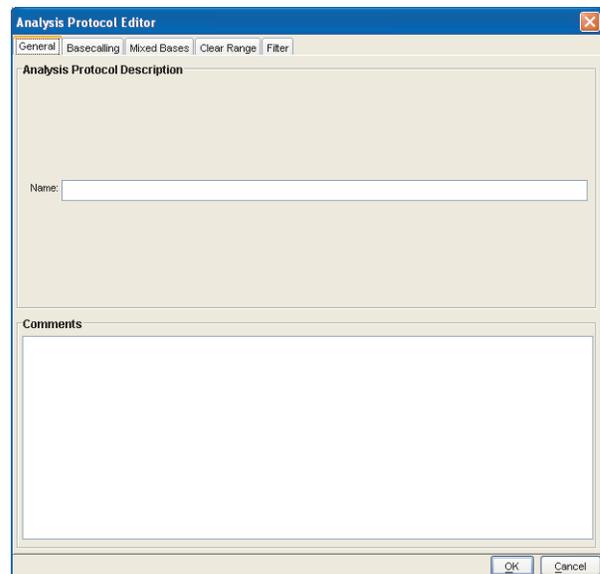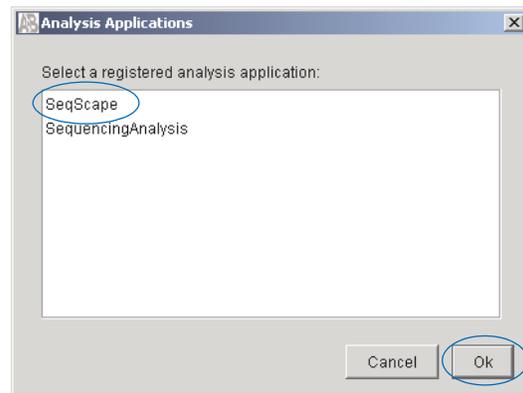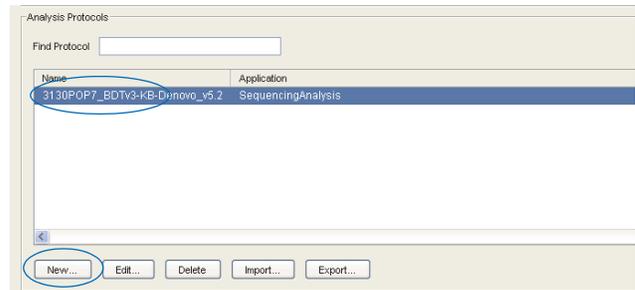
Notes

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

  a. Select the appropriate basecaller and DyeSet primer based on the chemistry, capillary array length and polymer type you are using. The dye set primer files displayed are based on the basecaller selected. See "3130/3130xl Genetic Analyzer Basecaller and DyeSet/Primer Files" on page 66 for a table on basecallers and dyeset/primer files.

  Note: SeqScape Software and the 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	Displays data as processed traces scaled uniformly. The average peak height in the region of strongest signal is about equal to a fixed value. The profile of the processed traces is similar to that of the raw traces.
⊙ Flat Profile	Displays data as processed traces scaled semi-locally. The average peak height in any region is about equal to a fixed value. The profile of the processed traces is flat on an intermediate scale (> about 40 bases). **Note:** This option is applied to data 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 setting	Function using KB basecaller
⊙ Call all bases and assign QV	Assigns a base to every position, as well as the QV.
⊙ Assign 'N' for bases with QV < 15	Assigns Ns to bases with QVs less than the set point. The QV will still be displayed.

Notes

5. Select and fill in the **Mixed Bases** tab:

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

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

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

   Note: Do not use less than 15% as your detection limit.
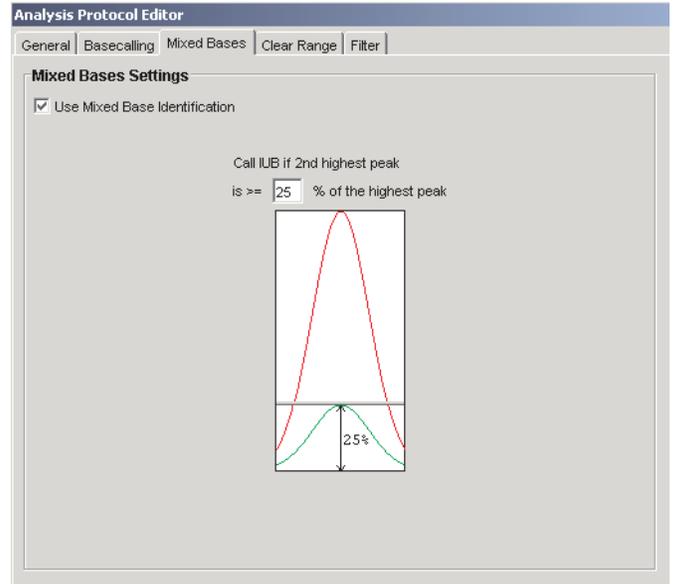
6. Select the **Clear Range** tab, then, if desired, select one or more stop points for data analysis. Select the recommended settings, which are the default settings:

   a. Use Quality values, fewer than **4** bases out of **20** have QVs less than **20**.

   b. Use reference trimming.

Note: The clear range is the region of the sequence that remains after excluding the low-quality or error-prone sequence at both the 5´ and 3´ ends.
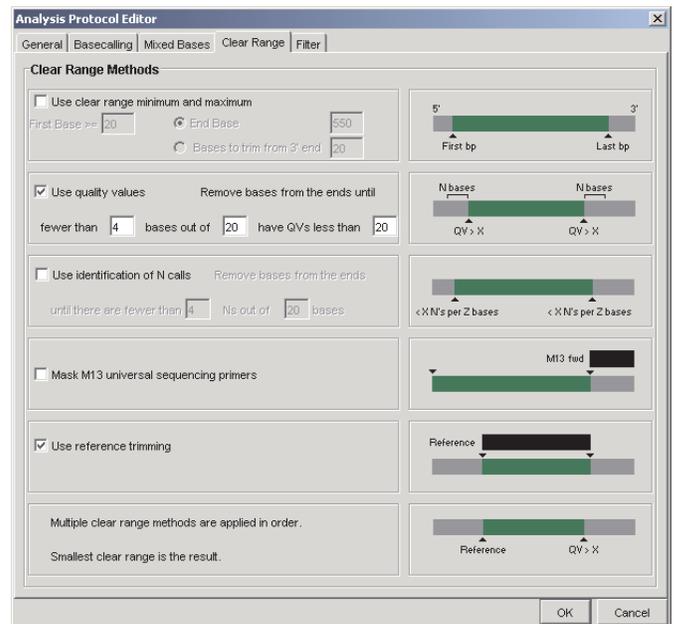
7. Select the **Filter** tab, then, if desired, change one or more of the settings.Select the recommended settings, which are the default settings:

    a. Maximum Mixed Bases (%): **20**

    b. Maximum Ns (%): **10.0**

    c. Minimum Clear Length (bp): **50**

    d. Minimum Sample Score: **20**
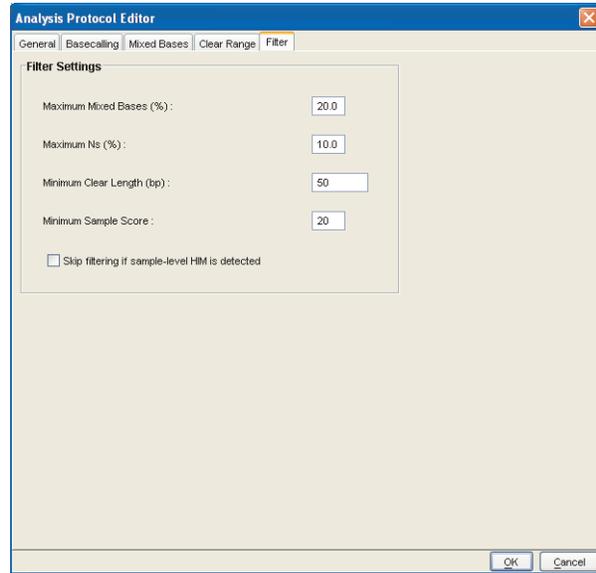
8. Click [OK] to save the protocol and close the Analysis Protocol Editor dialog box.

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.
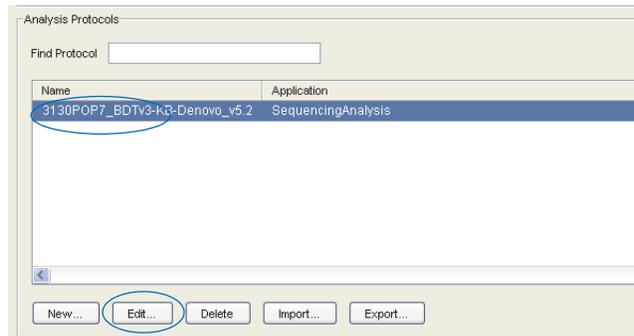
Deleting an Analysis Protocol

IMPORTANT! Do not delete an analysis protocol during a run while it is being used for that run or Autoanalysis will not be performed. 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 .
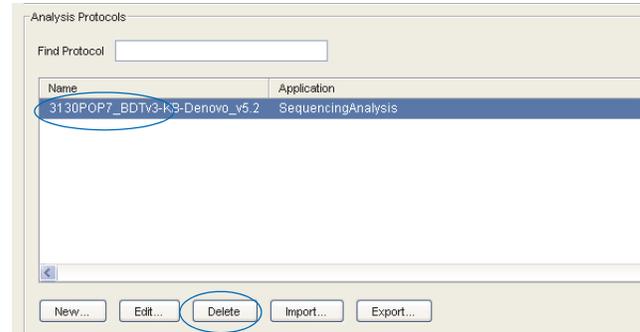
Exporting an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, highlight the protocol you want to export.

2. Click Export to display the standard File Export dialog box.

3. Browse to the desired folder location.

4. Click **Save**.

Importing an Analysis Protocol

1. Click Import to display the standard File Import dialog box.

2. Browse to the .xml file to import and click **Open**.

   Note: For multiple applications, select the appropriate application to associate with the analysis protocol.

Notes

Results Group for SeqScape Software

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 name, sort, and deliver samples that result from a run.

Creating a Results Group

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖫 **Results Group**.

2. Click New... .

The Results Group Editor window displays.
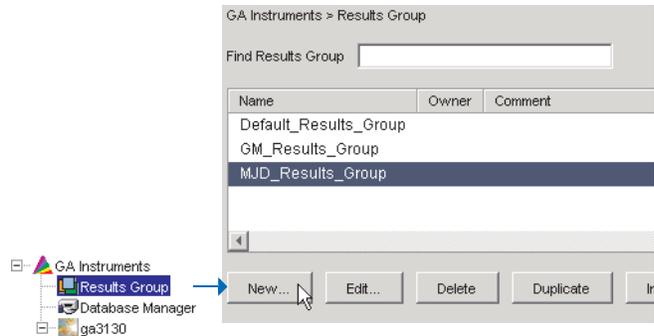
3. Complete the General tab:

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

    b. Optional: Type a Results Group Owner. The owner name can be used in naming and sorting sample files.

    c. Optional: Type a Results Group Comment.

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

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

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

    c. Type a valid SeqScape Software Login ID and Password in the text boxes.
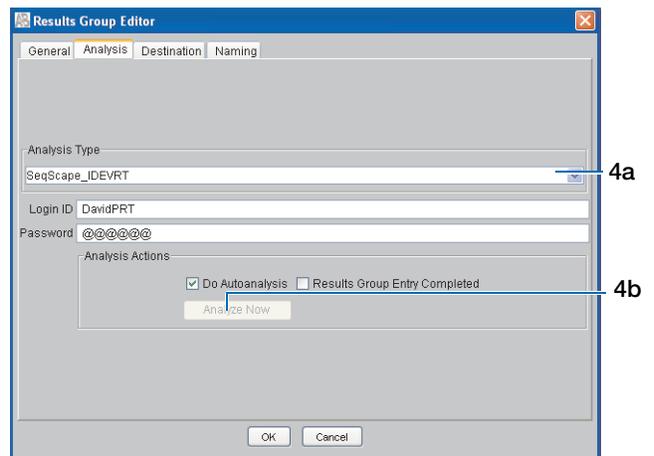
    Note: Failure to use the proper login and password causes your samples not to be analyzed automatically.

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

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

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

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

If it…	Then a message box displays …
Passes	Test succeeded: <"path">.
Fails	Test failed:<"path">.
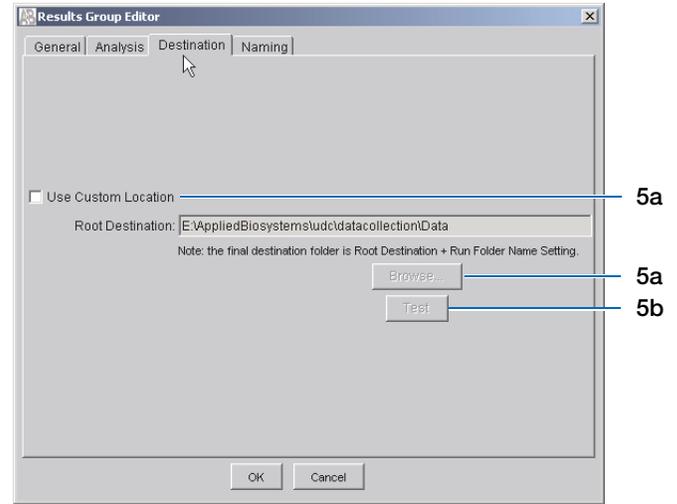
6. Select the **Naming** tab, then define custom names for the sample file and the run folder name. See "Optional: Completing Sample File Name Format Pane" on page 73 for more detailed information.

7. Click OK to save and close the Results Group Editor.
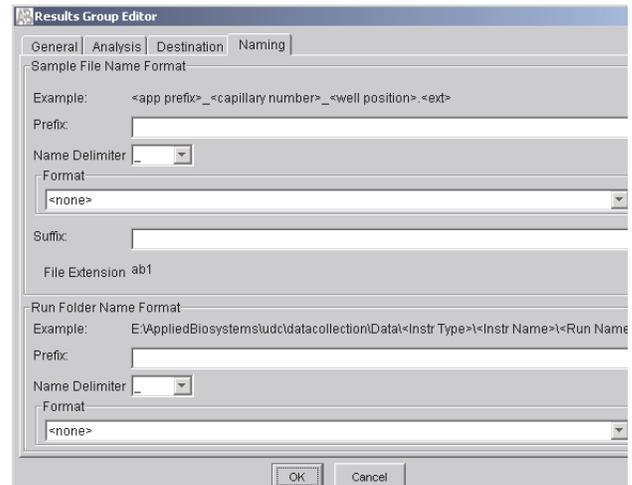
*Sample File Destinations

Locations where sample files are placed during extraction:

- Default Destination, default folder naming: E:\AppliedBiosystemsUDC\DataCollection\data*<instrument type>*\ *<instrument name>*\run folder
- Default Destination, custom folder naming: E:\AppliedBiosystemsUDC\DataCollection\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 customer folder\subfolders, etc.

