Applied Biosystems 3500/3500xL Genetic Analyzer User Guide

User Guide

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Preface

Safety information

Note: For general safety information, see this Preface and Appendix F, "Safety" on page 315. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see Appendix F, "Safety" on page 315 for the complete alert on the chemical or instrument.

Safety alert words

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

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

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

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

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

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments* (see "Safety symbols" on page 315).

MSDSs

The MSDSs (Material Safety Data Sheets) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see "MSDSs" on page 329.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Safety labels on instruments

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

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**CAUTION!** Potential slipping hazard.	**ATTENTION!** Risque potentiel d'avoir un sol glissant.
	**CAUTION!** Hot surface.	**ATTENTION!** Surface brûlante.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems.
	**CAUTION!** Class 2(II) visible and/or invisible radiation present. Do not stare directly into the beam or view directly with optical instruments.	**ATTENTION!** Rayonnement visible ou invisible d'un faisceau. Ne pas regarder le faisceau directement ou au travers d'un instrument optique.
	DANGER! Class 3B (III) visible and/or invisible radiation present. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau de Classe 3B (III) en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Sharp object.	**AVERTISSEMENT!** Objet Sharp.

About the product

The Applied Biosystems 3500/3500xL Genetic Analyzers is an automated 8 and/or 24 capillary instrument designed for a wide range of sequencing and fragment analysis applications.

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Purpose of this guide

The *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* provides step-by-step instructions for preparing and analyzing a sample. It is designed to help you learn how to use the instrument.

CAUTION! 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 (Applied Biosystems).

Audience

This user guide is written for principle investigators and laboratory staff who are planning to operate and maintain the Applied Biosystems 3500/3500xL Genetic Analyzers.

Assumptions

The *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* assumes that your 3500 or 3500xL analyzer has been installed by an Applied Biosystems service representative.

This guide also assumes you have the following background:

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

How to use this guide

Text conventions

This guide uses the following conventions:

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

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

  Before changing reagents, *always* determine what chemicals have been used in the instrument.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

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

User attention words

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

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

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

Table of Acronyms

The following table explains the acronyms used in the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide*.

Acronym	Definition
ABC	Anode Buffer Container
BDT	BigDye® Terminator Kit
BDX	BigDye® Xterminator™
Cap	Capillaries
CBC	Cathode Buffer Container
CV/Fitting	Check Valve pouch attachment fitting
EPT	ElectroPhoresis Telemetry
FR	Factory Repeating
MicroSeq® kit (or other product name)	Microbial Sequencing
NIC	Network Interface Card
NT	Nucleoid Type Nucleotide Base Color (A, G, C, T)
Pe	Probability of error
QV	Quality Value

Acronym	Definition
GM	GeneMapper® Software
GMIDx	GeneMapper® IDx
POP™	Polymer (Brand name of the polymer)
PPS	Power Protection System
SAE	Security, Administration, Electronic signature

How to obtain support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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

How to obtain more information

For detailed information on preparing for installation, refer to the *Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide* (4401689).

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

Instrument and Software Description 1

System description

The 3500 or 3500xL analyzer is shipped with the following system components:

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

Instrument description

The Applied Biosystems 3500/3500xL Genetic Analyzers are fluorescence based DNA analysis instrument using capillary electrophoresis technology with 8- or 24-capillaries.

For detailed dimensions of the instrument, refer to the *Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide* (4401689).

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

Instrument interior components

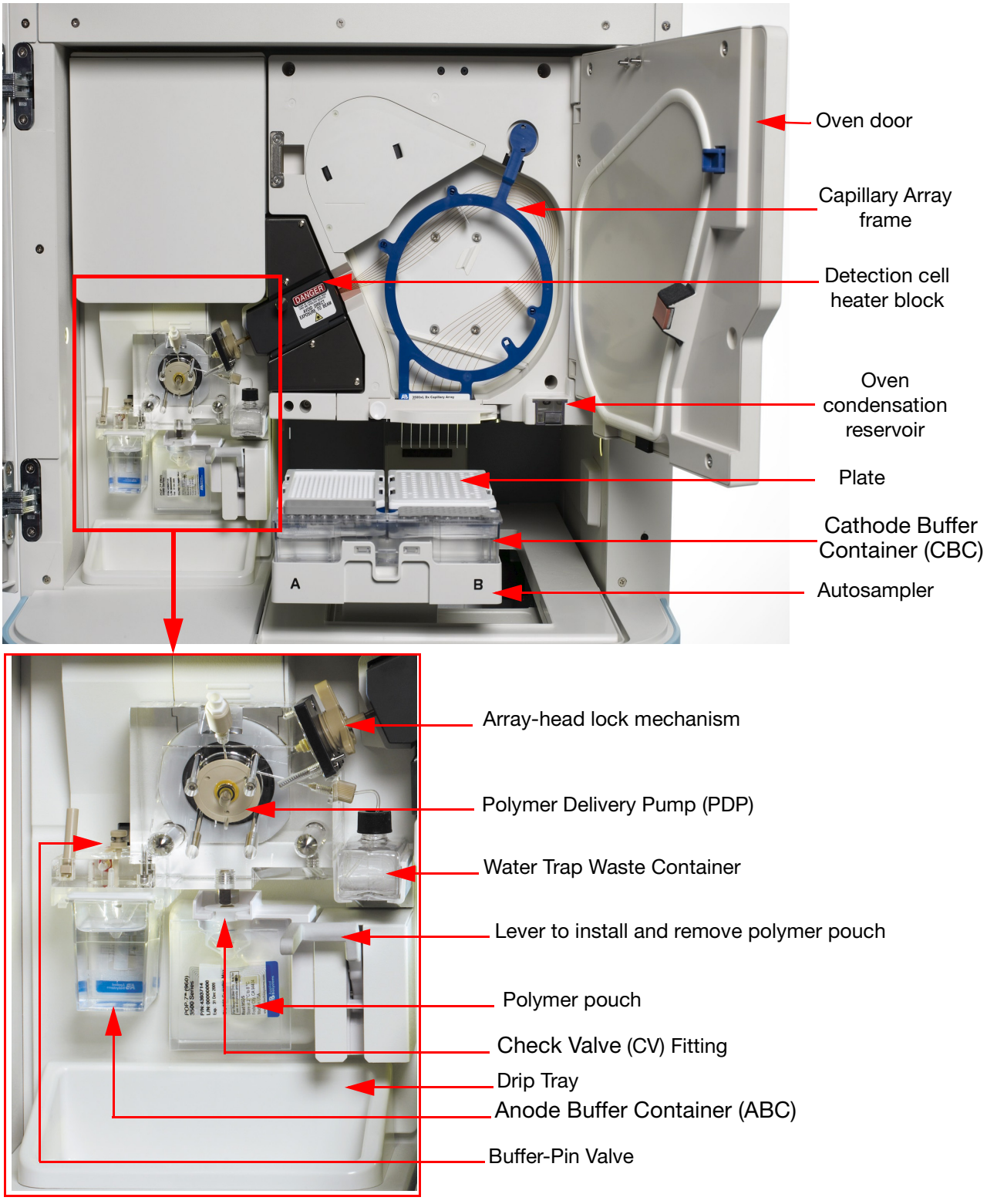


Figure 1 Instrument interior components

Instrument parts and functions

Table 1 Instrument parts and functions

Part	Function
Autosampler	Holds the sample plates and Cathode Buffer Container (CBC) and moves to align the plates and CBC with the capillaries.
Oven	Maintains uniform capillary array temperature.
Oven condensation reservoir	Collects condensation from the oven.
Pump block	Includes the displacement pump chamber, polymer chambers, piston water seal, syringe fitting array attachment point (array port), the lower polymer block, and the CV/Fitting (Check Valve pouch attachment fitting).
Detection cell heater block	Holds the detection cell in place for laser detection and maintains the detection cell temperature of 50 °C.
Polymer Delivery Pump (PDP)	Pumps polymer into the array and allows for automated maintenance procedures.
Lower polymer block	Contains the buffer valve, anode electrode, buffer gasket, and holds the anode buffer container.
Radio Frequency Identification (RFID)	RFID tags to read the following information for primary instrument consumables: • Lot numbers • Serial numbers • Dates (expiration) • Capacity (usage) The primary consumables are: • Capillary Array • Cathode Buffer Container (CBC) • POP™ Polymer • Anode Buffer Container (ABC)
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 and 36 cm length). **Note:** The 36 cm capillary is for HID applications, only.
Anode Buffer Container (ABC)	The Anode Buffer Container (ABC) contains 1× running buffer to support all electrophoresis applications on the instrument. It has a built-in overflow chamber to maintain constant fluid height.
Cathode Buffer Container (CBC)	The Cathode Buffer Container (CBC) contains 1× running buffer to support all electrophoresis applications on the instrument.
Polymer pouch	Supplies polymer to the Polymer Delivery Pump.
Conditioning reagent	The pouch 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.

Theory of operation

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

The 3500 or 3500xL analyzer is fully automated, from sample loading to primary data analysis, for sequencing, fragment analysis, and HID (human identification) analysis.

Note: In this document, primary analysis for sequencing is referred to as basecalling. Primary analysis for fragment or HID procedures is referred to as sizing.

Preparing samples

When DNA samples are prepared for sequencing, fragment analysis, or HID analysis on the 3500 or 3500xL analyzer, fluorescent dyes are attached to the DNA. For most applications, the sample is denatured so that only single-strand DNA is present.

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 99.
- **Spectral calibration** – Generates a matrix for each capillary that compensates for dye overlap and is used to convert the 20-color data into 4-, 5-, or 6-dye data. For more information, refer to "Spectral calibration" on page 103.

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 voltage.

  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 insure 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. The Peltier can heat and cool the oven to maintain sub-ambient temperatures, which are useful for non-denaturing applications such as SSCP (Single-strand conformation polymorphism).
- 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 provide20-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. For fragment and HID analysis applications, up to 6 dyes can be used in a single run for higher throughput.

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.

If the autoanalysis functionality has been set up, the system transfers the sample data to a secondary analysis software application for further processing. Alternatively, you can manually transfer the sample data to a secondary analysis software application for further processing.

Normalization

Overview of the normalization feature

For fragment analysis and HID applications, the 3500 Series Data Collection Software 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.

