

USER GUIDE

applied
biosystems®
by *life* technologies™

Applied Biosystems® 3730/3730xl DNA Analyzer

Getting Started

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Revision E

life
technologies™

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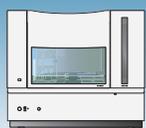
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About This Guide

IMPORTANT! Before using this product, read and understand the information the “Safety” section in this document.

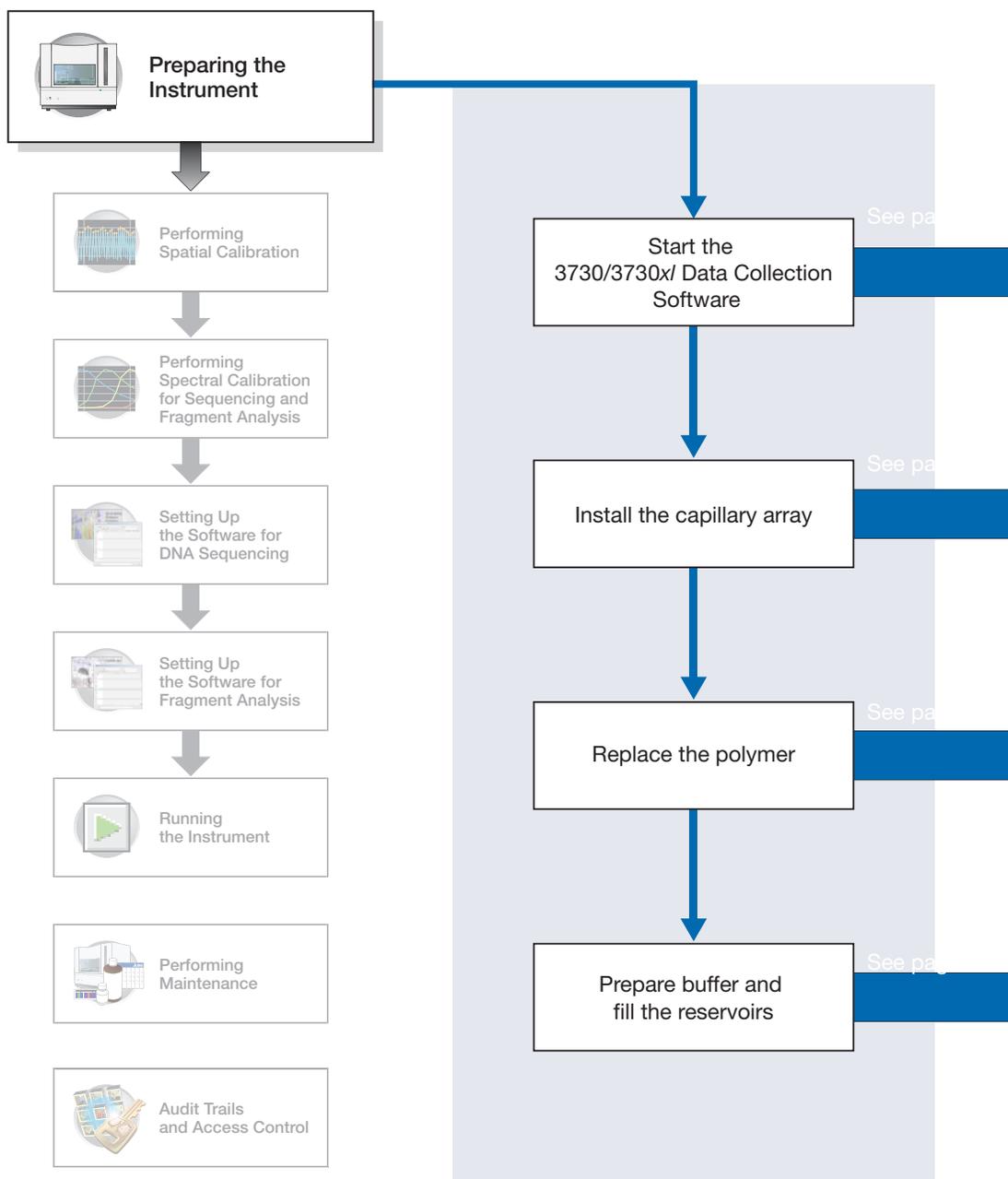
Revision history

Revision	Date	Description
E	September 2014	Update laser information in Safety section.



Preparing the Instrument

1

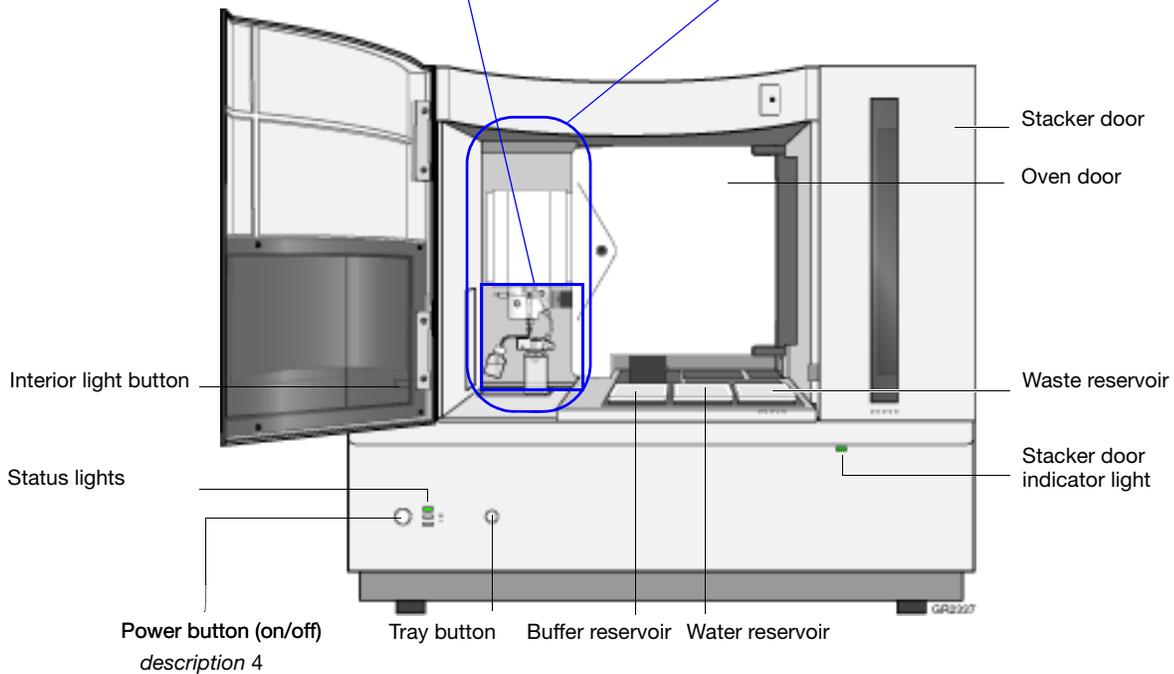
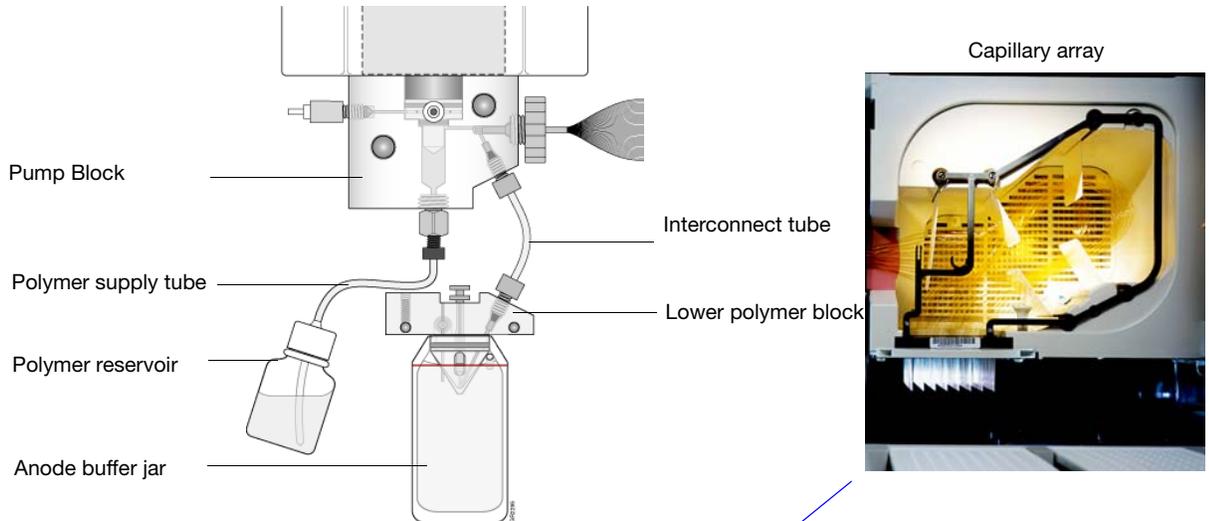


Notes _____



Instrument and Parts

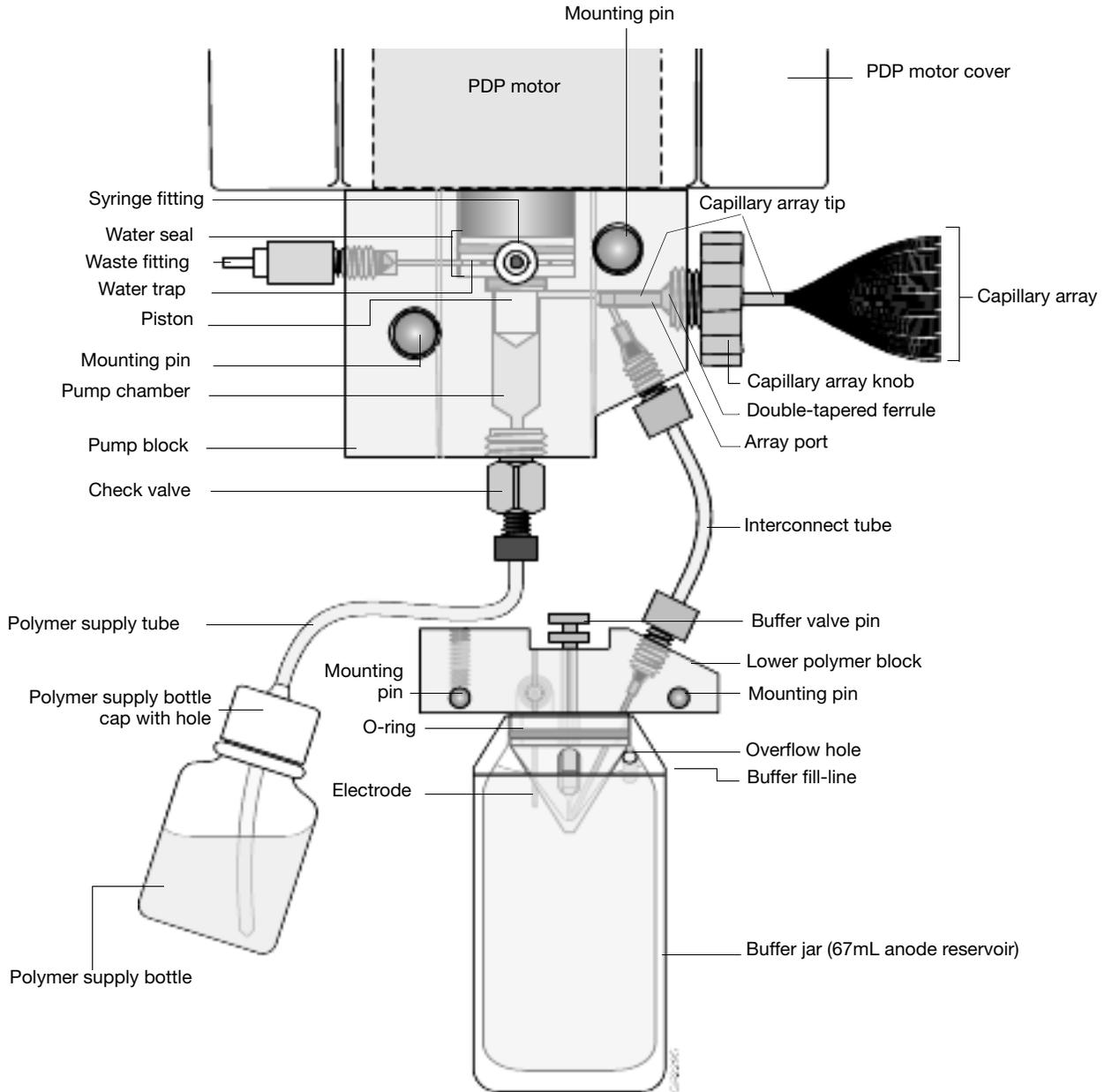
Polymer Delivery Pump (PDP)



Notes _____



Polymer Delivery Pump Detail



Notes _____



Overview

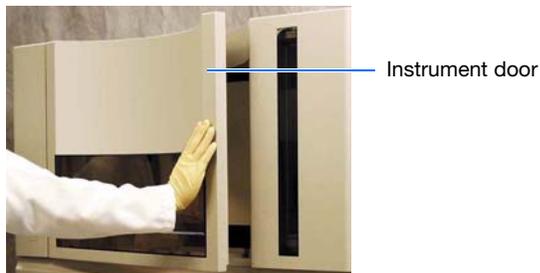
This chapter explains how to prepare the instrument for a run by installing the capillary array, buffer, and reservoirs.

Powering On the Computer and 3730/3730xl Analyzer Instrument

1. Press the power button on the monitor to power it on.
2. Press the power button on the computer to power it on.
3. In the **Log On to Windows** dialog box:
 - a. In the **User Name** field, enter your user name.
 - b. In the **Password** field, enter your password.
 - c. Click .
4. Close the oven door.
5. Close the stacker drawer.



6. Close the instrument door.



7. Wait until the monitor displays the desktop of the Windows® operating system.
8. Press the power button on the 3730/3730xl Analyzer instrument to power it on.

Notes _____



The Status Lights

Status	Status Light	Action
<ul style="list-style-type: none"> The instrument is ready An automated wizard operation is in progress with the instrument door closed 	Solid Green 	Go to page 9.
<ul style="list-style-type: none"> A run is in progress 	Flashing Green 	
<ul style="list-style-type: none"> The instrument cannot communicate with the computer. 	Solid Yellow 	Go to page 7.
<ul style="list-style-type: none"> The instrument is downloading firmware The instrument is performing diagnostics The oven door is open The instrument door is open The buffer reservoir is not installed The capillary array is not installed An automated wizard operation is in progress with the instrument door open 	Flashing Yellow 	Go to page 7.
<ul style="list-style-type: none"> The instrument has detected a problem 	Solid Red 	Go to page 7.

Notes _____



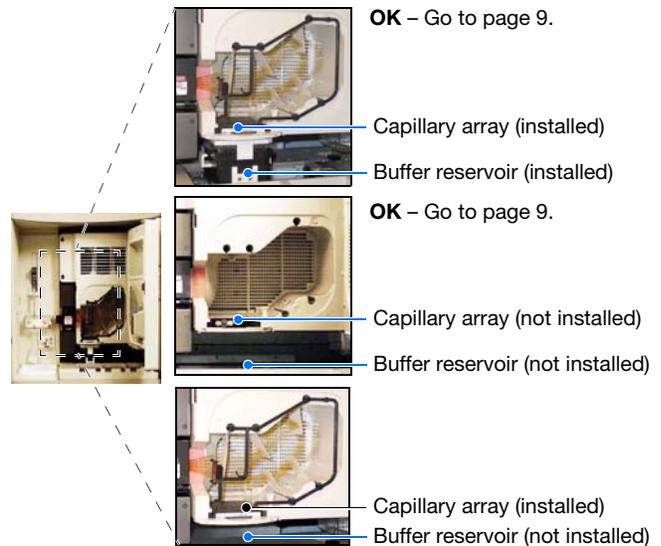
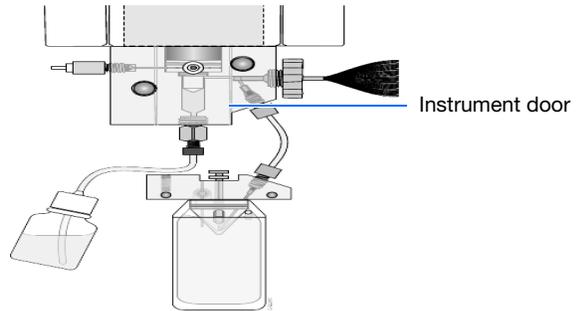
Troubleshooting Instrument Status Lights

Flashing Yellow



To determine the source of the problem:

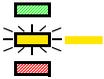
1. Press on the instrument door to ensure that it is closed. If the 3730/3730xl Analyzer instrument displays the green status light, then the instrument door was open. Go to page 9
2. If the 3730/3730xl Analyzer instrument continues to display the flashing yellow light:
 - a. Open the instrument door.
 - b. Press on the oven door to verify that it is closed.
 - c. Close the instrument door.
 - d. If the 3730/3730xl Analyzer instrument displays the green status light, then the oven door was open. Go to page 9
3. If the 3730/3730xl Analyzer instrument continues to display the flashing yellow light:
 - a. Open the instrument door.
 - b. Open the oven door.
 - c. Check that the buffer reservoir and capillary array are installed.
 - d. Close the oven door.
 - e. Close the instrument door.



Notes _____



Solid Yellow Light

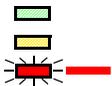


To determine the source of the problem, verify that the:

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

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

Solid Red Light



To determine the source of the problem:

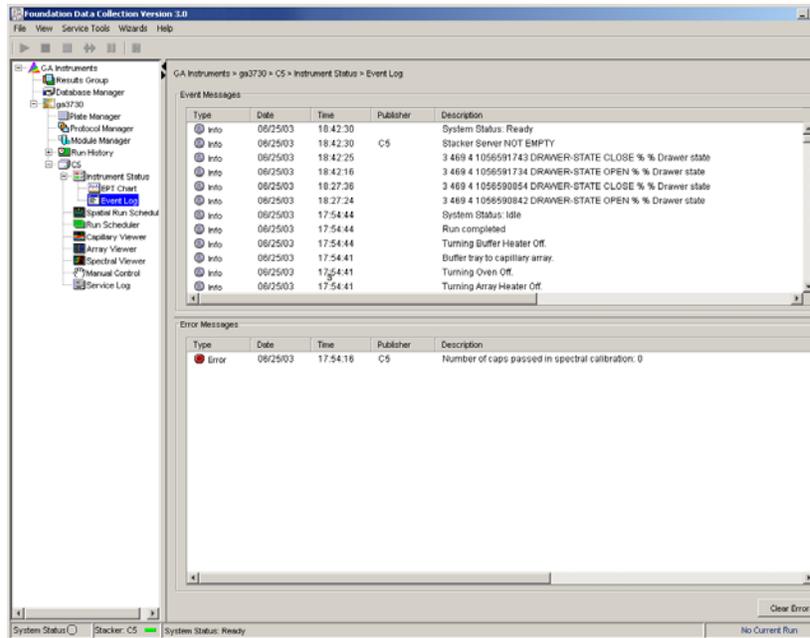
1. If the instrument continues to display the solid red light:
 - a. Power off the instrument.
 - b. Wait for 30 seconds.
 - c. Power on the instrument.
2. If the instrument continues to display the solid red light:
 - a. Start the 3730/3730xl Analyzer Data Collection Software as explained page 9.
 - b. In the navigation pane of the Data Collection Software, double-click **GA Instruments > ga3730 > instrument name > Instrument Status > Event Log**.

Notes _____



Chapter 1 Preparing the Instrument

Troubleshooting Instrument Status Lights



- c. In the Event Log view, find the last message in the log file.
 - d. Using the error code, perform the required tasks to fix the problem.
3. If the instrument continues to display the solid red light, contact technical support or your service representative for further assistance.

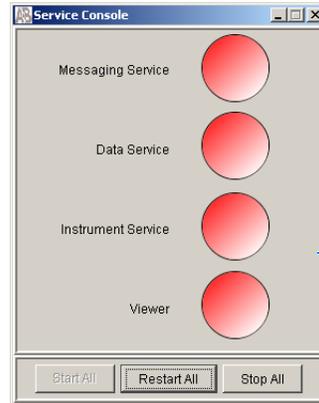
Notes _____



Starting the 3730/3730xl Analyzer Data Collection Software

1. Select  **start** > **All Programs** > **Applied Biosystems** > **Unified Data Collection** > **Run Unified Data Collection v3.0.**

The data collection software opens the Service Console dialog box.

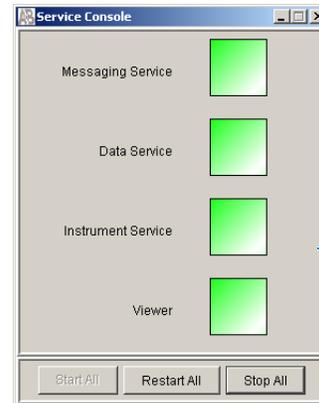
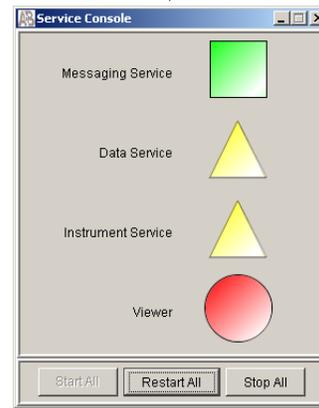


Red circles indicate that applications of the data collection software are not running.

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



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



Applications of the data collection software are running

Notes _____



Installing the Capillary Array

WARNING **CHEMICAL HAZARD.**

POP 7™ polymer may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

WARNING **CHEMICAL HAZARD.**

Running Buffer with EDTA causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Required Materials

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

Guidelines for Capillary Use

- Do not bend the capillaries
- Store capillary arrays using a buffer reservoir and the header shipping cover. For storage information refer to the *Maintenance and Troubleshooting Guide* (PN 4359473).

Installing a New or Used Capillary Array

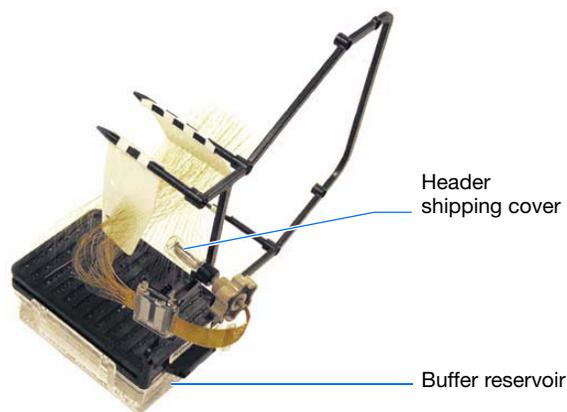
IMPORTANT! Wear gloves when you handle the capillary array.



CAUTION Failure to use the Install Array wizard when changing capillary arrays can result in degraded analysis data.

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

Notes





3. On the toolbar, select **Wizards > Install Array Wizard**.
4. Install the array as instructed by the Array wizard.
5. Perform a spatial calibration (see page 22).



Notes _____



Replacing the Polymer

Note: You can omit this section if you have installed a capillary array using the Install Array wizard during the initial activation of the instrument.



WARNING CHEMICAL HAZARD.

POP 7™ polymer may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Use for research and development purposes only.

Required Materials

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

Guidelines for Polymer Use

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

When to Replace the Polymer

Replace the polymer on the instrument:

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

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

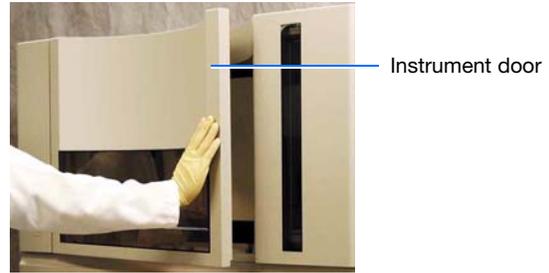
Notes _____



 **CAUTION** Wear gloves when you handle polymer.



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



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



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

Notes _____



Preparing Buffer and Filling the Reservoirs

WARNING CHEMICAL HAZARD.

Running Buffer with EDTA causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Required Materials

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

Buffer Storage

The 1X run buffer can be stored at:

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

When to Change the Buffer

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

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

Notes _____



Preparing the 1× Run Buffer

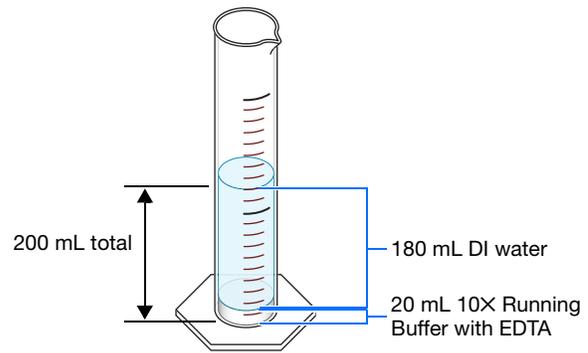
IMPORTANT! Wear gloves when you handle running buffer with EDTA.



WARNING CHEMICAL HAZARD.

Running Buffer with EDTA causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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

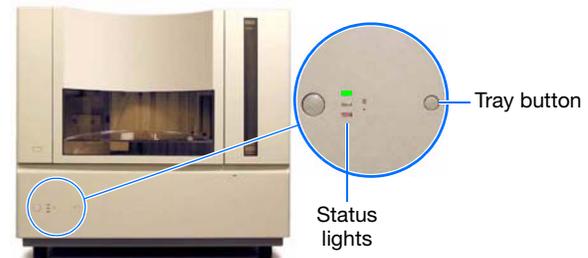


Filling the Water and Buffer Reservoirs

IMPORTANT! Wear gloves when you handle the reservoir.



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



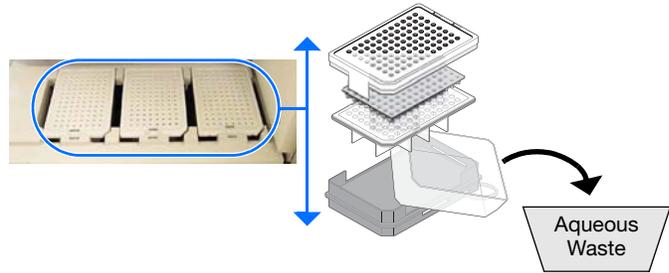
Notes



Chapter 1 Preparing the Instrument

Preparing Buffer and Filling the Reservoirs

4. Unplug the buffer reservoir. Remove the buffer, water, and waste reservoir assemblies from the instrument.
5. Disassemble each reservoir assembly then empty the contents of the reservoirs into an aqueous waste container.
6. Rinse each reservoir using deionized water.
7. Dry the reservoirs using lint-free wipes.



DI H₂O ≤40 °C

8. Fill then assemble the reservoirs.

Buffer Reservoir Assembly

- a. Add 80 mL 1X run buffer to the Buffer reservoir.
- b. Assemble the reservoir assembly as shown below:

GR2210

Water and Waste Reservoir Assemblies

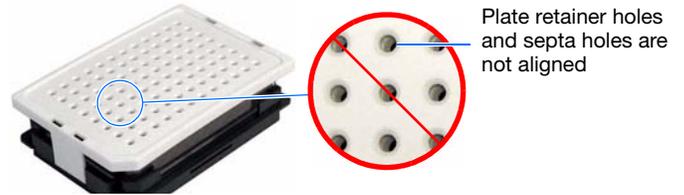
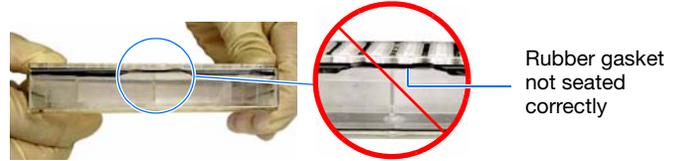
- a. Add 80 mL high-quality deionized water to each reservoir.
- b. Assemble each reservoir assembly as shown below:

Notes _____



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

- Septa fit snugly and flush on the reservoir cap
- Rubber gasket around the edge of the reservoir cap is seated correctly
- Holes of the plate retainer and the septa strip are aligned



10. Dry the reservoirs using lint-free wipes.



Notes _____



Placing Reservoirs into the Instrument



WARNING CHEMICAL HAZARD.

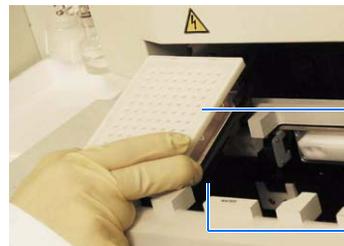
Running Buffer with EDTA causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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



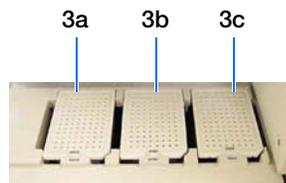
Heater outlet
Plate base cable

2. Move the buffer reservoir to the Buffer position (left) making sure the cable is out of the way of the autosampler.

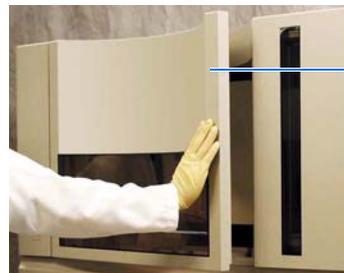


Buffer reservoir
Buffer position

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



4. Close the instrument door.



Instrument door

Notes _____



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



Filling the Anode Buffer Jar

! WARNING CHEMICAL HAZARD.

Running Buffer with EDTA causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

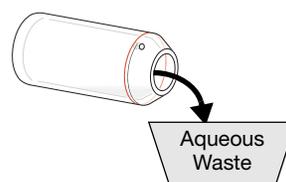
Replace the anode buffer:

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

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



1. Remove the anode buffer jar by pulling it down and twisting it slowly.
2. Empty the anode buffer jar into an aqueous waste container.
3. Rinse the anode buffer jar using deionized water.
4. Rinse the anode buffer jar using 1× run buffer:
 - a. Add 5 mL 1× run buffer to the anode buffer jar.
 - b. Tilt the anode buffer jar 90°.



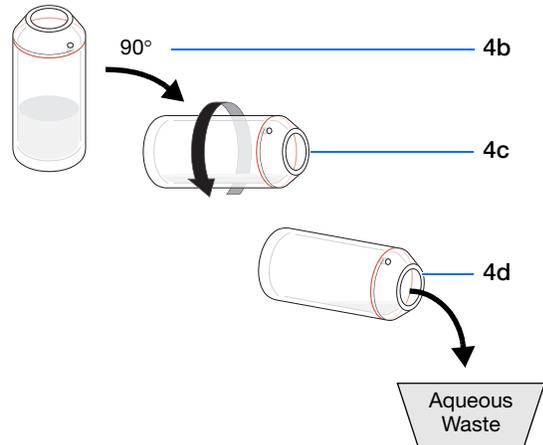
Notes _____



Chapter 1 Preparing the Instrument

Placing Reservoirs into the Instrument

c. Rotate the jar to rinse the interior with buffer.



d. Empty the anode buffer jar into an aqueous waste container.

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

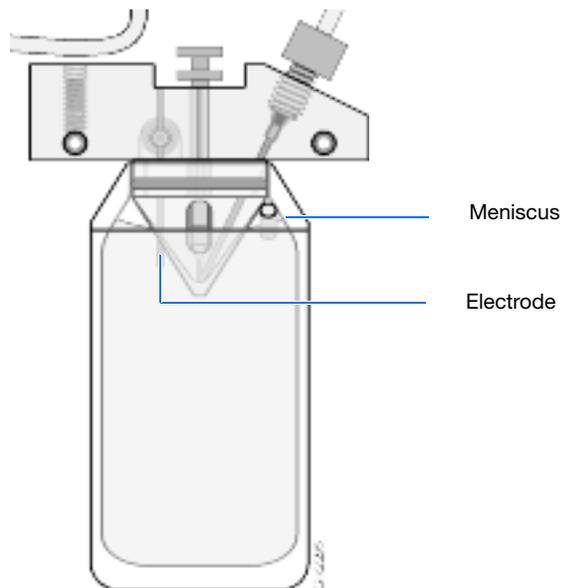
6. Put the anode buffer jar on the instrument with the overflow hole facing you.

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

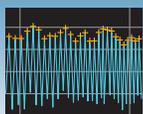
7. Verify that the electrode is immersed in the buffer.

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

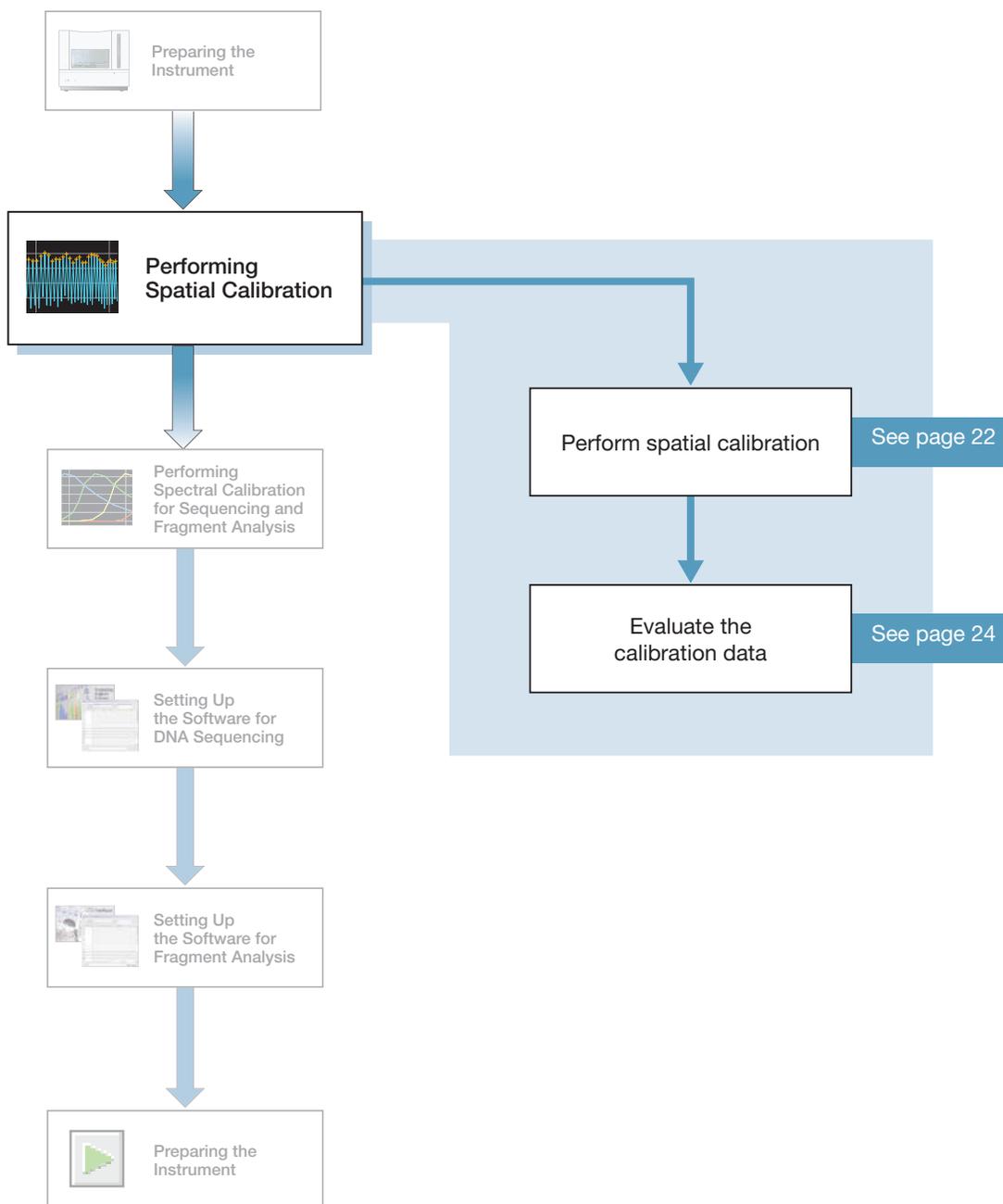
IMPORTANT! Replace buffer if excess polymer is expelled into the anode jar.



Notes _____

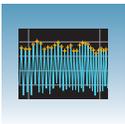


Performing Spatial Calibration



2

Notes



Overview

What a Spatial Calibration Tells You

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

When to Perform a Spatial Calibration

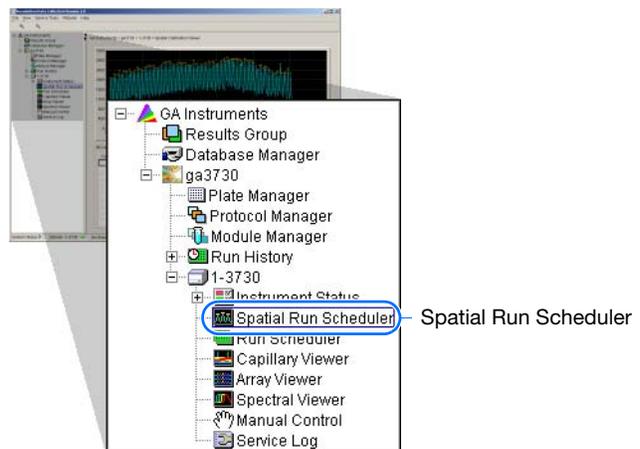
Perform a spatial calibration after you:

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

Performing Spatial Calibration

1. In the navigation pane of the Data Collection Software, double-click

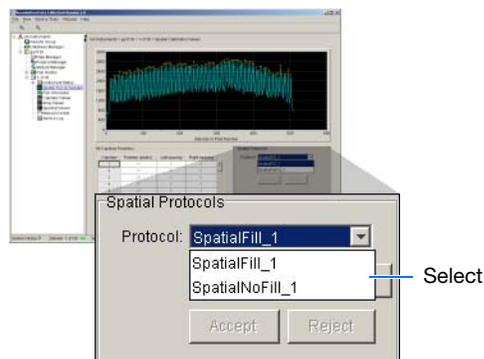
GA Instruments > ga3730 > instrument name > Spatial Run Scheduler.



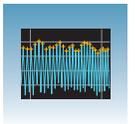
2. In the Spatial Run Scheduler view, do one of the following:

- If the capillaries contain fresh polymer, select **Protocol > SpatialNoFill**.
- Otherwise, select **Protocol > SpatialFill**.

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



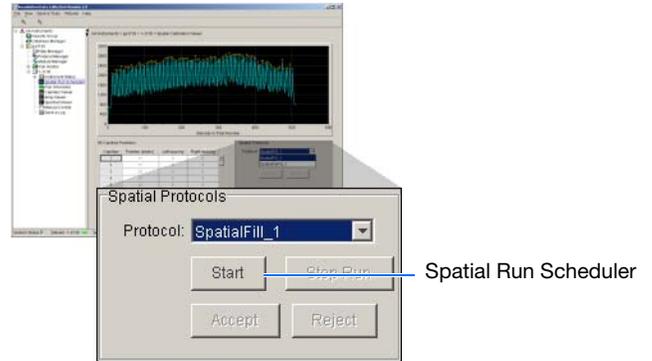
Notes _____



3. Click .

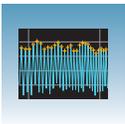
The approximate calibration run times are:

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



4. Evaluate the calibration as explained on page 24.

Notes _____



Evaluating the Calibration Data

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

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

Are the peaks in the profile approximately the same height?

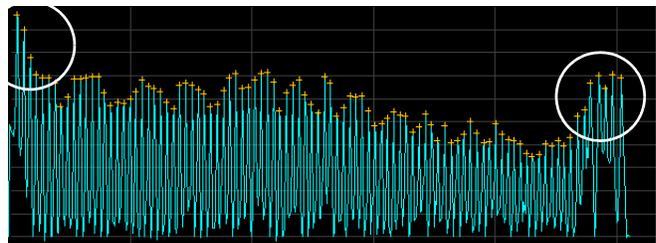
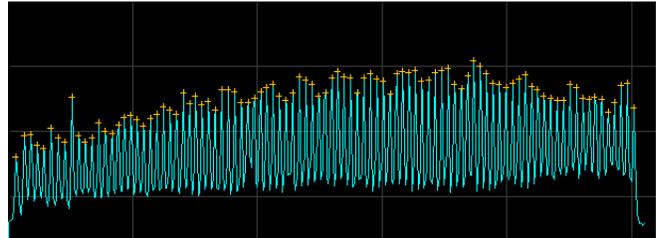
Yes – Go to step 2 on page 25.

No – How does the peak height vary?

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

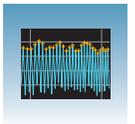
Go to step 2 on page 25.

Irregular – If the peak heights are irregular, go to “If the Calibration Fails” in the *Maintenance and Troubleshooting Guide PN 4359473*.



Magnifying the Spatial Profile	
<p>a. Click and drag the cursor to create a box around the area of interest.</p>	
<p>b. Release the mouse button. The data collection software displays the selected region.</p> <p>c. Press R to reset the view.</p>	

Notes _____



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

Does a cross appear at the top of each peak?

Yes – Go to step 3.

No – Where in the profile is the peak located?

- Left side of the profile

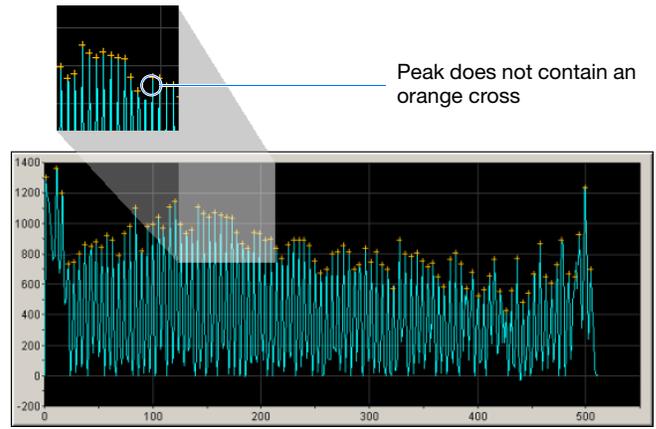
If using a 96-capillary array, a small peak may appear in the left side of the profile.