Notes
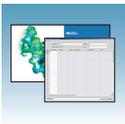
Importing and Exporting a Results Group

Note: Importing or exporting of SeqScape software Results Groups are not supported for transfer between separate computers.

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖳 **Results Group**.

2. Click   Import   to open a File Import dialog box.

3. Navigate to the file you want to import.

    Note: Import file type is .xml (XML file).

4. Click   Open .

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

Exporting a Results Group

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖳 **Results Group**.

2. Click the Results Group name to select it.

3. Click   Export .

    A standard File Export dialog box displays with the chosen Results Group name.

4. Navigate to the location where you want to save the exported file.

5. Click   Save .

    Note: If there is a name conflict with a Results Group that already exists at the save location, the Results groups can be duplicated to copy settings into a similar Results Group without the risk of user error when copying it manually (see "Duplicating a Results Group" on page 100).

Duplicating a Results Group

1. Click the Results Group to select it.

Notes

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.

Filling Out a SeqScape Software Plate Record

Creating a SeqScape Software Plate Record

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

2. Click New... .

The New Plate Dialog dialog box opens.

3. Complete the New Plate dialog box:

   a. Type a name for the plate.

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

   c. Select **SeqScape_***computer name* in the Application drop-down list.

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

   e. Type a name for the owner.

   f. Type a name for the operator.

   g. Click OK .

   The SeqScape Plate Editor opens.

Notes _____

default

default

default

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default

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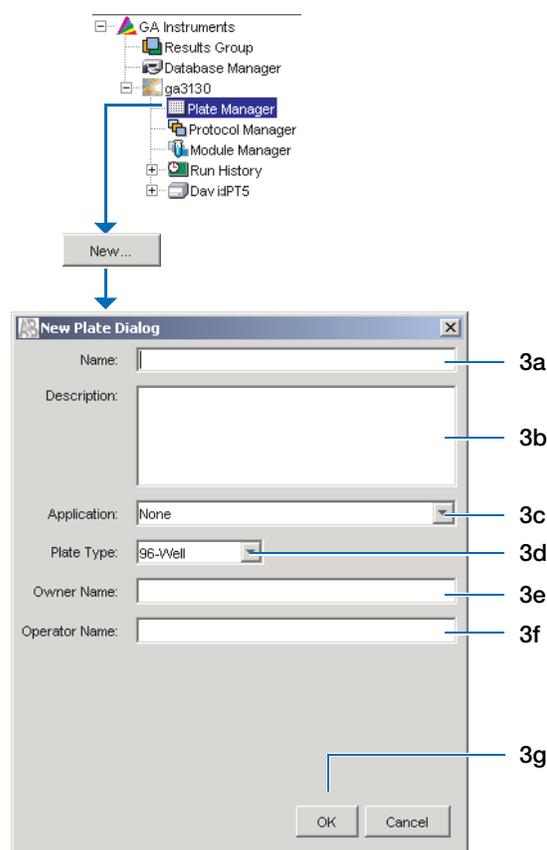
Completing a SeqScape Software Plate Record

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

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

3. In the **Priority** column, change the priority value, if desired. A lower number for each 4 or 16 set, receives a higher run priority. (See the *Applied Biosystems 3130/3130xl Maintenance, Troubleshooting, and Reference Guide* for changing priority values).

4. In the **Project** column, select a project from the drop-down list or create a new project with the corresponding project template.

   Based on the project you select, the project template is filled in automatically.

5. In the **Specimen** column, select or create a specimen.

6. In the **Results Group 1** column, select a group from the drop-down list (see page 98).

7. In the **Instrument Protocol 1** column of the row, select a protocol from the drop-down list (see page 88).

8. In the **Analysis Protocol 1** column of the row, select a protocol from the drop-down list (see page 93).

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

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

     Based your plate type (96- or 384-well) and capillary array (16 or 4 capillaries), the software automatically fills in the appropriate well numbers for a single run.

Notes

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

- For the different samples and protocols – Complete the entries manually.

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

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

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

11. Complete the columns for the additional runs.

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

> **Note:** After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database.The plate record can be searched for, edited, exported, or deleted in the Plate Manager.

For sample preparation and running the instrument, see Chapter 7, "Running the Instrument."

Edit	
Fill Down	Ctrl+D
Copy	Ctrl+C
Paste	Ctrl+V
Clear row(s)	Shift+Delete
Fill Down Special	Alt+D
Add Sample Run	Alt+A

5

Notes

Notes

Autoanalysis and Fragment Analysis

```
┌─────────────────────┐
│  Preparing the      │
│  Instrument         │
└─────────────────────┘
          │
┌─────────────────────┐
│  Performing a       │
│  Spatial Calibration│
└─────────────────────┘
          │
┌─────────────────────┐
│  Performing a       │
│  Spectral Calibration│
└─────────────────────┘
          │
┌─────────────────────┐
│  Autoanalysis       │
│  and Sequencing     │
│  Analysis           │
│  Software           │
└─────────────────────┘
          │
┌─────────────────────┐
│  Autoanalysis       │
│  and                │
│  SeqScape Software  │
└─────────────────────┘
          │
┌─────────────────────┐         ┌──────────────────────┐
│  Autoanalysis       │────────▶│  Create protocols     │  See page 112
│  and                │         │  for automation       │
│  Fragment Analysis  │         └──────────────────────┘
└─────────────────────┘                    │
          │                     ┌──────────────────────┐
┌─────────────────────┐         │  Create a             │  See page 123
│  Running the        │         │  GeneMapper Software  │
│  Instrument         │         │  Plate Record         │
└─────────────────────┘         └──────────────────────┘
```

6
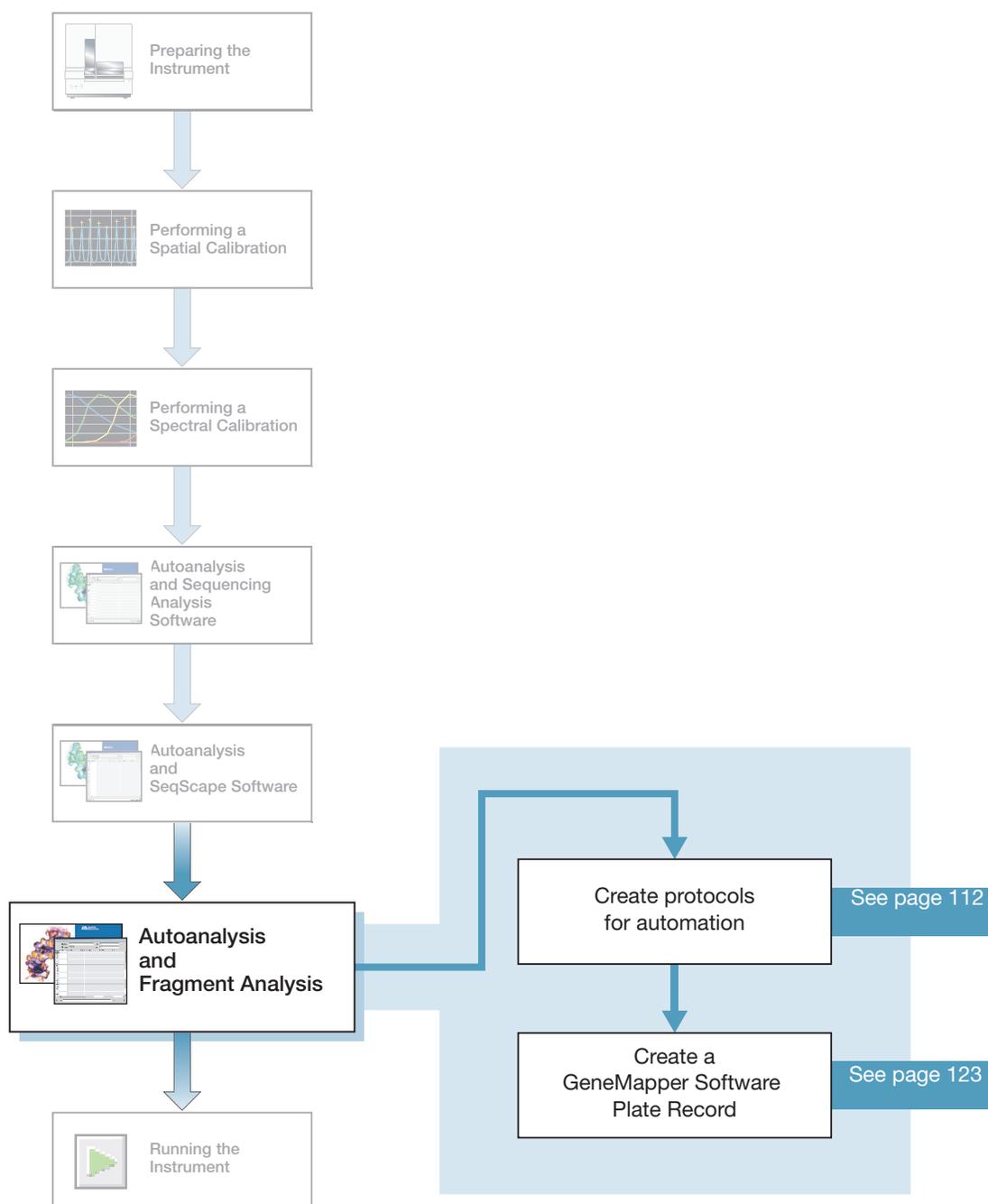
Notes _____

GeneMapper Software

Analyze your fragment analysis samples automatically using autoanalysis or manually.

> **Note:** This chapter is written for both GeneMapper® Software v3.7 or higher and GeneMapper® *ID* Software v3.2. Graphic examples are from GeneMapper software v3.7.

GeneMapper Software v3.7 and GeneMapper *ID* Software v3.2

Perform autoanalysis of fragment analysis samples with features of the 3130/3130*xl* Data Collection and GeneMapper software using the same instrument computer that collected the sample files or on a remote computer.

- Autoanalysis can be performed on the same instrument computer that collected the sample files or on another computer using the remote analysis feature of GeneMapper software.

- After autoanalysis, if you wish to edit/review results on another computer, then transfer the GeneMapper software project, analysis methods, size standards, panel, and bin set information to the other GeneMapper software database. All components need to be exported and imported individually.

- When completing the plate record, fill in the instrument protocol and other information for Data Collection software to complete the run.

- When creating a new Results Group for a set of samples to be autoanalyzed, check the Do Autoanalysis check box and enter the appropriate GeneMapper Software user name and password. For remote analysis, define a default location for sample file storage.

File-Naming Convention

Some alphanumeric characters are not valid for user names or file names. Do not use the invalid characters below:

spaces

\ / : * ? " < > |

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

Manual Analysis

For information on manual analysis, see the *GeneMapper Software v3.7 User Guide* (PN 4359413) or *GeneMapper ID Software V3.1 User Guide* (PN 4338775), or the User Bulletin titled *GeneMapper ID Software v3.2*, Subject: *New Features and Installation Procedures for GeneMapper ID Software v3.2* (PN 4352543).

Notes

About Fragment Analysis and Data Collection	When GeneMapper software is installed on a computer that has 3130/3130*xl* genetic analyzer Data Collection software, two applications are available through the Results Group Editor (see page 115):

- GeneMapper-Generic
- GeneMapper-<Computer Name>

GeneMapper-Generic

Use GeneMapper-Generic to generate .fsa files, without performing autoanalysis. When completing the Sample Sheet, fill in basic information for the Data Collection software to complete the run; all other GeneMapper software related fields are text entries. Text entries are useful if you are using other software analysis applications or 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 another 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>

Use GeneMapper-<Computer Name> for autoanalysis. The Size Standard, Analysis Method, and Panel columns in the Sample Sheet window read directly from the GeneMapper software database. Create these components in GeneMapper software prior to setting up the plate record for a run. New entries cannot be created for these columns once you are in the plate editor dialog box. If you create a new GeneMapper software component while the plate record dialog box is open, the columns will not update. The plate record must be closed and reopened to update the GeneMapper software components. For more information see, "Creating Protocols for Automated Fragment Analysis" on page 112.

6

Notes _____

About GeneMapper Software Plate Records

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

Plate records are data tables in the instrument database that store information about the plates and the samples such as:

- 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 where run modules specify information about how samples are run (in Instrument protocol)

When to Create a Plate Record

Create a plate record for each plate of samples for the following types of runs:

- Spectral calibrations
- Sequencing Analysis Software
- SeqScape Software analysis
- GeneMapper® software
- Mixed (sequencing and fragment analysis samples - see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* for creating a mixed plate record)

For runs to begin, you must create a plate record for and link it to a plate loaded on the instrument. However, you can create plate records for new plates while a run is in progress.