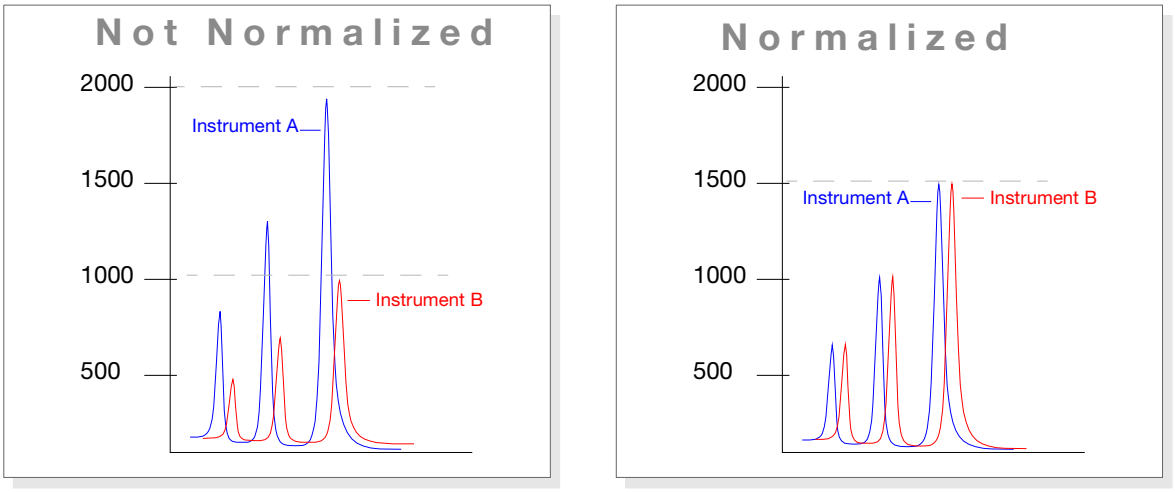


Figure 2 Comparison of fragment analysis results with and without the normalization feature

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 Data Collection Software provides three normalization size-standard definition files that you can specify for primary analysis of samples prepared with the GS600 LIZ v2 size standard:

- **Fragment:**
  - 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.
- **HID:**
  - GS600(80-400)LIZ+Normalization

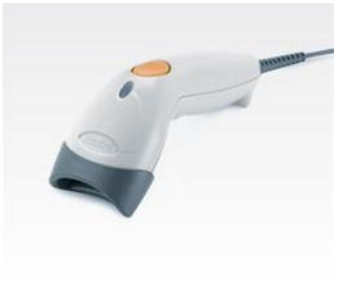
Materials for routine operation

Contact your local Applied Biosystems service representative (or go to **www.appliedbiosystems.com**, then click **Products**) to order the materials for 3500 or 3500xL analyzer.

External barcode scanner

An external barcode scanner can be used with the 3500 or 3500xL analyzer to scan the plate template.

Applied Biosystems recommends the Symbol LS 1203 handheld barcode scanner (shown), which connects to the instrument computer.



The scanner allows you to scan barcodes into any text box in the 3500 Series Data Collection Software.

For details on installation, use, decoding capabilities, and product specifications see the product documentation.

Uninterruptible Power Supply (UPS)

Loss of power during a run can result in lost data. To address concerns with loss of power in the laboratories, Applied Biosystems recommends the use of an Instrumentation Power Protection System (IPPS) with the 3500 or 3500xL analyzer.

If your laboratory has a backup-power generator, a battery-powered backup Power Protection System (PPS) is required to provide power for the amount of time that it takes for your laboratory's backup power to begin generating power and stabilize.

Note: The instrument, computer, and monitor must all be connected to the PPS.

If your laboratory does not have a backup-power generator, a PPS can provide protection from power disruptions of a limited duration. For longer durations, optional battery cabinets can be added to the base PPS unit. A base unit PPS rated for 800W can provide over 20 minutes of backup protection, and over 2 hours when a single battery cabinet was added.

Note: Battery output can be affected by temperature and the age of battery so these backup times are not guaranteed.

Instrument reagents and consumables

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

Anode buffer container (ABC)

The ABC (PN 4393927) contains 1✕ running buffer to support all electrophoresis applications on the 3500 or 3500xL analyzer.

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

For the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. Anode Buffer Container (ABC).

Store the ABC at 2 °C to 8 °C until ready to use. The sealed ABC is stable at this temperature until the expiration date shown on the label. Once the seal is peeled, the running buffer is stable at ambient temperature for up to 7 days. Ensure that the seal remains in place until just prior to use 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. When notified of the limit by the instrument software, you have to replace the ABC with a new one before you can proceed further.

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

See "Change the anode buffer container (ABC)" on page 237 for instructions on how to change the ABC.

Cathode buffer container (CBC)

The CBC (PN 4408256) contains 1✕ running buffer to support all electrophoresis applications on the 3500 or 3500xL analyzer.

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

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

For the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. Cathode Buffer Container (CBC).

Store the CBC at 2 °C to 8 °C until ready to use. The sealed CBC is stable at this temperature until the expiration date shown on the label. Once the seal is peeled, the running buffer is stable at ambient temperature for up to 7 days. Ensure that the seal remains in place until just prior to use 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)/50 injections on a 3500xL (24-capillary), whichever comes first. When notified of the limit by the instrument software, you have to replace the ABC with a new one before you can proceed further.

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

See "Change the cathode buffer container (CBC)" on page 238 for instructions on how to change the CBC.

Polymers

The polymer for 3500 or 3500xL analyzer is available as a ready to use pouch with either POP-4™, POP-6™ or POP-7™ polymer as the separation matrix.

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 on a 3500 (8-capillary) instrument, do not use that polymer on a 3500xL (24-capillary) instrument.

The pouch has adequate polymer to support the stated number of samples (384 or 960) or injections and additional volume to handle a limited number of 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 direct installation on to the instrument.

For the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. POP-4™, POP-6™, and POP-7™ polymers.

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 247 for instructions on how to change polymers.

IMPORTANT! If you remove a polymer pouch for storage, place a Pouch Cap (PN 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.

Applications

- POP-6™ and POP-7™ polymers are recommended for sequencing and fragment analysis applications.
- POP-4™ polymer is recommended for HID/Forensic applications.

Table 2 Polymers used for all applications

Polymer type	Part number	Instrument used	On-instrument life or whichever comes first‡	Pouch limits	
				Cannot exceed§	User option to continue#
POP-4™ (960)	4393710	3500 (8-capillary)	Lower of 7 days or 960 samples or 120 Injections	Expiry date, Sample limit and/or Injections limit	7-day limit
POP-6™‡‡ (960)	4393712	3500xL (24-capillary)	Lower of 7 days or 960 samples or 50 Injections		
POP-7™ (960)	4393714				
POP-4™ (384)	4393715	3500 (8-capillary)	Lower of 7 days or 384 samples or 60 Injections	Expiry date, Sample limit and/or Injections limit	7-day limit
POP-6™ ‡‡ (384)	4393717	3500xL (24-capillary)	Lower of 7 days or 384 samples or 20 Injections		
POP-7™ (384)	4393708				

‡ The polymer pouch includes additional volume to accommodate a limited number of installation and wizard operations. However, if the number of wizard operations exceeds a certain limit, the number of remaining samples or injections will be reduced. For example, if you run the total bubble remove option in the bubble remove wizard more than four times, or run other wizards operations excessively, the number of remaining samples or injections will be reduced. Refer to the polymer gage on the dashboard for the up-to-date number of remaining samples or injections at any given point.

§ Replace the pouch before proceeding further.

Applied Biosystems has verified the polymer for a maximum of 7 days on the instrument.

‡‡ Ambient temperature must be in the range of 15 °C to 25 °C. Sustained use at higher temperatures may result in shorter read lengths than specified.

Conditioning reagent

The conditioning reagent (PN 4393718) for 3500 or 3500xL analyzer is available as 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.

Note: Use of the conditioning reagent is dictated by the instrument wizards. Install the pouch when requested to do so by the wizard.

CAUTION! Expired pouches cannot be used on the instrument. Once installed on the instrument, the pouch is good for a one-time use, only.

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

See "Use the conditioning reagent" on page 250 for instructions on how to use the conditioning reagent.

Hi-Di™ Formamide

Hi-Di™ Formamide (pack of four) 5-ml tube (PN 4440753) is a highly deionized formamide, formulated with a stabilizer, ready for use as an injection solvent for all applications on the 3500 or 3500xL analyzer.

For the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. Hi-Di™ Formamide.

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

CAUTION! Expired Hi-Di™ Formamide cannot be used on the instrument.

Applications

The Hi-Di™ Formamide is used for sequencing analysis, fragment analysis, and HID/Forensic applications. To determine the exact, and necessary, volume of formamide for each specific application, follow the provided protocols and product inserts.

Table 3 Hi-Di™ Formamide used for all applications

Hi-Di™ Formamide name	Instrument	Part number	On-instrument life and usage
Hi-Di™ Formamide - 5-ml bottle (pack of four)	3500 (8-capillary) 3500xL (24-capillary)	4440753	24 hours

Capillary arrays

The capillary array for 3500 or 3500xL analyzer is installed on the instrument and ready to use.

CAUTION! SHARP The load-end of the capillary array has small but blunt ends and it could lead to piercing injury.

See "To change the capillary array" on page 252 for instructions on how to change the capillary array.

Applications

- The 36 cm capillary array is used for HID/Forensic applications.
- The 50 cm capillary array is used for sequencing and fragment analysis applications.

Table 4 Capillary arrays used for all applications

Capillary array name	Part number	Instrument used	On-instrument life	RFID-controlled limits
				User option to continue‡
8-Capillary, 36 cm	4404683	3500	160 injections	Under user option to continue (160 injections and expiry date)
8-Capillary, 50 cm	4404685			
24-Capillary, 36 cm	4404687	3500xL		Under user option to continue (160 injections and expiry date)
24-Capillary, 50 cm	4404689			

‡ Applied Biosystems has verified the array for 160 injections.

Overview of the 3500 Series Data Collection Software

About the software

Table 5 3500 Series Data Collection Software applications supported

Application	Supports
Sequencing	• Direct sequencing for mutation detection • Comparative sequencing with and without references • Microbial sequence identification
Fragment analysis	• Microsatellite • AFLP® (amplified fragment length polymorphism) • SNaPshot® kit • LOH (loss of heterozygosity) • MLPA® (Multiplex ligation-dependent probe amplification)
HID	• Forensic DNA casework • Databasing • Paternity testing

During a run, the software:

- Controls the instrument and generates sample data files:
  - Sequencing (.ab1)
  - Fragment analysis (.fsa)
  - HID (.hid)
- Performs primary analysis and reporting that evaluate the quality of the data:
  - Sequencing – Basecalling and trimming
  - Fragment analysis and HID – Peak detection and sizing
- (Optional) Performs secondary analysis (auto-analysis) with the following Applied Biosystems software applications:
  - Sequencing – SeqScape® Software v2.7 (or later) or MicroSeq® ID Analysis Software v2.2 (or later)
  - Fragment analysis – GeneMapper® Software v4.1 (or later)
  - HID – GeneMapper® *ID-X* Software v1.2 (or later)

Note: You can also manually import sample data files in to the secondary analysis software applications above. Sample data files generated by the 3500 Series Data Collection Software are also compatible with Applied Biosystems Variant Reporter™ Software (v1.1 or later) and Sequence Analysis (SeqA) Software (v5.4 or later).

