USER GUIDE

applied biosystems® by life technologies™

Applied Biosystems® 3730/3730*xl* DNA Analyzer

GETTING STARTED GUIDE

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life technologies™

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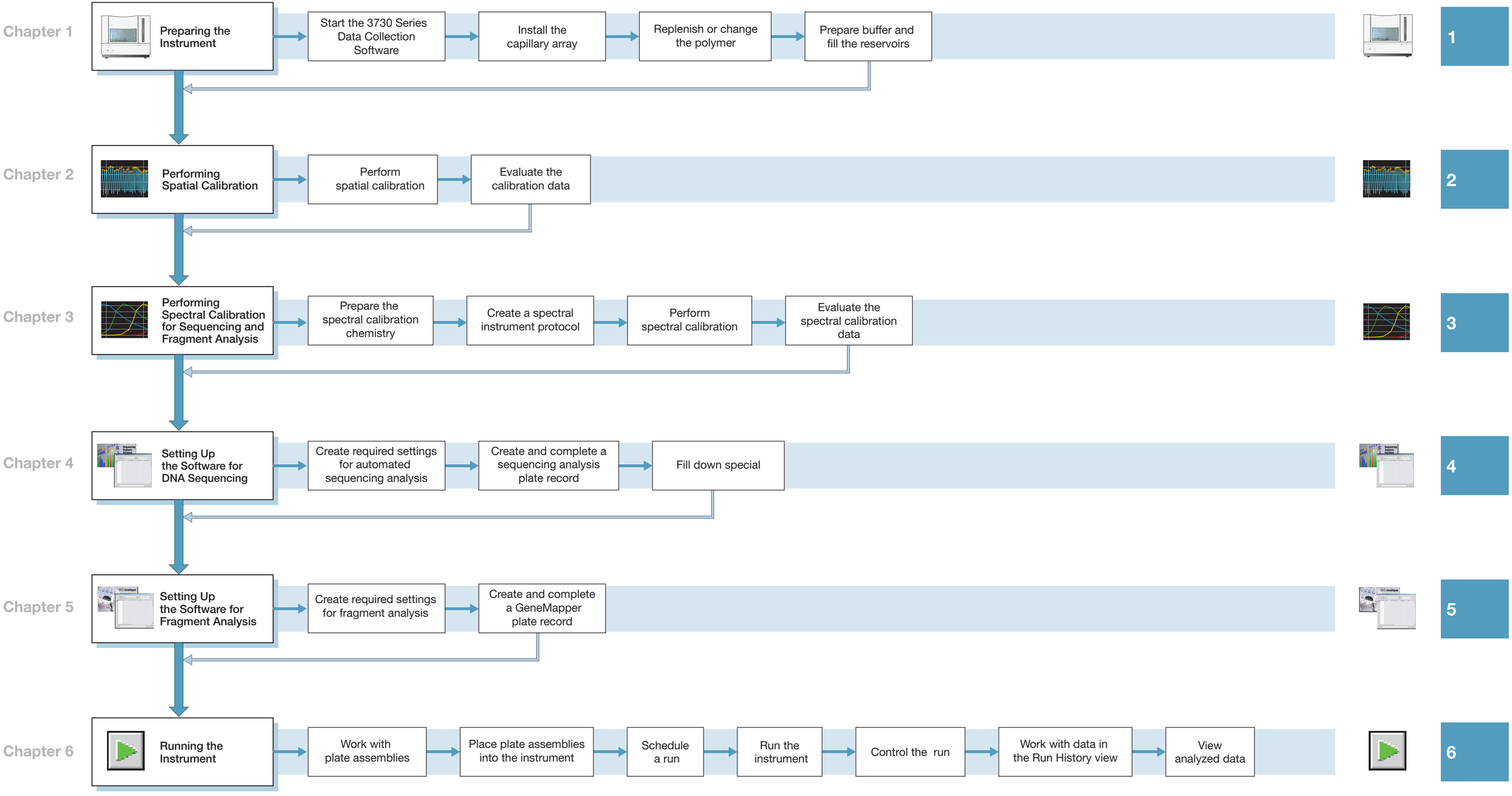
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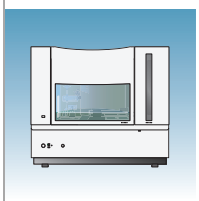
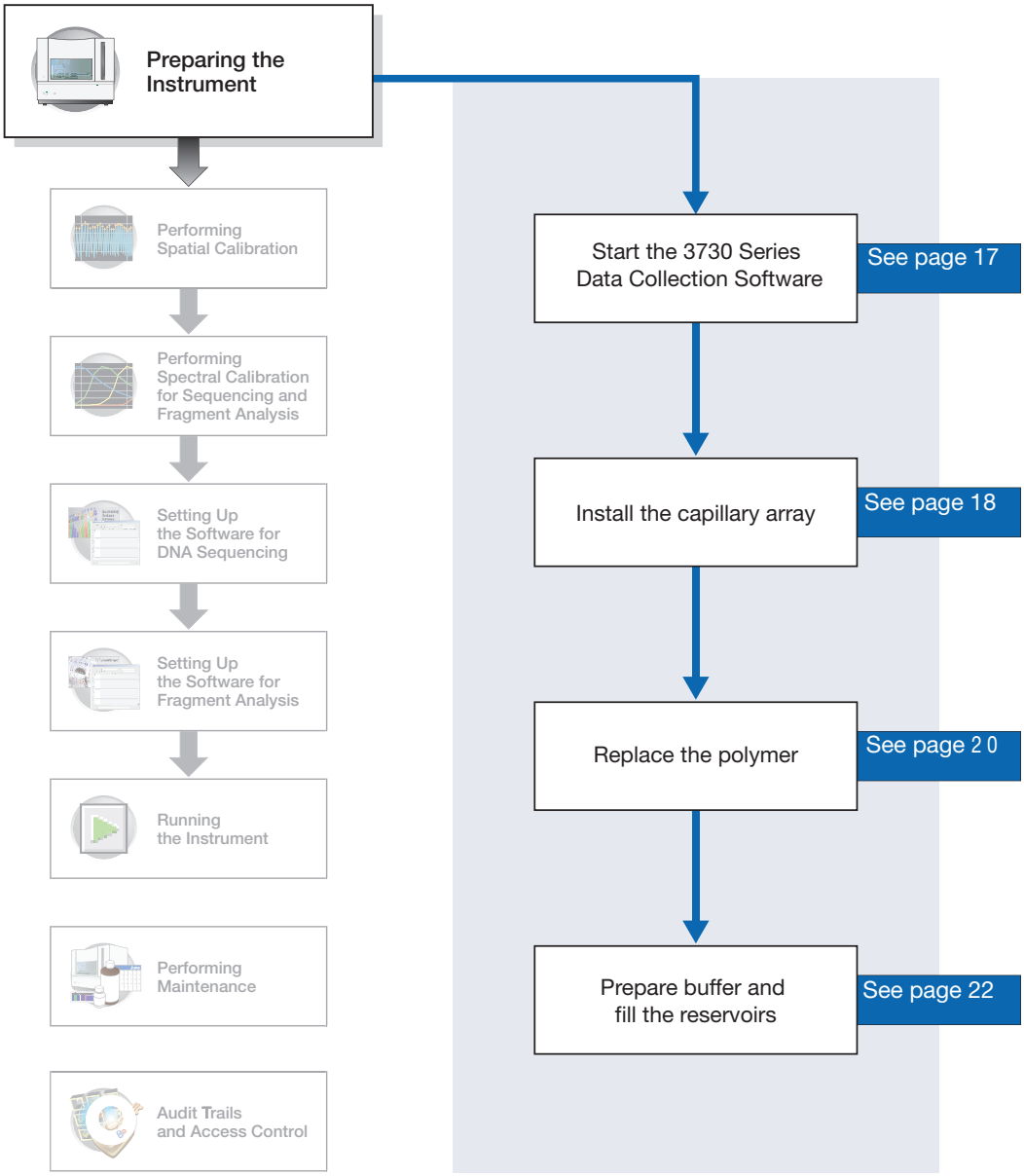


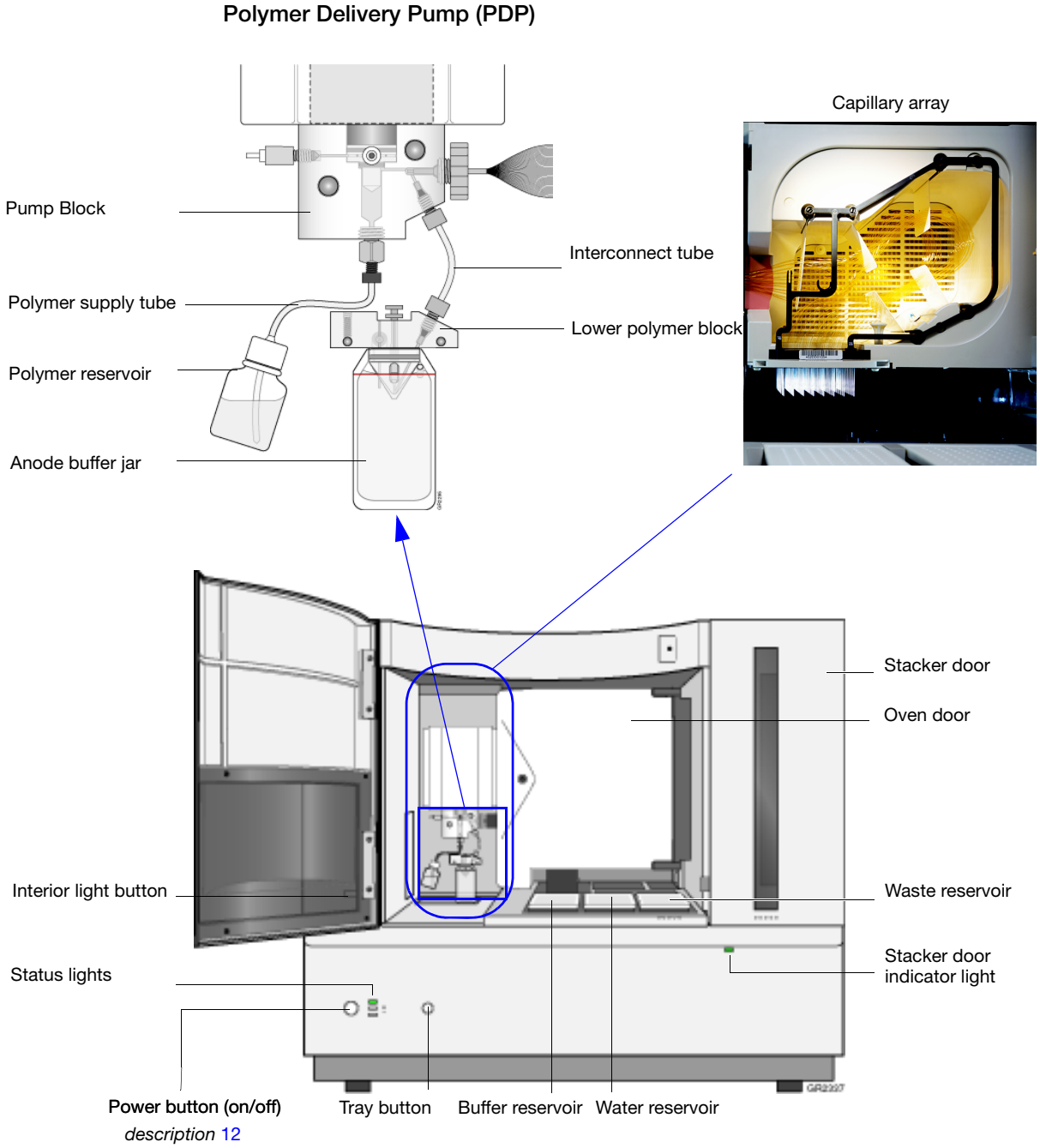
Preparing the Instrument

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.



Notes

Instrument and Parts



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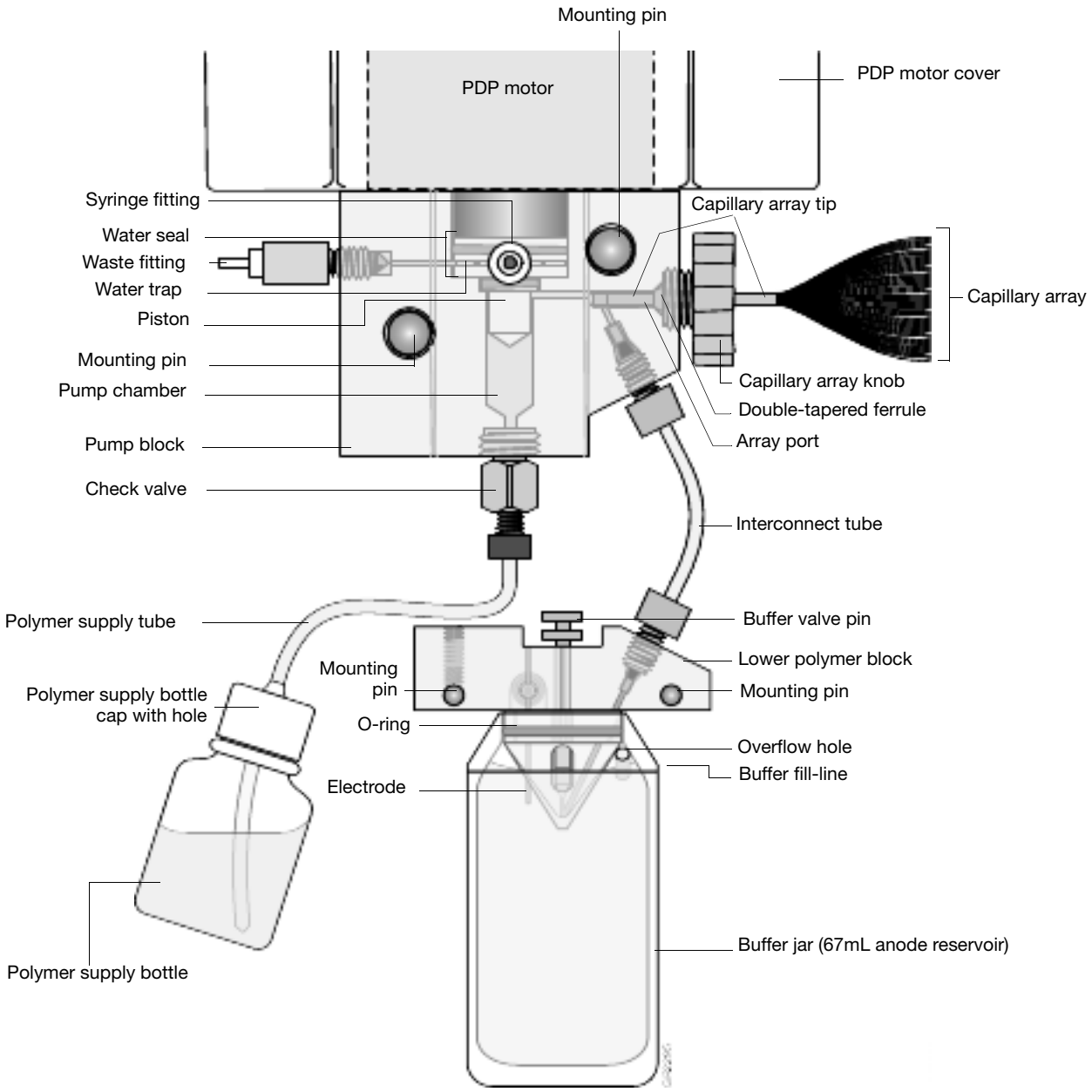
Polymer Delivery Pump Detail



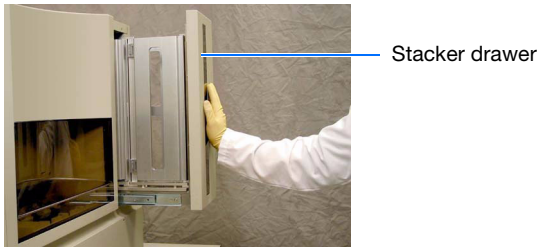
Notes

Overview

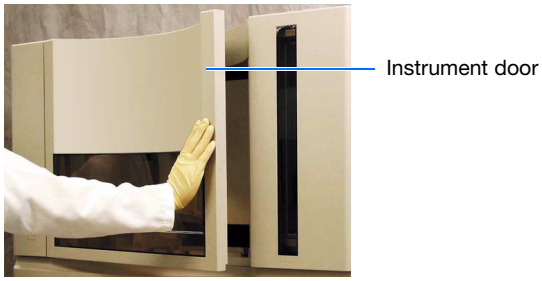
This chapter explains how to prepare the instrument for a run by installing the capillary array, buffer, and reservoirs.

Powering On the Computer and 3730/3730*xl* 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 OK.
4. Close the oven door.
5. Close the stacker drawer.

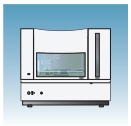


6. Close the instrument door.

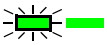
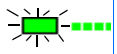
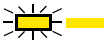
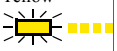
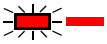


7. Wait until the monitor displays the desktop of the Windows® operating system.
8. Press the power button on the 3730/3730*xl* Analyzer instrument to power it on.

Notes



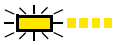
The Status Lights

Status	Status Light	Action
• The instrument is ready • An automated wizard operation is in progress with the instrument door closed	Solid Green 	Go to page 17.
• A run is in progress	Flashing Green 	—
• The instrument cannot communicate with the computer	Solid Yellow 	Go to page 15.
• 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 14.
• The instrument has detected a problem	Solid Red 	Go to page 15.

Notes

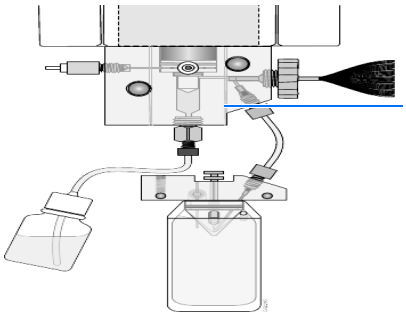
Troubleshooting Instrument Status Lights

Flashing Yellow



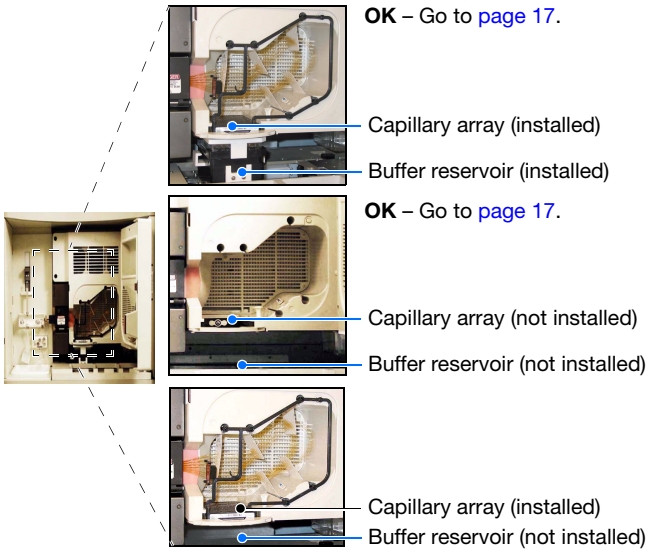
To determine the source of the problem:

1. Press on the instrument door to ensure that it is closed. If the 3730/3730*xl* Analyzer instrument displays the green status light, then the instrument door was open. Go to page 17.



2. If the 3730/3730*xl* 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 3730/3730*xl* Analyzer instrument displays the green status light, then the oven door was open. Go to page 17.

3. If the 3730/3730*xl* 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.



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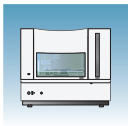
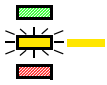


Solid Yellow Light

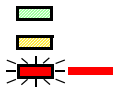


To determine the source of the problem, verify that the:

1. Monitor displays the desktop of the Windows operating system.
2. Ethernet cable is connected to the back of the 3730/3730*xl* 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 Life Technologies technical support or your service representative for further assistance.

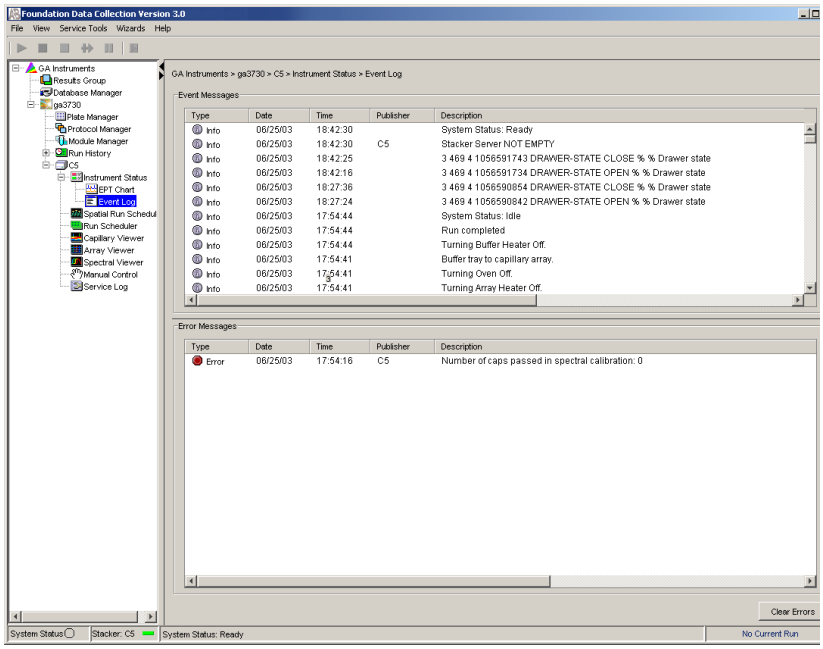
Solid Red Light



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 Series Data Collection Software as explained page 17.
   b. In the navigation pane of the Data Collection Software, double-click **GA Instruments** > **ga3730** > **instrument name** > **Instrument Status** > **Event Log**.

Notes



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 Life Technologies technical support or your service representative for further assistance.

Notes

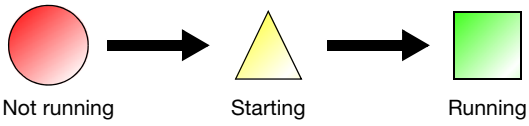
Starting the 3730 Series Data Collection Software

1. Select start > **All Programs** > **Applied Biosystems** > **Unified Data Collection** > **Run Unified Data Collection 4**.

   The Data Collection Software opens the Service Console dialog box.

   Note: The 3730 Series Data Collection Software 4 requires a license to run. Refer to Appendix D, Managing Data Collection Software Licenses on page 189 for more details.

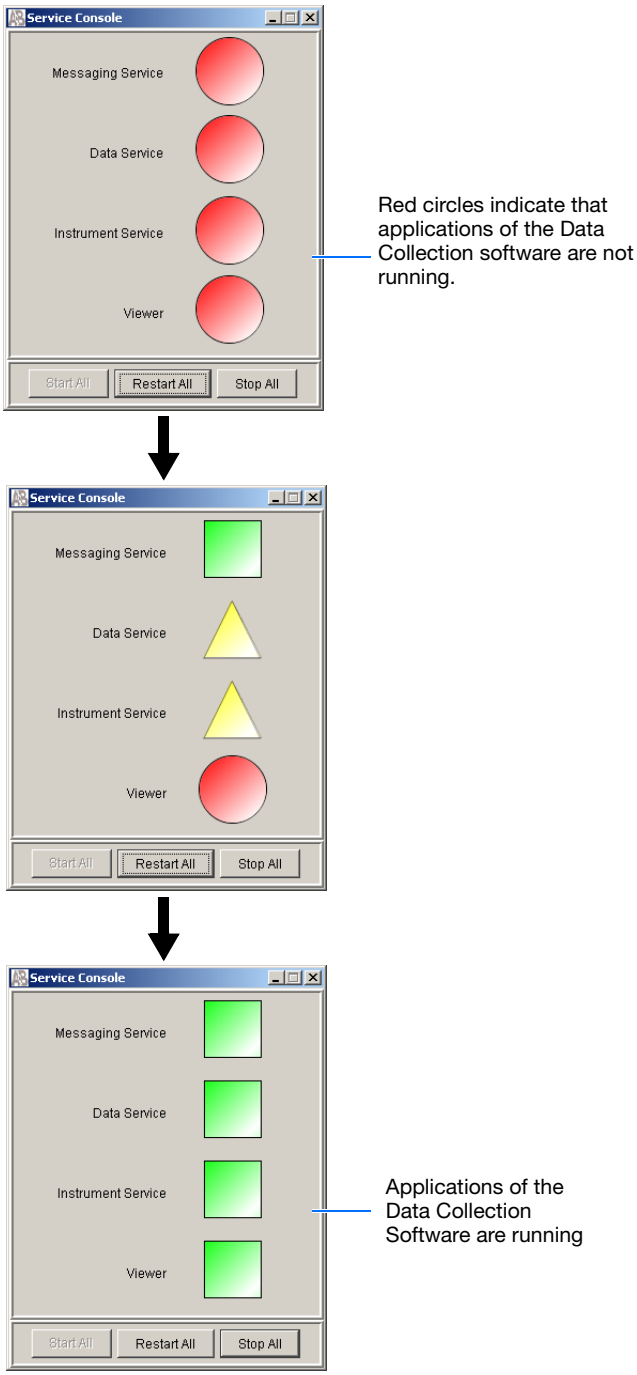
2. Wait for the Service Console dialog box to open the applications of the Data Collection Software.

   When the Data Service component displays the yellow triangle, do not press Start All or Stop All; if you do press either, you will need to re-boot the computer.

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

   Note: Ensure that all Data Collection Services are running before you launch the AB Navigator tool for security, audit trail and electronic signature features described in the AB Navigator Software Administrator Guide (Part no. 4477853). All services are running when the Service Console contains four green squares.



Notes

Installing the Capillary Array

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

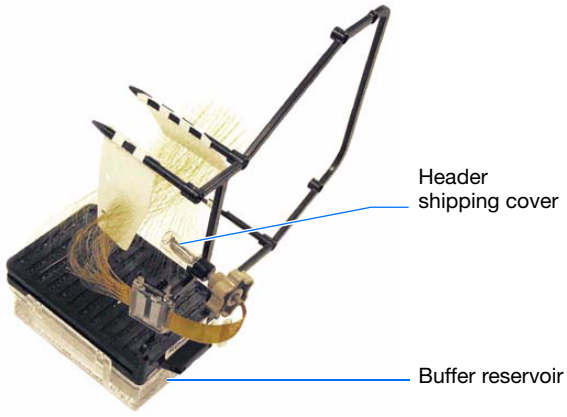
WARNING **CHEMICAL HAZARD.** **Running Buffer with EDTA** causes eye, skin, and respiratory tract irritation. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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* (Part no. 4477797).



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.

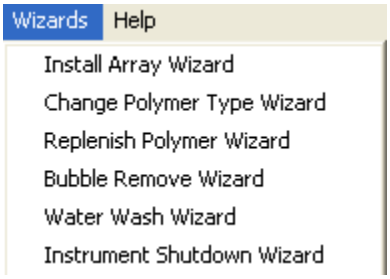
1. Close the instrument door.
2. In the Data Collection software, select **GA Instruments > ga3730 > instrument name >.**

Notes

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

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

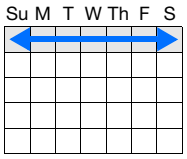
5. Perform a spatial calibration (see page 32).



Notes

Replenishing or Changing Polymer Type

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



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

Before Using the Polymer

1. Remove the polymer from 4°C storage.
2. Loosen the cap and bring the polymer to room temperature.
3. To dissolve deposits, tighten the cap and gently swirl the polymer.

Replenishing the Polymer

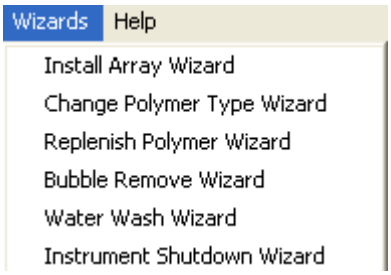
IMPORTANT! Wear gloves while handling polymer, the capillary array, septa, or buffer reservoirs.

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

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

Notes

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



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

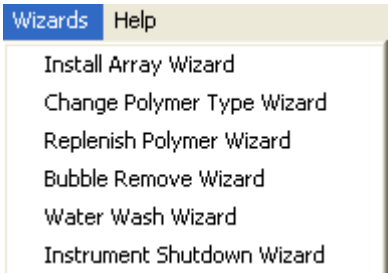
Changing Polymer Type

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

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

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

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



Notes

Preparing Buffer and Filling the Reservoirs

WARNING **CHEMICAL HAZARD**. **Running Buffer with EDTA** causes eye, skin, and respiratory tract irritation. Read the SDS, 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
- 10× Genetic Analyzer Running Buffer with EDTA, 20 mL
- Graduated cylinder, 250-mL
- Gloves, silicone-free, powder-free

Buffer Storage

The 1× run buffer can be stored at:

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

Notes

When to Change the Buffer

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

Note: When replacing all liquids, you should not simply 'top off'. Replacement is critical.

Note: Clean the reservoirs weekly in warm water followed by a rinse with deionized water.

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

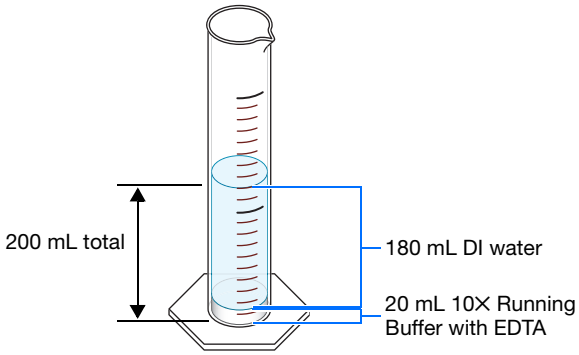
Notes

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 SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

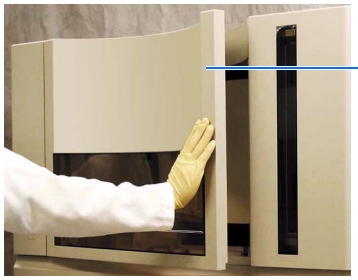
1. Pour 20 mL 10× running buffer with EDTA into a graduated cylinder.
2. Add 180 mL deionized water to bring the total volume to 200 mL.
3. Mix well and set aside.



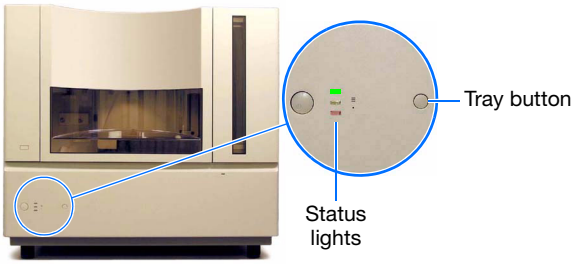
Filling the Water and Buffer Reservoirs

IMPORTANT! Wear gloves when you handle the reservoir.

1. Close the instrument door.



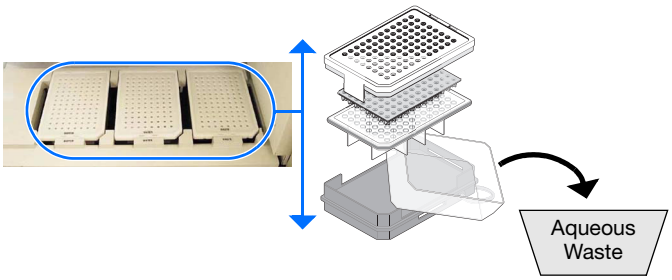
2. Press the Tray button to bring the autosampler to the forward position.
3. Wait for the autosampler to stop moving and for the green status light to illuminate before you open the instrument door.



Notes

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.

Note: Be sure to clean the buffer jar, as well as water, waste, and buffer reservoirs weekly in warm water followed by a rinse with deionized water.

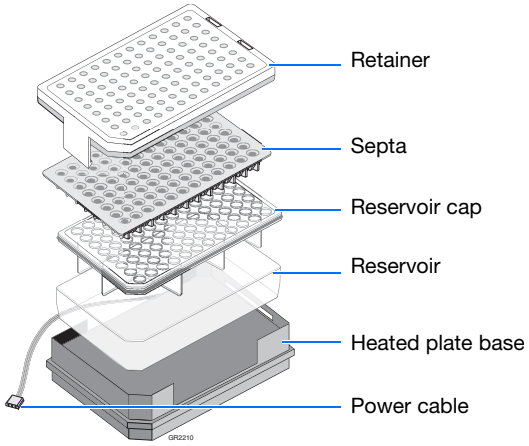
7. Dry the reservoirs using lint-free wipes.

8. Fill then assemble the reservoirs.

Buffer Reservoir Assembly

a. Add 80 mL 1× run buffer to the Buffer reservoir.

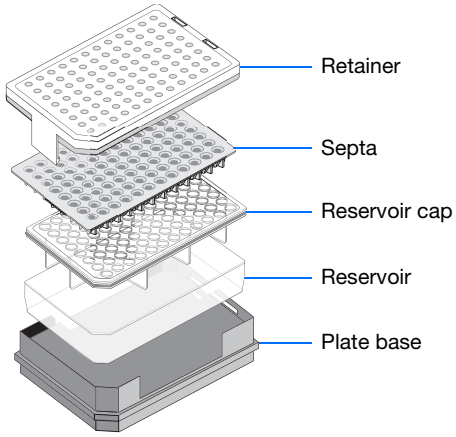
b. Assemble the reservoir assembly as shown below:



Water and Waste Reservoir Assemblies

a. Add 80 mL high-quality deionized water to each reservoir.

b. Assemble each reservoir assembly as shown below:



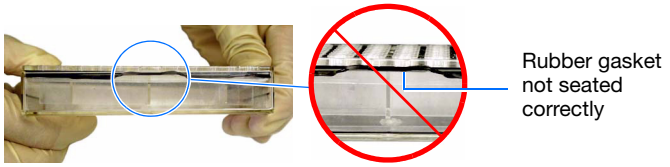
Notes

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

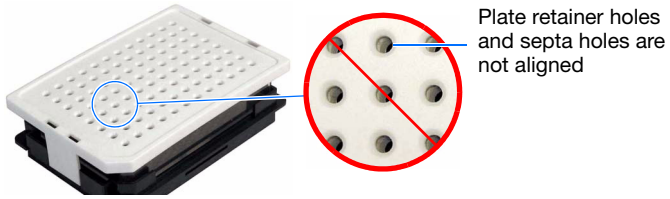
- Septa fit snugly and flush on the reservoir cap

  Note: Inspect septa weekly and replace any that are worn or discolored.

- Rubber gasket around the edge of the reservoir cap is seated correctly



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



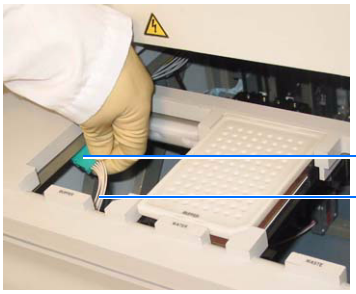
10. Dry the reservoirs using lint-free wipes.

Notes

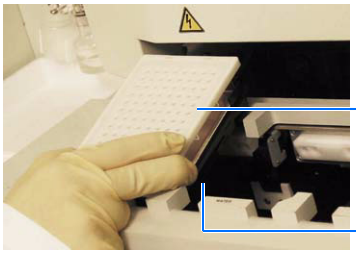
Placing Reservoirs into the Instrument

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

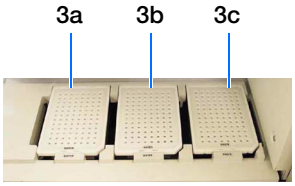
1. Connect the Buffer reservoir plate base cable into the heater outlet within the instrument.



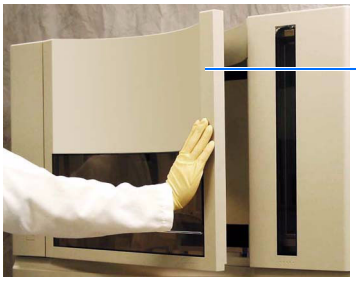
2. Move the buffer reservoir to the Buffer position (left) making sure the cable is out of the way of the autosampler.



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



4. Close the instrument door.



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 SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

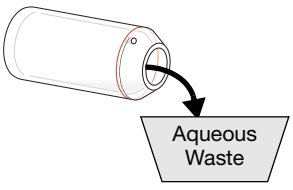
Replace the anode buffer:

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

Note: Complete replacement of all liquids is critical; do not simply 'top off' liquids. Be sure to clean the buffer jar, as well as water, waste, and buffer reservoirs weekly in warm water followed by a rinse with deionized water.

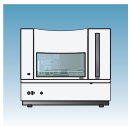
IMPORTANT! Wear gloves when you handle the anode buffer jar.

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

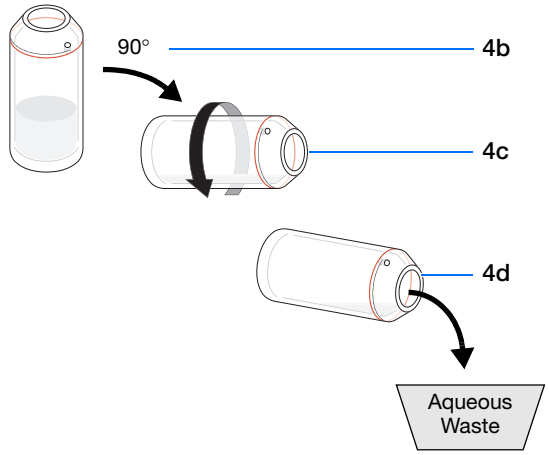


2. Empty the anode buffer jar into an aqueous waste container.
3. Rinse the anode buffer jar using deionized water.

Notes



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

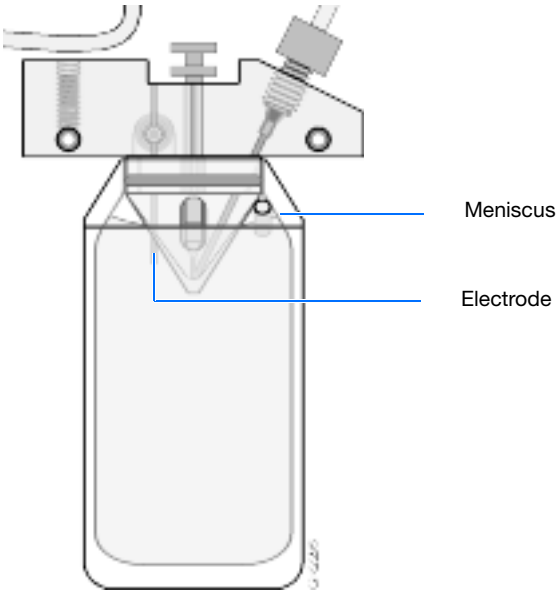


5. Add 67 mL 1X run buffer to the jar.
6. Put the anode buffer jar on the instrument with the overflow hole facing you.

   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.

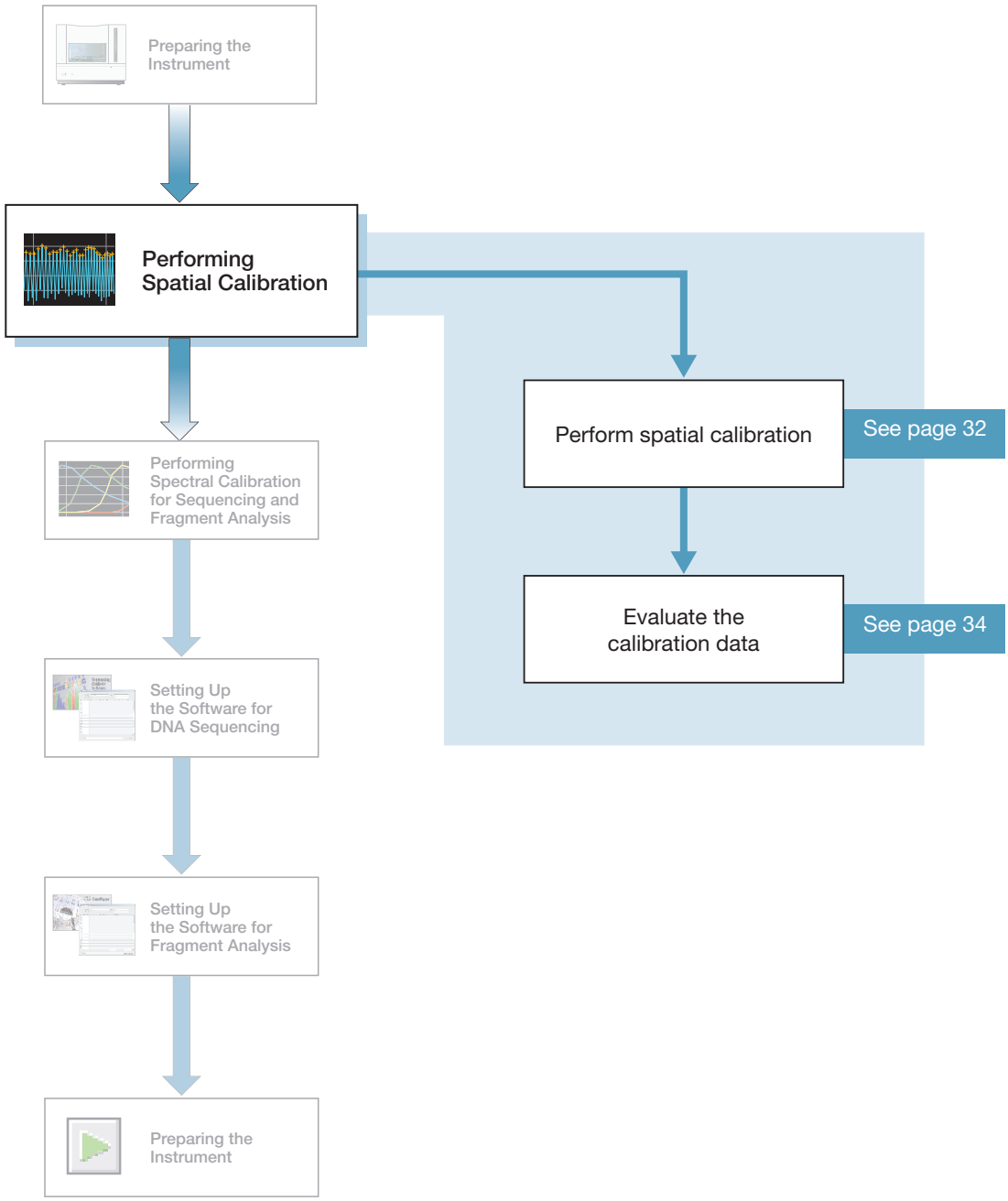
IMPORTANT! Replace buffer if excess polymer is expelled into the anode jar.



Notes

Notes

Performing Spatial Calibration



Notes

Overview

What a Spatial Calibration Tells You

The 3730 Series 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

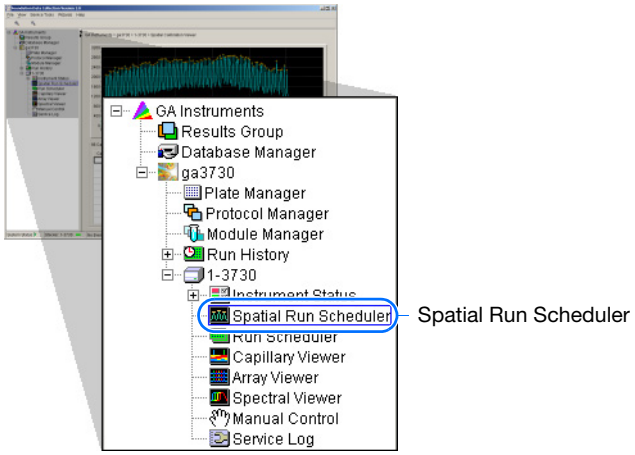
For all dye sets, perform a spatial calibration after you:

- 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)
- Move the array detection cell

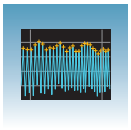
Note: Failure to perform a new spatial calibration can result in poor data quality.

Performing Spatial Calibration

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

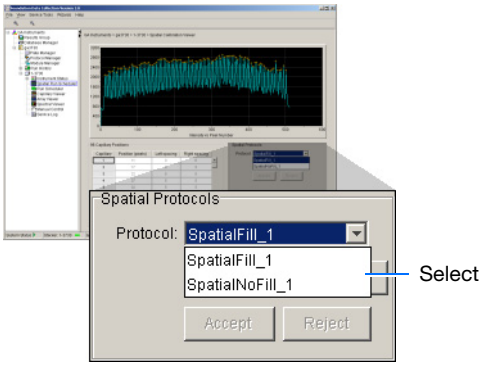


Notes



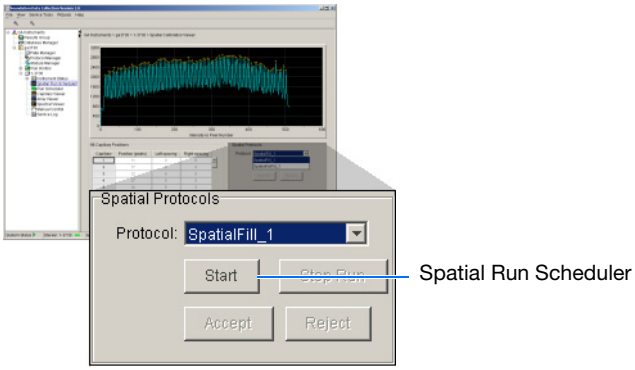
2. In the Spatial Run Scheduler view, do one of the following:
   - If the capillaries contain fresh polymer, select **Protocol** > **SpatialNoFill**.
   - Otherwise, select **Protocol** > **SpatialFill**.