Files needed to create a GeneMapper software plate record:

Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	112
Results Group	Defines the file type, the file name, autoanalysis, and file save locations that are linked to sample injections.	115

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

Elements of a GeneMapper software plate record
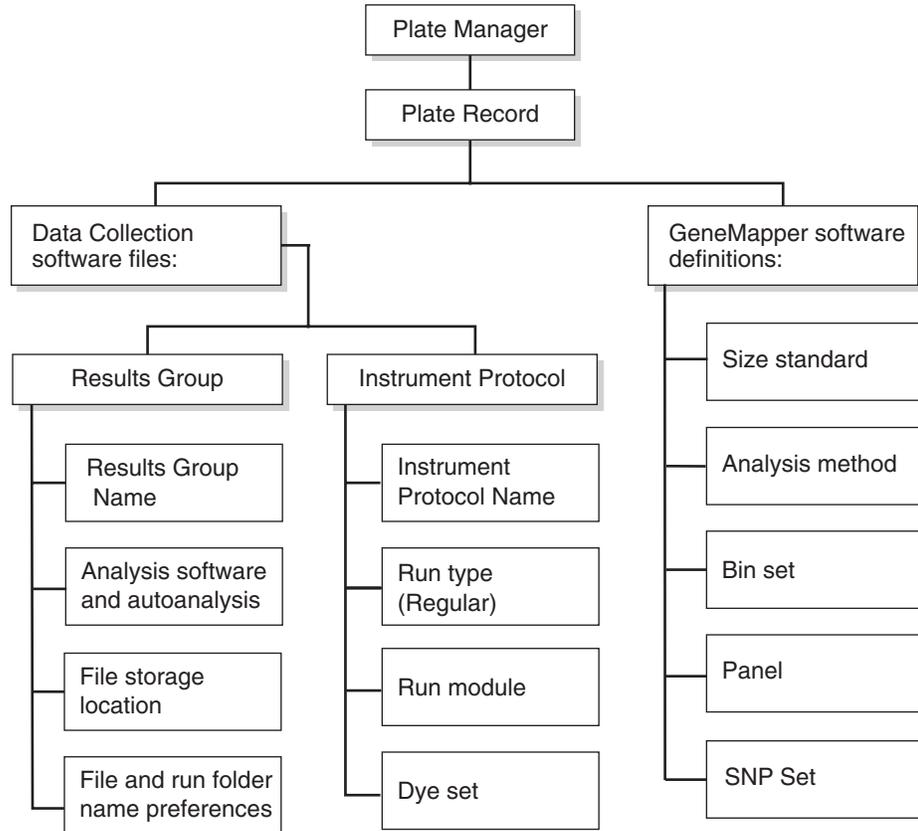
```
                          ┌─────────────────┐
                          │  Plate Manager  │
                          └─────────────────┘
                                   │
                          ┌─────────────────┐
                          │  Plate Record   │
                          └─────────────────┘
                                   │
              ┌────────────────────┴──────────────────────┐
    ┌─────────────────────┐                    ┌───────────────────────┐
    │  Data Collection    │                    │  GeneMapper software  │
    │  software files:    │                    │  definitions:         │
    └─────────────────────┘                    └───────────────────────┘
              │                                            │
       ┌──────┴──────────┐                          ┌──────────────┐
┌──────────────┐ ┌────────────────────┐             │ Size standard│
│ Results Group│ │ Instrument Protocol│             └──────────────┘
└──────────────┘ └────────────────────┘                    │
       │                   │                          ┌──────────────┐
┌──────────────┐  ┌──────────────────┐                │Analysis method│
│ Results Group│  │ Instrument       │                └──────────────┘
│ Name         │  │ Protocol Name    │                       │
└──────────────┘  └──────────────────┘                ┌──────────────┐
┌──────────────┐  ┌──────────────────┐                │ Bin set      │
│Analysis software│ │ Run type       │                └──────────────┘
│and autoanalysis│  │ (Regular)      │                       │
└──────────────┘  └──────────────────┘                ┌──────────────┐
┌──────────────┐  ┌──────────────────┐                │ Panel        │
│ File storage │  │ Run module       │                └──────────────┘
│ location     │  └──────────────────┘                       │
└──────────────┘  ┌──────────────────┐                ┌──────────────┐
┌──────────────┐  │ Dye set          │                │ SNP Set      │
│File and run folder│ └──────────────┘                └──────────────┘
│name preferences│
└──────────────┘
```

Notes _____
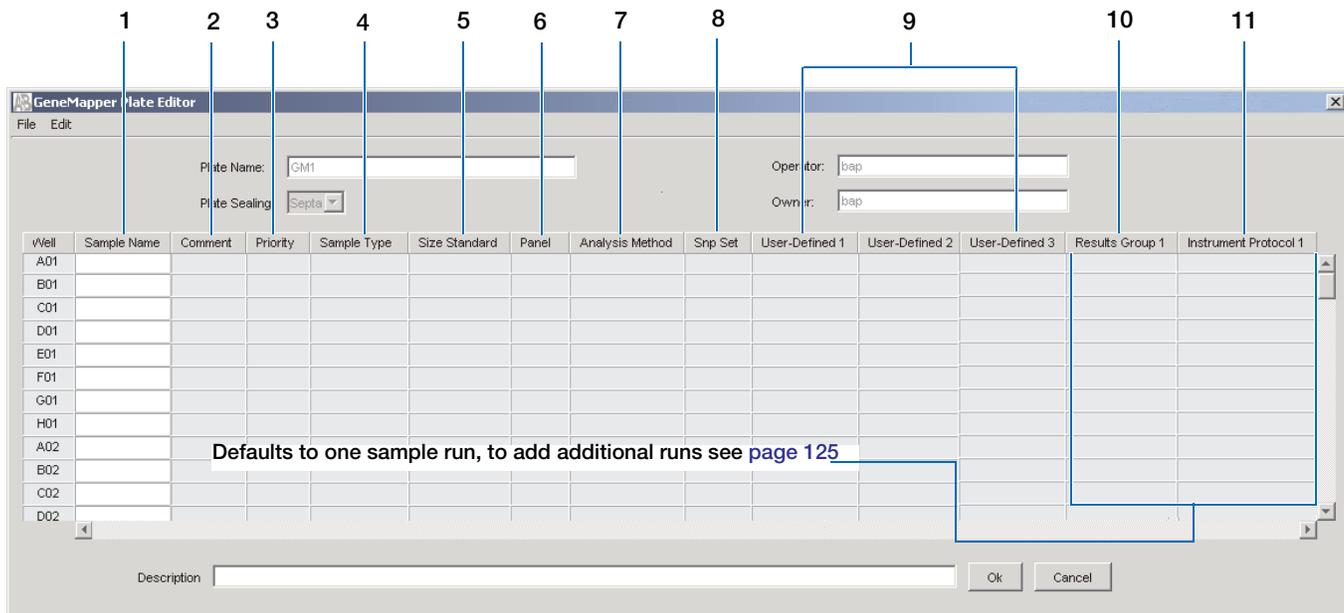
Blank GeneMapper software plate record

Columns inserted in a plate record for a fragment analysis run

Number and Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Priority	A default value of 100 to each sample. Changing the value to a smaller number causes that set of 16 or 4 samples to run to before the others in the injection list.
4. Sample Type	Use to identify the sample as Sample, Positive Control, Allelic Ladder or Negative Control.
5. Size Standard **IMPORTANT!** For GeneMapper-<Computer Name> ONLY: Create the Size Standard, Panel, and Analysis Method in GeneMapper software before creating a new plate	• GeneMapper-Generic (optional): Manually enter size standards in the text field • GeneMapper-<Computer Name>: Select a saved size standard from the drop-down list
6. Panel **IMPORTANT!** For GeneMapper-<Computer Name> ONLY: Create the Size Standard, Panel, and Analysis Method in GeneMapper software before creating a new plate	• GeneMapper-Generic (optional): Manually enter panels in the text field • GeneMapper-<Computer Name>: Select a saved panel from the drop-down list

Notes

Number and Column	Description

7. Analysis Method
 	
IMPORTANT! For GeneMapper <Computer Name> ONLY:
 	
Create the Size Standard, Panel, and Analysis Method in GeneMapper software before creating a new plate
	• GeneMapper-Generic (optional):
	
	  Manually enter analysis methods in the text field
	• GeneMapper-<Computer Name>:
	
	  Select a saved analysis method from the drop-down list

8. SNP Set **IMPORTANT!** For GeneMapper <Computer Name> ONLY: The SNP set, created in the GeneMapper software, links a SNP name to a marker name.	• GeneMapper-Generic (optional): Manually enter SNP set in the text field • GeneMapper-<Computer Name>
9. Three User-defined columns	Optional text entries
10. Results Group	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 select a Results Group for each sample entered in the Sample Name column. See "Results Group for Fragment Analysis" on page 115.
11. Instrument Protocol	Options: • 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 select an Instrument Protocol for each sample entered in the Sample Name column. See "Results Group for Fragment Analysis" on page 115.

Notes _____

Creating Protocols for Automated Fragment Analysis

If the appropriate fragment analysis protocols and results group have been created, proceed to "Creating a GeneMapper Software Plate Record" on page 123.

Instrument Protocol for Fragment Analysis

An instrument protocol contains all the settings necessary to run the instrument:

- Protocol name
- Type of run
- Run module
- Dye set

Creating an Instrument Protocol

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▦ **ga3130***xl* or **ga3130** > ▣ **Protocol Manager**.

2. In the Instrument Protocols section, click **New...** to open the Protocol Editor.

3. Complete the Protocol Editor:
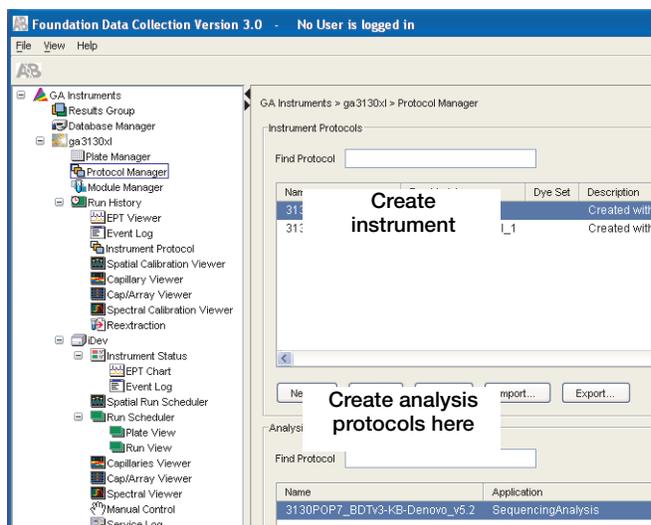
   a. Type a name for the protocol.

   b. Type a description for the protocol (optional).
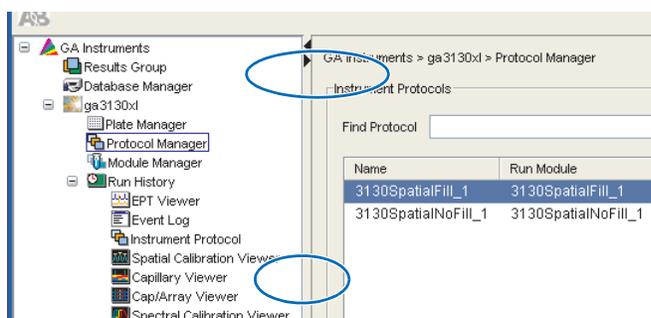
   c. Select **Regular** in the Type drop-down list.

Protocol Editor	✕
Name:	— 3a
Description:	— 3b
Type: REGULAR	— 3c
Run Module: FragmentAnalysis22_POP4_1	— 3d
Dye Set: E5 📁	— 3e
	— 3f
OK Cancel	
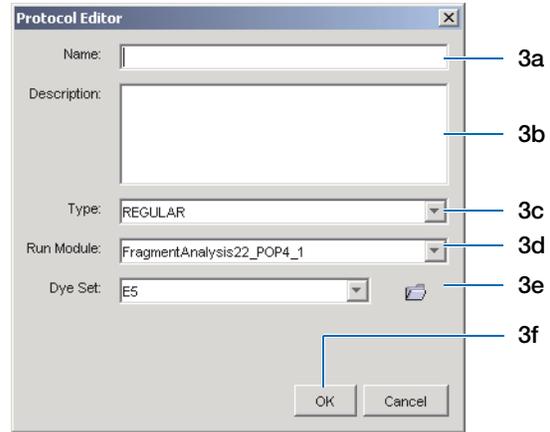
   d. Using the table below, select the run module for your run. To customize a run module, see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* on modifying a module.

Application or Kit	Capillary Array Length (cm)	Run Module
SNaPshot® Multiplex System	22	SNP22_POP4_1
	36	SNP36_POP4_1
• LMS v2.5	22	FragmentAnalysis22_POP4_1
• ABI PRISM® Mouse Mapping Set v1.0	36	FragmentAnalysis36_POP4_1
• Custom oligos		FragmentAnalysis36_POP7_1
• Stockmarks	50	FragmentAnalysis50_POP4_1
• AFLP®		FragmentAnalysis50_POP6_1
		FragmentAnalysis50_POP7_1
• AmpFℓSTR® COfiler®	36	HIDFragmentAnalysis36_POP4_1
• AmpFℓSTR Profiler Plus®		
• AmpFℓSTR SGM Plus®		
• AmpFℓSTR Profiler Plus *ID*		
• AmpFℓSTR SEfiler™		
• AmpFℓSTR Yfiler™		
• Other 4-Dye AmpFℓSTR		
• AmpFℓSTR Identifiler		
• Other 5-Dye AmpFℓSTR		

Notes _____

_____

6

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

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

f. Click OK .

Importing an Instrument Protocol

1. Click Import in the Instrument Protocols pane of the Protocol Editor window to display the standard File Import dialog box.

2. Navigate to the location of the .xml file you want to import.

3. Select the .xml file and click **Open**.

Notes

Exporting an Instrument Protocol

1. In the Instrument Protocols pane, highlight the protocol you want to export.

2. Click [Export] to display the standard File Export dialog box.

3. Browse to the desired folder location.

4. Click [Save] .

Results Group for Fragment Analysis

A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. It is called a Results Group because it is used to 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...] to open the Results Group Editor window.
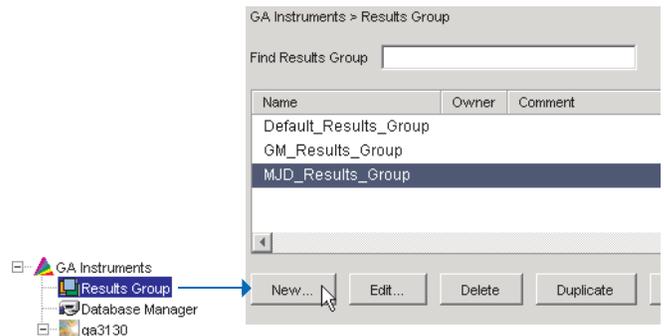
3. Complete the General tab:

    a. Type a unique Results Group Name. The name can be used in naming and sorting sample files.

    b. Optional: Type a Results Group Owner. The owner name can be used in naming and sorting sample files.

    c. Optional: Type a Results Group Comment.
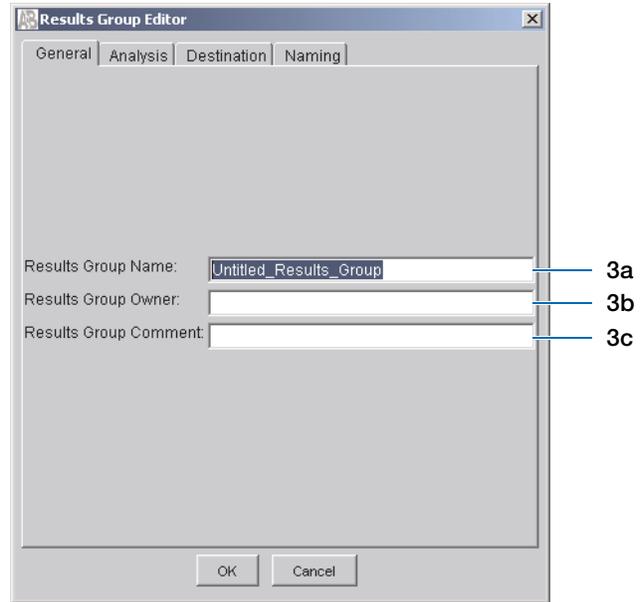
4. Select the **Analysis** tab, then:

    a. Click **Analysis Type** and then select one of the following:

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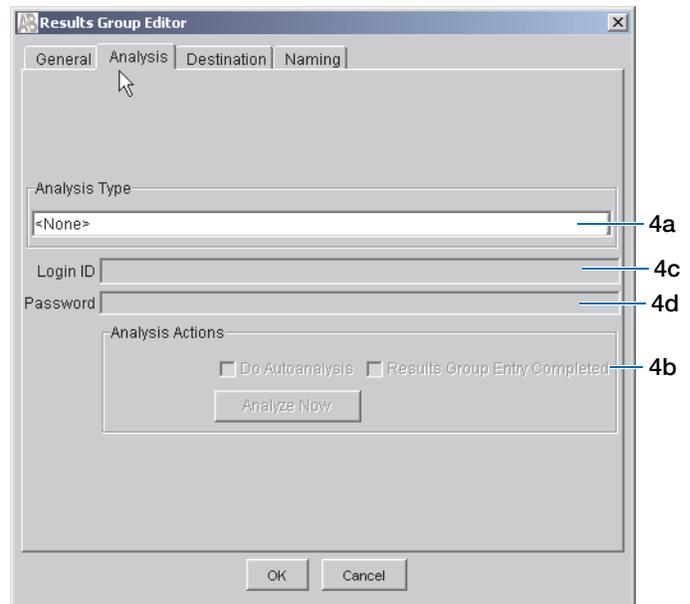
b. Select an option in the Analysis Actions section using the table below.

