

USER GUIDE

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by *life* technologies™

Applied Biosystems® 3500 / 3500xL Genetic Analyzer

3500 Series Software 2

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About This Guide



CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the “Safety” appendix in this document.

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

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.



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.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety” appendix for descriptions of the symbols.

1

Instrument and Software Description

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Instrument description

Overview

The Applied Biosystems® 3500 / 3500xL Genetic Analyzer with 3500 Series Software 2 (3500 Series Genetic Analyzer) is a fluorescence-based DNA analysis instrument using capillary electrophoresis technology with 8- or 24-capillaries.

For information about instrument dimensions and connections, refer to Appendix C, Instrument Specifications.

Both the 8-capillary model and the 24-capillary xL model are shipped with the following system components:

- Capillary Electrophoresis instrument.
- 3500 (8-capillary) or 3500xL (24-capillary) array and POP™ polymer
- DNA sequencing and/or fragment analysis reagents, and other consumables for system qualification.
- Dell® computer workstation with flat-screen monitor.
- Integrated 3500 Series Software 2 for instrument control, data collection, quality control, and basecalling and sizing of samples where appropriate.



IMPORTANT! The protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer (Life Technologies).

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

Antivirus software requirements

The computer provided with the instrument does not include antivirus software because customer preferences and network requirements vary. Therefore, you need to install antivirus software of your choice to protect the computer against viruses.

We recommend Norton Antivirus, which has been tested and approved for use with the Applied Biosystems 3500 / 3500xL Genetic Analyzer .



CAUTION! Do not install on the computer additional software other than antivirus software. Changes to the configured software could void the instrument warranty and cause the system to be nonoperational.

Instrument interior components

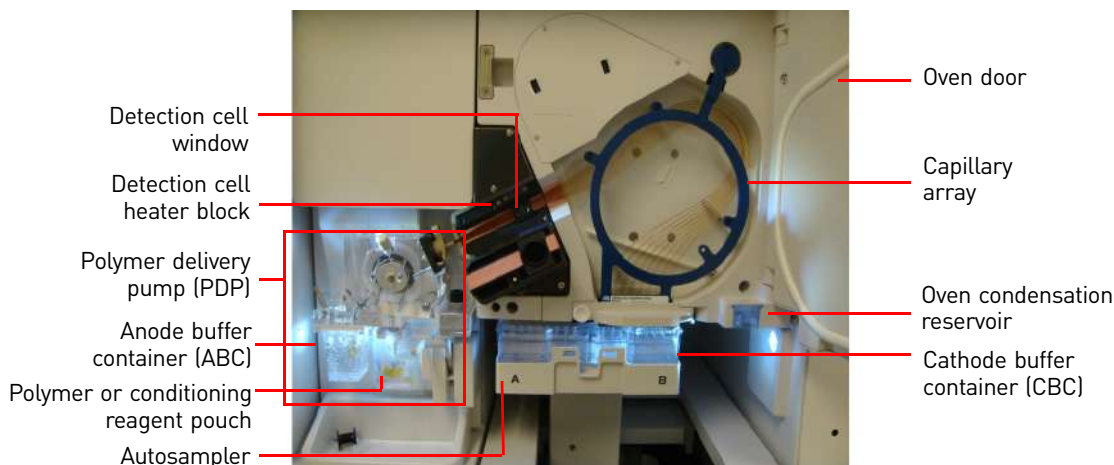


Figure 1 Instrument interior

Instrument parts and functions

Table 1 Instrument parts and functions

Part	Function
Anode buffer container (ABC)	The anode buffer container (ABC) contains 1X running buffer to support all electrophoresis applications on the instrument. It has a built-in overflow chamber to maintain constant fluid height.
Autosampler	Holds the sample plates and cathode buffer container (CBC) and moves to align the plates and CBC with the capillaries.
Capillary array	Enables the separation of the fluorescent-labeled DNA fragments by electrophoresis. It is a replaceable unit composed of 8 or 24 capillaries (50 cm length).
Cathode buffer container (CBC)	The cathode buffer container (CBC) contains 1 running buffer to support all electrophoresis applications on the instrument.
Detection cell heater block and detection cell window	Holds the detection cell in place for laser detection and maintains the detection cell temperature of 50°C.
Oven/oven door	Maintains uniform capillary array temperature.
Oven condensation reservoir	Collects condensation from the oven.
Polymer delivery pump (PDP)	Pumps polymer into the array and allows for automated maintenance procedures. Includes the displacement pump chamber, polymer chambers, piston water seal, capillary array port, check valve fitting, water trap waste container, buffer valve, anode electrode, buffer gasket, and holds the anode buffer container
Polymer pouch or conditioning reagent pouch	<ul style="list-style-type: none">• Polymer pouch – Supplies polymer to the polymer delivery pump.• Conditioning reagent pouch – Used for priming the polymer pump, washing the polymer pump between polymer type changes, and during instrument shut down. It has adequate volume for a one-time use.
Radio frequency identification (RFID)	<p>RFID tags on primary instrument consumables:</p> <ul style="list-style-type: none">• Capillary array• Cathode buffer container (CBC)• POP™ polymer• Anode buffer container (ABC) <p>The instrument reads and records the following consumable information:</p> <ul style="list-style-type: none">• Lot numbers• Serial numbers• Dates (expiration)• Capacity (usage) <p>RFID tags are read and written in response to a user action (for example, running a wizard or starting a run). All dashboard values are updated when RFID tags are read and written. The days on Instrument is also updated automatically every 10 minutes.</p>

Theory of operation

The 3500 Series Genetic Analyzer is a fluorescence-based DNA analysis system that uses proven capillary electrophoresis technology with 8- or 24-capillaries.

The 3500 Series Genetic Analyzer is fully automated, from sample loading to primary data analysis, for sequencing and fragment analysis.

Note: In this document, primary analysis for sequencing is referred to as basecalling. Primary analysis for fragment analysis and HID analysis is referred to as sizecalling.

Preparing samples

When DNA samples are prepared for sequencing, fragment analysis, or HID analysis on the 3500 Series Genetic Analyzer, 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, refer to “Spatial calibration” on page 105.
- **Spectral calibration** – Generates a matrix for each capillary that compensates for dye overlap and is used to convert the 20-color data into 4-dye data. For more information, refer to “Spectral calibration” on page 109.

During a run

During a run, the system:

- Prepares the capillary by pumping fresh polymer solution under high pressure from the polymer delivery pump to the waste position in the cathode buffer container (CBC).
- Electrokinetically injects the sample into the capillary using a low-voltage for a few seconds.
- Washes the capillary tips in the rinse position of the CBC, then returns the capillary to the buffer position of the CBC.
- Ramps the voltage up to a constant level.

A high electric field is created between the ground end of the anode buffer container (ABC) and the negative voltage 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. The oven has a Peltier heat unit and fan-circulated air.

In the detection cell, the dyes 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.

- Captures the fluorescent light on the instrument optics while blocking the laser light. The light passes through a transmission grating, which spreads the light out. The light is imaged onto a cooled, scientific-grade CCD array. For each capillary, 20 zones on the CCD are collected to provide 20-color data for each capillary.
- Converts the 20-color 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.

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 and HID analysis, the software uses the internal size standard to assign a fragment size and a sizing quality value to each peak.

Materials for routine operation

Contact your local Life Technologies service representative, or go to lifetechnologies.com, then click **Products**, to order the materials for the 3500 Series Genetic Analyzer.

Instrument reagents and consumables

For information on limits and expiration, see “Consumables usage limits and expiration” on page 22.

For application-specific reagents, consumables, and run modules, see Appendix A, Application Reagents and Run Modules.

Anode buffer container (ABC)

The ABC (Part no. 4393927) contains 1X running buffer to support all electrophoresis applications on the 3500 Series Genetic Analyzer.

The ABC is provided in a ready-to-use, disposable container with a radio frequency identification (RFID) tag incorporated into the label. The container has a built-in overflow chamber to maintain constant fluid height.

Store the ABC at the temperature specified on the product insert until ready to use. The sealed ABC is stable at this temperature until the expiration date shown on the label. Once open, the buffer is stable at ambient temperature for up to 7 days. Do not open until just before installing on the instrument.

To ensure optimal performance, the use of the ABC is limited to either 7 days after the first installation or 120 injections on a 3500 (8-capillary)/50 injections on a 3500xL (24-capillary), whichever comes first. After 7 days, the software displays a message indicating that you must replace the ABC.

For more details see the product insert included in the product package.

See “Change the anode buffer container (ABC)” on page 225 for instructions on how to change the ABC.

Cathode buffer container (CBC)

The CBC (Part no. 4408256) contains 1 running buffer to support all electrophoresis applications on the 3500 Series Genetic Analyzer.

The CBC is provided in a ready-to-use, disposable container with a radio frequency identification (RFID) tag incorporated into the label. The container has two separate sides:

- The side containing 24 holes provides the cathode buffer for electrophoresis.
- The side containing 48 smaller holes provides the liquid for rinsing the capillary tips and collecting wash waste between injections.

Store the CBC at the temperature specified on the product insert until ready to use. The sealed CBC is stable at this temperature until the expiration date shown on the label. Once opened, the buffer is stable at ambient temperature for up to 7 days. Do not open until just before installing on the instrument.

To ensure optimal performance, the use of the CBC is limited to either 7 days after the first installation or 120 injections on a 3500 (8-capillary) or 50 injections on a 3500xL (24-capillary), whichever comes first. After 7 days, the software displays a message indicating that you must replace the CBC.

For more details see the product insert included in the product package.

See “Change the cathode buffer container (CBC)” on page 226 for instructions on how to change the CBC.

Polymers

The polymer for 3500 Series Genetic Analyzer is provided in a ready-to-use pouch containing POP polymer.

IMPORTANT! Do not reuse a polymer pouch that has been installed on another type of instrument. For example, if you install a new polymer pouch on a 3500 (8-capillary) instrument, do not reuse that polymer on a 3500xL (24-capillary) instrument.

The pouch contains adequate polymer to support the stated number of samples (384 or 960) or injections and additional volume to handle installations and setup related wizard operations. Incorporated into the label is a radio frequency identification (RFID) tag.

Note: The top part of the pouch fitment is sealed with a plastic film, which should be removed prior to installation on to the instrument.

Store the polymer at 2°C to 8°C until ready to use. The sealed polymer is stable at this temperature until the expiration date shown on the label.

For more details see the product insert included in the product package.

See “Change polymer type” on page 234, for instructions on how to change the polymer type.

IMPORTANT! If you remove a polymer pouch for storage, place a pouch cap (Part no. 4412619) onto the pouch, then place an empty pouch (or conditioning reagent) on the connector to prevent desiccation of any residual polymer on the connector. Follow the instructions in the wizard to ensure the proper operation of the pouch and the instrument.

Polymer type	Part no.
POP-6™ (960)	4393712
POP-6™ (384)	4393717
POP-7™ (960)	4393714
POP-7™ (384)	4393708
POP-4® (960) Validated for HID applications	4393710
POP-4® (384) Validated for HID applications	4393715

Conditioning reagent

The conditioning reagent (Part no. 4393718) for 3500 Series Genetic Analyzer is provided in a ready-to-use pouch. It is used for priming the polymer pump, washing the polymer pump between polymer type changes, and during instrument shut down. It has adequate volume for a one-time use.

Maintenance wizards in the software indicate when to install conditioning reagent.

For more details see the product insert included in the product package.

Hi-Di™ Formamide

Hi-Di™ Formamide (pack of four) 5-ml tube (Part no. 4440753) is a highly deionized formamide, formulated with a stabilizer, ready for use as an injection solvent for all applications on the 3500 Series Genetic Analyzer.

IMPORTANT! Keep the formamide formulation frozen at –15° to –25°C to maintain long term storage stability. If necessary, the formulation may be stored at 2°C to 8°C for up to 1 week. However, the quality of the formulation may decrease if not stored frozen and if exposed to air through frequent sampling. If frequent sampling is required, dispense and freeze small aliquots into smaller tubes, to minimize freeze-thaw cycles, sampling, and exposure to air and room temperature.

For more details, see the product insert included in the product package.

Capillary arrays

The capillary array for 3500 Series Genetic Analyzer is installed on the instrument and ready to use.



WARNING! SHARP The load-end of the capillary array has small but blunt ends that can lead to piercing injury.

See “Change the capillary array” on page 238 for instructions on how to change the capillary array.

Capillary array name	Part no.
8-Capillary, 36 cm	4404683
8-Capillary, 50 cm	4404685
24-Capillary, 36 cm	4404687
24-Capillary, 50 cm	4404689

HID applications have been validated on 36-cm capillary arrays only.

Consumables usage limits and expiration

Anode and Cathode buffers

Instrument	On-instrument limits (the first limit met applies)	Recommendation
8-capillary	7 days or 120 injections [†]	Do not use after the expiration date on the package.
24-capillary	7 days or 50 injections	

[†] An injection is an instance of 8 or 24 samples (depending on instrument configuration) processed simultaneously under the same conditions.

The software does not allow you to run when the buffers exceed the 7-day on-instrument limit. The 7-day limit on buffers minimizes deleterious effects caused by evaporation and ion depletion.

Capillary array

Capillary array	Guaranteed for	Recommendation
8-capillary	160 injections [†]	Replace after 160 injections.
24-capillary		Do not use after the expiration date on the package.

[†] An injection is an instance of 8 or 24 samples (depending on instrument configuration and assuming all wells contain sample) processed simultaneously under the same conditions.

To ensure optimal performance, replace the capillary array after 160 injections. Although the software allows you to run beyond 160 injections or the capillary array expiration date, use at your own risk. Life Technologies has verified the array for 160 injections.

Polymer

Instrument		On-instrument limits (the first limit met applies) (see “How the polymer sample and injection counters calculate usage” on page 23 for more information)	Recommendation
8-cap	960 sample pouch	960 samples or 120 injections [†]	Replace after 7 days on instrument.
	384 sample pouch	384 samples or 60 injections	Do not use after the expiration date on the package.
24-cap	960 sample pouch	960 samples or 50 injections	
	384 sample pouch	384 samples or 20 injections	

[†] An injection is an instance of 8 or 24 samples (depending on instrument configuration and assuming all wells contain sample) processed simultaneously under the same conditions.

To ensure optimal performance, replace polymer after 7 days. Although the software allows you to run with polymer that has been on the instrument for more than 7 days or with polymer that is expired, use at your own risk. LifeTechnologies has verified the polymers for up to 7 days only on the instrument.

Each polymer pouch includes additional volume to accommodate for the volumes used during installation, when running wizards, and during routine operation.

Note: Excessive use of wizards reduces the number of remaining samples and injections, based on how many times specific wizards are run. For example, if you run the total bubble remove option in the Remove Bubbles wizard more than four times or run other wizards excessively, including multiple pouch installations, the number of remaining samples and injections is reduced.

How the polymer sample and injection counters calculate usage

The Polymer Sample Counter decrements only for wells that contain sample, but the Polymer Injection counter decrements for each injection, regardless of whether all wells contain sample. The sample limit and the corresponding injection limit may not coincide. The first limit that is reached depends on whether you use partial or full injections.

Instrument configuration: 24-capillary, 960 sample polymer pouch		
Partial injection example (not all wells contain sample)	1 injection with 24 samples + 49 injections with 1 sample = 73 samples, 50 injections	The 50 injection count limit is reached before the 960 sample count limit.
Full injection example (all wells contain sample)	40 injections with 24 samples = 960 samples, 40 injections	The 960 sample count limit is reached before the 50 injection count limit.

Conditioning reagent

Conditioning reagent is single-use.

Overview of the 3500 Series Software 2

About the software

During a run, the software:

- Controls the instrument and generates sample data files:
 - Sequencing (.ab1)
 - Fragment analysis (.fsa)
 - HID analysis (.hid)
- Performs primary analysis and provides reports that evaluate the quality of the data:
 - Sequencing – Basecalling and trimming
 - Fragment and HID analysis – Peak detection and sizing.



Parts of the software

Dashboard

The first screen that is displayed when you start the 3500 Series Software 2 is the Dashboard (Figure 2 on page 25).

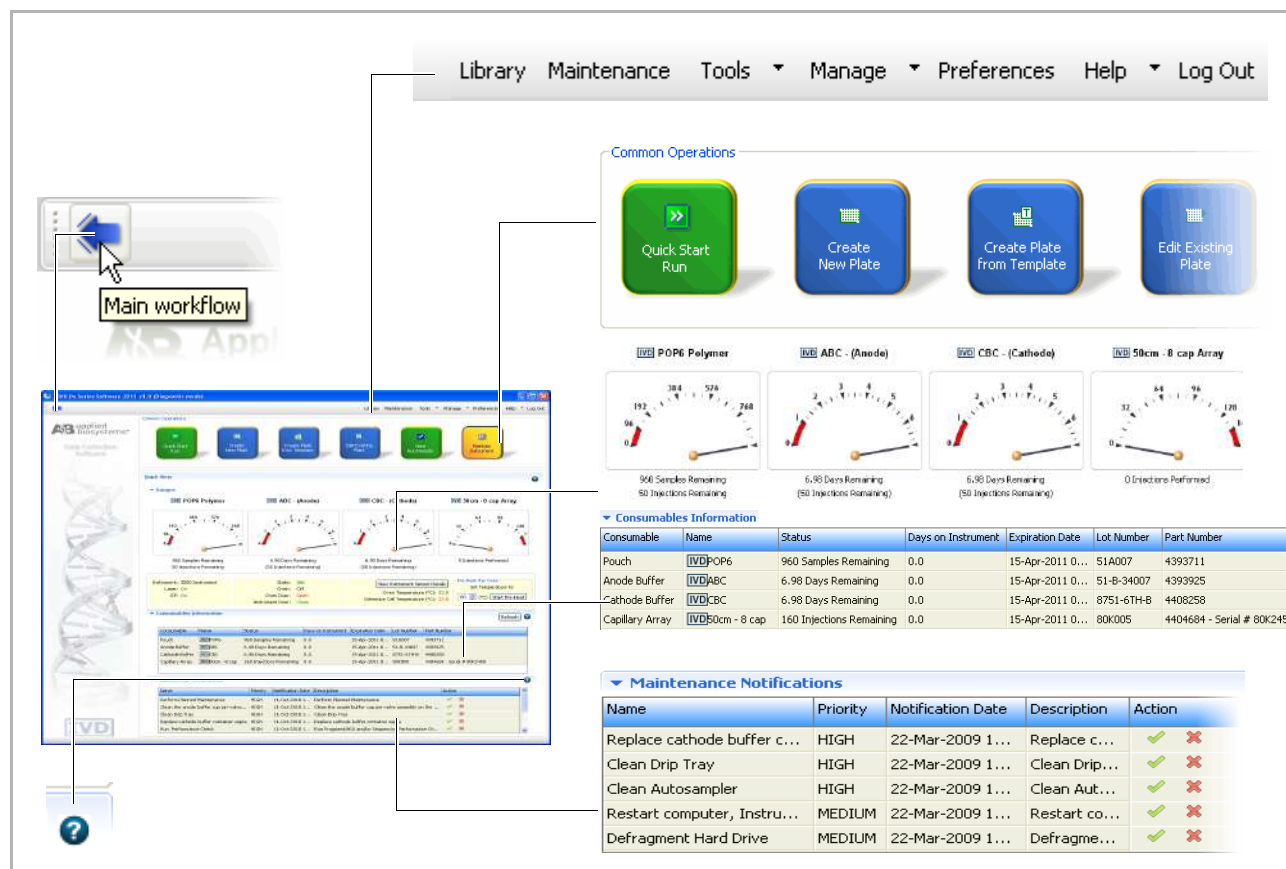


Figure 2 3500 Series Software 2 Dashboard overview

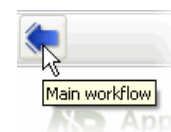
The Dashboard gives you quick access to the information and tasks you need to set up and run:

- **Main workflow arrow** – Advances to the set up, load, and run plates, and view results screens.
- **Menu bar** – Accesses all other parts of the software. The menu bar is displayed on all screens.
- **Common operations** – Allows you to quick-start (load a plate that is set up), create or edit plates, view results, and access the Maintenance workflow.
- **Quick view** – Displays gauges that show the remaining usage of consumables and gives the status of instrument conditions. Consumable usage is automatically tracked by the instrument by RFID tags.
- **Consumables information** – Gives details for the installed consumables and indicates if any consumable is about to expire based on RFID tags.
- **Maintenance notifications** – Lists the scheduled maintenance tasks.
- **Help icon** – Displays a help topic specific to a screen or an area of the screen. All screens include icons.

For more information, see “Check system status in the Dashboard” on page 36.

Main workflow

Click the Main workflow arrow at the top left of the Dashboard to access the Main workflow.

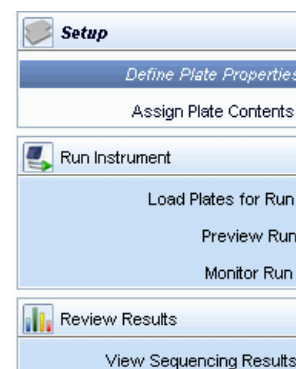


The Main workflow contains the screens where you set up, load, and run plates, and view results.

Select a task in the navigation pane to access each screen.

The Main workflow navigation pane is designed as a task workflow. Each screen contains a button that you can click to advance to the next screen in the workflow.

You can select **Dashboard** or any other menu item at any time to advance from the Main workflow.



The Main workflow is described in Chapter 3, “Set Up and Run” on page 51, and Chapter 4, “Review Results” on page 85.

Library workflow

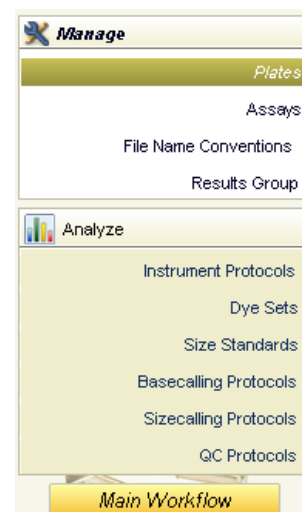
Select **Library** in the menu bar to access the Library workflow.



The Library workflow contains screens where you manage plates, assays, file name conventions, and results groups that you use to acquire and process data.

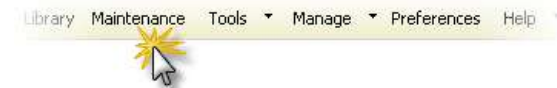
You can click **Main Workflow** or select **Dashboard** or any other menu item at any time to advance from the Library workflow.

The Library workflow is described in Chapter 6, “Manage Library Resources” on page 143.



Maintenance workflow

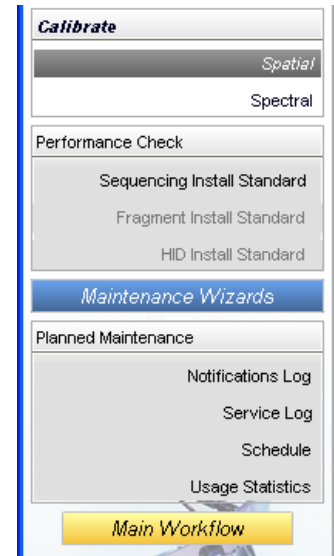
Select **Maintenance** in the menu bar to access the Maintenance workflow.



The Maintenance workflow contains the screens where you calibrate, check performance, run maintenance procedures, and access records about instrument maintenance and service.

You can click **Main Workflow**, or select **Dashboard** or any other menu item at any time to advance from the Maintenance workflow.

The Maintenance workflow is described in Chapter 8, “Maintain the Instrument” on page 219.

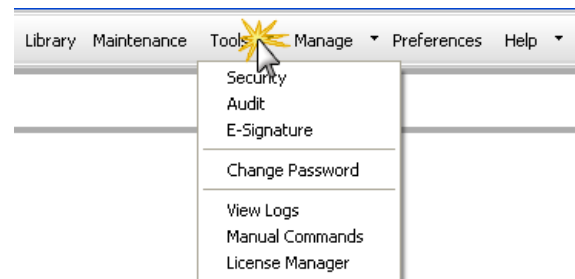


Tools menu

Select **Tools** in the menu bar to access 3500 Series Software 2 tools.

Tools provided are:

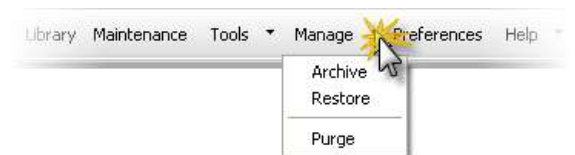
- Security, Audit, and E-signature
- Change Password
- View Logs provides reports of instrument runs
- Manual Commands to troubleshoot instrument performance
- License Manager to renew the software license



The SAE module is described in Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.

Manage menu

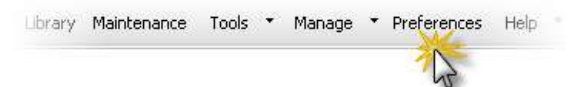
Select **Manage** in the menu bar to access Archive, Restore, and Purge functions.



Archive, Restore, and Purge are described in Chapter 7, Use Security, Audit, and E-Sig Functions (SAE Module).

Preferences menu

Select **Preferences** in the menu bar to access the parameters to set defaults.



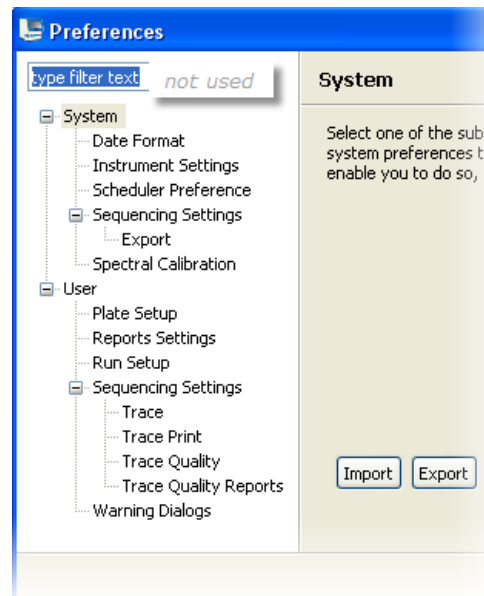
Preferences allow you to set system and user defaults for settings such as the date format, sample data file storage location, export file formats for sequencing data, and a variety of sequencing-specific settings.

System defaults apply to all users.

User defaults apply to:

- **All users** – If your system does not include the SAE module.
- **Each logged-in user** – If your system includes the SAE module.

Preferences are described in Chapter 2, “Start the System” on page 31.

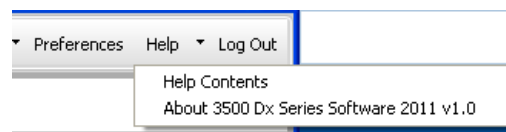


Help menu

Select **Help** in the menu bar to access 3500 Series Software 2 Help.

The Help menu provides quick access to brief information about how to perform tasks on a screen.

For details about tasks and other information, refer to the chapters in this user guide.



Navigate the software

From the Dashboard

To advance from the Dashboard to:

- **Main workflow** – Click .
- **Other screens in the software** – Select items from the menu bar.



From the Main workflow

To advance from the Main workflow to:

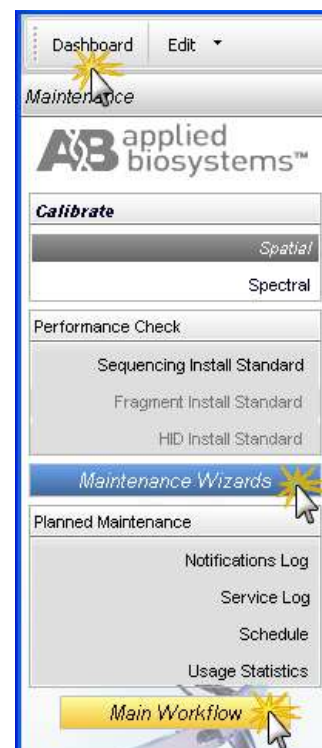
- **Dashboard** – Click **Dashboard**.
- **Other screens in the Main workflow** – Select items in the navigation pane.
- **Other screens in the software** – Select items from the menu bar.



From the Library or Maintenance workflows

To advance from the Maintenance or Library workflow to:

- **Dashboard** – Click **Dashboard**.
- **Other screens in the workflow** – Select items in the navigation pane.
- **Main workflow** – Click **Main Workflow** in the navigation pane.
- **Other screens in the software** – Select items from the menu bar.



Assays, protocols, file name conventions, and results groups

The 3500 Series Software 2 uses the following elements to specify settings for data collection. Note that an instrument protocol is part of an assay (described below).

New concept	Specifies settings for...
Primary Analysis protocols and templates: <ul style="list-style-type: none"> – Sequencing – basecalling – Fragment and HID analysis–sizecalling 	<ul style="list-style-type: none"> • Basecalling • Sizecalling
File Name Convention and templates	File naming
Results Group and templates	Naming, sorting, and customizing the folders in which sample data files are stored
Assay and assay templates	Data collection and processing. It contains: <ul style="list-style-type: none"> • Instrument protocol (dye set and run configuration) • Primary analysis (basecalling or sizecalling) protocol

New concept	Specifies settings for...
Plate template	Plate parameters. Can also contain: <ul style="list-style-type: none">• Assays• File name conventions• Results groups

Note: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).

Use the software without an instrument

You can install the 3500 Series Software 2 on a computer that is not connected to an instrument (see “Minimum Computer Requirements” on page 283). You can use this stand-alone version of the software to:

- Create plates, protocols, and other library items, then import them into a version of the software that is installed on the instrument computer
- Review completed results

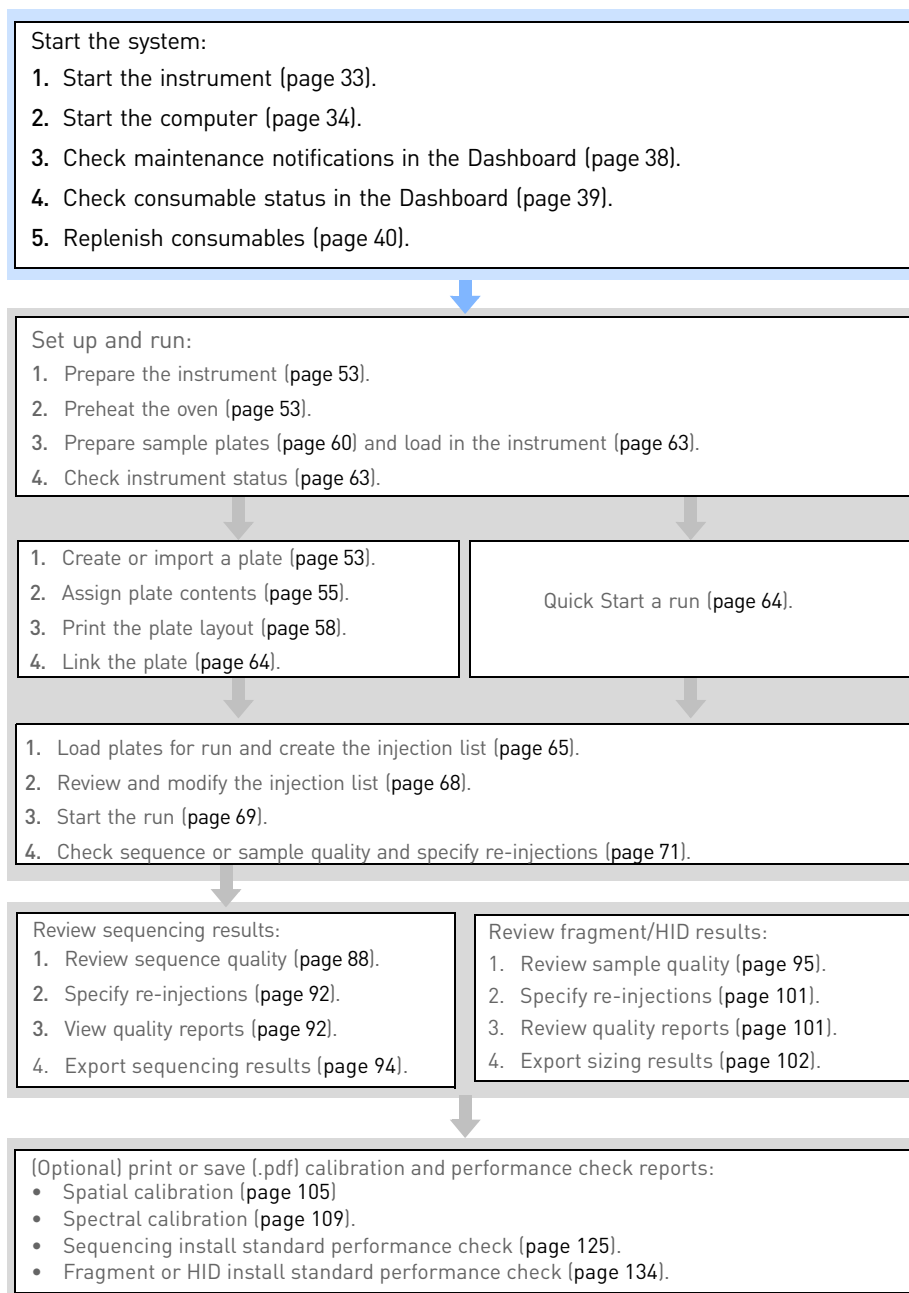
IMPORTANT! Do not select instrument-related functions in the stand-alone version of the software.

2

Start the System

- Workflow 32
- Start the instrument 33
- Start the computer 34
- Check system status in the Dashboard 36
- Set preferences 41

Workflow



Start the instrument

1. Verify that the instrument is connected to the appropriate power supply.



CAUTION! Do not unpack or plug in any components until the Life Technologies service representative has configured the system for the proper operating voltage.

See the *Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide* (Part no. 4401689) for details.

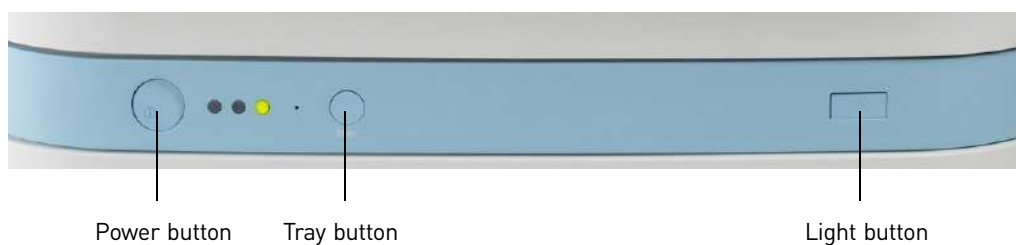
Note: The purpose of the Site Prep Guide is to help you prepare your site for installation of the 3500 Series Genetic Analyzer. For specific details about your system, please refer to this user guide.

IMPORTANT! Do not rename the computer after the 3500 Series Software 2 has been installed. The instrument computer has been assigned a unique name. Changing the name may cause the 3500 Series Software 2 to malfunction.

2. Inspect instrument interior. Ensure that:
 - a. Oven door is closed.
 - b. No objects are left inside the instrument.

IMPORTANT! Misplaced objects left inside the instrument can cause damage.

3. Close instrument door.
4. Power on the instrument.



- a. Press the power on/off button on the front of the instrument and wait for the green status light to turn on.

Note: If the door is open during power on, the yellow light will continue to flash until you close the doors. See indicator descriptions below.

- b. If desired, press the Light button to turn on the interior light.

- c. Check the instrument status. Ensure the green status light is on and not flashing before proceeding. The table below explains the status indicator lights for the instrument.

Indicator	Status
All lights off	Instrument off
Green light	Operational (awaiting run)
	Pause run, terminate run, stop injection button (in SW) pressed by user. Note: You can only abort an injection when the green light is flashing, not when it is solid green.
Green light (blinking)	Operational (Run in progress)
Amber light (blinking)	Power-up self-test in progress
	Run paused
	Door open
	Run failure that doesn't require restart of instrument
Amber light	Standby
Red light	Self-test failed
	Instrument failure
	Requires a restart of the instrument and computer

Start the computer

IMPORTANT! Do not start the computer until the green status light is on and not flashing.

1. Power on the computer and the monitor.
2. In the Log On to Windows dialog box:
 - a. Enter the user name.
 - b. If applicable, enter a password.
Note: If the computer is connected to a network, you do not need to log on to the network before starting the instrument.
 - c. Click **OK**. Wait until the computer completes start up.

Log on to Windows

Follow the prompts to log on to the Windows operating system.

Start the software


Step one: Start the Server Monitor

1. After you log in to Windows, wait ~1 to 2 minutes.
2. Look in the Windows taskbar at the bottom right of the desktop.


If the  Server Monitor icon is displayed, go to “Step two: Start the 3500 Series Software 2” below.




3. Select **Start ▶ Programs ▶ Applied Biosystems ▶ 3500 ▶ Server Monitor**.

The  Server Monitor icon is displayed in the taskbar, then a status bubble is displayed.

It takes ~ 1 minute for the Server Monitor to start up.

When the  Server Monitor icon is displayed, go to “Step two: Start the 3500 Series Software 2” below.

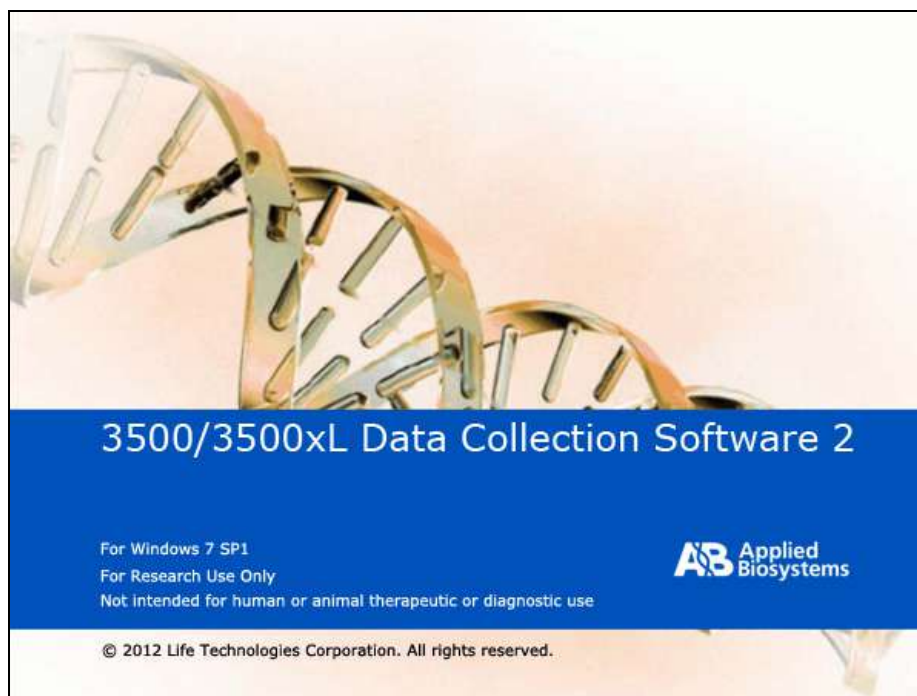


IMPORTANT! If the Server Monitor icon does not change to , you cannot start the software. See “Software troubleshooting – general” on page 264 for help.

Step two: Start the 3500 Series Software 2

Select **Start ▶ Programs ▶ Applied Biosystems ▶ 3500 ▶ 3500**.

The 3500 Series Software 2 splash screen appears. This screen will remain active for a few seconds until the 3500 Login dialog box opens.



Log in

In the 3500 Series Software 2 Login dialog box:

1. Enter your User Name and Password. See your 3500 Series Genetic Analyzer system administrator for log in information.

Note: For information on creating user accounts, see Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module).”



2. Click **OK**.

The 3500 Series Software 2 starts and the Dashboard is displayed (Figure 3 on page 37).

Check system status in the Dashboard

The Dashboard displays gauges, instrument information, consumable information, and maintenance notifications that provide a quick overview of the usage of each consumable and the status of the instrument.

Consumable containers include RFID tags that identify the consumable and allow the software to monitor the number of runs or days remaining, the number of days on the instrument, the expiration date, lot number and part numbers.

Gauges

Instrument
information

Consumables

Maintenance
notifications

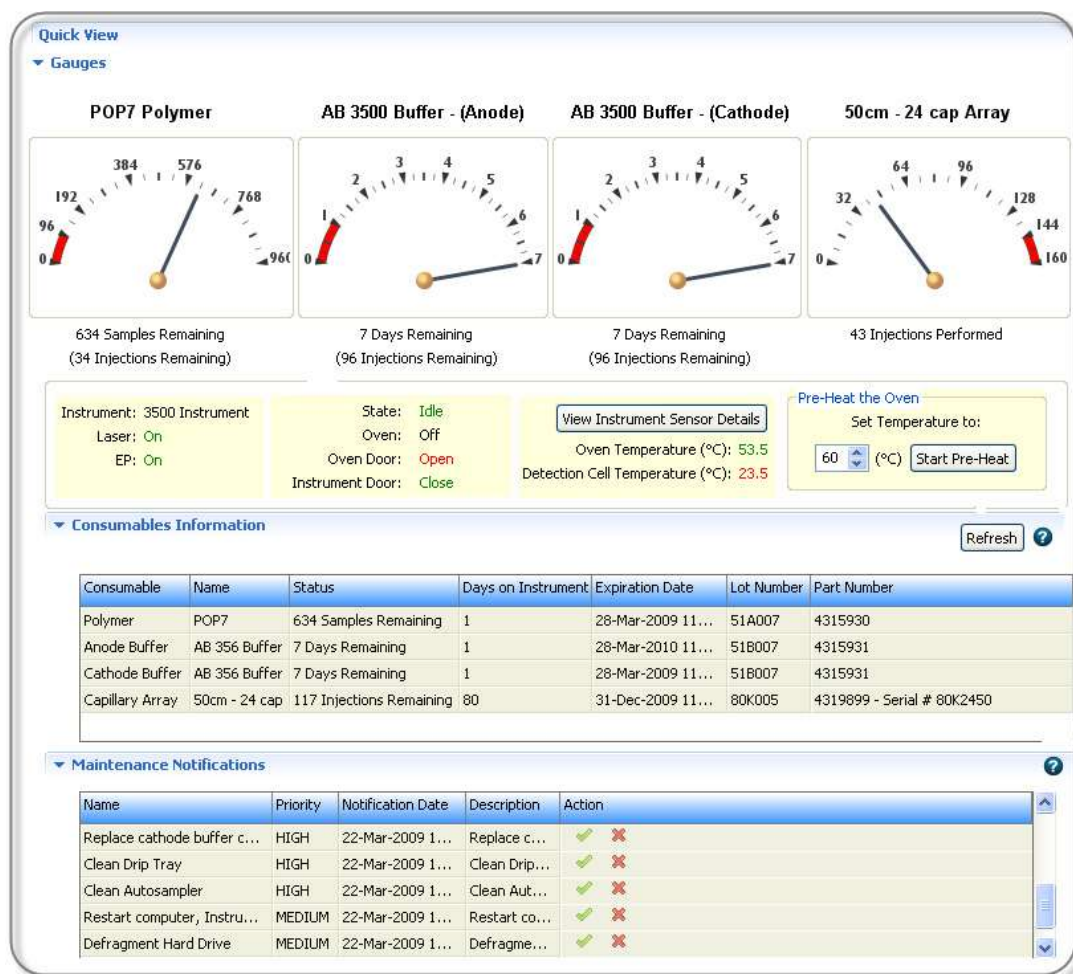


Figure 3 Dashboard

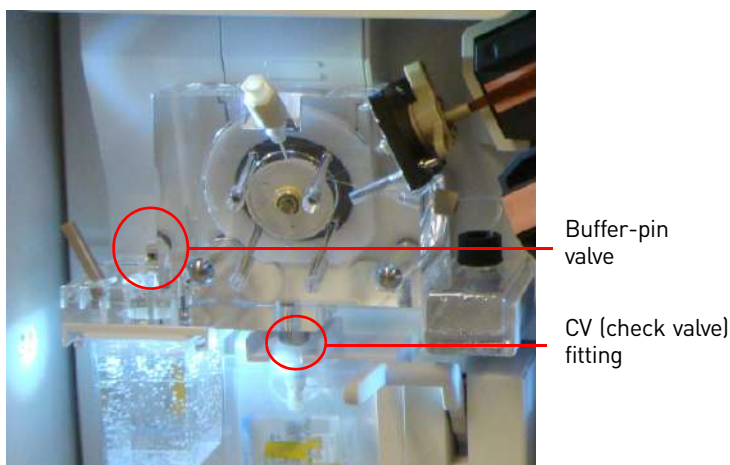
Check maintenance notifications

The Maintenance Notification section displays reminders for the tasks scheduled in the maintenance calendar (see “As-Needed instrument maintenance tasks” on page 222). You can set the time to trigger maintenance notifications in Preferences (see “Set general preferences” on page 42).

1. Review the Maintenance Notifications pane.

Maintenance Notifications				
Name	Priority	Notification Date	Description	Action
Flush Pump Trap	HIGH	23-Aug-2010 12:00:0...	Flush Pump Trap	✓ ✕
Clean the anode buffer cup pin-valve...	HIGH	06-Sep-2010 12:00:0...	Clean the anode buffer...	✓ ✕
Replace cathode buffer container septa	HIGH	23-Aug-2010 12:00:0...	Replace cathode buffer...	✓ ✕
Clean Drip Tray	HIGH	23-Aug-2010 12:00:0...	Clean Drip Tray	✓ ✕
Check Disk Space	HIGH	23-Aug-2010 12:00:0...	Check Computer for Dis...	✓ ✕

2. Perform any scheduled maintenance tasks, then click ✓ to mark it as complete, (or click ✕ to mark it as dismissed if you do not perform the task). Actions are recorded in the Notifications Log (for more information, see “Review the Maintenance Notifications Log” on page 224).
3. Perform any daily, monthly, or quarterly maintenance tasks that are not listed in the Maintenance Notifications pane (see “Service Log and Usage Statistics 247” on page 219). When you complete a task, click ✓ to mark it as complete, click ✕ to mark it as dismissed. Actions are recorded in the Notifications Log (for more information, see “Review the Maintenance Notifications Log” on page 224).
4. Inspect the instrument interior. See “Start the instrument” on page 33.
 - a. If you see any spills, clean immediately.
 - b. If you see any leaks and dried residue around the Buffer-Pin Valve, check valve, and array locking lever. If leaks persist, contact Life Technologies.



Check consumable status

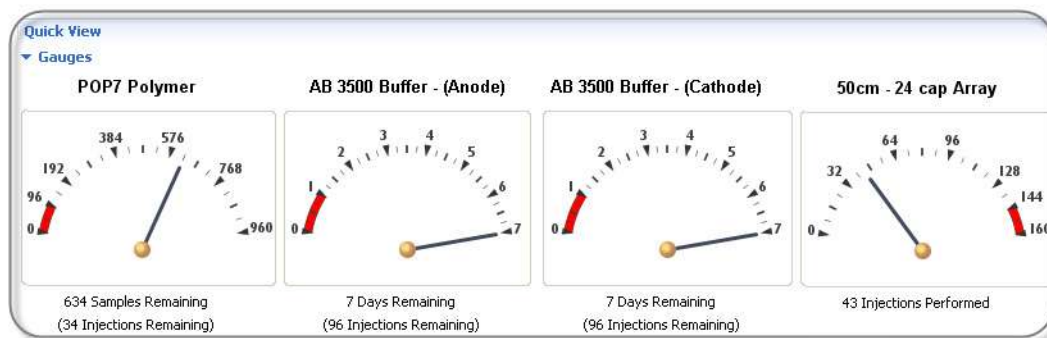
1. Click **Refresh** to update consumable status.

The Consumables pane displays expiration dates and lot numbers (from the RFID tags on the consumable containers).

Consumables Information Refresh ?						
Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP7	634 Samples Remaining	1	28-Mar-2009 11...	51A007	4315930
Anode Buffer	AB 356 Buffer	5 Days Remaining	1	28-Mar-2010 11...	51B007	4315931
Cathode Buffer	AB 356 Buffer	5 Days Remaining	1	28-Mar-2010 11...	51B007	4315931
Capillary Array	50cm - 24 cap	117 Injections Remaining	80	31-Dec-2009 11...	80K005	4319899 - Serial # 80K2450

2. Check the consumables gauges for the number of injections, samples, or days remaining for a consumable.

When <10% of the specified use of the consumable remains, the gauge moves into the red warning range. The consumable also displays in red in the Consumables pane.



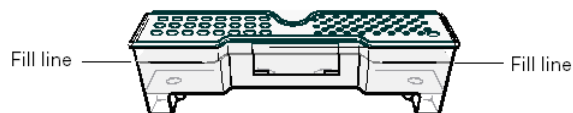
IMPORTANT! We recommend that you add a maintenance notification to your calendar for polymer and buffer replacement. Set the notification to display two days before the polymer expiration date.

Consumables usage limits and expiration

For information, see “Consumables usage limits and expiration” on page 22.

Check buffer fill levels

Check the fill levels on buffers. Verify that buffer level is at the top of the fill line and check that seal is intact. The meniscus must line up at or above the fill line.



IMPORTANT! Do not use if the buffer level is too low or if the seal has been compromised. Ensure that the buffer level is at or above the fill line and the seals is intact.

Replenish consumables

As needed, see:

- “Replenish polymer” on page 233.
- “Change polymer type” on page 234.

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

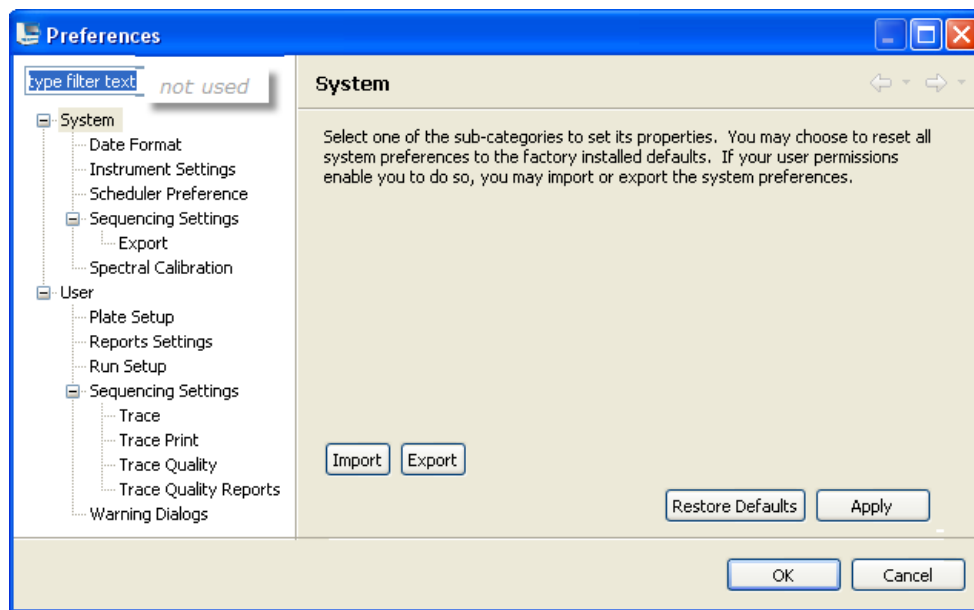
- “Change the anode buffer container (ABC)” on page 225.
- “Change the cathode buffer container (CBC)” on page 226.
- “Fill capillary array with fresh polymer” on page 237.
- “Change the capillary array” on page 238.

Go to Chapter 3, “Set Up and Run” on page 51.

Set preferences

Overview

To access the Preferences dialog box, select **Preferences** in the toolbar. You can optionally set any or all preferences.



Note: The “type filter text” field at the top of the dialog box is not used.

System preferences

These settings apply to all users:

- Date Format
- Instrument Settings (instrument name and message boxes)
- Scheduler Preference (trigger time for maintenance notifications)
- Sequencing Export Settings
- Spectral Calibration (number of allowed borrowing events)

User preferences

These settings are saved individually per user if your the SAE module is enabled on your system. These settings apply to all users if the SAE module is disabled on your system.

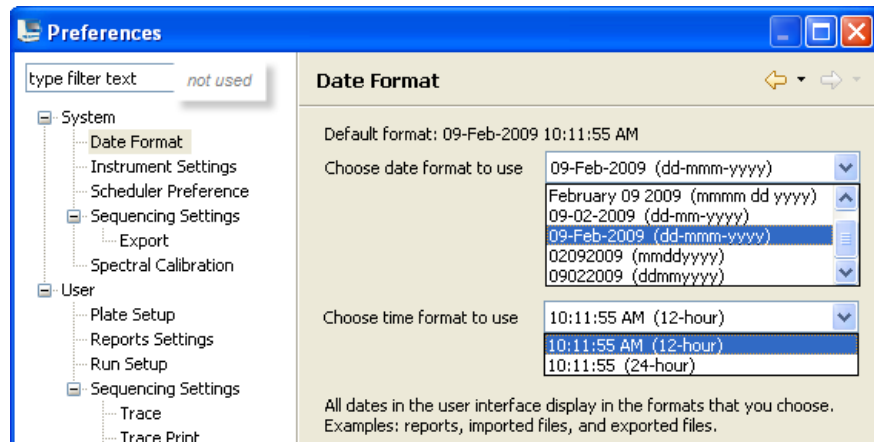
Note: For information on the SAE module, see Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.

- Plate Setup
- Reports Settings
- Run Setup
- Sequencing Settings (review and report settings)

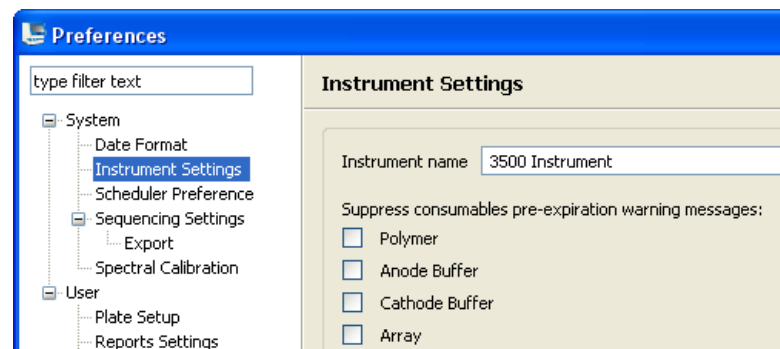
Set general preferences

System preferences

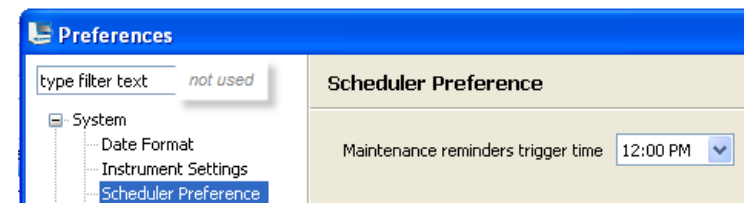
1. In the Preferences dialog box, click the following items:
 - **Date Format** to set the date and time format for the software.



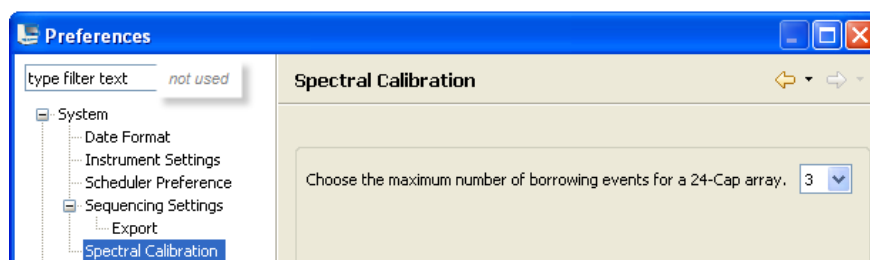
- **Instrument Settings** to:
 - Set the instrument name (appears in the Dashboard, reports, file name conventions, instrument sensor details, view sequencing results).
Note: If you have multiple instruments, you can assign each instrument a unique instrument name.
 - Suppress the messages that are displayed when you start a run that indicate the number of days left before a consumable expires or should be replaced.



- **Scheduler Preference** to set the time to trigger maintenance notifications displayed in the Dashboard (see “Check maintenance notifications” on page 38).



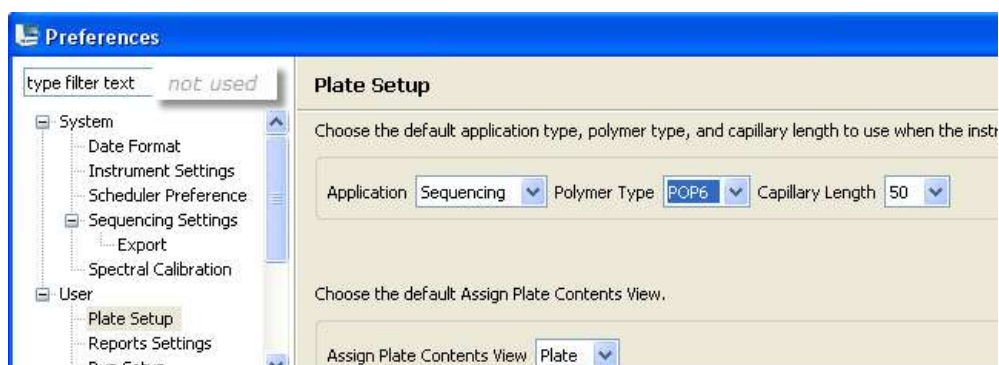
- **Spectral Calibration** to decrease the number of allowed borrowing events for spectral calibration (see “What you see during a spectral calibration” on page 117).



2. Click **Apply** to save the system preferences (see “System preferences” on page 42).

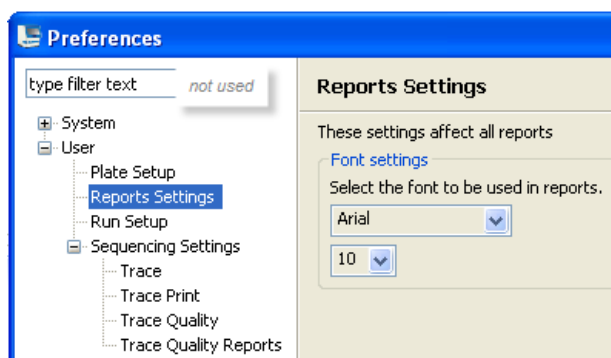
User preferences

1. In the Preferences dialog box, click the following items as needed:
 - Plate setup to set the default settings for:
 - Plate type and attributes when you create a plate.
 - Plate type in the Open Plate dialog box.



- **Reports Settings** to set the default font and size reports.

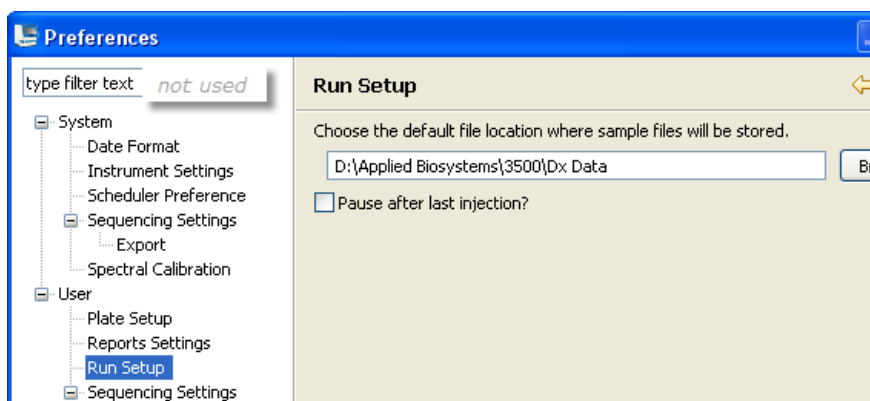
Note: You can override this setting in each report view.



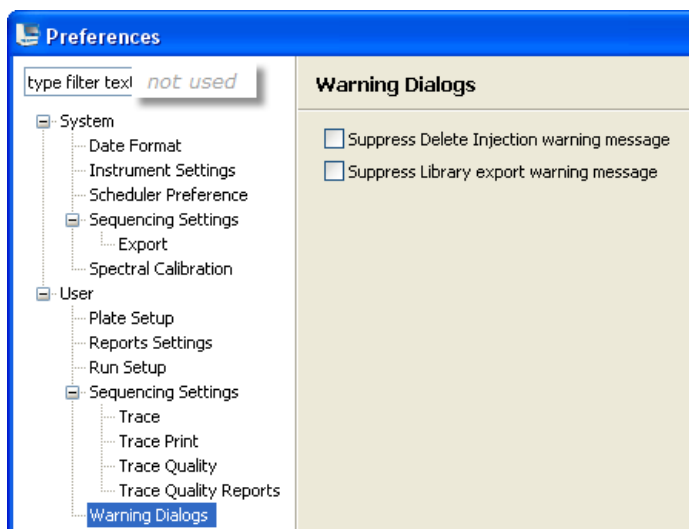
- **Run Setup** to set:
 - The default storage location for data files in file name conventions and results groups.

Note: You can override this setting in file name conventions and results groups.

- **Pause After Last Injection** – When enabled, allows reinjection of the last injection by pausing after the last injection is complete (before completing the run).



- **Warning Dialogs** to suppress warning messages for deleting an injection or exporting a library item.



2. Click **Apply** to save the user preferences (see “User preferences” on page 41).

Table settings user preferences

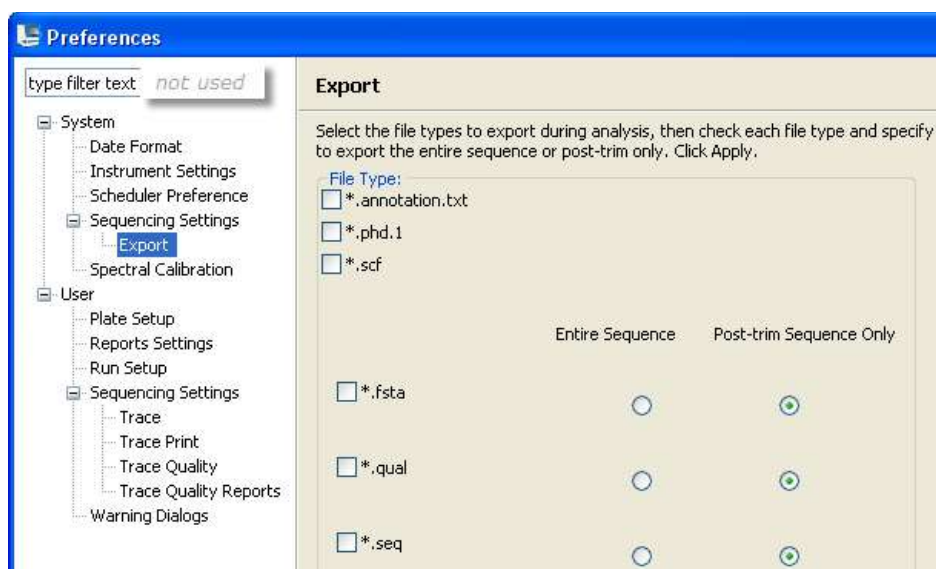
Users can also save user preferences while viewing tables. The Table settings dialog box – Determines the columns displayed in a table and the order of the columns.

Set sequencing preferences

Export (system preference)

Export preferences set the defaults for the file types to automatically export during a sequencing run. Exported files are stored in the same directory as the .ab1 files.

1. In the Preferences dialog box, click **Export** under System Sequencing Settings to display the Export pane.



2. Select the file types to export. Exported files are stored in the same directory as the .ab1 files.

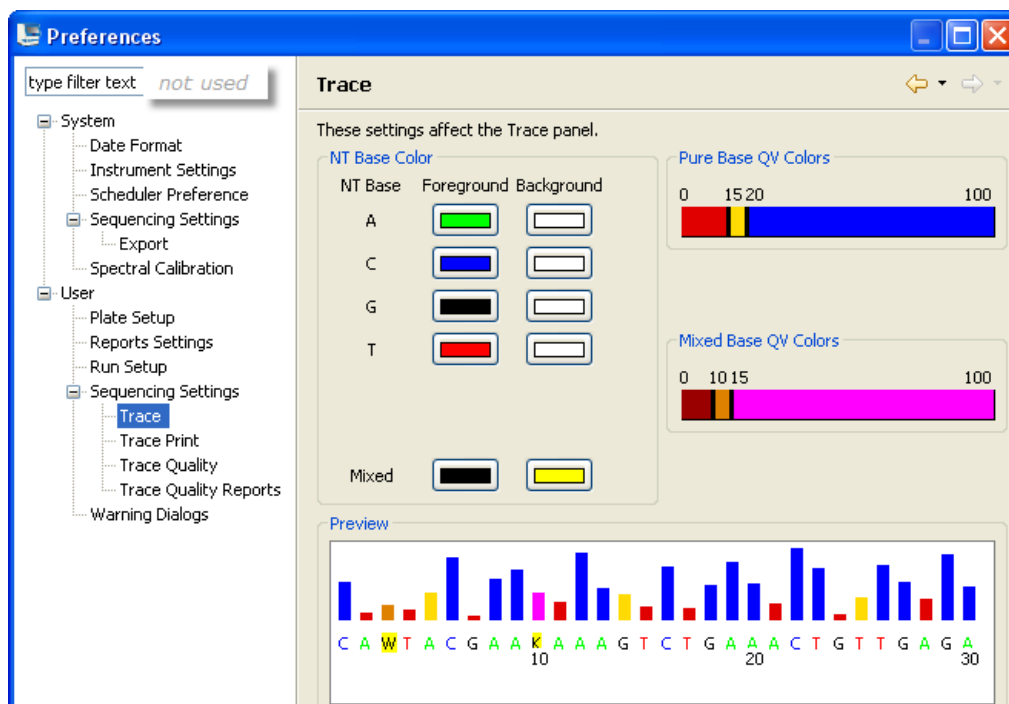
File type	Description
*.annotation.txt	Information from the Annotation tab in the sequencing trace view such as data collection time, run time start finish
*.phd.1, *.scf	Sequencing files
*.fasta, *.qual, *.seq	Reference files – specify Entire Sequence or Post-trim Sequence Only

3. Click **Apply** to save the system preferences (see “System preferences” on page 42).

Trace (user preference)

The Trace preference settings determine the default settings for color representation of nucleotide and quality value bars in the Trace View in View Sequencing Results.