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



3. Click Start.

   The approximate calibration run times are:
   - 48-cap/36cm array with fill, 4 minutes.
   - 96-cap/36cm array with fill, 3 minutes.
   - No fill, 2 minutes.



4. Evaluate the calibration as explained on page 34.

Notes

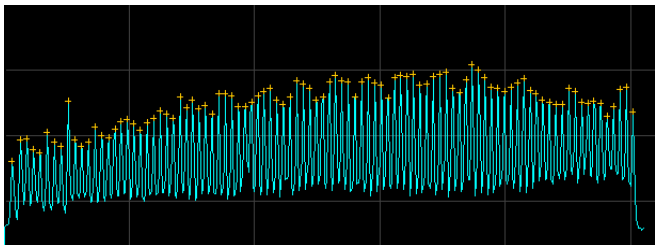
Evaluating the Calibration Data

Note: Examples of passing spatial calibration profiles start on page 37.

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

   Are the peaks in the profile approximately the same height?

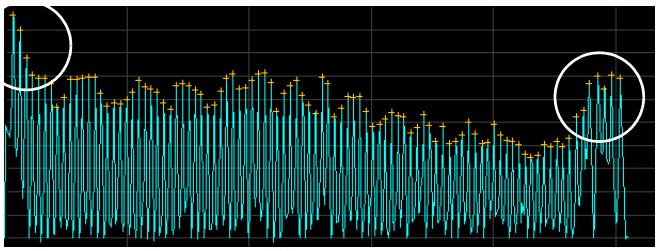
   Yes – Go to step 2 on page 35.



   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.

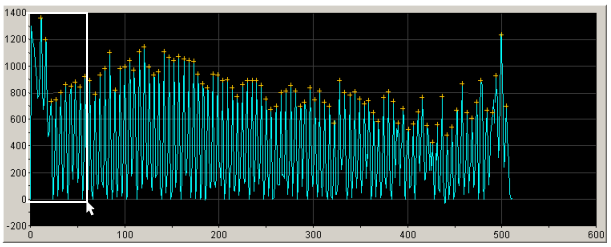
     Go to step 2 on page 35.



   Irregular – If the peak heights are irregular, go to "If the Calibration Fails" in the *Maintenance and Troubleshooting Guide* (Part no. 4477797).

Magnifying the Spatial Profile

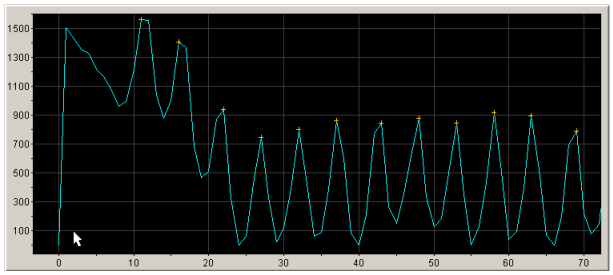
a. Click and drag the cursor to create a box around the area of interest.



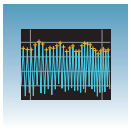
b. Release the mouse button.

   The Data Collection Software displays the selected region.

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



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:

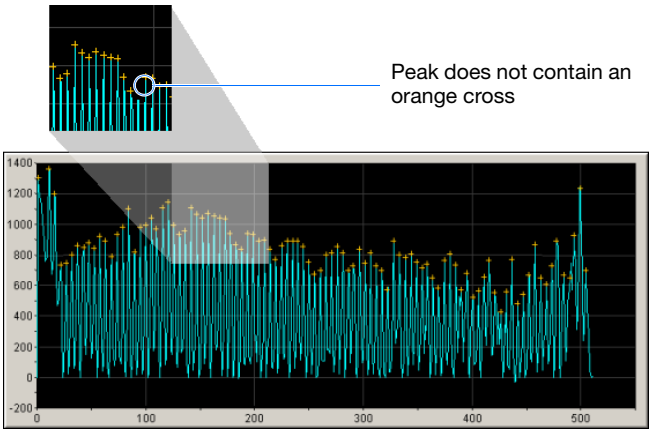
  If using a 96-capillary array, a small peak may appear in the left side of the profile.

  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* (Part no. 4477797).



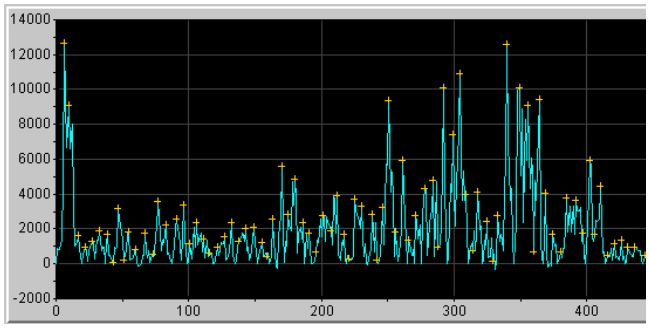
Elements of a poor spatial

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* (Part no. 4477797).

No – Go to step 4.

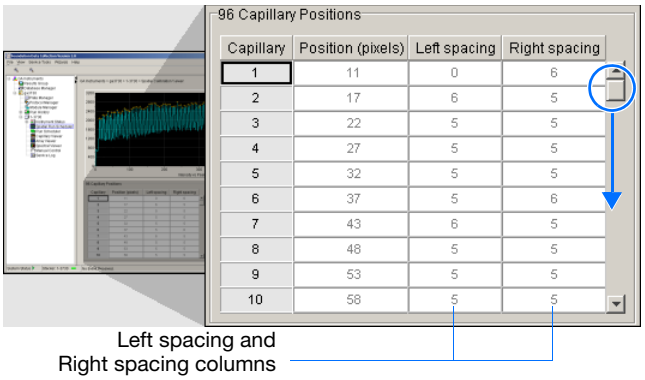


Notes

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

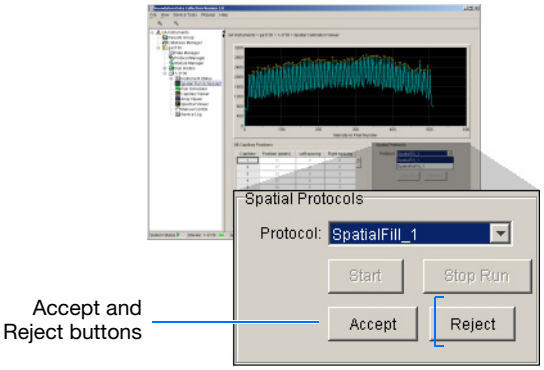
   Note: Values greater than those stated above are acceptable if you are able to see a corresponding gap in the capillaries in the detection cell.

   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.

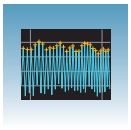


5. Accept or reject the spatial calibration as follows:

   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* (Part no. 4477797).

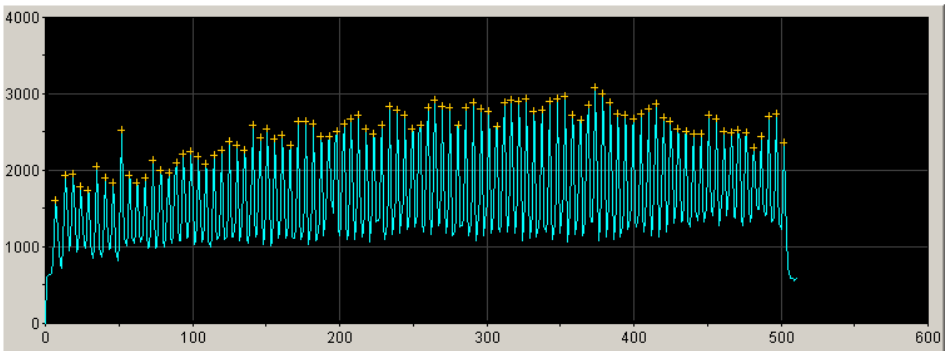


Notes



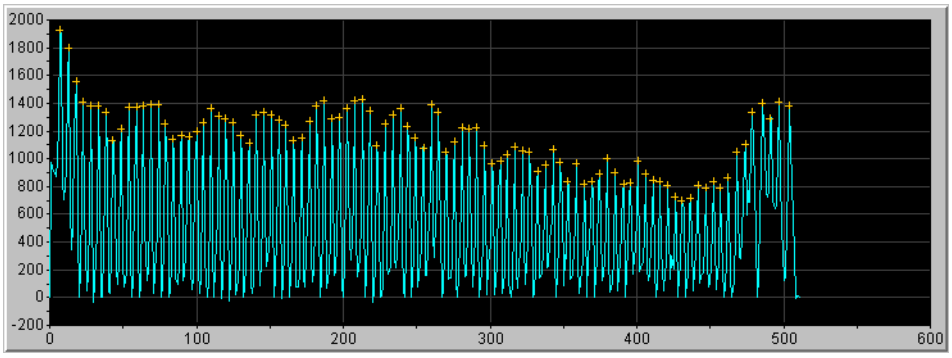
Examples of Passing Spatial Profiles

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

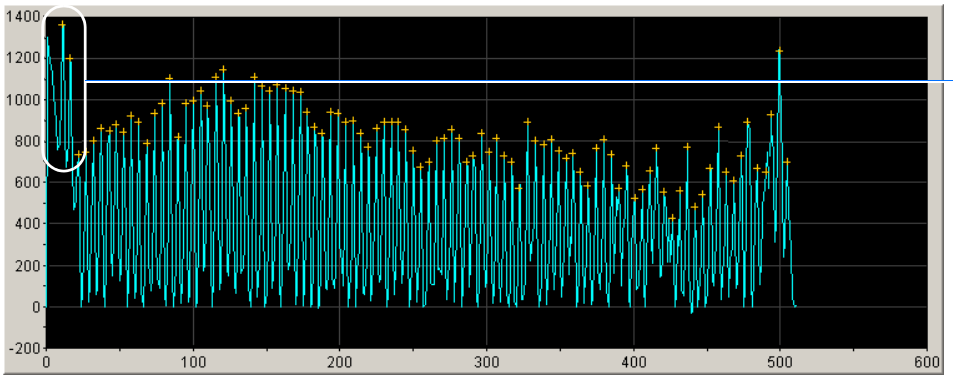


Passing Profile #1

This example shows a typical passing profile.



Passing Profile #2



Passing Profile #3

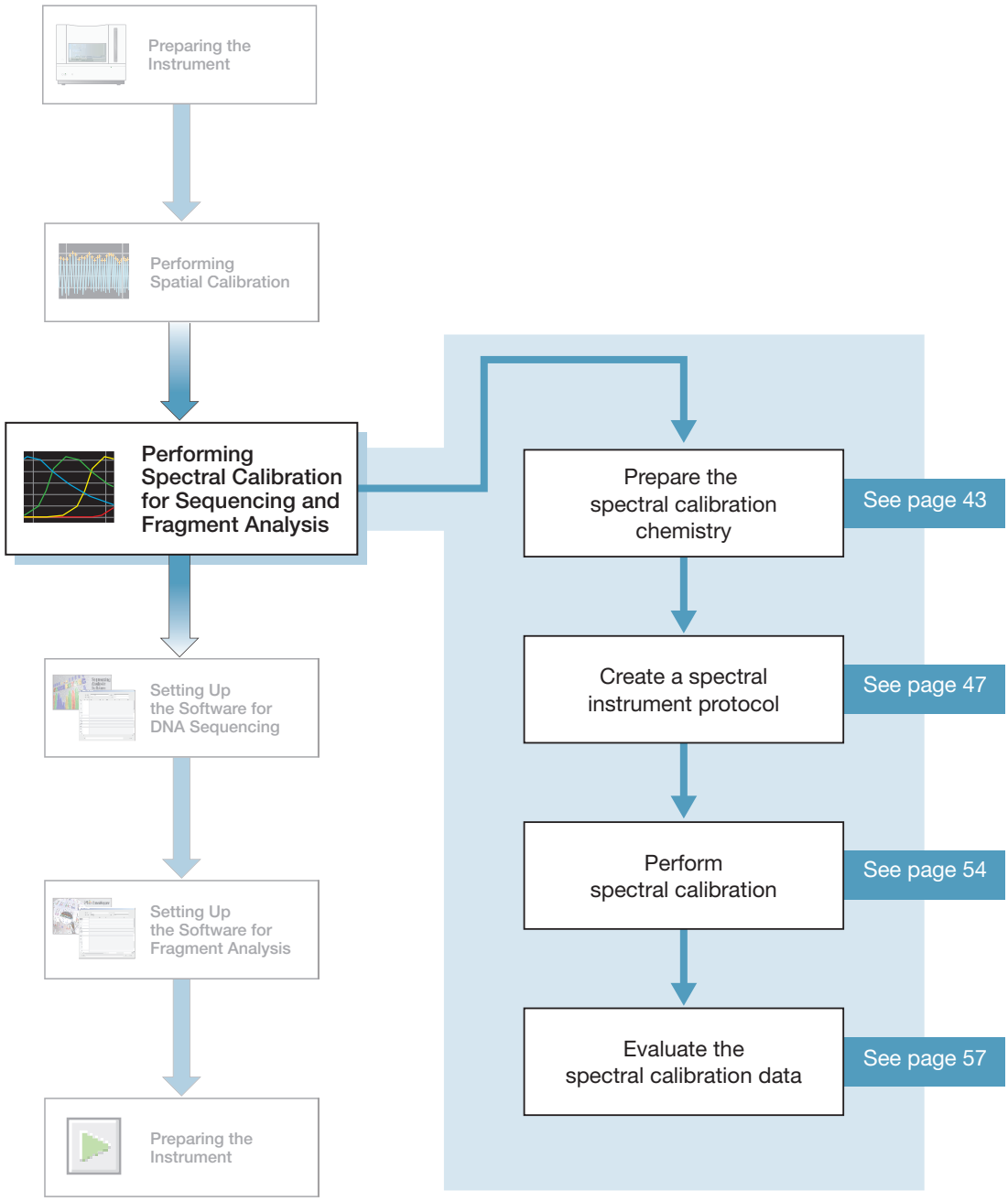
Background artifact

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

Notes

Notes

Performing Spectral Calibration For Sequencing and Fragment Analysis



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 43 and, "Dye Sets: G5, G5-RCT, Any4Dye, Any4Dye-HDR, and Any5Dye" on page 165.
- Array type (48- or 96-capillary)
- Array length (36- 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:

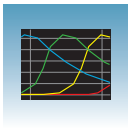
- Whenever you use a new dye set on the instrument
- After the laser or CCD camera has been realigned/replaced by a service engineer
- If you see a decrease in spectral separation (pull-up and/or pull-down peaks)
- If you alter any condition (dye set, array type, array length, or polymer type)

Note: Life Technologies recommends that you run a spectral calibration each time a new capillary array is installed. In the 3730 Series Data Collection Software, if you install an array that is the same length as the previously installed array, the active spectral calibration still persists. For optimal data quality, perform a new spectral calibration before you perform regular runs.

Changing Capillary Array Lengths

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

Notes



- 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 65 for information on how to switch calibrations.

Required Materials

Catalog numbers are located in Appendix Aon page 155.

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.

Notes

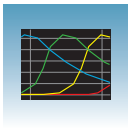
Select Dye Sets and Calibration Standards

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

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

Fragment Analysis Chemistry	Dye Set	Calibration Standards
Fragment Analysis	G5	DS-33
Fragment Analysis	G5-RCT	DS-33
SNaPshot® Multiplex Kit	Any5Dye	DS-02

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 SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

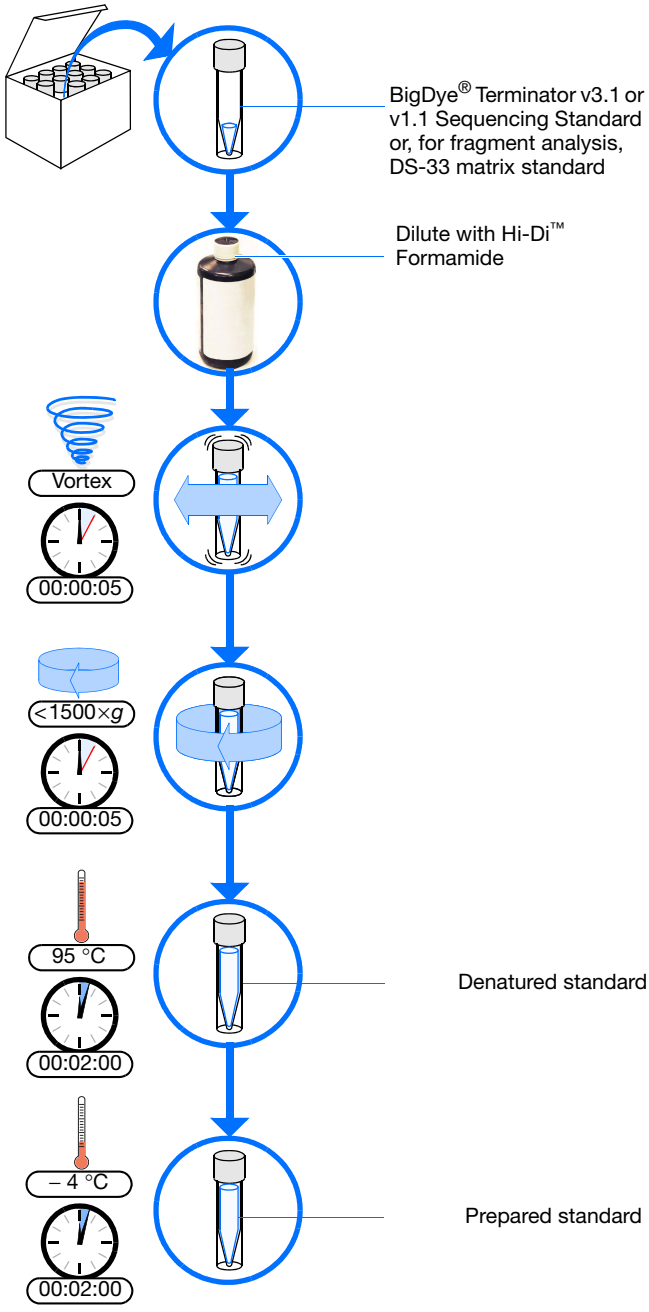
1. Dilute the spectral calibration standard with Hi-Di™ Formamide according to the insert instructions.

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

6. Vortex thoroughly and then briefly centrifuge the mixture.

Sealing and Preparing the Plate Assemblies

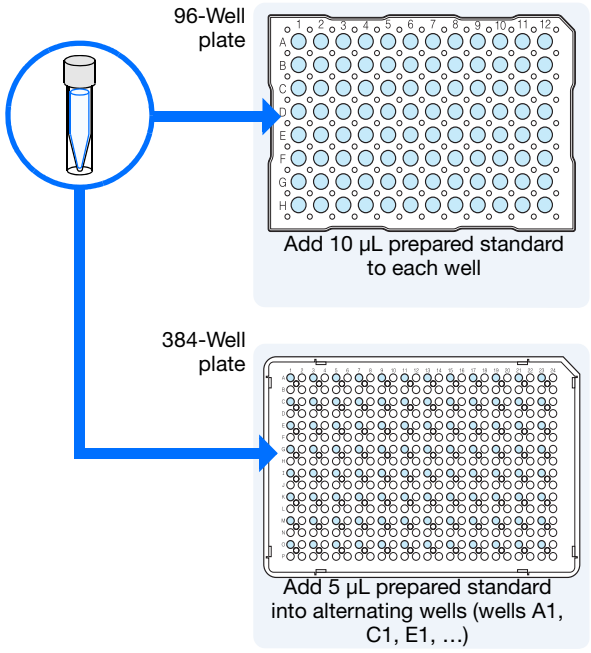
WARNING **WARNING** Do not use warped or damaged plates.

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

   If using a:

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

     See page 137 for load maps.



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

   With a septum:

   a. Inspect the septa and be sure to replace any that are worn or discolored.

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

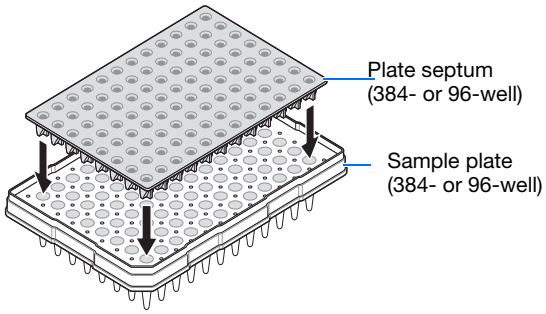
   c. Lay the septum flat on the plate.

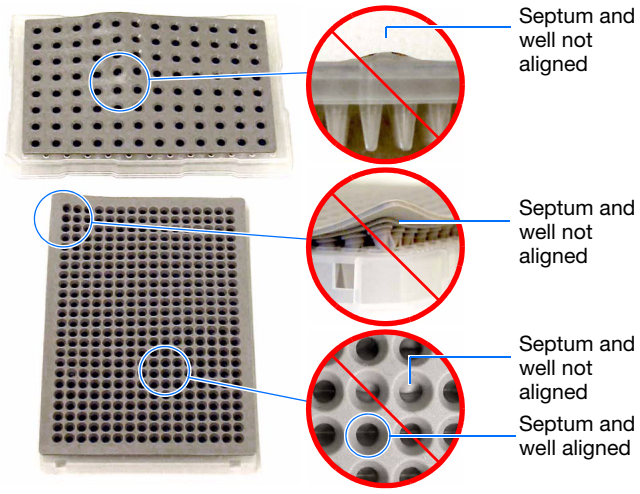
   d. Align the holes in the septum strip with the wells of the plate, then firmly press downward onto the plate. Ensure that:

      - 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.

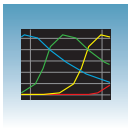
   With heat-seal:

   a. Follow your thermal sealer instrument instructions.

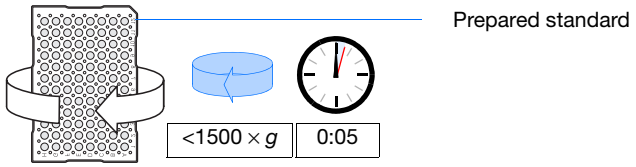




Notes

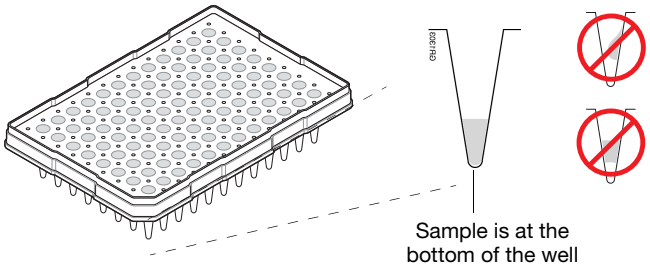


3. Briefly centrifuge the plate.



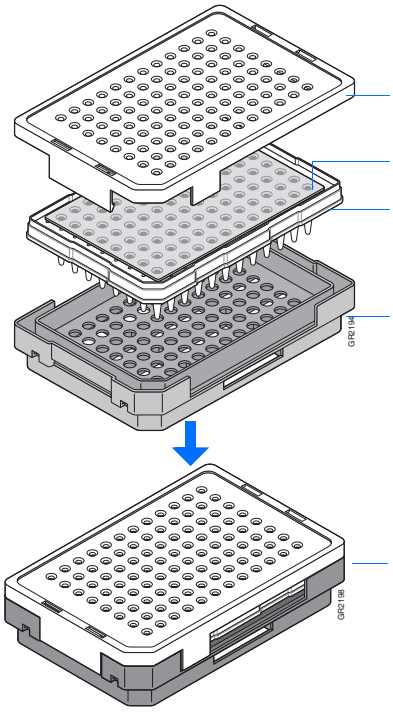
4. Remove the plate from the centrifuge and verify that each sample is positioned correctly in the bottom of its well.

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



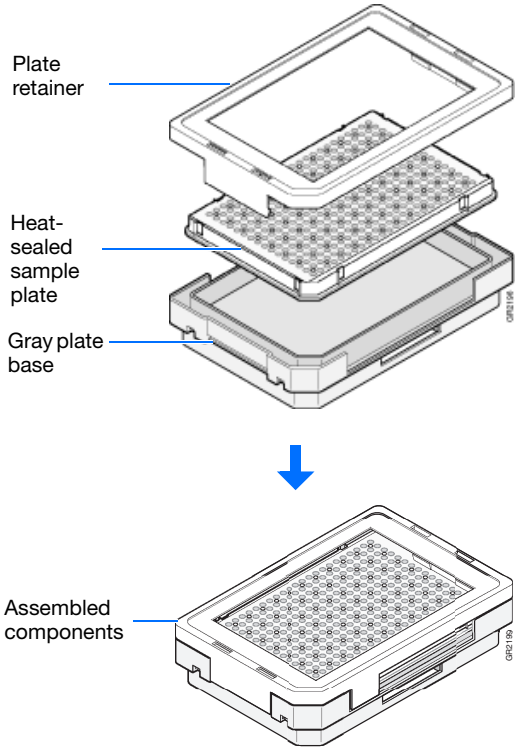
5. Assemble the plate assembly as shown in the following figures (see Appendix A, "Catalog List," on page 163 for catalog numbers).

Septum Assembly



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.

Heat-Sealed Assembly



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.

7. Make sure when you assemble a plate that the retainer clip is flush with the plate base. A simple way to ensure that they are flush is to run your finger along the edge.

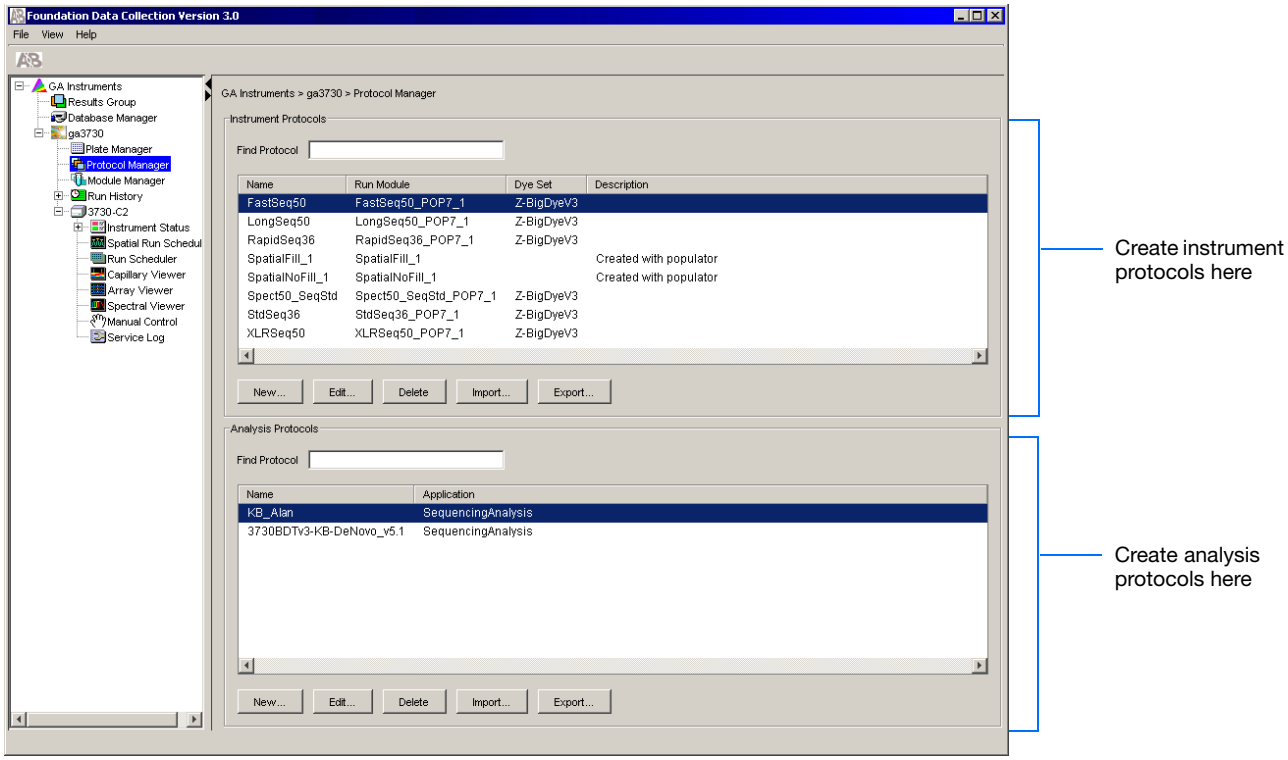
Important Heat Seal Recommendations

- Use 3-mil Life Technologies heat seal film (Cat. no. 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 3730/3730*xl* DNA Analyzer.
- Do *not* use heat-seal film containing adhesives or metals as these may damage the instrument's piercing needles.

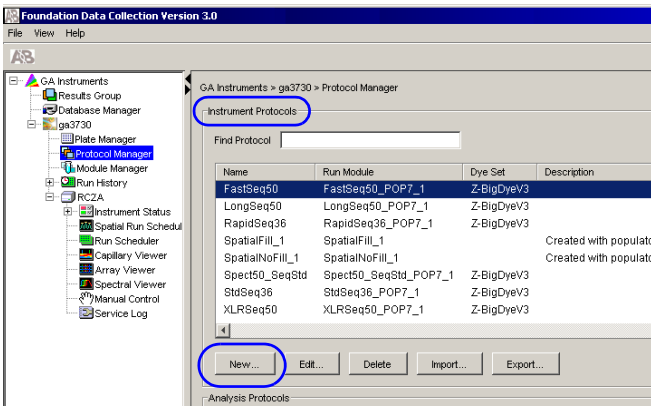
Notes

Creating a Spectral Instrument Protocol

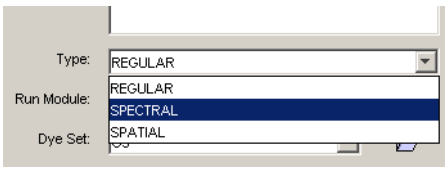
1. In the navigation pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **Protocol Manager**.



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



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



Notes

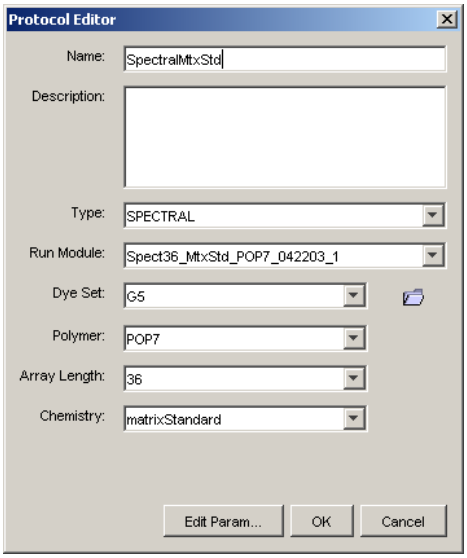
4. The Protocol Editor now displays additional drop-down lists. Select from the following:

   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**

   or

   - For a 50-cm capillary array, use:
     - Run module: **Spect50_MtxStd_POP-7™_1**
     - Chemistry: **matrixStandard**



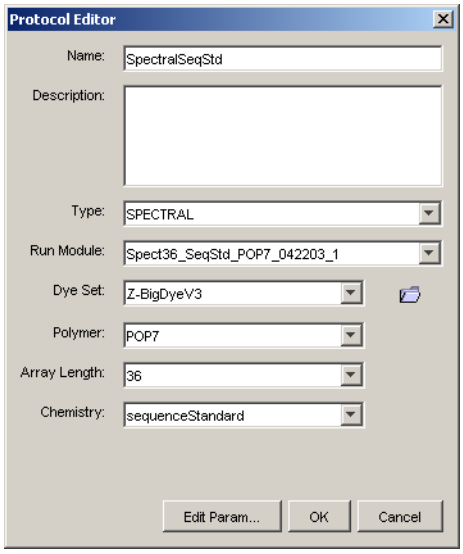
IMPORTANT! The array length you select must match the array length information from the Install Array wizard.

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**

or

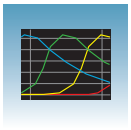
- For a 50-cm capillary array, use:
  - Run module: **Spect50_SeqStd**
  - Chemistry: **sequenceStandard**



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.

Notes



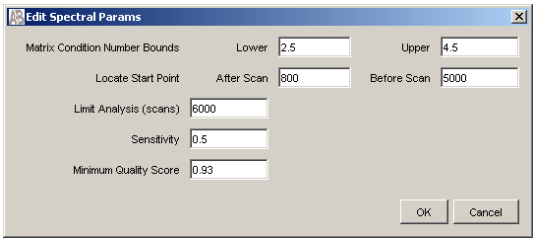
Use the following table to select the correct chemistry file for the spectral calibration samples you use.

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: 1–10	Upper: 3–20
Locate Start Point	After Scan: 100–5000	Before Scan: 100–5000
Limit Analysis (scans)	400–20,000	
Sensitivity	0–0.9	
Minimum Quality Score	0.80–0.99	

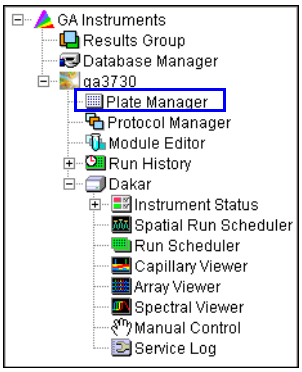
† These ranges are dye-set independent

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

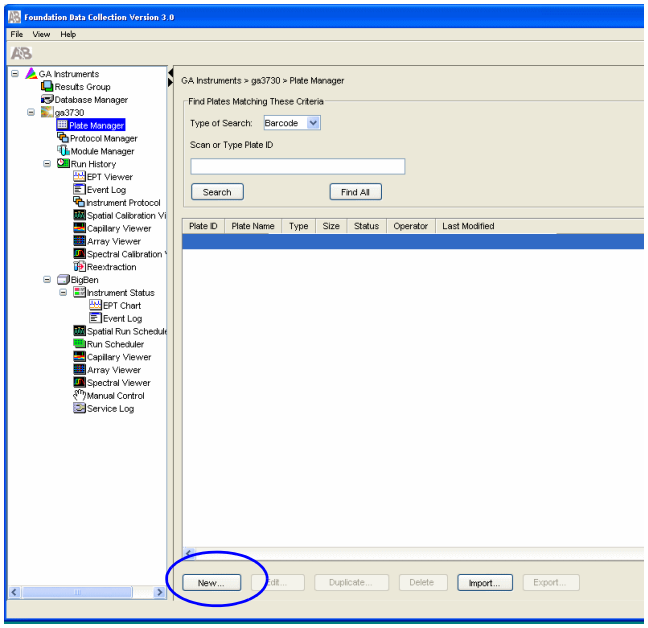
Notes

Creating a Spectral Calibration Plate Record

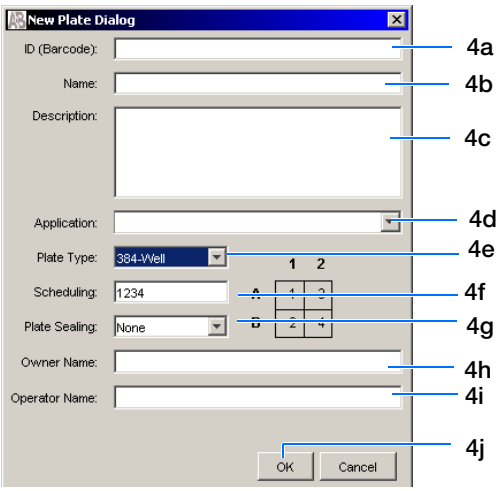
1. In the navigation pane of the Data Collection Software, double-click **GA Instruments** > **ga3730** > **instrument name** > **Plate Manager**.



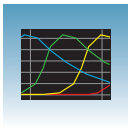
2. Click **New** to create a new plate.



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 135.



Notes



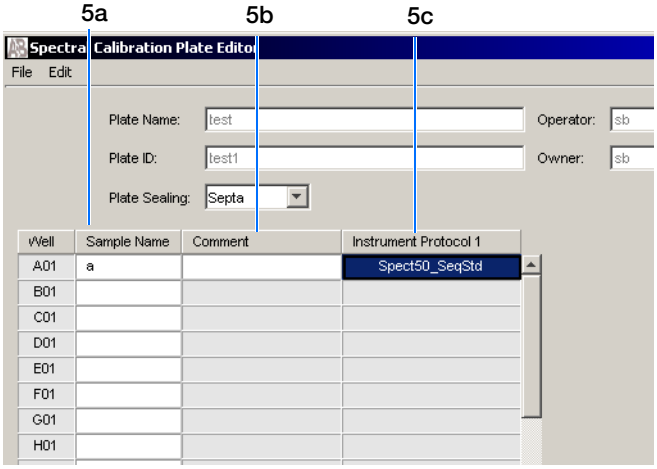
g. In the Plate Sealing drop-down list, select **Septa** or **Heat Seal**.

h. Enter a name for the owner.

i. Enter a name for the operator.

j. Click OK.

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



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

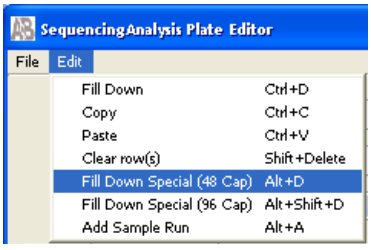
a. In the Sample Name column of row A01, enter a sample name, then click the next cell.

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)**

Notes

7. Click OK.

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

Note: If multiple cells are selected for copying, select the same number of corresponding target cells before you execute the Paste command.

Note: The Plate Editor Copy and Paste functionality is supported only within one plate editor. To copy and paste the contents of one plate to another plate, use the "Duplicate..." button on the Plate Manager dialog box.

Note: If you use the duplicate plate function, all the information in the plate to be duplicated must be valid. Otherwise, an empty plate is created.

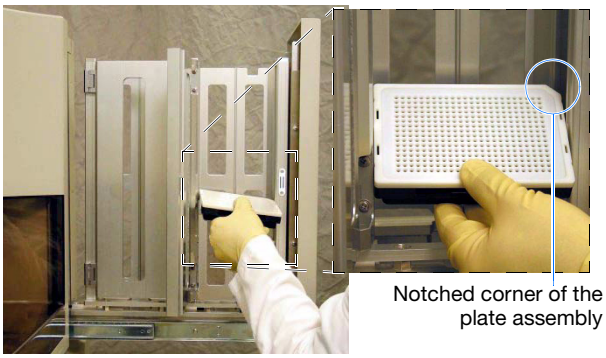
Notes

Loading the Plate into the Instrument

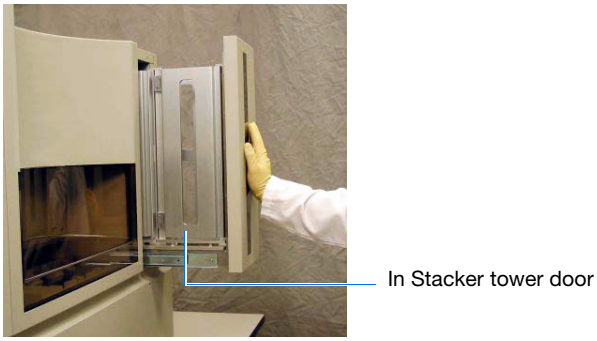
1. The name of the plate record you just created is displayed in the Input Stack window of the Data Collection Software, and is ready to run.
2. Open the stacker drawer.
3. Open the In Stack tower door.

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.



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

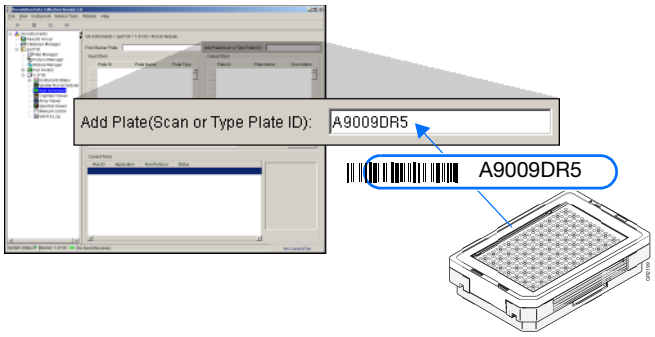


Notes

Running the Spectral Calibration Plate

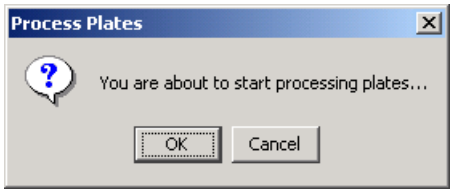
1. In the navigation pane of the Data Collection Software, double-click
   GA Instruments > **ga3730** > **instrument name** > **Run Scheduler**.

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 OK.

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.

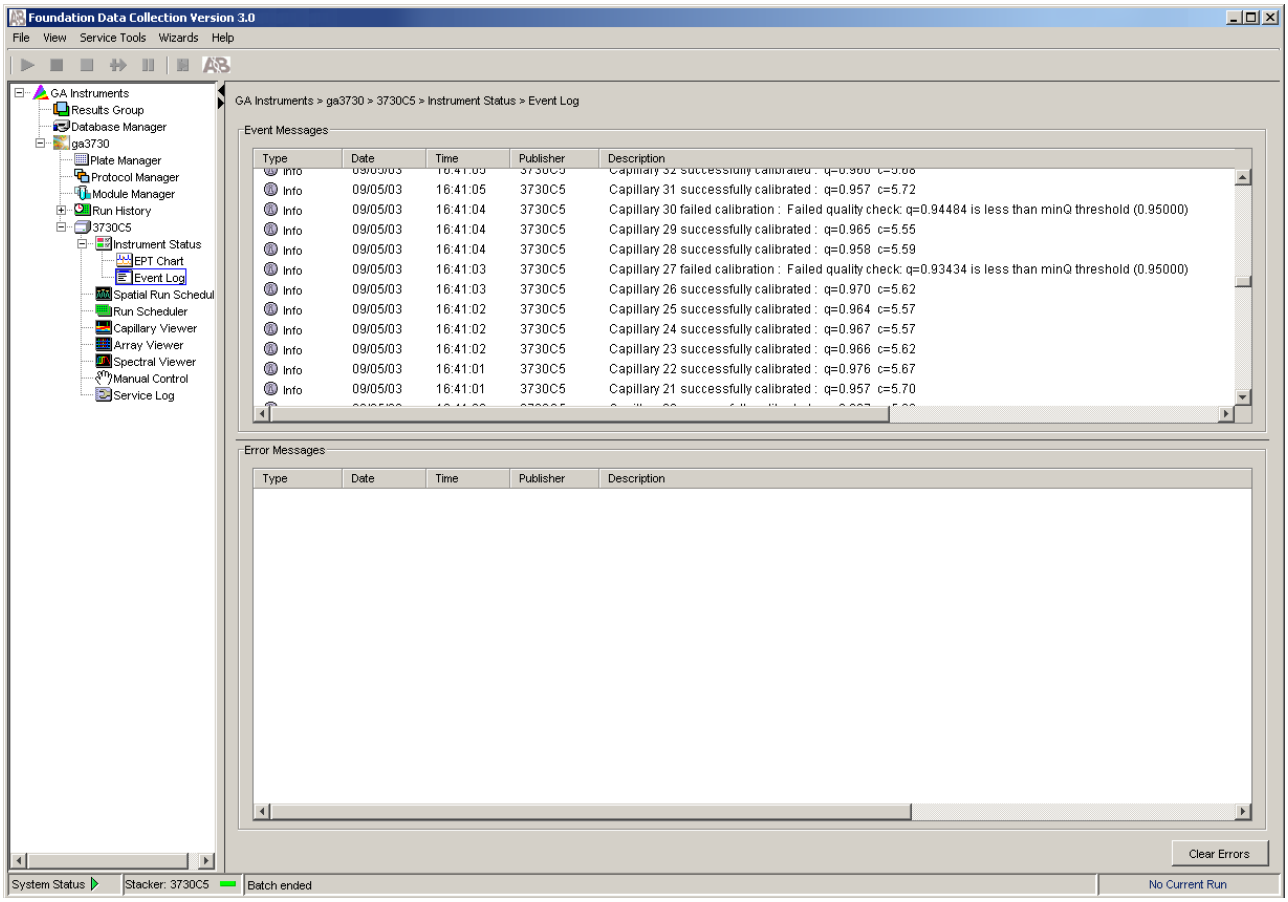
6. When the run is finished, remove the plate from the instrument.

Notes

Viewing the Pass/Fail Status After the Run

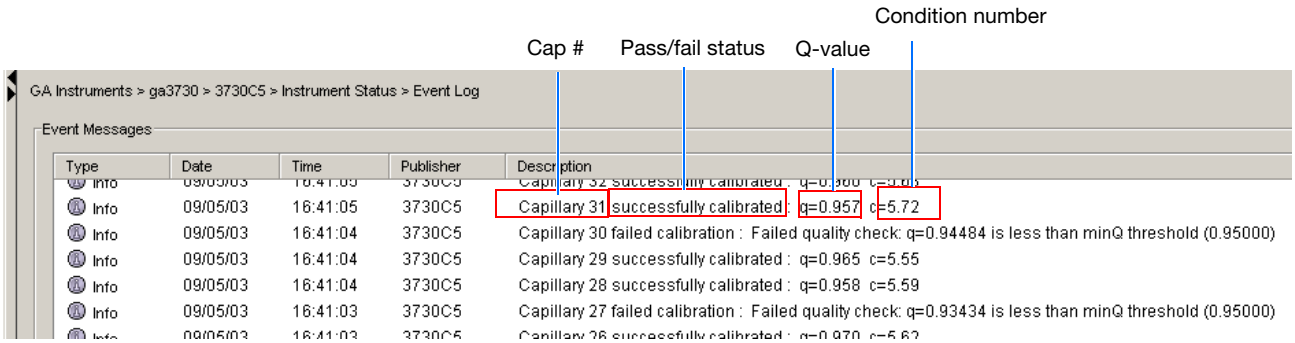
After the instrument completes the spectral calibration run, the pass or fail status of each capillary is recorded in the Events Messages section of the Instrument Status window.

