appliedbiosystems

3730xl DNA Analyzer

USER GUIDE

3730xl Data Collection Software 5

Windows™ 10 Operating System

Publication Number 100077621

Revision C

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

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. 100077621

Revision	Date	Description
C	11 February 2020	Update to include the D dye set, maintenance items, and other minor edits.
B	10 January 2019	Updated the cover; added information about low temperature effects and spectral and spatial calibration requirements.
A	1 October 2018	New document.

Contents

1 Instrument and software description

Overview

The Applied Biosystems™ 3730xl DNA Analyzer with 3730xl Data Collection Software 5 is a fluorescence-based DNA analysis instrument. It uses capillary electrophoresis technology with 48 or 96 capillaries.

The instrument is designed for high-throughput, unattended operation. Automation features include an integrated plate stacker and internal bar code reader.

IMPORTANT! The protection provided by the equipment may be impaired if any of the following conditions occur.

- The instrument is operated outside the environment and use specifications
- The user provides inadequate maintenance
- The equipment is used in a manner not specified by the manufacturer (Thermo Fisher Scientific)

IMPORTANT! Observe current good laboratory practices when using this instrument.

Parts of the instrument

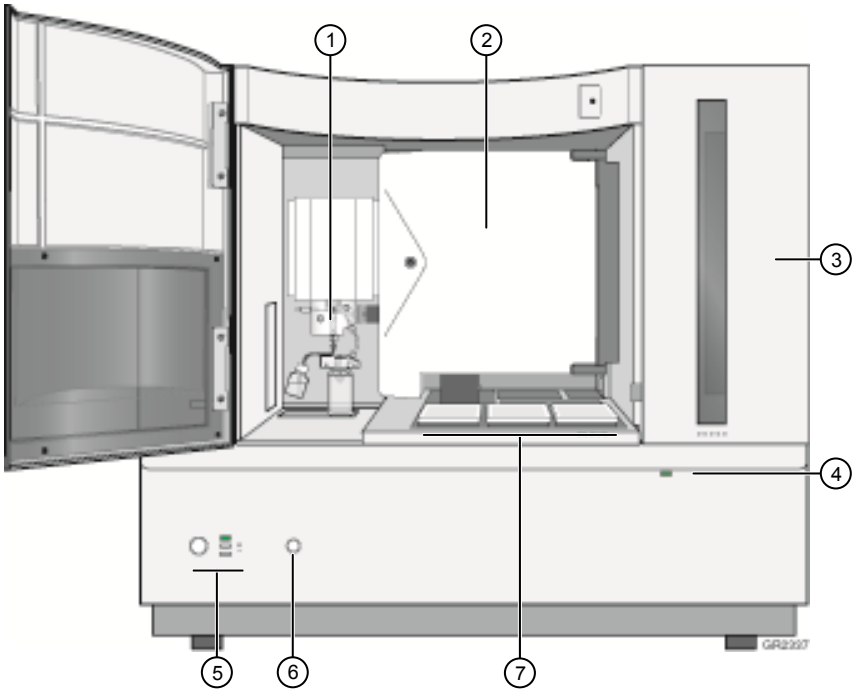


Figure 1 3730xl DNA Analyzer

1. Polymer delivery pump (PDP). See Figure 3.
2. Oven door (The detector cell and capillary array are inside this compartment.)
3. Stacker (automated plate handler)
4. Stacker door indicator light
5. Power button and status lights
6. Tray button
7. Buffer, water, and waste reservoirs

Instrument interior components

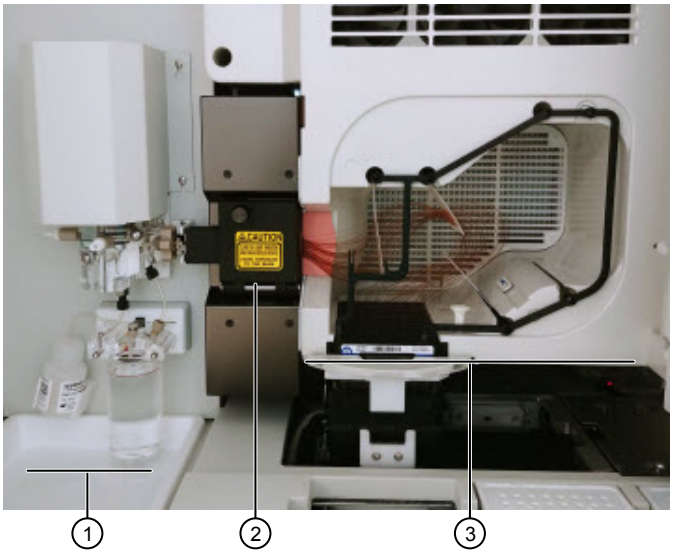


Figure 2 Polymer delivery pump, detection cell, and capillary array (view with oven door open)

① Polymer delivery pump
② Detection cell (behind door)
③ Capillary array

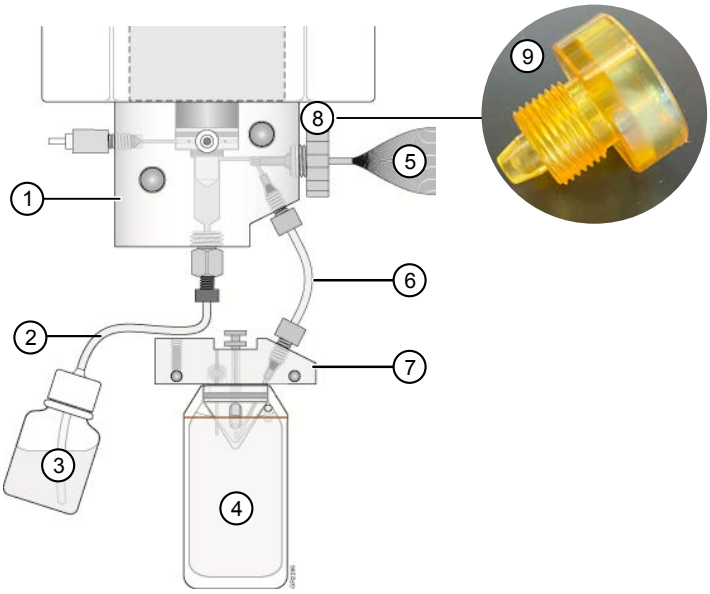


Figure 3 Polymer delivery pump (PDP)

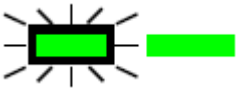
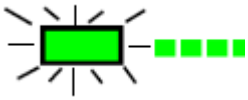
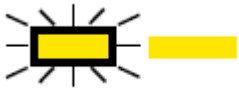
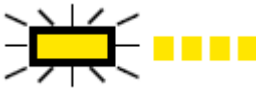
For the associated catalog numbers see Appendix E, “Catalog numbers”.

① Pump block
② Polymer supply tube
③ Polymer reservoir
④ Anode buffer jar
⑤ Capillary array
⑥ Interconnect tube
⑦ Lower polymer block
⑧ Capillary array knob
⑨ Array port plug (replaces the capillary array knob during shutdown)

Instrument front panel indicators

① Status indicators
② Stacker indicators

Table 1 Status lights

Status Light	Status
Solid Green 	• The instrument is ready • An automated wizard operation is in progress with the instrument door closed
Flashing Green 	• A run is in progress
Solid Yellow 	• The instrument cannot communicate with the computer. See Appendix A, “Troubleshooting”. • The buffer reservoir or capillary array is not installed
Flashing Yellow 	• The instrument is performing diagnostics • The instrument door or oven door is open • An automated wizard operation is in progress with the instrument door open • The instrument is downloading firmware

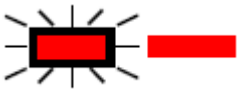
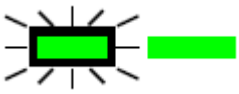
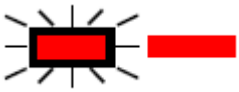
Status Light	Status
Solid Red 	• The instrument has detected a problem. See Appendix A, “Troubleshooting”. • Restart the instrument and computer. See “Power on the computer and the instrument” on page 27.

Table 2 Stacker indicators

Status Light	Status
Solid Green 	• **In Stack**— At least one plate is in the In stack, *and* • **Out Stack**— Empty, no plates in stack
Solid Red 	• **In Stack**—Empty, no plates in stack *and* • **Out Stack**— Full, remove plates before you can start another run

Consumables and usage limits

Consumable	Storage	On-instrument limit
Polymer	2–8°C	7 days
1X run buffer (prepared from 3730 Running Buffer (10X)) Used in the anode buffer jar and the buffer reservoir	• Shipped at room temperature. Stable for 1 week. • 2–8°C for up to 1 month	48 hours **IMPORTANT!** Do not add buffer to the anode buffer jar or the buffer reservoir. Always replace with fresh buffer.
Capillary array	Room temperature	300 injections

Important notice regarding use of consumables that exceed supported limits

Thermo Fisher Scientific does not recommend the use of consumables that exceed supported limits. The recommended limits are designed to promote the production of high quality data and minimize instrument downtime. Reagent and consumable lifetime minimum performance are based on testing and studies that use reagents and consumables that have not exceeded supported limits.

The use of consumables beyond the supported limits may impact data quality or cause damage to the instrument or capillary array. The cost of repairing such damage is NOT covered by any Thermo Fisher Scientific product warranty or service plan. Customer use of expired consumables is at customer's own risk and without recourse to Thermo Fisher Scientific. For example, product warranties do not apply to defects resulting from or repairs required due to misuse, neglect, or accident including, without limitation, operation outside of the environmental or use specifications or not in conformance with Thermo Fisher Scientific instructions for the instrument system, software, or accessories.

Please see your specific service contract or limited product warranty for exact language regarding coverage and ask yourThermo Fisher Scientific representative if you have further questions.

Computer and software requirements

Computer/software	Requirement
Computer	**IMPORTANT!** Do not modify the instrument hardware or software without notifying Thermo Fisher Scientific. Any modifications must be made by Thermo Fisher Scientific under change control. The computer provided with the instrument contains validated software and settings. Do not change the static IP settings. For minimum computer requirements, see "Instrument specifications" on page 142. **IMPORTANT!** Do not rename the computer after the 3730xl Data Collection Software 5 is installed. The instrument computer has been assigned a unique name. Changing the name may cause the software to malfunction. Because of the Ethernet cable connection between the instrument and the computer, the computer must be located within 9 feet of the instrument. **IMPORTANT!** The instrument communicates with the computer by Ethernet connection. Do not make any changes to the computer ethernet/internet connections during a run or during calibration.
Operating system	Operating system: Windows™ 10 Enterprise 2016 LTSB, 64-bit (requires 5 partitions) Consult with a Thermo Fisher Scientific representative before updating the Windows™ operating system or firewall settings.
Instrument firmware	Instrument firmware is to be updated only by a Thermo Fisher Scientific representative.
Antivirus software requirements	No antivirus software is provided with the Data Collection software. The following applications are compatible with the software: • Symantec Endpoint Protection 12 • McAfee Endpoint Security version 10.5 • Windows Defender Antivirus (comes as part of the Windows™ 10 installation)
Other software	⚠ **CAUTION!** Do not install additional software on the computer other than antivirus software. Changes to the configured software could void the instrument warranty and cause the instrument software to be non-operational.

Theory of operation

Preparing samples

When DNA samples are prepared for sequencing and fragment analysis on the instrument, fluorescent dyes are attached to the DNA.

Preparing the instrument

Two calibrations are required to prepare the instrument for sample runs:

- **Spatial calibration**—Determines the position of the image from each capillary on the CCD array. For more information, see "Perform spatial calibration" on page 92.
- **Spectral calibration**—Generates a matrix for each capillary that compensates for dye overlap and is used to convert the 20-color data into 4-, 5-, or 6-dye data. For more information, see "Perform spectral calibration for sequencing and fragment analysis" on page 98.

During a run

During a run, the instrument performs the following steps.

- Prepares the capillaries by pumping fresh polymer under high pressure from the polymer delivery pump to the waste reservoir.
- Electrokinetically injects the sample into the capillaries by briefly applying low voltage.
- Washes the capillary tips in the water reservoir, then returns the capillary to the buffer reservoir.
- Ramps the voltage up to a constant level.

  A high electric field is created between the ground end of the electrode in the anode buffer jar and the negative voltage that is applied to the load header of the capillary array. This field pulls the negatively charged DNA through the separation polymer. The smaller fragments migrate faster than the larger fragments and reach the detector first.

  To ensure optimal separation and maintain denaturation of the DNA, the capillaries are thermally controlled in the oven and in the detection cell.

  In the detection cell, the dyes that are attached to DNA are excited by a narrow beam of laser light. The laser light is directed into the plane of the capillaries from both the bottom and top. A small amount of laser light is absorbed by the dyes and emitted as longer wavelength light in all directions.
- CCD captures the fluorescent light on the instrument optics while blocking the laser light. The light passes through a transmission grating, which spreads out the light. The light is imaged onto a cooled CCD array.
- Computer converts the data into multi-dye data for the entire run. For sequencing applications, 4 different dyes are used to determine the 4 bases A, G, C, and T. For fragment analysis applications, up to 6 dyes can be used in a single run for higher throughput.
- Stores the data in an internal database, then creates sequence analysis (AB1) and fragment analysis (FSA) data files by "autoextracting" data from the database.

Results

The software generates an electropherogram (intensity plot) for each dye based on the migration of DNA fragments over the run and generates primary analysis results:

- For sequencing applications, the electropherogram is adjusted to compensate for slight mobility differences due to the dyes, then basecalling is performed and quality values are assigned.
- For fragment analysis, the software uses the internal size standard to assign a fragment size and a sizing quality value to each peak.

Overview of the software

3730xl Data Collection Software 5 manages instrument setup, controls instrument operations, allows real-time data visualization, and performs diagnostics. The Connect cloud-based platform feature allows you link the instrument to your Connect cloud-based platform account and upload data.

Use the **Navigation pane** (Figure 4) to set up your run, view and interrogate results, or to determine the amount of free space remaining in your database.

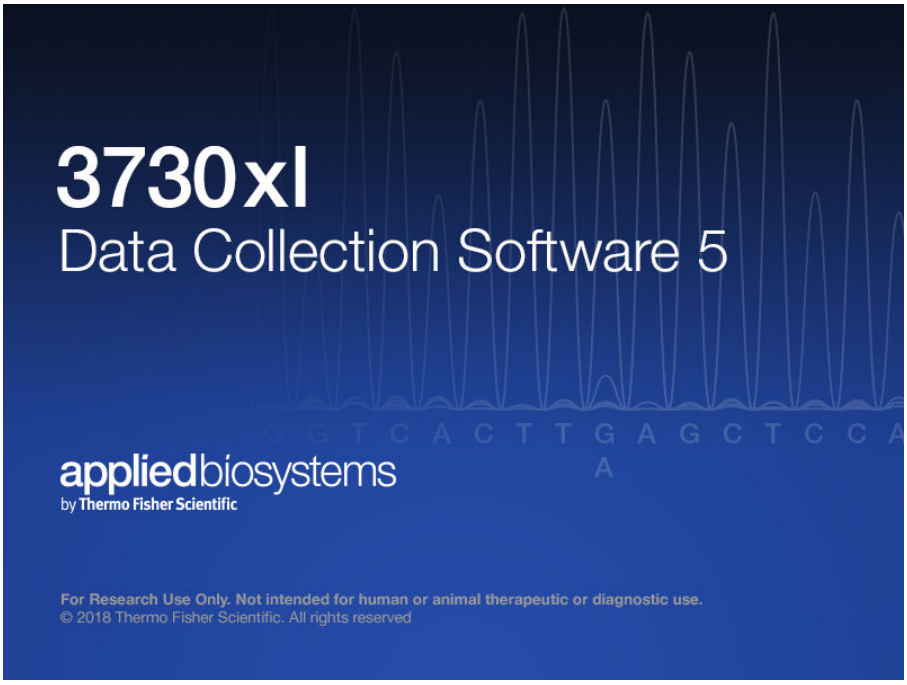


Figure 4 Navigation pane

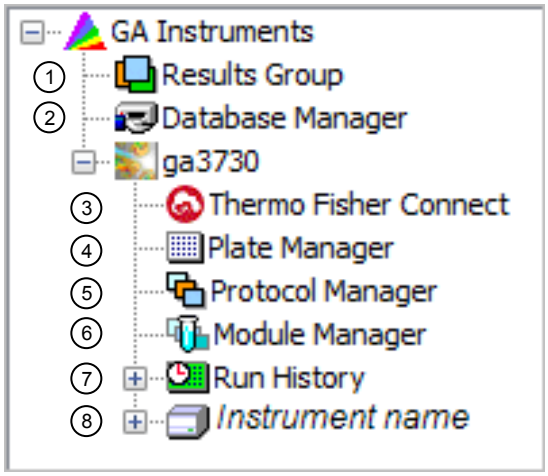


Figure 5 Navigation pane expanded

① **Results Group**—Determine the naming and storage location of results. See "Create a results group (using defaults)" on page 55.

② **Database Manager**—Manage the database; view number of runs in the database and available disk space. See "Maintain adequate space for database and sample data storage" on page 131.

③ **Thermo Fisher Connect**—Link to your Connect cloud-based platform account to set up, view, and analyze data. See Chapter 3, "Use the instrument with the Connect cloud-based platform".

④ **Plate Manager**—Create and manage plate records. See Chapter 4, "Create results groups and plate records using default settings" and "Create and manage plate records" on page 77.

⑤ **Protocol Manager**—Create and manage instrument protocols, which specify run modules, dye sets, capillary length, polymer, and chemistry type. See Chapter 7, "Create and manage instrument protocols, run modules, and analysis protocols".

⑥ **Module Manager**— Create and manage run modules, which specify instrument settings. See Chapter 7, "Create and manage instrument protocols, run modules, and analysis protocols".

⑦ **Run History**—View previous runs. See "View data from a completed run (Run History)" on page 71.

⑧ ***Instrument name***—View the status of the instrument. See "Check instrument status" on page 67.

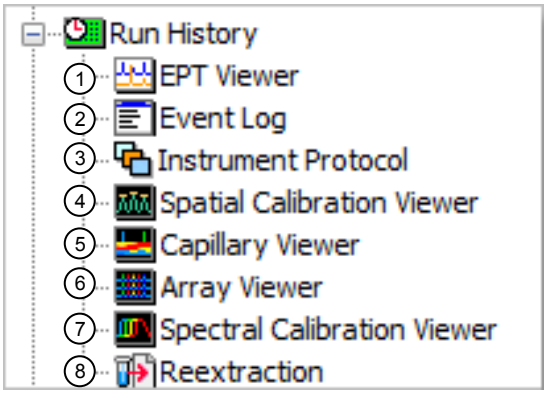


Figure 6 Run History pane expanded

① **EPT Viewer**—View EPT data from a specific run.
② **Event Log**—View events from a specific run (such as, when temperature was changed).
③ **Instrument Protocol**—View the run module settings for an instrument protocol.
④ **Spatial Calibration Viewer**—View the spatial calibration from a specific run.
⑤ **Capillary Viewer**—View the signal intensity for individual capillaries.
⑥ **Array Viewer**—View the signal intensity for the entire array.
⑦ **Spectral Calibration Viewer**—View the spectral calibration from a specific run.
⑧ **Reextraction**—Check autoextraction success or failure and re-analyze samples with different settings. See “Check the autoextraction status and manually re-extract” on page 72.

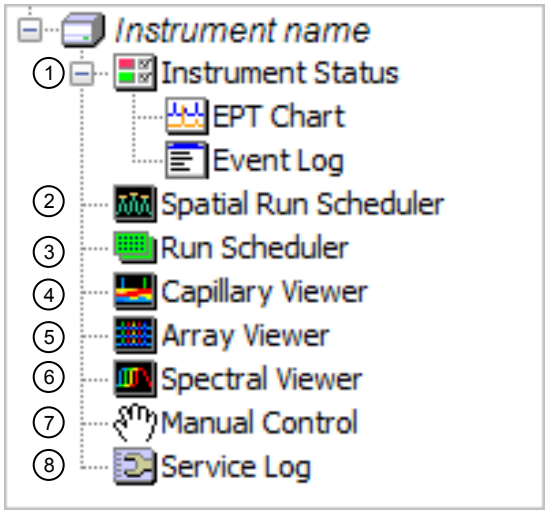


Figure 7 *Instrument name* **pane expanded**

① **Instrument Status**—EPT Viewer and Event Log for the current run. See "Check instrument status" on page 67.

② **Spatial Run Scheduler**—Run a spatial calibration. See "Perform spatial calibration" on page 92.

③ **Run Scheduler**—Set up runs for manual operation and monitor runs. See "Run the instrument in manual mode" on page 61.

④ **Capillary Viewer**—View data for each capillary in the current run. See "View data in the Capillary Viewer" on page 69.

⑤ **Array Viewer**—View data for the entire capillary array. See "View data in the Array Viewer" on page 70.

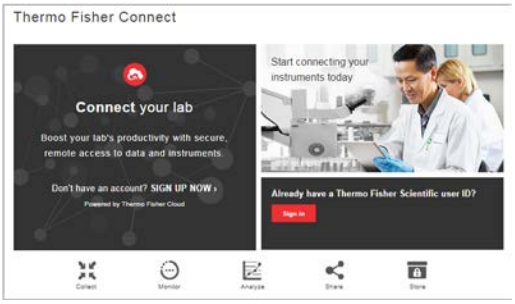
⑥ **Spectral Viewer**—Run spectral calibration, override calibration. See "Perform spectral calibration for sequencing and fragment analysis" on page 98.

⑦ **Manual Control**—Send a manual command to adjust parameters for electrophoresis, the laser, autosampler, polymer delivery pump, oven, or other system or miscellaneous parameters.

⑧ **Service Log**—View the service history.

Use the instrument with the Connect cloud-based platform

The new Connect cloud-based platform feature allows you to connect the instrument to your Connect cloud-based platform account.



- Automatically transfer data files from the instrument to your Connect cloud-based platform account.
- Receive instrument status email notifications.
- View instrument status on InstrumentConnect or a mobile device.

For more information, see Chapter 3, "Use the instrument with the Connect cloud-based platform".

Secondary analysis software

Secondary analysis software is available on the Connect and for desktop computers.

Visit **thermofisher.com/cloud** for the latest available secondary analysis applications.

Connect secondary analysis apps

Analysis	App	Description
Sequencing	Quality Check (QC) module 	• Automatically checks sequence trace quality. • Provides a results summary that is based on quality parameter settings. • Auto-flags lower-quality traces for further inspection.
	Variant Analysis (VA) module 	• Finds variants in samples that are sequenced on Applied Biosystems™ genetic analyzers. • Reports variants at genomic coordinates. • Allows export of variant calls in standard Variant Call Format.
	Next-generation Confirmation (NGC) module 	• Confirms next-generation sequencing (NGS) variants using CE technology. • Allows visualization of the variants that are detected by both NGS and CE platforms. • Allows export of confirmed variants in standard Variant Call Format.
Fragment analysis	Sizing Analysis Module Peak Scanner™ Software 	Performs peak sizing.
	Microsatellite Analysis Software 	Analyzes a mixture of DNA fragments, separated by size, and determines the microsatellite alleles present in the sample.

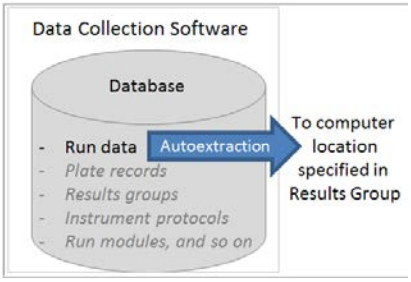
Desktop secondary analysis software for Windows™ 10 operating system

IMPORTANT! Older versions of the desktop secondary analysis software do not run on the Windows™ 10 operating system. Older versions may or may not be able to analyze data files that are generated by the 3730xl Data Collection Software 5. Contact Support for information on obtaining the latest versions of software if you did not buy these applications with the instrument.

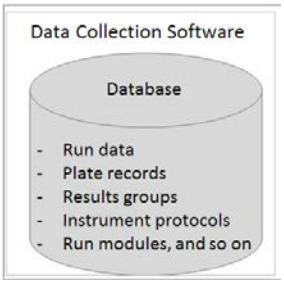
Analysis	Software	Minimum version required
Sequencing	(Required) Sequencing Analysis Software	7
	SeqScape™ Software	4
	Variant Reporter™ Software	3
Fragment analysis	(Required) GeneMapper™ Software	6

Data Collection software terminology

- **Autoextraction and re-extraction**—*Autoextraction* is the automatic process that creates data files at the end of a run (AB1 files for sequence analysis, FSA files for fragment analysis). Re-extraction is a manual process that you can perform after a run is complete. See "Autoextraction and re-extraction" on page 72.



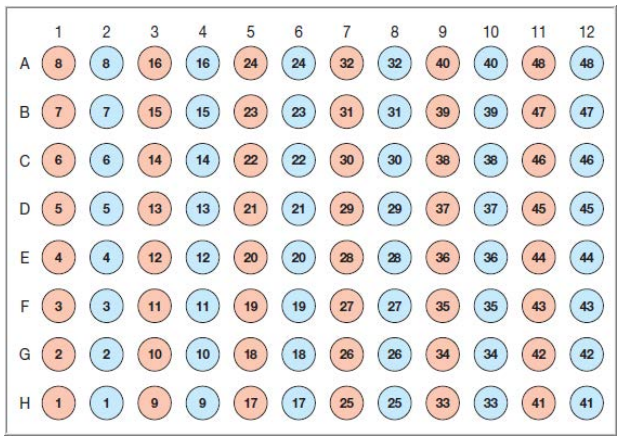
- **Batch Run**—All plates in the **Input Stack** list.
- **Database**—Internal database in the Data Collection Software that stores plate records, results groups, instrument protocols, run modules, analysis protocols, and data that is collected on the instrument.



  Data is autoextracted from the database to generate data files at the end of a run (AB1 files for sequence analysis, FSA files for fragment analysis). Data files are stored in the location specified in the results group specified in the plate record associated with the data. Data files are not stored in the database.

- **Plate record**—Defines the following information for a plate.
  - Plate attributes, sample information, and the type of application
  - Instrument run conditions (instrument protocol)
  - File-naming convention, result-folder–naming convention, analysis type, and data file storage location (results group)
- **Run**—One injection. Depending on number of wells and capillaries, multiple runs (injections) may occur for a plate.

  For example, a 96-well plate on a 48-capillary instrument will require 2 runs (injections) to sample all wells on the plate.



Injection 1

Injection 2

Workflow for a typical run

This workflow assumes that:

- Spatial and spectral calibrations have been done (see page 92)
- Instrument and analysis protocols have been developed and are available for selection (see page 85)

Prepare to run the instrument

Prepare the instrument (page 27)

▼

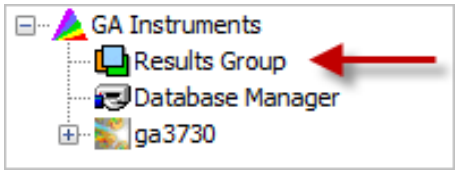
***(Optional)* Link the instrument to the Connect cloud-based platform** (page46)

▼

Prepare plates (page 36)

▼

Create a results group (page 55)



▼

Create a plate record (page 55)

Run the instrument

Run in auto mode (page 61)

▼

—

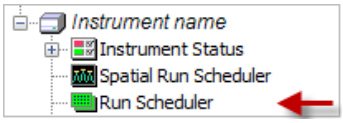
Place plates in stacker in *any order*

▼

Run in manual mode (page 61)

▼

Schedule runs



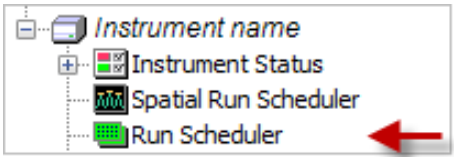
Place plates in stacker in *run order*

▼

Start run

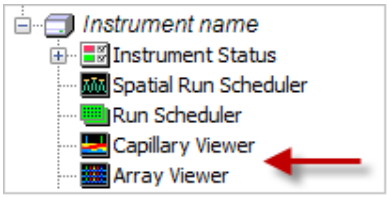
▼

Monitor run status (page 65)



▼

View data from the run (page 69)



▼

Review results in secondary analysis software on the Connect cloud-based platform or on desktop software (page 23)

2 Prepare the instrument

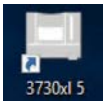
IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Power on the computer and the instrument

1. Close the oven, stacker, and instrument doors.
2. Power on the instrument.
3. Power on the monitor and computer.
4. 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**.

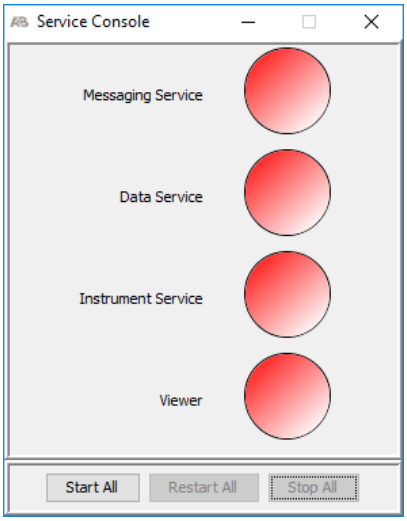
Start the software

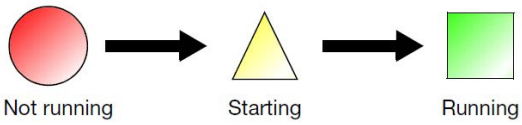
1. In the desktop, double-click the 3730xl Data Collection Software 5 icon.



The **Service Console** dialog box is displayed and services start.

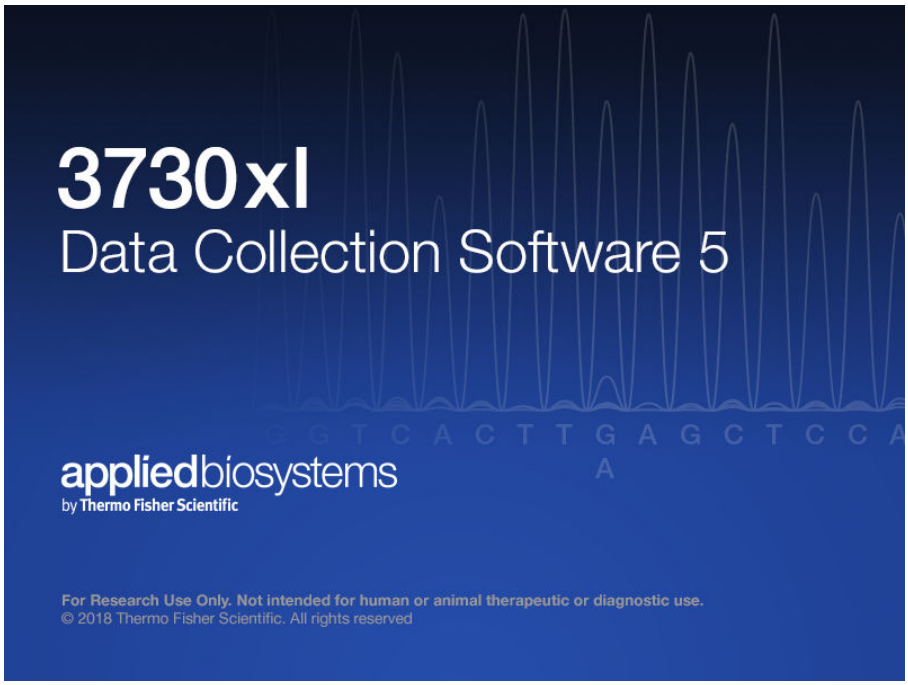
Note: If the dialog box is not displayed, click the **Service Console** tab in the Windows™ task bar.





If all services do not display after 1–2 minutes, see “Service console troubleshooting” on page 138.

2. When all services display , the main screen of the software is displayed (Viewer).



Note: Ensure that all Data Collection Services are running before you launch the AB Navigator Software for security, audit trail, and electronic signature features. For more information, see *AB Navigator Software Administrator Guide* (Pub. No. 4477853).

Prepare buffer and fill the reservoirs

Required materials

- Retainer, buffer/water/waste
- Septa
- Reservoir caps
- Reservoir, buffer/water/waste
- Plate base, water/waste
- Plate base, buffer
- Water, deionized, 180 mL plus, 160 mL for water and waste reservoirs
- 3730 Running Buffer (10X) , 20 mL
- Graduated cylinder, 250-mL
- Gloves, silicone-free, powder-free

1X run buffer storage

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

When to change the 1X run buffer

Replace the 1X run buffer in the anode buffer jar and the buffer reservoir every 48 hours, or before each batch of runs.