Parts of the software

Dashboard

The first screen that is displayed when you start the 3500 Series Data Collection Software is the Dashboard (Figure 3).

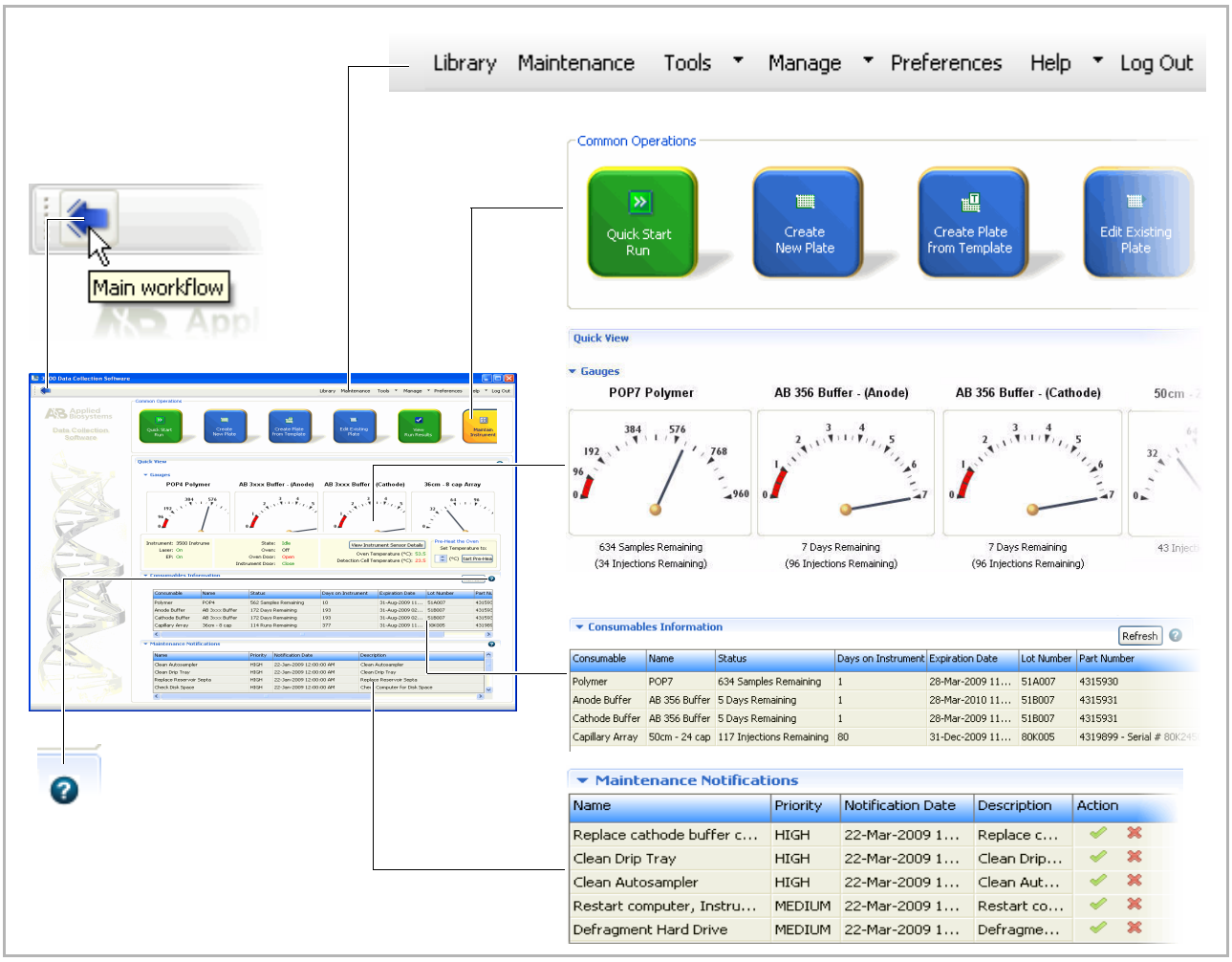


Figure 3 3500 Series Data Collection Software Dashboard

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

- **Main workflow arrow** – Advances to the screens where you set up, load, and run plates, and view results.
- **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 radio-frequencing identification (RFID) tags.
- **Consumables information** – Gives details for the installed consumables and indicates in red 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.

For more information, see "Check system status in the Dashboard" on page 26.

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.

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.

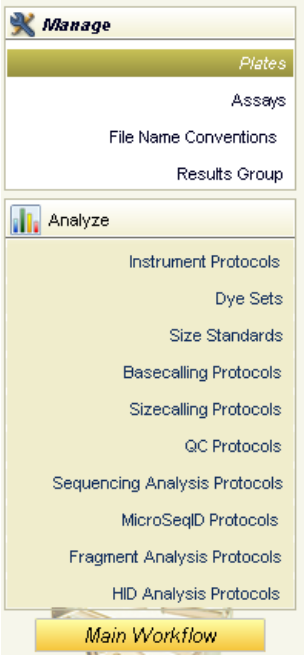
Select a task in the navigation pane to access each screen.

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 41, and "Review Results" on page 79.

Library workflow

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:

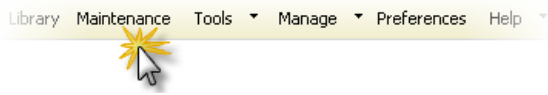
- Items that you select when you set up for a run: plates, assays, filename conventions, and results groups
- Items that you select when you create an assay:
  - Instrument protocols
  - Primary analysis protocols – Basecalling (sequencing), sizecalling (fragment analysis), QC (HID analysis)
  - Optional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

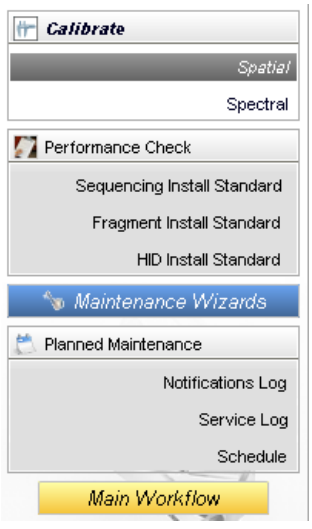
You can click **Main Workflow**, or select **Dashboard** or any other menu item at any time to advance from the Library workflow.

The Library workflow is described in "Manage Library Resources" on page 139.

Maintenance workflow

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





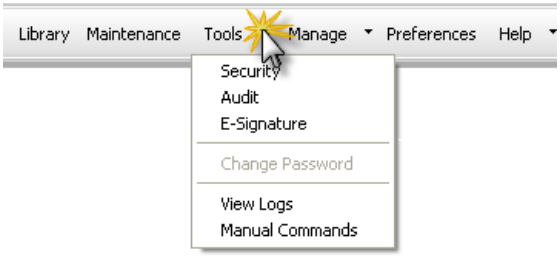
The Maintenance workflow contains the screens where you calibrate, check instrument performance, run maintenance procedures, and access records about instrument maintenance and service.

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 229.

Tools menu

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



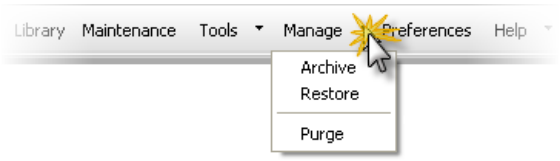
Tools provided are:

- Security, Audit, and E-signature (if your system includes the SAE module)
- Change Password that allows you to change passwords.
- View Logs that provides reports of instrument runs.
- Manual Commands that you can use to troubleshoot instrument performance.

The SAE module is described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 197.

Manage menu

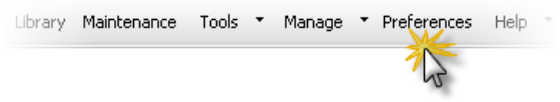
Select **Manage** in the menu bar to access archive, restore, and purge functions

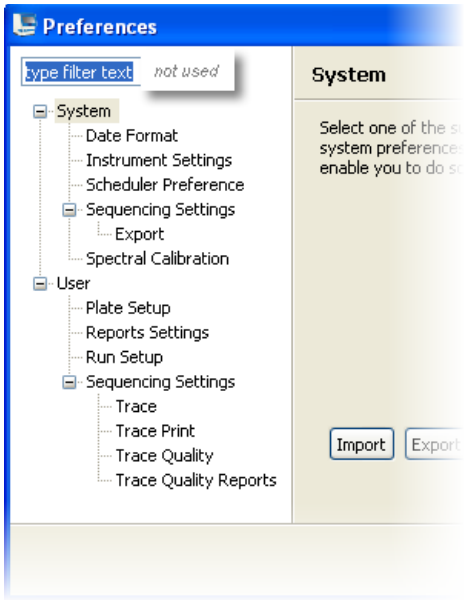


Archive, restore, and purge are described in Chapter 8, Maintain the Instrument.

Preferences menu

Select **Preferences** in the menu bar to access the parameters for which you can 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 21.

Help menu

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

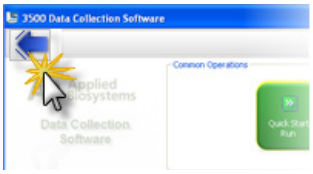
The Help provides quick access to brief information about how to perform tasks on a screen. For details about tasks and other information, refer to the chapters in this user guide.

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

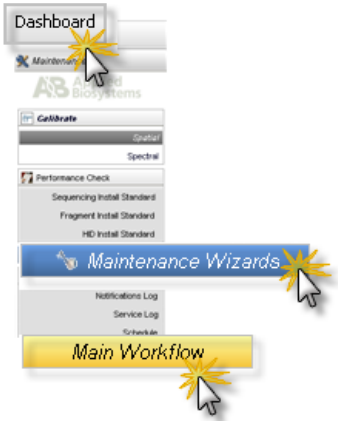
To advance from the Main workflow to:

- **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.