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



Notes

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



Dye set G5 status results

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

- Q-value:
  - > 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	2.5–4.5
E_BigDyeV1	3.0–5
Fragment Analysis	
G5	9.5–14.5
G5-RCT	9.5–14.5

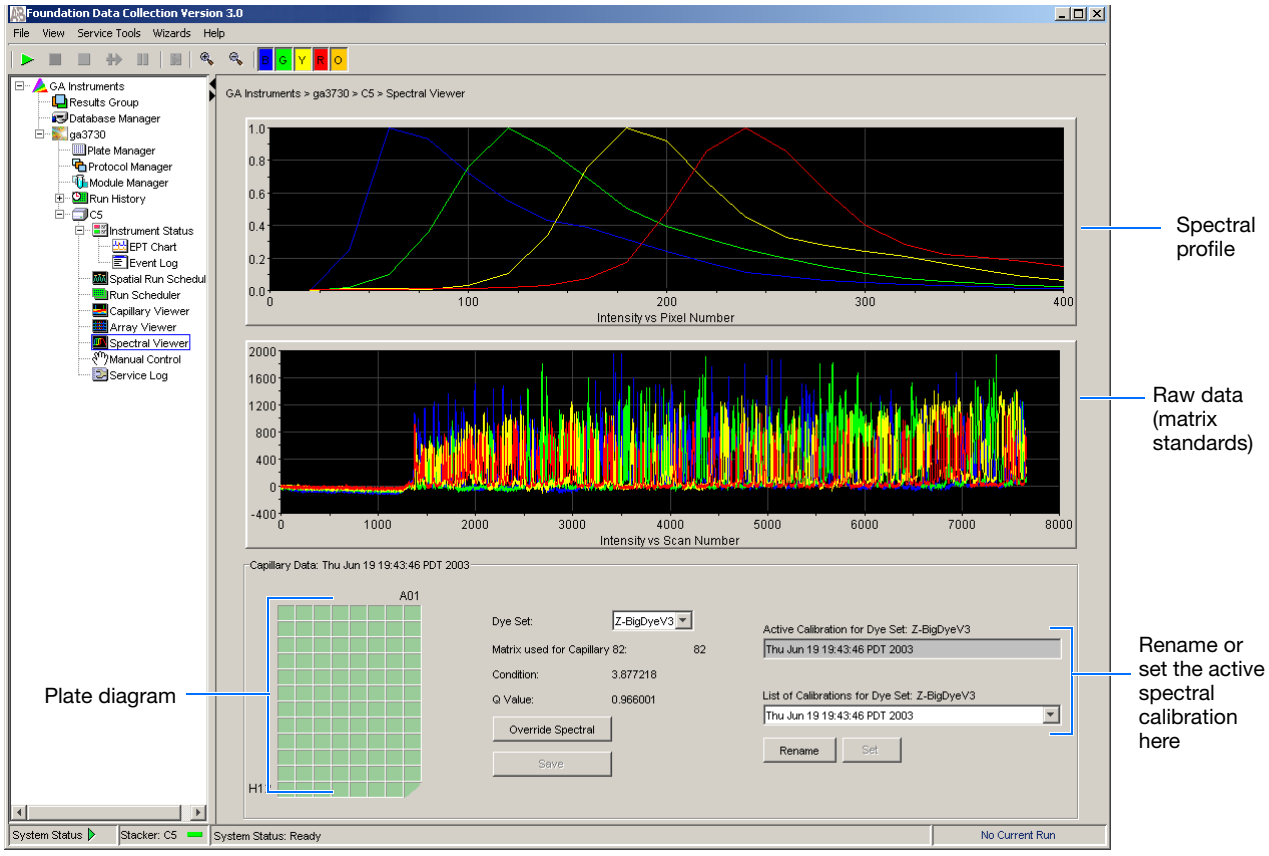
Notes

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.

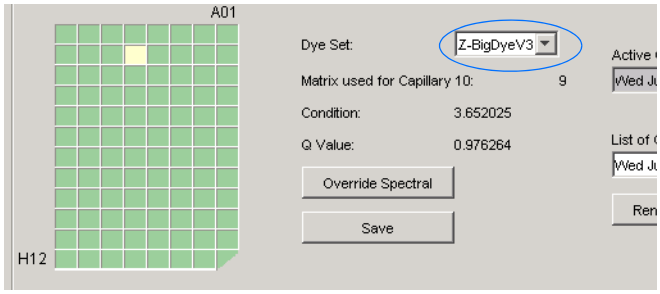
Note: Pages 61 and 62 contain examples of passing sequencing spectral calibration profiles, and page 63 contains an example of a passing fragment analysis spectral calibration profile.

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

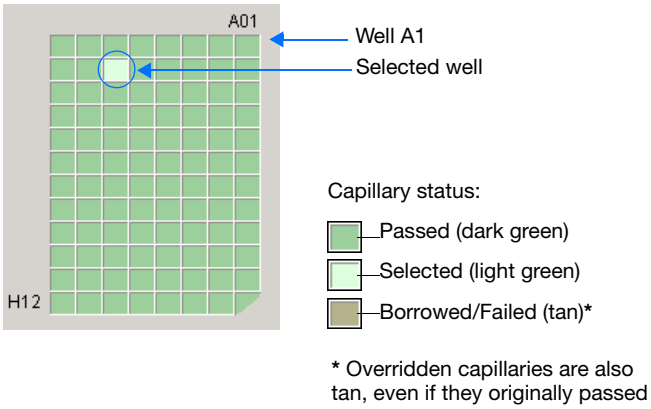


Notes

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



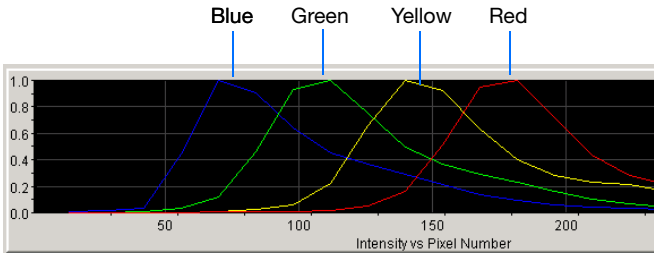
3. Select a well on the plate diagram to view the spectral results of the associated capillary.



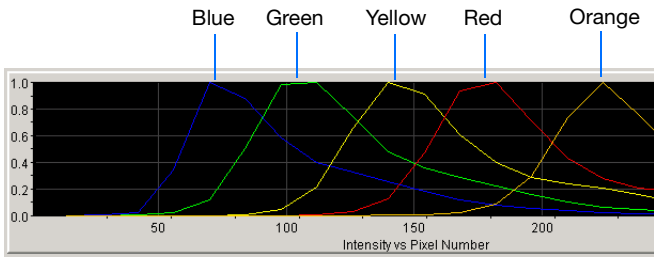
4. Evaluate the spectral calibration profile for the selected capillary:

   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 a.
   - The calibration run has failed–go to page 67.

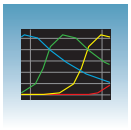


Example of a 4-dye spectral calibration profile

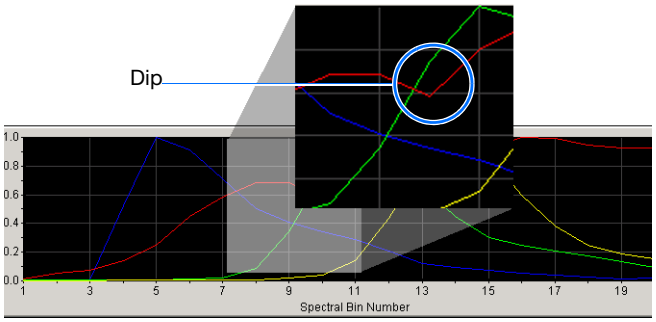


Example of a 5-dye spectral calibration profile

Notes



a. 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 60).



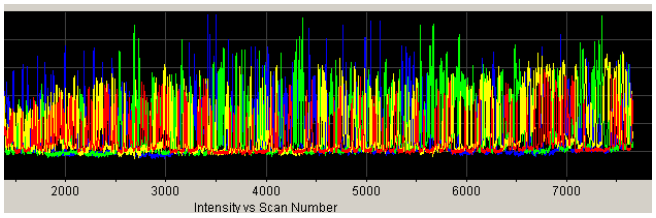
If the peaks in the spectral profile are:

- Separate and distinct–the capillary has passed. Go to step 5.
- Not separate and distinct–the calibration run has failed. Go to page 67.

a. 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



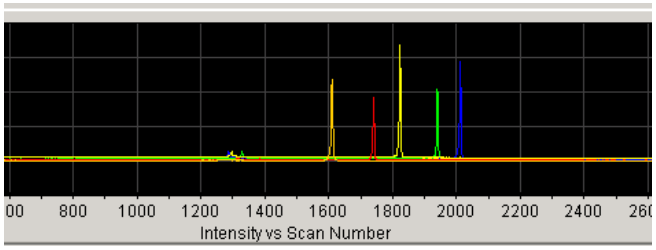
Example of a 4-dye sequencing raw data profile

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 67.

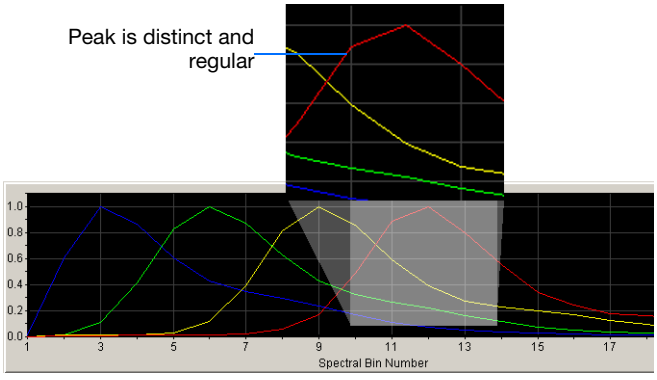
No: Go to step 5.

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



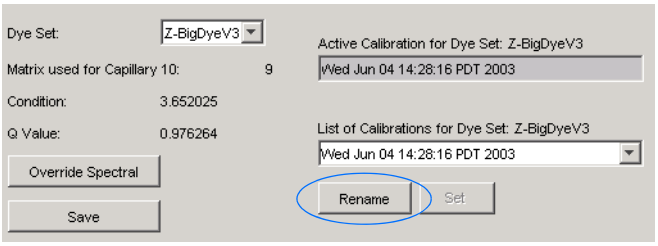
Example of a 5-dye fragment analysis raw data profile

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



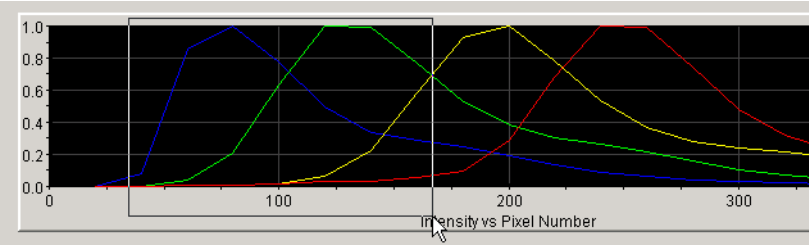
Notes

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



Tip: Magnifying the Spectral Profile

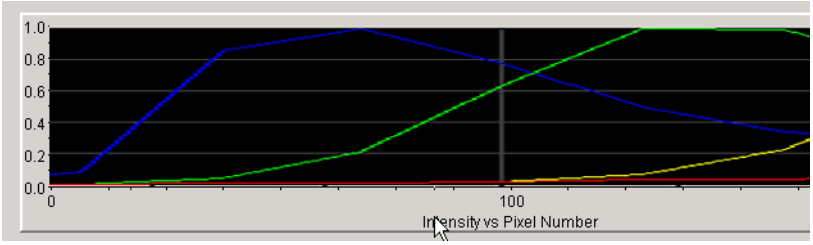
1. In the navigation pane of the Data Collection Software, click **GA Instruments** > **ga3730** > **instrument name** > **Spectral Viewer**.
2. In the profile or raw data display, click-drag the cursor to create a box around the area of interest.



Selecting an area to magnify in a spectral profile

3. Release the mouse button.

   The Data Collection Software displays the selected region.
4. Press **R** to reset the view.

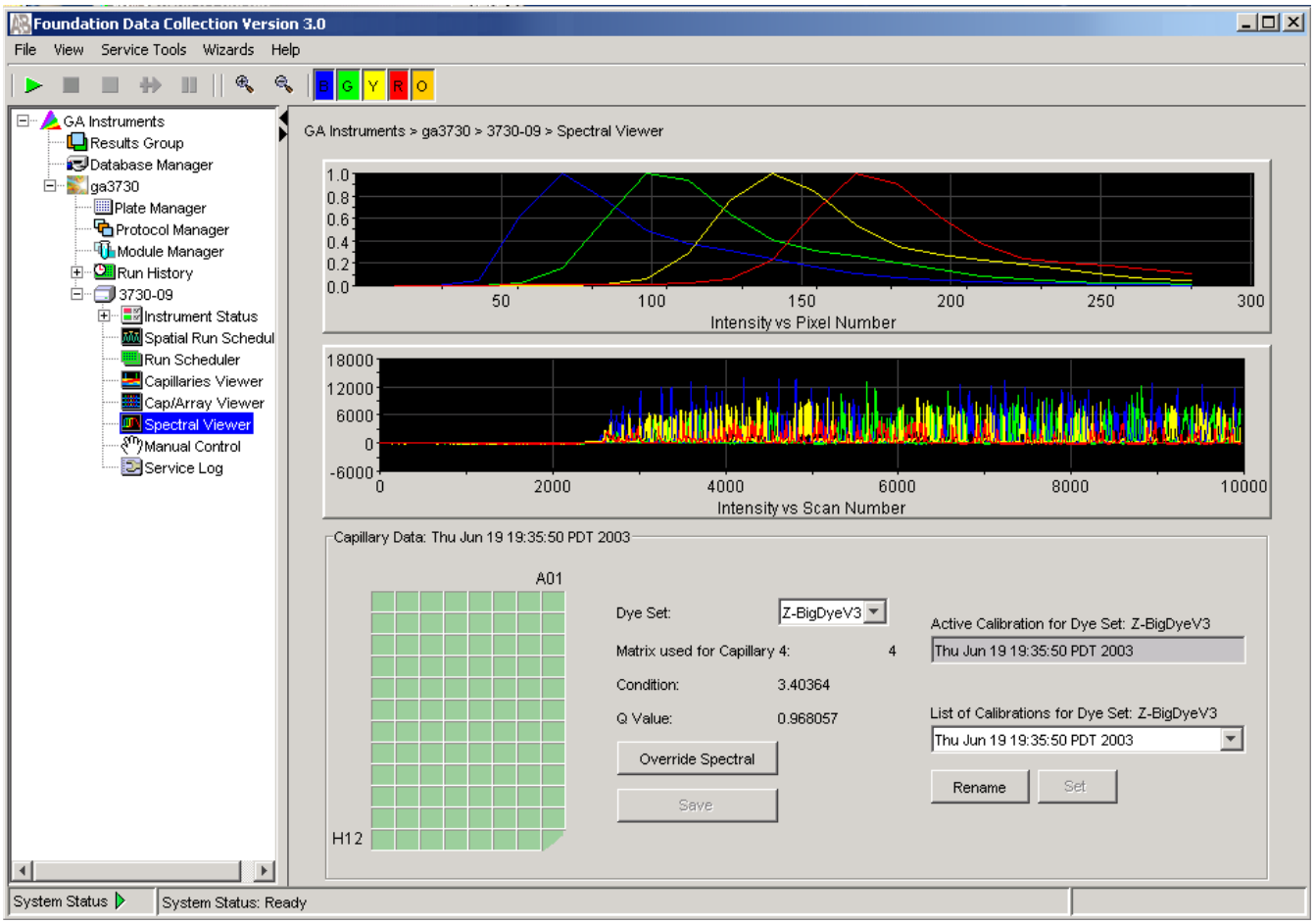


Magnified area of that spectral profile

Notes

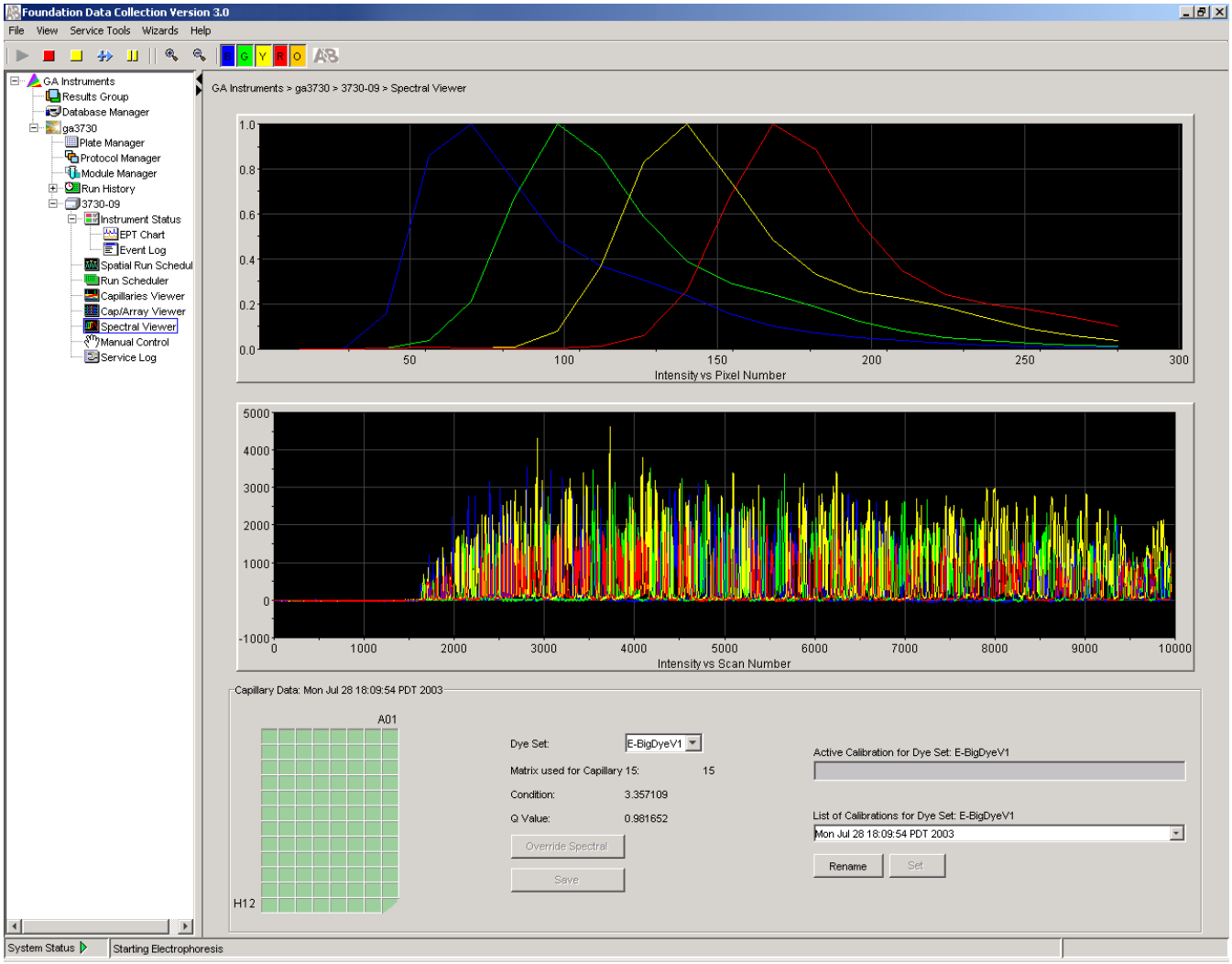
Examples of Passing Sequencing Spectral Calibrations

Dye Set Z Created from a Sequencing Standard



Notes

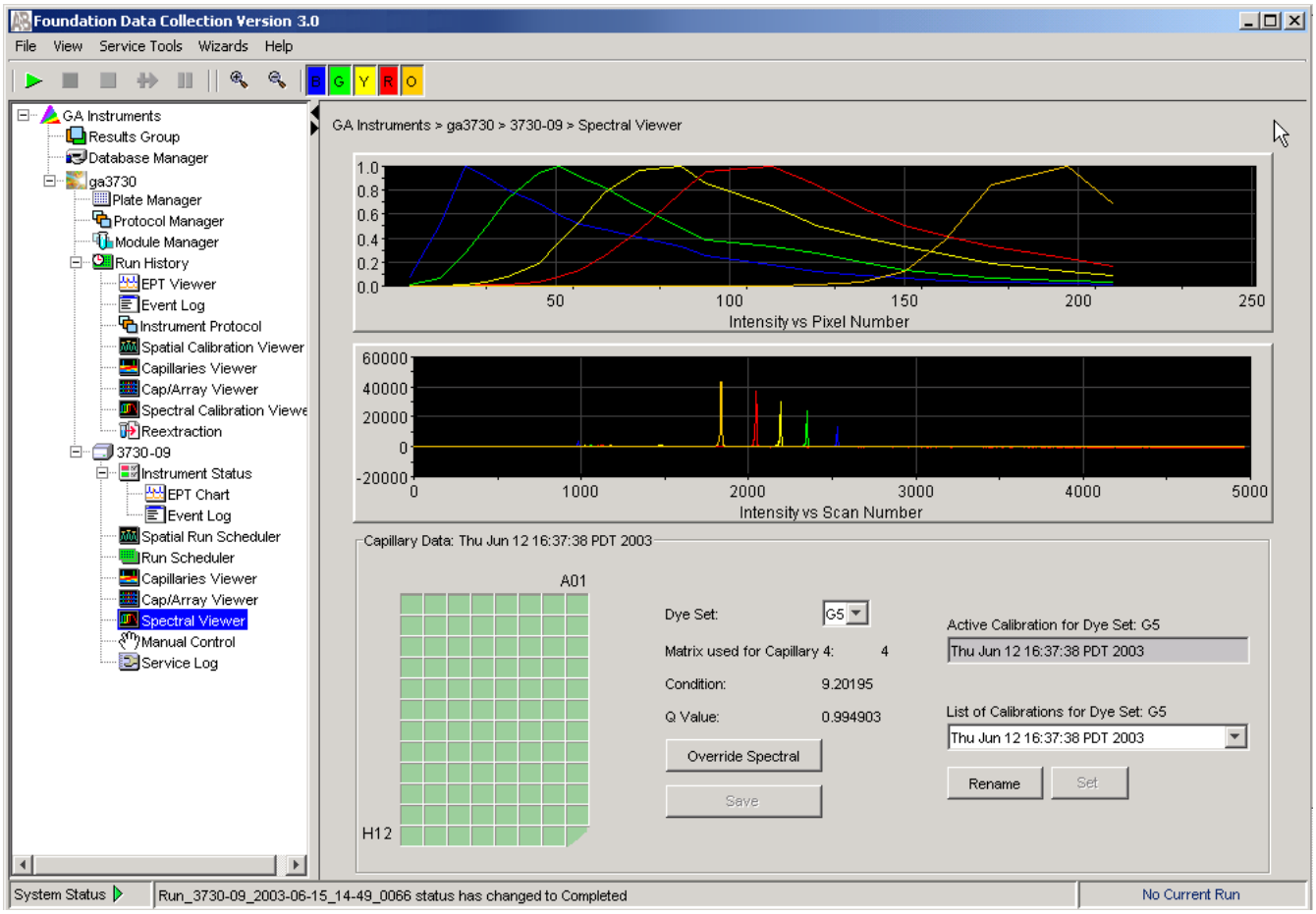
Dye Set E Created from a Sequencing Standard



Notes

Example of a Passing Fragment Analysis Spectral Calibration

Dye Set G5 Created from Matrix Standard Set DS-33



Notes

Spectral Viewer

Selecting Active Spectral Calibrations

For best quality data, Life Technologies 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! When you install an array that is a different length or type (48 vs. 96) from what you were using previously, all spectral calibrations are voided. If a previous spectral calibration for the new array/new condition does not exist, you must run a new spectral calibration. If a previous calibration exists, go to the Spectral Viewer and choose a past calibration to set as the active spectral calibration before you proceed with regular runs, even though spectral profiles are displayed; to do so, follow the directions described next, in "To select a previous spectral calibration:" on page 65.

IMPORTANT! You cannot link or run a plate unless the dye set used in the plate has been set in the Spectral Viewer. Furthermore, when a plate is running, the Set Active Spectral Calibration function is inactive. Spectral Calibrations can be set only during the idle or ready mode.

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, array length, and polymer type.

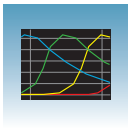
When a new *spatial* calibration is saved, the current spectral calibration for DyeSet G5-RCT is deactivated. Dye sets G5, E, and Z are not deactivated. If you wish to continue without a spectral recalibration, you can set an active spectral using the following instructions.

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.

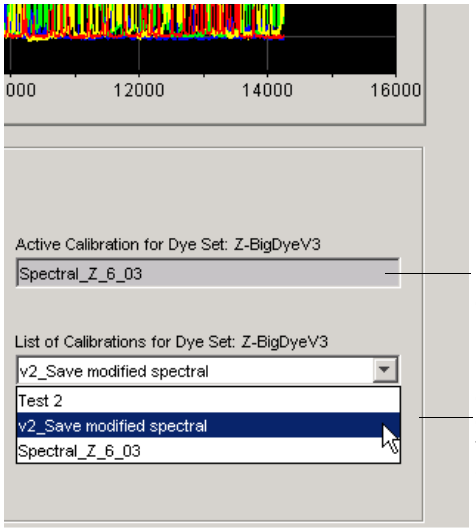
Notes



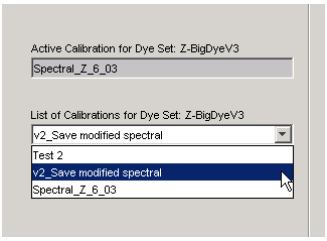
Because each dye set can have its own active calibration, there is no need to manually set the active calibration if you are performing runs with various dye sets.

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.

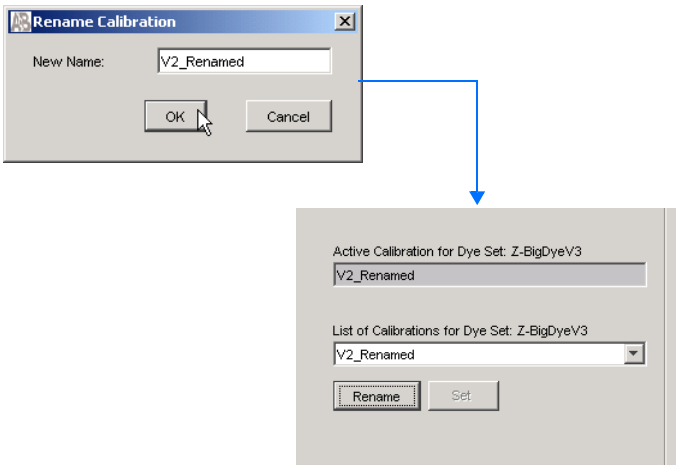


3. Select the spectral calibration you want to use for future runs.

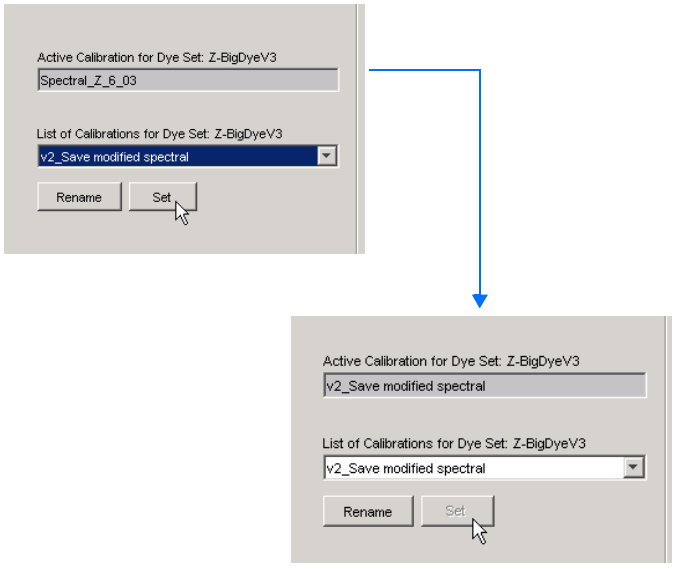


Notes

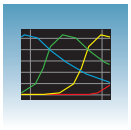
4. Click **Set** to display your chosen spectral calibration in the Active Calibration text box.



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



Notes

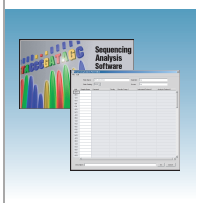


Troubleshooting

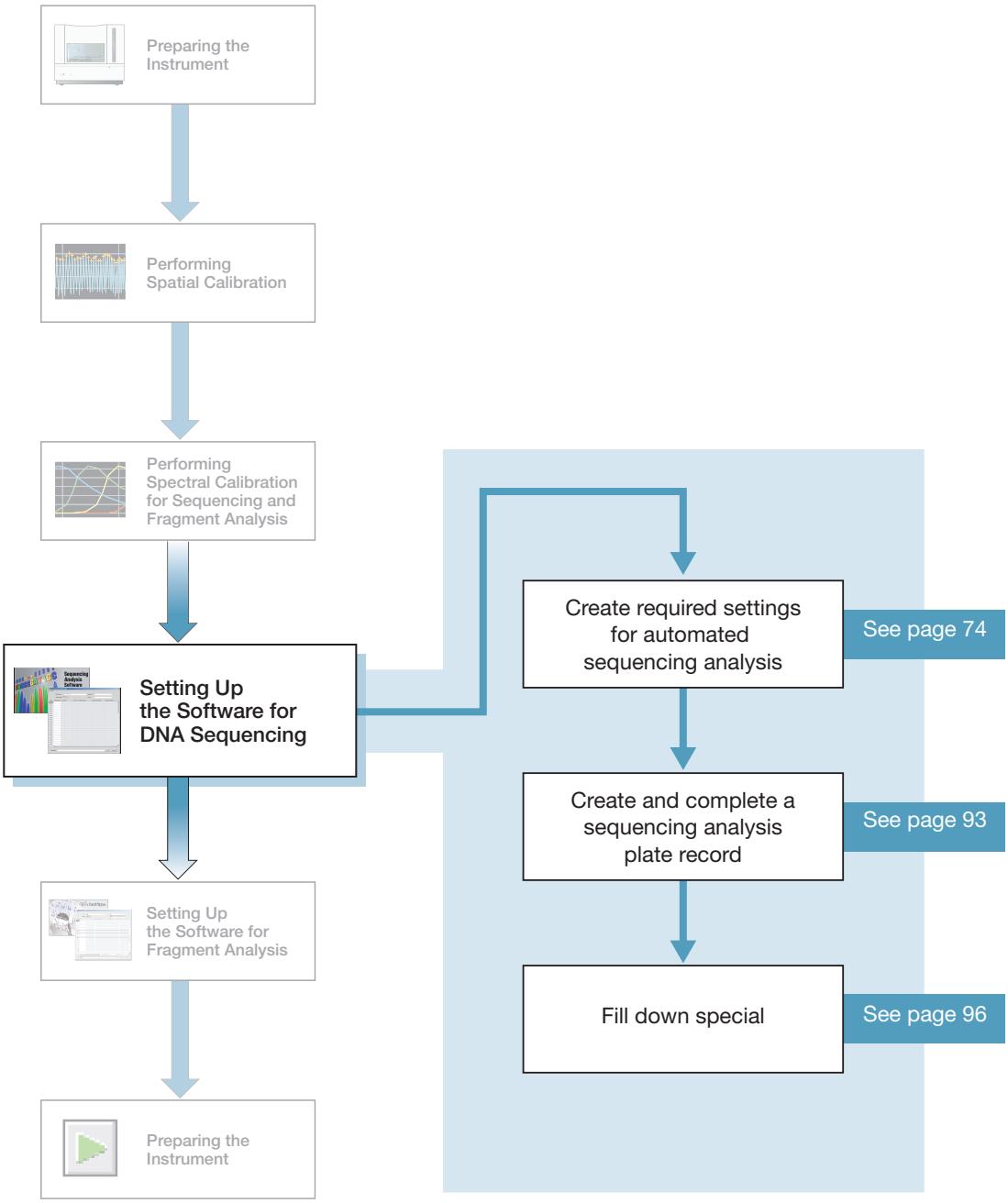
Troubleshooting spectral calibration		
Observation	**Possible Cause**	**Recommended Action**
No signal.	Incorrect sample preparation.	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide. **WARNING CHEMICAL HAZARD. Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Air bubbles in sample tray.	Centrifuge samples to remove air bubbles.
If the spectral calibration fails, or if a message displays “No candidate spectral files found”.	Clogged capillary.	Refill the capillaries using manual control. Look for clogged capillaries during capillary fill on the cathode side.
	Insufficient filling of array.	Check for broken capillaries and refill the capillary array.
	Expired spectral standards.	Check the expiration date and storage conditions of the spectral standards. If necessary, replace with a fresh lot.
Spikes in the data.	Expired polymer.	Replace the polymer with a fresh lot using the Change Polymer wizard. **WARNING CHEMICAL HAZARD. POP-7™ polymer** cause eye, skin, and respiratory tract irritation. Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Air bubbles, especially in the polymer.	• Refill the capillaries using the Bubble Remove wizard. • Properly bring the polymer to room temperature. • Replace expired polymer.
	Possible contaminant in the polymer.	Replace the polymer using the Change Polymer wizard.

Notes

Notes



Setting Up the Software for DNA Sequencing



Notes

Plate Records and Sequencing Analysis

Overview

A plate record is similar to a sample sheet or an injection list that you may have used with other Applied Biosystems® instruments. 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 87
- Mobility file (in Analysis Protocol), see page page 80
- 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 74
- Name of the Analysis Protocol (Analysis protocols specify how data is analyzed at the end of the run; see page page 80)

Important Notes

- A unique name must be assigned to the instrument computer before 3730 Series Data Collection Software is installed.
- Do not rename the computer once 3730 Series Data Collection Software has been installed. Doing so *will* cause the 3730 Series 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 (Autoanalysis by SeqScape® is no longer supported)

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.

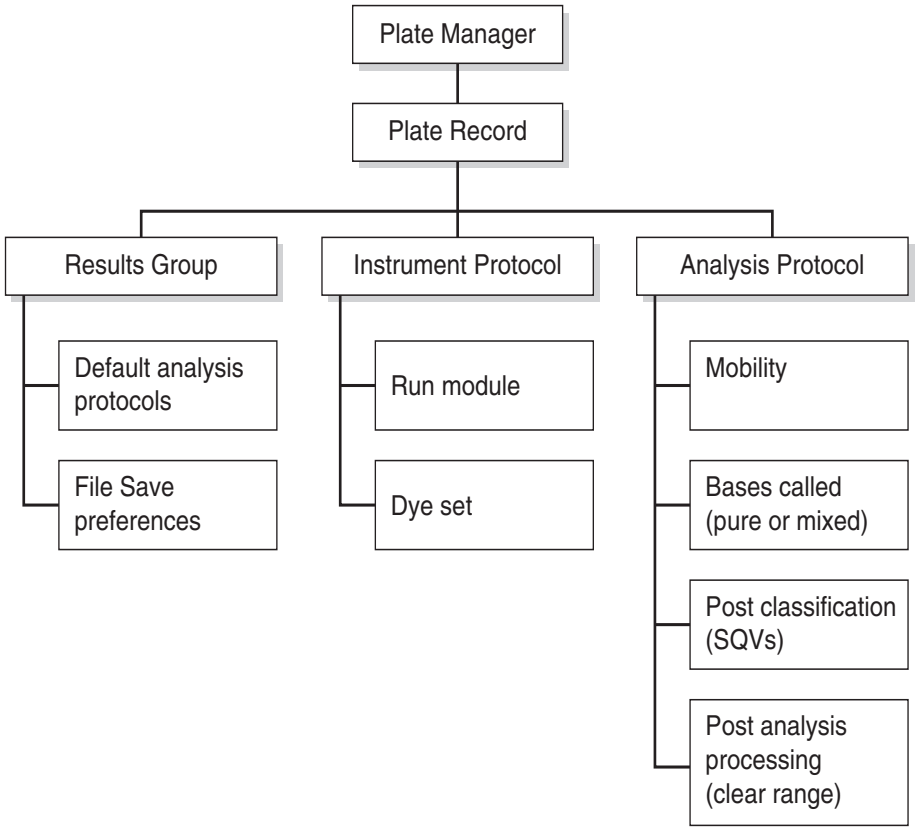
Notes

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 following table and flow chart describe what each file specifies.

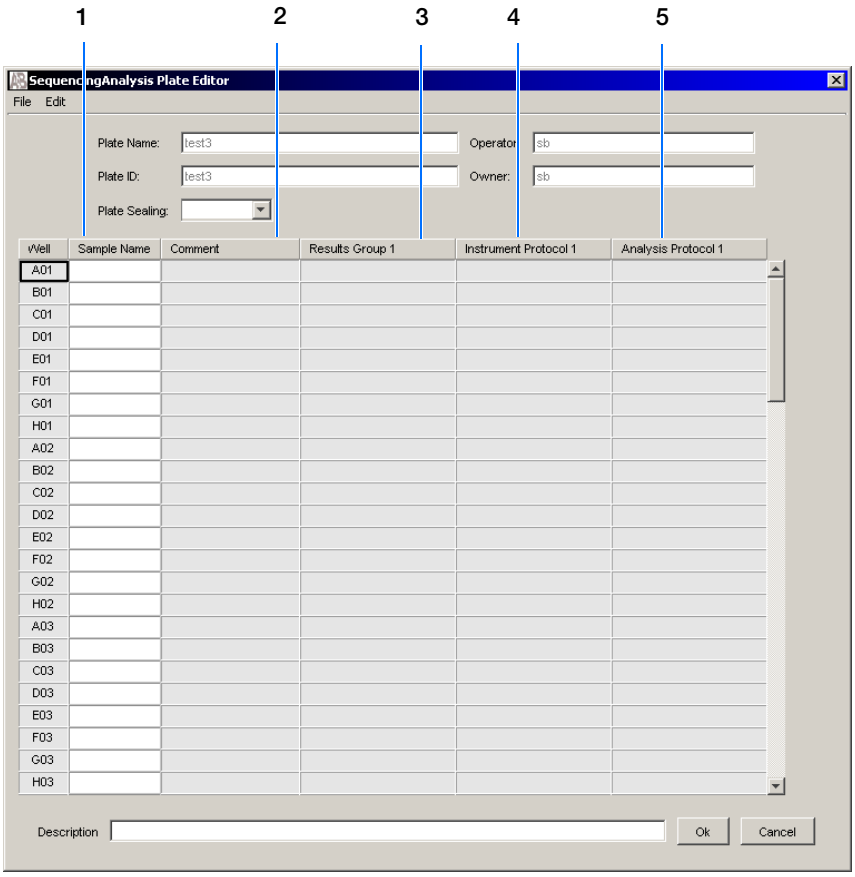
Parameters	Description	See Page
Instrument Protocol	Contains everything needed to run the instrument.	74
Analysis Protocol	Contains everything needed to analyze sequencing data.	79
Results Group	Defines the file type, the file name, file save locations, analysis software and autoanalysis by DNA Sequencing Analysis Software 6.	85



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. Autoanalysis by SeqScape® is no longer supported; use autoanalysis with DNA Sequencing Analysis Software 6.

Notes



Default is one sample run. To add additional runs, see page 95.

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	(*Optional*) Comments about the sample
(3.) Results Group	Options are: • New – Opens the Results Group Editor dialog box • Edit – Opens the Results Group Editor dialog box for the results group listed in the cell • None – Sets the cell to have no selected results group • Select one of the available results groups from the list **Note:** You must have a results group selected for each sample entered in the Sample Name column. See, "Results Groups" on page 85.

Notes

Name	Description
(4.)Instrument Protocol	• New–Opens the Protocol Editor dialog box. • Edit–Opens the Protocol Editor dialog box for the instrument protocol listed in the cell. • None–Sets the cell to have no selected protocol. • List of instrument protocols–In alphanumeric order. **Note:** You must have an Instrument Protocol selected for each sample entered in the Sample Name column. See, "Creating an Instrument Protocol" on page 74.
(5.) Analysis Protocol	• New–Opens the Analysis Protocol Editor dialog box. • Edit–Opens the Analysis Protocol Editor dialog box for the instrument protocol listed in the cell. • None–Sets the cell to have no selected protocol. • List of Analysis Protocols–In alphanumeric order **Note:** You must have an Analysis Protocol selected for each sample entered in the Sample Name column. See, "Creating an Analysis Protocol" on page 80.

Notes

Creating Required Settings for Automated Sequencing Analysis

If Settings Already Exist

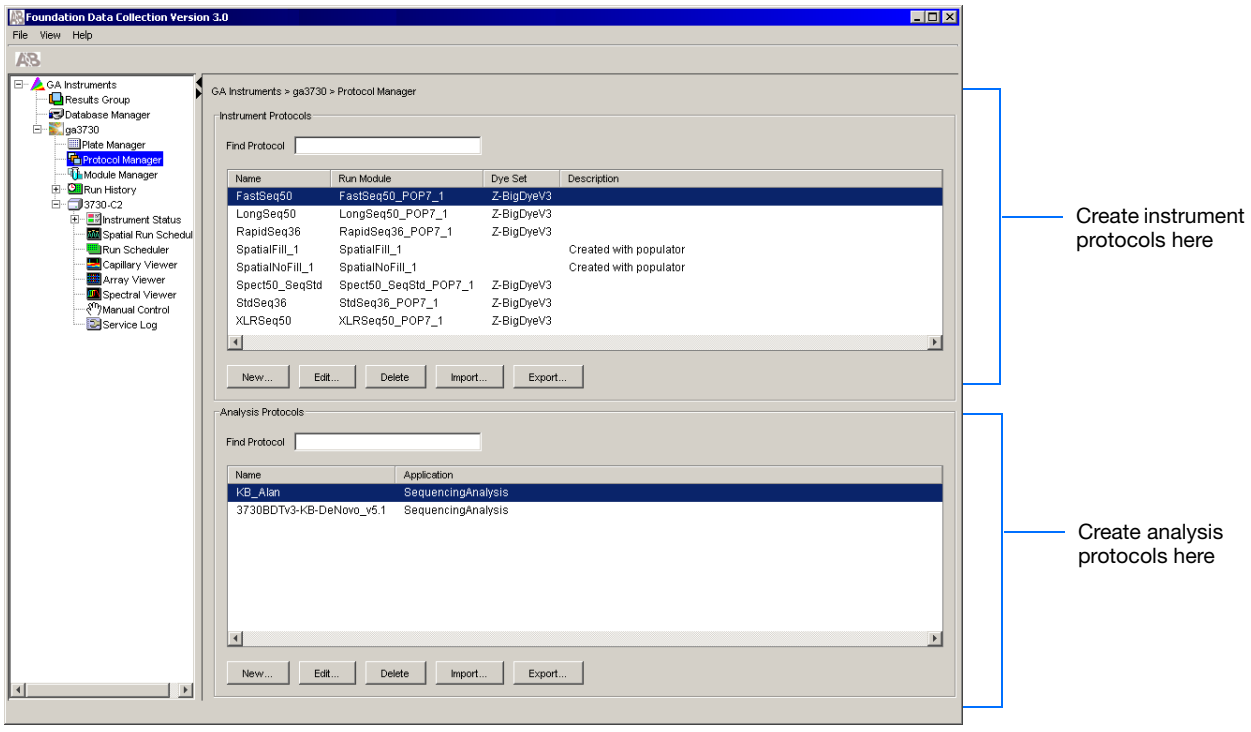
If the appropriate instrument protocol, analysis protocol, and results group have been created, proceed to "Creating and Completing a Sequencing Analysis Plate Record" on page 93.

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.