1. In the Preferences dialog box, click **Trace** under User Sequencing Settings to display the Trace pane.



2. Specify the following settings:

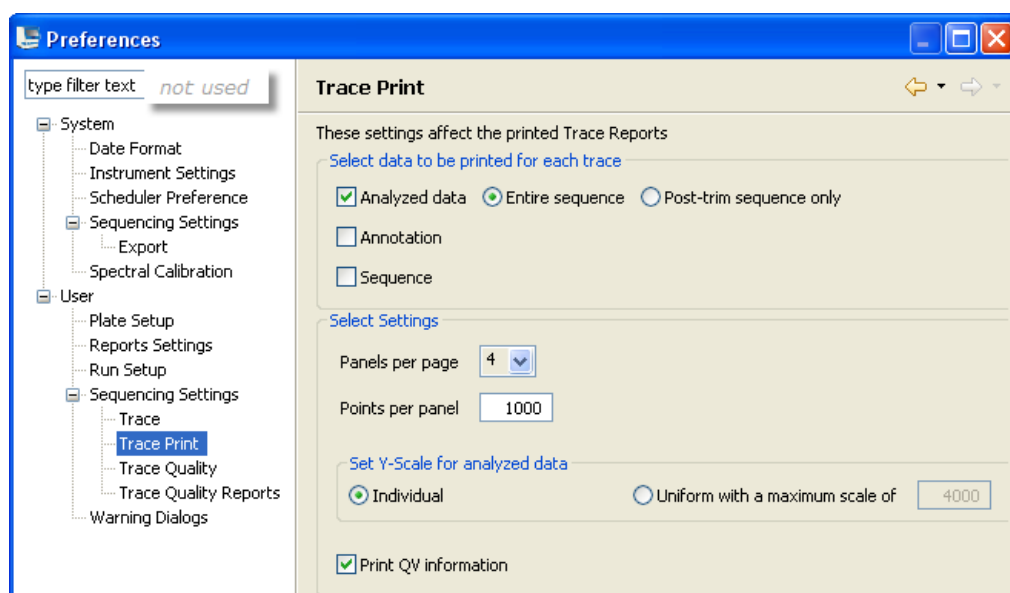
Setting	Description
NT (nucleotide) Base Color	Click an NT or Mixed base Foreground or Background color block, then select a color for the letter annotation or the highlight color for the letter annotation. <div> <div>NT Base</div> <div>Foreground</div> <div>Background</div> <div>A</div> <div></div> <div></div> <div>A</div> </div>
Pure Base and Mixed Base QV Colors	Sets the colors and ranges for pure and mixed base QVs (quality values) displayed in the Trace View: <ol style="list-style-type: none"> Click a pure base or mixed base color bar to select a new color. Place the mouse pointer over a slider, then drag to set a new range. Set the range appropriate for the expected amplicon size and data collection run time. <p>We recommend that you set the following ranges for QVs:</p> <ul style="list-style-type: none"> Pure bases: Low QV < 15, Medium QV = 15 to 19, High QV = 20+ (default) Mixed bases: Low QV < 5, Medium QV = 5 to 10, High QV > 10 (investigate to determine the best range for your application) <div> <div>0</div> <div>15</div> <div>20</div> <div></div> <div></div> <div></div> </div>

3. Click **Apply** to save the user preferences (see “User preferences” on page 43).

Trace Print (user preference)

Trace Print preferences determine settings for sequencing trace reports.

1. In the Preferences dialog box, click **Trace Print** under User Sequencing Settings to display the Trace Print pane.



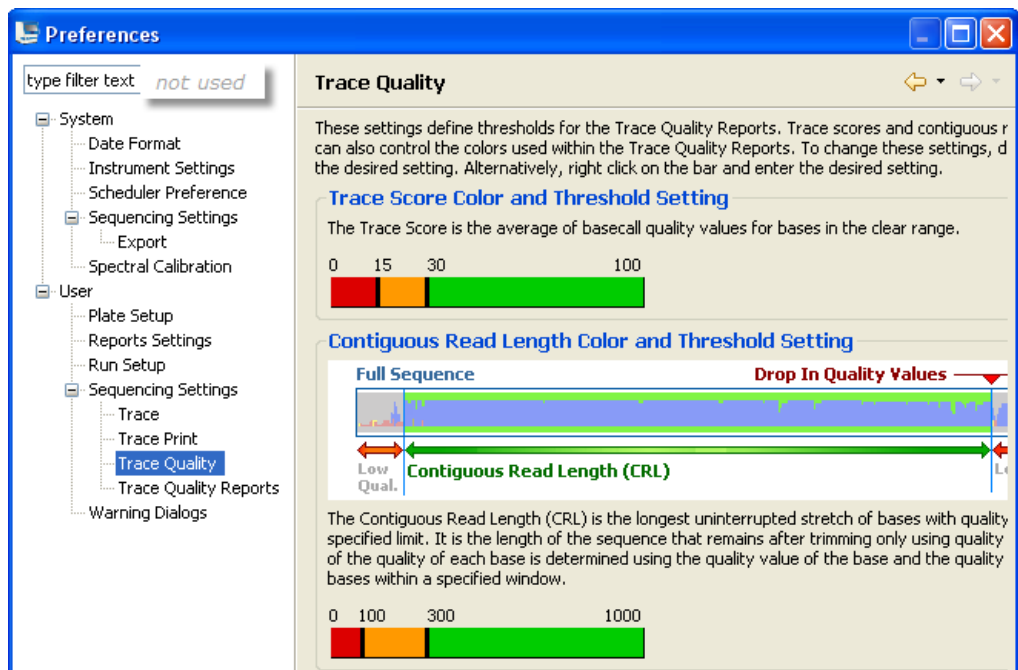
2. Specify the type of trace data, specific print settings, and Y-Scale preference to display in the Trace Report.
3. Click **Apply** to save the user preferences (see “User preferences” on page 43).

Trace Quality (user preference)

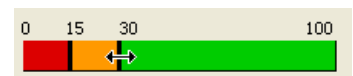
Trace Quality preferences control the quality ranges for:

- **QC report** – Trace Score and CRL
- **Plate report** – Trace Score

1. In the Preferences dialog box, click **Trace Quality** under User Sequencing Settings to display the Trace Quality pane.



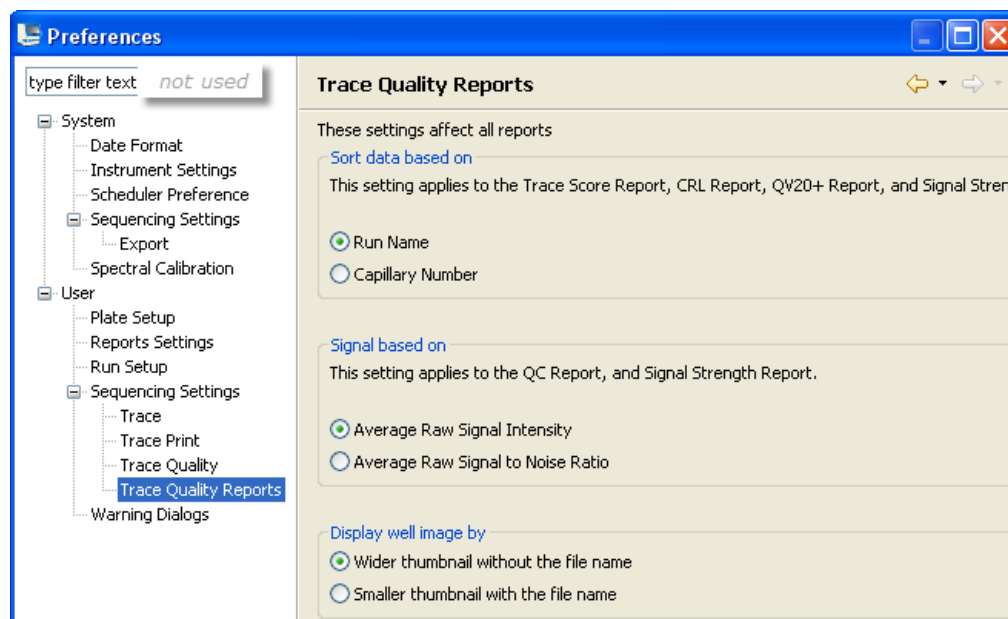
2. Set colors and ranges:
 - a. Click a color bar to select a new color.
 - b. Place the mouse pointer over a slider, then drag to set a new range.
3. Click **Apply** to save the user preferences (see "User preferences" on page 43).



**Trace Quality Report
(user preference)**

Trace Quality Report preferences determine the content and formatting used in QC, Plate, Trace Score, CRL, QV20+, and Signal Strength reports.

1. In the Preferences dialog box, click **Trace Quality Report** under User Sequencing Settings to display the Trace Quality Report pane.



2. Specify the following settings:

Setting	Description
Sort data	Sort data in Trace Score, CRL, QV20+, and Signal Strength Reports based on: <ul style="list-style-type: none"> • Run Name • Capillary Number
Signal based on	Base signal in QC and Signal Strength Reports based on: <ul style="list-style-type: none"> • Average Raw Signal Intensity • Average Raw Signal to Noise Ratio
Display well image by	Specify the thumbnail option for Plate Reports: <ul style="list-style-type: none"> • Wider thumbnail without file name • Smaller thumbnail without file name

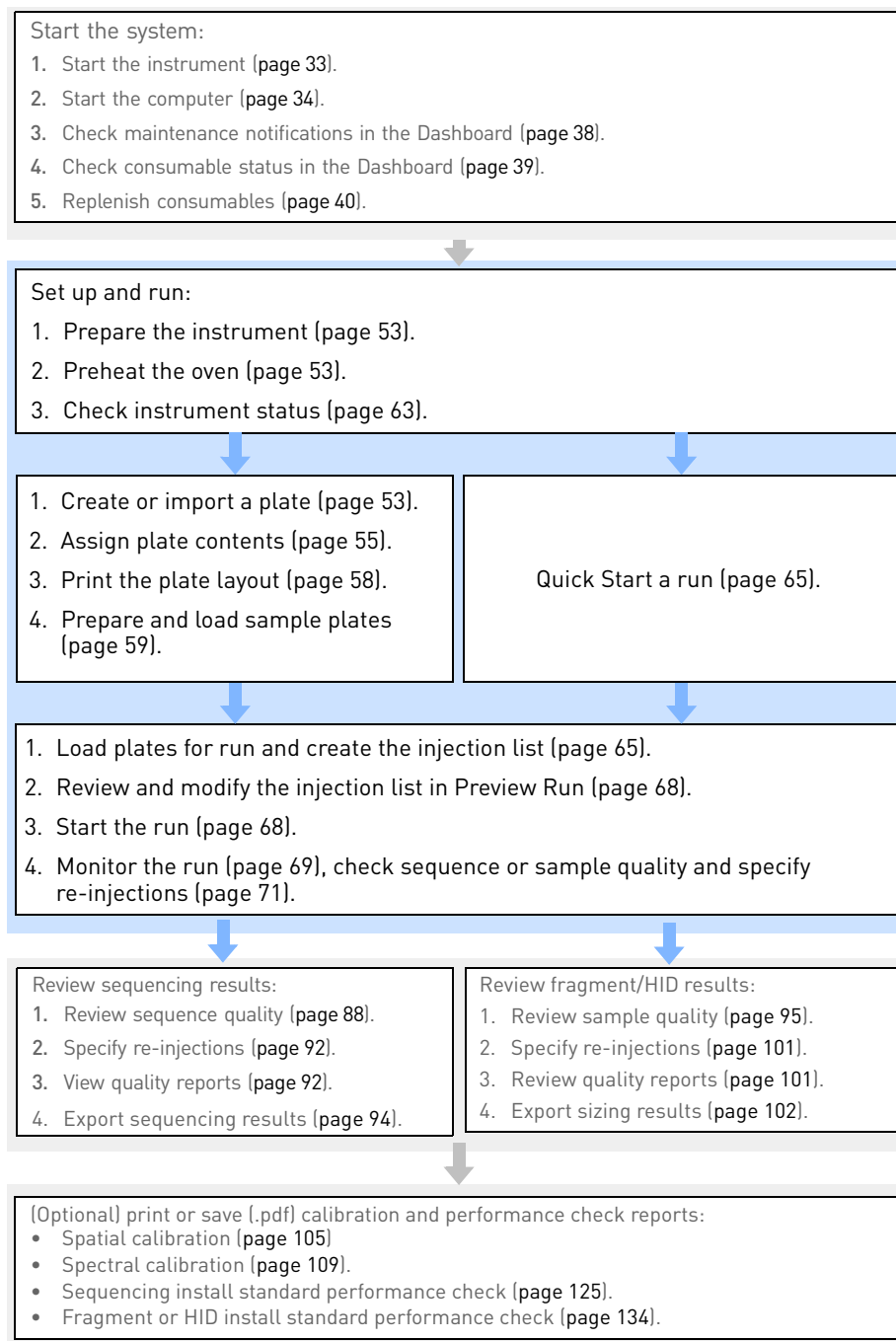
3. Click **Apply** to save the user preferences (see “User preferences” on page 43).

3

Set Up and Run

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Workflow



Prepare the instrument

1. In the Dashboard, check consumable status (“Check consumable status” on page 39). Ensure that:
 - Consumables are not expired
 - Adequate buffer levels are at the fill lines (“Check buffer fill levels” on page 40).
2. Check the Oven Temperature for the instrument protocol in the assay you will run (select the instrument protocol in the library, then click **Edit**), set the oven temperature, then click **Start Pre-heat**:

Pre-heat the oven and detection cell while you prepare for a run (detection cell temperature is set by the software). Preheating helps mitigate subtle first-run migration rate effects. The preheat function automatically turns off after 2 hours of instrument inactivity.




We recommend that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold.
3. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed (see “Remove bubbles from the polymer pump” on page 238).

Create a plate

Note: If you are running a stand-alone version of the 3500 Series Software 2 (a version that is not installed on the instrument computer), you can create plates, then export them for use on the instrument computer.

Select a plate template

The software includes plate templates that you can use as a starting point to create a plate (sequencing examples shown). Plate template names reflect the run module associated with the plate. The run module contains data collection settings.

 Seq_Std_BDTv3.1_xL-POP7	Sequencing	For the analysis of the Sequencing Install Standard (BigDye Terminator v3.1...
 Std_Seq_xL-POP6	Sequencing	For read lengths of 600 bp or greater and a run time of 2 hours - 24 capill...
 Std_Seq_xL-POP7	Sequencing	For read lengths of 850 bp or greater and a run time of 2 hours - 24 capill...

Appendix A, “Application Reagents and Run Modules” on page 249, lists the run time and size range collected for each run module.

You can also create your own templates. In addition to defining plate parameters, a plate template can also contain a list of the appropriate assays for an application. For more information, see “Create a plate template” on page 82.

Create a plate from a template

1. In the Dashboard, click **Create Plate From Template** to display the Open Plate Template from Library dialog box:
 - a. Select a plate template:
 - b. Find templates by selecting an attribute, entering the text to search for, then clicking **Go**. (Click **Clear** to clear the field and enter different search criteria).
 - c. Select the template, then click **Open**.



 A screenshot of the "Plate Details" dialog box. At the top, there is a toolbar with four icons and labels: "New Plate", "Open Plate", "Save Plate", and "Close Plate". Below this, the title "Plate Details" is displayed. The form contains several fields:

- * Name: A text input field with the placeholder "Enter plate name".
- * Number of Wells: Three radio buttons labeled "96", "96-FastTube", and "384". The "96" button is selected.
- * Plate Type: A dropdown menu currently showing "Sequencing".
- * Capillary Length: A dropdown menu showing "50" and a unit "cm".
- * Polymer: A dropdown menu showing "POP7".



2. In the Define Plate Properties screen, enter the plate name and select the number of wells on the plate.

IMPORTANT! Enter only alphanumeric characters in fields on this screen. Special characters in these fields may not be correctly displayed in other software screens.

- **96** – Select if you are using a 96-well standard reaction plate or 8-strip standard tubes with a retainer.
 - **96-Fast** – Select if you are using a 96-well Fast reaction plate or 8-strip fast tubes with a retainer.
 - **384** – Select if you are using a 384-well reaction plate (24-capillary instruments only).
3. Set remaining plate properties, then select **Save**.
 4. Select **Save**.
 5. Click **Assign Plate Contents**, then go to "Assign plate contents" on page 55.

Import a plate

1. Do either of the following:
 - Create a plate on another 3500 Series Software 2 system, then export (see "Import and export a plate" on page 81).
 - Create a plate import file (see "Create a plate import file" on page 81).

2. Access the Assign Plate Contents screen: Click the **Main workflow arrow** , in the Dashboard, then select **Assign Plate Contents** in the navigation pane.
3. Click  **Import**, then select the plate import file.
4. Click .



Assign plate contents

You assign the following information to the wells in a plate before you can run the plate:

- **Sample names and sample types** (required) – Identifies the well positions of each sample for data collection and processing.
- **Assay** (required) – Specifies the parameters that control data collection and primary analysis (basecalling or sizing). All named wells on a plate must have an assigned assay. For more information on assays, see “Assays library” on page 150.
- **Filename convention** (optional) – Specifies file naming. For more information, see “File name convention overview” on page 154.
- **Results group** (optional) – Specifies sample data file storage. For more information on assays, see “Results group overview” on page 158.

Access the Assign Plate Contents screen


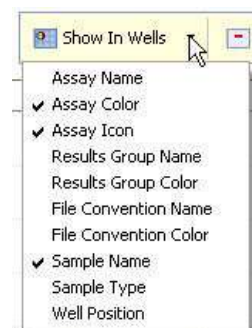
1. Access the Assign Plate Contents screen (Figure 4 on page 56) from:
 - The Define Plate Properties screen by clicking **Assign Plate Contents** (described above).
 - The navigation pane by selecting **Assign Plate Contents** in the navigation pane.
 - The Dashboard by clicking the **Main workflow arrow** , then selecting **Assign Plate Contents** in the navigation pane.
2. Click **Show In Wells** to specify the attributes to display in wells.



Figure 4 on page 56 shows the Plate View of the Assign Plate Contents screen.



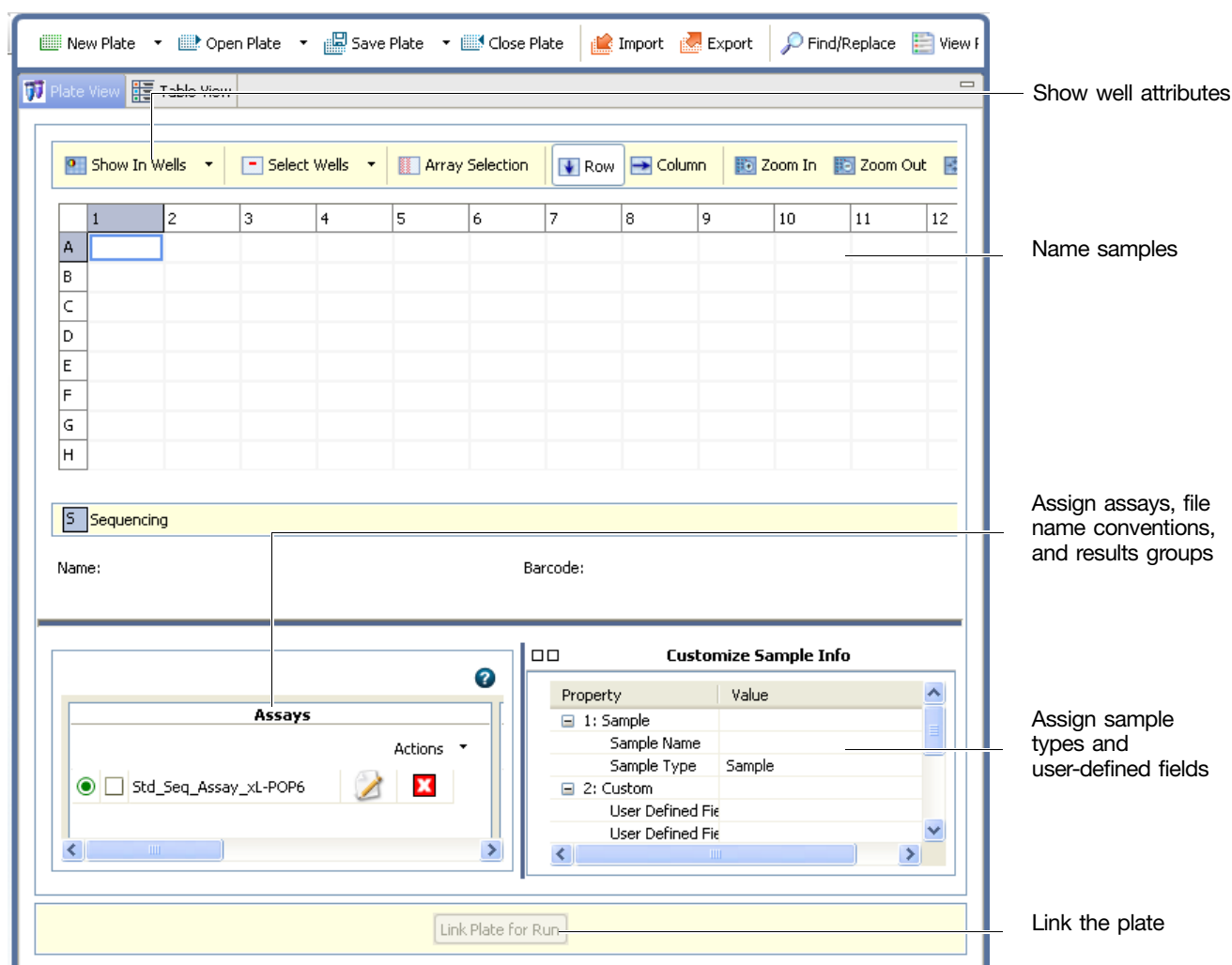


Figure 4 Plate View of the Assign Plate Contents screen

Name samples and assign sample types in the plate view

This section provides one way to name samples and assign sample types. For other ways to name samples, see “Use the Plate View” on page 77 and “Use the Table View” on page 78.

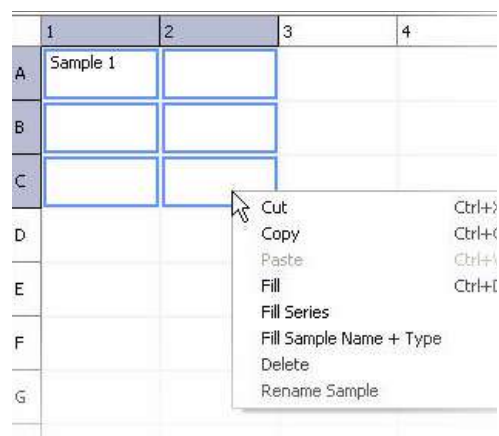
Procedure

1. Click a well, then type a sample name directly into the well, then press **Enter**.



2. Click-drag multiple wells.
3. Right-click and select **Fill** or **Fill Series** to populate the selected fields.

Note: To use Fill Series, type a number as the last character of the named well). You can copy and paste sample names instead of using fill commands.

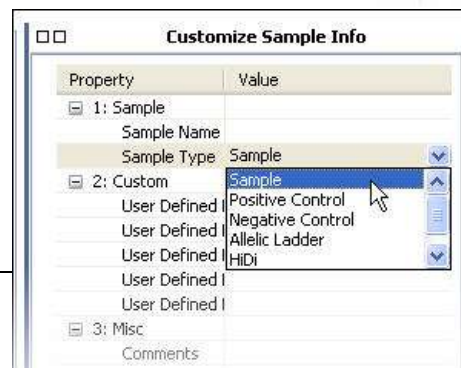


4. At the bottom right of the Assign Plate Contents screen, expand the Customize Sample Info pane.
5. In the plate view, click-drag to select wells of interest.

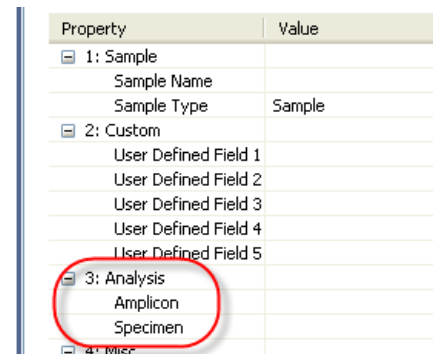


6. Specify the Sample Type for the selected wells, then press **Enter**.
7. (Optional) Specify User Defined Fields and Comments. User Defined Fields contain additional attributes you can assign to a plate and are displayed only in Table View.

IMPORTANT! Enter only alphanumeric characters in User-Defined fields. Special characters in these fields may not be correctly displayed in other software screens.



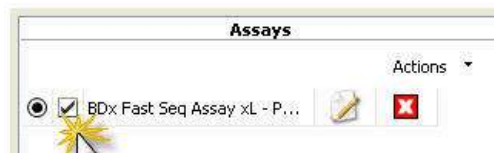
8. For sequencing assays, specify amplicon and specimen.
9. Repeat to assign the Sample Type for all named wells.
10. Go to "Assign assay, file name convention, and results group in the Plate View" on page 58.



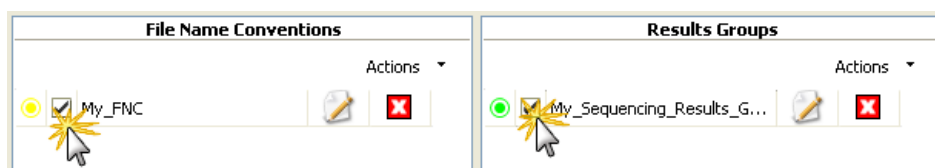
Assign assay, file name convention, and results group in the Plate View

Note: If a file name convention or results group you created is not listed for the plate, go to “Add assays, file name conventions, and results groups to a plate” on page 80.

1. Select the wells for which to specify an assay.
2. Enable the checkbox next to the assay name to assign it to the selected wells.



3. Repeat for file name conventions and results group.



4. Select **Save Plate**.
5. Go to “Print the plate layout” on page 58.

How file location in file name conventions and results groups work

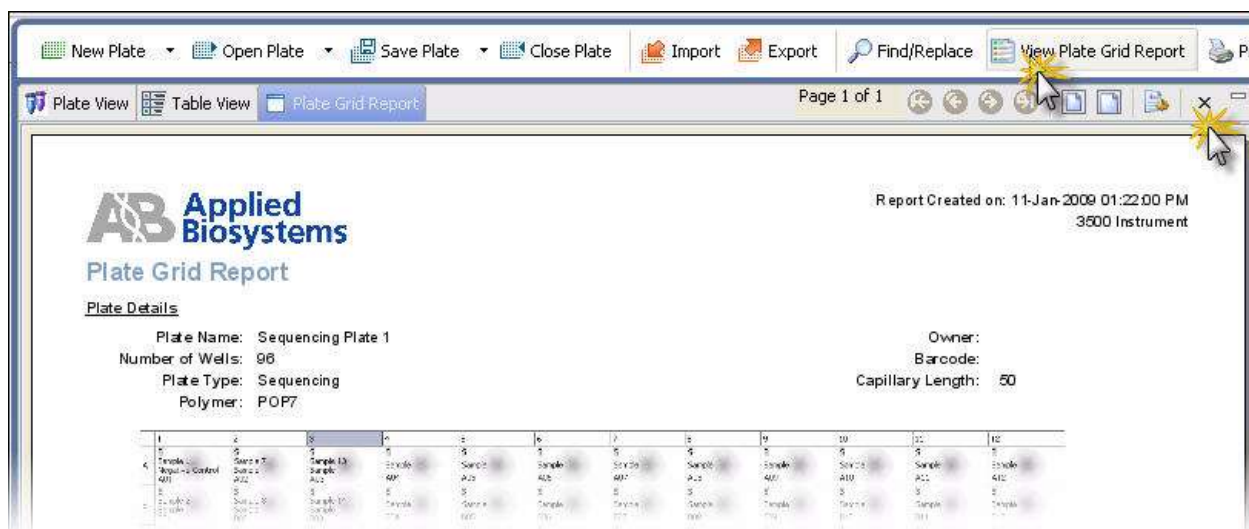
If you do not specify a file name convention, data files are named in this format:
<sample name>_<well>.

If you do not specify a results group, files are stored in the location specified in the file name convention or in Preferences ► User ► Run (see “User preferences” on page 41).

If you specify both a file name convention and a results group, files are stored in the location specified in the results group.

Print the plate layout

1. In the Assign Plates for Run screen, click **View Plate Grid Report**.



2. Select **Print Preview** or **Print** as needed.
3. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.
4. Close the report.
5. Go to "Prepare and load sample plates" on page 59.



Prepare and load sample plates

IMPORTANT! Do not use warped or damaged plates.



Capillary-to-plate mapping

The capillary-to-plate mapping for the default injection order is shown below. If you change the injection order in the injection list, mapping differs from the examples shown below.

8-capillary: 96-well plate

	Cap	3	4	5	6	7	8	9	10	11	12
A	1	3	4	5	6	7	8	9	10	11	12
B	2	3	4	5	6	7	8	9	10	11	12
C	3...	3	4	5	6	7	8	9	10	11	12
D	1	3	4	5	6	7	8	9	10	11	12
E	2	3	4	5	6	7	8	9	10	11	12
F	1	3	4	5	6	7	8	9	10	11	12
G	2	3	4	5	6	7	8	9	10	11	12
H	1	3	4	5	6	7	8	9	10	11	12

8-capillary: 384-well plate

Not supported on the 3500 Dx Genetic Analyzers (8-capillary)

24-capillary: 96-well plate

	1	2	3	4	5	6	7	8	9	10	11	12
Cap	1	2	3	4	5	6...						
A	1	2	3	4	5	6	7	8	9	10	11	12
B	1	2	3	4	5	6	7	8	9	10	11	12
C	1	2	3	4	5	6	7	8	9	10	11	12
D	1	2	3	4	5	6	7	8	9	10	11	12
E	1	2	3	4	5	6	7	8	9	10	11	12
F	1	2	3	4	5	6	7	8	9	10	11	12
G	1	2	3	4	5	6	7	8	9	10	11	12
H	1	2	3	4	5	6	7	8	9	10	11	12

24-capillary: 384-well plate

Cap	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	1	3	2	3	3	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
B	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
C	4	3	5	3	6...		5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
D	2	4	2	4	2		6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
E	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
F	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
G	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
H	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
I	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
J	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
K	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
L	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
M	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
N	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1
O	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	1
P	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	1

Allelic ladder run requirements

We recommend that you inject one allelic ladder for each set of 24 samples:

- **8-capillary instruments** – One allelic ladder per 3 injections
- **24-capillary instruments** – One allelic ladder per 1 injections

Allelic ladders that are injected under the same conditions are recommended to accurately genotype samples in the secondary analysis software (GeneMapper® ID-X Software v1.2 or later).

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed that can, in turn, cause sizing variation. We recommend the frequency of allelic ladder injections described above to account for normal variation in fragment migration speed. However, during internal HID validation studies, verify the required allelic ladder injection frequency to ensure accurate genotyping of all samples in your laboratory environment.

Results group for one allelic ladder per run folder

For a 24-capillary instrument, create a results group that specifies an injection folder, then select this results group for all injections on the plate.

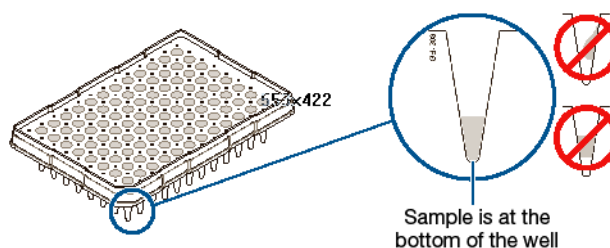
For an 8-capillary instrument, create one results group for each set of three injections on the plate (each results group specifies a results group name folder). For more information, see “Results group example 2: store one allelic ladder per run folder (8-capillary instruments)” on page 163.

Prepare sample plates

1. Pipette samples into the plate according to the plate layout (see “Print the plate layout” on page 58).
2. Briefly centrifuge the plate.
3. Verify that each sample is positioned correctly in the bottom of its well.

IMPORTANT! If the reagents 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.



4. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.

Prepare the plate assembly

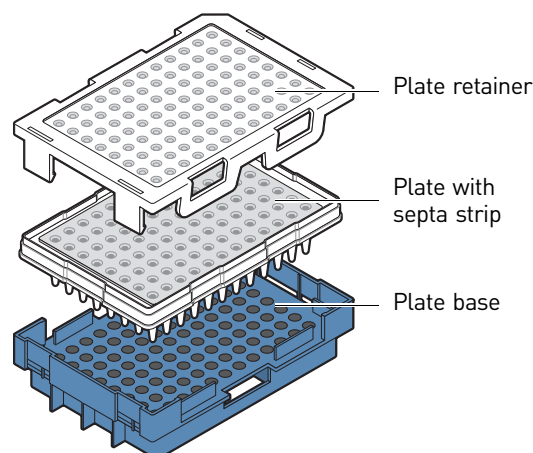
Prepare the plate assembly on a clean, level surface. Do not heat plates that are sealed with septa.

For information on plates and tubes that can be used on the Applied Biosystems 3500 / 3500xL Genetic Analyzer, see Appendix D, "Part Numbers" on page 287.

96-well plate assembly

IMPORTANT! Use the correct plate base for standard plates. Using the wrong plate base may affect performance.

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate. Listen for the light clicking sound that occurs when the septa is pressed down firmly into position.
2. Place the sample plate into the plate base.
3. Snap the plate retainer (cover) onto the plate, septa, and plate base.
4. Verify that the holes of the plate retainer and the septa strip are aligned. If holes are not aligned, re-assemble and then assemble the plate assembly.



If the reagents 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.

IMPORTANT! The array tips will be damaged if the plate retainer and septa strip holes do not align correctly.

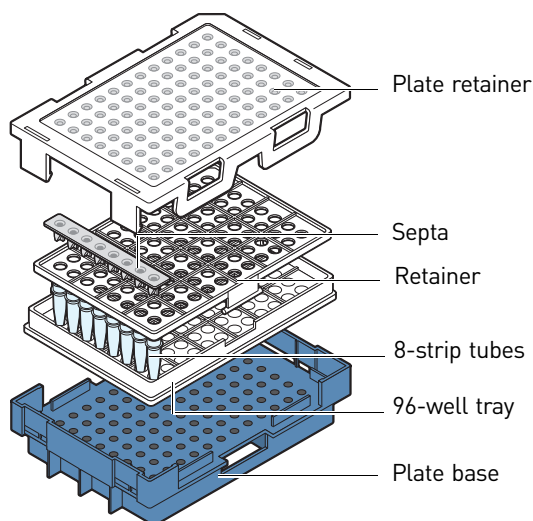
8-strip tube standard or fast assembly

IMPORTANT! Use the correct plate base for 8-tube standard or fast strips. Using the wrong plate base may affect performance.

1. Place the tubes in the 96-well tray.
2. Place the retainer on the tubes.

3. Align the holes in the septa strip with the retainer, then firmly press down.
4. Place the tray-tube-retainer assembly into the plate base.
5. Snap the plate retainer (cover) onto the plate, septa, and plate base.
6. Verify that the holes of the plate retainer and the septa strip are aligned. If holes are not aligned, re-assemble and then assemble the plate assembly.

If the reagents 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.



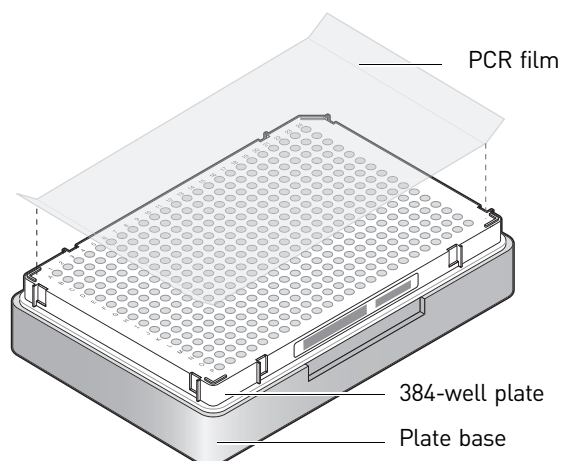
IMPORTANT! The array tips will be damaged if the plate retainer and septa strip holes do not align correctly.

384-well plate assembly

IMPORTANT! Use the correct plate base for 384-well plates. Using the wrong plate base may affect performance.

1. Place the sample plate into the plate base.
2. Seal the plate with PCR film.

If the reagents 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.



Load the plate in the instrument

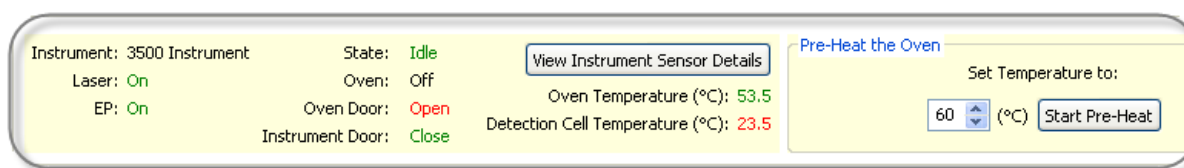
1. Click the **Tray** button on the front panel to move the autosampler to the front position.
2. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
3. Ensure that the pins in the autosampler are properly aligned with the holes at the bottom of the plate base, and that the tabs click into place on the base.
4. Close the instrument door to re-initialize the instrument.

Note: It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed.

Check instrument status

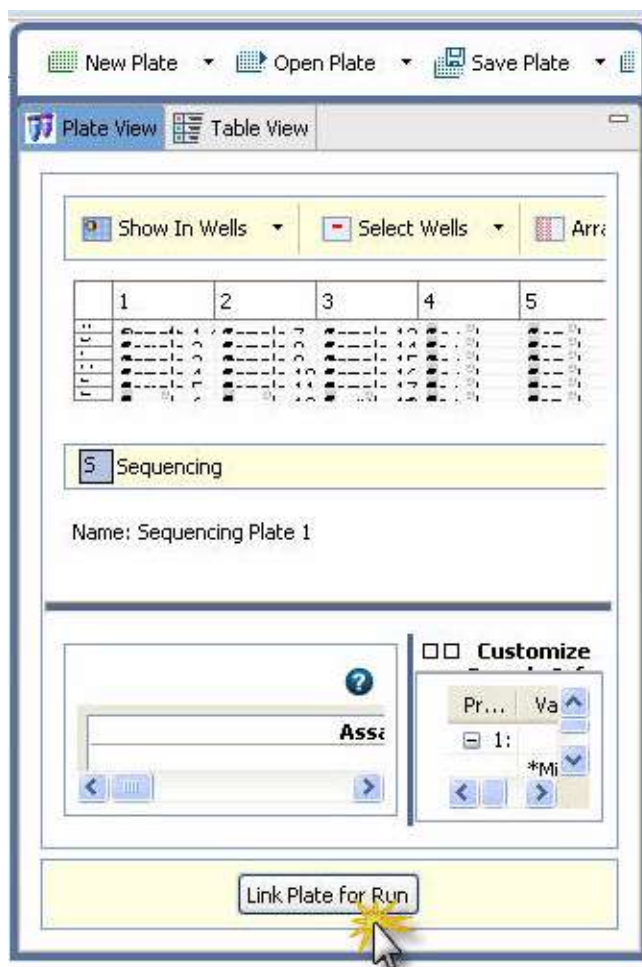
Check instrument status in the Dashboard. Temperatures are displayed in red as they warm to the set-points. When temperatures are at the set point they are displayed in green. Temperatures may fluctuate slightly when they reach the set point as they stabilize.

We recommend that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold. Pre-heating mitigates subtle first-run migration rate effects. (If you start the run when red indicators are shown, the run does not start until all indicators are green.)



Link the plate

1. In the Assign Plates for Run screen, click **Link Plate for Run**.



2. Go to "Load plates for run and create the injection list" on page 65.
Note: By default, plate A position is selected.

Quick Start a run

You can start a run in the Dashboard by selecting a plate with plate contents already assigned.

Load the plate in the instrument before proceeding (see "Load the plate in the instrument" on page 63).


1. In the Dashboard, click **Quick Start Run** to display the Select Plate from Library dialog box.
2. Select a plate, then click **Load Plate**.
3. Click **Start Run** from the Load Plates on Run Screen.



Load plates for run and create the injection list

Load the plate in the instrument (see “Load the plate in the instrument” on page 63) and link the plate (“Link the plate” on page 64) before proceeding.

1. Access the Load Plates for Run screen (Figure 5 on page 65) from:

- The **Assign Plate Contents** screen by clicking **Link Plate for Run**.
- The navigation pane by selecting **Load Plates for Run** in the navigation pane.
- The Dashboard by clicking the **Main workflow arrow** , then selecting **Load Plates for Run** in the navigation pane.

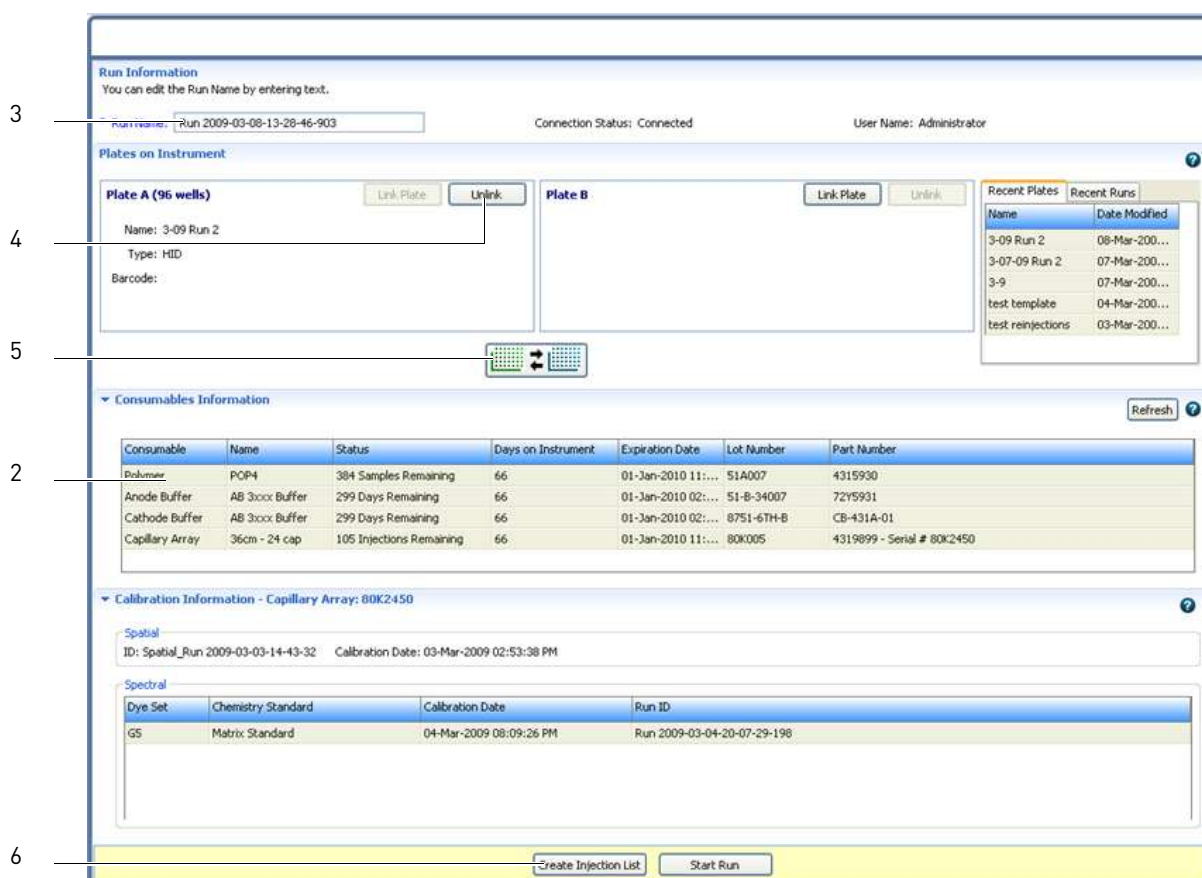


Figure 5 Load Plates for Run


2. Review the consumables information and the calibration information and ensure the status is acceptable for a run.

3. Enter a Run Name or use the default run name: <Start Instrument Run Date/Time Stamp> YYYY-MM-DD-hh-mm-ss-SSS (milliseconds), for example, “Run 2009-02-05-15-03-42-096” where the run start date is February 5 2009 and the run start time is 15:03:42:096.

Note: An instrument run begins when you click Start Run (on the Load Plates for Run screen) and ends when the last injection on the last plate has completed. For example, if you link two plates, then start the run, both plates and any duplicate injections or re-injections are part of the same instrument run. An injection is an instance of 8 or 24 samples (depending on instrument configuration) processed simultaneously under the same conditions.

When you access the Load Plates for Run screen by clicking Load Plates for Run on the Assign Plate Contents screen, the plate is automatically linked (indicated by the active Unlink button).



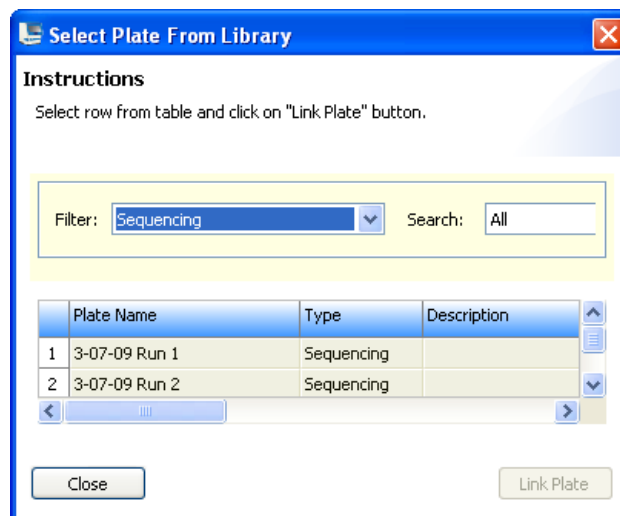
4. If needed, click **Unlink**, then follow the steps in “If a plate is not linked” below.
5. As needed, click **Switch Plates** () to assign the plate to the other position in the autosampler.
6. Click either of the following:
 - **Create Injection List** – Displays the Preview Run screen where you can modify the injection list before starting the run. Go to “Review and modify the injection list in Preview Run” on page 68.
 - **Start Run** – Displays the Monitor Run screen. Go to “Monitor the run” on page 69.

If a plate is not linked If you access the Load Plates for Run screen from the navigation pane, a plate may not be linked (indicated by the active Link Plate button).



To link a plate:


1. Click **Link Plate** to display the Select Plate from Library dialog box.



2. Select a plate, then click **Link Plate**.
3. Do either of the following:
 - Click **Create Injection List**, then go to "Review and modify the injection list in Preview Run" on page 68.
 - or
 - Click **Start Run**, then go to "Monitor the run" on page 69.

Review and modify the injection list in Preview Run

The Preview Run screen allows you to modify the injection list before you start the run.

1. Access the Preview Run screen (Figure 6 on page 68) from:
 - The Load Plates for Run screen by clicking **Create Injection List**.
 - The navigation pane by selecting **Preview Run**.
 - The Dashboard by clicking the **Main workflow arrow** , then selecting **Preview Run** in the navigation pane.
2. Click the icon above the plate to specify the attributes to display in the plate view.
3. Click the plate tabs to display Plate A or Plate B.



2. Injection List

3. 4 injections created - 4 in Plate A - 0 in Plate B

Type	Assay	Instrument Protocol	Plate
1	IF+Norm_POP4_xl	HID36_POP4xl_GS	3-09 Run 2
2	IF+Norm_POP4_xl	HID36_POP4xl_GS	3-09 Run 2
3	IF+Norm_POP4_xl	HID36_POP4xl_GS	3-09 Run 2
4	IF+Norm_POP4_xl	HID36_POP4xl_GS	3-09 Run 2

4. Plate A Plate B

	1	2	3	4	5	6	7	8	9	10	11	12
A	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
D	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample

Name: 3-09 Run 2 Barcode:

Legend
Duplicate Injection Re-Injection

Consumables Information

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Polymer	POP4	384 Samples Remaining	66	01-Jan-2010 11:...	51A007	4315930
Anode Buffer	AB 3:00x Buffer	299 Days Remaining	66	01-Jan-2010 02:...	51-B-34007	72Y5931
Cathode Buffer	AB 3:00x Buffer	299 Days Remaining	66	01-Jan-2010 02:...	0751-6TH-B	CB-431A-01
Capillary Array	36cm - 24 cap	105 Injections Remaining	66	01-Jan-2010 11:...	80K005	4319899 - Serial # 80K2450





Start the run

Start Run

Figure 6 Preview Run screen

The Preview Run screen contains an injection list and a plate view. The injection list is linked to the plate view. Click an injection to select the associated wells in the plate view.

IMPORTANT! If the injection list is blank, make sure that you clicked Create Injection List on the Load Plates for Run screen.

4. To modify the injection list at any time before a run or during a run, select an injection, then click  **Move Up**,  **Move Down**, and  **Delete** as needed.
5. To specify a duplicate injection (a replicate injection that uses the same instrument protocol as the original injection), select an injection, then click .

Sample data files for each duplicate injection can be saved in a separate folder in the results group folder if specified in the results group.

Note: To use a different protocol for a replicate injection, specify a re-injection in the Monitor Run screen after you start the run.

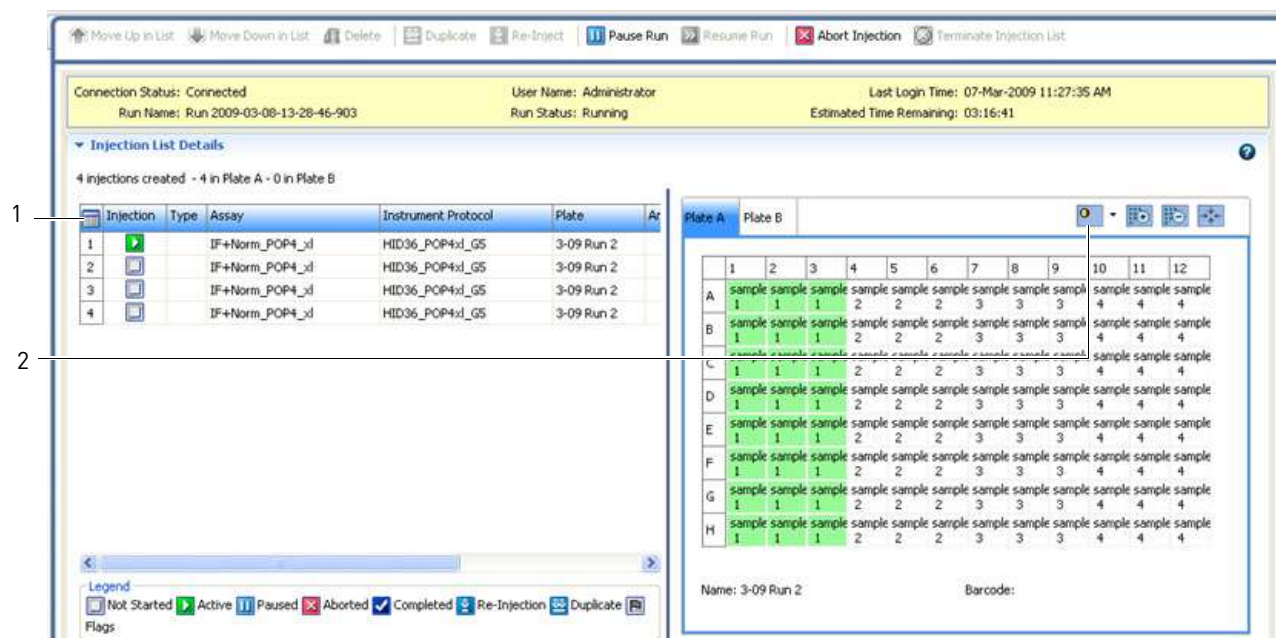
Start the run

When the injection list is configured, click **Start Run**. The Monitor Run screen is automatically displayed.

Note: By default, you can specify a re-injection before the run completes. To allow re-injections after a run is complete, set the Pause After Last Injection preference (see “User preferences” on page 43, Run Setup).

Monitor the run

The Monitor Run screen (Figure 7) is automatically displayed when you click Start Run in the Load Plates for Run screen or the Preview Run screen. The current injection is highlighted in green in the plate view. The injection list is linked to the plate view. Click an injection to select the associated wells in the plate view. A selected injection is highlighted in yellow in the plate view.


Figure 7 Monitor Run screen

Note: Samples with assays that specify more than one instrument protocol are listed one time in the injection list for each instrument protocol.

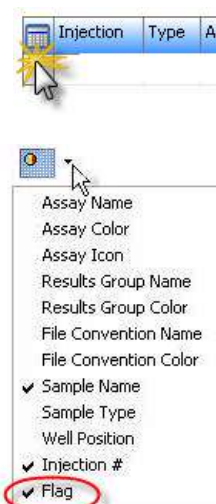
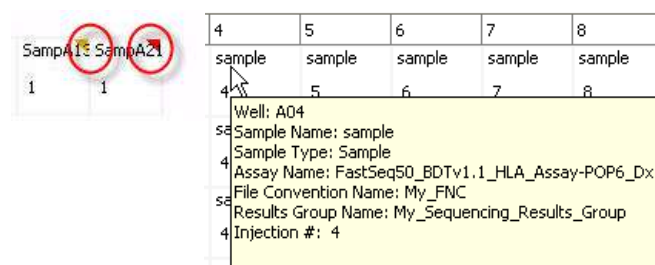
1. Click the Table Settings button, then specify the columns to show or hide in the injection list.

2. Optional:



- Click the icon above the plate to specify the attributes to display in the plate view. In addition to the attributes available in Preview Run, a Flag attribute is available.




If you select the Flags attribute, green marks are displayed for wells with Average QV values that are within range, yellow marks are displayed for wells with Average QV values that are in the suspect range, and red marks are displayed for wells with Average QV values that are out of range.

- Place the mouse pointer over a well to display sample details.



Check sequence or sample quality and specify re-injections

When an injection is complete, it is flagged with  in the Injection and Analysis columns. If the software detects a problem with offscale data or low quality samples, the injection is also flagged with .

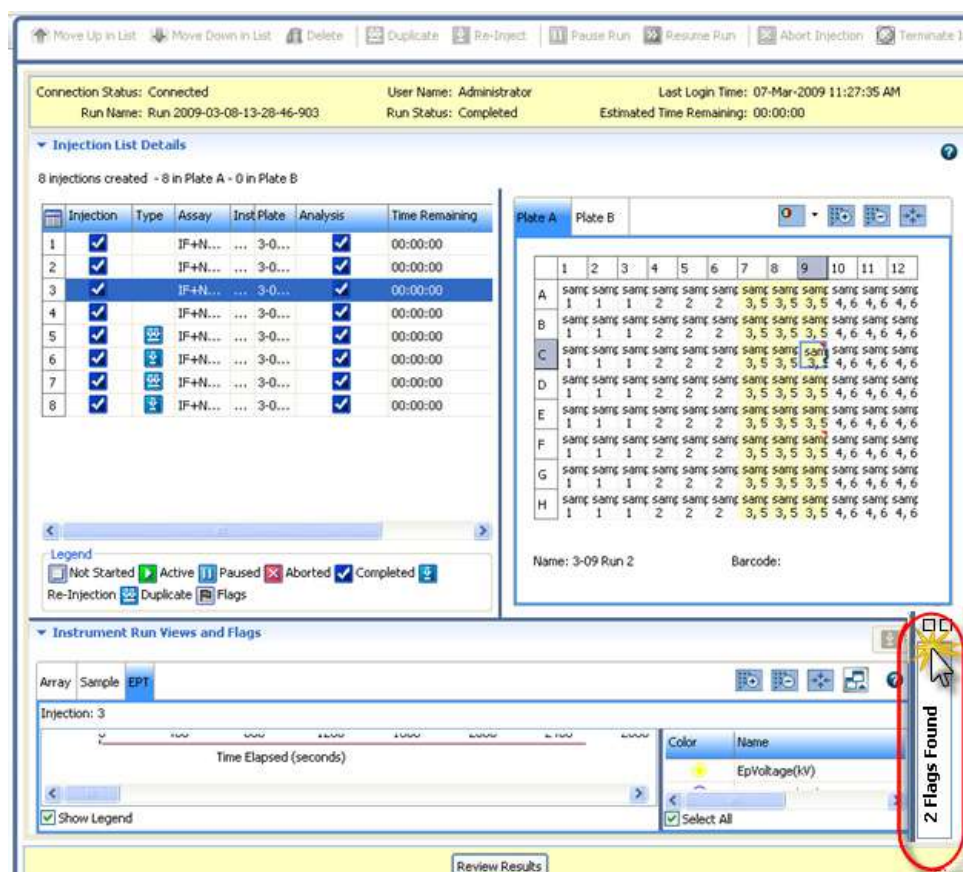
Injection	Type	Assay	Inst Plate	Analysis	Time Remaining	Flags
1		IF+N...	3-0...		00:00:00	

Note: If the Injection, Analysis, or Flags columns are not displayed, you can click the Table Settings button, then show them in the injection list.



Check sequence or sample quality

1. Expand the Flag pane at the bottom right of the screen.







The screenshot shows the software interface with the Injection List Details pane expanded. The list shows 8 injections, all with a checkmark in the Analysis column and a flag icon in the Flags column. The Flag pane at the bottom right is expanded, showing a table of sample quality data. The table has columns for Injection, Offscale, SQ/QV, Cap #, and Sample Name. The first two rows show data for injection 3, with Offscale values of 9 and 18, and Sample Names of 'sample'.

The flag table displays a quick preview of sample quality and identifies samples that may need investigation.




☐ 2 Flags Found

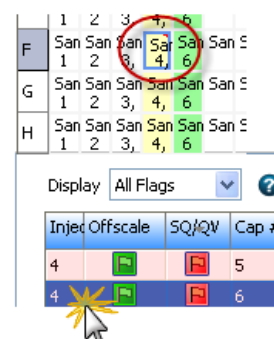
Display: All Flags

Inj	Offscale	SQ/QV	Cap #	Sample Name
3			9	sample
3			18	sample

The flag table is linked to the plate view. Click a flag to select the associated well in the plate view:

Note: If no samples are listed in this pane, no flags were found and the samples have passed quality checks.
















-  All samples passed
-  At least one sample is in the suspect range and requires review
-  At least one sample is offscale or is in the suspect range



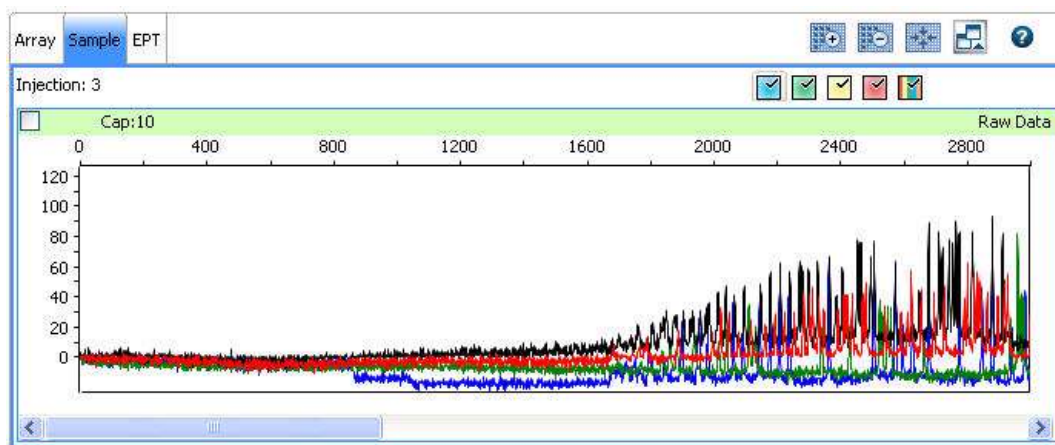
- To filter the flag table, select a flag type.

To sort the table, double-click column headers.

The flags you may see in the flag table are:

Flag/Symbols	Description
Offscale   (green or red)	 (red) At least one data point in the analysis range has saturated the CCD camera. Note: In the View Results screen, an offscale sample is flagged with  .
Average Quality Value (sequencing)    (green, yellow, red)	 (yellow) or  (red) The Average Quality Value (based on CRL, Trace Score, and QV20+ results) is in the Suspect or Fail range. For information, see "Basecalling protocol – QV settings" on page 179.
Sizing Quality (fragment/HID)    (green, yellow, red)	 (yellow) or  (red) The Sizing Quality is in the Suspect or Fail range. For information, see, Table 9 on page 179 or Table 11 on page 184. IMPORTANT! 

- Click a row in the flag table, then click the Sample tab in Instrument Run Views to display the associated data in the Sample view.








Specify re-injections

A re-injection physically re-injects all samples in the capillary array. You can specify whether to collect data for all or only selected samples in the array, and the instrument protocol to use for the re-injection (you can select the original instrument protocol, modify the original, or select a new instrument protocol for the re-injection).

By default, you can specify a re-injection before the run completes. To allow re-injections after a run is complete, set the Pause After Last Injection preference (see “User preferences” on page 43, Run Setup).

1. Select the injections or wells to re-inject:

Note:  Re-inject is grayed if you select an injection that contains more than one results group, or if you select flags in the flags table that correspond to samples with different results groups. To enable  Re-inject, select samples that specify the same results group.

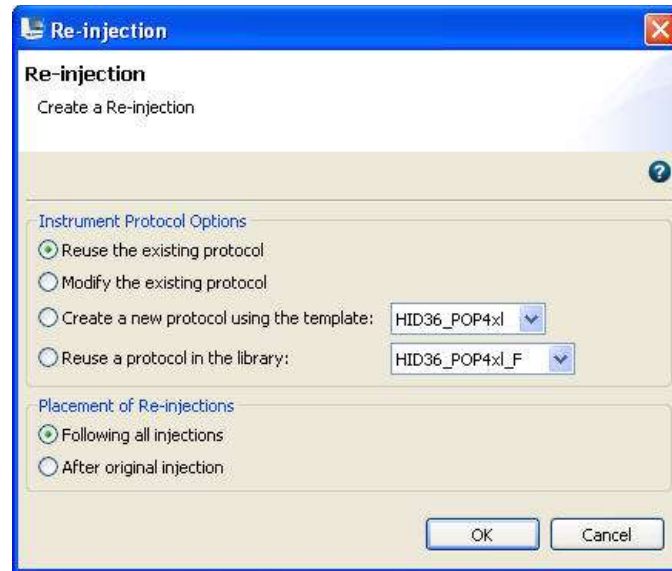
To collect data for all wells in an injection	<ol style="list-style-type: none"> 1. Select the injection in the injection list. 2. Click  Re-inject.
To collect data for only specific wells (Samples with assays that specify more than one instrument protocol are listed one time in the injection list for each instrument protocol)	<ol style="list-style-type: none"> 1. Select the injection. 2. Select in the array view the capillary that corresponds to the well or sample of interest (see “Array view” on page 83). 3. Click  Re-inject.
To collect data for only samples that contain flags	<ol style="list-style-type: none"> 1. Select the samples in the flag table (see “Check sequence or sample quality” on page 71). 2. Click  Re-inject.

Note: If you are running an HID plate, see “Re-injections of HID allelic ladder samples” on page 74.

2. In the Re-injection dialog box, select options, then click OK:

- The protocol to use for the re-injection: original, modified, new, or one from the library
- When to make the re-injection

Note: Sample data files for each re-injection can be saved in a separate folder in the results group folder if specified in the results group.



If you select a protocol other than the original

If you select a protocol other than the original, the software:

- Creates a copy of the assay specified for the re-injected well (Original_Assay-1).
- Adds the new or modified instrument protocol to Original_Assay-1.
- Assigns Original_Assay-1 to the re-injected well only.
- Saves the plate (the software does not save the copy of the assay to the library).

How re-injections are displayed in the plate view

If the Injection Number attribute is selected for display in the plate view, the number of the original injection and the re-injection are shown.

	1	2
A	sample 1 1, 2	
B	sample 2 1, 2	
C	sample 3	

Sample 1 selected for re-injection

Re-injection number listed for all samples in the re-injection

Note: If you select only specific wells for the re-injection (which physically re-injects all samples for the capillary array but collects data only for the selected wells), the re-injection number is displayed for all samples in the re-injection, not just the samples selected for data collection.

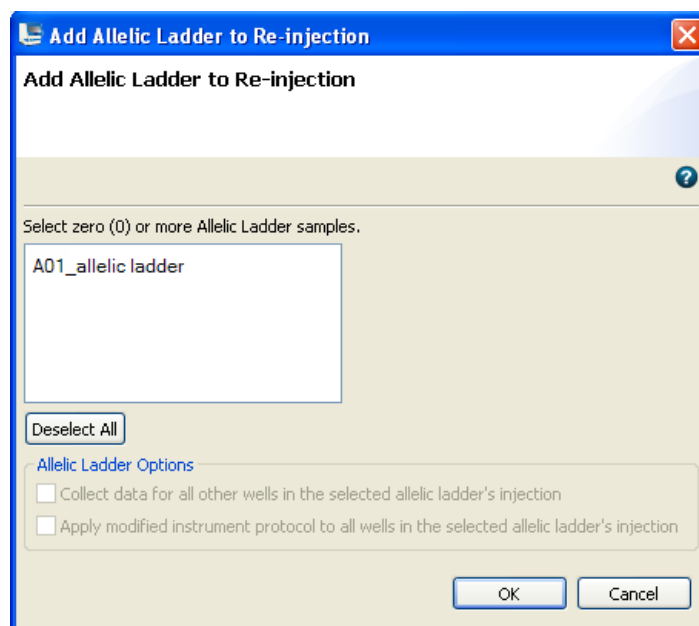
Re-injections of HID allelic ladder samples

If you select to re-inject a sample that includes an allelic ladder in its results group, but the allelic ladder is not part of the injection, the software prompts you to select one or more allelic ladder samples to re-inject.

For example:

- You are running an 8-capillary instrument, and you have specified one results group for each set of three injections (for more information, see "Results group example 2: store one allelic ladder per run folder (8-capillary instruments)" on page 163)
- The allelic ladder sample is in Injection 1.
- You select for re-injection a sample that is in injection 2.
- The software prompts you to select one or more allelic ladder samples to re-inject.

The allelic ladders available to select are from the same plate and within the same results group as the original injection. If the results group does not contain an allelic ladder sample, the software does not prompt you to select one for re-injection.



In the Add Allelic Ladder to Re-injection dialog box:

1. Select one or more allelic ladder samples.

IMPORTANT! The software does not display the well location of allelic ladder samples in this dialog box. To identify allelic ladder samples for re-injection, include the well position in the allelic ladder sample name when you assign plate contents.