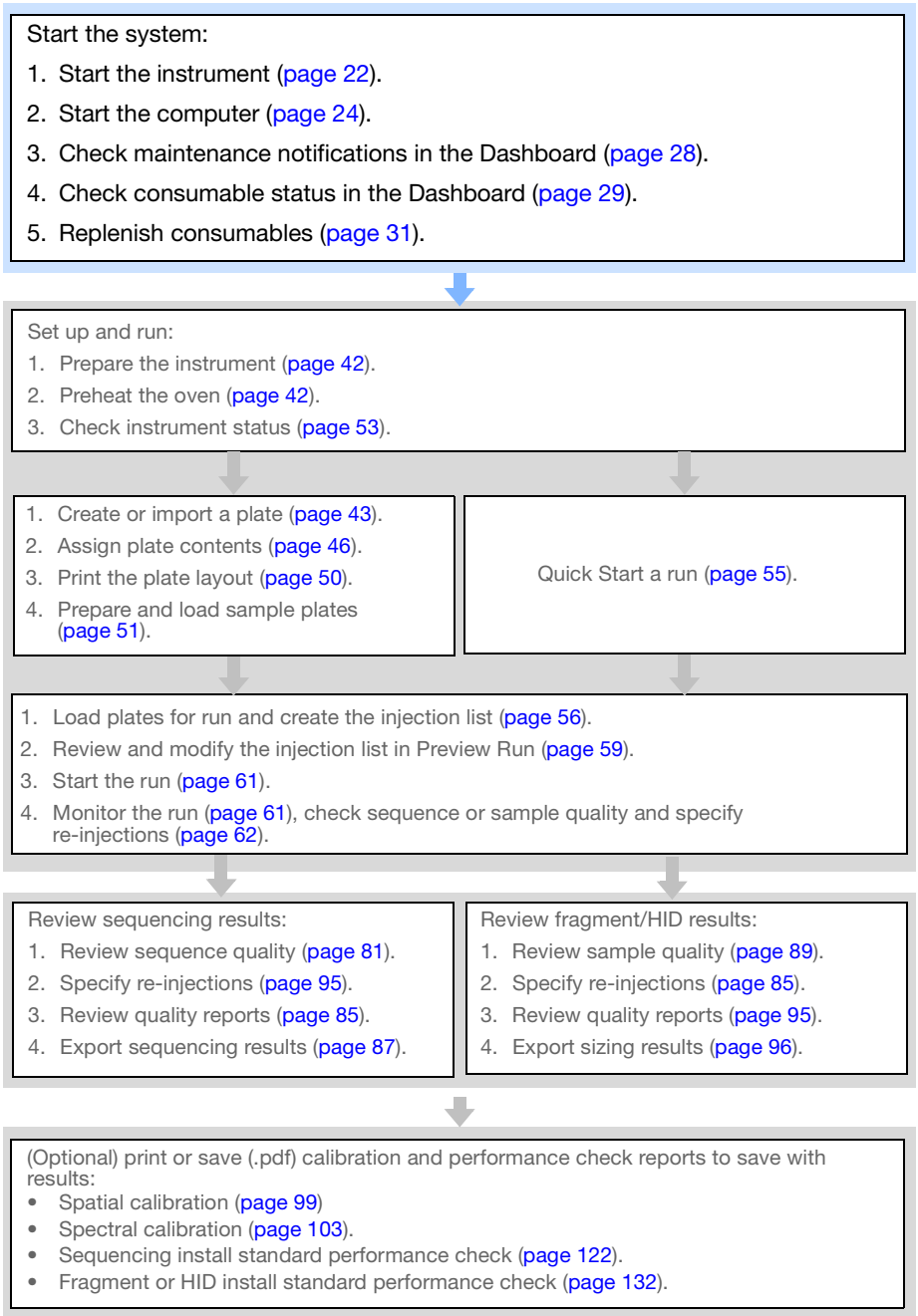
Use the software without an instrument

You can install the 3500 Series Data Collection Software on a computer that is not connected to an instrument. You can use this stand-alone version of the software to create plates, protocols, and other library items, and to review completed results.

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

Start the System 2

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 Applied Biosystems service representative has configured the system for the proper operating voltage.

   See the *Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide* (4401689) for details.

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

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

2. Inspect instrument interior. Ensure that:
   a. The 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. Turn on the instrument. Press the power on/off button on the front of the instrument and wait for the green status light to turn on.

   a. Press the Tray button on the outside of the instrument to bring the autosampler to forward position. Wait until the autosampler stops at the forward position.

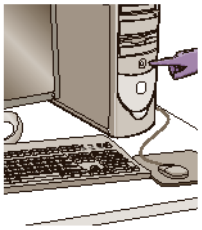
      Note: When the door is open, the yellow status light blinks while the instrument performs self-check and the autosampler adjusts.

b. 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.

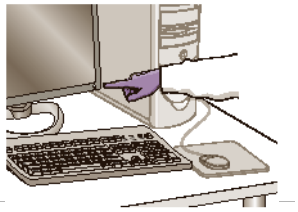
Indicator	Status
All lights off	Instrument off
Green light	Operational (awaiting run)
	Pause run, terminate run, stop injection button (in SW) pressed by user. **Note:** You can only abort an injection when the green light is flashing, not when it is solid green.
Green light (blinking)	Operational (Run 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

1. Power on the computer.



2. Power on the monitor.



3. In the Log On to Windows dialog box:
   a. Enter the user name.
   b. If applicable, enter a password.

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

   c. Click **OK**. Wait until the computer finishes booting.

IMPORTANT! The status icon, on the right lower-corner of your screen, shows when the 3500 Server Monitor is active by displaying the icon shown here.

IMPORTANT! Do not close this icon. Doing so will prevent proper functioning of the software.

Log on to Windows

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

Launch the application

Step one: Launch the Daemon

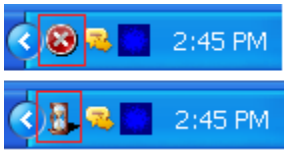
If the Daemon does not start automatically, launch the Daemon:
Start ▸ Programs ▸ Applied Biosystems ▸ 3500 ▸ Daemon

Note: It will take approximately 15 seconds for Daemon to populate.

Step two: Launch the Server Monitor

If the Server Monitor does not start automatically, launch the Server Monitor:
Start ▸ Programs ▸ Applied Biosystems ▸ 3500 ▸ Server Monitor

Note: It will take approximately 2 minutes for the Server Monitor to set up. During this time, you will see the status icon transition from a red circle, with an ✕ in the middle (indicating that not all 3500 services are loaded) to the shape of an hour-glass on your desktop, next to the clock.



When Server Monitor set up is complete, the icon in the shape of an hour-glass will disappear and a checkmark icon appears indicating that the 3500 Server Monitor has started and all 3500 services loaded.

Step three: Launch the 3500 application

Launch the application:

Start ▸ Programs ▸ Applied Biosystems ▸ 3500

Splash screen

After you launched the 3500 application, the 3500 Series Data Collection Software splash screen appears. This screen will remain active for a few seconds until the 3500 Log In dialog box opens.

After the 3500 Series Data Collection Software splash screen disappears, one of the following occurs:

- The Dashboard is displayed (go to "Check system status in the Dashboard" on page 26)
- The Login dialog box is displayed (go to "Log In" on page 26)

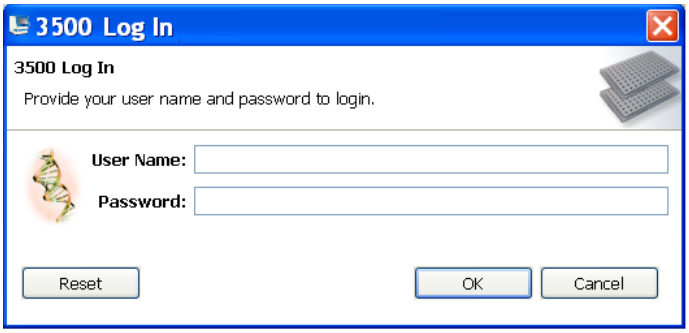
Log In

Security, Audit, and E-Signature

The Security, Audit, E-Signature (SAE) module is an optional component of the 3500 Series Data Collection Software. Researchers have the option to purchase this feature and enable/disable the functionality for SAE. If the SAE feature is enabled, see Chapter 7, Use Security, Audit, and E-Sig Functions (SAE Module) for user configurations.

After the 3500 Series Data Collection Software splash screen disappears, log in from the Dashboard:

1. Enter the User Name and Password in the 3500 Log In dialog box.



2. Click **OK**.

The 3500 Series Data Collection Software splash screen re-appears. This screen will remain active for a few seconds and the 3500 Series Data Collection Software opens.

The 3500 Series Data Collection Software launches and the Dashboard appears.

IMPORTANT! If you accidentally close any of the services (via 3500 Server Monitor), the system will not work. To open a closed service, place the cursor on the status icon, click the right-mouse button, go to Services, and click the service that is closed.

Check system status in the Dashboard

Dashboard, a quick glance

The first screen that is displayed when you start the 3500 Series Data Collection Software is the Dashboard (Figure 4).

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 radio frequency identification (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.

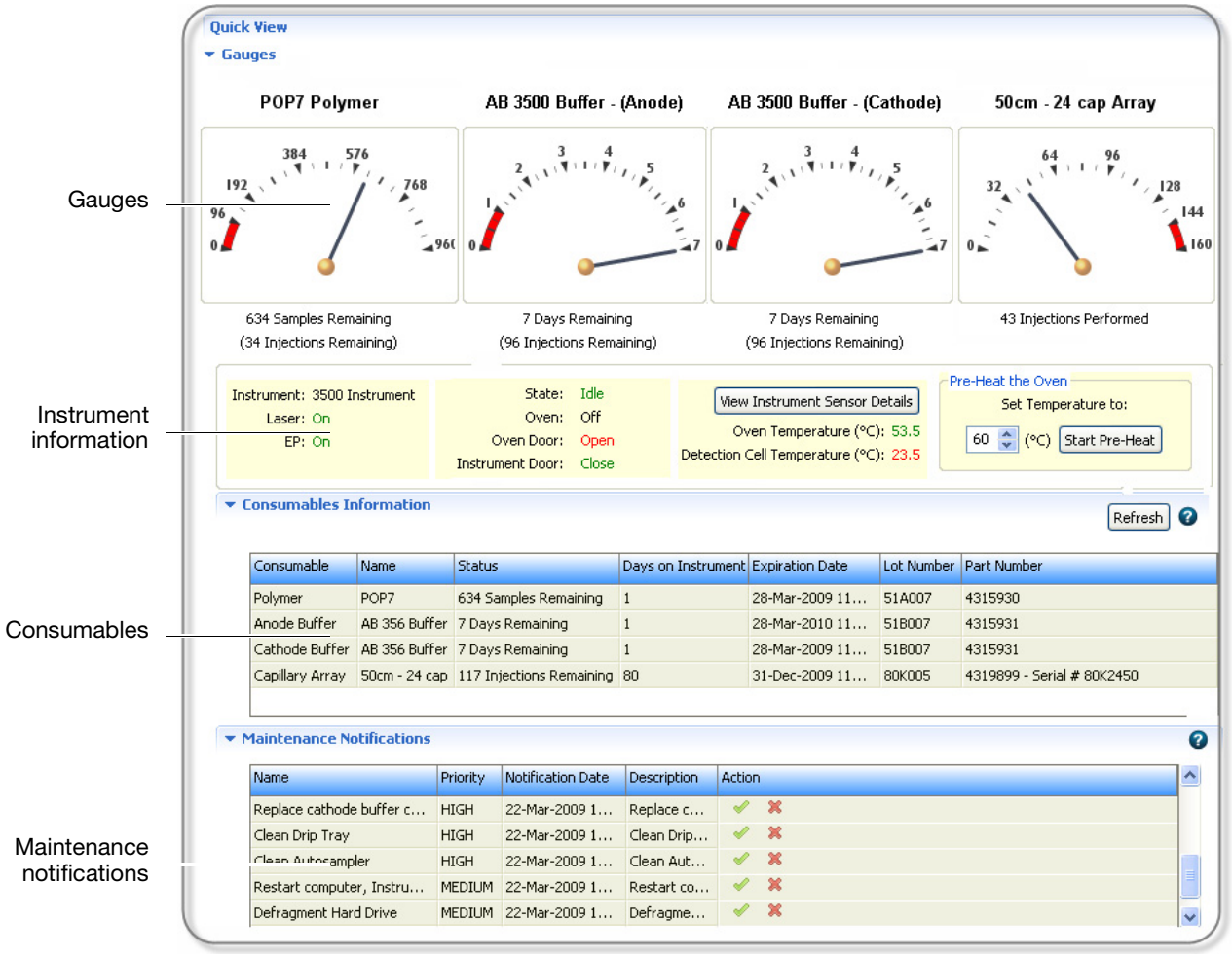
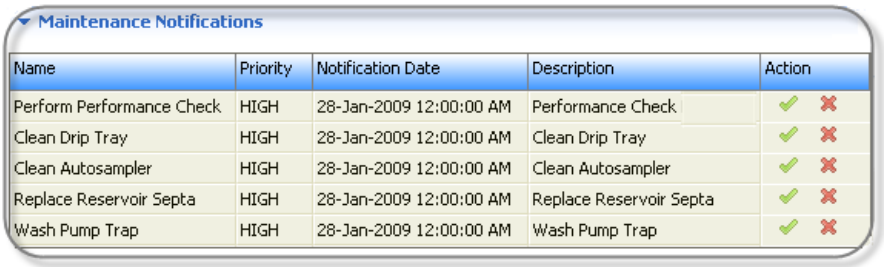