The peak is normal, go to step 3.

- After the first peak

The data collection software did not locate the peak correctly.

Move an orange cross to cover the peak. See, “To move an orange cross” in the *Maintenance and Troubleshooting Guide PN 4359473*.



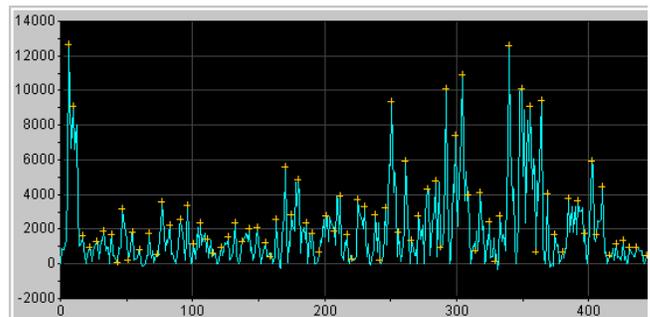
3. Check the profile for irregular peaks.

Does the profile contain any irregular peaks?

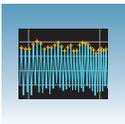
Yes – The calibration run has failed. Go to “If the Calibration Fails” in the *Maintenance and Troubleshooting Guide PN 4359473*.

No – Go to step 4.

Elements of a poor spatial



Notes _____



4. Examine each row of the 96 Capillary Position table. Typical values for the **Left spacing** and **Right spacing** columns are:

- 4 to 8 pixels for a 96-capillary array
- 9 to 11 pixels for a 48-capillary array

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

Be sure to account for all capillaries (e.g., 96 capillary positions for 96 capillary array).

- If *not*, verify that all peaks have crosses. If each peak does not each have a cross, see the Troubleshooting table below.
- If *yes*, go to step 5.

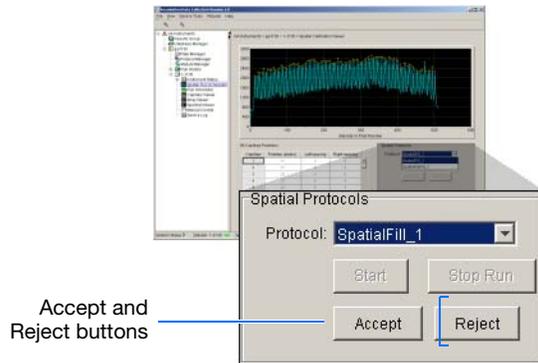
5. Accept or reject the spatial calibration as follows:

If the calibration:

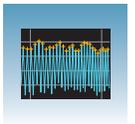
- Passed, click **Accept** writes the calibration data to the database.
- Failed, click **Reject**, then go to “If the Calibration Fails” in the *Maintenance and Troubleshooting Guide PN 4359473*.

Capillary	Position (pixels)	Left spacing	Right spacing
1	11	0	6
2	17	6	5
3	22	5	5
4	27	5	5
5	32	5	5
6	37	5	6
7	43	6	5
8	48	5	5
9	53	5	5
10	58	5	5

Left spacing and Right spacing columns

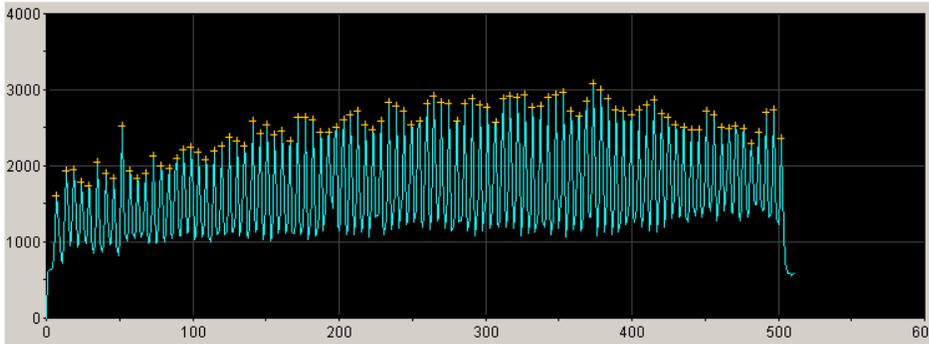


Notes _____



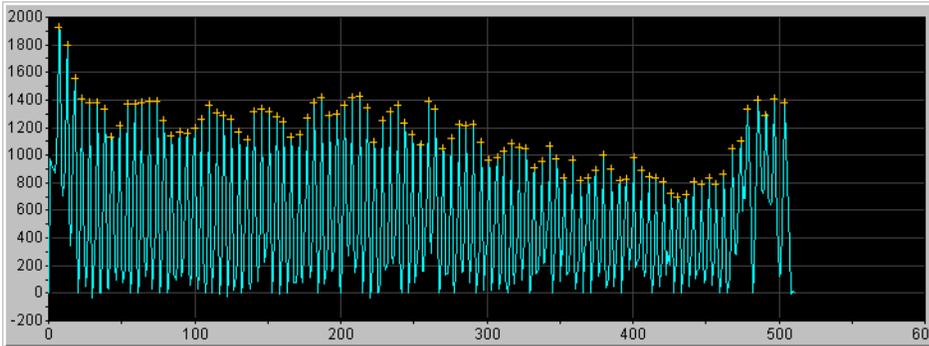
Examples of Passing Spatial Profiles

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

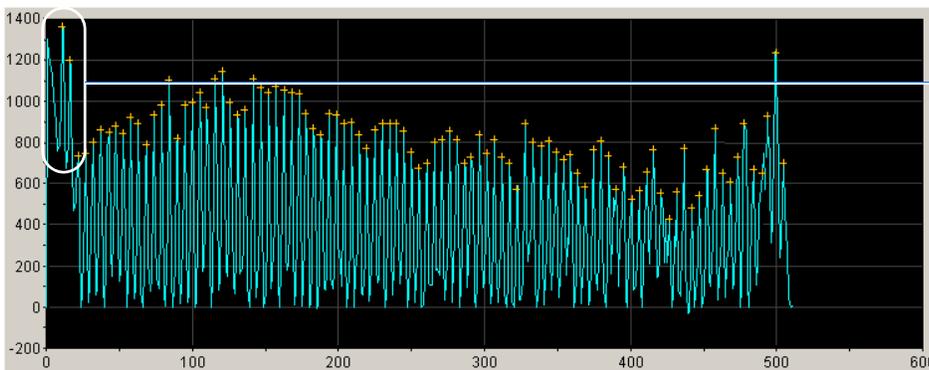


Passing Profile #1

This example shows a typical passing profile.



Passing Profile #2

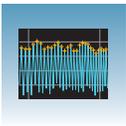


Passing Profile #3

Background artifact

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

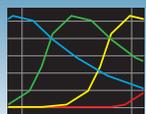
Notes



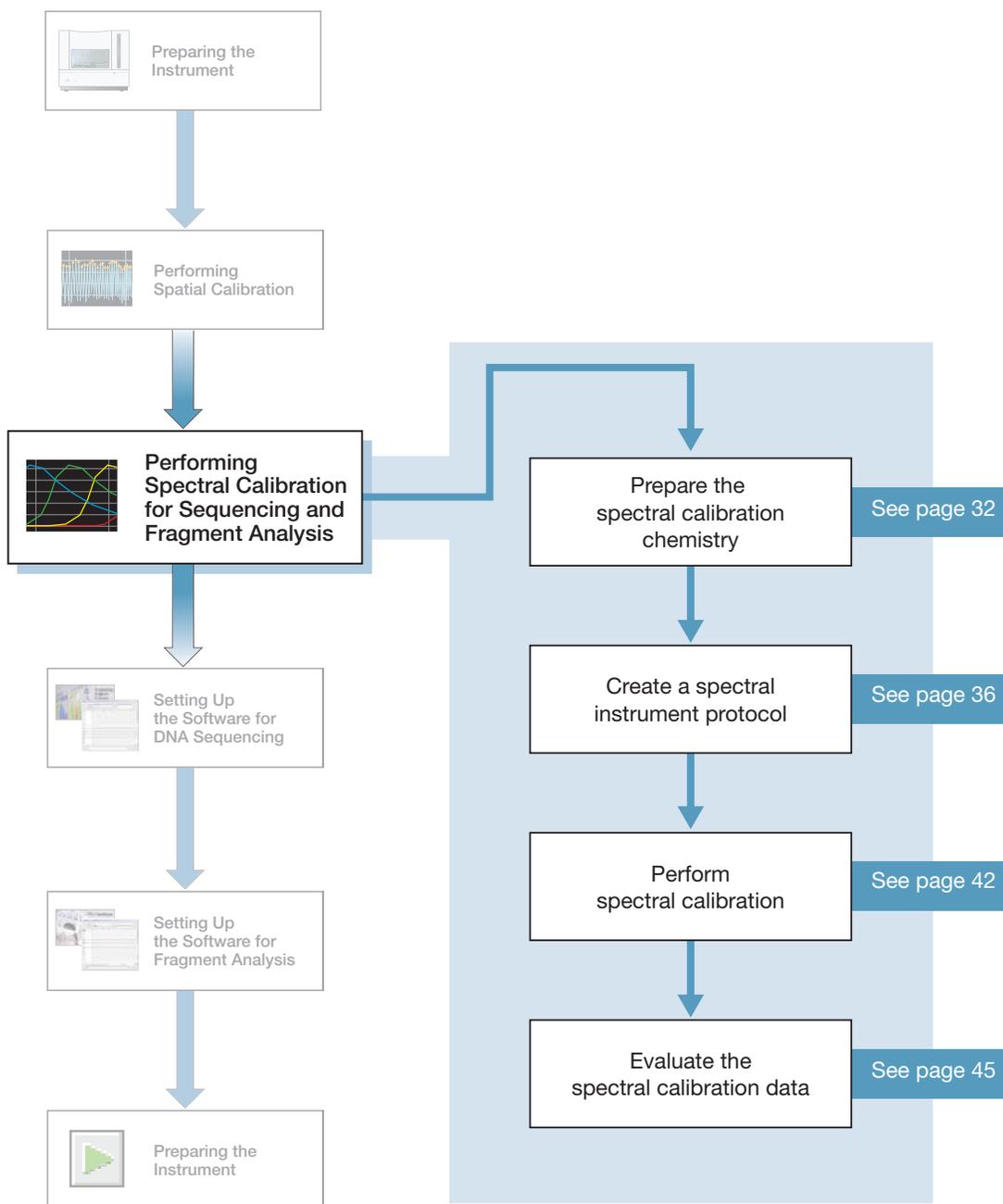
Chapter 2 Performing Spatial Calibration

Evaluating the Calibration Data

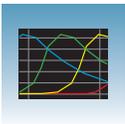
Notes _____



Performing Spectral Calibration For Sequencing and Fragment Analysis



Notes _____



Overview

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

IMPORTANT! Do not run your computer's Internet Connection wizard during a spectral calibration.

Note: A spectral calibration algorithm checks dye order. If the algorithm determines that the dyes are not in the correct order, the error message is "failed calibration due to bad data: Bad dye order detected." It is possible for the major peaks of the matrix standard to appear in the correct order and still receive this error message.

Spectral calibrations are performed with a specific combination of:

- Dye set (G5, G5-RCT, Any4Dye, Any4Dye-HDR, Any5Dye, E or Z). For further information see, "Preparing the Spectral Calibration Chemistry" on page 32 and, Appendix B, Dye Sets: G5, G5-RCT, Any4Dye, Any4dye-HDR, and Any5Dye.
- Array type (48-capillary or 96-capillary)
- Array length (36-cm or 50-cm)

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

When to Perform the Calibration

Perform a spectral calibration:

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

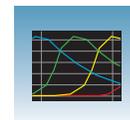
Changing Capillary Array Lengths

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

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

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

Notes _____



Required Materials

Part numbers are located in Appendix A

Description

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

Two Types of Calibration Standards

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

- For Fragment Analysis–Matrix standards are four or five fragments of varying size that are individually labeled with one of the four or five dyes of a set.
- For Sequencing–Sequencing Standards are standard sequencing reaction fragments of varying size that are individually labeled with one of the four dyes.

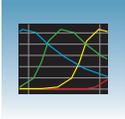
Select Dye Sets and Calibration Standards

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

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

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

Notes



Preparing the Spectral Calibration Chemistry

WARNING CHEMICAL HAZARD.

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

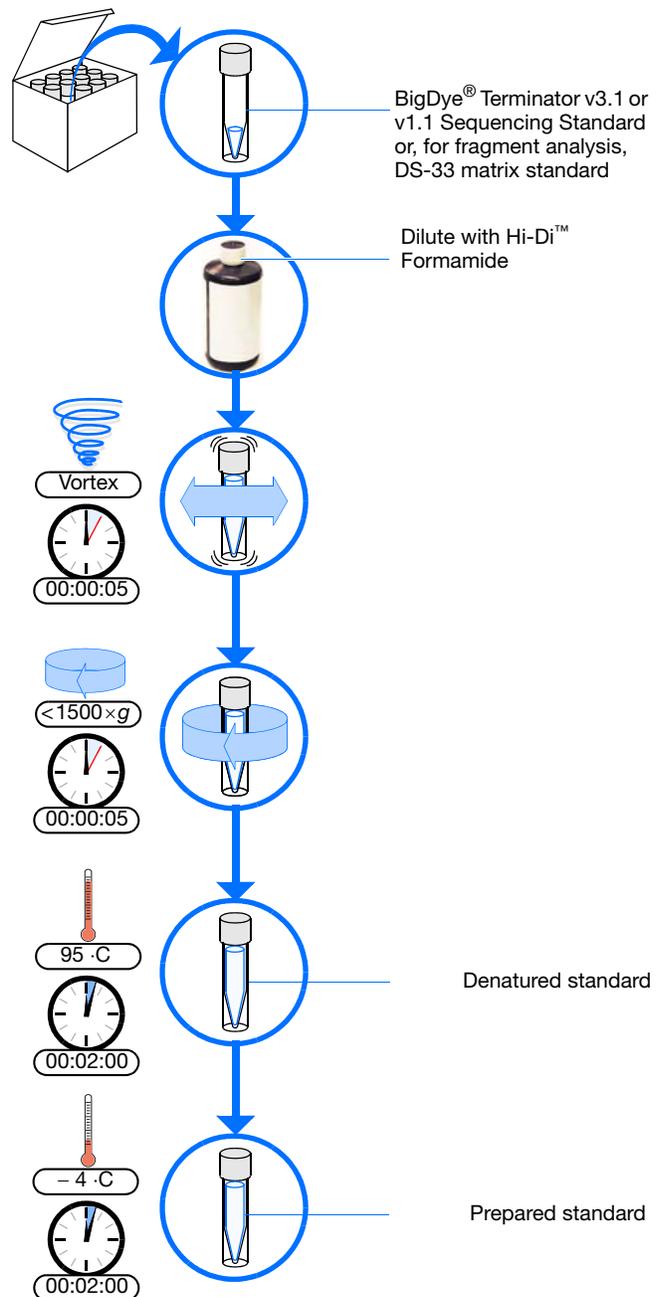
1. Dilute the spectral calibration standard with Hi-Di™ Formamide according to the insert instructions.

2. Vortex thoroughly.

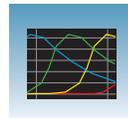
3. Briefly centrifuge the mixture.

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

5. Cool the tubes on ice for 2 minutes.



Notes _____



- Vortex thoroughly and then briefly centrifuge the mixture.

Sealing and Preparing the Plate Assemblies

WARNING Do not use warped or damaged plates.




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

If using a:

- **48-capillary, 96-well plate** – Add 10 μL of denatured standard to each well.
- **384-well plate** – Add 5 μL of denatured standard into alternating wells of the plate. See page 127 for load maps.

- Seal the plate with a septum or heat-seal:

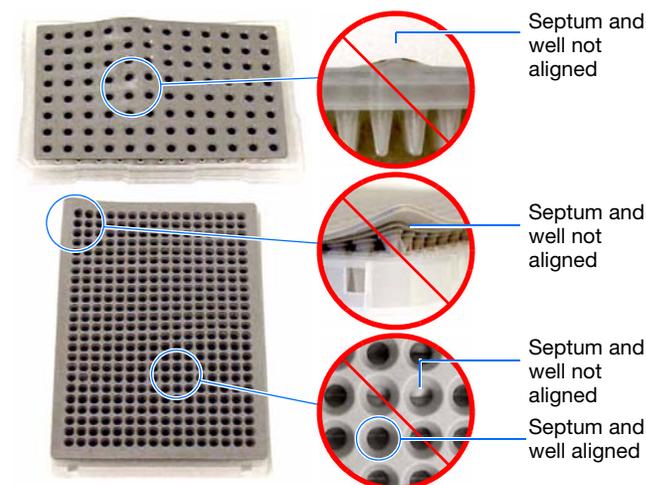
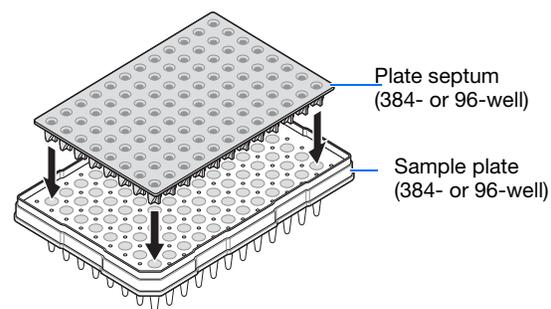
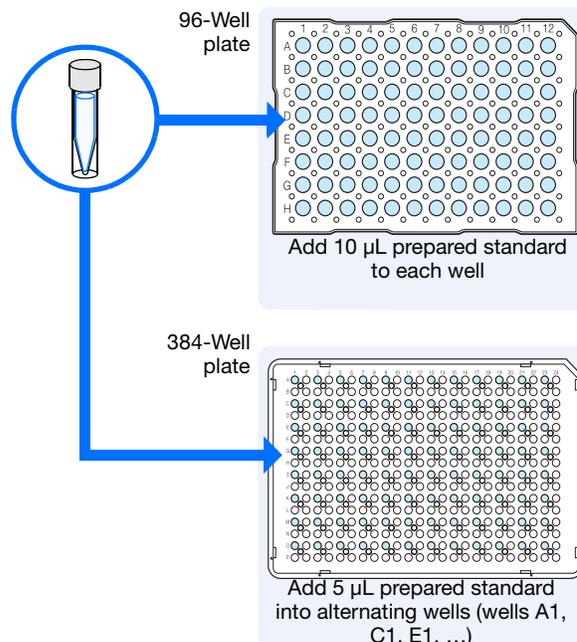
With a septum:

- Place the plate on a clean, level surface.
- Lay the septum flat on the plate.
- Align the holes in the septum strip with the wells of the plate, then firmly press downward onto the plate. Ensure that:

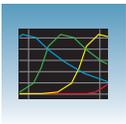
- The septa lie flat against the plate. You should not feel any lumps or raised edges.
- The septa are inserted straight into the wells. You should not see any bent or crooked duckbills when viewing the plate from above.

With heat-seal:

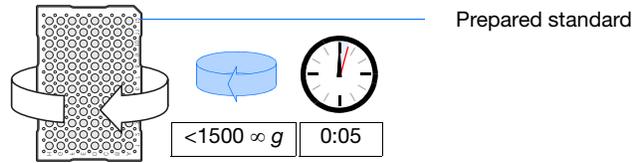
- Follow your thermal sealer instrument instructions.



Notes _____

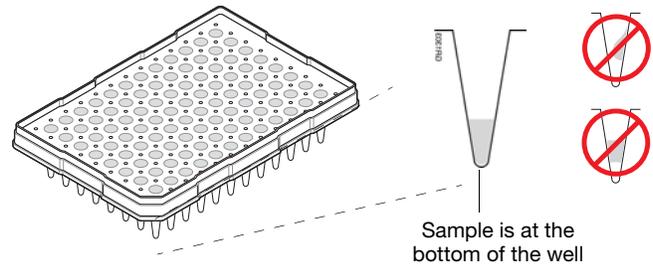


3. Briefly centrifuge the plate.

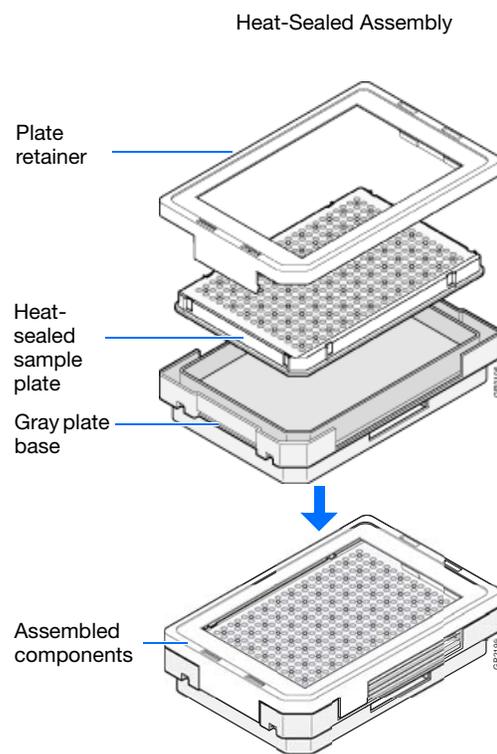
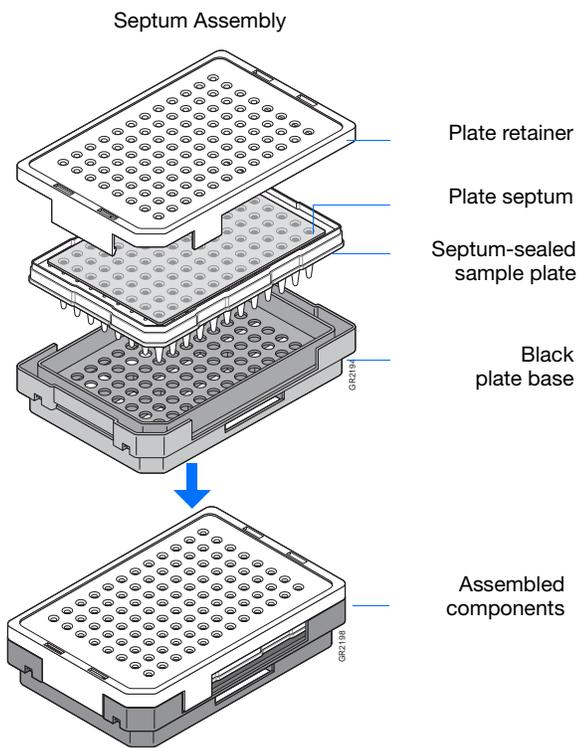


4. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.

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



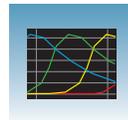
5. Assemble the plate assembly as shown below (see Appendix A, “Parts List,” for part numbers).



WARNING Use only **black** plate bases with septa-sealed plates. If you are using MicroAmp™ Fast 96-Well Reaction Plates (0.1 ml), use only **blue** plate bases and matching retainer.

WARNING Use only **gray** plate bases with heat-sealed plates. If you are using MicroAmp™ Fast 96-Well Reaction Plates (0.1 ml), use only **dark green** plate base and matching retainer.

Notes



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

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

Important Heat Seal Recommendations

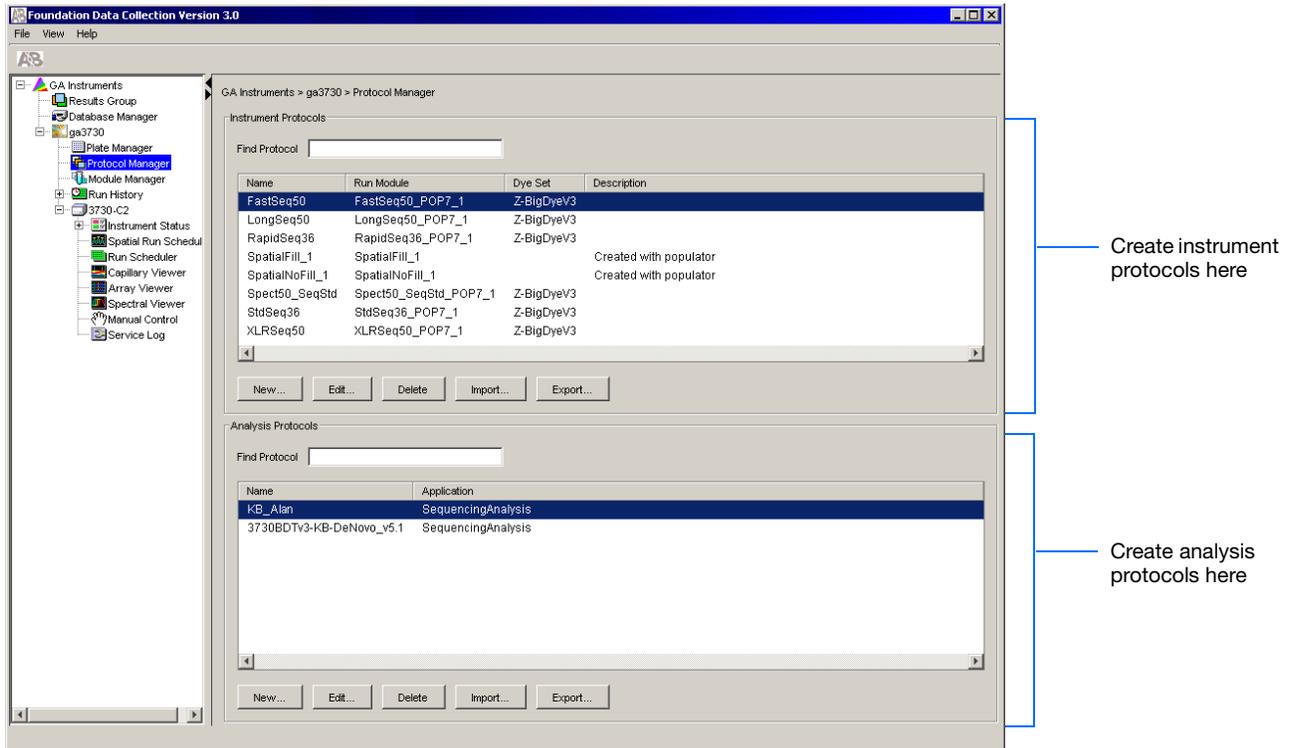
- Use 3-mil Applied Biosystems® heat seal film (PN 4337570). This film is 3-mil before, and 1-mil after, heating.
- *Do not* use heat seal film thicker than 1-mil, after heating, on the 3730/3730xl DNA Analyzer.
- *Do not* use heat-seal film containing adhesives or metals as these may damage the instrument's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

Notes _____

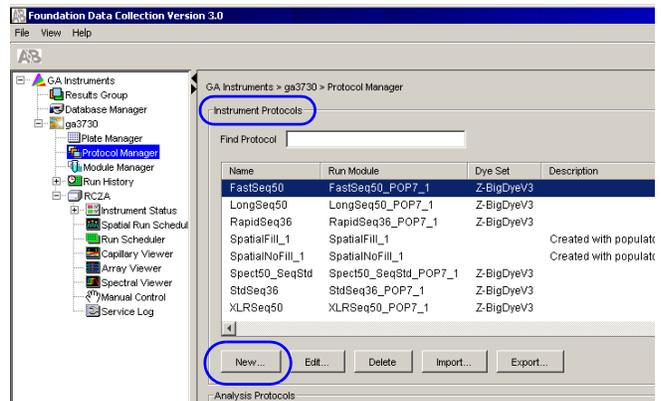


Creating a Spectral Instrument Protocol

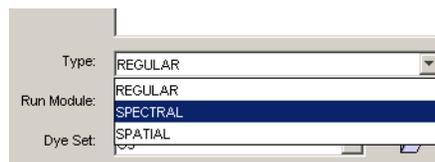
1. In the navigation pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.



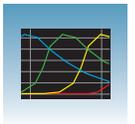
2. In the Instrument Protocols pane, click **New...**.
The Protocol Editor opens.



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



Notes



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

If you are using a *matrix standard* for spectral calibration, you can use a 36-cm or 50-cm array length:

For a 36-cm capillary array, use:

- Run Module: **Spect36_MtxStd_1**
- Chemistry: **matrixStandard**

For a 50-cm capillary array, use:

- Run module: **Spect50_MtxStd_POP7_1**
- Chemistry: **matrixStandard**

IMPORTANT! The array length you select must match the array length information from the Install Array wizard.

If you are using a *sequencing standard* for spectral calibration, you can use a 36-cm or 50-cm array length:

For a 36-cm capillary array, use:

- Run module: **Spect36_SeqStd_1**
- Chemistry: **sequenceStandard**

For a 50-cm capillary array, use:

- Run module: **Spect50_SeqStd**
- Chemistry: **sequenceStandard**

Note: The Chemistry file for fragment analysis dye sets automatically defaults to the matrix standard.

IMPORTANT! The array length you select must match the array length information from the Install Array wizard.

Protocol Editor

Name: SpectralMtxStd

Description:

Type: SPECTRAL

Run Module: Spect36_MtxStd_POP7_042203_1

Dye Set: G5

Polymer: POP7

Array Length: 36

Chemistry: matrixStandard

Edit Param... OK Cancel

Protocol Editor

Name: SpectralSeqStd

Description:

Type: SPECTRAL

Run Module: Spect36_SeqStd_POP7_042203_1

Dye Set: Z-BigDyeV3

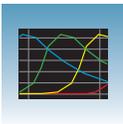
Polymer: POP7

Array Length: 36

Chemistry: sequenceStandard

Edit Param... OK Cancel

Notes



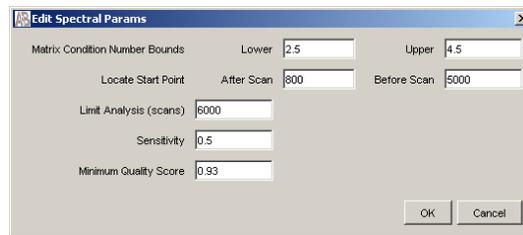
Use the following table to select the correct chemistry file for the spectral calibration samples you use

Dye Sets, Standards, And Chemistry Files

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

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

1. (Optional) Click **Edit Param** to display the Spectral Params dialog box.
2. Use this dialog box to edit the selection criteria for passing or failing spectral calibrations.

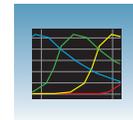


Valid Data Ranges

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

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

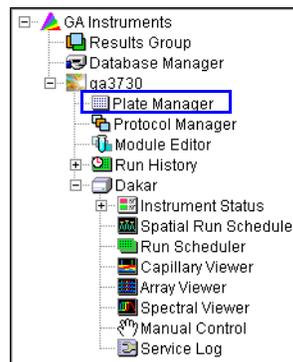
Notes _____



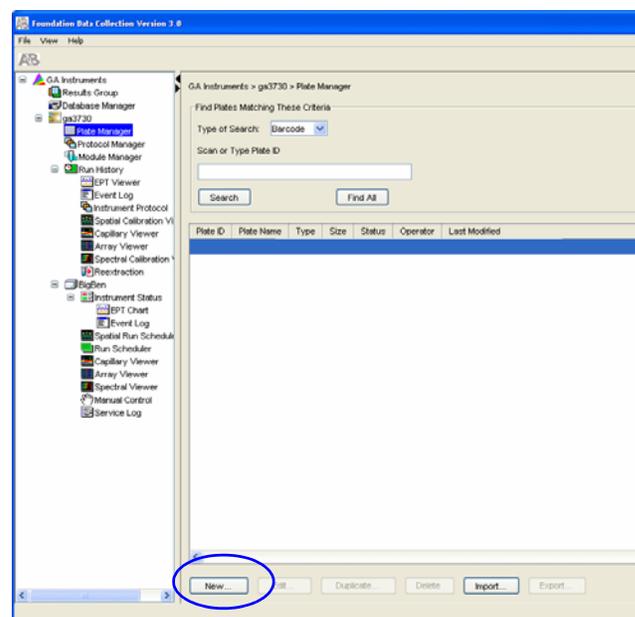
Creating a Spectral Calibration Plate Record

1. In the navigation pane of the Data Collection Software, double-click

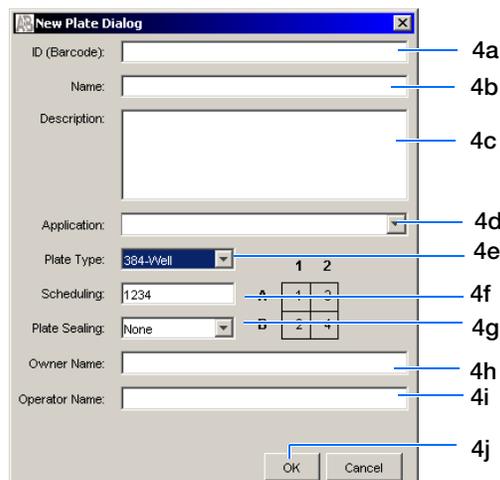
GA Instruments > ga3730 >
 instrument name > Plate Manager.



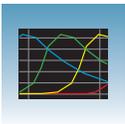
2. Click **New** to create a new plate.



3. Complete the New Plate dialog box:
 - a. Enter ID or Barcode number
 - b. Enter a name for the plate.
 - c. (Optional) Enter a description for the plate record.
 - d. In the Application drop-down list, select **Spectral Calibration**.
 - e. In the Plate Type drop-down list, select **96-Well** or **384-Well**.
 - f. Enter desired scheduling. For more information see, “Globally Modifying a Run Schedule” on page 125.



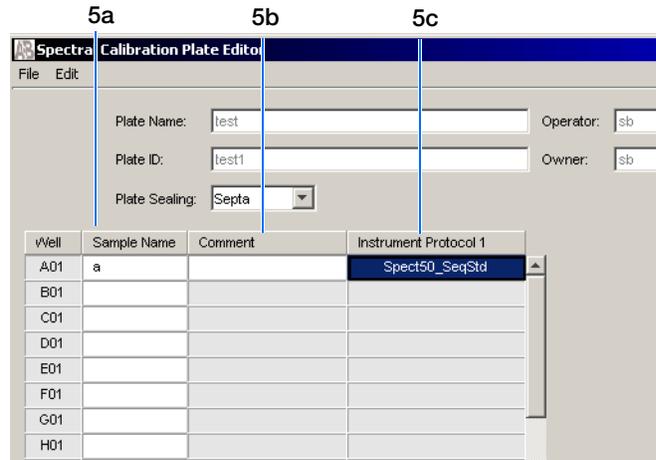
Notes



- g. In the Plate Sealing drop-down list, select **Septa** or **Heat Seal**.
 - h. Enter a name for the owner.
 - i. Enter a name for the operator.
 - j. Click .
4. In the Spectral Calibration Plate Editor, enter the following information:

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

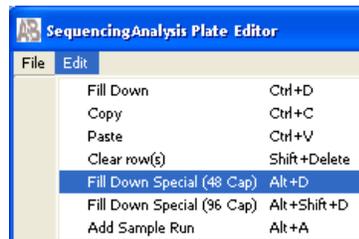
- a. In the Sample Name column of row A01, enter a sample name, then click the next cell.
- b. In the Comments column of row A01, enter any additional comments or notations for the sample at the corresponding position of the plate.
- c. In the Instrument Protocol 1 column of row A01, select a protocol from the drop-down list.



- 5. Select the entire row.
- 6. Select **Edit > Fill Down Special**.

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

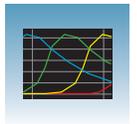
- 96 capillary/96-well plate: **Fill Down**
- 48 capillary/96-well plate: **Fill down Special (48 Cap)**
- 96 capillary/384-well plate: **Fill down Special (96 Cap)**
- 48 capillary/384-well plate: **Fill down Special (48 Cap)**



- 7. Click .

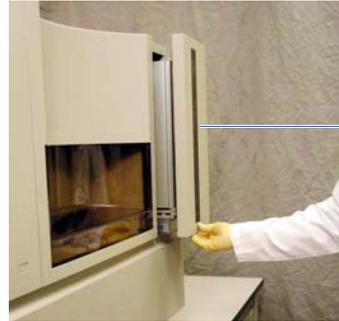
You have successfully created a plate record for the spectral calibration plate.

Notes _____



Loading the Plate into the Instrument

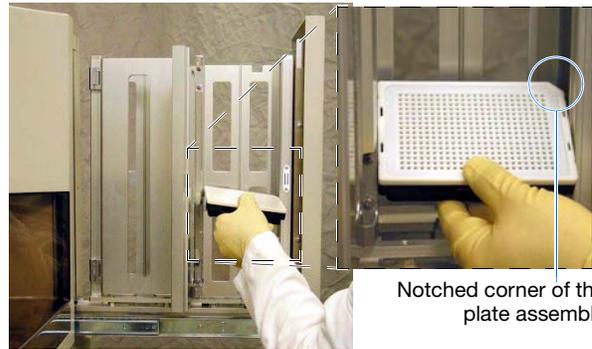
1. The name of the plate record you just created is displayed in the Input Stack window of the Data Collection software, and is ready to run.
2. Open the stacker drawer.
3. Open the In Stack tower door.



Stacker drawer

4. Place the plate assembly into the stacker.

IMPORTANT! The plate must be oriented so that the notched corner of the plate assembly is at the rear-right corner of the stacker.



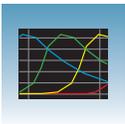
Notched corner of the plate assembly

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



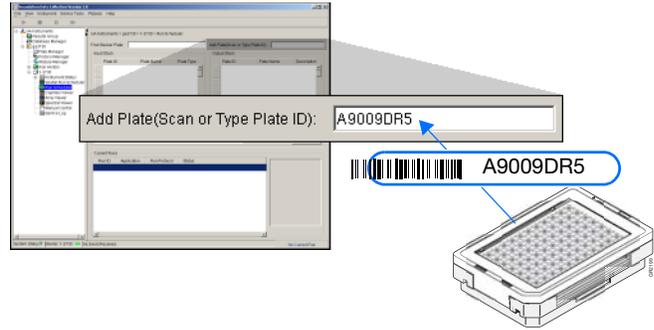
In Stacker tower door

Notes _____

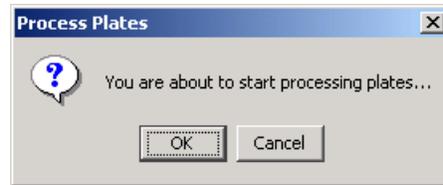


Running the Spectral Calibration Plate

1. In the navigation pane of the Data Collection Software, double-click
GA Instruments > **ga3730** >
instrument name > **Run Scheduler**.
2. In the Run Scheduler view:
 - In the Add Plate field, scan the bar code of a plate to add it to the input stack.
or,
 - Type the plate ID then press **Enter** to add it to the input stack.



3. In the toolbar of the Data Collection Software window, click to begin the run.
4. The Processing Plates dialog box opens.
5. Click .



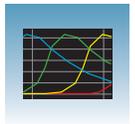
Note: The instrument may pause before running the plate to raise the oven temperature.

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

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

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

Notes _____



Viewing the Pass/Fail Status After the Run

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

1. In the navigation pane of the Data Collection Software, select
GA Instruments > ga3730 > instrument name > Instrument Status > Event Log.

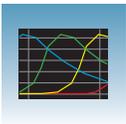
GA Instruments > ga3730 > Instrument Status > Event Log

Type	Date	Time	Publisher	Description
Info	09/05/03	16:41:03	3730C5	Capillary 32 successfully calibrated : q=0.900 c=5.66
Info	09/05/03	16:41:05	3730C5	Capillary 31 successfully calibrated : q=0.957 c=5.72
Info	09/05/03	16:41:04	3730C5	Capillary 30 failed calibration : Failed quality check: q=0.94484 is less than minQ threshold (0.95000)
Info	09/05/03	16:41:04	3730C5	Capillary 29 successfully calibrated : q=0.965 c=5.55
Info	09/05/03	16:41:04	3730C5	Capillary 28 successfully calibrated : q=0.958 c=5.59
Info	09/05/03	16:41:03	3730C5	Capillary 27 failed calibration : Failed quality check: q=0.93434 is less than minQ threshold (0.95000)
Info	09/05/03	16:41:03	3730C5	Capillary 26 successfully calibrated : q=0.970 c=5.62
Info	09/05/03	16:41:02	3730C5	Capillary 25 successfully calibrated : q=0.964 c=5.57
Info	09/05/03	16:41:02	3730C5	Capillary 24 successfully calibrated : q=0.967 c=5.57
Info	09/05/03	16:41:02	3730C5	Capillary 23 successfully calibrated : q=0.966 c=5.62
Info	09/05/03	16:41:01	3730C5	Capillary 22 successfully calibrated : q=0.976 c=5.67
Info	09/05/03	16:41:01	3730C5	Capillary 21 successfully calibrated : q=0.957 c=5.70

System Status | Stacker: 3730C5 | Batch ended | No Current Run

3

Notes



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

Type	Date	Time	Publisher	Description
Info	09/05/03	16:41:03	3730C5	Capillary 32 successfully calibrated : q=0.960 c=5.00
Info	09/05/03	16:41:05	3730C5	Capillary 31 successfully calibrated : q=0.957 c=5.72
Info	09/05/03	16:41:04	3730C5	Capillary 30 failed calibration : Failed quality check: q=0.94484 is less than minQ threshold (0.95000)
Info	09/05/03	16:41:04	3730C5	Capillary 29 successfully calibrated : q=0.965 c=5.55
Info	09/05/03	16:41:04	3730C5	Capillary 28 successfully calibrated : q=0.958 c=5.59
Info	09/05/03	16:41:03	3730C5	Capillary 27 failed calibration : Failed quality check: q=0.93434 is less than minQ threshold (0.95000)
Info	09/05/03	16:41:03	3730C5	Capillary 26 successfully calibrated : q=0.970 c=5.67

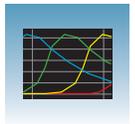
Dye set G5 status results

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

- Q-value:
 - > 0.95 for matrix standards
 - > above 0.93 for sequence standards
- Condition number range, indicated below, for each dye set:

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

Notes _____



Evaluating the Spectral Calibration Data

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

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

1. In the navigation pane of the Data Collection Software, select
GA Instruments > ga3730 >
instrument name > Spectral Viewer.