If You Select …	Then …	Use with Setting from Automated Processing Tab (page 118)
Do Autoanalysis	Samples are analyzed after each run of 16 or 4 samples.	**When every run completes**
Do Autoanalysis and Results Entry Group Complete	Samples are analyzed after all pending 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.

Note: 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. Autoanalysis will fail if the incorrect username and password are entered.

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 v3.7*	complete steps a-b.
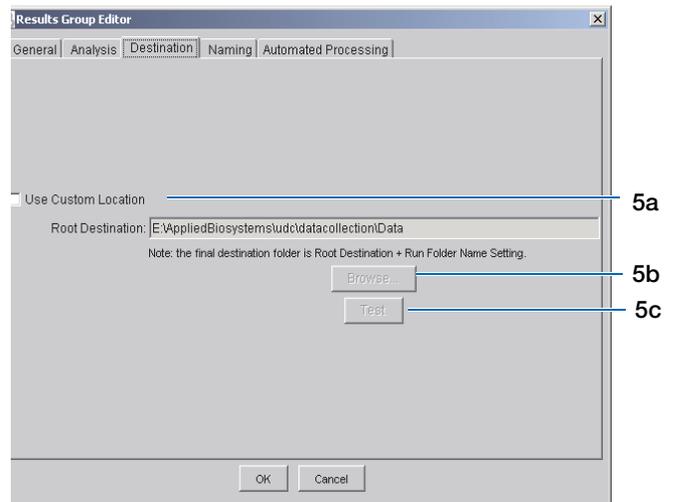
5a
5b
5c

*Sample File Destinations

Locations where sample files are placed during extraction:

• Default Destination, default folder naming: E:\AppliedBiosystemsUDC\DataCollection\data*<instrument type>*\ *<instrument name>*\run folder

• Default Destination, custom folder naming: E:\AppliedBiosystemsUDC\DataCollection\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 customer folder\subfolders, etc.

Notes

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…	Then a message box displays …
Passes	Test succeeded: <"path">.
Fails	Test failed:<"path">.

6. Select the **Naming** tab to customize sample file and run folder names.

Note: The default sample file naming for fragment analysis is: frag_<capillary number>_<wellposition>.fsa

Note: Sample name, run folder name, and path name, *combined*, can total no more than 250 characters.

For defining the elements of the Naming tab, see page 119.

Sample File Name Format

Run Folder Name Format pane
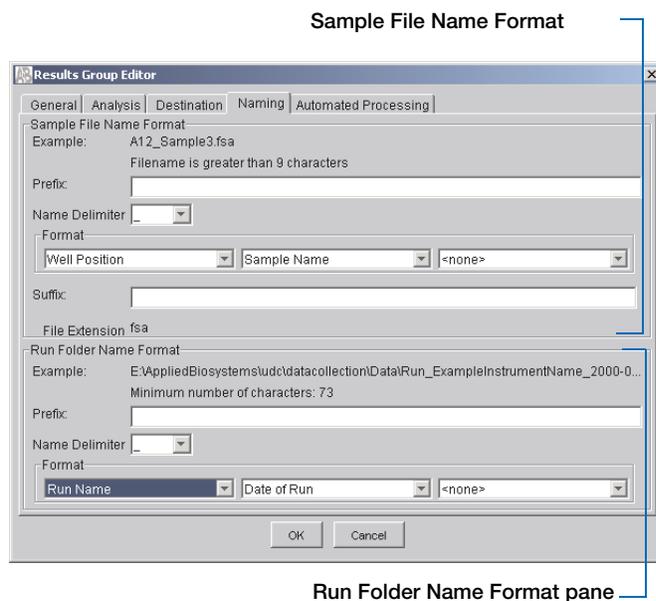
7. Select the **Automated Processing** tab.

In "Autoanalysis is performed" select when you want your samples autoanalyzed using the table below.

Select an autoanalysis option

If You **Select** …	Then …	Use with Settings from Analysis Tab (page 117)
Only when the result group is complete	Samples are analyzed after all pending samples using the same results group have been run.	**Do Autoanalysis and Results Entry Group Complete**
When every run completes	Samples are analyzed after each run of 16 or 4 samples.	**Do Autoanalysis**

8. Click OK to save the Results Group.

Notes

Completing the Sample File Name Format Pane

1. Type a prefix for the file name in the **Prefix** box. Anything that you type here is shown in the Example line.

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.
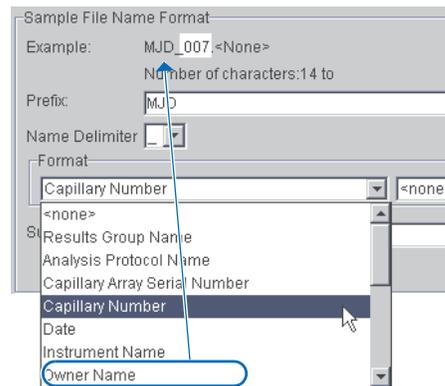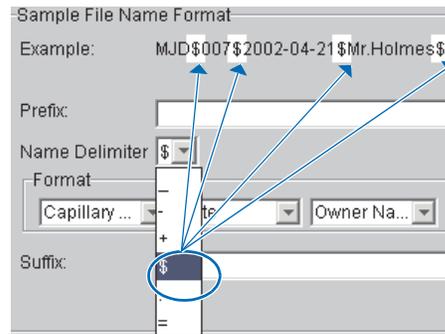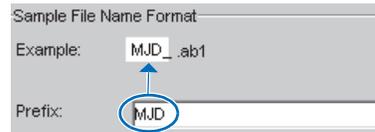
    Note: All the samples from a single run can be placed in the same run or results folder; the name of every sample from a single run should be different. Most of the Format options will not be different between samples; select at least one of the options to make the sample names unique within a run.

    If a unique identifier is not included in the name, a warning message displays.Select from the elements to make a unique sample file name. Examples are:
    – Well position
    – Capillary number
    – Run sequence number
    – Plate Quadrant

    Note: Some identifiers, such as well position, capillary number and plate quadrant are not unique in multiple sample instances. Plate Quadrant is not a unique identifier for a 96-well plate.
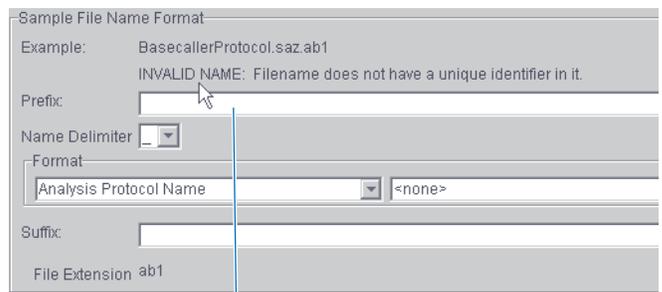
Warning message

Notes

As you select the elements for the file name, they are placed in the Example line. An additional element drop-down menu displays allowing you the option of selecting an additional element.
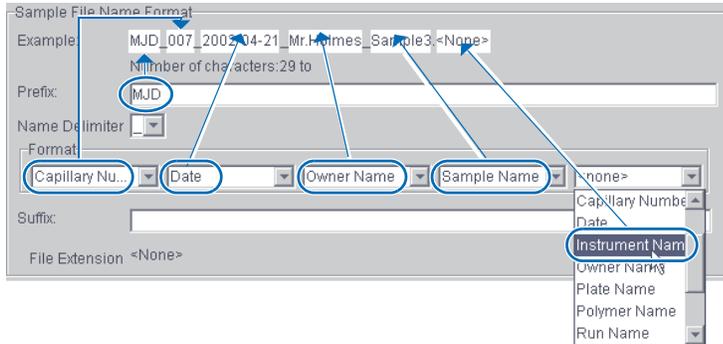
The names of the Format elements eventually truncate, but the Example field remains visible (up to 72 characters).
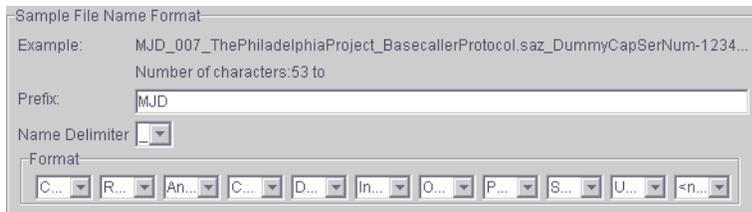
4. Click the Suffix box (optional) and type the suffix for the file name.

   The File Extension field displays the file extension generated from the Analysis Type specified on the Analysis tab (see page 116). For example, Sequencing Analysis produces sample files with an .ab1 extension.
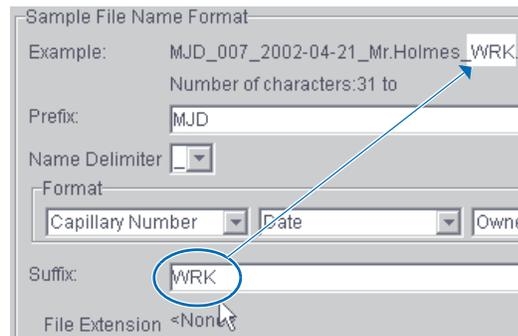
Run Folder/Sub-Folder Name Format Pane

Follow the same steps described above for the Sample File Name Format pane (see page 119) to change the sub-folder name within the run folder.

Notes

Importing and Exporting a Results Group

Importing or exporting of GeneMapper software Results Groups are not supported for transfer between separate computers.

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.
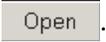
    Note: Import file type is .xml (XML file).

4. Click   Open .

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

Exporting a Results Group

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖳 **Results Group**.

2. Click the Results Group name to select it.

3. Click   Export .

    A standard file export dialog box displays with the chosen Results Group name.

4. Navigate to the location where you want to save the exported file.

5. Click   Save .

    Note: If there is a name conflict with a Results Group that already exists at the save location, the Results groups can be duplicated in order to copy settings into a similar Results Group without the risk of user error when copying it manually (see "Duplicating a Results Group" on page 122).

6

Notes _____

Duplicating a Results Group

1. Click the Results Group.

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.

Creating a GeneMapper Software Plate Record

Creating the Plate Record for Autoanalysis

1. In the tree pane of the Data Collection software, click ![] **GA Instruments** > ![] **ga3130***xl* or **ga3130** > ![] **Plate Manager**.

2. Click ![New...] to open New Plate Dialog.

3. Complete the information in the New Plate Dialog:

    a. Type a name for the plate.

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

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

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

    e. Type a name for the owner.

    f. Type a name for the operator.

    g. Click ![OK] .
        The GeneMapper software Plate Editor opens.
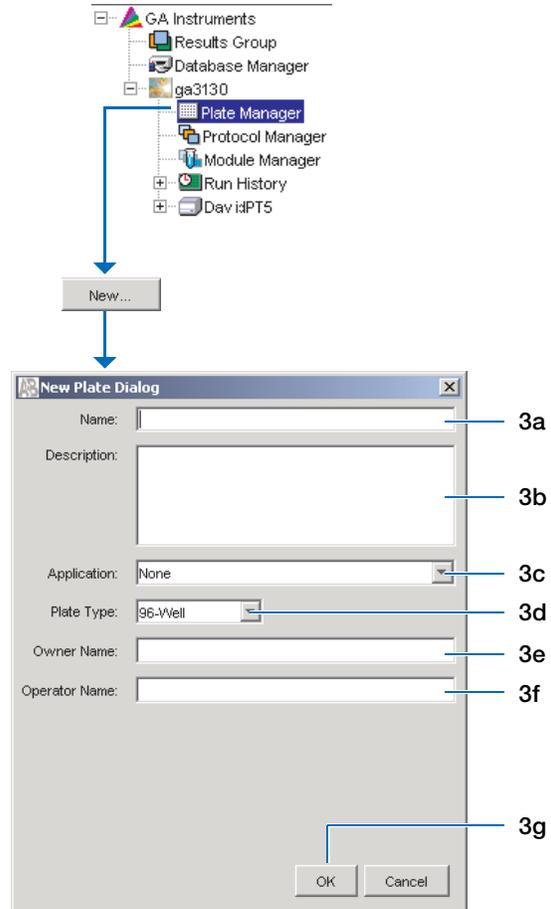
Completing a GeneMapper Software Plate Record for Autoanalysis

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

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

3. In the Priority column, change the priority value, if desired.

4. In the Sample Type column, select a sample type from the drop-down list.

5. In the Size Standard column, select a size standard from the drop-down list.

6. In the Panel column, select a panel from the drop-down list.

7. In the Analysis Method column, select a method from the drop-down list.

8. In the Snp Set column, select a SNP set from the drop-down lis if applicable, otherwise select None.

9. Enter text for User-Defined columns 1 to 3.

10. In the Results Group 1 column, create a new Results Group or select a group from the drop-down list (see page 115).

11. In the Instrument Protocol 1 column, create a new instrument protocol or select a protocol from the drop-down list (see page 112).