Figure 4 Dashboard

Check maintenance notifications

The Maintenance Notification section displays reminders for the tasks scheduled in the maintenance calendar (see "Use the maintenance calendar" on page 232). You can set the time to trigger maintenance notifications in Preferences (see "Set general preferences" on page 33).

1. Review the Maintenance Notifications pane.



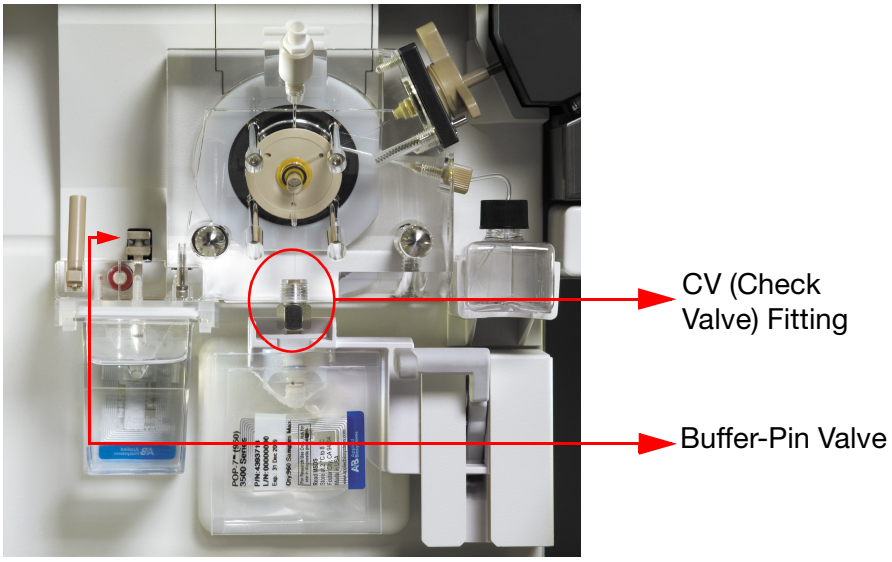
2. Perform any scheduled maintenance tasks, then click to mark it as complete, (or click to mark it as dismissed if you do not perform the task). Actions are recorded in the Notifications log (for more information, see "Review the Maintenance Notifications Log" on page 257).

3. Perform any daily, monthly, or quarterly maintenance tasks that are not listed in the Maintenance Notifications pane (see Chapter 8, Maintain the Instrument).

4. Inspect the instrument interior. See "Start the instrument" on page 22.

   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 Applied Biosystems.

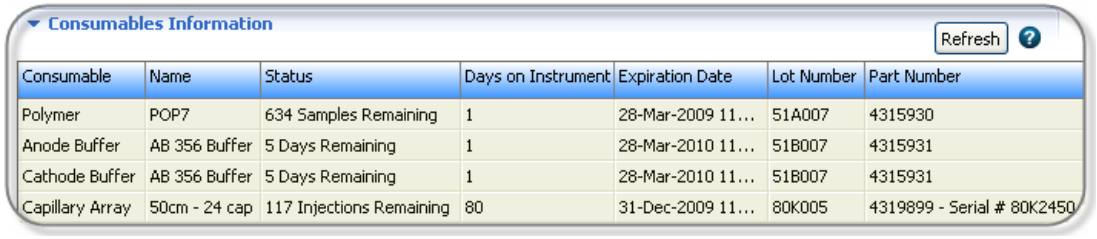


Check consumable status

IMPORTANT! The Days Remaining for buffers updates only when you click Refresh or start a run. As part of daily startup, click **Refresh** to update consumable status.

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

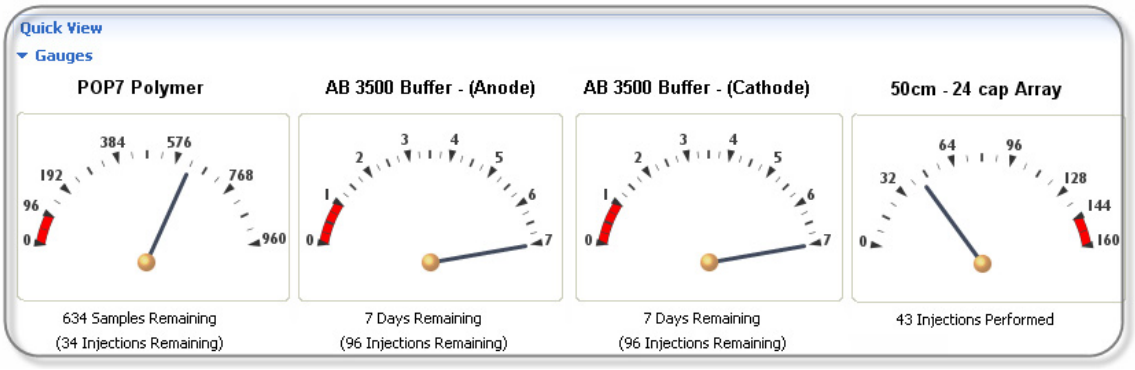
   The Consumables pane displays expiration dates and lot numbers (read from the RFID tags on the consumable containers).



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. Table below lists specifications for each 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! Applied Biosystems recommends that you add a maintenance notification to your calendar for polymer and buffer replacement. Set the notification to display two days before the polymer should be replaced.

Consumable			On-instrument limits (the first limit met applies)	Notes
Polymer‡§	8-cap	960 sample pouch	960 samples or 120 injections	Use within 7 days of installation on instrument. The software allows you to continue running past 7 days. However, Applied Biosystems has verified the polymers for up to 7 days only on the instrument.
		384 sample pouch	384 samples or 60 injections	
	24-cap	960 sample pouch	960 samples or 50 injections	
		384 sample pouch	384 samples or 20 injections	
Buffers	8-cap		7 days or 120 injections	To ensure optimal buffer performance, the software requires buffer replacement after 7 days.
	24-cap		7 days or 50 injections	
Capillary Array			160 injections	The software allows you to continue running after 160 injections. However, Applied Biosystems has verified the arrays for up to 160 injections.

‡ 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. Note that the initial injection limit is higher than the initial sample limit.

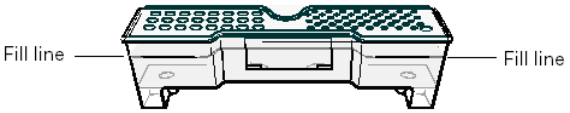
Example: 960 sample pouch on 24-cap:
If all wells contain sample for all injections: 960/24 = 40 injections.
If all wells do not contain sample for all injections: 960/<24 = 40+ injections, up to a maximum of 50 injections and a maximum of 960 samples.

A polymer pouch includes additional volume to accommodate the volume used during installation and by wizards. However, 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.

§ Ambient temperature must be in the range of 15 °C to 25 °C POP-6™. Sustained use at higher temperatures may result in shorter read lengths than specified.

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.



IMPORTANT! Do not use if the buffer level is too low or 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 245.
- "Change polymer type" on page 247.

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

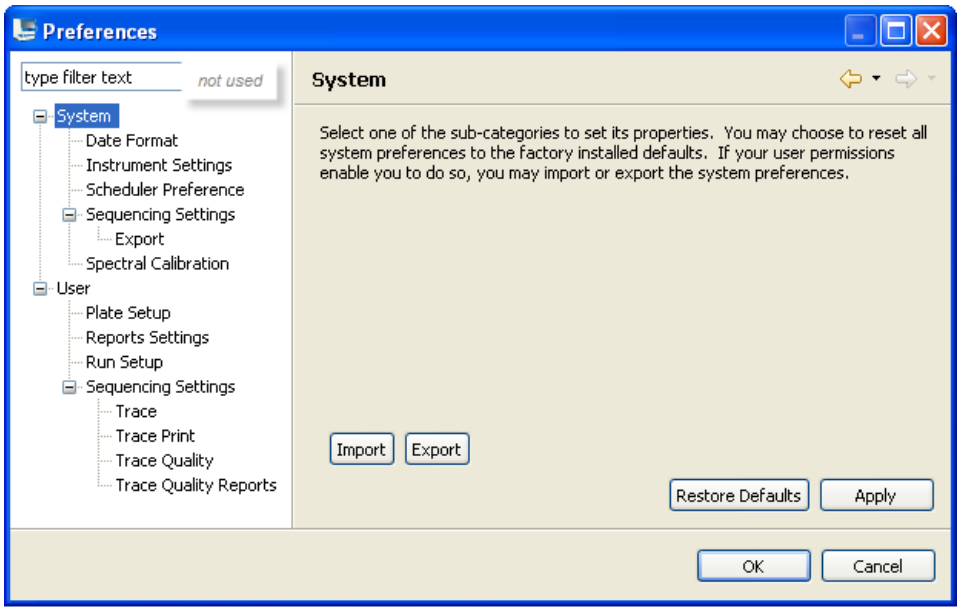
- "Change the anode buffer container (ABC)" on page 237.
- "Change the cathode buffer container (CBC)" on page 238.
- "Fill capillary array with fresh polymer" on page 251
- "To change the capillary array" on page 252.