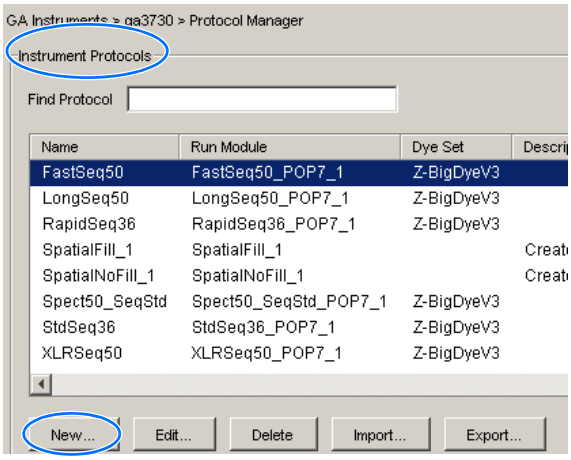
Creating an Instrument Protocol

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

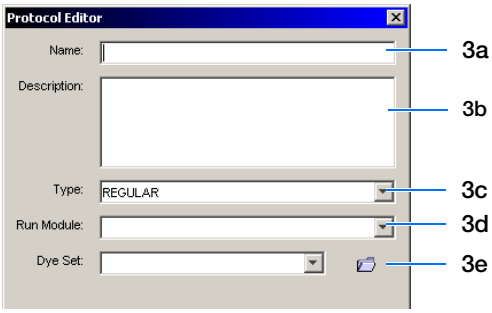


Notes

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. (*Optional*) Type a description for the protocol.
   c. Select **Regular** in the Type drop-down list.
   d. Using the information in the table below, select the correct run module for your run.



> **Note:** To customize a run module, see "Tip: Customizing Run Modules" on page 77.

Sequencing Run Modules	Capillary Array Length (cm)	Sequencing Run	Approximate Run Times† (min)	KB Basecaller QV20 LOR (Bases)§
XLRSeq50_POP-7™	50	Extra long read	180	900
LongSeq50_POP-7™	50	Long read	120	850
FastSeq50_POP-7™	50	Fast read	60	700
StdSeq36_POP-7™	36	Standard read	60	700
RapidSeq36_POP-7™	36	Rapid read	35	550
TargetSeq36_POP-7™	36	Short read	20‡	400‡
LongSeq50_POP-6™	50	Long read	150	600
StdSeq36_POP-6™	36	Standard read	60	500

† These approximate run times assume oven temperature has reached run temperature
‡ Time stated for 400 bases. Module can be customized to run 200-400 bases.
§ Length of read with 98.5% basecalling accuracy, and less than 2% N's, using pGEM-32f (+) as template.

Notes

Note: If the BigDye Xterminator® Purification Kit was used for sequencing reaction clean up, choose the run modules modified for BDx, as marked by 'BDx'. For additional information, refer to Appendix A in the *BigDye Xterminator® Purification Kit Protocol* (Part no. 4374408) for the appropriate run modules.

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

Dye Set	Chemistry
E_BigDyeV1	BigDye® Terminator v1.1 Cycle Sequencing Kit
Z_BigDyeV3	BigDye® Terminator v3.1 Cycle Sequencing Kit
Z_BigDyeV3	BigDye® Direct Cycle Sequencing Kit

f. Click OK.

Notes

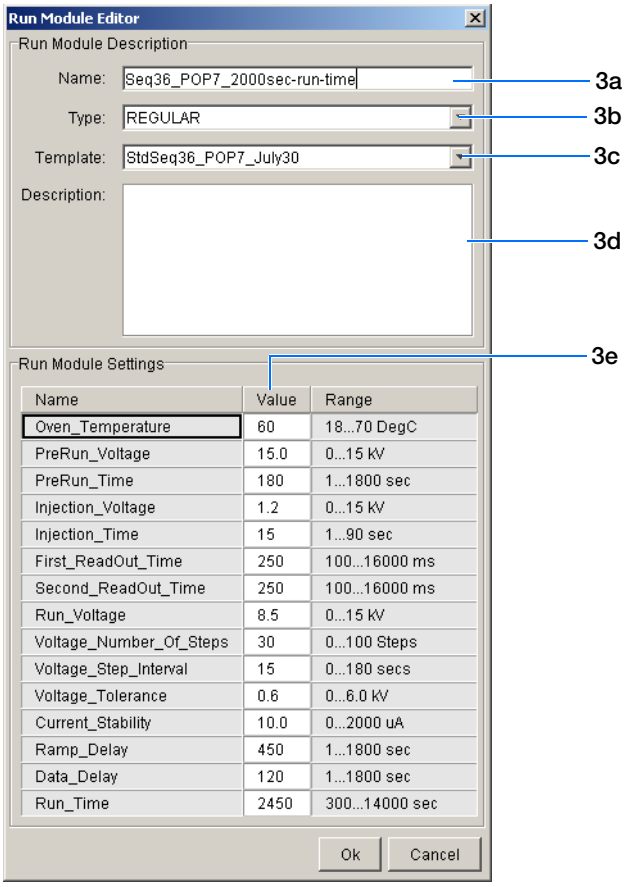
Tip: Customizing Run Modules

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 3730/3730*xl* 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°C–70°C	Temperature setting for main oven throughout run.
PreRun_Voltage	0–15 kV	Pre run voltage setting before sample injection.
PreRun Time	1–1800 sec	Prerun voltage time.
Injection_Voltage	0–15 kV	Injection voltage setting for sample injection.
Injection_Time	1–90 sec	Sample injection time.
First_ReadOut_time	100–16000 millisec	The interval of time for a data point to be produced. First_ReadOut_time should be equal to Second_ReadOut_time.
Second_ReadOut_Time	100–16000 millisec	The interval of time for a data point to be produced. Second_ReadOut_time should be equal to First_ReadOut_time.
Run_Voltage	0–15 kV	Final run voltage.
Voltage_Number_Of_Steps	0–100 steps	Number of voltage ramp steps to reach Run_Voltage. Life Technologies recommends that you do not change this value unless advised otherwise by Life Technologies support personnel.
Voltage_Step_Interval	0–180 sec	Dwell time at each voltage ramp step. Life Technologies recommends that you do not change this value unless advised otherwise by Life Technologies support personnel.
Voltage_Tolerance	0.1–6 kV	Maximum allowed voltage variation. Life Technologies recommends that you do not change this value unless advised otherwise by Life Technologies support personnel. If it goes beyond tolerance and shuts off, contact Life Technologies tech support.
Current_Stability	0–2000 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 Life Technologies support personnel.
Ramp_Delay	1–1800 sec	Delay During Voltage Ramp. Life Technologies recommends that you do not change this value unless advised otherwise by Life Technologies support personnel.
Data_Delay	1–1800 sec	Time from the start of separation to the start of sample data collection.
Run_Time	300–14000 sec	Duration data is collected after Ramp_Delay.

Notes

Analysis Protocols

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

- Protocol name – The name, description of the analysis protocol, and the sequence file formats to be used.
- Basecalling settings – The basecaller, DyeSet file, and analysis stop point to be used.
- Mixed Bases – (*Optional*): 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 by DNA Sequencing Analysis Software 6 will not be performed if you do so.

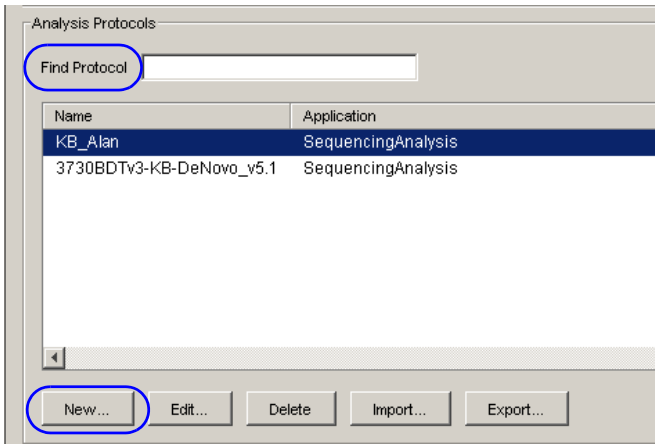
Notes

Creating an Analysis Protocol

Refer to Appendix C, "KB™ Basecaller Software v1.4.1," on page 175 and the *DNA Sequencing Analysis Software 6* (Part no. 4474239) for more information regarding analysis protocols

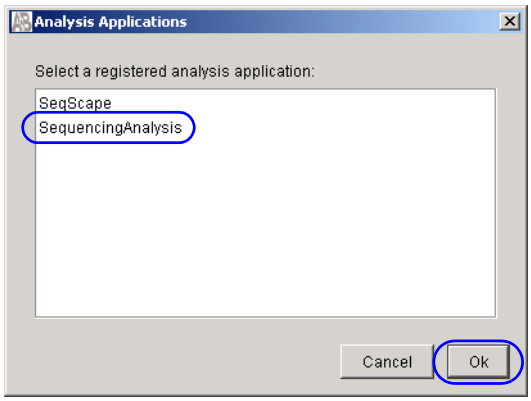
1. In the Analysis Protocol section of the Protocol Manager, click New....

   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.

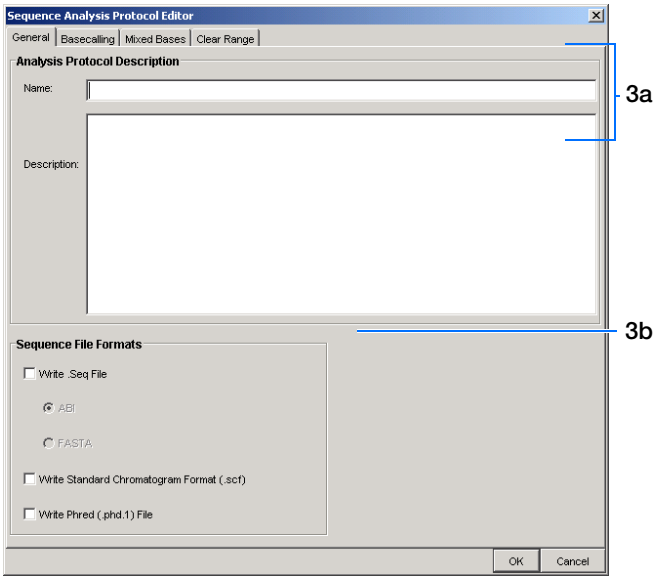


Notes

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

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

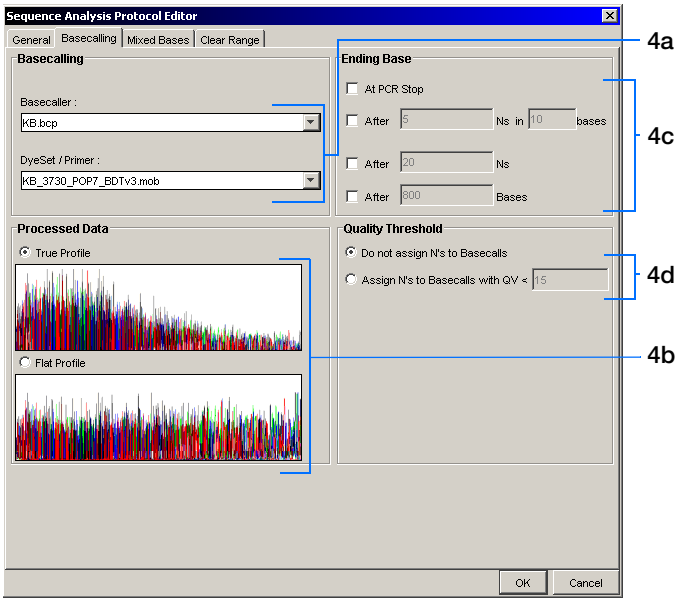
   b. Select the appropriate Sequence File formats settings.



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. • ABI format is used with Applied Biosystems® software. • FASTA format is used with other software
Write Standard Chromatogram Format file (.scf)	When selected, the software creates a .scf file that can be used with other software. When created, the .scf extension is not appended to the file name.
Write Phred (.phd.1) File	When selected and the KB basecaller is used, the software creates a .phd.1 file that can be used with other software.

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

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



Note: See Appendix C, "KB™ Basecaller Software v1.4.1," on page 175 for a comparison of Basecaller options.

Note: Select Sequencing Analysis Software and 3730 Series Data Collection Software 4 filter .mob file choices to match the chosen .bcp file.

Notes

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

Option	Function
True Profile	Used to display data as processed traces scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. The profile of the processed traces will be very similar to that of the raw traces.
Flat Profile	Used to display the data as processed traces scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces 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
Call all bases and assign QV	When using the KB™ basecaller, use this option to assign a base to every position, as well as the QV.
Assign 'N' for bases with QV < 15	When using the KB™ basecaller, use this option to assign Ns to bases with QVs less than the set point. The QV is still displayed.

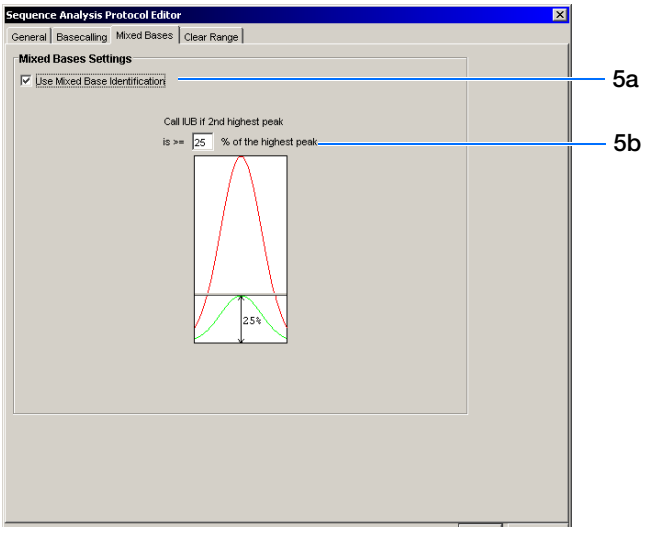
5. Select the **Mixed Bases** tab.

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

a. For data containing any mixed bases, select **Use Mixed Base Identification**.

b. The User can set the secondary peak threshold, as a percentage of the primary peak, for consideration as a mixed base by the basecalling algorithm. Reaching this threshold is a necessary but not sufficient condition for arriving at a mixed base determination. Set the percentage by entering a value into the " =__%" field or by dragging the horizontal line above or below the 25% default setting.

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



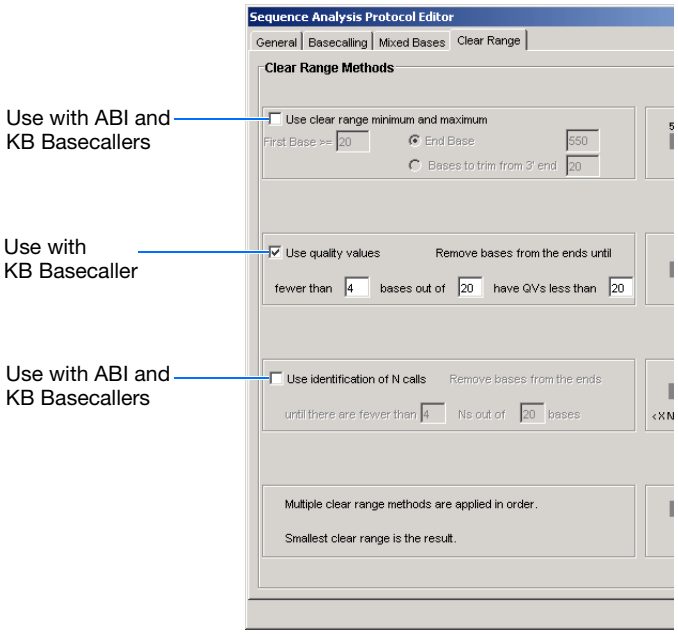
Notes

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.

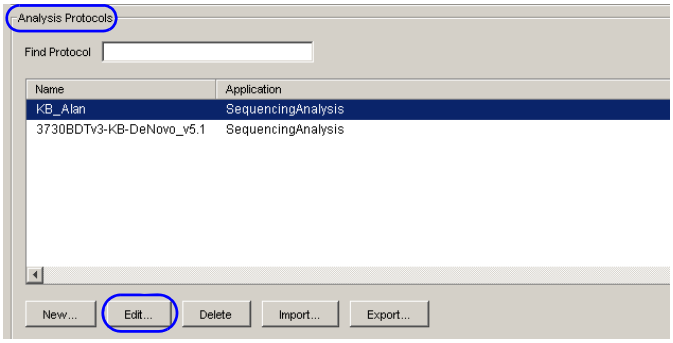
7. Click OK to save the protocol and close the Sequence Analysis Protocol Editor.



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.



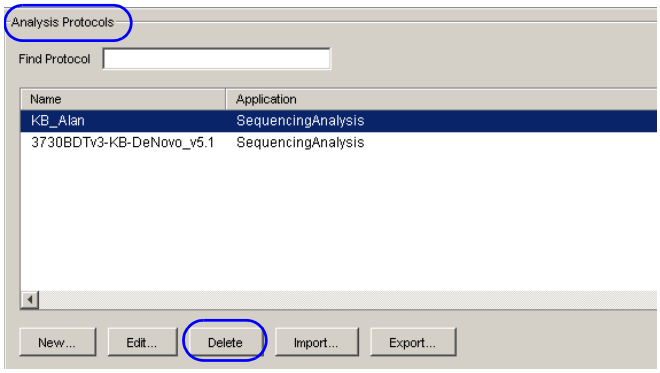
4

Notes

Deleting an Analysis Protocol

IMPORTANT! Do not delete an Analysis Protocol during a run while it is being used for that run. Autoanalysis by DNA Sequencing Analysis Software 6 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.

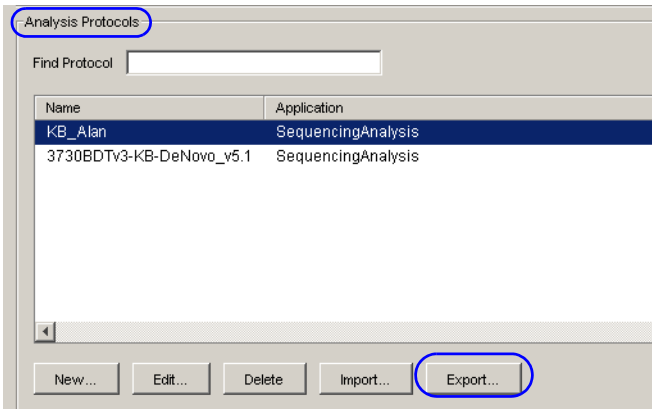


Note: To reuse a plate after deleting the analysis protocol associated with it, either re-create the analysis protocol with the same name or assign the plate a unique plate name.

Exporting and Importing Analysis Protocols

Exporting an Analysis Protocol

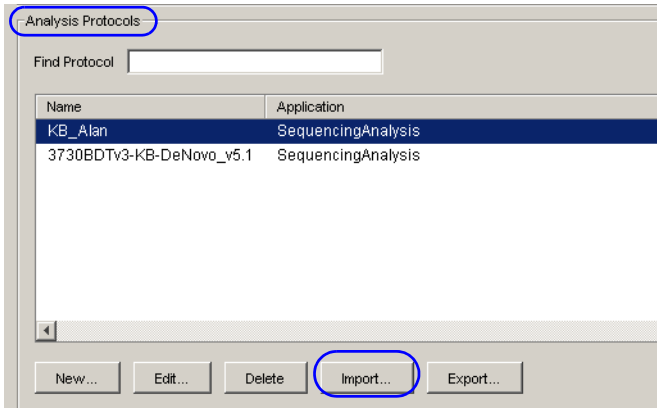
1. In the Analysis Protocols pane in the Analysis Protocol Manager, select the protocol you want to export.
2. Click Export. A standard file export dialog box opens.
3. Navigate to the destination folder.
4. Click **Save**.



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**.



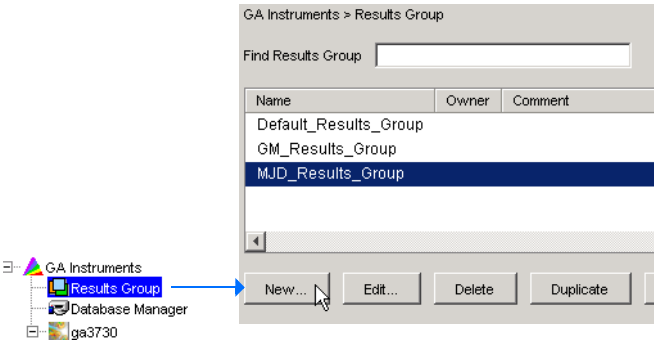
Results Groups

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.

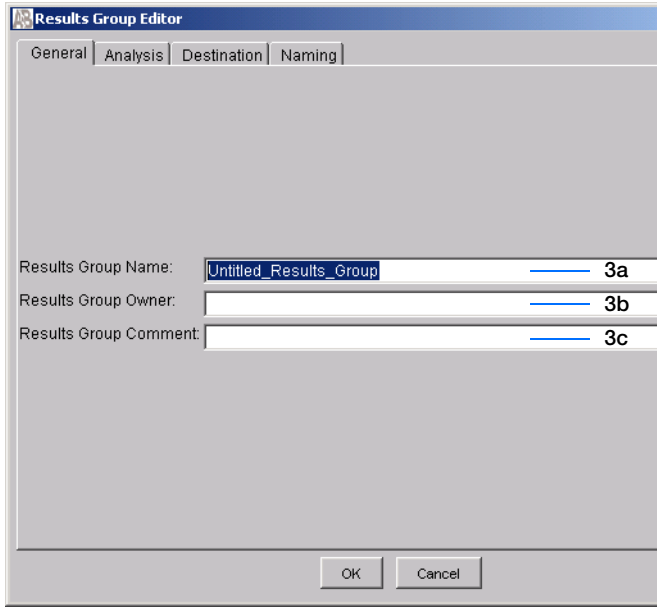
Creating a Results Group

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

   The Results Group Editor window opens.



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 70 for a list of accepted characters).
   b. (*Optional*) Type a Results Group Owner. The owner name can be used in naming and sorting sample files.
   c. (*Optional*) Type a Results Group Comment.

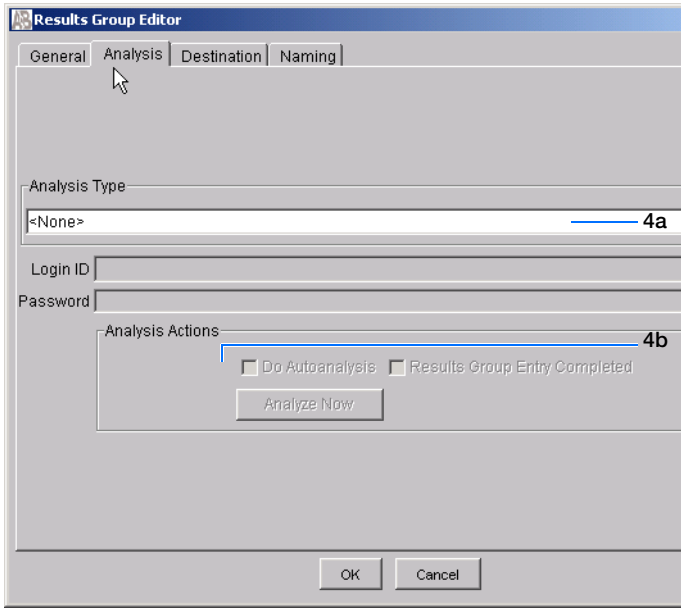


Notes

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

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

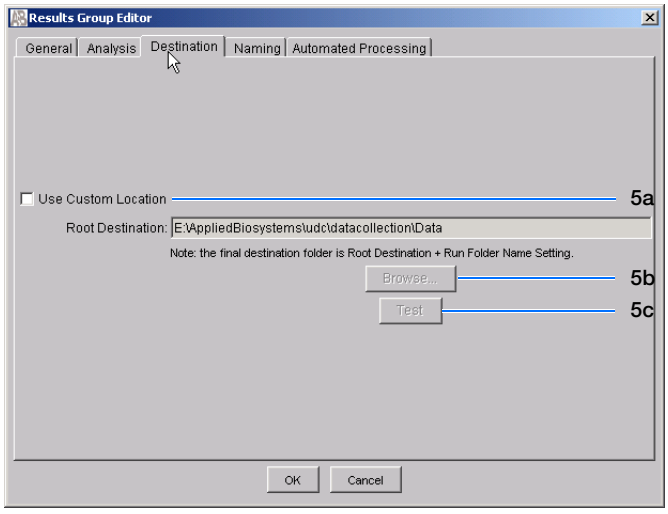
   b. In the Analysis Actions section, select **Do Autoanalysis**, if you want your data automatically analyzed after a run by DNA Sequencing Analysis Software 6.



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

Note: Autoanalysis by SeqScape® is no longer supported.

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 5b

Note: The Results Group Destination tab, and Data Collection Software in general, does not recognize remote storage locations unless they have been mapped to a local drive letter using the Map Network Drive feature of the operating system. Specify the mapped drive letter location in the Results Group Destination tab.

   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.

Notes

– 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:

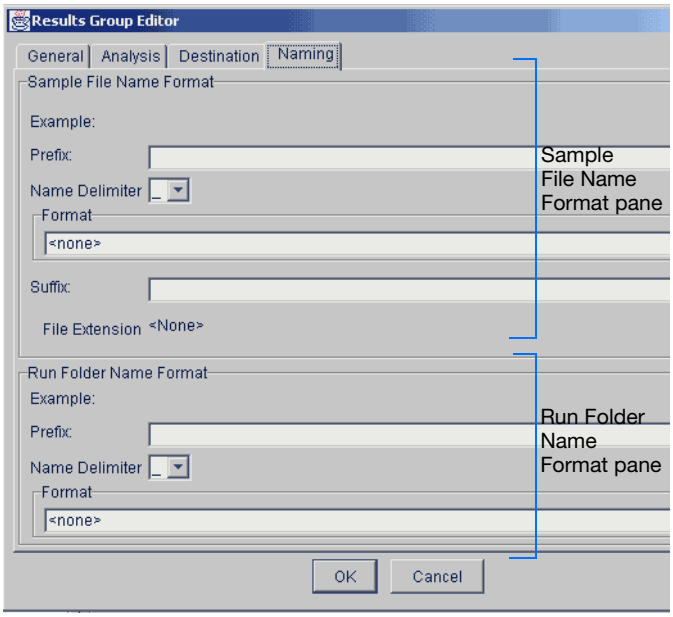
- Default Destination, default folder naming: Data / instrument type / instrument name / run folder (No ProcessedData folder)
- Default Destination, custom folder naming: Data/top custom folder/subfolders, and so on.
- Custom Destination, default folder naming: Destination/instrument type/instrument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

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 70 for accepted characters.

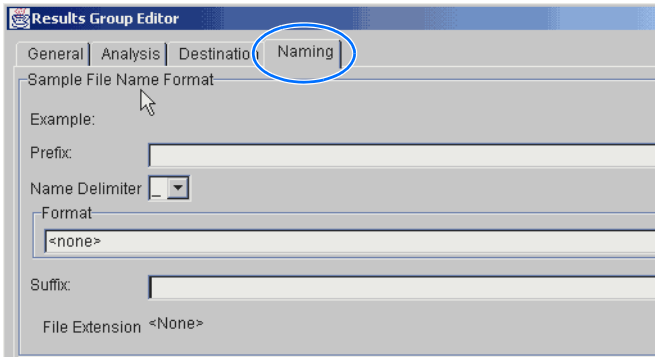
   The elements of the Naming tab are discussed in the following sections.



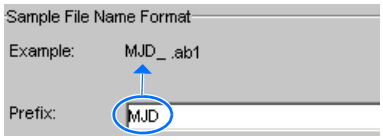
Sample File Name Format Pane

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

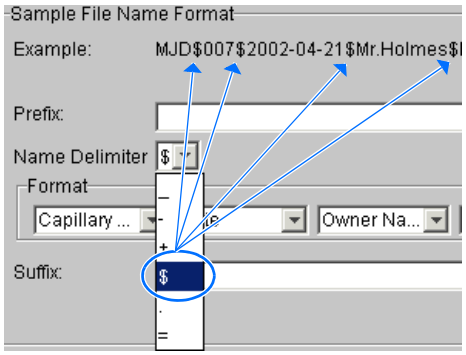
1. (*Optional*) In the Naming tab, select the **Prefix** box to type a prefix for the file name. Anything that you type here is shown in the Example line (see figure below).



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.

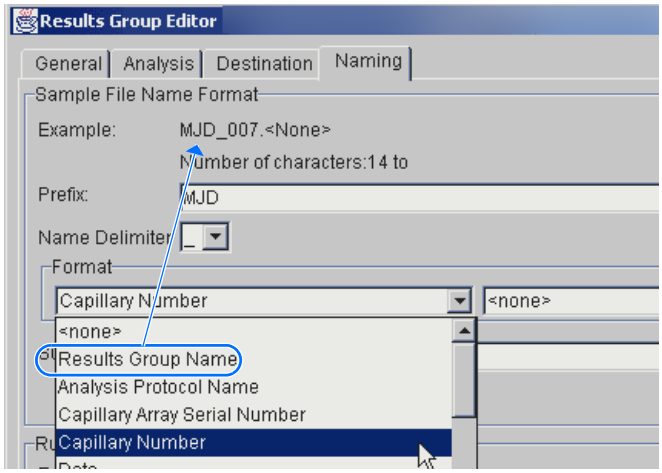


3. Click the Format list, then select the components that you want in the sample name.

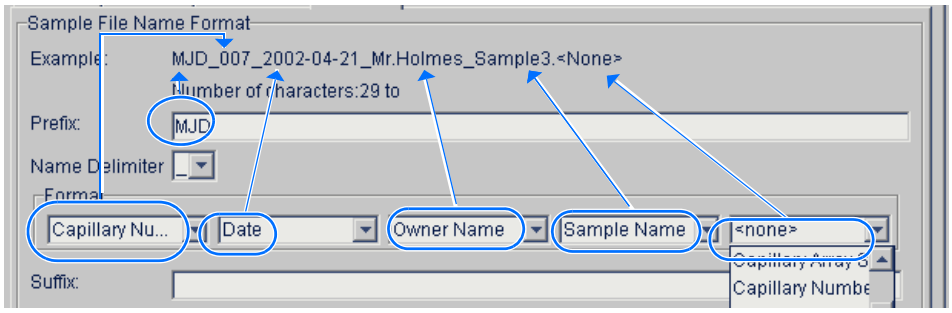
   Note: Generally, all the samples from a single run are placed in the same run or results folder, so the name of every sample from a single run should be different from each other. However, most of the Format options are not different between samples, you need to take care to select at least one of the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

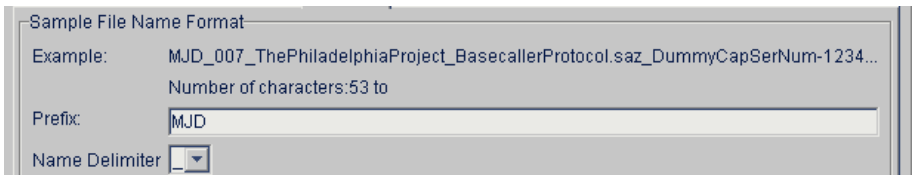
   As you continue to select elements for the file name, additional elements are displayed.



Notes

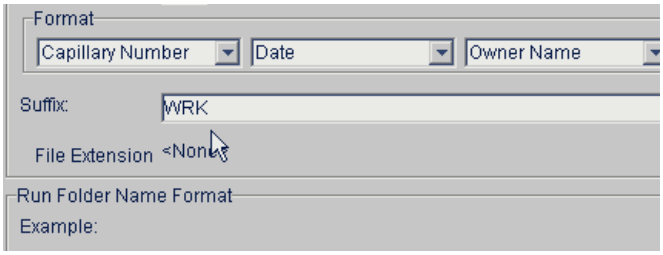


The names of the Format elements are eventually shortened, but the Example field remains visible (up to 72 characters).



4. (*Optional*) Select 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 86). 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.

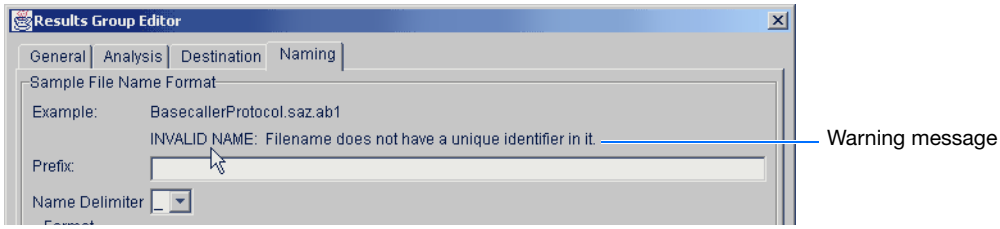
Format Elements (Unique Identifiers)

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 87) 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 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 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 Export. 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 Save.

   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.

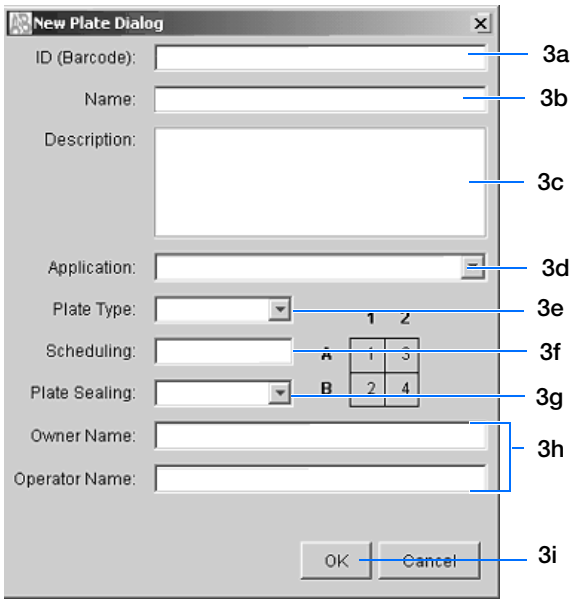
Notes

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. Schedule the plate. For more information, see "Scheduling Runs" on page 133.
   g. Select **heat seal** or **septa**.
   h. Type a name for the owner and operator.
   i. Click OK . The Sequencing Analysis Plate Editor opens.

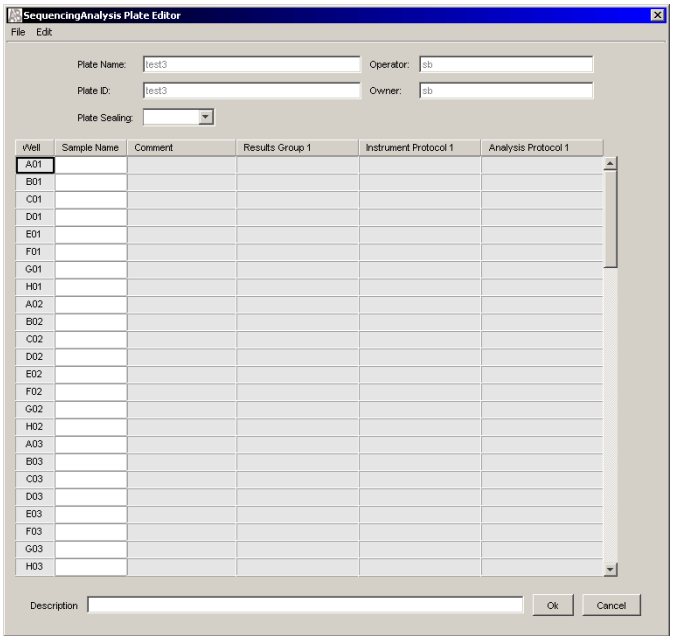


Notes

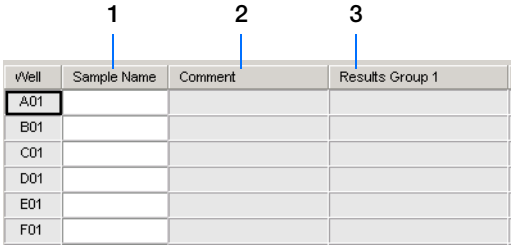
Completing a Sequencing Analysis Plate Record

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

Note: Importing Excel files is not supported.



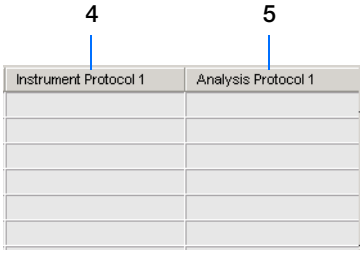
1. In the Sample Name column of a row, enter a sample name, then click the next cell. The value 100 is automatically displayed in the Priority column.



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 85).

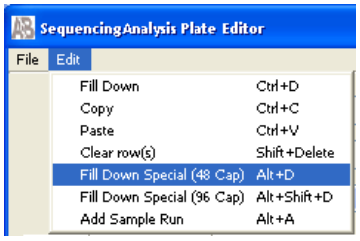
4. In the Instrument Protocol 1 column, select a protocol from the drop-down list (see page 74).



5. In the Analysis Protocol 1 column, select a protocol from the drop-down list (see page 80).

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 96)
   - 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:



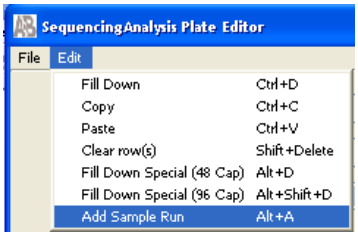
Notes

  - 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.

7. 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.



8. Complete the columns for the additional runs.

9. Click OK.

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

Note: If multiple cells are selected for copying, select the same number of corresponding target cells before you execute the Paste command.

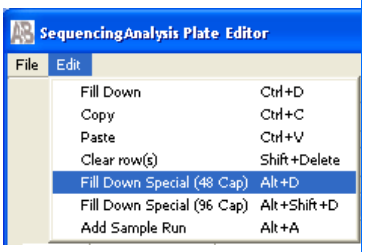
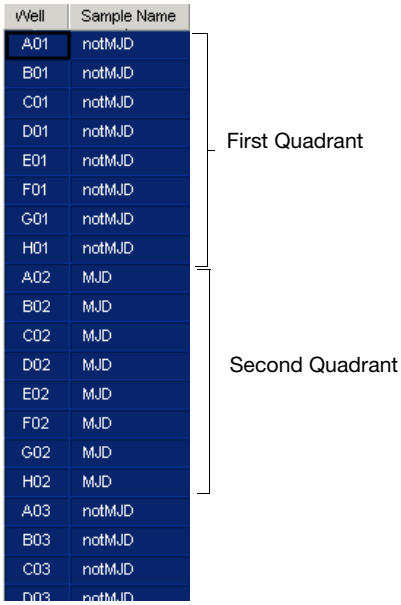
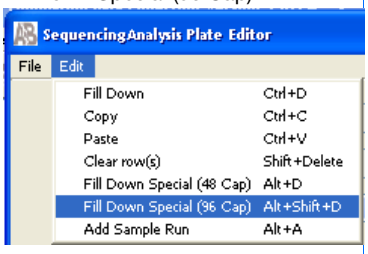
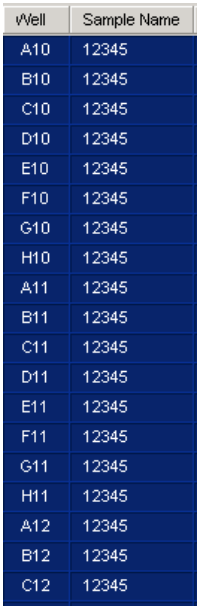
Note: The Plate Editor Copy and Paste functionality is supported only within one plate editor. To copy and paste the contents of one plate to another plate, use the "Duplicate..". button on the Plate Manager dialog box.

Note: If you use the duplicate plate function, all the information in the plate to be duplicated must be valid. Otherwise, an empty plate is created.

Notes

Fill Down Special

The following table illustrates the Fill Down Special feature.

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

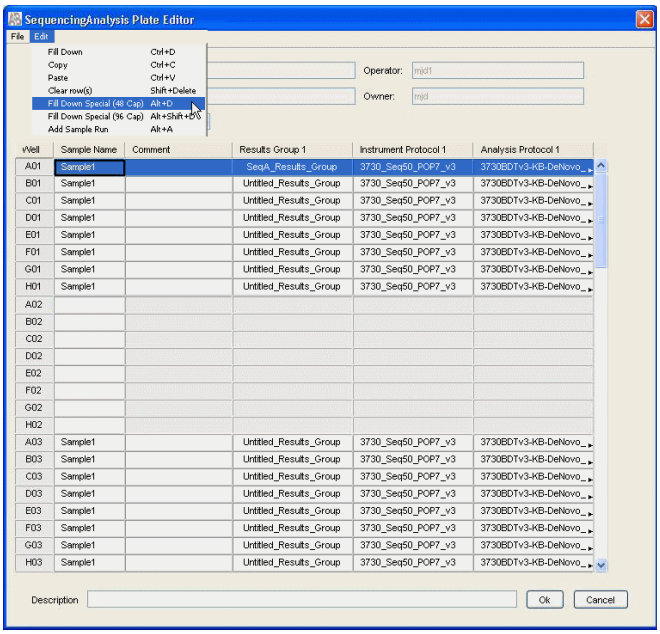
Notes

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

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

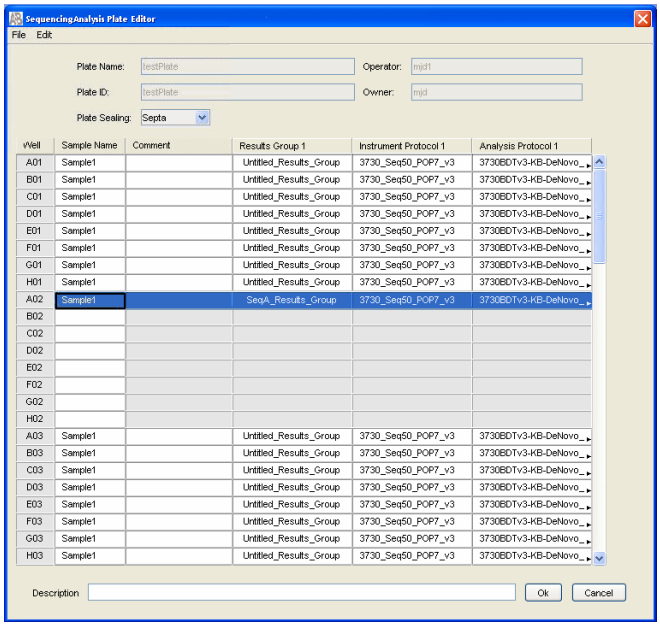
To use the fill down special function:

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.

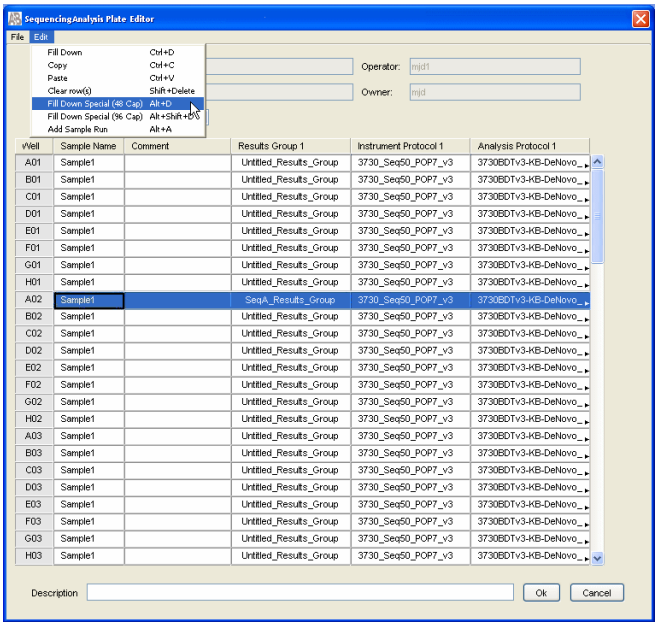


Notes

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



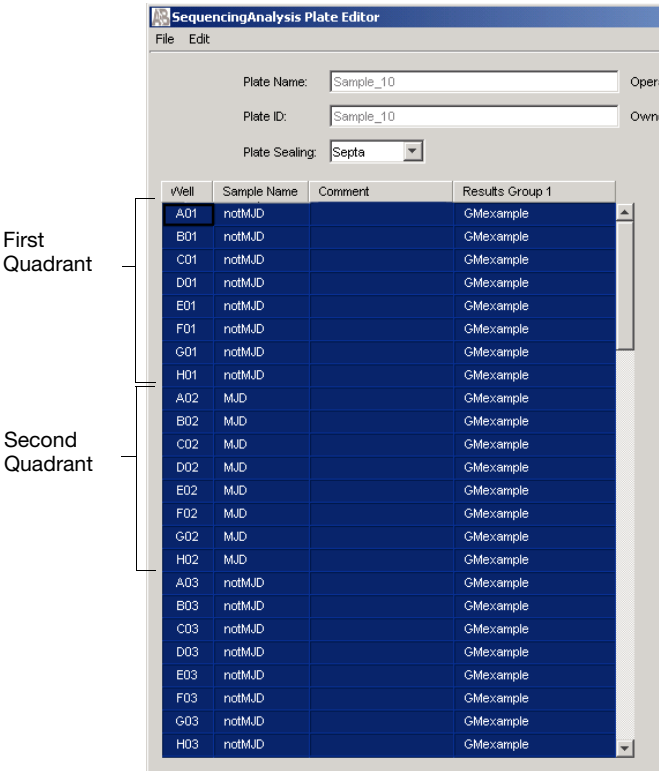
5. Select **Edit > Fill Down Special (48 Cap)** to fill the plate record with the second load pattern.



Notes

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

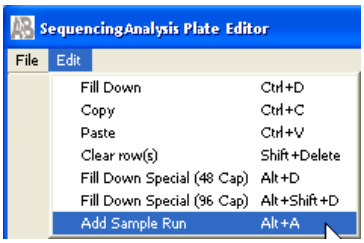
When you use the Fill Down Special (96 Cap) function on a 384-well plate, the fill-down pattern appears as in the adjoining illustration to the right.