2. Select whether to collect data for the remaining samples in the allelic ladder re-injection.
3. Select whether to apply a modified instrument protocol to the allelic ladder re-injections, or whether to use the original instrument protocol for the allelic ladder re-injection(s). You will select the modified protocol in the next screen.

IMPORTANT! Allelic ladders that are injected under the same conditions are recommended to accurately genotype samples in the secondary analysis software (GeneMapper® ID-X Software v1.3 or later).

4. Click **OK**.
5. Specify the remaining re-injection settings as described in “Specify re-injections” on page 73.

Two re-injections are added to the injection list. The first re-injection collects data for the selected sample. The second re-injection collects data for the allelic ladder.

Review completed injections in Review Results

You can review results for any completed injections. Select the injection, then click **Review Results**. The samples for the injection are loaded in the Samples Table in Review Results. For more information, see Chapter 4, “Review Results” on page 85.

Start and stop a run




Start a run

You can start a run in the:

- Load Plates for Run screen (see “Load plates for run and create the injection list” on page 65).
- Preview Run screen (see “Start the run” on page 69).


Pause and resume a run


As needed, click:




-  **Pause** – Pauses the run after the current injection completes (the  symbol is not displayed in the injection list because the injection continues to completion).
-  **Resume** – Resumes the run.

Abort or terminate

As needed, click:

-  **Abort** – Immediately aborts the current injection and pauses the instrument run. You can resume the run or terminate the injection list. Do not click Delete to stop an injection.

IMPORTANT! You can stop the current injection only when the front panel indicator is blinking green. If you click  **Abort** when the front panel indicator is solid green, the physical injection is already completed (although the software is still processing the information) and a message is displayed indicating that there is no injection in process.



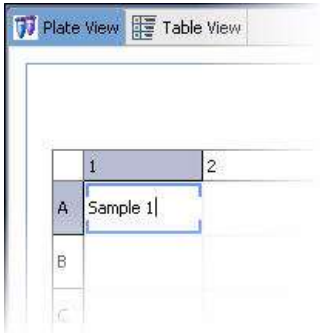
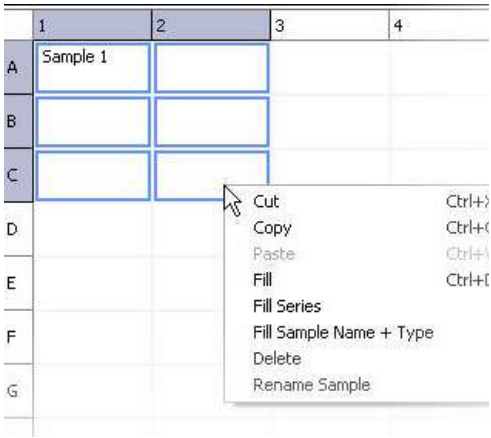
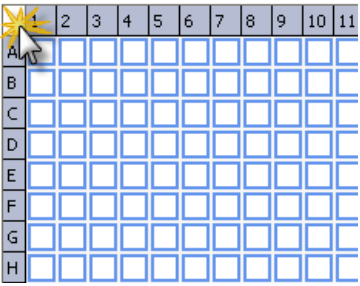
-  **Terminate injection list** – Stops the instrument run. Terminate is active only after you click  **Pause** or  **Abort**.

More features in Assign Plate Contents

Use the Plate View

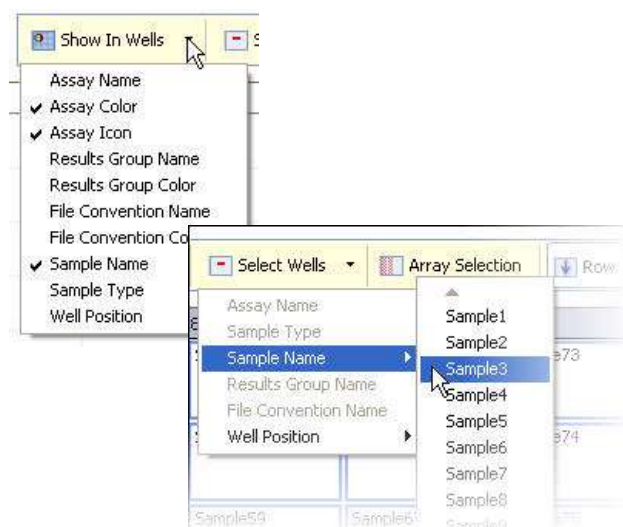
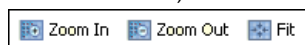
Name samples in the Plate View

To name samples in the Plate View:

To name one sample	<ul style="list-style-type: none"> Click a well, then type a sample name directly into the field, then press Enter. or Copy and paste a name from another well. <p>To set the direction for the cursor when you press Enter:</p> <ul style="list-style-type: none"> Click  Row to set the Enter key to move the cursor vertically to the next row. Click  Column to set the Enter key to move the cursor horizontally to the next column. 
To name multiple samples	<ol style="list-style-type: none"> Click a named well. Click-drag multiple wells. Right-click and select Fill or Fill Series to populate the selected fields <p>Note: To use Fill Series, type a number as the last character of the named well). You can also copy and paste sample names.</p> 
To name all wells at one time	<ol style="list-style-type: none"> Select all wells. Select assays, file name conventions, and results group for the plate. Enter name and select sample type (in the Customize Sample Info pane) for the whole plate. 

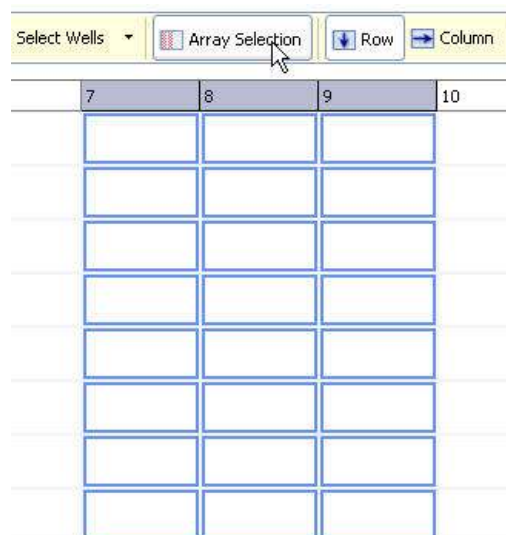
Customize the plate view

- Click **Show In Wells** to specify the attributes to display in wells.
- Click **Select Wells** to select wells with a specific attribute.
- Click **Zoom In**, **Zoom Out**, and **Fit** as needed.



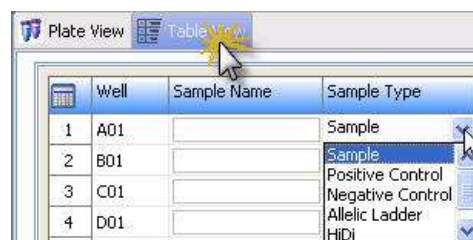
View the capillary/plate map

Click **Array Selection** to select wells by injection. Click again to turn off array selection.



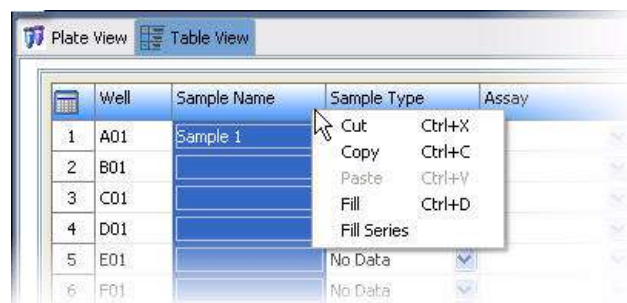
Use the Table View

1. Click **Table View**.
2. Click the Sample Name field, then type a name.
3. Click next to each field, then select a setting.



4. Right-click a column header, then select **Fill** or **Fill Series** to populate the selected fields (to use Fill Series, type a number as the last character of the named well).

Note: You can double-click column headers to sort columns. Multi-column sorting is supported (see “Multi-column sorting” below).



Sort and customize tables

Multi-column sorting You can sort any table in the software. Multi-column sorting is supported:

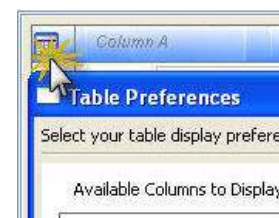
- Double-click a column header to sort the column.
- Alt+Shift-click another column header to sort another column.
- Alt+Shift-click a third column header to sort a third column.

Numbers in the column headers reflect sort order.



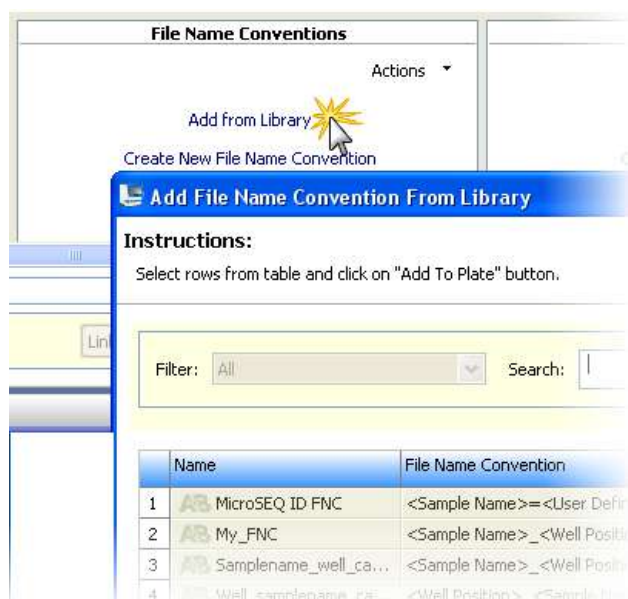
Customize tables

You can customize any table in the software. Click the Table Settings button, then specify the columns to show or hide.



Add assays, file name conventions, and results groups to a plate

To add an assay, file name convention, or results group from the library, click **Add from Library** at the bottom of the Assign Plate Contents screen.




Create a plate for importing

Create a plate import template

The 3500 Series Software 2 allows you to import plate information from files that you create in an application other than the 3500 Series Software 2.

To create a template for importing plate information, set up a plate in the 3500 Series Software 2, then export it to create a file that contains the correct header and column information for importing:

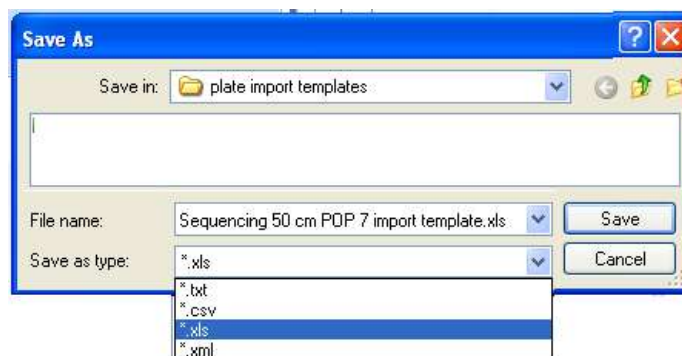
1. In the Dashboard, click **Create Plate from Template**.
2. In the Open Plate Template from Library dialog box:
 - a. Select a filter to display the plate template type of interest.
 - b. Select a plate template, then click **Open**.
3. Enter a name for the plate, then specify the capillary length and polymer type for the plate.
4. Click **Assign Plate Contents**.
5. In the Assign Plate Contents screen, click  **Export**.



Note: Before you click Export, you can assign other plate elements to the plate import template as described in “Assign plate contents” on page 55.

6. Select a file type for the plate import template.
7. Enter a name and location for the plate record template.
8. Click **Save**.

The figure below shows the format of the exported plate.



	A	B	C	D	E	F	G	H	
1	3500 Plate Layout File Version 1.0								
2									
3	Plate Name	Application Type	Capillary Length (cm)	Polymer	Number of Wells	Owner Name	Barcode Number	Comments	
4	plate import template	Sequencing	50	POP7	96				
5									
6	Well	Sample Name	Assay	Results Group	File Name Convention	Sample Type	User Defined Field 1	User Defined Field 2	User Defined Field 3
7	A01								
8	B01								
9	C01								
10									

Create a plate import file

1. Open a plate import template (see “Create a plate import template” on page 80).
2. Save the plate import template under a new name.
3. Enter sample names (required).
4. (Optional) Enter information for the remaining columns.
Note: If you specify assay, results group, or file name convention names, the names you enter must exactly match the names of existing items in the library.
5. Save the plate import file.

Edit a plate

You can edit a plate from:

- **Library** – Select a plate, then click **Edit**.
- **Dashboard** – Click **Edit Existing Plate**.
- **Define Plate Properties screen** – Select **Open Plate ▶ Edit Existing Plate**.
- **Assign Plate Contents screen** – Select **Open Plate ▶ Edit Existing Plate**.

Import and export a plate

You can import and export plates from:


- **Plates library** – Plates in .xml format for use on another 3500 Series Genetic Analyzer instrument. See “Import and export a library entry” on page 146.
- **Define plate properties** – Plates in .txt, .csv, and .xls format – Files you create that contain plate information in a specific format.
- **Assign Plate Contents** – Plates in .txt, .csv, and .xls format – Files you create that contain plate information in a specific format.

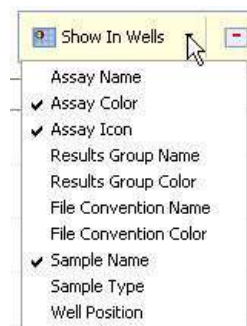
Create a plate template

A plate template contains default settings that you can edit when you create a plate from the template.

1. Create a plate (see “Create a new plate” on page 148).
2. (Optional) Add sample names and sample types (see “Name samples and assign sample types in the plate view” on page 56).
3. (Optional) Add the assays, file name conventions, and results groups appropriate for this plate template’s application (see “Add assays, file name conventions, and results groups to a plate” on page 80).

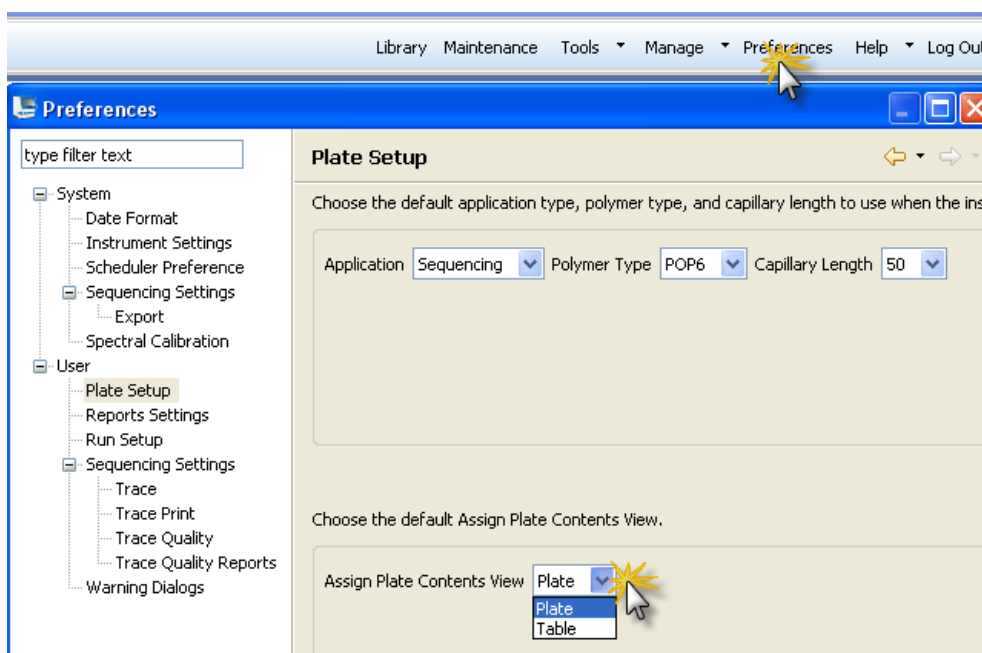
Adding assays, file name conventions, and results groups to the plate template automatically displays these items in the Assign Plate Contents screen when you open the plate template. You do not have to add these items from the library for each plate you create.

- (Optional) Click **Show In Wells** to specify the attributes to display in wells in the template.
4. Select **Save Plate ▶ Save As Template**. The software displays the template icon  below the plate layout.



Specify the default plate type for the Open Plate dialog box

Specify the default plate type for the Open Plate dialog box in Preferences.



Save electronic version of reports

When you print any report, you can select **CutePDF Writer** as the printer, to save the report to .pdf.

More features in Load Plate for Run

Link a plate from the recent plates or recent runs tab

Instead of clicking Link to select a plate, you can click-drag a plate from the Recent Plates tab (pending plates) or the Recent Runs tab (processed plates).



More features in Monitor Run

Review the Instrument Run views

Select an injection, then click an instrument run view tab. As needed:

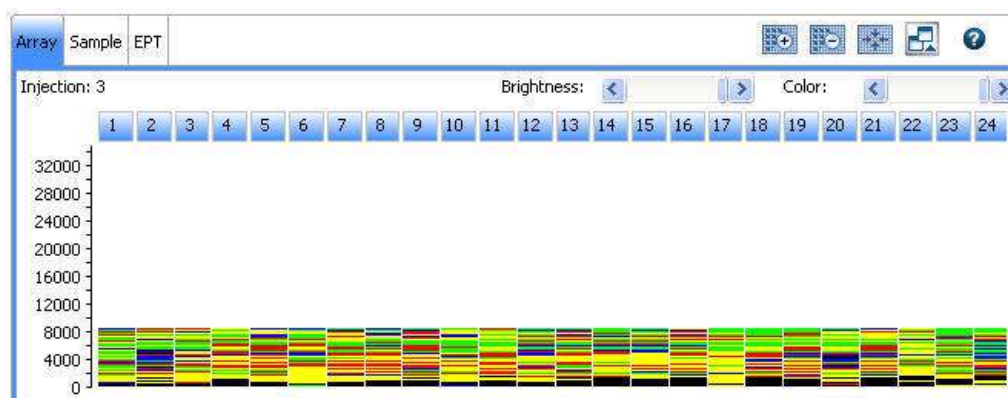
- Click to zoom in and out
- Click to detach a view and display it in a separate window that you can move around on the screen.

To locate a detached view, click the 3500 task bar icon.



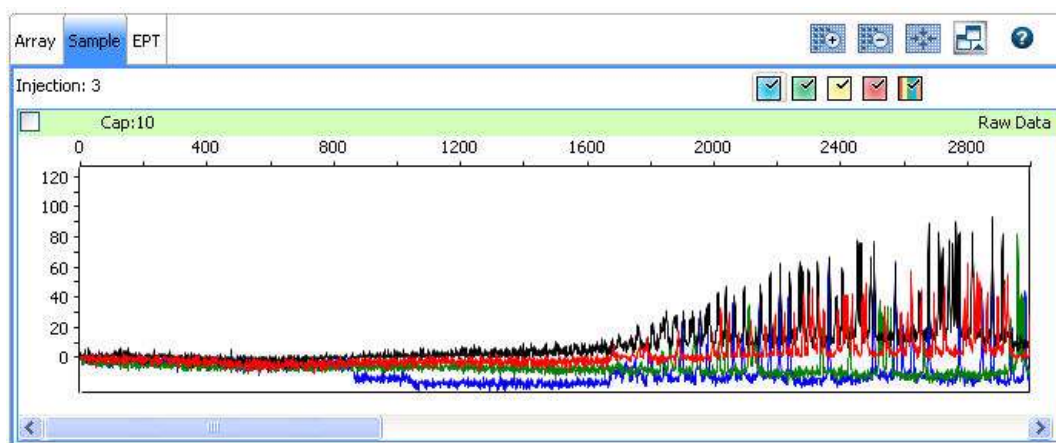
Array view

The Array view shows the color data (based on the dominant fluorescence color) for each capillary as a function of instrument scan number (time). Adjust the brightness and color by using the slider bars above the view.



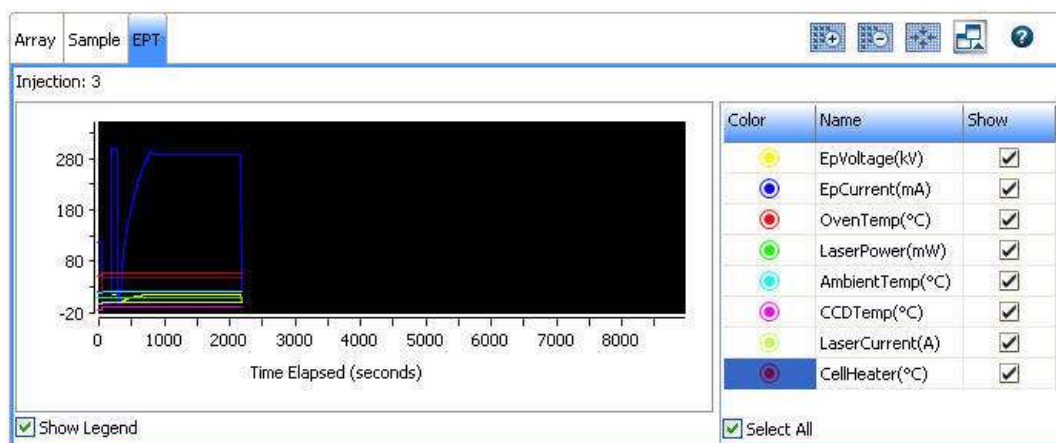
Sample view

The Sample view shows the relative dye concentrations as a function of instrument scan number (time) for the selected capillary. You can select and deselect the dye colors to display.



EPT view

The EPT view (ElectroPhoresis Telemetry) shows instrument data conditions (laser power, temperatures, electrophoresis voltage) as a function of time. In the legend to the right of the EPT view, you can select and deselect the traces to display in the view.

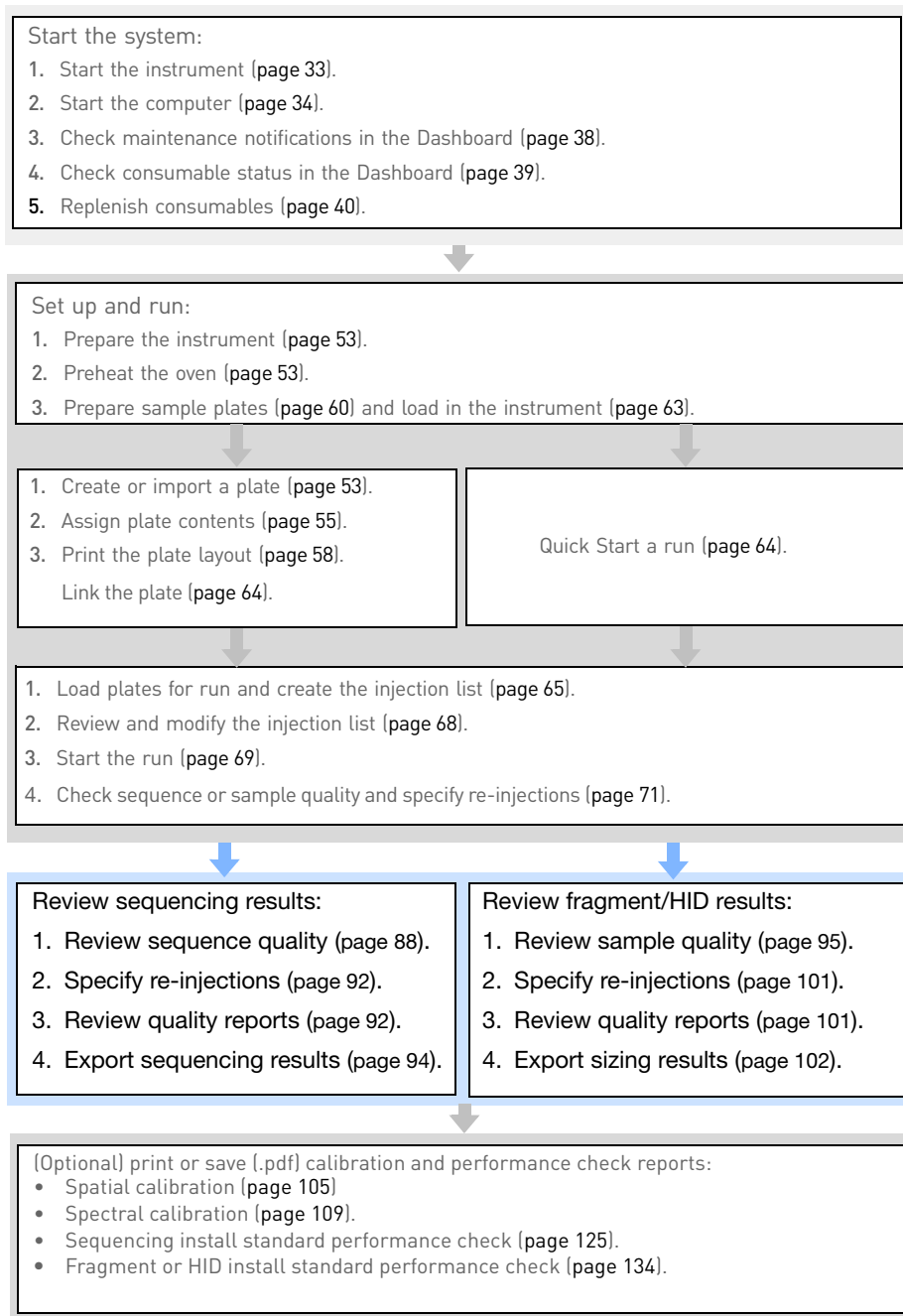


4

Review Results

- Workflow 86
- Review Sequencing Results. 87
- Review Fragment/HID Analysis results 94
- More features in Review Results 102
- Modify sequence, fragment analysis, or HID data 103

Workflow



Review Sequencing Results

Access the View Sequencing Results screen

Access the View Sequencing Results screen from:

- The Monitor Run screen by clicking **Review Results**.
- The navigation pane by selecting **View Sequencing Results**.
- The Dashboard by clicking View Run Results.

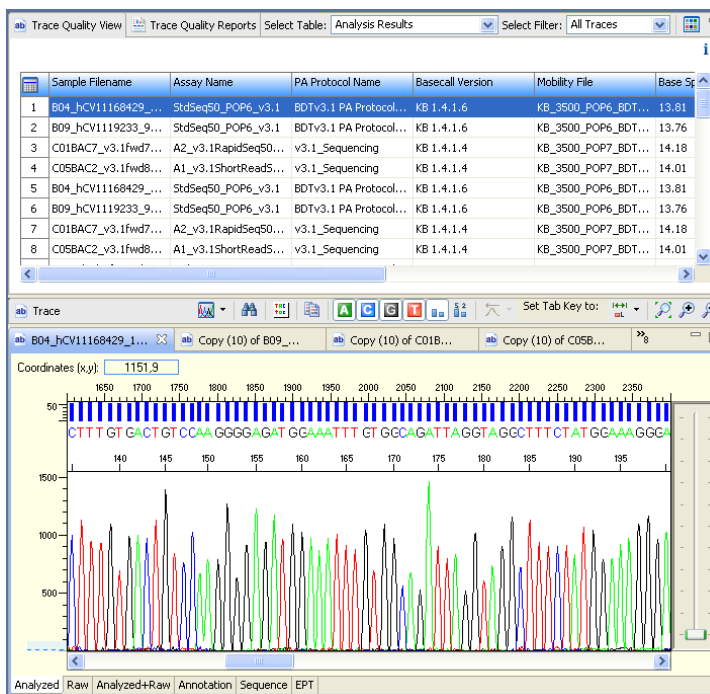


Review results for the currently running plate

If you access the View Sequencing Results screen while an instrument run is in progress, the Trace Quality View lists results for completed injections in the current run.


Select one or more samples, then click Open Trace to display their data in the Trace pane.

Note: The basecaller version listed in the basecalling protocol is limited to a 3-digit number. The version listed in sequencing results is a 4-digit number. The fourth digit is an internal number used by the software.



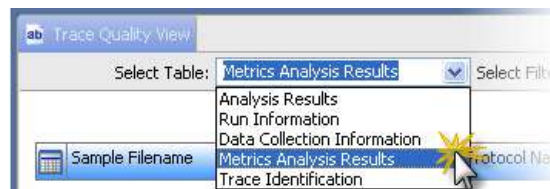
Review previously run samples

If you access the View Sequencing Results screen when no run is in progress and no plate is linked, no samples are listed. (If the plate from the most recent run is linked, the results from that plate are displayed.)

To view results for samples other than those from the most recent run, click  **Import**, then select the samples to review.

Review sequence quality

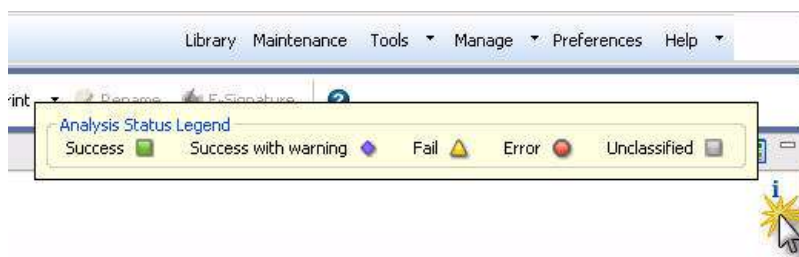
1. Display Metric Analysis results to review sample basecalling and trimming results.
2. Click the Table Settings button, then specify the columns to show or hide.
3. Double-click column headers to sort columns. Multi-column sorting is supported (see “Sort” on page 103).
4. Review the results:








Result	Description
Trace Score	The average basecall quality value (QV) of bases in the clear range sequence of a trace. The clear range is the region of the sequence that remains after excluding the low-quality or error-prone sequence at the 5' and 3' ends. The clear range is calculated by the KB basecaller using QVs.
CRL	The longest uninterrupted segment of bases with a Quality Value (QV) ≥ 20 . In addition to evaluating the QV of a base call, the software considers the QV of adjacent bases within ± 20 bases, before including a base in the continuous read length.
QV20+	The total number of bases in the entire trace that have basecaller quality values equal to or greater than 20.
Trace Score Quality CRL Quality QV20 Quality	Pass/fail/check determined by the settings in the Basecalling protocol QV Settings tab.
PUP Score	A measure of noise as calculated as the ratio of the fluorescence signal of the highest secondary peak to the fluorescent signal of the main called base.

5. Review warnings:
 - a. Scroll to the right of the Metric Analysis table to display the Warning column.

- b. Display the Analysis Status legend.




- c. Review warnings:

Result	Description
 Success	Basecalling and trimming successful.
 Success with warning	Basecalling successful with some anomalies. Warning messages are listed in the Warning/Error Message column (default position is the last column in the table).
 Fail	Basecalling and trimming failed, no results generated.
 Error	Basecalling and trimming failed due to internal software error, no results generated.
 Unclassified	No analysis performed.

6. (Optional) Click **Minimize** and **Restore** to collapse and expand the samples table.

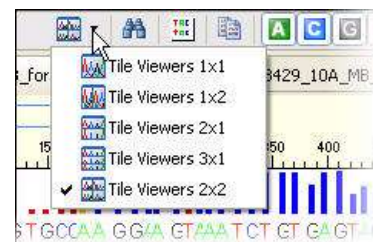


Review traces

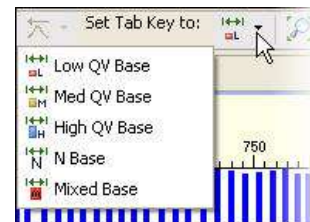
1. Select the samples of interest in the samples table, then click  **Open Trace**.
2. Select items from the trace toolbar to manipulate the trace as needed. Place the mouse pointer over a button for the description of the button.



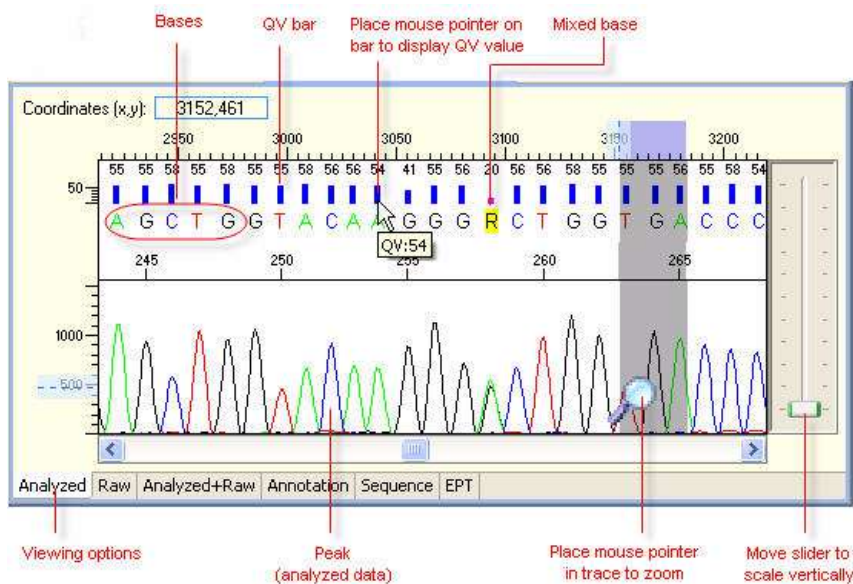
3. (Optional) Modify trace display:
 - Use the Tile Viewer options to display up to four traces at a time.
 - Set trace colors in Preferences (see "Set sequencing preferences" on page 45).



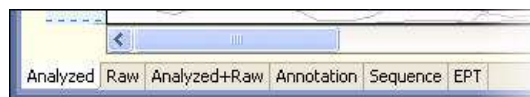
4. Set the category of base for the Tab key.



5. Review traces: press **Tab** to review bases from left to right in a trace. **Shift+Tab** to move right to left.



6. Click the tabs at the bottom of the trace pane for different views of the data.



Understand Quality Values (QVs)

Quality value ranges

We recommend the following ranges for QVs (set in Preferences, see “Set sequencing preferences” on page 45):

- **Pure bases** – Low QV < 15, Medium QV = 15 to 19, High QV = 20+ (default)
- **Mixed bases** – Low QV < 5, Medium QV = 5 to 10, High QV >10 (investigate to determine the best range for your application)

Pure base versus mixed base QVs

Pure bases and mixed bases have the same probability of error (Pe) for the associated basecall ($10^{-q/10}$). Note the following:

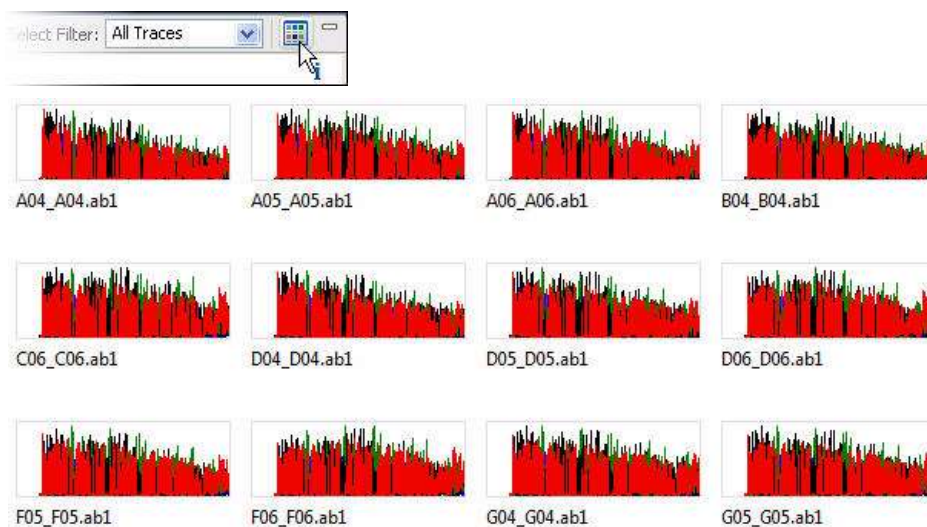
- High-quality pure bases typically have QVs of 20 or higher.
- The distribution of quality values for mixed bases differs dramatically from that of pure bases.
- For mixed bases, quality values greater than 20 are rare.
- Review all mixed base calls.

Quality values (QV) and probability of error (Pe)

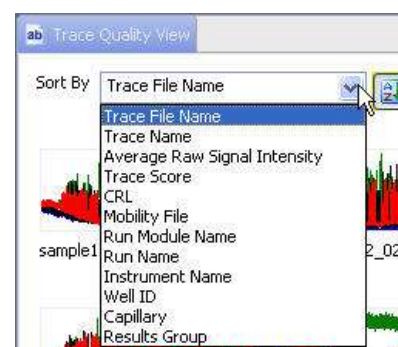
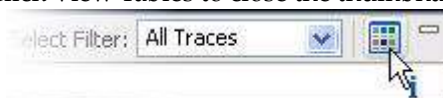
QV	Pe	QV	Pe
1	79.0%	30	0.10%
5	32.0%	35	0.032%
10	10.0%	40	0.010%
15	3.2%	45	0.0032%
20	1.0%	50	0.0010%
25	0.32%	60	0.00010%

Display thumbnails

1. Click View Thumbnails to display results as thumbnails.



2. Sort as needed.
3. To compare signal across all samples on a plate, select **Uniform Y Scaling**.
4. Click View Tables to close the thumbnail pane.



Specify re-injections


To re-inject, select a sample, then click  Re-inject.

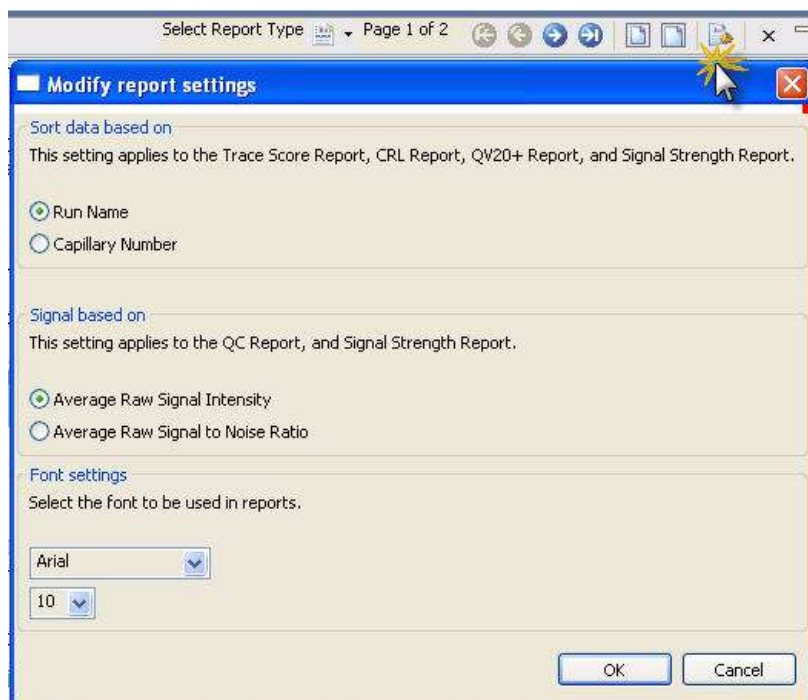
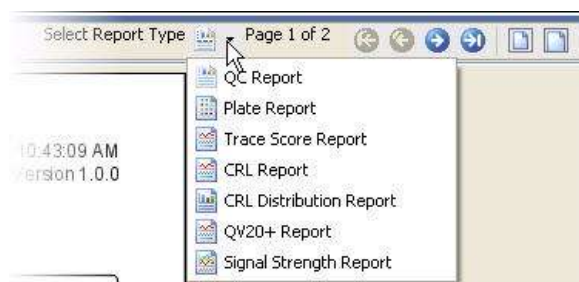
To allow re-injections after a run is complete, set the Pause After Last Injection preference before you start a run (see “User preferences” on page 43).

If you do not set the Pause After Last Injection Preference, you can specify a re-injection before a run is complete.


View, print, and save (.pdf) trace quality reports

View Trace Reports

1. Click  **View Trace Reports** to see the available reports for traces and print the reports you want. You can set defaults for the reports in Preferences (see “Set sequencing preferences” on page 45).
2. Select the report type and review the content of each report. See “Report options” on page 93.
3. Modify report settings as needed. You can specify additional report settings in Preferences (see “Trace (user preference)” on page 46, “Trace Quality (user preference)” on page 48, and “Trace Quality Report (user preference)” on page 49).



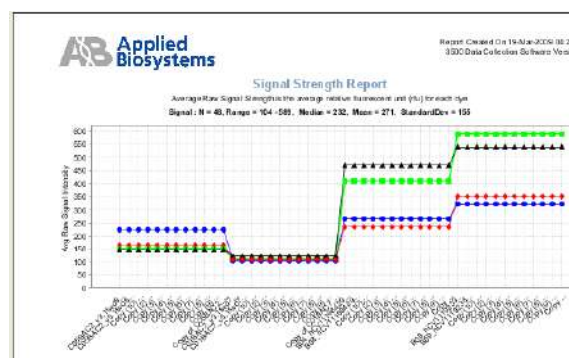
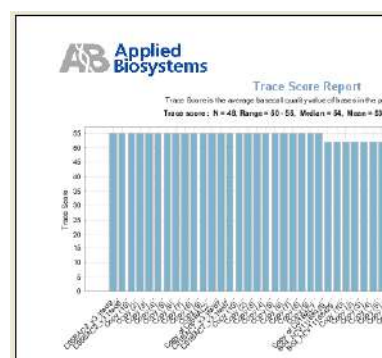
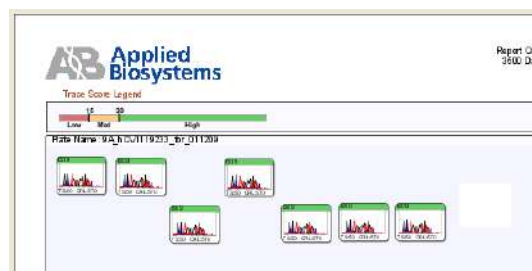
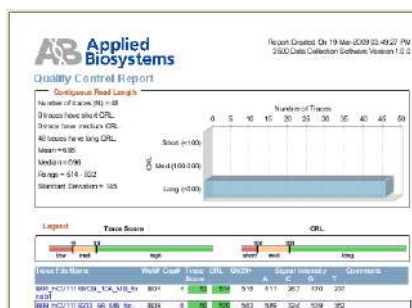
4. Double-click different elements in the QC report to open the Trace view and display the associated sample.

- To print the report, click  **Print**, then preview or print.
- To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.
- Close the report.



Report options

- QC** – One-page bar chart that shows trace score statistics and results for each selected sample.
- Plate** – One-page per plate for all selected samples that shows the well-location thumbnail raw data traces with color-coded headers that reflect Trace Score quality.
- Trace Score, CRL, and QV20+** – One-page bar chart that shows trace score, CRL, or QV20+ statistics and results for each selected sample.
- CRL Distribution** – One-page bar chart that shows CRL statistics and CRL results distribution for all selected samples.
- Signal Strength** – One-page graph that shows with average sequencing dye signal strength for all selected samples.



Export sequencing results

1. Filter the table of interest.
2. Select an export option: Results, Reports, or Traces.
3. Select the export options and the location for the export file, then click **OK**.



The file(s) are exported to the specified location with the following naming conventions:

- **Results** – *export_ReportName.txt*
- **Reports** – *ReportName.** (* is the format you selected: .txt, .xls, .pdf, .html)
- **Traces** – *FileName.** (* is the export format you selected: .annotation.txt, .phd.1, .scf, .fsta, .qual, .seq)

Review Fragment/HID Analysis results

Access the View Fragment/HID Results screen

Access the View Fragment/HID Results screen from:

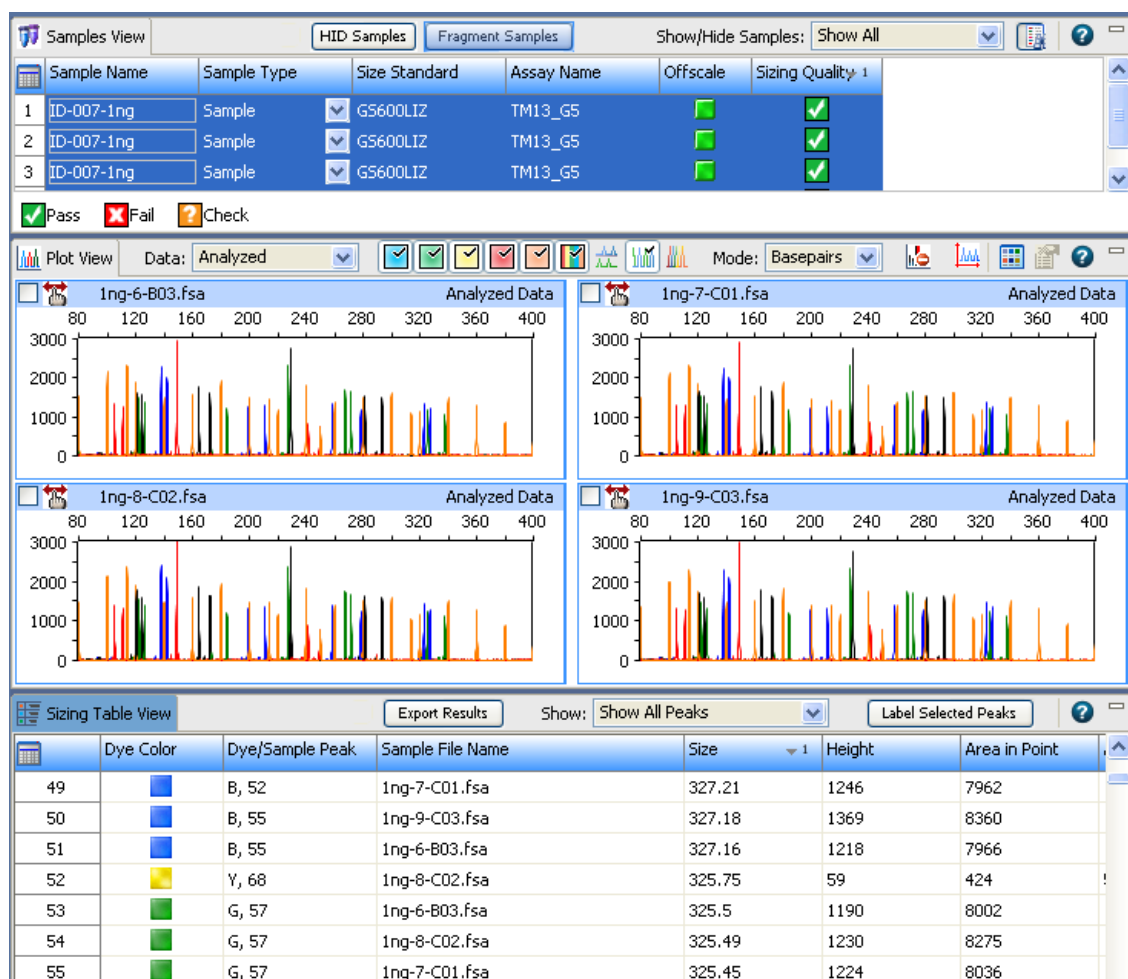
- The Monitor Run screen by clicking **Review Results**.
- The navigation pane by selecting **View Fragment/HID Results**.
- The Dashboard by clicking **View Run Results**.



Review results for the currently running plate

If you access the View Fragment/HID Results screen while an instrument run is in progress, the samples table lists results for completed injections in the current run.

Select one or more samples in the samples table to display their data in the plot view and sizing table view.

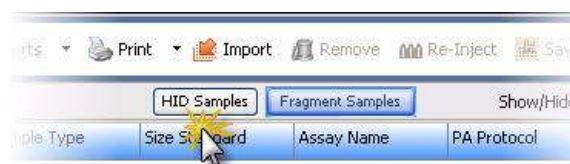


Review previously run samples

If you access the View Fragment/HID Results screen when no run is in progress and no plate is linked, no samples are listed. (If the plate from the most recent run is linked, the results from that plate are displayed.)

To view results for samples other than those from the most recent run, click **Import**, then select the samples to review.

Note: By default, the Fragment Samples view is selected. If you are importing HID files, click **HID Samples**.



Review sample quality

1. In the samples view, click the Table Settings button, then specify the columns to show or hide.
2. Double-click Offscale, Pull-Up (fragment), Broad Peak (HID), and SQ columns to sort suspect and failing flags to the top of the table.



Multi-column sorting is supported (see “Sort” on page 103).

Offscale	Spectral PullUp	Sizing Quality

Flag/Symbols	Description
Offscale 	At least one data point in the analysis range has saturated the CCD camera. Note: In the Monitor Run screen, an offscale sample is flagged with .
Spectral Pull-Up (fragment analysis only) 	At least one peak contains a pull-up peak. A pull-up peak is identified when the peak height of the minor peak is $\leq X\%$ of and within $\pm Y$ data point of the major peak, where X and Y are values you specify. See Chapter 6, Manage Library Resources.
Broad Peak (HID analysis only) 	At least one peak exceeds the Broad Peak threshold. Broad peaks affect Sizing Quality. See Chapter 6, Manage Library Resources. Note: The value displayed when you place the mouse pointer over a Broad Peak flag is an internal value and does not reflect the peak width.
Normalization Limit 	<ul style="list-style-type: none"> – Sample was collected with a normalization size standard, sample Normalization Factor is within range. – Sample was collected with a normalization size standard, sample Normalization Factor is not within range. No Data – Normalization is enabled, but Sizing Quality is . NO – Sample was not collected with a normalization size standard. N/A – Sample was not collected on a 3500 Series Genetic Analyzer instrument. For more information, see “Review normalized data” on page 97. Note: If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range.
Sizing Quality 	The Sizing Quality is in the Fail or Suspect range. Place the mouse pointer over a flag to display the Sizing Quality value for the sample. See Chapter 6, Manage Library Resources.

Note: If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range.


- Click a flag in the samples table, or select samples in the samples table to display the associated data in the Plot View and Sizing Table View.
- (Optional) Modify the sample view:
 - Right-click the Size Standard field to view the size standard for a sample.
 - Click **Minimize** and **Restore** to collapse and expand the samples table.



Review normalized data

Normalization corrects for instrument, capillary, and injection variability. When specified in the primary analysis protocol, the software calculates a normalization factor for each sample. The normalization factor is used as a multiplier to adjust the peak height of the sample peaks relative to the GS600 LIZ® V2 size standard peaks.

A sample is normalized if it is collected with a normalization size standard (specified in the primary analysis protocol [sizecalling or QC] in the assay).


Note: If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range. Ensure that you use the normalization size standard appropriate for your application. For more information, see “Normalization size standards provided” on page 173.

How normalization is applied

To normalize, the software:

1. Determines if the data was collected on the 3500 Series Genetic Analyzer instrument.
2. Determines if the sample was collected with a normalization size standard definition file (normalization is enabled).
3. If normalization is enabled, the software calculates a Normalization Factor for the sample using multiple size standard fragments. The Normalization Factor is calculated by dividing the Normalization Target by the observed average peak height of the size standard fragments in the samples.
4. Compares the sample Normalization Factor to the thresholds (set in the instrument protocol).
5. If the calculated Normalization Factor is within the Normalization Factor range, multiplies the peak heights of the sample by the calculated Normalization Factor.
6. If the calculated Normalization Factor is outside the Normalization Factor range, multiplies the peak heights of the sample by the maximum or minimum Normalization Factor threshold setting (for example, if the Normalization Factor range is 0.3 to 3.0 and the calculated Normalization Factor is 5, the software applies a Normalization Factor of 3.0).
7. Indicates the normalization state of the sample in the Normalization Limit column in the Samples View.

Normalization factor in secondary analysis

If normalization is applied in the 3500 Series Software 2, the calculated Normalization factor is stored with the raw data and is applied to the raw data in the GeneMapper® Software 5 and the GeneMapper® ID-X Software v1.2 or later secondary analysis software. You can turn normalization off and on in the analysis method used in the GeneMapper® 5 and GeneMapper® ID-X Software v1.2 or later secondary analysis software. If normalization is not applied in the 3500 Series Software 2 (either a normalization size standard was not used, or Sizing failed ) , normalization cannot be applied in the secondary analysis software.

Review plots

1. Select the samples of interest in the samples table.
2. Select items from the plot toolbar to manipulate the plot as needed. Place the mouse pointer over a button for the description of the button.

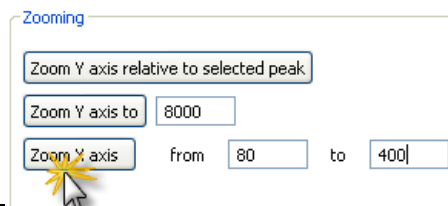


IMPORTANT! If you first view a 4-dye sample, then view a 5-dye sample, you must manually select the fifth dye. It is not automatically selected when you switch to a 5-dye sample.


3. Apply scaling settings to plots:
Enter the range for Y axis and X axis, then click the Zoom buttons.

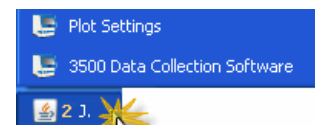


IMPORTANT! You must open Plot Settings each time you access the View Results screen, then click **Zoom**. Scaling settings are not automatically applied when you access this screen, or when you click Apply.

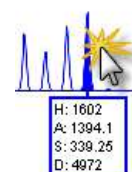
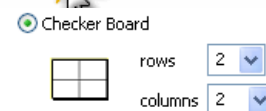


To apply scaling settings to all samples in the samples table, select all of the samples in the samples table to display them in the plot view, specify the scaling settings, click **Zoom**, then click **Page Up** and **Page Down** in the plot view to move through the samples.





If the  button is grayed, it indicates that the Plot Settings dialog is open. Click the 3500 task bar icon, then select Plot Settings.

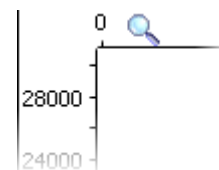


4. Display multiple plots as needed: in the Plot Settings Display tab, select **Checkerboard**.
5. Click a peak to label it (to label all peaks, see "Label peaks" on page 99).






Zoom

1. Place the mouse pointer *above the top* of the plot or *to the left* of the plot at the start of the area you want to zoom, then click to turn the pointer to .
2. With the  still *above* the plot or *to the left* of the plot, click-drag to the end of the area you want to zoom. Do not drag the  inside the plot area. Doing so changes  back to a pointer and does not zoom as expected.






Change plot settings


Click  (Plot Settings) in the Plot View toolbar. For information on plot settings, click  in the plot settings tabs.

If the  button is grayed, it indicates that the Plot Settings dialog is open. Click the 3500 task bar icon, then select Plot Settings.

Overlay samples

1. Select samples from the Samples View to display the plots.
2. Click  **Overlay All**. When  Combine Dyes is selected, the plot view displays one plot with all samples and all dyes. When  Separate Dyes is selected, the plot view displays on plot per dye. Each dye plot contains all samples.

Label peaks

1. Select samples from the Samples View to display the plots.
2. Click  (Plot Settings) in the Plot View toolbar.



3. In the Plot Settings dialog box, select the **Labels** tab.
4. If you have already specified default labeling preferences, under Labelling Options:
 - a. Enable **Show Peak Labels**.
 - b. Click **Label Peaks**.
 - c. Click **Apply**.

IMPORTANT! You must open Plot Settings each time you access the View Results screen, then click **Label Peaks**. Labelling settings are not automatically applied when you access this screen, or when you click Apply.

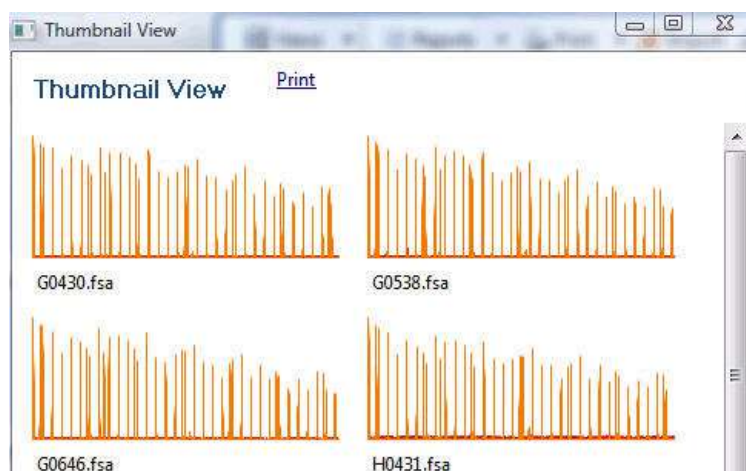
If you have not specified default label settings:

- d. Under Labels to Show, select the needed labels.
- e. Under Labelling Options:
 - Enable **Show Peak Labels**.
 - To label all peaks with the selected labels, click **Label Peaks** (make sure **All** is selected).
 - To label selected peaks, select the category from the Label Peaks list (Height, Area, Size), specify the range to label for the selected category (for example, if you select Height, specify the height range of the peaks to label), then click **Label Peaks**.
 - Enable **Retain Labels**.

- f. Click **Save to Preferences** to save these settings for future use. You can change preferences at any time.
- g. Click **Apply**.

View thumbnails

Click View Thumbnails to display the traces for the samples selected in the samples view, and the dyes selected in the plot view.



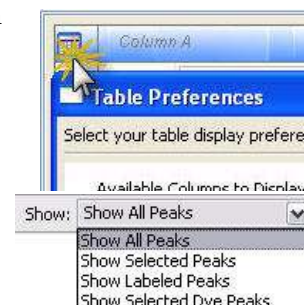
Review sizing

The Sizing Table View displays:


- **For fragment samples** – All dyes
- **For HID samples** – Size standard dye only (orange or red)

Set up the sizing table


1. Select the samples of interest in the samples table to display plots.
2. In the sizing table, click the Table Settings button, then specify the columns to show or hide.
3. Filter the table as needed.
4. Double-click column headers to sort columns. Multi-column sorting is supported (see “Sort” on page 103).
5. Selecting rows in the sizing table, then click **Label Selected Peaks**.




Examine the size standard plot

1. In the Plot View toolbar, deselect all dye colors except the size standard dye color (red or orange).
2. In the sizing table, select the size standard peaks of interest.
3. Click **Label Selected Peaks** to label the size standard peaks in the Plot View.
Note: If labels are not displayed, click  (Plot Settings) in the Plot View toolbar, then select Show Labels in the Labels tab. Click **Save to Preferences** to retain this setting.
4. Ensure that all size standard peaks are present and correctly labeled.


Overlay the sizing curve

1. Click  (Plot Settings) in the Plot View toolbar.
2. Select **Overlay Sizing Curve** in the Display tab.


Specify re-injections

Before the run completes, select a sample with suspect or failing flags, then click  **Re-inject**. For information on making a re-injection before a run completes, see “Specify re-injections” on page 73.

View, print, and save (.pdf) sample quality reports

1. Select the samples of interest in the samples table.
2. Click  **Reports** to see the available reports for traces and print the reports you want.
3. Select the report type. Reports are displayed in the Sizing Table View at the bottom of the screen.
4. Modify report settings as needed.

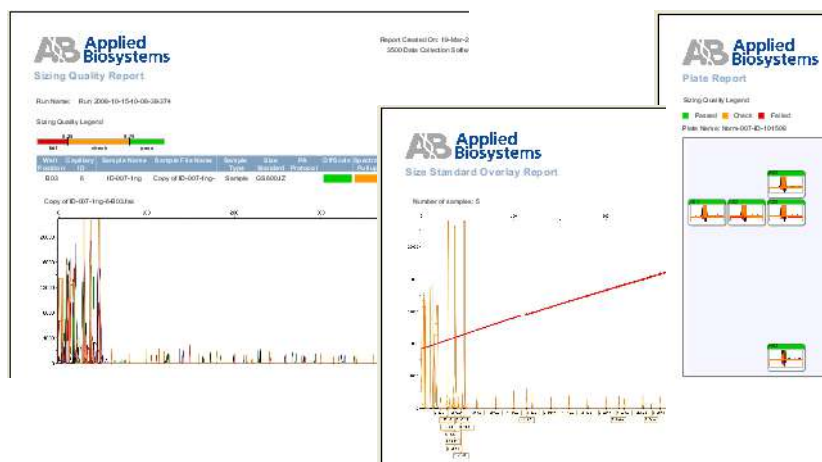


5. To print the report, click  **Print**, then preview or print.
6. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.
7. Close the report.



Report options

- **Sizing** – One page per selected sample that shows the quality ranges set in the sizingcalling or QC protocol, the quality values for the sample, and the electropherogram for the sample. Plot zooming is not retained in the report.
- **Overlay** – One page for all selected samples that shows the size standard dyes overlaid with the size standard curves.
- **Plate** – One page per plate for all selected samples that shows the well-location thumbnail traces with color-coded headers that reflect sizing quality. Plot zooming is not retained in the report.




Export sizing results

1. Set up the sizing table as described above. All rows and columns displayed in the sizing table are exported.
2. Click **Export Results**.

More features in Review Results

Use Rename

Note: Changes to sample names are tracked only if your system includes the SAE module and auditing is enabled on your system.

1. In the Sample Name column, select the samples to rename, or click the Sample Name column header to select the entire column.
2. Click  **Rename**.
3. In the Search field, enter the sample name to change.
4. In the Rename field, enter the new name.
5. Click Search, then click Rename.

Sort

Double-click column headers to sort. Multi-column sorting is supported:

- Double-click a column header to sort the column.
- Alt+Shift-click another column header to sort another column.
- Alt+Shift-click a third column header to sort a third column.

Numbers in the column headers reflect sort order.



Modify sequence, fragment analysis, or HID data

To edit, modify, or further analyze sequence, fragment analysis, or HID data, import the sample data files into a secondary analysis software application such as:

- **Sequencing** – SeqScape® Software 3 (or later), MicroSeq® ID Analysis Software v3.0 (or later), Variant Reporter® Software 2 (or later), and Sequence Analysis (SeqA) Software 6 (or later)
- **Fragment analysis** – GeneMapper® Software 5 (or later)
- **HID** – GeneMapper® ID-X Software v1.2 (or later)

5

Calibrate and Check Performance

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■ Sequencing install standard performance check	125
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Section 5.1 Calibrate

Spatial calibration

The 3500 Series Software 2 uses images collected during the spatial calibration to establish a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera.

When to perform a spatial calibration

Perform a spatial calibration after you:

- Remove or replace the capillary array for maintenance purposes
- Replace the capillary when the capillary array expires (the expiration date is indicated on the packaging and the RFID tag)

Note: When the instrument reads the information from a newly installed capillary array, it requires that you run a spatial calibration and a spectral calibration before you can run plates.

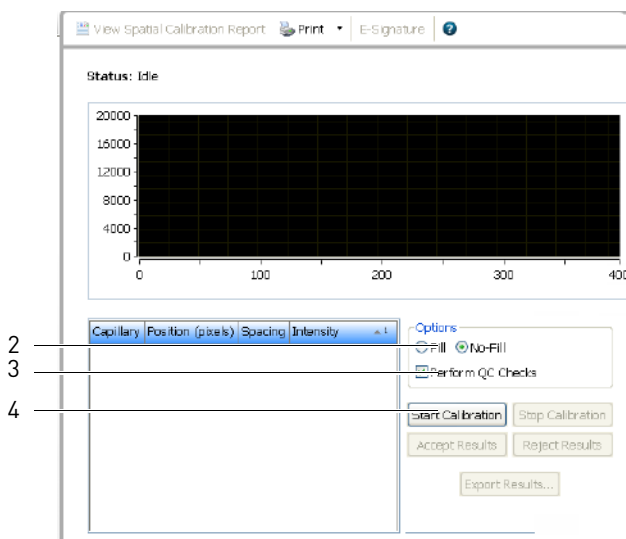
- Have a service engineer perform an optical service procedure, such as realigning or replacing the laser or CCD camera or mirrors on the instrument
- Open the detector door or move the detection cell
- Move the instrument

Perform a spatial calibration

IMPORTANT! Do not open the instrument door during a spatial calibration run. Doing so will stop the run and require you to restart the 3500 Series Software 2.

1. Access the Spatial Calibration screen:
Select **Maintenance**, then select **Spatial Calibration** in the navigation pane.

Note: The screen does not display results unless you have previously performed a spatial calibration.



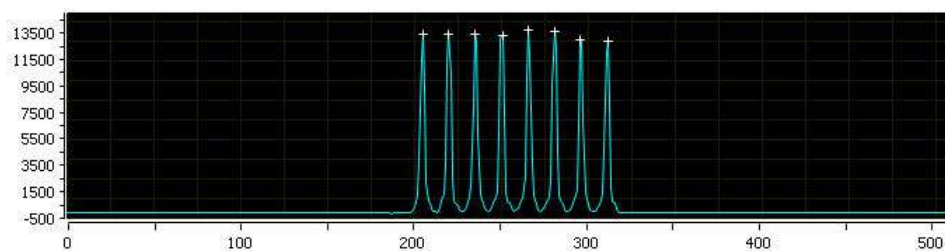
2. Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.

(Optional) Select **Perform QC Checks** if you want the system to check each capillary against the specified range for spacing and intensity. During the calibration, the software calculates:

Attribute	Calculation	Threshold
Average peak height	$\frac{\text{sum of all peak heights}}{\text{number of peaks}}$	<ul style="list-style-type: none"> 8-cap: 6400 RFU 24-cap: 3000 RFU
Individual peak height	Peak height	1000 RFU
Uniformity (peak height similarity)	$\frac{\text{standard deviation}}{\text{average peak height}}$	0.2
Capillary spacing	max spacing – min spacing	2 pixels

3. Click **Start Calibration**.

The display updates as the run progresses.



A Spatial QC Check error message is displayed if:

- The average peak height or individual peak height is below the threshold
- Uniformity or capillary spacing exceeds the threshold

Evaluate the spatial calibration profile

When the run is complete:

1. Evaluate the spatial calibration profile to ensure that you see:
 - One sharp peak for each capillary. Small shoulders are acceptable.
 - One marker (+) at the apex of every peak. No off-apex markers.
 - An even peak profile (all peaks about the same height).

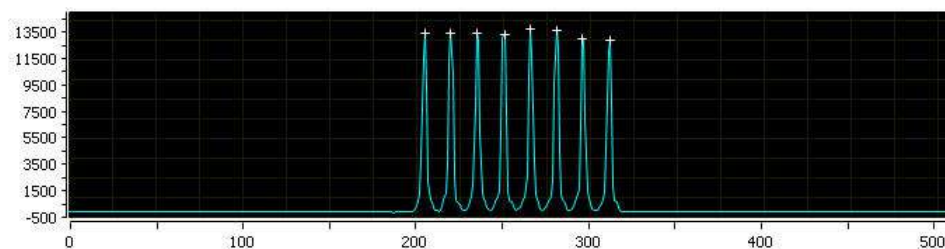
2. If the results meet the criteria above, click **Accept Results**.