The screenshot shows the 'Spectral Viewer' window in the 'Foundation Data Collection Version 3.0' software. The interface is divided into several sections:

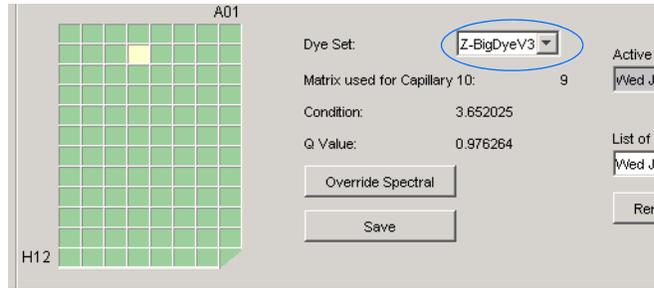
- Navigation Pane (Left):** A tree view showing the hierarchy: GA Instruments > Results Group > Database Manager > ga3730 > C5 > Spectral Viewer.
- Spectral Profile Graph (Top):** A line graph titled 'Intensity vs Pixel Number' with the x-axis ranging from 0 to 400. It displays four distinct curves in blue, green, yellow, and red, representing different dye channels.
- Raw Data Graph (Middle):** A bar chart titled 'Intensity vs Scan Number' with the x-axis ranging from 0 to 8000. It shows a dense series of multi-colored peaks (blue, green, yellow, red) representing raw data from matrix standards.
- Plate Diagram (Bottom Left):** A grid representing a 96-well plate. The top row is labeled 'A01' and the left column is labeled 'H1'. A blue box highlights a specific well in the grid.
- Calibration Settings (Bottom Right):** A control panel for the selected well. It includes:
 - Dye Set:** A dropdown menu set to 'Z-BigDyeV3'.
 - Active Calibration for Dye Set:** A text field containing 'Thu Jun 19 19:43:46 PDT 2003'.
 - List of Calibrations for Dye Set:** A dropdown menu also showing 'Thu Jun 19 19:43:46 PDT 2003'.
 - Buttons:** 'Override Spectral', 'Save', 'Rename', and 'Set'.

Blue arrows on the right side of the screenshot point to the 'Spectral profile' graph, the 'Raw data (matrix standards)' graph, and the 'Rename or set the active spectral calibration here' section.

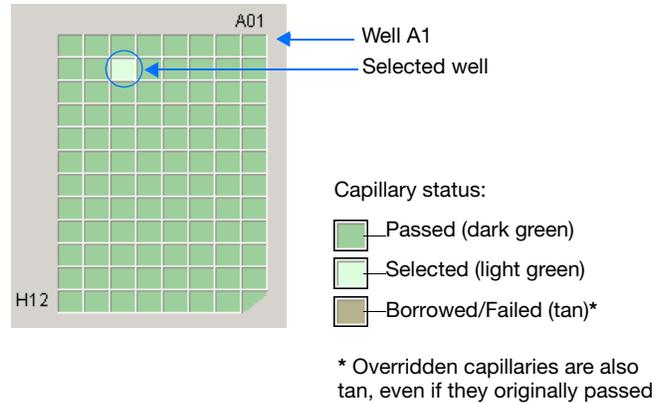
Notes



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

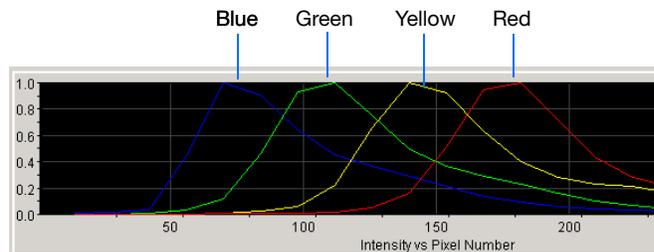


3. Select a well on the plate diagram to view the spectral results of the associated capillary.

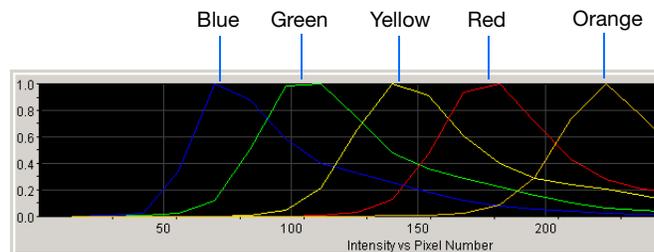


4. Evaluate the spectral calibration profile for the selected capillary:

- a. Verify that the order of the peaks in the spectral profile from left to right are:
 - 4-dye–blue–green–yellow–red
 - 5-dye–blue–green–yellow–red–orange
 If the peaks in the profile:
 - Are in the correct order–go to step c.
 - The calibration run has failed–go to page 55.

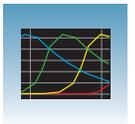


Example of a 4-dye spectral calibration profile



Example of a 5-dye spectral calibration profile

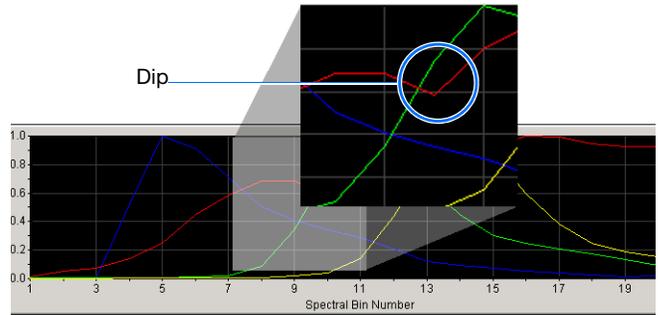
Notes _____



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

If the peaks in the spectral profile are:

- Separate and distinct—the capillary has passed. Go to step 5.
- Not separate and distinct—the calibration run has failed. Go to page 55.



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

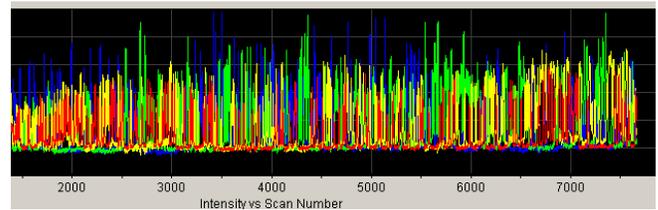
Fragment Analysis

– 5-dye: orange-red-yellow-green-blue

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

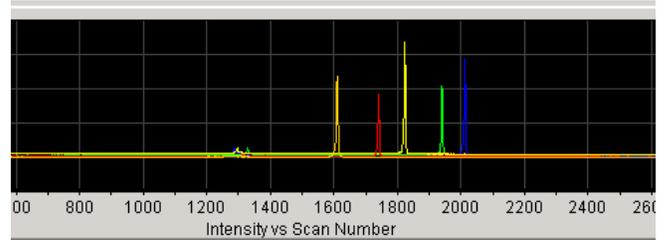
Yes: The calibration run has failed. Go to page 55.

No: Go to step 5.



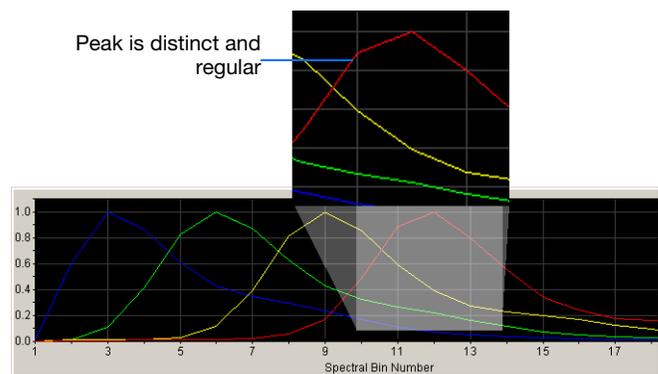
Example of a 4-dye sequencing raw data profile

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

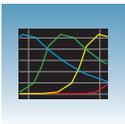


Example of a 5-dye fragment analysis raw data profile

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

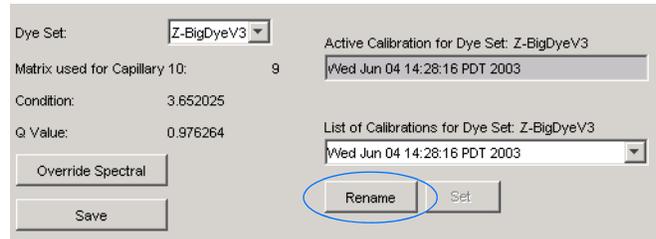


Notes _____



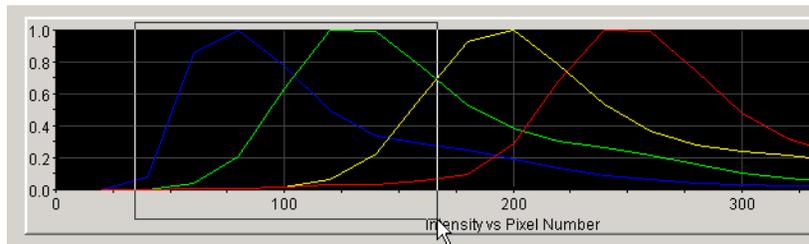
6. Rename the spectral run. The spectral file default name is the day, date and time of the run.

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

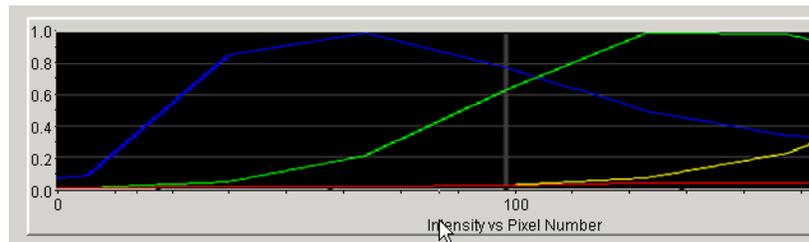


Tip: Magnifying the Spectral Profile

1. In the navigation pane of the Data Collection Software, click **GA Instruments > ga3730 > instrument name > Spectral Viewer**.
2. In the profile or raw data display, click - drag the cursor to create a box around the area of interest.
3. Release the mouse button.
The data collection software displays the selected region.
4. Press **R** to reset the view.

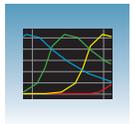


Selecting an area to magnify in a spectral profile



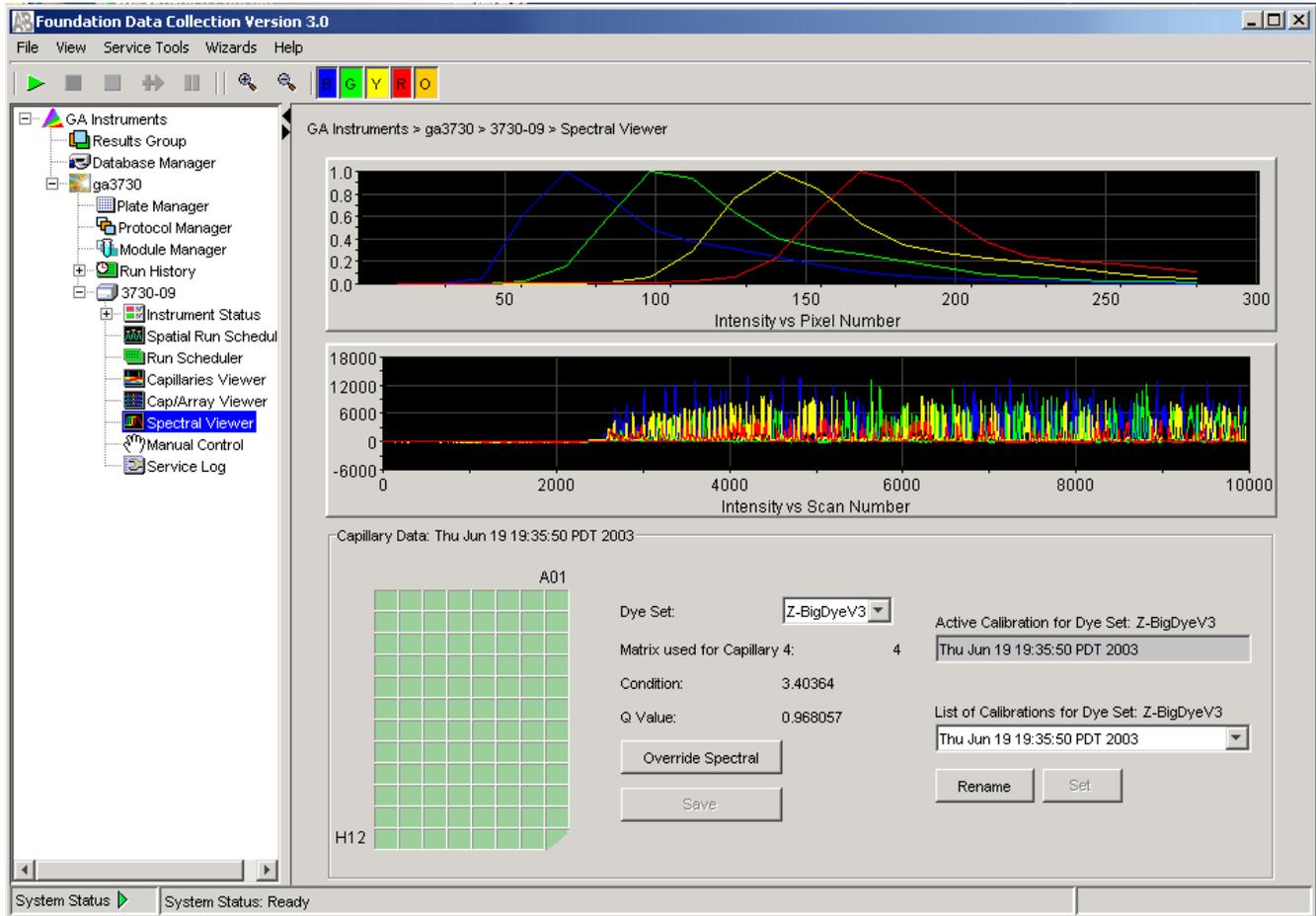
Magnified area of that spectral profile

Notes _____



Examples of Passing Sequencing Spectral Calibrations

Dye Set Z Created from a Sequencing Standard



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Notes



Dye Set E Created from a Sequencing Standard

GA Instruments > ga3730 > 3730-09 > Spectral Viewer

Intensity vs Pixel Number

Intensity vs Scan Number

Capillary Data: Mon Jul 28 18:09:54 PDT 2003

A01

H12

Dye Set: E-BigDyeV1

Matrix used for Capillary 15: 15

Condition: 3.357109

Q Value: 0.981852

Active Calibration for Dye Set: E-BigDyeV1

List of Calibrations for Dye Set: E-BigDyeV1

Mon Jul 28 18:09:54 PDT 2003

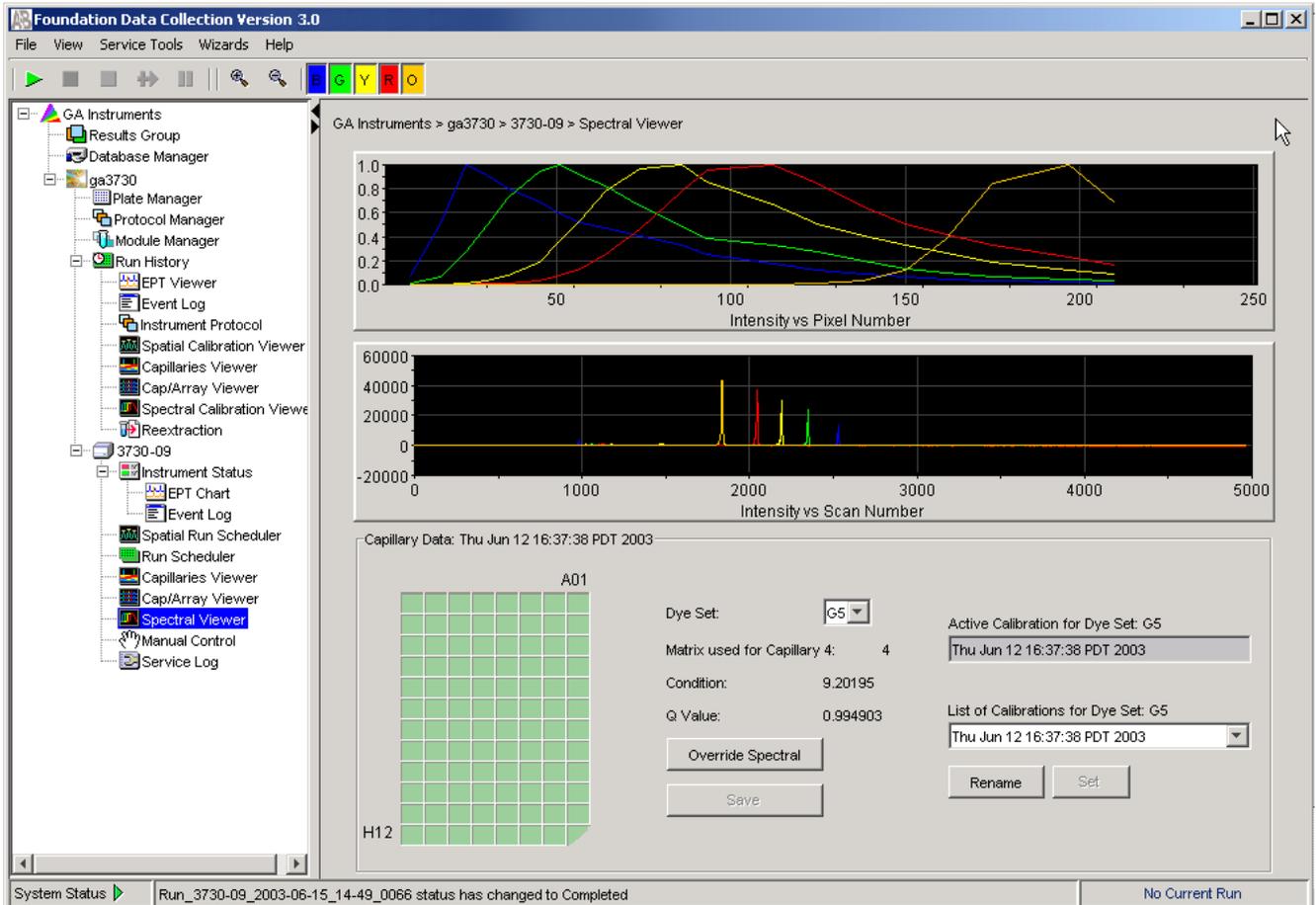
System Status Starting Electrophoresis

Notes



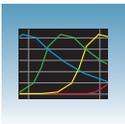
Example of a Passing Fragment Analysis Spectral Calibration

Dye Set G5 Created from Matrix Standard Set DS-33



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Notes



Spectral Viewer

Selecting Active Spectral Calibrations

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

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

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

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

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

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

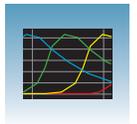
All calibrations for your current dye set are listed in the List of Calibrations drop-down list. Therefore, you can choose a spectral calibration to use from the list before you begin a new run.

Note: An asterisk * precedes failing calibrations.

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

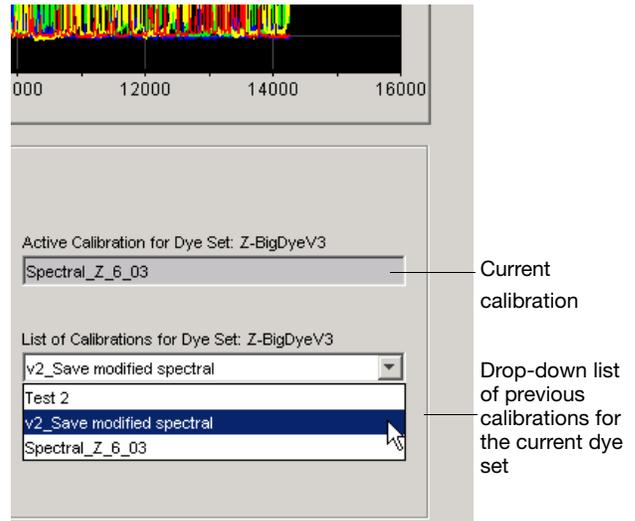
Because each dye set can have its own active calibration, there is no need to manually set the active calibration if you are performing runs with various dye sets.

Notes _____

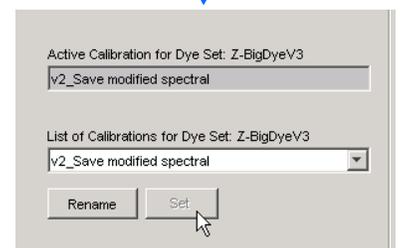
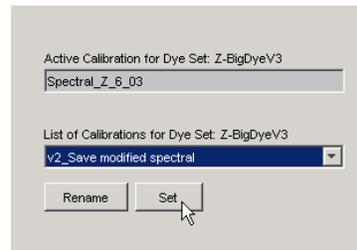
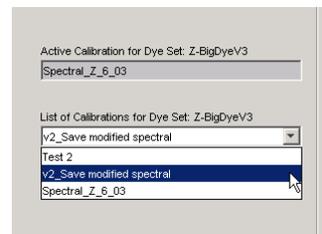


To select a previous spectral calibration:

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



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

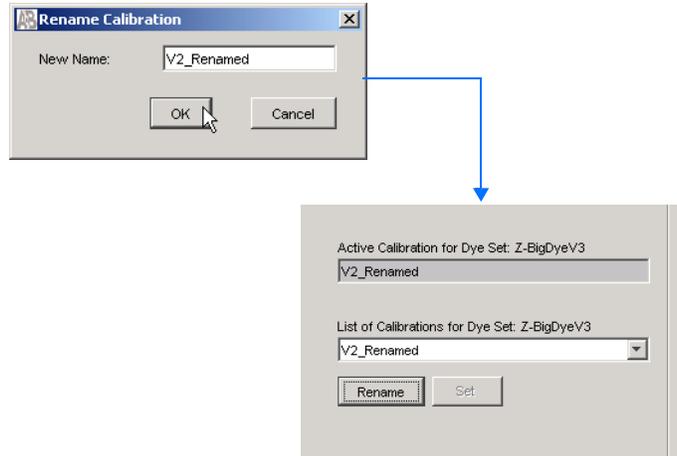


3

Notes

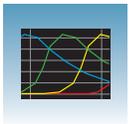


4. Click **Set** to display your chosen spectral calibration in the Active Calibration text box.



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

Notes _____



Troubleshooting

Troubleshooting spectral calibration		
Observation	Possible Cause	Recommended Action
No signal.	Incorrect sample preparation.	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide.
	Air bubbles in sample tray.	Centrifuge samples to remove air bubbles.
If the spectral calibration fails, or if a message displays “No candidate spectral files found.”	Clogged capillary.	Refill the capillaries using manual control. Look for clogged capillaries during capillary fill on the cathode side.
	Insufficient filling of array.	Check for broken capillaries and refill the capillary array.
	Expired spectral standards.	Check the expiration date and storage conditions of the spectral standards. If necessary, replace with a fresh lot.
Spikes in the data.	Expired polymer.	Replace the polymer with a fresh lot using the Change Polymer wizard.
	Air bubbles, especially in the polymer.	<p> WARNING CHEMICAL HAZARD. POP-7™ polymer cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</p> <ul style="list-style-type: none"> • Refill the capillaries using the Bubble Remove wizard. • Properly bring the polymer to room temperature. • Replace expired polymer.
	Possible contaminant in the polymer.	Replace the polymer using the Change Polymer wizard.

3

Notes _____

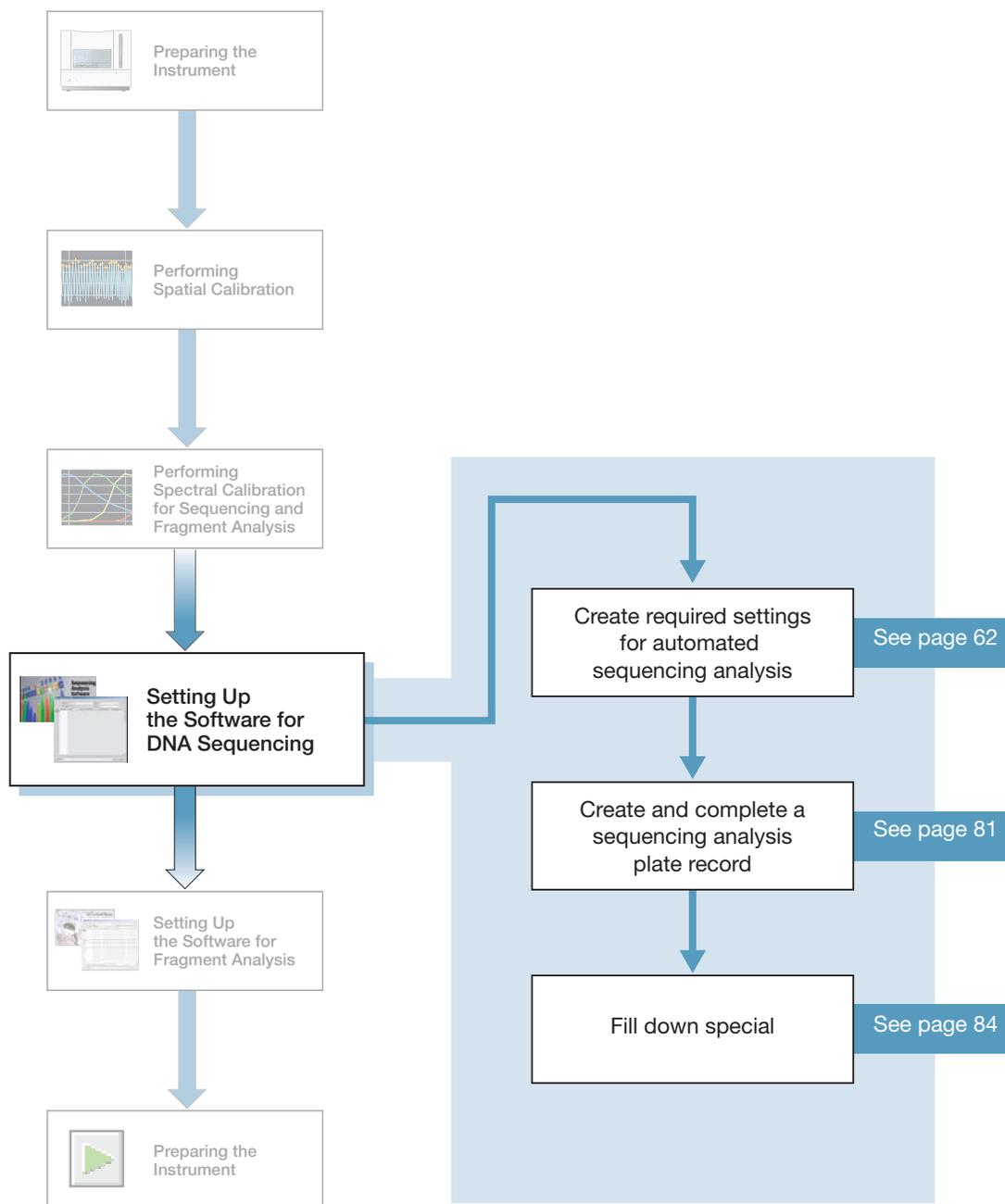


Chapter 3 Performing Spectral Calibration For Sequencing and Fragment Analysis

Troubleshooting

Notes _____

Setting Up the Software for DNA Sequencing



Notes



Plate Records and Sequencing Analysis

Overview A plate record is similar to a sample sheet or an injection list that you may have used with other Life Technologies instruments. Plate records are data tables in the instrument database that store information about the plates and the samples they contain. A plate record contains the following information:

- Plate name, type, and owner
- Position of the sample on the plate (well number)
- Sample
- Name, see page page 75
- Mobility file (in Analysis Protocol), see page page 67
- Comments about the plate and about individual samples
- Name of the run module and Dye set information (run modules specify information about how samples are run) (in Instrument Protocol), see page 62
- Name of the Analysis Protocol (Analysis protocols specify how data is analyzed at the end of the run; see page page 67)

Important Notes

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

File-Naming Convention

Alphanumeric characters that are not valid for user names or file names are:
spaces

\ / : * ? " < > |

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

When to Create a Plate Record

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

- Spectral calibrations
- Sequencing analysis
- SeqScape analysis

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

Notes _____

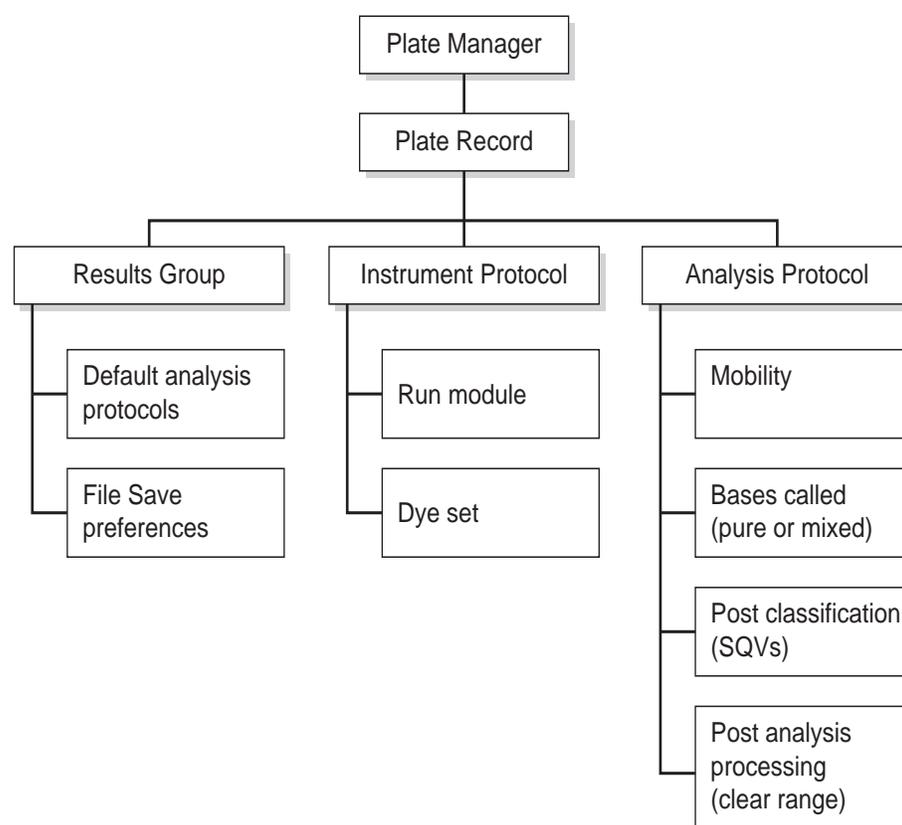


Sequencing Analysis Plate Record

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

The table and the flow chart below describe what each file specifies.

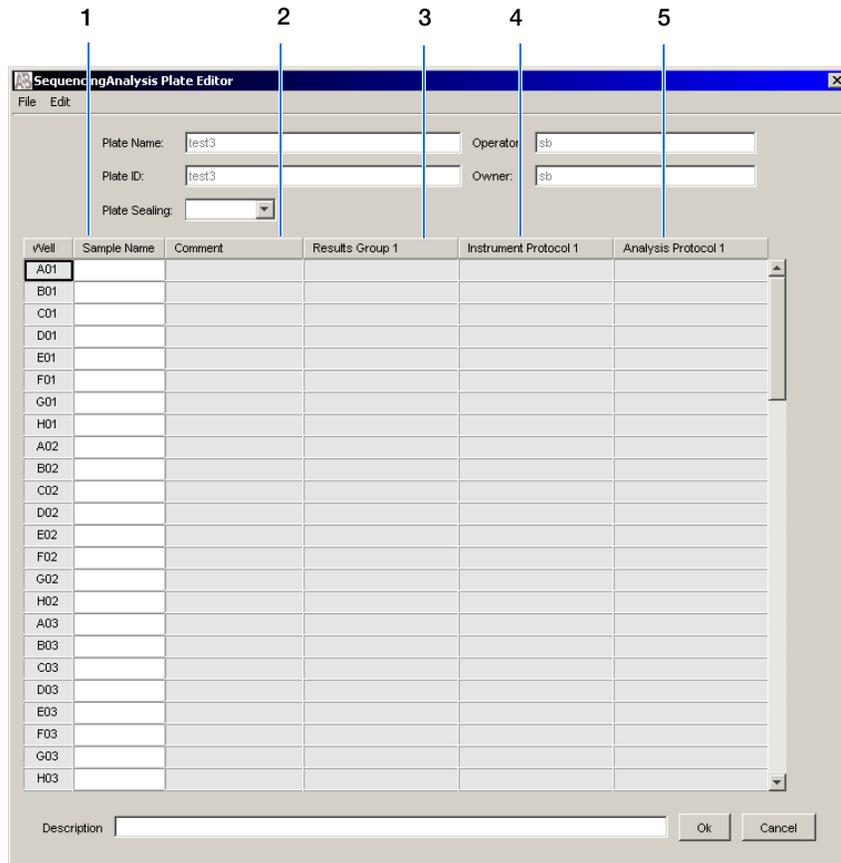
Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	62
Analysis Protocol	Contains everything needed to analyze sequencing data.	66
Results Group	Defines the file type, the file name, file save locations, analysis software and autoanalysis.	72



Elements of a sequencing analysis plate record

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

Notes _____



Default is one sample run. To add additional runs, see page 83.

Blank sequencing analysis plate record

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

Name	Description
(1.) Sample Name	Name of the sample
(2.) Comment	Comments about the sample (optional)
(3.) Results Group	Options are: <ul style="list-style-type: none"> • 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 <p>Note: You must have a results group selected for each sample entered in the Sample Name column.</p> <p>See, “Results Groups” on page 72.</p>

Notes _____



Name	Description
(4.) Instrument Protocol	<ul style="list-style-type: none"> • 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 alphanumeric order. <p>Note: You must have an Instrument Protocol selected for each sample entered in the Sample Name column.</p> <p>See, “Creating an Instrument Protocol” on page 62.</p>
(5.) Analysis Protocol	<ul style="list-style-type: none"> • 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 alphanumeric order <p>Note: You must have an Analysis Protocol selected for each sample entered in the Sample Name column.</p> <p>See, “Creating an Analysis Protocol” on page 67.</p>

Notes _____



Creating Required Settings for Automated Sequencing Analysis

If Settings Already Exist

If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to “Creating and Completing a Sequencing Analysis Plate Record” on page 81.

Instrument Protocols

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

Creating an Instrument Protocol

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

Foundation Data Collection Version 3.0

GA Instruments > ga3730 > Protocol Manager

Instrument Protocols

Name	Run Module	Dye Set	Description
FastSeq50	FastSeq50_POP7_1	Z-BigDyeV3	
LongSeq50	LongSeq50_POP7_1	Z-BigDyeV3	
RapidSeq36	RapidSeq36_POP7_1	Z-BigDyeV3	
SpatialFill_1	SpatialFill_1		Created with populator
SpatialNoFill_1	SpatialNoFill_1		Created with populator
Spect50_SeqStd	Spect50_SeqStd_POP7_1	Z-BigDyeV3	
StdSeq36	StdSeq36_POP7_1	Z-BigDyeV3	
XLRSeq50	XLRSeq50_POP7_1	Z-BigDyeV3	

Analysis Protocols

Name	Application
kB_Alan	SequencingAnalysis
3730BDTV3-kB-DeNovo_v5.1	SequencingAnalysis

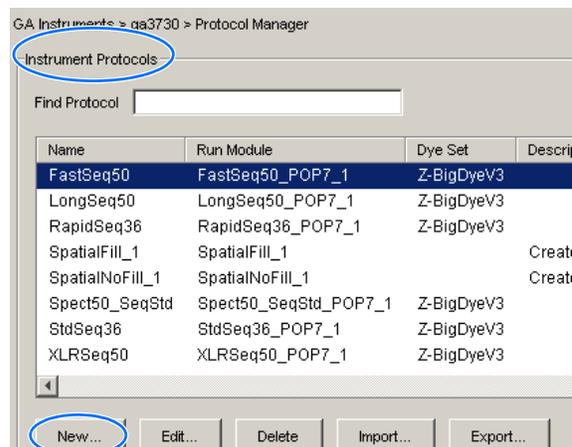
Create instrument protocols here

Create analysis protocols here

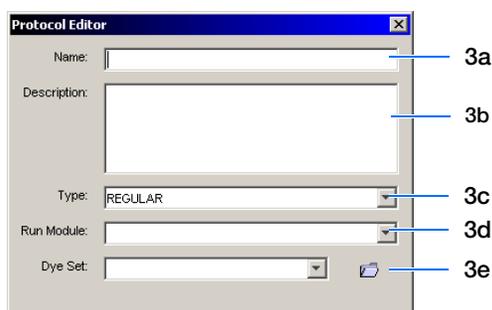
Notes



2. In the Instruments Protocols section, click **New...**. The Protocol Editor opens.



3. Complete the Protocol Editor:
- Type a name for the protocol.
 - Type a description for the protocol (optional).
 - Select **Regular** in the Type drop-down list.
 - Using the information in the table below, select the correct run module for your run.



Note: To customize a run module, see “Tip: Customizing Run Modules” on page 64.

Sequencing Run Modules	Capillary Array Length (cm)	Sequencing Run	Approximate Run Times† (min)
XLRSeq50_POP7	50	Extra long read	180
LongSeq50_POP7	50	Long read	120
FastSeq50_POP7	50	Fast read	60
StdSeq36_POP7	36	Standard read	60
RapidSeq36_POP7	36	Rapid read	35
TargetSeq36_POP7	36	Short read	20‡

† Approximate run times assume oven temperature has reached run temperature
‡ Time stated for 400 bases. Module can be customized to run 200-400 bases.

Note: If the BigDye® Xterminator™ Purification Kit was used for sequencing reaction clean up, refer to Appendix A in the BigDye® Xterminator™ Purification Kit Protocol for the appropriate run modules.

Notes



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

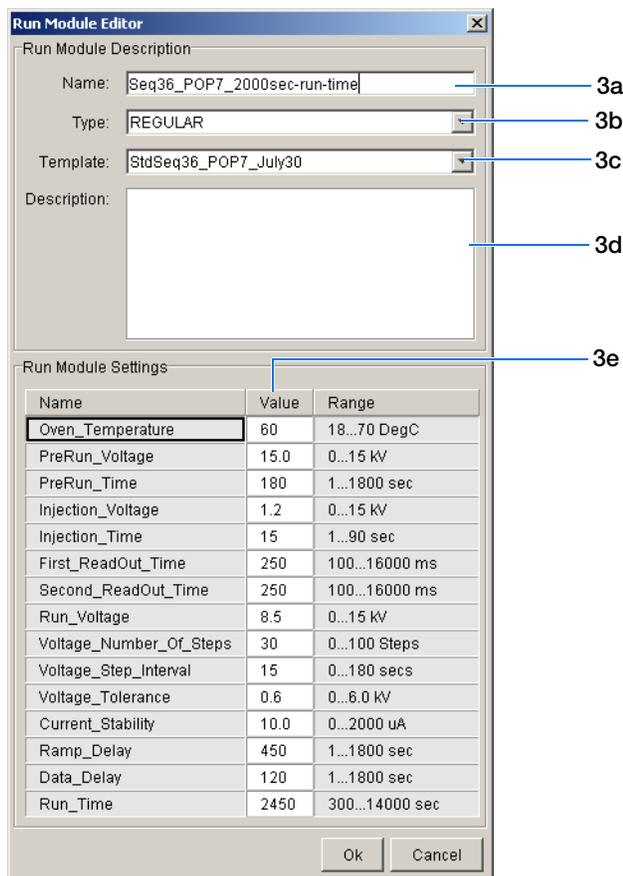
Dye Set	Chemistry
E_BigDyeV1	BigDye® v1.1 Terminator
Z_BigDyeV3	BigDye® v3.1 Terminator

- f. Click **OK**.

Tip: Customizing Run Modules

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

1. Click **GA Instruments > ga3730 > instrument name > Module Manager**.
 2. Click **New...**
The Run Module Editor dialog box opens.
 3. Complete the Run Module Editor dialog box:
 - a. Enter a name for your new module.
 - b. In the Type drop-down list, select the type of module (Regular, Spatial or Spectral).
 - c. In the Template drop-down list, select a template module as a basis for the new module.
- Note:** You cannot edit a default module installed with 3730/3730xI Analyzer Data Collection software.
- d. (Optional) Enter a description of your new run module.