When you replace the 1X run buffer, do not add fresh buffer to old buffer. Discard old buffer, then add fresh buffer.

IMPORTANT! Using old 1X buffer can lead to loss of resolution and data quality.

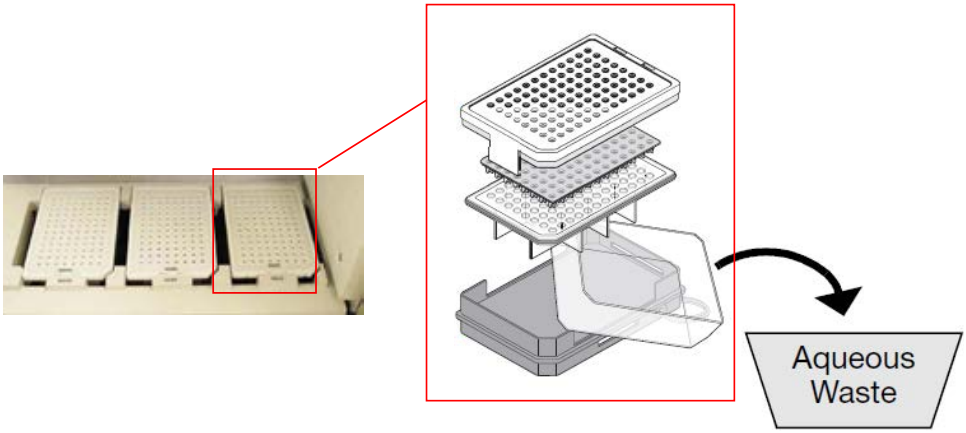
Prepare the 1X running buffer

1. Pour 20 mL 3730 Running Buffer (10X) into a graduated cylinder.
2. Add 180 mL deionized water to bring the total volume to 200 mL.
3. Mix well, then set aside.

1X running buffer can be stored at room temperature for up to one week, or at 2–8°C for up to 30 days.

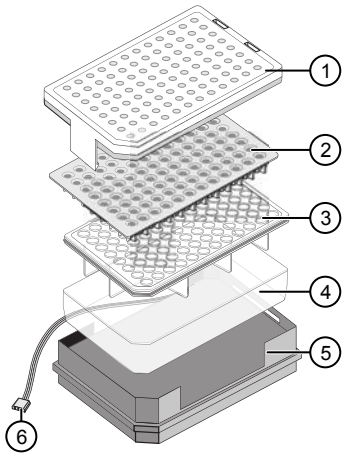
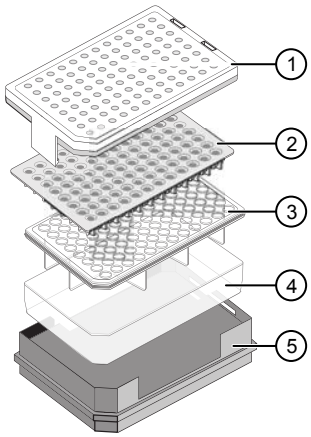
Fill the water and buffer reservoirs

1. Close the instrument door.
2. Press the **Tray** button to bring the autosampler to the forward position (see Figure 1).
3. Wait for the autosampler to stop moving and for the green status light to illuminate before you open the instrument door.
4. Unplug the buffer reservoir. Remove the buffer, water, and waste reservoir assemblies from the instrument.
5. Disassemble each reservoir assembly then empty the contents of the reservoirs into an aqueous waste container.



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

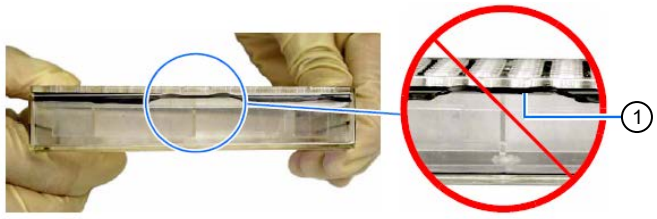
8. Fill, then assemble the reservoirs.

Buffer reservoir assembly	Water and waste reservoir assemblies
1. Add 80 mL 1X run buffer to the Buffer reservoir. 2. Assemble the reservoir assembly as shown below.  ① Retainer ② Septum ③ Reservoir cap ④ Reservoir ⑤ Heated plate base ⑥ Power cable	1. Add 80 mL high-quality deionized water to each reservoir. 2. Assemble each reservoir assembly as shown below.  ① Retainer ② Septum ③ Reservoir cap ④ Reservoir ⑤ Plate base

For catalog numbers see Appendix E, “Catalog numbers”.

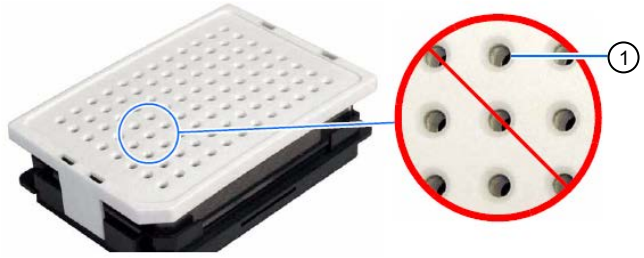
Note: The heater is used to heat the buffer for fragment analysis applications. Disconnecting the cable is not recommended.

9. To prevent damage to the capillary array, inspect each reservoir for the following conditions.
   - Septa fit snugly and flush on the reservoir cap
   - Rubber gasket surrounding the reservoir cap is seated correctly



① Rubber gasket not seated correctly

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

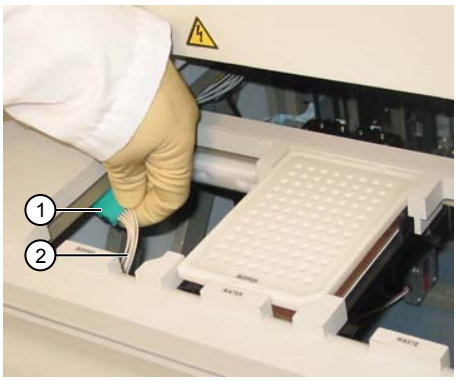


① Plate retainer holes and septa holes are not alignted

10. Dry the exterior of the reservoirs using lint-free wipes.

Place reservoirs into the instrument

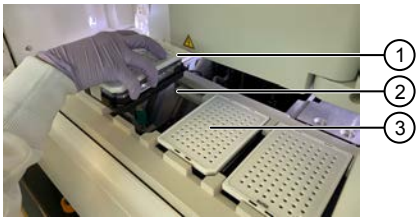
1. Connect the Buffer reservoir plate base cable into the heater outlet inside the instrument.



① Heater outlet
② Plate base cable

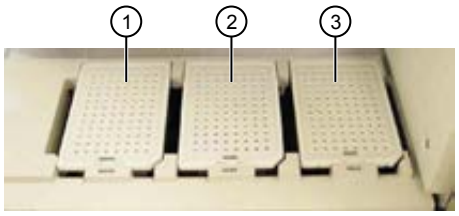
2. Move the cable out of the way of the autosampler. Move the buffer reservoir to the Buffer position (left).

   Note: The heater is used to heat the buffer for fragment analysis applications. Disconnecting the cable is not recommended.



① Buffer reservoir
② Left reservoir position
③ Middle reservoir position

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



① Buffer reservoir
② Water reservoir
③ Waste reservoir

4. Close the instrument door.

Fill the anode buffer jar

Replace the anode buffer at the following times.

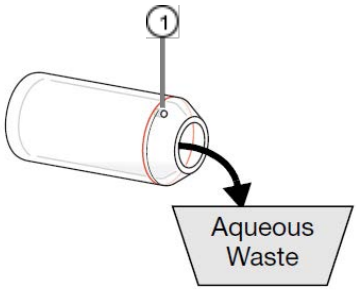
- Before each group of scheduled runs, or at least every 48 hours
- After you replenish polymer
- After you replace the capillary array
- After you run the **Bubble Remove** wizard
- Every time that you change the buffer reservoir

IMPORTANT! The operations list above introduce polymer into the anode buffer jar.

When you replace the 1X run buffer, do not add fresh buffer to old buffer. Discard old buffer, then add fresh buffer.

IMPORTANT! Using old 1X buffer can lead to loss of resolution and data quality.

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



① Overflow hole

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

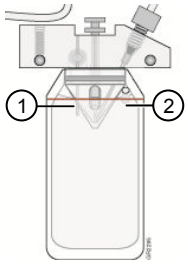
d. Empty the anode buffer jar into an aqueous waste container.

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.

7. Verify that the meniscus of the buffer is just below the red fill line on the jar.

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



① Electrode
② Meniscus

Check the polymer level

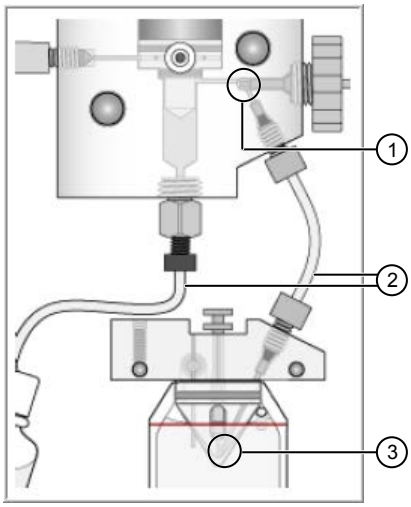
Check the level of polymer, then replenish if needed. Replace polymer if it has been on the instrument for more than 1 week. See “Replenish or change polymer type” on page 119.

Note: When you replace the polymer, do not add fresh polymer to the old polymer. Discard the old polymer.

IMPORTANT! Allow polymer to reach room temperature before installing on the instrument.

Check for bubbles in the pump system

1. Check for bubbles at any point where the pump channels join.



① Array ferrule tip
② All tubing
③ Elbow (internal channel) of the lower pump block

2. If bubbles are present, run the **Bubble Remove Wizard**. For more information, see “Typical conditions for using maintenance wizards” on page 117.

IMPORTANT! When this wizard runs, it introduces polymer into the anode buffer jar. Replace the anode jar buffer after you run this wizard. See “Fill the anode buffer jar” on page 33.

Check the active spectral calibration

Ensure that the active calibration is appropriate for the dye set and capillary length you are running.

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

① Dye set for the run
② Active spectral calibration

2. Select the dye set for the run.

3. If the active spectral calibration is not appropriate for your application, see “Select a previous calibration as the active spectral calibration” on page 111.

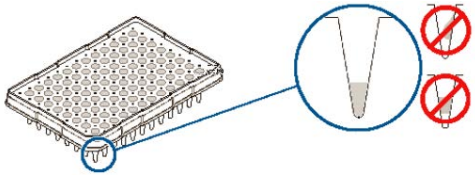
Prepare and load sample plates

Prepare sample plates as needed for your application. Refer to the following topics to help you determine sample placement in wells.

- “Default injection positions—48-capillary arrays” on page 37
- “Default injection positions—96-capillary arrays” on page 39
- “Default injection order on plates” on page 59

1. Briefly centrifuge the plate.
2. Ensure that each sample is positioned correctly in the bottom of its well.

   IMPORTANT! If the contents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

3. Store the plate on ice and protected from light until you prepare the plate assembly and load the plate in the instrument.

After you load samples in plates, see “Seal and assemble plates” on page 41.

Default injection positions—48-capillary arrays

96-Well plate, 48 capillaries

2 injections are required to sample all wells on the plate.

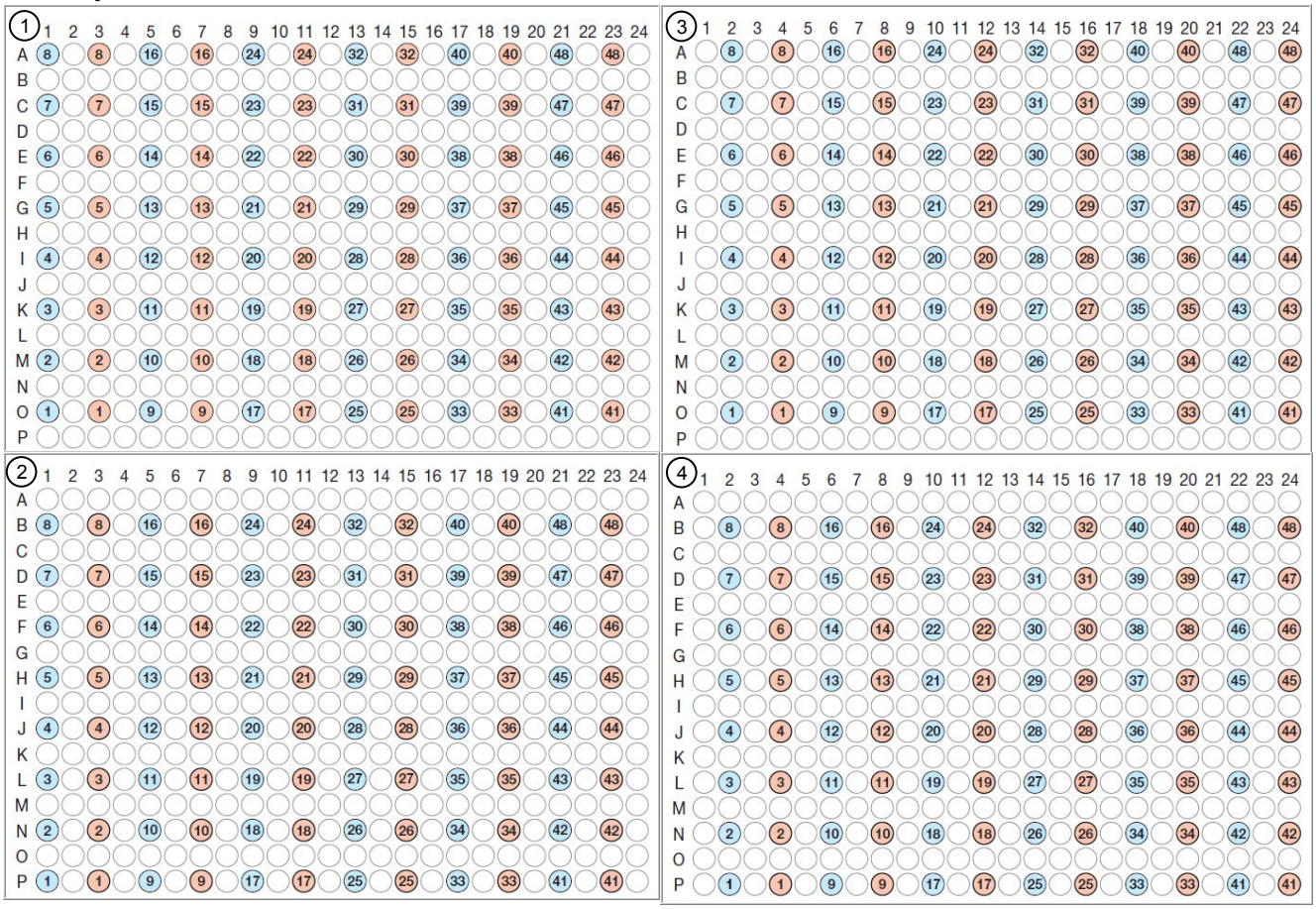
	1	2	3	4	5	6	7	8	9	10	11	12
A	8	8	16	16	24	24	32	32	40	40	48	48
B	7	7	15	15	23	23	31	31	39	39	47	47
C	6	6	14	14	22	22	30	30	38	38	46	46
D	5	5	13	13	21	21	29	29	37	37	45	45
E	4	4	12	12	20	20	28	28	36	36	44	44
F	3	3	11	11	19	19	27	27	35	35	43	43
G	2	2	10	10	18	18	26	26	34	34	42	42
H	1	1	9	9	17	17	25	25	33	33	41	41

Injection 1

Injection 2

384-Well plate, 48 capillaries

2 injections from each quadrant are required to sample all wells on the plate.



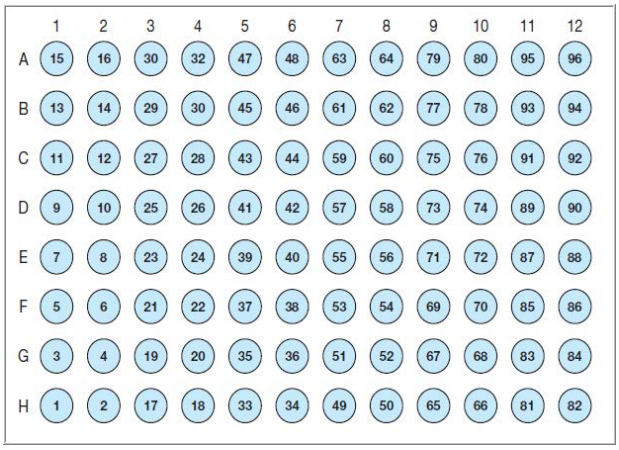
- Injection 1
- Injection 2
- No injection

① Quadrant 1
② Quadrant 2
③ Quadrant 3
④ Quadrant 4

Default injection positions—96-capillary arrays

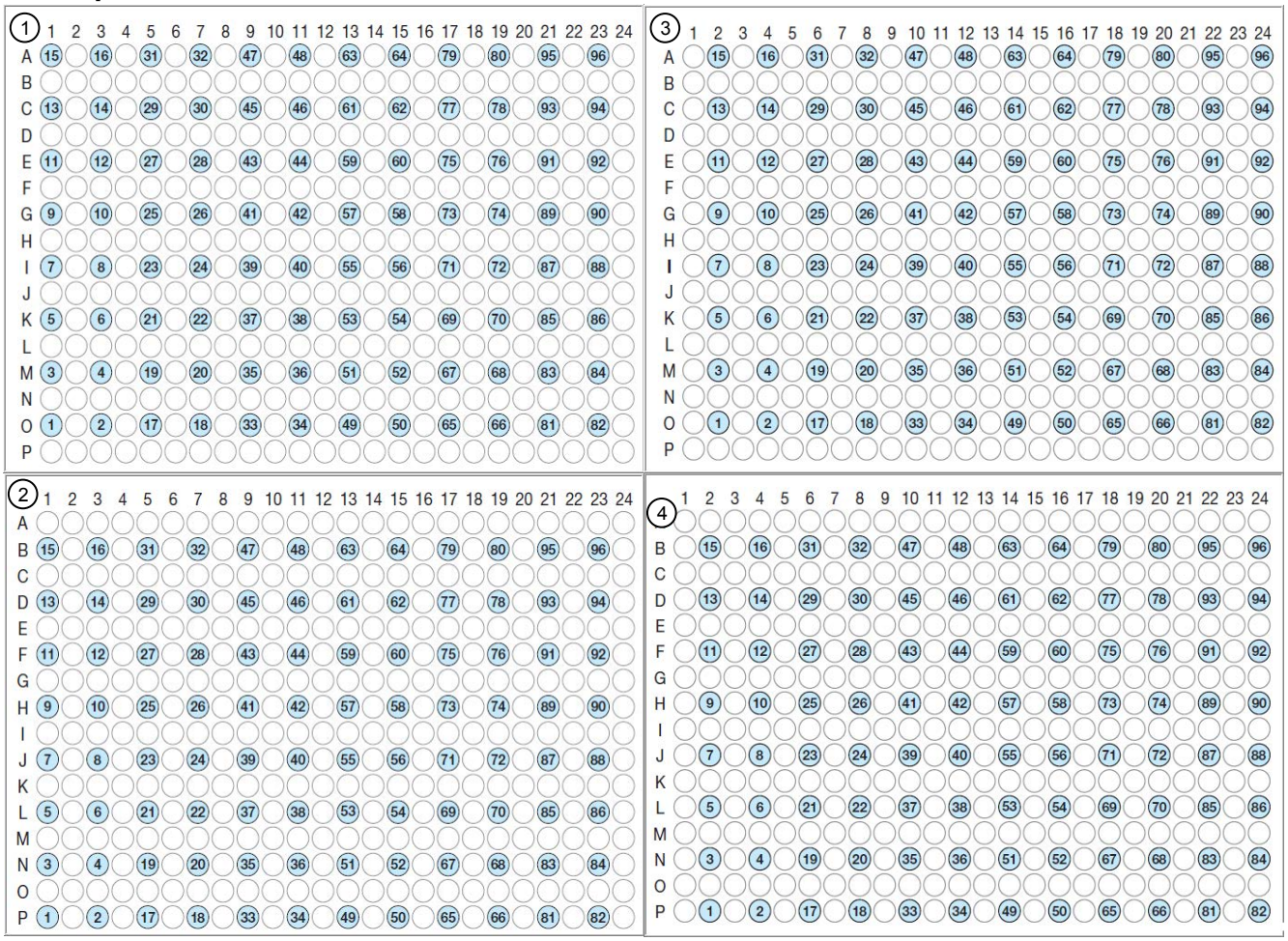
96-Well plate, 96 capillaries

1 injection



384-Well plate, 96 capillaries

1 injection from each quadrant is required to sample all wells on the plate.



Injection 1

No injection

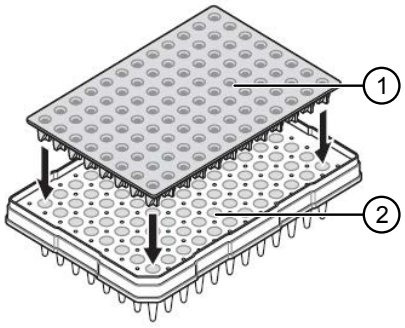
① Quadrant 1
② Quadrant 2
③ Quadrant 3
④ Quadrant 4

Seal and assemble plates

Seal and prepare the plate assemblies

WARNING! Do not use warped or damaged plates.

Seal the plate with a heat-seal or septum.

Option	Description
Heat seal	Follow your thermal sealer instrument instructions.
Septum seal	 ① Septum ② Plate 1. Place the plate on a clean, level surface. 2. Place a new septum flat on the plate. 3. Align the holes in the septum strip with the wells of the plate, then firmly press downward onto the plate. Ensure that the septum is seated and aligned (see Figure 8). • The septa lie flat against the plate. Ensure that there are no lumps or raised edges. • The septa are inserted straight into the wells. Ensure that no well openings are obscured by a pinched well.

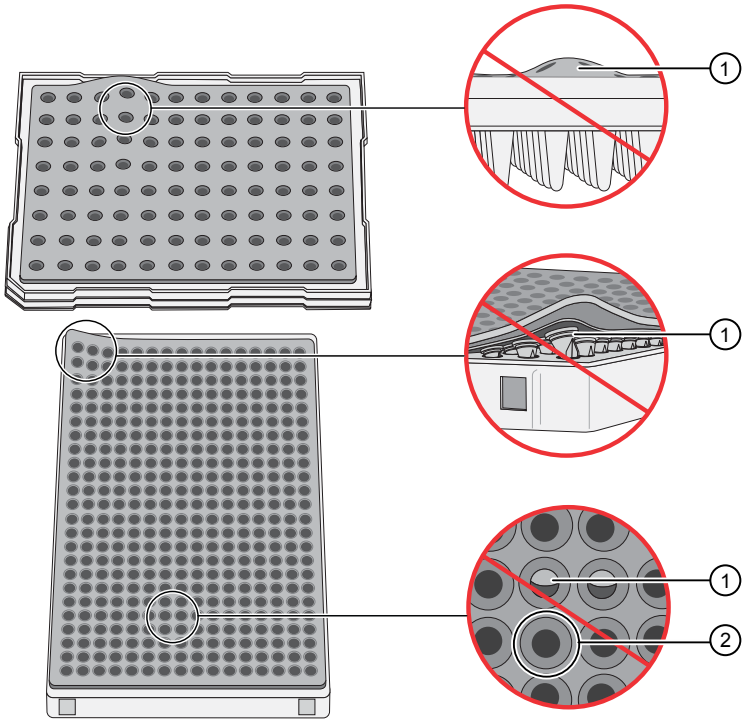


Figure 8 Examples of bad and good septa alignment

① Septum and well are not aligned ② Septum and well are properly aligned

Prepare the plate assemblies

1. Prepare the plate assembly as shown in the following figures.

Table 3 Thermo Fisher Scientific plates, seals, and base colors

Plate type	Seal	Plate base color
96 or 384 well standard plate	Septa	Black
	Heat seal	Gray
Fast 96 well plate	Septa	Blue
	Heat seal	Dark green

Note: Colors may change. Please refer to the product information sheet.

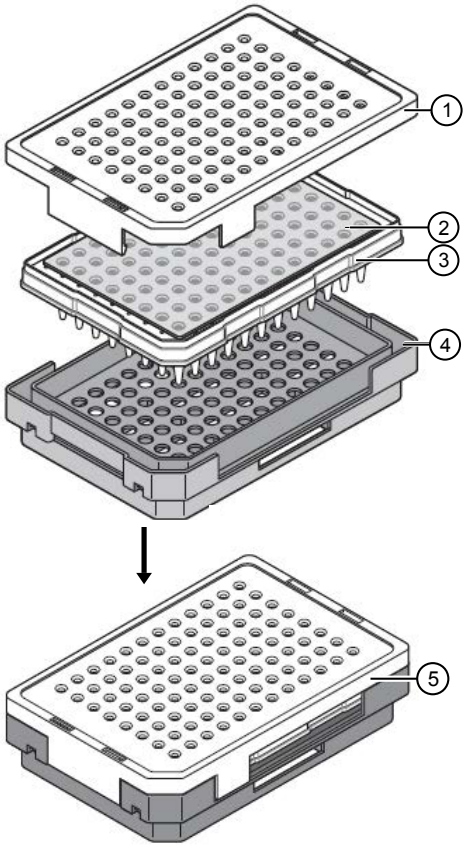


Figure 9 Septum-sealed plate assembly

① Plate retainer
② Plate septum
③ Septum-sealed sample plate
④ Black plate base
⑤ Assembled components

IMPORTANT! 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.

For catalog numbers see Appendix E, “Catalog numbers”.

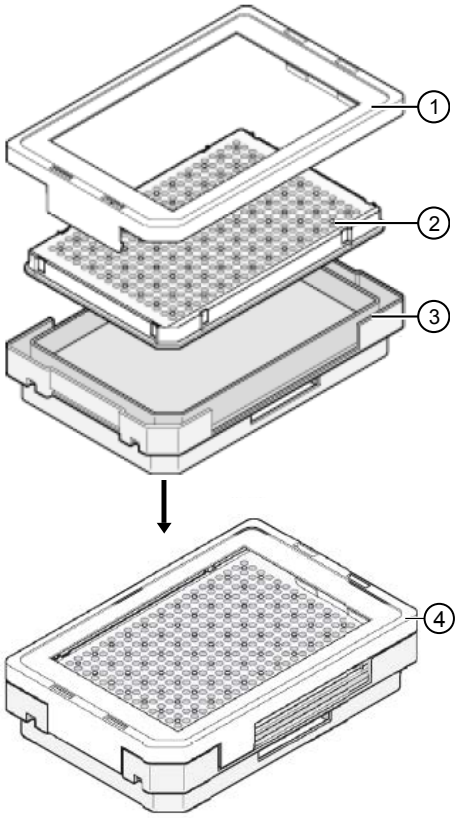


Figure 10 Heat-sealed plate assembly

① Plate retainer	③ Gray plate base
② Heat-sealed sample plate	④ Assembled components

IMPORTANT! 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.

For catalog numbers see Appendix E, “Catalog numbers”.

2. For a septa-sealed plate, 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.

3. 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

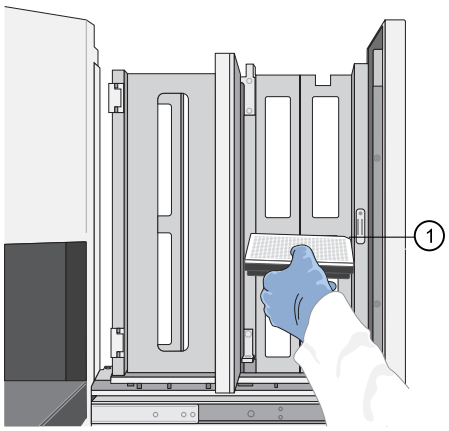
- Use 3 mm Thermo Fisher Scientific heat seal film (Cat. No. 4337570). This film is 3 mm thick before heating and 1 mm thick after heating.
- Do not use heat seal film that is thicker than 1 mm after heating.
- Do not use heat-seal film that contains adhesives or metals. These seals can damage the internal needles that pierce the film before the injection.

Place 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. Ensure that the notched corner of the plate assembly is at the rear right corner of the stacker. The bottom plate assembly is run first.

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



① Notched corner

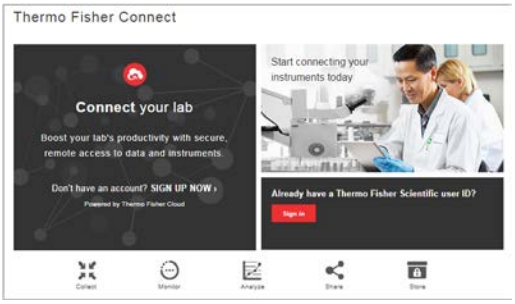
4. Close the metal In Stack tower door.
5. Close the Stacker drawer.

3 Use the instrument with the Connect cloud-based platform

Connect cloud-based platform features

This option is *not* available if **Access Control Administration** is enabled in the AB Navigator Software.

If the instrument is connected to a network you can use the Connect cloud-based platform feature. The Connect cloud-based platform provides the following functions.

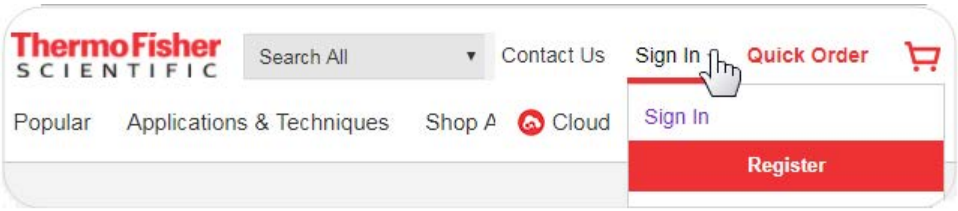


- Automatically transfer data files from the instrument to your Connect cloud-based platform account.
- Receive instrument status email notifications.
- View instrument status on InstrumentConnect or a mobile device.

IMPORTANT! The instrument communicates with the computer by Ethernet connection. Do not make any changes to the computer ethernet/internet connections during a run or during calibration.

Register and obtain a Connect cloud-based platform account

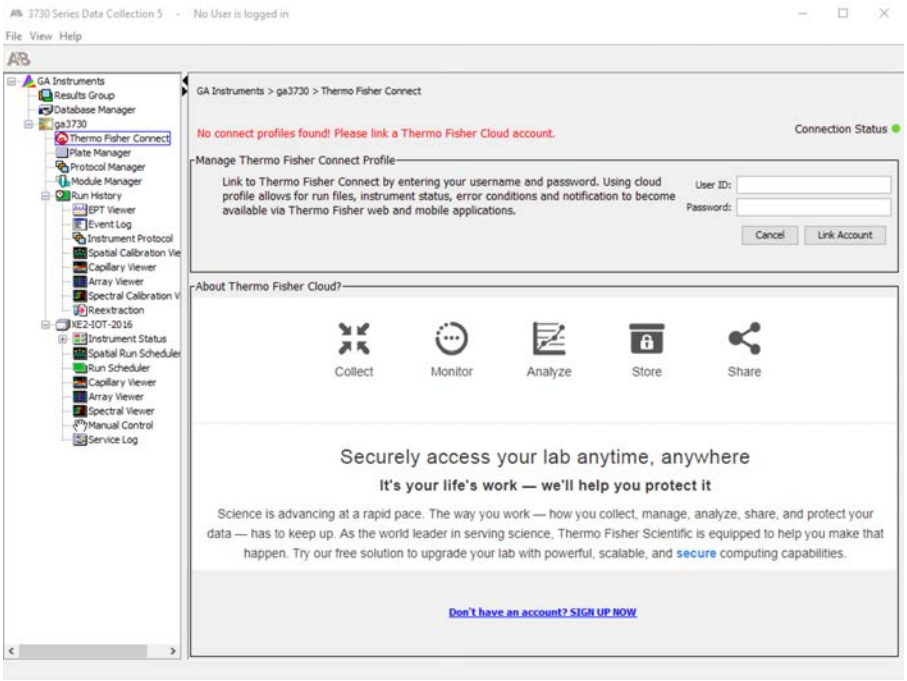
1. Go to **www.thermofisher.com**.
2. On the home page, select **Sign In** ▸ **Register**.