	1	2	3	4	5
Well	Sample Name	Comment	Priority	Sample Type	Size Standard
A01					
B01					
C01					
D01					
E01					
F01					

6	7	8	9	
Panel	Analysis Method	Snp Set	User-Defined 1	User-Defined 2

9	10	11
User-Defined 3	Results Group 1	Instrument Protocol 1

Notes

12. To complete the plate record:

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

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

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

- For the different samples and protocols – Complete the entries manually.

13. To do more than one run, select **Edit > Add Sample Run**.

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

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

14. Complete the columns for the additional runs.

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

> **IMPORTANT!** After clicking OK in the Plate Editor, the plate record is stored in the Plate Manager database and can now be located, edited, exported, or deleted.

Notes

Running the Instrument

Preparing the Instrument

Performing a Spatial Calibration

Performing a Spectral Calibration

Autoanalysis and Sequencing Analysis Software

Autoanalysis and SeqScape Software

Autoanalysis and Fragment Analysis

Running the Instrument

Prepare samples and plate assemblies	See page 128
Place Plate onto Autosampler	See page 128
Link the Plate	See page 128
Schedule a run	See page 129
Run the instrument	See page 130
Set up for continuous operation	See page 134
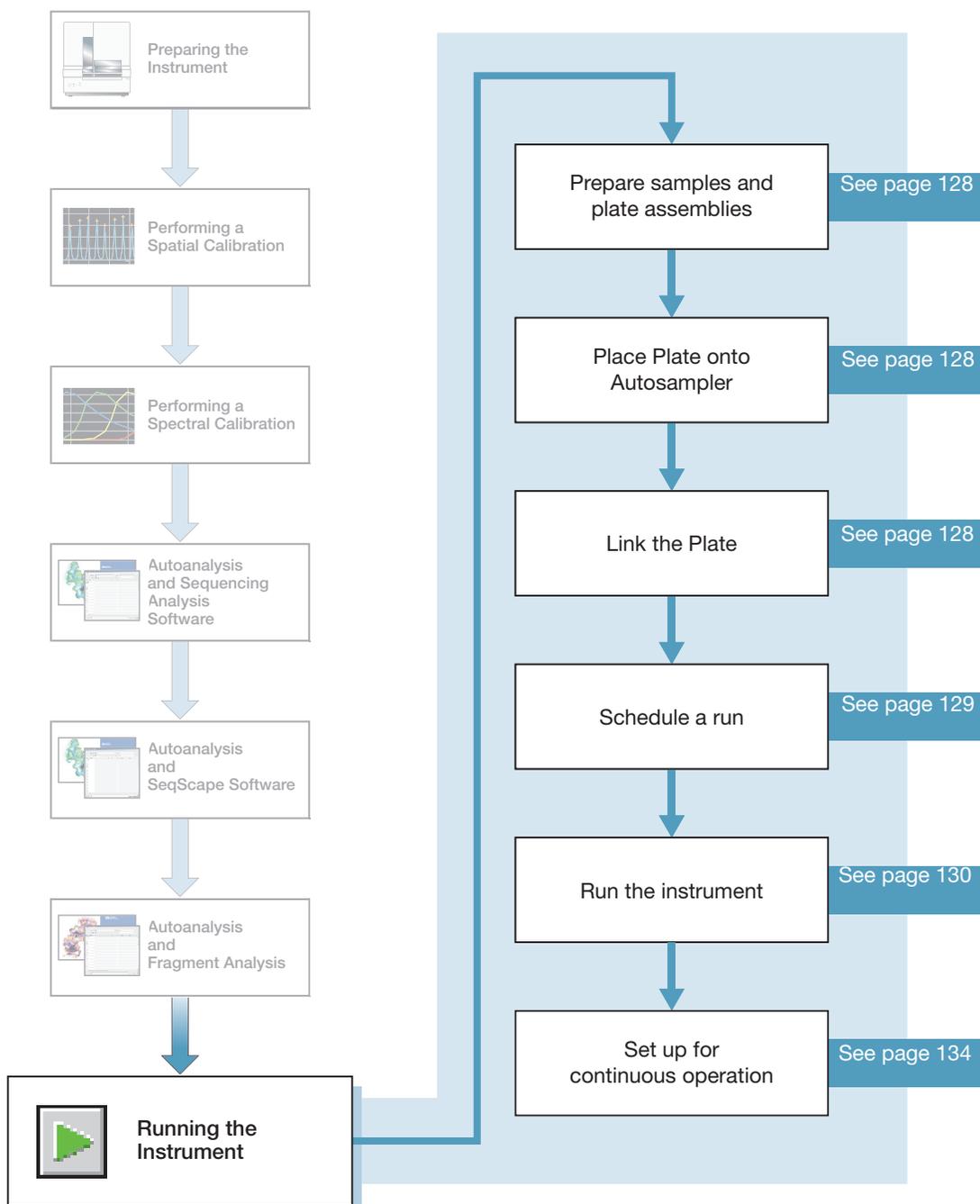
Notes

Preparing Samples and Plate Assemblies

Review Table

Generalized sample preparation and plate assembly procedures are discussed in Chapter 3, "Performing a Spectral Calibration," on page 27.

Topic...	See
Sample Preparation	"Preparing the Calibration Standard" on page 31 and "Loading Samples" on page 32 to review handling samples. Follow your individual kit protocol to prepare sample load volume: 10 to 30 µL for a 96-well plate 5 to 15 µL for a 384-well plate
Sealing plates and plate assembly	"Seal the plate:" on page 33.
Placing plate assemblies in the instrument	"Placing the Plate Assembly into the Instrument" on page 39.
Searching for plate records to link to a plate	"Running the Spectral Calibration Plate" on page 40.

Linking and Unlinking a Plate

Link a plate on the autosampler to your newly created plate record before running the plate.

Select the desired plate record, then click the plate position indicator corresponding to the plate position in the instrument. The plate position (A or B) displays in the link column.

Note: The 3130 genetic analyzer has only one plate position (B) to link a plate record.

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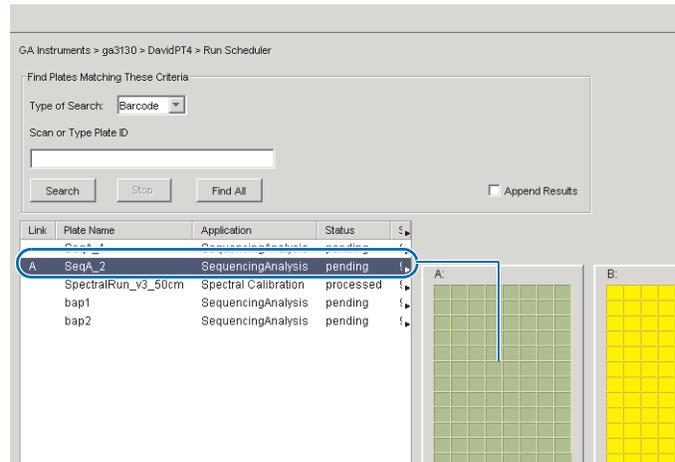
Notes

Unlinking a Plate Record

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

2. Click [Unlink] .
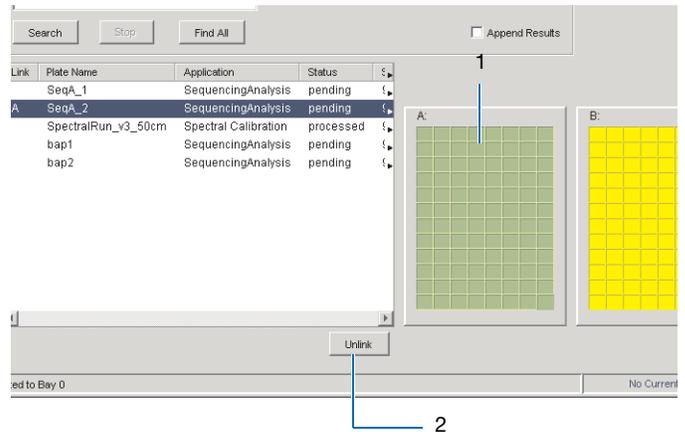
Viewing the Run Schedule

For more information on run scheduling and sample injection order, see "Plate Mapping" on page 161.

To verify that runs are scheduled correctly:

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▦ **ga3130***xl* or **ga3130** > ▭ *instrument name* > ▦ **Run Scheduler** > ▦ **Run View**.

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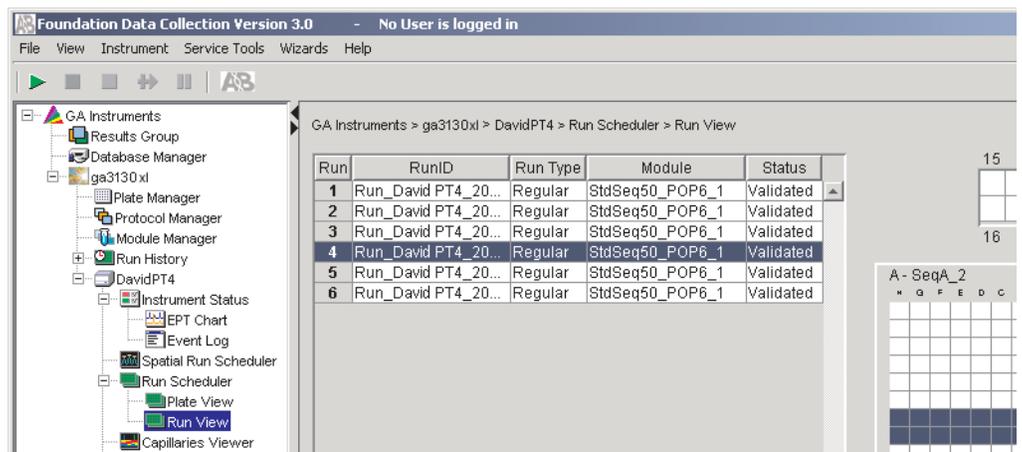
Notes

Running the Instrument

Starting the Run

1. Verify that the active spectral calibration matches your dye set and capillary array length for all scheduled runs.

   To change the active spectral calibration, see "Activating a Spectral Calibration" on page 47.

   To create a new spectral calibration, see "Creating a Spectral Instrument Protocol" on page 35.

2. Click the green button in the toolbar.

3. The Processing Plates dialog box opens, then click ⬛ OK ⬛.
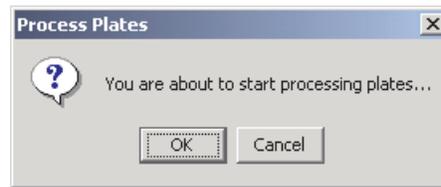
   The software automatically performs a run validation:

   • If the validation passes, the run starts
   • If any of the validation test fails, the run does not start. Check the event log for information.

Notes

Starting the Autoanalysis Manager for SeqScape and/or GeneMapper Software

Use the Autoanalysis Manager (AAM) software with SeqScape® and/or GeneMapper® software to automatically analyze the data.

1. Select **Start > Programs > Applied Biosystems > Autoanalysis Manager > Autoanalysis Manager 3.0**.

   Note: Autoanalysis Manager does not start automatically. Open Autoanalysis Manager to receive messages from the data collection software for autoanalysis in SeqScape and/or GeneMapper software.

   The Autoanalysis Manager window opens.
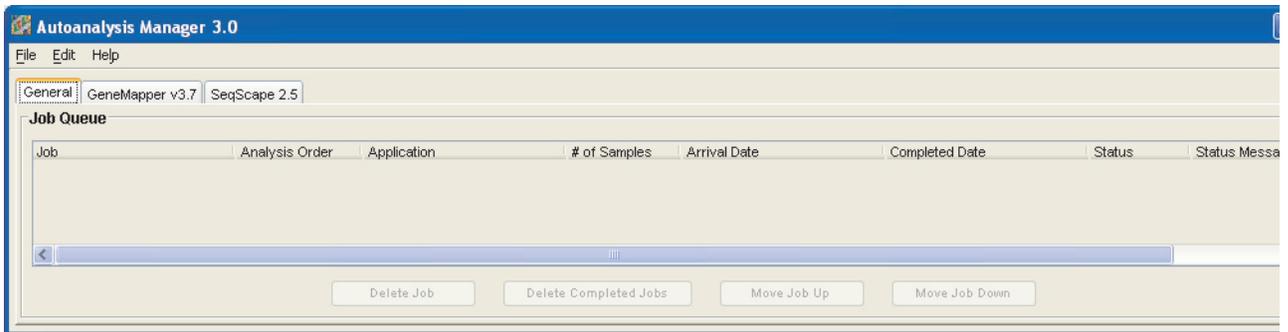
2. Quit the SeqScape and/or GeneMapper software.

   No other interaction with the AAM software is needed until the runs finish. See "Using Autoanalysis Manager Software" on page 155 on how to use the Autoanalysis Manager.

7

Notes _____

Basic Run Module Steps

Automated module steps when the run starts

Module Steps	Approximate Time
Turn Oven On	N/A
Wait for oven to equilibrate Initialize autosampler	1 min 40 sec
Fill Array	3-4 min
PreRun	3 min
Inject samples	10 to 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:

- Oven unheated: ~25 min
- Oven at set temperature: ~6.5 min

To customize a run module, see the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting and Reference Guide*.

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

DNA Sequencing Run Times

Approximate run times of common DNA sequencing analysis runs

Type of Run	Run Module	Run Time (min)
Ultra rapid	UltraSeq36_POP4	40
	UltraSeq36_POP7	35
Rapid	RapidSeq36_POP6	60
	RapidSeq36_POP7	
Fast	FastSeq50_POP7	60
Standard	StdSeq50_POP4	100
	StdSeq50_POP6	150
	StdSeq50_POP7	120
Long read	LongSeq80_POP4	210
	LongSeq80_POP7	170

Notes

Fragment Analysis Run Times

Approximate run times of common fragment analysis runs

Type of Run	Run Modules	Run Time (min)
High Throughput, Small Size Fragment Analysis	FragmentAnalysis22_POP4	20
	SNP22_POP4	20
Standard Fragment Analysis	FragmentAnalysis36_POP4	45
	HIDFragmentAnalysis36_POP4	45
	SNP36_POP4	30
	FragmentAnalysis36_POP7	35
	FragmentAnalysis50_POP4	65
	FragmentAnalysis50_POP6	90
	FragmentAnalysis50_POP7	50

For the latest size standard modules (for example, run modules for the LIZ 1200 system), go to http://www.appliedbiosystems.com, then click the link for Support, then Software Downloads, then GeneScan™ Size Standards, then Run Modules.