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

Notes



Editable Run Module Parameters

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

Notes



Analysis Protocols

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

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

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

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

Notes _____

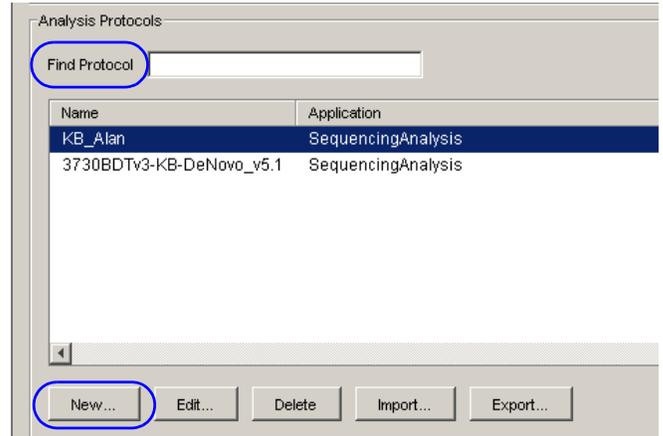


Creating an Analysis Protocol

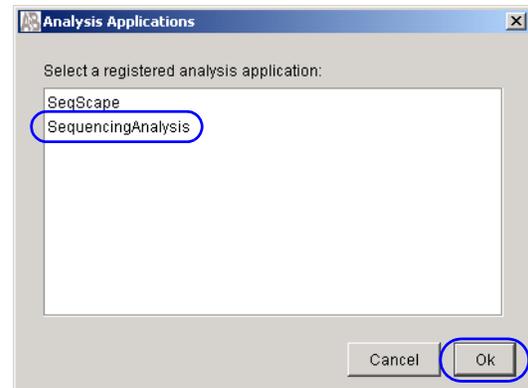
Refer to the Applied Biosystems® *DNA Sequencing Analysis Software v5.1 User Guide* (P/N 4346366), chapter 8, for more information regarding analysis protocols

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

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



2. Select **Sequencing Analysis**, then click **OK**.
The Analysis Protocol Editor opens.



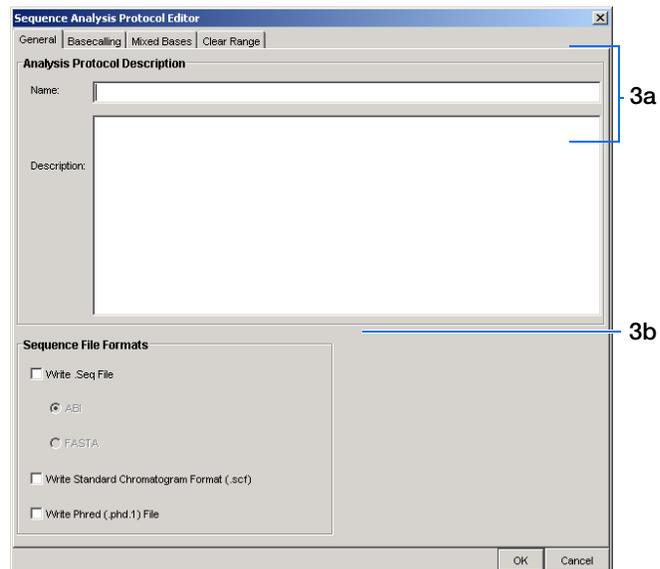
Notes _____



3. Select the **General** tab, then:

- a. Enter a unique name and description for the new protocol.
- b. Select the appropriate Sequence File formats settings.

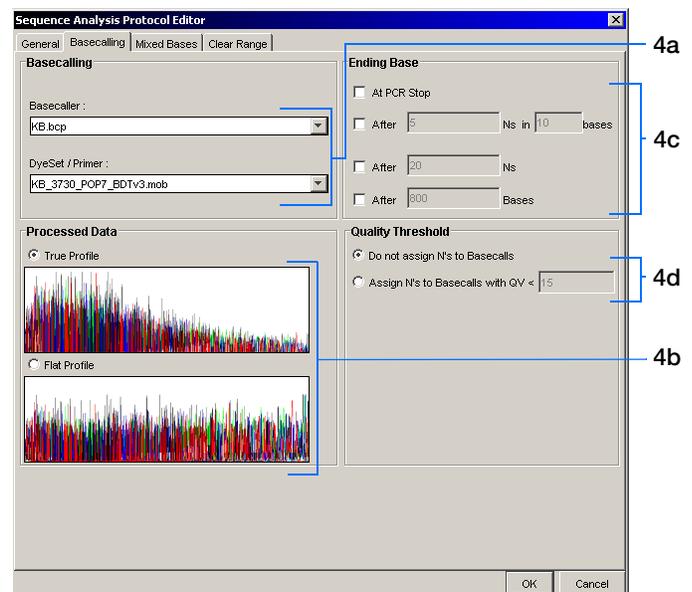
Option	If checked, the software creates...
Write .Seq File check box	a .seq file for printing the sequence as text file or for using the file in other software. <ul style="list-style-type: none"> • ABI format is used with Life Technologies software. • FASTA format is used with other software
Write Standard Chromatogram Format file (.scf)	When selected, the software creates a .scf file that can be used with other software. When created, the .scf extension is not appended to the file name.
Write Phred (.phd.1) File	When selected and the KB basecaller is used, the software creates a .phd.1 file that can be used with other software.



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

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

Note: Sequencing Analysis Software v5.2 and 3730/3730xl Analyzer Data Collection software filter .mob file choices to match the chosen .bcp file.



Notes _____



- b. In the Processed Data pane, select **True** or **Flat Profile**.

Option	Function
<input type="radio"/> True Profile	Used to display data as processed traces scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. The profile of the processed traces will be very similar to that of the raw traces.
<input type="radio"/> Flat Profile	Used to display the data as processed traces scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces is 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.

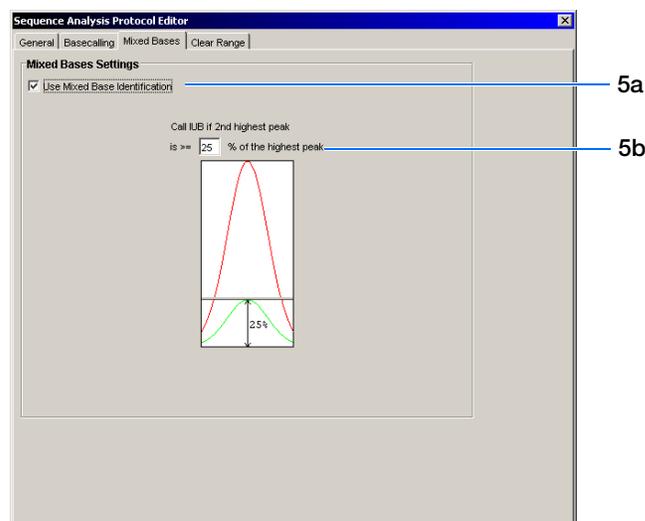
Option	Function
<input type="radio"/> Call all bases and assign QV	When using the KB basecaller, use this option to assign a base to every position, as well as the QV.
<input type="radio"/> Assign 'N' for bases with QV < 15	When using the KB basecaller, use this option to assign Ns to bases with QVs less than the set point. The QV is still displayed.

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

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

- a. For mixed bases only, select **Use Mixed Base Identification**.
 b. Use the default setting of 25% or change the detection level by entering a new value or dragging the % line up or down.

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



Notes

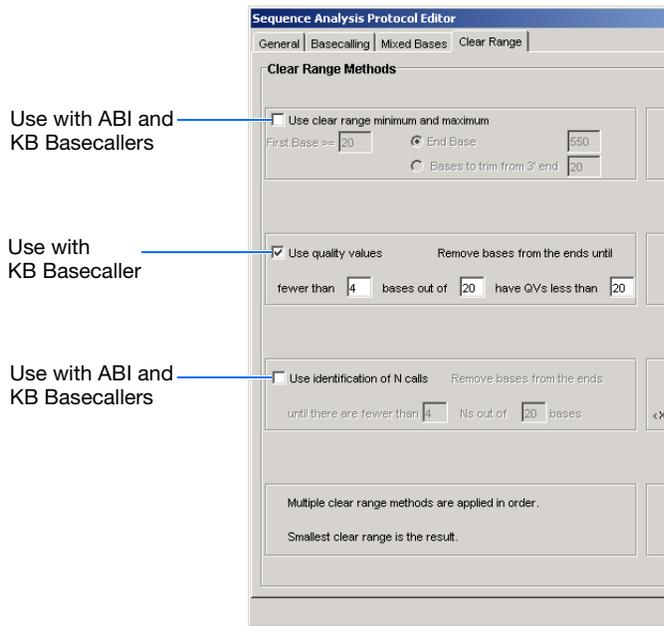


6. Select the **Clear Range** tab.

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

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

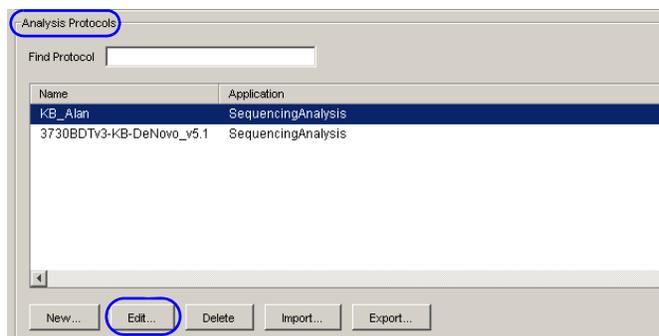
7. Click **OK** to save the protocol and close the Sequence Analysis Protocol Editor.



Editing and Deleting Analysis Protocols

Editing an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, select 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.



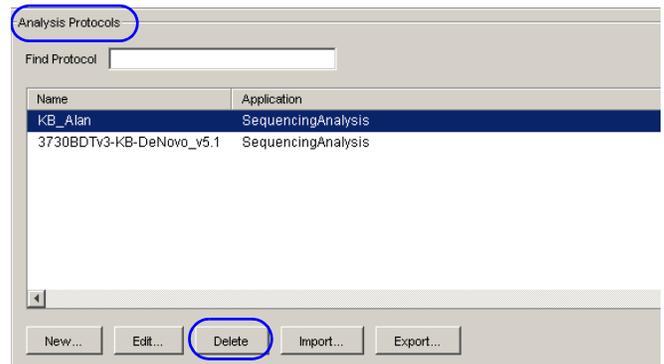
Notes _____



Deleting an Analysis Protocol

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

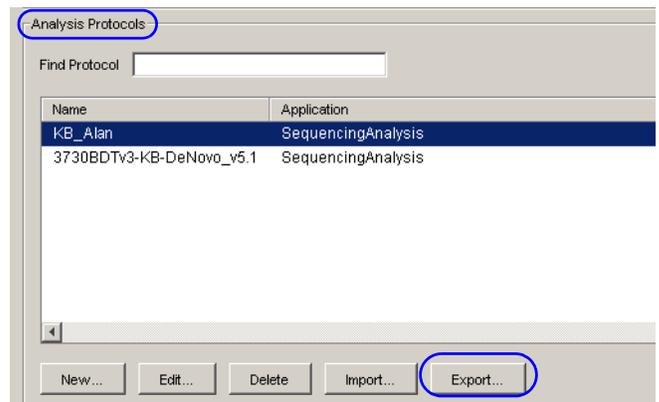
1. In the Analysis Protocols pane in the Analysis Protocol Manager, select the protocol you want to delete.
2. Click **Delete**. The Deletion Confirmation dialog box opens.
3. Click **Yes**.



Exporting and Importing Analysis Protocols

Exporting an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, select the protocol you want to export.
2. Click **Export**. A standard file export dialog box opens.
3. Navigate to the destination folder.
4. Click **Save**.

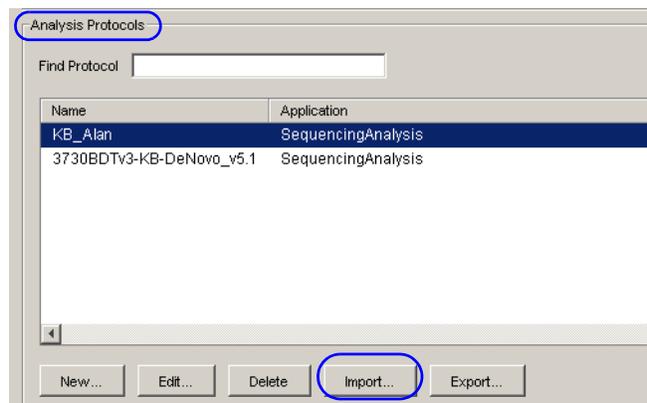


Notes



Importing an Analysis Protocol

1. In the Analysis Protocols pane in the Analysis Protocol Manager, select the protocol you want to import.
2. Click **Import** . a standard file export dialog box opens.
3. Click **Save**.

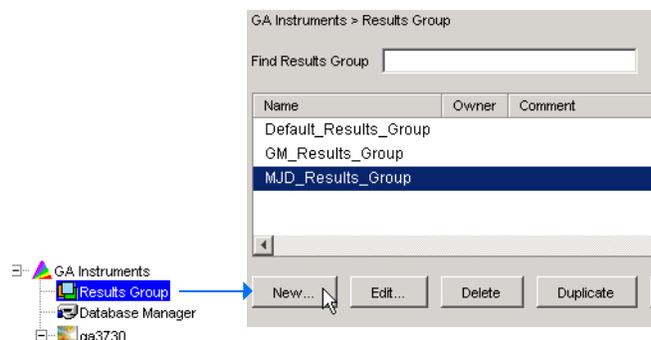


Results Groups

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

Creating a Results Group

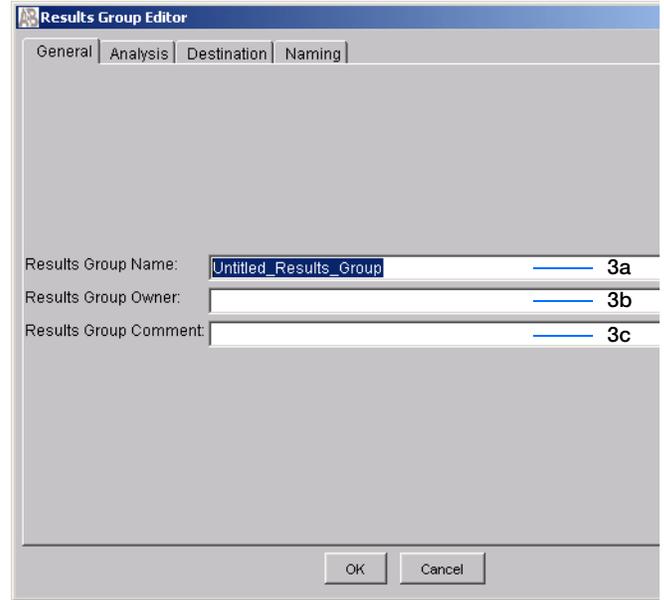
1. In the navigation pane of the Data Collection Software, click **GA Instruments** > **Results Group**.
2. Click **New...** .
The Results Group Editor window opens.



Notes _____

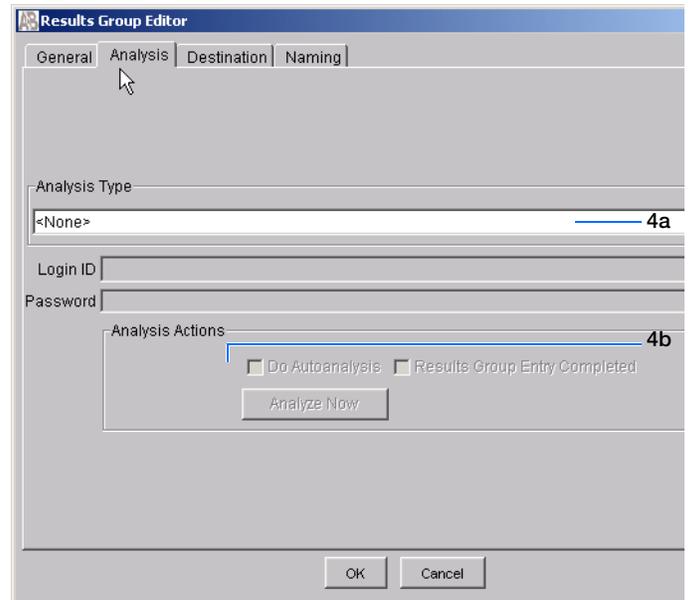


3. Select the General tab, then:
 - a. Type a Results Group Name. The name can be used in naming and sorting sample files. It must be unique (see page for a list of accepted characters).
 - b. Type a Results Group Owner (optional). The owner name can be used in naming and sorting sample files.
 - c.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



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

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



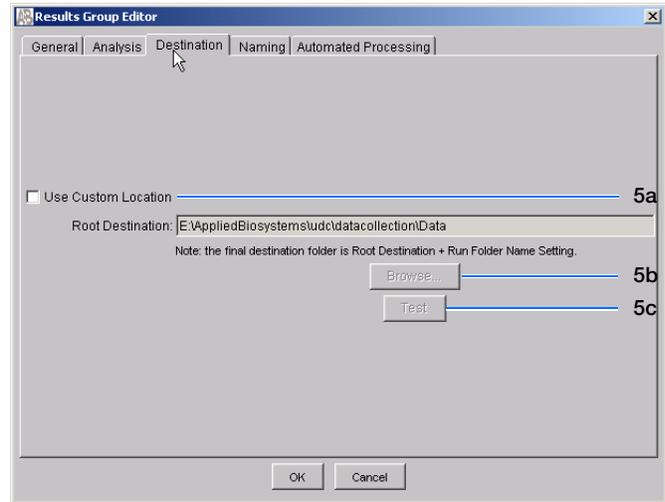
Notes _____



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

To use ...	Then ...
default location	skip to step 1
custom location	complete step a and step b below

- a. Click **Use Custom Location**, then click **Browse...** to navigate to a different save location.
- b. Click **Test** to test the Location path name connection:
 - If it passes, “Path Name test successful” is displayed.
 - If it fails, “Could not make the connection. Please check that the Path Name is correct.” is displayed. Click **Browse** then select a different location.



Sample File Destinations

Locations Where Sample Files Are Placed During Extraction:

- Default Destination, default folder naming: Data / instrument type / instrument name / run folder (No ProcessedData folder)
- Default Destination, custom folder naming: Data/top custom folder/subfolders, and so on.
- Custom Destination, default folder naming: Destination/instrument type/instrument name/run folder
- Custom Destination, custom folder naming: Destination/top custom folder/subfolders, and so on.

Notes _____



1. Select the **Naming** tab.

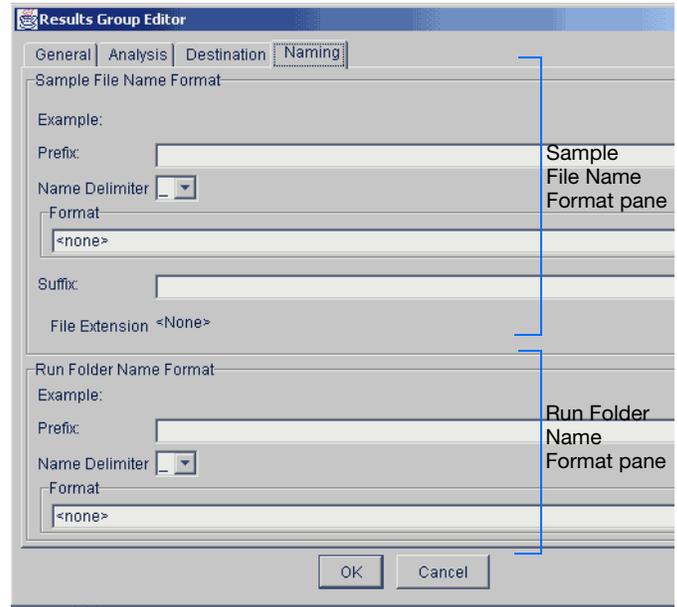
Use the Naming tab to customize sample file and run folder names.

Note: Sample name, run folder name, and path name, *combined*, can total no more than 250 characters. See page page 58 for accepted characters.

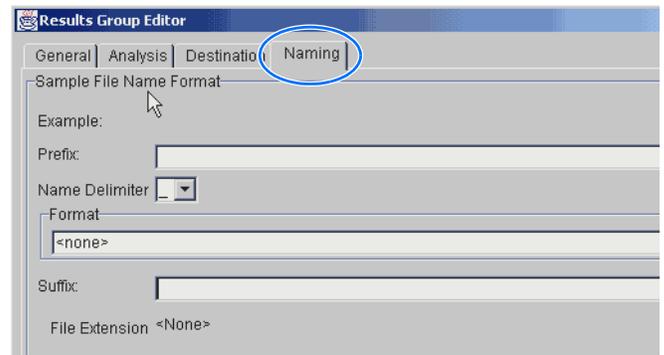
The elements of the Naming tab are discussed in the following sections.

Sample File Name Format Pane

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



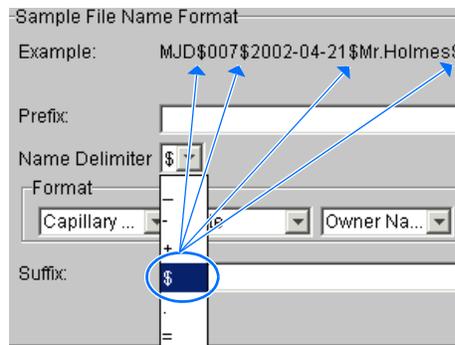
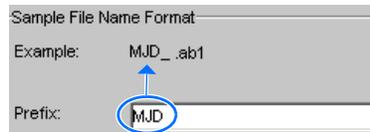
1. In the Naming tab, select the **Prefix** box (optional) to type a prefix for the file name. Anything that you type here is shown in the Example line (see figure below).



Notes



2. Click the **Name Delimiter** list then select the symbol that will separate the Format elements in the file name (see step 3 below). You can select only one delimiter symbol.

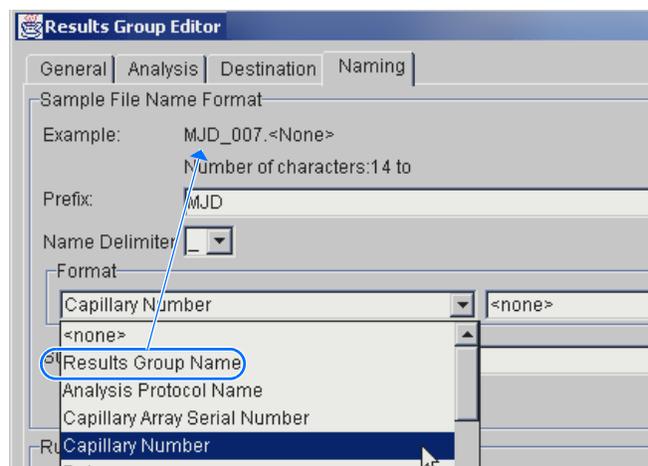


3. Click the Format list, then select the components that you want in the sample name.

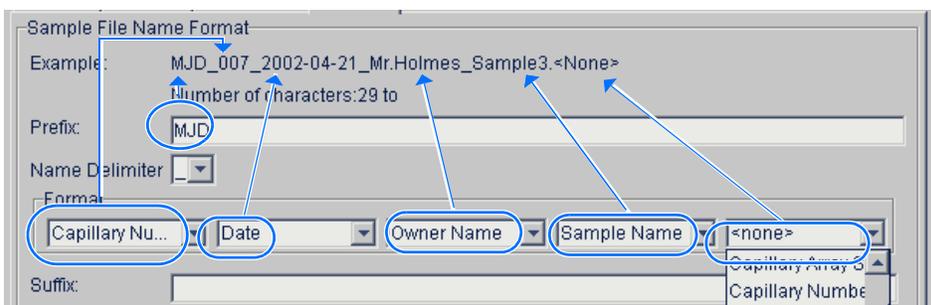
Note: Generally, all the samples from a single run are placed in the same run or results folder, so the name of every sample from a single run should be different from each other. However, most of the Format options are not different between samples, you need to take care to select at least one of the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

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

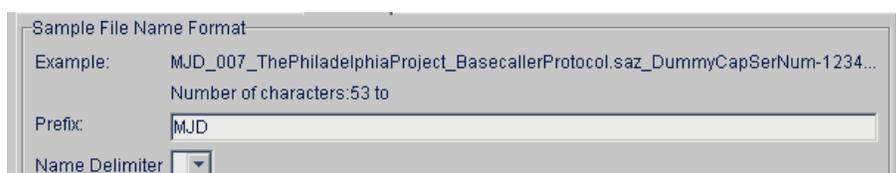
As you continue to select elements for the file name, additional elements are displayed.



Notes _____

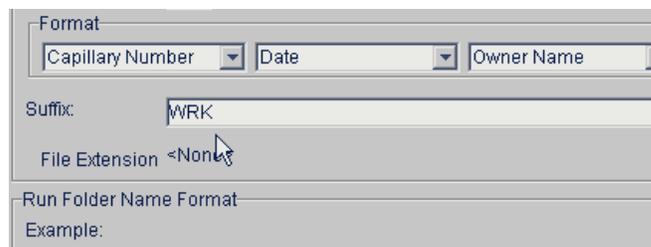


The names of the Format elements are eventually shortened, but the Example field remains visible (up to 72 characters).



4. Select the Suffix box (optional), then type the suffix for the file name.

The File Extension field displays the file extension generated from the Analysis Type specified on the Analysis tab (page page 73). For example, Sequencing Analysis produces sample files with an .ab1 extension.



Saving a Results Group

Click **OK** in any tab after you select all the elements within the Results Group.

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

Format Elements (Unique Identifiers)

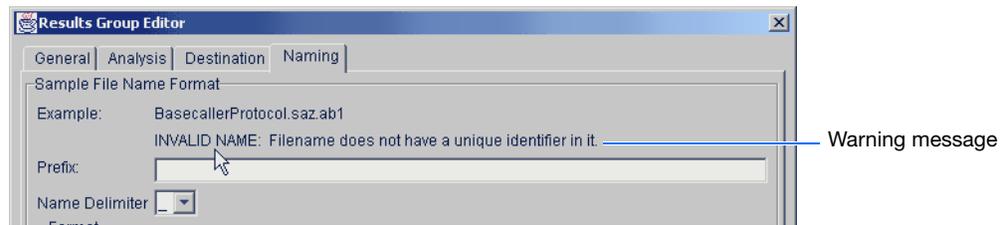
Although you can save a results group by selecting a minimum of one Format element, selecting just the minimum may not provide enough information for you to identify the file or folder later.

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

Notes



If you select elements from the Format lists that do not create unique Sample file or Run folder names, a warning message is displayed below the Example line (see next figure).



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

Run Folder/Sub-Folder Name Format Pane

Follow the same steps described above for the Sample File Name Format pane (page page 75) to specify the run folder name within the run folder.

Notes _____



Importing and Exporting a Results Group

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

Note: Importing Excel files is not supported.

Importing a Results Group

1. In the navigation pane of the Data Collection Software, select  **GA Instruments** >  **Results Group**.
2. Click . A standard File Import dialog box opens.
3. Navigate to the file you want to import.

Note: Import file type is .xml (extensible markup language).

4. Click .

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

Exporting a Results Group

1. In the navigation pane of the Data Collection Software, select  **GA Instruments** >  **Results Group**.
2. Click the Results Group name to select it.
3. Click . A standard file export dialog box opens with the chosen Results Group name.
4. Navigate to the location where you want to save the exported file.
5. Click .

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

Notes _____



Duplicating a Results Group

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

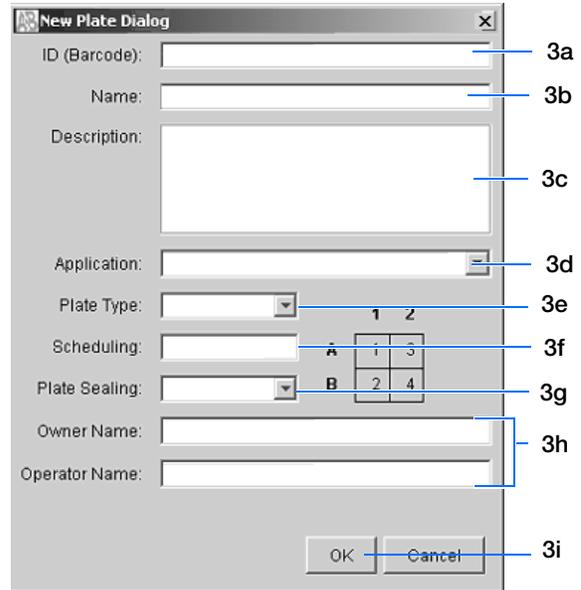
Note: When you import or duplicate a Results Group, the software prompts you to type a name for the new Results Group and for the analysis application type.

Notes _____



Creating and Completing a Sequencing Analysis Plate Record

1. In the navigation pane of the Data Collection Software, select **GA Instruments** > **ga3730** > **Plate Manager**.
2. Click **New...**. The New Plate Dialog dialog box opens.
3. In the New Plate Dialog:
 - a. Type a plate ID or barcode.
 - b. Type a name for the plate.
 - c. (Optional) Type a description for the plate.
 - d. Select your sequencing application in the Application drop-down list.
 - e. Select **96-well** or **384-well** in the Plate Type drop-down list.
 - f. Select **96-well** or **384-well** in the Plate Type drop-down list.
 - g. Select **heat seal** or **septa**.
 - h. Type a name for the owner and operator.
 - i. Click **OK**. The Sequencing Analysis Plate Editor opens.



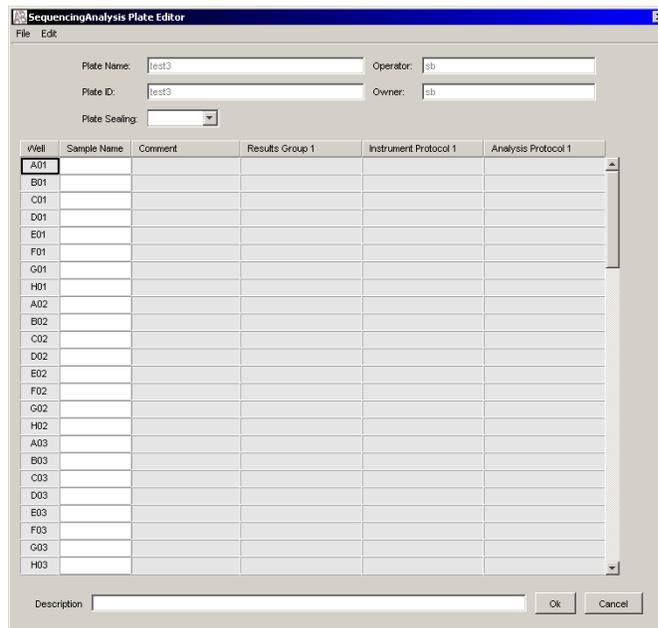
Notes



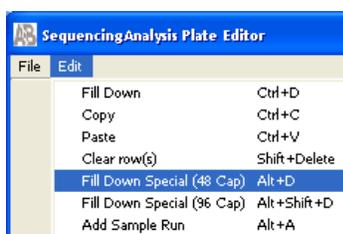
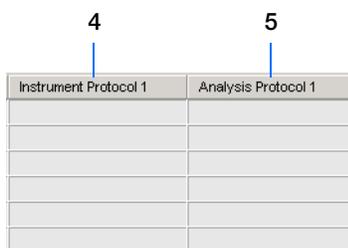
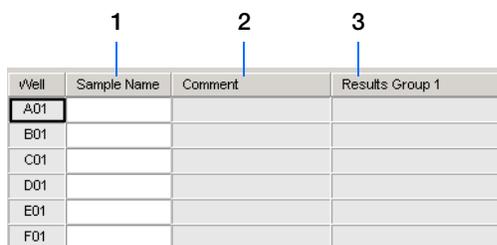
Completing a Sequencing Analysis Plate Record

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

Note: Importing Excel files is not supported.



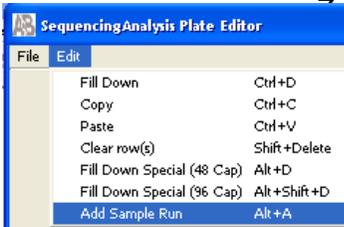
1. In the Sample Name column of a row, enter a sample name, then click the next cell. The value 100 is automatically displayed in the Priority column.
2. In the Comments column, enter any additional comments or notations for the sample.
3. In the Results Group 1 column, select a group from the drop-down list (see page 72).
4. In the Instrument Protocol 1 column, select a protocol from the drop-down list (see page 62).
5. In the Analysis Protocol 1 column, select a protocol from the drop-down list (see page 67).
6. To complete the rest of the plate record based on the samples loaded in your plate, do one of the following:
 - For the same samples and protocols – Select the entire row, then select **Edit > Fill Down Special** (see “Fill Down Special” on page 84)
 - Based on the plate type (96- or 384-well) and capillary array (48 or 96 capillaries) you are using, select the appropriate fill down option:



Notes



- 96 capillary/96-well plate: **Fill Down**.
- 48 capillary/96-well plate: **Fill down Special (48 Cap)**.
- 96 capillary/384-well plate: **Fill down Special (96 Cap)**.
- 48 capillary/384-well plate: **Fill down Special (48 Cap)**.
- For the same samples and protocols – Select the entire row, then select **Edit > Fill Down**.
- For the different samples and protocols, complete the plate editor manually.



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

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

To add additional runs, select **Edit > Add Sample Run** again.

Complete the columns for the additional runs.

9. Click .

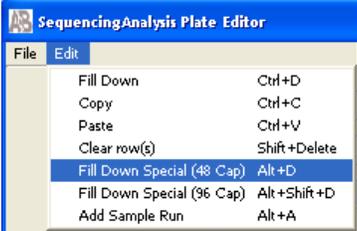
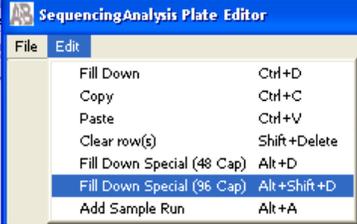
IMPORTANT! After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database, then the plate record can be searched for, edited, exported, or deleted.

Notes _____



Fill Down Special

The following table illustrates the Fill Down Special feature.

If You Choose ...	Then ...																																										
<p>Fill Down Special (48 Cap)</p> 	<p>The fill down pattern matches the 48-capillary load pattern.</p> <table border="1" data-bbox="516 506 703 1098"> <thead> <tr> <th>Well</th> <th>Sample Name</th> </tr> </thead> <tbody> <tr><td>A01</td><td>notMJD</td></tr> <tr><td>B01</td><td>notMJD</td></tr> <tr><td>C01</td><td>notMJD</td></tr> <tr><td>D01</td><td>notMJD</td></tr> <tr><td>E01</td><td>notMJD</td></tr> <tr><td>F01</td><td>notMJD</td></tr> <tr><td>G01</td><td>notMJD</td></tr> <tr><td>H01</td><td>notMJD</td></tr> <tr><td>A02</td><td>MJD</td></tr> <tr><td>B02</td><td>MJD</td></tr> <tr><td>C02</td><td>MJD</td></tr> <tr><td>D02</td><td>MJD</td></tr> <tr><td>E02</td><td>MJD</td></tr> <tr><td>F02</td><td>MJD</td></tr> <tr><td>G02</td><td>MJD</td></tr> <tr><td>H02</td><td>MJD</td></tr> <tr><td>A03</td><td>notMJD</td></tr> <tr><td>B03</td><td>notMJD</td></tr> <tr><td>C03</td><td>notMJD</td></tr> <tr><td>D03</td><td>notMJD</td></tr> </tbody> </table> <p>First Quadrant</p> <p>Second Quadrant</p>	Well	Sample Name	A01	notMJD	B01	notMJD	C01	notMJD	D01	notMJD	E01	notMJD	F01	notMJD	G01	notMJD	H01	notMJD	A02	MJD	B02	MJD	C02	MJD	D02	MJD	E02	MJD	F02	MJD	G02	MJD	H02	MJD	A03	notMJD	B03	notMJD	C03	notMJD	D03	notMJD
Well	Sample Name																																										
A01	notMJD																																										
B01	notMJD																																										
C01	notMJD																																										
D01	notMJD																																										
E01	notMJD																																										
F01	notMJD																																										
G01	notMJD																																										
H01	notMJD																																										
A02	MJD																																										
B02	MJD																																										
C02	MJD																																										
D02	MJD																																										
E02	MJD																																										
F02	MJD																																										
G02	MJD																																										
H02	MJD																																										
A03	notMJD																																										
B03	notMJD																																										
C03	notMJD																																										
D03	notMJD																																										
<p>Fill Down Special (96 Cap) *</p>  <p>* Especially useful for 384-well plates</p>	<p>The fill down pattern matches the 96-capillary load pattern.</p> <table border="1" data-bbox="516 1167 703 1766"> <thead> <tr> <th>Well</th> <th>Sample Name</th> </tr> </thead> <tbody> <tr><td>A10</td><td>12345</td></tr> <tr><td>B10</td><td>12345</td></tr> <tr><td>C10</td><td>12345</td></tr> <tr><td>D10</td><td>12345</td></tr> <tr><td>E10</td><td>12345</td></tr> <tr><td>F10</td><td>12345</td></tr> <tr><td>G10</td><td>12345</td></tr> <tr><td>H10</td><td>12345</td></tr> <tr><td>A11</td><td>12345</td></tr> <tr><td>B11</td><td>12345</td></tr> <tr><td>C11</td><td>12345</td></tr> <tr><td>D11</td><td>12345</td></tr> <tr><td>E11</td><td>12345</td></tr> <tr><td>F11</td><td>12345</td></tr> <tr><td>G11</td><td>12345</td></tr> <tr><td>H11</td><td>12345</td></tr> <tr><td>A12</td><td>12345</td></tr> <tr><td>B12</td><td>12345</td></tr> <tr><td>C12</td><td>12345</td></tr> </tbody> </table>	Well	Sample Name	A10	12345	B10	12345	C10	12345	D10	12345	E10	12345	F10	12345	G10	12345	H10	12345	A11	12345	B11	12345	C11	12345	D11	12345	E11	12345	F11	12345	G11	12345	H11	12345	A12	12345	B12	12345	C12	12345		
Well	Sample Name																																										
A10	12345																																										
B10	12345																																										
C10	12345																																										
D10	12345																																										
E10	12345																																										
F10	12345																																										
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H10	12345																																										
A11	12345																																										
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G11	12345																																										
H11	12345																																										
A12	12345																																										
B12	12345																																										
C12	12345																																										

Notes

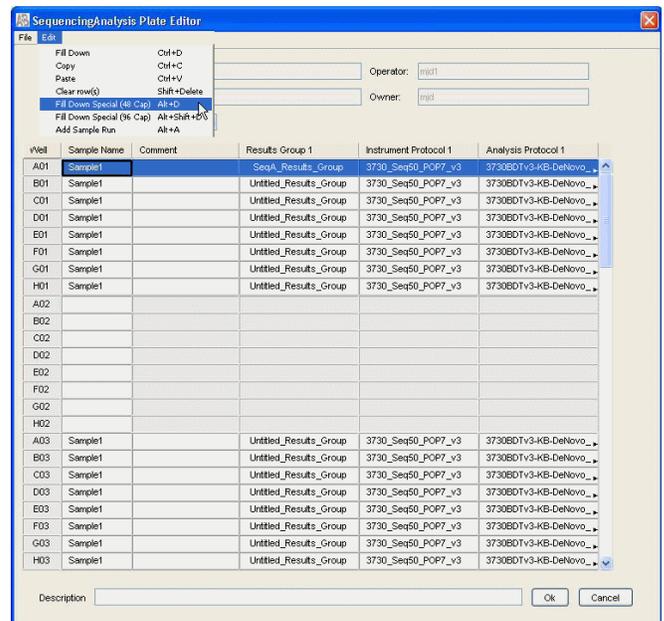


Fill Down Special for a 48 Cap/96-Well Plate

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

To use the fill down special function:

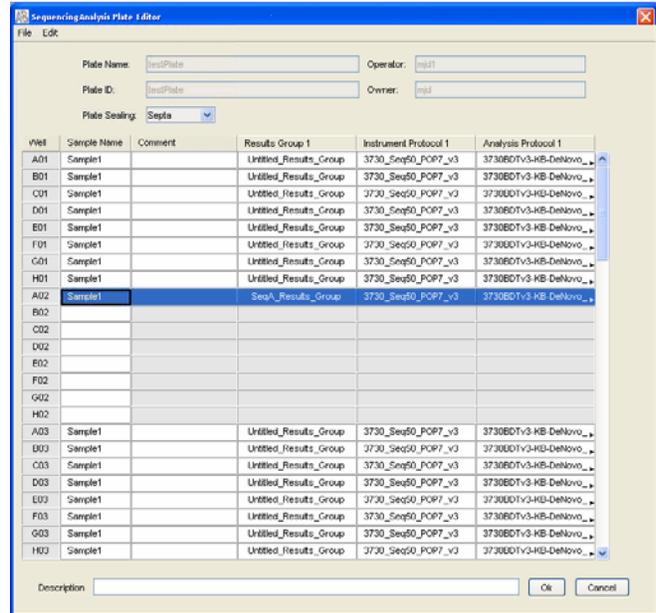
1. In the Plate Manager, double-click the plate of interest to open the Plate Editor.
2. Type the sample name, complete all columns, then click-drag the entire row to select it.
3. Select **Edit > Fill Down Special (48 Cap)** to fill the plate record with the first load pattern.



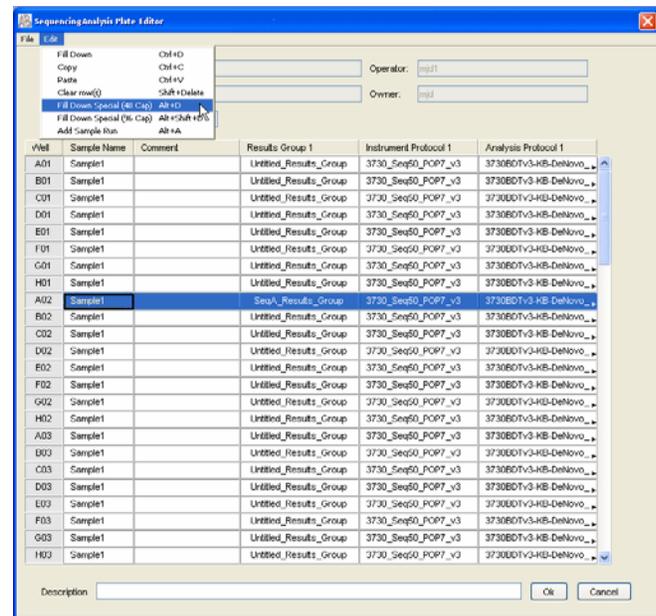
Notes



- Click A02, type the name of sample 2, complete all columns, then click-drag the entire row to select it.



- Select **Edit > Fill Down Special (48 Cap)** to fill the plate record with the second load pattern.