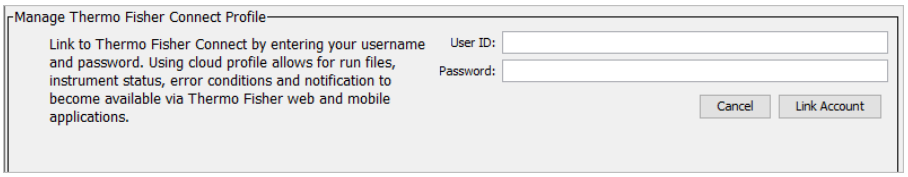
3. Fill in all information, then click **Create account**.

Connect the instrument to your Connect cloud-based platform account

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Thermo Fisher Connect**.



2. Enter the **User ID** and **Password** for your Connect cloud-based platform account, then click **Link Account**.



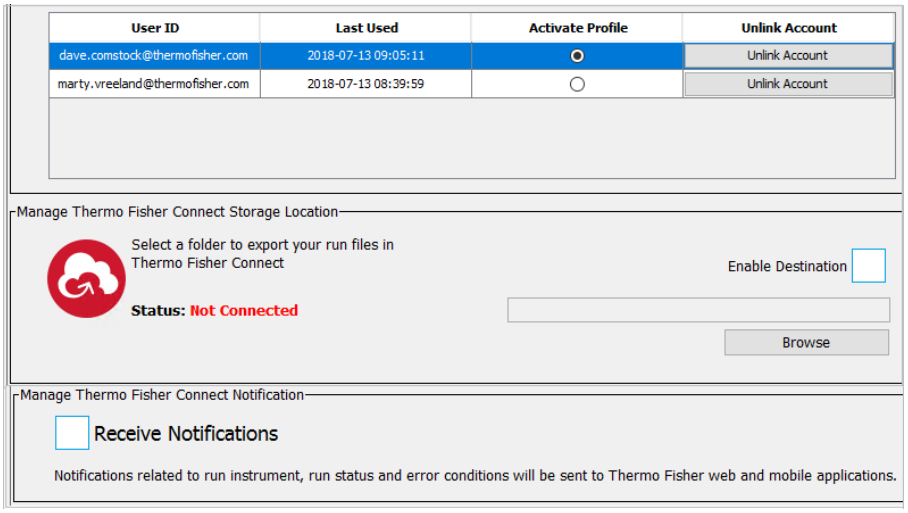
An email is sent to the email address associated with your account, and the instrument is listed in the screen on the Connect cloud-based platform (see “Monitor a run from InstrumentConnect” on page 49).

Set up the data storage location and email notifications

When an instrument is linked to your Connect cloud-based platform account, you can store run data in your Connect cloud-based platform account. You can also have email notifications sent to your Connect cloud-based platform account email address.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Thermo Fisher Connect**.

2. In the middle of the screen, click your **User ID** to connect the instrument to your Connect cloud-based platform account.



3. Select the **Enable Destination** checkbox, then select the storage location in your Connect cloud-based platform account.

   When a run is complete, the data is stored in the results group folder that is specified in the plate record. The data will be automatically uploaded your account whenever your **User ID** is the active ID.

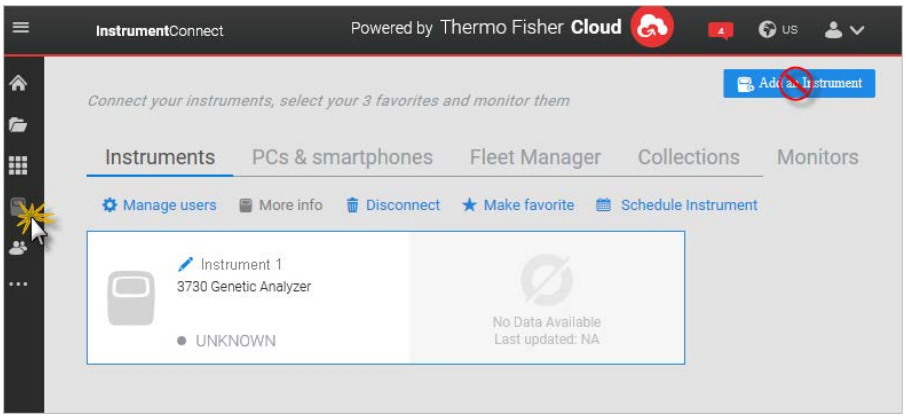
4. Select the **Receive Notifications** checkbox.

   Run status emails will be sent to your Connect cloud-based platform account email address.

Monitor a run from InstrumentConnect

1. Sign in to **thermofisher.com/cloud**.

2. Click **Connect Your Lab**, then click to access InstrumentConnect.



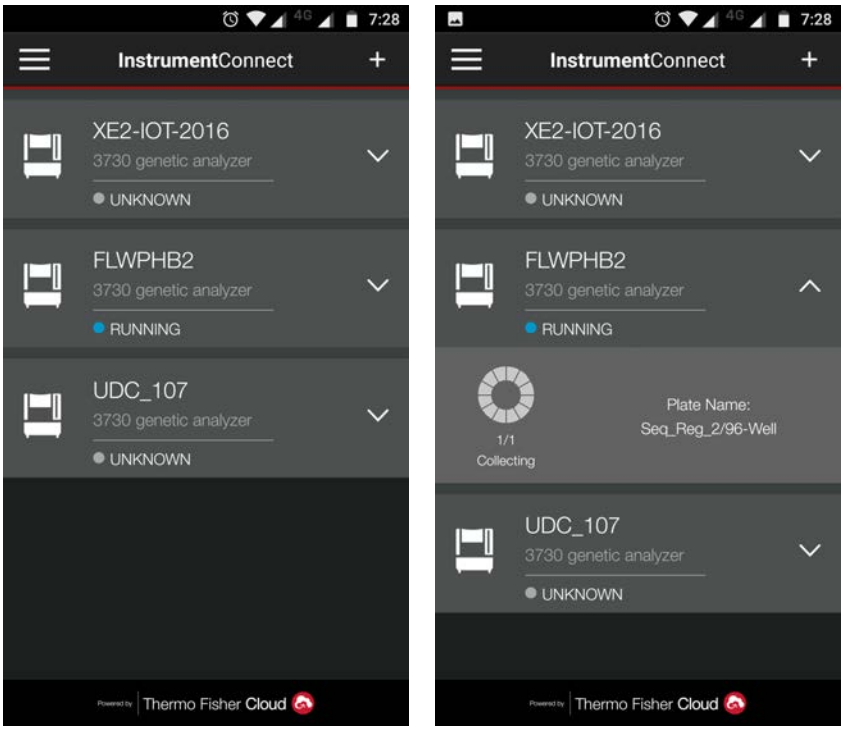
Note: The **Add an Instrument** function is not supported for the instrument. See “Connect the instrument to your Connect cloud-based platform account” on page 47.

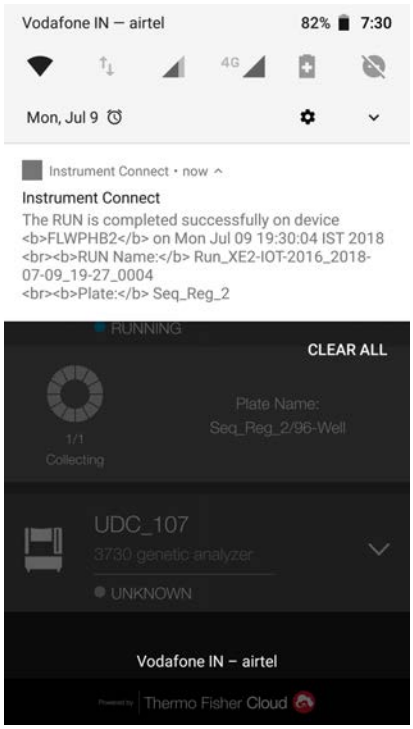
3. Click the instrument to display instrument status.

Monitor a run from a mobile device

The instrument must be connected to your Connect cloud-based platform account before you can monitor it. See “Connect the instrument to your Connect cloud-based platform account” on page 47,

1. On your mobile device, download the InstrumentConnect from the Apple Store or from Google™ Play.
2. On your mobile device, launch InstrumentConnect.
3. Touch the instrument to monitor.





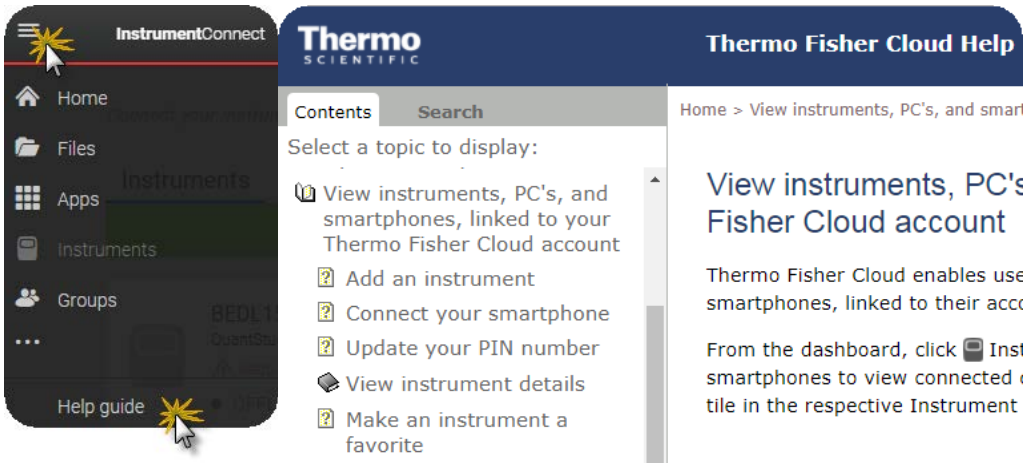
View notifications from the instrument on your Connect cloud-based platform account

1. In any screen in the Connect cloud-based platform, click .

2. Click a notification, then click **Dismiss** or **Dismiss all** to dismiss the notification.

For more information on using InstrumentConnect

In the top left of any screen in InstrumentConnect, click ☰, then select **Help guide**.



Connect cloud-based platform administrators for an instrument

First user who links is assigned administrator role

The first user who links the instrument to their Connect cloud-based platform account is assigned administrator role for the instrument.

Additional instrument administrators can be assigned, and user roles can be changed after linking.

Instrument administrator functions

An administrator can perform the following tasks from InstrumentConnect.

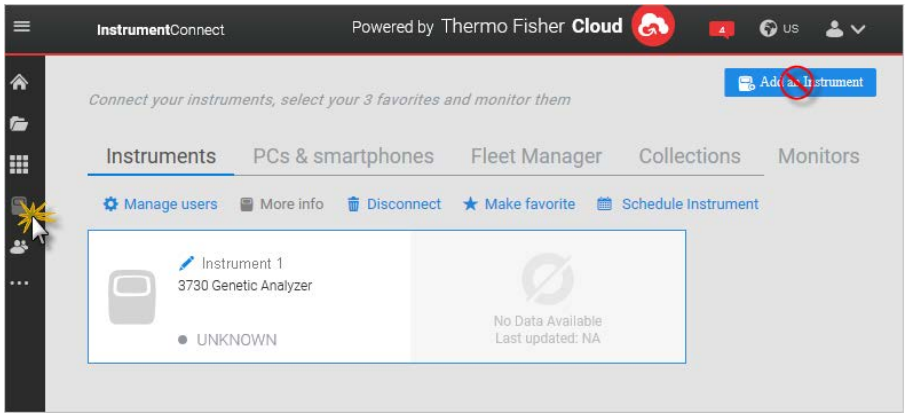
- Access the **Manage users** function to see a list of all accounts that are linked to the instrument.
- Assign administrator role to one or more users.
- Remove an account from an instrument.
- Disconnect the instrument from InstrumentConnect.
- Change the instrument name.

Manage the users and administrators of your instrument

Any user with administrator role can manage users for an instrument or disconnect an instrument from InstrumentConnect.

If an administrator...	The software...
Assigns Administrator role to a user	Allows the user to perform all administrator functions (see “Instrument administrator functions” on page 52).
Removes a user	Unlinks the instrument from their Connect cloud-based platform account.
Disconnects the instrument	• Unlinks the instrument from all Connect cloud-based platform accounts. • Removes the instrument from InstrumentConnect.

1. Sign in to **thermofisher.com/cloud**.
2. Click **Connect Your Lab**, then click to access InstrumentConnect.
3. Select the instrument.



Note: The **Manage users** and other administrator functions are not enabled until you select an instrument.

4. To assign the Administrator role to a user or to remove a user, click **Manage users**, then perform the following tasks as needed.

To...	Do this...
Assign the Administrator role to an additional user	Select the **Admin** checkbox, then click **Close**.
Remove a user	Click , then click **Confirm**.

Disconnect individual users from an instrument

You cannot disconnect individual users from an instrument.

To *disconnect* a user, or to unlink the instrument from a Connect cloud-based platform account, you must disconnect the instrument from InstrumentConnect. Doing so unlinks *all* accounts and removes the instrument, and all user data for that instrument, from InstrumentConnect.

You can *remove* a user from the instrument. However, doing so deletes the user data from the instrument.

For more information, see “Manage the users and administrators of your instrument” on page 53.

4 Create results groups and plate records using default settings

This chapter contains simple procedures to create a results group and plate record for a run. It assumes that instrument and analysis protocols have been developed and are available for selection.

For information on creating a results group and plate record with more advanced settings, see Chapter 6, “Create and manage plate records and results groups”.

For information on creating instrument and analysis protocols, see Chapter 7, “Create and manage instrument protocols, run modules, and analysis protocols”.

Create a results group (using defaults)

This procedure creates a results group that uses the default destination and naming conventions. For more information on results groups, see the following sections.

- “Overview of results groups” on page 80.
- To define a custom destination or to specify custom naming conventions, see “Create a results group (detailed procedure)” on page 81.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **Results Group**.
2. Click **New**.
3. In the **General** tab, enter a **Results Group Name**.
4. Click the **Analysis** tab, then select the **Analysis Type**.

   For more information, see “Overview of results groups” on page 80.

   For information on autoanalysis, see the documentation for the secondary analysis software.
5. Click **OK**.

Create a plate record

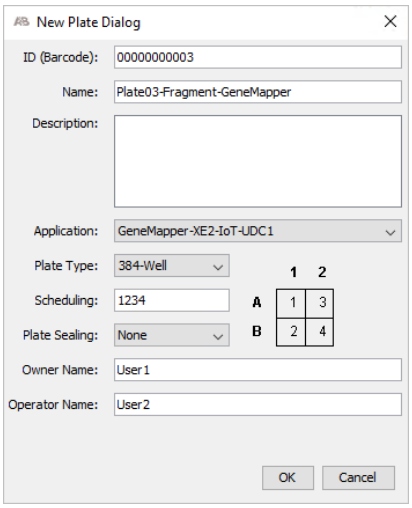
Note: You can create plate records and add plates to the stacker during a run.

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

2. Click **New**.

   You can also import a plate record. See “Import a plate record” on page 79.

3. Enter the plate information in the **New Plate Dialog** box, then click **OK**.

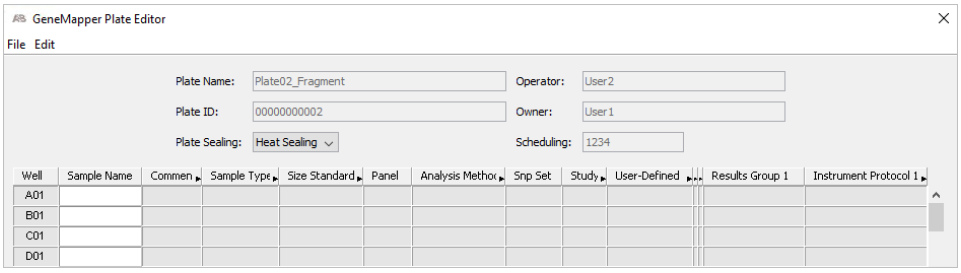


   - **Plate attributes**—Barcode, name, owner, operator, application, number of wells, and sealing option. (For more information, see “Overview of plate records” on page 74.)

     IMPORTANT! If you are creating the plate record to run in auto mode, scan or enter the barcode of a physical plate to link it to this plate record.

   - **(*Optional*)**—Change the default order of injections for the plate quadrants (384-well plates or 96-well plates on 48-capillary instruments). For more information, see “Default injection order on plates” on page 59.
   - **Sample and run information**—Sample name, type, comment, and the settings to use for the run (described below).

4. Enter a sample name in the **Sample Name** field, then press **Enter**.



5. *(Optional)* To add reinjections for a plate, select **Edit**, then **Add Sample Run**.

6. *(Optional)* Enter a comment.

7. In the **Results Group** field, select the field, select ⌄, then do either of the following.
   - Select an existing results group from the list.
   - Click **New** or **Duplicate** to create a new results group

   For information on results groups, see "Overview of results groups" on page 80.

8. In the **Instrument Protocol** field, click the field, select ⌄, then select a protocol.

   For information on instrument protocols, see Chapter 7, "Create and manage instrument protocols, run modules, and analysis protocols".

9. For the remaining fields, select click the field, select ⌄, then select an appropriate item for your application.

   The settings in these lists are provided by the secondary analysis software. For information on these settings, see the software help in the secondary analysis software.

10. Fill in the remaining rows by manually entering and selecting information, by using **Fill Down** commands, or by copy/pasting.

Fill Down	Copy/paste
Select the entire row, select **Edit** ▸ **Fill Down Special**, then select a fill down option. For 48-capillary instruments, select **Fill down Special (48 Cap)**. For 96-capillary instruments: • 96-well plate: Select **Fill Down**. • 384-well plate: **Fill down Special (96 Cap)**.	• If you copy multiple cells, select the same number of corresponding target cells before you paste. • You can copy/paste within one protocol. **Note:** Use the **Duplicate** function in the **Plate Manager** to copy information from an existing protocol to a new protocol.

11. Click **OK**.

5 Run the instrument

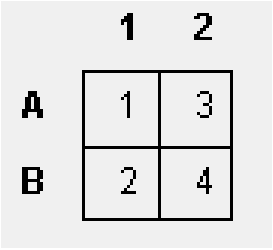
Understanding run modes and default injection positions

Manual and auto run mode features

Manual run mode	Auto run mode
For attended operation.	For unattended operation.
Plates do not require a barcode.	Plates require a barcode.
A plate record is not linked to a plate.	A plate record is linked to a plate by barcode.
You must set up the run in **Run Scheduler** before you start a run.	You do not have to set up the run in **Run Scheduler** before you start a run.
The first plate record in the **Run Scheduler Input Stack** list is used for the first plate that runs. The second plate record in the list is used for the next plate, and so on.	No plate records are listed in the **Run Scheduler Input Stack** list. The software finds and uses the plate record with a barcode that matches the plate.
Plates are run in the order in which they are placed in the stacker (bottom plate runs first).	
The currently running plate is listed in the **Auto Sampler** field.	
Completed plates are listed in the **Output Stack** list.	

Default injection order on plates

For plates that require more than one injection to sample all wells, the plate is divided into quadrants. The default injection order is quadrant A1, quadrant B1, quadrant A2, quadrant B2.



You can change this default injection order in two places.

- In a new plate record before a run
- In the **Run Scheduler** (See “Change injection order of plate quadrants” on page 63)

The wells that are injected in a quadrant depend on the plate size and the instrument.

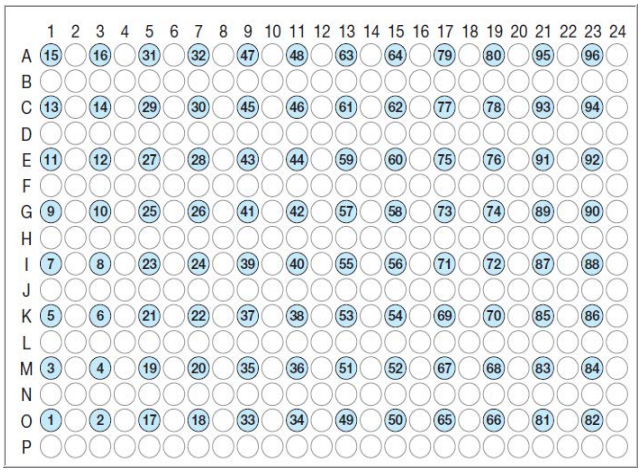


Figure 11 Example: Quadrant 1 on a 384-well plate

Table 4 Default injection order for all plate and capillary options

Number of capillaries	Plate size	Default priority	Plate quadrant	First well in the injection
96	384-well	1	Q1	Well A1
		2	Q2	Well B1
		3	Q3	Well A2
		4	Q4	Well B2
48	96-well	1	Q1, injection 1	Well A1
			Q1, injection 2	Well A2
48	384-well	1	**Q1,** injection 1	Well A1
			Q1, injection 2	Well A3
		2	**Q2,** injection 1	Well B1
			Q2, injection 2	Well B3
		3	**Q3,** injection 1	Well A2
			Q3, injection 2	Well A4
		4	**Q4,** injection 1	Well B2
			Q4, injection 2	Well B4
Note: With 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 injection numbers.				

For more information, see the following sections.

- “Default injection positions—48-capillary arrays” on page 37
- “Default injection positions—96-capillary arrays” on page 39

Run the instrument in auto mode

1. Select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Run Scheduler**.

2. At the top of the screen, select **Instrument** ▸ ***Instrument name***, then select **Auto**.

   Note: The **Instrument** menu is available only when the **Run Scheduler** is displayed.

3. Load the barcoded plates into the input stack in the stacker.

   See “Move a plate in the Input stack during a run” on page 64.

   The plates will be run in the order in which they are placed in the stacker. The bottom plate runs first.

4. Click (Run).

   As the plates are moved to the autosampler, plate barcodes are scanned and their plate records are used for the run.

   IMPORTANT! Ensure that barcodes have been scanned or manually entered for each plate. If the plate barcode scanned by the instrument does not match a barcode in the plate record, the plate will be moved to the **Output Stack** without being run.

   During an auto run:

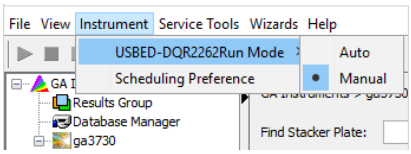
   - No plates are listed in the **Input Stack**
   - The currently running plate is listed in the **Auto Sampler** field
   - Completed plates are listed in the **Output Stack**

Run the instrument in manual mode

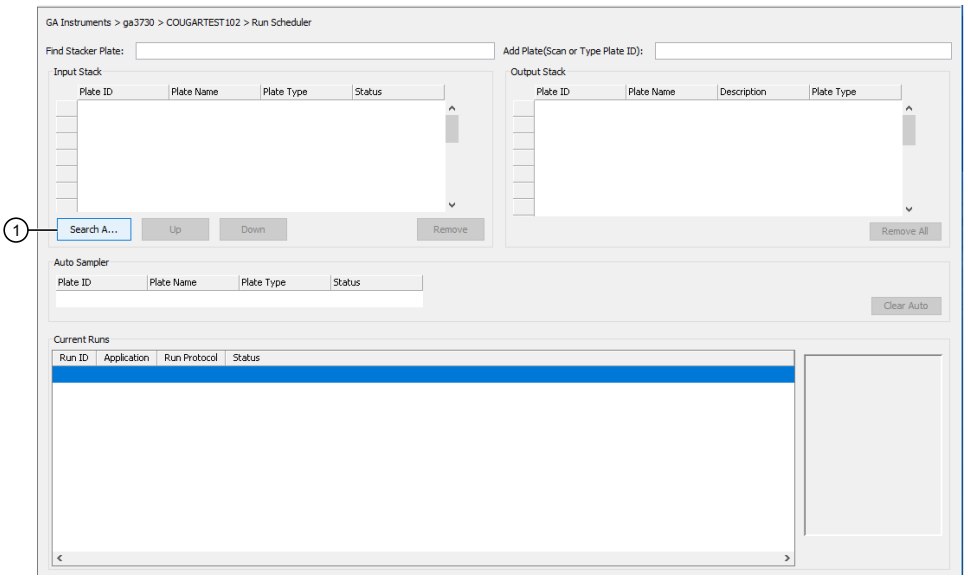
1. Select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Run Scheduler**.

2. At the top of the screen, select **Instrument** ▸ ***Instrument name*** **Run Mode**, then select **Manual**.

   Note: The **Instrument** menu is available only when the **Run Scheduler** is displayed.



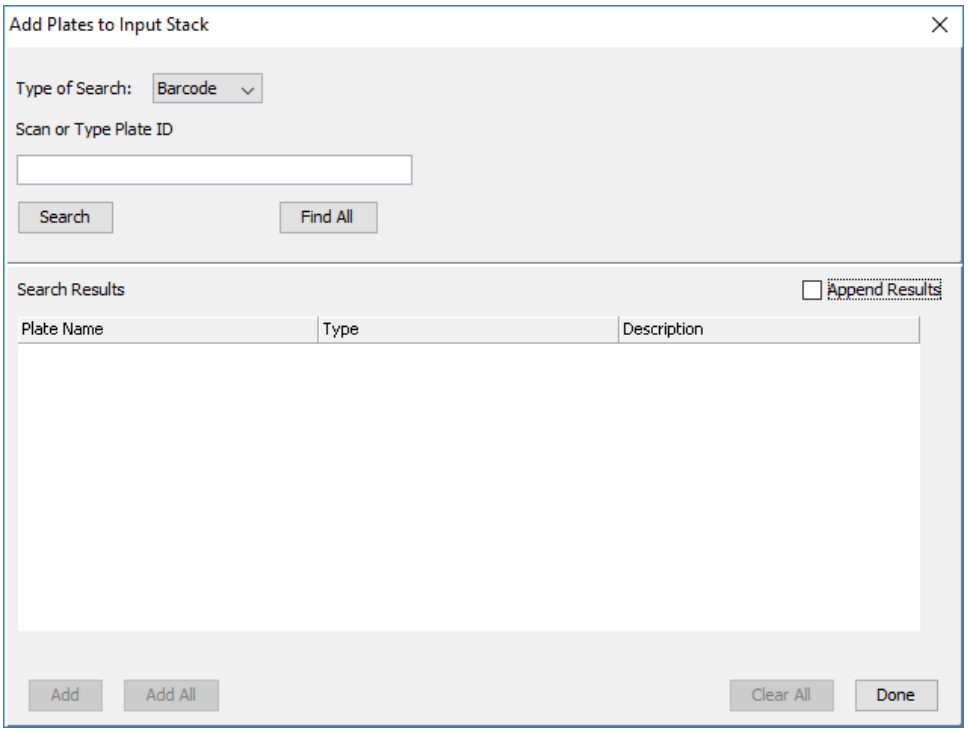
3. Click **Search A... [Search plate(s) and add to the input stack]** to search for plate records.



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

4. Click **Find All** or type the name of the plate or scan the **Plate ID**, then click **Search**.

You can also change the **Type of Search** to **Advanced** and enter more specific plate criteria to search for. For more information, see “Search for a plate record” on page 77.



5. Select the plate records to add, then click **Add**.

6. When the needed plate records are listed, click **Done**.

7. As needed, click **Up**, **Down**, or **Remove** to organize the list of plate records in the **Input Stack** list.

8. Load the plates in the In Stack in the order to match the corresponding plate record. The bottom plate runs first and uses the first plate record in the list.

   IMPORTANT! The order of the plate records must match the stack order of the plates in the In Stack. If the order does not match, data files will contain the wrong plate record information.

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

9. Click (Run).

   During a manual run, the following steps occur.

   - Plates are listed in the **Input Stack**
   - The currently running plate is listed in the **Auto Sampler** field
   - Completed plates are listed in the **Output Stack**

For more information, see “Monitor the status of the run” on page 65.

Change injection order of plate quadrants

You can change the run order of quadrants. Changing the order of the quadrants overrides the setting in the plate record and is applied to all plates.

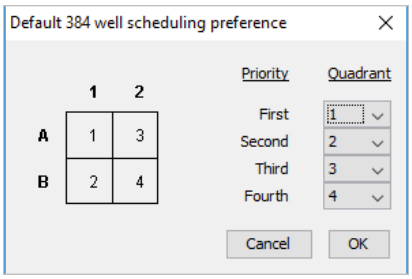
1. Select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Run Scheduler**.

2. At the top of the screen, select **Instrument** ▸ **Scheduling Preference**.

   Note: The **Instrument** menu is available only when the **Run Scheduler** is displayed.

3. Select the quadrant priority (run order) from the **Quadrant** list.

   You can select any run order. For information on the order of well injection, see the following sections.

   - “Default injection positions—48-capillary arrays” on page 37
   - “Default injection positions—96-capillary arrays” on page 39
   - “Default injection order on plates” on page 59

Move a plate in the Input stack during a run

You can move a physical plate in the In Stack tower to change the plate run order.

It is not necessary to pause the run to move a plate.

1. Remove the plates from the In Stacl tower. Place them back in with the plate of interest closest to the bottom of the stack.

   For more information, see “Place plate assemblies into the instrument” on page 45.

2. If you are running the instrument in manual mode, change the order of the plate records in the Input Stack list. The order must correspond to the plate order in the In Stack tower.

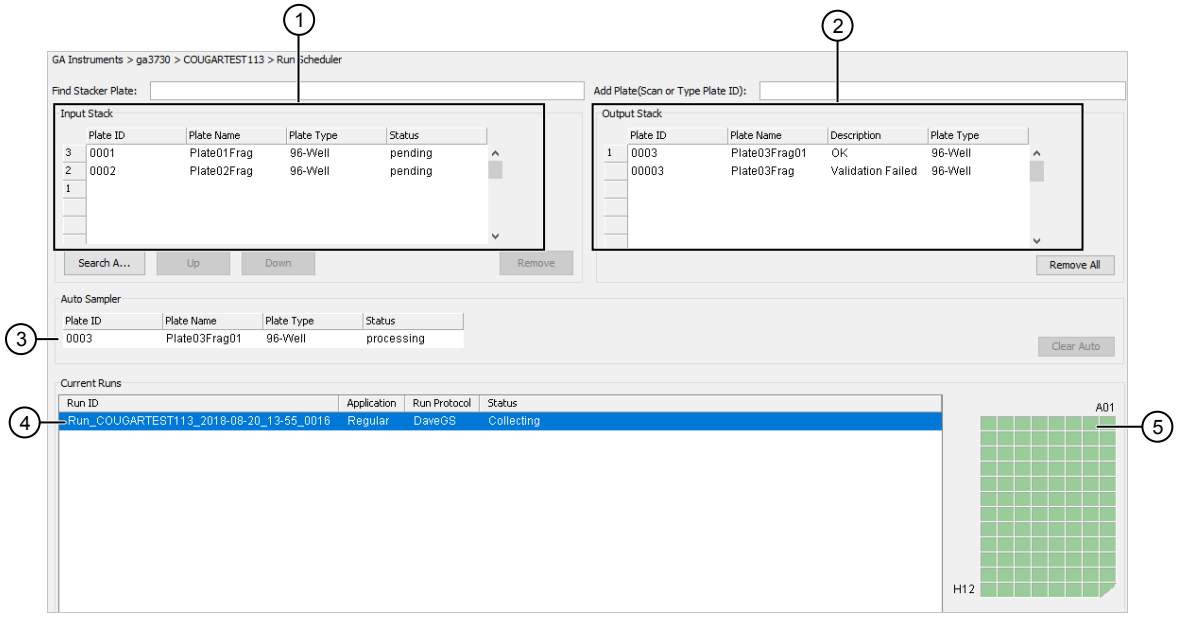
Controlling the run

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

To …	Click …
Start the run	
Stop the current run	
Stop after the current run	
Skip to next run (stops the current run and begins next scheduled run)	
Pause the current run	
Resume after pause (start the next scheduled run)	

Monitor the status of the run

1. Select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Run Scheduler**.



① **Input stack**—In manual run mode, lists the plate records for the plates to be run. In auto run mode, lists "Unknown".

② **Output stack**—Validated plates. Plates that pass validation are listed as **OK** and will be run. Plates that fail validation are listed as **Validation failed** and will not be run.

③ **Autosampler**—The plate currently in the autosampler. The runs for the current plate are listed under **Current Runs**.

④ **Current runs**—All injections set up for the plate in the plate record

⑤ Plate—The wells to be sampled when the selected injection is run are .