If the results do not meet the criteria above, click **Reject Results**, then go to “Spatial calibration troubleshooting” on page 266.

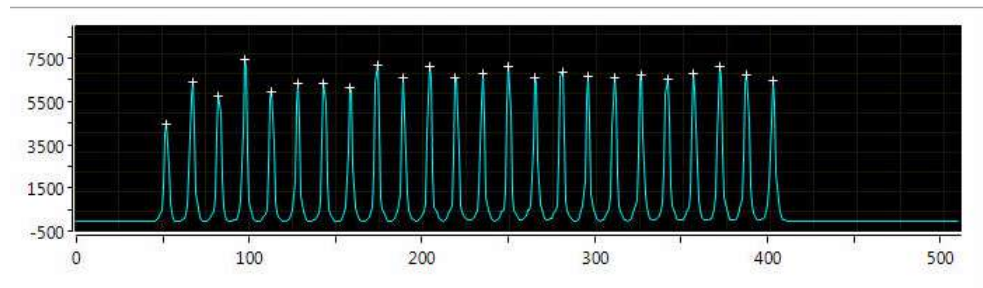
IMPORTANT! Do not log off or close the software before clicking Accept Results. Spatial calibration results are not saved until you click Accept Results.

Example spatial profiles

8-capillary



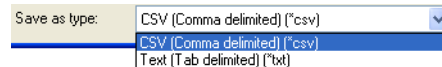
24-capillary



Export spatial calibration results

To export spatial calibration results:

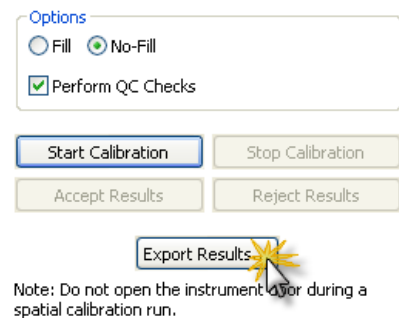
1. Click **Export Results**.
2. Enter an export file name.
3. Select the export file type.



4. Click **Save**.


The export file contains the following results:

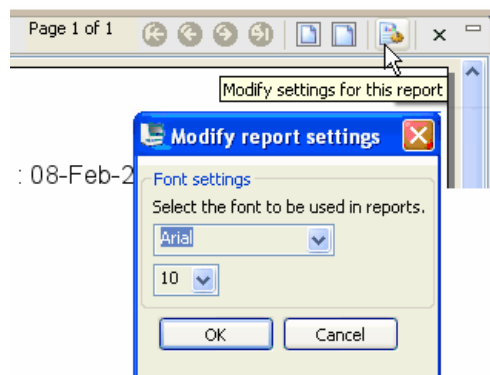
- Capillary Number
- Position (pixels)
- Spacing
- Intensity




View and print a spatial calibration report

Note: Spatial and spectral calibration reports include the date on which a capillary array is installed for the first time on the instrument. Install standard reports use the most recent install date if a capillary array was removed and re-installed on the instrument.

1. Click  **View Spatial Calibration Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.





3. To print the report, click  **Print**.
4. Close the report.



Save historical calibration reports (.pdf) for record keeping

IMPORTANT! After performing a calibration, save the calibration report electronically for record keeping. The software does not save historical calibration results. Only the most recent spatial calibration is maintained in the software.

1. Click  **View Spatial Calibration Report**.
2. Click  **Print**.
3. In the Printer dialog box, select **CutePDF Writer** as the printer.
4. Specify a name and location for the report.

Spectral calibration

A spectral calibration creates a de-convolution matrix that compensates for dye overlap (reduces raw data from the instrument) in the dye data stored in each sample file.

IMPORTANT! To calibrate a custom dye set using AnyDye, first create the dye set, then select the name of the custom dye set from the Dye Set list. The AnyDye selection in the Dye Set list contains default settings. It does not correspond to custom dye sets created with the AnyDye dye set template.

When to perform a spectral calibration

Perform a spectral calibration for each dye set/polymer type combination you will use:

- Sequencing dye set/polymer type
- Fragment dye set/polymer type
- HID dye set/polymer type

Perform a spectral calibration when you:

- Use a dye set that you have not previously calibrated
- Replace the capillary array for main ten ace purposes
- Replace the capillary when the capillary array expires (the expiration date is indicated on the packaging and the RFID tag)

Note: When the instrument reads the information from a newly installed capillary array, it requires that you run a spatial calibration and a spectral calibration before you can run plates.

- Have a service engineer perform an optical service procedure, such as realigning or replacing the laser or CCD camera or mirrors on the instrument

- See a decrease in spectral separation (pull-up/pull-down in peaks) in the raw or analyzed data

Note: If you are using the v3.1 sequencing standard or v1.1 sequencing standard and want to run a performance check and a spectral calibration, you can skip this process, and run the Sequencing Install Standard performance check. If you select Keep Spectral Calibration Data in the Performance Check, the software runs a spectral calibration for dye set E or Z during a sequencing check and allows you to save the spectral calibration data. For information, see “Sequencing install standard performance check” on page 125.

Estimated run time

Standard	Polymer Type	Run Time (min)
Matrix standard	Any	≤30
Sequencing standard	POP-7™ polymer	≤40
	POP-6™ polymer	≤135

Prepare for the spectral calibration


Prepare the instrument

1. If you have not already done so, perform a spatial calibration (see “Spatial calibration” on page 105).
2. In the Dashboard, check consumable status (“Check consumable status” on page 39). Ensure that:
 - Consumables are not expired
 - Adequate injections remain for consumables
3. Ensure that the buffer levels are at the fill lines (“Check buffer fill levels” on page 40).
4. Set the oven temperature, then click **Start Pre-heat**:

Standard	Polymer	Preheat Temperature
Sequencing	POP-6™	50°C
	POP-7™	60°C
Matrix	POP-4®	60°C
	POP-6™	60°C
	POP-7™	60°C

Pre-heat the oven and detection cell while you prepare for a run (detection cell temperature is set by the software). Preheating helps mitigate subtle first-run migration rate effects. The pre-heat function automatically turns off after 2 hours. We recommend that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold.

5. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed (see “Remove bubbles from the polymer pump” on page 238).

Prepare the spectral
calibration plate**IMPORTANT!** Do not use warped or damaged plates. 

1. Prepare the spectral calibration standard as described in the product insert. See Appendix A, Application Reagents and Run Modules for spectral calibration standard part numbers.

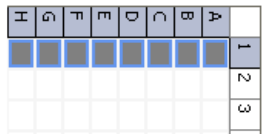
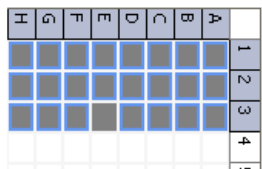
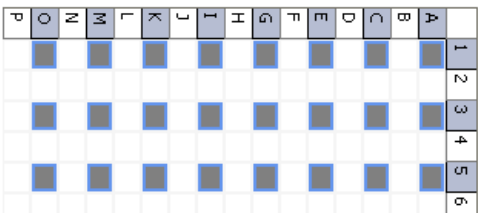
Note: If peaks are offscale for G5, F, and E5 dye sets, dilute the matrix standard and repeat the calibration.

Note: For the BigDye® Direct DNA PCR Amplification/ Clean-up/Cycle Sequencing kit, use the Z dye set.

Dye set	Standard
E	BigDye® Terminator™ (BDT) v1.1 Sequencing Standard
	BigDye® Terminator™ (BDT) v1.1 Matrix Standard
Z	BigDye® Terminator™ (BDT) v3.1 Sequencing Standard
	BigDye® Terminator™ (BDT) v3.1 Matrix Standard
F	DS-32 Matrix Standard
E5	DS-02 Matrix Standard
G5	DS-33 Matrix Standard
J6	DS-36 Matrix Standard

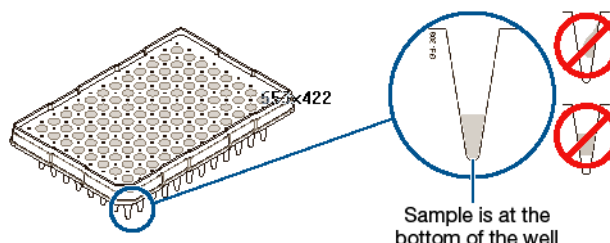
2. Load the calibration standards in injection position 1 in the spectral calibration plate:

IMPORTANT! You do not create a plate for the spectral calibration. The software uses predetermined well positions for the calibration standard. If you do not place calibration standards in the positions indicated, the calibration will fail.

8-capillary 96-well plate	A1 through H1	
24-capillary 96-well plate	A1 through H1, A2 through H2, and A3 through H3	
24-capillary 384-well plate Note: 384-well plates are not supported on 8-capillary instruments.	Columns 1, 3, and 5 in rows A, C, E, G, I, K, M, O	

- **96** – Supports 96-well standard reaction plate. 8-strip standard tubes are also supported with appropriate retainers.
 - **96-Fast Tube** – Supports 96-well Fast reaction plate. 8-strip fast tubes are also supported with appropriate retainers
3. Briefly centrifuge the plate containing the standards.
 4. Verify that each sample is positioned correctly in the bottom of its well.

IMPORTANT! If the reagents 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.



5. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.
6. Prepare the plate assembly as described in:
 - “96-well plate assembly” on page 61
 - “8-strip tube standard or fast assembly” on page 61
 - “384-well plate assembly” on page 62

Load the plate in the instrument

1. Click the **Tray** button on the front panel to move the autosampler to the front position.
2. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
3. Close the instrument door to re-initialize the instrument.

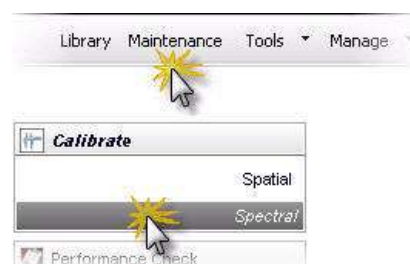
Perform a spectral calibration

IMPORTANT! Do not change electronic signature settings during a spectral calibration.

IMPORTANT! If you change polymer type, spectral calibrations for the original polymer type are not retained.

1. Access the Spectral Calibration screen:
Select **Maintenance**, then select **Spectral Calibration** in the navigation pane.

Note: The screen does not display results until you perform a spectral calibration. To view previous calibration data, click **History View**.



2 — Calibration Settings

3 — Number of Wells: 96 96-Fast 384 Chemistry Standard: Sequencing Standard

4 — Plate Position: A B Dye Set: E

5 — ☒ Allow Borrowing

6 — Capillary Run Data

Current Instrument Consumables
Polymer Type: POP6 Capillary Length: 50cm

Start Run

Status: Ready 0%

Capillary	1	2	3	4	5	6	7	8
Run 1								
Run 2								
Run 3								
Overall								

Passed Failed Borrowed Not Calibrated

Quality Value: Condition #: Status: Message:

Intensity vs Scan Number

Raw Data

Intensity vs Pixel Number

Accept Reject

2. Select the number of wells (refers to the number of wells on the plate, not the number of wells filled with sequencing standard) in the spectral calibration plate and specify the plate location in the instrument.

Note: You do not create a plate for the calibration. The software uses predetermined positions for the calibration. You cannot specify standard location on the plate.

3. Select the chemistry standard and the dye set that you are running the calibration for.

Note: If the dye set list is empty, ensure that your instrument is configured with a compatible polymer type and capillary length for the selected chemistry standard.

IMPORTANT! To calibrate a custom dye set using AnyDye, first create the dye set (see “Create a new dye set” on page 169), then select the name of the custom dye set from the Dye Set list. The AnyDye selection in the Dye Set list contains default settings. It does not correspond to custom dye sets created with the AnyDye dye set template.

4. In the Comments field, enter the name of the dye set you are calibrating.

IMPORTANT! The E-Signature function creates a record when a spectral calibration is performed, but does not record the dye set calibrated. To include the dye set calibrated in the E-Signature record, enter the dye set in the Comments field.

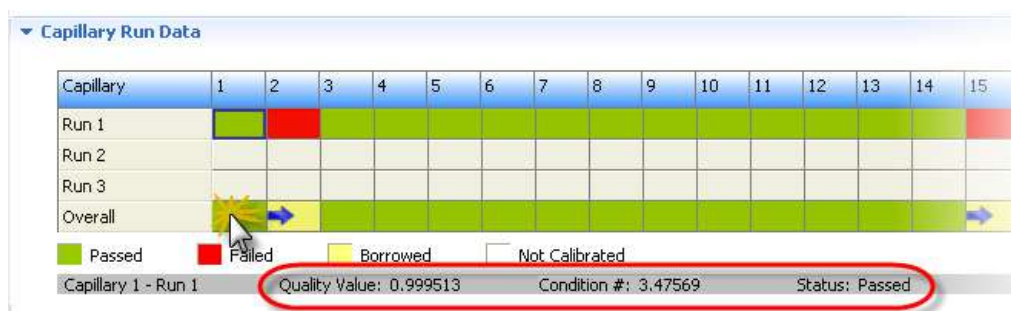
5. (Optional) Select **Allow Borrowing**. Selecting this option instructs the software to automatically replace information from failed capillaries with information from an adjacent passing capillary with the highest Quality value. For more information, see “What you see during a spectral calibration” on page 117.
6. Click **Start Run**. The following occurs:
 - The system sets up three injections (see “What you see during a spectral calibration” on page 117 for information on the number of injections performed).
 - The Capillary Run Data display updates after each injection is complete.
 - The status bar updates during Run 1.

IMPORTANT! The status bar does not update during Run 2 or Run 3.

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed.

To display the result for each capillary (spectral data, Quality Value, and Condition Number) below the run results table, click a capillary in the table.

Note: The results displayed when you click a borrowed capillary are the passing results borrowed from the adjacent capillary. To determine the reason that a capillary fails, view the spectral calibration report. See “View and print a spectral calibration report” on page 123.



For all spectral calibration injections (even capillaries that are green in the Overall row), evaluate the data as described in the next section.

Spectral Quality Values and Condition Numbers

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 peak(s) causes 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 (see "Evaluate the spectral calibration data" on page 116).

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.

The ranges that the software uses to determine if a capillary passes or fails are:

Dye Set	Quality Value Minimum	Condition Number Maximum
AnyDye	0.8 (default)	20.0 (default)
E	0.95	5.5
E5	0.95	6.0
F	0.95	8.5
G5	0.95	13.5
J6	0.95	8.0
Z	0.95	5.5

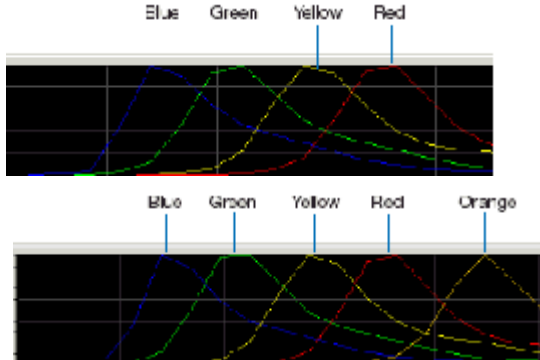
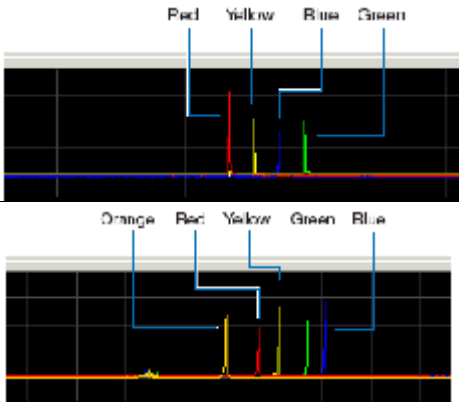
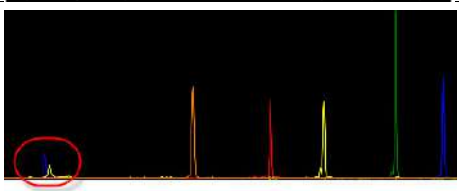
Evaluate the spectral calibration data

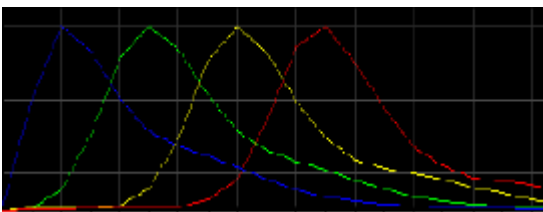
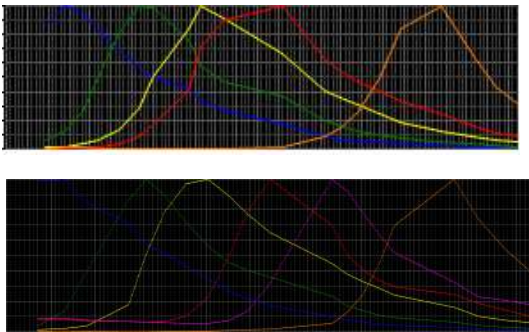
IMPORTANT! Do not accept a spectral calibration until you examine the data for all capillaries.

When a spectral calibration completes successfully, the Overall row displays green, red, or yellow results.





For each capillary:

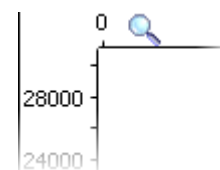
1. Click a capillary to display the spectral and raw data for a capillary.
2. Check that the data meet the following criteria:


Attribute	Acceptance Criteria	Example
Order of the peaks in the spectral profile from left to right	<ul style="list-style-type: none"> • 4-dye: blue-green-yellow-red • 5-dye: blue-green-yellow-red-orange 	
Order of the peaks in the raw data profile from left to right	<ul style="list-style-type: none"> • Sequencing (matrix standard only): <ul style="list-style-type: none"> – 4-dye: red-yellow-blue-green • Fragment analysis/HID: <ul style="list-style-type: none"> – 4-dye: red-yellow-green-blue – 5-dye: orange-red-yellow-green-blue 	
Extraneous peaks in the raw data profile	<p>None</p> <p>Note: The E5 profile may include extraneous peaks outside the matrix peak region which can be ignored.</p>	

Attribute	Acceptance Criteria	Example
Peak morphology in the spectral profile	<ul style="list-style-type: none"> No gross overlaps, dips, or other irregularities Peaks separate and distinct 	
	<p>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).</p>	

3. As needed, zoom on the spectral profile traces to determine if the data meet the criteria:

- Place the pointer *above the top* of the plot or *to the left* of the plot at the start of the area you want to zoom, then click to turn the pointer to .
- With the  still *above* the plot or *to the left* of the plot, click-drag to the end of the area you want to zoom. Do not drag the  inside the plot area. Doing so changes  back to a pointer and does not zoom as expected.



You can also click zoom and fit buttons to zoom.   

4. If the data for all capillaries meet the criteria above, click **Accept Results**.

5. If any capillary data does not meeting the criteria above, click **Reject Results**, then go to "Spectral calibration troubleshooting" on page 267.

What you see during a spectral calibration

A spectral calibration can run up to three injections. The number of injections performed depends on:

- The number of capillaries that pass or fail during an injection
- Whether you select the Allow Borrowing option

Note: The first time you perform a spectral calibration (for each dye set) after installing a new capillary array, you may notice pull-down peaks (or mirror image peaks). While the run is in progress, these pull-down peaks will eventually correct themselves. Once the run completes the electropherogram, the pull-down peaks disappear.

Capillary information sharing

A spectral calibration can share capillary information:

- **Between injections** – If a capillary in an injection does not meet the spectral Quality Value and Condition Number limits shown on page 115, the software automatically uses the information from that capillary in a different injection.
- **Within an injection** – If a capillary in an injection does not meet the spectral Quality Value and Condition Number limits shown on page 115 and the Allow Borrowing option is selected, the software can also use the information from a capillary to the left or the right of that capillary, if the values are higher than those for that capillary in a different injection.

Spectral calibration with Borrowing disabled

☐ Allow Borrowing

When Borrowing is *disabled*, all capillaries must pass (meet the spectral Quality Value and Condition Number limits) for the calibration to pass.

Injection 1	<ul style="list-style-type: none"> • The software evaluates the Quality Value and Condition Number of all capillaries. • If all capillaries pass, the calibration is complete, and injections 2 and 3 are not performed. • If any capillaries fail, injection 2 is performed.
Injection 2	<ul style="list-style-type: none"> • The software evaluates the Quality Value for each capillary across injections 1 and 2 and uses the information from the capillary with the highest Quality Value. • If all capillaries now pass, the calibration is complete and injection 3 is not performed. • If the same capillary fails in both injection 1 and 2, injection 3 is performed.
Injection 3	<ul style="list-style-type: none"> • The software evaluates the Quality Value for each capillary across injections 1, 2, and 3 and the information from the capillary with the highest Quality Value. • If all capillaries now pass, the calibration passes. • If the same capillary fails in injection 1, 2, or 3, the calibration fails.

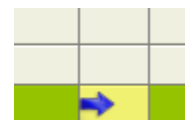
Spectral calibration with Borrowing enabled



When Borrowing is *enabled*, all capillaries have to pass (meet the spectral Quality Value and Condition Number limits) within the borrowing limits:

- 8-capillary instruments – One adjacent-capillary borrowing event allowed
- 24-capillary instruments – Up to three adjacent-capillary borrowing events allowed (the number of allowed borrowing events can be decreased in Preferences).

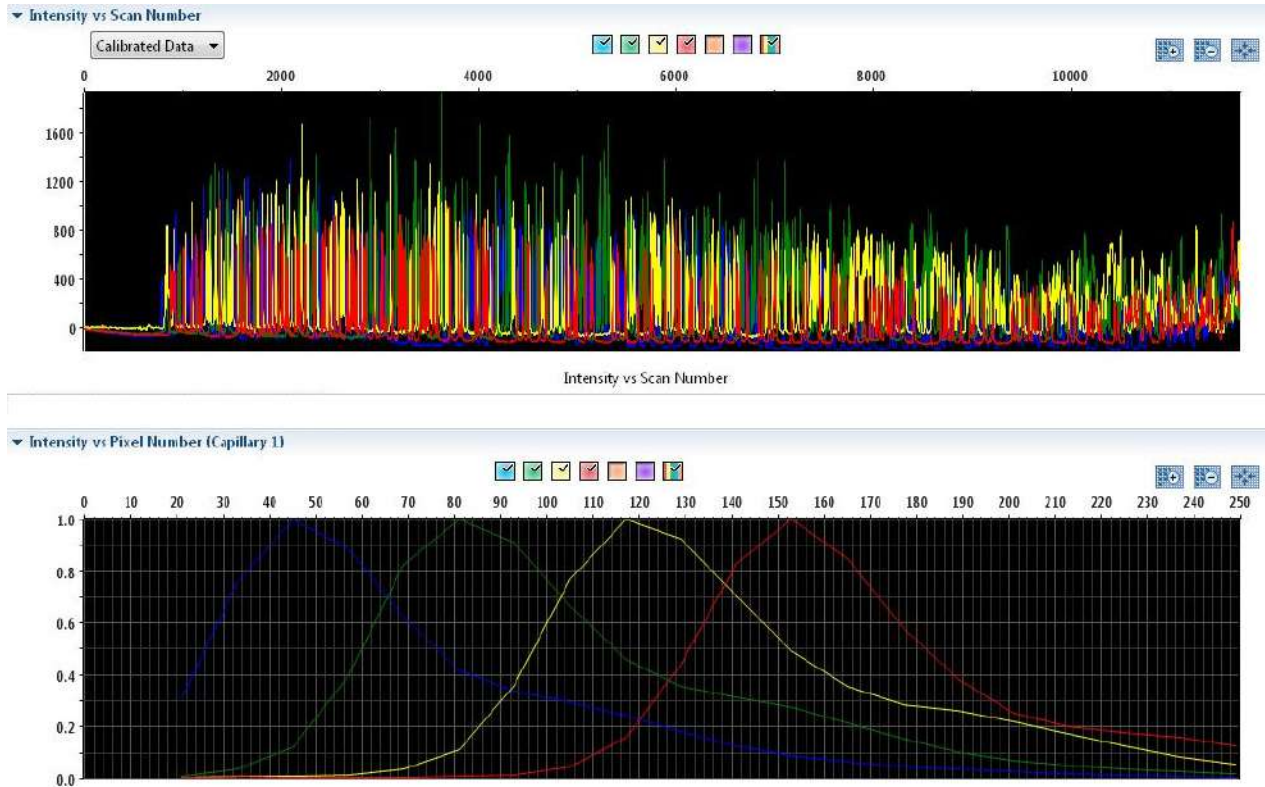
The software identifies a borrowed capillary with an arrow pointing from the capillary from which the data is borrowed.



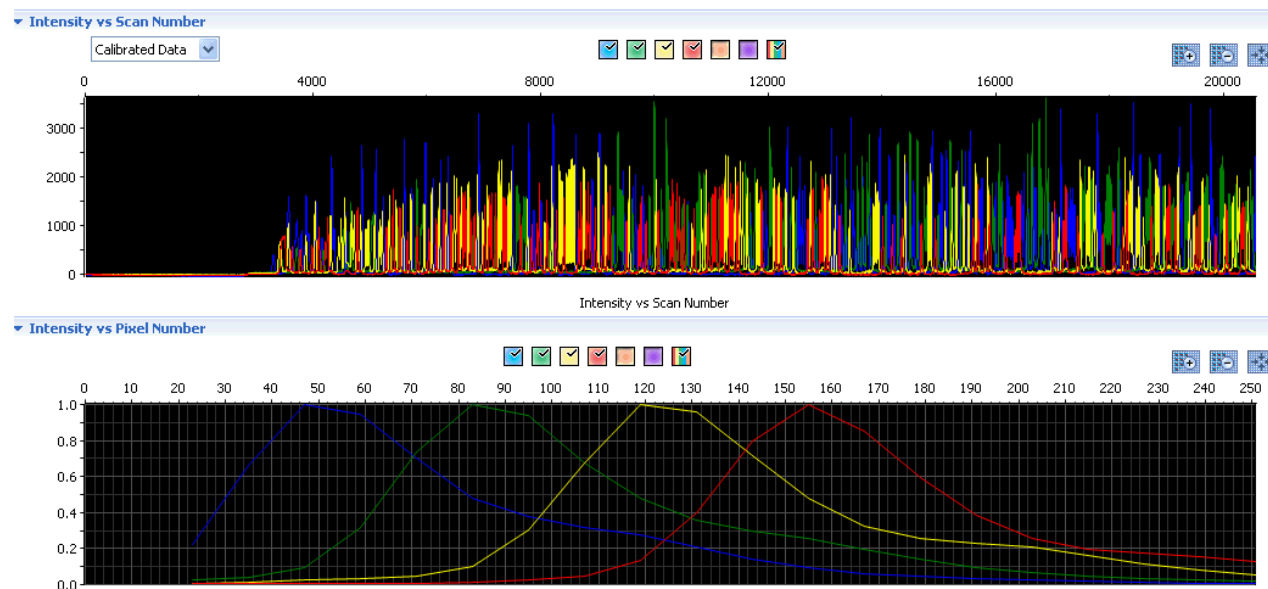
Injection 1	<ul style="list-style-type: none"> • The software evaluates the Quality Value and Condition Number of all capillaries. • If all capillaries pass, the calibration is complete, and injections 2 and 3 are not performed. • If any capillaries fail, the software borrows from an adjacent capillary. • If, after borrowing, >1 or > 3 capillaries fail, injection 2 is performed.
Injection 2	<ul style="list-style-type: none"> • The software evaluates the quality values between adjacent capillaries in injection 2 and for each capillary across injections 1 and 2 and the information with the highest Quality Value for each capillary. • If all capillaries pass, the calibration is complete and injection 3 is not performed. • If, after borrowing, >1 or > 3 capillaries from injection 1 or 2 do not pass, injection 3 is performed.
Injection 3	<ul style="list-style-type: none"> • The software evaluates the quality values between adjacent capillaries in injection 3 and for each capillary across injections 1, 2, and 3, then the information with the highest Quality Value for each capillary. • If all capillaries now pass, the calibration passes. • If after borrowing, >1 or > 3 capillaries from injection 1, 2, or 3 do not pass, the calibration fails.

Example spectral calibration data

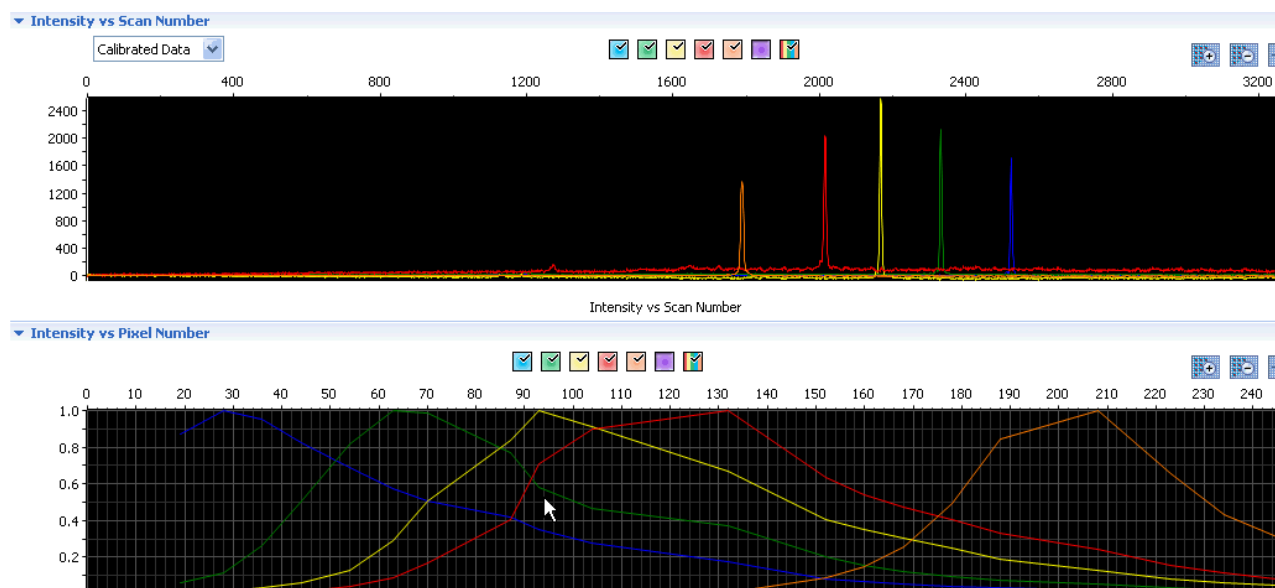
Dye Set E created
from Sequencing
Standard



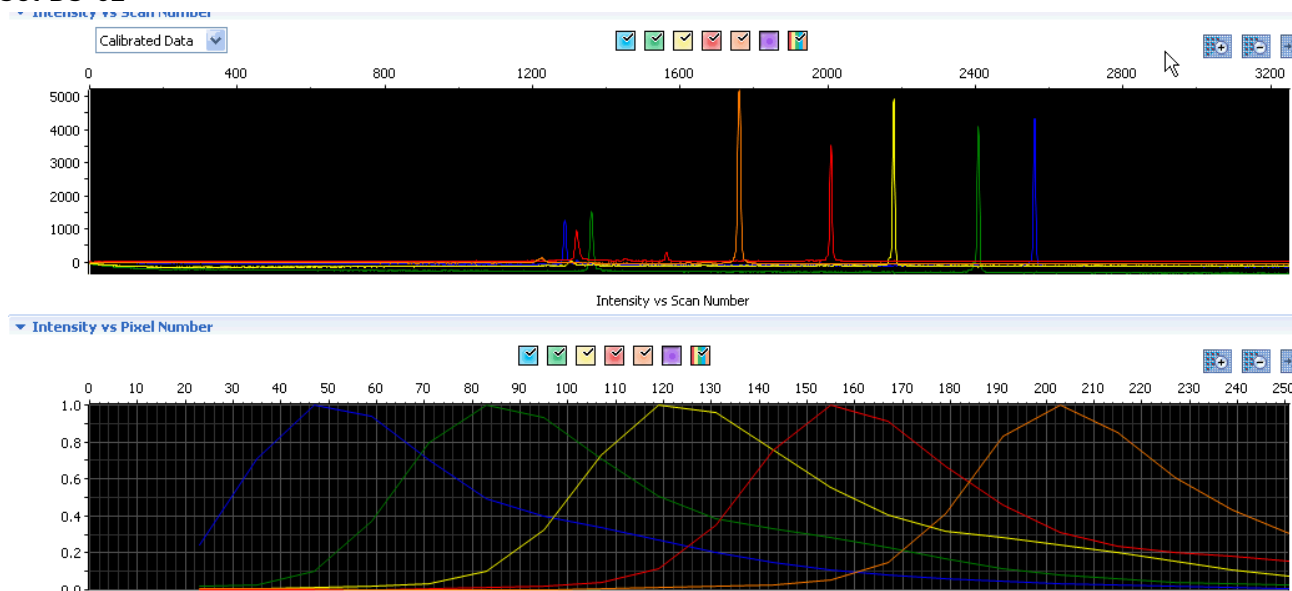
Dye Set Z created from Sequencing Standard



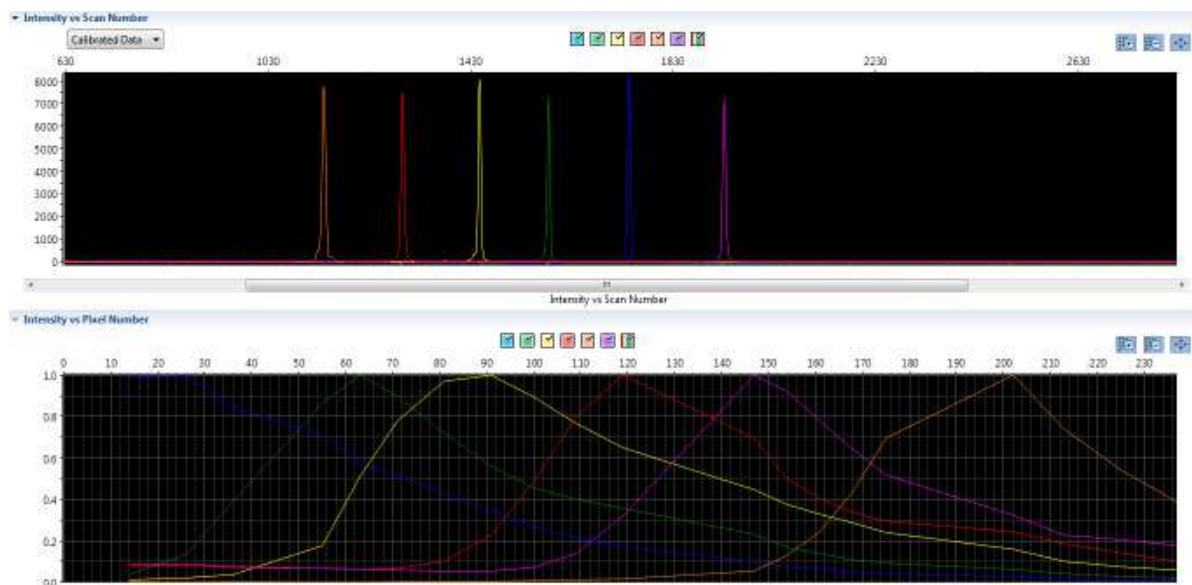
Dye Set G5 created from Matrix Standard Set DS-33



Dye Set E5 created from Matrix Standard Set DS-02




Dye Set J6 created from Matrix Standard Set DS-36



Export spectral calibration results

To export spectral calibration results:


1. Click  **Export Spectral Calibration Results**.
2. Specify an export file name and location.
3. Click **Save**.

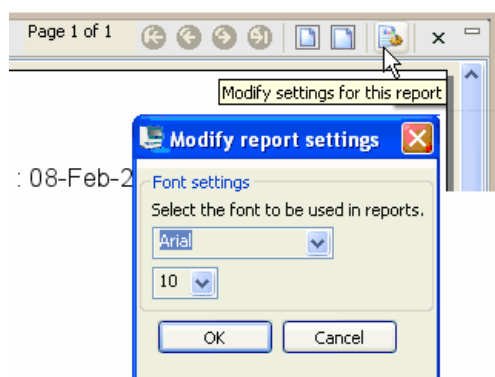
The export file contains the following results:


- Capillary Number
- Condition Number
- Scan Number
- Borrowed From Capillary
- Quality Value
- Peak Height
- Reason For Failure
- Run From Injection

View and print a spectral calibration report

Note: Spatial and spectral calibration reports include the date on which a capillary array is installed for the first time on the instrument. Install standard reports use the most recent install date if a capillary array was removed and re-installed on the instrument.

1. Click  **View Spectral Calibration Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.





3. To print the report, click  **Print**.
4. Close the report.



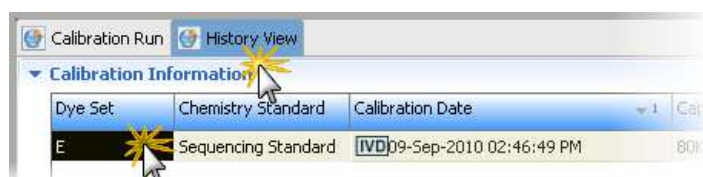
Save historical calibration reports (.pdf) for record keeping

IMPORTANT! After performing a calibration, save the calibration report electronically for record keeping. Only the most recent spectral calibration for each dye set is maintained in the software.

1. Click  **View Spectral Calibration Report**.
2. Click  **Print**.
3. In the Printer dialog box, select **CutePDF Writer** as the printer.
4. Specify a name and location for the report.

View the spectral calibration history

Only the most recent spectral calibration for each dye set is maintained in the software. Select **History View**, then select a dye set to view the associated calibration history.



Section 5.2 Performance check

The Performance check allows you to periodically self-check the instrument system.

Sequencing install standard performance check

When to perform

When your instrument is installed, the service engineer runs a sequencing install standard performance check.

We recommend that you run the sequencing install standard performance check monthly to verify that the instrument meets read length specifications.

The Sequencing Install Performance check has an option to include and save the spectral calibration. If you select this option and you accept the sequencing install standard results, you do not need to run the spectral calibration (described in “Spectral calibration” on page 109) for E dye set.

Estimated run time

- General sequencing – 45 minutes
- MicroSeq[®] ID – 2 hours
- BDTv1.1POP6 – >2 hours

Prepare for the sequencing install standard performance check

Prepare the instrument

1. In the Dashboard, check consumable status (“Check consumable status” on page 39). Ensure that:
 - Consumables are not expired
 - Adequate injections remain for consumables
2. Ensure that the buffer levels are at the fill lines (“Check buffer fill levels” on page 40).
3. Set the oven temperature, then click **Start Pre-heat**:


Standard	Polymer	Preheat Temperature
Sequencing	POP-6™	50°C
	POP-7™	60°C
Matrix	POP-4 [®]	60°C
	POP-6™	60°C
	POP-7™	60°C

Pre-heat the oven and detection cell while you prepare for a run (detection cell temperature is set by the software). Preheating helps mitigate subtle first-run migration rate effects. The pre-heat function automatically turns off after 2 hours.

We recommend that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold.

4. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed (see “Remove bubbles from the polymer pump” on page 238).

Prepare the installation standard plate

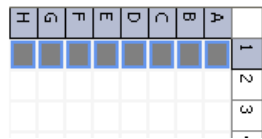
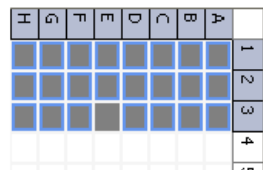
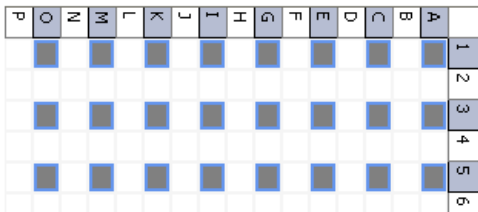
IMPORTANT! Do not use warped or damaged plates. 

1. Prepare the sequencing install standard as described in the product insert. See Appendix A, Application Reagents and Run Modules for standard part numbers.

Application	Standard
General sequencing (POP-7™ polymer, 50-cm capillary)	BigDye® Terminator (BDT) v3.1 Standard
MicroSEQ® ID applications (POP-6™ polymer, 50-cm capillary)	BigDye® Terminator (BDT) v1.1 Standard

2. Load the standards in injection position 1 in the spectral calibration plate:

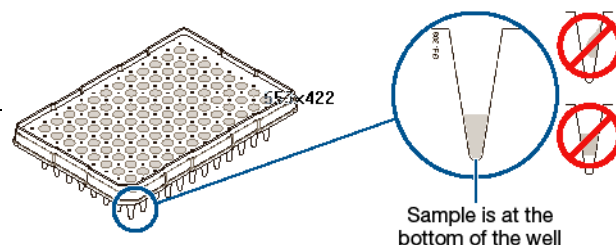
IMPORTANT! You do not create a plate for the spectral calibration. The software uses predetermined well positions for the calibration standard. If you do not place calibration standards in the positions indicated, the calibration will fail.

8-capillary 96-well plate	A1 through H1	
24-capillary 96-well plate	A1 through H1, A2 through H2, and A3 through H3	
24-capillary 384-well plate Note: 384-well plates are not supported on 8-capillary instruments.	Columns 1, 3, and 5 in rows A, C, E, G, I, K, M, O	

- **96** – Supports 96-well standard reaction plate. 8-strip standard tubes are also supported with appropriate retainers.
 - **96-Fast Tube** – Supports 96-well Fast reaction plate. 8-strip fast tubes are also supported with appropriate retainers.
3. Briefly centrifuge the plate containing the standards.

4. Verify that each sample is positioned correctly in the bottom of its well.

IMPORTANT! If the reagents 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.



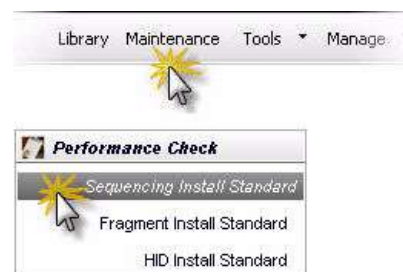
5. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.
6. Prepare the plate assembly as described in:
 - “96-well plate assembly” on page 61
 - “8-strip tube standard or fast assembly” on page 61
 - “384-well plate assembly” on page 62

Load the plate in the instrument

1. Click the **Tray** button on the front panel to move the autosampler to the front position.
2. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
3. Close the instrument door to re-initialize the instrument.

Run the sequencing install standard performance check

1. Access the Sequencing Install Standard screen (Figure 8 on page 128): Select **Maintenance**, then select **Sequencing Install Standard** in the navigation pane.
2. Select the chemistry type.
3. Select the number of wells and plate position in the instrument.



Note: You do not create a plate for the performance check. The software uses predetermined positions for the run. You cannot specify standard location on the plate.

4. (Optional) If you have not already run a spectral calibration, select **Keep Spectral Calibration Data** to save the sequencing install standard run (if it passes) as a spectral calibration.

Note: The spectral calibration record will only be saved if Keep Spectral Calibration Data option is checked on the screen. If you decide to uncheck the option, create a separate spectral calibration from the Maintenance menu.

5. Click Start Run.

IMPORTANT! Do not accept a sequencing installation standard run until you examine the data.

The screenshot displays the 'Run View' tab of the Sequencing Install Standard screen. It is divided into several sections:

- Calibration Settings:** Includes a dropdown for 'Chemistry Type' (BDTv1.1POP6), radio buttons for 'Number of Wells' (96, 96-Fast, 384), radio buttons for 'Plate Position' (A, B), and a checked checkbox for 'Keep Spectral Calibration Data'.
- Scoring Settings:** Includes a 'CRL Pass/Fail Threshold' (600), 'Read Length Start' (20), and 'Read Length End' (619).
- Current Instrument Consumables:** Shows 'Polymer Type: POP6' and a 'Start Run' button.
- Capillary Run Data:** A table with columns for Capillary (1-8), Median, and SD. The first row is 'Spectral Calibration Run'. Below it are rows for 'Contiguous Read Length (CRL)', 'CRL Pass/Fail', 'Comparison with Ref Sequence', 'CRL Basepair Accuracy', 'Basepair Accuracy', and 'Read Length'.
- Intensity vs Scan Number:** A graph showing 'Raw Data' with a scale from 0 to 32000. The graph area is currently black.

Annotations 2 through 5 point to the following elements:

- 2: Chemistry Type dropdown
- 3: Number of Wells radio buttons
- 4: Plate Position radio buttons
- 5: Keep Spectral Calibration Data checkbox

Figure 8 Sequencing Install Standard screen

What you see during a run

The system performs one run, then evaluates:

- Spectral data, if you specified to keep spectral data
- Sequence data

The Capillary Run Data display (Figure 9 on page 129) updates after the run is complete:

- The spectral calibration status is displayed in the first row of the run results table. Passing and failing capillaries in the performance run are shown in green and red respectively for the CRL criteria. Borrowed capillaries (spectral only) are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. The spectral result for each capillary is displayed below the run results table.
- The sequencing install standard status is displayed in the third row of the run results table (CRL Pass/Fail).
- The Quality Value and Condition Number for each capillary is displayed below the table.

Note: The values shown in this figure are examples only.

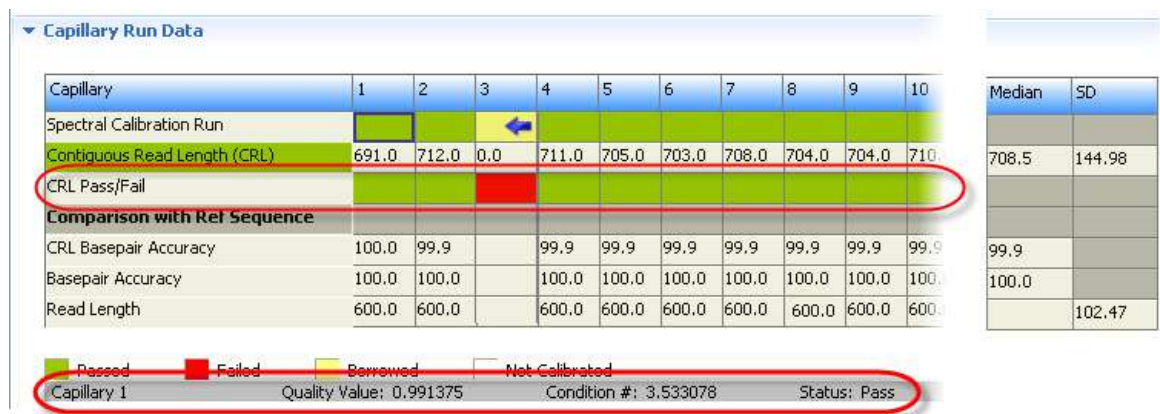


Figure 9 Sequencing install standard – Capillary Run Data

How the software determines passing and failing capillaries for the spectral calibration

The software evaluates the Quality Value and Condition Number for each capillary (for more information, see “Spectral Quality Values and Condition Numbers” on page 115).

Borrowing is automatically enabled: 1 borrowing event is allowed for 8-capillary instruments, up to 3 borrowing events for 24-capillary instruments. For more information, see “Capillary information sharing” on page 118. The number of borrowing events can be decreased – see “User preferences” on page 41.

Thresholds used by the software for pass/fail are:

Dye Set	Quality Value Minimum	Condition Number Maximum
E	0.95	5.5
Z	0.95	5.5

How the software determines passing and failing capillaries for the sequencing performance check

The software calculates the Contiguous Read Length for each capillary. Capillaries that are below the threshold fail. The remaining results that the software displays are for information only.

Result	Description
Contiguous Read Length (CRL)	The longest uninterrupted segment of bases with an average Quality Value (QV) ≥ 20 . In addition to evaluating the QV of a base call, the software considers the QV of adjacent bases within a ± 20 -bp moving average to determine a contiguous read length based on quality values: the software starts from the 5' end and calculates the average QV across a moving window size of 20, sliding 1 bp at a time, to the 3' end. The resulting longest contiguous segment is determined as the CRL.
CRL Pass/Fail	<ul style="list-style-type: none"> • BDTv1.1POP6 – Capillaries with a CRL <600 bp fail. • General sequencing – Capillaries with a CRL ≤ 500 bp fail. • MicroSeq® ID – Capillaries with a CRL ≤ 600 bp fail.
For information only – Based on alignment of the base-called sample sequence with the known reference of the sequencing install standard	
CRL Basepair Accuracy	CRL accuracy is determined by base-pair comparison between the base-called sample and the known reference sequence for the install standard within the contiguous read length region calculated (as described in the CRL definition above).
Read Length	The length of read (in bases) at which base calling accuracy is $\geq 98.5\%$. The read length value for this information is derived from basecall-accuracy, not from quality value.
Basepair Accuracy (Read Length Accuracy)	Basepair Accuracy is determined by base-pair comparison between the sample and the known reference sequence for the install standard in the read length range (see the Scoring settings at the top of the screen for read length range) with $\geq 98.5\%$ accuracy in the called sequence when compared to the reference sequence).
CRL Median and SD	Median and standard deviation for all capillaries.

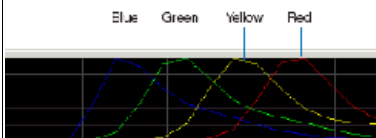
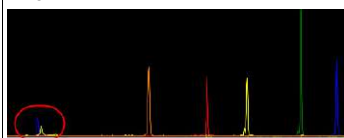
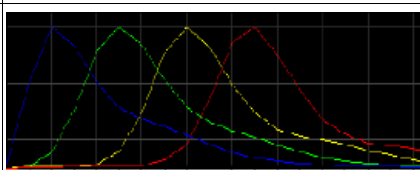
Evaluate sequencing install standard data

When a sequencing install standard run completes successfully, the CRL Pass/Fail row displays green or red results.

For each capillary:

1. Click a capillary to display the spectral and raw data profiles for a capillary.



2. Check that the data meet the following criteria:

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	
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.	E5: 
Peak morphology in the spectral profile (intensity vs pixel)	<ul style="list-style-type: none"> No gross overlaps, dips, or other irregularities Peaks separate and distinct Peak apexes are separate and distinct (the tails will overlap) 	

3. (Optional) Review the CRL accuracy to determine discrepancies from the reference sequence. For

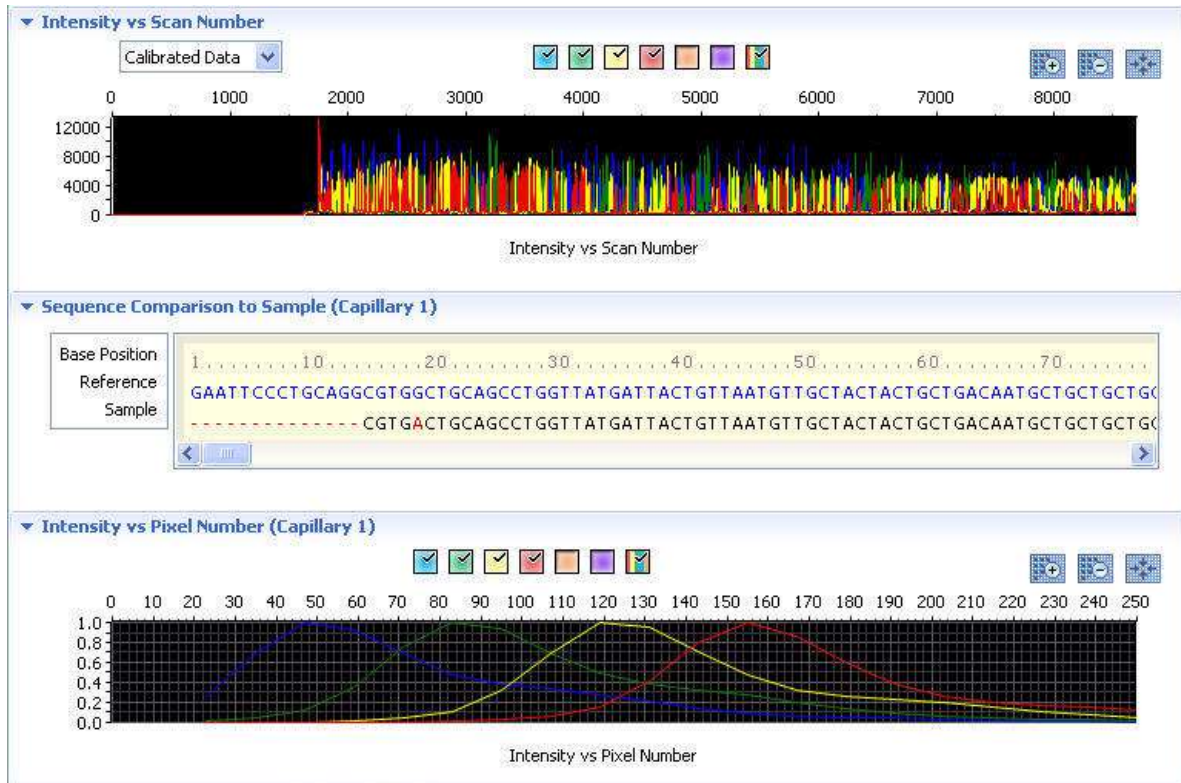
- BDTv1.1POP6: 20 to 619 bp.
- General sequencing: 40 to 539 bp
- MicroSeq® ID: 20 to 619 bp

If you observe large discrepancies (for example, 5 to 10 contiguous miscalled bases in the middle of a sequence), review the data. If you see a raw data peak larger than the adjacent peaks with baseline pull-up in all 4-dye color channels, it may indicate the presence of a bubble. Check the pump, run the Remove Bubbles wizard (see “Remove bubbles from the polymer pump” on page 238), then repeat the run as needed.

- If the data for the required number of capillaries meets the criteria above (at least 7 capillaries for 8-capillary instruments, at least 21 capillaries for 24-capillary instruments), click **Accept Results**.
- If the data for the required number of capillaries do not meet the criteria above (7 capillaries for 8-capillary instruments, 22 capillaries for 24-capillary instruments):
 - (Optional) If you want to generate a report for the failed calibration, click  **View Summary Report** or  **View Detail Report** before you click Reject Results. To save the report electronically, select **CutePDF** as the printer.
 - Click **Reject Results**. For troubleshooting information, see “Sequencing install standard troubleshooting” on page 270.

IMPORTANT! If you reject results, the spectral calibration is not saved.

Example sequencing install standard results



View previously run sequencing install standards



Select **History View**, then select an install standard to view the associated calibration information.

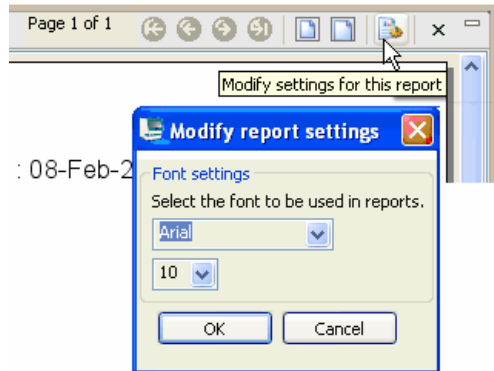
View and print a sequencing install standard report


IMPORTANT! Ensure that all dyes are selected before viewing the report. The report may contain incomplete data if all dyes are not selected.

Note the following:

- Install standard reports include the most recent install date if a capillary array was removed, then re-installed on the instrument. Spatial and spectral calibration reports include the date on which a capillary array is installed on the instrument for the first time.
- The sorting in the Install Standard screen is not applied to the report.
- You can generate a report for a failed installation standard run before you click Reject Results.




1. Click  **View Summary Report** or  **View Detail Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.



3. To print the report, click  **Print**.
4. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.
5. Close the report.



Save historical performance check reports (.pdf) for record keeping

1. Click  **View Summary Report** or  **View Detail Report**.
2. Click  **Print**.
3. In the Printer dialog box, select **CutePDF Writer** as the printer.
4. Specify a name and location for the report.

Fragment analysis or HID Install standard performance check

When to perform

When your instrument is installed, the service engineer runs a fragment analysis or HID install standard install performance check.

You can also run the fragment or HID install standard performance check monthly to verify that the instrument conforms to fragment analysis sizing precision, sizing range, and peak height specifications.

IMPORTANT! The performance check is application-specific. If you change polymer and capillary length, you must perform a new performance check.

Estimated run time 30 minutes

Prepare for the fragment or HID install standard performance check

Prepare the instrument

1. If you have not already done so, perform a spatial calibration (see “Spatial calibration” on page 105).
2. In the Dashboard, check consumable status (page 39). Ensure that:
 - Consumables are not expired
 - Adequate injections remain for consumables
3. Ensure that the fluid levels are at the fill lines (“Check buffer fill levels” on page 40).
4. Set the oven temperature to 60°C, then click **Start Pre-heat**.
 Pre-heat the oven and detection cell while you prepare for a run (detection cell temperature is set by the software). Preheating helps mitigate subtle first-run migration rate effects. The pre-heat function automatically turns off after 2 hours. We recommend that you pre-heat the oven for at least 30 minutes before you start a run if the instrument is cold.
5. Check the pump assembly for bubbles and run the Remove Bubble wizard if needed (see page 238).

Prepare the installation standard plate

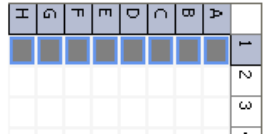
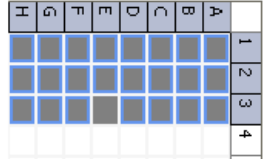
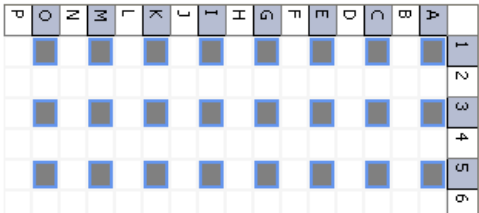
IMPORTANT! Do not use warped or damaged plates. 

1. Prepare the installation standard as described in the product insert. See Table 47 on page 290 for standard part numbers.

Application	Installation Standard
Fragment analysis: <ul style="list-style-type: none"> • G5 dye set, POP-7™ polymer, 36 or 50 cm capillary • G5 dye set, POP-4® polymer, 36 or 50 cm capillary 	GeneScan™ Installation Standard DS-33
HID (G5 dye set, POP-4® polymer, 36 cm capillary)	AmpFSTR® Identifiler® Allelic Ladder

2. Load the standards in injection position 1 in the plate:

IMPORTANT! You do not create a plate for the performance check. The software uses predetermined positions for the performance check run. You cannot specify standard location on the plate. If you do not place standards in the positions indicated, the calibration will fail.

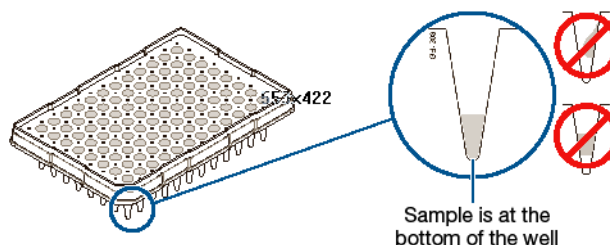
8-capillary 96-well plate	A1 through H1	
24-capillary 96-well plate	A1 through H1, A2 through H2, and A3 through H3	
24-capillary 384-well plate 384-well plates are not supported on 8-capillary instruments.	Columns 1, 3, and 5 in rows A, C, E, G, I, K, M, O	

3. Briefly centrifuge the plate containing the standards.

4. Verify that each sample is positioned correctly in the bottom of its well.

IMPORTANT! If the reagents 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.



5. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.
6. Prepare the plate assembly as described in:
 - "96-well plate assembly" on page 61
 - "8-strip tube standard or fast assembly" on page 61
 - "384-well plate assembly" on page 62

Load the plate in the instrument

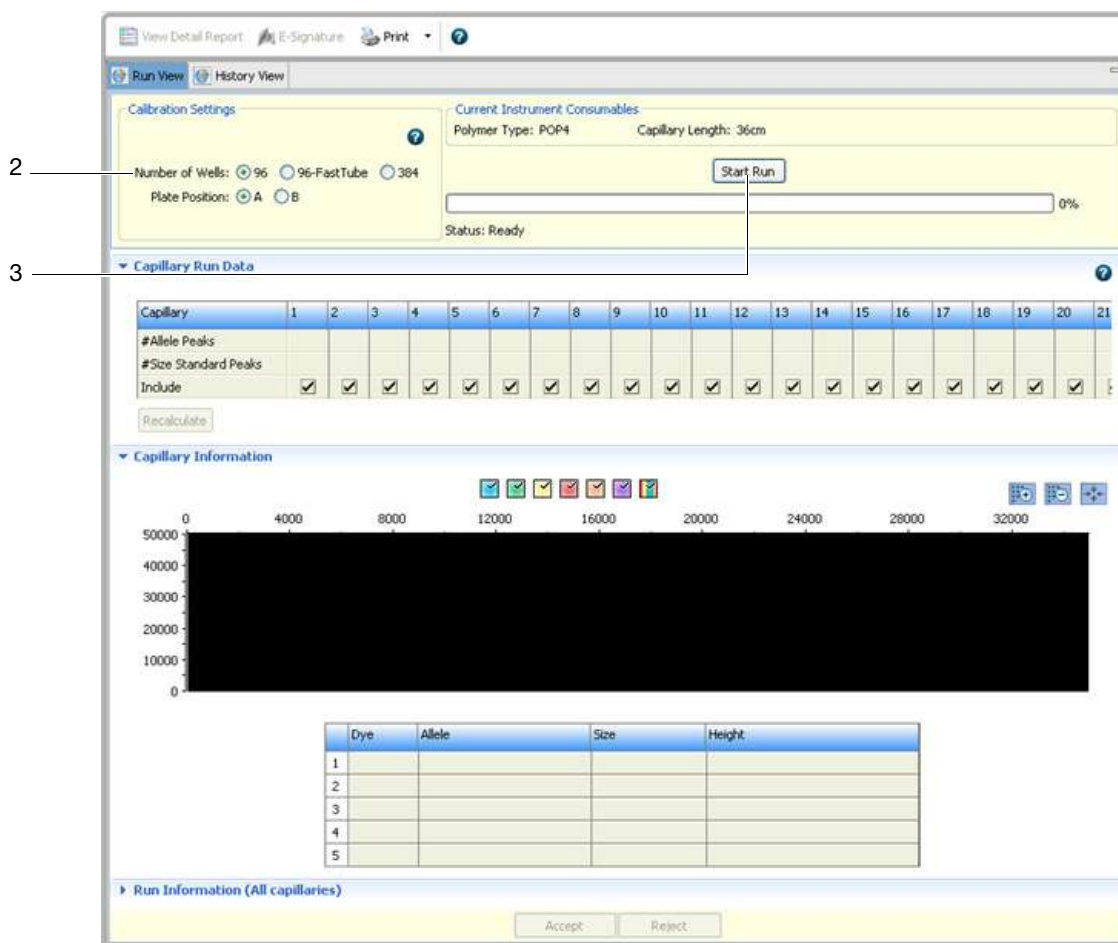
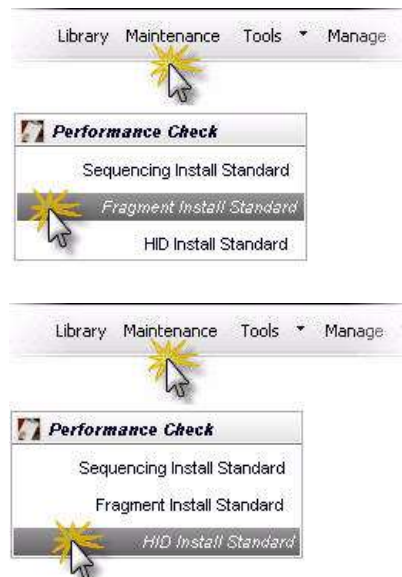
1. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
2. Close the instrument door to re-initialize the instrument.

Run the fragment analysis or HID install standard performance check

1. Access the Fragment Install Standard or the HID install standard screen: Select **Maintenance**, then select **Fragment Install Standard** or **HID Install Standard** in the navigation pane.
2. Select the plate type and plate position in the instrument.

Note: You do not create a plate for the performance check. The software uses predetermined positions for the run. You cannot specify standard location on the plate.

3. Click **Start Run**.

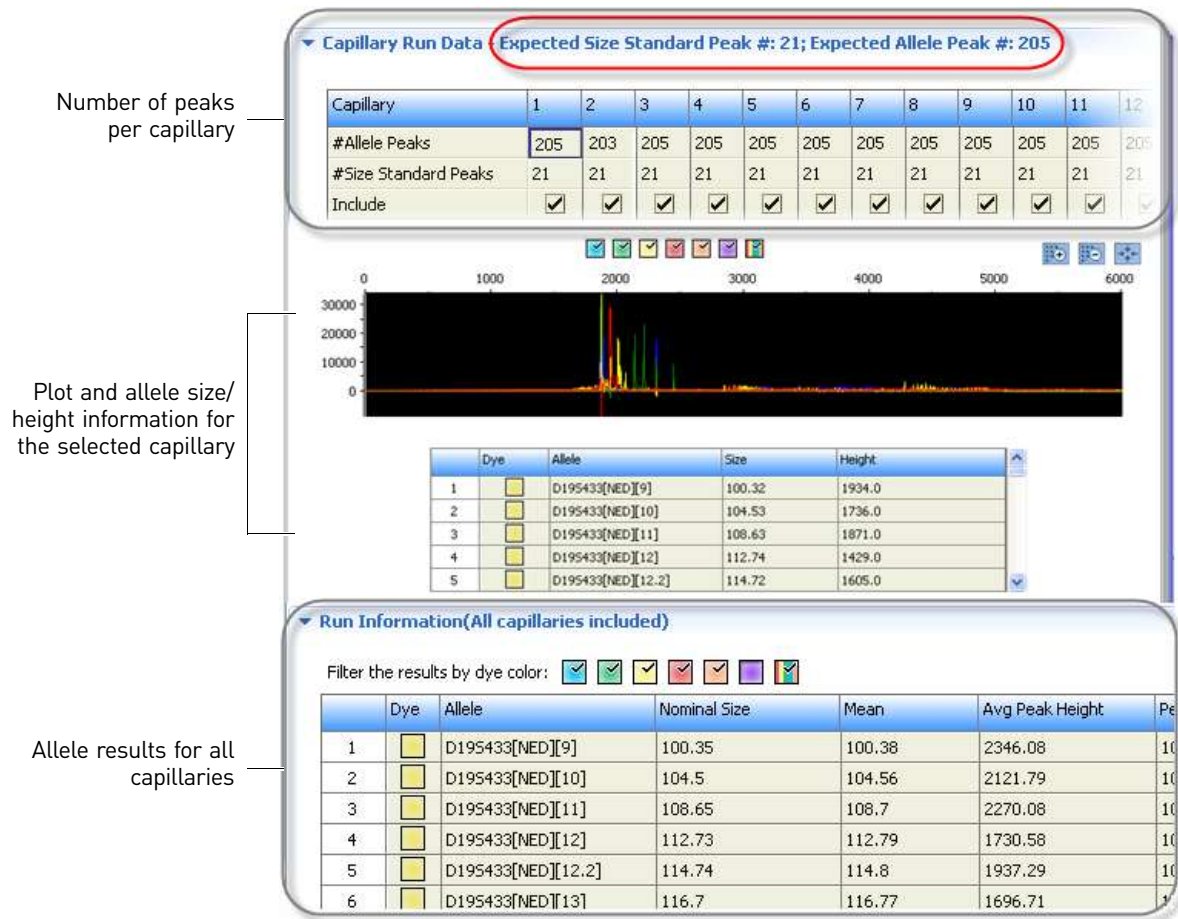


What you see during a run

The system performs one run and indicates the number of observed allele and size standard peaks.