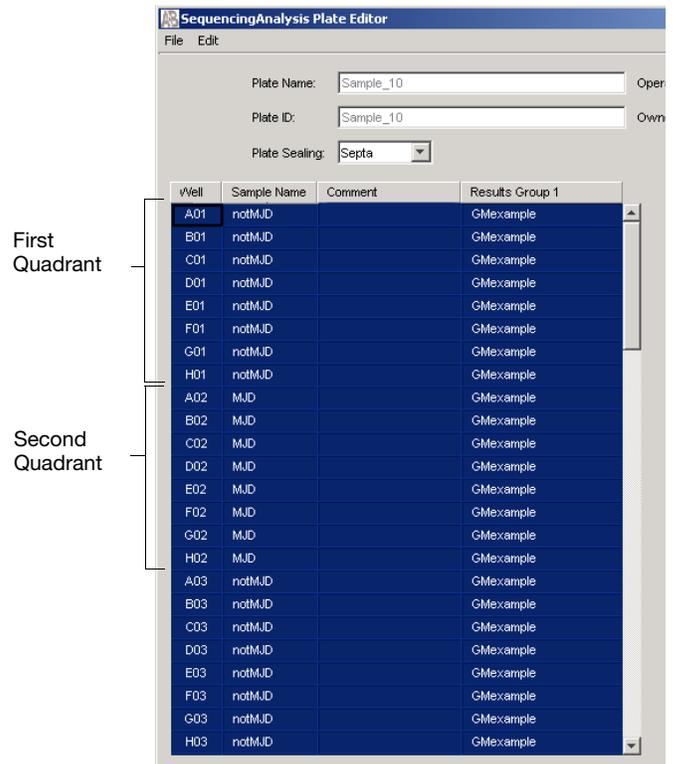


Notes _____



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

When you use the Fill Down Special (96 Cap) function on a 384-well plate, the fill-down pattern appears as in the adjoining illustration to the right.



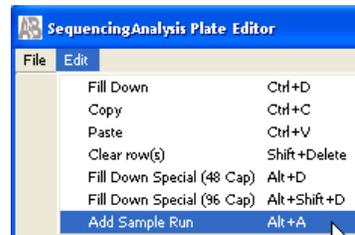
Adding a Sample Run

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

To add a sample run **Select Edit > Add Sample Run.**

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

To run the plate(s), see “Running the Instrument” on page 117.



Notes



Chapter 4 Setting Up the Software for DNA Sequencing

Fill Down Special

SequencingAnalysis Plate Editor

File Edit

Plate Name: 384 Operator: sc

Plate ID: 384 Owner: sc

Plate Sealing: Heat Sealing Scheduling: 1234

vWell	Sample Name	Comment	Results Group 1	Instrument Protocol 1	Analysis Protocol 1
A01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
B01					
C01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
D01					
E01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
F01					
G01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
H01					
I01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
J01					
K01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def
L01					
M01	sample		SeqA	RapidSeq	3730BDTv3-KB-Def

SequencingAnalysis Plate Editor

File Edit

Plate Name: Sample_10 Operator: m

Plate ID: Sample_10 Owner: m

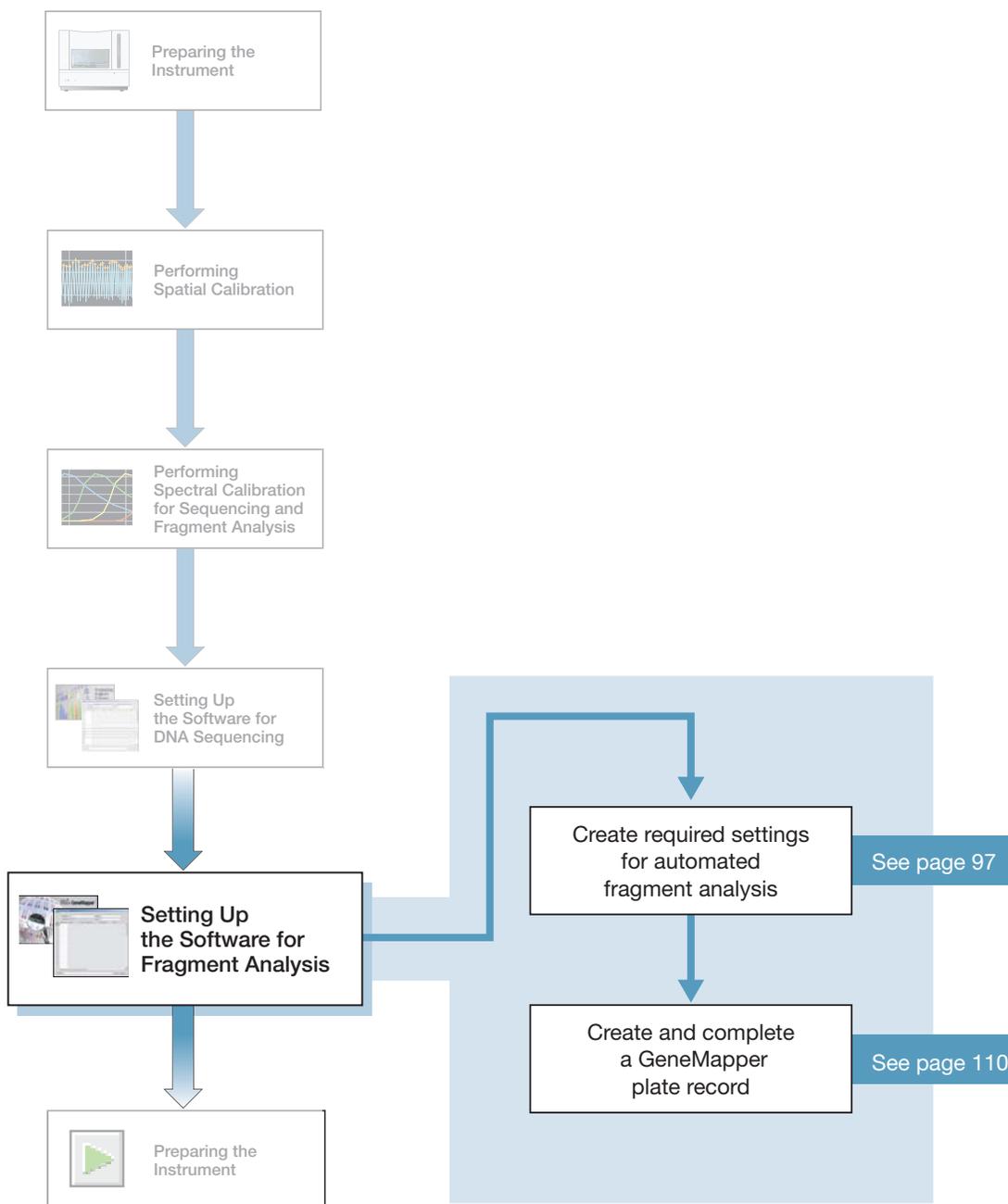
Plate Sealing: Septa

vWell	Instrument Protocol 1	Analysis Protocol 1	Results Group 2	Instrument Protocol 2	Analysis Protocol 2
A01					
B01					
C01					
D01					
E01					
F01					
G01					
H01					
A02					
B02					
C02					
D02					
E02					
F02					
G02					
H02					
A03					
B03					
C03					
D03					
E03					
F03					
G03					

Description | Ok | Cancel

Notes _____

Setting Up the Software for Fragment Analysis



Notes



3730/3730xl Analyzer Data Collection and GeneMapper® Software

IMPORTANT! Do not rename the computer after 3730/3730xl Analyzer Data Collection software is installed. Doing so causes the 3730/3730xl Analyzer Data Collection software to malfunction.

File-Naming Convention

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

spaces \ / : * ? " < > |

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

Autoanalysis

You may choose to perform autoanalysis of fragment analysis samples by using the 3730/3730xl Analyzer Data Collection, and GeneMapper® software.

GeneMapper® Software v3.7

You can perform Autoanalysis on the same instrument that collected the sample files or on a remote computer.

Manual Analysis

For information on manual analysis, refer to *GeneMapper Software Version 3.7 User Guide* (PN 4359413)

Fragment Analysis and Data Collection

When GeneMapper® software is installed on a computer that has 3730/3730xl DNA Analyzer Data Collection Software, you can access through the Results Group Editor (see page 102):

- GeneMapper-Generic
- GeneMapper-<Computer Name>

GeneMapper-Generic

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

Notes _____



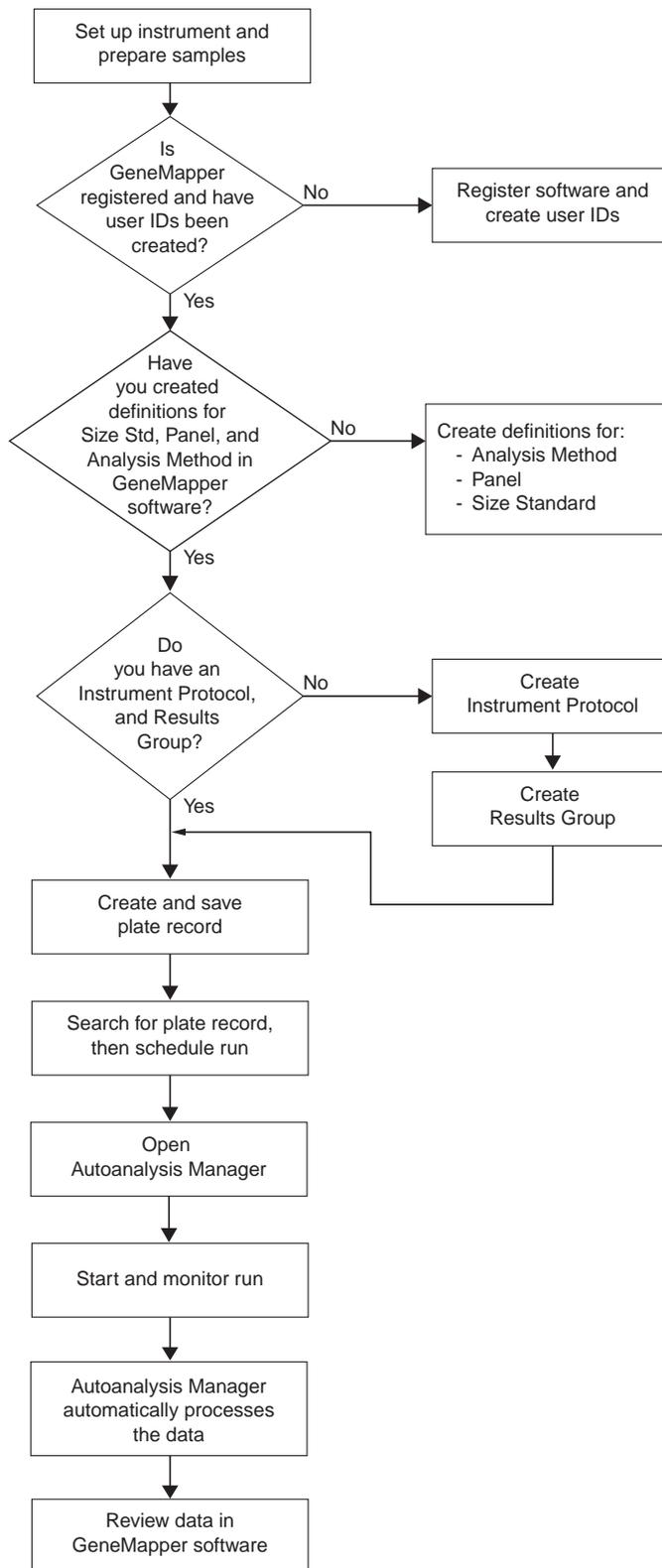
**GeneMapper-
<Computer
Name>**

GeneMapper-<Computer Name> is for autoanalysis. The Size Standard, Analysis Method, and Panel columns in the Sample Sheet window read directly from the GeneMapper® software database. These components must be created in GeneMapper software prior to setting up the plate record for a run. There is no way to create a new entry for these columns once inside the plate editor dialog box. If you create a new GeneMapper software component while the plate record dialog box is open, the columns will not update. The plate record must be closed and reopened to update the GeneMapper software components. For more information see, “Setting Up a Run for Autoanalysis” on page 136.

Notes _____



Workflow for Autoanalysis Using GeneMapper® Software



Notes _____



GeneMapper® Software Plate Records

Overview Plate records are data tables in the instrument database that store information about the plates and the samples they contain. A plate record contains:

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

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

When to Create a Plate Record You must create a plate record for each plate of samples for:

- Spectral calibrations
- Fragment analysis

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

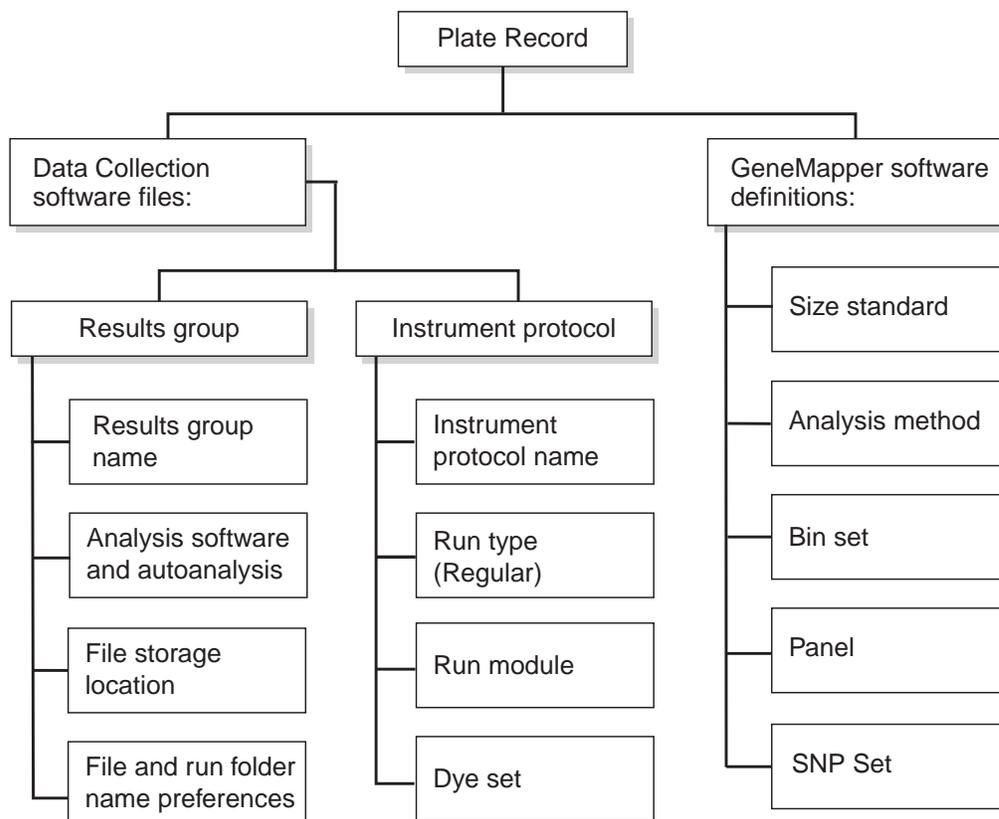
Parameters	Description	See Page
Instrument protocol	Contains everything needed to run the instrument.	97
Results group	Defines the file type, the file name, autoanalysis, and file save locations that are linked to sample injections.	102

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

Notes _____



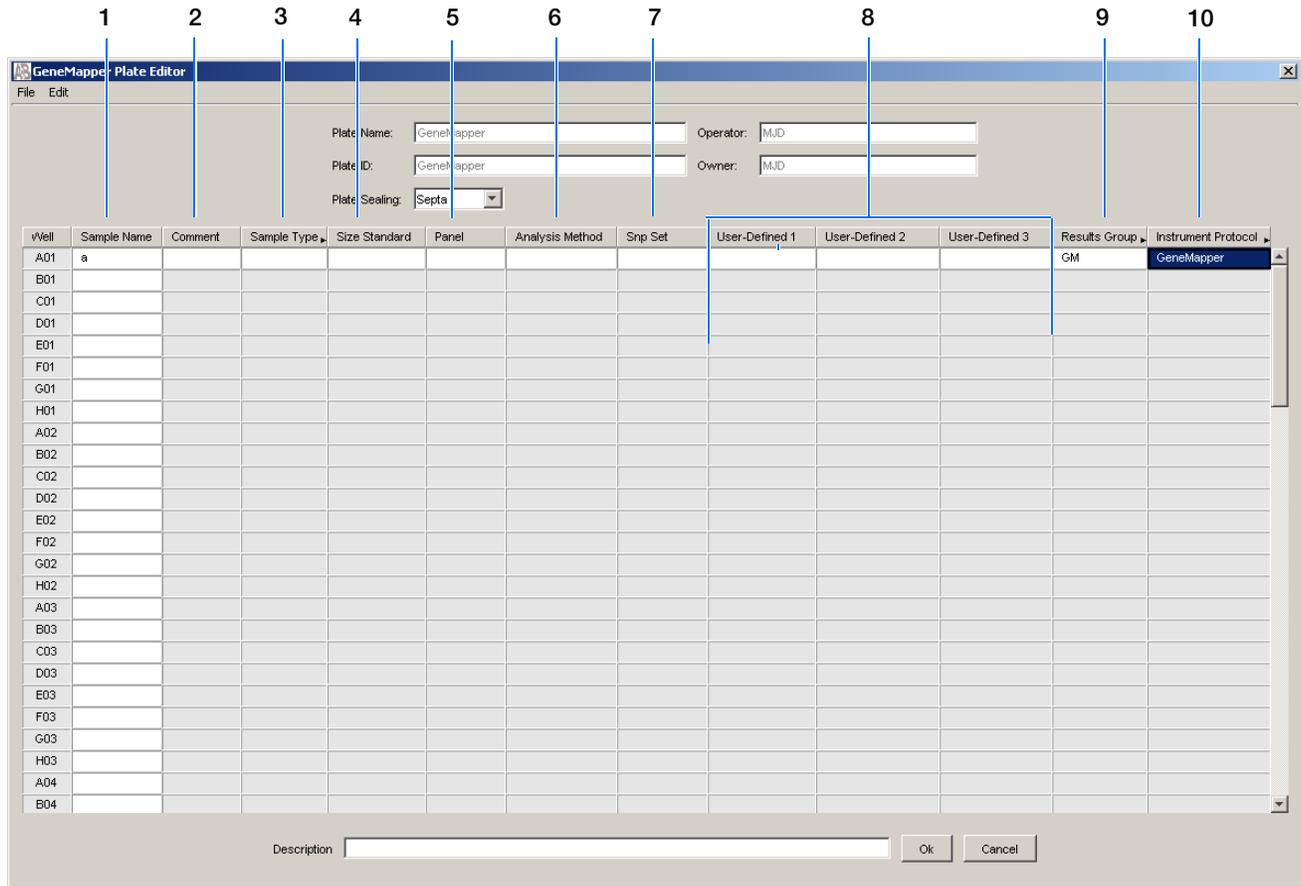
Components of a GeneMapper® Software Plate Record



Notes _____



Descriptions for numbers 1 to 10 are in the table below



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

The following table describes columns 1-10 inserted in a plate record for a fragment analysis run (see figure above).

Table 5-1 Components of the plate record

Column	Description
1. Sample Name	Name of the sample
2. Comment	Comments about the sample (optional)
3. Sample Type	Use to identify the sample as Sample, Positive Control, Allelic Ladder, or Negative Control.
4. Size Standard IMPORTANT! For GeneMapper-<Computer Name> ONLY: Size Standard, Panel, and Analysis Method must be created in GeneMapper® software before creating a new plate	<ul style="list-style-type: none"> GeneMapper-Generic (optional): Manually enter size standards in the text field GeneMapper-<Computer Name>: Select a saved size standard from the drop-down list

Notes



Table 5-1 Components of the plate record

Column	Description
5. Panel IMPORTANT! GeneMapper-<Computer Name> ONLY: For Size standard, panel, and analysis method must be created in GeneMapper software before creating a new plate	<ul style="list-style-type: none"> GeneMapper-Generic (optional): Manually enter panels in the text field* GeneMapper-<Computer Name>: Select a saved panel from the drop-down list
6. Analysis Method IMPORTANT! For GeneMapper <Computer Name> ONLY: Size standard, panel, and analysis method must be created in GeneMapper software before creating a new plate	<ul style="list-style-type: none"> GeneMapper-Generic (optional): Manually enter analysis methods in the text field* GeneMapper-<Computer Name>: Select a saved analysis method from the drop-down list
7. Snp IMPORTANT! GeneMapper <Computer Name> ONLY: For Size standard, panel, and analysis method must be created in GeneMapper software before creating a new plate	<ul style="list-style-type: none"> GeneMapper-Generic (optional): Manually enter analysis methods in the text field* GeneMapper-<Computer Name>: Use for SNPlex system chemistry; select a saved SNP set from the drop-down list
8. 3 User-defined columns	Optional text entries
9. Results group	Some options: <ul style="list-style-type: none"> 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 <p>Note: You must have a results group selected for each sample entered in the Sample Name column. See, “Results Groups” on page 102.</p>
10. Instrument protocol	<ul style="list-style-type: none"> 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. <p>Note: You must have an instrument protocol selected for each sample entered in the Sample Name column.</p> <ul style="list-style-type: none"> See, “Instrument Protocols” on page 97.

Notes _____



Creating Required Settings for Automated Fragment Analysis

If the Settings Already Exist

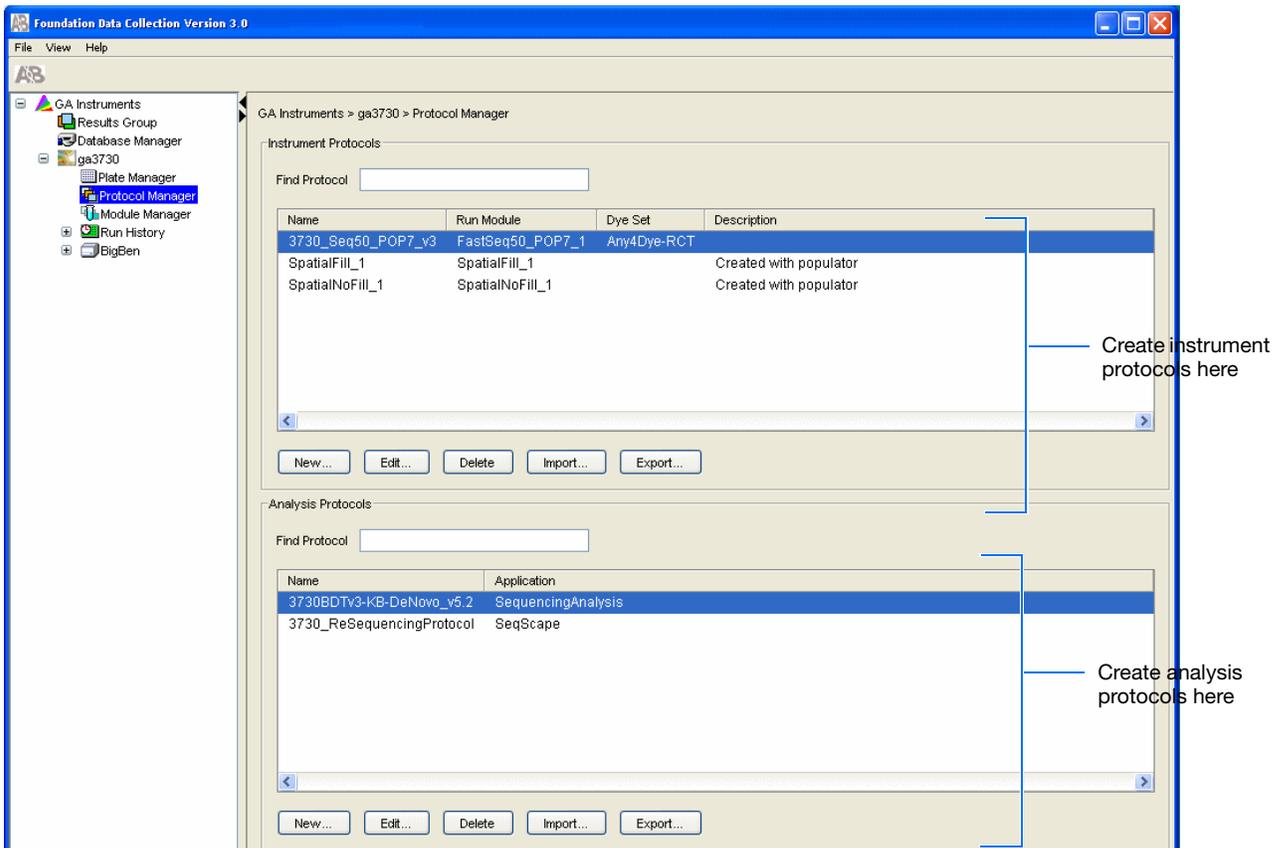
If the appropriate data collection and fragment analysis files have been created, go to “Creating and Completing a GeneMapper Plate Record” on page 110.

Instrument Protocols

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

Creating an Instrument Protocol

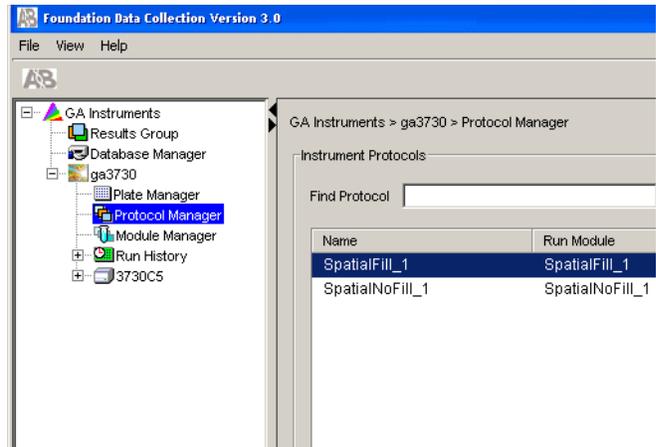
1. In the navigation pane of the Data Collection Software, select **GA Instruments** > **ga3730** > **Protocol Manager**.



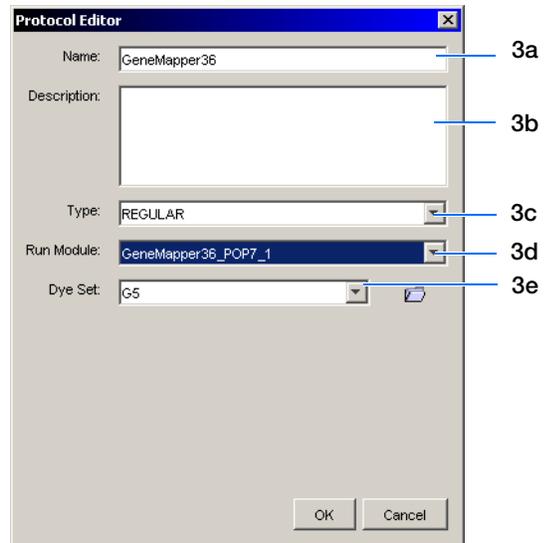
Notes



2. In the Instruments Protocols section, click **New...**. The Protocol Editor opens.



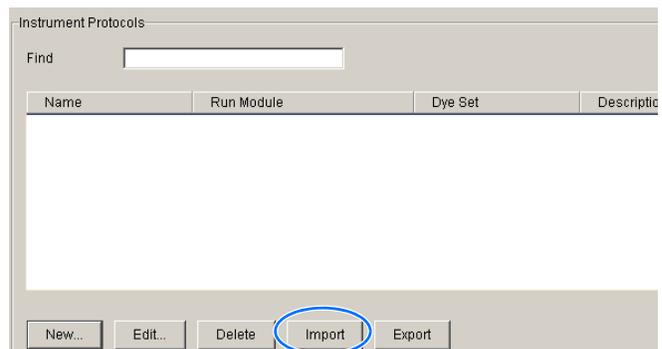
3. Complete the Protocol Editor:
 - a. Type a name for the protocol.
 - b. Type a description for the protocol (optional).
 - c. Select **Regular** in the Type drop-down list.



- d. Select **GeneMapper36_POP7**.
 - e. Select **G5**.
 - f. Click **OK**.

Importing an Instrument Protocol

1. In the Protocol Editor window select **Import** in the Instrument Protocols pane, if you want to use an existing instrument protocol.



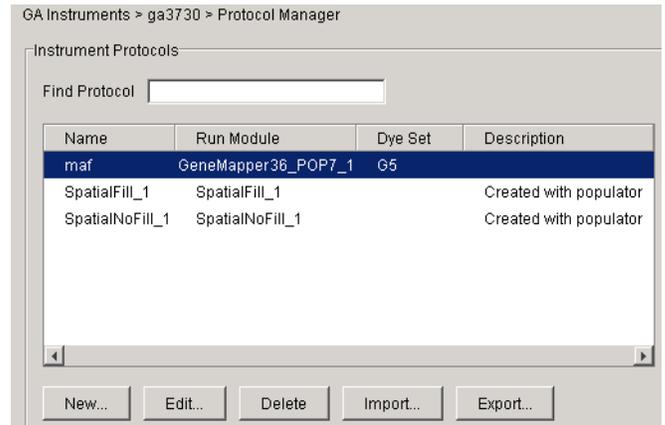
Notes _____



- Navigate to the protocol you want to import.

Note: Import file type is .xml (extensible markup language).

- Double-click the protocol to import it.
- The imported files are displayed alphabetically in the Instrument Protocol pane.



Fragment Analysis Run Modules

Select one run module:

Run Module	Capillary Length
GeneMapper36_POP7	36 cm
GeneMapper50_POP7	50 cm
HTSNP36_POP7_V3 (SNPlex)	36 cm
HTSNP50_POP7 (SNPlex)	50 cm

Notes _____



Customizing Run Modules

If you need to modify default run modules to suit your particular needs:

1. Select GA Instrument

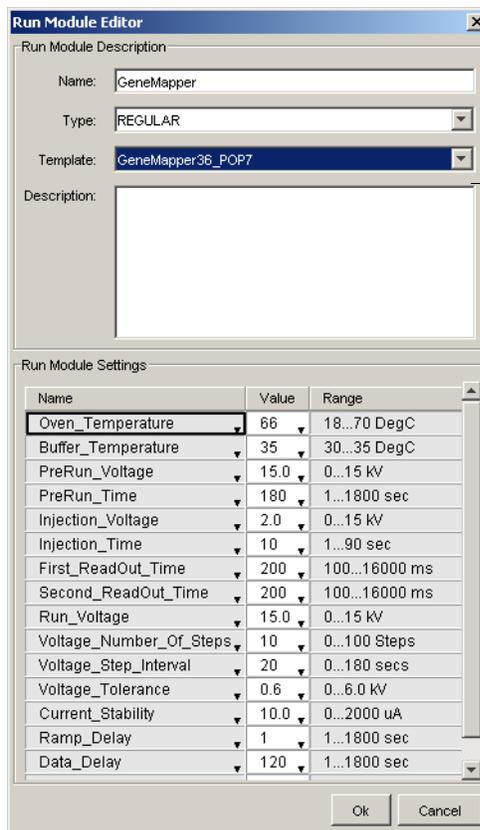
> **ga3730** > **Module Manager**.

2. Click **New...**.

3. Select a template module as a basis for the new module.

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

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



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

Notes _____



The Run Module Parameters that you can edit:

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

Notes

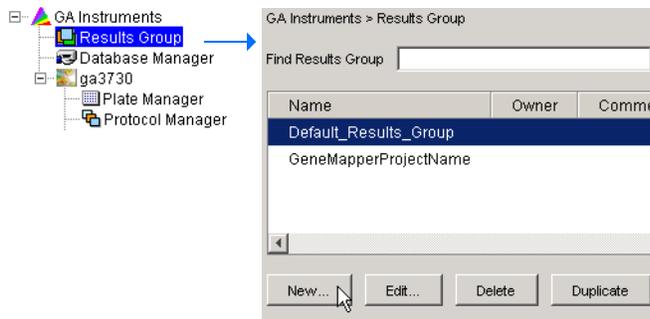


Results Groups

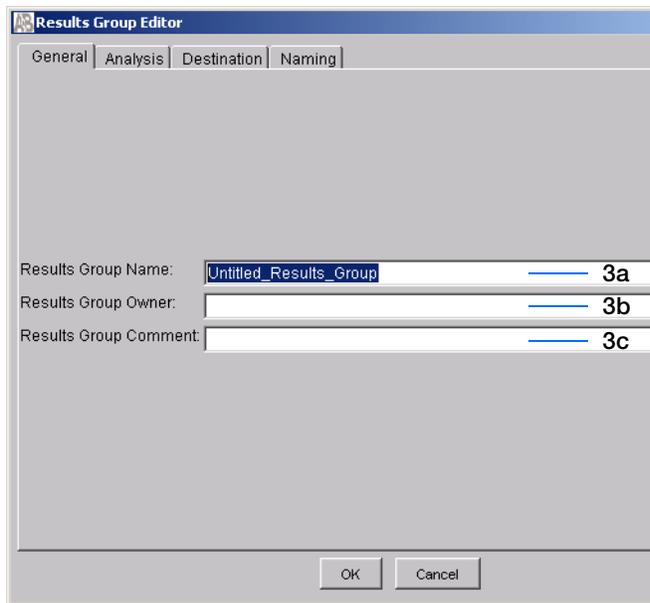
A Results Group is a component within Data Collection that organizes samples and certain user settings under a single name. A Results Group is used to prepare samples for analysis and to name, sort, and deliver samples that result from a run.

Creating a Results Group for Autoanalysis

1. In the navigation pane of the Data Collection Software, select **GA Instruments > Results Group**.
2. Click **New**. The Results Group Editor window opens.



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



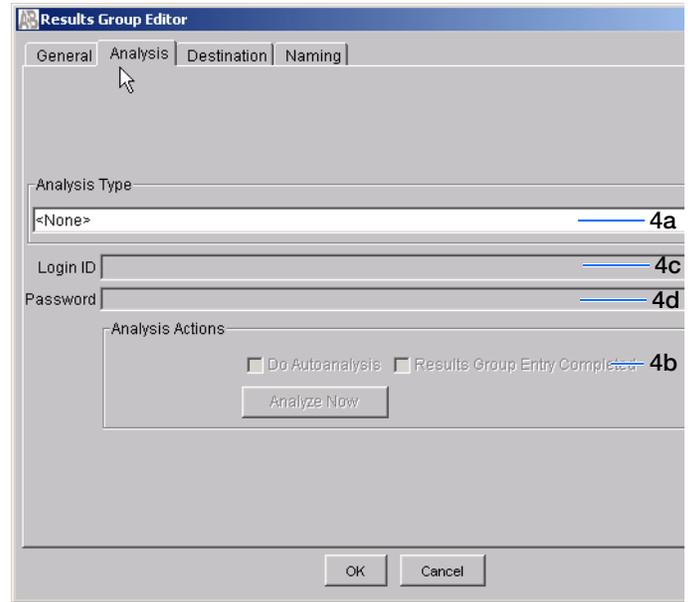
Notes _____



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

- a. Click the Analysis Type, then select one of the following:

If You Select ...	Then ...
None	Only raw data files are generated
GeneMapper-Generic	Autoanalysis is not available and only .fsa files are generated
GeneMapper-<Computer Name>	<ul style="list-style-type: none"> • Autoanalysis of completed runs is available • Automated Processing tab is available <p>Steps b, c, and d below apply only to GeneMapper-<Computer Name> (not GeneMapper-Generic).</p>



- b. If you selected GeneMapper-<Computer Name> in step a, select:
- **Do Autoanalysis**—To analyze samples after each run of 48 or 96 is complete.
 - **Do Autoanalysis** and **Results Entry Group Complete**—To analyze samples after all samples using the same results group have been run.

c. Type the Login ID.

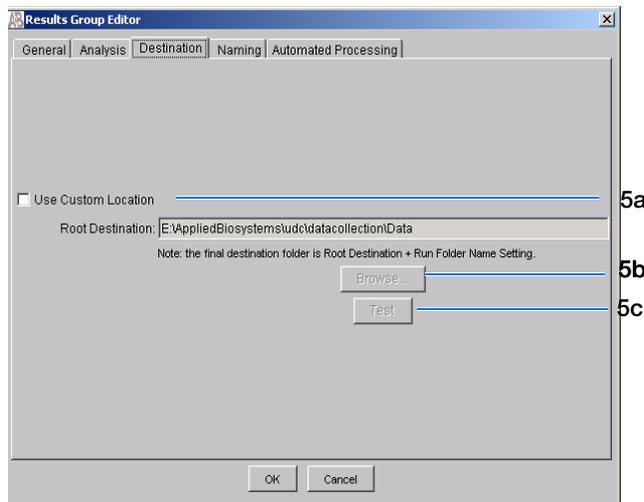
d. Type the login password.

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

Notes



5. Select the **Destination** tab, then use the default destination or define a new location for data storage. To use a:
 - Default location–Skip to step 6.
 - Custom location–Complete step a and step b below.
 - a. Click **Use Custom Location**, then click **Browse...** to navigate to a different save location.
 - b. Click **Test** to test the Location path name connection:
 - If the test passes, “Path Name test successful,” displays.
 - If the test fails, “Could not make the connection. Please check that the Path Name is correct,” displays. Click **Browse**, then select a different location.



Sample File Locations

Locations Where Sample Files Are Placed During Extraction:

- Default Destination, default folder naming: Data / instrument type / instrument name / run folder (No ProcessedData folder)
- Default Destination, custom folder naming: Data/top custom folder/subfolders, and so on.
- Custom Destination, default folder naming: Destination/instrument type/instrument name/run folder
- Custom Destination, custom folder naming: Destination/top custom folder/subfolders, and so on.

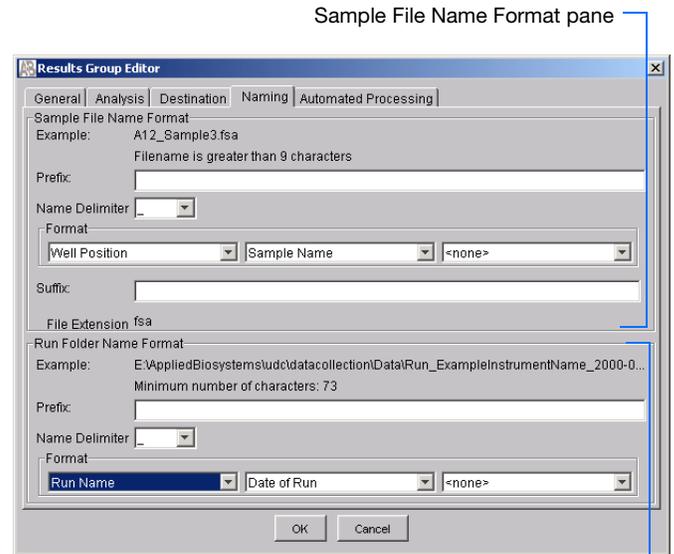
Notes _____



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

Note: Sample name, run folder name, and path name, *combined*, can total no more than 250 characters. See page 90 for accepted characters.

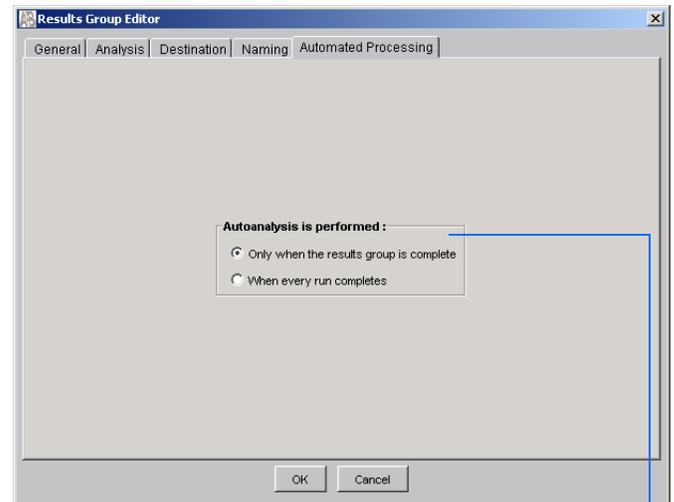
The elements of the Naming tab are discussed in the following sections, see page 106.



Run Folder Name Format pane

2. Select the **Automated Processing** tab.

Note: The Automated Processing tab is available only if you selected GeneMapper-
<Computer Name> in step 4 on page 103



Select an autoanalysis option

In the “Autoanalysis is performed” section of the Results Group Editor, when you want your samples autoanalyzed select:

- **Only when the result group is complete**—If you want samples to be analyzed after all samples that use the sample results group have been run.
- **When every run completes**—If you want samples to be analyzed after each run of 48 or 96 samples.

3. Click  to save the Results Group.

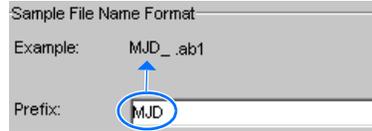
Notes



Sample File Name Format Pane

To complete the Sample File Name Format pane:

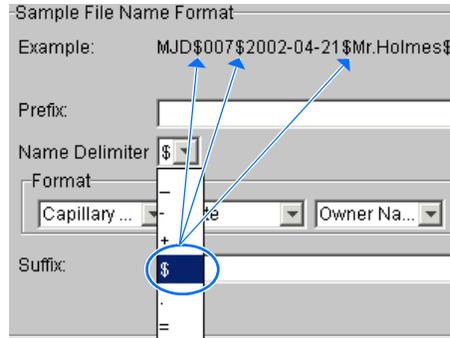
1. (Optional) Select the **Prefix** box then type a prefix for the file name. Anything that you type here is shown in the Example line (see graphic below).
2. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3 below). You can only choose one delimiter symbol.