For more information, see “Default injection positions—48-capillary arrays” on page 37 and “Default injection positions—96-capillary arrays” on page 39.

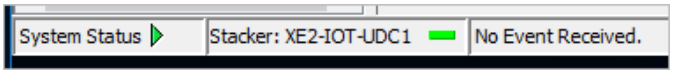
Status	Description
Input stack	
Completed	Run data for all injections in the plate record is added to the database and autoextraction of data files is starting.
Processed	Data files for all injections in the plate record are autoextracted from the database to the location specified in the results group. A processed plate cannot be run again. To run the plate again, add samples to the plate record. See “Add reinjections to a plate record” on page 66.
Pending	Plate is waiting to be validated. The software checks that the settings in the plate record match the settings on the instrument.
Output stack	
OK	Plate has passed validation and will be run.

Status	Description
Validation failed	Plate has been validated and will not be run. For more information, see the following sections. • "Display the Event Log" on page 135 • "Plate validation fails when you start a run" on page 137
Autosampler	
Processing	Data is being collected from the plate.
Current runs	
Collecting	Injection is in process.
Validated	Injection has been validated and will be run.

Table 5 Current Run fields

Field	Description
Run ID	The internal number assigned by the software
Application	Instrument protocol type selected in the instrument protocol
Run protocol	Instrument protocol name selected in the plate record

2. Monitor the **System status** indicator in bottom left of screen.
   - —Instrument is running
   - flashing—Check the **Event Log**.
     See "View the Event Log" on page 68 or "Display the Event Log" on page 135.
   - —Instrument has stopped



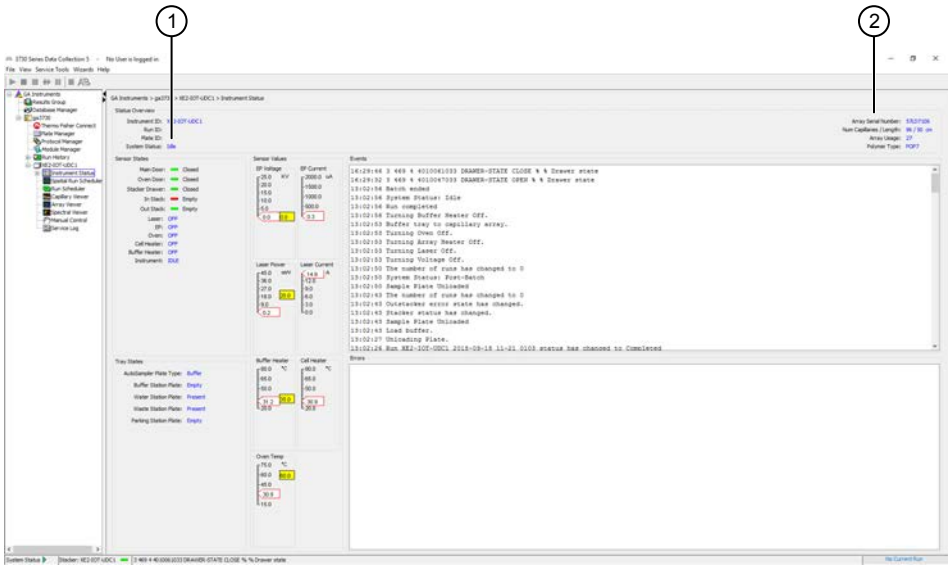
Add reinjections to a plate record

To add a reinjection, edit the plate record, then run the plate again.

1. In the navigation pane of the Data Collection Software, double-click **GA Instruments** ▸ **ga3730** ▸ **Plate Manager**.
2. Select a plate record, then click **Edit**.
3. Select **Edit** ▸ **Add Sample Run**.
   Columns for **Results Group 2** and **Instrument Protocol 2** are added to the plate record.
4. Specify the settings for the new injection.

Check instrument status

In the navigation pane of the Data Collection software, select **GA instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Instrument Status**.



1 System status
2 Capillary array information

Events box

Displays instrument and calibration information.

- 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
- Information for service engineers

Errors box

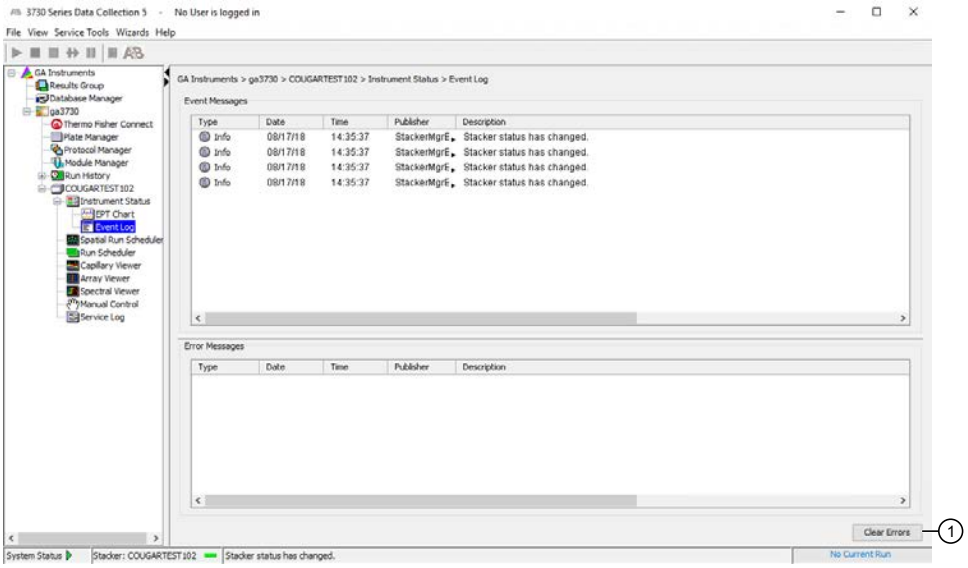
Displays errors that have occurred during the current run.

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

View the Event Log

Use the **Event Log** to view a record of instrument events and error messages.

1. In the navigation pane of the Data Collection Software, click **GA instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Instrument Status** ▸ **Event Log**.



① Clear Errors

2. To delete error messages, select all error messages, then click **Clear Errors**.
   Error messages are retained in the **Runviewer** event log files that are stored in
   The system status indicator flashes red until all errors are cleared.

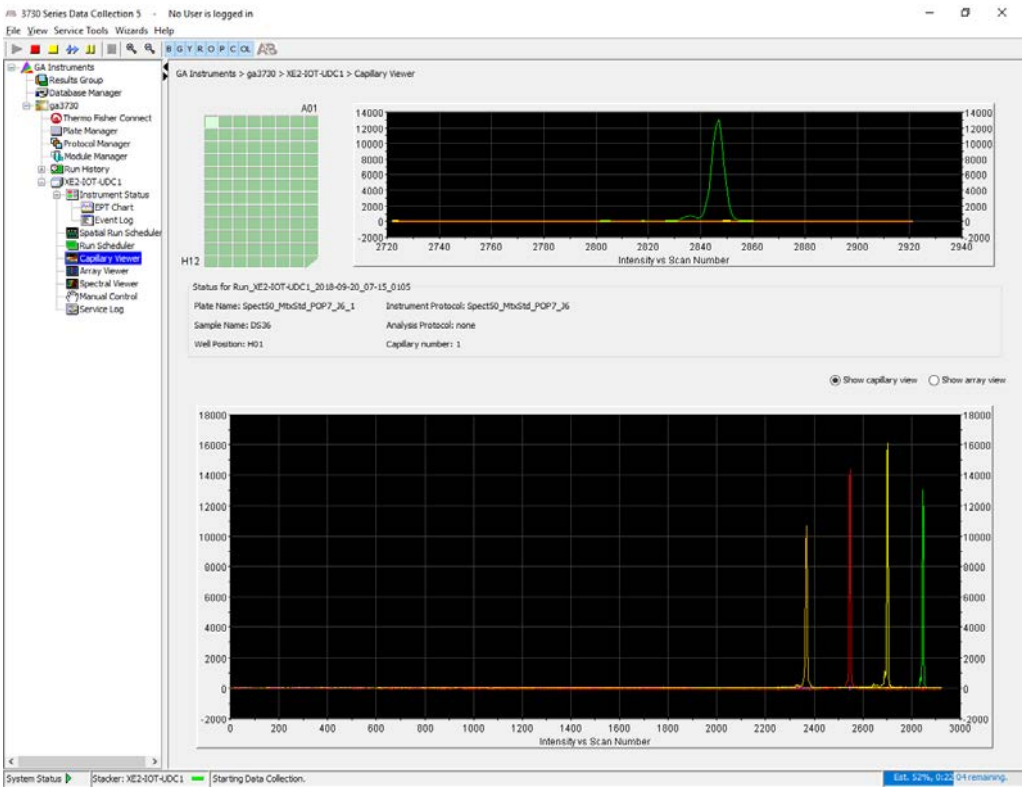
View data from the run

View electropherogram data

View 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*** ▸ **Capillary Viewer**.



Electropherogram plots

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

How to zoom

To zoom an area of an electropherogram:

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

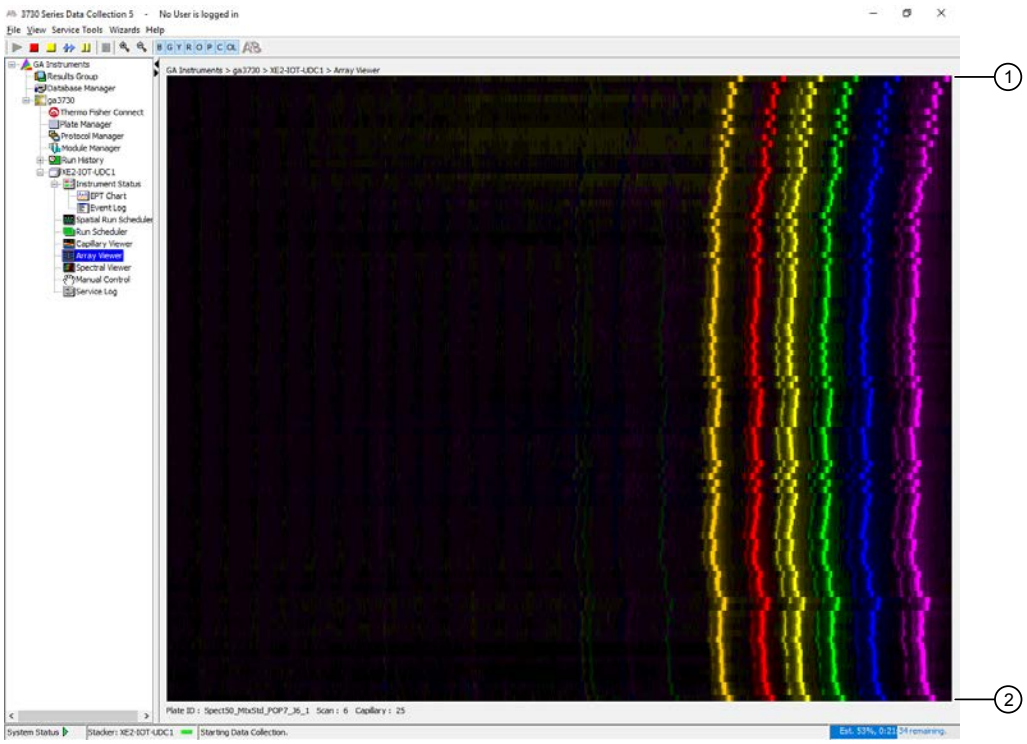
Display or hide color

Click individual colors in the color bar to view or hide the color in the Array View.

View data in the Array Viewer

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

In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Array Viewer**.



① Capillary 1
② Capillary 48 or 96

How to zoom

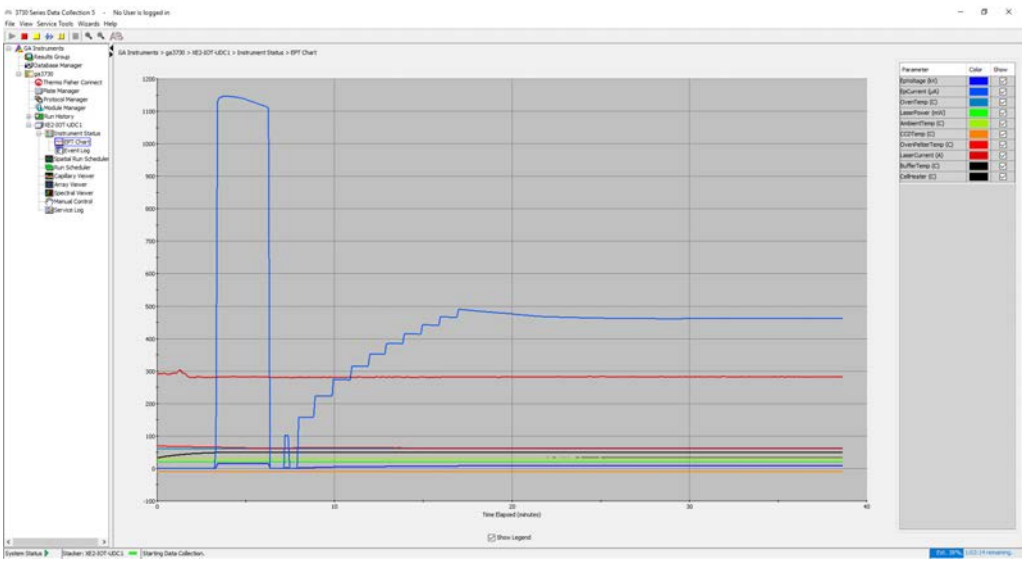
1. To expand the view, click-drag the mouse over the area of interest.
2. Click to return to full view.

Display or hide color

Click individual colors in the color bar to view or hide the color in the Array View.

View electrophoresis conditions (EPT)

In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Instrument Status** ▸ **EPT Chart**.



View data from a completed run (Run History)

You can view the injection run history for processed plates that are stored in the database.

Note: You cannot view run history for plates that have been deleted from the database using the **Database Manager**.

1. Select **GA Instruments** ▸ **ga3730** ▸ **Run History**.
2. Search for the plate record of interest.
   See “Search for a plate record” on page 77.
3. Select the run of interest, then select any of the icons that are listed below.

Run History views	Icon
EPT Viewer	
Spatial Calibration Viewer	
Capillary Viewer	
Array Viewer	
Spectral Calibration Viewer	
Reextraction	

Check the autoextraction status and manually re-extract

Autoextraction and re-extraction

After a run is complete, the data from each capillary is autoextracted from the database and a data file is created (AB1 files for sequence analysis, FSA files for fragment analysis).

Samples can be re-extracted with the same settings, or with different analysis protocols or results group.

You can re-extract data for several reasons.

- To extract data and create data files if autoextraction did not occur.

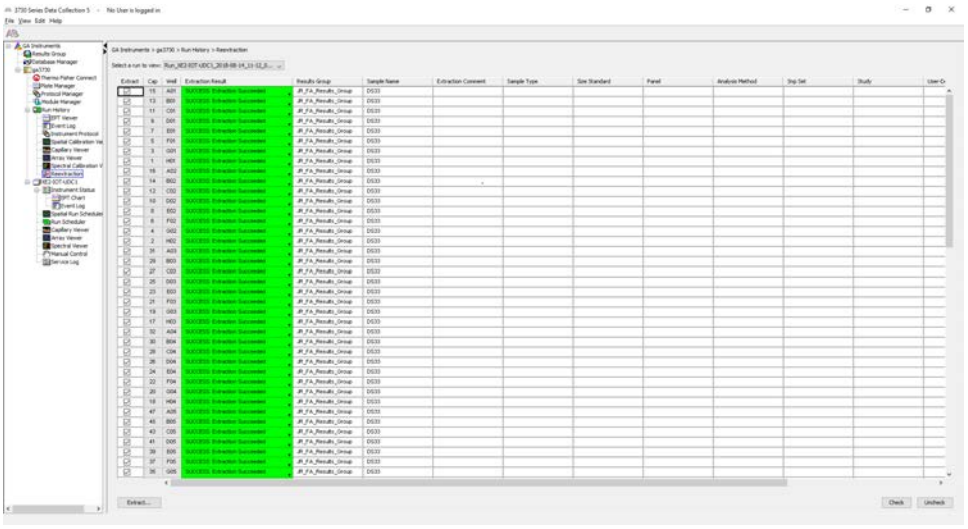
  Example: If a network destination location that is specified in the results group is not available, data is not autoextracted and data files are not created.
- To save data files with different names or in different locations than autoextracted files (extract with a different results group).
- To save data files with different analysis settings than autoextracted files.

Re-extract runs

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Run History**.
2. Search for the plate record of interest. See “Search for a plate record” on page 77.

   All completed runs from the plate are listed and can be reextracted. Pending runs from the plate are not listed.
3. Select a run.
4. Click (Reextraction) in the navigation pane.

   The **Reextraction** window opens. Click-drag the **Result** column to display messages.



Color	Value
Red	Extraction or analysis failed
Yellow	Warnings for extraction or analysis “FAILURE: Analysis Fail Bad Data; Error Number=nnnnn WARNING…"
Green	Successful extraction (with no analysis intended), or successful extraction and analysis.

(Sequence analysis only): The **Score** column represents the quality values for an entire sequence. Quality values are assigned only to analyzed samples when using the **KBBasecaller**. The **Score** column is blank if:

- Analysis was not performed
- Analysis failed
- **ABIBasecaller** was used for analysis. The **ABIBasecaller** does not assign a score.

5. Select the checkboxes in the **Extract** column for the samples to re-extract.

6. As needed, select different results group and other settings.

7. Click **Extract**.

   Reextraction creates a new sample file and does not replace the previously saved sample file. For example, if the first extracted file name is, “sample01.ab1” then the second first extracted file is assigned “sample01.**2**.ab1”.

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

6 Create and manage plate records and results groups

This chapter contains detailed information on plate records and results groups. For simple instructions on how to create a plate record or results group, see Chapter 4, “Create results groups and plate records using default settings”.

Overview of plate records

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

The first screen in a plate record specifies the following information.

- **Plate attributes**—Barcode or uniqu ID, name, owner, operator, number of wells, and sealing option.
- **Scheduling (injection order by plate quadrant)**—Allows you to specify the order of injection by quadrant on 384-well plates or 96-well plates (48-capillary array). For more information, see “Default injection order on plates” on page 59.

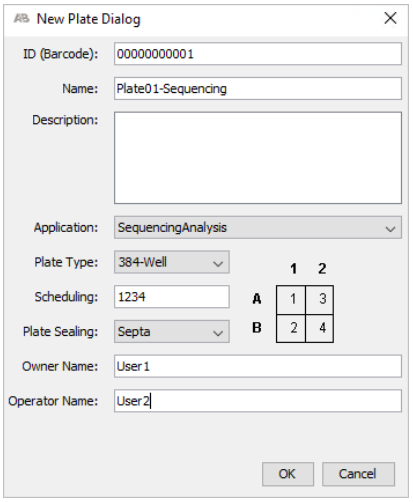


Figure 12 Plate attributes and injection order

- **Application**—The type of run the plate record will be used for. The options listed are determined by the secondary analysis software that is installed on the computer. The **Application** determines the required entries in the next screen of the plate record (see Table 6).

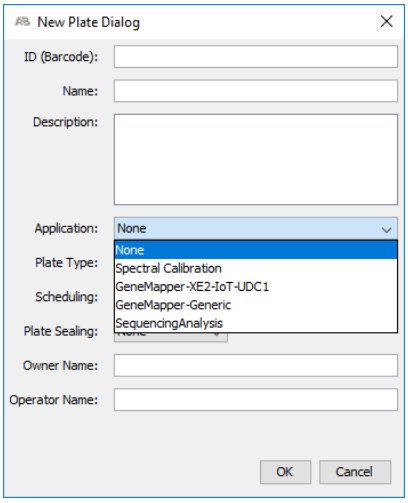


Figure 13 Application

The second screen in a plate record specifies the following information.

- **Sample information**—**Sample Name** and **Comment**.
- **Instrument Protocol**—Contains the settings needed to run the instrument. See “Create an instrument protocol” on page 86.
- **Results Group**—Contains the settings that determine sample file naming and storage location. See “Overview of results groups” on page 80.

The remaining fields that are listed are determined by the **Application** you select for the plate record. These fields are imported from the secondary analysis software associated with the plate record **Application**. All fields contain lists of available items that have been created in the secondary analysis software.

For information on these fields, see the user documentation or software help provided with the secondary analysis software.

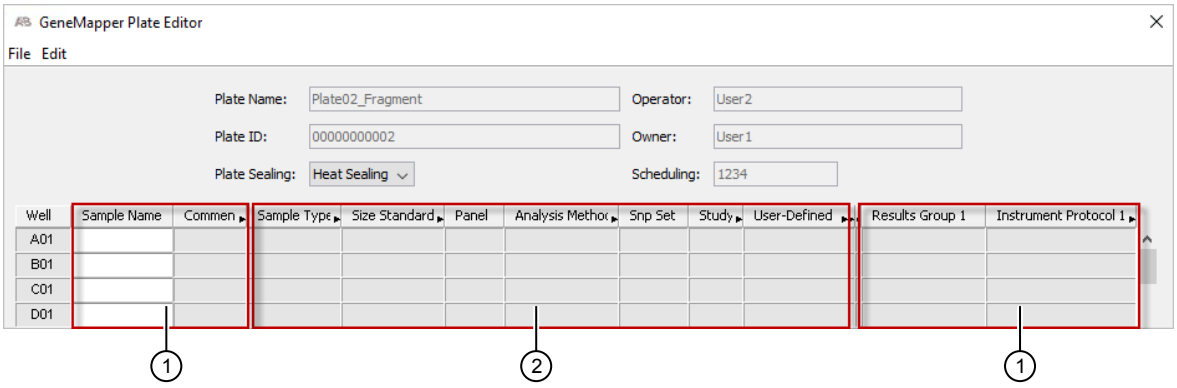


Figure 14 Plate record required fields

① Fields required by Data Collection Software
② Fields required by secondary analysis software (this example shows fields required by GeneMapper™ Software)

If this secondary analysis software is installed on the Data Collection computer	Then this Plate Record Application is available
GeneMapper™ Software 6	**GeneMapper-***Computer name* (the name of the Data Collection computer)
	GeneMapper-Generic Allows you to run fragment analysis and generate FSA files without analysis information.
Sequencing Analysis Software 7	**Sequencing Analysis**
SeqScape™ Software 4	**SeqScape-***Computer name* (the name of the Data Collection computer)

Table 6 Plate record Application and required fields

Sequencing Analysis	SeqScape-*Computer name*	GeneMapper-*Computer name*	GeneMapper-Generic
Analysis Protocol	• **Project** • **Project Template** • **Specimen** • **Analysis Protocol**	• **Size Standard** • **Panel** • **Analysis method** • **User Defined fields 1–3**	No required fields. All fields are optional text fields that you can fill in as needed.

Create and manage plate records

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

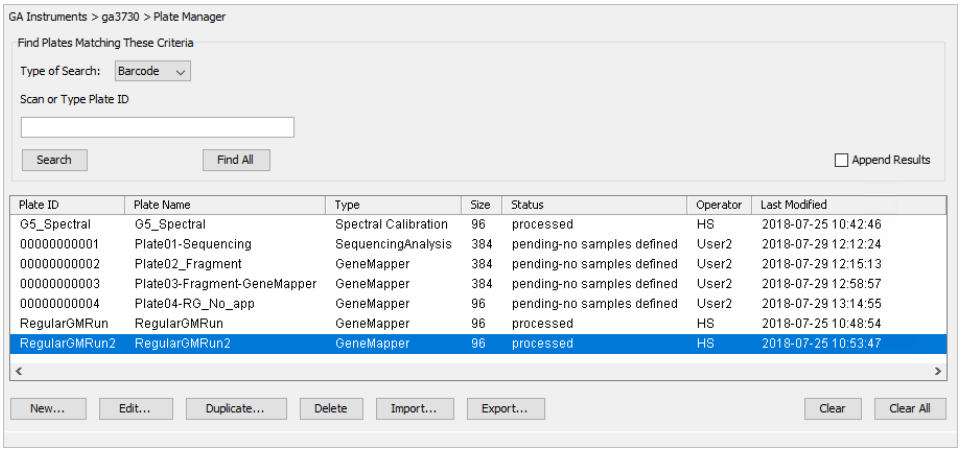
2. Use the following functions if needed.

Function	Description
Search	See “Search for a plate record” on page 77.
New	See “Create a plate record” on page 56.
Edit	Opens an existing plate record and allows changes.
Duplicate	Copies information from an existing plate record to a new plate record. **Note:** If any of the original fields are not valid, the plate record will be blank. For example, the results group is missing from the plate record.
Delete	Deletes a saved plate record.
Import	See “Import a plate record” on page 79.
Export	Saves a plate record as a TXT file in `E:\Users\`*`username`*`\Documents`.

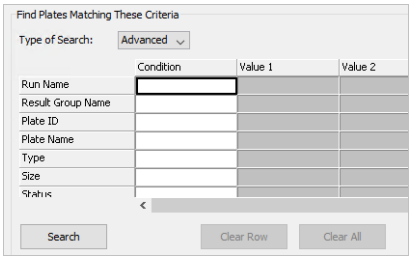
3. Add replicate injections with the same or different results group or instrument protocol: Select **Edit** ▸ **Add Sample Run**.
   Columns for **Results Group 2** and **Instrument Protocol 2** are added to the plate record.

Search for a plate record

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



2. Use the search functions if needed.

Function	Procedure
Show all plate records	Click **Find All**.
Find a plate by **Plate ID** (barcode)	Enter a **Plate ID**, then click **Search**.
Find a plate by plate record attributes. • **Results Group Name** • **Plate Name** • Application (**Type**) • Number of wells (**Size**) • Status (**Pending–no samples defined**, **Pending**, **Processing**, and **Processed**) • **Plate Owner** • **Instrument Operator** • **Date Last Modified**	1. From the **Type of Search** list, select **Advanced**.  2. Click the **Condition** field, then select the relevant search condition. The conditions that are displayed depends on the attribute that is selected. **=** (equal), **<** (less than), **>** (greater than), **Not Equal**, **Contains**, **Starts With**, **Ends With**, or **Between**. 3. In the **Value** field, enter the attributes of interest. (**Value 2** is active only when the **Between** search condition is selected.) 4. Click **Search**.
Add plate records to the search list	Select the **Append Results** checkbox.
Remove plate records from the search list	Click **Clear** or **Clear All**.

Import a plate record

To create a template for an import plate record file, export a plate. See “Create and manage plate records” on page 77.

Note: The **Container name**, **Plate ID**, and **Sample Names** need to be changed before you can use the file for import. You can use a spreadsheet program to create the import file, but you must save the final file as a tab-delimited text file (TXT).

IMPORTANT! If you specify an instrument protocol or results group name, the entry must exactly match a name that currently exists in the Data Collection Software, including capitalization. If entries do not match exactly, an error is displayed and the plate record is not imported.

---

1. In the **Plate Manager**, click **Import**, then navigate to the plate record TXT file to import.
2. Select the file of interest, then click **Open**.
   A progress screen opens. Click **OK** when the import is complete.

Overview of results groups

A results group determines how data is processed, how data files are named, and where data files are saved.

A results group specifies the following information.

- **Analysis Type** determines the analysis parameters that are applied to the data. The options that are listed are determined by the secondary analysis software that is installed on the computer (see Table 7).
- **Destination** in which to save files. Default location is:
  `E:\AppliedBiosystems\UDC\DataCollection\Data`
- **Naming** for data files. Default naming convention is:
  `<app prefix>_<capillary number>_<well position>`
  where `<app prefix>` is the analysis type you specify for the results group.
- **Naming** for run folders. Default structure and naming convention is:
  `<Instr Type>\<Instr Name>\<Run Name>` (This string is appended to the directory specified in the location to save files.)
  where `<Run Name>` is the plate record name.

Table 7 Results group Analysis Type and settings that are applied to or saved with the data

Sequencing Analysis	SeqScape-*Computer name*	GeneMapper-*Computer name*	GeneMapper-Generic
Analysis Protocol settings are applied to the data to determine ACGT sequence	Settings are saved with the data. • **Project** • **Project Template** • **Specimen** • **Analysis Protocol**	Settings are saved with the data. • **Size Standard** • **Panel** • **Analysis method** • **User Defined fields 1–3**	Text entries are saved with the data.

Create and manage results group

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **Results Group**.
2. Use the following functions if needed.

Function	Description
New	See “Create a results group (detailed procedure)” on page 81.
Edit	Opens an existing results group and allows changes.
Duplicate	Copies information from an existing results group to a new results group.
Delete	Deletes a saved results group.
Import	Imports an XML file.
Export	Saves as an XML file in `E:\Users\`*`username`*`\Documents`.

3. Click **OK**.

Create a results group (detailed procedure)

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **Results Group**.
2. Click **New**.
3. In the **General** tab, enter a **Results Group Name** and an optional owner and comment.
4. Click the **Analysis** tab, then select the **Analysis Type**.

   For more information, see “Overview of results groups” on page 80.

   For information on autoanalysis, see the documentation for the secondary analysis software.
5. Click the **Destination** tab, then use the default destination or define a new location for data storage.

   The destination specifies the location to which data files are autoextracted or manually extracted.

To use	Do this
Default location	No changes are needed. See “Overview of results groups” on page 80 for default storage location.
Custom location	1. Select the **Use Custom Location** checkbox, then click **Browse** and navigate to a location. 2. Click **Test** to ensure the location is accessible. **Note:** The maximum length for the complete path is 250 characters (includes sample name, run folder name, and destination path name).

Note: You cannot specify remote storage locations directly from this field. Before specifying a remote location, map the remote location to a local drive letter (Map Network Drive feature in the Windows™ operating system). You can specify a mapped drive as a destination.

IMPORTANT! Do not specify C: drive locations for data storage. C: drive is used for the operating system. The software does not perform pre-run disk space checks on C: drive.

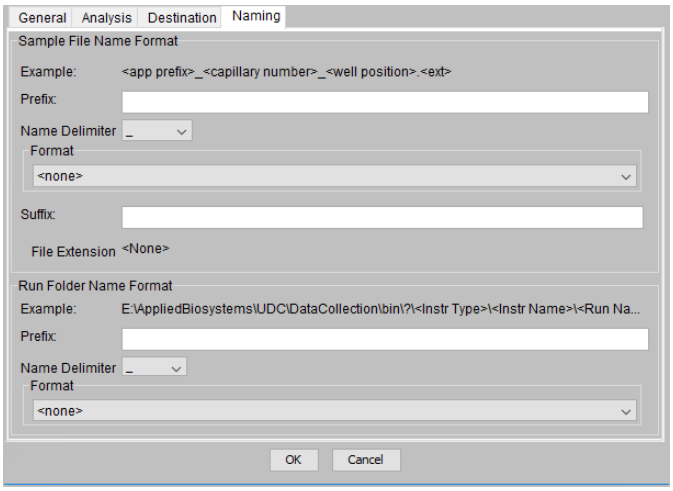
6. Click the **Naming** tab. You can use the default naming conventions for sample files and Results Group folders or define new conventions.

To use	Do this
Default naming convention	No changes are needed. See “Overview of results groups” on page 80 for default storage location.
Custom naming conventions	1. Select the **Use Custom Location** checkbox, then click **Browse** and navigate to a location. 2. Click **Test** to ensure the location is accessible.

Specify custom sample file naming in a results group

1. Create a results group as described in “Create a results group (detailed procedure)” on page 81.

2. Click the **Naming** tab.



3. Specify the following settings if needed.

IMPORTANT! The maximum length for the complete path is 250 characters (includes sample name, run folder name, and destination path name).

The software warns you if your selection exceeds the maximum, but allows you to save the file name convention.

Specify at least one unique identifier for **Sample File Name Format**. If you do not, the **Example** displays **INVALID NAME**.

Note: Even if you create a custom run folder location, a separate default run folder is generated that contains the log file. The log file can be used for troubleshooting.

Field	Description
Example	Interactively displays the attributes you select. The <ext> attribute is determined by the **Analysis Type** you select in the **Analysis** tab.
Prefix	Text you can type.
Delimiter	Symbols you can include in the file name: dash (-), dot (.), underscore (_), plus (+), dollar ($).