Well	Sample Name	Comment	Results Group 1
A01	notMJD		GMexample
B01	notMJD		GMexample
C01	notMJD		GMexample
D01	notMJD		GMexample
E01	notMJD		GMexample
F01	notMJD		GMexample
G01	notMJD		GMexample
H01	notMJD		GMexample
A02	MJD		GMexample
B02	MJD		GMexample
C02	MJD		GMexample
D02	MJD		GMexample
E02	MJD		GMexample
F02	MJD		GMexample
G02	MJD		GMexample
H02	MJD		GMexample
A03	notMJD		GMexample
B03	notMJD		GMexample
C03	notMJD		GMexample
D03	notMJD		GMexample
E03	notMJD		GMexample
F03	notMJD		GMexample
G03	notMJD		GMexample
H03	notMJD		GMexample

Adding a Sample Run

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



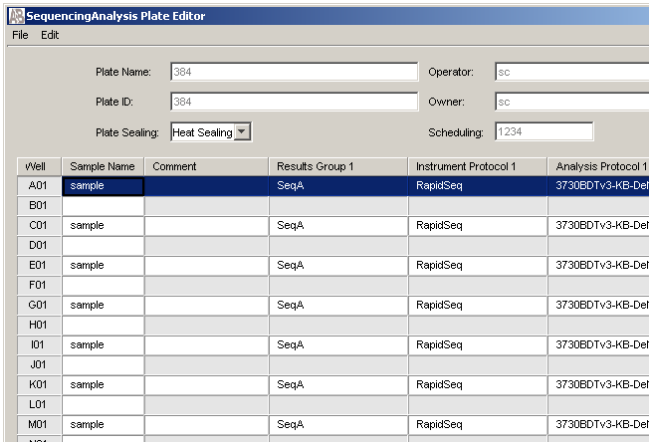
To add a sample run **Select Edit > Add Sample Run**.

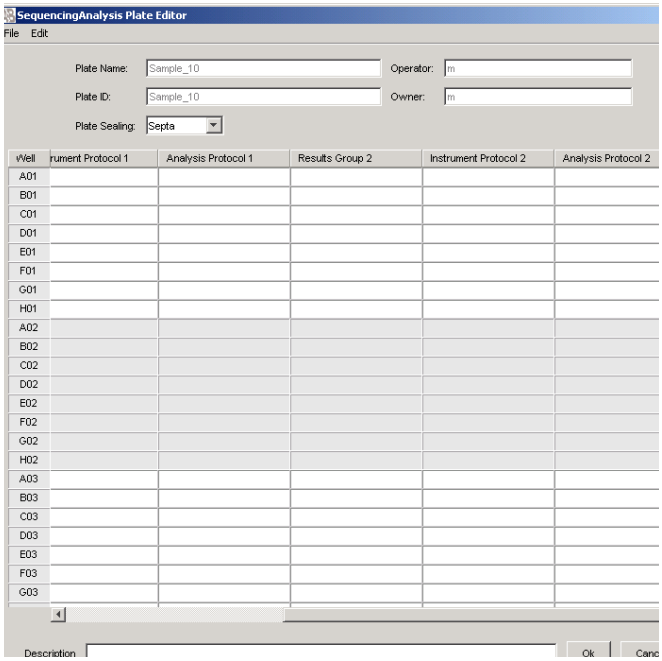
- Results Group
- Instrument Protocol
- Analysis Protocol (sequencing only)

To run the plate(s), see "Running the Instrument" on page 127.

Note: When you add another sample run to a processed plate, confirm that all the information in the processed runs is valid. Otherwise, that data will not be validated, and a new sample run cannot be created.

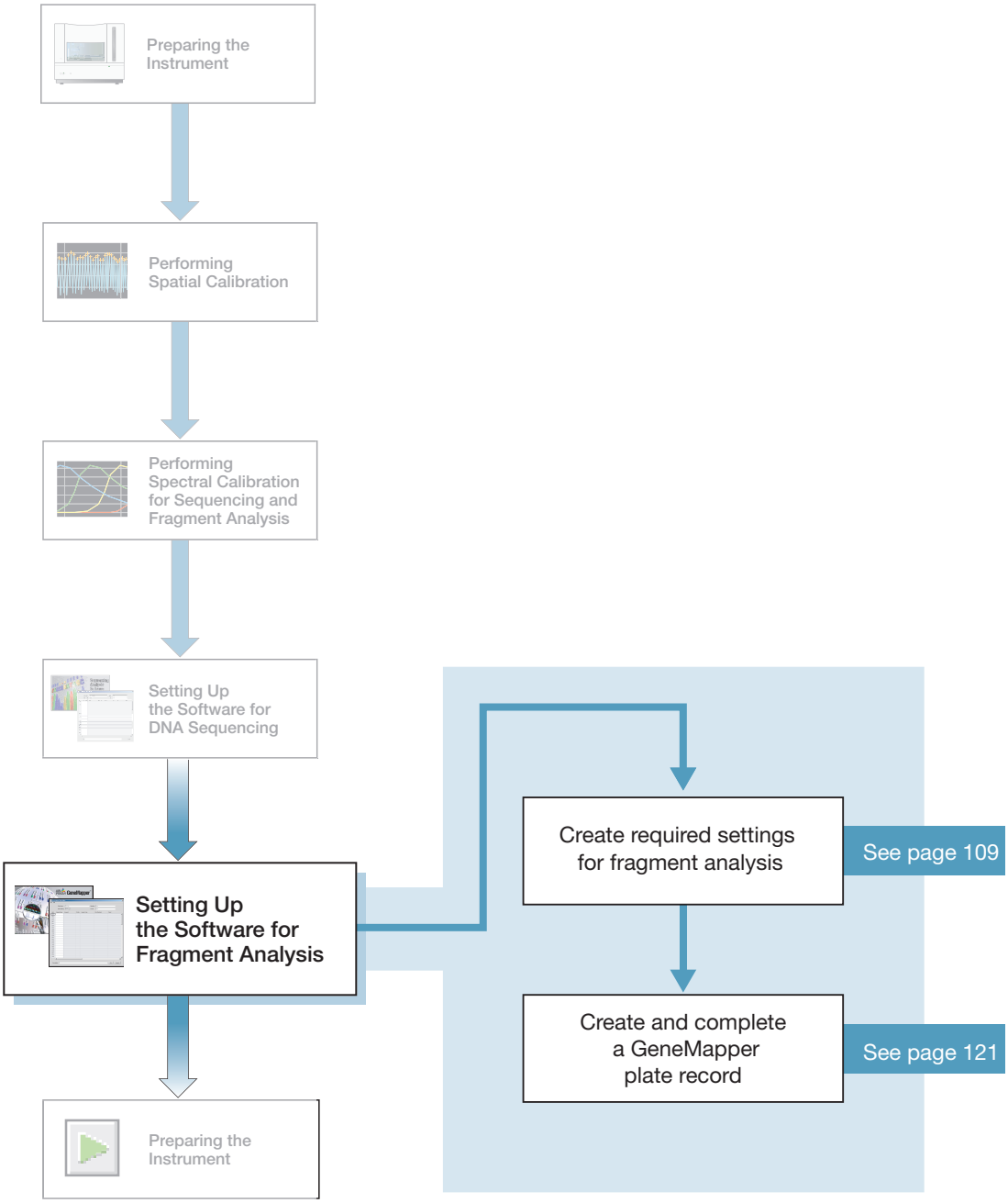
Notes


SequencingAnalysis Plate Editor
File Edit
Plate Name: 384
Operator: sc
Plate ID: 384
Owner: sc
Plate Sealing: Heat Sealing
Scheduling: 1234
Well
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
Well
rument 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
Canc

Notes

Setting Up the Software for Fragment Analysis



Notes

3730/3730*xl* Analyzer Data Collection and GeneMapper Software

IMPORTANT! Do not rename the computer after 3730 Series Data Collection Software is installed. Doing so causes the 3730 Series 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.

Note: Autoanalysis by GeneMapper® Software is no longer supported.

Data Analysis

For information on data analysis, refer to *GeneMapper® Software 5 Online Help* (Part no. 4474202)

Fragment Analysis and Data Collection

When GeneMapper® software is installed on a computer that has 3730 Series Data Collection Software, you can access GeneMapper® through the Results Group Editor (see page 114):

- GeneMapper-Generic
- GeneMapper-<Computer Name>

GeneMapper-Generic

GeneMapper-Generic enables you to generate .fsa files. 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.

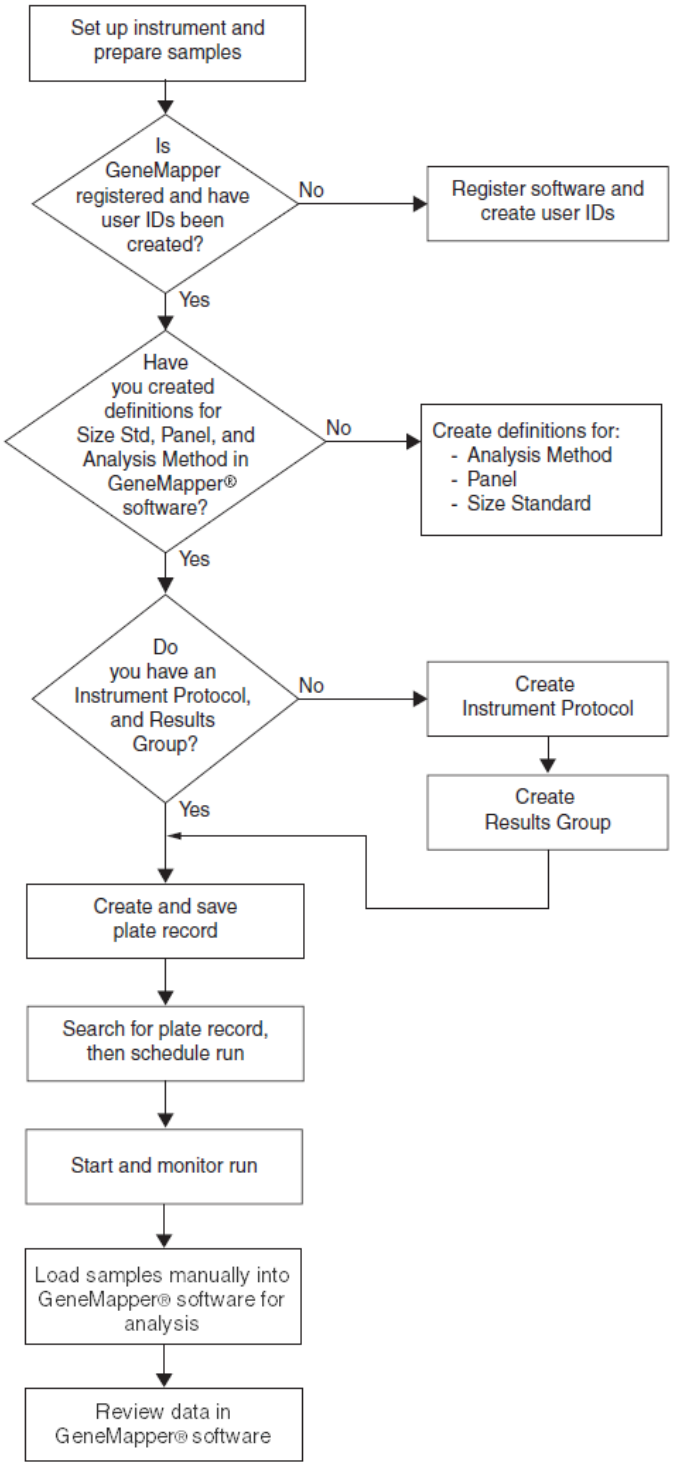
Notes

GeneMapper-<Computer Name>

GeneMapper-<Computer Name> permits the Size Standard, Analysis Method, and Panel columns in the Sample Sheet window to be 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.

Notes

Workflow with GeneMapper-<Computer Name> option 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 Applied Biosystems® 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.

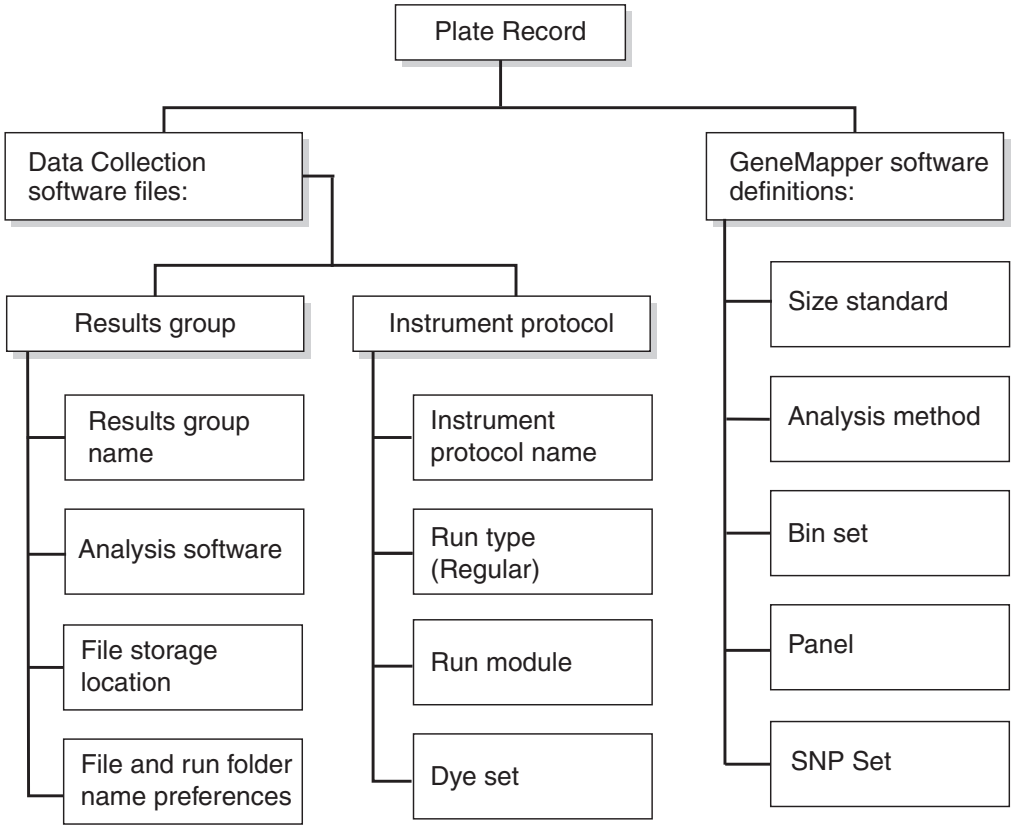
Parameters	Description	See Page
Instrument protocol	Contains everything needed to run the instrument.	99
Results group	Defines the file type, the file name, and file save locations that are linked to sample injections.	104

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

Note: Autoanalysis by GeneMapper® is no longer supported.

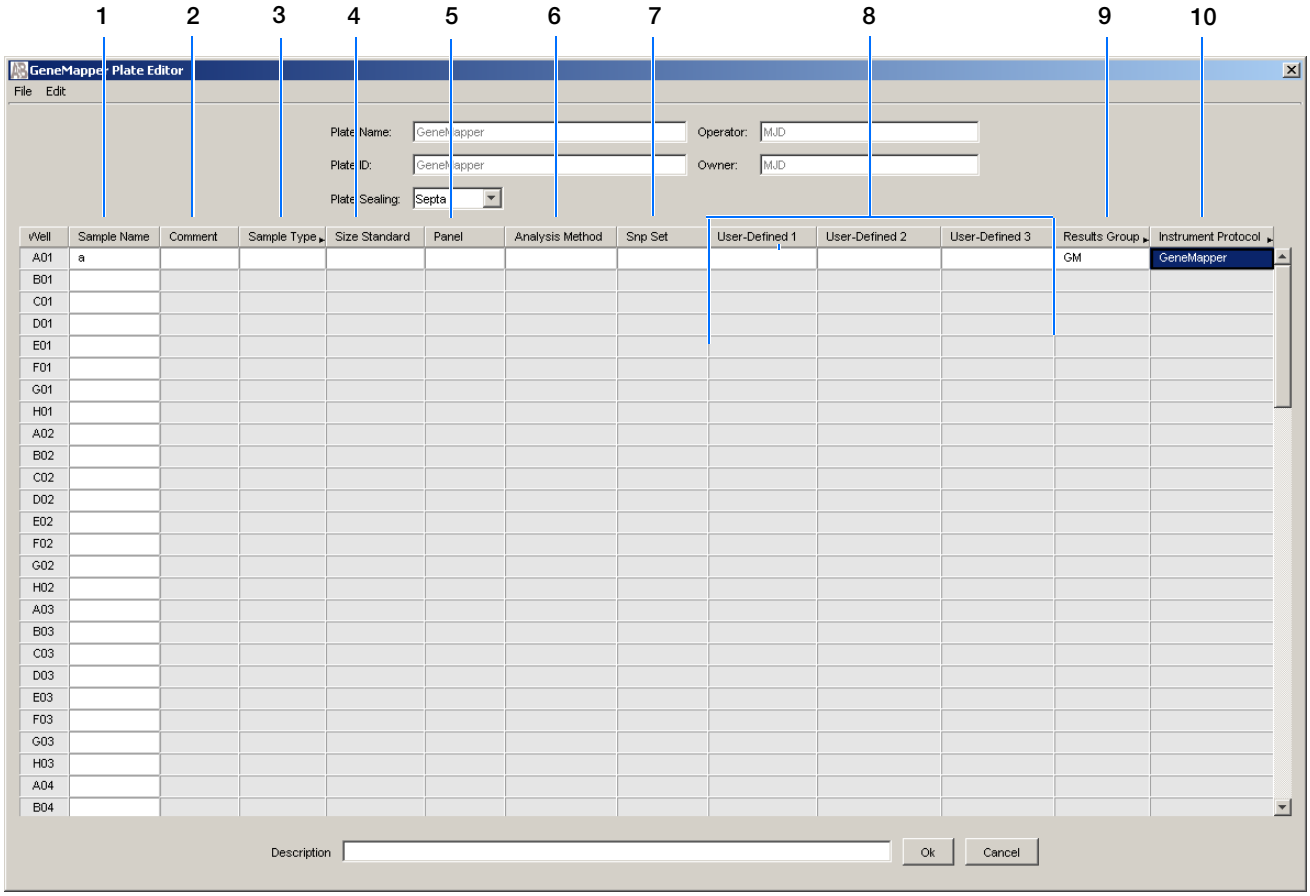
Notes

Components of a GeneMapper® Software Plate Record



Notes

Descriptions for numbers 1–10 are in the following table.



Default is one sample run. To add additional runs, see page 115.

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

Table 5-1 Components of the plate record

Column	Description
1. Sample Name	Name of the sample
2. Comment	(*Optional*) Comments about the sample
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 in order to make them available in Data Collection Software	• (*Optional*) GeneMapper-Generic: 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!** For GeneMapper-<Computer Name> ONLY: Size standard, panel, and analysis method must be created in GeneMapper software before creating a new plate	• (*Optional*) GeneMapper-Generic: 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	• (*Optional*) GeneMapper-Generic: Manually enter analysis methods in the text field* • GeneMapper-<Computer Name>: Select a saved analysis method from the drop-down list
7. Snp	Optional field, typically left blank
8. 3 User-defined columns	Optional text entries
9. Results group	Some options: • New: Opens the Results Group Editor dialog box • Edit: Opens the Results Group Editor dialog box for the results group listed in the cell • None: Sets the cell to have no selected results group • Select one of the available Results groups from the list **Note:** You must have a results group selected for each sample entered in the Sample Name column. See, "Results Groups" on page 114.
10. Instrument protocol	• New: Opens the Protocol Editor dialog box. • Edit: Opens the Protocol Editor dialog box for the instrument protocol listed in the cell. • None: Sets the cell to have no selected protocol. • List of Instrument Protocols: In alpha-numeric order. **Note:** You must have an instrument protocol selected for each sample entered in the Sample Name column. • See, "Instrument Protocols" on page 109.

Notes

Creating Required Settings for Fragment Analysis

If the Settings Already Exist

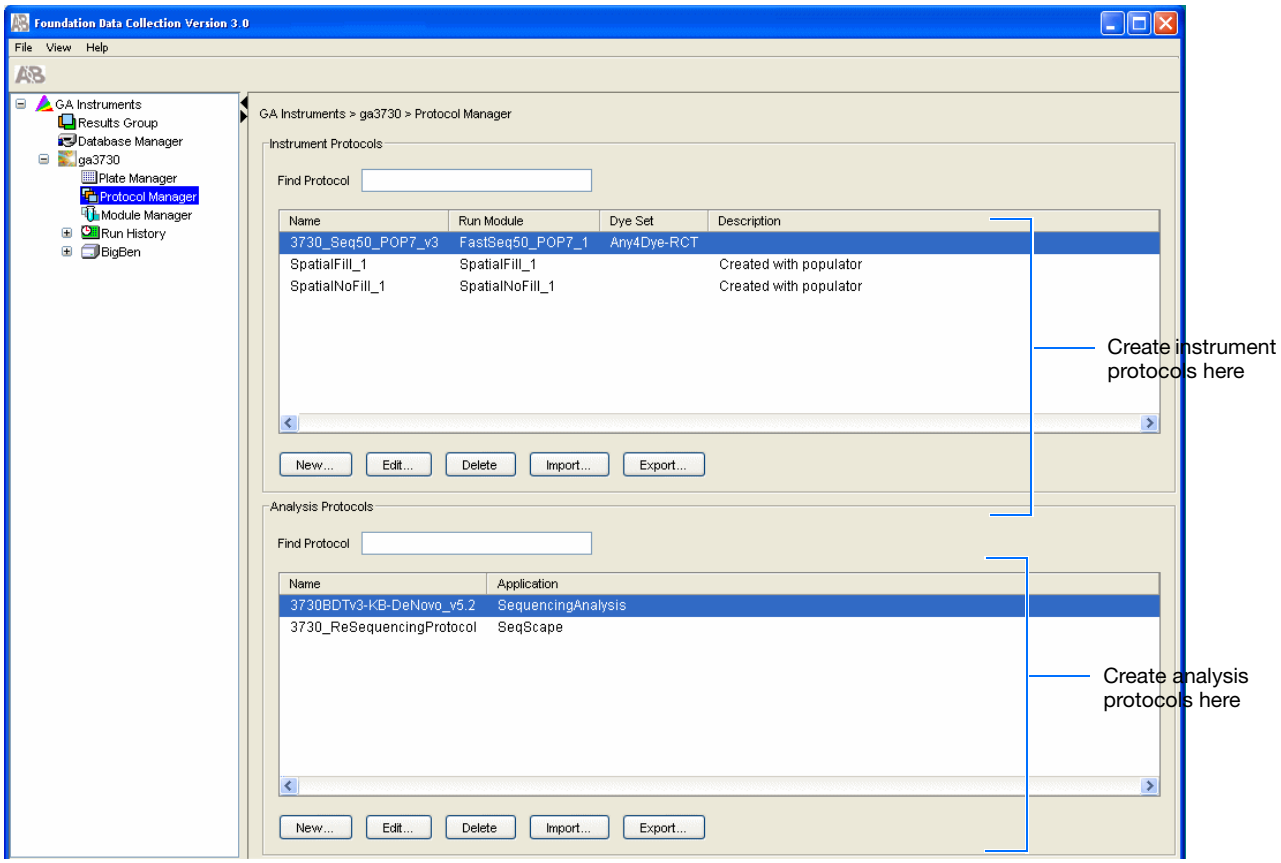
If the appropriate data collection and fragment analysis files have been created, go to "Creating and Completing a GeneMapper® Software Plate Record" on page 121.

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.

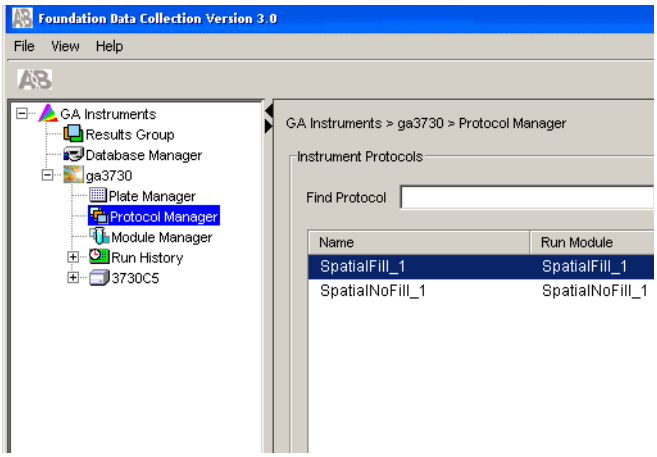
Creating an Instrument Protocol

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

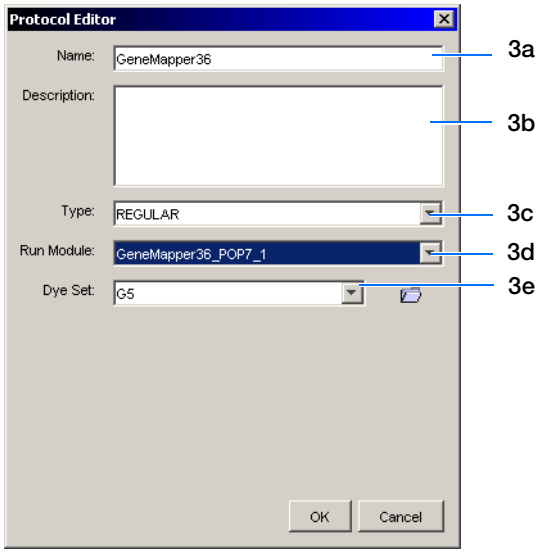


Notes

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



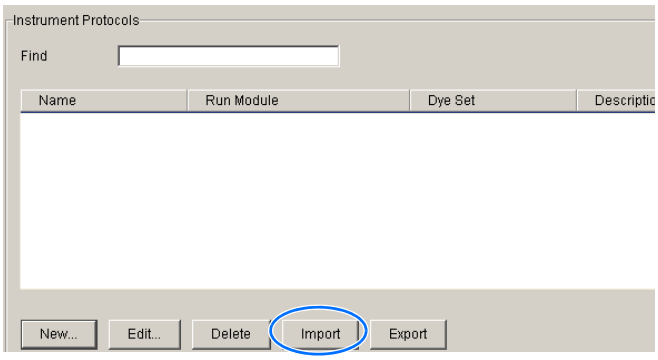
3. Complete the Protocol Editor:
   a. Type a name for the protocol.
   b. (*Optional*) Type a description for the protocol.
   c. Select **Regular** in the Type drop-down list.



   d. Select **GeneMapper36_POP-7™.**
   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.



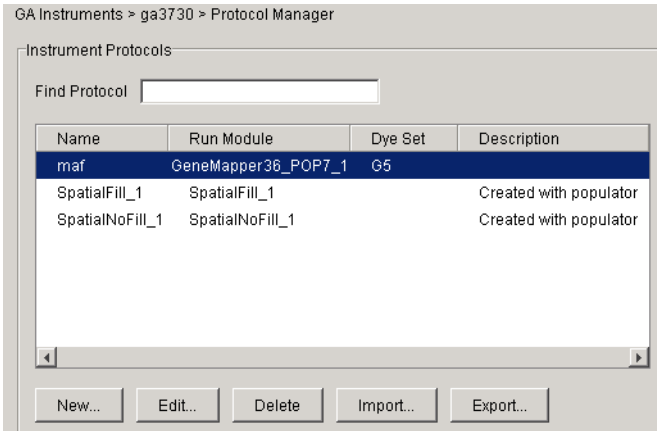
Notes

2. Navigate to the protocol you want to import.

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

3. Double-click the protocol to import it.

4. The imported files are displayed alphabetically in the Instrument Protocol pane.



Fragment Analysis Run Modules

Select one run module:

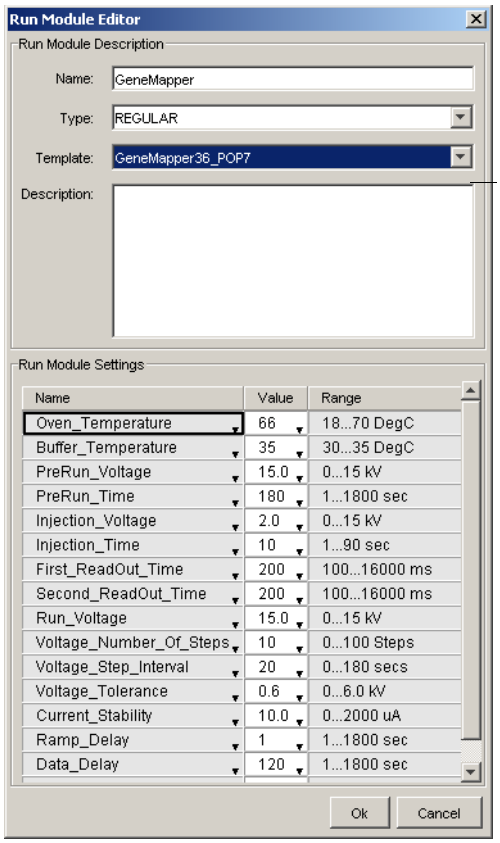
Run Module	Capillary Length
HTSNP36_POP-7™_V3 (SNaPshot®)	36 cm
HTSNP50_POP-7™_V3 (SNaPshot®)	50 cm
GeneMapper36_POP-7™	36 cm
GeneMapper50_POP-7™	50 cm
GS1200LIZ_36_POP-7™	36 cm
GS1200LIZ_50_POP-7™	50 cm

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.



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

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

Notes

The Run Module Parameters that you can edit:

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

Notes

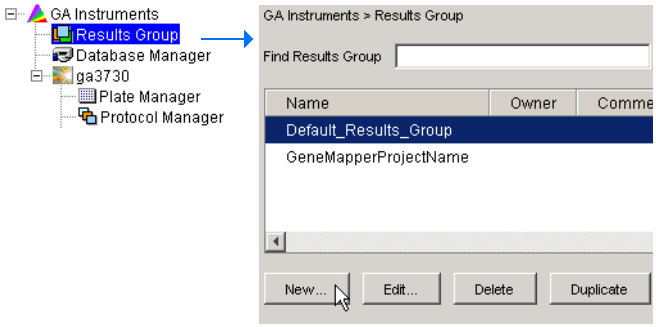
Results Groups

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

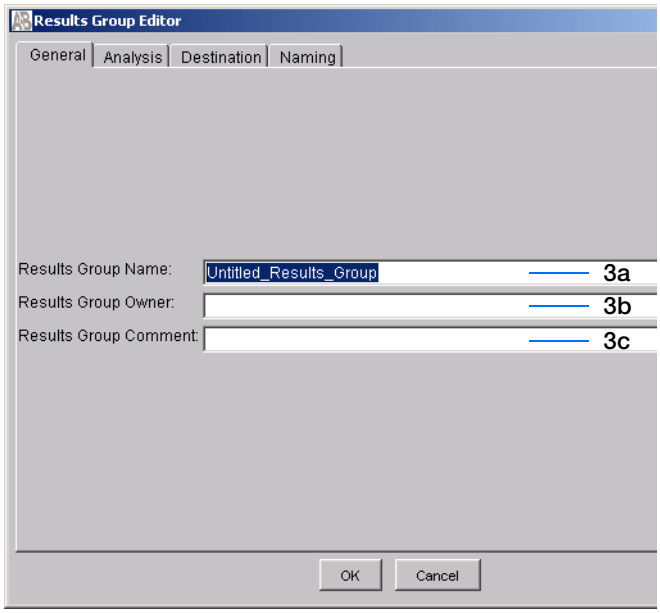
Creating a Results Group

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. (*Optional*) Type a Results Group Owner. The owner name can be used in naming and sorting sample files.
   c. (*Optional*) Type a Results Group Comment.



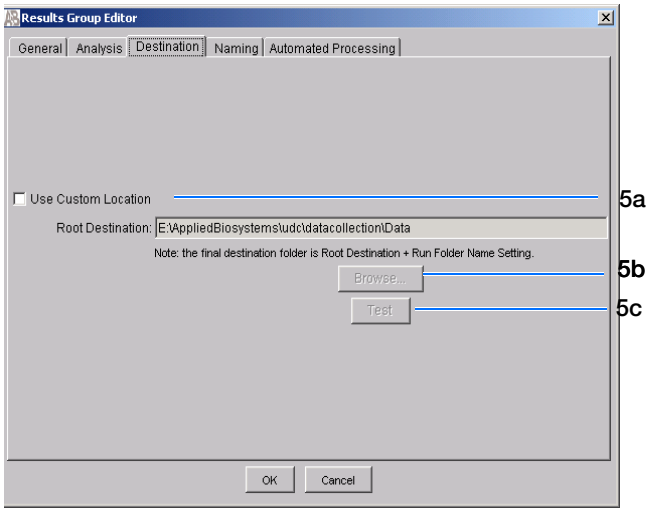
4. Skip the **Analysis** tab, because autoanalysis by GeneMapper® is no longer supported.

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.



Note: The Results Group Destination tab, and Data Collection Software in general, does not recognize remote storage locations unless they have been mapped to a local drive letter using the Map Network Drive feature of the operating system. Specify the mapped drive letter location in the Results Group Destination tab.

Sample File Locations

Locations Where Sample Files Are Placed During Extraction:

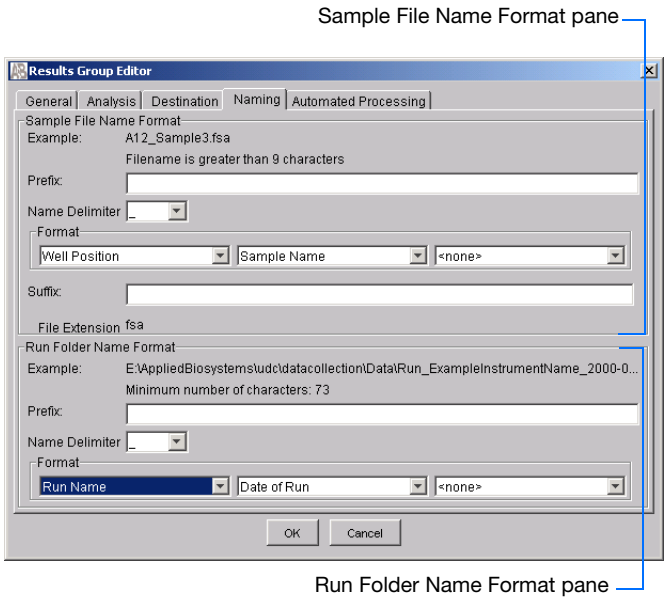
- Default Destination, default folder naming: Data / instrument type / instrument name / run folder (No ProcessedData folder)
- Default Destination, custom folder naming: Data/top custom folder/subfolders, and so on.
- Custom Destination, default folder naming: Destination/instrument type/instrument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

Notes

6. 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 102 for accepted characters.

   The elements of the Naming tab are discussed in the following sections, see page 117.



7. Skip the **Automated Processing** tab, because Autoanalysis by GeneMapper® is no longer supported.

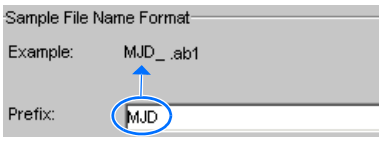
8. Click OK to save the Results Group.

Notes

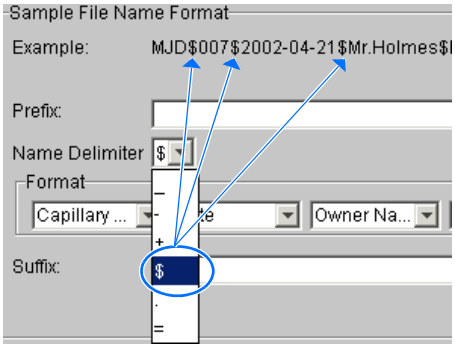
Sample File Name Format Pane

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 the following graphic).



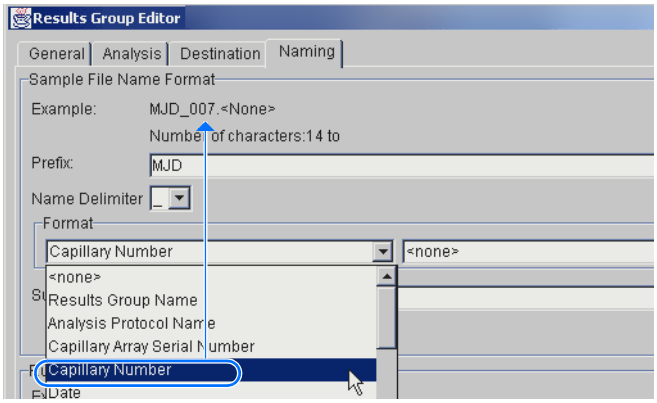
2. Click the **Name Delimiter** list choose the symbol that will separate the Format elements in the file name (see step 3). You can only choose one delimiter symbol.



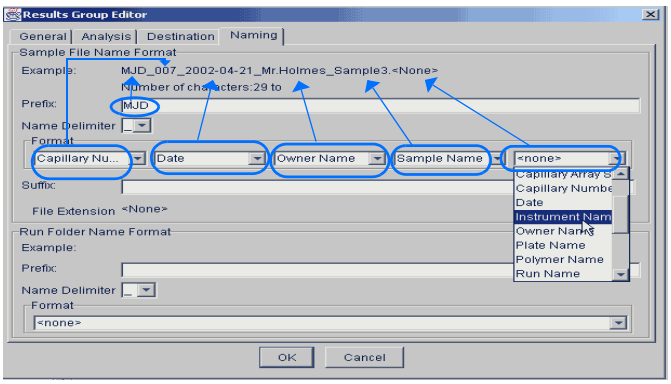
3. Click the **Format** list and then select the components that you want in the sample name.

   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.



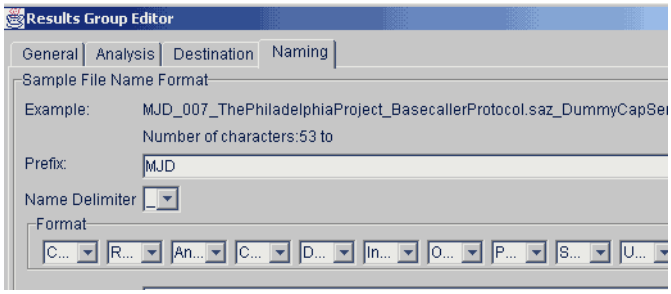
Note: An additional drop-down list of formats is displayed after you select a format option.



Notes

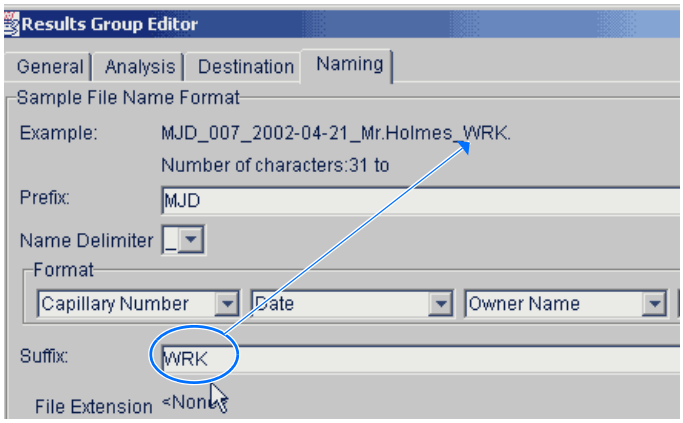
The names of the Format elements are eventually shortened, but the Example field remains visible (up to 72 characters).

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 114). 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 117) to change the sub-folder name within the run folder.

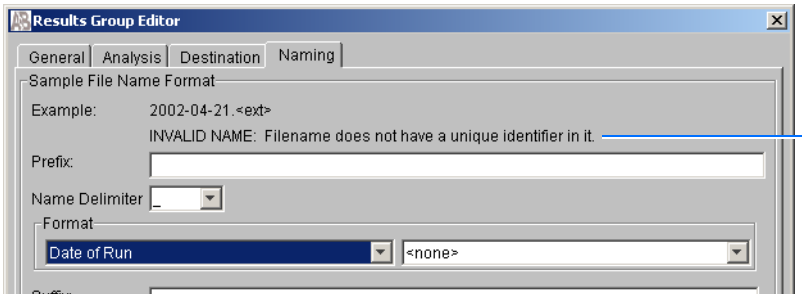
Notes

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 the following figure).



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).

Notes

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.

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

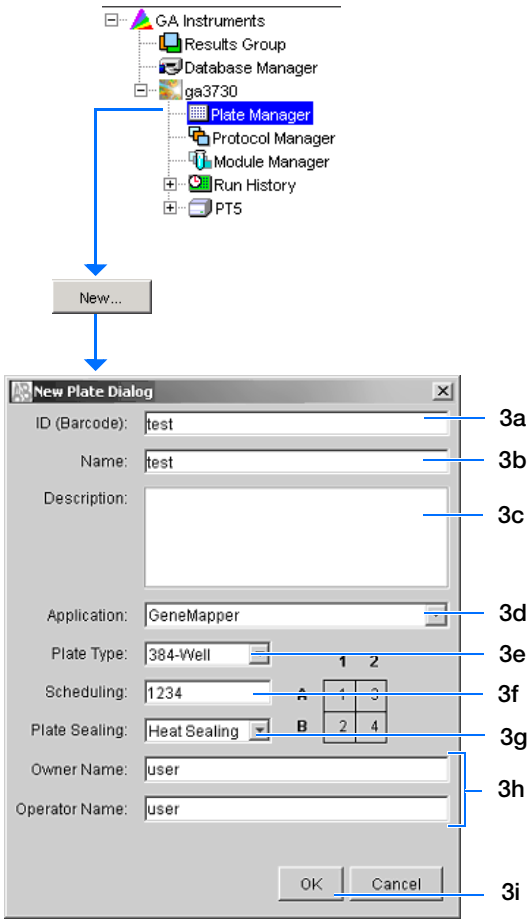
Creating and Completing a GeneMapper® Software Plate Record

Creating the GeneMapper® Software Plate Record

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. (*Optional*) Type a description for the plate.
   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 133.
   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.



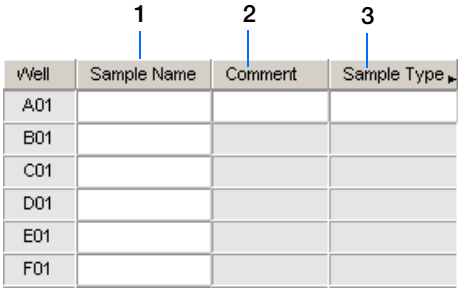
Completing a GeneMapper Software Plate Record

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.

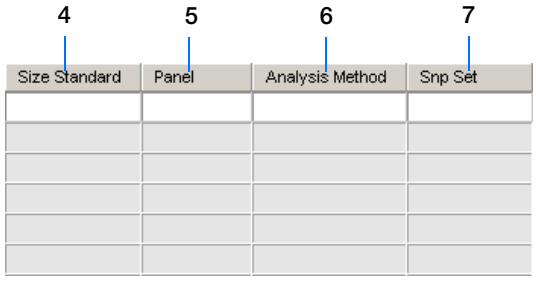
3. In the Sample Type column, select a sample type from the drop-down list.

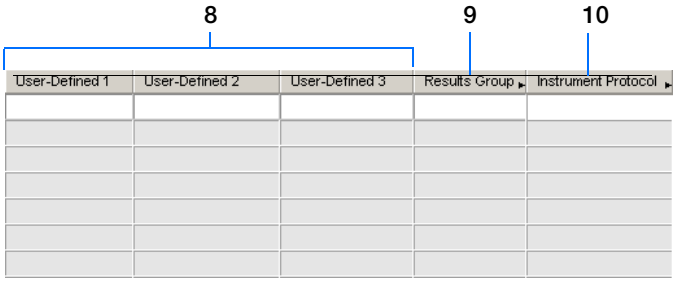
4. In the Size Standard column, select a size standard from the drop-down list.



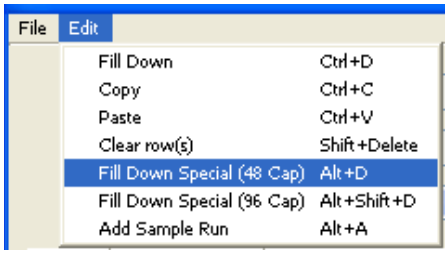
Notes

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. (*Optional*) 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.





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 124.
    - 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.

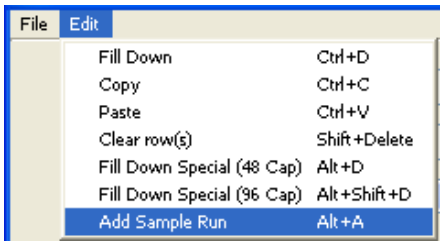


Notes

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 126.



13. Complete the columns for the additional runs.

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

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

Note: If multiple cells are selected for copying, select the same number of corresponding target cells before you execute the Paste command.

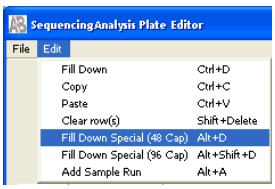
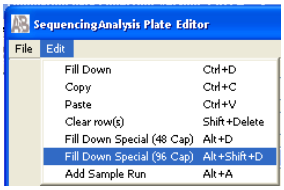
Note: The Plate Editor Copy and Paste functionality is supported only within one plate editor. To copy and paste the contents of one plate to another plate, use the "Duplicate..." button on the Plate Manager dialog box.

Note: If you use the duplicate plate function, all the information in the plate to be duplicated must be valid. Otherwise, an empty plate is created.