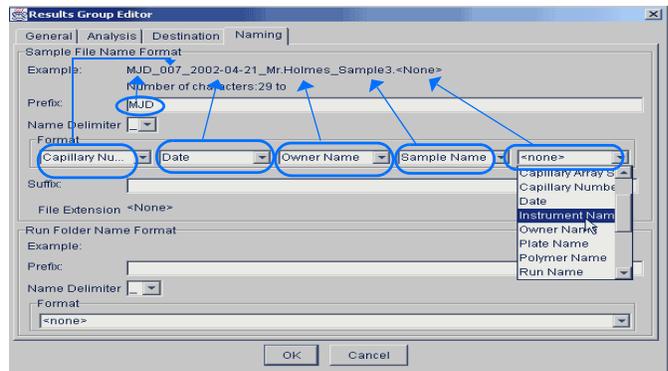
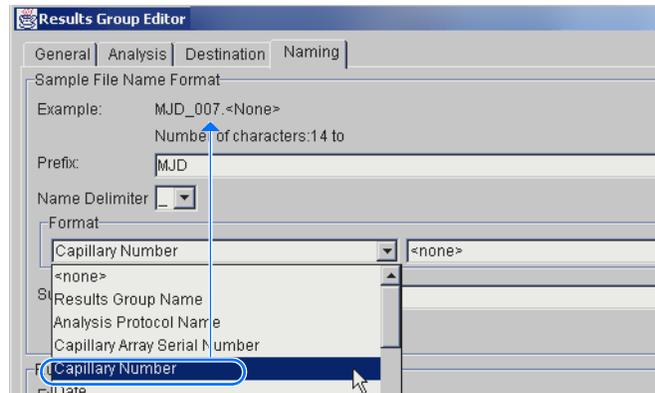
3. Click the **Format** list and then select the components that you want in the sample name.

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

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



Note: An additional drop-down list of formats is displayed after you select a format option.



Notes



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

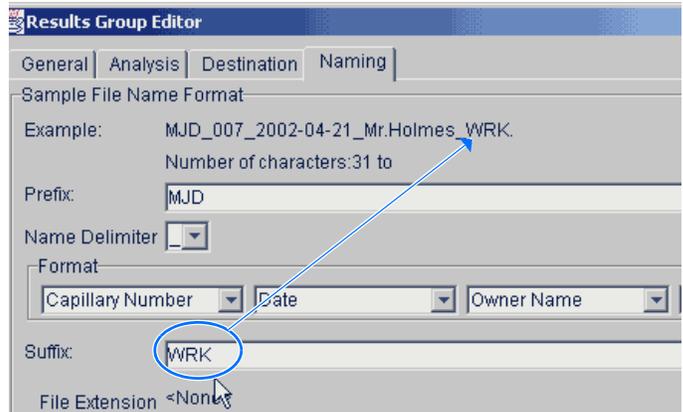
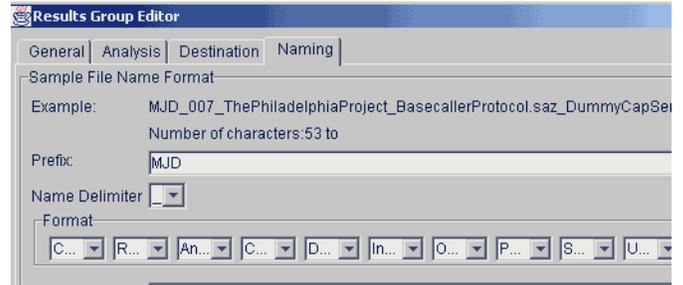
Note: To view the shortened format elements, place the cursor on the edge of the window until it turns into a double-arrow. Drag the arrow to expand the window horizontally.

4. (Optional) Click the Suffix box then type the suffix for the file name.

The File Extension field displays the file extension generated from the Analysis Type specified on the Analysis tab (page 103). For example, fragment analysis produces sample files with an .fsa extension.

Run Folder/Sub-Folder Name Format Pane

Follow the same steps described above for the Sample File Name Format pane (page 106) to change the sub-folder name within the run folder.



Notes _____

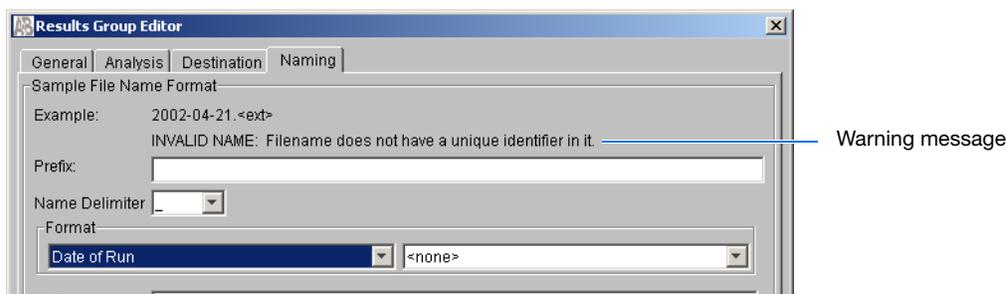


Format Elements (Unique Identifiers)

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

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

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



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

Importing and Exporting a Results Group

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

Note: Importing Excel files is not supported.

Importing a Results Group

1. In the navigation pane of the Data Collection Software, select **GA Instruments > Results Group**.
2. Click **Import**. A standard File Import dialog box opens.
3. Navigate to the file you want to import.

Note: Import file type is .xml (extensible markup language).

4. Click **Open**.

Note: When you duplicate a Results Group, the software prompts you to type a name for the new Results Group and for the analysis application type.

Notes



Exporting a Results Group

1. In the navigation pane of the Data Collection Software, select **GA Instruments** > **Results Group**.
2. Select the Results Group name.
3. Click **Export**. A standard file export dialog box opens, displaying the chosen Results Group name.
4. Navigate to where you want to save the exported file.
5. Click **Save**.

Note: If a results group with the same name already exists at the save location, you can duplicate the results groups to copy settings into a similar results group without the risk of user error.

Duplicating a Results Group

1. Click the results group to select it.
2. Click **Duplicate**.

Note: When you duplicate a results group, the software prompts you to type a name for the new Results Group and for the analysis application type.

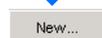
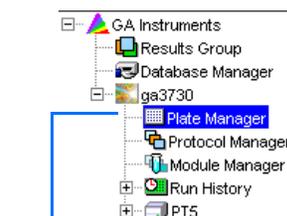
Notes _____



Creating and Completing a GeneMapper® Software Plate Record

Creating the GeneMapper® Software Plate Record for Autoanalysis

1. In the navigation pane of the Data Collection Software, select **GA Instruments** > **ga3730** > **Plate Manager**.
2. Click **New...**. The New Plate Dialog dialog box opens.
3. Complete the information in the New Plate Dialog:
 - a. Type a plate ID.
 - b. Type a name for the plate.
 - c. Type a description for the plate (optional).
 - d. Select your GeneMapper application in the Application drop-down list.
 - e. Select **96-well** or **384-well** in the Plate Type drop-down list.
 - f. Schedule the plate. For more information, see “Scheduling Runs” on page 123.
 - g. Select **Heat Sealing** or **Septa**.
 - h. Type a name for the owner and the operator.
 - i. Click **OK**. The GeneMapper Software Plate Editor opens.



The New Plate Dialog box contains the following fields and controls:

- 3a: ID (Barcode) field with value 'test'
- 3b: Name field with value 'test'
- 3c: Description text area
- 3d: Application dropdown menu with 'GeneMapper' selected
- 3e: Plate Type dropdown menu with '384-Well' selected
- 3f: Scheduling field with value '1234' and a small grid below it
- 3g: Plate Sealing dropdown menu with 'Heat Sealing' selected
- 3h: Owner Name field with value 'user'
- 3h: Operator Name field with value 'user'
- 3i: OK and Cancel buttons

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.
2. In the Comment column, enter any additional comments or notations for the sample.
3. In the Sample Type column, select a sample type from the drop-down list.
4. In the Size Standard column, select a size standard from the drop-down list.

vWell	1 Sample Name	2 Comment	3 Sample Type
A01			
B01			
C01			
D01			
E01			
F01			

Notes _____



5. In the Panel column, select a panel from the drop-down list.
6. In the Analysis Method column, select a method from the drop-down list.
7. In the Snp Set column, select a SNP set from the drop-down list.
8. Enter text for User-Defined columns 1 to 3.
9. In the Results Group 1 column, select a group from the drop-down list.
10. In the Instrument Protocol 1 column, select a protocol from the drop-down list.

4	5	6	7
Size Standard	Panel	Analysis Method	Snp Set

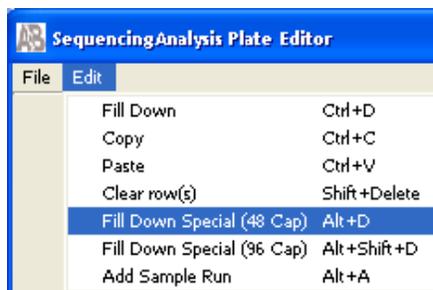
8			9	10
User-Defined 1	User-Defined 2	User-Defined 3	Results Group ▶	Instrument Protocol ▶

Notes _____



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

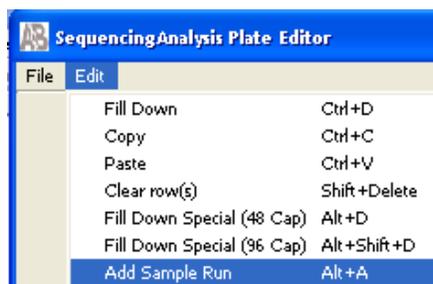
- For the same samples and protocols – Select the entire row, then select **Edit > Fill Down Special**. For more information see, “Filling Down the Plate Record” on page 113.
- Based on the plate type (96- or 384-well) and capillary array (48, 50, or 96 capillaries) you use—Select the appropriate fill down option:
 - 96 capillary/96-well plate: **Fill Down**
 - 48 capillary/96-well plate: **Fill down Special (48 Cap)**
 - 96 capillary/384-well plate: **Fill down Special (96 Cap)**
 - 48 capillary/384-well plate: **Fill down Special (48 Cap)**
- For the different samples and protocols, complete the plate editor manually.



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

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

To add additional runs select **Edit > Add Sample Run**, again (for more information see, “Adding a Sample Run” on page 115).



13. Complete the columns for the additional runs.

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

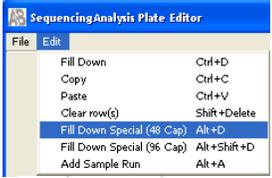
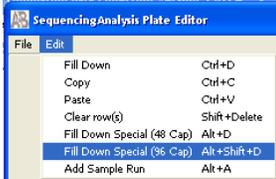
IMPORTANT! After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database. After the plate record is in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

Notes _____



Filling Down the Plate Record

The Fill Down Special function allows you to fill a plate record based on the load pattern of the capillary array that you use, as shown in the table below.

If You Choose ...	Then ...																																																		
<p>Fill Down Special (48 Cap)</p> 	<p>The fill down pattern matches the 48-capillary load pattern.</p> <table border="1" data-bbox="797 495 954 989"> <thead> <tr> <th>Well</th> <th>Sample Name</th> </tr> </thead> <tbody> <tr><td>A01</td><td>notMJD</td></tr> <tr><td>B01</td><td>notMJD</td></tr> <tr><td>C01</td><td>notMJD</td></tr> <tr><td>D01</td><td>notMJD</td></tr> <tr><td>E01</td><td>notMJD</td></tr> <tr><td>F01</td><td>notMJD</td></tr> <tr><td>G01</td><td>notMJD</td></tr> <tr><td>H01</td><td>notMJD</td></tr> <tr><td>A02</td><td>MJD</td></tr> <tr><td>B02</td><td>MJD</td></tr> <tr><td>C02</td><td>MJD</td></tr> <tr><td>D02</td><td>MJD</td></tr> <tr><td>E02</td><td>MJD</td></tr> <tr><td>F02</td><td>MJD</td></tr> <tr><td>G02</td><td>MJD</td></tr> <tr><td>H02</td><td>MJD</td></tr> <tr><td>A03</td><td>notMJD</td></tr> <tr><td>B03</td><td>notMJD</td></tr> <tr><td>C03</td><td>notMJD</td></tr> <tr><td>D03</td><td>notMJD</td></tr> <tr><td>E03</td><td>notMJD</td></tr> <tr><td>F03</td><td>notMJD</td></tr> <tr><td>G03</td><td>notMJD</td></tr> <tr><td>H03</td><td>notMJD</td></tr> </tbody> </table> <p>First Quadrant</p> <p>Second Quadrant</p>	Well	Sample Name	A01	notMJD	B01	notMJD	C01	notMJD	D01	notMJD	E01	notMJD	F01	notMJD	G01	notMJD	H01	notMJD	A02	MJD	B02	MJD	C02	MJD	D02	MJD	E02	MJD	F02	MJD	G02	MJD	H02	MJD	A03	notMJD	B03	notMJD	C03	notMJD	D03	notMJD	E03	notMJD	F03	notMJD	G03	notMJD	H03	notMJD
Well	Sample Name																																																		
A01	notMJD																																																		
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E02	MJD																																																		
F02	MJD																																																		
G02	MJD																																																		
H02	MJD																																																		
A03	notMJD																																																		
B03	notMJD																																																		
C03	notMJD																																																		
D03	notMJD																																																		
E03	notMJD																																																		
F03	notMJD																																																		
G03	notMJD																																																		
H03	notMJD																																																		
<p>Fill Down Special (96 Cap) *</p>  <p>* Especially useful for 384-well plates</p>	<p>The fill down pattern matches the 96-capillary load pattern.</p> <table border="1" data-bbox="797 1056 954 1539"> <thead> <tr> <th>Well</th> <th>Sample Name</th> </tr> </thead> <tbody> <tr><td>A10</td><td>12345</td></tr> <tr><td>B10</td><td>12345</td></tr> <tr><td>C10</td><td>12345</td></tr> <tr><td>D10</td><td>12345</td></tr> <tr><td>E10</td><td>12345</td></tr> <tr><td>F10</td><td>12345</td></tr> <tr><td>G10</td><td>12345</td></tr> <tr><td>H10</td><td>12345</td></tr> <tr><td>A11</td><td>12345</td></tr> <tr><td>B11</td><td>12345</td></tr> <tr><td>C11</td><td>12345</td></tr> <tr><td>D11</td><td>12345</td></tr> <tr><td>E11</td><td>12345</td></tr> <tr><td>F11</td><td>12345</td></tr> <tr><td>G11</td><td>12345</td></tr> <tr><td>H11</td><td>12345</td></tr> <tr><td>A12</td><td>12345</td></tr> <tr><td>B12</td><td>12345</td></tr> <tr><td>C12</td><td>12345</td></tr> </tbody> </table>	Well	Sample Name	A10	12345	B10	12345	C10	12345	D10	12345	E10	12345	F10	12345	G10	12345	H10	12345	A11	12345	B11	12345	C11	12345	D11	12345	E11	12345	F11	12345	G11	12345	H11	12345	A12	12345	B12	12345	C12	12345										
Well	Sample Name																																																		
A10	12345																																																		
B10	12345																																																		
C10	12345																																																		
D10	12345																																																		
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F10	12345																																																		
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A11	12345																																																		
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C11	12345																																																		
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F11	12345																																																		
G11	12345																																																		
H11	12345																																																		
A12	12345																																																		
B12	12345																																																		
C12	12345																																																		

To use the fill the plate record based on the 48 capillary load pattern:

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

Notes _____



4. Click position A02, type the sample information, then select the entire row.

Well	Sample Name	Comment	Sample Type	Size Standard	Panel	Analysis Method	Snp Set	User-Defined 1	User-Defined 2	User-Defined 3	Results Group	Instrument Protocol
A01	a										GM	GeneMapper
B01	a										GM	GeneMapper
C01	a										GM	GeneMapper
D01	a										GM	GeneMapper
E01	a										GM	GeneMapper
F01	a										GM	GeneMapper
G01	a										GM	GeneMapper
H01	a										GM	GeneMapper
A02												
B02												
C02												
D02												
E02												
F02												
G02												
H02												
A03	a										GM	GeneMapper
B03	a										GM	GeneMapper
C03	a										GM	GeneMapper
D03	a										GM	GeneMapper
E03	a										GM	GeneMapper
F03	a										GM	GeneMapper
G03	a										GM	GeneMapper
H03	a										GM	GeneMapper
A04												
B04												

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

Notes



Filling Down a 96 Cap/384-well Plate Record

When you use the Fill Down Special (96 Cap) feature on a 384-well plate, the fill down pattern appears as shown below.

Well	Sample Name	Comment	Sample Type	Size Standard	Panel	Analysis Method	Snp Set	User-Defined 1	User-Defined 2	User-Defined 3	Results Group 1	Instrument Protocol 1
A01	a										GM	GeneMapper
B01												
C01	a										GM	GeneMapper
D01												
E01	a										GM	GeneMapper
F01												
G01	a										GM	GeneMapper
H01												
I01	a										GM	GeneMapper
J01												
K01	a										GM	GeneMapper
L01												
M01	a										GM	GeneMapper
N01												
O01	a										GM	GeneMapper
P01												
A02												
B02												
C02												

Adding a Sample Run

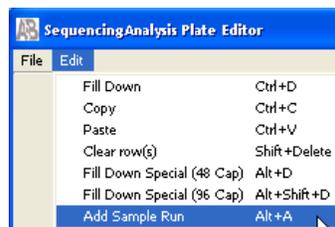
By adding additional sample runs, you can run samples that have different variables (different run modules, for example).

Adding a sample run opens an additional:

- Results group
- Instrument protocol

To add a sample run, select **Edit > Add Sample Run**.

To run the plate(s), see “Running the Instrument” on page 117.



Notes



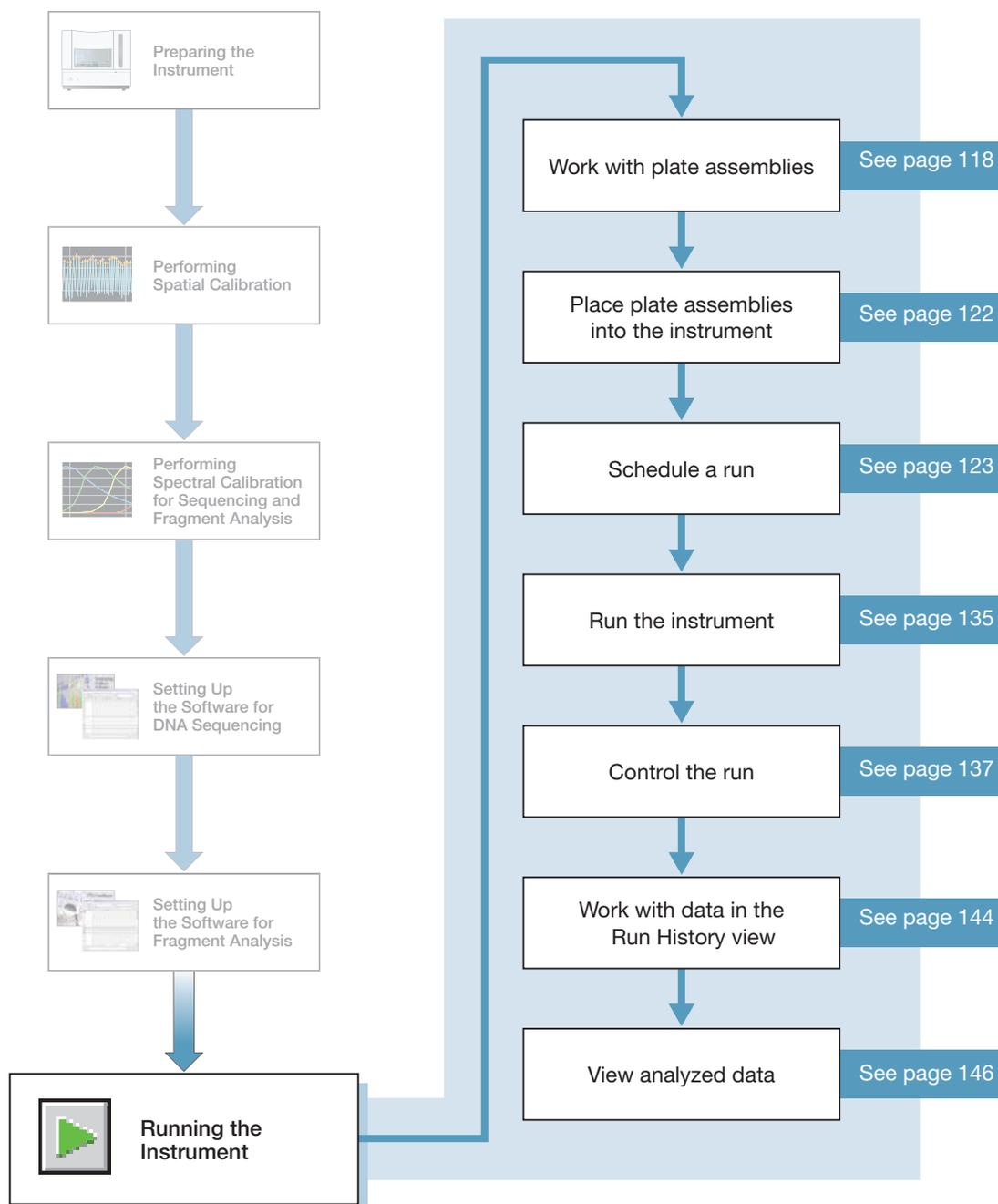
Chapter 5 Setting Up the Software for Fragment Analysis

Filling Down the Plate Record

Notes _____



Running the Instrument



Notes _____



Working with Plate Assemblies

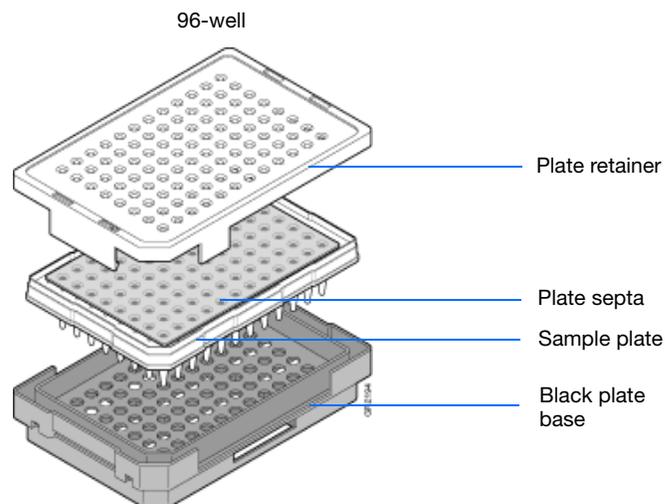
Plate Assembly Components

WARNING Do not use warped or damaged plates.

Materials Required for Each Septa Assembly:

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

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



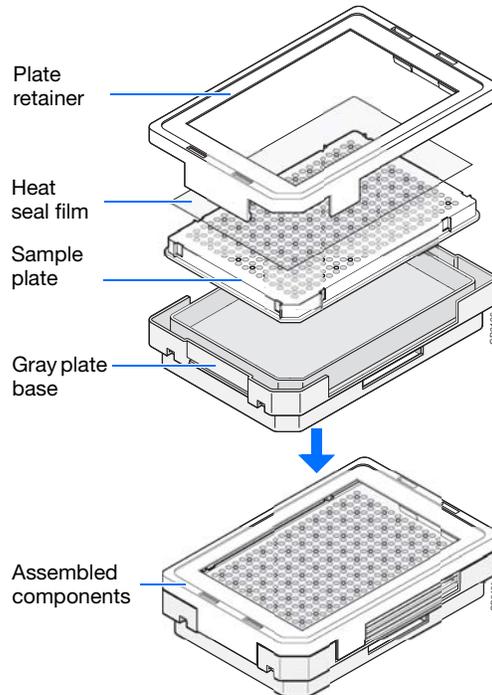
Notes _____



Materials Required for Each Heat-Sealed Assembly

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

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



Heat Seal Film Guidelines

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

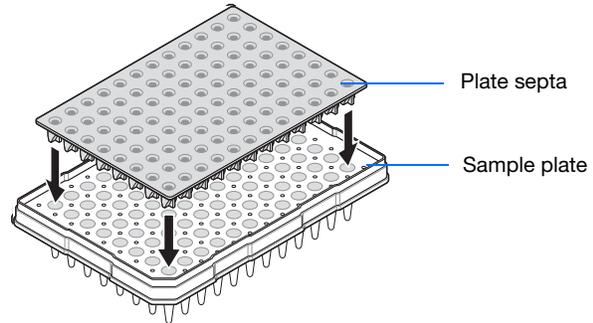
Notes _____



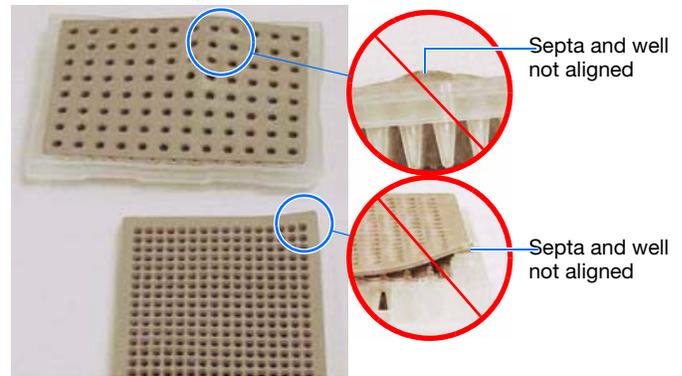
Preparing a Septum-Sealed Plate Assembly

1. Seal the plate:

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

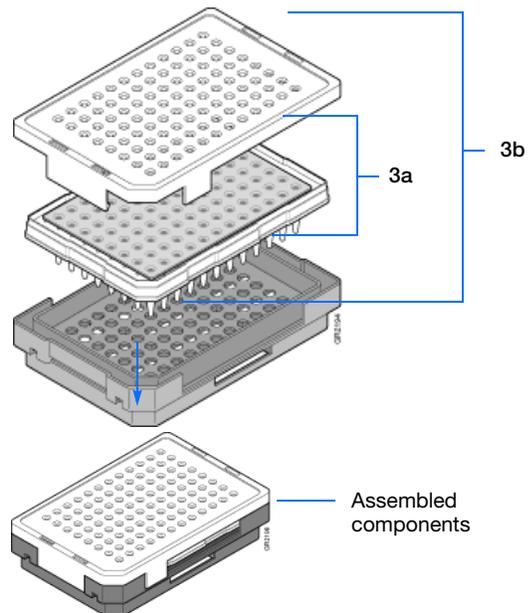


2. To prevent damage to the capillary array, inspect the plate and septa to verify that the septum fits snugly and flush on the plate.



3. Assemble the plate assembly:

- a. Place the sample plate into the plate base.
- b. Snap the plate retainer onto the plate and plate base.

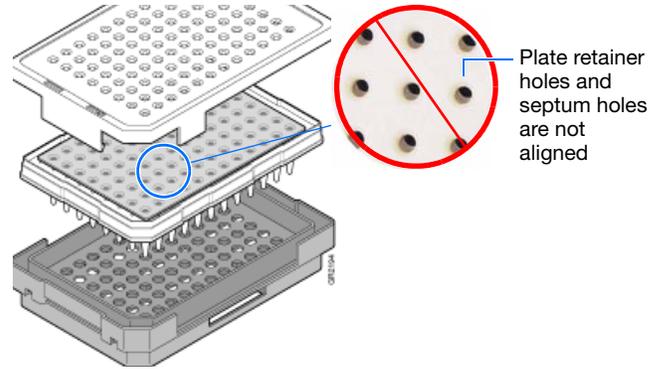


Notes _____



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

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

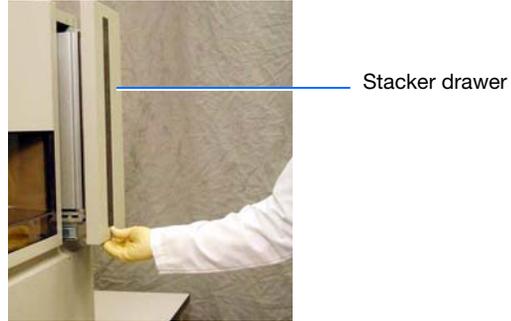


Notes _____

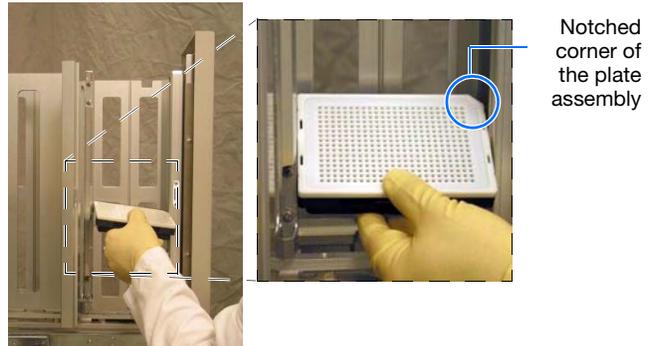


Placing Plate Assemblies into the Instrument

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

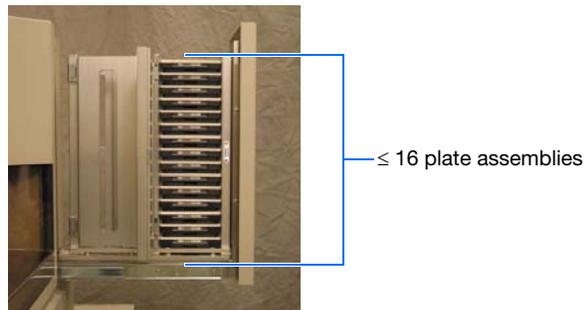


3. Place the plate assemblies into the stacker in any order, making sure that each plate is oriented so that the notched corner of the plate assembly is at the rear right corner of the stacker.



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

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

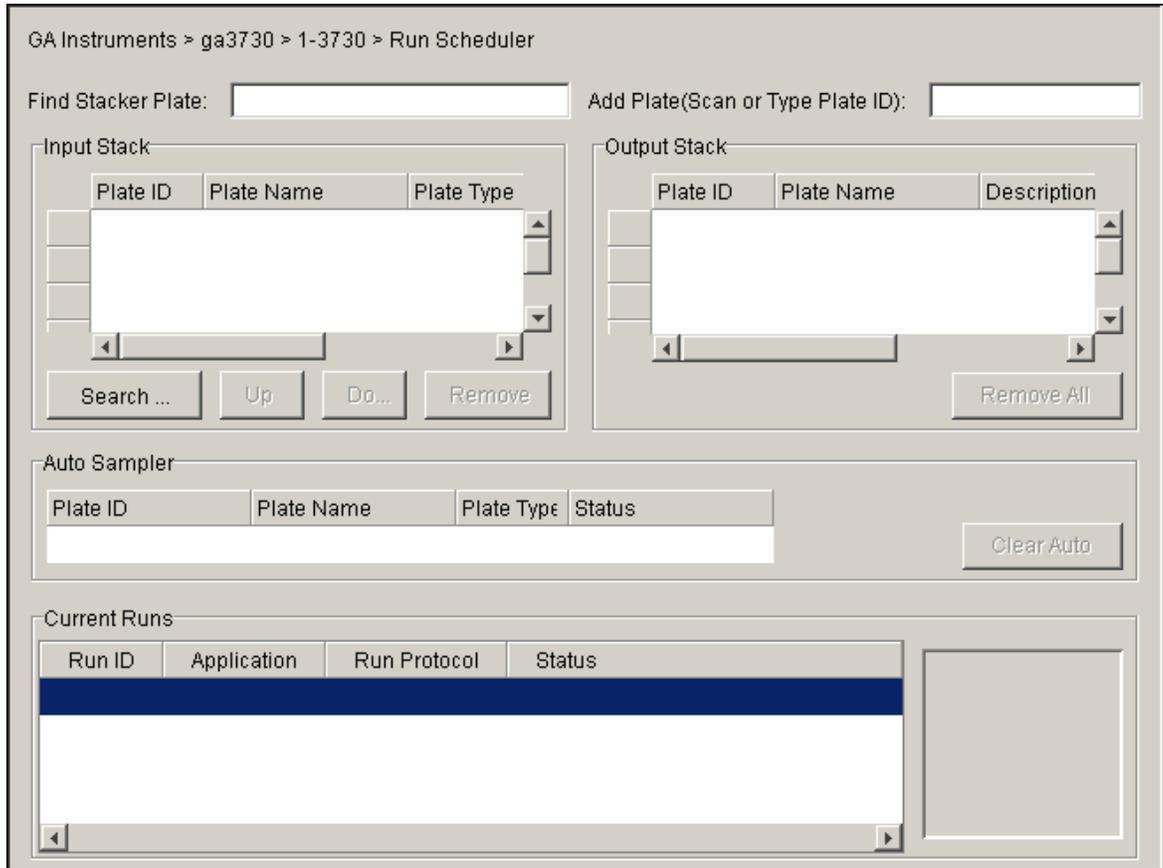


Notes _____



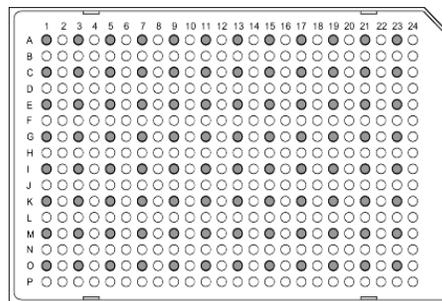
Scheduling Runs

In the navigation pane of the Data Collection Software, select
GA Instruments > ga3730 > instrument name > Run Scheduler.



384-Well Plate Mapping and Default Run Scheduling

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



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

Notes



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

Note: The analysis module of a sample does not affect the order in which the sample quadrant runs.

Default Run Priorities and Load Positions

For information on setting up a plate record for:

- Sequencing—see page 58.
- Fragment analysis—see page 93.

The following table indicates the default run priorities and load positions.

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

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

Notes _____

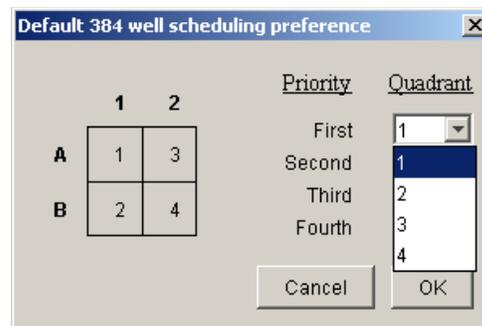


Globally Modifying a Run Schedule

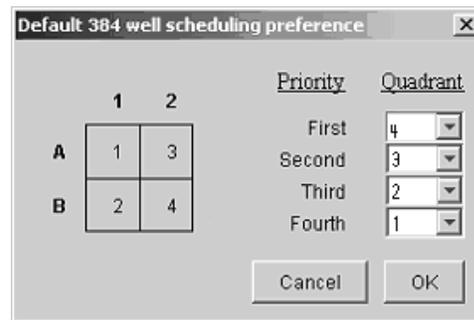
You can change the run order of quadrants and then apply it to all 384-well plates.

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

1. Click your instrument name in the navigation pane.
2. Select **Instrument > Scheduling Preference**.
The Default 384 well scheduling preference dialog box opens.
3. Select the quadrant priority (run order) from the Quadrant list.



You can select any run order. The example to the right shows a 4-3-2-1 quadrant priority (run order). With a 384-well and a 96-capillary array, the samples run in the order B2, A2, B1, A1...



Locally Modifying a Run Schedule

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

1. In the Plate Manager, click **New Plate**.
Note: For information about the Plate Manager, see page 81 for sequencing, and page 110 for fragment analysis.
2. Select **384-Well** from the Plate Type list.
The Scheduling box is activated.

Notes _____



Chapter 6 Running the Instrument

Scheduling Runs

3. Type the run priority in the Scheduling box.
4. Click **OK**.

Type run priorities here

New Plate Dialog

ID (Barcode): test

Name: test

Description:

Application: GeneMapper-Generic

Plate Type: 384-Well

Scheduling: 1234

Plate Sealing: Heat Sealing

Owner Name: user

Operator Name: user

OK Cancel

	1	2
A	1	3
B	2	4

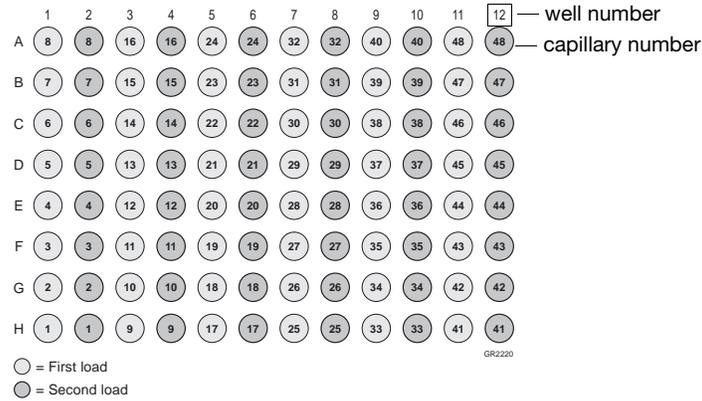
Notes _____



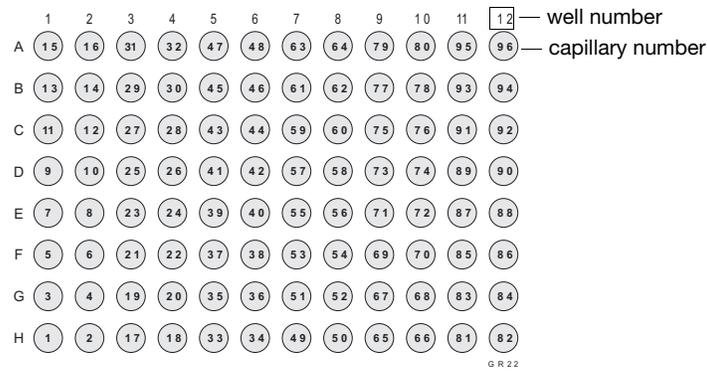
Default Load Maps

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

96-Well Plate, 48 Capillaries



96-Well Plate, 96 Capillaries

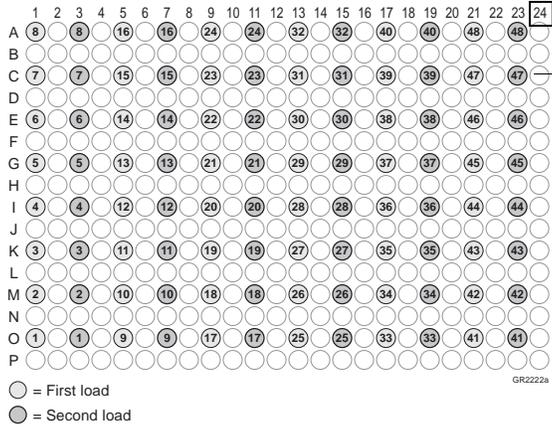


Notes _____

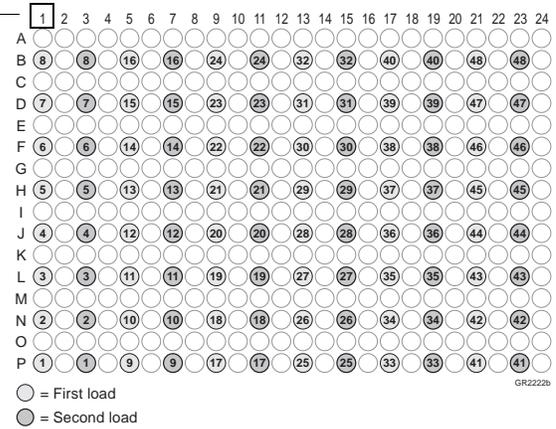


384-Well Plate, 48 Capillaries

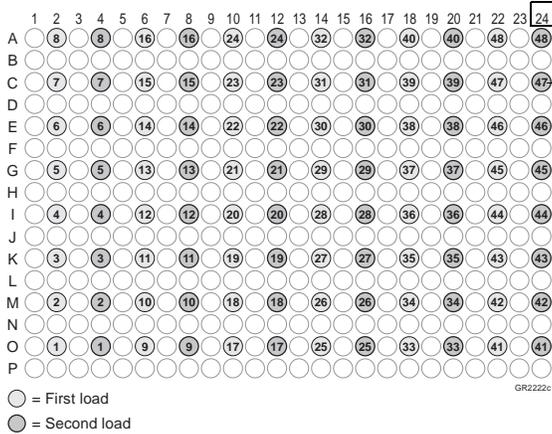
First quadrant pickup



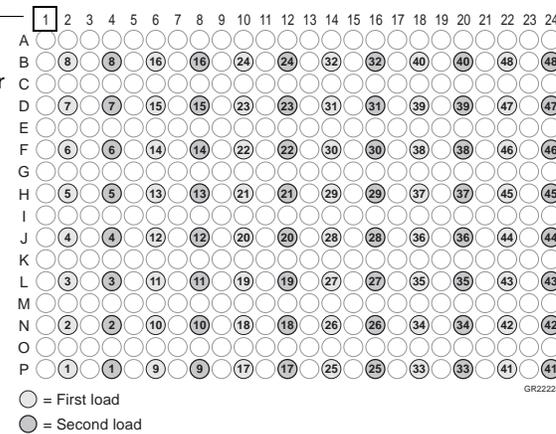
Second quadrant pickup



Third quadrant pickup



Fourth quadrant pickup

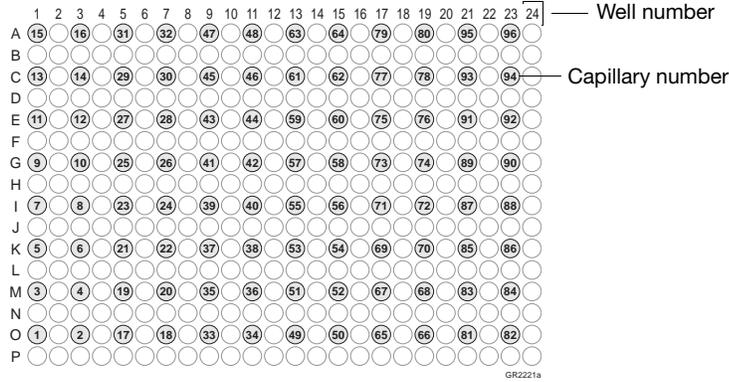


Notes

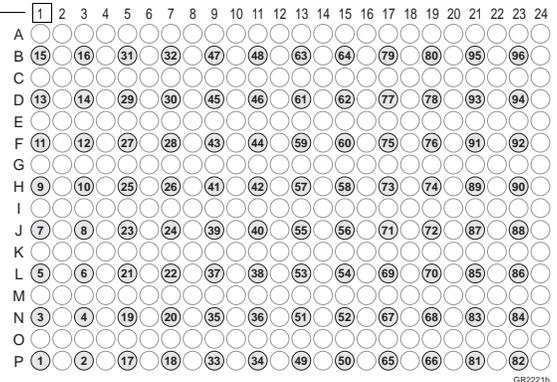


384-Well Plate, 96 Capillaries

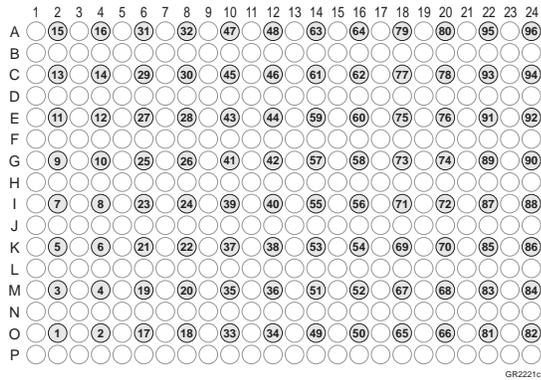
First quadrant pickup



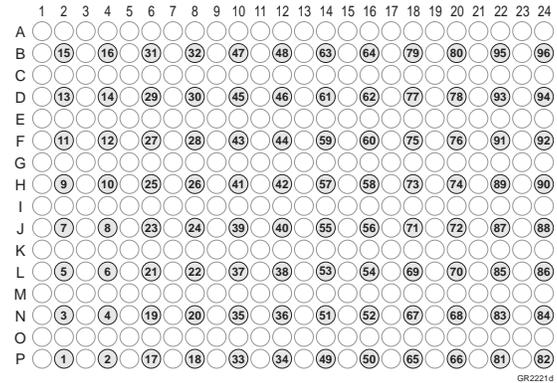
Second quadrant pickup



Third quadrant pickup



Fourth quadrant pickup



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

Notes



Barcode Readers

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

Internal Barcode Reader

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

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

Note: All Applied Biosystems® barcoded plates for the 3730/3730*xl* Analyzer use code 128 format.