Field	Description
Format	Attributes you can add to the file name. • **Analysis Protocol Name** • **Capillary Array Serial Number** • **Capillary Number** (**Sample File Name Format** only) • **Date of Extraction** • **Date of Run** • **Instrument Name** • **Owner Name** (Owner of the run) • **Plate ID** • **Plate Name** • **Plate Quadrant** • **Polymer Name** • **Results Group Name** • **Run Name** • **Run Number** (generated by the software) • **Run Sequence Number** (generated by the software) • **Sample GUID** (**Sample File Name Format** only) • **Sample Name** (**Sample File Name Format** only) • **Time of Extraction** (**Sample File Name Format** only) • **Time of Run** (**Sample File Name Format** only) • **Unique Time Stamp Integer** (**Sample File Name Format** only) • **Subfolder Delimiter** (**Run File Name Format** only) • **User Name** (the user logged into the software if you are using the AB Navigator Software) • **Well Position** (**Sample File Name Format** only)

4. Click **OK**.

7 Create and manage instrument protocols, run modules, and analysis protocols

Create and manage instrument protocols

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Protocol Manager**.
2. Use the following functions if needed.

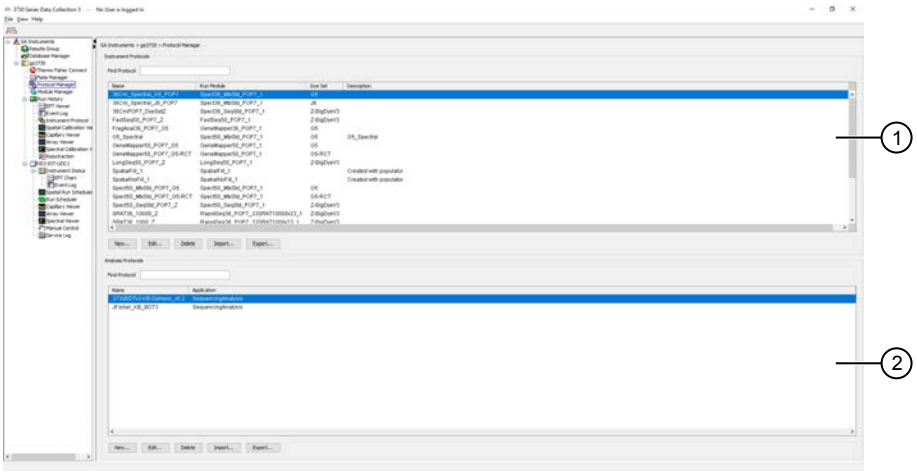
Function	Description
New	See “Create an instrument protocol” on page 86.
Edit	Opens an existing protocol and allows changes.
Duplicate	Copies information from an existing protocol to a new protocol.
Delete	Deletes a saved protocol.
Import	Imports an XML file.
Export	Saves as an XML file in `E:\Users\username\Documents`.

3. Click **OK**.

Create an instrument protocol

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

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



① Instrument protocols
② Analysis protocols

2. In the **Instruments Protocols** section, click **New**.
3. Enter a protocol name and optional description.
4. Select **Regular** in the **Type** dropdown list.
5. Select the run module and dye set for the protocol (see Appendix C, “Run modules”).

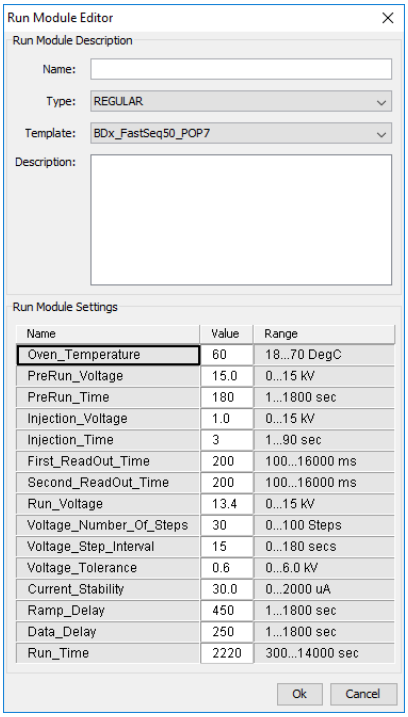
   Note: If you will use BigDye XTerminator™ Purification Kit for sequencing reaction clean-up, select the run modules marked as 'BDx'.
6. Click **OK**.

Create run module

A run module contains the settings that are necessary to run the instrument (protocol name, type of run, and dye set).

Note: You cannot edit a default module installed with the Data Collection Software.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Module Manager**.
2. Click **New**.
3. Enter a run module name and optional description.
4. Select **Regular** in the **Type** dropdown list.
5. In the **Template** dropdown list, select a template module.
6. Edit the settings as needed. See “Run module parameters” on page 88.
7. Click **OK**.



Run module parameters

Note: Not all parameters are editable in all run modules.

Parameter Name	Range	Comment
Oven_Temperature	18℃–70℃	Temperature setting for the main oven throughout the run.
Buffer_Temperature	30°C–35℃	Temperature setting for buffer heater throughout the run (improves data quality in fragment analysis and HID runs).
PreRun_Voltage	0–15 kV	Pre run voltage setting before sample injection.
PreRun Time	1–1,800 seconds	Prerun voltage time.
Injection_Voltage	0–15 kV	Injection voltage setting for sample injection.
Injection_Time	1–90 seconds	Sample injection time.
First_ReadOut_time	100–16,000 milliseconds	The interval of time for a data point to be produced. First_ReadOut_time should be equal to Second_ReadOut_time.
Second_ReadOut_Time	100–16,000 milliseconds	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[1]	0–100 steps	Number of voltage ramp steps to reach Run_Voltage.
Voltage_Step_Interval[1]	0–180 seconds	Dwell time at each voltage ramp step.
Temperature_Step	0.0–1.0 seconds	Dwell time at each temperature ramp step.
Voltage_Tolerance[1]	0.1–6 kV	Maximum allowed voltage variation.
Current_Stability[1]	0–2,000 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.
Ramp_Delay[1]	1–1,800 seconds	Delay During Voltage Ramp.
Data_Delay	1–1,800 seconds	Time from the start of separation to the start of sample data collection.
Run_Time	300–14,000 seconds[2]	Run time during which data is collected after Ramp_Delay.

[1] Do not change this value unless you are instructed to do so by Support.

[2] At temperatures of <20°C, contiguous read lengths or fragment analysis reads may be shorter. In those cases, you can choose to extend the run time. Increase the run time in increments of 300 seconds to find the optimum setting.

Overview of analysis protocols (sequence analysis)

An analysis protocol contains all the settings that are necessary for sequence analysis with Sequencing Analysis Software or SeqScape™ Software.

- **Basecalling settings**—The basecaller, DyeSet file, and analysis stop point to be used.
- **Mixed Bases**—(*Optional*) Mixed base identification and 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 or SeqScape™ Software, it will be visible as an option in the plate record for that field.

Create and manage analysis protocols

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Protocol Manager**.
2. Use the following functions if needed.

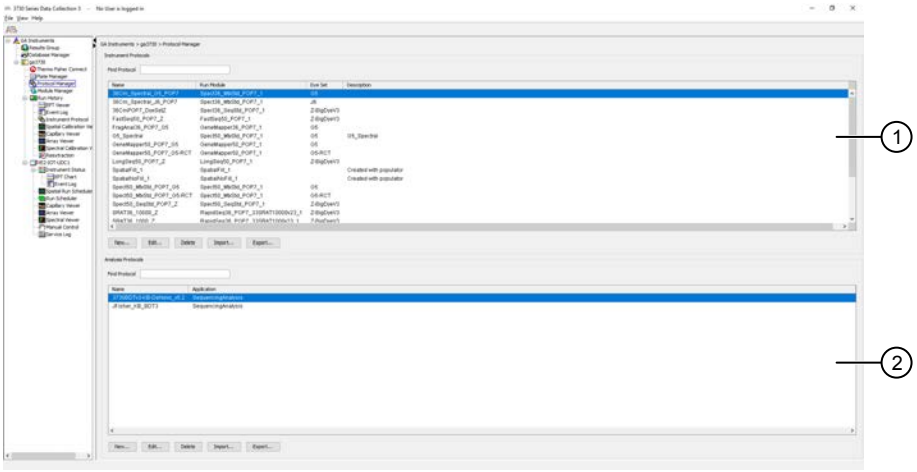
Function	Description
New	See “Create an analysis protocol” on page 90.
Edit	Opens an existing protocol and allows changes.
Duplicate	Copies information from an existing protocol to a new protocol.
Delete	Deletes a saved protocol. **IMPORTANT!** Do not delete the analysis protocol during a run. If deleted, autoanalysis by Sequencing Analysis Software or SeqScape™ Software will not be performed.
Import	Imports an XML file.
Export	Saves as an XML file in `E:\Users\username\Documents`.

3. Click **OK**.

Create an analysis protocol

An analysis protocol contains all the settings necessary to analyze sequencing data.

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



① **Instrument Protocols**
② **Analysis Protocols**

2. Click **New** in the **Analysis Protocols** pane.
3. Edit the settings as needed. See “Analysis protocol parameters” on page 90.
4. Click **OK**.

Analysis protocol parameters

Option	If checked, the software creates…
Sequence File Formats	• **Write SEQ File**—A SEQ file for printing the sequence as text file or for using the file in other software. – ABI format that is used with Applied Biosystems™ software. – FASTA format that 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. 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.
Basecaller and **Dye Set**	Select the appropriate Basecaller and DyeSet/Primer based on the chemistry and capillary array length you are using.

Option	If checked, the software creates…
Processed Data	Determines scaling of the processed traces. This parameter does not affect the accuracy of the basecalling. • **True Profile**—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. The profile of the processed traces will be very similar to that of the raw traces. • **Flat Profile**—The processed traces are scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces will be flat on an intermediate scale (> about 40 bases).
Ending base	Last base on which to perform basecalling: • **At PCR Stop** • **After X number of Bases** • **After X number of Ns in X number of Bases** • **After X number of Ns** **Note:** If you have PCR products with sequences that end while data is still being collected, select the **At PCR Stop** check box.
Quality Threshold	• **Do not assign N's to Basecalls** —When using the KB™ basecaller, use this option to assign a base to every position, as well as the QV. • **Assign N's to Basecalls 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.
Mixed Bases	**Note:** This function is active with the KB™ Basecaller only. When enabled, determines the secondary peak height ratio where the secondary peak is considered a potential mixed base. Reaching the threshold is a necessary but not sufficient condition for the basecalling algorithm to call a mixed base.
Clear range methods	• **Use clear range minimum and maximum**—Specifies the first and last base in the range to consider, or trims the specified number of bases from the 3′ end. • **Use quality values**—Sets a window with a specified number of allowed low-quality bases by removing bases until there are < X number of bases per Z number of bases with QV < Y. • **Use identification of N cells**—Sets a window with a specified number of allowed ambiguous base calls (Ns) by removing bases until there are < X number of Ns per Y number of bases.

8 Calibrate the instrument

Perform spatial calibration

Spatial calibration description and run time

The software uses images collected during spatial calibration to establish a relationship between the signal emitted by each capillary and the position where it is detected by the CCD camera.

Estimated run time is <5 minutes.

When to perform a spatial calibration

For all dye sets, perform a spatial calibration after you perform any of the following actions.

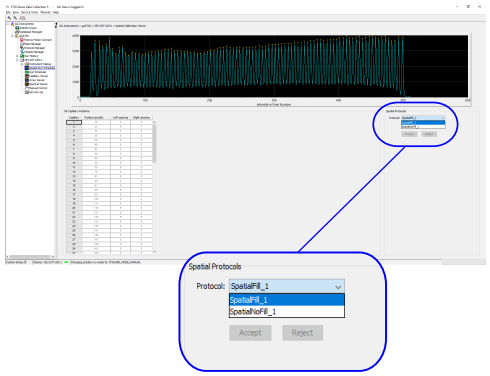
- Change or replace the capillary array
- Remove the capillary array from the detection cell block (even to adjust it)
- Move the instrument

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

Perform spatial calibration

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Spatial Run Scheduler**.
2. In the **Spatial Run Scheduler** view, select one of the following options
   - **Protocol** ▸ **SpatialNoFill** if the capillaries contain fresh polymer.
   - **Protocol** ▸ **SpatialFill**.

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



3. Click **Start**.
4. Evaluate the calibration. See “Evaluate the spatial calibration data” on page 93.

Evaluate the spatial calibration data

1. Examine the peaks of the spatial calibration.

   A profile is acceptable if the peaks meet the following conditions.

   - Peaks in the profile are approximately the same height.
   - The peak height increases at the beginning and the end of the spatial profile.

   Note: If you are using a 96-capillary array, a small peak may appear in the left side of the profile. The peak is normal.

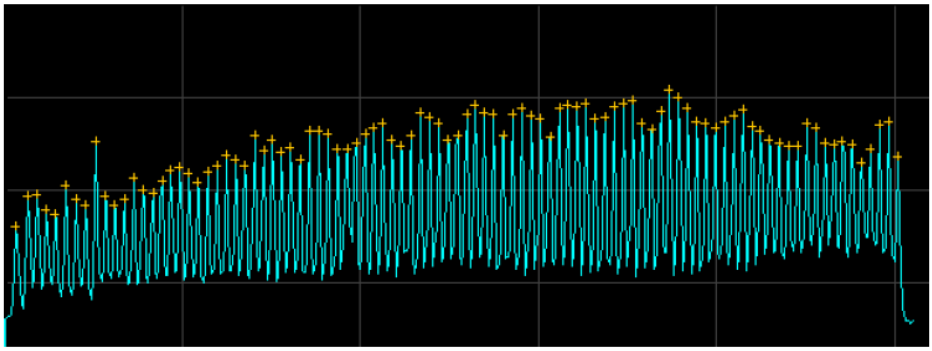


Figure 15 Passing spectral profiles

For additional information, see “Examples of passing and failing spatial profiles” on page 95.

A profile is not acceptable if the peak heights are irregular. See “Spatial calibration troubleshooting” on page 139.

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

   Expand the profile if needed. See “Magnify the spatial profile” on page 97.

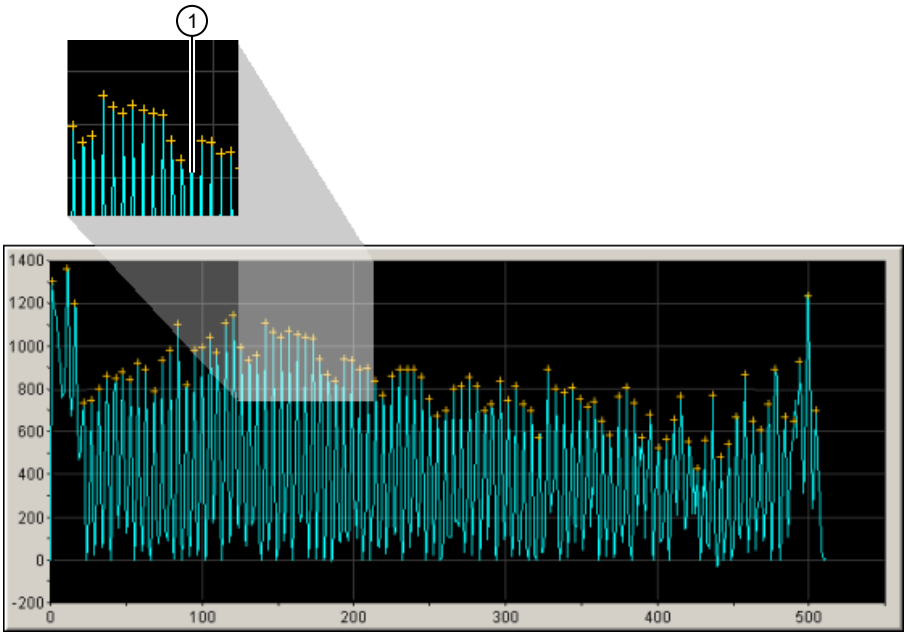


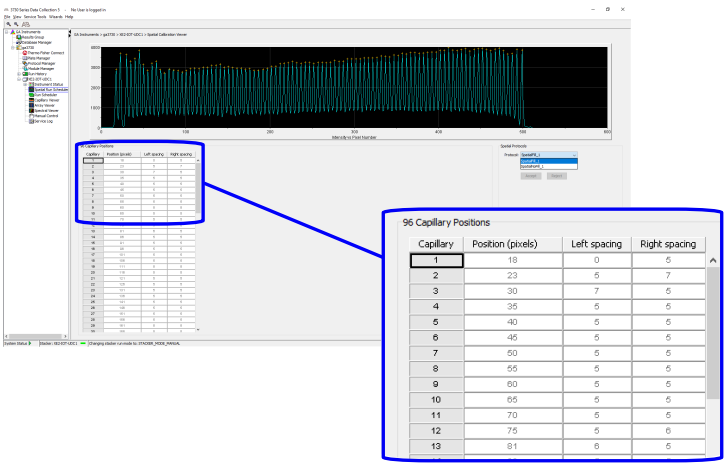
Figure 16 Peak that does not have orange cross

① No orange cross

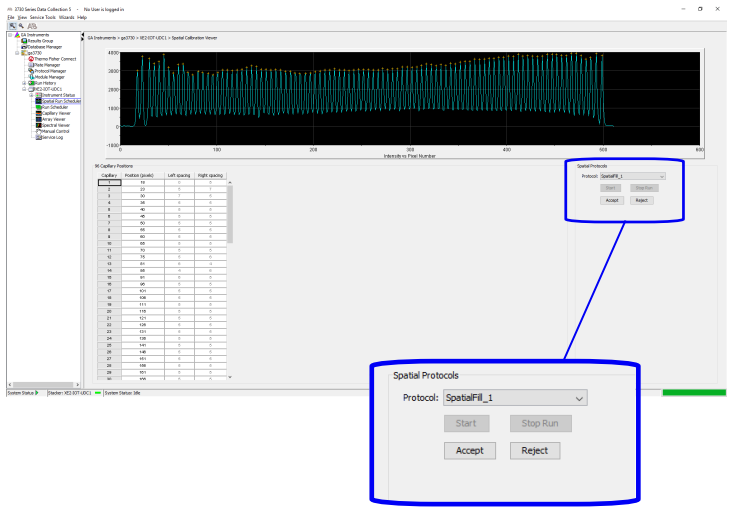
3. Examine each row of the 96 Capillary Position table. Typical values for the **Left spacing** and **Right spacing** columns are:
   - 4–8 pixels for a 96-capillary array
   - 9–11 pixels for a 48-capillary array

   Note: Higher values are acceptable if a corresponding gap in the capillaries in the detection cell are visible.

   Examine every position for the capillary array.



4. Click **Accept** or **Reject**.



Examples of passing and failing spatial profiles

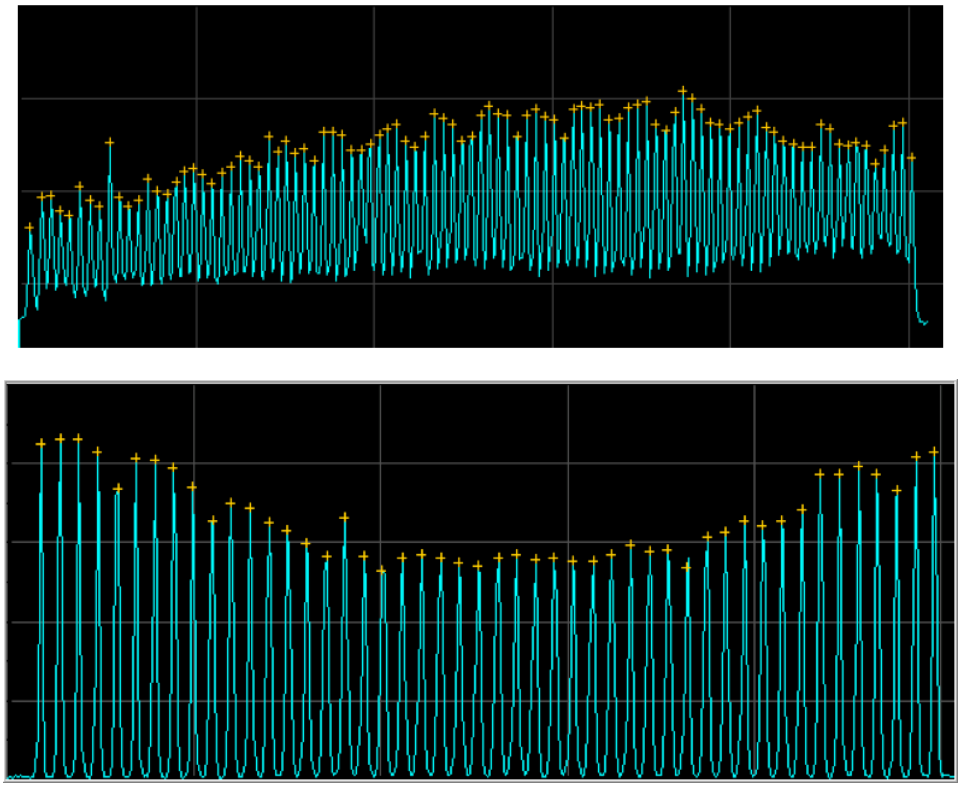


Figure 17 Typical passing profile

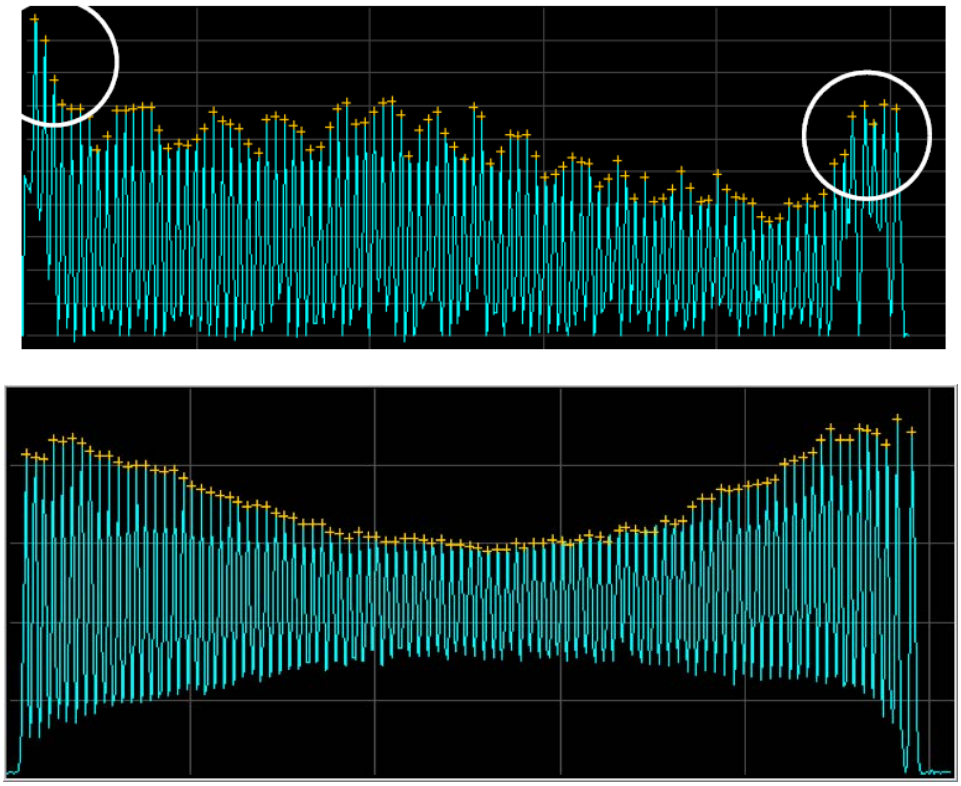


Figure 18 Passing profiles with higher peak heights at the beginning and the end of the profile

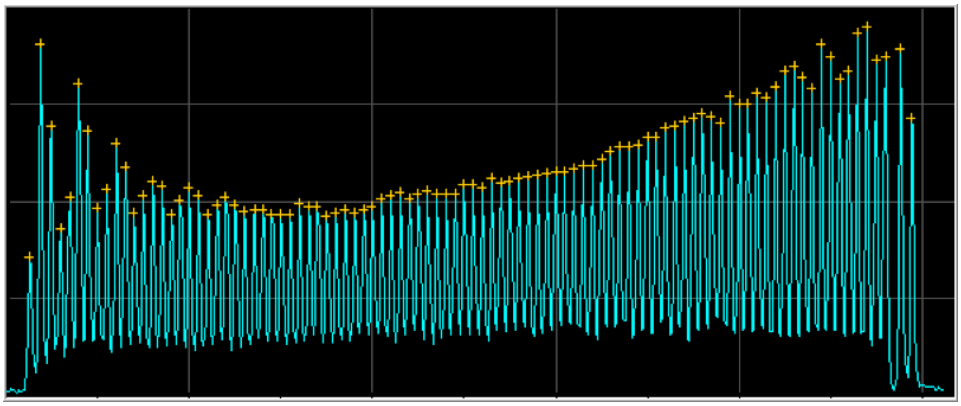


Figure 19 Passing profile with low signal at the beginning of the profile

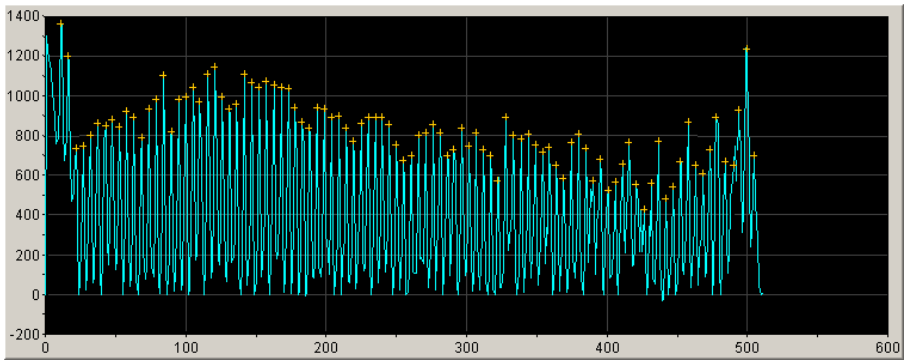


Figure 20 Passing profile with high background at the beginning of the profile

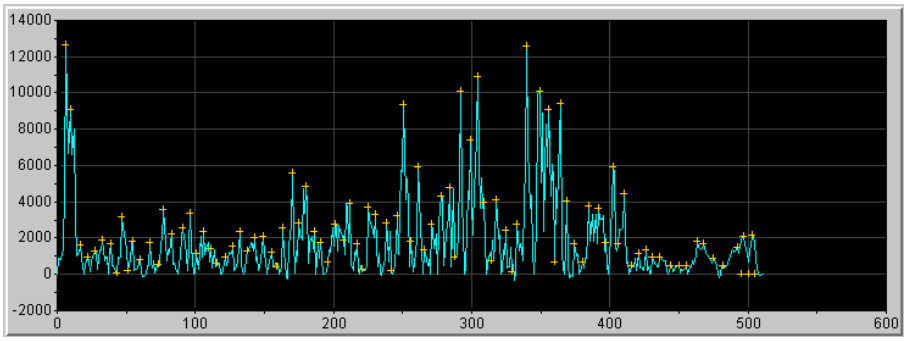
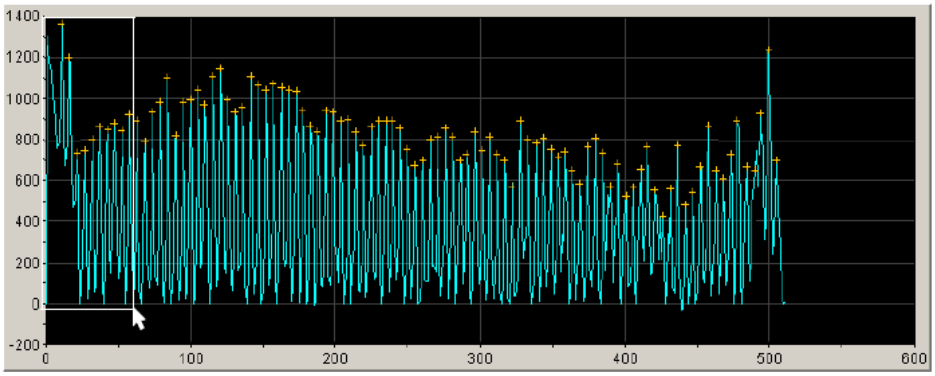
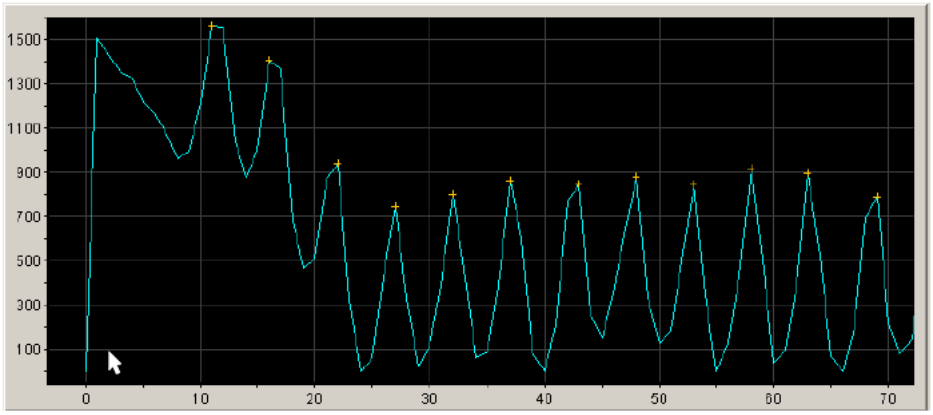


Figure 21 Failing spectral profile

Magnify the spatial profile

1. Click-drag the cursor to create a box around the area of interest.
2. Release the mouse button.





Perform spectral calibration for sequencing and fragment analysis

Spectral calibration definition and run time

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 that is stored in the sample files.

Note: Preheating the oven via manual control can reduce run time and allow system consumables to equilibrate. This results in spectral calibrations with fewer capillary failures, and improved sequencing and fragment analysis performance for the first injection run of the day.

Application	Standard	Polymer	Capillary array length	Run module	Run Time (min) [1]
Sequencing	Sequencing standard	POP-7™	36 cm	Spect36_SeqStd_POP7	60
			50 cm	Spect50_SeqStd_POP7	115
		POP-6™	36 cm	Spect36_SeqStd_POP6	65
			50 cm	Spect50_SeqStd_POP6	145
Fragment analysis	Matrix standard	POP-7™	36 cm	Spect36_MtxStd_POP7	50
			50 cm	Spect50_MtxStd_POP7	60

[1] The software may take up to 30 minutes to calculate the matrices after the run is complete.

When to perform the calibration

- If you see a decrease in spectral separation (pull-up and/or pull-down peaks)
- For each dye set–capillary array type–capillary array length combination
- After the laser or CCD camera has been realigned/replaced by a service engineer
- Change or replace the capillary array
- Remove the capillary array from the detection cell block (even to adjust it)
- If you alter any condition (dye set, array type, array length, or polymer type)

Supported dye sets and calibration standards

Table 8 Sequencing dye sets and standards

Kit used for sequencing	Dye Set	Calibration Standards
BigDye™ Terminator v3.1 Cycle Sequencing Kit	Z_BigDyeV3	3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v3.1
BigDye™ Direct Cycle Sequencing Kit	Z_BigDyeV3	
BigDye™ Terminator v1.1 Cycle Sequencing Kit	E_BigDyeV1	3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v1.1

Table 9 Fragment analysis dye sets and matrix standards

Dye Set	Calibration Standards
D	DS-30 or DS-31
G5	DS-33
G5-RCT (reduced cross-talk)[1]	
J6	DS-36
Any4Dye, Any5Dye	Custom
Any4Dye-HDR (high dynamic range)[2, 3]	Custom
Any5Dye	DS-02 for SNaPshot™ applications Custom

[1] Use for fragment analysis applications on 96-capillary arrays.