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 following table.

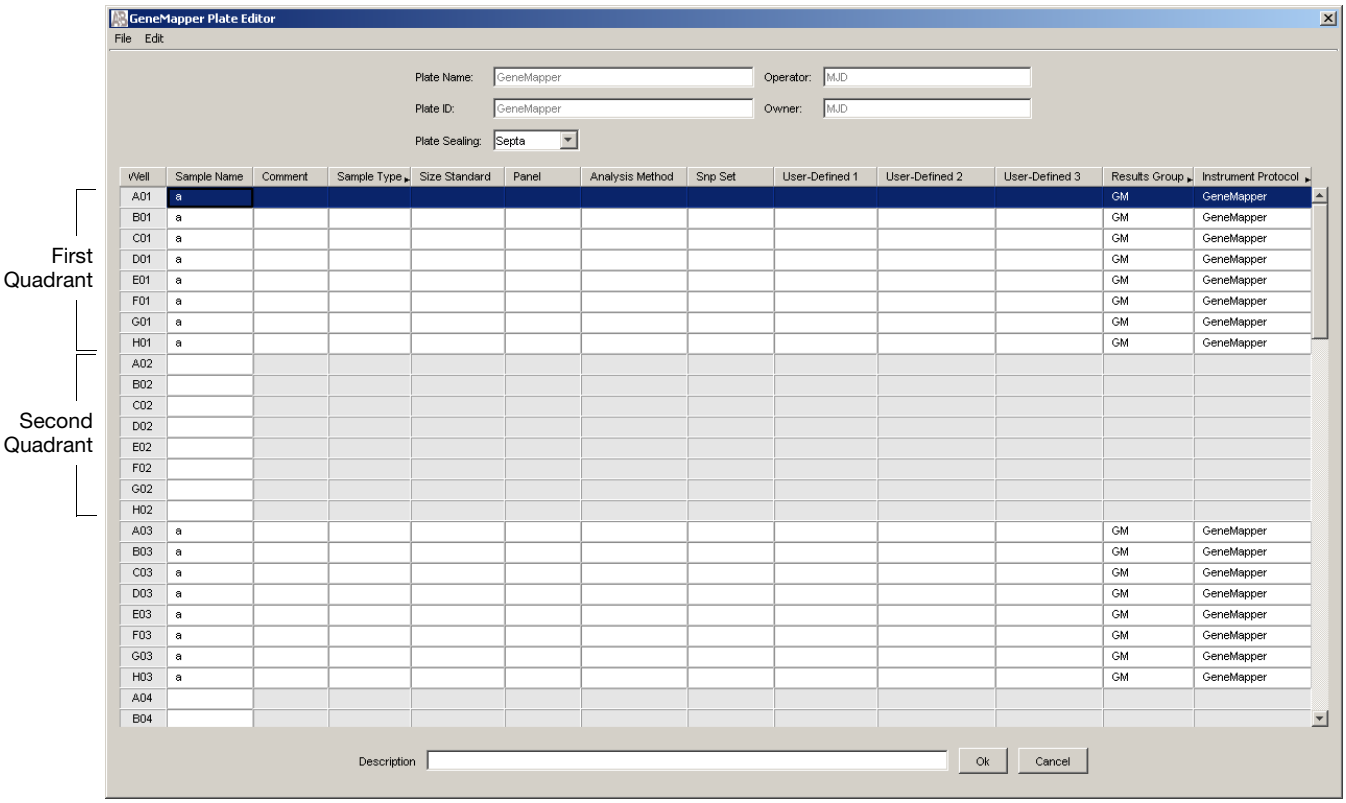
If You Choose ...	Then ...
Fill Down Special (48 Cap) 	The fill down pattern matches the 48-capillary load pattern. Well / Sample Name A01 notMJD B01 notMJD C01 notMJD D01 notMJD E01 notMJD F01 notMJD G01 notMJD H01 notMJD A02 MJD B02 MJD C02 MJD D02 MJD E02 MJD F02 MJD G02 MJD H02 MJD A03 notMJD B03 notMJD C03 notMJD First Quadrant Second Quadrant
Fill Down Special (96 Cap) *  * Especially useful for 384-well plates	The fill down pattern matches the 96-capillary load pattern. 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

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.



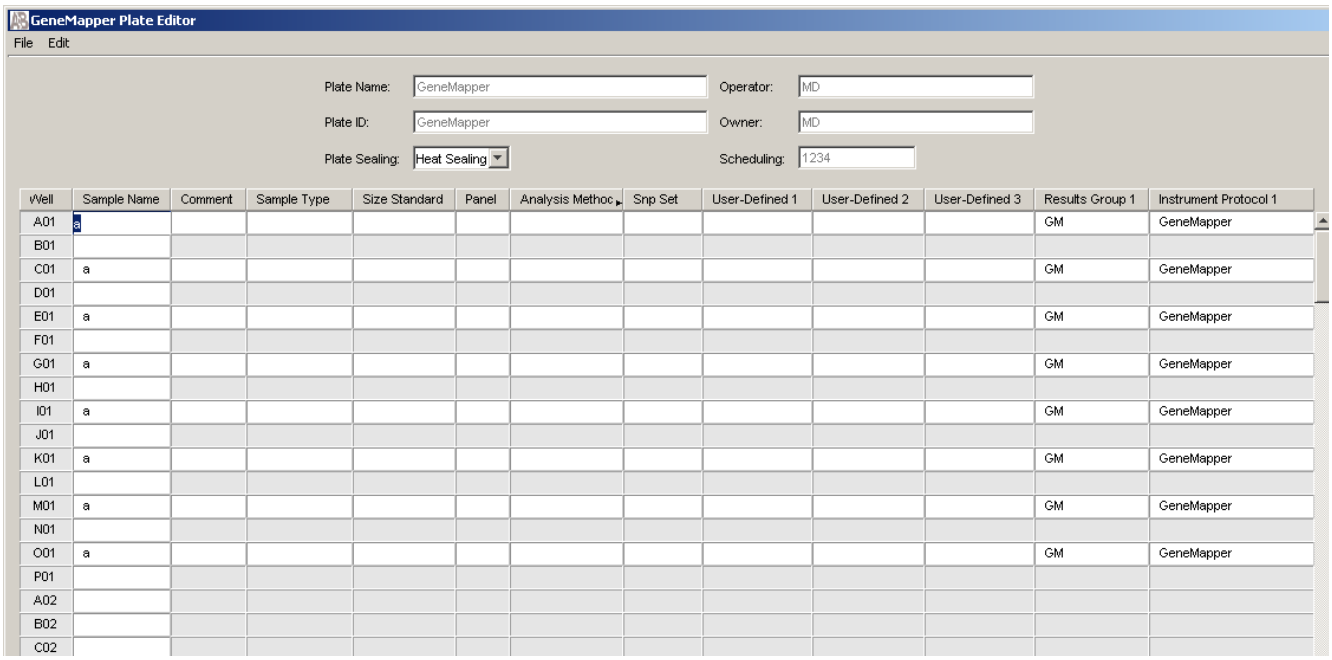
Well	Sample Name	Comment	Sample Type	Size Standard	Panel	Analysis Method	Snp Set	User-Defined 1	User-Defined 2	User-Defined 3	Results Group	Instrument Protocol
A01	a										GM	GeneMapper
B01	a										GM	GeneMapper
C01	a										GM	GeneMapper
D01	a										GM	GeneMapper
E01	a										GM	GeneMapper
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 the preceding figure).

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 in the following figure.



Adding a Sample Run

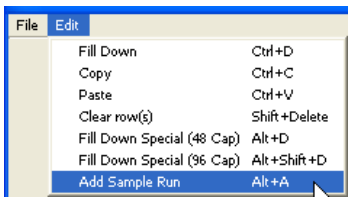
By adding additional sample runs, you can run samples that have different variables (different run modules, for example).

Adding a sample run opens an additional:

- Results group
- Instrument protocol

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

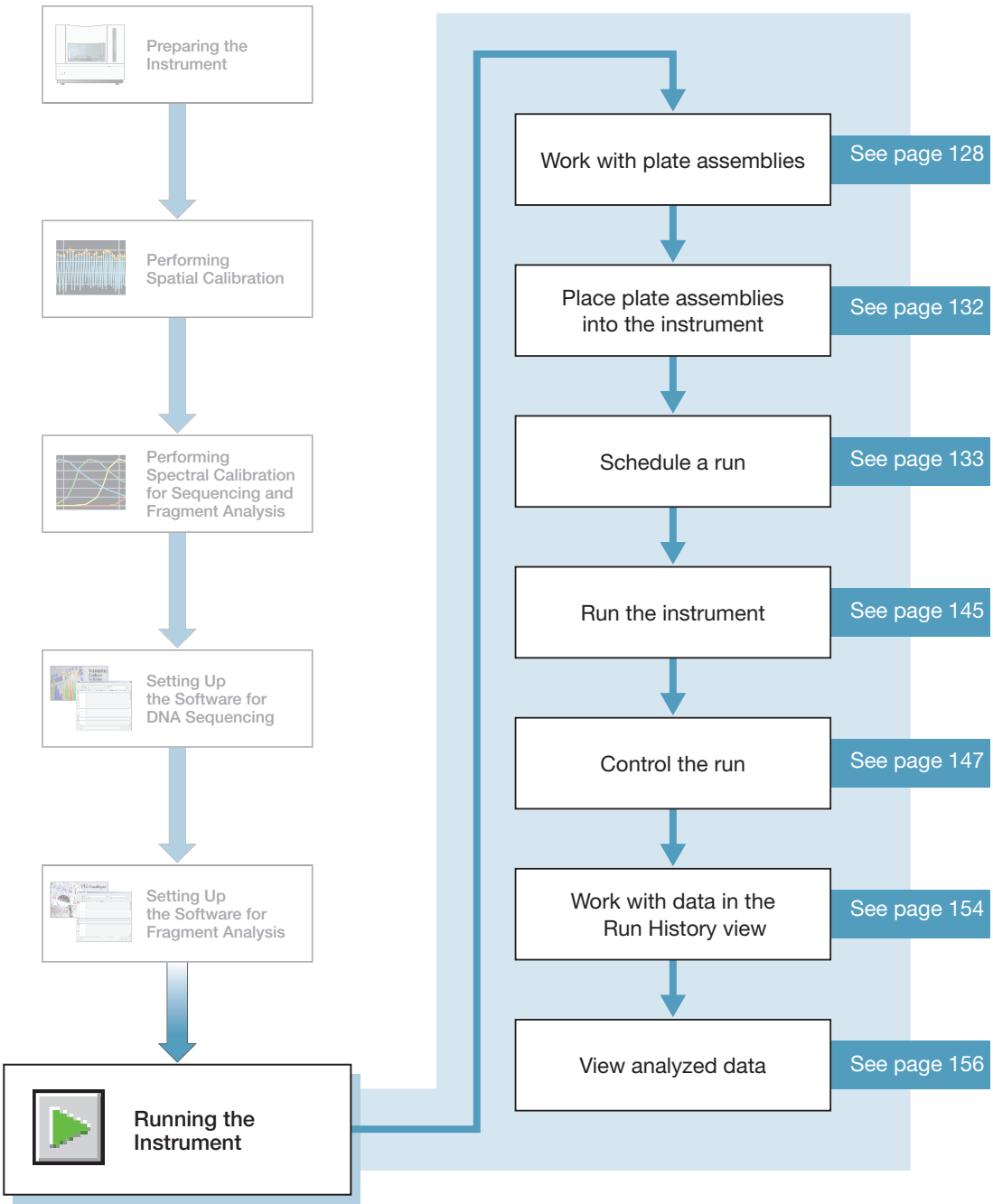
To run the plate(s), see "Running the Instrument" on page 127.



Note: When you add another sample run to a processed plate, confirm that all the information in the processed runs is valid. Otherwise, that data will not be validated, and a new sample run cannot be created.

Notes

Running the Instrument



Notes

Working with Plate Assemblies

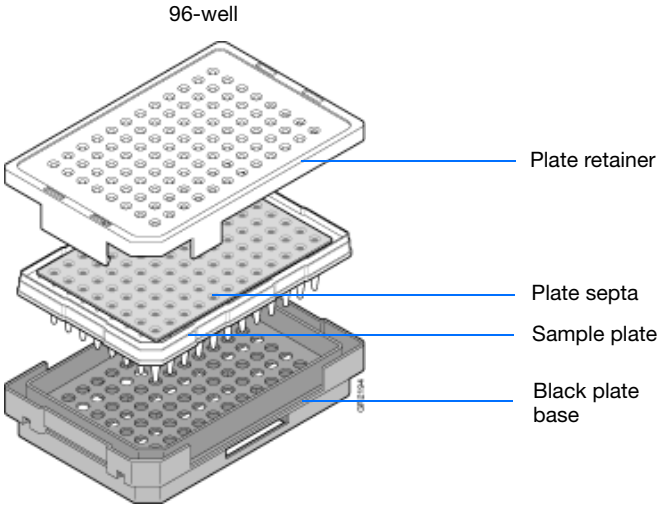
Plate Assembly Components

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.

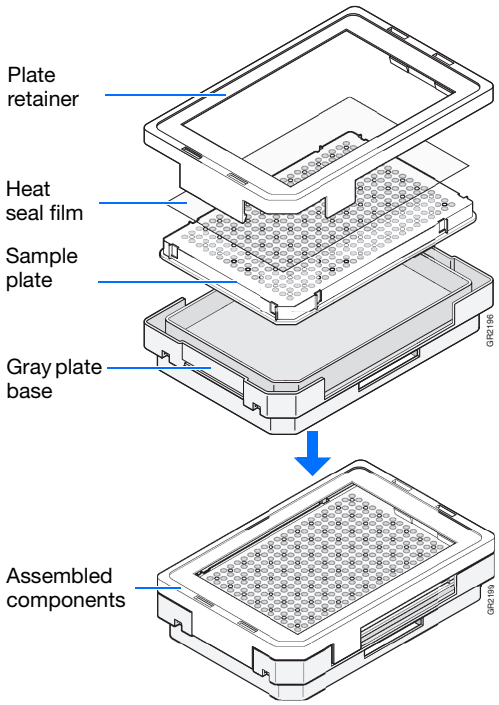


Notes

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.



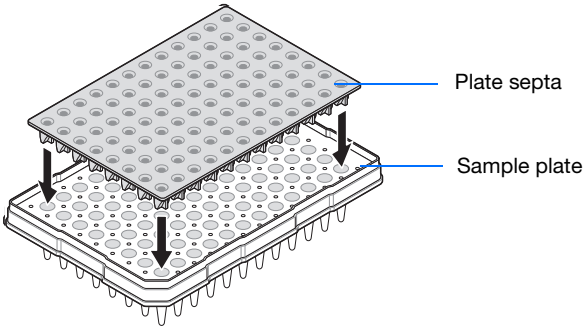
Heat Seal Film Guidelines

- Use 3-mil heat seal film (Catalog no. 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 3730/3730*xl* DNA Analyzer.
- Do *not* use heat-seal film containing adhesives or metals because they may damage the instrument's piercing needles

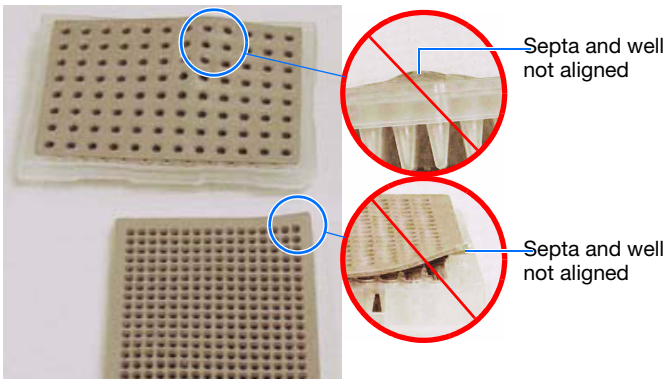
Notes

Preparing a Septum-Sealed Plate Assembly

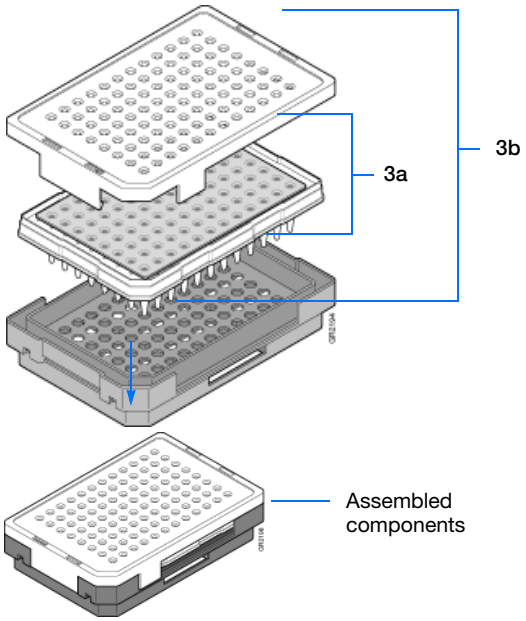
1. Seal the plate:
   a. Place the plate on a clean, level surface.
   b. Inspect septa weekly and be sure to replace any that are worn or discolored.
   c. Lay the septum flat on the plate.
   d. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.



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.



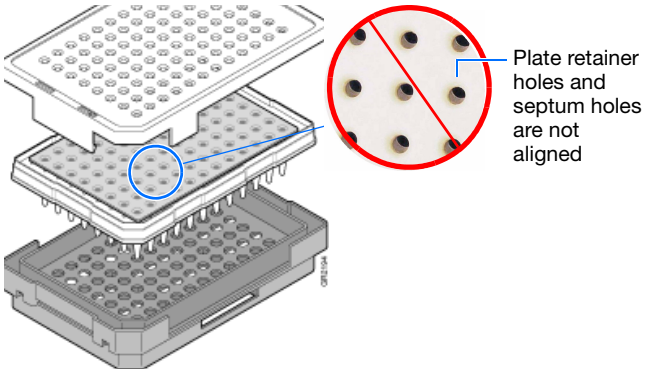
3. Assemble the plate assembly:
   a. Place the sample plate into the plate base.
   b. Snap the plate retainer onto the plate and plate base.
   c. Make sure when you assemble a plate that the retainer clip is flush with the plate base. A simple way to ensure that they are flush is to run your finger along the edge.



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.



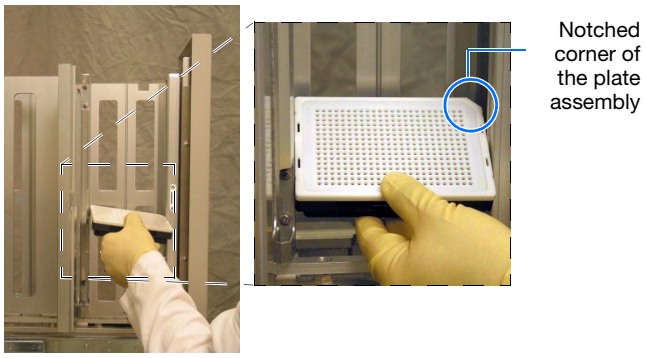
Notes

Placing Plate Assemblies into the Instrument

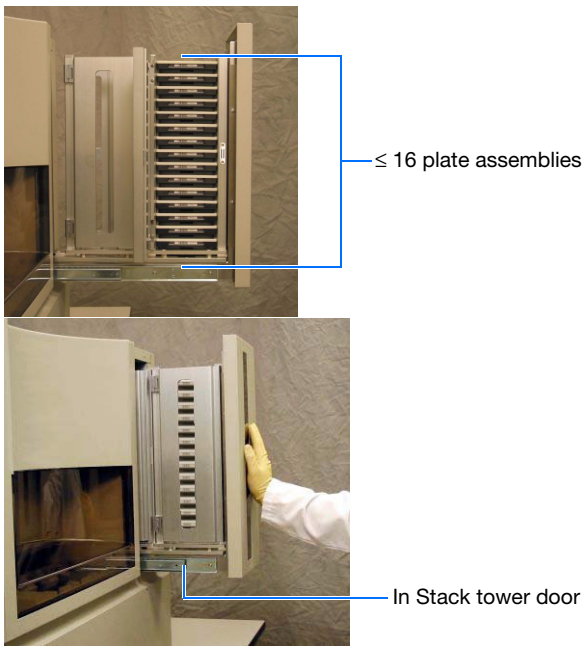
1. Open the stacker drawer.
2. Open the door of the In Stack tower.

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.



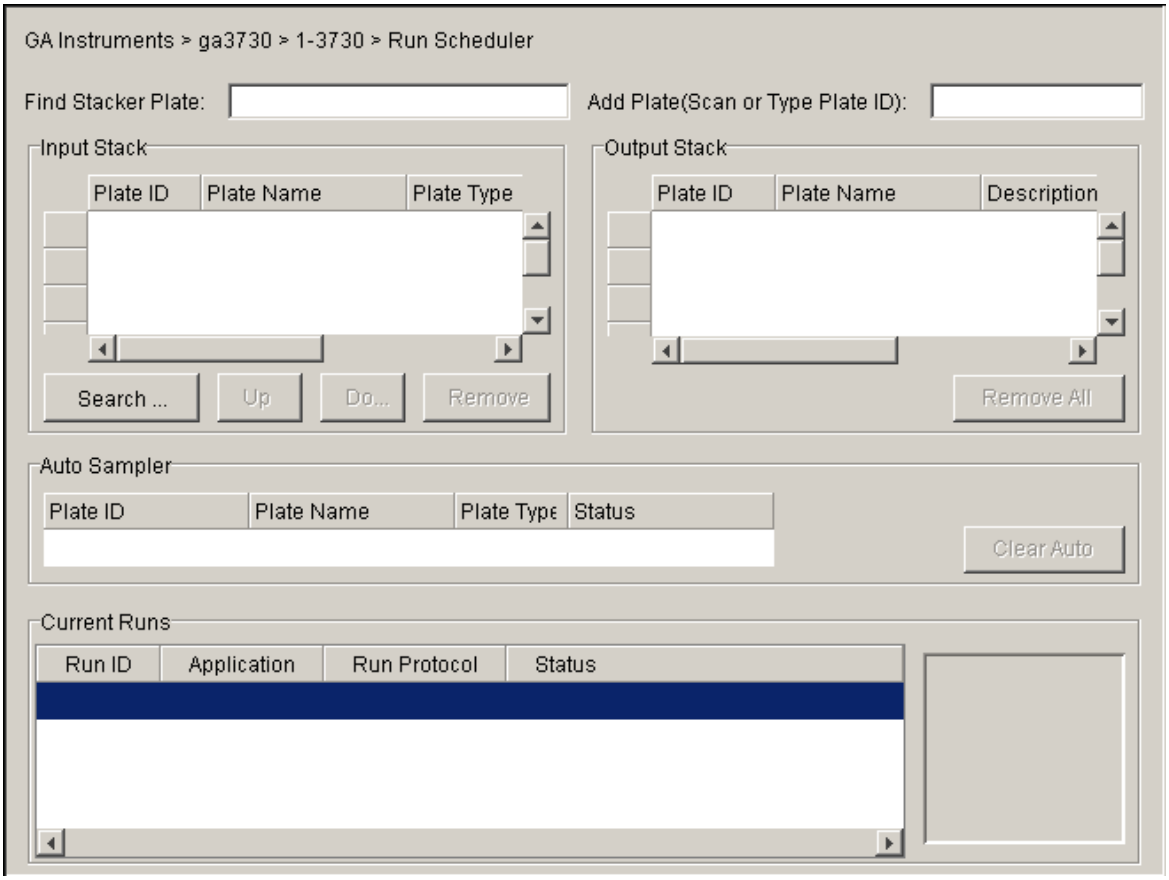
4. Close the metal In Stack tower door.
5. Close the Stacker drawer.



Notes

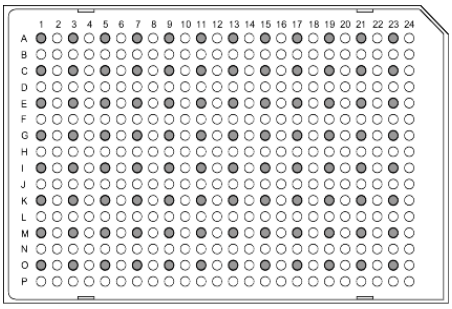
Scheduling Runs

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 70.
- Fragment analysis – See page 105.

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

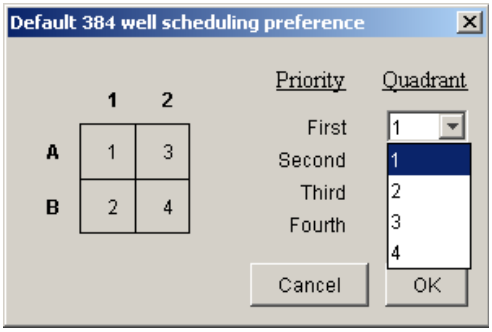
Globally Modifying a Run Schedule

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

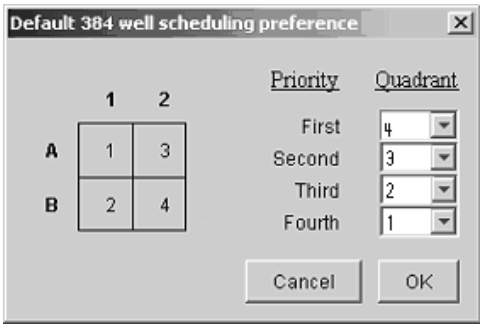
To modify the run order for all 384-well plates:

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:

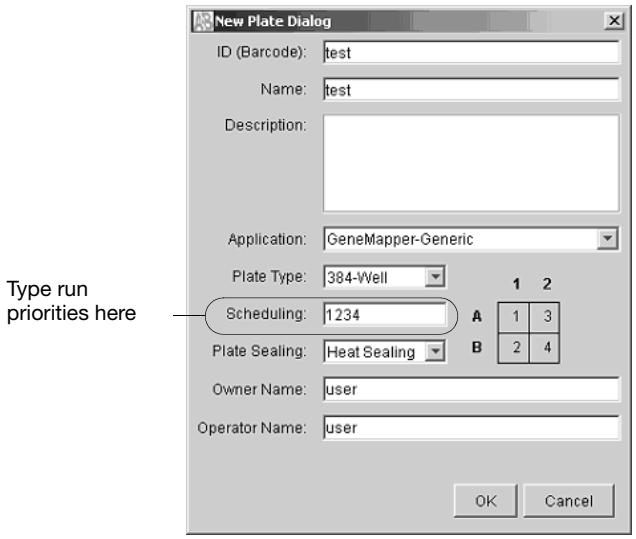
1. In the Plate Manager, click **New Plate**.

   Note: For information about the Plate Manager, see page 93 for sequencing, and page 121 for fragment analysis.
2. Select **384-Well** from the Plate Type list.

   The Scheduling box is activated.

Notes

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

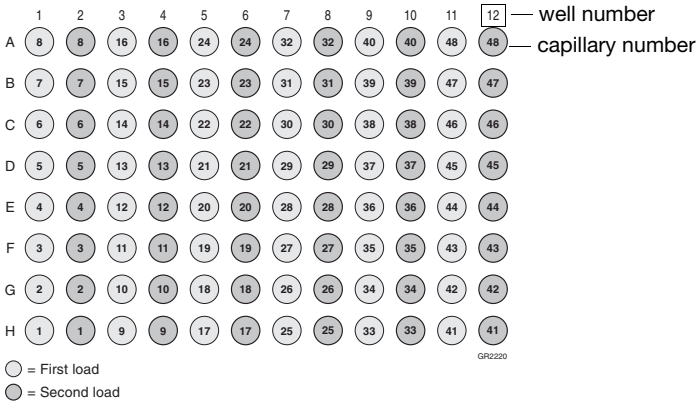


Notes

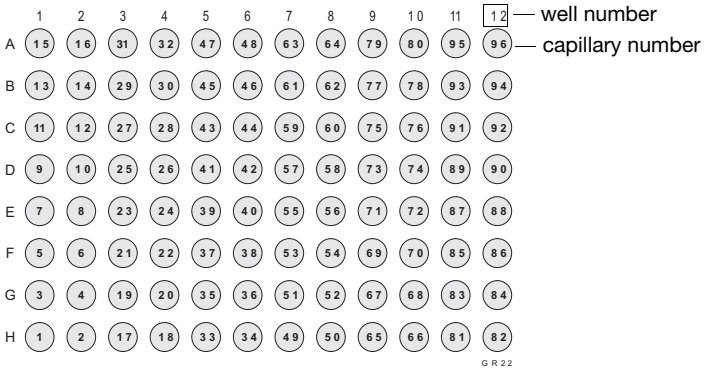
Default Load Maps

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

96-Well Plate, 48 Capillaries



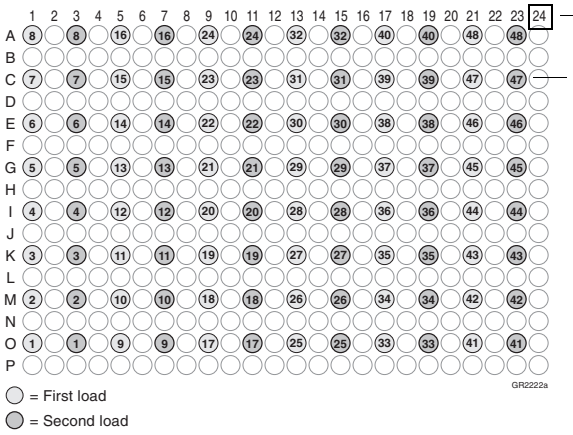
96-Well Plate, 96 Capillaries



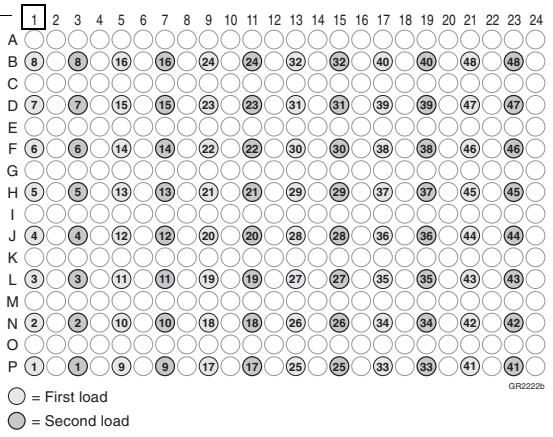
Notes

384-Well Plate, 48 Capillaries

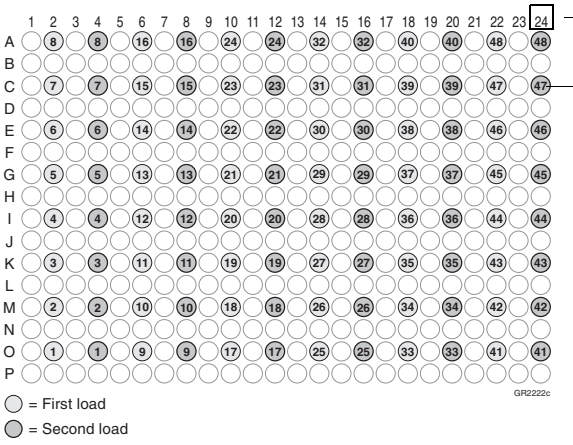
First quadrant pickup



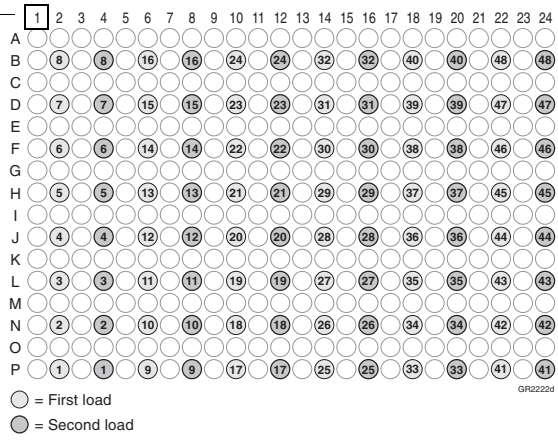
Second quadrant pickup



Third quadrant pickup



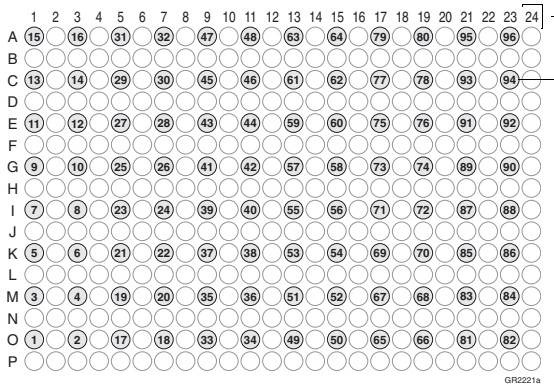
Fourth quadrant pickup



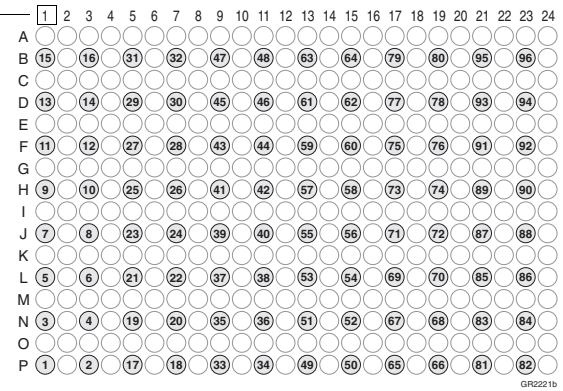
Notes

384-Well Plate, 96 Capillaries

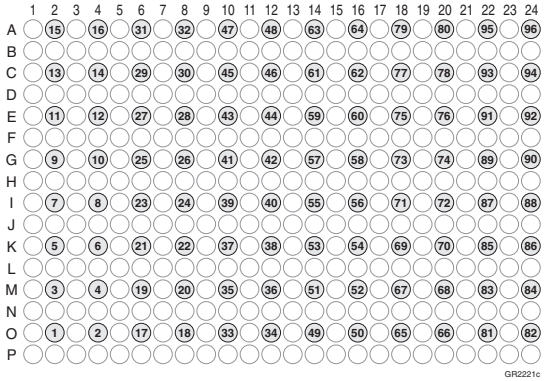
First quadrant pickup



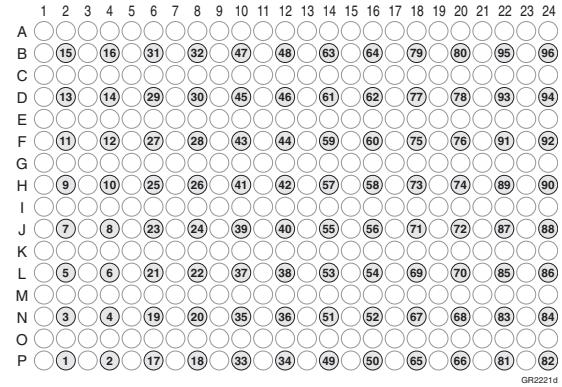
Second quadrant pickup



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:

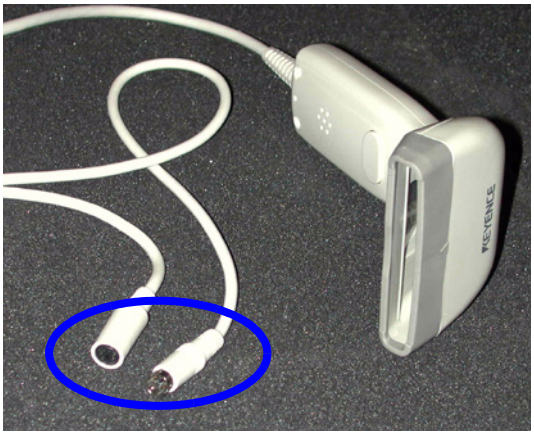
- Code 128
- Code 39
- Code 93
- LOGMARS
- EAN-8

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

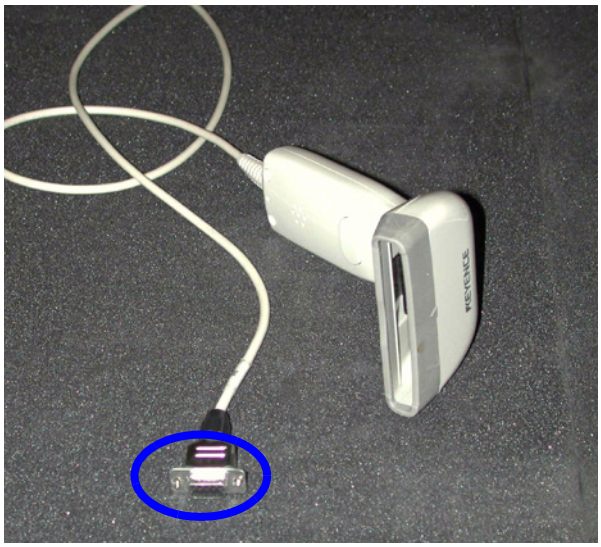
KEYENCE BL-80VE



An external barcode reader can also be used with the 3730/3730*xl* Analyzer. The KEYENCE BL-80VE (see the preceding photo) 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 in the following sections. Access either mode by selecting in the navigation pane:

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

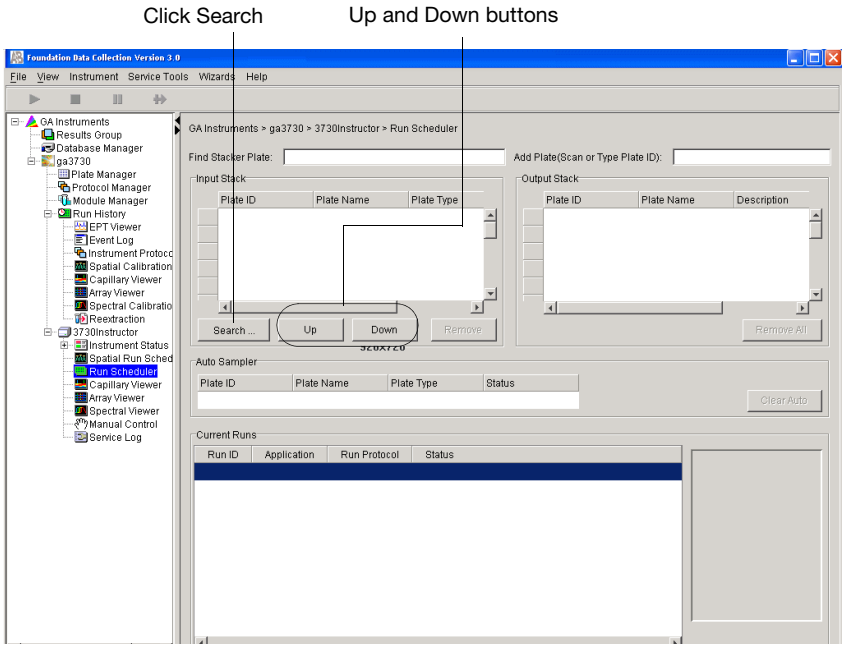
Note: You must be in the Run Scheduler view to see the instrument run mode menu.

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)

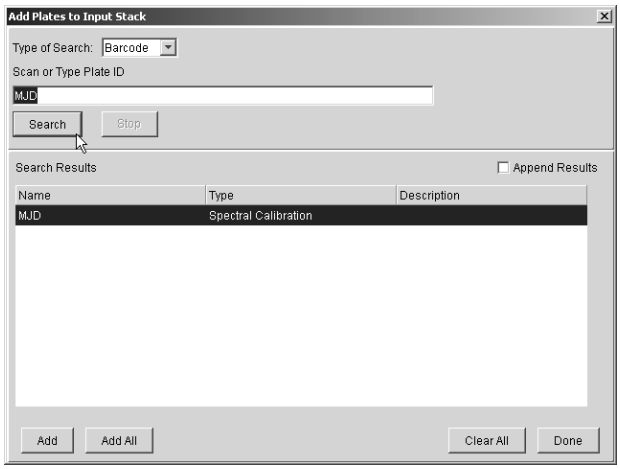
1. In the navigation pane, select **Instrument > Instrument Name > Manual mode**.
2. Click **Search** in the Run Scheduler to search for plate record(s).



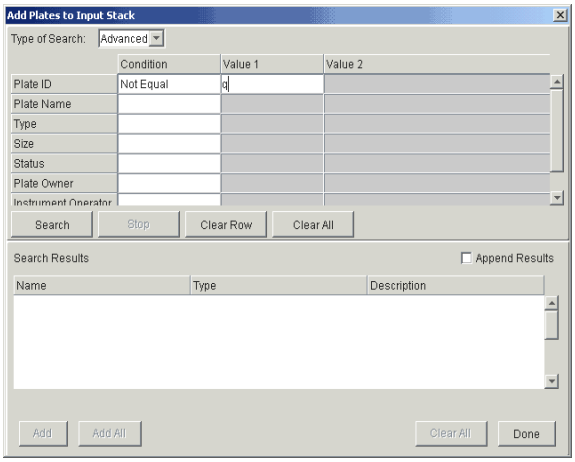
The **Add Plates to In Stack** dialog box opens.

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:

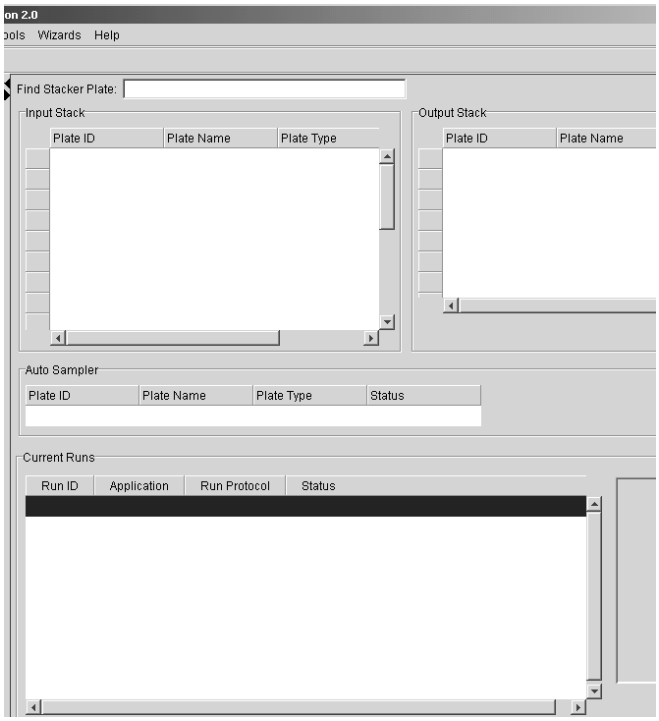
1. Select **Run Scheduler > Instrument Name > Auto mode**.

   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

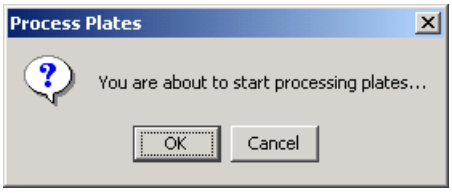
1. Verify that the active spectral calibration matches your dye set and capillary array length.

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* (Part no. 4477797).
     – 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_POP-7™	20‡
Rapid read DNA sequencing	36	RapidSeq36_POP-7™	35
Standard read DNA sequencing	36	StdSeq36_POP-7™	60
Fast DNA sequencing	50	FastSeq50_POP-7™	60
Long read DNA sequencing	50	LongSeq50_POP-7™	120
Extra Long DNA sequencing	50	XLRSeq50_POP-7™	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_POP-7™	32
Fragment Analysis	50	GeneMapper50_POP-7™	43
SNPlex® Genotyping	36	HTSNP36_POP7_V3	15
SNPlex® Genotyping	50	HTSNP50_POP-7™	25

Notes

Controlling the Run

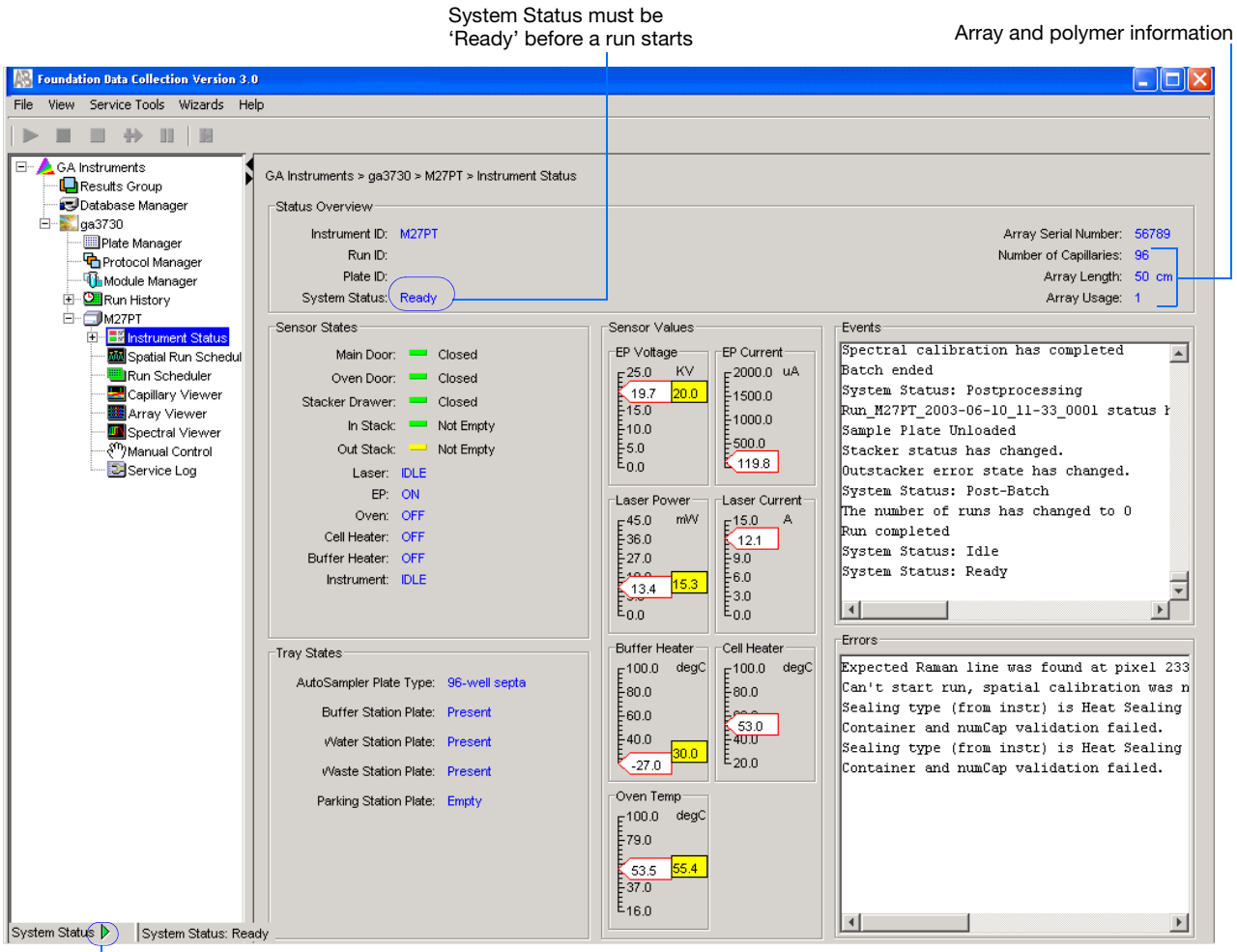
You can use the toolbar at the top of the Data Collection Software window to control the run.