Go to Chapter 3, "Set Up and Run" on page 41.

Set preferences

Overview

Preferences are user-definable default settings. 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)
- Scheduler preference (trigger time for maintenance notifications)
- Sequencing export settings
- Spectral calibration (number of allowed borrowing events)

User preferences

These settings apply to all users if your system does not include the SAE module, but are saved individually per user if your system includes the SAE module:

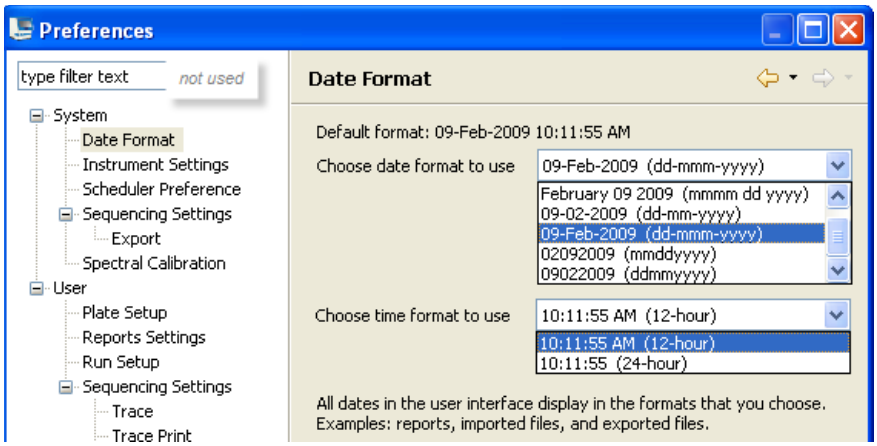
Note: For information on the SAE module, see Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 197.

- Plate setup
- Reports settings
- Run setup
- Sequencing (review and report settings)

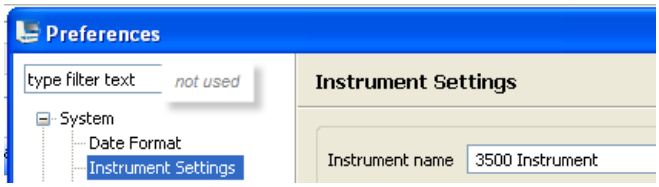
Set general preferences

System preferences

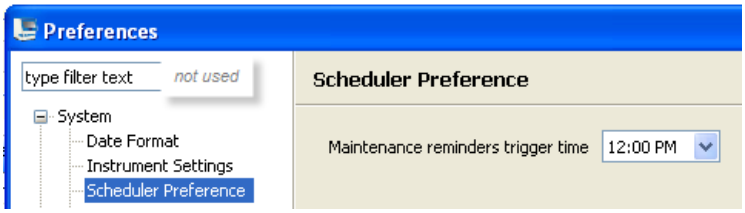
1. In the Preferences dialog box, click the following items:
   - **Date Format** to set the date and time format for the software.



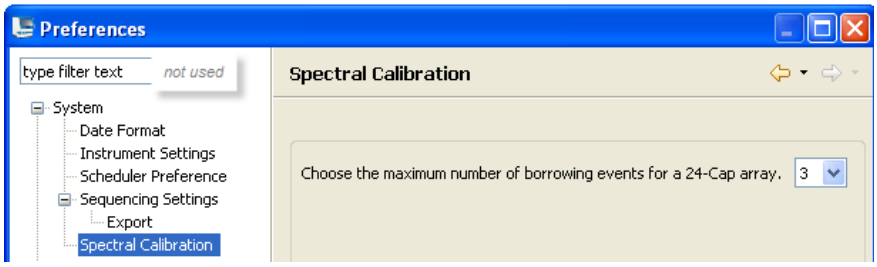
   - **Instrument Settings** to set the instrument name (appears in the Dashboard, reports, file name conventions, instrument sensor details, view sequencing results).



   - **Scheduler Preference** to set the time to trigger maintenance notifications displayed in the Dashboard (see "Check maintenance notifications" on page 28).



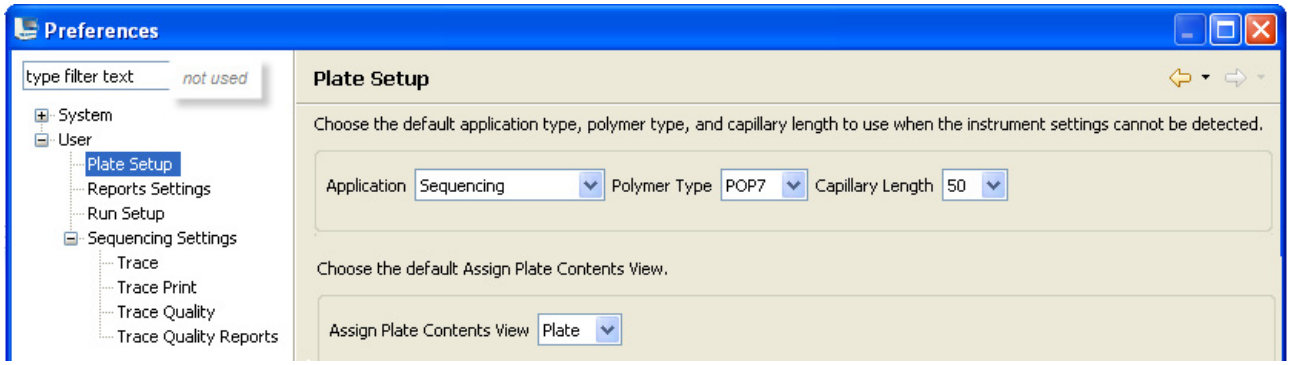
- **Spectral Calibration** to decrease the number of allowed borrowing events for spectral calibration (see "What you see during a spectral calibration" on page 112).



2. Click **Apply** to save the system preferences (see "System preferences" on page 32).

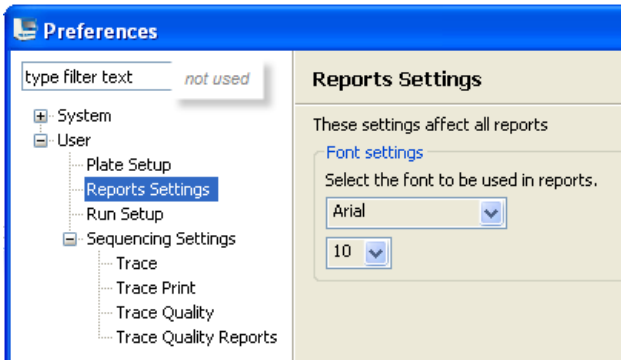
User preferences

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.



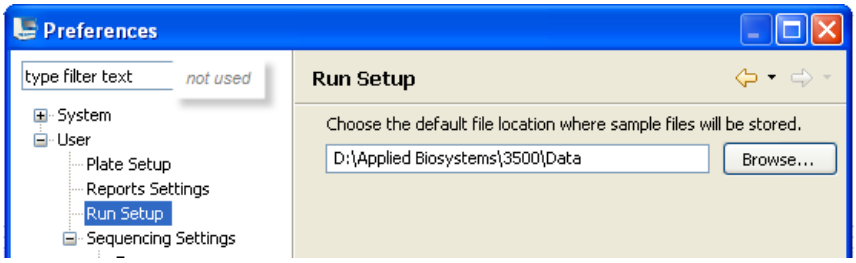
- **Reports settings** to set the default font and size reports.

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.



2. Click **Apply** to save the user preferences (see "User preferences" on page 33).

Table and plot settings user preferences

Users can also save user preferences while viewing tables and plots:

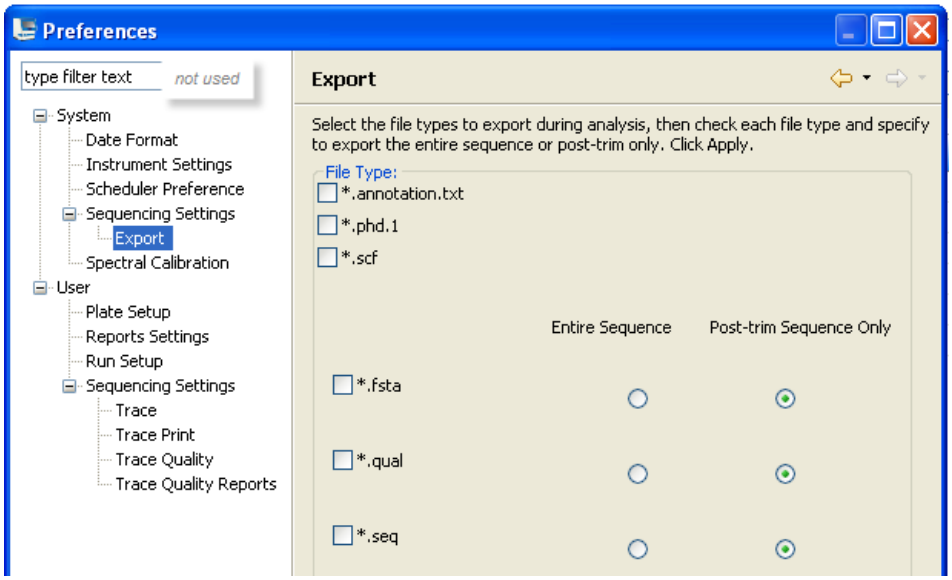
- **Table settings dialog box** – Determines the columns displayed in a table and the order of the columns.
- **Plot settings dialog box** – Determines the settings applied to plots.

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
*.fsta, *.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 33).