Controlling the Run

Using the Toolbar

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

Click ...	Description
Start Run	Starts the run
Stop	Stops the current run, and all other scheduled runs
Stop After Current Run	Completes the current run, then stops all other scheduled runs

Click ...	Description
Skip to Next Run	Stops the current run, then starts the other scheduled runs
Pause Run	Pauses the current run[a]

a Pausing the instrument for too long, especially after sample injection, will adversely affect data quality.

Set Up for Continuous Operation

Overview Use the continuous run feature to create and link a plate during a run. You can:

- Run one or more plates
- Remove the plate(s) once samples have run
- Link and run additional plates

When using the continuous run feature:

- Only mount or unmount plates when the instrument is paused.
- Create the plate record before pausing the instrument to make linking more efficient, reducing long pauses.
- Link new plates after a run has resumed.

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

If the plate is in use, see "Adding, Replacing, or Removing a Plate During a Run" on page 135.

IMPORTANT! Pausing the instrument for too long, especially after sample injection, will affect data quality.

Notes

Adding, Replacing, or Removing a Plate During a Run

Replace a completed plate or add a new plate to an unused plate bay (3130*xl* genetic analyzer only).

1. Prepare your plate and create the plate record.

2. Click ▮▮ (Pause).

In the Pause dialog box, read the pause warning, then click OK to pause the run.
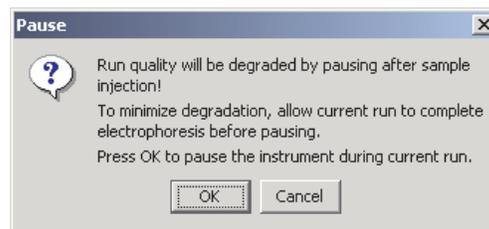
The Resume run dialog box opens when the run is paused.

IMPORTANT! *Do not* click OK to resume the run. Temporarily ignore the dialog box.

3. Remove the old plate, if applicable.

    a. Press the Tray button to bring the autosampler forward.

    b. Open the instrument door.

    c. Remove the old plate.

4. Mount the new plate.

5. Close the door.

6. Click **OK** in the Resume Run dialog box.

7. In the Completed Run dialog box, click OK to continue if the samples have been injected, or click Cancel to abort the run and return the instrument to an idle state.

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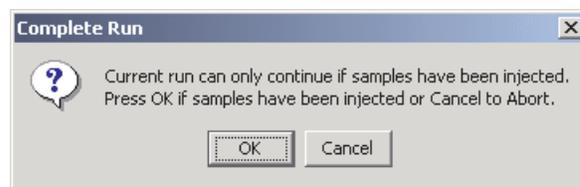
Notes _____

_____

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

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

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

Viewing Data During a Run

**Run Scheduler >
Plate View**
In the tree pane of the Data Collection software, click
▲ **GA Instruments** > 🖼 **ga**3130*xl* or **ga3130** > ▭ *instrument name* > 🖥 **Run
Scheduler** > 🖥 **Plate View**.

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

Run Scheduler >   In the tree pane of the Data Collection software, click
Run View   🔺 **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > ▭ *instrument name* > ▦ **Run Scheduler** > ▦ **Run View** to monitor the status of the scheduled runs.

Note: For default load maps, see Appendix A, "Plate Mapping."

Instrument Status

In the tree pane of the Data Collection software, click
GA Instruments > **ga**3130*xl* or **ga3130** > *instrument name* > **Instrument Status** to monitor the status of the instrument or the current run.

Open the Event Log to monitor system messages

System Status must be 'Ready' before a run starts

Array and polymer information

System Status changes from green to flashing red when errors occur, see Event Log.

Notes

Instrument Condition Sensor States Pane

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

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

Events Box

The Events box lists the:

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

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

Errors Box

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

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

Notes

**Instrument Status
> EPT Chart**

In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > 🖥 *instrument name* > 📊 **Instrument Status** > 📊 **EPT Chart**. The EPT chart displays real-time electrophoresis (EP) data during a run.
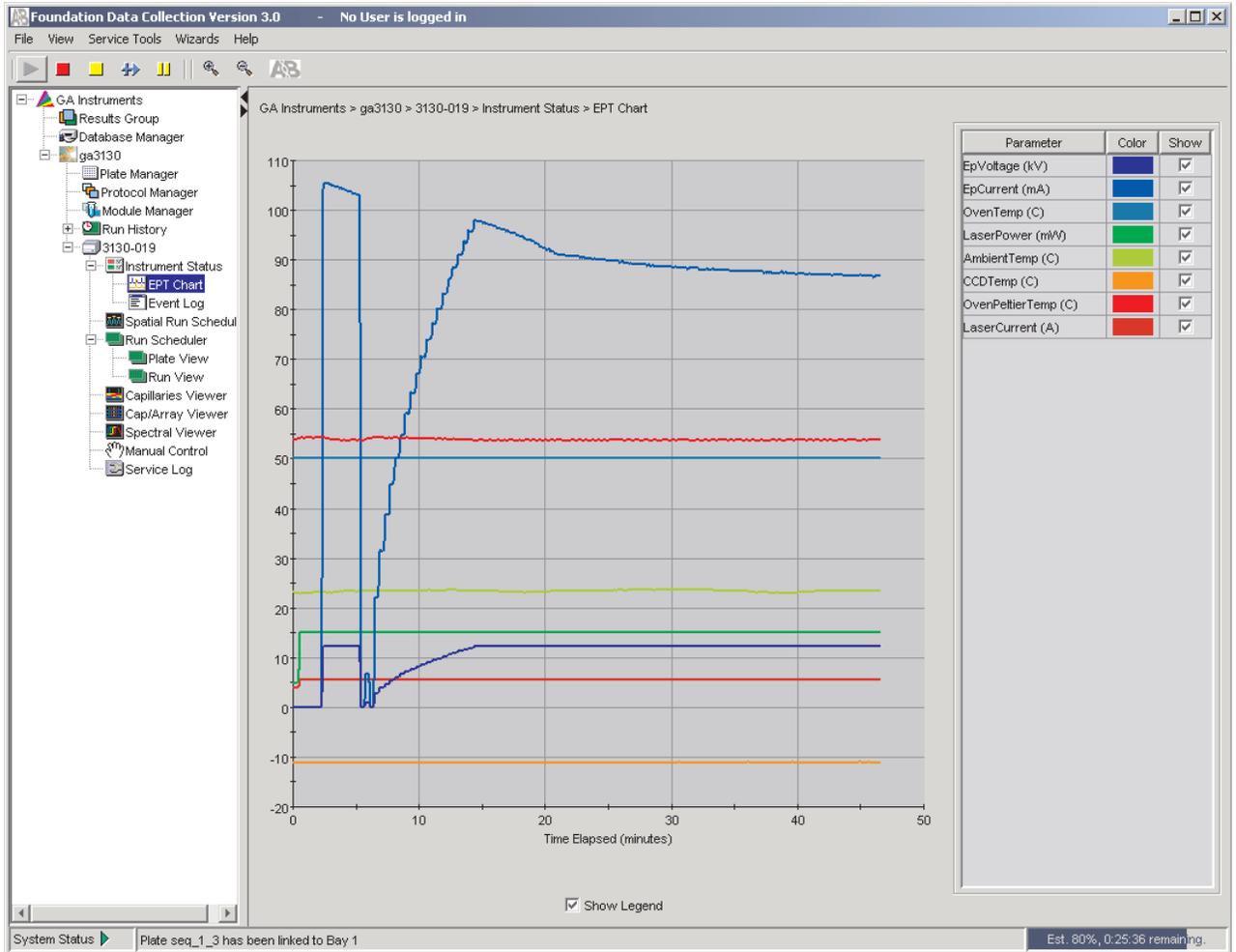
**Instrument Status
> Event Log**

In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▓ **ga3130***xl* or **ga3130** > ▭ *instrument name* > ▓ **Instrument Status** > ▤ **Event Log**. The Event log itemizes events such as errors and general information for all data collection steps.

Clear error messages by clicking **Clear Errors**. The System Status light flashes red until all errors are cleared. Take corrective action based on error message.

Note: This view can also be used to monitor spectral calibration results in real time to verify the capillary-by-capillary processing status.
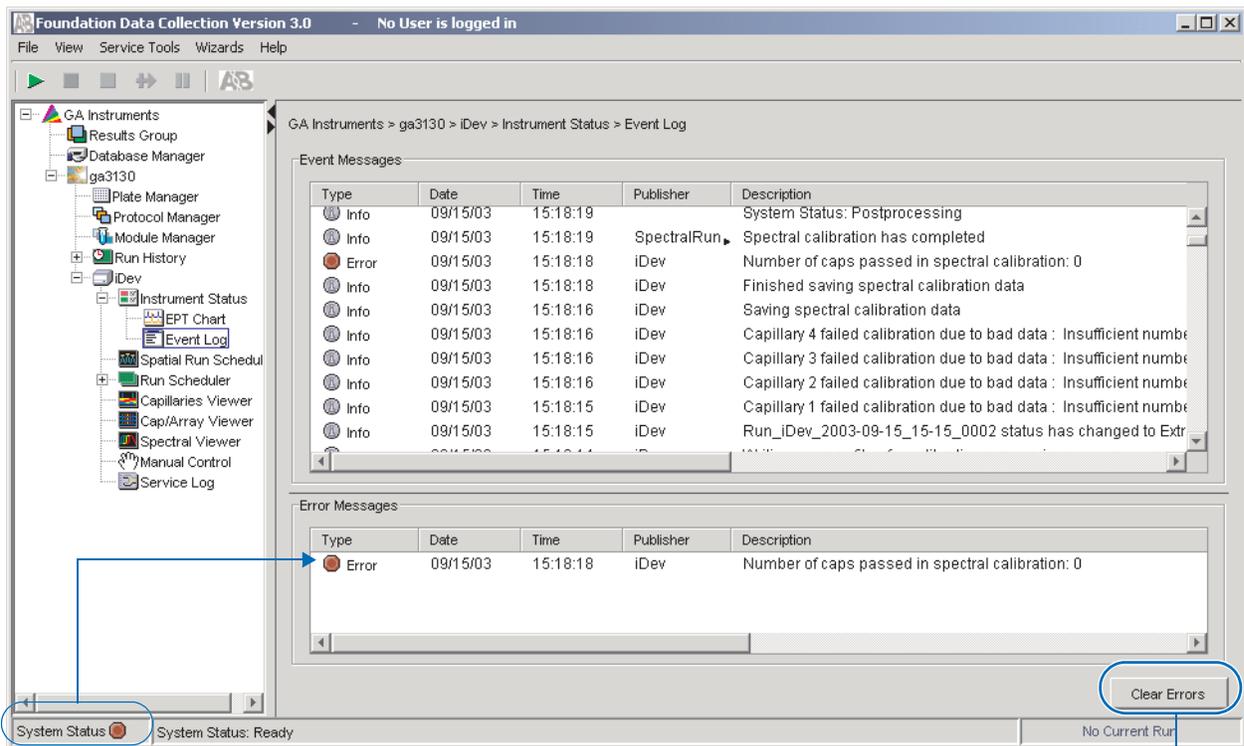
Clear Errors changes the System status from red to green (ready state).

Capillaries Viewer In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖼 **ga3130** or **ga3130xl** > 🗐 *instrument name* > 🖼 **Capillaries Viewer**. Use the Capillary Viewer to examine the quality of the raw data during a run for several capillaries at once.

Click individual colors to view or hide them

Select check boxes of capillaries to display

Raw, multicomponented data
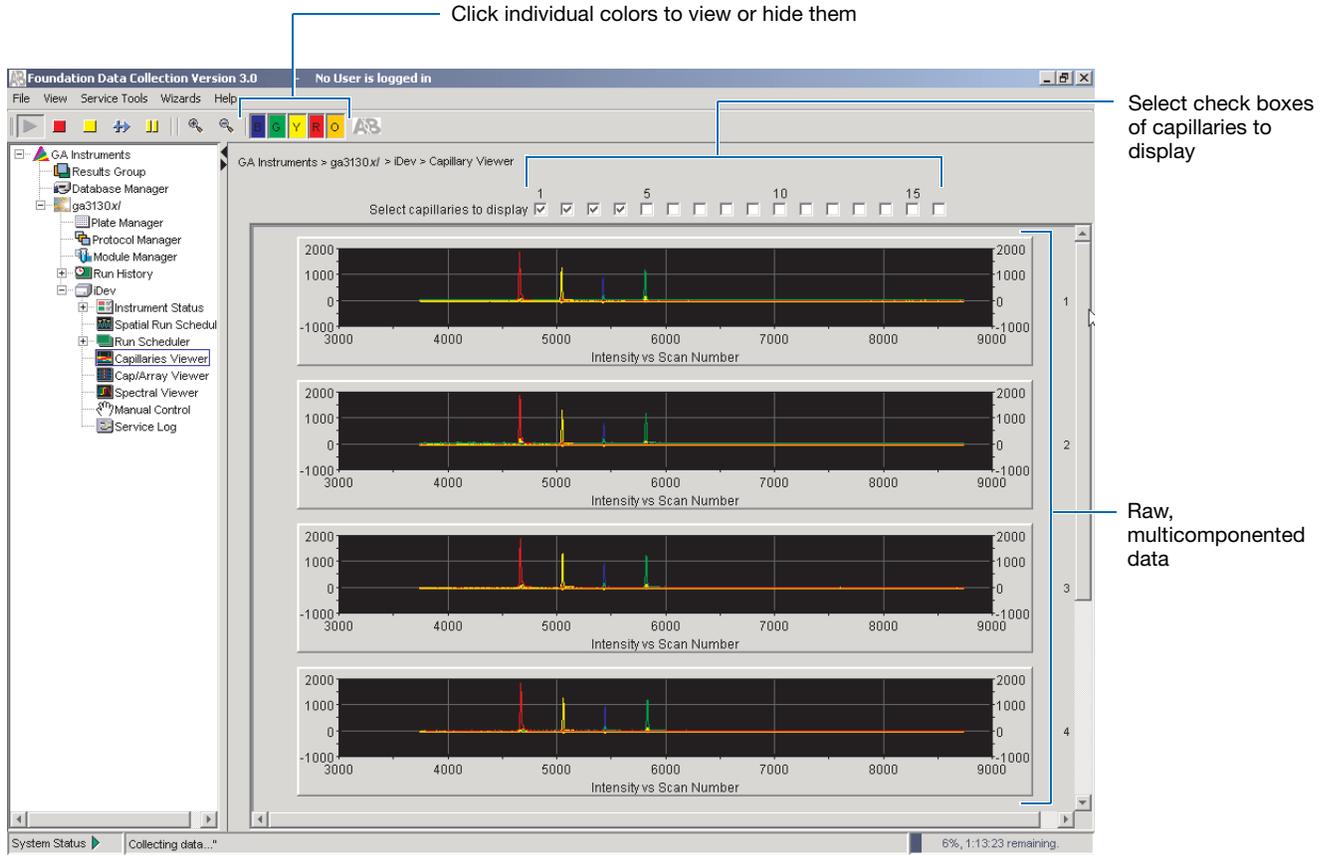
Check Boxes

Select the check boxes of the capillaries to view their electropherograms. The capillaries are displayed in the order in which the boxes are checked. The more boxes that are selected, the slower the refresh window rate.