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.



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

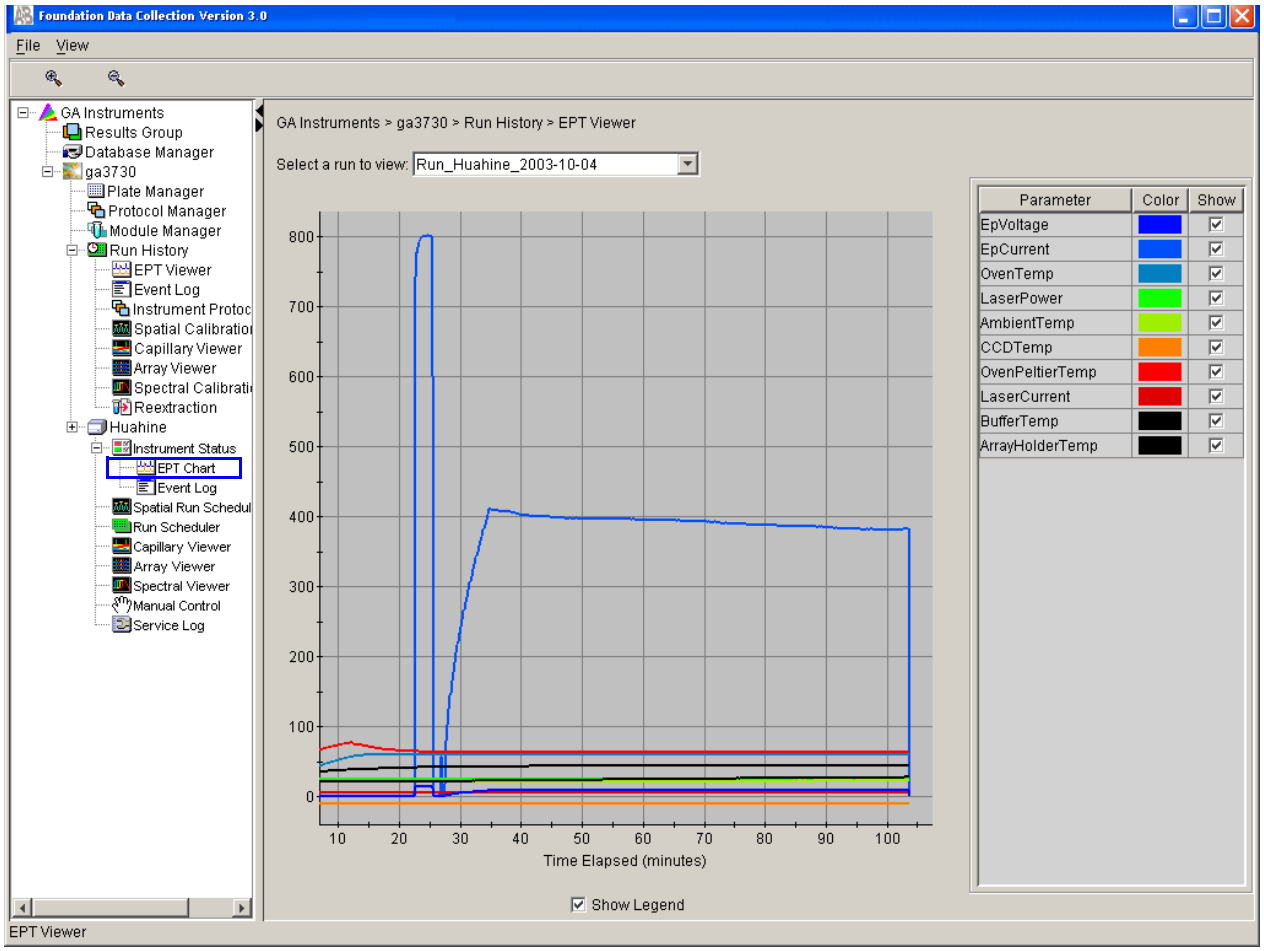
Some of the error messages provide information for service engineers. A “fatal” error usually requires that you restart the Data Collection Software.

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**.



Notes

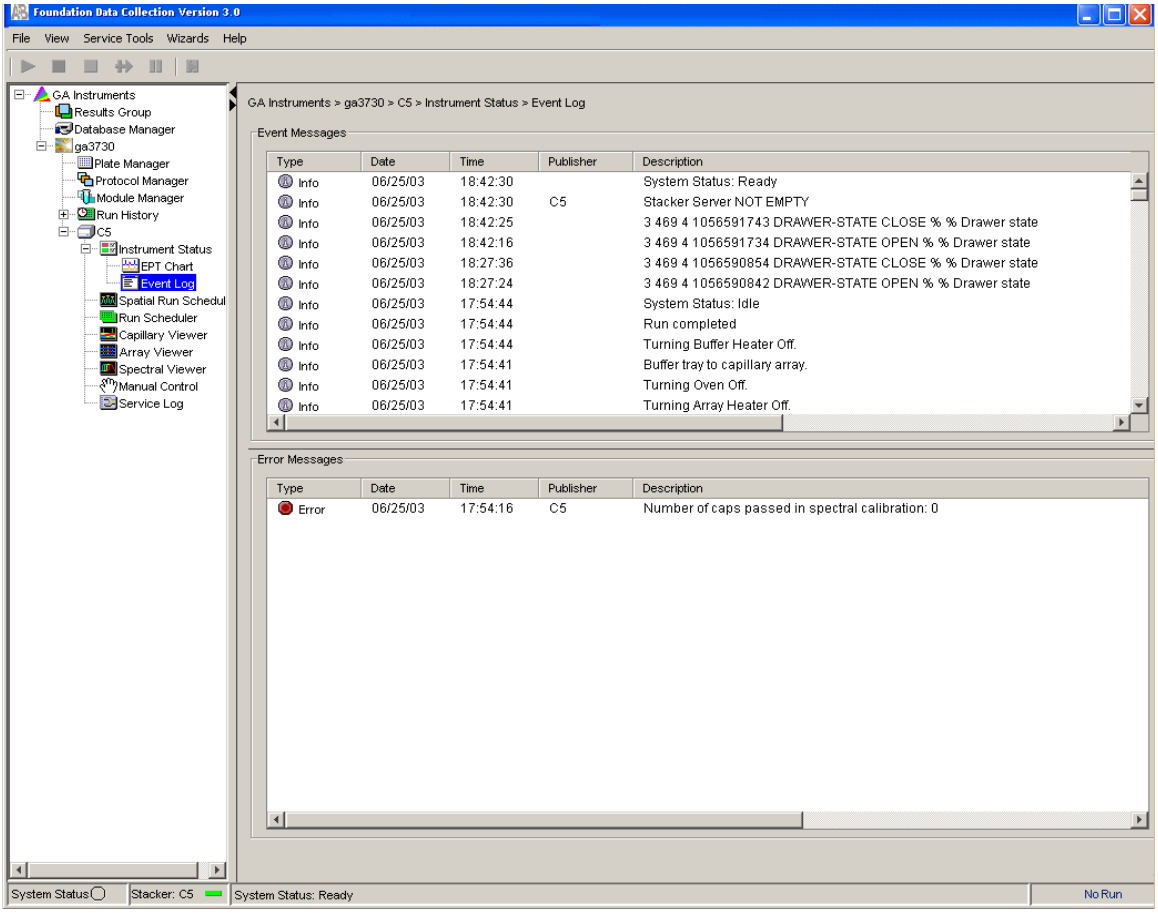
Viewing Event History

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.



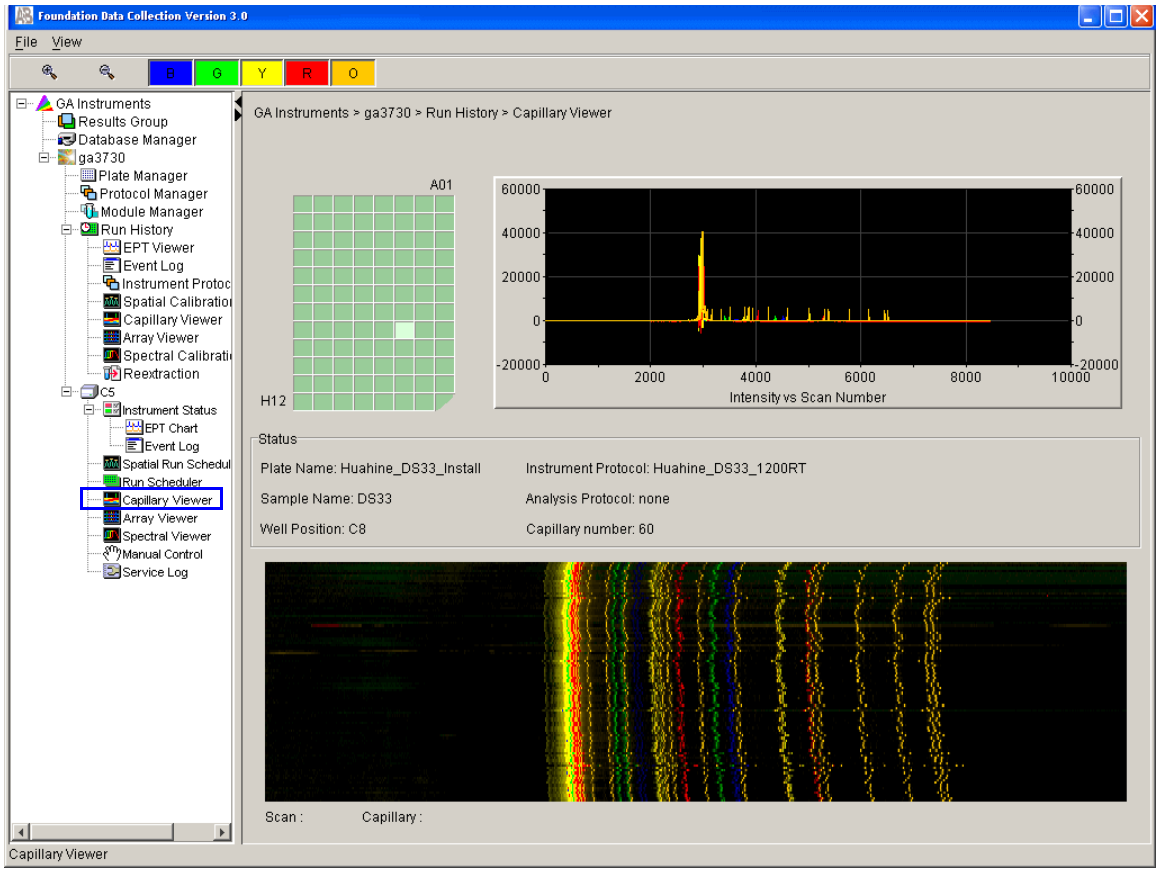
Note: If an error is generated while using manual control, reboot the instrument then restart the Data Collection Software to recover from the error stage.

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.

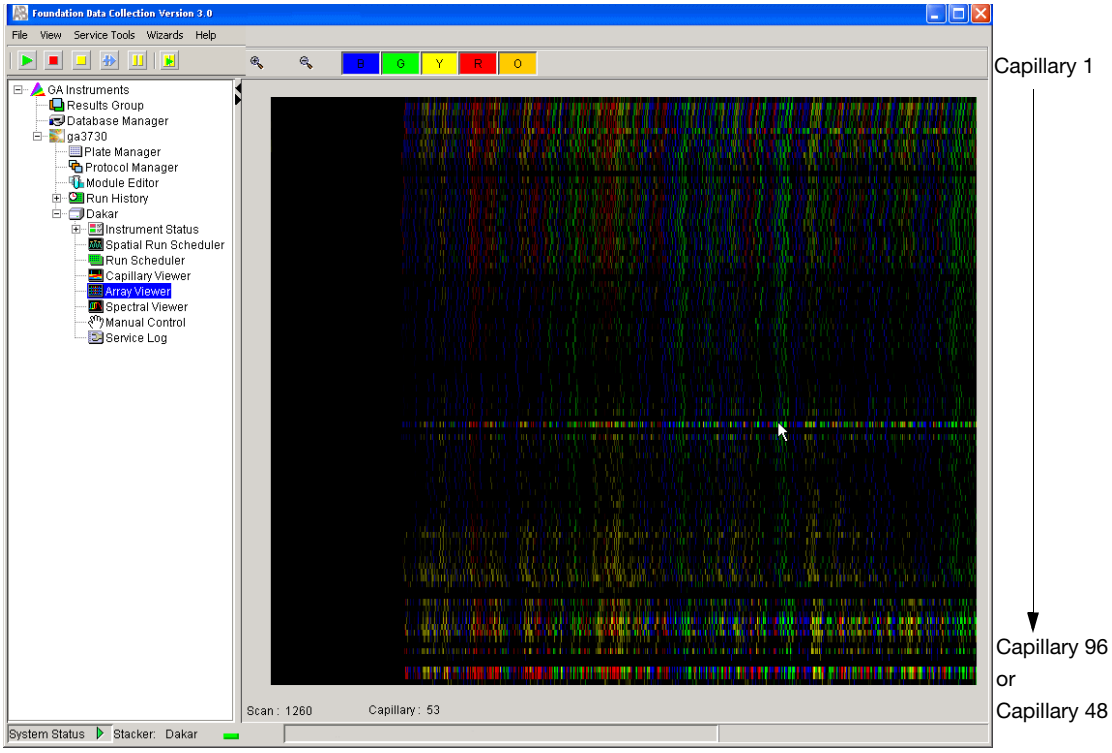
Click individual colors to view or hide them. 

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 3730/3730*xl* 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.	

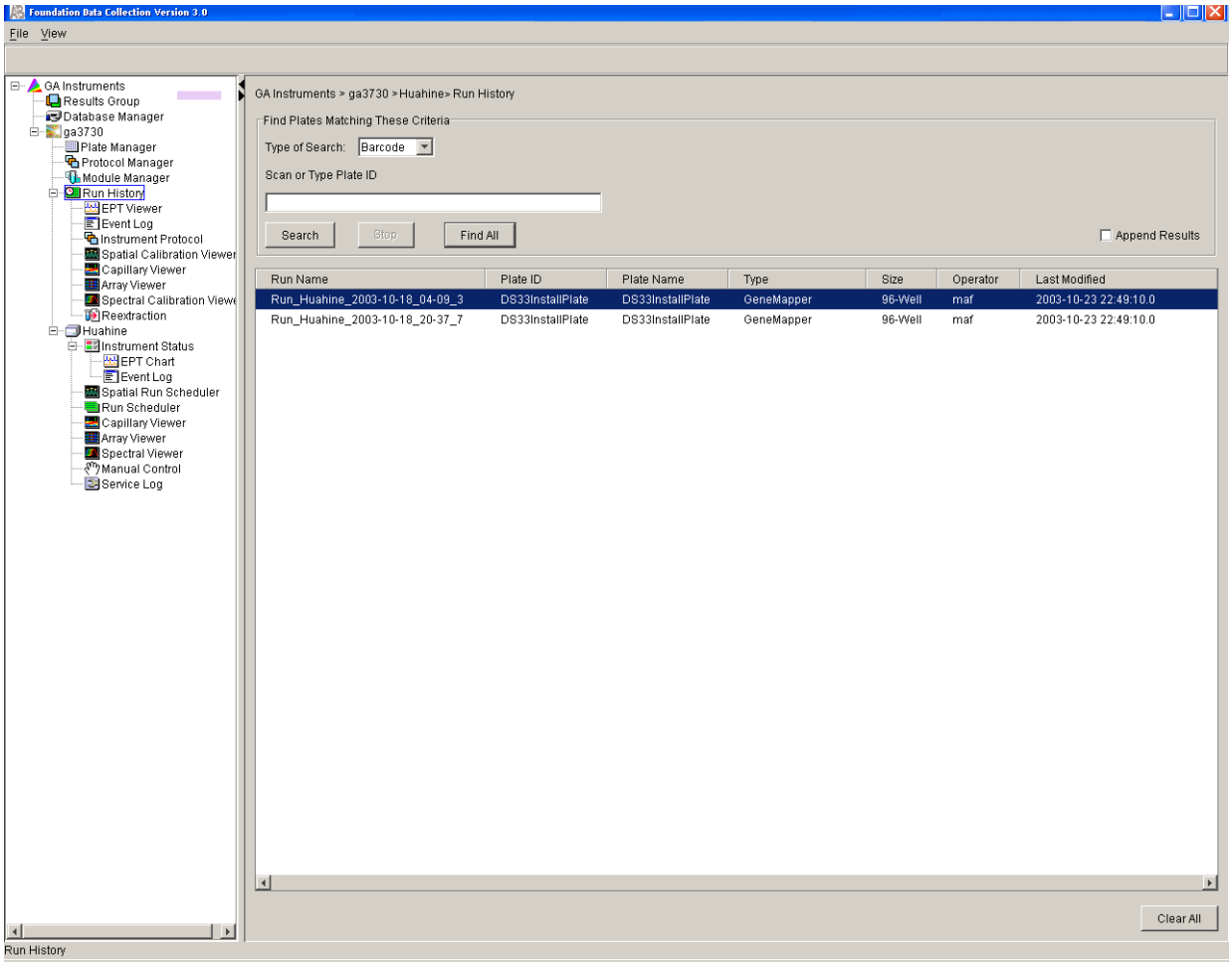
Viewing Data from a Completed Run

There are two formats for viewing data within the 3730/3730*xl* 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 3730/3730*xl* 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.

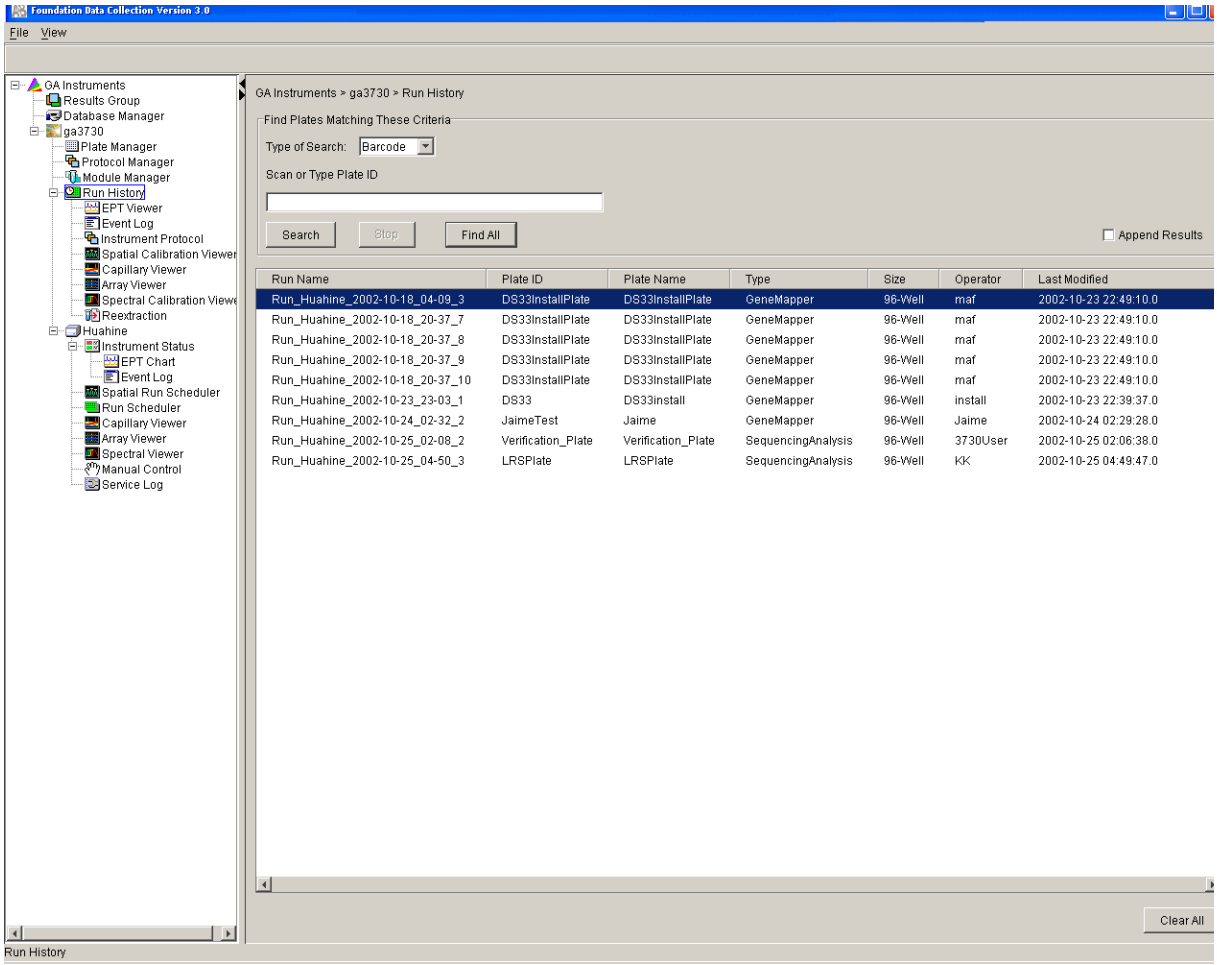
The auto extractor component of the 3730/3730*xl* Analyzer Data Collection automatically extracts data from stopped runs. If autoextraction fails, click Reextraction  to extract data.

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



4. Click (Reextraction) in the navigation pane. The Reextraction window opens.

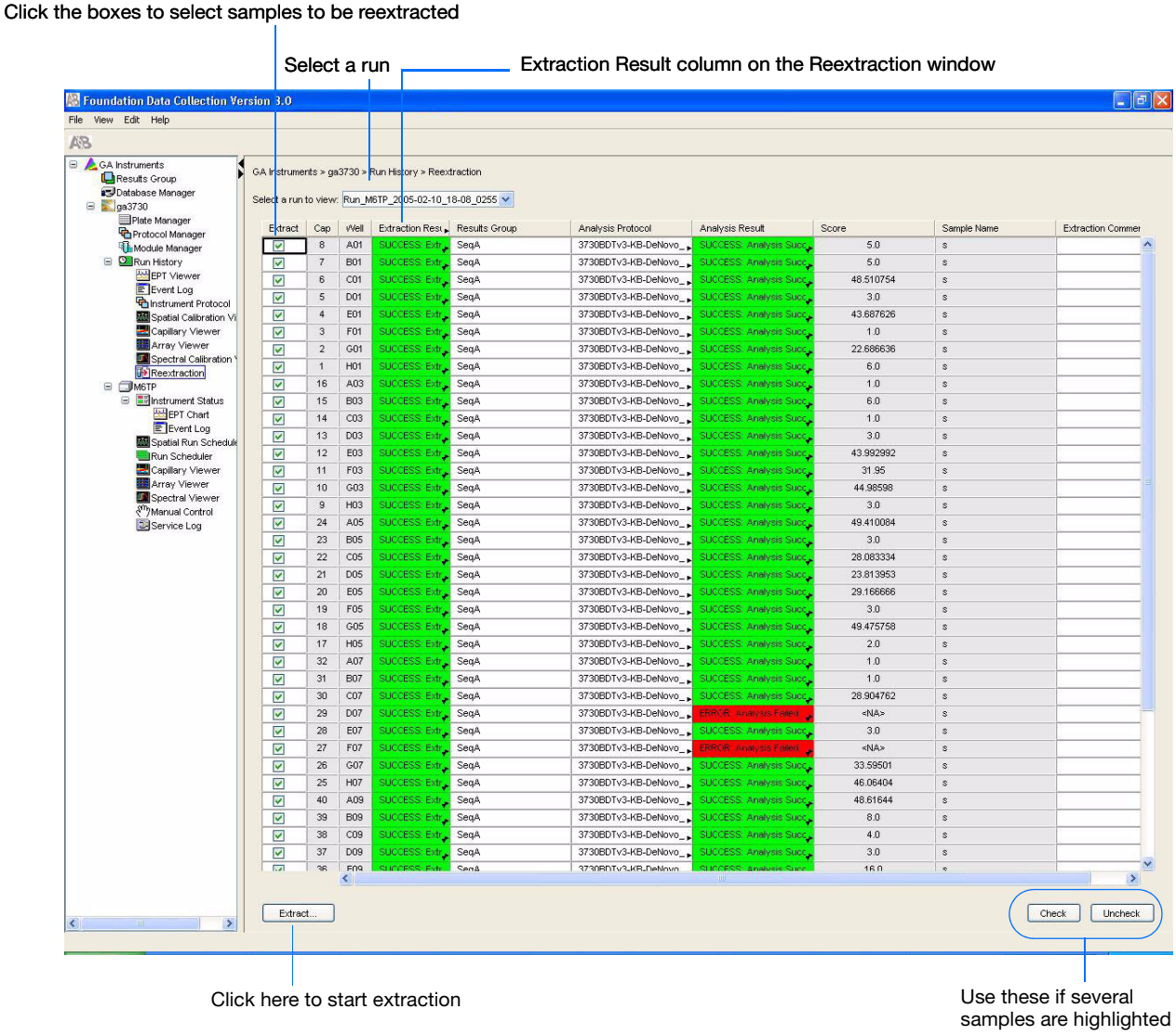
5. Select the checkboxes in the Extract column that correspond to the samples to be reextracted.

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.

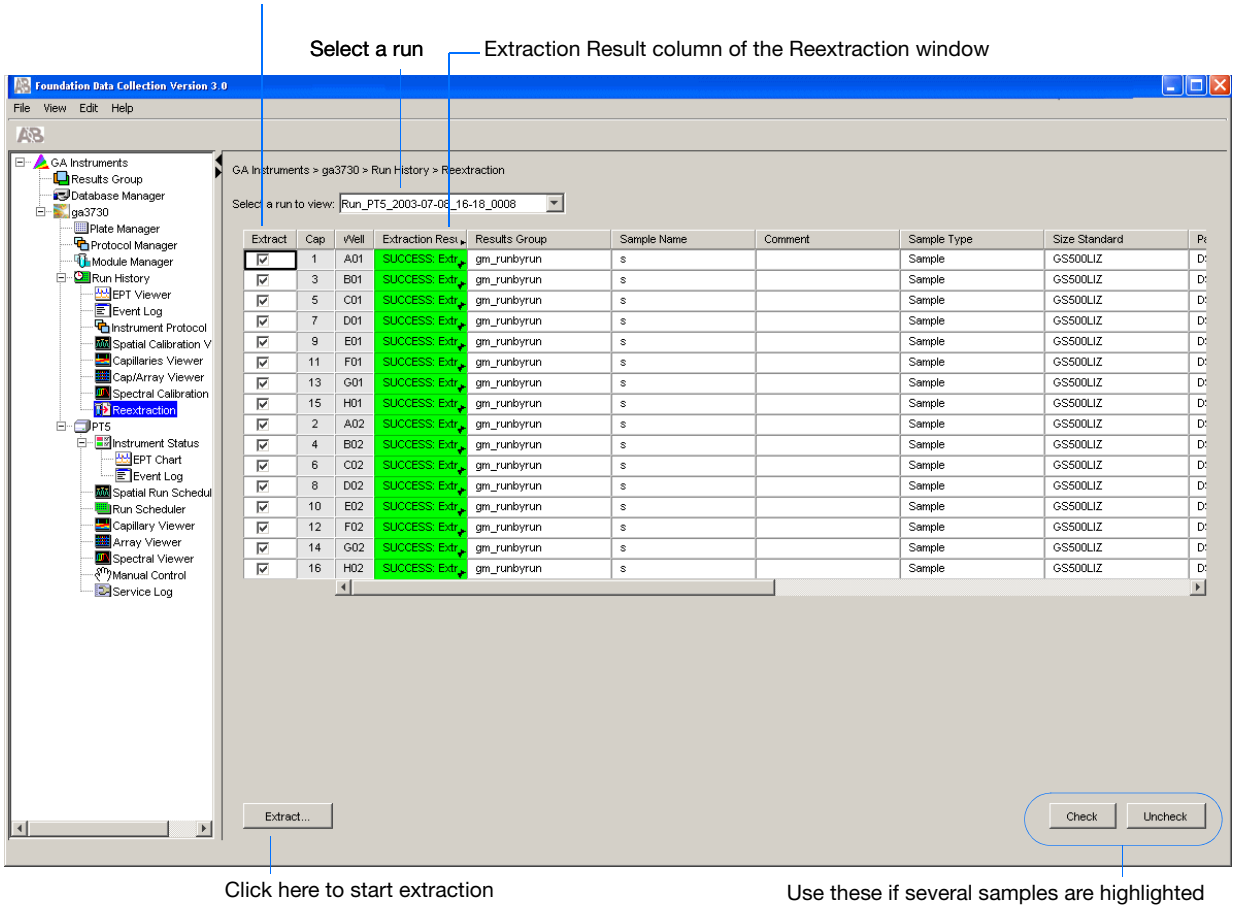
Notes

Reextraction Window for Sequencing Analysis



Notes

Reextraction Window for Fragment Analysis



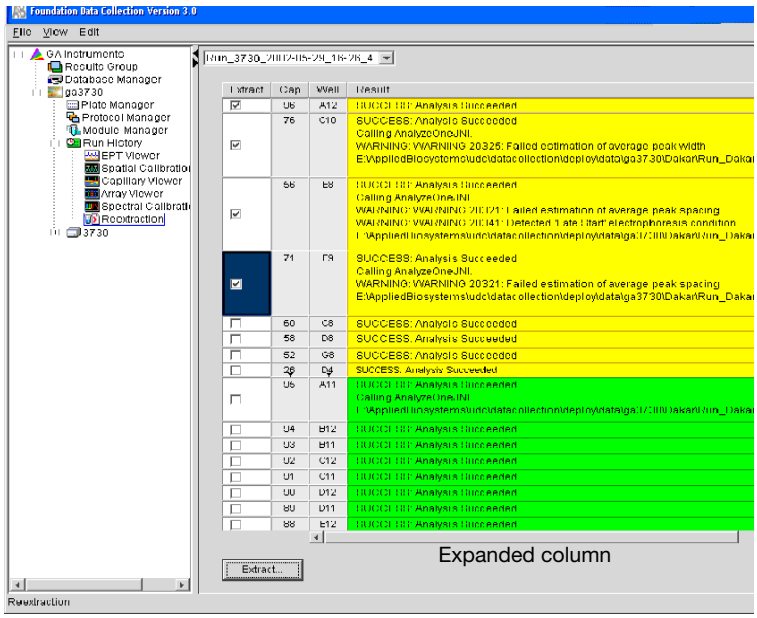
Notes

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.



Expanded column

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. 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. If desired, 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

Notes

Catalog List

Item	Cat. and 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
POP-6™ Polymer (1 bottle of 7ml each)†	4352757
POP-6™ Polymer (1 bottle of 3.5ml each)†	4363783
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

Notes

Item	Cat. and Part no.
FAST (0.1ml) 96-Well Plate Retainer for 3730 (septa-sealed)	4367472
FAST (0.1ml) 96-Well Plate Base for 3730 (septa-sealed)	4367469
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® 3730/3730*x*/ DNA Analyzer Getting Started Guide	4359476
Applied Biosystems® BigDye Xterminator® Purification Kit Protocol	4374408
AB Navigator Software Administrator Guide	4477853

† Call Technical Support for an adaptor to use POP-6 on the 3730 Series.

Notes

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

Supported Dye Sets

Sequencing Analysis Dye Sets for All Applications

Dye Set	Application Name
E_BigDyeV1	DNA sequencing with BigDye® Terminator v1.1 Cycle Sequencing Kit
Z_BigDyeV3	DNA sequencing with BigDye® Terminator v3.1 Cycle Sequencing Kit
Z_BigDyeV3	DNA sequencing with BigDye® Direct Cycle Sequencing Kit, with combined DNA PCR Amplification/Clean-up/Cycle Sequencing kit

Fragment Analysis Dye Sets for All Applications

Dye Set	Application Name
G5	DNA sizing for 5-dye chemistry
G5-RCT	DNA sizing for 5-dye chemistry
Any5Dye	SNaPshot® Multiplex System

Additional Dye Sets

Dye Set	Application Name
Any4Dye-HDR	DNA sizing and DNA sequencing
Any4Dye	DNA sizing and DNA sequencing

Notes

Dye Sets G5 and G5-RCT For Fragment Analysis

Overview

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

Dye Set G5-RCT

To reduce crosstalk for fragment analysis applications, a new dye set has been created for Data Collection Software 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.

Life Technologies supports:

- Fragment analysis on the 96-capillary array using G5-RCT only
- 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	**When to use/Advantages:** • All DNA sequencing applications using BigDye® Terminators v3.1 and v1.1 and BigDye® Direct. • Higher signal relative to the Any4Dye-HDR dye set • Optimized for the highest signal-to-noise ratio **Issues:** • More susceptible to samples within a plate with large variation in peak height relative to the Any4Dye-HDR dye set
Any4Dye	**When to use/Advantages:** • Use of unsupported dyes. Provides an open platform for system capable applications **Issues:** • 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)	**When to use/Advantages:** • 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 **Issues:** • Signal intensity is reduced by approximately half relative to the standard dye sets, along with a minimal reduction in the noise, resulting in a very slight decrease in the signal/noise ratio 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

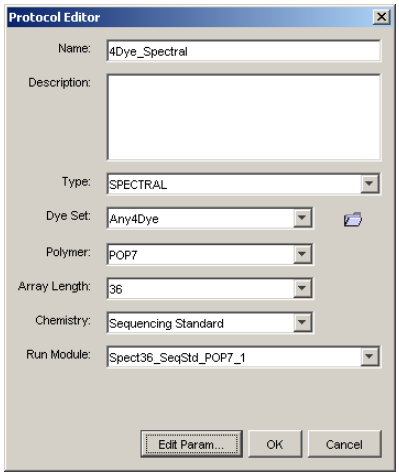
Notes

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

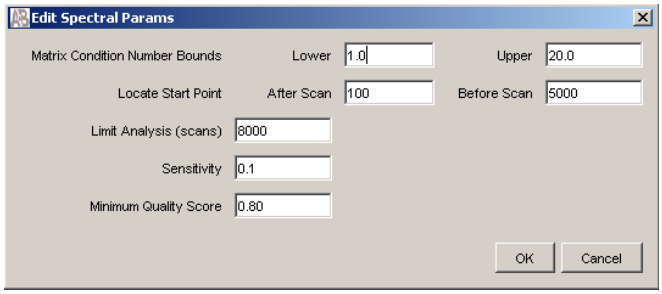
The steps to creating and running a customized 4- or 5-Dye Set are similar to running a supported dye set.

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.

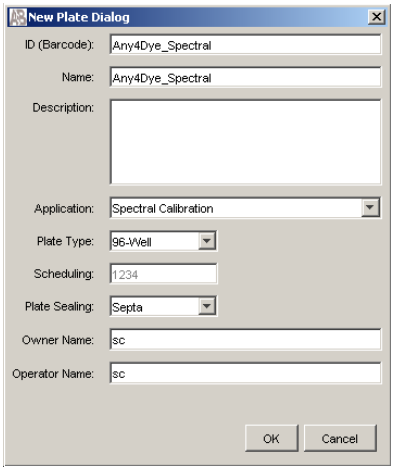


Note: Customize the Spectral parameters as needed. For more information, see step 1 on page 49.

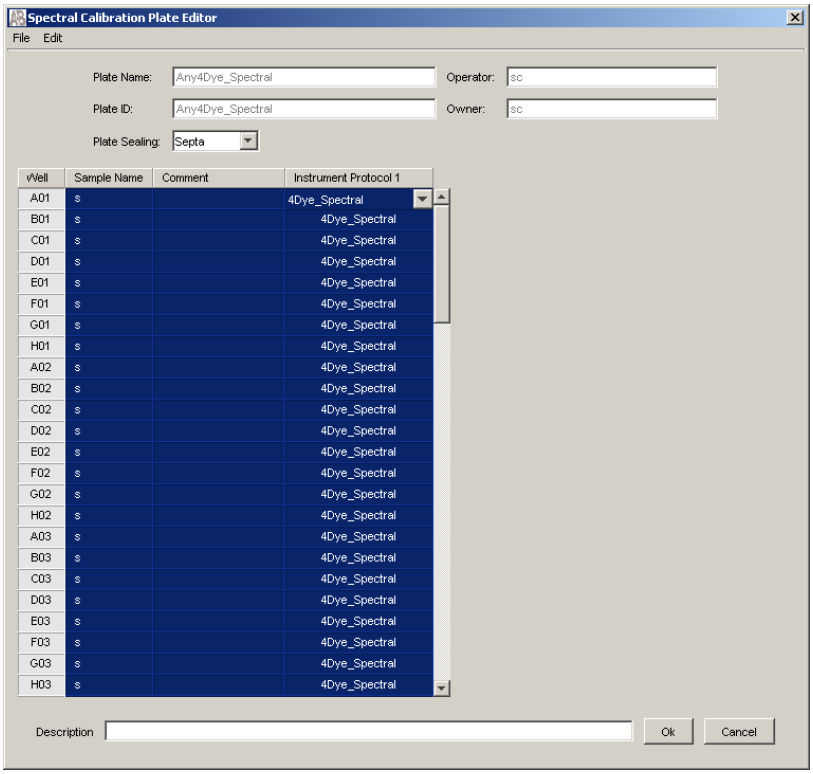


Notes

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 47.

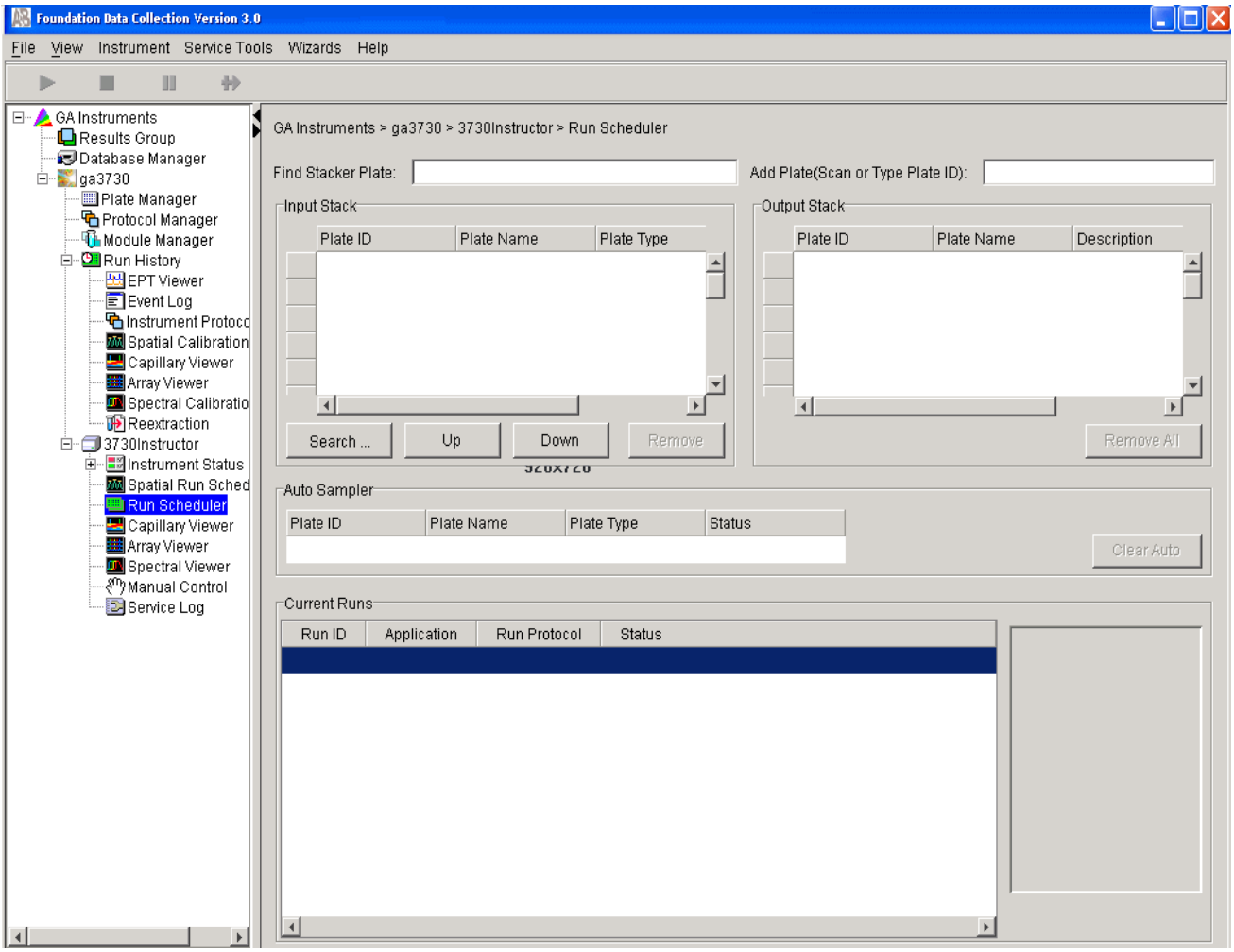


9. In the Plate Editor, select the Instrument Protocol that you just created in the previous steps, then click **OK** to save the plate.



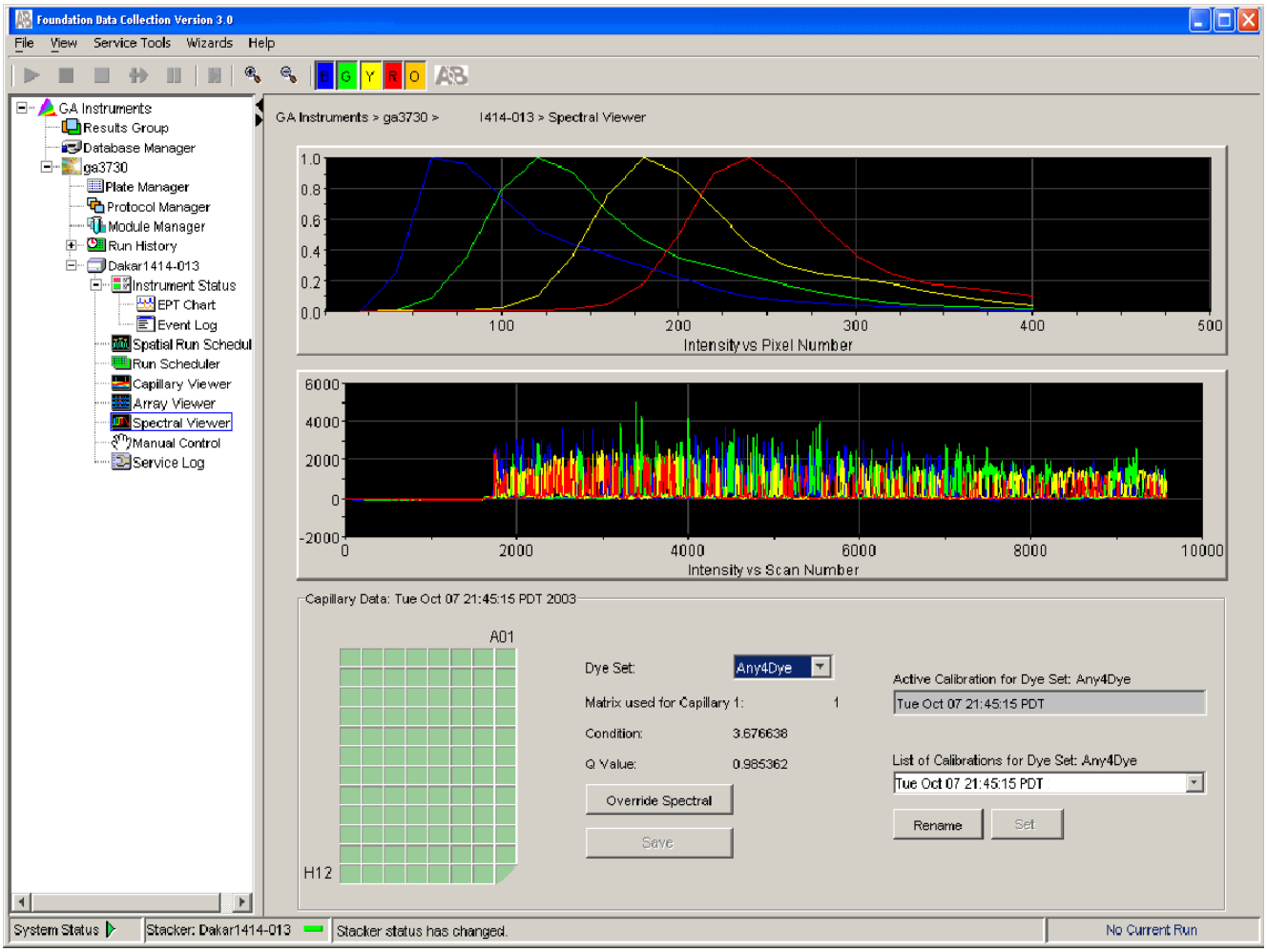
Notes

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.