Note: The barcode reader cannot read spaces or the characters \ / : * ? " < > |.

External Barcode Readers

KEYENCE BL-80VE

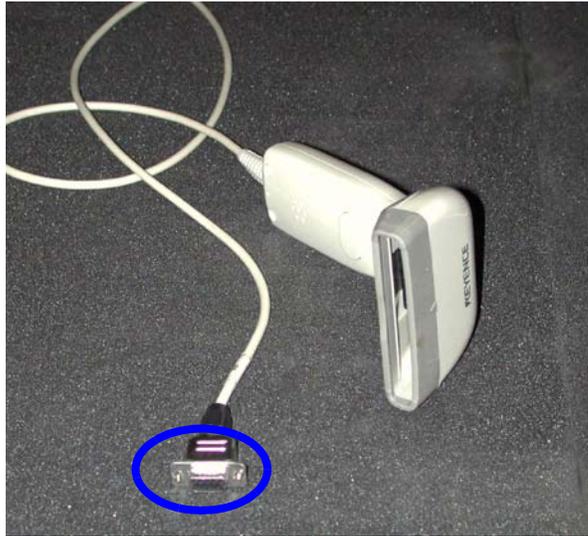


An external barcode reader can also be used with the 3730/3730*xl* Analyzer. The KEYENCE BL-80VE (see photo above) connects to the instrument computer keyboard. With this reader, you can scan barcodes into any text box in the Data Collection software.

Notes _____



KEYENCE 80RKE



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

Note: The 80RE is not supported for the 3730 or 3730*xl* DNA Analyzers.

Notes _____



Running the Instrument: Manual vs Auto Mode

Accessing Modes You can schedule a run or runs using either manual mode or auto mode. Both modes are described below. Access either mode by selecting in the navigation pane:

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

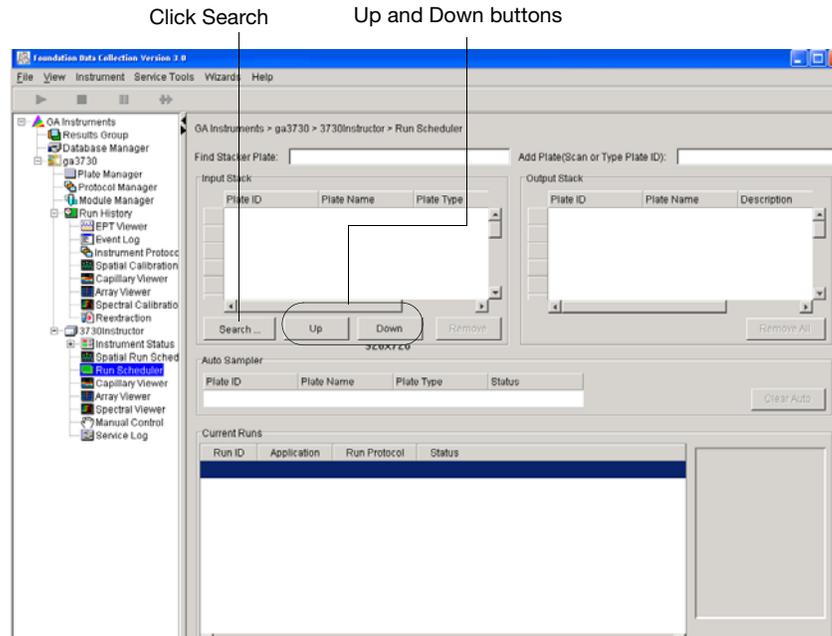
Note: You must be in the Run Scheduler view to see the instrument run mode menu.

Manual Mode Features

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

Scheduling Runs Using Manual Mode (Default)

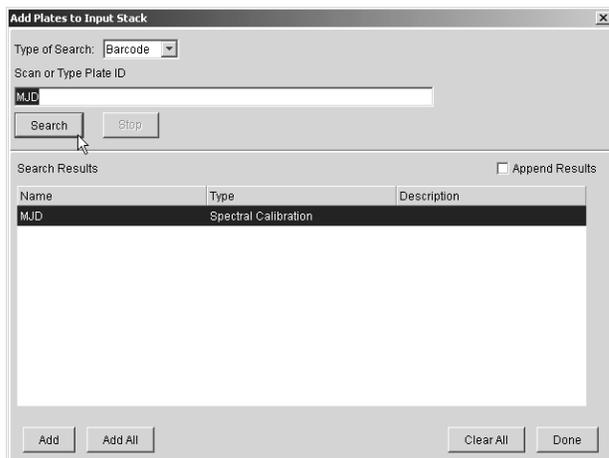
1. In the navigation pane, select **Instrument > Instrument Name > Manual mode**.
2. Click **Search** in the Run Scheduler to search for plate record(s).



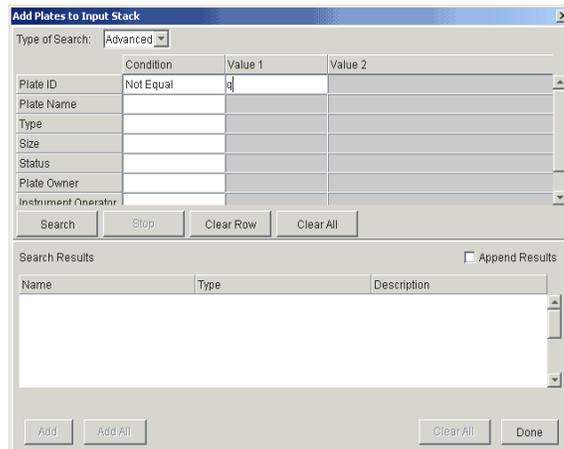
The **Add Plates to In Stack** dialog box opens.

3. Type the name of the plate(s) or scan the plate ID, then click **Search**.

Notes _____



Barcode search



Advanced search

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



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



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

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

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

7. Click  (**Run**).

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

Notes _____



Auto Mode Features

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

To schedule runs using the Auto mode:

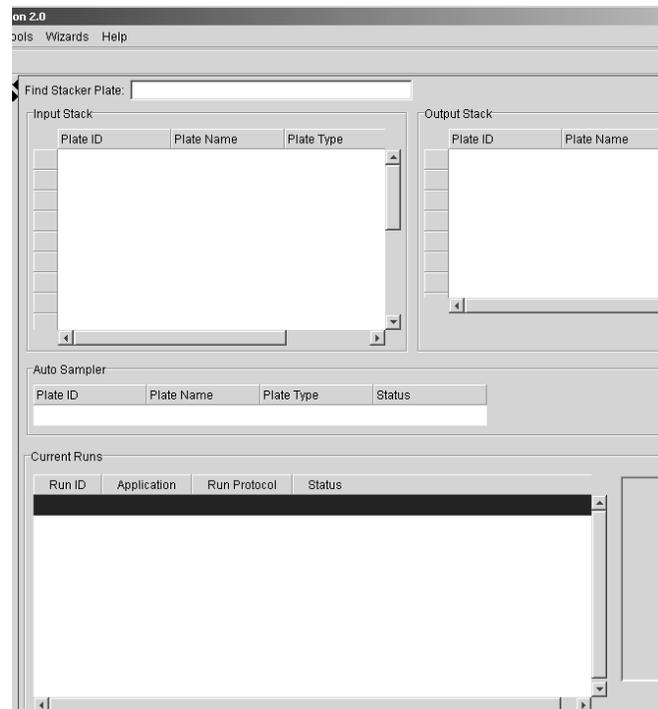
1. Select **Run Scheduler > Instrument Name > Auto mode.**

Notice that the Search, Up, and Down buttons are not available (as they are in Manual mode). Also, the Add Plate (Scan or Type Plate ID) option is not available in Auto mode.

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

3. Click  (Run).

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

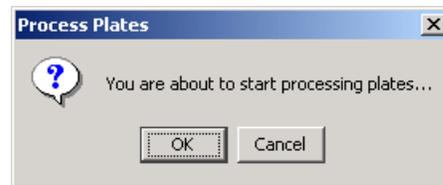


Notes _____



Starting the Run

1. Verify that the active spectral calibration matches your dye set and capillary array length.
2. If you want to review the run schedule before beginning the run, click
 **GA Instruments** >  **ga3730** >
 **instrument name** >  **Run Scheduler**
3. Select the green button in the toolbar.
The Processing Plates dialog box opens.
4. Click **OK**.



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

If the database or drive E is:

- Full—A warning is displayed. Do the following:
 - Delete unneeded files, see “Maintaining Adequate Space for Database and Sample Data Storage” in the Applied Biosystems® 3730/3730xl DNA Analyzer *Maintenance and Troubleshooting Guide*, PN 4359473.
 - Click the green button to start the run.
- Not full—The run starts.

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

Notes _____



DNA Sequencing Run Times

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

Application	Capillary Array Length (cm)	Run Module	Approximate Run Time [†] (min)
Short read DNA Sequencing	36	TargetSeq36_POP7	20 [‡]
Rapid read DNA sequencing	36	RapidSeq36_POP7	35
Standard read DNA sequencing	36	StdSeq36_POP7	60
Fast DNA sequencing	50	FastSeq50_POP7	60
Long read DNA sequencing	50	LongSeq50_POP7	120
Extra Long DNA sequencing	50	XLRSeq50_POP7	180

[†] Times assume oven is at temperature

[‡] Approximate time to run 400 bases. The run module can be customized to run 200-400 bases.

Fragment Analysis Run Times

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

Application	Capillary Array Length (cm)	Run Module	Approximate Run Time (min)
Fragment Analysis	36	GeneMapper36_POP7	32
Fragment Analysis	50	GeneMapper50_POP7	43
SNPlex™ Genotyping	36	HTSNP36_POP7_V3	15
SNPlex™ Genotyping	50	HTSNP50_POP7	25

Notes _____



Controlling the Run

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



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

Notes _____

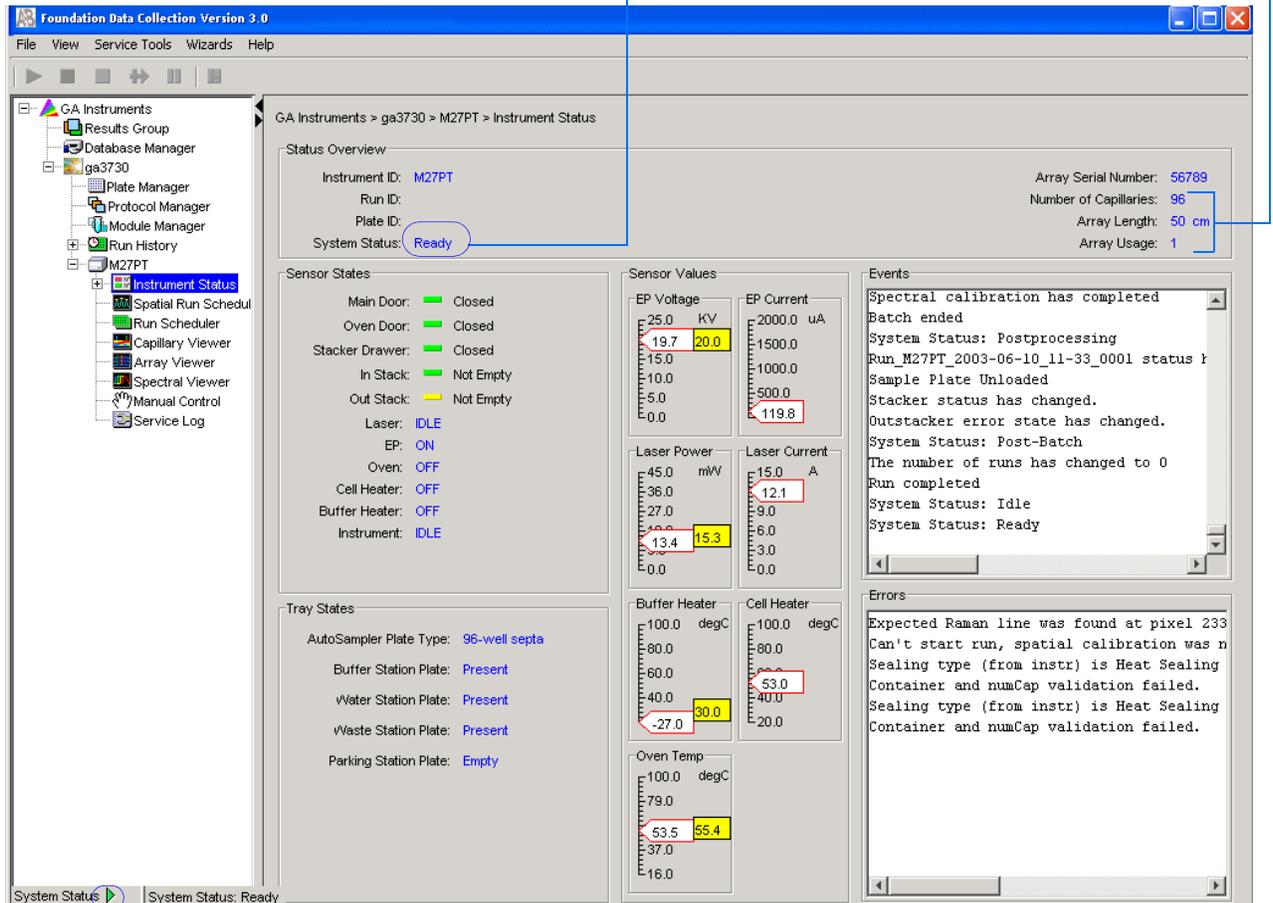


Monitoring the Status of the Run

In the navigation pane of the Data Collection Software, select  (Instrument Status) to view the status of the instrument or the current run.

System Status must be 'Ready' before a run starts

Array and polymer information



The screenshot displays the 'Instrument Status' window for instrument M27PT. The 'System Status' is 'Ready'. The 'Sensor States' section shows: Main Door: Closed, Oven Door: Closed, Stacker Drawer: Closed, In Stack: Not Empty, Out Stack: Not Empty, Laser: IDLE, EP: ON, Oven: OFF, Cell Heater: OFF, Buffer Heater: OFF, Instrument: IDLE. The 'Sensor Values' section shows: EP Voltage (25.0 KV), EP Current (2000.0 uA), Laser Power (45.0 mW), Laser Current (15.0 A), Buffer Heater (30.0 degC), Cell Heater (53.0 degC), and Oven Temp (55.4 degC). The 'Events' section shows: Spectral calibration has completed, Batch ended, System Status: Postprocessing, Run_M27PT_2003-06-10_11-33_0001 status I, Sample Plate Unloaded, Stacker status has changed, Outstacker error state has changed, System Status: Post-Batch, The number of runs has changed to 0, Run completed, System Status: Idle, System Status: Ready. The 'Errors' section shows: Expected Raman line was found at pixel 233, Can't start run, spatial calibration was n, Sealing type (from instr) is Heat Sealing, Container and numCap validation failed, Sealing type (from instr) is Heat Sealing, Container and numCap validation failed.

System Status changes from green to flashing red when errors occur.

Notes



Events Box Displays the:

- Recent actions of the instrument
- Status of each capillary (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 Displays 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.

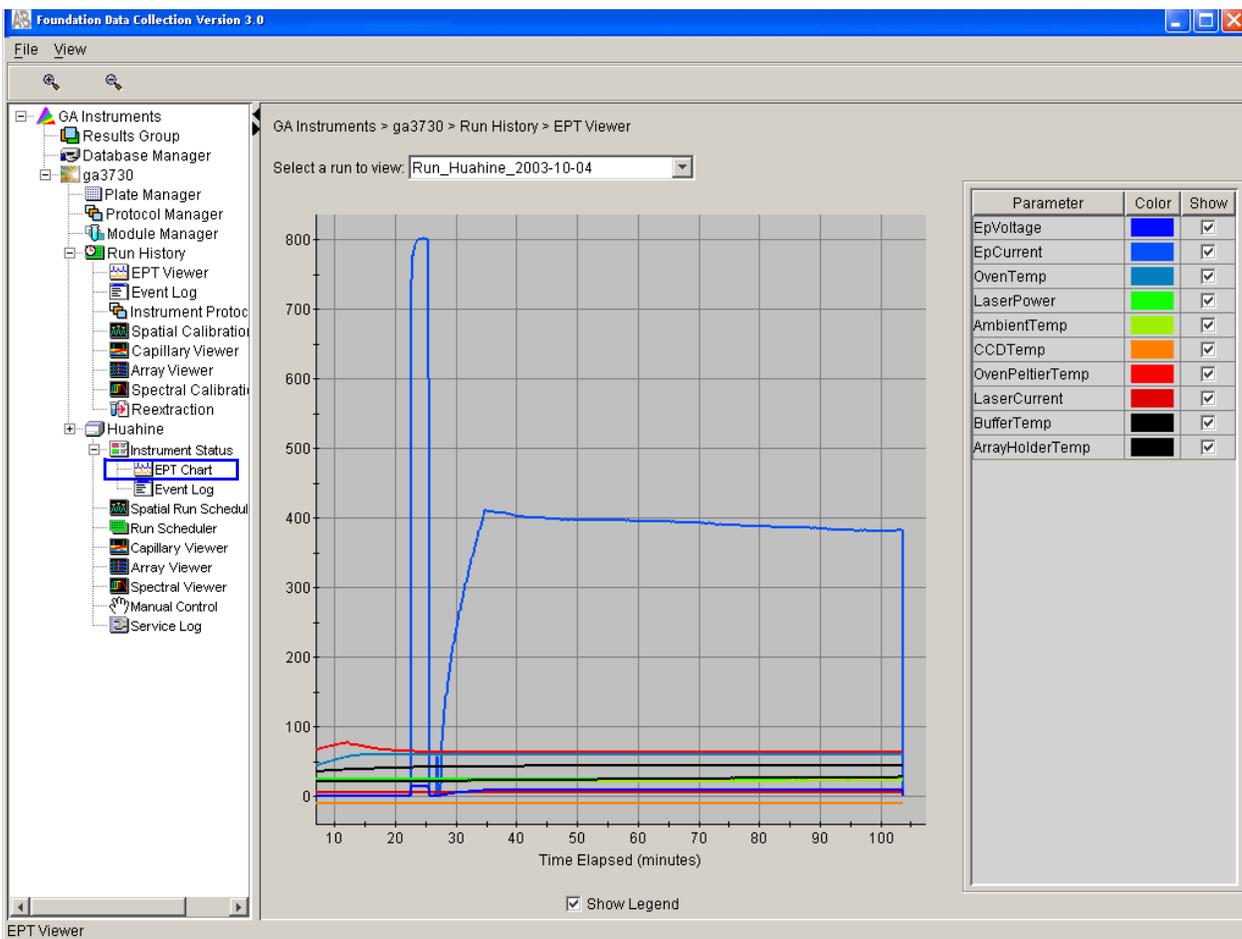
Notes _____



Viewing Real-Time Electrophoresis Data

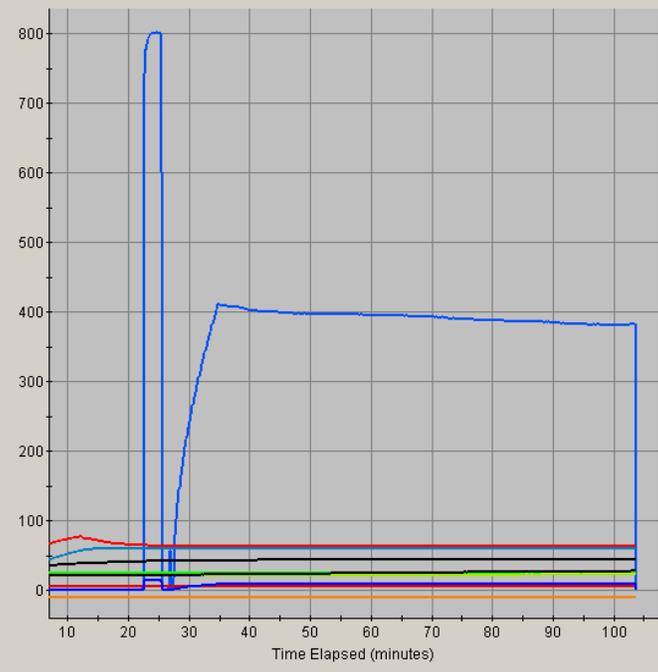
Use the EPT Viewer to view real-time electrophoresis (EP) data during a run.

To access the viewer, in the navigation pane of the Data Collection Software, select **GA Instruments** > **ga3730** > **instrument name** > **Instrument Status** > **EPT Chart**.



GA Instruments > ga3730 > Run History > EPT Viewer

Select a run to view: Run_Huahine_2003-10-04



Parameter	Color	Show
EpVoltage	Blue	<input checked="" type="checkbox"/>
EpCurrent	Blue	<input checked="" type="checkbox"/>
OvenTemp	Blue	<input checked="" type="checkbox"/>
LaserPower	Green	<input checked="" type="checkbox"/>
AmbientTemp	Green	<input checked="" type="checkbox"/>
CCDTemp	Orange	<input checked="" type="checkbox"/>
OvenPeltierTemp	Red	<input checked="" type="checkbox"/>
LaserCurrent	Red	<input checked="" type="checkbox"/>
BufferTemp	Black	<input checked="" type="checkbox"/>
ArrayHolderTemp	Black	<input checked="" type="checkbox"/>

Notes



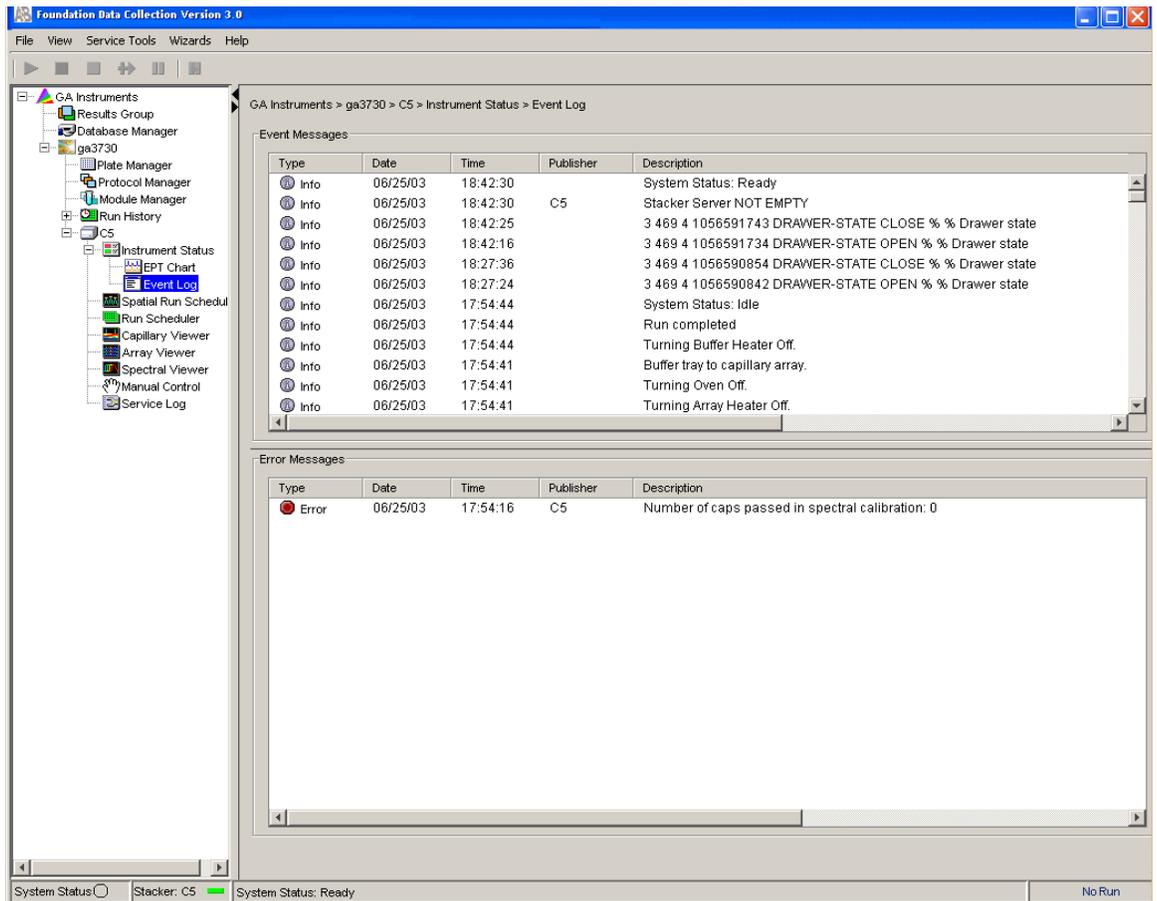
Viewing Event History

Use the Event log window to view a record of operational events, as shown in the next figure.

To access the Event Log window, in the navigation pane of the Data Collection Software, click **GA Instruments > ga3730 > instrument name > Instrument Status > Event Log.**

IMPORTANT! To delete error messages, select all error messages, then click **Clear Errors**. The system status light flashes red until all errors are cleared.

Note: Using the Event Log window, you can also verify the capillary-by-capillary processing status during a spectral calibration run.



Note: If an error is generated while using manual control, reboot the instrument then restart the Data Collection Software to recover from the error stage.

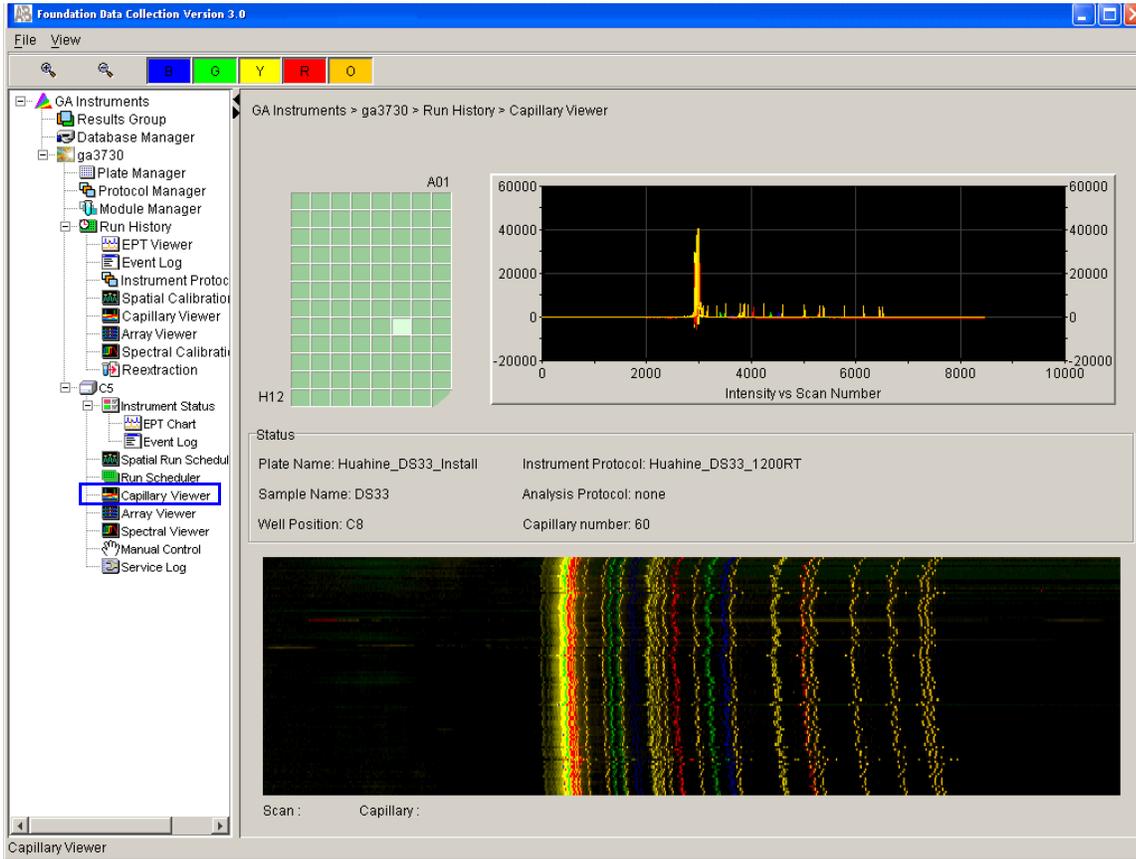
Notes



Viewing Electropherogram Data

Viewing Data in the Capillary Viewer

Use the Capillary Viewer to examine the quality of electropherogram data from multiple capillaries during a run. In the navigation pane of the Data Collection Software, select GA Instruments > ga3730 > instrument name > Instrument Status > Capillary Viewer.



Electropherogram Displays

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

How to Zoom

To zoom an area of an electropherogram:

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

Click individual colors to view or hide them.



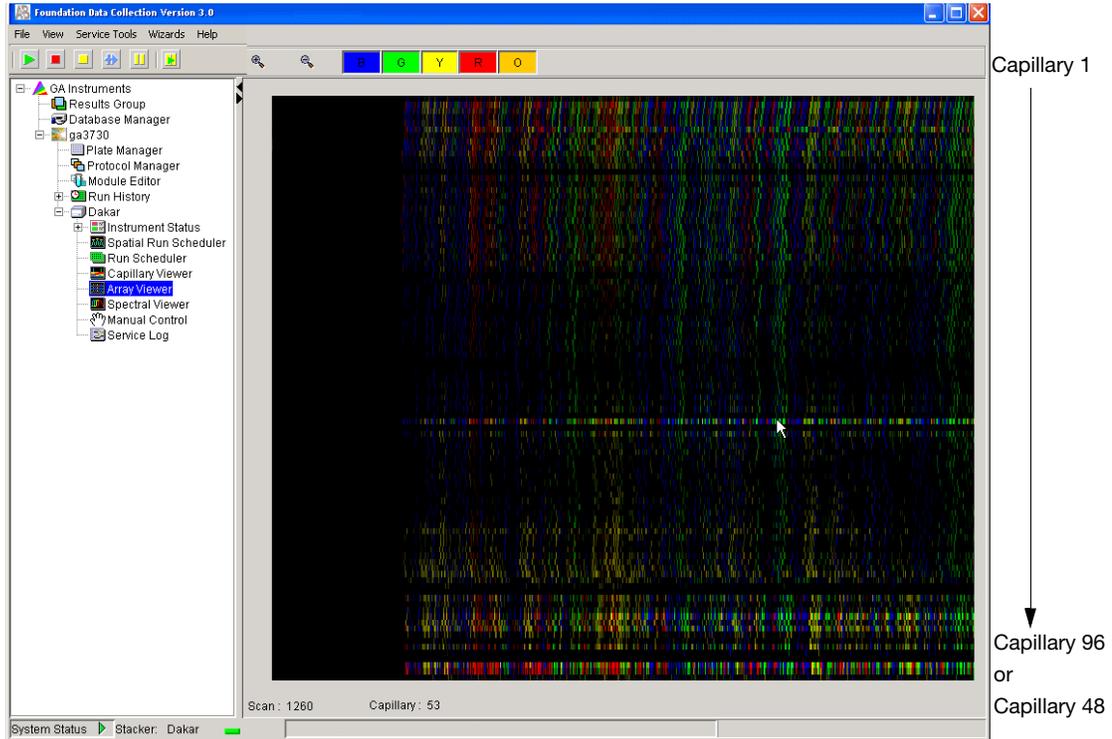
Notes



Viewing Data in the Array Viewer

Use the Array Viewer during or after a run to examine the quality of your data from all capillaries. You can view all the capillaries (vertical axis) as a function of time/data point (horizontal axis).

To open the Array Viewer window in the navigation pane of the Data Collection Software, select **GA Instruments** > **ga3730** > **instrument name** > **Array Viewer**.



How to Zoom

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

Displaying or Hiding Color



Click individual colors in the color bar to view or hide the color in the Array View (same in Capillary Viewer).

Notes _____



Viewing the Run History Data

Run History Components

To view the Run History utility can be used only with completed runs stored in the local 3730/3730xl Analyzer Data Collection database. It does not provide real-time viewing of collecting runs.

In the navigation pane, click the icon next to the function to launch it.

Run History Views	Icon
EPT Viewer Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Spatial Calibration Viewer	
Capillary Viewer Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Array Viewer Note: If Cleanup Database has been used, you cannot view processed data in Run History.	
Spectral Calibration Viewer	
Reextraction Note: If Cleanup Database has been used, you cannot view processed data in Run History.	

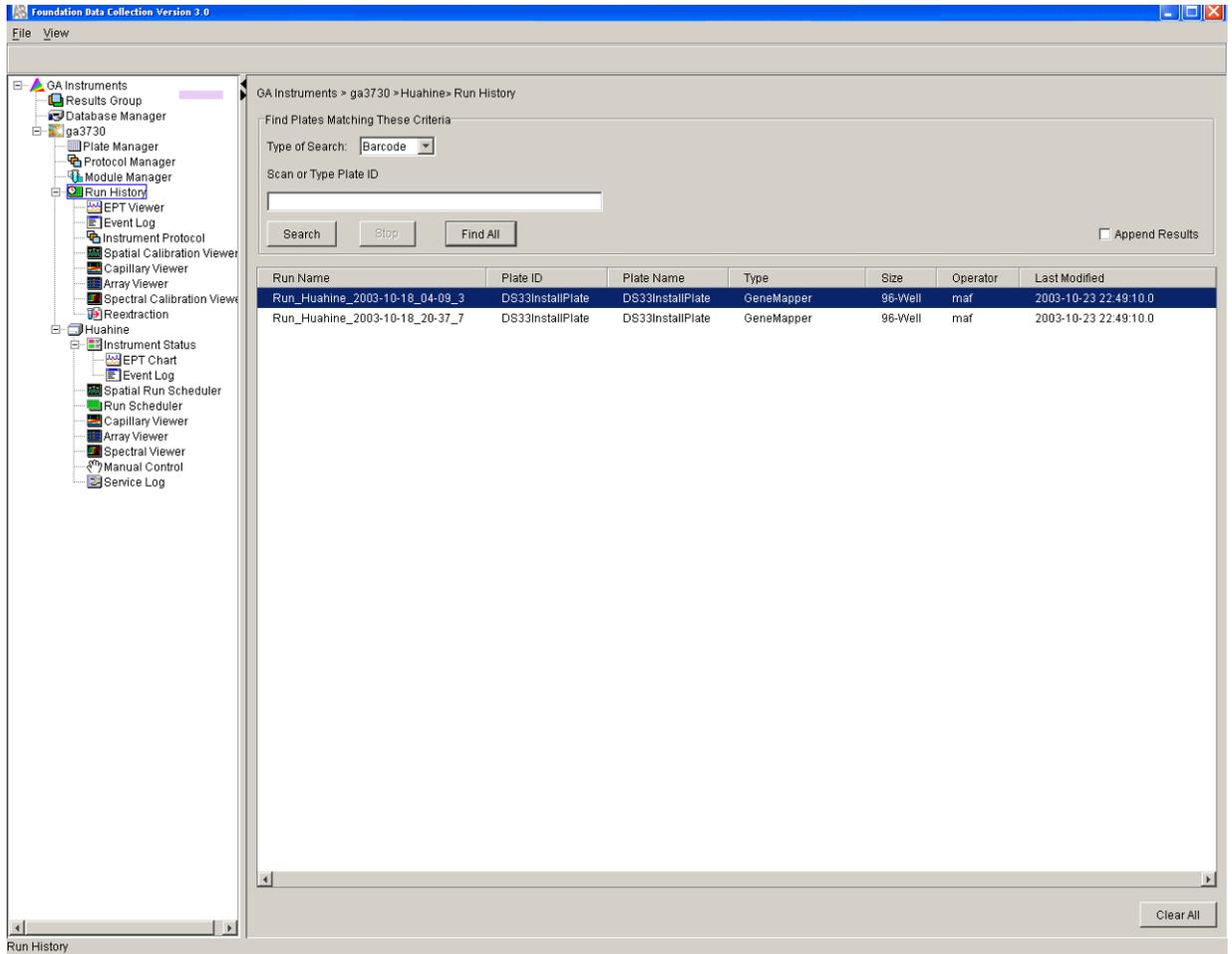
Viewing Data from a Completed Run

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

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

1. In the navigation pane of the 3730/3730xl Analyzer Data Collection software, select (**Run History**).

Notes _____



2. Search for the run you want to use by either Barcode or Advanced search.
3. After choosing the run, select the **Array Viewer** or the **Capillary Viewer** in the navigation pane.

Notes



Viewing the Results of Autoextraction

After a run is completed extraction and analysis are performed automatically, according to the settings in the Plate Editor and the Results group. The results of extraction and analysis can be viewed in the Reextraction Panel. Samples can be extracted again with the same settings, or with different Analysis Protocols or different Results Groups. This can be useful for several reasons:

- The destination location may not have been available during extraction.
- Some samples may have failed analysis and a different Analysis Protocol might be more successful.
- Samples might be saved in different locations, or with no analysis at all to save space.
- Sample files are created based on the your destination and folder naming selections.

Runs Stopped Before Complete Autoextraction

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

The auto extractor component of the 3730/3730xl Analyzer Data Collection automatically extracts data from stopped runs. If autoextraction fails, click Reextraction  to extract data.

Selecting and Queuing Samples for Reextraction

You can queue individual samples for reextraction. This is especially useful for experimenting with different analysis protocols for samples that have failed initial extraction.

1. Click  (Run History).
2. Enter the plate ID for a plate that has been run, then click **Search**. All completed runs from that plate appear in the window and can be reextracted. Pending runs from the plate do not appear in the window.
3. Select a run from the list.

Notes _____



Run Name	Plate ID	Plate Name	Type	Size	Operator	Last Modified
Run_Huahine_2002-10-18_04-09_3	DS33InstallPlate	DS33InstallPlate	GeneMapper	96-Well	maf	2002-10-23 22:49:10.0
Run_Huahine_2002-10-18_20-37_7	DS33InstallPlate	DS33InstallPlate	GeneMapper	96-Well	maf	2002-10-23 22:49:10.0
Run_Huahine_2002-10-18_20-37_8	DS33InstallPlate	DS33InstallPlate	GeneMapper	96-Well	maf	2002-10-23 22:49:10.0
Run_Huahine_2002-10-18_20-37_9	DS33InstallPlate	DS33InstallPlate	GeneMapper	96-Well	maf	2002-10-23 22:49:10.0
Run_Huahine_2002-10-18_20-37_10	DS33InstallPlate	DS33InstallPlate	GeneMapper	96-Well	maf	2002-10-23 22:49:10.0
Run_Huahine_2002-10-23-03_1	DS33	DS33Install	GeneMapper	96-Well	install	2002-10-23 22:39:37.0
Run_Huahine_2002-10-24_02-32_2	JaimeTest	Jaime	GeneMapper	96-Well	Jaime	2002-10-24 02:29:28.0
Run_Huahine_2002-10-25_02-08_2	Verification_Plate	Verification_Plate	SequencingAnalysis	96-Well	3730User	2002-10-25 02:06:38.0
Run_Huahine_2002-10-25_04-50_3	LRSPlate	LRSPlate	SequencingAnalysis	96-Well	KK	2002-10-25 04:49:47.0

4. Click  (Reextraction) in the navigation pane. The Reextraction window opens.
5. Select the checkboxes in the Extract column that correspond to the samples to be reextracted.
6. Click **Extract** to start the reextraction.