The Capillary Run Data display updates after the run is complete. The number of observed size standard and allele peaks is shown. Results for each allele are shown at the bottom of the screen in the Run Information table.

Note: The example shown below is for the HID install standard.



How the software determines passing and failing capillaries for the fragment/HID performance check

The software evaluates peaks in the data for each capillary. To be identified as a possible allele, peaks must be within the following ranges (nominal allele size, or reference bin size, is hard-coded):

Fragment Analysis	HID Analysis
<ul style="list-style-type: none"> All markers between ± 0.4 bp or ± 0.5 bp of nominal size for the allele 	<ul style="list-style-type: none"> All markers except TH01: ± 0.7 bp of nominal size for the allele TH01: <ul style="list-style-type: none"> Seven markers are ± 0.7 bp of nominal size for the allele Three markers are ± 0.5 bp of nominal size for the allele

For all peaks that are within the nominal size range, the software calculates the Average Peak Height and the Sizing Precision. Peaks that meet the thresholds below pass.

Result	Description	Threshold
Minimum Peak Height	Minimum peak height for observed allele peaks of the included capillaries.	<ul style="list-style-type: none"> Fragment: > 175 RFU HID: > 400 RFU
Sizing Precision	Standard deviation of the observed allele fragment sizes.	<0.15 for expected alleles
Pass/Fail	Alleles with a sizing precision and minimum peak height that do not meet thresholds fail. Review the data for failed alleles as described below.	

Result	Description
For information only	
Nominal Size	Expected allele fragment peak size (bp).
Mean	Average fragment size for the observed allele peaks.
Peak Height % > Min	Percentage of observed allele peaks with a peak height above the minimum threshold.
Sizing Accuracy	Difference between the allele size and the mean allele size.

Evaluate fragment/HID install standard data

- Examine the number of size standard and allele peaks found for each capillary.

Note: The number of expected peaks shown below is for the HID install standard.

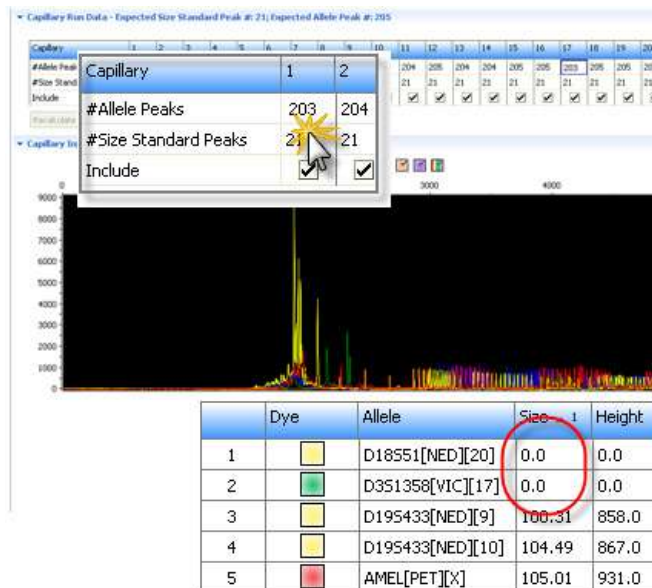
Expected	Capillary Run Data Expected Size Standard Peak #: 21; Expected Allele Peak #: 205												
Observed	Capillary	1	2	3	4	5	6	7	8	9	10	11	12
	#Allele Peaks	205	203	205	205	205	205	205	205	205	205	205	205
	#Size Standard Peaks	21	21	21	21	21	21	21	21	21	21	21	21
	Include	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

2. If the expected number of alleles and size standard peaks are found, click **Accept Results**.

If the expected number of alleles and size standard peaks are not found, troubleshoot as described below.

Troubleshoot

1. Click a capillary with fewer than the expected number of peaks to display detailed information for each allele in the table below the plot.
2. Double-click the Size column to sort results and identify the alleles that were not found.
 A "0" Size value indicates that an allele falls outside the expected size window (Nominal Size ± 0.7 bp or ± 0.5 for TH01).
3. Troubleshoot failing data:



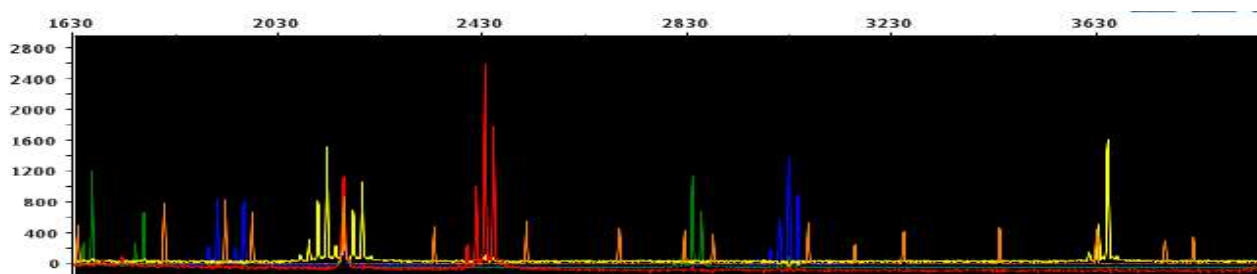
- a. Analyze the install standard data files in your secondary analysis software (GeneMapper® Software 5 or later; GeneMapper® ID-X Software v1.3 or later using Identifiler® kit panels and bins).
- b. Evaluate the failed data and examine the alleles not found by the 3500 Series Software 2.
- c. If the alleles are properly called in the secondary analysis software, you can:
 - Deselect the Include checkmark for a capillary.
 - Click **Recalculate**.
 - Accept the install standard results.

Note: The GeneMapper® ID-X Software may identify alleles not identified by the 3500 Series Software 2 because of the bin-offsetting feature (which uses the observed alleles in the allelic ladder samples to adjust the reference bin locations for samples).

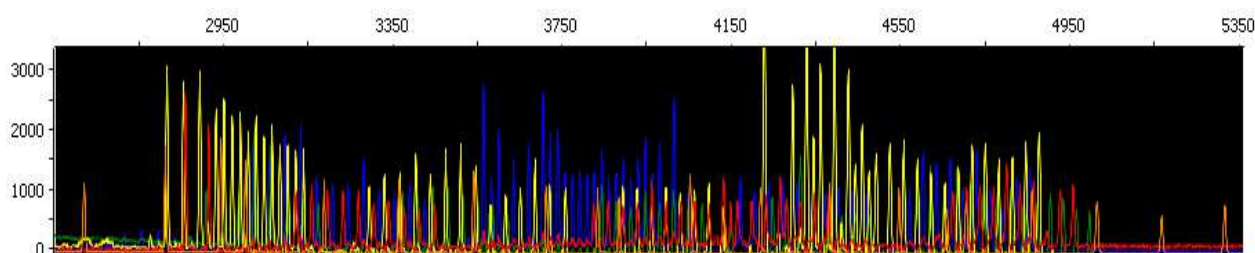
If the alleles are not properly called:

- (Optional) Click **View Detail Report** to save a record of the failed run. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer. For more information, see "Save historical performance check reports (.pdf) for record keeping" on page 142.
- Click **Reject Results**.
- Rerun the install standard to determine if the problem may be caused by sample preparation, a poor injection, a capillary issue, or a system problem (which may require instrument service). For more information, see "Fragment/HID install standard troubleshooting" on page 271.

Example fragment install standard results



Example HID install standard results



View previously run install standards


Select **History View**, then select an install standard to view the associated calibration information.

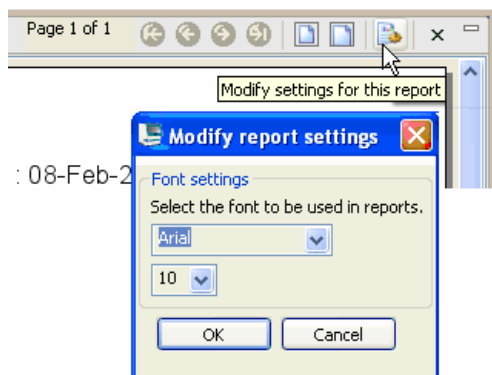
View and print a fragment or HID install standard detail report


IMPORTANT! Ensure that all dyes are selected before viewing the report. The report will contain incomplete data if all dyes are not selected.

Note the following:

- Install standard reports include the most recent install date if a capillary array was removed, then re-installed on the instrument. Spatial and spectral calibration reports include the date on which a capillary array is installed on the instrument for the first time.
- The sorting in the Install Standard screen is not applied to the report.
- To generate a report for a failed installation standard run, you must do so before you click Reject Results.

1. Click  **View Detail Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.





3. To print the report, click  **Print**.
4. Close the report.



Save historical performance check reports (.pdf) for record keeping

IMPORTANT! After performing a performance check, save the performance check report electronically for record keeping. The software does not save historical calibration results. Only the most recent spectral calibration for each dye set is maintained in the software.

1. Click  **View Detail Report**.
2. Click  **Print**.
3. In the Printer dialog box, select **CutePDF Writer** as the printer.
4. Specify a name and location for the report.

6

Manage Library Resources

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Overview of libraries

The Library workflow contains:

- Items that you select when you set up a run:
 - Plates
 - Assays
 - Optional filename conventions
 - Optional results groups
- Items that you select when you create an assay:
 - Instrument protocols
 - Primary analysis protocols – Basecalling (sequencing), sizecalling (fragment analysis), QC (HID analysis)
- Items you select when you create instrument sizecalling and QC protocols:
 - Dye sets
 - Size standards

HID-Validated, Factory-provided Protocols:




The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).

Factory-provided, template, and locked items

The 3500 Series Software 2 libraries include factory-provided items that are optimized for different applications (for example, instrument protocols with specific run modules and primary analysis protocols with specific settings).¹ You can use the factory-provided items directly. If the factory-provided items do not suit your needs, you can do one of the following:

- Duplicate and modify a factory-provided item, and save the item with a new name.
- Create a new item.

Entries in the library may be flagged with the following symbols:

-  Factory-provided. Cannot be edited or deleted.
-  Template.
-  Locked (applies to file name conventions and results groups only). If the SAE module is enabled on your system, this item can be unlocked and modified by the user who created it, the administrator, or another user with unlock permissions. For information, see Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.

General library procedures

Access libraries

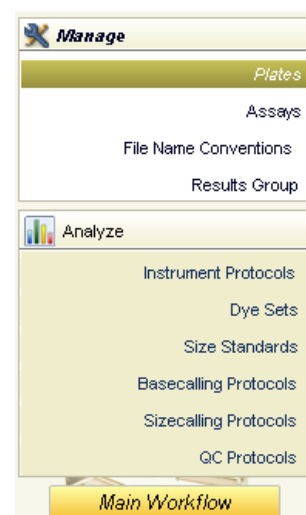
Select **Library** in the menu bar to access the Library workflow.



The Library workflow contains the screens where you manage assays, protocols, and other items that you use to acquire and process data.

The Library workflow contains:

- Items that you select when you set up for a run: plates, assays, filename conventions, and results groups
- Items that you select when you create an assay:
 - Instrument protocols
 - Primary analysis protocols – Basecalling (sequencing), sizecalling (fragment analysis), QC (HID analysis)
- Items you select when you create instrument, sizecalling, and QC protocols:
 - Dye sets
 - Size standards





You can click **Main Workflow**, or select **Dashboard** or any other menu item at any time to advance from the Library workflow.

¹ HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).



Create a new entry from a factory-provided, template, or locked entry

IMPORTANT! Auditing of an item depends on whether it is created directly from the library or from within another item (for example, you can create an assay directly from the library, or within a plate in the Assign Plate Contents screen). For more information on auditing, see “Review the object audit history” on page 202.

1. Select the factory-provided entry in the library.
2. Click  **Duplicate**.
3. Enter a name for the item.
4. Select the item, then click  **Edit**.
5. Modify parameters as needed (see the appropriate section for information).
6. Click **Save**.

Delete a library entry

IMPORTANT! Auditing of an item depends on whether it is deleted directly from the library or from within another item (for example, you can delete an assay directly from the library, or within a plate in the Assign Plate Contents screen). For more information on auditing, see “Review the object audit history” on page 202.

Note: You cannot delete  or  factory-provided items.

Select an item, then click  **Delete**.


Deleting a library entry does not affect existing items that contain the entry. (When you select an item to include in a higher-level item, a *copy* of that item is included in the higher-level item. For example, when you select an instrument protocol to include in an assay, a copy of the instrument protocol is included in the assay. If you delete the instrument protocol, the copy of the instrument protocol in the assay remains intact.)

For information on how deleted items are tracked in auditing, see “Audit action” on page 203.

Edit a library entry



IMPORTANT! Auditing of an item depends on whether it is edited directly from the library or from within another item (for example, you can edit an assay directly from the library, or within a plate in the Assign Plate Contents screen). For more information on auditing, see “Review the object audit history” on page 202.

Note: To edit a plate template, select the template from the main workflow. Go to Define Plate Properties screen ► Open Plate ► select Edit Existing Template.

1. Select an item, then click  **Edit**.
2. Modify parameters as needed.
3. Click **Save**.

Import and export a library entry

You can import and export .xml files for use with other 3500 Series Genetic Analyzer instruments:

- **Import** – Click  **Import**, then select the .xml file to import. If any items in the import file exist in the library, the software displays a message and gives you the option to replace or skip the item.
- **Export** – Select one or more entries, then click  **Export**, then specify a location for the export file.



To select multiple entries, Shift-click to select contiguous entries, Ctrl-click to select non-contiguous entries.

IMPORTANT! You must save a plate before you export it.

View audit and e-signature histories for library entries

Note: An administrator can also view audit and e-signature histories in the SAE module. For information, see Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.

To view the audit or e-signature history for a library entry:

1. Select the item in the library.
2. Click  **View Audit History** or  **View E-Signature History** (active only if the selected item is enabled for e-sig).

Note: Factory-provided items do not list creation date in the audit history. If you duplicate a factory-provided item, the new item contains an audit history that starts with the duplication date listed as the creation date.

3. For more information, see “Display audit histories” on page 202.

Sort, filter, and search library entries

Sort

Double-click column headers to sort. Multi-column sorting is supported:

- Double-click a column header to sort the column.
- Alt+Shift-click another column header to sort another column.
- Alt+Shift-click a third column header to sort a third column.

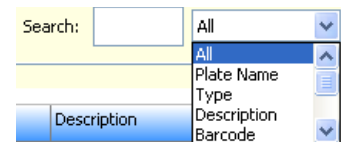
Numbers in the column headers reflect sort order.



Search

In each library, you can select a category to search, then enter the text to search for. The list of categories corresponds to the column headers in each library.

Click **Go** to search. Click **Clear** to remove the search criteria.

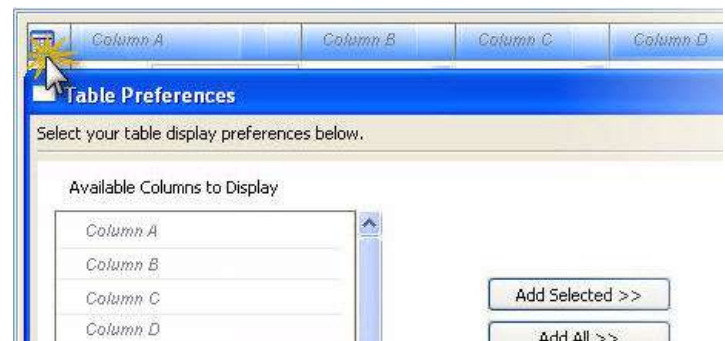


Customize a library table

Click the Table Settings button, then specify the columns to show or hide.

Click:

- **Apply** – To use the settings for this session only.
- **Save to Preferences** – To save for future use by all users. If your system includes the SAE module, preferences are saved for the logged-in user.
- **Restore Defaults** – To restore factory default settings.



Plates library

The Plates library contains all plates that have been saved in the software (plates that have been run and plates that have not yet been run).

Plate overview

Plate definition

A plate associates sample attributes (sample information and analysis information) with a well position. A plate defines how samples are analyzed during capillary electrophoresis and how sample files are named and stored after analysis.

When you create a plate, you specify:

- Plate type (sequencing, fragment, mixed, or HID)
- Number of wells, capillary length, and polymer type

When you set up a plate for a run, you add assays, optional file name conventions, and optional results groups to wells in the plate. If you add these items from the library, a *copy* of the items is added to the plate, and can be modified independently from the original items stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Plate templates

The Plates library includes templates that defined with the appropriate application type, polymer, and capillary length that you can use to create new plates.

Plate template names reflect the run module associated with the plate (a plate specifies an assay, an assay specifies an instrument protocol, and an instrument protocol specifies a run module which contains data collection settings). Appendix A, “Application Reagents and Run Modules” on page 249, lists the run time and size or base range collected for each run module.

Create a new plate

Note: If you are running a stand-alone version of the 3500 Series Software 2 (a version that is not installed on the instrument computer), you can create plates, then export them for use on the instrument computer.

1. Access the Plates library.


2. Click  **Create**.

The software switches to the Main workflow and displays the Define Plate Properties screen (Figure 10 on page 149).

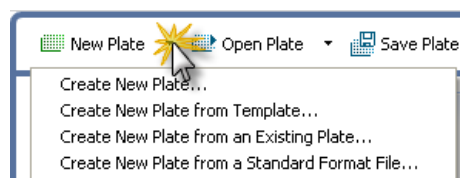
Note: You can also access the Define Plate Properties screen from the Dashboard and the Assign Plate Properties screen.



3. To create a new plate, specify settings (Table 2 on page 149).

To create a new plate based on an existing plate, click  **New Plate**, then select an option. Select a plate, click **Open**, then specify settings.

For information on other Create New Plate options, see “Create a plate from a template” on page 54.



4. Select a Save option.



Figure 10 Define Plate Properties

Table 2 Define Plate Properties

Setting	Description
Plate Details	
Name	Plate name. Names must be unique.
Number of Wells	<ul style="list-style-type: none"> • 96 well – For standard 96-well plates standard reaction plates and 8-strip standard tubes with retainers. • 96 Fast tube – For Fast 96-well plates and 8-strip tubes with retainers. • 384 well – For 384-well plates (24-capillary instruments only)
Plate Type	<ul style="list-style-type: none"> • Sequencing • Fragment analysis • Mixed (Seq + Frag) • HID
Capillary Length and Polymer	Capillary length and polymer type with which the plate will be used
Owner, Barcode, Description (optional)	Optional text entries You can use these entries to search for plates in the Plates library and in run logs (Tools ► View Logs).

Assays library

Assay overview

An assay contains the instrument protocol (dye set and run module) and primary analysis protocol needed to collect data and basecall or sizecall a sample. Assays, File Name Conventions, and Results Groups may already be listed in the plate template when you create a plate from a template.

Note: If no assay is listed, add at least one assay.

An assay contains:

- One or more instrument protocols appropriate for the sample type/dye set for which the assay will be used
- A primary analysis protocol that depends on your application:
 - **Sequencing** – Basecalling protocol
 - **Fragment** – Sizecalling protocol
 - **HID** – QC protocol¹

Assays are required by all application types. You must assign an assay to all named sample wells on a plate before you can link a plate and run it.

When you create an assay, you add one or more instrument protocols and a primary analysis protocol. If you add these items from the library, a *copy* of the items is added to the assay, and can be modified independently from the original items stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

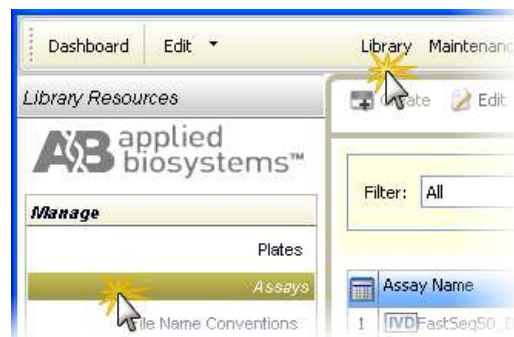
Create a new assay

If factory-provided assays do not suit your needs, you can create new assays:

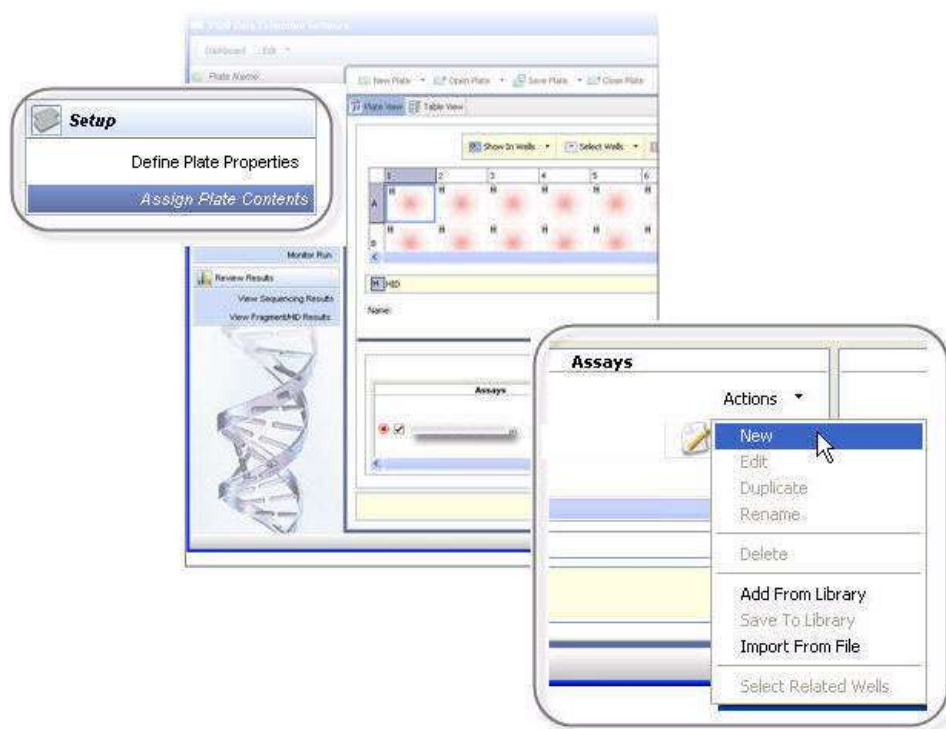
1. Access the Assays library.

2. Click  **Create**.

Note: You can also create an assay from the Assign Plate Contents screen.



¹ HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).



3. In the Create New Assays dialog box, select an application type: Sequencing, Fragment, or HID. The screen changes depending on the application type you select (Figure 11 on page 152 shows the sequencing screen).
4. Specify settings (see Table 3 on page 152).
5. Save the assay:
 - If you are creating the assay from the Library, click **Save**.
 - If you are creating the assay from the Assign Plate Contents screen, click **Apply to Plate** or **Save to Library**.

Figure 11 Create New Assay – sequencing – the highlighted area changes based on the Application Type

Table 3 Assay settings

Setting	Description
Assay Name	Name of the assay.
Locked	Prevents the item from being edited.
Color	Color code for the assay when it is displayed in the Assign Plate Contents screen (if Assay Color is selected for Show In Wells).
Application Type	<ul style="list-style-type: none"> Sequencing Fragment analysis HID

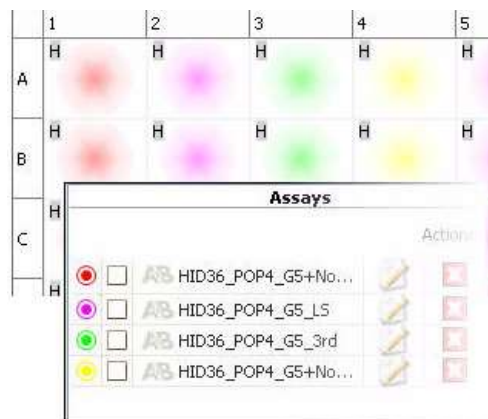
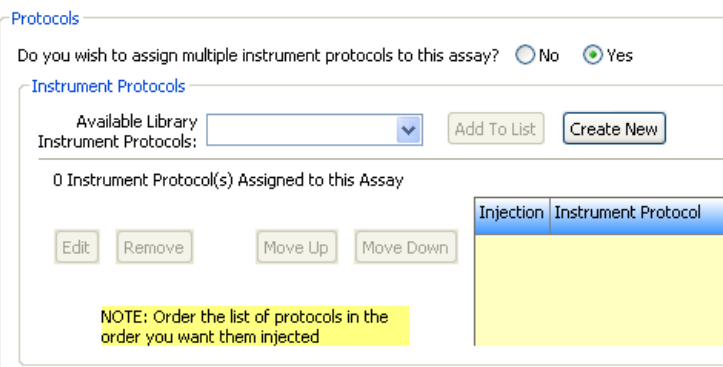


Table 3 Assay settings (continued)

Setting	Description
Do you wish to assign multiple instrument protocols to this assay?	<p>When you select Yes, allows you to select or create additional instrument protocols for the assay. The software creates one injection for each instrument protocol specified in an assay.</p> 
Instrument Protocol	<p>Instrument protocol for data collection.</p> <p>For information, see “Instrument protocol library” on page 166.</p>
Sequencing	
<ul style="list-style-type: none"> Basecalling Protocol 	<p>Protocol for primary analysis (basecalling and trimming) and quality determination.</p> <p>For information, see “Basecalling protocols library (primary analysis – sequencing)” on page 176.</p>
Fragment	
<ul style="list-style-type: none"> Sizecalling Protocol 	<p>Protocol for primary analysis (peak detection and sizing) and quality determination.</p> <p>For information, see “Sizecalling protocols library (primary analysis – fragment)” on page 180.</p>
HID	
<ul style="list-style-type: none"> QC Protocol 	<p>Protocol for primary analysis (peak detection and sizing) and quality determination.</p> <p>For information, see “QC protocols library (primary analysis – HID)” on page 185.</p>

File name conventions library

File name convention overview

A File Name Convention (FNC) specifies the naming convention for sample data files. It is an optional component in a plate.

If you do not specify a file name convention, data files are named in this format:

<sample name>_<well>

The file extension is determined by the application you run:


- **Sequencing** – .ab1 (you can also set Preferences to export additional file formats. See “Set sequencing preferences” on page 45.)
- **Fragment analysis** – .fsa
- **HID** – .hid

Note: The file location specified in a file name convention is used only if a results group is not specified for a well.

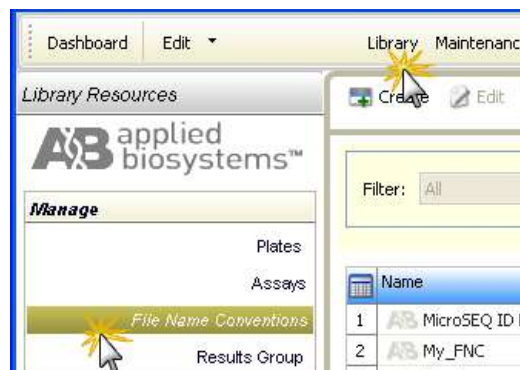
When you set up a plate for a run, you can optionally add file name conventions to the plate. If you add this item from the library, a *copy* of the item is added to the plate, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new file name convention

If factory-provided file name conventions do not suit your needs, you can create new file name conventions:

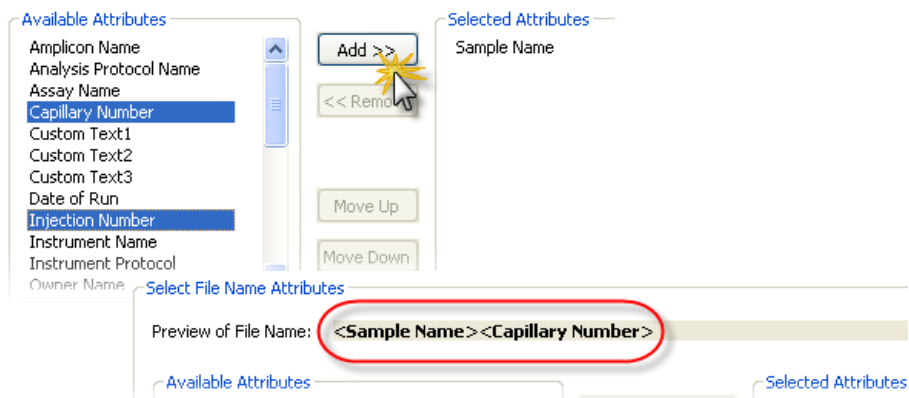
1. Access the File Name Conventions library.
2. Click  **Create**.

Note: You can also create a file name convention from the Assign Plate Contents screen.





3. In the Create New File Name Conventions dialog box (Figure 12 on page 156), select attributes and delimiters (see Table 4 on page 157).
As you select attributes, the software displays a preview of the file name.



4. To add delimiters between items in the Selected Attributes list:
 - a. Ctrl-click or Shift-click to select two or more attributes.
 - b. Select a delimiter.
 - c. Select the Add between attributes check box.
 - d. Click **Add**.
5. Save the file name convention:
 - If you are creating the file name convention from the Library, click **Save**.
 - If you are creating the file name convention from the Assign Plate Contents screen, click **Apply to Plate** or **Save to Library**.

Create New File Name Convention

Setup a File Name Convention

Name is a required field. Provide a unique value.

* Name: ☐ Locked Color: Black

Select File Name Attributes

Preview of File Name: **<Sample Name>**

Available Attributes

- Amplicon Name
- Analysis Protocol Name
- Assay Name
- Capillary Number**
- Custom Text1
- Custom Text2
- Custom Text3
- Date of Run
- Injection Number
- Instrument Name
- Instrument Protocol
- Owner Name

Delimiters

Select a delimiter: Plus (+)

☒ Add between attributes Add >>

Selected Attributes

Sample Name

Add >> << Remove

Move Up Move Down

Add a custom value to available attributes (optional)

Custom Text 1: Custom Text 2: Custom Text 3:

Select File Location

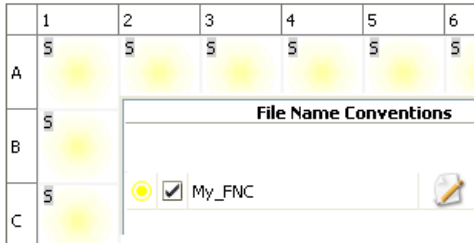
☒ Default File Location C:\Applied Biosystems\3500\Data

☐ Custom File Location Browse...

Close Save

Figure 12 Create New File Name Convention

Table 4 File name conventions settings

Setting	Description
Name	Name of the file name convention. Names must be unique.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when the SAE module is enabled on your system (described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 191.
Color	<p>Color code for the file name convention when it is displayed in the Assign Plate Contents screen (if File Name Convention Color is selected for Show In Wells).</p> 
Preview of name	Interactively displays the attributes you select.
Available attributes	<ul style="list-style-type: none"> • Amplicon Name (from Customize Sample Info in sequencing assays) • Analysis Protocol Name – (primary analysis protocol) • Assay Name • Capillary Number • Custom Text fields (up to 3) • Date of Run • Injection Number • Instrument Name • Instrument Protocol • Owner Name (plate owner) • Plate Name • Polymer Type • run name • Sample Type • Specimen Name (from Customize Sample Info in sequencing assays) • Time of Run (run start time) • Unique Time Stamp Integer – (numeric string in milliseconds that does not correspond to the current time) • User-defined Fields (up to 5; specified in Assign Plate Contents, see page 57) • User Name (available only when security is enabled in the SAE module) • Well Position <p>IMPORTANT! The maximum allowed length of a file name, including the path, is 240 characters. The software warns you if your selections will possibly exceed the maximum, but allows you to save the file name convention. However, you will see a pre-check validation error when you start a run if the file name will exceed 240 characters.</p>
delimiters	Symbols you can include in the file name: Dash (-), Dot (.), Underscore (_), Plus (+), Dollar (\$).
Custom text	Text to display for the custom text attribute fields.
File location	<p>The file location in the file name convention is used only if no results group is specified for a well.f</p> <p>The Results Group file location overrides the File Name Convention file location.</p>

Results Group library

Results group overview

A Results Group is used to name, sort, and customize the folders in which sample data files are stored. It is an optional component in a plate.

Note: The file location specified in a results group overrides the file location in the file name convention specified for a well.

When you set up a plate for a run, you can optionally add results groups to wells in the plate. If you add this item from the library, a *copy* of the item is added to the plate, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Allelic ladder location (HID analysis)

To accurately genotype samples, the GeneMapper® ID-X Software requires at least one allelic ladder sample per run folder. (Multiple allelic ladder samples in a single run folder can also be used for analysis.)

We recommend that you run one allelic ladder for 24 a set of samples:


- **8-capillary instruments** – One allelic ladder per 3 injections
- **24-capillary instruments** – One allelic ladder per 1 injection

Note: Run HID validation studies to determine the required number of allelic ladders for your application.

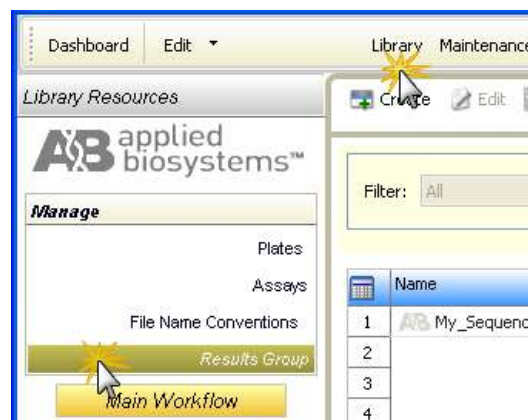
See “Results group example 2: store one allelic ladder per run folder (8-capillary instruments)” on page 163 for a results group example that places three injections in each run folder for 8-capillary instruments.

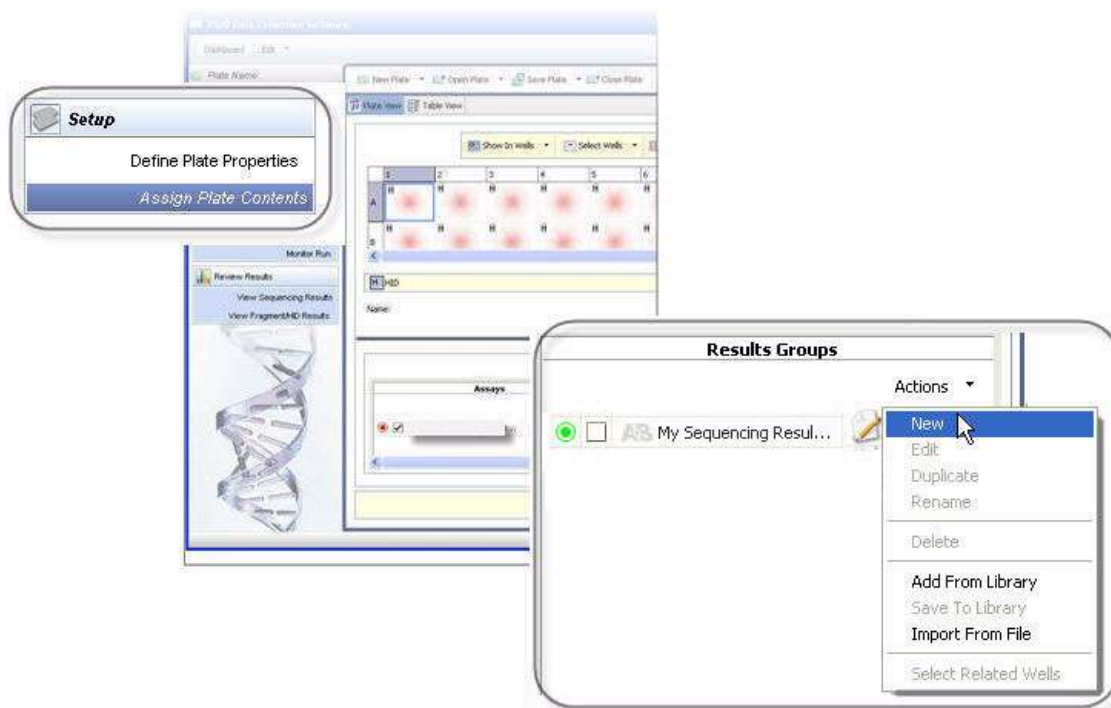
Create a new results group

If factory-provided results groups do not suit your needs, you can create new results groups:

1. Access the Results Group library.
2. Click  **Create**.

Note: You can also create a results group from the Assign Plate Contents screen.





3. In the Create Results Group dialog box (Figure 13 on page 160), select attributes and delimiters (see Table 5 on page 160).

As you select attributes, the software displays a preview of the results group name.



4. To add delimiters between items in the Selected Attributes list:
 - a. Ctrl-click or Shift-click to select two or more attributes.
 - b. Select a delimiter.
 - c. Select the Add between attributes check box.
 - d. Click **Add**.
5. Save the results group:
 - If you are creating the results group from the Library, click **Save**.
 - If you are creating the results group from the Assign Plate Contents screen, click **Apply to Plate** or **Save to Library**.

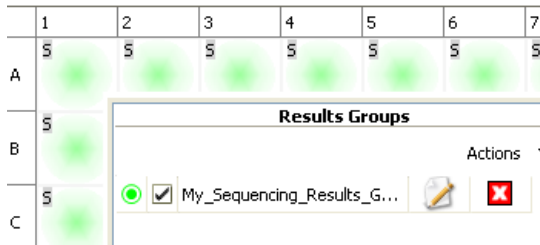
The Results Group file location overrides the File Name Convention file location.

Figure 13 Create New Results Group

Table 5 Results Group settings

Setting	Description
Name	Name of the results group. Names must be unique. The Results Group Name is a required attribute, you cannot remove this attribute from the Selected Attribute list.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when the SAE module is enabled on your system (described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 191

Table 5 Results Group settings (continued)

Setting	Description
Color	Color code for the results group when it is displayed in the Assign Plate Contents screen (if Results Group Color is selected for Show In Wells). 
Preview of name	Interactively displays the attributes you select.
Available attributes	<ul style="list-style-type: none"> Results Group Name (required) Assay Name Injection Number IP Name (instrument protocol) Logged-in User Name (available only when security is enabled in the SAE module) PA Protocol Name (primary analysis=basecalling protocol) Plate Name Prefix Start Instrument Run Date/time Stamp Suffix
delimiters	Symbols you can include in the results group name: Dash (-), Dot (.), Underscore (_), Plus (+), Dollar (\$).
Prefix/suffix text	Text to display for the prefix or suffix text attribute fields.
Select re-injection folder option	<ul style="list-style-type: none"> Store reinjection sample files in a separate reinjection folder (same level as injection folders) Store reinjection sample files with original sample files (same level)
Select folder option	<p>Location:</p> <ul style="list-style-type: none"> Default file location (specified in Preferences ► User ► Run Setup) Custom location <p>Sub-folder options:</p> <ul style="list-style-type: none"> Include an instrument run name folder (run name can be user-defined in the Load Plates for Run screen) Include a results group name folder Include an injection folder

Results group example 1: store files by plate name

Two default, factory-provided, results groups are provided that store sample data files by plate name:

- Figure 14 on page 162 shows the factory-provided PN_Injfolder_RG results group and the folders created when it is used. This results group creates a folder for each injection.
- Figure 15 on page 162 shows the factory-provided PN_RG results group and the folders created when it is used. This results group does not create a folder for each injection. All samples for a plate are stored in the same folder. If you include two plates in a run, a separate folder is created for each plate.

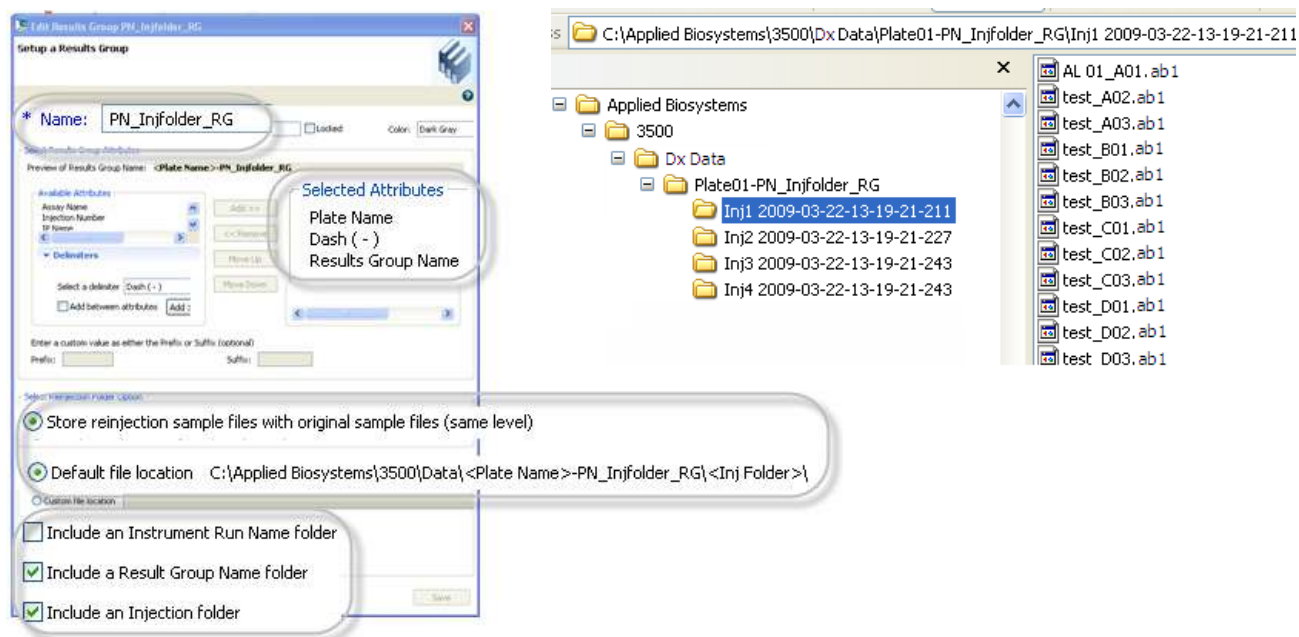


Figure 14 PN_Injfolder_RG results group

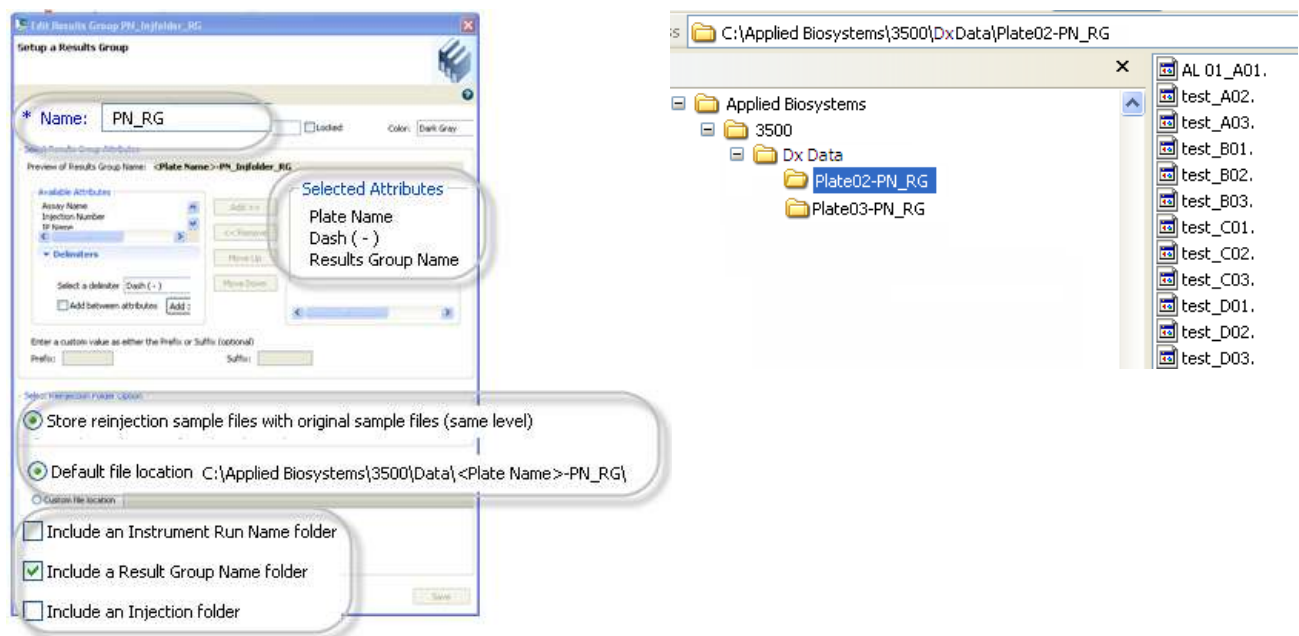


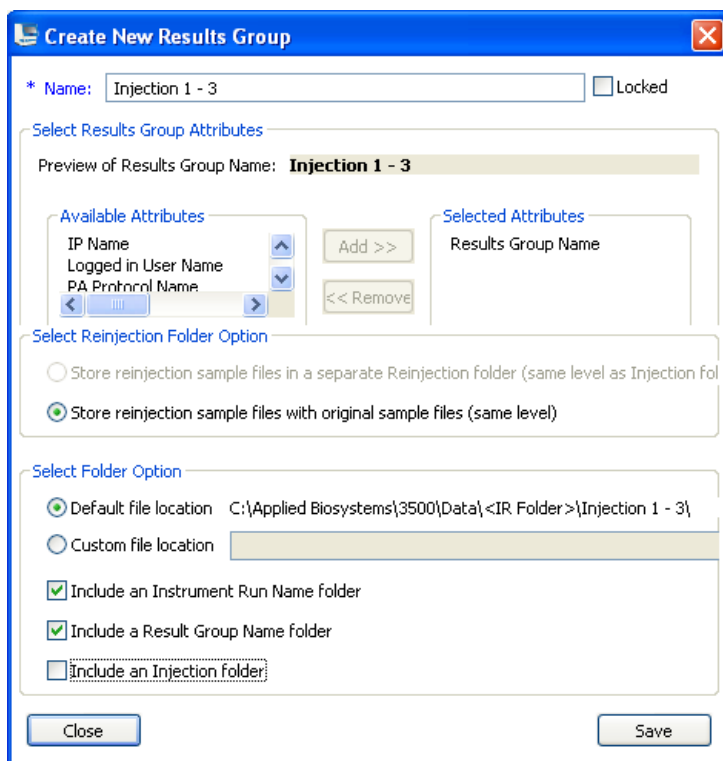
Figure 15 PN_RG results group

Results group example 2: store one allelic ladder per run folder (8-capillary instruments)

We recommend that you run one allelic ladder for each set of 24 samples (see “Allelic ladder location (HID analysis)” on page 158).

To store one allelic ladder per run folder on an 8-capillary instrument, create one results group for each set of three injections on the plate. Each results group specifies a results group name folder. Because you assign one results group to a set of three injections, all 24 sample data files, including the allelic ladder, are stored in the same results group folder.

The example below shows one results group; for a full 96-well plate, create three more with the same settings, but different names, for example, Injection 4 - 6, Injection 7 - 9, and Injection 10 - 12.



Results group example 3: store re-injections in separate folders

Figure 16 on page 164 shows an example results group that specifies a sample file storage location of:

C:\Example\instrument run (IR) folder\result group name folder[results group name+start instrument run date/time stamp+logged in user name]\injection name or re-injection name folder.

The numbers in the figure relate the elements in the results group with the elements in the file hierarchy created by a run that uses this results group (Figure 19 on page 165).

* Name: ☐ Locked

Select File Name Attributes

Preview of File Name: **<<Sample Name>>.<<Analysis Protocol Name>>.<<Unique Time Stamp Integer>>**

Available Attributes

- Amplicon Name
- Assay Name
- Capillary Number
- Custom Text1
- Custom Text2
- Custom Text3
- Date of Run

Add >>

<< Remove

Selected Attributes

- Sample Name
- Dot (.)
- Analysis Protocol Name
- Dot (.)
- Unique Time Stamp Integer

Figure 18 File name convention example

Figure 19 on page 165 shows the folders and files generated by the results group, file name convention, run name, and injections shown in Figure 16 on page 164, Figure 17 on page 164, and Figure 18 on page 165.

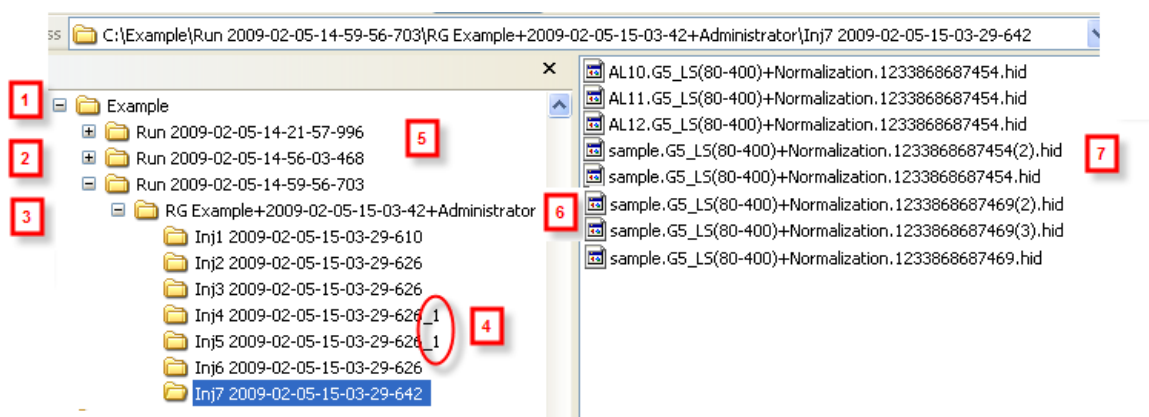


Figure 19 Folder hierarchy and file naming example

1	File location from results group <input checked="" type="checkbox"/> Custom file location <input type="text" value="C:\Example"/>
2	Instrument Run Name folder from results group <input checked="" type="checkbox"/> Include an Instrument Run Name folder
3	Results group Name folder from results group <input checked="" type="checkbox"/> Include a Result Group Name folder
4	Injection folder from results group <input checked="" type="checkbox"/> Include an Injection Folder Duplicate injections indicated with _n where n is the number of duplicates.
5	Run name (default or user-defined) from injection list Run Name: Run 2009-02-05-14-59-56-703
6	Results group name syntax from results group RG Example+<Start Instrument Run Date/Time Stamp>+<Logged in User Name>
7	File name syntax from file name convention <Sample Name>.<Analysis Protocol Name>.<Unique Time Stamp Integer>

Instrument protocol library

Instrument protocol overview

An instrument protocol contains the parameters that control the instrument during data acquisition. An instrument protocol is a required element of an assay for all applications.

When you create an assay, you add one or more instrument protocols to the assay. If you add these items from the library, a *copy* of the items is added to the assay, and can be modified independently from the original items stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new instrument protocol

If factory-provided instrument protocols do not suit your needs, you can create new instrument protocols:


1. Access the Instrument Protocols library.
2. Click  **Create**.
3. In the Create New Instrument Protocol dialog box (Figure 20 on page 167), select an application type: Sequencing, Fragment, or HID. The run module selection list is filtered based on the application you select.
4. Specify settings (Table 6 on page 167).
5. Save the assay:
 - If you are creating the assay from the Library, click **Save**.
 - If you are creating the assay from the Assign Plate Contents screen, click **Apply to Plate** or **Save to Library**.





Figure 20 Create New Instrument Protocol – normalization parameters circled in red are displayed for fragment analysis and HID applications only

Instrument protocol settings

Table 6 Instrument protocol settings

Setting	Description
Application Type	<ul style="list-style-type: none"> Sequencing Fragment analysis HID
Capillary Length, Polymer, Dye set	Capillary length, polymer type, and dye set with which the protocol will be used
Run module	Factory-provided modules that specify instrument control parameters. For more information, see “Run modules” on page 251.
Protocol name	Name of the protocol. Names must be unique.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when your system includes the SAE module (described in Chapter 7, “Use Security, Audit, and E-Sig Functions [SAE Module]” on page 191.

Table 6 Instrument protocol settings *(continued)*

Setting	Description
Description	Optional text entry.
Oven temperature (°C)	Temperature setting for main oven throughout run.
Run voltage (kVolts)	Final sample electrophoresis separation run voltage.
Prerun voltage (kVolts)	Pre run voltage setting before sample injection.
Injection voltage (kVolts)	Injection voltage setting for sample injection.
Run time (sec)	Length of time data is collected after voltage is ramped up to the run voltage and the run starts.
PreRun time (sec)	Prerun voltage time.
Injection time (sec)	Sample injection time.
Data delay (sec)	Time from the start of separation to the start of sample data collection.
<i>Advanced options – Do not change unless advised otherwise by Life Technologies support personnel</i>	
Voltage tolerance (kVolts)	Maximum allowed voltage variation.
Voltage # of Steps (nk)	Number of voltage ramp steps to reach Run Voltage.
Voltage step interval (sec)	Dwell time at each voltage ramp step.
First read out time (ms)	The interval of time for a data point to be produced. First ReadOut time should be equal to Second ReadOut time.
Second read out time (ms)	The interval of time for a data point to be produced. Second ReadOut time should be equal to First ReadOut time.
Fragment and HID protocols only: Normalization parameters – Leave at default settings (for information on how these parameters are used, see “Review normalized data” on page 97).	
Normalization Target	<p>The expected average RFU for the subset of peaks in the GS600 LIZ® v2 size standard used for normalization.</p> <p>The default value for each run module has been experimentally determined based on the average peak height of selected peaks in the GS600 size standard with a specific injection time.</p> <p>IMPORTANT! If you change the injection time in an instrument protocol, adjust the Normalization Target proportionately. For example, for an instrument protocol with an injection time of 10 seconds and a Normalization Target of 2000: if you change the injection time to 15 seconds (50% increase), change the Normalization Target to 3000 (50% increase).</p>
Normalization Factor Thresholds	<p>The passing range for Normalization Factor (default range is 0.3 to 3.0).</p> <p>IMPORTANT! Increasing the factor threshold above 3.0 may cause amplification of noise.</p> <p>If the calculated Normalization Factor is outside the Normalization Factor range, the software multiplies the peak heights of the sample by the low or high Normalization Factor threshold setting (for example, if the Normalization Factor range is 0.3 to 3.0 and the calculated Normalization Factor is 5, the software applies a Normalization Factor of 3.0).</p>
Normalization Factor	<p>Average peak height of the subset of peaks in the GS600 LIZ® v2 size standard used for normalization divided by the Normalization Target.</p> <p>Samples are flagged with  in results if Normalization Factor is within threshold range, or with  if it is out of threshold range.</p>

Dye sets library

Dye set overview

A dye set defines the following for an instrument protocol:

- Dye color(s)
- Order of the dye peaks in the standard
- Spectral analysis parameters

When you create an instrument protocol, you add a dye set to the protocol. If you add this item from the library, a *copy* of the item is added to the assay, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new dye set

If factory-provided dye sets do not suit your needs, you can create new dye sets:


1. Access the Dye Sets library.
2. Click  **Create**.
3. In the Create New Dye Set dialog box (Figure 21 on page 170).
4. Specify settings (Table 7 on page 170).
5. Click **Save**.



Figure 21 Create New Dye Set

Table 7 Dye set settings


Setting	Description
Dye Set Name	Name of the dye set. Names must be unique.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when your system includes the SAE module (described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 191.
Chemistry	The standard for which you are creating the dye set: Sequencing Standard or Matrix standard
Dye Set Template	Factory-provided template upon which to base the dye set. The Any Dye template can be used for applications that do not use all of the dye colors contained in the matrix standard kits used for spectral calibration. For information, see "Create a new dye set using the AnyDye template" on page 171.

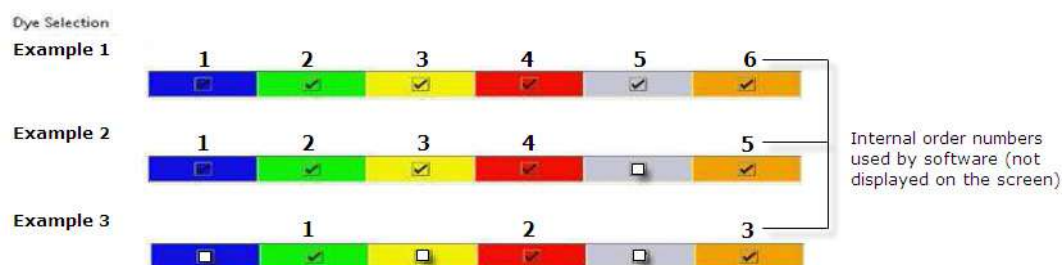
Table 7 Dye set settings

Setting	Description
Arrange Dyes	Displays the dyes and the peak order for the dye set template selected. Editable only for AnyDye template: <ul style="list-style-type: none"> • Dye Selection – Specifies the dyes to use for calibration • Reduced Selection – Specifies the dyes used in the samples. For example, if you use the 5 dye kit and have samples with only blue peaks, you can “reduce” or deconvolute with blue and orange (size standard) dyes only.
Parameters	Specifies the Quality Value, Condition Number, Scan, and Sensitivity requirements for the dye set.
Notes	Optional text entry.

Create a new dye set using the AnyDye template

If factory-provided dye sets do not suit your needs, you can create new dye sets:

1. Access the Dye Sets library.
2. Click  **Create**.
3. Enter a dye set name.
4. Select a chemistry and the AnyDye dye set template.
5. Select the dye colors to use and set the calibration peak order:
 - a. Select the dye colors to use, which specifies the order number of the dye used internally by the software. Note that when you deselect a dye, the order number of the dye used internally by the software changes.
In Example 1 with all dyes selected, internal order number is Blue (1), Green (2), Yellow (3), Red (4), Purple (5), Orange (6).
In Example 2 with the Purple dye deselected, internal order number is Blue (1), Green (2), Yellow (3), Red (4), Orange (5) - the internal order number of Orange changes to 5.
In Example 3 with the Blue, Yellow, and Purple dyes deselected, internal order number is Green (1), Red (2), Orange (3) - the internal order number of Green changes to 1, Red changes to 2, and Orange changes to 3.



- b. Specify the order of the peaks in the calibration standard you are using. Use the internal order number of the dye based on the dyes selected.

IMPORTANT! The Calibration Peak Order fields do not correspond to the dye colors displayed above the Calibration Peak Order fields.

In Example 1, if the order of the peaks in the calibration standard you are using is Orange, Red, Yellow, Blue, Green, Purple, specify for Calibration Peak Order: 6 (Orange), 4 (Red), 3 (Yellow), 1 (Blue), 2 (Green), 5 (Purple).

In Example 2, if the order of the peaks in the calibration standard you are using is Orange, Red, Yellow, Blue, Green, specify for Calibration Peak Order: 5 (Orange), 4 (Red), 3 (Yellow), 1 (Blue), 2 (Green).

In Example 3, if the order of the peaks in the calibration standard you are using is Orange, Red, Green, specify for Calibration Peak Order: 3 (Orange), 2 (Red), 1 (Green).

Example 1

Arrange Dyes

	1	2	3	4	5	6
Dye Selection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Reduced Selection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Calibration Peak Order	6	4	3	1	2	5

Example 2

Arrange Dyes

	1	2	3	4	5
Dye Selection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Reduced Selection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Calibration Peak Order	5	4	3	1	2

Example 3

Arrange Dyes

	1	2	3
Dye Selection	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Reduced Selection	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Calibration Peak Order	0	3	0

6. Expand the Parameters section, then specify remaining settings.

7. Click **Save**.

Size standards library

Size standard overview

A size standard defines the sizes of known fragments. It is used to generate a standard curve. The standard curve is used to determine the sizing of unknown samples.

When you create a sizecalling (fragment) or QC (HID) protocol, you add a size standard to the protocol. If you add this item from the library, a *copy* of the item is added to the protocol, and can be modified independently from the original items stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

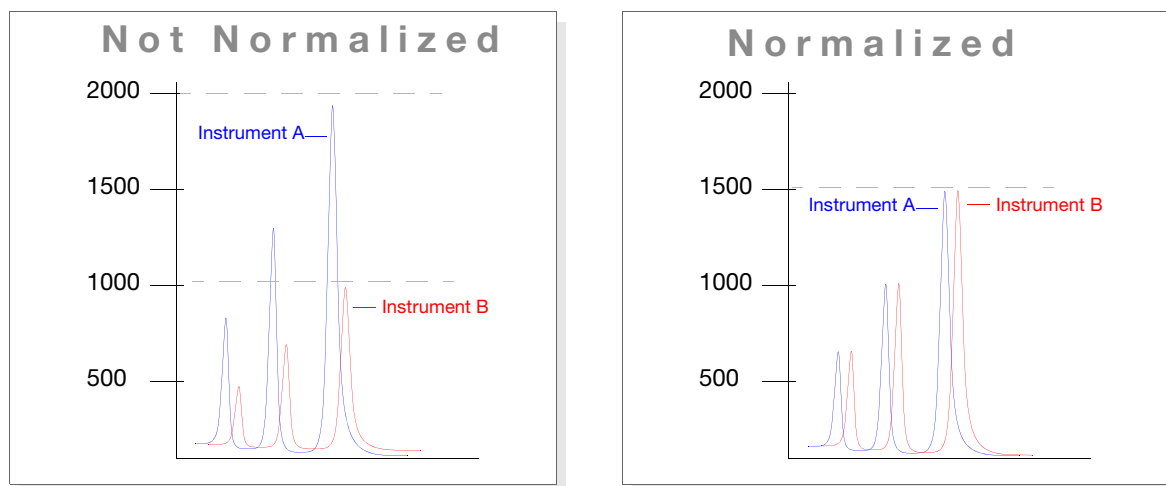
Normalization size standards provided

The library contains factory-provided normalized size standards that you can use to normalize fragment analysis and HID data:

- Fragment analysis (POP-6™ and POP-7™ polymer):
 - GS600LIZ+Normalization
 - GS600(60-600)LIZ+Normalization – For applications that have primer peaks that obscure the 20 and 40-mer peaks of the GS600 size standard.
- Fragment analysis (POP-4® polymer):
 - GS600(80-400)LIZ+Normalization
- HID¹:
 - GS600(80-400)LIZ+Normalization

Normalization corrects for instrument, capillary, and injection variability. For each sample, the software calculates a normalization factor based on a threshold setting. The normalization factor is used as a multiplier to adjust the peak height of the sample peaks relative to the GS600 LIZ® v2.0 size standard peaks.

IMPORTANT! Normalization is not applied to samples with failing sizing quality. Select a size standard definition file appropriate for your application that accurately sizes samples. For example, if your application includes small fragments that may be obscured by primer peaks, or large fragments that may not be present due to slower migration rates, specify a size standard definition file that eliminates these fragments from sizing.



For more information, see “Review normalized data” on page 97.

¹ HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ▶ Software Downloads ▶ 3500 Series Genetic Analyzers for Human Identification).

Create a new size standard

If factory-provided size standards do not suit your needs, you can create new size standards:



1. Access the Size Standards library.
2. Click  **Create**.
3. In the Create New Size Standard dialog box (Figure 21 on page 170), enter a size standard name.
4. (Optional):
 - Select the Locked check box. When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when your system includes the SAE module (described in Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.
 - Enter a description.
5. Select a dye color.
6. Enter sizes in the list on the left. Separate sizes with a comma, space, or return.
7. Click **Add Sizes**.
8. Click **Save**.



Figure 22 Create New Size Standard

Modify a factory-provided normalization size standard

1. Select a factory-provided normalization size standard (indicated in the name with "+Normalization.")
2. Click  **Duplicate**.
3. Edit the copy of the normalized size standard. The size standard peaks used to normalize the data are displayed in gray and are not editable.
4. Click **Save**.

Basecalling protocols library (primary analysis – sequencing)

Basecalling protocol overview

A basecalling protocol is the required primary analysis protocol for sequencing applications.


A basecalling protocol defines the settings used by the sequencing basecallers to assign base calls to each detected peak and assign a quality value:

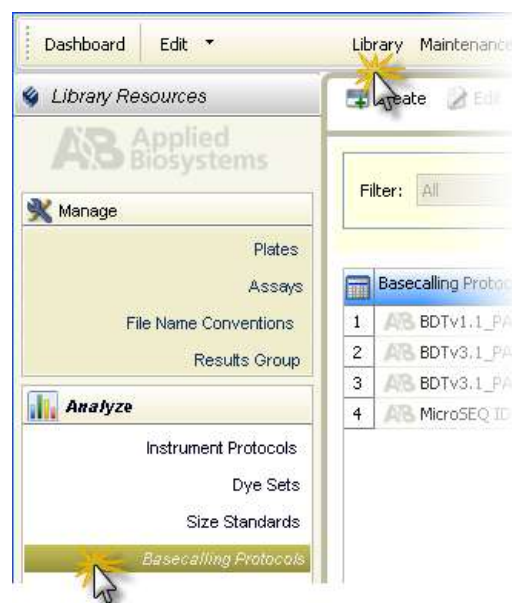
- Analysis settings
- Ranges for the sequencing quality flags displayed in View Results

When you create a sequencing assay, you add a basecalling protocol to the assay. If you add this item from the library, a *copy* of the item is added to the assay, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new basecalling protocol

If factory-provided basecalling protocols do not suit your needs, you can create new basecalling protocols:

1. Access the Basecalling Protocols library.
2. Click  **Create**.
3. In the Analysis Settings tab of the Create New Basecalling Protocol dialog box (Figure 23 on page 177), specify settings (see Table 8 on page 177).
4. Click **QV Settings**. In the QV Settings tab of the Create New Basecalling Protocol dialog box (Figure 23 on page 177), then specify settings and Table 8 on page 177).
5. Click **Save**.