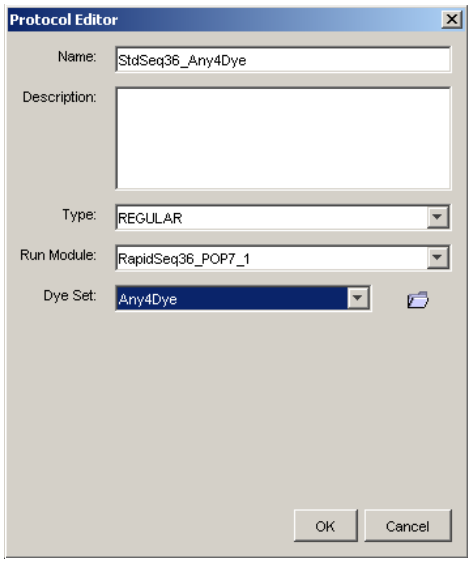


Notes

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.

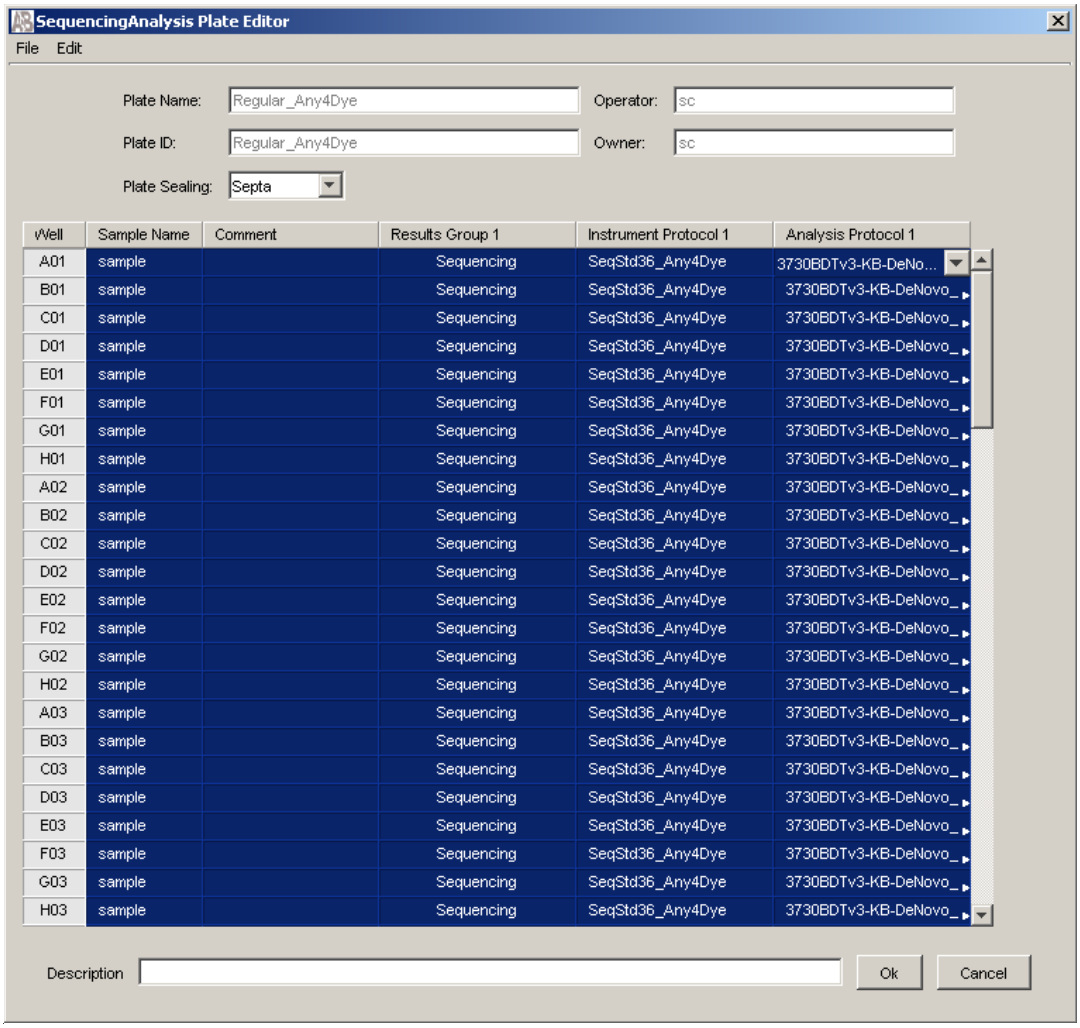
1. In the Protocol Editor, create a regular instrument run protocol for the Any4Dye dye set, then choose the appropriate default run module template. (You can create a customized run module in the Module Editor if desired).



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

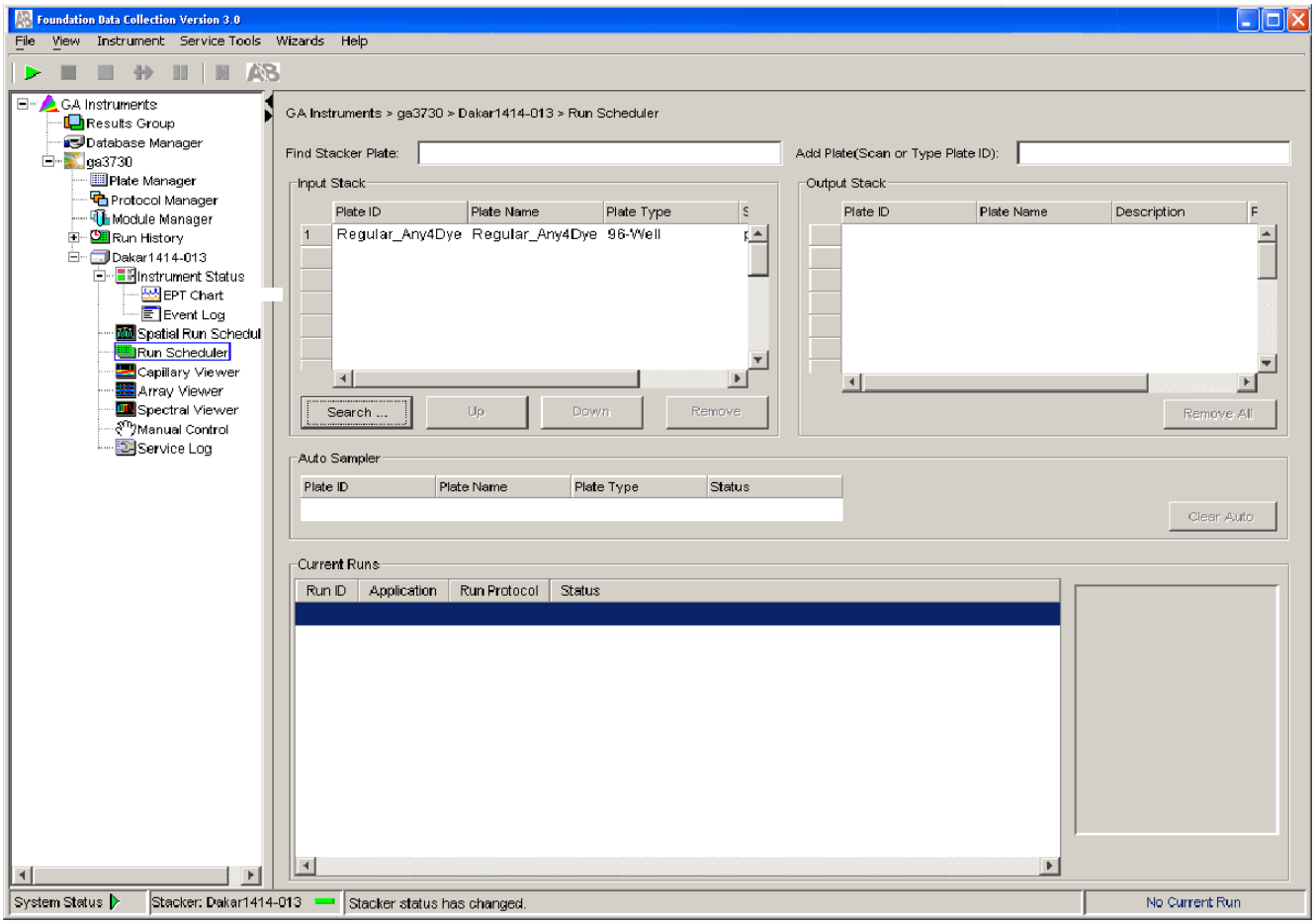
Notes

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



Notes

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



Notes

KB™ Basecaller Software v1.4.1

April 2012

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Executive Summary

Applied Biosystems® KB™ Basecaller Software v1.4.1 reduces manual data review time and increases the read length of high-quality bases in sequences. This algorithm accurately extracts more bases out of the sequencing data generated on Applied Biosystems® DNA Analyzers and Genetic Analyzer Instrument and chemistry platforms. KB™ Basecaller Software v1.4.1 supports all BigDye® Terminator v3.1 and v1.1 and BigDye® Direct chemistries and run modules available on Applied Biosystems® instruments.

- 310 Genetic Analyzer
- 3100/3100-*Avant* Genetic Analyzers
- 3130/3130*xl* Genetic Analyzers
- 3730/3730*xl* DNA Analyzers
- 3500 Dx and 3500 Dx/3500xL Dx Genetic Analyzers
- 3500 and 3500/3500xL Genetic Analyzers.

Notes

Software integration

KB™ Basecaller Software v1.4.1 is integrated with:

- Sequencing Analysis Software 6 and v5.4
- SeqScape® Software 3 and v2.7
- Variant Reporter™ Software 2 and v1.1
- 3130 Series and 3730 Series Data Collection Software 4
- 3500 Series Data Collection Software
- MicroSEQ® ID Analysis Software v2.2

KB™ Basecaller Software v1.4.1 is *not* integrated with:

- MicroSeq® ID software versions 2.1 and older
- Any versions of Data Collection Software for the 310 and 3100/3100-*Avant*
- 3130/3130*xl* and 3730/3730*xl* Data Collection Software versions before v3.1
- Sequencing Analysis Software before v5.4
- SeqScape® Software versions before v2.7
- Variant Reporter™ Software versions before v1.1.

During the co-installation of Sequencing Analysis Software 6 and SeqScape® Software 3 with Data Collection Software 4, KB™ Basecaller Software v1.4.1 is installed into your Data Collection Software 4 on the same computer.

Testing on more than 50,000 sequencing samples shows that version 1.4.1 of the algorithm offers many advantages, including longer, accurate read lengths.

Details of the test and validation process are in the poster *Longer Reads and More Robust Assemblies with the* KB™ Basecaller.

IMPORTANT! Life Technologies strongly recommends using the KB™ Basecaller.

Benefits of using the KB™ Basecaller

Some benefits of using the KB™ Basecaller include:

- Increased length of read
- Per-base quality value predictions using an equation that is standardized by Phred software
- Optional detection of mixed-base with quality values
- Analysis of short PCR products
- Accurate start point detection
- Increased accuracy in regions of low signal-to-noise or anomalous signal artifacts
- Detection of failed samples
- Trimming of data using per-base quality value
- Per-sample quality value that helps to determine the quality of each read

Notes

- Optional detection of PCR stop
- Optional assignment of Ns
- Optional generation of .phd.1 files

Increased length of read

KB™ Basecaller accurately extracts more bases than ABI Basecaller from the 3′ and 5′ ends of a sequence. Tests on genomic BAC samples, performed on data generated using 3730/3730*xl* instruments, indicate an improvement of approximately 100 bases in length-of-read as compared to the same data analyzed by the ABI Basecaller and Phred software (v0.020425.c). The gain in read length varies depending on the run module used to collect the data. The accuracy of start point estimation and the first 50 bases of called sequence is substantially increased. Typically, ~10 more correct calls on average are identified at the 5′ end as compared to the ABI Basecaller.

Per-base quality value predictions

The KB™ Basecaller assigns quality values to every basecall. The quality prediction algorithm is calibrated to return Q values that conform to the industry-standard relation established by the Phred software. The KB™ Basecaller and its output are, therefore, interchangeable in processes requiring Phred software for output.

Quality value calibration was performed using a set of correct-sequence annotated sample files, representative of production sequencing data generated on capillary electrophoresis platforms. Over 52.1 million basecalls were used to calibrate KB™ Basecaller Quality Values and over 32.9 million distinct basecalls were used to test the calibration.

Accuracy in start point detection

Improved start point detection contributes to better mobility shift corrections and greater basecalling accuracy in the first 50 bases. Because the KB™ Basecaller detects the start point accurately, you do not need to manually set start points for each sample.

Optional detection of mixed-base with quality values

The KB™ Basecaller can detect mixed base positions, and assign two-base (R, Y, K, M, S, W) IUB codes and quality values to those positions. Quality values are assigned to mixed basecalls using an algorithm similar to that for pure bases.

The definition conforms to the Phred relation. Quality values for mixed bases are inherently lower than those of pure bases due to the higher error risk of interpreting more complex signals. Note that when using the ABI Basecaller or ABI Basecaller and Phred software, a separate analysis stage is required to determine mixed bases.

Increased accuracy in regions of low signal-to-noise or anomalous signal artifacts

The KB™ Basecaller increases the accuracy of sequence reads from low-signal regions or from data that are partially contaminated by a secondary sequence or by other sources of “chemistry noise”.

Basecalling errors caused by anomalous chemistry and/or instrument signals such as dye blobs and fluorescent spikes are substantially reduced. These artifacts often occur in otherwise high-quality “clear-range” data. They result in the loss of high-quality bases that are downstream from the noise region. Tests indicate that KB™ Basecaller distinguishes between target DNA peaks and the most common artifacts better than ABI Basecaller.

Notes

Analysis of short PCR products

The KB™ Basecaller has been tested for accuracy in basecalling and quality value estimates on PCR products as short as 100 bases. Although KB™ Basecaller may be able to basecall products with less than 100 bases, these types of sample files were not tested.

Detection of failed samples

The KB™ Basecaller indicates the gross sample quality of each analysis as "Success without warnings," "Success with warnings," or "Failure due to poor data quality". A common failure mode is no signal–insufficient detection of DNA peaks. For failed samples, the KB™ Basecaller uses "NNNNN" as the sequence, indicating that the sample quality is very low and may need to be omitted from further analysis. Failed samples are flagged in reports in the analysis software. Note that this behavior is different from the ABI Basecaller, which *always* tries to call bases, resulting in sequences of many Ns.

Option to trim data using per-base quality value

You can use software with KB™ Basecaller to automatically determine the clear range region by trimming the ends using the per-base quality values. The parameters used for trimming are similar to those in other tools used by the genome community.

Per-sample quality value (QV) evaluates quality of reads

Software with the KB™ Basecaller uses the QV from the KB™ Basecaller to trim and determine a sample score. The sample score is the average QV in the clear range, or, if no clear range is determined, in the entire read. This single number value is a measure of the quality of the data. The sample score appears in reports generated by Sequencing Analysis Software, SeqScape® Software, Sequence Scanner Software, Variant Reporter™ Software, and/or MicroSeq® ID Software.

Optional detection of PCR stop

You can set the KB™ Basecaller to end basecalling at a PCR stop. Note that samples with enzymatic failure may have signal properties similar to those in PCR stop conditions. The KB Basecaller may not be able to distinguish between these two conditions.

Optional assignment of Ns

By default, the KB™ Basecaller does not generate Ns. However, you may choose to reassign Ns to bases with QVs below a user-specified threshold for both pure and mixed base positions.

Optional generation of .phd.1 files

.phd.1 files can be generated by autoanalysis or in analysis software.You can use the .phd.1 files for further analysis by downstream software such as Phred software.

Notes

Future support of ABI and KB™ Basecallers

Life Technologies will continue to provide technical support for the ABI Basecaller. However, further development and defect fixes will occur only on the KB™ Basecaller. If you encounter a defect in the ABI Basecaller, please use the KB™ Basecaller instead. In future releases, ABI Basecaller support files are removed from the software wherever they duplicate support in the KB™ Basecaller.

Features in KB™ Basecaller Software v1.4.1

- A basecalling algorithm that supports Applied Biosystems® 310, 3100/3100-*Avant*, 3130/3130*xl*, 3730/3730*xl*, 3500/3500xL, and 3500 Dx/3500xL Dx Genetic Analyzers
- Improvements over all earlier versions of KB™ Basecaller (v1.0, v1.1, v1.1.1, v1.1.2, v1.2, v1.3, and v1.4)

Note: Basecalling results with KB™ Basecaller Software v1.4.1 may differ slightly from results obtained with previous versions of KB™ Basecaller.

Notes

Comparison of the ABI and KB™ Basecallers

Question	ABI Basecaller	KB™ Basecaller
What does the software do?	• Processes raw traces • Provides processed traces • Provides AGCTN calls	• Processes raw traces • Provides processed traces • Provides pure bases only *or* • Provides pure and mixed calls • Provides quality values • Generates .phd.1 and .scf files • Provides a sample score
What are the resulting basecalls?	One option available: Only mixed bases are assigned as Ns. Further processing (either manual or using additional software) is required to assign IUB codes to the Ns or pure bases.	Four options are available. The software can assign an: • ACGT and Q value to each peak. • ACGT and Q value to each peak. Any peak with a Q value below a defined threshold is reassigned an N. • ACGT or a mixed base and a Q value to each peak. • ACGT or a mixed base and a Q value to each peak. Any peak with a Q value below a defined threshold is reassigned an N.
How are failed samples handled (for example, no signals, chemistry failure)?	Attempts to call all bases so a sample results with many Ns.	Assigns five Ns to the entire sample to indicate that the sample failed analysis. The analysis report flags these files.
Baseline in processed data	Appears smoother than in KB™ Basecaller.	Appears less smooth than in ABI KB™ Basecaller.
What are the steps to process data?	Calls bases on Windows OS.	Calls bases and estimates QVs on Windows OS.
Data and future support	Supports the 310, 3100, 3100-*Avant*, Applied Biosystems® 3130/3130*xl* and 3730/3730*xl* instruments. Further development has stopped.	Applied Biosystems® 310, 3100/3100-*Avant*, 3130/3130*xl,* 3730/3730*xl,* 3500/3500xL, and 3500 Dx/3500xL Dx Genetic Analyzers. Development is ongoing.

Notes

Differences between the ABI and KB™ Basecallers

Question	Answer	
	ABI Basecaller	KB™ Basecaller
Can the KB™ Basecaller basecall short PCR products?		The KB™ Basecaller has been tested for accuracy in basecalling and quality value estimation on PCR products as short as 100 bases. Although it may be possible to basecall products with less than 100 bases, such sample files have not been tested. Samples shorter than 100 bases may not contain enough signal information to basecall the sample file.
Why is the baseline less smooth when the data are analyzed with the KB™ Basecaller?	Processed signals or traces from the ABI Basecaller appear smoother than those from the KB™ Basecaller because each software application uses an algorithm that processes the signals differently. The ABI Basecaller assigns only AGCT and Ns to each peak. Therefore, you must manually search for mixed bases or use a secondary software to complete the task. To facilitate this secondary process, the ABI Basecaller subtracts an aggressive baseline estimate to show a cleaner baseline in the processed signals.	The KB™ Basecaller can determine pure and mixed bases. Therefore, second-stage processing, which allows less aggressive baseline subtraction, is not needed. The processed traces have a higher baseline. If you have mixed bases, turn on the mixed-base detection option and allow KB™ Basecaller to call mixed bases. Use the mixed base calls and the associated QVs to review mixed bases – do not look only at the baseline.
What is the signal to noise value found with data analyzed with the KB™ Basecaller?	The signal-to-noise value is the average of the signal intensity of the A, C, G, or T base divided by the average of the noise for that base. The ABI Basecaller calculates only the signal intensity. The signal-to-noise value is more indicative of data quality than the signal intensity value alone. Both properties are important in determining quality.	KB™ Basecaller calculates the information and presents the data in the Annotation view and analysis report.

Notes

Question	Answer	
	ABI Basecaller	KB™ Basecaller
What scaling options are available with the KB™ Basecaller?	The ABI Basecaller uses a scaling method closer to the "True profile" option than the "Flat profile" option.	The KB™ Basecaller can display scaled data in two ways: • True profile scaling With this method, the processed traces are scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value (for example, 1000). The profile of the processed traces is very similar to that of the raw traces. • Flat profile scaling The processed traces are scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value (for example, 1000). The profile of the processed traces is flat on an intermediate scale (> about 40 bases). You must decide which option is better suited to your circumstances. The sequence and QVs called by the KB™ Basecaller are independent of the selected scaling option.
Does the KB™ Basecaller produce more usable sample files than the ABI Basecaller?		Tests show that medium- and high-quality data result in more usable bases (longer read length) when analyzed by the KB™ Basecaller than by the ABI Basecaller. For very poor-quality data (samples with no, low, or noisy signal), the KB™ Basecaller does not provide more bases but instead fails the samples. By calling a string of "NNNNN" for the failed samples (instead of a sequence containing low QVs), the KB™ Basecaller indicates that the sample is unusable.
Can the KB™ Basecaller analyze data generated on the ABI PRISM® 373, 377, or 3700 instruments?		No, the KB™ Basecaller is calibrated to basecall and estimate the basecall quality for BigDye® Terminator chemistries on 310, 3100, 3100-*Avant*, and 3130/3130*xl* Genetic Analyzers, 3730/3730*xl* DNA Analyzers, and 3500/3500xL and 3500/3500 Dx/3500xL Dx Genetic Analyzers. Life Technologies has stopped support for the 373, 377, and 3700 instruments and data analysis.

Notes

Question	Answer	
	ABI Basecaller	KB™ Basecaller
How can I determine which basecaller was used to analyze each sample file?		The Annotation view for each sample file and for the print header displays the basecaller name and version number. When displaying samples files, files analyzed by the KB™ Basecaller have QV value bars displayed above the electropherogram.
Are there any known incompatibilities when a sample file is analyzed with the KB™ Basecaller?		Life Technologies does not know of any incompatibility issues when a sample file (.ab1) is analyzed with the KB™ Basecaller and used in third-party software.

Notes

FAQs: Processing data with Phred software and .phd.1 Files

Question	Answer
Can I analyze sample files with the KB™ Basecaller and then reprocess them with Phred software?	In principle, yes, but this is not recommended. The resulting quality values from Phred software are not calibrated—i.e., it is possible that Phred will over or under-predict quality in certain circumstances because it has not been trained on the type of processed electropherogram produced by the KB™ Basecaller. (Phred has been trained using the ABI Basecaller to produce the processed traces.) In addition, Phred replaces (and ignores) the initial called sequence. Reprocessing KB-analyzed samples with Phred, on average, degrades the accuracy of the analysis in terms of actual sequence error. Analysis improvements in KB™ Basecaller outlined above are lost. Studies by Life Technologies indicate that running Phred software on sample files processed by the KB™ Basecaller degrades the quality of the results. Analysis with KB™ Basecaller can generate .phd.1 files, which are interchangeable with any processes that currently depend on Phred.
Which Applied Biosystems®software generates .phd.1 files?	The following software products have KB™ Basecaller (version varies for each software) integrated and can generate .phd.1 files: • ABI PRISM® 3100-*Avant* Data Collection Software v2.0 • ABI PRISM® 3100 Data Collection Software v2.0 • Applied Biosystems® 3130/*xl* and 3730/*xl* Data Collection Software v3.0 and later • Sequencing Analysis Software v5.2 and later • SeqScape® Software v2.5 and later • MicroSeq® ID Software v1.0 and later • Variant Reporter™ Software v1.0 and later

Notes

FAQs: Quality values

Question	Answer
How do I use quality values to review data?	When analyzing data with pure bases, Life Technologies Corporation recommends that you use the following settings: **Pure bases** – Low QV = <15, Medium QV= 15–19, High QV= 20+ (default) When reviewing data with pure bases, use the QVs to briefly review bases with high QV(>20). Pay close attention to bases with medium QVs because you may need to make edits. Quickly review low-QV bases, although you will likely discard these bases from further analysis. Mixed base quality values will be lower than pure bases. For mixed bases, review all mixed basecalls. You may want to accept basecalls with quality values as low as 1. **Mixed bases** – Low QV = <5, Medium QV = 5–10 (investigate to determine the best range for your application) In all cases, keep in mind that, by definition, the predicted probability of error for a particular basecall is $10^{-q/10}$.
What are the differences between quality values of mixed bases and pure bases?	Pure bases and mixed bases have the same probability of error for the associated basecall (10-q/10). Note the following: • High-quality pure bases typically have QVs of 20 or higher. • The distribution of quality values for mixed bases differs dramatically from that of pure bases. • For mixed bases, quality values greater than 20 are rare. • Accurate mixed basecalls may be assigned quality values as low as 1, because the probability of error with mixed bases is higher. Review all mixed basecalls.
Can I trim my data using quality values?	Yes. When using Data Collection, you can set trimming using QVs in the analysis protocols. When using Sequencing Analysis Software, SeqScape® Software, MicroSeq® ID Software or Variant Reporter™ Software, you can set trimming using QVs in the Analysis settings.

Notes

<table>
<tr><th>Question</th><th>Answer</th></tr>
<tr><td>Is there a table that shows each quality value and its corresponding probability of error?</td><td>The following table shows each quality value and its corresponding probability of error. For a more extensive table, look in the Help menu or the Sequencing Analysis Software or the SeqScape® Software user guides.
<table>
<tr><th>QV</th><th>Pe</th><th>QV</th><th>Pe</th></tr>
<tr><td>1</td><td>79.0%</td><td>35</td><td>0.032%</td></tr>
<tr><td>5</td><td>32/0%</td><td>40</td><td>0.010%</td></tr>
<tr><td>10</td><td>10.0%</td><td>41</td><td>0.0079%</td></tr>
<tr><td>15</td><td>3.2%</td><td>45</td><td>0.0032%</td></tr>
<tr><td>20</td><td>1.0%</td><td>50</td><td>0.0010%</td></tr>
<tr><td>21</td><td>0.79%</td><td>60</td><td>0.00010%</td></tr>
<tr><td>25</td><td>0.32%</td><td>99</td><td>0.00000000013%</td></tr>
<tr><td>30</td><td>0.10%</td><td></td><td>–</td></tr>
</table></td></tr>
<tr><td>Where can I see quality value bars and numbers?</td><td>Sequencing Analysis Software, SeqScape® Software, MicroSeq® ID Software, and Variant Reporter™ Software allow you to display or hide quality value (QV) bars in displays and printouts. You can customize the color and range for low, medium, and high quality values. For QVs ≤ 50, the length of a bar is proportional to the corresponding quality value. Quality values above 50 will have the same color and QV bar length as those defined for a QV of 50. To see the quality value for a particular base, place the computer mouse over the QV bar.

In SeqScape® Software, MicroSeq® ID Software, and Variant Reporter™ Software, the per-base quality values also appear in the reports corresponding to bases identified as mutations.</td></tr>
<tr><td>Why are the quality value bars displayed in gray?</td><td>A quality value is assigned to a specific basecall. When you change a basecall, the quality value does not apply to the new base, and therefore, it is displayed as a gray bar.

Also when you reassign Ns to bases below a certain QV, the QV bar does not apply to the N basecall, and therefore it is displayed as a gray bar.</td></tr>
<tr><td>Are quality value bars printed for the Electropherogram or Sequence views?</td><td>You can show or hide the QV bars when printing the Electropherogram and Sequence views of the sample file. QV bars are not printed if you print more than seven panels per page (due to space limitations). The quality value numbers cannot be printed.</td></tr>
<tr><td>Which Life Technologies software can display the quality values?</td><td>Sequencing Analysis Software v5.X, Sequencing Analysis Software 6, SeqScape® Software v2.X, SeqScape® Software 3, MicroSeq® ID Software v1.X, v2.X, Variant Reporter™ Software v1.X, and Variant Reporter™ 2 can display quality values.</td></tr>
<tr><td>Can I view quality values from KB™ Basecaller with other software?</td><td>Quality value graphics from KB™ Basecaller are customized for processing by other Life Technologies software. The KB™ Basecaller allows other Life Technologies software to perform additional functions, such as clear range trimming and more streamlined editing.</td></tr>
</table>

Notes

Miscellaneous FAQs

Some frequently asked questions regarding Ns, spacing values, and providing feedback are shown below.

Question	Answer
When do Ns appear in samples analyzed by the KB™ Basecaller Software?	When using the KB™ Basecaller, the sequence “NNNNN” appears in the sample file when the sample fails analysis. Omit this file from further analysis. The Analysis Report in Sequencing Analysis Software will also flag these files. In addition to pure and mixed bases shown with QV bars, N’s and gray QV bars are also shown when you reassign Ns to all bases before the user-specified QV threshold. This allows you to view the longer read length and more accurate basecalling of KB™ Basecaller while still viewing data with software that does not display QVs.
Why does the spacing value sometimes appear in red?	When the ABI Basecaller fails to determine a spacing value for a sample file, it uses a default value of 12.00 for all run conditions. This number appears as in red in the Sample Manager, and the Annotation view displays “–12.00”.
Why does the spacing value sometimes have a negative value?	When the KB™ Basecaller fails to determine a spacing value for a sample file, it uses a default value specific to the instrument/polymer/chemistry/run condition used to generate the sample file. This value appears in red in the Sample Manager. The Annotation view displays –1 times this value.
How can I provide feedback to the KB™ Basecaller product team?	Email information to your local Life Technologies applications support representative at **www.lifetechnologies.com/support**. If applicable, please include sample files and details (including analysis settings) on how to reproduce your observation.

Notes

Conference posters and reference

Posters

- ABRF 2007 – *Improved Accuracy for Mutation and SNP Detection: Variant Reporter™ Software*, Ming Li et. al.
- ESHG 2007 – Direct Sequencing Quality Control
- AGBT 2004 – Longer Reads with the KB™ Basecaller
- ABRF 2004 – Integrated Sequencing Analysis Solutions using the KB™ Basecaller from Applied Biosystems
- ESHG 2009 Performance of the KB™ Basecaller for a New Sequencing System

These posters and other literature can be found at:

www.lifetechnologies.com

Click **Support**, then **Products and Technical Literature**. Search with the keyword *KB*.

Reference

B. Ewing and P. Green, Genome Research, 8:186-194, 199.

Notes

Managing Data Collection Software Licenses

Manage software licenses

The 3730 Series Data Collection Software 4 requires a license to run.

IMPORTANT! If you replace or add a network card in the computer running the software, or relocate the software to a new computer, contact Life Technologies to update your license for the new network card or computer.

Obtain and activate a software license

The 3730 Series Data Collection Software 4 Software Activation dialog box is displayed when you start the software if no license is installed and activated on your computer.

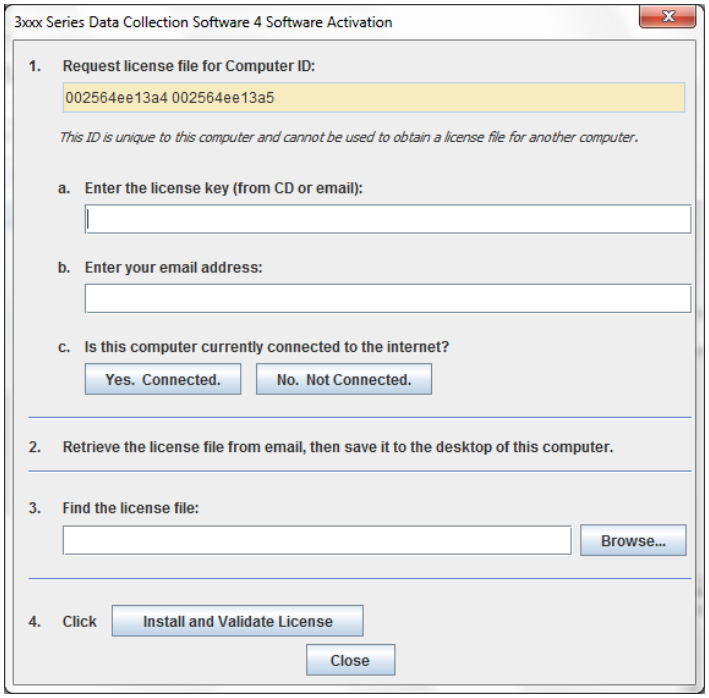
This task is typically performed by the Life Technologies service representative during installation of the instrument.

1. Ensure that all network cards in the computer are enabled.

   IMPORTANT! You can run the 3730 Series Data Collection Software 4 using only the network cards enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

Notes

2. Display the Software Activation dialog box by starting the 3730 Series Data Collection Software 4.



3. Obtain the license key. The license key is provided on the 3730 Series Data Collection Software 4 CD case, or in an email from Life Technologies.

4. Request the software license file by performing steps **1a**, **1b**, and **1c** as listed on the activation screen.

IMPORTANT! Keep a record of the email address used to activate the software license. You must use the same email address to renew the software license when it expires.

5. Obtain the software license file from your email.

6. Make a copy of the software license file and keep in a safe location.

7. Copy the software license file to the desktop of the 3730 Series Data Collection Software 4 computer.

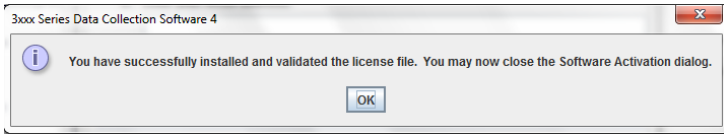
Notes

8. If the Software Activation dialog box has closed, start the 3730 Series Data Collection Software 4 to open it.

9. Click **Browse**, then navigate to the software license file saved on your computer.

10. Click **Install and Validate License**. A message is displayed when the license is installed and validated.

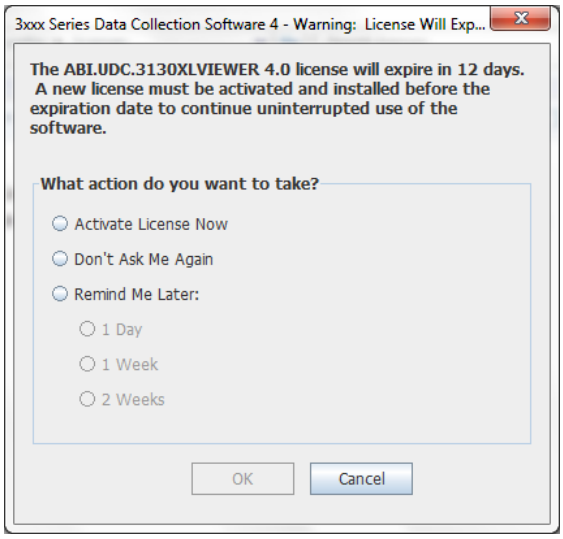
11. Click **Close**.



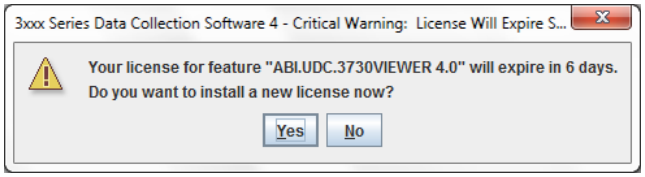
Renew a software license

1. Ensure that all network cards in the computer are enabled.

2. Display the Software License Renewal dialog box by doing either of the following:
   - Select **Activate License Now** in the Warning: License Will Expire Soon dialog box that is displayed 8–30 days prior to expiration.

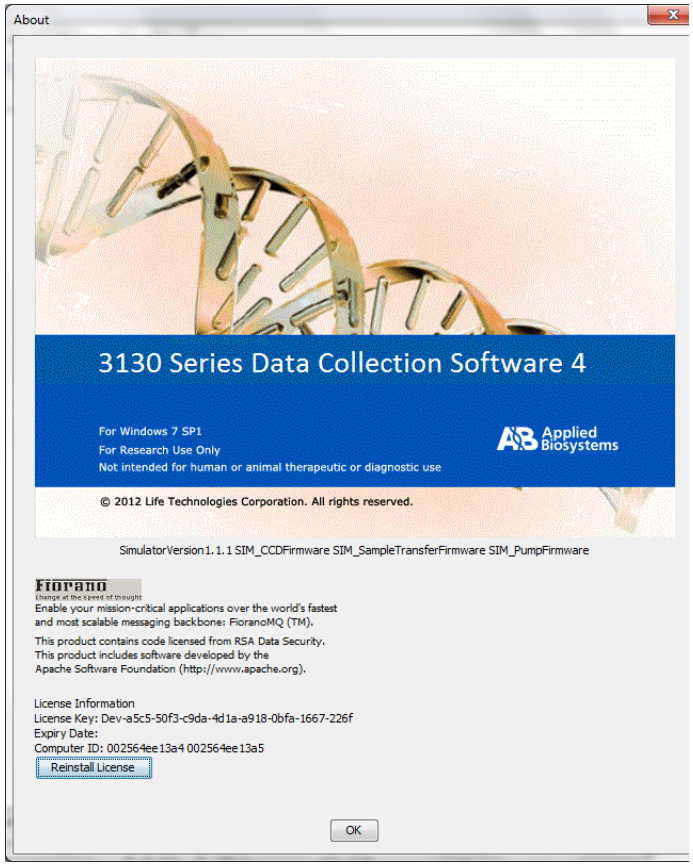


   - Click **Yes** in the Critical Warning: License Will Expire Soon dialog box that is displayed within 7 days of expiration.



Notes

3. Choosing to **Activate/Install License Now** will result in the display of 3730 Series Data Collection Software 4 box, shown here for the 3130. Click **Reinstall License** in the Lower Left Corner.



Notes

4. Complete the License Renewal dialog box as described below:

5. Enter the email address used to activate the software license.

   IMPORTANT! You must use the same email address to activate and renew the software license. If you do not have the activation email address available, enter any email address, click the licensing link in the Software Renewal dialog box, then click **Contact Support** in the License Renewal web page displayed.

6. Request the renewed software license file by performing step **1c** as listed on the renewal screen.

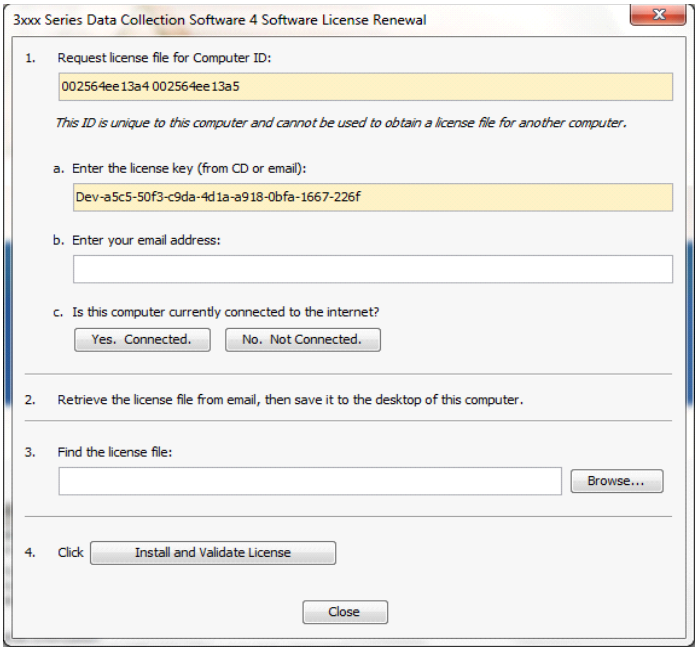
7. Obtain the renewed software license file from your email.

8. Copy the renewed software license file to the desktop of this computer.

9. Click **Browse**, then navigate to the renewed software license file saved on your computer.

10. Click **Install and Validate License**. A message is displayed when the license is installed and validated.

11. Click **Close**.



Notes

Notes

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

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words described:

- CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, hot surface	Attention, surface chaude
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Laser radiation	Rayonnement laser

Symbol	English	Français
	Caution, piercing hazard	Attention, danger de perforation
	Potential biohazard	Danger biologique potentiel
	Ultraviolet light	Rayonnement ultraviolet
	On	On (marche)
	Off	Off (arrêt)
	On/Off	On/Off (marche/arrêt)
	Standby	En attente
	Earth (ground) terminal	Borne de (mise à la) terre
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Terminal that can receive or supply alternating or direct current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant continu ou alternatif
	Do not dispose of this product in unsorted municipal waste CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. CAUTION! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.

Safety Alerts on Instruments

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation	Location on Instrument
DANGER! Class 3B (III) visible and/or invisible laser radiation present when open and interlocks defeated. Avoid exposure to beam.	**DANGER!** Rayonnement laser visible ou invisible de classe 3B (III) présent en position ouverte et avec les dispositifs de sécurité non enclenchés. Éviter toute exposition au faisceau.	Detection cell cover 

Instrument Safety

General

CAUTION **Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION **Solvents and Pressurized fluids.** Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric 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 and electrical sparks may result.

Physical injury

CAUTION **Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of

obstructions.
•Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
•Participants should coordinate lift and move intentions with each other before lifting and carrying.
•For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION **Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical

WARNING **Fuse Installation.** Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

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

WARNING **Voltage Selector Switch.** Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

WARNING **Ensure appropriate electrical supply.** For safe operation of the instrument:
•Plug the system into a properly grounded receptacle with adequate current capacity.
•Ensure the electrical supply is of suitable voltage.
•Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING **Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

WARNING **Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Overvoltage Rating

The Applied Biosystems® 3730/3730*xl* DNA Analyzer has an installation (overvoltage) category of II, and is classified as portable equipment.

Cleaning and decontamination

CAUTION **Cleaning and Decontamination.** Using a cleaning or decontamination method not specified by the manufacturer may result in damage to the equipment. For the protection of others, ensure the instrument is properly decontaminated prior to having the instrument serviced at your facility or before sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan. Decontamination forms may be requested from customer service.

Laser

WARNING **LASER HAZARD.** Under normal operating conditions, the Applied Biosystems® 3730/3730*xl* DNA Analyzer are categorized as a Class I laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 3B laser. Lasers can burn the retina, causing permanent blind spots. Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.
To ensure safe laser operation:
•Never look directly into the laser beam.
•Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
•The system must be installed and maintained by an Life Technologies Technical Representative.
Life Technologies Technical Representatives are instructed to:
•Remove jewelry and other items that can reflect a laser beam into your eyes or those of others
•Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing.
DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.
Note the laser warnings provided in "Safety Alerts on Instruments" on page 197.

CAUTION **LASER HAZARD, Bar Code Scanner**. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Laser Classification

The 3730/3730*xl* DNA Analyzer uses a laser. Under normal operating conditions, the instrument laser is categorized as a Class I laser. When safety interlocks are disabled during certain servicing procedures, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

The Applied Biosystems® 3730/3730*xl* DNA Analyzer has been tested to and complies with 21 CFR, 1040.10 and 1040.11, as applicable.

The 3730/3730*xl* DNA Analyzer laser has been tested to and complies with standard EN60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide."

Safety and Electromagnetic Compatibility (EMC) Standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010 UL 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-081 EN 61010-2-081 UL 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007, as applicable	U.S. FDA Health and Human Services (HHS) "Radiological health performance standards for laser products" and "Radiological health performance standards for specific purpose laser products"

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2002/96/EC	European Union "WEEE Directive" – Waste electrical and electronic equipment

Chemical safety

WARNING **GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the 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.

WARNING **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING **4L Reagent and Waste Bottle Safety.** 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.

Biological hazard safety

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Document title	Pub. Part no.
Applied Biosystems® 3730/3730xl DNA Analyzer Maintenance and Troubleshooting Guide	4477797
Applied Biosystems® 3730/3730xl DNA Analyzer Quick Reference Card	4477852
Applied Biosystems 3730/3730xl DNA Analyzer and 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4477853

Portable document format (PDF) versions of this guide and the documents listed above are also available on the Applied Biosystems® 3730 Series Data Collection Software 4 CD.

Note: To open the user documentation included on the Applied Biosystems® 3730 Series Data Collection Software 4 CD, use the Adobe® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "Obtaining Support" on page 204.

Obtaining SDSs

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

Note: For the SDSs of chemicals not distributed by Life Technologies Corporation, contact the chemical manufacturer.

Obtaining Support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Computer Configuration

Life Technologies Corporation supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Life Technologies Corporation 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 Corporation. Life Technologies Corporation 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

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

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