Note: Reextracted sample files are saved in the original folder that data was extracted to, unless you modify the results group settings.

Notes



Reextraction Window for Sequencing Analysis

Click the boxes to select samples to be reextracted

Select a run

Extraction Result column on the Reextraction window

Extract	Cap	Well	Extraction Result	Results Group	Analysis Protocol	Analysis Result	Score	Sample Name	Extraction Comment
<input checked="" type="checkbox"/>	8	A01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	5.0	s	
<input checked="" type="checkbox"/>	7	B01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	5.0	s	
<input checked="" type="checkbox"/>	6	C01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	48.510754	s	
<input checked="" type="checkbox"/>	5	D01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	4	E01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	43.687626	s	
<input checked="" type="checkbox"/>	3	F01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	1.0	s	
<input checked="" type="checkbox"/>	2	G01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	22.686636	s	
<input checked="" type="checkbox"/>	1	H01	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	6.0	s	
<input checked="" type="checkbox"/>	16	A03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	1.0	s	
<input checked="" type="checkbox"/>	15	B03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	6.0	s	
<input checked="" type="checkbox"/>	14	C03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	1.0	s	
<input checked="" type="checkbox"/>	13	D03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	12	E03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	43.992992	s	
<input checked="" type="checkbox"/>	11	F03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	31.95	s	
<input checked="" type="checkbox"/>	10	G03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	44.98598	s	
<input checked="" type="checkbox"/>	9	H03	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	24	A05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	49.410084	s	
<input checked="" type="checkbox"/>	23	B05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	22	C05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	28.083334	s	
<input checked="" type="checkbox"/>	21	D05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	23.813953	s	
<input checked="" type="checkbox"/>	20	E05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	29.166666	s	
<input checked="" type="checkbox"/>	19	F05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	18	G05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	49.475758	s	
<input checked="" type="checkbox"/>	17	H05	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	2.0	s	
<input checked="" type="checkbox"/>	32	A07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	1.0	s	
<input checked="" type="checkbox"/>	31	B07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	1.0	s	
<input checked="" type="checkbox"/>	30	C07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	28.904762	s	
<input checked="" type="checkbox"/>	29	D07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	ERROR: Analysis Failed	<NA>	s	
<input checked="" type="checkbox"/>	28	E07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	27	F07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	ERROR: Analysis Failed	<NA>	s	
<input checked="" type="checkbox"/>	26	G07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	33.59501	s	
<input checked="" type="checkbox"/>	25	H07	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	46.06404	s	
<input checked="" type="checkbox"/>	40	A09	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	48.61644	s	
<input checked="" type="checkbox"/>	39	B09	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	8.0	s	
<input checked="" type="checkbox"/>	38	C09	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	4.0	s	
<input checked="" type="checkbox"/>	37	D09	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	3.0	s	
<input checked="" type="checkbox"/>	36	E09	SUCCESS: Ext...	SeqA	3730BDTV3-KB-DeNovo...	SUCCESS: Analysis Succ...	16.0	s	

Click here to start extraction

Use these if several samples are highlighted

Notes



Reextraction Window for Fragment Analysis

Click the check boxes to select samples to be reextracted

Select a run — Extraction Result column of the Reextraction window

Extract	Cap	Well	Extraction Result	Results Group	Sample Name	Comment	Sample Type	Size Standard	Plate
<input checked="" type="checkbox"/>	1	A01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	3	B01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	5	C01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	7	D01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	9	E01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	11	F01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	13	G01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	15	H01	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	2	A02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	4	B02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	6	C02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	8	D02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	10	E02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	12	F02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	14	G02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:
<input checked="" type="checkbox"/>	16	H02	SUCCESS: Extr	gm_runbyrun	s		Sample	GSS00LIZ	D:

Click here to start extraction — Use these if several samples are highlighted

Notes _____



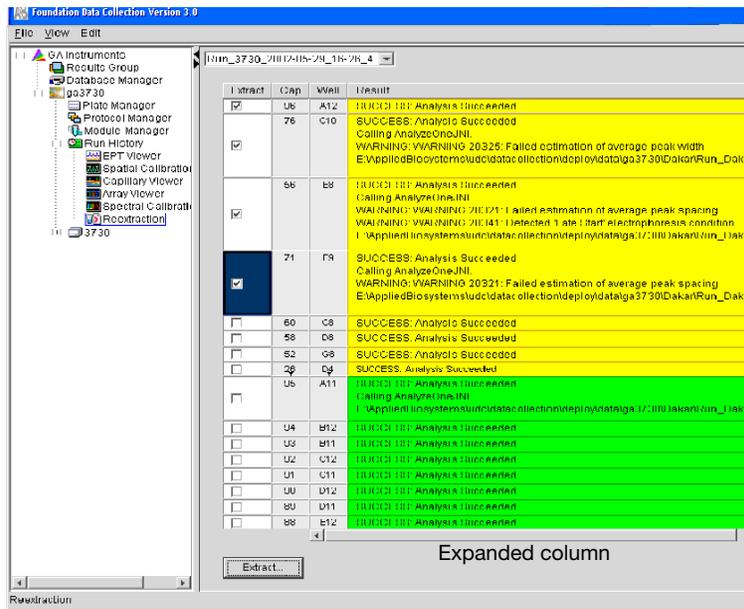
Results Column of the Reextraction Window

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

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

* Note: The text message for samples that produce yellow is: "FAILURE: Analysis Fail Bad Data; Error Number=nnnnn WARNING..."

The Results column, by default, shows only the beginning of any processing message. The entire message returned from extraction and autoanalysis can be viewed by expanding the cell.



Quality Column of the Reextraction Window

The Quality column represents the quality values for an entire sequence. Quality values are assigned only to analyzed samples when using the KB™ Basecaller. The Quality column is empty (white) if:

- Analysis was not performed
- Analysis failed
- ABI Basecaller was used for analysis. ABI basecaller does not assign quality values.

Notes



Results Group and Analysis Protocol Columns

The Results Group and the Analysis Protocol (Analysis Method in the GeneMapper® software) can be edited and the changes used for reextraction.

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

Sorting The Samples

The samples can be sorted according to any of the column properties by holding down the Shift key while clicking on the column header. Shift-clicking a column a second time sorts the column contents in the reverse order. This is most useful for sorting by capillary number, by well position, by results, by quality, and by the Extract column. For example, it is often useful to bring all the samples that failed analysis or extraction to the top of the column where they can be examined without having to scroll down to each sample individually.

Reextracting Selected Samples

1. Expand the Results column cells for any yellow or red results, to see a description of the warning or failure.
2. You can select a new Results Group, or edit the current one. This allows you to turn off autoanalysis, change the samples and folder naming options, the location where they are placed, the owner of the Results Group, and so on.
3. You can change the analysis protocol to experiment with different ways of analyzing the sample, using a different basecaller for example.
4. Select the check box in the Extract column for the samples you wish to extract again.
5. Click **Extract**.

IMPORTANT! Reextraction creates a 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 naming options that are used for reextraction are identical to those used previously, a number is added to the filename. For example, if the first sample is, “sample01.ab1” then the second sample would be, “sample01.2.ab1.”

Notes _____



Chapter 6 Running the Instrument

Viewing the Results of Autoextraction

Notes _____

Parts List

Item	Part No
3730 36-cm capillary array	4331247
3730 50-cm capillary array	4331250
3730xl 36-cm capillary array	4331244
3730xl 50-cm capillary array	4331246
3700/3730 BigDye Terminator v3.1 Sequencing Std	4336943
3700/3730 BigDye Terminator v1.1 Sequencing Std	4336799
Matrix Standard Set DS-33	4345833
HiDi™ Formamide, 25 mL	4311320
POP-7 Polymer (1 bottle of 25ml each)	4363929
POP-7 Polymer (10 bottles of 25ml each)	4363935
POP-7 Polymer (30 bottles of 25ml each)	4335611
Buffer (10X) with EDTA - 500 mL	4335613
Buffer (10X) with EDTA - 4L	4318976
96-Well sample plates w/barcode	4306737
96-Well sample plates, no bar code	N801-0560
96-Well plate septa	4315933
96-Well plate base (septa sealed)	4334873
96-Well plate base (heat sealed)	4334875
96-Well plate retainer (septa sealed)	4334869
96-Well and 384-well Plate Retainer (heat sealed)	4334865
FAST (0.1ml) 96-Well Plate Retainer for 3730 (septa-sealed)	4367472
FAST (0.1ml) 96-Well Plate Base for 3730 (septa-sealed)	4367469

Notes _____

Item	Part No
FAST (0.1ml) 96-Well Plate Retainer for 3730 (heat-sealed)	4367474
FAST (0.1ml) 96-Well Plate Base for 3730 (heat-sealed)	4367473
384-Well Sample plates with barcode	4309849
384-Well plate septa	4315934
384-Well plate base (septa-sealed)	4334874
384-Well plate base (heat-sealed)	4334877
384-Well plate retainer (septa-sealed)	4334868
Heat seal film, 3-mil	4337570
Applied Biosystems® 3730/3730x/ DNA Analyzer Getting Started Guide	4359476
Applied Biosystems® BigDye® Xterminator™ Purification Kit Protocol	4374408
AB Navigator Software Administrator Guide	4359472
Applied Biosystems® Data Collection Software v2.0 Upgrade to v3.0 Procedure	4363191
GeneMapper® Software 3.7 update CD (no charge)	4363136

Notes _____

Dye Sets: G5, G5-RCT, Any4Dye, Any4dye-HDR, and Any5Dye

Dye Sets G5 and G5-RCT For Fragment Analysis

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

Dye Set G5-RCT To reduce crosstalk for fragment analysis applications, a new dye set has been created for Data Collection Software v3.0, called dye set G5-RCT. G5-RCT uses the same chemistry as dye set G5 (6-FAM[™], VIC[®] NED[™], PET[®], LIZ[®] dyes). This dye set reduces signal, but reduces potential crosstalk to a greater degree, so the reduction in signal-to-noise ratio is less pronounced than the reduction in signal overall. Higher concentration peaks can be used without going offscale, this results in a higher dynamic range for the G5-RCT dye set.

Recommendations for Using G5 or G5-RCT Dye set G5-RCT may be especially useful for users performing fragment analysis with a 96 capillary array, as well as users interested in applications with a high dynamic range (large peaks much higher than small peaks). For most other conditions, users prefer the G5 dye set.

We support:

- Fragment analysis on the 96-capillary array using G5-RCT only
- SNPlex[™] System analysis
- G5 and G5-RCT on the 48-capillary array.

Notes _____

Refer to the following table for more information about the advantages and issues to consider for each dye set.

Dye Set	Features
Standard Z, E Dye Sets	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> • De Novo Sequencing using BigDye • Terminator v3.1 or v1.1. Higher signal relative to the Any4Dye-HDR dye set • Optimized for the highest signal-to-noise ratio <p>Issues:</p> <ul style="list-style-type: none"> • More susceptible to samples within a plate with large variation in peak height relative to the Any4Dye-HDR dye set
Any4Dye	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> • Use of unsupported dyes. Provides an open platform for system capable applications <p>Issues:</p> <ul style="list-style-type: none"> • Performance of system has not been tested nor can the performance be guaranteed • More susceptible to samples within a plate with large variation in peak height relative to the Any4Dye-HDR dye set
Any4Dye-HDR (High Dynamic Range)	<p>When to use/Advantages:</p> <ul style="list-style-type: none"> • High dynamic range when samples within a plate have a large variation in peak height • Resequencing/Mutational Profiling applications • 4-Dye Fragment Analysis applications • Use of unsupported dyes. Provides an open platform for system capable applications <p>Issues:</p> <ul style="list-style-type: none"> • Signal is reduced by approximately ¼, along with a minimal reduction in the noise, resulting in a slight decrease in the signal/noise when compared to data generated using the standard dye sets • Essential that spectral calibrations are performed each time the capillary array is replaced or moved within the detection cell

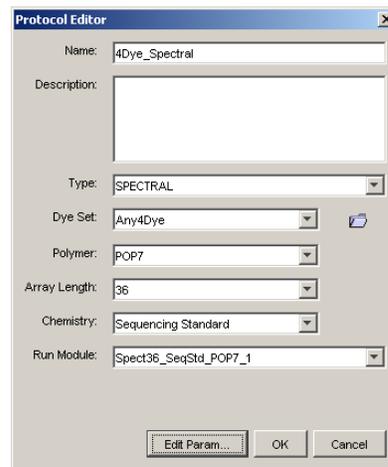
Notes _____

Creating a Spectral Calibration for Dye Sets Any4Dye, Any4Dye-HDR, or Any5Dye

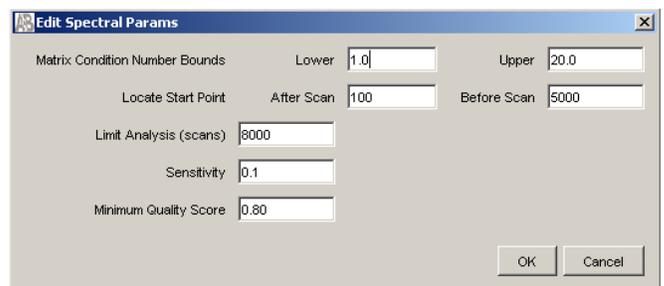
The steps to creating and running a customized 4- or 5- DyeSet are similar to running a supported dye set.

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

1. In the navigation pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.
2. In the Instrument Protocols pane, click **New...**. The Protocol Editor opens.
3. In the Protocol Editor, create a spectral protocol for the 4Dye dye set, specifying the appropriate protocol parameters.
4. Click **OK** to save the spectral protocol.

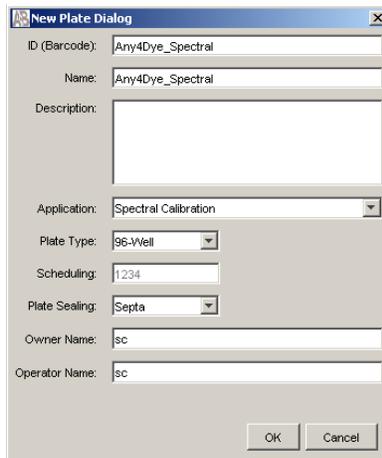


Note: Customize the Spectral parameters as needed. For more information see, step 1 on page 38.

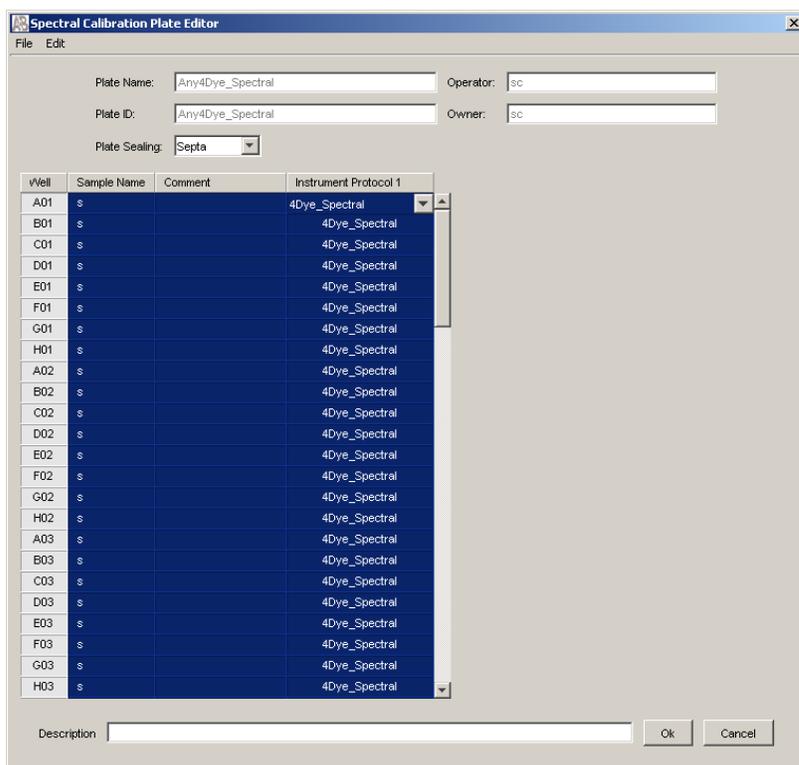


Notes

5. Click **New** in the Plate Manager to display the New Plate Dialog box.
6. Create a spectral plate for the Any4Dye dye set by completing the New Plate Dialog box.
7. Click **OK**.
8. Create an instrument protocol. For more information, see page 36.

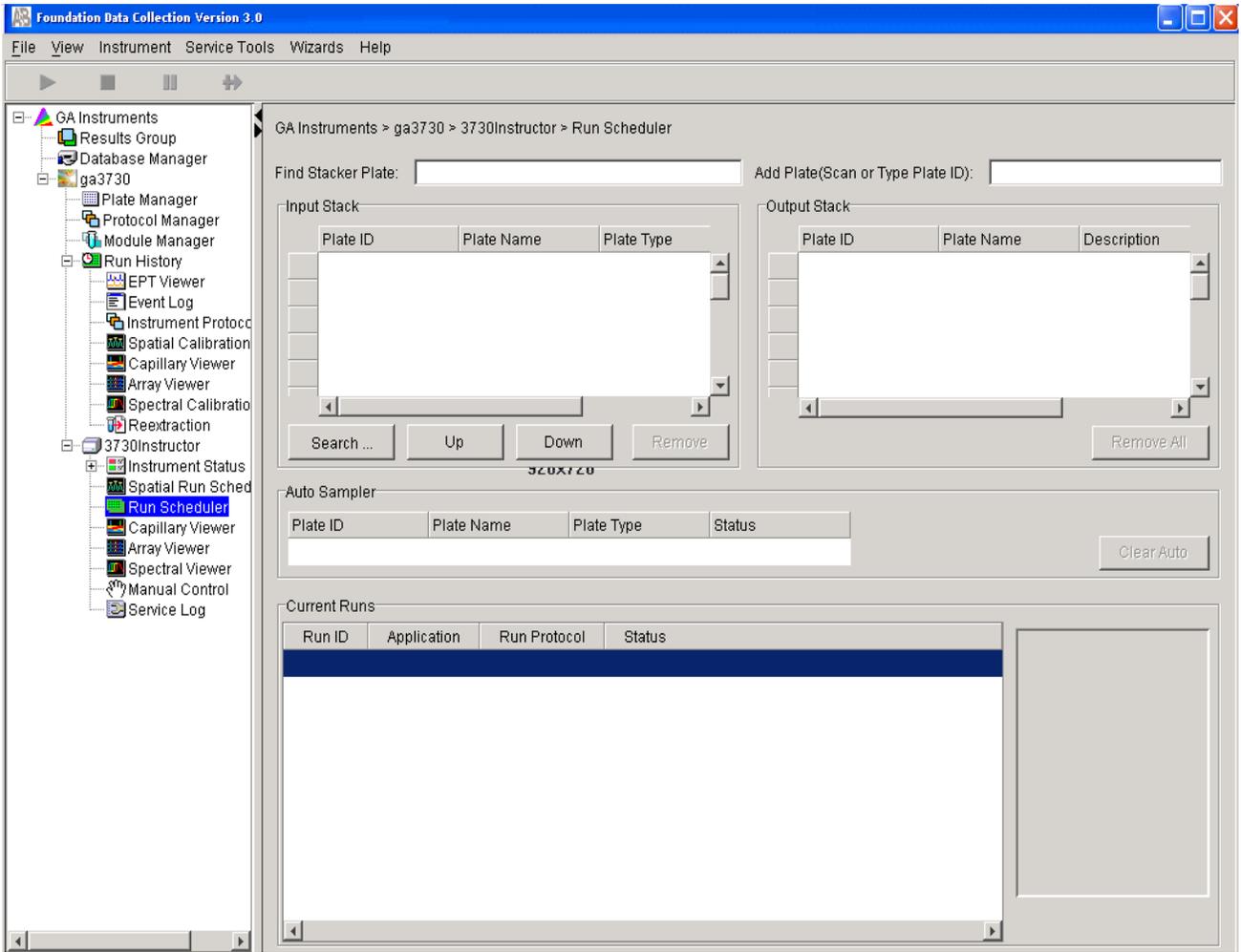


9. In the Plate Editor, select the Instrument Protocol that you just created in the previous steps, then click **OK** to save the plate.



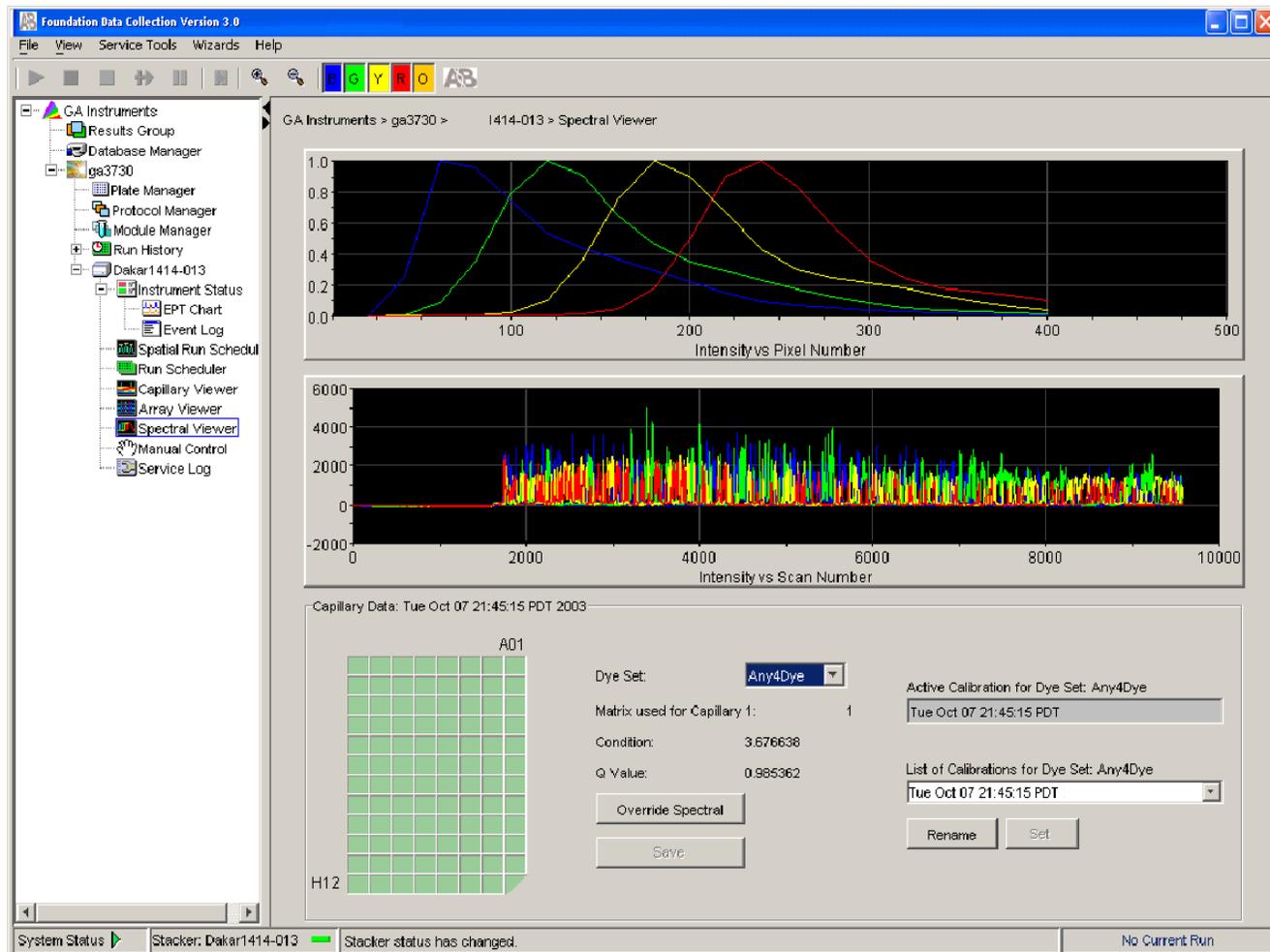
Notes

10. In the Run Scheduler, add the spectral plate to the Input Stack, then run the plate.



Notes

11. Verify that spectral matrices for all capillaries meet acceptance criteria (pass). Override individual capillaries and rename calibration as needed.



Notes

Regular Runs Using Any4Dye or Any5Dye Dye Sets

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

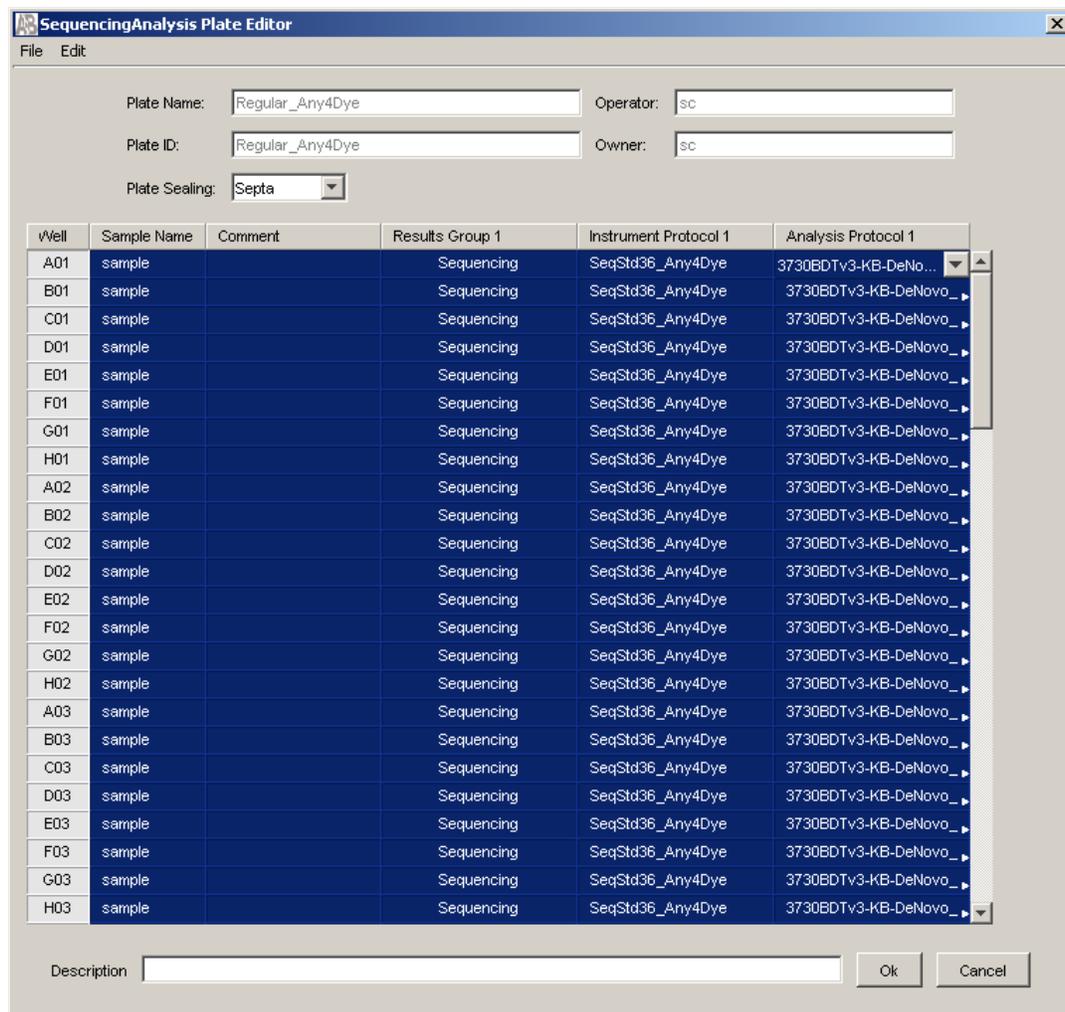
1. In the Protocol Editor, create a regular instrument run protocol for the Any4Dye dye set, then choose the appropriate default run module template. (You can create a customized run module in the Module Editor if desired).



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

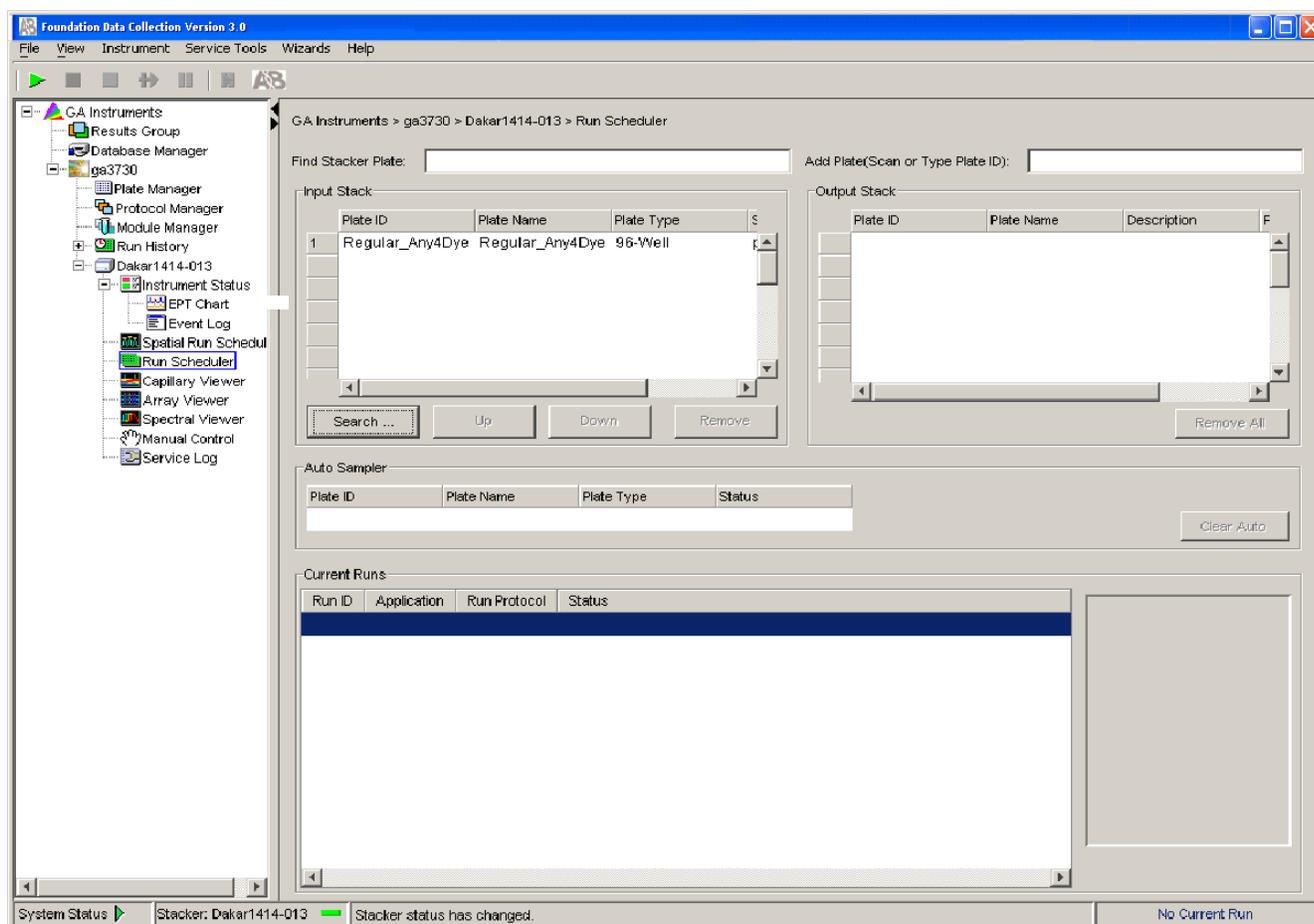
Notes _____

- In the Plate Editor, select the instrument protocol that you created in step 1, then click **OK** to save the plate.



Notes _____

- In the Run Scheduler, add this plate to the Input Stack, then run the plate.



Notes _____

Notes _____

Instrument Warranty Information

Computer Configuration

Life Technologies supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Life Technologies reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Life Technologies. Life Technologies also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty Life Technologies warrants that all standard components of the Applied Biosystems® 3730/3730xl DNA Analyzer will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Life Technologies will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Life Technologies at its published rates. Life Technologies also provides service agreements for post-warranty coverage. Life Technologies reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Life Technologies warrants that all optional accessories supplied with its Applied Biosystems 3730/3730xl DNA Analyzer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Life Technologies will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Life Technologies will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Life Technologies warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Life Technologies warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Life Technologies within the ninety (90) day warranty period, Life Technologies will replace the defective media.

Life Technologies does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Life Technologies personnel. For all hardware and software installed by the buyer or anyone other than Life Technologies, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Life Technologies; and modification or repair of the product not authorized by Life Technologies.

THE FOREGOING PROVISIONS SET FORTH LIFE TECHNOLOGIES' SOLE AND EXCLUSIVE REPRESENTATIONS, WARRANTIES, AND OBLIGATIONS WITH RESPECT TO ITS PRODUCTS, AND LIFE TECHNOLOGIES MAKES NO OTHER WARRANTY OF ANY KIND WHATSOEVER, EXPRESSED OR IMPLIED, INCLUDING WITHOUT LIMITATION, WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, WHETHER ARISING FROM A STATUTE OR OTHERWISE IN LAW OR FROM A COURSE OF DEALING OR USAGE OF TRADE, ALL OF WHICH ARE EXPRESSLY DISCLAIMED.

Warranty Limitations

THE REMEDIES PROVIDED HEREIN ARE THE BUYER'S SOLE AND EXCLUSIVE REMEDIES. WITHOUT LIMITING THE GENERALITY OF THE FOREGOING, IN NO EVENT SHALL LIFE TECHNOLOGIES BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE (INCLUDING WITHOUT LIMITATION, ANY TRADE PRACTICE, UNFAIR COMPETITION, OR OTHER STATUTE OF SIMILAR IMPORT) OR ON ANY OTHER BASIS, FOR DIRECT, INDIRECT, PUNITIVE, INCIDENTAL, MULTIPLE, CONSEQUENTIAL, OR SPECIAL DAMAGES SUSTAINED BY THE BUYER OR ANY OTHER PERSON OR ENTITY, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT LIFE TECHNOLOGIES IS

Notes _____

ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING WITHOUT LIMITATION, DAMAGES ARISING FROM OR RELATED TO LOSS OF USE, LOSS OF DATA, FAILURE OR INTERRUPTION IN THE OPERATION OF ANY EQUIPMENT OR SOFTWARE, DELAY IN REPAIR OR REPLACEMENT, OR FOR LOSS OF REVENUE OR PROFITS, LOSS OF GOOD WILL, LOSS OF BUSINESS, OR OTHER FINANCIAL LOSS OR PERSONAL INJURY OR PROPERTY DAMAGE.

NO AGENT, EMPLOYEE, OR REPRESENTATIVE OF LIFE TECHNOLOGIES HAS ANY AUTHORITY TO MODIFY THE TERMS OF THIS LIMITED WARRANTY STATEMENT OR TO BIND LIFE TECHNOLOGIES TO ANY AFFIRMATION, REPRESENTATION, OR WARRANTY CONCERNING THE PRODUCT THAT IS NOT CONTAINED IN THIS LIMITED WARRANTY STATEMENT, AND ANY SUCH MODIFICATION, AFFIRMATION, REPRESENTATION, OR WARRANTY MADE BY ANY AGENT, EMPLOYEE, OR REPRESENTATIVE OF LIFE TECHNOLOGIES WILL NOT BE BINDING ON LIFE TECHNOLOGIES, UNLESS IN A WRITING SIGNED BY AN EXECUTIVE OFFICER OF LIFE TECHNOLOGIES.

THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM LIFE TECHNOLOGIES AND IS NOT TRANSFERABLE.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, Claims, and Returns

- Damages** If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Life Technologies without first securing an inspection report and contacting Life Technologies Technical Support for a Return Authorization (RA) number.
- Claims** After a damage inspection report is received by Life Technologies, Life Technologies will process the claim unless other instructions are provided.
- Returns** Do not return any material without prior notification and authorization.
- If for any reason it becomes necessary to return material to Life Technologies, contact Life Technologies Technical Support or your nearest Life Technologies subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Life Technologies representative.

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Appendix C Instrument Warranty Information
Damages, Claims, and Returns

Notes _____

Obtain SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com.

Note: For the SDSs of chemicals not distributed by LifeTechnologies, contact the chemical manufacturer.

Obtain support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the Support page, you can:

- Access worldwide telephone and fax numbers to contact LifeTechnologies Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order LifeTechnologies user documents, SDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training and available instrument service options

Limited Product Warranty

LifeTechnologies and/or its affiliate(s) warrant their products as set forth in their the LifeTechnologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Safety



WARNING GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
- All testing should be performed in accordance with local, regional and national acceptable laboratory accreditation standards and/or regulations.

Symbols on Instruments

Electrical Symbols

The following electrical symbols may be displayed on instruments.

Symbol	Description
	Indicates the On position of the main power switch.
	Indicates the Off position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the Standby condition. Hazardous voltage may be present if this switch is on standby.
	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 safety symbols may be displayed on instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see “Safety Labels on Instruments” on page 174). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

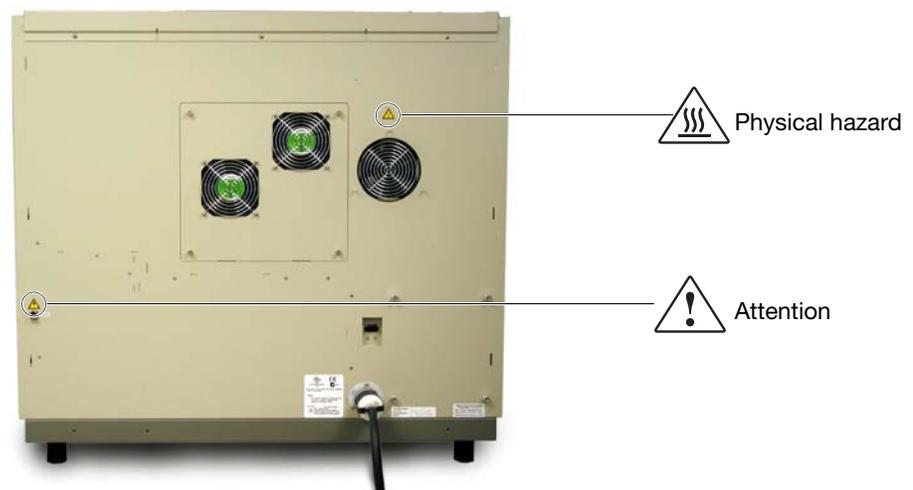
Symbol	Description	Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.		Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard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

Safety Labels on Instruments

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

English	Français
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	ATTENTION Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	ATTENTION Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	ATTENTION Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	AVERTISSEMENT Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems® lamp.	AVERTISSEMENT Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems®.
CAUTION Hot surface.	ATTENTION Surface brûlante.
DANGER High voltage.	DANGER Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Life Technologies 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 Life Technologies.
DANGER Class 3b laser present when open and interlock defeated. Do not stare directly into beam.	DANGER de 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 II laser radiation present. Avoid exposure to the beam.	DANGER de Class II rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.
DANGER Class II laser radiation present when open. Avoid exposure to the beam.	DANGER de Class II rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	ATTENTION Parties mobiles.

Locations of Laser Warnings The 3730/3730x1 DNA Analyzer contains laser warnings at the locations shown below:



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 Life Technologies 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.

Operating the Instrument

Ensure that anyone who operates the instrument has:

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

Chemical Safety

Chemical Hazard Warnings

 **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.

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

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

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

Chemical Safety Guidelines

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

Chemical Waste Safety

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

Chemical Waste Safety Guidelines

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

Waste Profiles

A waste profile for the 3730/3730xl DNA analyzer is provided in the *3730/3730xl DNA Analyzer Site Preparation Guide*.

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

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

Waste Disposal

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

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

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

Electrical Safety



DANGER

ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the 3730/3730xl DNA Analyzer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses



WARNING

FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.



WARNING

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

Power  **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 3730/3730x1 DNA Analyzer system has 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 3730/3730xl DNA Analyzer. Disconnect power before servicing the 3730/3730xl DNA Analyzer.



DANGER

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

Solvents and Pressurized Fluids



WARNING

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



WARNING

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

- Be aware that Radel[®] tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.
- Always wear eye protection when in proximity to pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use Radel[®] tubing that has been severely stressed or kinked.
- Do not use Radel[®] tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause Radel[®] tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Biological Hazard Safety



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

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

Additional information about biohazard guidelines is available at:

<http://www.cdc.gov>

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

Laser Safety

Laser Classification

The 3730/3730xl DNA Analyzer uses a laser. Under normal operating conditions, the instrument laser is categorized as a Class 1 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 3730/3730xl DNA Analyzer laser has been tested to and complies with the “Radiation Control for Health and Safety Act of 1968 Performance Standard CFR 1040.”

The 3730/3730xl DNA Analyzer laser has been tested to and complies with standard EN60825-1: 1994+All: 1996 7 A2: 2001 or EN 60825-1, “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 Life Technologies 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 1 rating.
- Do not remove safety labels or disable safety interlocks.

Laser specifications

This instrument uses a 25 mW, multi-line, single mode Argon-ion laser. Wave length 488 nm, 514.5 nm, Output power 25 mW, Beam divergence 1 mrad.

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.



CAUTION Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Bar Code Scanner Laser Safety

Laser Classification The bar code scanner included with the 3730/3730x1 DNA Analyzer is categorized as a Class II laser.

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



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

Computer Workstation Safety

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



CAUTION MUSCULOSKELETAL AND REPETITIVE MOTION

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

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

Safety and Electromagnetic Compatibility (EMC) Standards

U.S. and Canadian Safety Standards



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

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

Canadian EMC Standard

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

European Safety and EMC Standards

Safety



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

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”

Australian EMC Standards

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



Safety

Safety and Electromagnetic Compatibility (EMC) Standards

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