[2] 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

[3] When using this dye set, perform spectral calibrations each time the capillary array is replaced or moved within the detection cell

Required materials

- 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

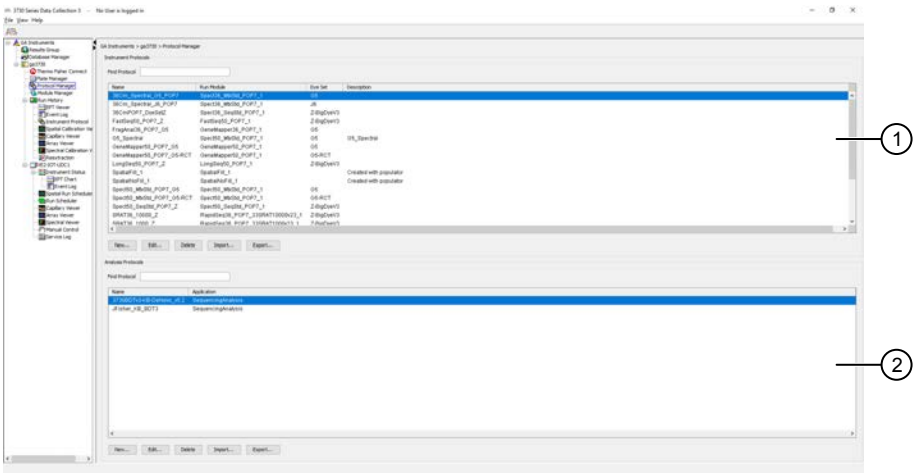
- Vortex
- Gloves

Prepare the spectral calibration standard

1. Prepare the matrix or sequencing standard appropriate for your application as described in the product insert. See Appendix E, “Catalog numbers” for catalog numbers.
2. Dispense the calibration chemistry into the wells specified for the plate record you create (see “Create a spectral calibration plate” on page 101).
3. Seal and assemble the plate (see “Seal and assemble plates” on page 41).

Create a spectral instrument protocol

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



① Instrument protocols
② Analysis protocols

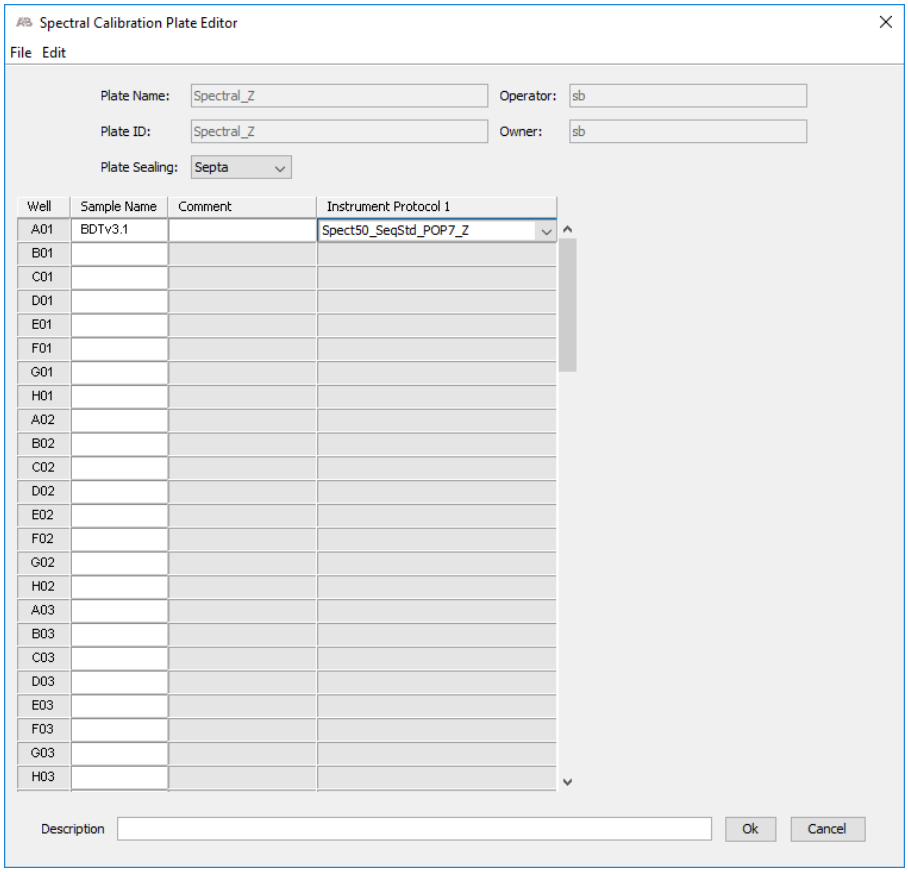
2. In the **Instrument Protocols** pane, click **New**.
3. Select **Spectral** from the **Run Module** dropdown list.
4. Select the dye set, polymer, and array length.
5. Select the run module and the chemistry.

Calibration type	Run Module for the installed capillary length	Chemistry
Matrix	Spect36_MtxStd_1 Spect50_MtxStd_POP-7™_1	Matrix Standard
Sequencing	Spect36_SeqStd_1 Spect50_SeqStd	Sequence Standard

Note: Do not change settings in the **Edit Parameters** dialog box.

Create a spectral calibration plate

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ **Plate Manager**.
2. At the bottom of the screen, click **New**.
3. Enter plate record information, then select **Spectral Calibration** in the **Application** dropdown list.
4. In row A01, enter a sample name, click the next cell, then enter a comment if needed.
5. In column 1 of row A01, select the instrument protocol you created for the spectral calibration.



6. Fill in the remaining rows by manually entering and selecting information, by using **Fill Down** commands, or by copy/pasting.

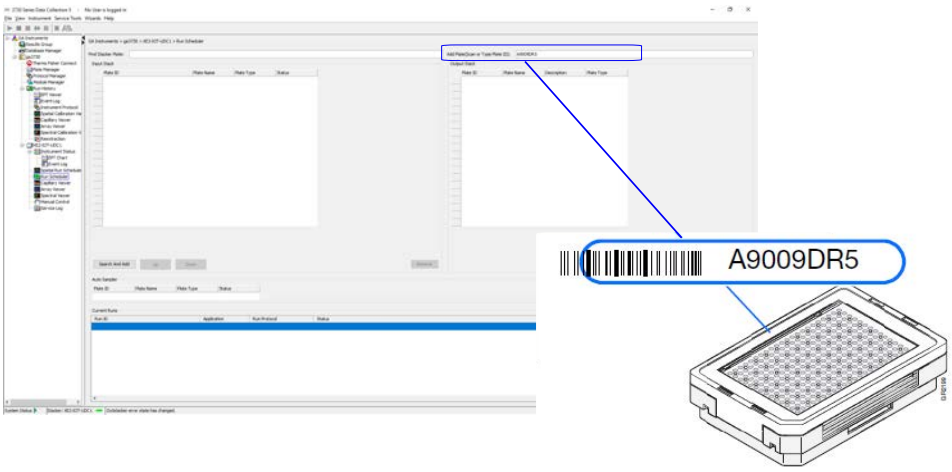
Fill Down	Copy/paste
Select the entire row, select **Edit** ▸ **Fill Down Special**, then select a fill down option. For 48-capillary instruments, select **Fill down Special (48 Cap)**. For 96-capillary instruments: • 96-well plate: Select **Fill Down**. • 384-well plate: **Fill down Special (96 Cap)**.	• If you copy multiple cells, select the same number of corresponding target cells before you paste. • You can copy/paste within one protocol. **Note:** Use the **Duplicate** function in the **Plate Manager** to copy information from an existing protocol to a new protocol.

7. Click **OK**.

Run the spectral calibration plate

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Run Scheduler**.

2. In the **Add Plate** field, type the **Plate ID** then press **Enter** or scan the bar code of a plate to add it to the input stack.



3. Place the plate assembly into the instrument. See “Place plate assemblies into the instrument” on page 45.

4. In the toolbar of the **Data Collection Software** window, click . Click **OK** in the **Processing Plates** dialog box.

You can view the capillary-by-capillary processing status during a spectral calibration run in the **Event Log**. See “View the Event Log” on page 68.

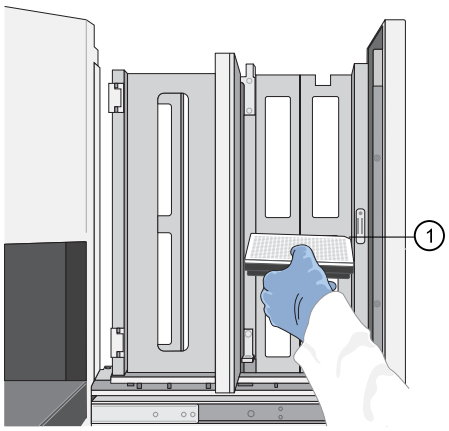
5. When the run is finished, remove the plate from the instrument.

Place 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. Ensure that the notched corner of the plate assembly is at the rear right corner of the stacker. The bottom plate assembly is run first.

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



① Notched corner

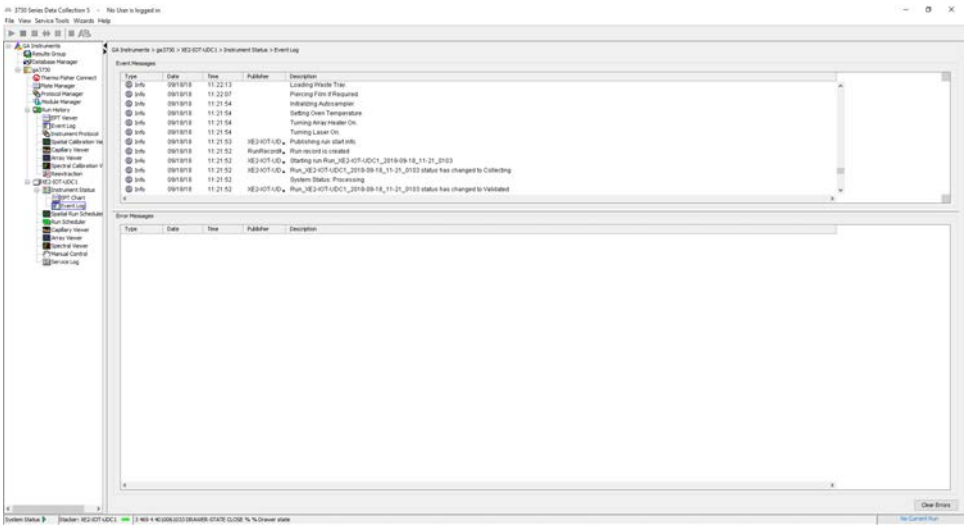
4. Close the metal In Stack tower door.

5. Close the Stacker drawer.

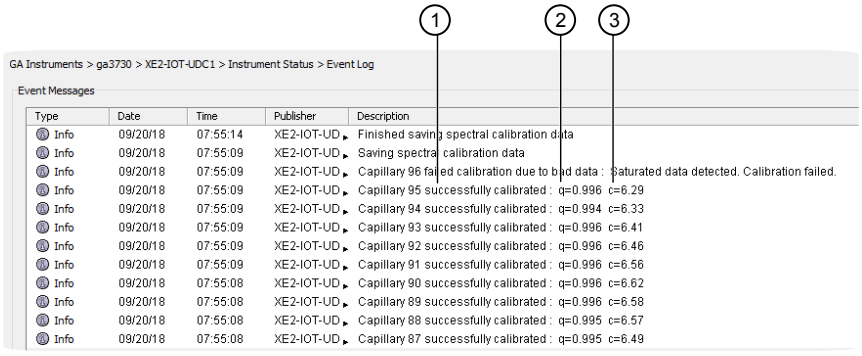
View the Pass/Fail status after the run

After the instrument completes the spectral calibration run, the pass or fail status of each capillary is listed in the Event Log.

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



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



① Pass/fail status
② q value (Spectral Quality Value)
③ c value (Condition Number)

Spectral Quality Value

A spectral Quality Value reflects the confidence that the individual dye emission signals can be separated from the overall measured fluorescence signal. It is a measure of the consistency between the final matrix and the data from which it was computed. A Quality Value of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.

In rare cases, a high Quality Value can be computed for a poor matrix. This can happen if the matrix standard contains artifacts, leading to the creation of one or more extra peaks. The extra peaks cause the true dye peak to be missed by the algorithm, and can lead to a higher Quality Value than would be computed with the correct peak. Therefore, it is important to visually inspect the spectral calibration profile for each capillary.

Condition Number

A Condition Number indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set.

If there is no overlap in a dye set, the Condition Number is 1.0 (ideal conditions), the lowest possible value. The condition number increases with increasing peak overlap.

Quality (Q) value and condition number passing ranges

Dye Set	Quality Value minimum	Default Condition Number Range
Z_BigDyeV3	>0.93	1.0–4.5
D	>0.95	1.0–6.5
E_BigDyeV1		1.0–5.0
G5		1.0–13.5
G5-RCT		
J6	>0.8	1.0–8.0
Any4Dye		1.0–20.0
Any4Dye-HDR		
Any5Dye		

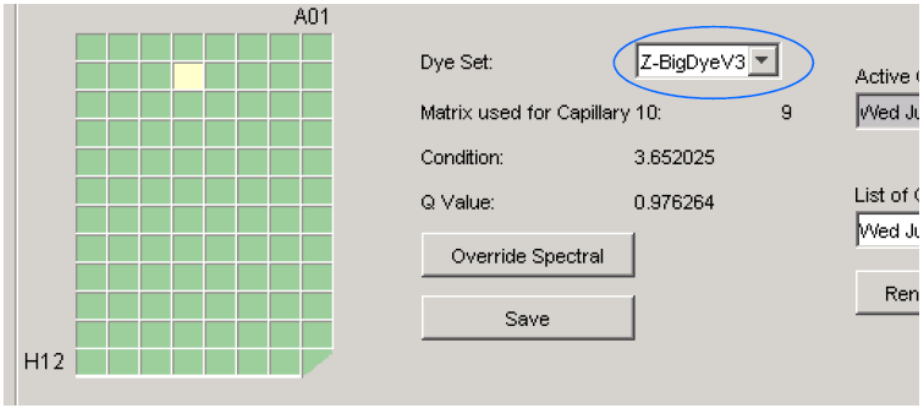
Evaluate the spectral calibration data

IMPORTANT! Review and evaluate the spectral calibration profile for each capillary, even if the Q value and Condition numbers are within range.

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

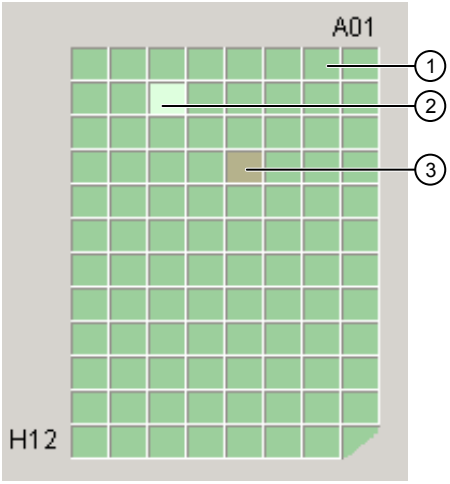
① Spectral profile
② Raw data (matrix standards)
③ Rename or set the active calibration

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



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

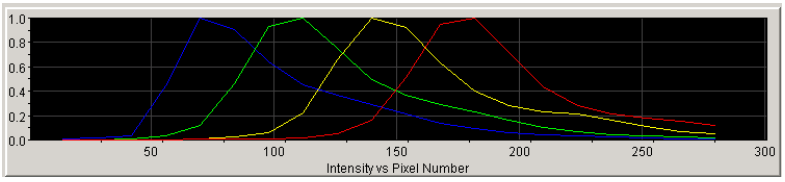
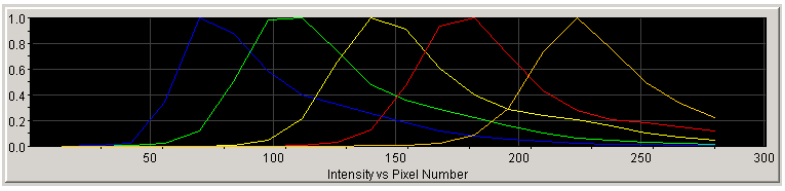
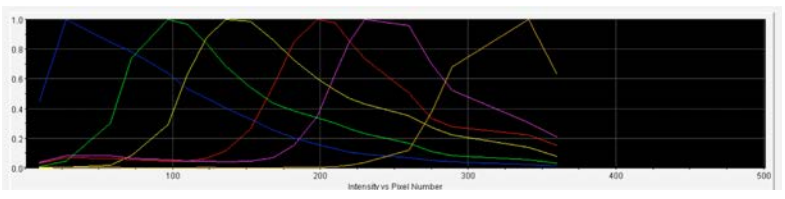
   Status— Passed (dark green), Selected (light green), Borrowed/failed (tan). If you override a capillary, the status is set to .

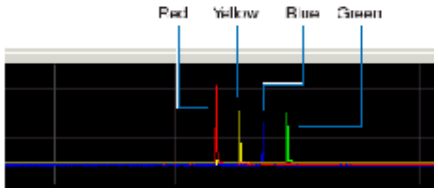
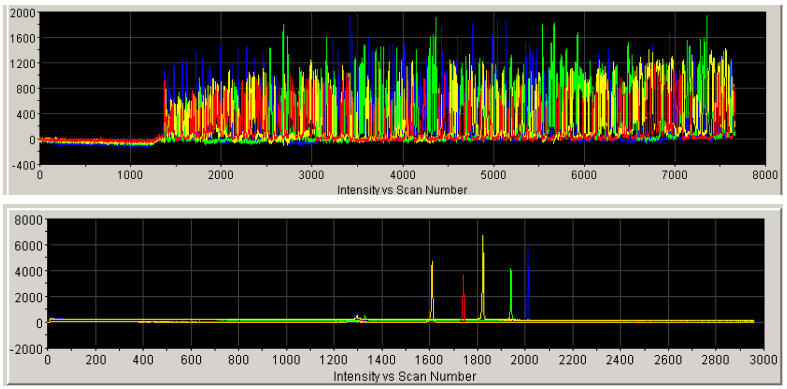
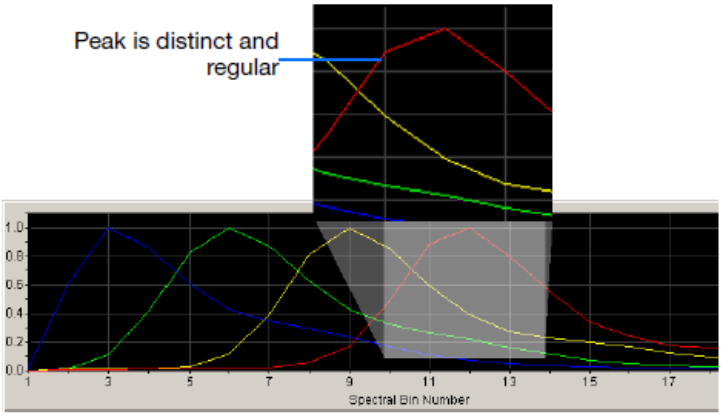
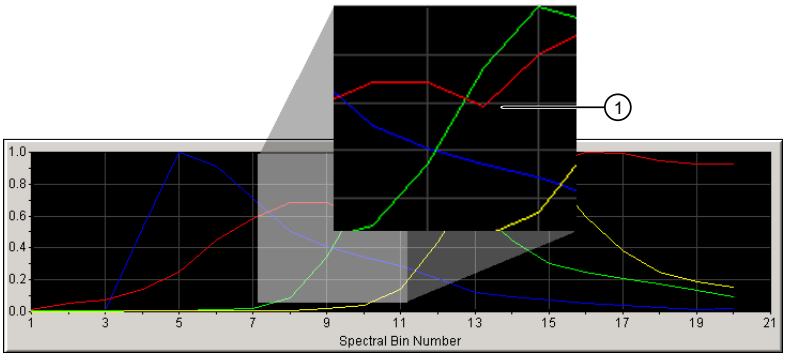


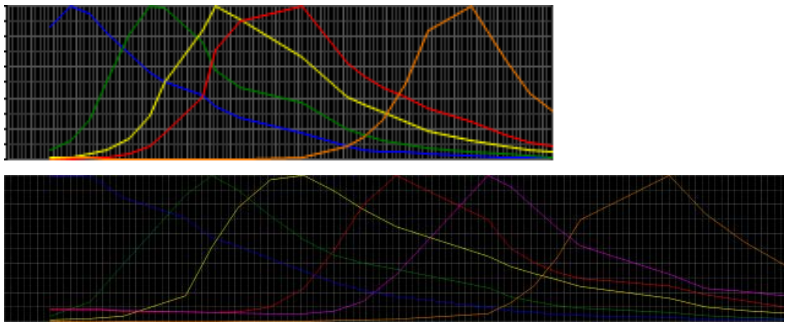
① Well A01—Passed
② Well B06—Selected
③ Well D04—Borrowed, failed, or overridden

4. If necessary, borrow calibration data from another capillary:
   a. Select a failed capillary.
   a. Click **Override**.
   b. Select a passing capillary from which to borrow the calibration.
5. Evaluate the spectral calibration profile for each capillary. Ensure that the profile meets the criteria listed in the following table.

   See also“Magnify the spatial profile” on page 97.

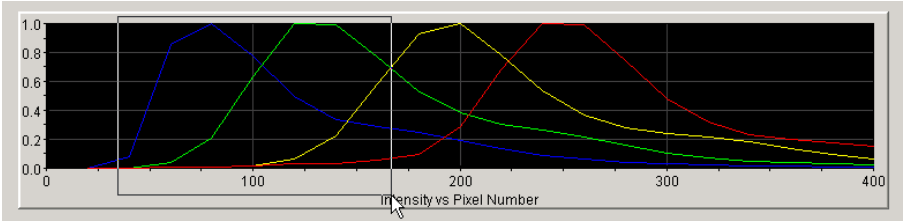
Attribute	Acceptance criteria	Example
Order of the peaks in the spectral profile (intensity vs pixel) from left to right	4-dye: blue-green-yellow-red	
	5-dye: blue-green-yellow-red-orange	
	6-dye: blue-green-yellow-red-purple-orange	

Attribute	Acceptance criteria	Example
Order of the peaks in the raw data profile from left to right	Sequencing (matrix standard only): 4-dye: red-yellow-blue-green	
Order of the peaks in the raw data profile from left to right	Fragment analysis: • 4-dye: red-yellow-green-blue • 5-dye: orange-red-yellow-green-blue	
Extraneous peaks in the raw data profile (intensity vs scan)	None **Note:** The E5 profile may include extraneous peaks outside the matrix peak region, which can be ignored.	
Peak morphology in the spectral profile (intensity vs pixel)	• No gross overlaps, dips, or other irregularities • Peaks separate and distinct • Peak apexes are separate and distinct (the tails will overlap)	 ① Dip

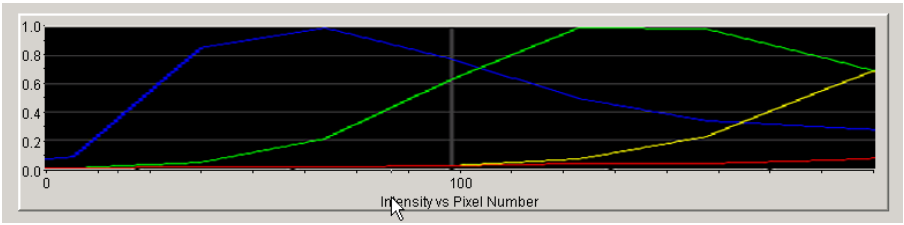
Attribute	Acceptance criteria	Example
	Note: The peak morphology of G5 (shown to the right, top), F, and J6 (shown to the right, bottom) may not be as rounded and symmetrical as the peak morphology for other dye sets (shown above) due to the effect of variable binning (a feature that reduces signal variation between dyes of different fluorescent efficiencies).	

Magnify the spectral profile

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



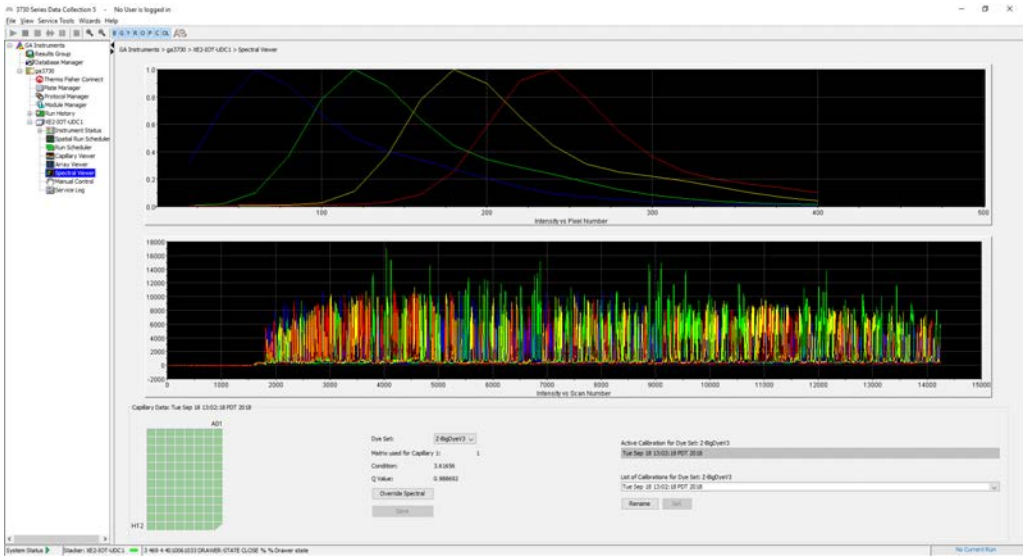
2. Release the mouse button.



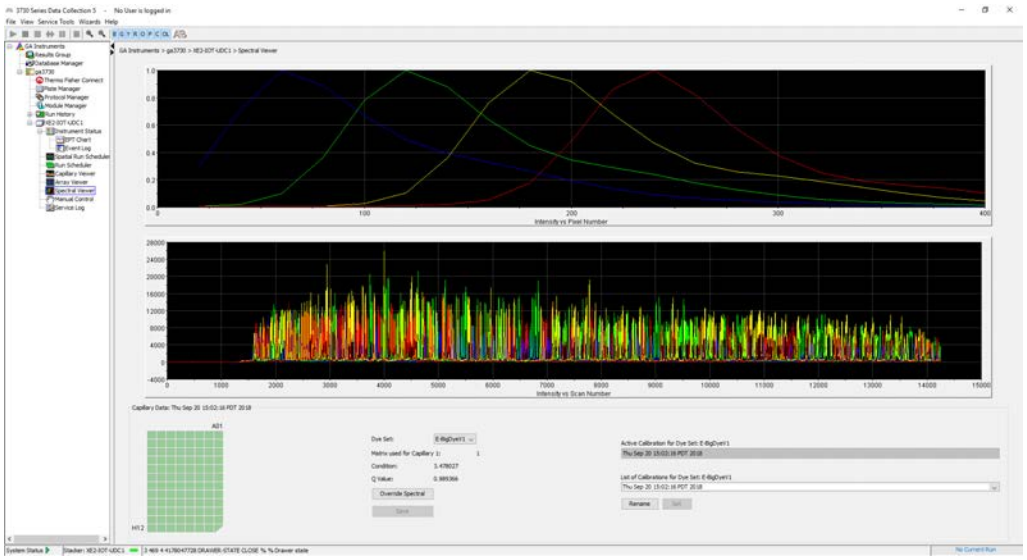
3. Press **R** to reset the view.

Examples of passing sequencing spectral calibrations

Dye set Z created from a sequencing standard

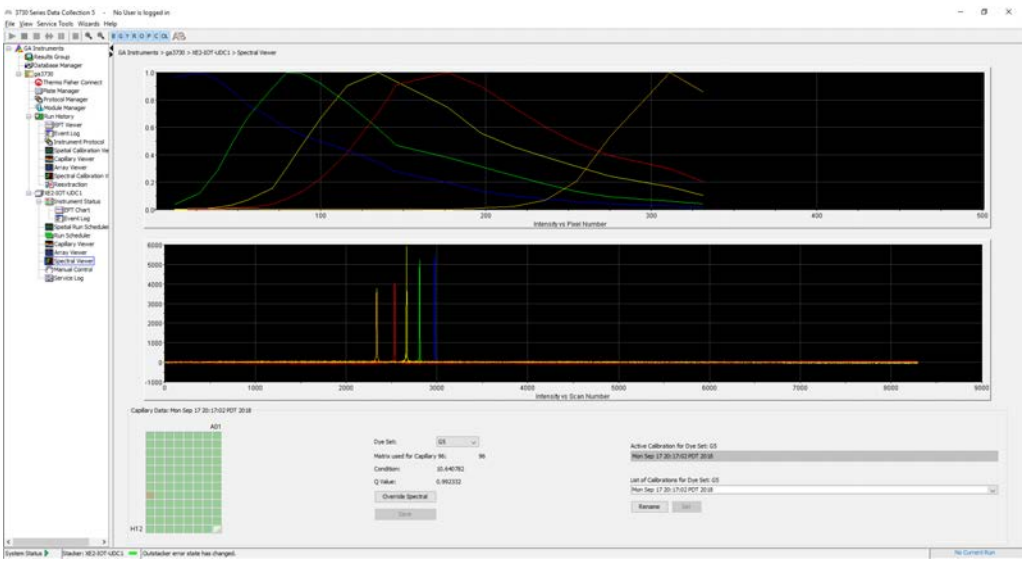


Dye set E created from a sequencing standard



Example of a passing fragment analysis spectral calibration

Dye set G5 created from matrix standard set DS-33

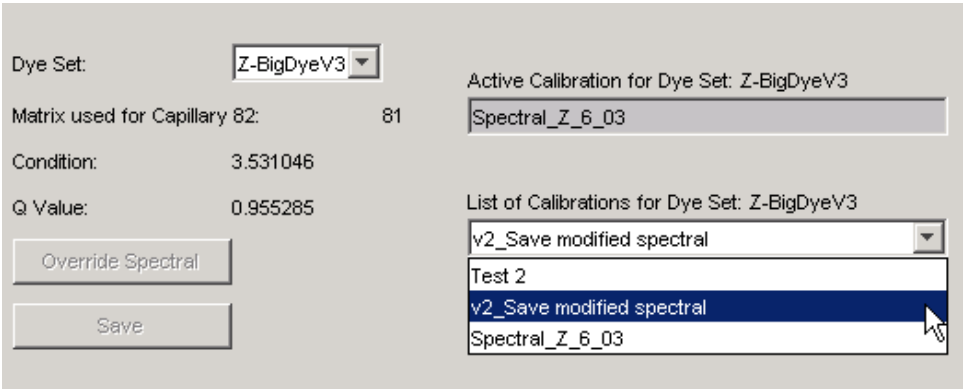


Select a previous calibration as the active spectral calibration

- Best practice is to perform a spectral calibration every time you install a new capillary array.
- There may be circumstances where you want to reuse a calibration that was generated using a different capillary array.

  Note: You must select the calibration before data is collected. After data is collected, you cannot reapply a different spectral calibration.
- Do not reuse a previous calibration when you use the G5-RCT dye set. Because of the way the spectral calibration is generated, it is specific to the capillary array used to generate the calibration.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name*** ▸ **Spectral Viewer**.
   Failed calibrations are marked with an asterisk *.
2. Select the dye set of interest.
3. Click the **List of Calibrations** list in the lower right-pane.