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.:

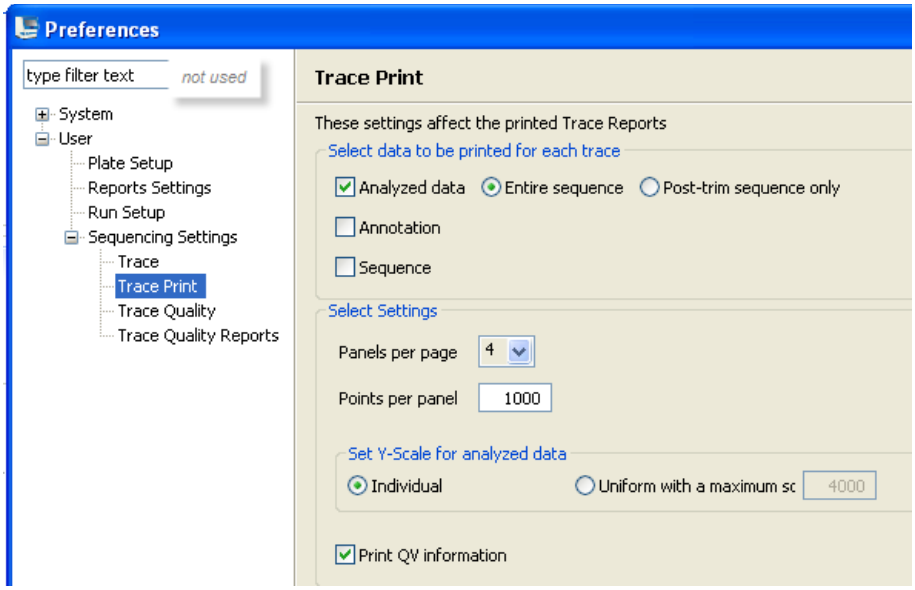
Setting	Description
NT (nucleotide) Base Color	Click an NT or Mixed base Foreground or Background color block, then select a color for the letter annotation or the highlight color for the letter annotation. 
Pure Base and Mixed Base QV Colors	Sets the colors and ranges for pure and mixed base quality value indicators (QVs) displayed in the Trace View (the default settings are recommended):  a. Click a pure base or mixed base color bar to select a new color. b. Place the mouse pointer over a slider, then drag to set a new range. Applied Biosystems recommends that you set the following ranges for QVs: • 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) **Note:** The predicted probability of error for a basecall is high QV >10.

3. Click **Apply** to save the user preferences (see "User preferences" on page 34).

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.

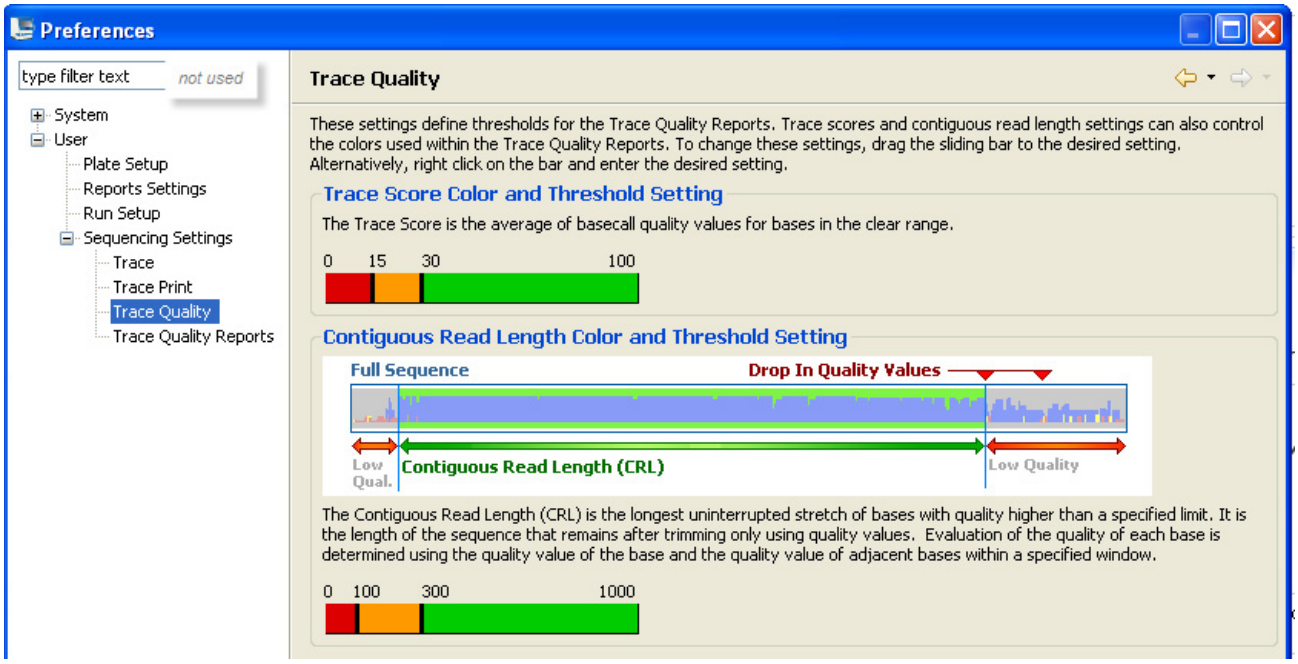
3. Click **Apply** to save the user preferences (see "User preferences" on page 34).

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.

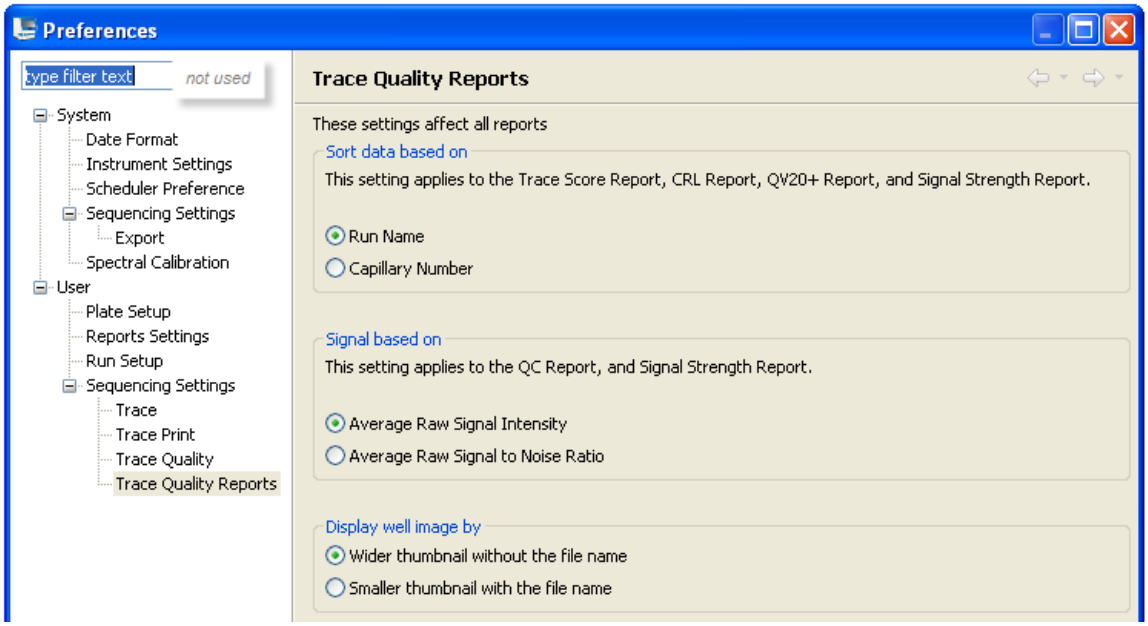
   b. Place the mouse pointer over a slider, then drag to set a new range.

3. Click **Apply** to save the user preferences (see "User preferences" on page 34).

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.



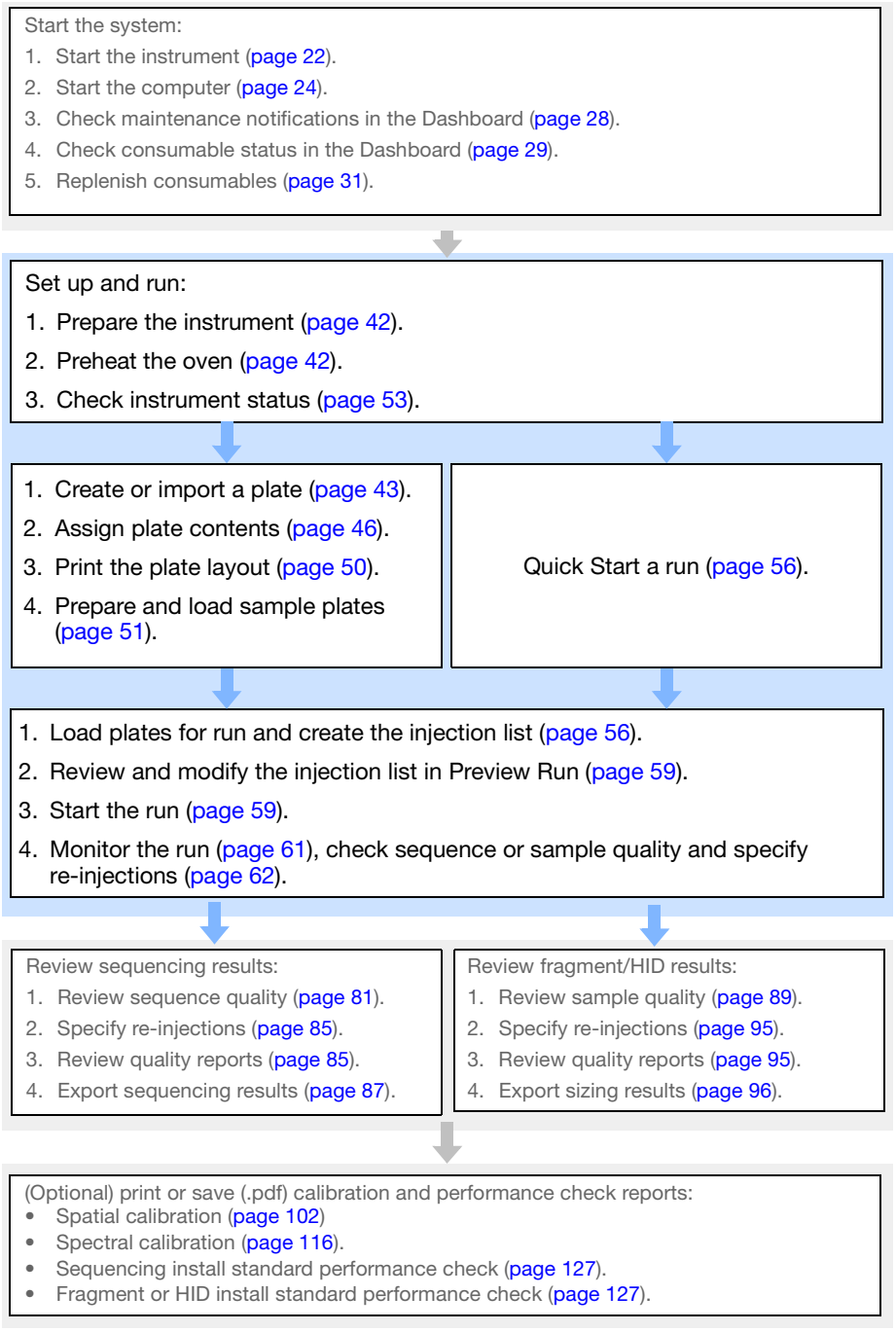
2. Specify the following settings.:

Setting	Description
Sort data	Sort data in Trace Score, CRL, QV20+, and Signal Strength reports based on: • Run Name • Capillary Number
Signal based on	Base signal in QC and Signal Strength reports based on: • Average Raw Signal Intensity • Average Raw Signal to Noise Ratio
Display well image by	Specify the thumbnail option for Plate reports: • Wider thumbnail without file name • Smaller thumbnail without file name

3. Click **Apply** to save the user preferences (see "User preferences" on page 34).

Set Up and Run 3

Workflow



Prepare the instrument

1. In the Dashboard, check consumable status (page 29). Ensure that:
   - Consumables are not expired
   - Adequate buffer levels are at the fill lines.
2. Set the oven temperature, then click **Start Pre-heat**:
   - 60 °C – POP-7™ and POP-4™ polymers
   - 50 °C – POP-6™ polymer

   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.