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

How to Zoom

To zoom in and out:

1. Select a rectangular area over the area of interest by holding down the mouse button. Release the mouse button to zoom in.

2. Click 🔍 to return to full view.

Notes

Cap/Array Viewer   In the tree pane of the Data Collection software, click ▲ **GA Instruments** > ▦ **ga3130** or **ga3130***xl* > ▭ *instrument name* > ▦ **Cap/Array Viewer**. Use the window during a run to examine the quality of your data, which is displayed as color data for the entire capillary array. You can view all the capillaries (vertical axis) as a function of time/scan numbers (horizontal axis).
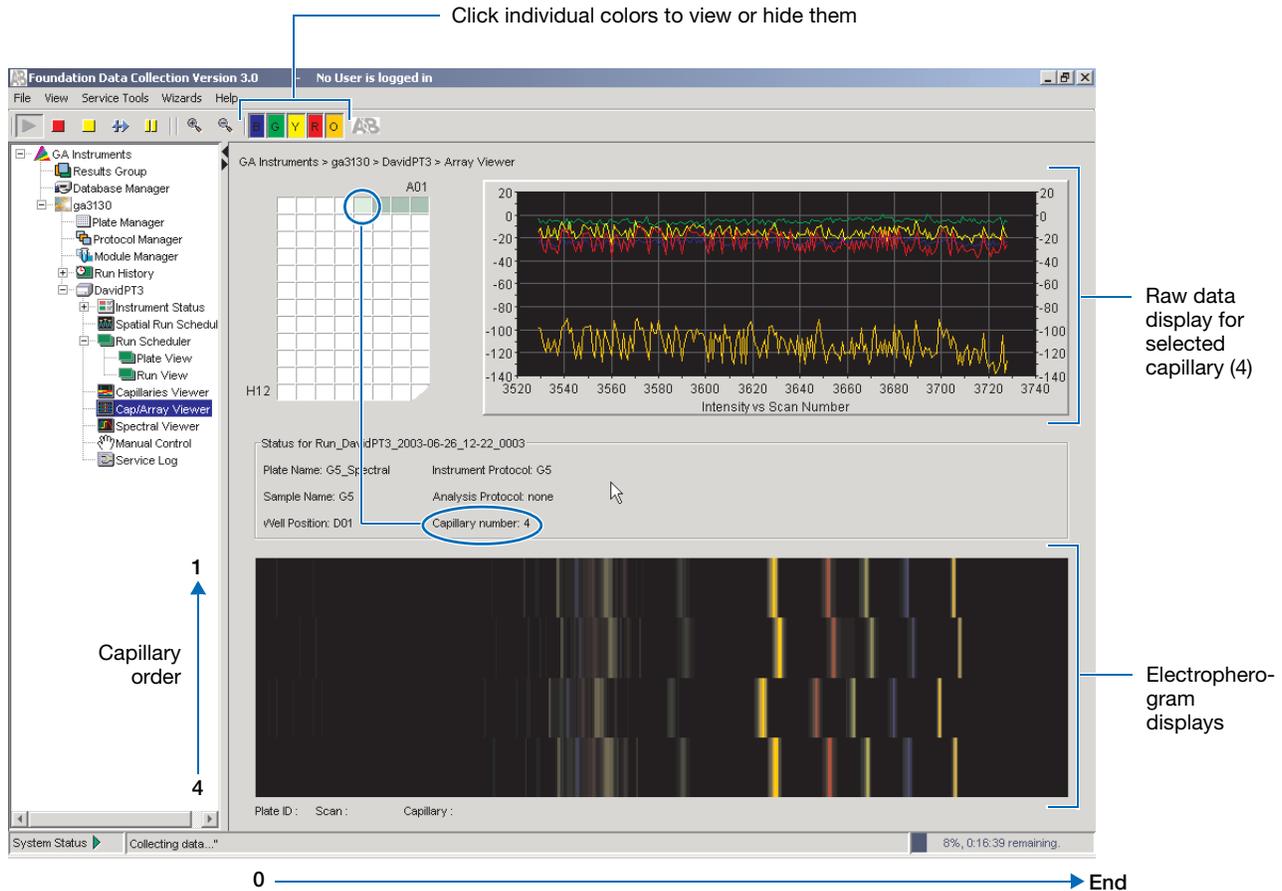
Click individual colors to view or hide them

Raw data display for selected capillary (4)

Capillary order

Electrophero-gram displays

How to Zoom

To zoom in and out:

1. Select a rectangular area over the area of interest by holding down the mouse button. Release the mouse button to zoom in.

2. Click ⚲ to return to full view.

Working with Data in The Run History View

Run History Components

Elements of the Run History Utility

Use the Run History utility only with completed runs stored in the local 3130/3130*xl* genetic analyzer 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	Element	Displays the...
	EPT Viewer	Electrophoresis data of the run, such as voltage, current, and oven temperature profiles.
	Event Log	Event messages associated with the run.
	Instrument Protocol	Instrument protocol and run module settings used for the run.
	Spatial Calibration Viewer	Spatial calibration associated with the run.
	Capillaries Viewer	Individual electropherogram for each sample in the run.
	Cap/Array Viewer	Array view of the run.
	Spectral Viewer	Spectral calibration used for the run.
	Reextraction	Extraction and analysis status for all samples in the run. See "Viewing Autoextraction Results" on page 147 for more details.

Note: If Cleanup Database has been used, you cannot view processed data in Run History.

7

Viewing Data from a Completed Run in the Data Collection Software

View data using two formats within the 3130/3130*xl* genetic analyzer Data Collection software under the Run History icon:

- In the Cap/Array Viewer window (in much the same way that you might view the gel file output from an ABI PRISM® slab gel instrument).

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

Viewing Data from a Completed Run

1. In the tree pane of the Data Collection software, click ▲ **GA Instruments** > 🖼 **ga3130***xl* or **ga3130** > 🖼 **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 run history elements, such as Cap/Array Viewer or the Capillary Viewer from the left tree pane to look at the run data.

Notes

Viewing Autoextraction Results

Overview

After a run is completed, extraction and analysis is performed automatically, using the settings in the Plate Editor and the Results Group. View the results of extraction and analysis in the Reextraction Panel. Reextract samples with the same settings, or with different Analysis Protocols or Results Groups. This process can be useful for many reasons:

- The destination location may not have been available during extraction.
- Some samples may have failed analysis and a different Analysis Protocol might be more successful.
- Samples might be saved in different locations, or with no analysis at all to save space.
- Sample names may need to be edited.

Sample File Destinations

Locate the Results Group's Naming Tab and check the Destination Tab and Run Folder Name Format to find the sample file destination.

Effects of Changes Made in the Reextraction Panel

Changes made in the Reextraction Panel to a Results Group, Analysis protocol, sample names, or Comments, also change in the original plate record. The original plate information is overwritten.

Notes

Selecting and Queuing Samples for Extraction

Queue individual samples for reextraction to experiment with different Analysis Protocols for samples that have failed initial extraction.

1. Click ![icon] (Run History).

2. Enter the plate name for a plate that has been completed, or click **Search**. Plates that have runs still pending cannot be reextracted. All the runs from that plate appear in the window.

3. Select a run from the list.

4. Click ![icon] (Reextraction) in the left tree pane to display the Reextraction window.

5. Click the check boxes in the Extract column to select the samples to be reextracted.

6. Click **Extract** to start the reextraction.

   Note: Reextracted sample files are saved in the original folder that data was extracted to unless this destination was changed through the Results Group.

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

Use check boxes to select samples to be reextracted

Select a run

Results of extraction and analysis

Click here to start extraction

Use these buttons if several samples are highlighted

Reextraction Window for Fragment Analysis

Use check boxes to select
samples to be reextracted

Select a run

Results of extraction

Foundation Data Collection Version 3.0   -   Administrator user is logged in

File   View   Edit   Help

GA Instruments > ga3130xl > Run History > Reextraction

Select a run to view: Run_DavidPT5_2003-07-08_16-18_0008

Extract	Cap	vWell	Extraction Resu	Results Group	Sample Name	Comment	Sample Type	Size Standard	Pi
☑	1	A01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	3	B01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	5	C01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	7	D01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	9	E01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	11	F01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	13	G01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	15	H01	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	2	A02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	4	B02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	6	C02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	8	D02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	10	E02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	12	F02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	14	G02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:
☑	16	H02	SUCCESS: Extr	gm_runbyrun	s		Sample	GS500LIZ	D:

GA Instruments
  Results Group
  Database Manager
  ga3130xl
    Plate Manager
    Protocol Manager
    Module Manager
    Run History
      EPT Viewer
      Event Log
      Instrument Protocol
      Spatial Calibration V
      Capillaries Viewer
      Cap/Array Viewer
      Spectral Calibration
      Reextraction
    DavidPT5
      Instrument Status
        EPT Chart
        Event Log
      Spatial Run Schedul
      Run Scheduler
        Plate View
        Run View
      Capillaries Viewer
      Cap/Array Viewer
      Spectral Viewer
      Manual Control
      Service Log

Extract... ← Click here to start extraction

Check   Uncheck

Use these buttons if several
samples are highlighted

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

Color	Value
Red	Extraction or analysis failed **Note:** Descriptive messages can be viewed by resizing the Results column to view all text.
Yellow	Warnings for extraction or analysis **Note:** Descriptive messages can be viewed by resizing the Results column to view all text.
Green	Successful extraction (with no analysis intended), or successful extraction and analysis.

The Results column, by default, shows only the beginning of any processing message. The entire message and the sample file location can be viewed by expanding the cell. There is a tooltip view for each sample results message.

Tooltip view. Access by placing the cursor over the sample of interest

Drag the cell's edge to expand the column

Foundation Data Collection Version 3.0 - **No User is logged in**

File View Edit Help

GA Instruments > ga3130xl > Run History > Reextraction

Select a run to view: Run_DavidPT2_2004-10-29_19-19_0005

Extract	Cap	lysis Protocol	Analysis Result
☑	1	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	3	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	5	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	7	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	9	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	11	0POP7_BDTv3-KB-De	ERROR: Analysis Failed Analysis failed due to poor data quality : (20321)Failed estimation of average peak spacing : (20325)Failed estimation of avera
☑	13	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded
☑	15	0POP7_BDTv3-KB-De	SUCCESS: Analysis Succeeded

GA Instruments
  Results Group
  Database Manager
  ga3130xl
    Plate Manager
    Protocol Manager
    Module Manager
    Run History
      EPT Viewer
      Event Log
      Instrument Protocol
      Spatial Calibration Vi
      Capillary Viewer
      Cap/Array Viewer
      Spectral Calibration
      Reextraction
    DavidPT2
      Instrument Status
        EPT Chart
        Event Log
        Spatial Run Schedule

Expanded column

Quality Column   The Quality column represents the quality values for an entire sequence. Quality Values are only assigned to analyzed samples when using the KB™ Basecaller.

Colors displayed 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.

Notes

Results Group and Analysis Protocol Columns

You can edit and make changes for reextraction to the Results Group and the Analysis Protocol (Analysis Method in the GeneMapper® software).

Note: Select an entire column in the Reextraction window by clicking on the column header. For example, clicking on the Extract column header selects all samples. Clicking the Uncheck or Check buttons at the bottom of the window, enables or disables the check boxes for each sample. The fill-down command (**Ctrl+D**) works the same here as in the Plate Editor for easier information input.

Sorting the Samples

1. Hold down the shift key while clicking on the column header to sort samples according to the column properties.

2. Shift-click again to sort them in reverse order. Sort by:
   - Capillary number
   - Well position
   - Results
   - Quality
   - Extract column

   Note: For example, bring all of the failed analysis or extraction samples to the top of the column. Now they can be viewed without having to scroll down to each sample individually.

7

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. Selecting a new Results Group allows you to turn off autoanalysis, change the samples and folder naming options, the location where they are placed, and the owner of the Results Group.

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**.

   Note: 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

Using Autoanalysis Manager Software

Use Autoanalysis Manager software with SeqScape and/or GeneMapper software. The Autoanalysis Manager software is installed by the SeqScape or GeneMapper software installation CD.

Autoanalysis occurs in the following sequence:

1. When data collection software finishes a run, the Message Service sends the message "Run Completed."

2. The Autoanalysis Manager receives the message, and the job is submitted. The job appears in the General tab.

3. The Autoanalysis Manager polls for jobs every 2 minutes and opens the automated processing version of SeqScape and/or GeneMapper software to analyze the data.

4. At the end of analysis, the status in the Autoanalysis Manager is updated.

Files Created   The data collection software stores the sample files in the location specified in the results group. The Autoanalysis Manager copies the files into the Data Store for SeqScape or GeneMapper processing.

To maintain sufficient storage space on your hard drive, delete or transfer unnecessary sample files to a different network or CD.

Autoanalyzed Samples   **Note:** If your GeneMapper or SeqScape software was opened during autoanalysis, then close your analysis application and relaunch the Autoanalysis Manager.

Once an internal message from the instrument is received by the Autoanalysis Manager, it opens the automated processing version of SeqScape and/or GeneMapper software to autoanalyze the samples. The standard user version of SeqScape and/or GeneMapper software must be closed in order for autoanalysis to begin.