Create New Basecalling Protocol

Setup a Basecalling Protocol

✖ Protocol Name is a required field. Provide a unique value.

* Protocol Name: ☐ Locked

Description:

Basecaller:

Analysis Settings ☒ QV Settings

☐ Mobility File
☐ Quality Threshold
☐ Mixed Bases Threshold
☐ Analyzed Data Scaling
☐ Clear Range Methods

Summary of current settings

Mobility File: KB_3500_POP7_BDTv3
 Quality Threshold: Do not assign N's to Basecall
 Mixed Base Threshold: 25.0%
 Scaling: True Profile
 Clear Range Methods: Use quality values.

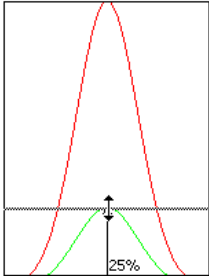
Close Save

Figure 23 Create New Basecalling Protocol – Analysis Settings

Table 8 Basecalling protocol – Analysis settings

Setting	Description
Name	Name of the protocol. Names must be unique.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when your system includes the SAE module (described in Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 191.
Description	Optional text entry.
Basecaller	Basecalling algorithm used to identify bases. Note: The basecaller version listed in the basecalling protocol is a 3-digit number. The version listed in sequencing results is a 4-digit number. The fourth digit is an internal number used by the software.
Mobility file	Compensates for mobility differences between dyes and primers, correcting the color code to the chemistry used to label the DNA during instrument processing.

Table 8 Basecalling protocol – Analysis settings (*continued*)

Setting	Description
Quality Threshold	<ul style="list-style-type: none"> Basecall Assignment (ambiguous bases): <ul style="list-style-type: none"> Do not assign N's to basecalls Assign N's to basecalls with QV<15 – Bases with a QV less than the threshold display N instead of the base letter Ending base – Last base on which to perform basecalling: <ul style="list-style-type: none"> At PCR Stop After X number of Bases After X number of Ns in X number of Bases After X number of Ns <p>Note: If you have short PCR products, select the At PCR Stop check box.</p>
Mixed bases threshold	<p>When enabled, allows the software to determine the secondary peak height where the base position is considered a potential mixed base.</p> <p>Adjust this parameter by dragging the bar in the display or typing in a numeric value.</p> <p><input checked="" type="checkbox"/> Use Mixed Base Identification</p> <p>Do not assign a mixed base when the secondary peak height is <= to <input type="text" value="25"/> %</p> 
Analyzed Data Scaling	<p>Determines scaling of the processed traces. This parameter does not affect the accuracy of the basecalling.</p> <ul style="list-style-type: none"> 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).
Clear range methods	<ul style="list-style-type: none"> 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.

Create New Basecalling Protocol

Setup a Basecalling Protocol

Protocol Name is a required field. Provide a unique value.

* Protocol Name: ☐ Locked

Description:

Basecaller: KB 1.4.1

Analysis Settings | **QV Settings**

Sequence Quality

	Fail If Value Is	Suspect Range	Pass if Value Is
Contiguous Read Length	< 100	100-300	>= 300
Trace Score	< 15	15-30	>= 30
QV20+	< 100	100-300	>= 300

Close Save

Figure 24 Create New Basecalling Protocol – QV Settings

QV settings are quality value ranges used in the following screens:

- **Monitor Run screen** – The state of the QV flag:
 - If all three values are in the pass range, the QV flag in Monitor Run is set to (green).
 - If any values are in the suspect range, the QV flag in Monitor Run is set to (yellow).
 - If any values are in the fail range, the QV flag in Monitor Run is set to (red).
- **View Sequencing Results ▶ Metric Analysis Results table** – The pass/check/fail status for Trace Score Quality, CRL Quality, and QV20+ Quality results.

Table 9 Basecalling protocol – QV settings

Setting	Description
Contiguous Read Length	The longest uninterrupted segment of bases with an average Quality Value (QV) ≥ 20 . In addition to evaluating the QV of a base call, the software considers the QV of adjacent bases within a ± 20 -bp moving average to determine a contiguous read length based on quality values: the software starts from the 5' end and calculates the average QV across a moving window size of 20, sliding 1 bp at a time, to the 3' end. The resulting longest contiguous segment is determined as the CRL.
Trace Score	The average basecall quality value (QV) of bases in the clear range sequence of a trace.
QV20+	The total number of bases in the entire trace with quality values ≥ 20 .

Sizecalling protocols library (primary analysis – fragment)

Sizecalling protocol overview


A sizecalling protocol is the required primary analysis protocol for fragment applications.

A sizecalling protocol defines peak detection, sizing, and quality values.

When you create a fragment assay, you add a sizecalling protocol to the assay. If you add this item from the library, a *copy* of the item is added to the assay, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new sizecalling protocol

If factory-provided sizecalling protocols do not suit your needs, you can create new sizecalling protocols:

1. Access the Sizecalling Protocols library.
2. Click  **Create**.
3. In the Analysis Settings tab of the Create New Sizecalling Protocol dialog box (Figure 25 on page 181), specify settings (see Table 10 on page 181).
4. Click **QC Settings**. In the QC Settings tab of the Create New Sizecalling Protocol dialog box (Figure 11 on page 184), then specify settings and Table 12 on page 184).
5. Click **Save**.

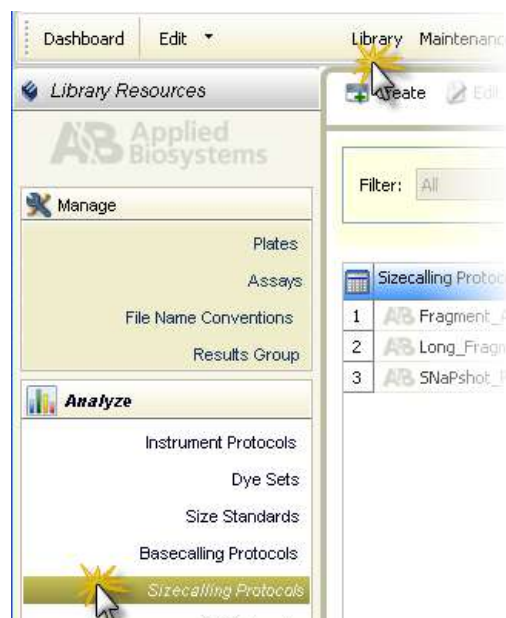


Figure 25 Create New Sizecalling Protocol – Analysis Settings

IMPORTANT! Normalization is not applied to samples with Size Quality flags. Specify analysis settings that accurately detect and size the size standard, and QC settings with appropriate pass fail ranges. The 3500 Series Software 2 does not support re-analyzing data with new settings.

Table 10 Sizecalling protocol – Analysis settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Size standard	Size standard definition in the software that corresponds to the dye set used in the chemistry. To apply normalization, select a normalization size standard (see “Normalization size standards provided” on page 173).

Table 10 Sizecalling protocol – Analysis settings (*continued*)

Setting	Description
Analysis Range	<p>Specify the range (in data points) to analyze:</p> <ul style="list-style-type: none"> • Full Range to analyze the entire scan region as collected by the genetic analysis instrument, including the primer peak. • Partial Range to analyze only data points within a specified range. Enter Start Point in data points after the primer peak and before the first required size standard peak. Enter a Stop Point after the last required size standard fragment. Start and Stop points may vary from instrument to instrument and platform to platform. Display raw data to determine the appropriate analysis range. <p>Data points outside the specified analysis range are ignored.</p> <p>Note: Ensure the Analysis Range contains all size standard fragments included in the Sizing Range specified below.</p>
Sizing Range	<p>Specify the size range (in base pairs) appropriate for the kit you are using:</p> <ul style="list-style-type: none"> • All Sizes for the software to analyze fragments of all sizes in the Analysis Range. • Partial Sizes for the software to analyze only fragments within a specified range. Enter a Start Size and a Stop Size appropriate for the size standard used.
Size Calling Method	<ul style="list-style-type: none"> • Local Southern - (default) Determines the fragment sizes using the reciprocal relationship between fragment length and electrophoretic mobility. • 3rd Order Least Squares - Uses regression analysis to build a best-fit size calling curve. • 2nd Order Least Squares - Uses regression analysis to build a best-fit size calling curve. • Cubic Spline Interpolation - Forces the sizing curve through all the known points of the selected size standard. • Global Southern Method - Compensates for standard fragments with anomalous electrophoretic mobility (similar to least squares methods).
Primer Peak	<p>If the primer peaks in your application obscure peaks of interest, select Present. Selecting Present instructs the algorithm to ignore primer peaks. Primer peaks are still displayed in the trace.</p> <p>Note: If this setting does not allow detection of the 20 and 40-mer peaks for samples that use the GS600 LIZ size standard, running samples with the GS600(60-600)LIZ+Normalization may allow detection of the peaks.</p>
Peak Amplitude Thresholds	<p>Specify the threshold (RFU) for peak detection for each dye color. Peaks below the threshold are not detected.</p> <p>For example, if you use the default values of 175, peaks with heights equal to or greater than 175 are detected. Peaks with heights below 175 are still displayed in the electropherogram plots but are not detected or labeled.</p> <p>Note: Set the peak amplitude thresholds to 175 in your GeneMapper® Software analysis method.</p>

Table 10 Sizecalling protocol – Analysis settings (*continued*)

Setting	Description
Smoothing	<p>Select an option to smooth the outline of peaks and reduce the number of false peaks detected:</p> <ul style="list-style-type: none"> • None (default) to apply no smoothing. Best if the data display sharp, narrow peaks of interest. • Light to provide the best results for typical data. Light smoothing slightly reduces peak height. • Heavy for data with very sharp, narrow peaks of interest. Heavy smoothing can significantly reduce peak height.
Baseline Window	<p>Specify a window to adjust the baseline signals of all detected dye colors to the same level for an improved comparison of relative signal intensity. Note the following:</p> <ul style="list-style-type: none"> • A small baseline window relative to the width of a cluster, or grouping of peaks spatially close to each other, can result in shorter peak heights. • Larger baseline windows relative to the peaks being detected can create an elevated baseline, resulting in peaks that are elevated or not resolved to the baseline.
Min. Peak Half Width	Specify the smallest half peak width at full height for peak detection. The range is 2 to 99 data points.
Polynomial Degree	<p>Polynomial Degree cannot be greater than Peak Window Size.</p> <p>Adjust to affect the sensitivity of peak detection. You can adjust this parameter to detect a single base pair difference while minimizing the detection of shoulder effects and/or noise.</p> <p>The peak detector calculates the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range.</p> <p>Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, captures more of the peak structure in the electropherogram.</p>
Peak Window Size	<p>Enter a window width in data points for peak detection sensitivity. If more than one peak apex is within the window, all are labeled as a single peak. Note the following:</p> <ul style="list-style-type: none"> • The maximum value is the number of data points between peaks. • The Peak Window Size setting is limited to odd numbers. <p>To increase peak detection sensitivity: Increase polynomial degree, decrease peak window size.</p> <p>To decrease peak detection sensitivity: Decrease polynomial degree, increase peak window size.</p>
Slope Thresholds Peak Start and End	<ul style="list-style-type: none"> • Peak Start - The peak starts when the first derivative (slope of the tangent) in the beginning of the peak signal before the inflection point becomes equal to or exceeds the "Peak Start" value. This threshold is set to 0 by default, which means that the peak will normally start at the leftmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak start point toward its center. The value entered must be non-negative. • Peak End - The peak ends when the first derivative (slope of the tangent) in the end of the peak signal after the inflection point becomes equal to or exceeds the "Peak End" value. This value is set to 0 by default, which means that the peak will normally end at the rightmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak end point toward its center. The value entered in this field must be non-positive.

Table 11 Sizecalling Protocol – QC Settings

IMPORTANT! Normalization is not applied to samples with Size Quality flags. The 3500 Series Software 2 does not support re-analyzing data with new settings.

Table 12 Sizecalling protocol – QC settings

Setting	Description
Size Quality	<p>Enter the Pass Range and the Low Quality Range for the SQ flag displayed in View Fragment Results.</p> <p>Results that are within the Pass range are flagged as (Pass). Results that are within the Low Quality range are flagged as (Low Quality). Results that are between the Pass and Low Quality ranges are flagged (Check).</p> <p>For example, with a Pass Range of 0.75 to 1.0 and a Low Quality Range of 0.0 to 0.25, any result above 0.75 is , any result at 0.25 or lower is , and any result between 0.26 to 0.74 is .</p> <p>How Size Quality is determined</p> <p>The Size Quality algorithm evaluates the similarity between the fragment pattern for the size standard dye specified in the size standard definition and the actual distribution of size standard peaks in the sample, calculates an interim SQ (a value between 0 and 1).</p>
Assume Linearity	Defines the expected linear range. Useful in large fragment size standards where non-linearity might be expected.
Pull-Up	<p>Enter the pull-up ratio and tolerance for pull-up peak identification.</p> <p>A pull-up peak is identified when the peak height of the minor peak is:</p> <ul style="list-style-type: none"> • ≤ X% (pull-up ratio) of the major peak <i>and</i> • Within ±Y data point (pull-up scan) of the major peak <p>When at least one peak is identified as a pull-up peak, the (Check) flag is displayed for the Spectral Pull-Up quality flag in View Fragment Results.</p>

QC protocols library (primary analysis – HID)¹

QC protocol overview

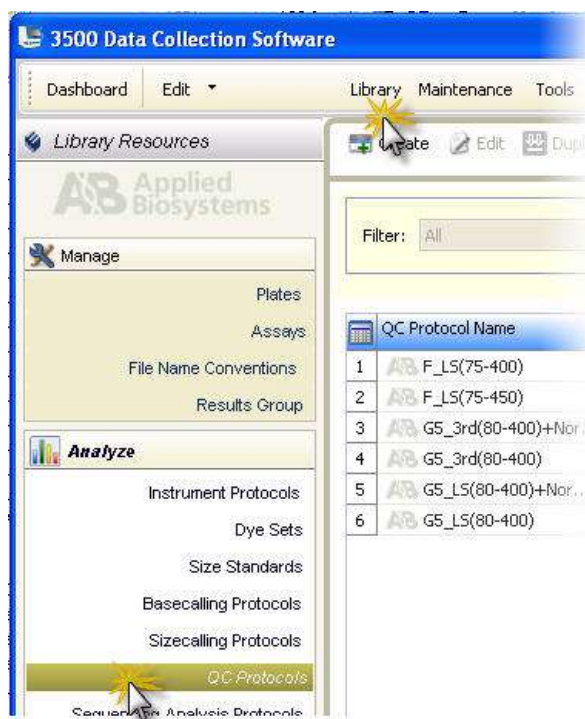
A QC protocol is the required primary analysis protocol for HID applications. A QC protocol defines peak detection, sizing, and quality values.


When you create an HID assay, you add a QC protocol to the assay. If you add this item from the library, a *copy* of the item is added to the assay, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see “Audit action” on page 203.

Create a new QC protocol

If factory-provided QC protocols do not suit your needs, you can create new QC protocols:

1. Access the QC Protocols library.



2. Click  **Create**.
3. In the Analysis Settings tab of the Create New QC Protocol dialog box (Figure 26 on page 186), specify settings (see Table 13 on page 186).
4. Click **QC Settings**. In the QC Settings tab of the Create New QC Protocol dialog box (Figure 14 on page 189), specify settings (Table 15 on page 190).
5. Click **Save**.

¹ HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).

Figure 26 Create New QC Protocol – Analysis Settings

IMPORTANT! The default values in the QC protocol templates (other than peak amplitude threshold values) have been optimized for each kit. You must optimize and validate peak amplitude threshold values during internal HID validation. If you modify other settings, ensure that the size standard is accurately detected and sized with the new settings. Normalization is not applied to samples with Size Quality flags. The 3500 Series Software 2 does not support re-analyzing data with new settings.

Table 13 QC protocol – Analysis settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Size standard	Size standard definition in the software that corresponds to the dye set used in the chemistry. To apply normalization, select a normalization size standard (see “Normalization size standards provided” on page 173).

Table 13 QC protocol – Analysis settings (*continued*)


Setting	Description
Analysis Range	<p>Select Full to collect data points for the entire scan region, including the primer peak. You can specify a limited analysis range in the GeneMapper® ID-X Software.</p> <p>Note: Note: If you select Partial, ensure that the Analysis Range contains all size standard fragments included in the Sizing Range specified below.</p>
Sizing Range	<p>Select Partial, then specify 80 to 400 to limit the fragment sizes evaluated for the size standard.</p> <p>If you specify sizes outside this range, the Sizing Quality may fail.</p>
Size Calling Method	<p>Select the method to determine the molecular length of unknown fragments appropriate for the AmpFSTR® kit you use. Refer to the user guide provided with the kit for information.</p> <ul style="list-style-type: none"> • Local Southern - (default) Determines the fragment sizes using the reciprocal relationship between fragment length and electrophoretic mobility. The unknown fragment is surrounded by two known-sized fragments above and one below, then two below and one above. The results are averaged and the size of the allele is determined. • 3rd Order Least Squares - Uses regression analysis to build a best-fit size calling curve. <p>Size calling options for kits other than those listed above are:</p> <ul style="list-style-type: none"> • 2nd Order Least Squares - Uses regression analysis to build a best-fit size calling curve. • Cubic Spline Interpolation - Forces the sizing curve through all the known points of the selected size standard. • Global Southern Method - Compensates for standard fragments with anomalous electrophoretic mobility (similar to least squares methods).
<p>IMPORTANT! If you modify peak detection settings, ensure that the size standard is accurately detected and sized with the new settings. Normalization is not applied to samples with  Size Quality flags. The 3500 Series Software 2 does not support re-analyzing data with new settings. For more information on peak detection parameters, see the GeneMapper® ID-X Software Reference Guide.</p>	
Smoothing	<p>Select an option to smooth the outline of peaks and reduce the number of false peaks detected:</p> <ul style="list-style-type: none"> • None to apply no smoothing. Best if the data display sharp, narrow peaks of interest. • Light (default) to provide the best results for typical data. Light smoothing slightly reduces peak height. • Heavy for data with very sharp, narrow peaks of interest. Heavy smoothing can significantly reduce peak height.
Baseline Window	<p>Specify a window to adjust the baseline signals of all detected dye colors to the same level for an improved comparison of relative signal intensity. Note the following:</p> <ul style="list-style-type: none"> • A small baseline window relative to the width of a cluster, or grouping of peaks spatially close to each other, can result in shorter peak heights. • Larger baseline windows relative to the peaks being detected can create an elevated baseline, resulting in peaks that are elevated or not resolved to the baseline.

Table 13 QC protocol – Analysis settings (*continued*)

Setting	Description
Peak Amplitude Thresholds	<p>IMPORTANT! Optimize these thresholds during internal HID validation.</p> <p>Specify the threshold (RFU) for peak detection for each dye color. Peaks below the threshold are not detected.</p> <p>For example, if you use the default values of 175, peaks with heights equal to or greater than 175 are detected. Peaks with heights below 175 are still displayed in the electropherogram plots but are not detected or labeled.</p> <p>Note: Ensure that the same peak amplitude thresholds are used in secondary analysis software such as GeneMapper® Software 5 (or later)</p>
Min. Peak Half Width	Specify the smallest half peak width at full height for peak detection. The range is 2 to 99 data points.
Polynomial Degree	<p>Adjust to affect the sensitivity of peak detection. You can adjust this parameter to detect a single base pair difference while minimizing the detection of shoulder effects and/or noise.</p> <p>The peak detector calculates the first derivative of a polynomial curve fitted to the data within a window that is centered on each data point in the analysis range.</p> <p>Using curves with larger polynomial degree values allows the curve to more closely approximate the signal and, therefore, captures more of the peak structure in the electropherogram.</p>
Peak Window Size	<p>Enter a window width in data points for peak detection sensitivity. If more than one peak apex is within the window, all are labeled as a single peak. Note the following:</p> <ul style="list-style-type: none"> • The maximum value is the number of data points between peaks. • The Peak Window Size setting is limited to odd numbers. <p>To increase peak detection sensitivity: Increase polynomial degree, decrease peak window size.</p> <p>To decrease peak detection sensitivity: Decrease polynomial degree, increase peak window size.</p>
Slope Thresholds Peak Start and End	<p>Not recommended for use with AmpFΦSTR® kit data.</p> <ul style="list-style-type: none"> • Peak Start - The peak starts when the first derivative (slope of the tangent) in the beginning of the peak signal before the inflection point becomes equal to or exceeds the “Peak Start” value. This threshold is set to 0 by default, which means that the peak will normally start at the leftmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak start point toward its center. The value entered must be non-negative. • Peak End - The peak ends when the first derivative (slope of the tangent) in the end of the peak signal after the inflection point becomes equal to or exceeds the “Peak End” value. This value is set to 0 by default, which means that the peak will normally end at the rightmost point where the slope of the tangent is closest to 0° (horizontal line). A value other than 0 moves the peak end point toward its center. The value entered in this field must be non-positive.

Setup a QC Protocol

Protocol Name is a required field. Provide a unique value.

* Protocol Name: ☐ Locked

Description:

Size Standard:

Sizecaller:

Analysis Settings **QC Settings**

Size Quality

Fail if Value is	Suspect Range	Pass if Value is
< 0.25	0.25 - 0.75	≥ 0.75

Broad Peak








Activate Broad Peak flag if value ≥

Close Save

Table 14 Create New QC Protocol – Analysis Settings

IMPORTANT! Normalization is not applied to samples with Size Quality flags. The 3500 Series Software 2 does not support re-analyzing data with new settings.

Table 15 QC Protocol – QC Settings

Setting	Description
Size Quality	<p>Enter the Pass Range and the Low Quality Range for the SQ flag displayed in View HID Results.</p> <p>Results that are within the Pass range are flagged as  (Pass). Results that are within the Low Quality range are flagged as  (Low Quality). Results that are between the Pass and Low Quality ranges are flagged  (Check).</p> <p>For example, with a Pass Range of 0.75 to 1.0 and a Low Quality Range of 0.0 to 0.25, any result above 0.75 is , any result at 0.25 or lower is , and any result between 0.26 to 0.74 is .</p>
Size Quality	<p>How Size Quality is determined</p> <p>The Size Quality algorithm evaluates the similarity between the fragment pattern for the size standard dye specified in the size standard definition and the actual distribution of size standard peaks in the sample, calculates an interim SQ (a value between 0 and 1).</p> <p>Weighting</p> <p>The Broad Peak (BD) threshold specified in the QC Protocol - QC Settings tab affects the SQ. To determine the final SQ value, the software:</p> <ul style="list-style-type: none"> Evaluates size standard peak widths in the sample in the dye color specified in the size standard definition. If the width of any size standard peak in the sizing range exceeds the broad peak threshold, applies a 0.5 weighting factor: Interim SQ × (1–0.5) <p>Note: The GeneMapper® <i>ID-X</i> Software allows you to set broad peak weighting. For more information, see the GeneMapper® <i>ID-X</i> Software Reference Guide.</p>
Broad Peak	<p>Enter the maximum peak width (in base pairs).</p> <p>When a peak width is greater than the threshold, the  (Check) flag is displayed for the BD (Broad Peak) quality flag in View HID Results.</p>

7

Use Security, Audit, and E-Sig Functions (SAE Module)

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Section 7.1 Administrators

Administrators overview of system security, auditing, and electronic signature

The Security, Audit, E-Signature module (SAE module) provides the following functionality:

- **System security** – Controls user access to the software. A default Administrator user account is provided, and additional user accounts and permissions can be user-defined.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the SAE settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. Provides reports for audited library items, SAE changes, and actions.

- **Electronic signature (e-sig)** – Determines if users are permitted, prompted, or required to provide a user name and password when performing certain functions. Can be configured so that a predefined list of functions can be performed only if the data used for the functions is signed (for example, you can run a plate only if the calibration data for the system has been signed. Can be configured to require multiple signatures and to require specific users or users with specific permissions to sign.

Example applications You can configure the SAE module in a variety of ways:

- Require users to log in, and leave audit and e-sig disabled.
- Allow only certain users to approve reviewed samples.

Configure the security system

Access the Security screen

The Security screen allows you to control restrictions and security policies for all user accounts, and set up notifications when certain security events occur.

To access the Security screen:



Figure 27 Security screen

Set account setup and security policies

Security policies apply to all user accounts.

1. Under Account Setup, specify user name limits.

Account Setup	User Password
User Name The length of user names must be between <input type="text" value="5"/> and <input type="text" value="32"/> characters. Define name spacing <input type="checkbox"/> Leading <input type="checkbox"/> Trailing <input type="checkbox"/> Consecutive Define name characteristics <input checked="" type="checkbox"/> Alpha <input checked="" type="checkbox"/> Numeric <input checked="" type="checkbox"/> Uppercase <input checked="" type="checkbox"/> Lowercase <input checked="" type="checkbox"/> Special	The length of user passwords must be between <input type="text" value="8"/> and <input type="text" value="32"/> characters. Define password spacing <input checked="" type="checkbox"/> Leading <input checked="" type="checkbox"/> Trailing <input checked="" type="checkbox"/> Consecutive Define password characteristics <input type="text" value="0"/> Alpha <input type="text" value="0"/> Numeric <input type="text" value="0"/> Uppercase <input type="text" value="0"/> Lowercase <input type="text" value="0"/> Special User may not reuse the previous <input type="text" value="3"/> passwords.

IMPORTANT! The software allows spaces in user names (Define name spacing). Use spaces in user names with caution. For information, see “Spaces in user names” on page 194.

2. Specify the *allowed* characters in user names: spaces and alpha, numeric, upper/lower case, and special characters (@, commas, periods, semicolons, dashes, underscores, and tildes).
3. Specify password limits.
4. Specify the *required* characters in passwords: spaces and alpha, numeric, upper/lower case, and special characters (any non-space, non-alpha, or non-numeric characters).
5. Specify password reuse. You cannot disable the password reuse restriction.
6. Under Security Policies, specify password expiration, account suspension, and session timeout settings.

Password Expiration	Account Suspension	Session Timeout
Passwords will expire <input checked="" type="radio"/> Yes <input type="radio"/> No every <input type="text" value="60"/> days. Notify user <input type="text" value="3"/> days before expiration.	Login attempts with an incorrect password will suspend the user account <input type="radio"/> Yes <input checked="" type="radio"/> No for the next <input type="text" value="24"/> Hours if consecutively failing <input type="text" value="3"/> time(s) within any <input type="text" value="60"/> minute	User sessions will be timed out if there is no user activity <input checked="" type="radio"/> Yes <input type="radio"/> No for <input type="text" value="60"/> minutes. (An instrument run is not considered user activity.)

Note: A session times out while a run is in progress if the timeout period is exceeded and there is no other user activity.

7. Click **Setup Messaging Notification** to specify when and how to notify the administrator of certain security events. For information, see “Set up messaging notifications” on page 194.
8. Click **Save Settings**.

The new settings are applied to the logged-in user the next time the user logs in.

Spaces in user names

If you allow spaces in user names, be aware of the following issues:

- Leading and trailing spaces in user names are difficult to detect on the screen or in printed reports.
- The number of consecutive spaces in a user name is difficult to determine on the screen or in printed reports.

Spaces in user names may cause confusion when searching for an audit or E-Sig record associated with a user name. To find a record associated with a user name, you must specify the user name exactly, including leading, consecutive, and trailing spaces.

Set up messaging notifications

1. In the Security screen (Figure 27 on page 192), click **Setup Messaging Notifications** to display the Setup Notifications dialog box.

	Event Name	Pop-up dialog	Message when Admin logs in
1	# of Failed Authentications over specified Time interval	<input type="checkbox"/>	<input type="checkbox"/>
2	Session Timeout For a User	<input type="checkbox"/>	<input type="checkbox"/>
3	Account Suspension For Failed Authentication	<input type="checkbox"/>	<input type="checkbox"/>
4	Notification For SAE Activation	<input type="checkbox"/>	<input type="checkbox"/>

2. Select the events for notification:
 - **Number (#) of failed authentications over specified time interval** – A user attempts to log in with an incorrect password. The message indicates the number of failed authentications.
 - **Session timeout for a user** – No activity occurred in a user account for the specified period of inactivity.
 - **Account suspension for failed authentication** – The user exceeds maximum number of allowed failed authentications (login attempts with an incorrect password).
 - **Notification for SAE activation** – Security has been enabled or disabled.

3. Select the notification method:

- **Pop-up dialog** – The software immediately displays a pop-up message to the current user if an event is triggered by the current user. The message instructs the user to inform a system administrator of the event.
- **Message when Admin logs in** – If an event triggers notification, the next time any user with an Administrator role logs in, the software displays a list those events, indicating the time each event occurred and the user who triggered the event.

The Administrator has the option of acknowledging the event, which removes it from the notification list.

4. Click OK.

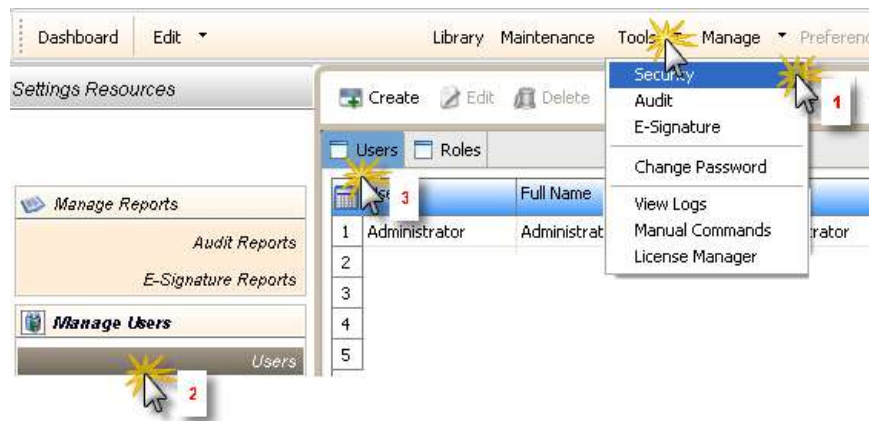
Manage user accounts

Create or edit a user account

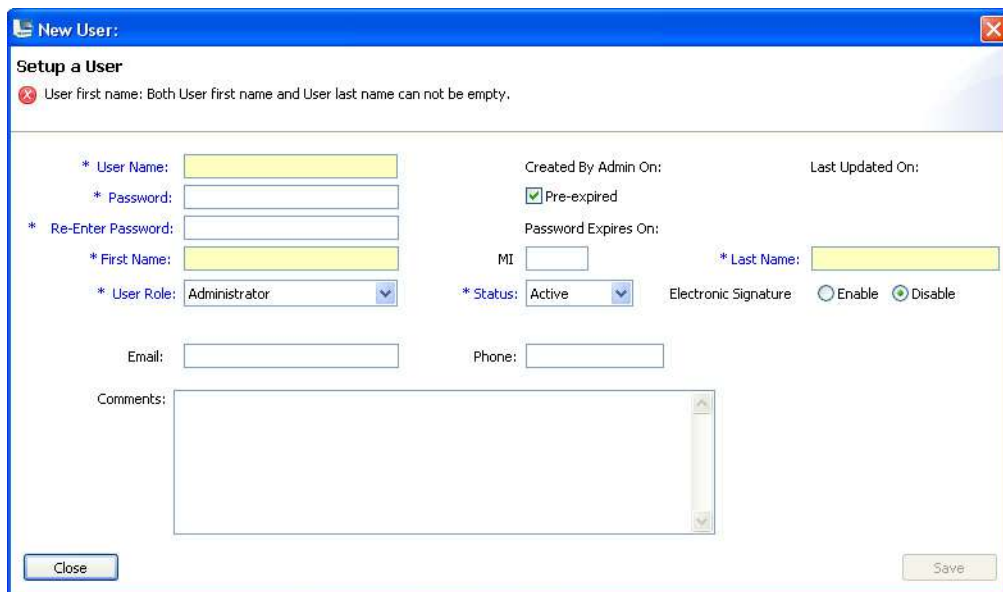
The software includes a default Administrator user account with permissions (defined by the account user role) to perform all functions in the software.

Create a user account

1. Access the Users screen.



2. Click  **Create** to display the New User dialog box.



New User:

Setup a User

⚠ User first name: Both User first name and User last name can not be empty.

* User Name:

* Password:

* Re-Enter Password:

* First Name:

* User Role:

Email:

Comments:

Created By Admin On:

☒ Pre-expired

Password Expires On:

MI:

* Last Name:

* Status:

Electronic Signature: ☐ Enable ☒ Disable

Phone:

Close Save

3. Enter User Name, Password, First Name, MI (middle initial – optional) and Last Name. Click a field to display the field limits, which are specified in Security settings.

Note: First Name, MI (middle initial), and Last Name are used to create User Full Name, which is displayed as the name of the logged-in user.

Note: You cannot change the User Name after you save the user account.


4. Select **Pre-expired** to require the user account to specify a new password at first log in. The Password Expires On date is specified in Security settings.
5. Select the user role (described in “Create or edit a user role” on page 197) and the electronic signature state (determines if a user account has permission to electronically sign objects). Leave the status set to Active.
6. (Optional) Enter email (for information only), phone, and comments.
7. Click **Save**.

If the Save button is grayed, it indicates an invalid entry in a field. Click a field to display the limits for the field, then enter a valid entry.

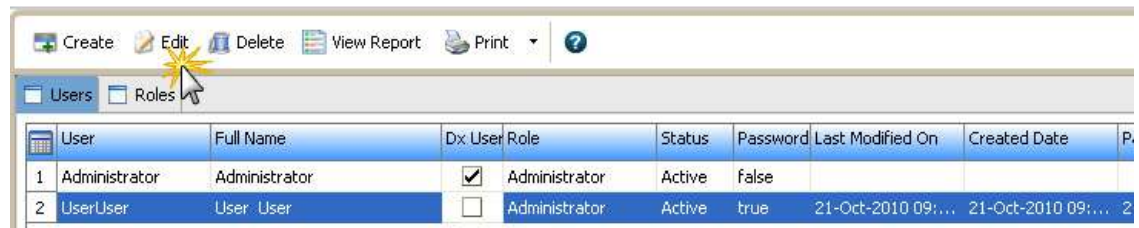
The Users screen displays the following information for each user account:

- User
- Full Name
- Role
- Status
- Password Expired (true=yes, false=no)
- Last Modified On
- Created Date
- Password Change Date (by either user or administrator)
- Email (for records only)
- Phone
- Comments

Edit a user account


1. In the Users screen, select a user account, then click  **Edit**.

Note: If you select multiple users, only Status and Role will be changed.




2. Edit settings as needed. You cannot edit the user name of an existing user.
3. Click **Save**.

Activate a suspended user account

1. Select the user.
2. Click  **Edit**.
3. Change the status from Suspended to Active.

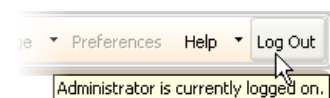
Delete (inactivate) a user account

You cannot delete a user because user records are required for auditing. To disable a user account, inactivate it.

1. Select the user.
2. Click  **Edit**.
3. Change the status from active to inactive.
4. Click **Save**.

Determine the name of the logged-in user

To display the full name of the logged-in user, place the mouse pointer on the Logout menu. The full name of the logged-in user is also displayed in the Load Plates for Run screen and the Monitor Run screen.




Create or edit a user role

User roles determine the permissions associated with a user account.

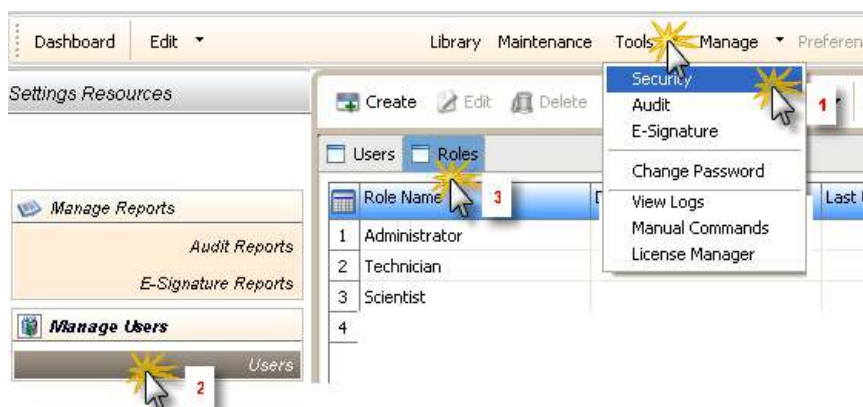
Three default user roles are included in the software. You can modify two of them, and can create your own roles with customized settings as needed:

- Administrator (cannot be edited or deleted)
- Scientist
- Technician

To determine the permissions for these roles or to edit these roles, select the role, then click  **Edit**.

Create a user role

1. Access the Roles screen.



2. Click **Create**.
3. Enter a role name and (optional) comment.
4. Select permissions (see Table 16 on page 198). To select all permissions in a category, select the checkbox next to the category.
5. Click **Save Role**.


Table 16 User role permissions

Category	Permissions
Setup	Create plate/plate template
Run	<ul style="list-style-type: none"> Edit default instrument name Manage injection list Duplicate injection Re-inject
Primary Analysis	<ul style="list-style-type: none"> Edit sample (names) Export sequencing results
<ul style="list-style-type: none"> Assay File name convention Results group Instrument protocol PA protocol SA protocol QC protocol Size standard Dye sets 	<ul style="list-style-type: none"> Create Edit Delete Import Export
Plates and templates	<ul style="list-style-type: none"> Edit Delete Import Export



Table 16 User role permissions *(continued)*

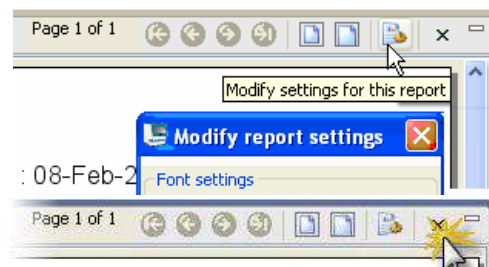
Category	Permissions
Locking/Unlocking	<ul style="list-style-type: none"> Assays File name convention Results group Instrument protocols PA protocols Size standards Dye sets
Preferences	<ul style="list-style-type: none"> Edit system preferences Export system preferences Import system preferences Export user preferences (all)
Calibrations	<ul style="list-style-type: none"> Perform spatial calibration Perform spectral calibration
Performance check	Run performance check install standards
Archiving	<ul style="list-style-type: none"> Archive Purge Restore
SAE configuration	<ul style="list-style-type: none"> Configure SAE Log in to timed-out user sessions

Edit a user role

1. In the Roles screen, select a user role, then click  **Edit**.
2. Edit settings as needed. You cannot edit the Administrator user role.
3. Click **Save**.

View and print a user report

1. Select the **User** or **Roles** tab. Click  **View Report**.
2. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.
3. To print the report, click  **Print**.
Close the report.



Save electronic copies (.pdf) of the report

To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.

Manage auditing


Access the Audit screen and enable or disable auditing

The Audit screen controls the auditing state (enabled/disabled), the events that are audited, and the reasons available to users when audit mode is set to Prompt or Required.

Auditing is enabled by default.

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions. No audit record is generated for the inactivation of audit and electronic signature functions when you disable security.

1. Access the Audit screen.
2. Click **Disable Audit** or **Enable Audit** (Figure 28 on page 200).

Note: When auditing is disabled, the  is not active in lower parts of the screen.

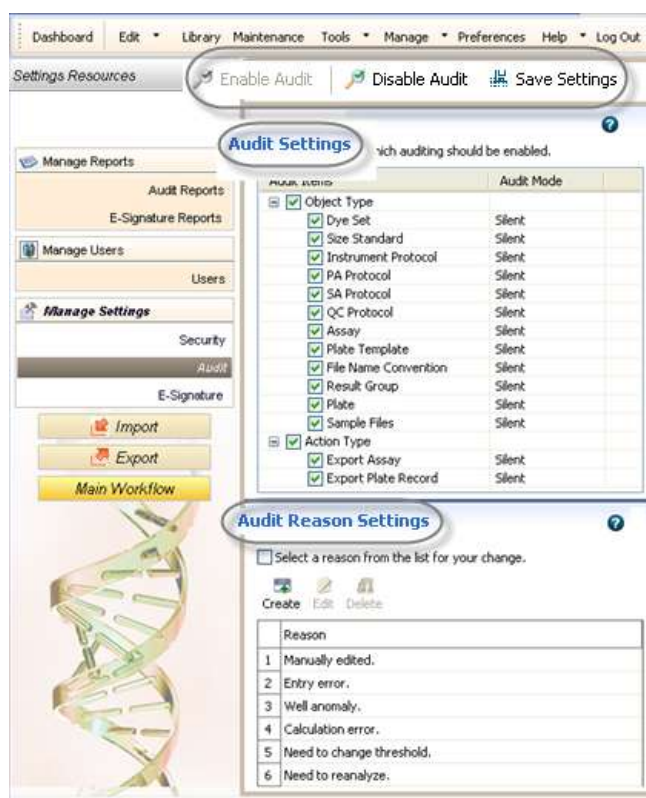


Figure 28 Audit – disable or enable

Select objects to audit

1. Select the objects and actions to audit and the mode for each enabled item.




Object Type (audit records displayed in Object Audit History)	Action Type (audit records displayed in Action Log)
<ul style="list-style-type: none"> • Dye set • Size standard • Instrument protocol • PA protocol (primary analysis) • Assay • Plate template • File name convention • Results group • Plate • Sample files 	<ul style="list-style-type: none"> • Export assay • Export plate record

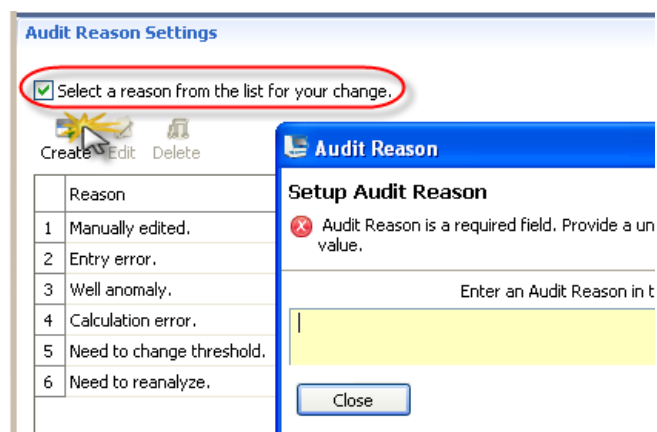
Note: For a list of items that the system audits silently in addition to the configurable items listed above, see “Generate audit reports” on page 202.

2. Set the Audit Mode for each item you enable for auditing:
 - **Prompt** – The event is audited, a reason prompt is displayed, but the user can cancel and continue without entering a reason.
 - **Required** – The event is audited, a reason prompt is displayed, and the user must specify a reason.
 - **Silent** – The event is audited, no reason prompt is displayed.
3. Click **Save Settings**.

Create audit reason settings

You can create, modify and delete the reasons that are available for selection in the Audit Reason dialog box (displayed when a user performs an audited action).

1. To require users to select a pre-defined reason in the Audit Reason dialog box (displayed when a user performs an audited action), enable the **Select a reason from the list for your change** checkbox. Users are not permitted to enter a reason.
2. As needed, click  **Create**, or select a reason, then click  **Edit** or  **Delete**.



Generate audit reports

Display audit histories

1. Access the Audit Reports screen.

Note: To access the Audit Reports screen, the user role for an account must specify the Configure SAE permission. Users without the Configure SAE permission can view object audit histories for individual entries in the libraries by selecting entries, then clicking View Audit History (see “View audit and e-signature histories for library entries” on page 146).



2. Select a tab to display:

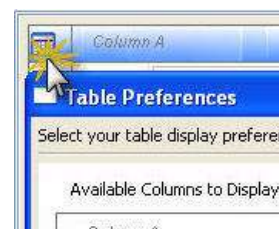
- **Object Audit History** – The most recent audit for all user objects (samples and objects in the Library) that have been audited.
- **System Configuration History** – SAE configuration records, including audit history for each user account.
- **Action log** – System-specified audit events.

3. (Optional):

- Sort the table. See “Multi-column sorting” on page 79.
- Specify filters (date range, user name, action, object or record type, object or record name, reason), then click **Go**.

Note: The Reason field in System Configuration History is not used.

- Select a record, then click **Show Object History** or **Show Audit Details**.
- In the history dialog box, select a record, then click **Show Audit Details**.
- Click **Table Settings**, then specify the columns to show or hide.



Review the object audit history

Audit records

The Object Audit History lists the most recent audit for the user objects listed below (samples and objects in the libraries) that have been audited.

- | | |
|----------------------------------|------------------------|
| • Dye set | • Assay |
| • Size standard | • Plate template |
| • Instrument protocol | • File name convention |
| • PA protocol (primary analysis) | • Results group |
| | • Plate |
| | • Sample files |

Audit action

Possible actions for all objects are update, create, and delete. Audit records are generated under the following conditions:

Action	Description
Update	<p>The auditing of updates depends on whether an object is modified or overwritten:</p> <ul style="list-style-type: none"> • Modified – A record is created when an object is modified. • Updated – A record is not created when an object is overwritten in the library. <p>Example: You create a plate, then create a results group from within the plate and save it to the library. You then open the plate, edit the results group from within the plate, then save it to the library. A message indicates that the results group already exists and asks if you want to overwrite it. You click Yes. This action is considered a creation of a new results group, not a modification of the existing results group. No Update record is created; a Create record is created.</p>
Create	<p>A record is created when you:</p> <ul style="list-style-type: none"> • Create an item in the library. • Create an item from within another item. • Modify an item from within another item, then overwrite the item in the library when you save it (as described in the “Updated” bullet above). <p>Note: An audit record is not created when a sample file is generated. However, an audit record is generated when a sample is renamed.</p>
Delete	<p>The auditing of deletions depends on the item deleted:</p> <ul style="list-style-type: none"> • Items in the library – A record is retained until it is deleted from the library. The deletion of the item from the library is not audited. For example, if you <i>delete</i> a size standard from the library, no audit record for the deletion is listed in the Object Audit Detail History. • Items within other items – The deletion of an item from within another item is audited.

Display the object history

To display the history for an object, select the object, then click **Show Object History**.

The object history shows the audit history for the object and for all objects contained in the selected object. For example, when you create an assay, a copy of the instrument protocol and the primary analysis protocol (and therefore dye set, and size standard) are included in the assay object. The objects contained within an object have audit histories distinct from the audit history of the objects stored in the Library.

Review the system configuration history

The System Configuration History lists SAE configuration records.

Note: The Reason field in System Configuration History is not used.

Table 17 Audit – system configuration history

Record Type	Action	Corresponds To
Security settings	Update	<ul style="list-style-type: none"> • Enable security • Disable security • Modify security policies: <ul style="list-style-type: none"> – Session timeout settings
Account settings	Update	<ul style="list-style-type: none"> • Modify user name settings • Modify password settings • Modify security policies: <ul style="list-style-type: none"> – Password expiration – Account suspension
Audit reason for change	Update	Modify reason for change
	Create	Create reason for change
	Delete	Delete reason for change
Audit settings	Update	<ul style="list-style-type: none"> • Enable auditing • Disable auditing
Audit type	Update	Modify audit settings
E-Signature function	Update	<ul style="list-style-type: none"> • Modify the authorities for a “prompt before” function • Modify the Enable state of either a “check after” or “prompt before” function
E-Signature settings	Update	<ul style="list-style-type: none"> • Enable e-signature • Disable e-signature
E-Signature type	Update	<ul style="list-style-type: none"> • Modify e-signature settings • Modify the enable state of an E-Signature Type
Role assignment	Create	<ul style="list-style-type: none"> • Create a new user account • Assign a different user role to an existing user account
	Delete	Assign a different user role to an existing user account
Role permissions	Update	Modify user role permissions
	Create	Create a user role - creates one role assignment record for each permission in a role
	Delete	Delete a user role - creates one role delete record for each permission in the deleted role
User account	Update	<ul style="list-style-type: none"> • Edit • Suspend
	Create	Create new user account
User role	Update	Modify user role
	Create	Create user role
	Delete	Delete user role

Review the action log

The action log lists system-specified audit events.

All items in the action log are audited silently, except for the items noted as configurable. Configurable items may include comments in the action log.

Table 18 Audit – action log

Category	Action
Assay	Assay exported successfully
Log In	<ul style="list-style-type: none"> User logged in Login failed User logged out
Maintenance Wizards	<ul style="list-style-type: none"> Remove Bubbles Wizard started Change Polymer Type Wizard started Change Array Wizard started Replenish Polymer Wizard started Fill Polymer Wizard started Water Wash Wizard started Instrument Shutdown Wizard started
Plate	Plate exported successfully
Run	<ul style="list-style-type: none"> Start Pause Resume Stop (Abort injection) Terminate (injection list)
SAE Configuration	<ul style="list-style-type: none"> Export
System Audit Records	<ul style="list-style-type: none"> Archive Purge Restore
System Action Records	<ul style="list-style-type: none"> Archive Purge Restore
User Profile	<ul style="list-style-type: none"> Export

View and print audit reports

1. Display the records of interest as described above.
2. Filter the list to decrease the time required to generate reports.

IMPORTANT! You cannot cancel a report after you click a view button.

3. Click  **View Audit Summary Report** or  **View Audit Detailed Report**.

System Configuration History Summary Report

#	Date	User Name	User Full Name	Record Type	Record Name	Action
1	28-Jan-2009 05:01:08 PM	Administrator	Administrator	Security Settings		Update
2	28-Jan-2009 05:00:57 PM	Administrator	Administrator	Security Settings		Update

System Configuration History Detailed Report

1 **Date :** 28-Jan-2009 05:01:08 PM **Action :** Update
User Name : Administrator **User Full Name :** Administrator
Record Type : Security Settings **Record Name :**

#	Record Type	Object Name	Old Value	Current Value	Action
1	Security Settings	Security On / Security Off	DISABLED	ENABLED	Update

2 **Date :** 28-Jan-2009 05:00:57 PM **Action :** Update
User Name : Administrator **User Full Name :** Administrator
Record Type : Security Settings **Record Name :**

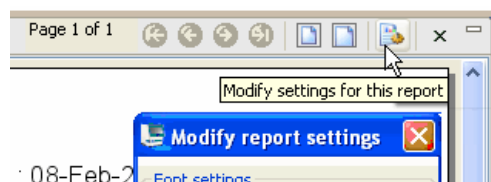
#	Record Type	Object Name	Old Value	Current Value	Action
1	Security Settings	Security On / Security Off	ENABLED	DISABLED	Update

4. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.

5. To print the report, click  **Print**.

6. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.

7. Close the report.





Archive, purge, and restore audit records


The audit archive function makes a copy of audit records. Purge makes a copy of audit records, and then deletes them. You can use the Restore function to restore purged audit records.

Archive and purge

To selectively archive or purge (delete) system configuration or action audit records:


1. Select records in the appropriate screen.
2. Click  **Archive Audit Records** or  **Purge Audit Records**.
3. If you select Archive:
 - Specify a location and name for the .asz archive file
 - (Optional) Click **Yes** to Purge (delete) the records after archive.

Restore

To restore system configuration or action audit records, click  **Restore**, then select the .asz file to restore.

Export audit records

As needed, you can export audit records to a .txt file for additional manipulation and reporting outside the 3500 Series Software 2.

1. Display the records of interest as described above.
2. Select the records to export.
3. Click  **Export Audit Records**.
4. Specify a name and location for the export .txt file.
5. Click **Save**.

Note: If you export audit records for samples that are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

Manage electronic signature

Access the E-Signature Settings screen and enable or disable e-sig

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions. No audit record is generated for the disabling of audit and electronic signature functions when you disable security.

1. Access the E-Signature Settings screen.
2. Click **Disable E-Sig** or **Enable E-Sig** (Figure 29 on page 208).





Figure 29 E-Sig – disable or enable

Select the actions that allow signature

IMPORTANT! Do not change electronic signature settings during a spectral calibration.

1. Select the checkbox next to an item in the E-Signature Type list to identify events for which to allow electronic signature (see Table 19 on page 209). This selection activates the E-Sig button for the selected items; it does require an electronic signature for these selections.
2. (Optional) For each item that you select, optionally:
 - a. From the top-right of the screen, select a function *after which* the system will prompt for electronic signature. This selection presents an e-sig prompt to users when they perform a function. Users can sign or can continue without signing.
 - b. From the bottom-right of the screen, select a function (start run) before which the system will check for required electronic signatures (see Table 20 on page 210). This selection presents an e-sig prompt to users when they start a run if the required signatures have not previously been made. Users must sign before they can continue. For “check before” functions, you can also:
 - Change the number of signatures required.

- Set a special authority for a signature: click the Authorities Required field, then select the user account or the user role to require for electronic signature of this function. By default, each required signature needs no special authority; any user can sign.
- Click **Apply**.

E-Signature Settings
Select the electronic signature types that should be allowed.

E-Signature Type

- ☒ Approve Dye Set
- ☐ Approve Size Standard
- ☒ Approve Spatial Calibration
- ☐ Approve Spectral Calibration
- ☐ Approve Instrument Protocol
- ☐ Approve Sizecall Protocol
- ☐ Approve Basecall Protocol
- ☒ Approve QC Protocol
- ☒ Approve Gene Mapper Protocol
- ☒ Approve Gene Mapper IDx Protocol
- ☒ Approve SeqScape Protocol
- ☒ Approve MicroSeq ID Protocol
- ☒ Approve Assay
- ☒ Approve Plate Template
- ☒ Approve Plate
- ☒ Approve Sample
- ☒ Approve Sequencing Install Standard Results
- ☒ Approve MicroSeqID Install Standard Results
- ☒ Approve BDTv1.1POP6 Install Standard Results
- ☒ Approve Fragment Install Standard Results
- ☒ Approve HID Install Standard Results

Select the functions after which the system will prompt for an electronic signature of the selected type

Function

- ☐ Accept Spatial Calibration

Select the functions before which the system will check for required electronic signatures

Function	# Signatures Required	Authorities Required
Start Run	1	Any

Select the minimum signatures that must exist for data to qualify as being signed.

Function Name: Run.Start

Signatures Required: 1

Authorities Required: Any

Apply Cancel

3. Click **Save Settings**.

By default, no E-Signature types are enabled.

Table 19 E-signature settings to prompt after

E-Signature Type	Function to Prompt After
Approve Dye Set	Save
Approve Size Standard	Save
Approve Spatial Calibration	Accept
Approve Spectral Calibration	Accept
Approve Instrument Protocol	Save
Approve Sizecall Protocol	Save
Approve Basecall Protocol	Save
Approve QC Protocol	Save
Approve Size Standard	Save
Approve GeneMapper Protocol	Not used
Approve GeneMapper IDX Protocol	Not used
Approve SeqScape Protocol	Not used
Approve MicroSeq ID Protocol	Not used

Table 19 E-signature settings to prompt after *(continued)*

E-Signature Type	Function to Prompt After
Approve Assay	Save
Approve Plate Template	Save
Approve Plate	Save
Approve Sample	Save
Approve Sequencing Install Standard Results	Accept
Approve MicroSeq ID Install Standard Results	Accept
Approve BDTv1.1 POP6 Install Standard Results	Accept
Approve Fragment Install Standard Results	Accept
Approve HID Install Standard Results	Accept

Table 20 E-signature settings to check before

E-Signature Type	Function to Check Before	Signatures and Authorities Required (defaults if enabled)
Approve Spatial Calibration	Start Run	1 signature, any authorities (any user, any user role)
Approve Spectral Calibration		
Approve Plate		
Approve Sequencing Install Standard Results		
Approve MicroSeq ID Install Standard Results		
Approve BDTv1.1 POP6 Install Standard Results		
Approve Fragment Install Standard Results		
Approve HID Install Standard Results		

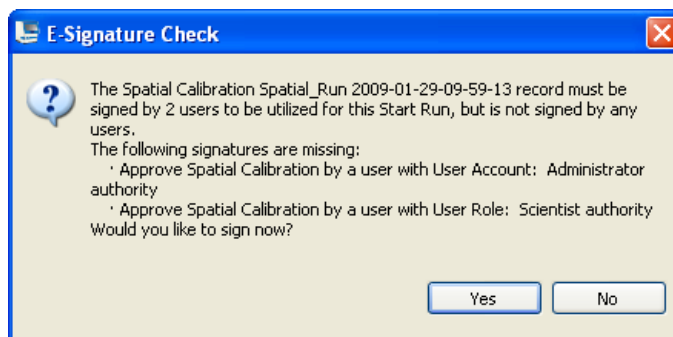
How the software prompts electronic signature before a run

If the system is configured to check that data is signed before starting a run and the data for the run is not signed, a message is displayed when the user clicks **Start Run**.

Example

The e-sig system is configured to require signatures from two users (one from the user account named Administrator, and the other from any user account with a scientist user role) for a spatial calibration before it can be used in a run. The spatial calibration has not been signed.

A user starts a run. The following message is displayed:



Before the run can start, the following users must sign:

- The Administrator user
- Any other user with the Scientist role specified and electronic signature enabled in their user account

If a user that does not meet the specified criteria signs, a message is displayed to indicate which users have e-signed.

Generate e-signature reports

Display e-signature records

1. Access the E-Signature Reports screen.
2. (Optional):
 - Specify filters (date range, user name, object type, object name), then click **Go**.
 - Select a record, then click **Show Object History**.
 - In the history dialog box, select a record, then click **Show E-Signature Details**.
 - Double-click column headers to sort. Multi-column sorting is supported (see "Multi-column sorting" on page 79).
 - Customize the table (see "Customize tables" on page 79).



3. The records that are displayed (if they are specified in E-Signature settings) are:

- Approve Dye Set
- Approve Size Standard
- Approve Spatial Calibration
- Approve Spectral Calibration
- Approve Instrument Protocol
- Approve Sizecall Protocol
- Approve Basecall Protocol
- Approve Qc Protocol
- Approve Assay
- Approve Plate Template
- Approve Plate
- Approve Sample
- Approve BDTv1.1POP6 Install Standard Results
- Approve Sequencing Install Standard Results
- Approve Microseq ID Install Standard Results
- Approve Fragment Install Standard Results
- Approve HID Install Standard Results

View and print e-signature reports

1. Display the records of interest as described above.

Note: Filter the list to decrease the time required to generate reports.

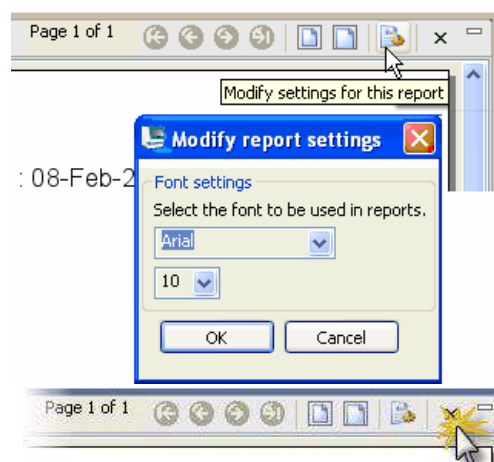
2. Click  **View E-Sig Summary Report** or  **View E-Sig Detailed Report**.

3. In the Report screen, click toolbar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.

4. To print the report, click  **Print**.

5. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.

6. Close the report.



Export e-sig records

As needed, you can export e-sig records to a .txt file for additional manipulation and reporting outside the 3500 Series Software 2.

1. Display the records of interest as described above.

2. Select the records.


3. Click  **Export E-Sig Records**.

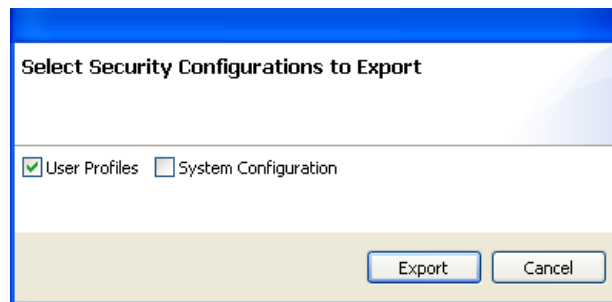
4. Specify a name and location for the export .txt file.

5. Click **Save**.

Export and import user accounts, security, audit, and electronic signature settings


Export

1. In any screen in the SAE module, click  **Export** in the navigation pane.
2. Select the items to export:

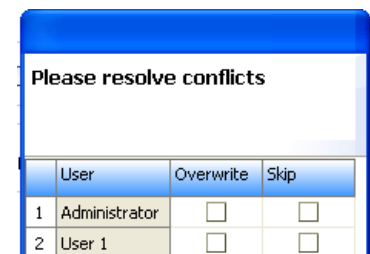


- **User Profiles** – Contains all settings in the following screens:
 - **Edit User** – All user accounts with Active status
 - **User Role** – All user roles and associated permissions (in case a user account specifies a user role that does not exist on the system into which you import the profiles)
 - **System Configuration** – Contains all settings in the following screens:
 - **Security** – Account setup and security policies
 - **Audit** – Objects selected for auditing, audit modes, and reasons
 - **E-Signature Settings** – Objects selected for E-Signature, functions, number of signatures, and authorities
 - **User Roles** – All user roles and associated permissions
3. Click **Export**.
 4. Specify the name and location for the exported .dat file, then click **Save**.
A message is displayed when the export completes.

Import

1. In any screen in the SAE module, click  **Import** in the navigation pane.
2. Select the .dat file to import, then click **Open**.
A message is displayed asking if you want to overwrite the current system configuration. Click **Yes**.

If any imported user accounts already exist on the system, you are prompted to overwrite or skip each account.



Section 7.2 Users

Users overview of System Security, Audit Trail and E-Signature

The Security, Audit, E-Signature (SAE) module provides the following functionality:

- **System security** – Controls user access to the software.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the SAE settings.
- **Electronic signature (e-sig)** – Requires users to provide a user name and password when performing certain functions.

Depending on the way that your administrator configures these features, you may see the following dialog boxes and prompts when you use the software.

Security

Log in

If security is enabled on your system, you must provide a user name and password to access the software.

Your access to functions in the software is based on the permissions associated with your user account. Functions for which you do not have permissions are grayed out.



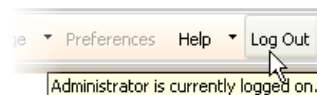
If your system is configured for password expiration, you will periodically be prompted to change your password. If your system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password for a specified number of times.

Permissions

If your user account does not have permission to perform any function in the software, menu commands are grayed.

Determine the name of the logged-in user

To display the full name of the logged-in user, place the mouse pointer on the Logout menu. The full name of the logged-in user is also displayed in the Load Plates for Run screen and the Monitor Run screen.



Change your password when it expires

When your password is about to expire, a message is displayed when you log in.

To change your password, select **Tools ▶ Change Password**.

Enter your current password, then enter the new password two times, then click **OK**.



Account suspension

If your system is configured to suspend a user account for failed logins, and you enter an incorrect user name and password for more than the allowed number of times, your user account is suspended, and the Log In dialog box indicates that your account is inactive.

There are two ways to activate a suspended account:

- You can wait until the suspension period ends.
- An administrator can change the account status from Suspended to Active.

Note: While a user is suspended, another user can click **Reset**, then log in and replace the suspended user.



Session timeout

If your system is configured to timeout and there is no user activity for the specified time, the Log In dialog box indicates that your user session has timed out. You must enter your user name and password to access the software.

Note: The administrator or another user with permission to log in to timed-out sessions can click **Reset**, then log in.



Audit

If your system is configured for auditing, you may be prompted to specify a reason when you make certain changes in the software.

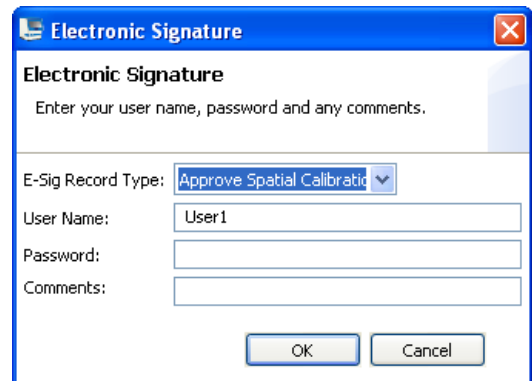
Based on your system configuration, you can either select a reason or enter a reason for change.




Electronic signature

If your system is configured for electronic signature, you may be prompted to provide your user name and password when you perform certain actions in the software.

If an item is set to require two signatures, the signers are not required to sign at the same time. When the first signer signs, the E-Sig status is set to Partially Signed. When the second signer signs, the E-Sig status is set to Signed.



You may also be permitted to sign objects such as plates, calibrations, or other library items. If electronic signature is enabled for items, any of the following may apply:

- The  E-Signature button is enabled in the library or the calibration.
- You are prompted to sign as described in “How the software prompts electronic signature before a run” on page 210.

- The Open Plates dialog box or the library displays an “Is signed” column that reflects the electronic signature status of an item.

Open Plate From Library

Instructions
Select row from table and click on "Open" button.

Filter: Search: All

	Plate Name	Type	Date Modified	Is Signed
1	Plate 01	HID	12-Apr-2009 05:13:13 PM	No
2	test well attributes	HID	13-Apr-2009 09:58:39 AM	No

8

Maintain the Instrument

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Maintenance schedule



WARNING! This section lists the common tasks required to maintain your instrument in good working condition. Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids used on this instrument, or parts that may come into contact with these fluids.

IMPORTANT! Use only the cleaning agents listed in this guide. Use of cleaning agents other than those listed in this guide may damage the instrument.

Review maintenance notifications

Review maintenance notifications list in the Dashboard daily, then perform the scheduled tasks.

Maintenance Notifications				
Name	Priority	Notification Date	Description	Action
Flush Pump Trap	HIGH	23-Aug-2010 12:00:0...	Flush Pump Trap	✓ ✕
Clean the anode buffer cup pin-valve...	HIGH	06-Sep-2010 12:00:0...	Clean the anode buffer...	✓ ✕
Replace cathode buffer container septa	HIGH	23-Aug-2010 12:00:0...	Replace cathode buffer...	✓ ✕
Clean Drip Tray	HIGH	23-Aug-2010 12:00:0...	Clean Drip Tray	✓ ✕
Check Disk Space	HIGH	23-Aug-2010 12:00:0...	Check Computer for Dis...	✓ ✕

When you complete a task, click to mark it as complete, click to mark it as dismissed.