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

5. Click **Set** to display the selected spectral calibration in the **Active Calibration** text box.

9 Maintenance

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

Maintenance schedules

Daily maintenance

Task	Frequency
Ensure that adequate levels of liquid are in the buffer, waste, and water reservoirs.	Before each run
Ensure the plate assemblies are properly assembled. **IMPORTANT!** The holes in the plate retainer must align with the holes in the septa, or the capillary tips will be damaged. Make sure the retainer clips are flush with the sides of the plate and with the plate base. A simple way to ensure that they are flush is to run your finger along the edge.	Before each run
Ensure the plate assemblies are properly positioned on the plate deck. Plates should sit snugly on the deck. **IMPORTANT!** Do not use warped plates.	Before each run

Task	Frequency
Check the level of buffer in the anode buffer jar. Ensure that the overflow hole is facing toward the front of the instrument and is not blocked.	Before each run
Replace the 1X run buffer in the buffer jar and the buffer reservoir on the instrument, and the water in the water reservoir. Ensure that the outside of the assemblies are dry. When you replace the 1X run buffer, do not add fresh buffer to old buffer. Discard old buffer, then add fresh buffer.	Every 48 hours
Check for bubbles in the pump block, lower polymer block, interconnect tube, polymer supply tube, and channels. Remove all bubbles using the **Bubble Remove** wizard. See “Typical conditions for using maintenance wizards” on page 117.	Daily or before each run
Check the loading-end header of the capillary array. Ensure that the capillary tips are not crushed or damaged.	Daily or before each run
Check the level of polymer in the bottle to ensure that the volume is sufficient for runs.	Daily or before each run
Clean the instrument surfaces.	Daily
Check for leaks around the array knob, interconnect tubing nuts, and check valve.	Daily

Weekly maintenance

Task	Frequency
Replace the polymer using the **Replenish Polymer** wizard or **Change Polymer Type** wizard.	Weekly
Check the storage conditions of the used arrays.	Weekly
Replace reservoir septa.	Weekly
Clean the buffer jar, water, waste, and buffer reservoirs with warm water, then rinse with distilled/deionized water.	Weekly
Flush the polymer delivery pump water trap. See “Flush the water seal trap” on page 122.	Weekly
Reboot the instrument and computer.	Weekly

Monthly maintenance

Task	Frequency
Run the **Water Wash** wizard (see "Typical conditions for using maintenance wizards" on page 117). Flush the array port during the **Water Wash** wizard, whether or not bubbles are present in the array port.	Monthly or as needed
Check disk space (see "Check available disk space on E: drive" on page 132).	Monthly

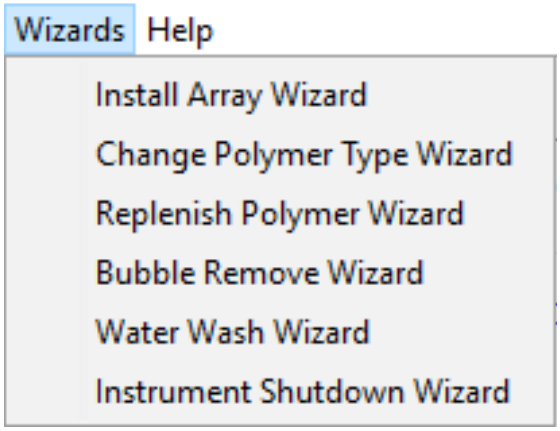
As-Needed maintenance

Task	Frequency
Clean the drip tray.	As needed
Change the capillary array using the Install Array wizard (see "Typical conditions for using maintenance wizards" on page 117).	As needed
Remove any dried polymer from the capillary tips. Use a lint-free wipe moistened with deionized water.	As needed
Disk defragmentation of the C and E drives. **IMPORTANT!** Do not defragment the D or F drives. Disk defragmentation can corrupt the database information located there and cause the 3730xl Data Collection Software 5 and the GeneMapper™ Software to stop working.	As needed

Wizards types and functions

To display the Wizard menu at the top of the screen, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name***.

There are six Wizards available.



Wizard	When to use
Install Array	• To install a capillary array • To replace an installed capillary array with another capillary array
Change Polymer Type	• To change to a different polymer type
Replenish Polymer	• To replenish the polymer supply • To replace the polymer in the polymer delivery pump with polymer of the same or different lot
Bubble Remove	• To remove bubbles in the polymer delivery pump chamber, channels, array ferrule, and tubing
Water Wash	• To wash the polymer delivery pump chamber, lower polymer block, channels, and tubing with water when the following conditions are met. – As part of a monthly maintenance protocol – To remove any suspected contaminants in the polymer delivery pump – To remove persistent bubbles (followed by the **Bubble Remove** wizard, if needed) – To replace old polymer in the polymer delivery pump
Instrument Shutdown	• To prepare the instrument for a shutdown >1 week • To remove the capillary array

Typical conditions for using maintenance wizards

Condition	Applicable Wizard/action	Description
The polymer has been in the pump longer than 1 week.	Use the **Water Wash** wizard (instead of **Change Polymer** wizard) to replace the polymer.	Using the **Water Wash** wizard ensures that the system is clean before fresh polymer is introduced. Certain polymer components may decompose, causing an increase in electrophoresis current in polymer that has been at room temperature for more than 1 week.
Bubbles move but are not completely cleared by the **Bubble Remove** wizard.	Use the **Bubble Remove** wizard *repeatedly* until the bubbles are gone.	—
You want to clear *persistent* bubbles.	• Run the **Water Wash** wizard. If bubbles are still present, run the **Bubble Remove** wizard. • Remove the polymer bottle. Run the **Bubble Remove** wizard to draw air into the pump chamber and other parts of the system. Reinstall the polymer bottle and repeat the **Bubble Remove** wizard to remove all bubbles.	• The **Water Wash** wizard includes refilling the pump with polymer. • After the pump is idle, bubbles that previously did not move are often cleared by running the **Bubble Remove** wizard.
Many or large bubbles are present in the pump chamber.	Run the **Water Wash** wizard.	—
No bubbles are present in the array port during the monthly water wash procedure.	Perform the Flush Array Port procedure using the **Water Wash** wizard as part of monthly maintenance, even if no bubbles are present.	Occasional flushing of the array port keeps this space filled with fresh solution.
You want to install a capillary array on an instrument without an array.	Use the **Install Array** wizard.	Filling the array helps to ensure complete changeover to polymer after the polymer delivery pump has been washed with water.
You want to remove or install a capillary array.	Carefully follow the instructions in the appropriate wizard (**Install Array** or **Instrument Shutdown** wizard).	—
You select **Discard** during installation of an array using the **Install Array** wizard.	The information for that array cannot be entered again on the instrument.	—

Condition	Applicable Wizard/action	Description
The instrument will be idle for >1 week.	Use the **Instrument Shutdown** wizard.	—
You are using the **Install Array** wizard to reactivate the instrument.	First power on the instrument power to activate the wizard menu in the Data Collection software.	The instrument must be powered on for the wizards to be available through the Data Collection software. If the instrument is turned off, the wizard names in the drop-down menu are grayed out.
You want to clean the polymer delivery pump.	Use the **Water Wash** wizard with deionized water at ≤40°C.	Hot water may damage the polymer delivery pump seals and joints. Do not use any solutions or fluids in the instrument other than water and polymer.

Effects of the instrument door state on wizard tasks

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

IMPORTANT! Whenever the door is closed, the autosampler initializes. Before starting a wizard, ensure that the autosampler has stopped moving and that the green status light is solid green.

Wizard-based task	Status of instrument door	Result
IMPORTANT! Do not open or close the instrument door while an automated procedure is in progress. Leave the door in the starting state (whether open or closed) until the automated procedure is complete.		
Begin an automated procedure	Open	The procedure continues when the door is closed, and after the autosampler moves to initialize. If you open the door again, the procedure pauses until the door is closed.
Begin an automated procedure	Closed	The procedure pauses if the door is opened. Close the door again to resume the procedure.
Click **Fill Array**	Open	The procedure does not start; the door must be closed.
Perform an automated procedure	Closed	The green status light remains on (not flashing)
Perform an automated operation	Open	The yellow status light flashes.

Manual control of autosampler content

Use the manual control of the autosampler feature with care.

When you use Manual Control to move the autosampler, watch all autosampler movements and positions.

If the autosampler reaches its maximum travel position, a fatal instrument error is generated. The software must be re-started after this error.

Clean the instrument

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

   IMPORTANT! Do not use organic solvents to clean the instrument or any of its components.

Replenish or change polymer type

Replace polymer that has been on the instrument for more than 1 week.

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.

Replenish the polymer

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

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name***.
2. At the top of the screen, select **Wizards** ▸ **Replenish Polymer Wizard**.

IMPORTANT! When this wizard runs, it introduces polymer into the anode buffer jar. Replace the anode jar buffer after you run this wizard. See “Fill the anode buffer jar” on page 33.

Change polymer type

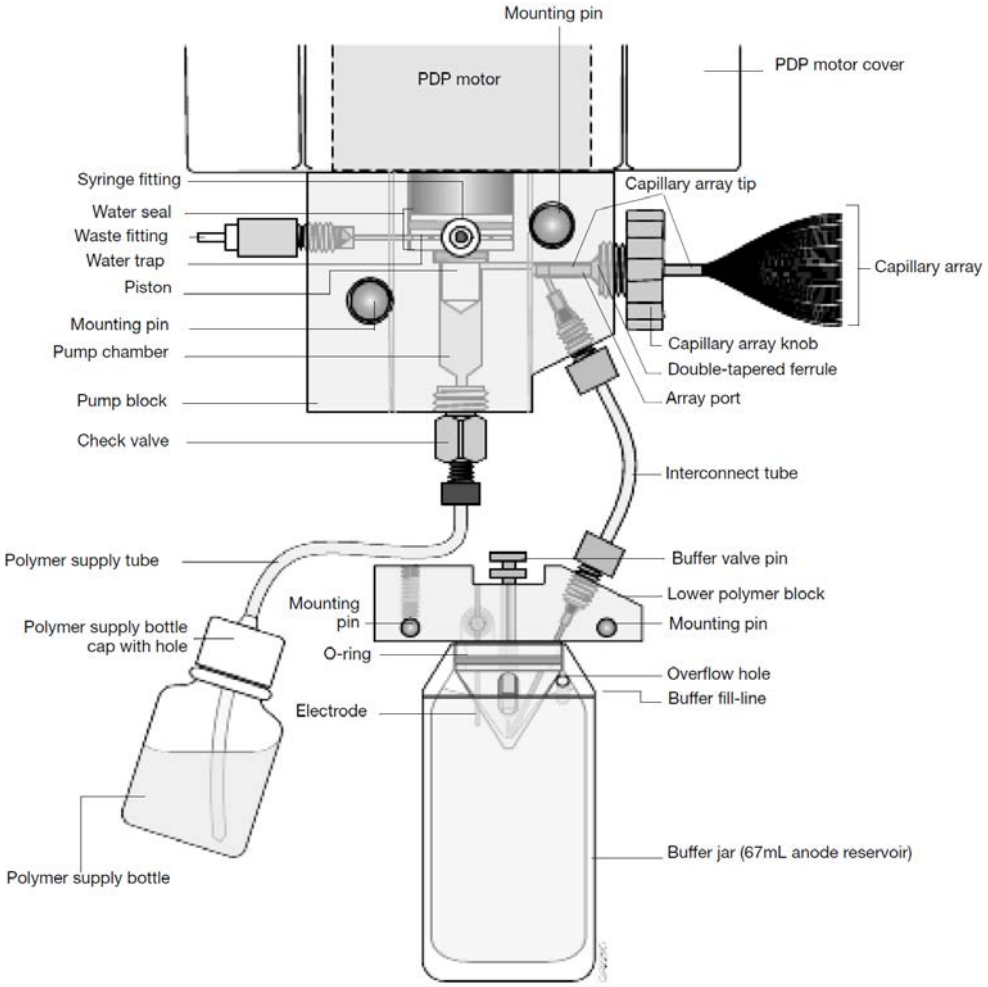
IMPORTANT! Wear gloves while handling polymer, the capillary array, septa, or buffer reservoirs.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name***.
2. At the top of the screen, select **Wizards** ▸ **Change Polymer Type** wizard.

IMPORTANT! When this wizard runs, it introduces polymer into the anode buffer jar. Replace the anode jar buffer after you run this wizard. See “Fill the anode buffer jar” on page 33.

Clean the polymer delivery pump (PDP) and lower polymer block

Parts of the polymer delivery pump



Guidelines

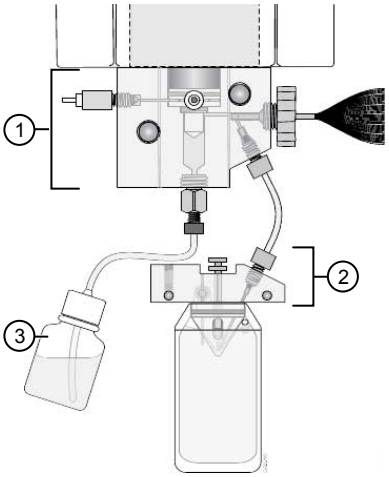
- Do not expose the polymer blocks to organic solvents.
- Do not use sharp or pointed instruments to remove dried polymer from the polymer blocks.
- Do not use water >40°C to clean the polymer blocks.

Frequency

- Clean the exterior 1 time per week, when you replenish polymer.
- Flush the polymer delivery pump water trap 1 time per week.
- Clean the polymer delivery pump chamber, channels, and tubing 1 time per month.

Clean the polymer delivery pump chamber, channels, and tubing

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name***.
2. Select **Wizards** ▸ **Water Wash Wizard**.
3. Inspect the channels of the pump and lower blocks for any contaminants. Repeat the **Water Wash** wizard until contaminants are removed.



① Pump block
② Lower block
③ 40°C DI water bottle. Use DI water only.

Flush the water seal trap

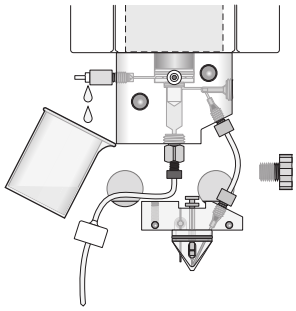
Flush the water trap of the polymer delivery pump 1 time per week. Flushing removes diluted polymer that may have passed through the water trap seals. Some air bubbles in the water trap are acceptable and do not affect performance. Leave the trap filled with either distilled or deionized water.

1. Fill the 20-mL syringe from the Polymer Block Cleaning Kit (Cat. No. 4335860) with distilled or deionized water. Expel any bubbles from the syringe.

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

2. Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.
3. Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
4. Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.

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



IMPORTANT! DO NOT USE EXCESSIVE FORCE. Take approximately 30 seconds to flush 5 mL of either distilled or deionized water through the trap.

6. Close the fittings by turning each clockwise until the fittings seal against the block. Close the Luer fitting first, then close the Exit fitting.

IMPORTANT! Do not over-tighten the fittings. The fittings require only enough tightening to prevent water leaks. Excessive tightening can damage the fittings.

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

Install the capillary array

Required materials

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

Install a new or used capillary array

IMPORTANT! Wear gloves when you handle the capillary array.

Use the **Install Array** wizard when changing capillary arrays to properly fill the capillaries with polymer.

1. In the navigation pane, select **GA Instruments** ▸ **ga3730** ▸ ***Instrument name***.
2. At the top of the screen, select **Wizards** ▸ **Install Array** wizard.
3. Follow the instructions in the wizard to install the capillary array.

IMPORTANT! When this wizard runs, it introduces polymer into the anode buffer jar. Replace the anode jar buffer after you run this wizard. See “Fill the anode buffer jar” on page 33.

4. Perform a spatial calibration (see “Perform spatial calibration” on page 92).

Store a capillary array

CAUTION! During storage, keep both ends of the capillary array immersed in 1X run buffer. If the capillary array dries out, it may be damaged.

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

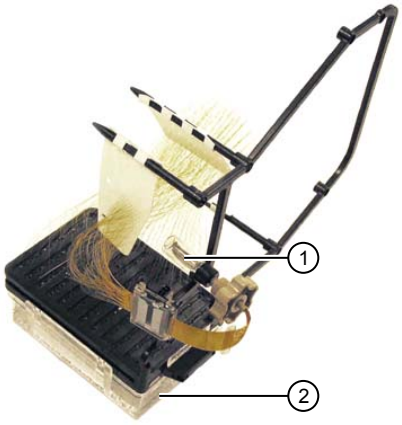
   IMPORTANT! Do not select **Discard Array** if you want to install the capillary array on the instrument again. If you click **Discard**, the software flags the array information as "discarded" will not allow you to enter the array information again.

2. Place 80 mL of 1X run buffer in the capillary array header shipping cover.

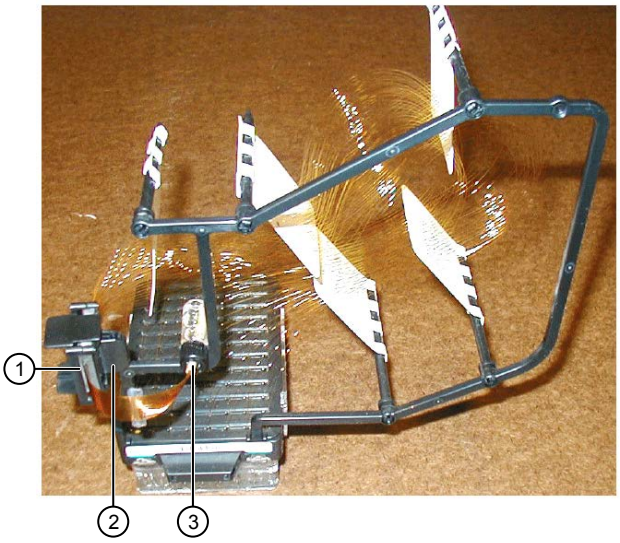
3. Lower the capillary tips of the array header into the shipping cover and lock the header onto the cover. The tips of the capillaries should be immersed in buffer.

4. Clip the detection cell window cover onto the detection cell.

5. Attach the detection cell with cover to the storage post on the capillary array frame.



① Shipping vial
② Header shipping cover



① Detection cell with cover
② Storage post on the capillary array frame
③ Vial with the array tip

6. If the array knob and ferrule are on the array tip, remove them and rinse them with deionized water. Dry the parts with a lab wipe.
7. Clean the array tip carefully with a lab wipe moistened with deionized water.
8. Attach the array tip shipping vial filled with 1X run buffer to the array tip. Loosen the vial cap slightly, insert the tip and then tighten the cap.
9. Clip the vial with the array tip onto the array frame.
10. Store the capillary array upright in a safe area.

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

Clean the detection cell

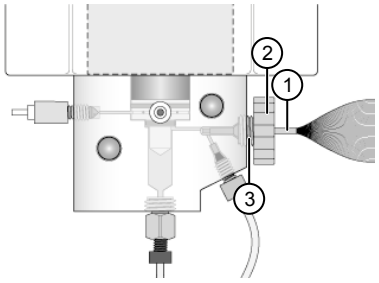
1. Open the instrument door and the oven door.
2. Open the detection cell door by turning the door knob to the left, then lowering the door.

1. Detection cell door knob
2. Capillary array knob
3. Capillary array

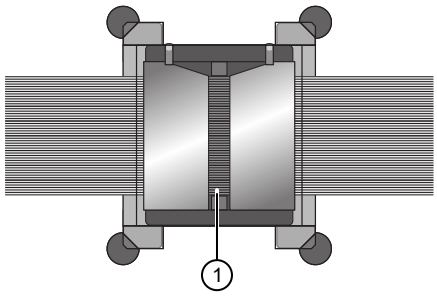
3. Turn the capillary array knob 1/4-turn clockwise. The pointer on the knob is pointed to the left.
4. Pull the pump and lower polymer blocks forward until the detection cell is out of the detection block.

5. Detach the tip of the capillary array from the pump block, then remove the capillary array knob and ferrule.

6. Clean the front surface of the detection cell.
   a. Add one drop of methanol to a sterile swab or lint-free wipe.
   b. Gently wipe the detection cell.



① Tip of the capillary array
② Capillary array knob
③ Ferrule



① Detection cell

7. Reinstall the tip of the capillary array, array knob, and ferrule in the pump block.

8. Push the pump and lower polymer blocks back against the pump panel. Make sure that the buffer valve lever engages the buffer pin valve.

9. Carefully place the detection cell into the detection block. Secure it by rotating the cam knob 1/4-turn counterclockwise. The pointer on the knob is pointed down.

10. Close the detection cell door, oven door, and instrument door.

Perform a short-term shutdown

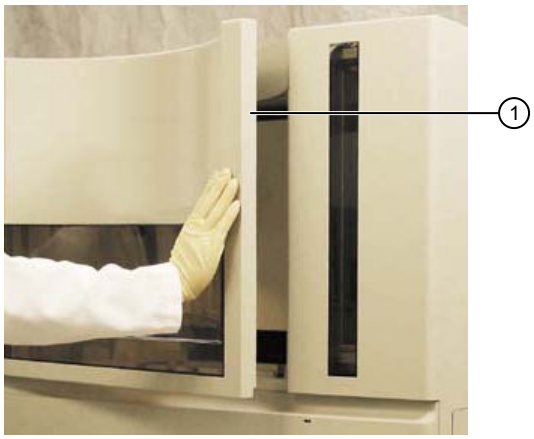
Perform the short-term shutdown procedure if the instrument will be idle for <1week.

Materials required

- 1X run buffer
- Deionized water
- Lab wipes
- Gloves

Perform a short-term shutdown

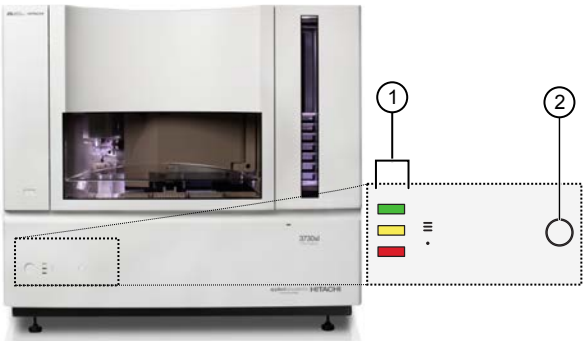
1. Close the instrument door.



① Instrument door

2. Press the tray button to bring the autosampler to the forward position.

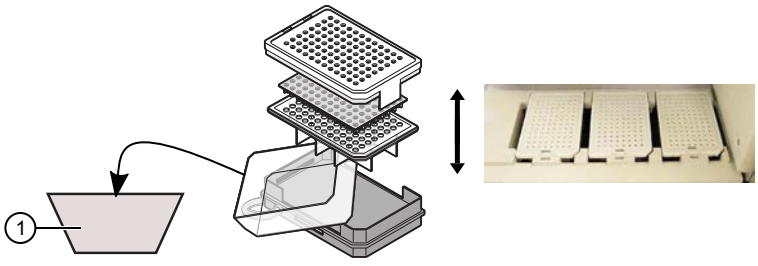
3. After the autosampler stops moving and the green status light illuminates, open the instrument door.



① Status lights
② Tray button

4. Remove the buffer, water, and waste reservoir assemblies from the instrument.

5. Disassemble each reservoir assembly and empty the contents of the reservoirs into an aqueous waste container.



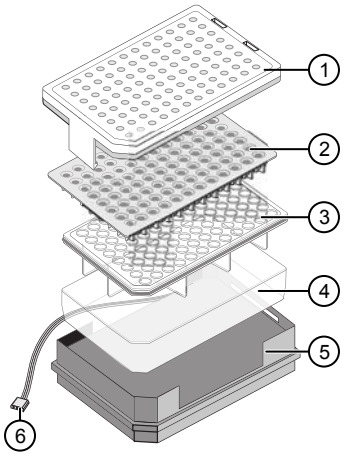
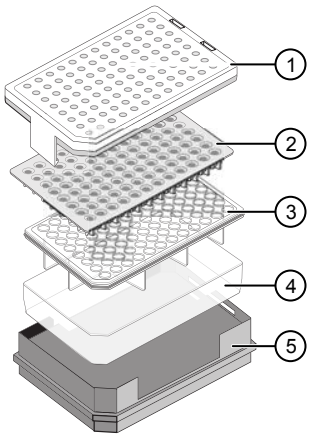
① Aqueous waste

6. Rinse each reservoir using deionized water.

7. Dry the reservoirs using lint-free wipes.

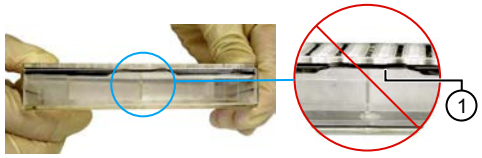
① DI H2O ≤40°C

8. Fill and assemble the reservoirs.
   a. Fill the buffer reservoir with 80 mL of 1X run buffer.
   a. Fill the water and the waste reservoirs with 80 mL of deionized water.
   b. Assemble each reservoir assembly as shown below.

Buffer reservoir assembly	Water and waste reservoir assemblies
1. Add 80 mL 1X run buffer to the Buffer reservoir. 2. Assemble the reservoir assembly as shown below.	1. Add 80 mL high-quality deionized water to each reservoir. 2. Assemble each reservoir assembly as shown below.
	
① Retainer ② Septum ③ Reservoir cap ④ Reservoir ⑤ Heated plate base ⑥ Power cable	① Retainer ② Septum ③ Reservoir cap ④ Reservoir ⑤ Plate base

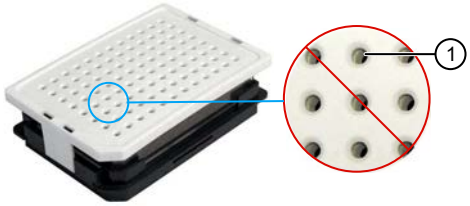
For catalog numbers see Appendix E, “Catalog numbers”.

9. To prevent damage to the capillary array, inspect each reservoir assembly and verify that the following conditions are met.
   - Septa fit snugly and flush on the reservoir
   - Rubber gasket around the edge of the reservoir cap is seated



① Rubber gasket not seated correctly

   - Plate retainer holes and the septa strip are aligned



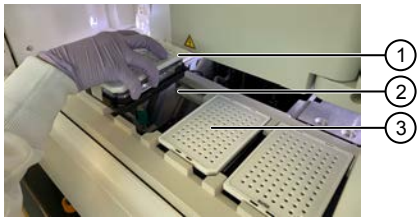
① Plate retainer holes and septa holes are not aligned

10. Dry the reservoirs using lint-free wipes.

① Lint-free wipe

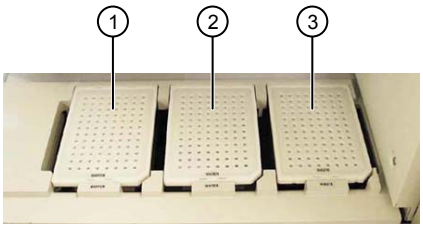
11. Connect the buffer reservoir plate base cable to the heater outlet inside the instrument.

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



① Buffer reservoir
② Buffer position

12. Place the water and waste reservoirs in the instrument. Load the three reservoirs in the following order (buffer, water, then waste).



① Buffer reservoir
② Water reservoir
③ Waste reservoir

13. Close the instrument door.

14. Press the instrument power button.

① Power button

15. Power off the computer:
    a. Select **Start** ▸ **Shutdown**.
    b. In the Shut Down dialog box, select **Shut down**.
    c. Click **OK**.
    d. Press the monitor power button.

For long term shutdown, use the Wizard. The Wizard will guide you through the following procedures.

1. Discarding and storing the capillary array
2. Washing the pump and channels
3. Setting up the pump for storage. For example, putting the array port plug in place (see Figure 3) and replacing polymer with water.

Maintain adequate space for database and sample data storage

Pre-run automatic disk space checks

Before a run or batch of runs, the Data Collection Software automatically checks free disk space.

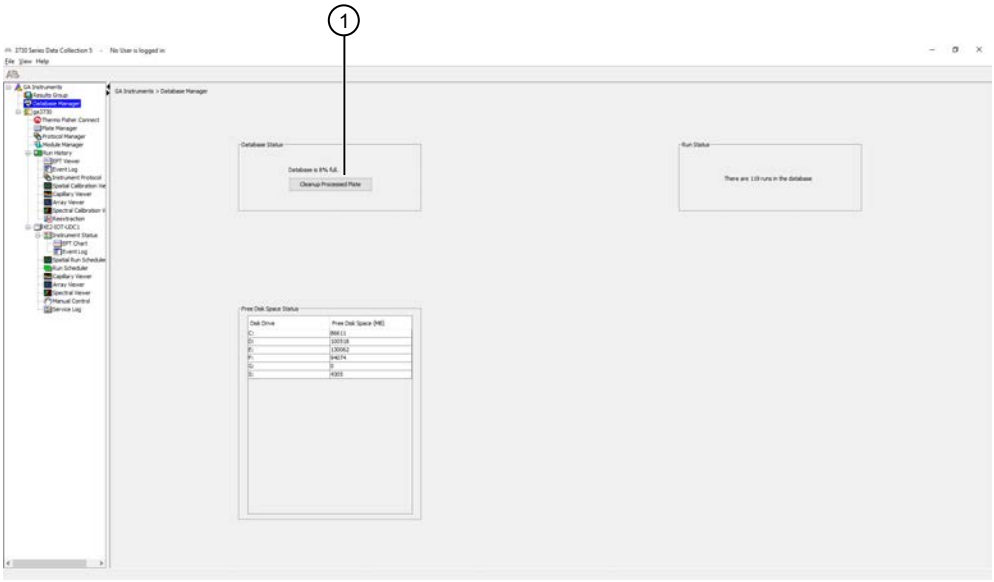
If sufficient disk space is not available to store the run data, the following occurs.

- An error message is displayed in the **Error Code** pane in the **Event Log**.
- The status bar displays a flashing  .

Full database error

To correct the error, click **Cleanup Processed Plates** in the Database Manager or go to the **Plate Manager** and manually delete the plates. Perform cleanup every 300 runs or 6 months in order to minimize cleanup time.

IMPORTANT! Runs can not be started until you delete data from the database. See “Delete data from the database” on page 132.

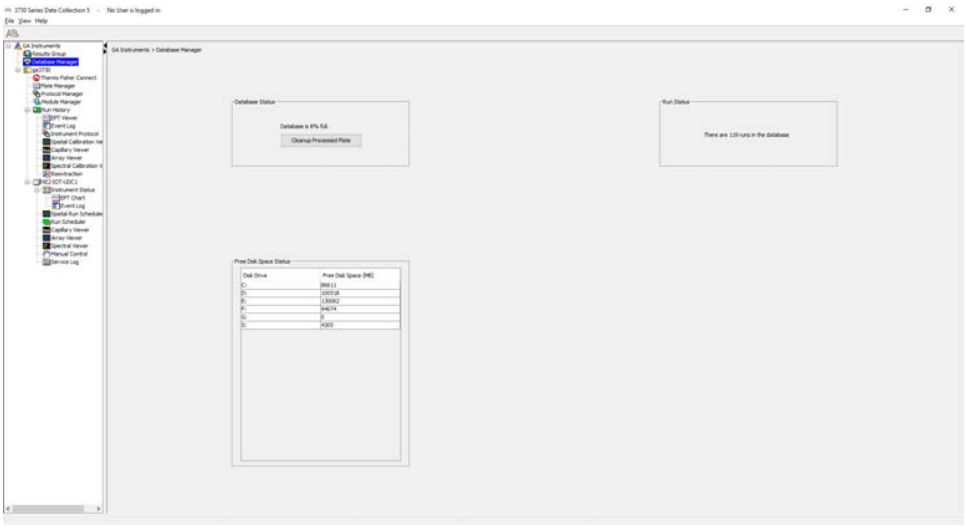


① Cleanup Processed Plates

Check available disk space on E: drive

Check disk space monthly.

1. In the navigation pane of the Data Collection Software, select **GA Instruments** ▸ **Database Manager**.



2. If there is insufficient space on E: drive, archive the sample files to another location, then delete the files from E: drive.

   Note: The software requires at least 2 GB of free disk space on E: for proper operation. Move files from the E:\drive to archival locations regularly.

   Note: Do not specify C: drive locations for data storage. C: drive is used for the operating system. The software does not perform pre-run disk space checks on C: drive.

Delete data from the database

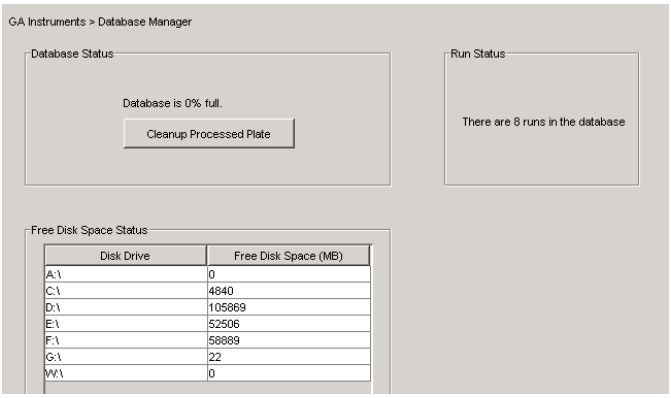
The **Cleanup Database** function deletes the following content from the database.

Deleted	Not deleted
• Sequence analysis and fragment analysis plate records with a status of "Processed" • All run data in the database that corresponds to the plate records	• Spectral calibration plate records • Sequence analysis and fragment analysis plate records with a status of "Completed" • Instrument protocols • Result groups • Analysis protocols

IMPORTANT! Do not delete spectral calibration plate records. Doing so permanently deletes the spectral information from the database and prevents you from re-extracting data from the plate record.

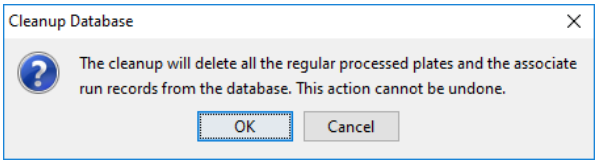
1. In the navigation pane of the Data Collection Software, click **GA Instruments** ▸ **Database Manager**.