Notes

Components

The Autoanalysis Manager has two or three tabs:

- General tab
- GeneMapper tab, if GeneMapper v3.7 or GeneMapper *ID* v3.2 software is installed
- SeqScape tab, if SeqScape software is installed

General Tab   The General tab shows the jobs that have been submitted and their status.
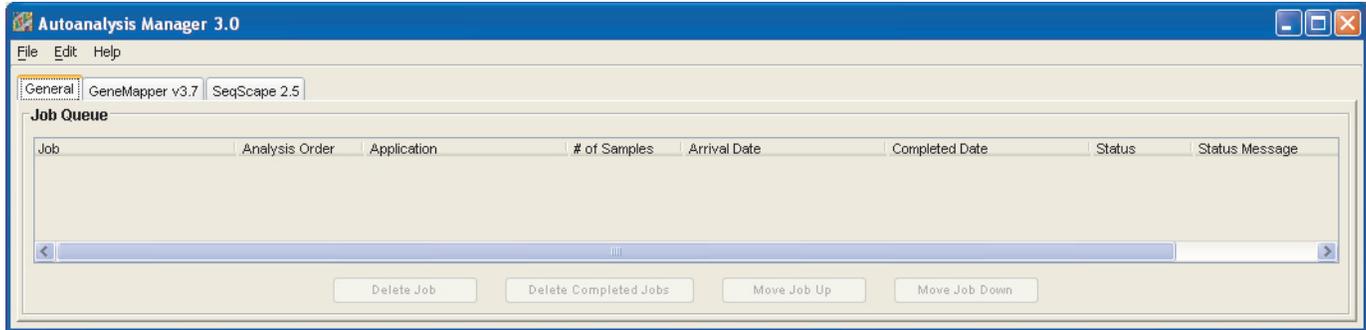
Command Buttons in the General Tab

Command Button Functions in the General Tab

Button Name	Function
Delete Job	Deletes an individual job/project from the Autoanalysis Manager list. Does not delete sample files, SeqScape or GeneMapper software project.
Delete Completed Jobs	Deletes all completed jobs/projects from the Autoanalysis Manager list. Only successful jobs are deleted. Does not delete sample files, SeqScape or GeneMapper software projects.
Move Job Up	The active job/project is always given a queue number of 1. Once job 1 is finished analyzing, job 2 becomes job 1 and all other numbers are changed accordingly. Use the Move Up/Down buttons if you want to rearrange the analysis order.
Move Job Down	

Notes

GeneMapper Software Tab

The GeneMapper software tab shows the jobs that have been submitted and their status.
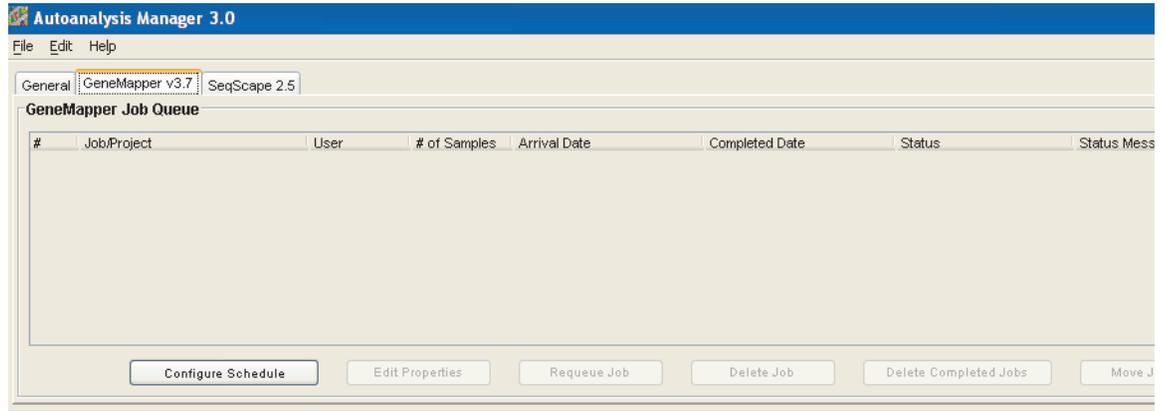
Command Buttons in the GeneMapper Software Tab

Command Button Functions in the GeneMapper Software Tab

Button Name	Function
Configure Schedule	• Next Analysis Time: Enables you to set a start time for autoanalysis. Before this time arrives, autoanalysis of projects will not occur. • Periods restricting automated analysis: Enables you to set times during which autoanalysis will not occur. Useful if you know that you are going to be reviewing data during a certain time period and don't want to be bothered by the "Runs ready for processing" dialog box. Runs build up in the queue until the restricting time period is over at which time the runs will be autoanalyzed. • Automatic Deletion of Completed Jobs: Enables you to set the software to automatically delete successfully completed jobs. Jobs that failed or have not been analyzed will not be deleted. Only the Autoanalysis Manager job is deleted, sample files and GeneMapper software projects are not.
Edit Properties	Enables you to change the following settings: • Job/Project Name • UserName: GeneMapper software UserName • Password: Matching password for GeneMapper software UserName • Queue position: Enter a new queue position number for the project

Notes

7

Button Name	Function
Requeue Job	• Samples that need to be autoanalyzed have queue numbers listed in the # column. • Samples that are already analyzed or failed, have a blank cell in the # column. To resubmit a job for autoanalysis, use the Requeue Job button to assign a queue number to that job.
Delete Job	• Deletes an individual job/project from the AutoAnalysis Manager list. Does not delete sample files or GeneMapper software project.
Delete Completed Jobs	• Deletes all completed jobs/projects from the AutoAnalysis Manager list. Only successful jobs are deleted. Does not delete sample files or GeneMapper software projects.

Notes

SeqScape Software Tab

The SeqScape software tab shows the jobs, project, and status information.
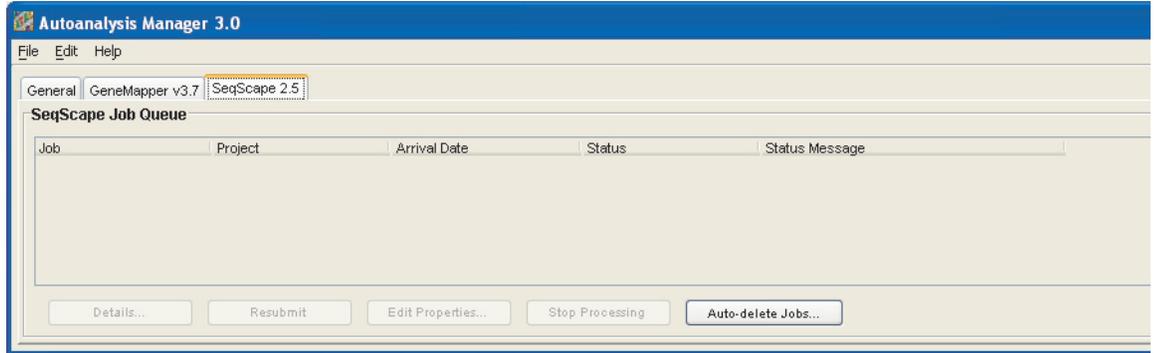
Command Button Functions in the SeqScape Software Tab

Button Name	Function
Details	Displays the project in the navigation pane
Resubmit	Submits a job for analysis
Edit Properties	Edits the name and password (active only if analysis failed)
Delete	Deletes a job from the Autoanalysis Manager
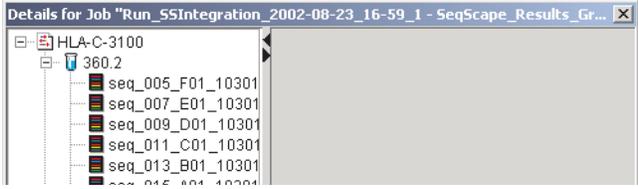
Viewing Analyzed Data

Locating Sample Files

Analyzed sample files are extracted into a run folder defined in the Naming tab of your Results Group, and placed in a location defined in the Destination tab.

The default location is:

E:\AppliedBiosystems\UDC\Data Collection\Data\ *<instrument type>\<instrument name>\<Run Name>*
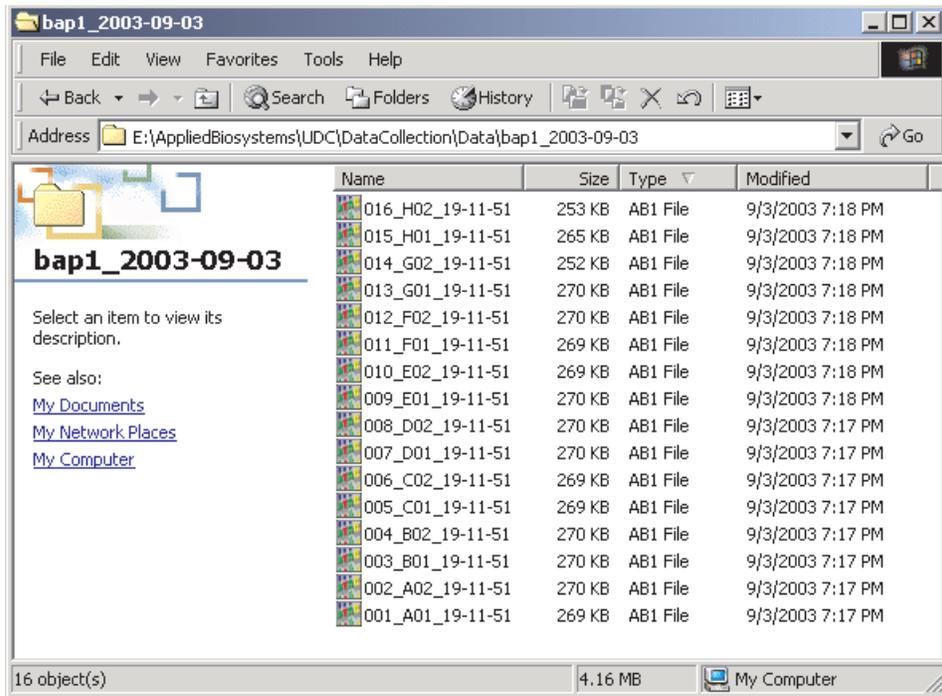
Locating Sample Files

Locate the reextracted data in the location defined by the Results Group or the default destination location.

Viewing Sample Files

Use the Sequencing Analysis, SeqScape, or the GeneMapper Software to view re-extracted sample electropherogram data, both raw, and analyzed.

Sample file type	File extension
Sequencing, SeqScape	.ab1
Fragment analysis	.fsa

Notes

Plate Mapping

Injection Scheduling

Samples are scheduled for injection based on their position in a 96-or 384-well plate and the capillary number. Study the appropriate plate mapping patterns described in "96-Well Plate Mapping" or "384-Well Plate Mapping" to arrange your samples according to your preferred injection priority.

The injection schedule system is based on the:

- Order of the linked plates (3130*xl* instrument only). See page 128 for linking plate information.
- Sample priority value in the plate record.

If all priorities are set to 100 (default), then runs are scheduled as outlined below. See the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* on "Run Priority Scheduling".

Capillary Array Map

The capillary numbers in the capillary array are shown in the layout below. The capillary array layout is the same for both 96- and 384-well plates.

Capillary Number Layout
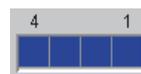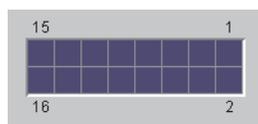
96-Well Plate Mapping

3130*xl* Genetic Analyzer

For a 96-well plate, injections are made from every well in two consecutive rows, starting with an odd row. A full 96-well plate requires six runs to inject all samples. See the examples below where samples (grey) and capillary number (number) positions can affect your number of runs.
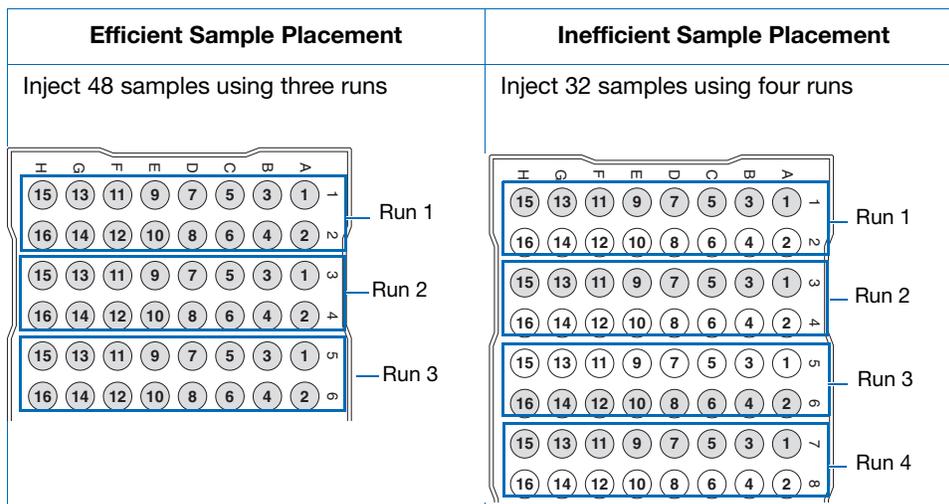
3130 Genetic Analyzer

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

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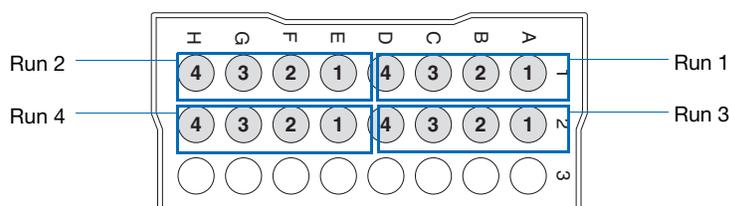
Notes

384-Well Plate Mapping

3130*xl* Genetic Analyzer

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

The example below is the injection pattern for the first four injections, starting with well A01. The light gray circles represent samples and the dark gray circles indicate the injection pattern.
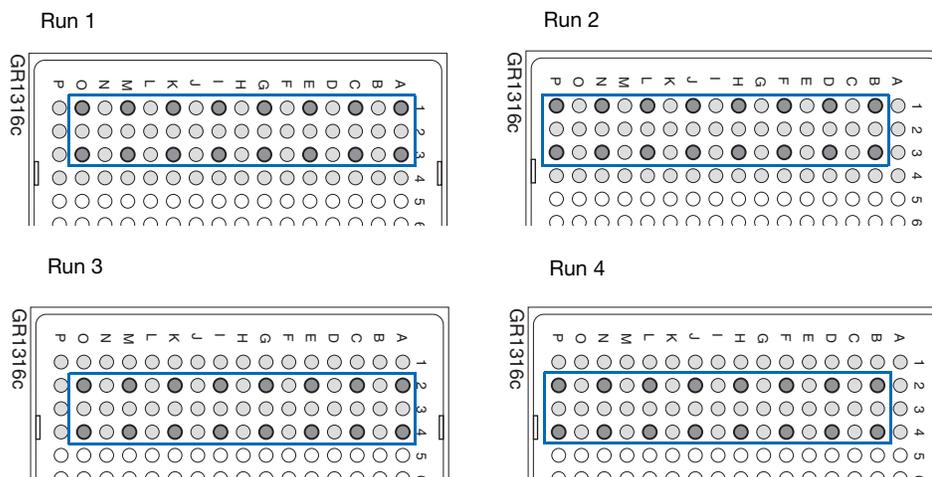
3130 Genetic Analyzer

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

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

Notes _____

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