Note: Completed and dismissed tasks are removed from the Maintenance Notification section, and they do not appear again unless they are repeating tasks. Dismissed tasks can be logged in the Notifications Log.

All actions are recorded in the Notification Log. See “Review the Maintenance Notifications Log” on page 224.

Daily instrument maintenance tasks

Clean the assemblies, anode buffer container, and cathode buffer container, and ensure that the outside of the assemblies is dry.

IMPORTANT! Use only the cleaning agents listed in this guide. Use of cleaning agents not listed in this manual can impair instrument function.

Task	Frequency	For information, see...
Click Refresh , then check consumables on the Dashboard – Refer to the gauges on the Dashboard to see the status for anode buffer container, cathode buffer container, and polymer.	Before each run	“Check consumables on the Dashboard” on page 224
Visually inspect the level of fluid inside the anode buffer container and the cathode buffer container. The fluid must line up with the fill line.		“Change the anode buffer container (ABC)” on page 225
Ensure that the plate assemblies are properly assembled. Align the holes in the plate retainer with the holes in the septa to avoid damaging capillary tips.		“Prepare the plate assembly” on page 61
Ensure that the plate assemblies and the cathode buffer container are positioned on the plate deck properly. They should sit securely on the deck.		“Load the plate in the instrument” on page 63
Ensure the array locking lever on the capillary array is secured.		Figure 30 on page 256
Check for bubbles in the pump block and channels. Use the Remove Bubble wizard to remove bubbles.	Daily or before each run	“Remove bubbles from the polymer pump” on page 238
Check the loading-end header to ensure that the capillary tips are not crushed or damaged.		“Change the capillary array” on page 238
Ensure that the pump block is in pushed back position.	Daily	Figure 31 on page 256.
Clean the instrument surfaces of dried residue, spilled buffer, or dirt.		“Routine instrument cleaning” on page 230
Check for leaks and dried residue around the Buffer-Pin Valve, check valve, and array locking lever. If leaks persist, contact Life Technologies.		“Check maintenance notifications” on page 38

Weekly instrument maintenance tasks

Task	Frequency	For information, see...
Check the storage conditions of the used arrays to ensure the array tip is covered in the reservoir.	Weekly	"Check stored capillary arrays" on page 228
Run the Wash Pump and Channels wizard.		"Wash the pump chamber and channels" on page 236
Use a lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.		Chapter 1, Instrument and Software Description
Restart the computer and instrument.		"Reset the instrument" on page 282.

Monthly instrument maintenance tasks

Task	Frequency	For information, see...
Flush the pump trap	Monthly or as needed	"Flush the water trap (pump trap)" on page 229
Empty the condensation container and the water trap waste container. The waste container is to the right of the pump block.		Chapter 1, Instrument and Software Description
Replace cathode buffer container septa.		"Change the cathode buffer container (CBC)" on page 226
Clean the autosampler		"Routine instrument cleaning" on page 230
Clean the drip tray		
Check disk space		"Monitor disk space" on page 242
Defragment the hard drive	Monthly Before fragmentation reaches 10%.	"Defragment the computer hard drive" on page 242

Quarterly maintenance tasks

Task	Frequency	For information, see...
Run performance check	Every three months	Chapter 5, Calibrate and Check Performance

Annual planned maintenance tasks

Call your Life Technologies representative to schedule annual planned maintenance.

As-Needed instrument maintenance tasks

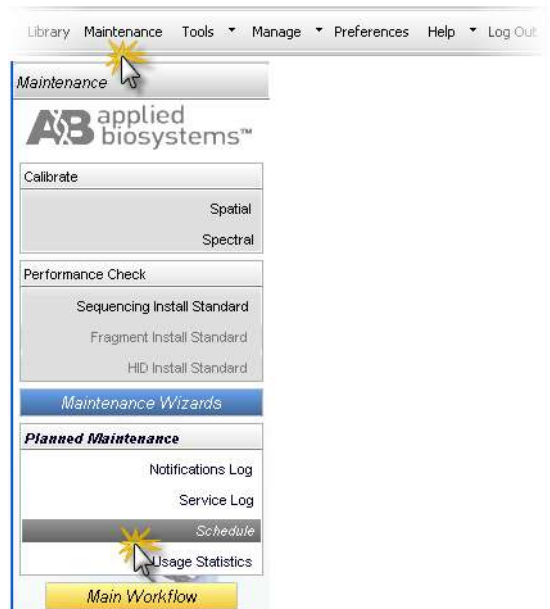
Task	Frequency	For information, see...
Change the tray.	As needed	"Routine instrument cleaning" on page 230
Remove dried polymer from the capillary tips with a lint-free wipe moistened with deionized water.		
Archive and purge library objects Dashboard ► Manage ► Archive or Purge		Chapter 6, Manage Library Resources

Use the maintenance calendar

The Maintenance calendar is a monthly or daily view of the routine maintenance tasks scheduled for your instrument. When a task is due to be performed, it is listed in the Maintenance Notifications list in the Dashboard (see "Review the Maintenance Notifications Log" on page 224).

View the calendar

You can access the maintenance calendar from the Dashboard or the menu bar:



Default calendar entries

A set of recommended tasks are scheduled in the calendar, flagged with FR (Factory Repeating) in the monthly view and F (Factory) in the daily view. User-specified repeating tasks are flagged with R (Repeating) in the monthly view, see picture below.

You cannot remove them from the calendar or alter the frequency at which the notifications for the tasks are displayed.

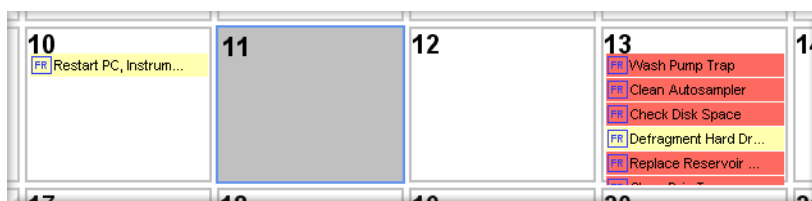
Additionally, we suggest that you add to the maintenance calendar:

- The regular maintenance tasks.
- A maintenance task to replace a consumable based on its installation date (for example, create a task to replace the polymer for two days before the polymer will expire)

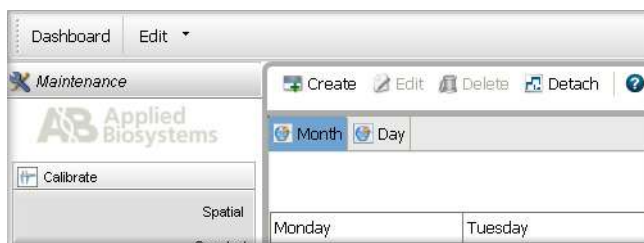
Create calendar entries

To create a new scheduled task, click **Create** and follow the prompts.

The following is an example of scheduled events in the calendar.

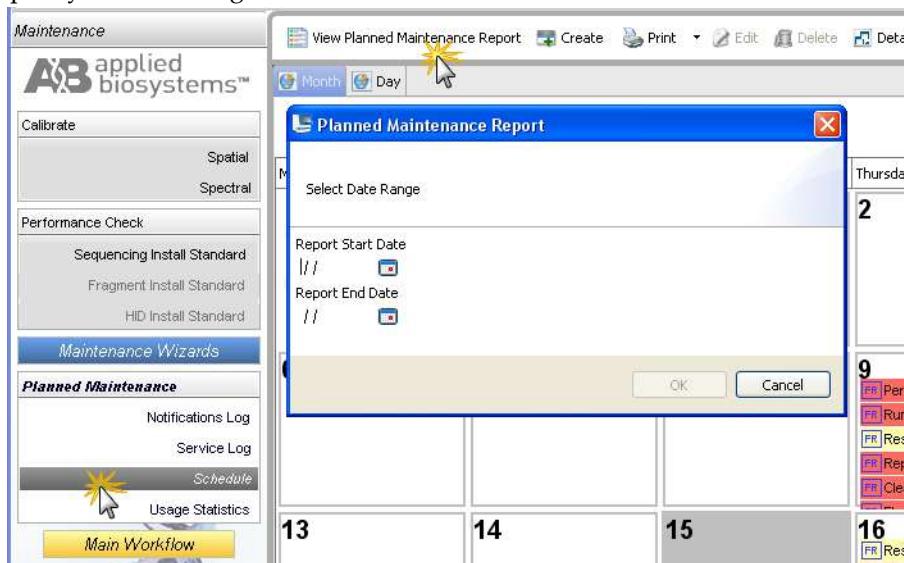


The Month and Day tabs allow you to view your schedule in different formats. Click **Detach** to move the calendar window.



View the Planned Maintenance Report

1. Access the maintenance calendar, then click **Planned Maintenance Report**.
2. Specify the date range, then click **OK**.

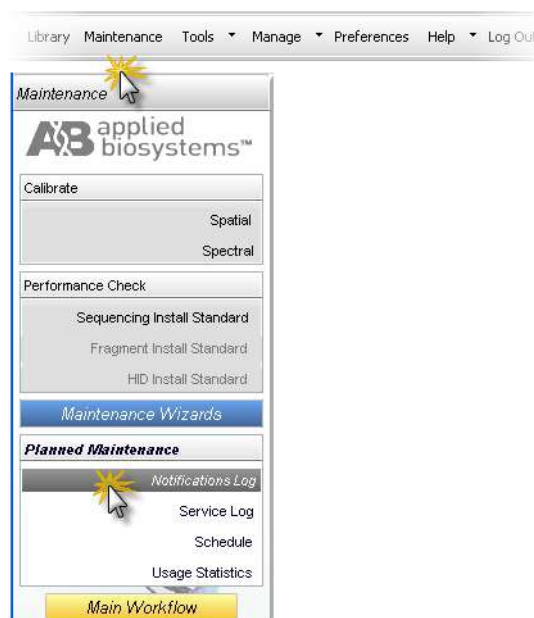


3. Select **Print** as needed.
4. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.
5. Close the report.



Review the Maintenance Notifications Log

1. The Notifications Log is a history of the action taken on maintenance notifications messages in the Dashboard (see “Check maintenance notifications” on page 38). Access the Notifications Log.
2. View the Notification Log Report and print as needed.
Note: Multi-column sorting is supported (see “Multi-column sorting” on page 79).
3. Print the Notifications log by clicking **Print**.



Instrument operational procedures

The day-to-day operation of the instrument involves performing the following tasks.

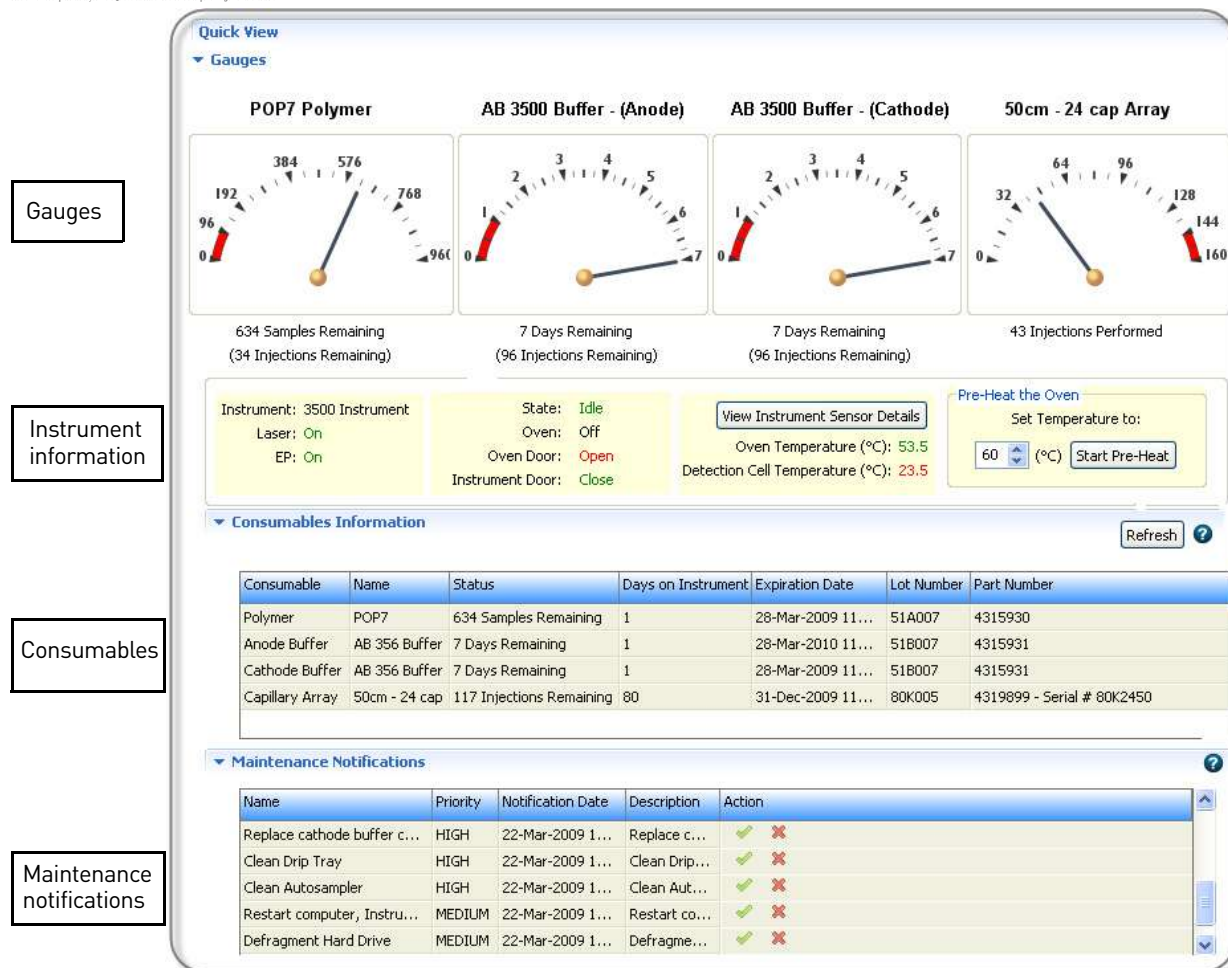
Check consumables on the Dashboard

- Change the anode buffer container (ABC)
- Change the cathode buffer container (CBC)
- Change the polymer
- Use the Conditioning Reagent
- Fill Capillary Array with fresh polymer
- Remove bubbles

The Quick View section of the Dashboard provides the necessary information that you need to operate the instrument.

The information shown within the Quick View is generated automatically, via the Radio Frequency Identification (RFID) reader.

Use the information presented to you in the Quick View section before and after performing a maintenance task.



Change the anode buffer container (ABC)

For details see “Instrument reagents and consumables” on page 19.

Contamination might cause poor-quality data. To prevent the contamination, use genuine packaged polymer, anode buffer, cathode buffer and conditioning reagent.

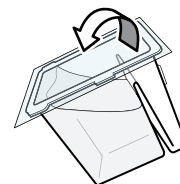
1. Remove the ABC from storage.
2. Check for expiration date on the ABC label to make sure it is not expired prior to or during intended use.
3. Allow refrigerated ABC to equilibrate to ambient temperature prior to first use. Do not remove the seal until you have completed step 5 below.

IMPORTANT! Ensure that all the buffer is moved to the larger side of the ABC prior to removing the seal.

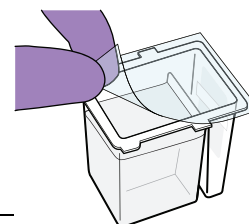
4. Verify that buffer level is at or above the fill line and check that seal is intact.

IMPORTANT! Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ± 1 mm is acceptable.

5. Invert the ABC, then tilt it slightly (as shown in the figure below) to make sure most of the buffer is in the larger side of the container. There should be less than 1 ml of the buffer remaining in the smaller side of the container.

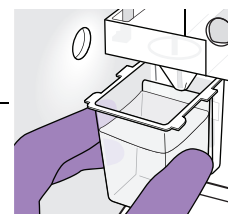


6. Verify that the buffer is at the fill line.
7. Peel off the seal at the top of the ABC.
8. Place the ABC into the Anode end of the instrument, below the pump.



IMPORTANT! The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.

9. Close the instrument door to re-initialize.
Note: If you do not close the instrument door to re-initialize, you need to click **Refresh** from the Dashboard.



10. Click **Refresh** from the Dashboard to update the screen.
11. Check the Quick View section of the Dashboard for updated status after changing the ABC.

Change the cathode buffer container (CBC)

For details see “Instrument reagents and consumables” on page 19.

Contamination might cause poor-quality data. To prevent the contamination, use genuine packaged polymer, anode buffer, cathode buffer and conditioning reagent.

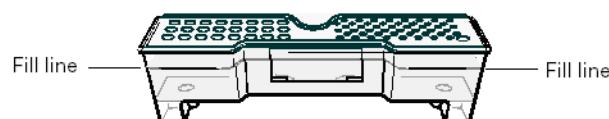
Use genuine parts and reagent. The use of inappropriate parts, or reagents, causes poor-quality data or damage the instrument.

1. Remove the CBC from storage.
2. Check for expiration date on the CBC label to make sure it is not expired prior to or during intended use.
3. Allow refrigerated CBC to equilibrate to ambient temperature before use.
4. Wipe away condensation on the CBC exterior with a lint-free lab cloth.
5. Verify that buffer level is at or above the fill line and check that seal is intact.

IMPORTANT! Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ± 0.5 mm is acceptable.

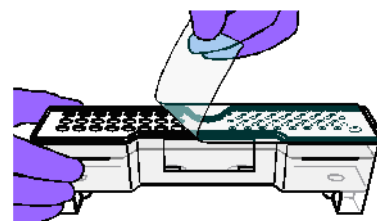
Note: The meniscus must be at or above the fill line.

6. Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles.



Note: If you do not tilt the CBC back and forth, the buffer sticks to the baffles, due to surface tension.

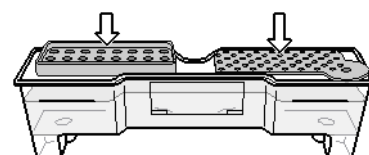
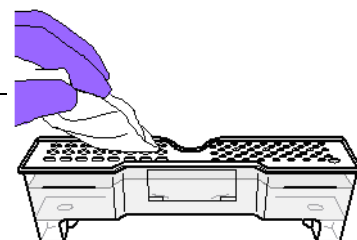
7. Verify that the buffer is at or above the fill line.
8. When ready to install CBC, place the container on a flat surface (such as a lab bench) and peel off the seal.
9. Wipe off any buffer on top of the CBC with a lint-free cloth. Ensure that the top of the container is dry.



IMPORTANT! Failure to perform this action may result in an arcing event and termination of the run.

10. Place the appropriate septa on both sides of the CBC:

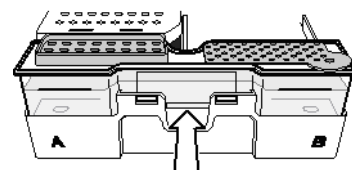
- a. Align the buffer septa (the part that is symmetrical) over the 24 holes of the CBC.
- b. Push the septa lightly into the holes to start and then push firmly to seat the septa.



11. Click the **Tray** button on the front panel to move the autosampler to the front position.
12. Install the CBC on the autosampler.

Note: When properly installed, it will click on the autosampler as the tabs are snapped in place.

13. Close the instrument door to re-initialize.
14. Click **Refresh** from the Dashboard to update the screen.
15. Check the Quick View section of the Dashboard for updated status after changing the CBC.



Check stored capillary arrays



WARNING! SHARP The load-end of the capillary array has small but blunt ends and it could lead to piercing injury.

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

When the capillary array is installed, electrodes at the bottom are inserted on the CBC. The electrodes at the top connect with the polymer delivery pump. We recommend you keep the electrodes on the bottom in the tray with 1X running buffer. For details see “Instrument reagents and consumables” on page 19.

IMPORTANT! Keep the loading-end of the capillary array 1X running buffer to prevent the polymer from drying in the capillaries. If fluid level is low, add distilled water (DI) to buffer solution.

Refer to the Install capillary wizard for instructions on how to store the capillary array.



1X running buffer and distilled water (DI)

Flush the water trap (pump trap)

The water trap must be flushed once per month to prolong the life of the pump and to clean any diluted polymer.

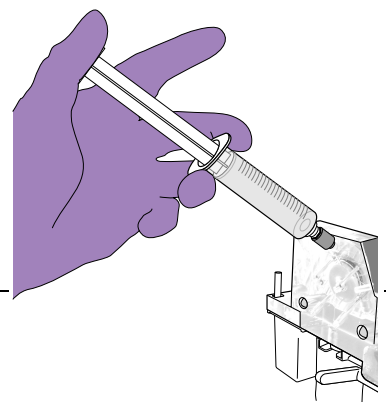
Flush with either distilled or deionized water and ensure that the water flows into the overflow container. Dispose the excess water (inside the overflow container). See “Chemical safety” on page 299.

Note: Leave the trap filled with either distilled or deionized water.

1. Fill the supplied 20 mL, all-plastic Luer lock syringe (in the PDP Cleaning kit, PN 4462858) with distilled or deionized water. Expel any bubbles from the syringe.

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

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



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

- Note:** Because the water trap volume is approximately 325 μ L, a relatively small volume of water is adequate for complete flushing. However, a larger volume only improves flushing as long as force and flow rate are kept within the limits given above.
4. Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.
 5. Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

Routine instrument cleaning

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

IMPORTANT! Use only the cleaning agents listed in this guide. Use of cleaning agents other than those listed in this guide may damage the instrument.

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

Note: The drip tray can be removed.

Maintaining the polymer delivery pump assembly

IMPORTANT! To minimize background fluorescence, use clean, powder-free, silicone-free latex gloves whenever you handle the pump assembly or any item in the polymer path.

The polymer delivery pump can be irreversibly damaged if:

- Polymer dries in the polymer channels of the pump assembly.
- The inside surface of the channel is scratched.
- The pump assembly is exposed to organic solvent.
- The pump assembly is exposed to temperatures greater than 40°C.
- There is arcing in the pump assembly.

Move and level the instrument

IMPORTANT! If you relocate the instrument, we recommend that you have an IQ and OQ performed. Contact Life Technologies to schedule the IQ OQ service.



WARNING! PHYSICAL INJURY HAZARD. Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. Two or three people are required to lift the instrument, depending upon instrument weight.

1. Remove the following components from the instrument:
 - Any plate assemblies from the autosampler.
 - CBC from the autosampler.
 - Capillary array: click **Shutdown the Instrument in the Maintenance Wizards**. See “Shutdown the instrument” on page 239.
 - Anode buffer reservoir.
2. Switch off the circuit breaker on the back of the instrument.
3. Disconnect the power cord and the Ethernet cable.

IMPORTANT! While moving the instrument, avoid any shock or vibration.

4. Move the instrument.
5. Turn the instrument legs to level the instrument.

To move the instrument corner...	Turn the leg...
up	right (clockwise)
down	left (counterclockwise)

6. Have an IQOQ performed before using the instrument. Contact Life Technologies to schedule the IQOQ service.

Use the Maintenance Wizards to perform operations

About Maintenance Wizards

To activate the Maintenance Wizards from the Dashboard, click **Maintain Instrument** toggle key. The Maintenance Wizards feature of the Data Collection software allows you to perform operations necessary for sustaining the instrument.

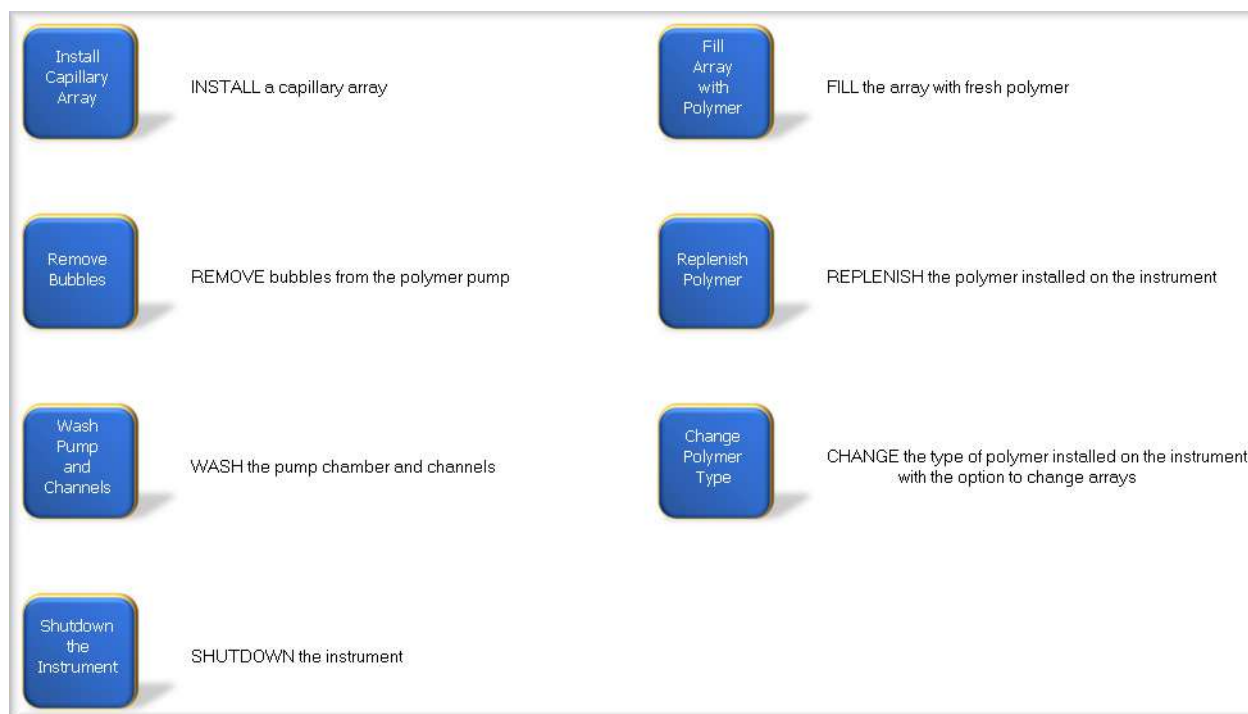


In no particular order, these operations include the following:

- Install a Capillary Array
- Remove bubbles from the polymer pump
- Wash the pump chamber and channels
- Fill the array with fresh polymer
- Replenish the polymer installed on the instrument
- Change the type of polymer installed on the instrument with the option to change the capillary array.
- Shutdown the Instrument.

IMPORTANT! Once started, Wizard operations cannot be canceled.

IMPORTANT! After performing a conditioning wash ensure that the buffer level inside the ABC is at or above fill line before proceeding to the next step except for the wash pump and channels wizard.



Replenish polymer

IMPORTANT! Do not use a polymer pouch that has been installed on one type of instrument on another type of instrument. For example, if you install a new polymer pouch originally on a 3500 (8-capillary) instrument, do not subsequently use that same polymer pouch on a 3500xL (24-capillary) instrument, or vice versa. Doing so may result in a lower number of samples/injections than specified.

For details see “Instrument reagents and consumables” on page 19.

If you are replacing the same polymer type only, follow the procedures below:

IMPORTANT! If you remove a polymer pouch for storage, place a Pouch Cap (Part no. 4462785) onto the pouch, then place an empty pouch (or conditioning reagent) on the connector to prevent desiccation of any residual polymer on the connector.

1. In the Maintenance Wizards screen, click **Replenish Polymer**.

Note: The Replenish Polymer Wizard takes 10 to 20 minutes to complete.

2. Follow the prompts in the Replenish Polymer Wizard window.

3. Click **Refresh** from the Dashboard to update the screen.

4. Check the Quick View section of the Dashboard for updated status after replenishing the polymer.



Change polymer type

IMPORTANT! Do not use a polymer pouch that has been installed on one type of instrument on another type of instrument. For example, if you install a new polymer pouch originally on a 3500 (8-capillary) instrument, do not subsequently use that same polymer pouch on a 3500xL (24-capillary) instrument, or vice versa. Doing so may result in a lower number of samples/injections than specified.

IMPORTANT! If you remove a polymer pouch for storage, place a Pouch Cap (Part no. 4462785) onto the pouch, then place an empty pouch (or conditioning reagent) on the connector to prevent desiccation of any residual polymer on the connector.

For details see “Instrument reagents and consumables” on page 19.

IMPORTANT! If the polymer dries on the fitment or in the pouch opening, the dried polymer prevents the pouch fitment from closing the internal cap properly. If that happens, the polymer pouch is no longer usable. When the pouch is removed, cover the fitment with a new, empty, or a conditioning pouch. To prevent drying, the pouch fitment must be covered with Pouch Cap (Part no. 4462785).

Note: To ensure optimal performance, replace polymer after 7 days. Although the software allows you to run with polymer that has been on the instrument for more than 7 days or with polymer that is expired, use at your own risk. Life Technologies has verified the polymers for up to 7 days only on the instrument.

1. Remove the polymer from storage 4°C.
2. Allow refrigerated polymer to equilibrate to ambient temperature before use.
3. Check for expiration date on the pouch label to make sure it is not expired prior to use.

IMPORTANT! Do not use if the pouch and/or the label is damaged or the top seal is missing.

4. Peel off seal at the top of the pouch fitment.

Note: You may occasionally notice a tiny droplet of polymer inside the fitment (residual from the pouch filling process). This is **not** expected to cause any performance issues.

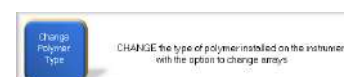
5. Slide the pouch fitment on to the slot of the lever assembly. Push the lever up to snap the pouch into the connector end of the instrument pump.

Note: The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.



6. If a partially used pouch is removed for later use, use the suggested cap to plug the fitment opening and store the pouch under recommended storage conditions.

7. From the Maintenance Wizards screen, click **Change Polymer Type**.



IMPORTANT! This feature allows you to change the type of polymer installed on the instrument with the option to change the Capillary Arrays.

Note: The Change Polymer Type Wizard takes 60 to 70 minutes to complete.

8. Follow the prompts in the Change Polymer Type Wizard window.

Note: Changing polymer requires the use of a Conditioning Reagent. See "Follow the prompts in the Wash Wizard window." on page 236.



9. Click **Refresh** from the Dashboard to update the screen.
10. Check the Quick View section of the Dashboard for updated status after changing the polymer.

Store partially used polymer

IMPORTANT! Do not use a polymer pouch that has been installed on one type of instrument on another type of instrument. For example, if you install a new polymer pouch originally on a 3500 (8-capillary) instrument, do not subsequently use that same polymer pouch on a 3500xL (24-capillary) instrument, or vice versa. Doing so may result in a lower number of samples/injections than specified.

If a partially used pouch is removed for later use, use the suggested Pouch Cap to plug the fitment opening and store the pouch under recommended storage conditions. The Pouch Cap is sold separately (Part no. 4462785).

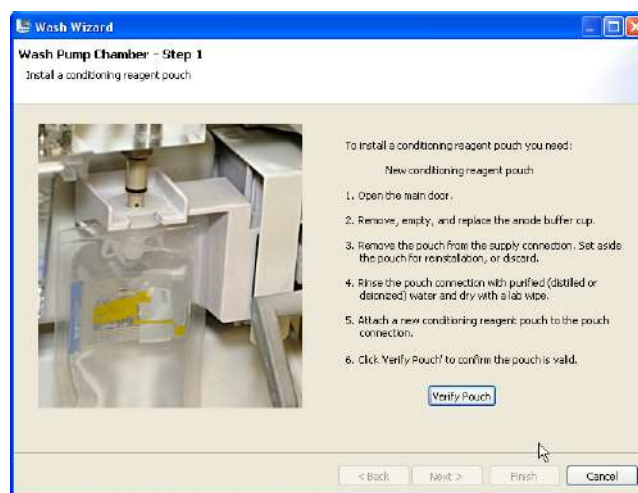
If you remove a polymer pouch for storage, place a Pouch Cap (Part no. 4462785) onto the pouch, then place an empty pouch (or conditioning reagent) on the connector to prevent desiccation of any residual polymer on the connector. If the polymer dries on the fitment or in the pouch opening, the dried polymer prevents the pouch fitment from closing the internal cap properly. If that happens, the polymer pouch is no longer usable.

IMPORTANT! Follow the instructions in the wizard to ensure the proper installation and operation of the pouch and the instrument.

Wash the pump chamber and channels

Note: The Wash Pump and Channels wizard takes over 40 minutes to complete.

1. From the Maintenance Wizards screen, click **Wash Pump and Channels**.
2. Follow the prompts in the Wash Wizard window.



Use the conditioning reagent

For details see “Instrument reagents and consumables” on page 19.

IMPORTANT! Once installed on the instrument, the pouch is good for a one-time use, only.

The use of the conditioning reagent is dictated by the instrument wizards. Although the software allows you to run with Conditioning Reagent that is expired, use at your own risk.

Contamination might cause poor-quality data. To prevent the contamination, use genuine packaged polymer, anode buffer, cathode buffer and conditioning reagent.

Use genuine parts and reagent. The use of inappropriate parts, or reagents, causes poor-quality data or damage the instrument.

Refer to Chapter 3, “Set Up and Run” on page 51 for instructions on priming the pump and initiating the run.

The Quick View section of the Dashboard provides the necessary information that you need for using the Conditioning Reagent.

Note: Install the pouch only when requested to do so by the wizard.

To place the conditioning reagent on the instrument

1. Check for expiration date on the label to make sure it is not expired prior to use.

IMPORTANT! Do not use if pouch/label is damaged or top seal is missing.

2. Peel off the seal at the top of the conditioning reagent pouch fitment.
3. Insert the pouch fitment on to the slot of the pump lever mechanism. Push the lever up to snap the pouch into the connector end of the instrument pump.

Note: The RFID label must be facing the instrument (and not you) to ensure that the RFID information is read accurately by the instrument.



4. Follow the wizard for further instructions.
5. Click **Refresh** from the Dashboard to update the screen.
6. Check the Quick View section of the Dashboard for updated status after changing the Conditioning Reagent.

Fill capillary array with fresh polymer

For details see “Instrument reagents and consumables” on page 19.

The filling of the capillary array with fresh polymer is dictated by the instrument wizards.

1. To fill capillary array with fresh polymer (same type of polymer), click **Fill the Array with fresh Polymer**.
2. Follow the prompts in the Fill Array Wizard window.
3. Click **Refresh** from the Dashboard to update the screen.
4. Check the Quick View section of the Dashboard for updated status after filling of the Capillary Array with fresh polymer.



Remove bubbles from the polymer pump

Remove bubbles from the polymer pump fluid path before each run. See “Daily instrument maintenance tasks” on page 220 for more information.

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

1. To remove bubbles from the polymer pump fluid path that travel from the polymer pouch through the pump, array port, and the anode buffer container, click **Remove Bubbles**.



Note: The Bubble Remove Wizard takes 5 to 15 minutes to complete.

2. Follow the prompts in the Bubble Remove Wizard window. Check the Quick View section of the Dashboard for updated status of the polymer pouch after removing bubbles from the polymer pump fluid path.



Change the capillary array



WARNING! SHARP The load-end of the capillary array has small but blunt ends and it could lead to piercing injury.

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

IMPORTANT! Check the loading-end header to ensure that the capillary tips are not crushed or damaged.

For details see “Instrument reagents and consumables” on page 19.

1. From the Maintenance Wizards screen, click **Install Capillary Array**.



Note: The Install Capillary Array Wizard takes 15 to 45 minutes to complete.

2. Follow the prompts in the Install Capillary Array Wizard window.
3. Check the Quick View section of the Dashboard for updated status of the capillary array.
4. Perform a spatial calibration (see "Spatial calibration" on page 105).



Shutdown the instrument

Use the Instrument Shutdown Wizard for short- and long-term shutdown.

1. From the Maintenance Wizards screen, click **Shutdown the Instrument**.

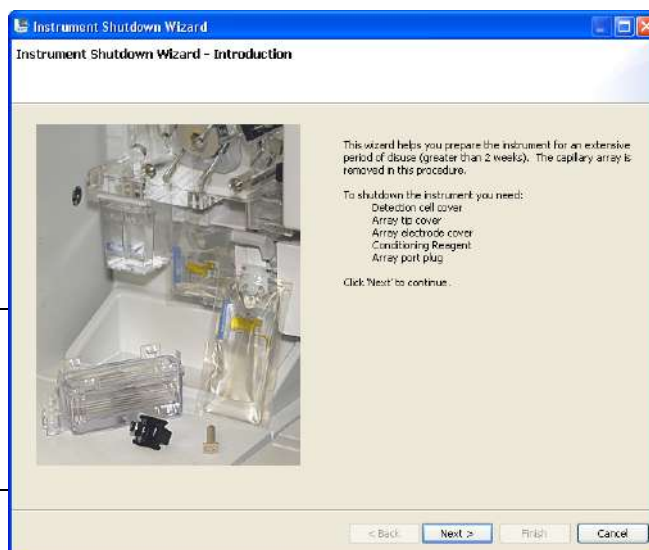
Note: The Instrument Shutdown Wizard takes 60 minutes to complete.



2. Follow the prompts in the Instrument Shutdown Wizard window.

Perform the appropriate shutdown procedure based on the information in the following table:

IMPORTANT! Place a conditioning reagent pouch onto the instrument when performing instrument shutdown



If the instrument will be unattended for...	Perform this shutdown procedure...
no more than 1 week	No action is required.
1 to 2 weeks	Keep the load-end of the capillary array in 1X buffer to prevent the polymer from drying in the capillaries. If fluid level is low, add DI water to buffer solution. Install the new CBC when ready to resume runs.

If the instrument will be unattended for...	Perform this shutdown procedure...
for more than 2 weeks	<ol style="list-style-type: none"> 1. Run the Install Capillary wizard and store the capillary array. 2. Remove the polymer pouch. 3. Install a conditioning pouch. 4. Run the Wash Pump and Channels wizard. 5. Clean any spills or residual polymer. 6. Run the Shutdown the Instrument wizard. 7. Unplug the instrument.

Computer maintenance

This section lists the common tasks required to maintain the computer for your 3500 Series Genetic Analyzer in good working condition.

Note: In the event of power being disrupted, the computer should be restarted.

For the computer troubleshooting issues, see Appendix B, “Troubleshoot” on page 255.

Backing up the datastore during software uninstall

IMPORTANT! Do not uninstall the software unless instructed to do so by Life Technologies.

When you uninstall the software, you are prompted to back up the datastore (the directory that contains all library items you created, such as plates and protocols).

Select a location other than the install directory for the datastore backup.

IMPORTANT! Do not back up the datastore to the installation directory. The installation directory is deleted during the uninstall.

Archive, purge, and restore data

IMPORTANT! The customer is responsible for validation of archive, restore, and purge functions.

- **Archive** – Makes a copy of the data in an external file that you can save in another location.
- **Purge** – Allows you to delete (purge) user-created items stored in the library. Factory-provided items are not purged. You have an option to archive the items, also.
- **Restore** – Restores archived data back to the system.

IMPORTANT! These functions affect items stored in the library (datastore). These functions do not affect sample data files.

Frequency

We recommend that you purge the library objects once every three months.

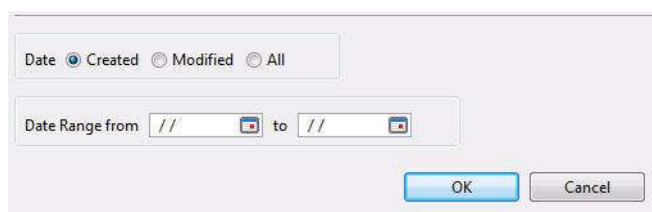
Archive library items

This function archives items stored in the library. To archive audit records, see “Archive, purge, and restore audit records” on page 206.

1. Access the Archive screen.



2. Specify the date category and range, then click **OK**.



3. Specify a location and file name for the archive (.dsz) file, then click Save.
A message is displayed when the archive is complete.

IMPORTANT! Do not specify <<install directory>>:\Applied Biosystems\3500\datastore as the archive location. If you do so, your archive can be deleted if you uninstall the software and do not back up the datastore.

If you specify a location to which you do not have permission to save, a warning message is displayed and gives you the option to save in another location.

Archive data files

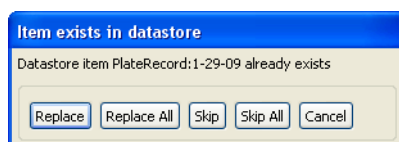
Use the Windows backup function (Start ► Control Panel ► Backup and Restore) to archive the data files

Note: If you export audit records for samples that are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

Restore

This function restores items stored in the library. To restore audit records, see “Archive, purge, and restore audit records” on page 206.

1. Access the Restore function.
2. Select the archive (.dsz) file to restore, then click **Open**.
If the archive file contains items that exist in the system, a message is displayed.



3. Select an option to continue.
A message is displayed when the restore is complete.

Purge

This function purges (deletes) user-defined items stored in the library. To purge audit records, see “Archive, purge, and restore audit records” on page 206.

1. Access the Purge function.
2. Click **Yes** in the Purge warning message stating that you are about to permanently delete all files in the library.
3. Specify the date category and range, then click **OK**.
4. Click **Yes** in the Purge warning message.



A message is displayed when all records are deleted.

Monitor disk space

Ensure that you have sufficient drive space by regularly:

- Archiving data
- Deleting unneeded files
- Emptying the trash
- Defragmenting the drives

Hard disk and status

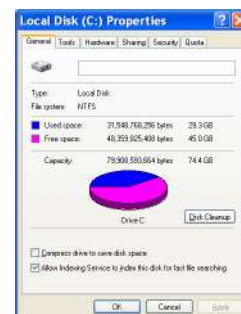
Manually check available disk space on Drive D.

To check the status, go to My Computer ► right-mouse click on C drive ► Select Properties ► Click **General** tab.

The Data Collection software will prompt you when it is 70 to 75% full. At 78% full, the software will not start a run

If there is insufficient space:

- Archive the sample files.
- Delete the sample file data from the drive D and empty the contents of the Recycle Bin.



Defragment the computer hard drive

This option can be set as a reminder in the scheduler. The fragmentation of files decreases the performance of both the Data Collection software and the computer operating system. Programs take a longer time to access files by performing multiple search operations of the fragments.

Go to Start ► Programs ► Accessories ► System Tools ► Disk Defragmenter and follow the prompts.

Note: You can click **Analyze** to see if you should defragment or not.

Check available space on all drives

Before a run, the Data Collection software checks free disk space. If adequate free disk space is not available to store the data, the Data Collection software displays the following message:

Remove data: the drive is getting full

View the errors that appear for generated errors and in the Event Log window. See Appendix B, “Troubleshoot” on page 255.

Also, check the status light in the bottom left-hand corner of the data collection window to see if it flashes red.

Manage software licenses

The 3500 Series Software 2 requires a license to run.

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

Obtain and activate a software license

The 3500 Series Software 2 Software Activation dialog box is displayed when you start the software if no license is installed and activated on your computer.

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

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

IMPORTANT! You can run the 3500 Series Software 2 using only the network cards enabled when you activate the software license. For example, if you activate the software when your wireless network card is disabled, you will not be able to run the software when the wireless network card is enabled.

2. Display the Software Activation dialog box by starting the 3500 Series Software 2 (see page 35).

3500 Series Data Collection Software 2 Software Activation

1. Request license file for Computer ID:

a088b42a1c44 5c260a57d79c

This ID is unique to this computer and cannot be used to obtain a license file for another computer.

a. Enter the license key (from CD or email):

b. Enter your email address:

c. Is this computer currently connected to the internet?

Yes. Connected. No. Not Connected.

2. Retrieve the license file from email, then save it to the desktop of this computer.

3. Find the license file:

Browse...

4. Click

Install and Validate License

Close

3. Obtain the license key. The license key is provided on the 3500 Series Software 2 CD case, or in an email from Life Technologies.
4. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen.

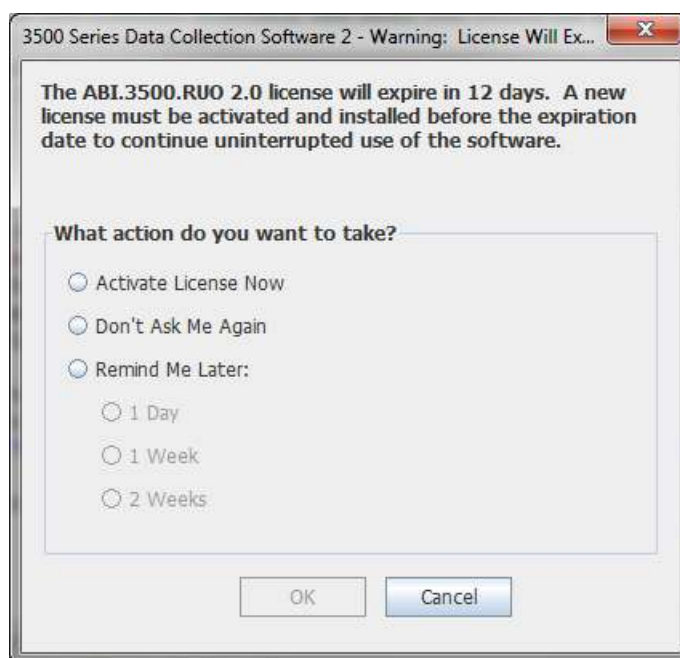
IMPORTANT! Keep a record of the email address used to activate the software license. You must use the same email address to renew the software license when it expires.

5. Obtain the software license file from your email.
6. Make a copy of the software license file and keep in a safe location.
7. Copy the software license file to the desktop of the 3500 Series Software 2 computer.
8. If the Software Activation dialog box has closed, start the 3500 Series Software 2 to open it (see page 35).
9. Click **Browse**, then navigate to the software license file saved on your computer.

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

Renew a software license

1. Ensure that all network cards in the computer are enabled.
2. Display the Software License Renewal dialog box by doing any of the following:
 - Select **Renew License Now** in the Warning: License Will Expire Soon dialog box (which is displayed starting ~30 days before license expiration).

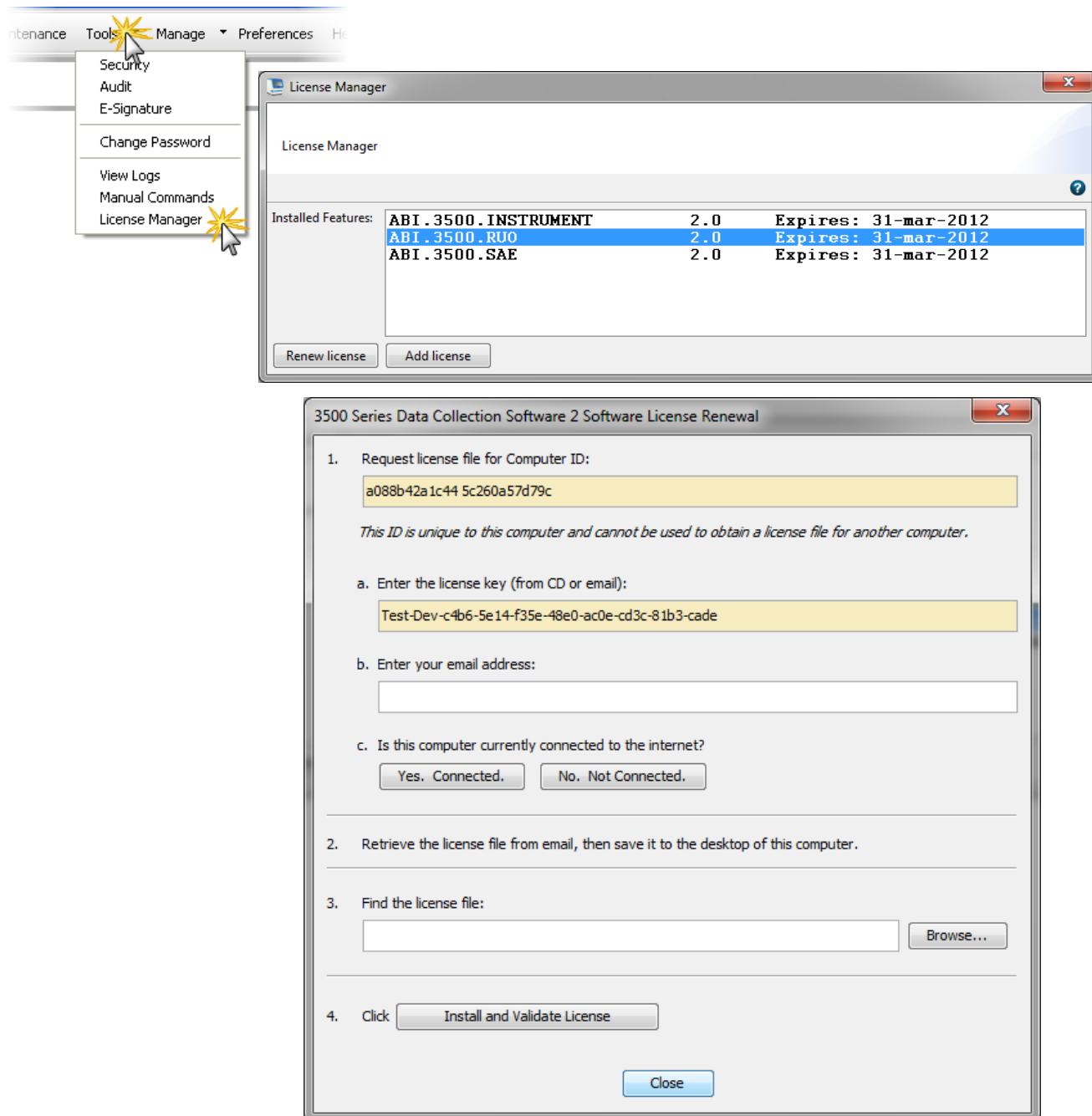


- Click **Yes** in the Critical Warning: License Will Expire Soon dialog box (which is displayed starting ~7 days before license expiration).



- From the Main Workflow or Dashboard, select Tools ► License Manager, then click any of the 3 licenses. Click **Renew License** to renew all three licenses.

Note: The Add License function is for future use.



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

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

4. Request the renewed software license file by performing step 1c as listed on the renewal screen.
5. Obtain the renewed software license file from your email.
6. Copy the renewed software license file to the desktop of this computer.
7. If the Software renewal dialog box has closed, access it from the License Manager (see page 246).
8. Click **Browse**, then navigate to the renewed software license file saved on your computer.
9. Click **Install and Validate License**. A message is displayed when the license is installed and validated.
10. Click **Close**.

Service Log and Usage Statistics

The Service Log and Usage Statistics functions are for use by Life Technologies service engineers at the time of service.



Application Reagents and Run Modules

Note: For reagent or consumable shelf-life expiration date, see the package label. For part numbers, see “Part Numbers” on page 287.

Sequencing analysis dye sets for all applications

Table 21 Dye Sets for various applications

Dye Set	Application Name
E (v1.1 BigDye® Terminator)	DNA sequencing
Z (v3.1 BigDye® Terminator)	DNA sequencing
Z (BigDye® Direct)	DNA PCR Amplification/ Clean-up/Cycle Sequencing kit

Fragment analysis dye sets for all applications

Table 22 Fragment analysis dye sets

Dye Set	Application
E5	SNaPshot® kit
G5	DNA sizing for 5-dye chemistry
J6	DNA sizing for 6-dye chemistry
F	DNA sizing for 4-dye chemistry
Any dye	DNA sizing

HID analysis dye sets¹

Table 23 AmpF/STR Kit Table

AmpF/STR® Kits	Dye set (use with HID Fragment Analysis 36_POP4 run module)
4-dye: <ul style="list-style-type: none"> • COfiler® Kit • Profiler® Kit • Profiler Plus® Kit • Profiler Plus® ID Kit • SGM Plus® Kit • Other 4-dye kits 	F
5-dye: <ul style="list-style-type: none"> • Identifiler® Kit • Identifiler® Direct Kit • Identifiler® Plus Kit • Minifiler™ Kit • NGM™ Kit • NGM™ SElect Kit • SEfiler™ Plus Kit • SinoFiler™ Kit • Yfiler® Kit • Other 5-dye kits 	G5
6-dye: <ul style="list-style-type: none"> • GlobalFiler™ Kit 	J6

¹ HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).

Run modules

Capillary array and polymer (sequencing analysis run modules)

Table 24 Capillary array and polymer (sequencing analysis run modules)

Run Module Type & Run Module Name	Configuration		23 hours Throughput [†]			Performance
	Capillary Length (cm)	Polymer Type	Run Time (min)	3500 (8-capillary)	3500xL (24-capillary)	Contiguous Read Length (CRL) [‡]
FastSeq50_POP6	50	POP-6™	≤90	≥122	≥368	≥600
RapidSeq50_POP6	50	POP-6™	≤65	≥168	≥504	≥450
Rapid sequencing RapidSeq50_POP7	50	POP-7™	≤40	≥280	≥840	≥500
Standard sequencing StdSeq50_POP6	50	POP-6™	≤135	≥80	≥240	≥600
Fast sequencing FastSeq50_POP7	50	POP-7™	≤65	≥168	≥504	≥700
Standard sequencing StdSeq50_POP7	50	POP-7™	≤125	≥88	≥264	≥850
Short read sequencing ShortReadSeq_POP7	50	POP-7™	≤30	≥368	≥1104	≥300
Rapid sequencing BigDye® XTerminator™ RapidSeq_BDX_50_POP7	50	POP-7™	≤40	≥280	≥840	≥500
Standard sequencing BigDye® XTerminator™ StdSeq_BDX_50_POP6	50	POP-6™	≤140	≥80	≥240	≥600
Fast sequencing BigDye® XTerminator™ FastSeq_BDX_50_POP7	50	POP-7™	≤65	≥168	≥504	≥700

Table 24 Capillary array and polymer (sequencing analysis run modules) *(continued)*

Run Module Type & Run Module Name	Configuration		23 hours Throughput [†]			Performance
	Capillary Length (cm)	Polymer Type	Run Time (min)	3500 (8-capillary)	3500xL (24-capillary)	Contiguous Read Length (CRL) [‡]
Standard sequencing BigDye® XTerminator™ StdSeq_BDX_50_POP7	50	POP-7™	≤125	≥88	≥264	≥850
Short read sequencing BigDye® XTerminator™ ShortReadSeq_BDX_POP7	50	POP-7™	≤30	≥368	≥1104	≥300
Microbial Sequencing MicroSeq_POP7	50	POP-7™	≤125	≥88	≥264	≥850
Microbial Sequencing MicroSeq_POP6	50	POP-6™	≤135	≥80	≥240	≥600

[†] Throughput (Samples / Day): The total number of samples run in 23 hours (0.5 hour for User interaction and 0.5hr for warm-up time).

[‡] The maximum number of contiguous bases in the analyzed sequence with an average QV ≥20, calculated over a sliding window 20 base pairs wide from an AB Long Read Standard sequencing sample. This calculation starts with base number 1. The read length is counted from the middle base of the 1st good window to the middle base of the last good window, where a “good” window is one in which the average QV ≥20.

Capillary array and polymer (fragment and HID analysis run modules)

Table 25 Capillary array and polymer (fragment and HID analysis run modules)

Run Modules Type & Run Modules Name	Configuration		23 hours Throughput [†]			Performance			
	Capillary Length (cm)	Polymer Type	Run Time (min)	3500	3500xL	Range [‡]	Sizing Precision [§]		
							50bp-400bp	401bp-600bp	601bp-1200bp
Fragment analysis FragmentAnalysis50_POP7	50	POP-7™	≤40	≥280	≥840	≤40 to ≥560	<0.15	<0.30	NA ^{††}
Fragment analysis FragmentAnalysis50_POP6	50	POP-6™	≤100	≥112	≥336	≤20 to ≥550	<0.15	<0.30	NA ^{††}
Long fragment analysis LongFragAnalysis50_POP7	50	POP-7™	≤125	≥88	≥360	≤40 to ≥700	<0.15	<0.30	<0.45
Fragment analysis FragmentAnalysis36_POP4	36	POP-4®	≤35	≥312	≥936	≤60 to ≥400	<0.15	NA ^{††}	NA ^{††}
Fragment analysis FragmentAnalysis36_POP7	36	POP-7™	≤30	≥368	≥1104	≤60 to ≥500	<0.15	NA ^{††}	NA ^{††}
HID HID36_POP4 ^{‡‡}	36	POP-4®	≤35	≥312	≥936	≤60 to ≥400	<0.15	NA ^{††}	NA ^{††}
SNaPshot® SNaPshot50_POP7	50	POP-7™	≤30	≥376	≥1104	≤40 to ≥120	<0.50	NA ^{††}	NA ^{††}

[†] Throughput (Samples / Day): The total number of samples run in 23 hours (0.5 hour for User interaction and 0.5hr for warm-up time).

[‡] Resolution Range: The range of bases over which the resolution (peak spacing interval divided by the peak width at half-max in a GeneScan™ 600 LIZ® or GeneScan™ 1200 LIZ® size standard sample sized with a third order fit) is ≥1. The table shows the resolution range in ≥90% of samples.

[§] Sizing Precision: Standard deviation of sizes for one allele in the DS-33 install standard sized with the GeneScan™ 600 LIZ® size standard across multiple capillaries in the same run. For one injection to pass, 100% of the alleles in that injection must meet the intra-run sizing precision specifications. The table shows the sizing precision of 100% of alleles in ≥90% of samples.

^{††} Not applicable because of the size of the fragments collected in the run.

^{‡‡} HID-Validated, Factory-provided Protocols: The latest validated HID assays and protocols can be downloaded from the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ► Software Downloads ► 3500 Series Genetic Analyzers for Human Identification).



Appendix A Application Reagents and Run Modules

Run modules

B

Troubleshoot

If you encounter any unforeseen and potentially hazardous event while operating the instrument, turn off the power, unplug the instrument, and call your Life Technologies service representative.

IMPORTANT! See “Safety” on page 293 for instrumentation and chemical safety information and guidelines.

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Section B.1 Troubleshooting tables

Figures 30 and 31 are provided below for reference in this section.

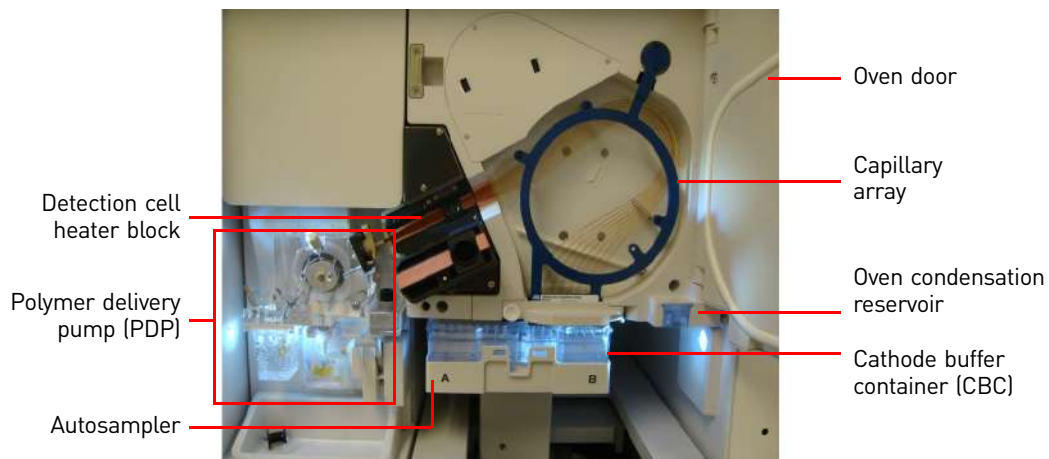


Figure 30 Instrument interior

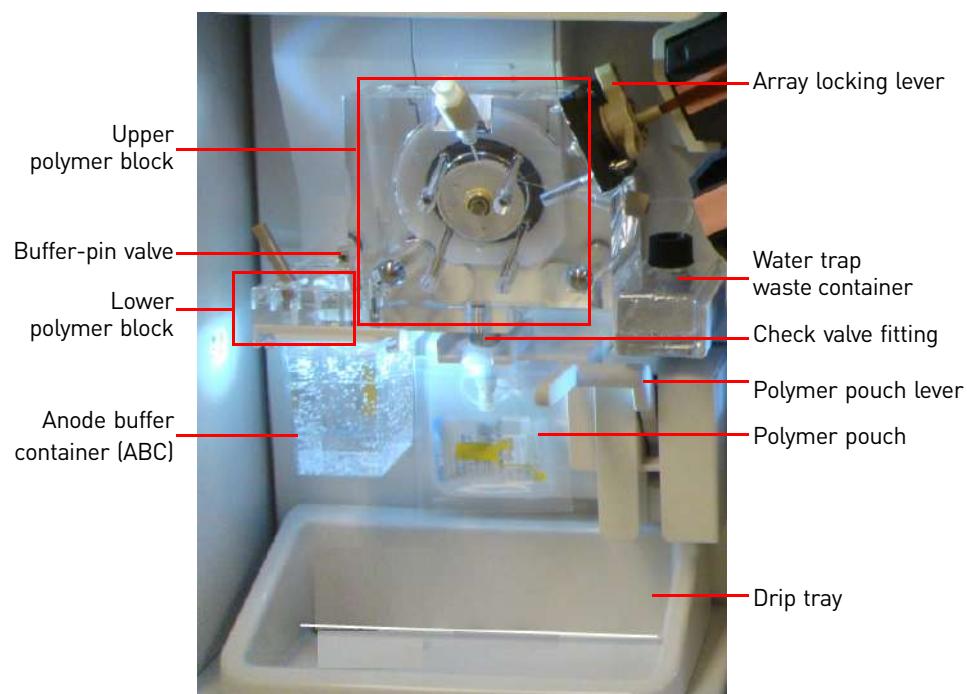


Figure 31 Polymer delivery pump (PDP)

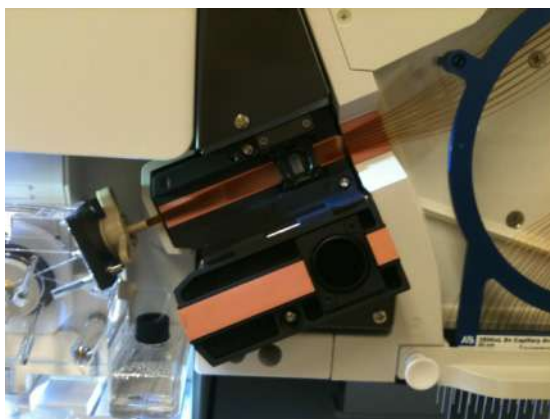


Figure 32 Detection cell and window

Restart the instrument and the computer

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

1. Exit the 3500 Series Software 2.
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. Do not log in.
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.
7. Look in the Windows taskbar at the bottom right of the desktop and make sure the Server Monitor icon is displayed. If it is not, go to “Step two: Start the 3500 Series Software 2” on page 35.
8. Start the 3500 Series Software 2.



Instrument troubleshooting

Table 26 Instrument troubleshooting

Symptom	Possible cause	Action
Amber light (blinking)	Run paused	Resume run.
	Door open	Close the instrument door.
	Run failure that does not require restart of instrument	Restart the instrument and the computer. (see page 257).
Instrument status light is blinking red	Instrument error	Restart the instrument and the computer. (see page 257).
Autosampler does not move the plate to a higher position	Array electrodes are bent. The plate is not aligned correctly resulting in the array tips missing center of septa. The plate retainer may not be snapped onto the plate base.	Ensure that the plate retainer, plate (or tube strip), and plate base are assembled correctly. Listen for a snap when the plate retainer and the plate base are clipped together. See "Prepare the plate assembly" on page 61. IMPORTANT! If array tips are bent, replace the array.
	The plate base is not sitting properly on the autosampler.	The plate base should sit flat on the autosampler. When placing the plate on the autosampler, ensure that the pins in the autosampler are properly aligned with the holes at the bottom of the plate base, and that the left and right sides are latched.
	The plate retainer is lifted off the plate base by array.	Securely clip the plate retainer and plate base together.
	The septa is stripped off the CBC.	Ensure that the septa is completely inserted into position. Listen for the light clicking sound that occurs when the septa is pressed down firmly into position.