   The Database Manager opens.



2. Click **Cleanup Processed Plates**.

   The **Cleanup Database** dialog box opens.



3. Click **OK**.

   Note: It may take several minutes to clean up the database.

Troubleshooting

Restart the instrument and the computer

Use this procedure if any of the following conditions occur.

- If communication errors are displayed
- If the front panel indicator is blinking red
- At the end of spatial calibration, if Accept/Reject buttons are dimmed
- If maintenance wizards are taking longer than expected
- If software operations are taking longer than expected

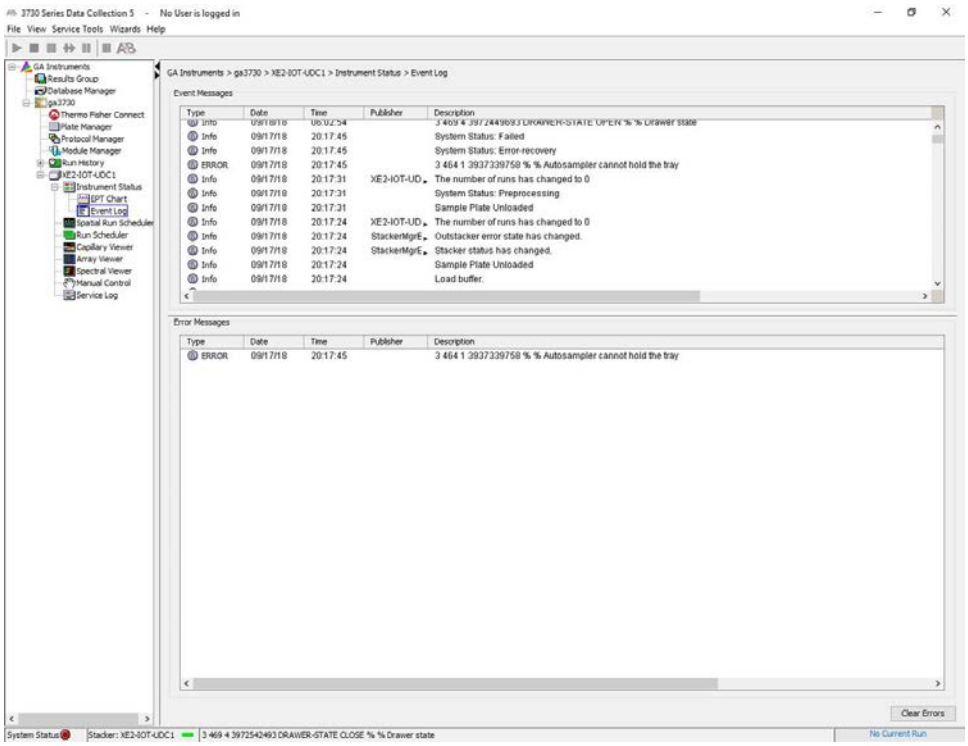
When you are instructed to restart the instrument and the computer:

1. Exit the Data Collection Software.
2. Power off the computer.
3. Make sure the instrument door is closed, then power off the instrument.
4. When the computer is completely powered off, wait 60 seconds, then power on the computer. Wait until the Windows™ login screen is displayed.
5. Power on the instrument and wait until the green status light on the front panel is on and not flashing before proceeding.

6. Log in to Windows™ operating system.
7. Start the Data Collection Software.

Display the Event Log

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



2. In the **Error Code** pane, find the error code for the last message in the log file.
3. Perform the required tasks to fix the problem.

Review a run log file

Run log files are stored in the default directory, not in the destination directory specified by the results group.

Navigate to `E:/AppliedBiosystems/UDC/DataCollection/Log`.

Hi-Di™ Formamide preparation and storage

Formamide is used to prepare samples, it is not installed on the instrument as are the other consumables listed in this section. It does not include an RFID tag on the label.

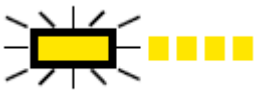
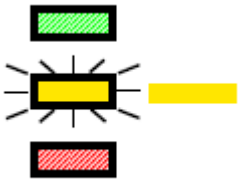
Storage

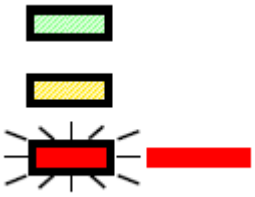
- –25°C to –15°C long term
- 2 to 8°C for ≤1 week

IMPORTANT! More than 8 freeze/thaw cycles or storage at 2 to 8°C causes breakdown of the formamide. This can lead to a change in odor, loss of resolution, and data artifacts.

If frequent sampling is required, dispense and freeze small aliquots of Hi-Di™ Formamide into smaller tubes. Minimize freeze-thaw cycles and exposure to air and room temperature because the quality of the material may decrease when exposed to air.

Front panel status light troubleshooting

Observation	Possible cause	Recommended action
Status light is flashing yellow 	The instrument door is open.	Close the instrument door.
	The oven door is open.	Close the oven door.
	The buffer reservoir or capillary array are not installed.	Install the buffer reservoir or capillary array.
Status light is solid yellow 	The instrument cannot communicate with the computer.	1. Ensure that the instrument door is closed. 2. Ensure that the Ethernet cable is connected to the back of the instrument and to the computer.
	A reservoir is not installed.	Ensure that buffer, water, and waste reservoirs are installed.

Observation	Possible cause	Recommended action
Status light is solid red 	Error condition on instrument.	1. Power off the instrument, wait for 30 seconds, then power on the instrument. 2. Ensure that the Ethernet cable is connected to the back of the instrument and to the computer. 3. Display the **Event Log** and troubleshoot the error codes (see page 135).

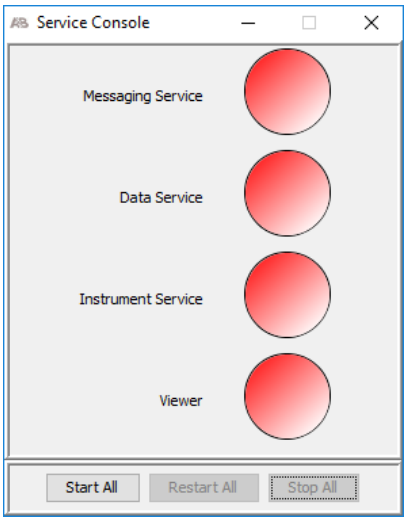
Instrument run troubleshooting

Observation	Possible cause	Recommended action
Plate validation fails when you start a run	The dye set for the active calibration does not match the dye set specified in the instrument protocol.	Change the active calibration to match the dye set that is specified in the instrument protocol.
		Edit the results group to specify an instrument protocol with a dye set that matches the active calibration.
		See also the **Event Log** message observations listed below.
	An object in the plate record (instrument protocol, analysis protocol, results group) has been deleted from the database.	Edit the plate record.
	Results group **Analysis** tab specifies autoanalysis, but no **Login ID** or **Password** is specified.	Edit the results group.
"Container and NumCap validation failed" message in **Event Log**	The plate record specifies a plate type (number of wells) that does not match the plate type that is detected by the instrument.	Edit the plate record to match the plate type.
"Sealing type (from instr) is Heat Sealing and does not match set by user Septa" message in **Event Log**	The plate record specifies a sealing type that does not match the base in which the plate is loaded.	Edit the plate record to specify the sealing type that matches the base in which the plate is loaded.
		Load the plate in the correct base. See “Prepare the plate assemblies” on page 42.
"Error getting plate info from database" message in **Event Log**	After the plate record was added to the **Run Scheduler**, the plate record was deleted in the **Plate Manager**.	Load a different plate record.

Re-extraction troubleshooting

Observation	Possible cause	Recommended action
Re-extraction fails	You used an analysis protocol that was previously used to process the plate record (it was deleted from the plate record, then added back to the plate record).	Use a new analysis protocol with the same settings, or create a new plate record for re-extraction.
	Unknown cause.	Review the run log file. See “Review a run log file” on page 135.

Service console troubleshooting

Observation	Possible cause	Recommended action
Service console: All indicators are red 	Services are starting.	Wait a few minutes.
Service console: Data Services is  for more than 1–2 minutes	The service is not starting.	Click **Stop All**, then restart the computer.
Service Console: Viewer is  after all other services are 	The service is not starting.	Click **Stop All**, then restart the software.
"The requested service has already been started. More help is available by typing NET HELPMSG 2182."	Ignore message.	Ignore message.

Spatial calibration troubleshooting

Observation	Possible cause	Recommended action
Spatial calibration fails	Bubbles are present in capillaries.	Run the **Bubble Remove** wizard. Observe the array port area to ensure bubbles are removed.
	Detection cell window is not clean.	Clean the detection cell (see page 125).
	Problem with capillary array.	Use the **Install Capillary Array** wizard to replace the capillary array (see page "Store a capillary array" on page 124).

Spectral calibration troubleshooting

Observation	Possible cause	Recommended action
No signal	Incorrect sample preparation.	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide (see "Hi-Di™ Formamide preparation and storage" on page 136).
	Air bubbles are present in the plate wells.	Centrifuge the plate to remove air bubbles.
Spectral calibration fails	Expired spectral standards.	Check the expiration date and storage conditions of the spectral standards. If necessary, replace with a fresh lot.
	Insufficient filling of the capillary array.	Check for broken capillaries.
Spectral calibration "No candidate spectral files found" message displayed	One or more of the internal files generated for each capillary was not stored in the database.	Check for clogged capillary.
Spikes in the data	Expired polymer.	Replace the polymer with a fresh lot using the **Change Polymer wizard**.
	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**.
Spectral calibration peaks are offscale	Calibration standard is too concentrated.	Dilute the matrix standard and repeat the calibration.
"Failed calibration due to bad data: Bad dye order detected"	Incorrect dye set was selected in the spectral calibration instrument protocol.	Adjust the spectral calibration instrument protocol, then repeat the spectral calibration.

Observation	Possible cause	Recommended action
"Failed calibration due to bad data: Bad dye order detected" *(continued)*	Problem with spectral calibration, even if dyes are in the correct order.	Repeat the spectral calibration.

General troubleshooting

Observation	Possible cause	Recommended action
Software does not start when you double-click the desktop icon 3730xL 5	The software window is minimized.	Click the Data Collection Software icon in the task bar to maximize the software window.
No signal	Incorrect sample preparation.	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide (see "Hi-Di™ Formamide preparation and storage" on page 136).
	Air bubbles are present in the plate wells.	Centrifuge the plate to remove air bubbles.
Spikes in the data	Expired polymer.	Replace the polymer with a fresh bottle using the **Change Polymer** wizard.
	Air bubbles, especially in the polymer.	Refill the capillaries using the **Bubble Remove** wizard.
		Bring the polymer to room temperature.
		Replace the polymer with a fresh lot using the **Change Polymer** wizard.
	Possible contaminant in the polymer.	Replace the polymer with a fresh lot using the **Change Polymer** wizard.
Instrument fittings are leaking	Fittings are loose.	Finger-tighten all fittings. Do not overtighten.
Brown or black debris is visible in the lower polymer block	Arcing is occurring in the pump system.	Clean the pump. See "Clean the polymer delivery pump (PDP) and lower polymer block" on page 121.
	Bubbles in the tubing or in the polymer block.	Use the **Bubble Remove** wizard.
Air bubbles in the pump	Fittings may be loose.	Finger-tighten all fittings. Do not overtighten.
		Run the **Bubble Remove wizard**.
		For persistent bubbles, replace the polymer with water, then run the **Bubble Remove wizard**. Replace the water with polymer, then run the wizard again.
Polymer is visible in the anode buffer jar when the capillaries are filling (polymer is "streaking" into buffer)	You ran any of these wizards: Bubble Remove, Replenish Polymer, Replace Polymer, Install Array	Replace the anode buffer. See "Fill the anode buffer jar" on page 33.
	The buffer pin valve is leaking.	Contact Support.

Observation	Possible cause	Recommended action
Polymer is visible in the anode buffer jar when the capillaries are filling (polymer is "streaking" into buffer) *(continued)*	The buffer pin valve needs to be adjusted.	Contact Support.
	Arcing is occurring in the pump system.	Clean the pump. See “Clean the polymer delivery pump (PDP) and lower polymer block” on page 121.
Polymer is leaking at fittings during pump fill stroke	The check valve is leaking.	Remove the polymer bottle to expose the check valve. Push 100 mL of DI-water through the check valve with a 50-cc syringe and adapter.
“Tray on deck does not match Tray Type in run setup” error message displayed	The plate record specifies a sealing type that does not match the base in which the plate is loaded.	Edit the plate record to specify the sealing type that matches the base in which the plate is loaded.
		Load the plate in the correct base. See “Prepare the plate assemblies” on page 42.
	Plate sensor malfunction.	Contact Support.
"Full database" message	Insufficient disk space.	See “Maintain adequate space for database and sample data storage” on page 131.

B

Instrument specifications

Instrument specifications

Table 10 3730xl DNA Analyzer physical dimensions, weight, and power consumption

Component	Height	Length (depth)	Width	Weight
Instrument	90 cm (35.5 in.)	65 cm (26.0 in.)	100 cm (40.0 in.) with door closed 170 cm (67.0 in.) with door open	~186 kg (411 lbs)
Computer	36.0 cm (14.2 in.)	41.7 cm (16.4 in.)	17.5 cm (6.9 in.)	~12.2 kg (27 lbs)
Monitor (compressed)	36.9 cm (14.5 in.)	18.0 cm (7.1 in.)	40.6 cm (16.0 in.)	~5.0 kg (11.0 lbs)
Keyboard	3 cm (2 in.)	44.5 cm (6 in.)	14.0 cm (17.5 in.)	1 kg (0.2 lbs)

- Access to all four sides of the instrument is required for servicing.
- Do not block access to the rear of the instrument.
- Minimum rear clearance is 30.5 cm (12 in.).
- If two instruments are placed back-to-back, allow 61 cm (24 in.) of clearance between the instruments.

Table 11 Operating specifications

Component	Specification
Laser	• Long-life, single-line 505 nm, solid-state laser excitation source • Laser Output power 20mW • Beam divergence 1.4 mrad
Barcode reader laser	• Laser Output power 85 µW • Wavelength 655 nm • Pulse duration 112 µs
LED	• Emitting color Natural White, maximum of 21 mW • Luminous Intensity 250 Cd
Electrophoresis voltage	Up to 20 kV

Component	Specification
Oven temperature	Active temperature control from 18°C to 70°C
Internal barcode reader	Supports the following formats: • Code 128 • Code 39 • Code 93 • LOGMARS • EAN-8 **Note:** All Applied Biosystems™ barcoded plates for the instrument use code 128 format.
Minimum computer requirements	• Hardware: OptiPlex™ XE2, with Intel™Core I7-47705, 3.1 GHz Processor • Operating system: Windows™ 10 Enterprise 2016 LTSB, 64-bit (requires 5 partitions) • Installed RAM: 16 GB • Hard drive: 2 x 500GB SATA 3.0Gb/s and 8MB Data Burst Cache

Environmental requirements

Condition	Requirement
Installation site	Indoor use only
Altitude	Safety tested up to 2,000 m (6,562 ft)
Electromagnetic interference	Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). Strong electromagnetic radiation may interfere with the proper operation of the device.
Transient/overvoltage category	Installation categories II
Vibration	The instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.
Pollution degree	II Install the instrument in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas.

Condition	Requirement
Operating conditions	• 15–30°C (59–86°F) (Room temperature should not fluctuate ±2°C during an instrument run)[1] • 20–80% relative humidity, noncondensing
Transport and storage conditions	–30 to +60°C (–22 to +140°F) Humidity: minimum 5%, maximum 95% (average in a year <80%)
Liquid waste collection	Dispose of the polymer, buffer, reagents and any liquid waste as hazardous waste in compliance with local and national regulations.
Other conditions	Ensure the room is away from any vents that could expel particulate material on the components. Avoid placing the instrument and computer adjacent to heaters, cooling ducts, or in direct sunlight.

[1] At temperatures of <20°C, contiguous read lengths or fragment analysis reads may be shorter. In those cases, you can choose to extend the run time. Increase the run time in increments of 300 seconds to find the optimum setting.

Electrical requirements

CAUTION! Do not unpack or plug in any components until they are configured for the proper operating voltage by the service representative.

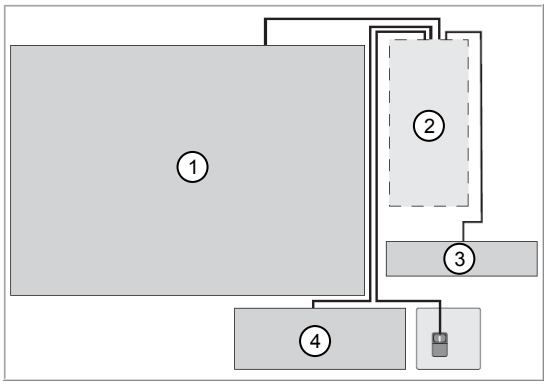
WARNING! For safety, the power outlet for the instrument must be accessible at all times. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

- Dedicated line and ground between the instrument and the main electrical service.
- Maximum power dissipation: 600 VA (not including computer and monitor)
- Mains AC line voltage tolerances must be up to ±10 percent of nominal voltage
- Instrument—AC 100–240 V ±10%, 50/60 Hz, 6.7 A, power rated 600 VA
- Maximum current—6.7 A

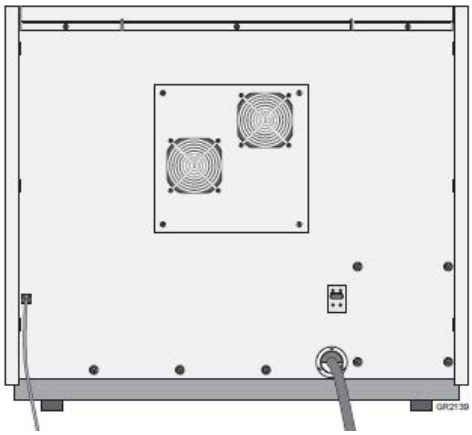
Device	Rated voltage	Circuit required	Rated frequency	Rated power
Instrument	100–240 ±10% VAC[1]	10 A	50/60 Hz	600 VA
Computer (desktop)	100–240 ±10% VAC	10 A	50/60 Hz	125 VA
Monitor				65 VA

[1] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

Power and communication connections



① Instrument
② Computer
③ Monitor
④ Keyboard



Because of the Ethernet cable connection between the instrument and the computer, the computer must be located within 9 feet of the instrument.

Run modules

Fragment analysis run times

Polymer	Capillary Array Length (cm)	Run Module	Approximate Run Time (min)
POP-7™	36	GeneMapper36_POP7_1	35
	50	GeneMapper50_POP7_1	45
	36	GS1200LIZ_36_POP7_v2_1	130
	50	GS1200LIZ_50_POP7_v2_1	135

Sequence analysis run times

Polymer	Capillary Array Length (cm)	Sequencing Run	Sequencing Run Modules	Approximate Run Times[1] (minutes)	KB™ Basecaller QV20 CRL (Bases)[2]
POP-7™	50	Extra long read	XLRSeq50_POP7	180	≥900
			BDx_XLRSeq50_POP7[3]		
	50	Long read	LongSeq50_POP7	120	≥850
			BDx_LongSeq50_POP7[3]		
	50	Fast read	FastSeq50_POP7	60	≥700
			BDx_FastSeq50_POP7[3]		
	36	Standard read	StdSeq36_POP7	60	≥700
			BDx_StdSeq36_POP7[3]		
	36	Rapid read	RapidSeq36_POP7	35	≥550
			BDx_RapidSeq36_POP7		
	36	Short read	TargetSeq36_ POP7	20 [4]	≥400[4]

Polymer	Capillary Array Length (cm)	Sequencing Run	Sequencing Run Modules	Approximate Run Times[1] (minutes)	KB™ Basecaller QV20 CRL (Bases)[2]
POP-6™	50	Long read	LongSeq50_POP6	150	≥600
	36	Standard read	StdSeq36_POP6	60	≥500

[1] These approximate run times assume oven temperature has reached run temperature

[2] Contiguous read length with 98.5% basecalling accuracy, and less than 2% N's, using pGEM™-32f (+) as template.

[3] Run the BDx utility after installation or after preventative maintenance is performed.

[4] Time stated for 400 bases. Module can be customized to run 200-400 bases.

IMPORTANT! Use BDX run modules only if you prepare samples with BigDye XTerminator™ Purification Kit. Use non-BDX run modules for samples purified with other methods.

D

Dye sets

Supported dye sets and calibration standards

Table 12 Sequencing dye sets and standards

Kit used for sequencing	Dye Set	Calibration Standards
BigDye™ Terminator v3.1 Cycle Sequencing Kit	Z_BigDyeV3	3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v3.1
BigDye™ Direct Cycle Sequencing Kit	Z_BigDyeV3	
BigDye™ Terminator v1.1 Cycle Sequencing Kit	E_BigDyeV1	3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v1.1

Table 13 Fragment analysis dye sets and matrix standards

Dye Set	Calibration Standards
D	DS-30 or DS-31
G5	DS-33
G5-RCT (reduced cross-talk)[1]	
J6	DS-36
Any4Dye, Any5Dye	Custom
Any4Dye-HDR (high dynamic range)[2, 3]	Custom
Any5Dye	DS-02 for SNaPshot™ applications Custom

[1] Use for fragment analysis applications on 96-capillary arrays.

[2] 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

[3] When using this dye set, perform spectral calibrations each time the capillary array is replaced or moved within the detection cell

Dye sets G5 and G5-RCT for fragment analysis

Fragment analysis using a 96-capillary array: cross-talk peaks

In fragment analysis applications using a 96-capillary array, a small peak in one color channel that occurs at exactly the same size as a major peak in another color channel can be incorrectly identified as an allele. This phenomena is referred to as "cross-talk" between the different spectral channels.

Reduced cross-talk—Dyeset G5-RCT

The G5-RCT (Reduced Cross Talk) dye set has been optimized to minimize cross talk peaks.

- RCT dye sets reduce potential crosstalk peaks, but also cause a decrease in overall signal intensity.
- RCT dye sets slightly reduce signal-to-noise ratio.
- Because of the decrease in overall signal intensity, a higher dynamic range is available when using RCT dye sets.
- The spectral calibration for an RCT dye set is specific to the capillary array on which is it run. Do not ovveride spectral calibration when you use an RCT dye set.

When to use RCT dye sets

- Fragment analysis with a 96-capillary array
- Applications with a high dynamic range (where the signal intensity of large peaks is much higher than the signal intensity of small peaks)

Catalog numbers

Plates, bases, retainers, and septa

IMPORTANT! 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.

IMPORTANT! 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.

Item	Cat. No.
96 well	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
Plate Septa, 96-well	4315933
Plate Base (septa seal), 96 well	4334873
Plate Base (heat seal), 96 well	4334875
Plate Retainer (septa seal), 96 well	4334869
96-Well and 384-Well Plate Retainer (heat seal)	4334865
FAST 96 well	
Plate Retainer for 3730l (septa seal), Fast (0.1mL), 96 well	4367472
Plate Base for 3730 (septa seal), Fast (0.1 mL), 96 well	4367469
Plate Retainer (heat seal) for 3730 Systems , Fast (0.1mL), 96 well	4367474
Plate Base (heat seal) for 3730 Systems, Fast (0.1 mL), 96 well	4367473
384 well	
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	4309849
Plate Septa, 384 well	4315934
Plate Base (septa seal) for 3730/3730*xl*, 384 well	4334874

Item	Cat. No.
Plate Base (heat seal) for 3730/3730*xl*, 384 well	4334877
Plate Retainer (septa seal) for 3730/3730*xl* systems, 384 well	4334868
Heat Seal Film for Sequencing and Fragment Analysis Sample Plates	4337570

Instrument consumables

Item	Cat. No.
Capillary arrays	
3730 DNA Analyzer 48-Capillary Array, 36-cm	4331247
3730 DNA Analyzer 48-Capillary Array, 50-cm	4331250
3730*xl* DNA Analyzer 96-Capillary Array, 36-cm	4331244
3730*xl* DNA Analyzer 96-Capillary Array, 50-cm	4331246
POP polymer	
POP-7™ Polymer 1 x 28 mL	4363929
POP-7™ Polymer 10 x 28 mL	4363935
POP-6™ Polymer 1 x 7 mL	4352757
Running buffer	
3730 Running Buffer (10X) 500 mL	4335613
Miscellaneous	
Hi-Di™ Formamide	4311320
Polymer Block Cleaning Kit	4335860
SNaPshot™ Multiplex Kit	4323159

Sequencing and fragment analysis calibration standards

Item	Cat. No.
Sequencing	
3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v3.1	4336943
3730/3730xl DNA Analyzer Sequencing Standards, BigDye™ Terminator v1.1	4336799
Fragment analysis	
DS-30 Matrix Standard Kit (Dye Set D)	4345827
DS-31 Matrix Standard Kit (Dye Set D with VIC™ dye)	4345829
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
DS-36 Matrix Standard Kit (Dye set J6, 6-dye)	4425042
DS-02 Matrix Standard Kit (Dye Set E5)	4323014

Fragment analysis size standards

Name	Part Number
DS-33 GeneScan™ Installation Standards with GeneScan™ 600 LIZ™ Size Standard v2.0	4376911
GeneScan™ 120 LIZ™ Size Standard	4324287
GeneScan™ 500 ROX™ Size Standard	401734
GeneScan™ 600 LIZ™ Size Standard v2.0	4408399
GeneScan™ 1200 LIZ™ Size Standard	4379950

F

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, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

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.

- **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.

Safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.

Symbol and description
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Location of safety labels

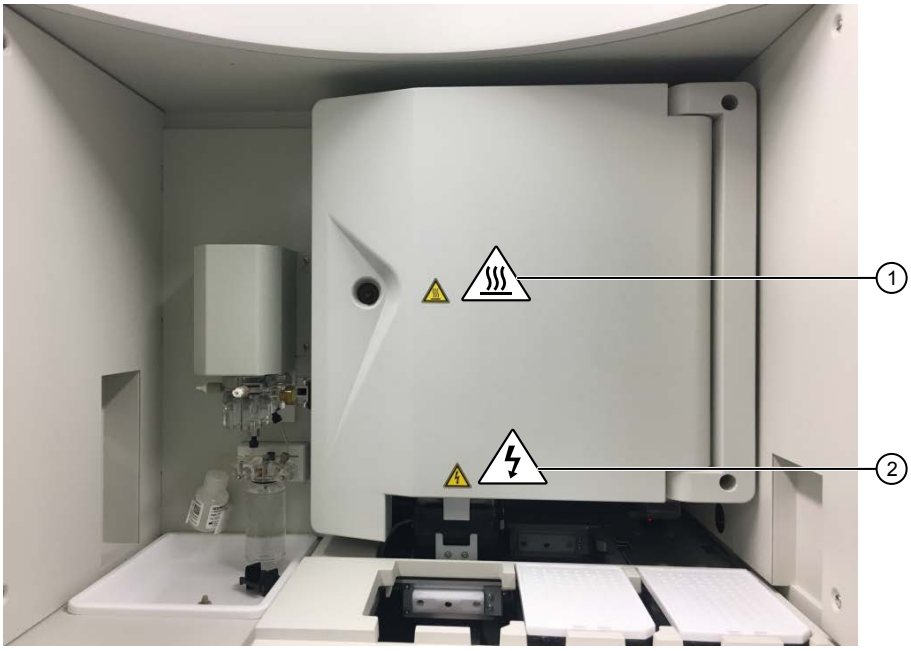


Figure 22 Safety symbols that are visible with the oven door closed

① Physical hazard
② Electric shock

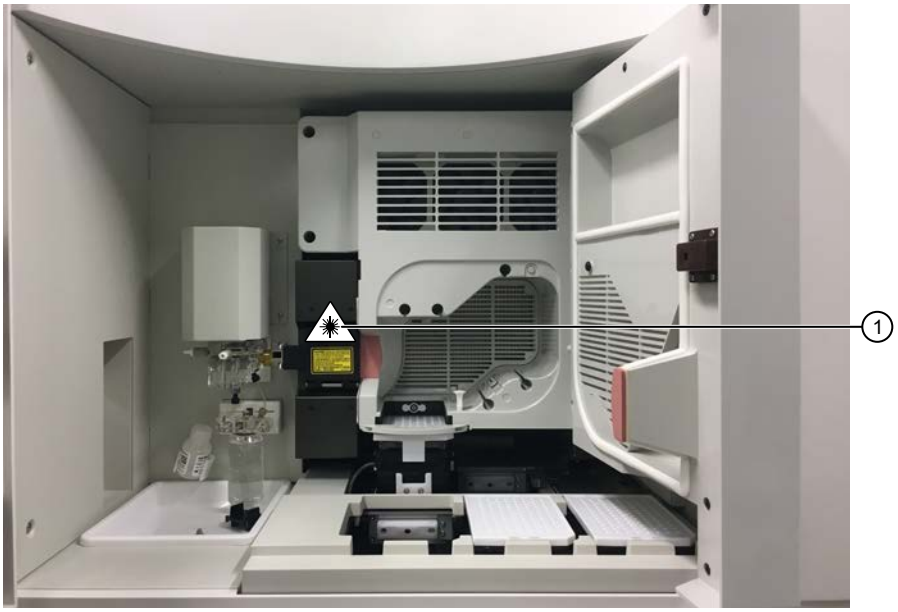


Figure 23 Safety symbols that are visible with the oven door open

① Laser

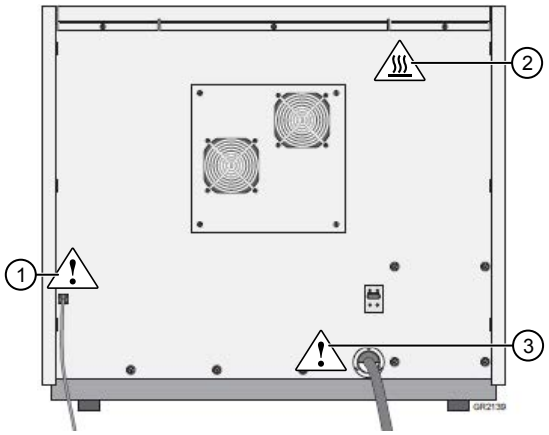


Figure 24 Safety symbols on the rear panel

① Attention
② Physical hazard
③ Attention

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Alternating current
	Both direct and alternating current

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.

Conformity mark	Description
CE	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with the WEEE Directive 2012/19/EU. ⚠ **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.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

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.

Physical injury

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 safety

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.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

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

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

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

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

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.

Laser safety

DANGER! LASER HAZARD. Under normal operating conditions, the 3730xl DNA Analyzer is categorized as a Class 1 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. 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 a Thermo Fisher Scientific Technical Representative.
- 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.

The following table lists laser safety symbols and alerts that may be present on the instrument.

Alert
DANGER! Class 3B (III) visible and/or invisible laser radiation present when open and interlocks defeated. Avoid exposure to beam.
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.

Alerte (français)
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.
MISE EN GARDE ! RISQUE LIÉ AU RAYONNEMENT LASER, Lecteur de code-barres. Le lecteur de code-barres inclut dans l'instrument est un appareil laser de classe 2. Pour éviter toute lésion oculaire, ne regardez pas directement le faisceau et ne le dirigez pas vers les yeux d'une autre personne.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/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	*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 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 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 standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Reference	Description
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures: • Reorient or relocate the receiving antenna. • Increase the separation between the equipment and receiver. • Connect the equipment into an outlet on a circuit different from that to which the receiver is connected. • Consult the dealer or an experienced radio/TV technician for help.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

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. 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.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l’utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d’utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).

- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
3730xl DNA Analyzer with 3730xl Data Collection Software 5 Quick Reference	100077622
3730xl DNA Analyzer Site Preparation Guide	100077623
Manage data before upgrading to 3730xl Data Collection Software 5	MAN0018320
DNA Sequencing by Capillary Electrophoresis Chemistry Guide Second Edition	4305080
DNA Sequencing by Capillary Electrophoresis Chemistry Guide Third Edition	COL02120 0716 **thermofisher.com/sangerhandbook**

Table 14 Documents for related products

Document	Publication number
AB Navigator Software Administrator Guide	4477853
BigDye XTerminator™ Purification Kit Protocol	4374408
SeqScape™ Software 3 User Guide	4474242
DNA Sequencing Analysis Software 6	4474239
GeneMapper™ Software v4.1 Quick Reference Guide	4403615

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support

- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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