Table 26 Instrument troubleshooting (continued)

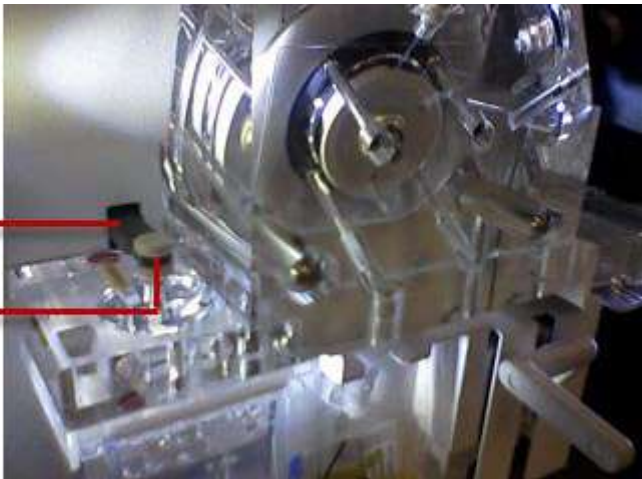

Symptom	Possible cause	Action
Polymer delivery pump (PDP) is extremely noisy and vibrating while running any wizard	Polymer delivery pump block is not pushed back into position after capillary array change	<p>Gently push the buffer-pin valve lever (yoke). If the lever does not move up and down freely, Restart the instrument and the computer. (see page 257).</p> <p>After the instrument has restarted, check the lever movement. If the lever does not move up and down freely, contact Life Technologies.</p> <p>If the lever moves up and down freely, push the upper polymer block all the way back against the wall.</p>
 <p>Figure 33 Buffer-pin valve lever (yoke)</p>		
	<p>The array locking lever is not in the correct position.</p> <p>IMPORTANT! If it the lever is not in correct position, you will receive "Leak error" message.</p>	<p>Lock the lever in the correct position. If this is not possible contact Life Technologies.</p>
<p>When you remove the heat seal from a new pouch, some residual seal remains on top of the pouch.</p> 	<p>The top seal of the pouch has become delaminated and left the polyethylene behind on the pouch cap.</p>	<p>Use a pipette tip to remove the entire seal from the pouch cap before installing on the instrument.</p>

Table 26 Instrument troubleshooting (*continued*)

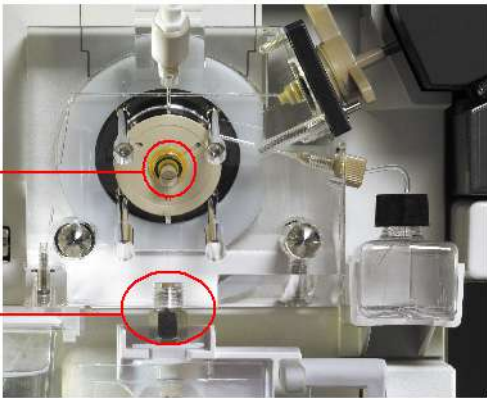
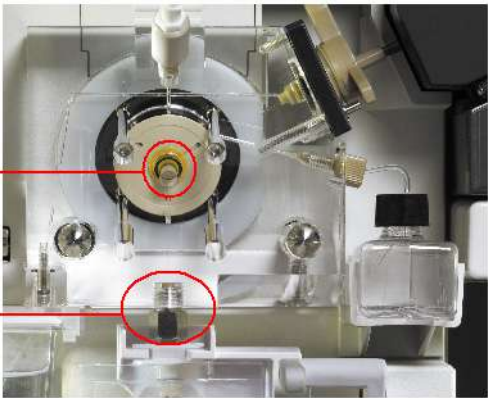
Symptom	Possible cause	Action
Polymer is not pumping properly - wizard fails - filling array	Check Valve is clogged Crystals present in polymer delivery pump path	Run the Wash Pump and Channels wizard. See “Flush the water trap (pump trap)” on page 229 and “Wash the pump chamber and channels” on page 236. If the problem persists, contact Life Technologies.
	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">Debris in pump chamber</div>  </div> <div style="margin-top: 10px;"> <div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">Check valve fitting</div>  </div> </div> <p>Figure 34 Polymer Delivery Pump</p>	
Buffer-pin valve does not move	Polymer crystallizations have formed around the buffer-pin valve	<p>If you see any crystals, leaks, and dried residue around the buffer-pin valve, clean the valve and the array locking lever immediately.</p> <p>Add DI water to the buffer solution to dissolve crystals.</p> <p>Note: Use the lint-free swabs, included in the PDP Cleaning kit (PN 4461875).</p> <p>If leaks persist, contact Life Technologies.</p>
	The vent hole behind the buffer-pin valve is clogged	<p>Clean the vent hole behind the buffer-pin valve with DI water.</p>
	The PDP block is not in the correct position	<p>See “Polymer delivery pump (PDP) is extremely noisy and vibrating while running any wizard” on page 259.</p> <p>If the problem persists, contact Life Technologies.</p>
	Buffer valve leakage	<p>Clean the buffer-pin valve.</p> <p>Perform maintenance tasks routinely as described in “Maintenance schedule” on page 219.</p>
Polymer crystals on the buffer-pin valve	Buffer valve leakage	<p>Clean the buffer-pin valve.</p> <p>Perform maintenance tasks routinely as described in “Maintenance schedule” on page 219.</p>

Table 26 Instrument troubleshooting (continued)

Symptom	Possible cause	Action
Fluid does not move through the polymer delivery pump and into the ABC from polymer or conditioning pouch	Blockage in fluid path or problem with polymer delivery pump	Contact Life Technologies.
Poor signal and resolution after replenishing polymer	The Check Valve is clogged (see Figure 34 on page 260).	Wash the channels using the PDP Cleaning kit (PN 4461875). If the problem persists, contact Life Technologies.
Any of the following visual or audible conditions: <ul style="list-style-type: none"> • Unstable current • Arc-detect errors • A crackling noise at the beginning of electrophoresis • A blue lightning symbol below the oven • An error message regarding electrical current • Electric discharge 	The buffer level is below the fill line.	Verify that buffer level is at or above the fill line.
	The buffer spilled on top of the CBC.	IMPORTANT! Ensure that the environment (humidity) is non-condensing.
	The buffer spilled on top of the Autosampler.	
	Condensation on the CBC.	Wipe away spills, moisture, and condensation with a lint-free lab cloth. If the problem persists, contact Life Technologies.
	Condensation around the septa.	
	Condensation on the lower part of the oven door, near the array header.	
	Condensation inside the oven.	Pipette the buffer from the smaller overflow chamber to the larger chamber. Ensure that the buffer is filled to within ± 1 mm of the fill line. When installing new ABC, tilt the container to move buffer to the larger side of the container as described "Change the anode buffer container (ABC)" on page 225.
	There is not enough fluid in larger chamber of ABC, or the anode buffer has spilled into smaller overflow chamber.	

RFID troubleshooting

Table 27 RFID troubleshooting

Symptom	Possible cause	Action
Unable to read RFID information. “Failure to Read from RFID tag”	Consumable package is improperly installed or label is defective. Polymer/Conditioning reagent pouch is not positioned properly.	Ensure that the RFID label is not visibly damaged and consumable package is properly installed. Ensure that label is close, and parallel, to the instrument. Reposition or re-install pouch, then click Refresh on the Dashboard.
		Restart the instrument and the computer. (see page 257).
		Install a new consumable (if available). If problem persists, contact Life Technologies.
	Malfunctioning RFID label or reader	Place a used CBC, ABC, pouch, or array on the instrument: <ul style="list-style-type: none">• If the instrument can read the RFID label, install a new CBC, ABC, pouch, or array.• If the instrument cannot read the RFID label, contact Life Technologies.

Error messages

Table 28 Error messages

Symptom	Possible cause	Action
“An error has been detected from the instrument.”	Instrument monitor circuit failure	Restart the instrument and the computer. (see page 257).
“Unable to transmit measurement data. Internal data buffer overflow.”	Communications error.	Restart the instrument and the computer. (see page 257).
Electric discharge message during runs.	The ABC buffer may be low.	Replace the ABC. Ensure that the ABC is being replaced per 3500 Series Software 2 notifications.
“Leak error” message.	The array locking lever is not in the correct position.	Secure the array locking lever (see Figure 30 on page 256).

Table 28 Error messages (continued)

Symptom	Possible cause	Action
“Leak error” occurs when capillary arrays are filled with fresh polymer or when replenishing polymer, causing the wizard to fail to complete.	Debris is clogging the check valve (CV) fitting (see Figure 34 on page 260).	<p>While wearing gloves, use a lint-free cloth and water to wipe the CV Fitting.</p> <p>Note: To prevent crystals from forming around the check valve, always install the Conditioning Reagent Pouch after removing a used or a partially used polymer pouch.</p> <p>Completely remove the top seal of the Polymer pouch or Conditioning Reagent Pouch before use.</p> <p>If the problem persists, contact Life Technologies.</p>
	The Yoke is not seated properly on the buffer-pin valve.	<p>Make sure the buffer-pin valve lever (yoke) is seated properly on the buffer-pin valve (see Figure 33 on page 259).</p> <p>If the lever does not move up and down freely, close the door. Restart the instrument and the computer. (see page 257).</p> <p>After the instrument has restarted, check the lever movement. If the lever does not move up and down freely, contact Life Technologies.</p> <p>If the lever moves up and down freely, push the upper polymer block all the way back against the wall.</p>
<ul style="list-style-type: none"> “Leak detected during polymer delivery” “Leak detected during bubble compression” The run aborts.	Bubbles in the polymer system.	Run the Remove Bubbles wizard to clear bubbles.
	Leak in the polymer system.	<p>Check for evidence of leaks.</p> <p>If polymer leak occurred, conduct a water wash and wash the pump trap using the PDP Cleaning Kit (PN 4461875) supplied with the instrument.</p>
	Buffer valve leakage.	<p>Check the buffer-pin valve and see if it closes correctly.</p> <p>Clean the buffer-pin valve.</p> <p>Ensure that the maintenance schedule is followed per 3500 Series Software 2 notifications.</p>
	Filling the array during install array.	Run Fill the Array with fresh Polymer wizard, or run Change Polymer Type wizard.
“Bubble” error	Bubbles present	Run the Remove Bubbles wizard.

Table 28 Error messages (continued)

Symptom	Possible cause	Action
"Java update scheduler" error message	The Java updater is unable to complete the update.	Close the Java update scheduler. Note: The Java update scheduler does not affect the performance of the 3500 Series Software 2 or the quality and accuracy of the data collected.
"Invalid Contents" message In Assign Plate Contents screen when you use Ctrl+D	The first row in you have selected to fill from is empty.	<ul style="list-style-type: none"> Enter sample name or select an assay in the first row in you have selected to fill from. Use the table view to add the assay to the samples
"Injection failed" message. After some of the injections complete.	Capillary RFID cannot be read.	Check the connection between the instrument and computer. Restart the instrument and the computer. (see page 257).
"Instrument is not connected" message after you start 3500 Series Software 2.	Bad connection between the computer and instrument.	Check the connection between the instrument and computer and restart both the instrument and computer.
"Internal buffer data overflow" message.		

Software troubleshooting – general

Table 29 Software troubleshooting – general



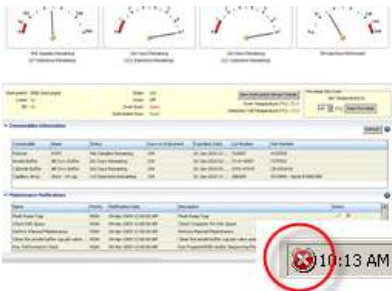

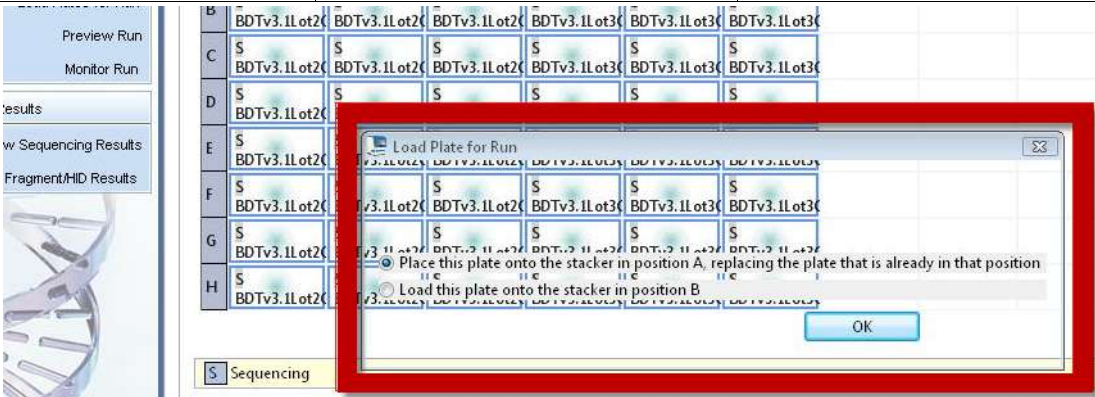
Symptom	Possible cause	Action
When you start the 3500 Series Software 2, "Windows cannot find 3500.exe" message is displayed.	The Norton Antivirus Sonar Protection feature is enabled on the instrument computer.	<ul style="list-style-type: none"> Disable the optional Sonar feature in Norton Antivirus software (contact your IT department for assistance). Contact Life Technologies.
3500 Series Software 2 status icon is  instead of  . 	One or more of the services are stopped.	<p>Right-click the status icon, then select Services. If any item does not display a checkmark, click the item to start the service.</p> 
Print dialog box is not displayed when you select or click Print	Dialog boxes are sometimes displayed behind the main screen	Minimize the main screen.
The Load plate for run message does not display correctly.	The window is not refreshing properly.	Click OK to dismiss the message and continue.

Table 29 Software troubleshooting – general (continued)

Symptom	Possible cause	Action
		
Save option is not available (only Save As) when you edit a plate template from the library	You must select a plate template from the main workflow to edit it.	Go to Define Plate Properties screen ► Open Plate ► select Edit Existing Template.
Specimen name and Amplicon name are specified in File Name Convention but not included in sample name.	The Specimen Name attribute is not functional. Even when selected, specimen name is not included in the file name.	<p>Enter the Specimen name and Amplicon name in the Sample Name field in the Assign Plate Contents screen, Customize Sample information section.</p> <p>To view Specimen Name and Amplicon Name in the Customize Sample Information section, a Sequencing assay must be assigned to a well.</p> <p>Note: The Specimen Name and Amplicon Name fields are available in the Plate View only, not the Table View of the Assign Plate Contents screen.</p>
Software is not behaving as expected	You open the instrument door after you start a run	Do not open the instrument door during a run.
	You restarted the instrument only, not the computer.	<p>Restart the instrument and the computer. (see page 257).</p> <p>Note: Restart the instrument and the computer as part of weekly maintenance.</p>

Dashboard troubleshooting

Table 30 Dashboard troubleshooting

Symptom	Possible Cause	Action
The Days Remaining value for buffer/polymer does not automatically update.	The Days Remaining for buffers updates only when you click Refresh or Start A Run .	Restart the instrument and the computer. (see page 257). Note: Restart the instrument and the computer as part of weekly maintenance.
When you click Refresh on the dashboard, and consumables information is listed as "Unknown."	Bad connection between the computer and instrument.	Check the connection between the instrument and computer.
Consumables status in the Dashboard is not updated.	Dashboard updates every ~10 minutes.	Click Refresh .
After installing new CBC or ABC, the consumables status in the Dashboard is not updated automatically.	Dashboard updates every ~10 minutes.	Click Refresh after changing or installing consumables.

Spatial calibration troubleshooting

Table 31 Spatial calibration troubleshooting

Symptom	Possible cause	Action
"Start" Spatial Calibration button is disabled.	Communication failure between the Data Collection Software and instrument	Check the connection between the instrument and computer. Restart instrument and computer.
Unusual peaks or a flat line for the spatial calibration.	Improper installation of the array window in the detection cell (see Figure 32 on page 257).	Run the Install a Capillary Array wizard to uninstall, then re-install the array. If the calibration fails again: 1. Fill the capillaries with polymer. 2. Repeat the spatial calibration.
	Broken capillary resulting in a bad array fill.	Check for a broken capillary, particularly in the detection cell area. If necessary, replace the capillary array using the Install Capillary Array wizard.
Persistently bad spatial calibration results.	Bad capillary array.	Replace the capillary array using the Install Capillary Array wizard, then repeat the calibration. If the problem persists, contact Life Technologies.
"Spatial Calibration Error" message.	Conditioning reagent is installed. The instrument cannot perform Spatial Calibration with Array fill.	Replace the conditioning reagent with polymer.

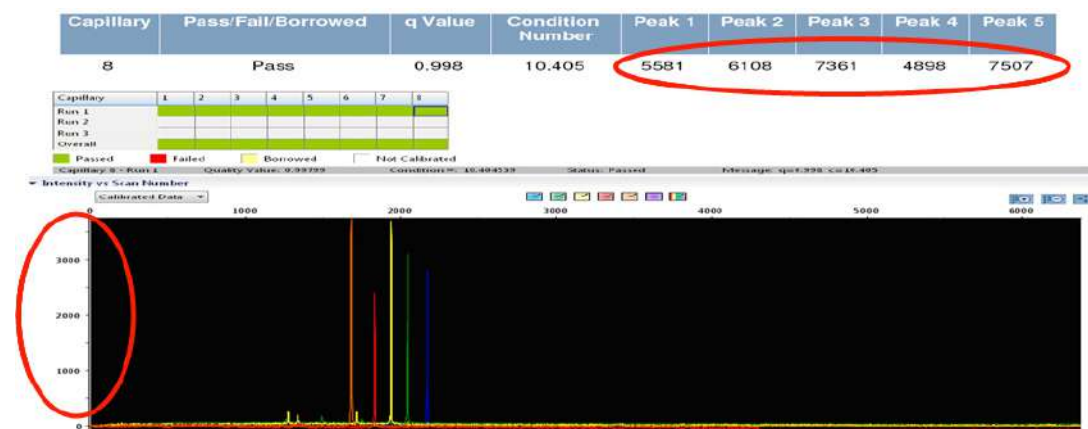
Table 31 Spatial calibration troubleshooting (continued)

Symptom	Possible cause	Action
Spatial calibration takes >5 minutes to complete, and green light goes from blinking to solid	Communication problem between the computer and instrument.	Restart the instrument and the computer. (see page 257).
	Oven is on.	Do not preheat the oven before running the spatial calibration.

Spectral calibration troubleshooting

Table 32 Spectral calibration troubleshooting

Symptom	Possible cause	Action
No signal	Incorrect preparation of sample	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide (see page 21 for storage conditions).
	Bubbles in sample wells	Centrifuge samples to remove bubbles.
	Capillaries are not aspirating sample	Check that sample volume is at least 10 µL. If sample volume is adequate, contact Life Technologies.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Contact Life Technologies.
Peak heights in the Spectral report are different from the values seen when viewing the spectral data in the electropherogram display.	The raw data electropherogram display in the software does not have the Run Scale Divisor applied to the data. The final peak height values displayed in the Spectral report have the Run Scale Divisor applied.	No action.



The Spectral peaks in the raw data view appear to be in the wrong order or there are extraneous peaks	Septa contamination.	Replace the CBC septa. IMPORTANT! Make sure to replace the CBC septa as part of monthly maintenance.
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Table 32 Spectral calibration troubleshooting (*continued*)

Symptom	Possible cause	Action
No history is stored for a failed run.	No history is stored for a failed run.	To retain a history for a failed run, generate a report <i>before</i> you click Reject Results . To generate a report, click View Summary Report or View Detail Report . To save the report electronically, select CutePDF as the printer.
Extra peaks or spikes in the raw data or "Bad dye order detected" error message.	Bubbles in the polymer system.	Run the Remove Bubbles wizard.
	Septa contamination.	Replace the CBC septa.
	Possible contaminant, crystal deposits, or precipitate.	Allow the polymer to come to room temperature. Do not heat to bring to room temperature. Replace the polymer if it has expired.
	Expired polymer.	Replace the polymer.

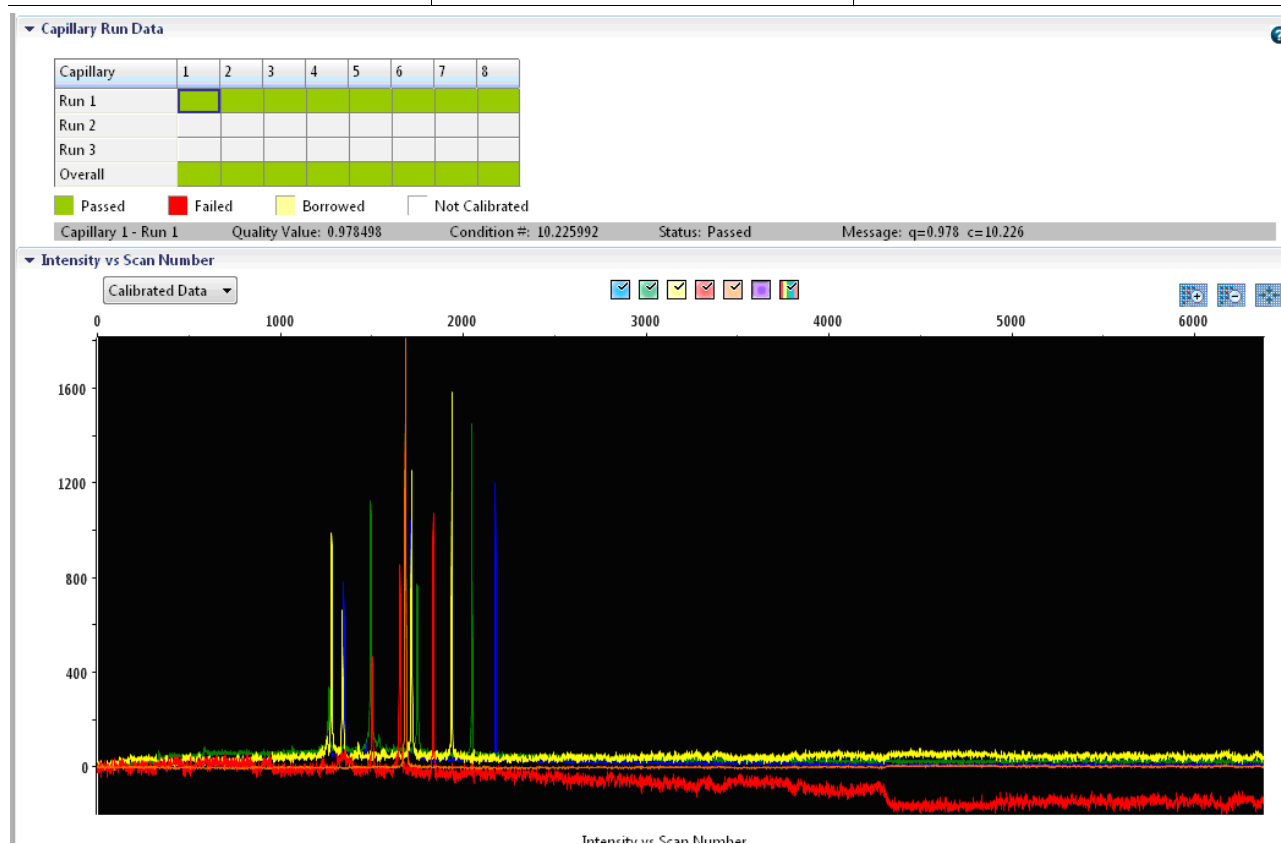
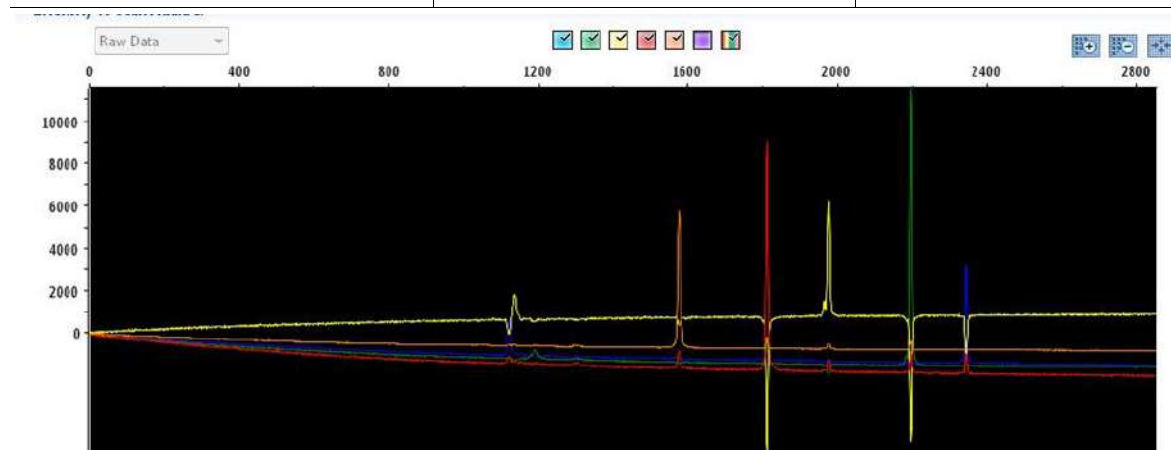


Table 32 Spectral calibration troubleshooting (continued)

Symptom	Possible cause	Action
Spectral calibration fails, or “No spectral files found” message is displayed.	Blocked capillary	Run the Fill Array with Polymer wizard to clear blockage.
	Insufficient filling of array.	Check for broken capillaries. Run the Fill Array with Polymer wizard.
	Expired calibration standards or old reagents.	Check the expiration date and storage conditions of the calibration standards and/or reagents. If necessary, replace with a fresh lot.
Data Error - One or more peaks fall below the minimum required amplitude of 750.	One or more peaks fall below the minimum required amplitude of 750.	Rerun the spectral standards.
Elevated baseline.	Poor spectral calibration.	Perform new spectral calibration.
Pull-down (mirror image) peaks (see the following figure)	The first time you perform a spectral calibration (for each dye set) after installing a new capillary array, you may notice pull-down peaks (or mirror image peaks). These pull-down peaks will eventually correct themselves once the run completes.	No action.



AnyDye Set Spectral Calibration fails.	Problem with spectral calibration	See “Spectral calibration” on page 109.
	AnyDye dye set is not set up correctly.	See “Create a new dye set using the AnyDye template” on page 171.

Sequencing install standard troubleshooting

Table 33 Sequencing install standard troubleshooting

Symptom	Possible cause	Action
No signal	Incorrect preparation of standard	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide (see page 21 for storage conditions).
	Bubbles in sample wells	Centrifuge samples to remove bubbles.
	Capillaries are not aspirating sample	Check that sample volume is at least 10 µL. If sample volume is adequate, contact Life Technologies.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Contact Life Technologies.
The Sequencing install standard (Performance check) fails: Failed capillaries <ul style="list-style-type: none"> One or more (for 8-capillary). Three or more (for 24-capillary). Accept button is not active, Reject button is active.	Blocked capillary	Run the Fill Array with Polymer wizard. Install a new capillary array.
	Insufficient filling of array.	Check for broken capillaries. Run the Fill Array with Polymer wizard.
	Expired sequencing standard or old reagents.	Check the expiration date and storage conditions of the sequencing standard and/or reagents. If necessary, replace with a fresh lot.
	Expired polymer.	Replace the polymer using the Replenish Polymer wizard.
	Bubbles in the polymer system.	Run the Remove Bubbles wizard.
	Possible contaminant or crystal deposits in the polymer.	Properly bring the polymer to room temperature; do not heat. Replace the polymer if it has expired.

Fragment/HID install standard troubleshooting

Symptom	Possible cause	Action
Fragment/HID report contains blank pages or incomplete information.	All dyes are not selected before you generate the report.	Select all dyes, then generate the report.
No signal	Incorrect preparation of sample	Replace samples with fresh samples prepared with fresh Hi-Di™ Formamide.
	Bubbles in sample wells	Centrifuge samples to remove bubbles.
	The capillary tips may not be touching the samples.	Check the volume of your samples. If no results, call your Life Technologies representative.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Call your Life Technologies representative.
Fragment/HID install standard (Performance check) fails.	Blocked capillary	Refill capillary array. You may have to install a fresh array or consider that capillary non-usable for purposes of planning your runs.
	Insufficient filling of array.	Check for broken capillaries and refill the capillary array.
	Expired matrix standards or old reagents.	Check the expiration date and storage conditions of the matrix standards and/or reagents. If necessary, replace with a fresh lot.
	Expired polymer.	Replace the polymer with a fresh lot using the Replenish Polymer Wizard.
	Bubbles in the polymer system.	Select the Bubble Remove Wizard to clear the bubbles.
	Possible contaminant or crystal deposits in the polymer.	Properly bring the polymer to room temperature; do not heat. Replace the polymer if it has expired.

Assign Plate Contents troubleshooting

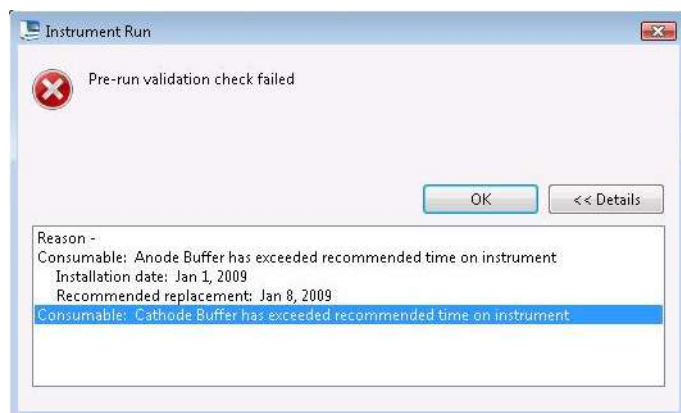
Table 34 Assign Plate Contents troubleshooting

Symptom	Possible Cause	Action
Error message is displayed when you export a newly created plate from the Assign Plate Contents screen.	Plate is not saved.	Save the plate, close the plate, open the plate, then export.

Link/load a plate troubleshooting

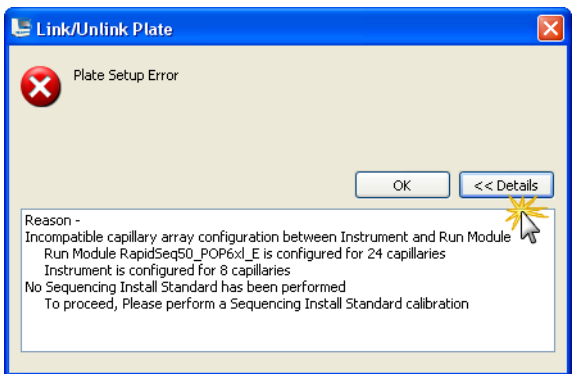
Table 35 Link a plate troubleshooting

Symptom	Possible cause	Action
Plate was linked, but now it is unlinked.	If you access the Load Plates for Run screen from the navigation pane, a plate may not be linked (indicated by the active Link button).	Access the Load Plates for Run screen from the navigation pane and click Link Plate .
"No plate in position A" message.	You physically loaded plate in position B (plate B position) and try to link plate.	Click Link Plates and link the plate directly to position B (plate B position).
"No plate detected" message	The plate is in position B.	Place the plate in position A. See "Load the plate in the instrument" on page 63.
		Manually link the plate to position B. See "Link the plate" on page 64.
	You selected Quick Start. Note: Quick Start expects the plate to be in position A.	Do not use Quick Start, instead open plate and link via the main workflow.
	The Autosampler has not completed initialization.	Wait for the green light to light on the front panel before linking the plate. It takes approximately 10 seconds for the instrument to initialize after the instrument door is closed.
	Malfunctioning plate sensor(s).	Contact Life Technologies.
Pre-run validation check does not display a date for a consumable.	The software does not display a date if it is identical to the preceding date. In the example below, the installation and recommended replacement dates for cathode buffer are identical to the dates for anode buffer.	No action.





Link/Unlink Plate error message.	Listed in Details.	Click Details to determine the cause of the error. When the plate is successfully loaded, the Load Plates for Run screen is displayed.
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Table 35 Link a plate troubleshooting (continued)

Symptom	Possible cause	Action
		
"No plate detected" message	The plate is in position B.	Place the plate in position A
Create Injection List and Start Run buttons dimmed	The Pause After Last Injection preference is set, and the instrument is paused	Go to Monitor Run and resume the run. When the run is complete, Create Injection List and Start Run buttons are active.

Monitor run troubleshooting

Symptom	Possible Cause	Action
The instrument run unexpectedly pauses.	RFID read/write error.	Click Refresh in the Dashboard. If consumables status does not refresh, restart the instrument and the computer. (see page 257).
Only some injections from a series of injections are completed. 3500 Series Software 2 never moves on to the next injection	Bad connection between the instrument and computer	Check the connection between the instrument. Restart the instrument and the computer. (see page 257).
Estimated Time Remaining in Monitor Run is longer than expected.	Estimated Time Remaining is the time remaining in the instrument run. This estimate is adjusted after the completion of every step in an injection.	To view time remaining per injection, scroll to the Time Remaining column in the Injection List Details.
Contents of tooltip in Flag list is truncated	Special characters were included when entering sample information	Use only alpha-numeric characters for sample information. Special characters in sample information fields may not be correctly displayed in other software screens.
Re-inject button is dimmed when you select an injection	Injection contains samples with assays that specify more than one instrument protocol.	Select in the injection list the injection with the instrument protocol of interest, select in the array view the capillary that corresponds to the well of interest, then click Re-inject.

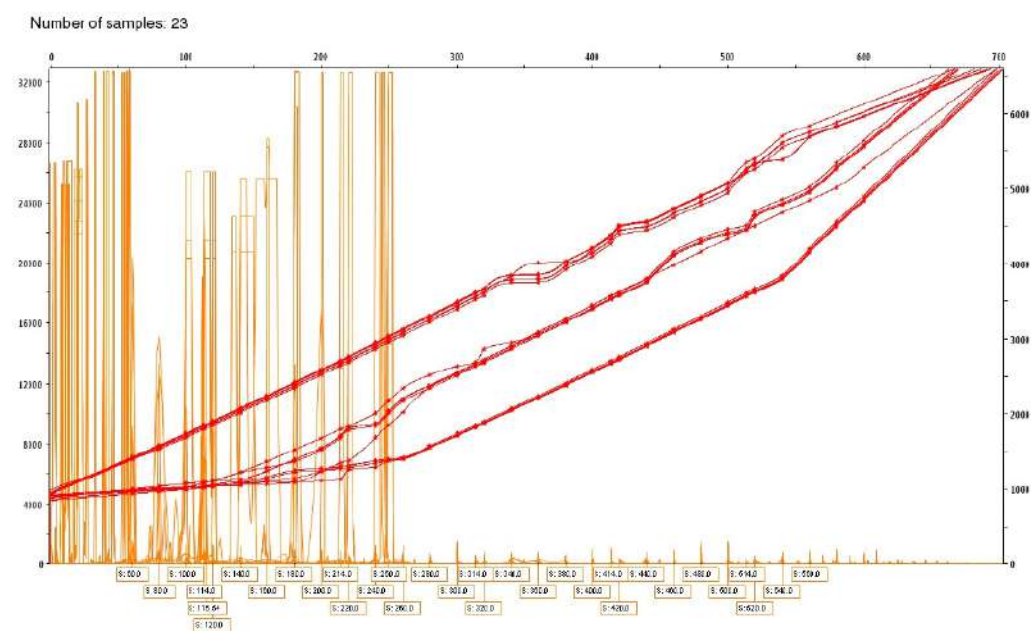
Symptom	Possible Cause	Action
 QV flag for sequencing data, but data quality is good	Contiguous Read Length of the amplicon is less than the Contiguous Read Length Pass value specified in Basecalling Protocol QV settings or Trace Quality preference settings.	<ul style="list-style-type: none"> If the expected read length of the amplicon is <300, adjust the Contiguous Read Length Pass value. If the expected read length of the amplicon ≥ 300, review the sample quality throughout the entire trace.
	Run Time in Instrument Protocol is too short for the amplicon.	Adjust Run Time.
 QV flag for sequencing data	Incorrect Mobility file for dye/polymer is selected in Basecalling Protocol.	Select the correct Mobility file for dye/polymer in Basecalling Protocol, then re-inject.
		Apply correct Basecalling Protocol in secondary sequence analysis software.

Review results troubleshooting

Symptom	Possible Cause	Action
Zoom errors in electropherogram graphical displays (Monitor Run, Review Results, Spectral and Performance Check): <ul style="list-style-type: none"> The zoom feature does not re-baseline the sample data view, or The X axis of the sample plot does not stay at the bottom of the screen. It moves up toward the region the user has zoomed in on, making data difficult to review 	The zoom feature does not re-baseline the sample data view.	No action.
Samples are not imported when you select multiple folders for import	At least one file is not in the correct format for import, therefore no files are imported	Select individual folders or files for import instead of multiple folders.
Plate Owner truncated in Annotation tab>Run Configuration	Special characters were included when entering plate information	Use only alpha-numeric characters for plate information. Special characters in plate information fields may not be correctly displayed in some software screens.
Sample files are not displayed when imported.	You imported (.hid) files and you did not click HID Samples.	Click HID Samples .
Peaks are not labeled when you access the screen.	Labels are not automatically applied.	See "Label peaks" on page 99.
x and y scaling plot settings are not applied when you click Apply.	Scaling settings are applied only when you click Zoom.	Click Zoom .

Symptom	Possible Cause	Action
The sizing quality result reported in the 3500 Series Software 2 differs from the sizing quality result for reported in the GeneMapper® ID-X Software.	You imported (.fsa) files instead of (.hid) files into the GeneMapper® ID-X Software. The 3500 Series Software 2 <i>does not</i> consider the presence of broad peaks when determining sizing quality for fragment analysis data, therefore the sizing quality result reported in the 3500 Series Software 2 will differ from the sizing quality result reported in the GeneMapper® ID-X Software, which considers broad peaks in sizing quality.	No action.
Sizing Overlay report displays data for all capillaries including those that fail sizing.	Plots displayed in Sizing Overlay Plot are based on the samples selected.	Select only the capillaries that pass sizing to include in the report.

Size Standard Overlay Report



Data/electropherogram troubleshooting

Table 36 Data/electropherogram troubleshooting

Symptom	Possible cause	Action
Signal too high.	Sample concentration is too high.	Dilute the sample.
		Decrease the injection time.
	Too much DNA added to the reaction, resulting in uneven signal distribution.	Optimize reaction conditions.
No signal.	Blocked capillary.	Run the Fill Array with Polymer wizard. Install a new capillary array.
	Bent capillary array tips or cracked or broken capillary array.	Visually inspect the capillary array, including the detector window area for signs of breakage. Replace the capillary array.
	Failed reaction	Repeat reaction.
Low signal.	Degraded formamide.	Use a fresh aliquot of Hi-Di™ Formamide (see page 21 for storage conditions).
	Not enough sample: Pipetting error.	Prepare new sample.
	Sample has high salt concentration.	Dilute or desalt samples.
	Insufficient mixing.	Vortex the sample thoroughly, and then centrifuge the tube to condense the sample to the bottom of the tube.
	Weak amplification of DNA.	Reamplify the DNA.
		Check DNA quality.
	Sample volume is <10 µL.	Check that sample volume is at least 10 µL.
Elevated baseline.	Autosampler out of calibration.	Contact Life Technologies.
	Possible contaminant in the polymer path.	Run the Wash Pump and Channels wizard.
	Possible contaminant or crystal deposits in the polymer.	Polymer has been on the instrument for more than the recommended 7 days.
		Replace the polymer if it has expired.
Loss of resolution.	Poor spectral calibration.	Perform new spectral calibration.
	Too much sample injected.	Dilute the sample and re-inject.
	Poor quality water.	Use distilled or deionized water.
	Degraded polymer.	Replace polymer.
	Capillary array used for more than 160 injections.	Replace the capillary array. Run the Install Capillary Array wizard.
	Degraded formamide.	Prepare fresh Hi-Di™ Formamide (see page 21 for storage conditions) for sample preparation.
	Sample has high salt concentration.	Dilute or desalt samples.

Table 36 Data/electropherogram troubleshooting

Symptom	Possible cause	Action
Poor resolution in some capillaries.	Insufficient filling of capillary array.	Tighten the connectors and array locking lever. Run the Fill Array with Polymer wizard and look for polymer leakage. Check for broken capillaries, run the Install Capillary Array wizard if needed.
	Poor quality samples.	Re-inject the same samples.
	Leak in system.	Check the sample preparation. Tighten the connectors and array locking lever.
No current.	Not enough buffer in ABC.	Tighten the connectors and array locking lever. Ensure that the buffer is filled up to the fill line. See "Check buffer fill levels" on page 40.
	Bubble(s) present in the lower polymer block and/or the array and/or channels.	Pause the run and inspect for bubbles in the tubing connectors. Run the Remove Bubbles wizard.
Elevated current.	Degraded polymer.	Run the Replenish Polymer wizard.
	Arcing in the lower polymer block.	Inspect the lower polymer block for discoloration or damage. Contact Life Technologies.
Fluctuating current.	Bubble in polymer block.	Pause run and inspect for bubbles hidden in the tubing connectors. Run the Remove Bubbles wizard.
	Slow leak	Check polymer blocks for leaks. Tighten the connectors and array locking lever.
	Not enough buffer in ABC.	Ensure that the buffer is filled up to the fill line. See "Check buffer fill levels" on page 40.
	Arcing	Check for moisture in and around the septa, the CBC, the oven, and the autosampler. Wipe condensation.
Poor performance of capillary array used for fewer than 100 runs.	Poor quality samples, possible cleanup problems.	Desalt samples.
	Improperly stored formamide.	Prepare fresh Hi-Di™ Formamide (see page 21 for storage conditions) for sample preparation.
	Leak in system.	Tighten the connectors and array locking lever.

Table 36 Data/electropherogram troubleshooting

Symptom	Possible cause	Action
Migration time becomes progressively slower.	Leak in system.	Tighten the connectors and array locking lever.
	Improper filling of the system with polymer.	Polymer delivery pump may need to be serviced. If the issue persists, contact Life Technologies.
Migration time becomes progressively faster.	Buffer valve leakage.	Ensure the buffer-pin valve is closed correctly.
Extra peaks in the electropherogram.	Data off scale.	Dilute the sample and re-inject the sample.
	Possible contaminant in sample.	Re-amplify the DNA.
	Sample re-naturation.	Heat-denature the sample in properly stored formamide (see page 21 for storage conditions) and immediately place on ice.
Electrophoresis current is unstable.	Bubbles in the polymer system.	Run the Remove Bubbles wizard.
Electrophoresis failure.	Buffer below fill line.	Ensure that the buffer is filled up to the fill line. See "Check buffer fill levels" on page 40.
	There is not enough fluid in larger chamber of ABC, or the anode buffer has spilled into smaller overflow chamber.	Pipette the buffer from the smaller overflow chamber to the larger chamber. Ensure that the buffer is filled to within ± 1 mm of the fill line. When installing new ABC, tilt the container to move buffer to the larger side of the container as described in "Change the anode buffer container (ABC)" on page 225.
Extra peaks in the electropherogram.	Data off scale.	Dilute the sample and re-inject the sample.
	Possible contaminant in sample.	Re-amplify the DNA.
	Sample re-naturation.	Heat-denature the sample in good quality formamide and immediately place on ice.

Audit troubleshooting

Table 37 Audit troubleshooting

Symptom	Possible Cause	Action
"Export did not complete successfully"	You exported records for samples that are not in their original location (samples have been deleted or moved).	Return sample data files to their original location, then export again.

Electronic signature troubleshooting

Symptom	Possible Cause	Action
The dye set calibrated is not listed in a spectral calibration E-Signature record.	The E-Signature function creates a record when a spectral calibration is performed, but does not record the dye set calibrated.	To include the dye set calibrated in the E-Signature record, enter the dye set in the Comments field.

Manual commands troubleshooting

Table 38 Manual commands troubleshooting

Symptom	Possible Cause	Action
When you select Tools ► Manual Commands, Set defined command for Consumables, then select a Read Command, the information displayed is not readable.	The feedback from Consumables Read Tag commands does not display valid information.	Refer to the Dashboard for consumables RFID tag information.

Section B.2 Troubleshooting procedures

View the log files

The 3500 Series Software 2 generates the following log files that you view using a text editor such as Wordpad:

- **3500UsageStatistics.txt**—Provides a summary of the number of plates run and number of run types

Stored in: <<install drive>>:\Applied Biosystems\3500\LogFiles (you can also view this log from the Maintenance workflow under Planned Maintenance>Usage Statistics)

- **3500ConsumableUpdates.txt**—Provides a summary of consumables installation information and dates.

Stored in: <<install drive>>:\Applied Biosystems\3500\LogFiles

View instrument sensor details

Click **View Instrument Sensor Details** in the Dashboard to display instrument information.

View Instrument Sensor Details

Run status of the instrument is displayed while a run is in progress.

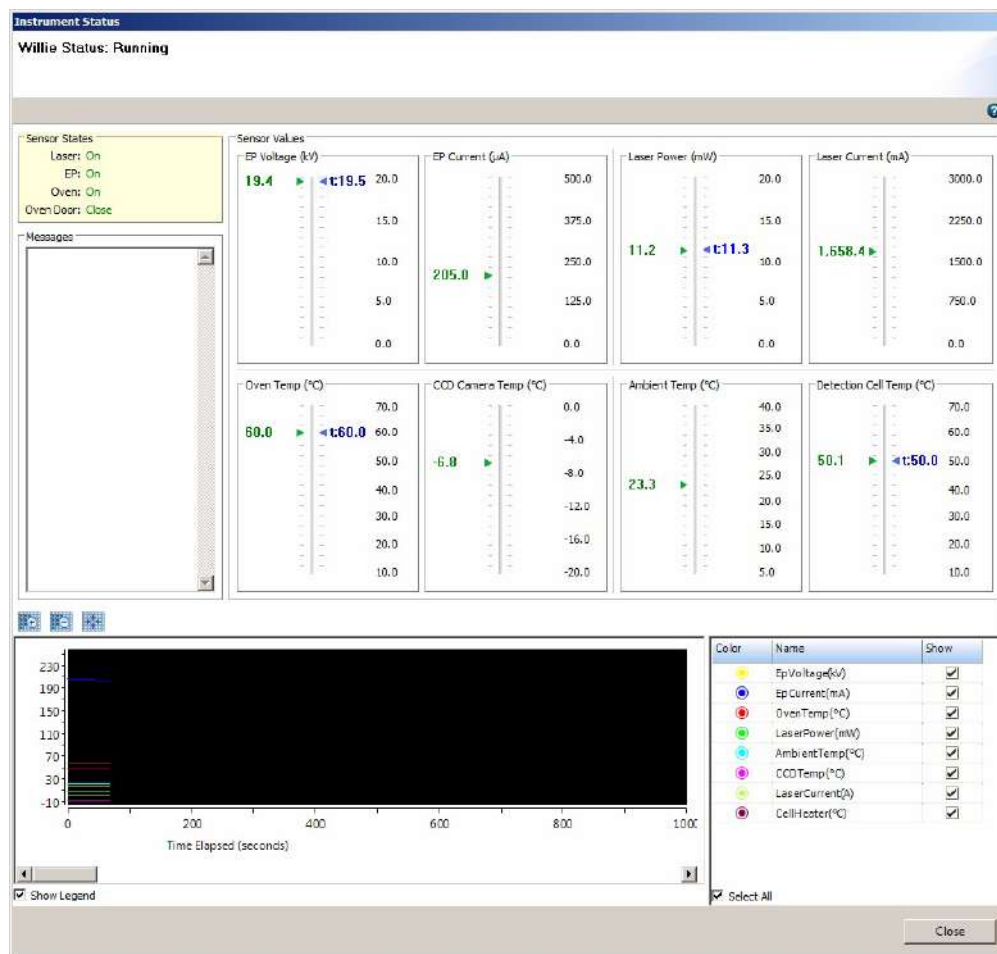
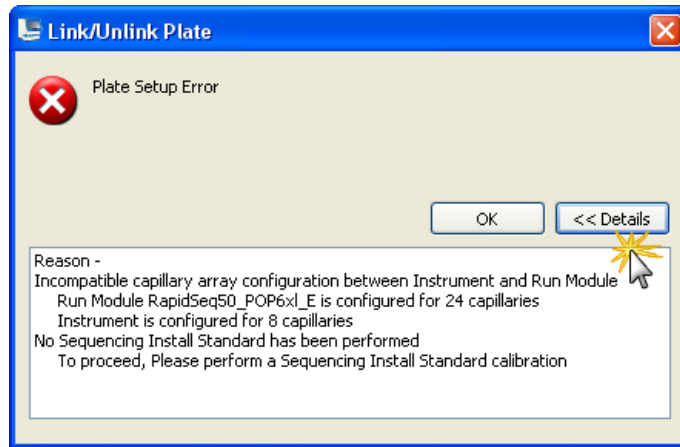


Figure 35 Instrument sensor details

Review error message details

Error messages in the 3500 Series Software 2 include a Details button. Click Details to display more information about an error message.



Reset the instrument

Reset the instrument when:

- There is a fatal error as indicated by the red status light
- The instrument does not respond to the Data Collection software

1. Shut down the computer.
2. Close the instrument doors.
3. Reset the instrument with the Reset button, as shown.

Note: The Reset button is accessible through a small hole to the left of the Tray button.



Reset button



Instrument Specifications

Instrument specifications

Table 39 Applied Biosystems 3500 / 3500xL Genetic Analyzer physical dimensions, weight, and power consumption

Parameter	Instrument Footprint	Recommended Clearance
Depth	61 cm (24 in.)	25.4 cm (10 in.) [†]
Width	61 cm (24 in.) (closed door) 122 cm (48 in.) (open door)	158 cm (62 in.) [‡]
Height	72 cm (28.3 in.)	0 cm (0 in.)
Weight	≈82 kg (180 lbs)	

[†] At the rear of the instrument to ensure adequate airflow and cooling.

[‡] For the instrument, computer, and computer monitor.

Table 40 Computer dimensions and weight

Parameter	Computer	Monitor	Keyboard
Depth	44.5 cm (17.52 in.)	19.3 cm (7.6 in.)	44.7 cm (17.5 in.)
Width	18.7 cm (7.36 in.)	44.7 cm (17.5 in.)	15.25 cm (6 in.)
Height	41 cm (16.1 in.)	36.6 cm (14.4 in.)	5 cm (2 in.)
Weight	10.9 kg (24 lbs)	6.9 kg (15.2 lbs)	0.09 kg (0.2 lbs)

Table 41 Applied Biosystems 3500 / 3500xL Genetic Analyzer operating specifications

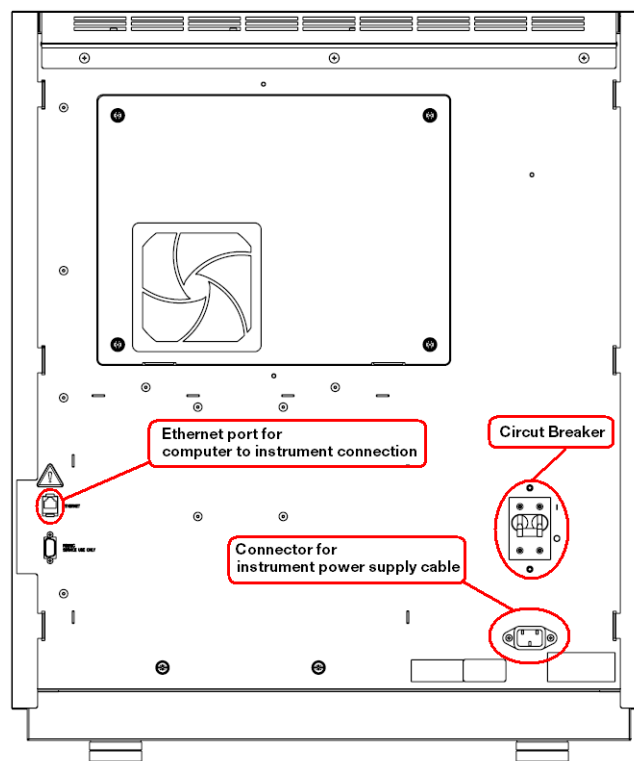
Component	Specification
Laser	Long-life, single-line 505 nm, solid-state laser excitation source
Electrophoresis Voltage	Up to 20 kV
Oven Temperature	Active temperature control from 18°C to 70°C
Minimum Computer Requirements	<ul style="list-style-type: none"> Hardware: Dell OptiPlex® XE, E8400 3 GHZ Processor Operating system: Windows 7 SP1, 32-bit Installed RAM: 4 GB Hard drive: 500GB SATA 3.0Gb/s and 8MB Data Burst Cache
Minimum monitor resolution	1280 x 1024 pixel resolution

Environmental requirements

Table 42 Environmental requirements

Condition	Requirement
Installation site	Indoor use only
Altitude	Safety tested up to 2000 m
Electrical ratings	Power cord with ground pin required <ul style="list-style-type: none"> Instrument – AC 100-240 V $\pm 10\%$, 50/60 Hz, 3.1 A, power rated 320 VA - FROM SPG Maximum current - 15A Maximum power dissipation - 417 VA, 371 W (approximately, not including computer and monitor) Computer – AC 100-240 V $\pm 10\%$, 50/60 Hz, 2.1 A, power rated 125 VA Monitor – AC 100-240 V $\pm 10\%$, 50/60 Hz, 1.5 A, power rated 65 VA
Mains AC line voltage tolerances	Up to ± 10 percent of nominal voltage
Transient category	Installation categories II
Pollution degree	2
Operating conditions	15 to 30°C (59 to 86°F) (Room temperature should not fluctuate $\pm 2^\circ\text{C}$ during an instrument run) 20 to 80% relative humidity, noncondensing
Transport and storage conditions	-30 to $+60^\circ\text{C}$ (-22 to $+140^\circ\text{F}$) Minimum 20% relative humidity, maximum 85% (non-condensing)

Power and communication connections







Part Numbers

Plates, bases, retainers, and septa

Table 43 Plates and tubes for use with the Applied Biosystems 3500 / 3500xL Genetic Analyzer

Part Description	General purpose supply, obtain from any laboratory supplier
	IMPORTANT! The customer is responsible for validation of all general purpose supplies before use, and for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.
Plates	96-well PCR microtiter plate, standard or optical-grade polypropylene, 0.1 mL or 0.2 mL, half- or semi-skirted design, with or without barcode.
	384-well PCR microtiter plate, standard or optical-grade polypropylene, 0.02 mL, fully-skirted design, with or without barcode
Tubes	8-strip PCR tubes, standard- or optical-grade polypropylene, 0.1 mL
	8-strip full-height PCR tubes, standard- or optical-grade polypropylene, 0.2 mL
Tube caps	8-tube PCR strip caps, domed, standard- or optical-grade polypropylene, for 0.1 mL or 0.2 mL 8-strip PCR tubes

Table 44 Bases, retainers, and septa for use with the Applied Biosystems 3500 / 3500xL Genetic Analyzer

Part Description	Part Number
Retainer and base (Standard), 96-well	4410228
Retainer and base (Fast), 96-well	4409530
Septa, 96-well	4412614

Instrument consumables

Note: For reagent or consumable storage conditions and shelf-life expiration date, see the package label or “Instrument reagents and consumables” on page 19

Table 45 Instrument consumables

Name	Part Number
Anode buffer container	4393927
Capillary array, 8-Capillary, 36 cm	4404683
Capillary array, 8-Capillary, 50 cm	4404685
Capillary array, 24-Capillary, 36 cm	4404687
Capillary array, 24-Capillary, 50 cm	4404689
Cathode buffer container	4408256
Conditioning reagent	4393718
Polymer, POP-6™ (960 sample pouch)	4393712
Polymer, POP-6™ (384 sample pouch)	4393717
Polymer, POP-7™ (960 sample pouch)	4393714
Polymer, POP-7™ (384 sample pouch)	4393708
Polymer, POP-4® (960 sample pouch)	4393710
Polymer, POP-4® (384 sample pouch)	4393715
Hi-Di™ Formamide - 5-mL bottle (pack of four)	4440753

Sequencing analysis reagents and consumables

Table 46 Sequencing analysis reagents and consumables

Name	Part Number
BigDye® Terminator (BDT) v1.1 Sequencing Install Standard (long read)	4462113
BigDye® Terminator (BDT) v3.1 Sequencing Install Standard (long read)	4404312
BigDye® Terminator (BDT) v3.1 Matrix Standard	4336974
BigDye® Terminator (BDT) v1.1 Matrix Standard	4336824
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 24 reactions	4337454
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 100 reactions	4337455
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 1000 reactions	4337456
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 5000 reactions	4337457
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 24 reactions	4337449
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 100 reactions	4337450
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 1000 reactions	4337451
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 5000 reactions	4337452
BigDye® Direct Cycle Sequencing Kit 24 reactions	4458689
BigDye® Direct Cycle Sequencing Kit 100 reactions	4458687
BigDye® Direct Cycle Sequencing Kit 1000 reactions	4458688

Fragment and HID analysis reagents

Table 47 Fragment analysis and HID standards

Name	Part Number
Fragment Analysis Matrix Standards (5-Dye) -DS-02	4323014
Fragment Analysis Matrix Standards (4-dye) - DS-32 (Dye Set F)	4345831
Fragment Analysis Matrix Standards (5-Dye) -DS-33 (Dye Set G5)	4345833
Fragment Analysis Matrix Standards (6-Dye) -DS-36	4426042
Fragment Analysis Installation kit (5-Dye) -DS-33 with GeneScan™ 600 LIZ® Size Standard v2.0	4376911
GeneScan™ 120 LIZ® Size Standard	4322362
GeneScan™ 500 ROX™ Size Standard	401734
GeneScan™ 600 LIZ® Size Standard v2.0 (required for Normalization, but may be used without Normalization)	4408399
GeneScan™ 1200 LIZ® Size Standard	4379950

E

Normalization

Overview of the normalization feature

For fragment analysis applications, the 3500 Series Software 2 includes a normalization feature for use with the GeneScan™ 600 LIZ® Size Standard v2.0 (GS600 LIZ v2). This feature attenuates signal variations associated with instrument, capillary array, sample salt load, and injection variability between capillaries and instruments. Normalization can be applied during primary analysis of the data.

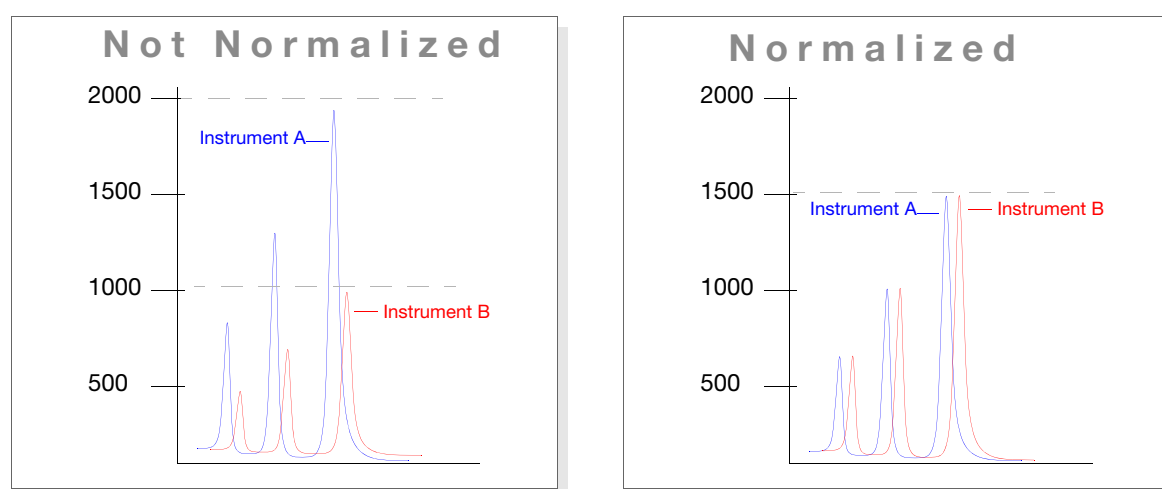


Figure 36 Comparison of fragment analysis results with and without the normalization feature

To use the normalization feature, prepare each sample with the GS600 LIZ v2 size standard, then specify the appropriate normalization size standard for file primary analysis. The GS600 LIZ® v2 reagent can function as an internal standard for signal-height normalization as well as a size standard for peak sizing.

When to use the normalization feature

The 3500 Series Software 2 provides three normalization size-standard definition files that you can specify for primary analysis of samples prepared with the GS600 LIZ v2 size standard and the G5 and J6 dye sets:

- Fragment (POP-6™ and POP-7™ polymer):
 - GS600LIZ+Normalization
 - GS600(60-600)LIZ+Normalization – For applications that have primer peaks that obscure the 20 and 40-mer peaks of the GS600 LIZ v2 size standard.
- Fragment (POP-4® polymer):
 - GS600(80-400)LIZ+Normalization
- HID (POP-4® polymer):
 - GS600(80-400)LIZ+Normalization

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, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
- All testing should be performed in accordance with local, regional and national acceptable laboratory accreditation standards and/or regulations.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with user attention words described in the “About This Guide” section to highlight important safety information. The following table gives the meaning of these symbols.



Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, hot surface	Attention, surface chaude
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Laser radiation	Rayonnement laser
	Caution, piercing hazard	Attention, danger de perforation
	Potential biohazard	Danger biologique potentiel
	Ultraviolet light	Rayonnement ultraviolet

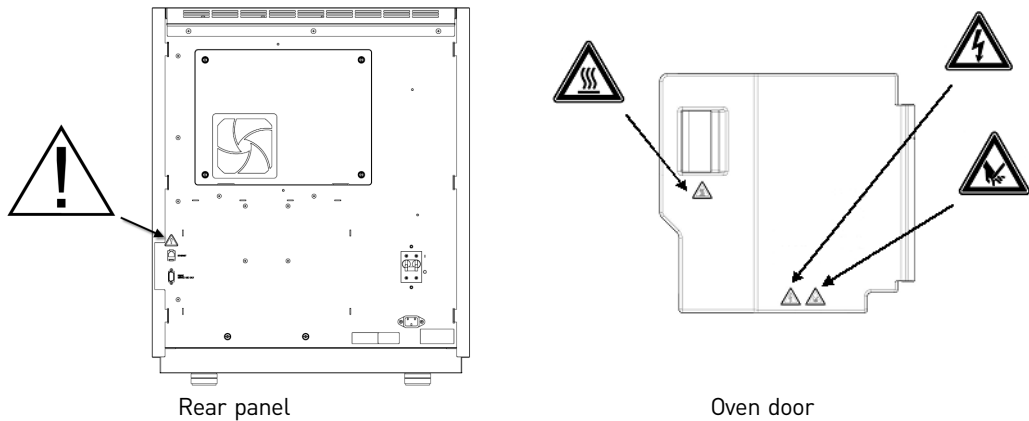
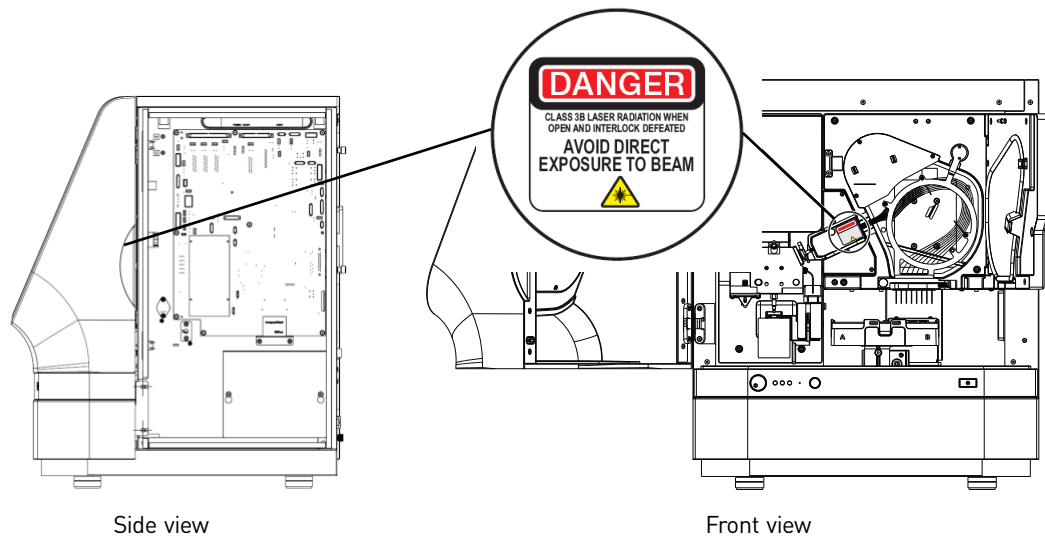


Symbol	English	Français
	On	On (marche)
	Off	Off (arrêt)
	On/Off	On/Off (marche/arrêt)
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif
	Terminal that can receive or supply alternating or direct current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant continu ou alternatif
	<p>Do not dispose of this product in unsorted municipal waste</p> <p>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.</p>	<p>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.</p> <p>CAUTION! Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.</p>
Conformity mark	Description	
	Indicates conformity with safety requirements for Canada and U.S.A.	

Safety alerts on this instrument

The following table shows the location of safety alerts found on the instrument. See “Symbols on this instrument” on page 293 for more information.

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



Instrument safety

General



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

Physical injury



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

Improper lifting can cause painful and permanent back injury.

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

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



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

Electrical



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

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



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



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

Cleaning or decontaminating the instrument



CAUTION! CLEANING AND DECONTAMINATION.

- If hazardous materials are spilled onto the instrument, the instrument should be appropriately decontaminated.
- Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.
- Care should be taken to not use decontamination or cleaning agents which would cause a hazard as a result of a reaction with parts of the equipment or with material contained in it.
- For the protection of others, ensure the instrument is properly decontaminated prior to having the instrument serviced at your facility or before sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan.
- Decontamination forms may be requested from customer service.

Laser



WARNING! LASER HAZARD. Under normal operating conditions, the <Short form of the primary product name> is categorized as a Class I laser product. However, removing the protective covers and (when applicable) defeating the interlock(s) may result in exposure to the internal Class 3B laser. Lasers can burn the retina, causing permanent blind spots. Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure. To ensure safe laser operation:

- Never look directly into the laser beam.
- Do not remove safety labels, instrument protective panels, or defeat safety interlocks.
- The system must be installed and maintained by a Life Technologies Corporation Technical Representative.

Life Technologies Corporation Technical Representatives are instructed to:

- Remove jewelry and other items that can reflect a laser beam into your eyes or those of others
- Wear proper eye protection and post a laser warning sign at the entrance to the laboratory if the laser protection is defeated for servicing

DO NOT operate the laser when it cannot be cooled by its cooling fan; an overheated laser can cause severe burns on contact.

Note the laser warnings provided in “Safety alerts on this instrument” on page 294.



Safety and electromagnetic compatibility (EMC) standards

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

Safety

Reference	Description
UL 61010-1 CSA C22.2 No. 61010-1	<i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i>
UL 61010-2-010	<i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</i>
21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007, as applicable	U.S. FDA Health and Human Services (HHS) “Radiological health performance standards for laser products” and “Radiological health performance standards for specific purpose laser products”

EMC

Reference	Description
FCC Part 18	U.S. Standard “Industrial, Scientific, and Medical Equipment”

Chemical safety



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

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



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



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

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

In the EU:

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

Documentation and Support


Related documentation

The following related documents are shipped with the system:


Document	Part no.	Description
<i>Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide</i> (Part no. 4401689) Note: The purpose of the Site Prep Guide is to help you prepare your site for installation of the 3500 Series Genetic Analyzer. For specific details about your system, please refer to this user guide.	4457393	Provides information about the space, environmental, and electrical requirements needed to support the 3500 Dx Series 2011 Genetic Analyzer.
<i>Polymer Delivery Pump (PDP) Cleaning Instructions for Use</i>	4457387	Provides information on using the PDP cleaning kit provided with the instrument (Part No. 4461875).

Note: For additional documentation, see “Obtain support” on page 302.

Obtain information from the Help system

The 3500 Series Software 2 interface has instructions guiding the user through basic tasks of the workflow and expanded help information for complex decisions and operations. Users can access these instructions by clicking the help icon .

The 3500 Series Genetic Analyzer has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click  in the screens of the 3500 Series Software 2 window.
- Select **Help** ▶ **Help Contents**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the contents
- Searching for a specific topic
- Searching an alphabetized index

Obtain HID assays and protocols

For the latest validated HID Assays and protocols, get the 3500 HID Updater Software at www.lifetechnologies.com (go to Technical Resources ▶ Software Downloads ▶ 3500 Series Genetic Analyzers for Human Identification).

Obtain SDSs

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

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

Obtain support

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

www.lifetechnologies.com/support

At the website, you can:

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

Limited product warranty

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

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