   Applied Biosystems recommends 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 page 251).

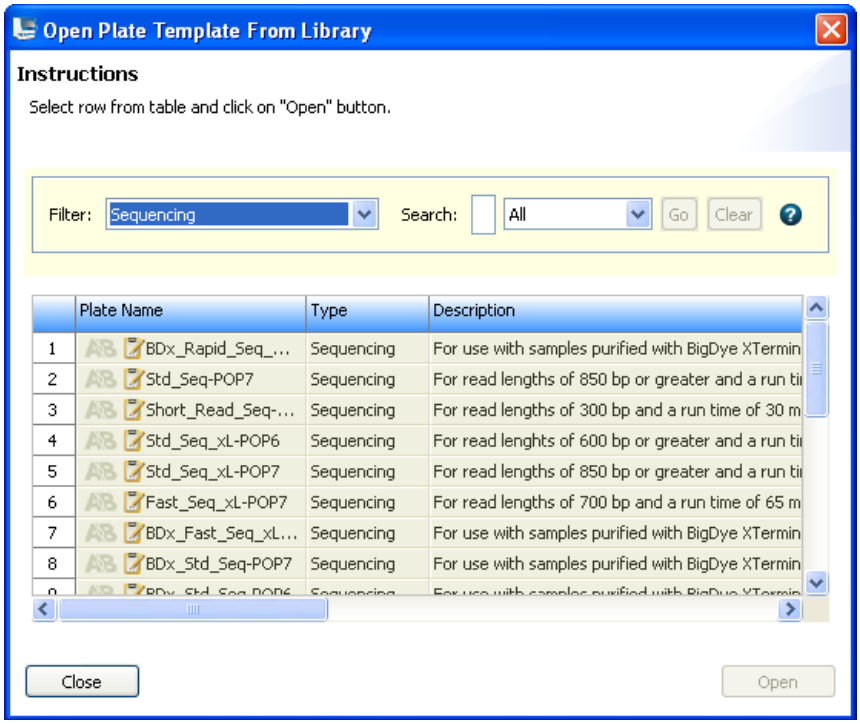
Create a plate

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

Create a plate from a template

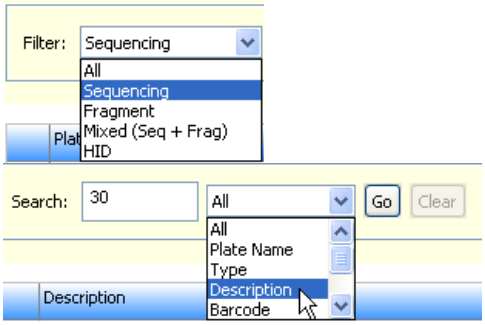
The software includes factory-provided plate templates that you can use as a starting point to create a plate (you can also create your own plate templates). In addition to pre-defined plate parameters, a plate template can also contain a list of the appropriate assays, file name conventions, and results groups for an application. For more information, see "Create a plate template" on page 75.

1. In the Dashboard, click **Create Plate From Template** to display the Open Plate Template from Library dialog box.



2. (Optional) Filter the templates listed:

   a. Select a template type.

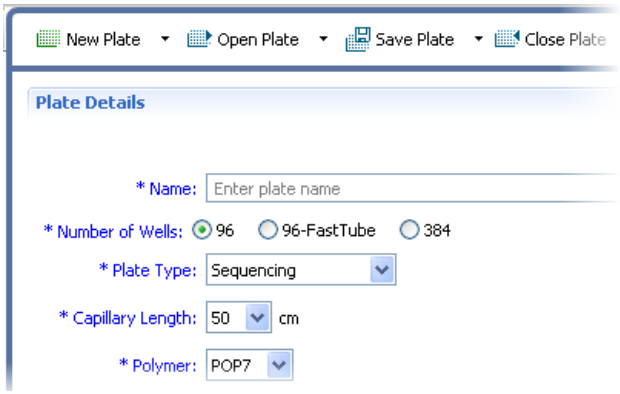


Note: You can set the default plate type for this filter in Preferences. See "Specify the default plate type for the Open Plate dialog box" on page 76.

   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).

3. Select the template, then click **Open**.

4. In the Define Plate Properties screen, select the plate type.



   - **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.

5. Set remaining plate properties, then select **Save**.

6. Click **Assign Plate Contents**, then go to "Assign plate contents" on page 46.

Import a plate

1. Do either of the following:
   - Create a plate on another 3500 Series Data Collection Software system, then export (see "Import and export a plate" on page 75).
   - Create a plate import file (see "Create a plate import file" on page 74).

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**.

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 147.
- **Filename convention** (optional) – Specifies file naming. For more information on assays, see "File name conventions library" on page 151.
- **Results group** (optional) – Specifies sample data file storage. For more information on assays, see "Result group library" on page 155.

Before you assign plate contents

1. Access the Assign Plate Contents screen (Figure 5 on page 47) 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. Create a plate. Select one of the following topics:
   - "Create a new plate" on page 144
   - "Create a plate from a template" on page 43
   - "Import a plate" on page 44
   - Or select **Open Plate ▸ Edit Existing Plate**

3. Click **Show In Wells** to specify the attributes to display in wells.

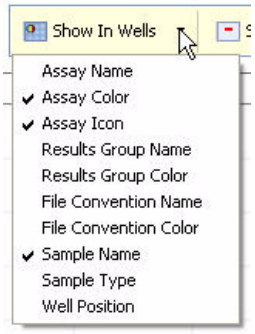


Figure 5 on page 47 shows the Plate View of the Assign Plate Contents screen.

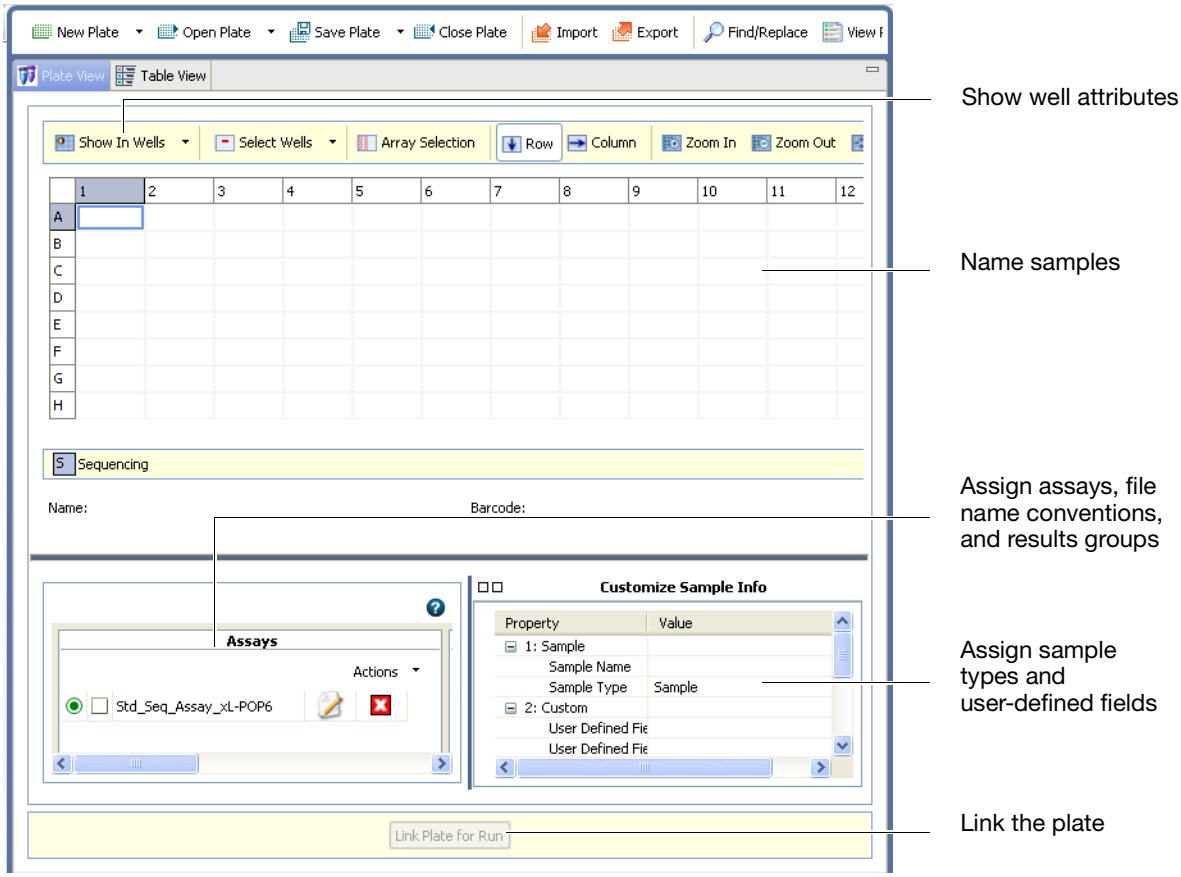


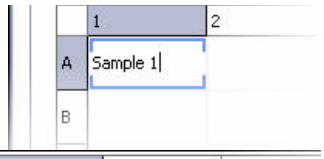
Figure 5 Assign Plate View of the Assign Plate Contents screen

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 70 and "Use the Table View" on page 71.

Procedure

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

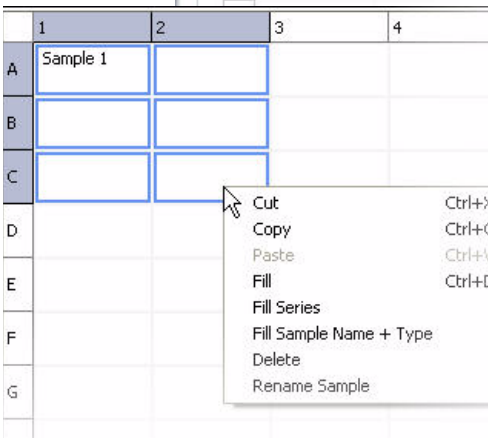


2. Click-drag multiple wells.

3. Right-click and select **Fill** or **Fill Series** to populate the selected fields.

   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.

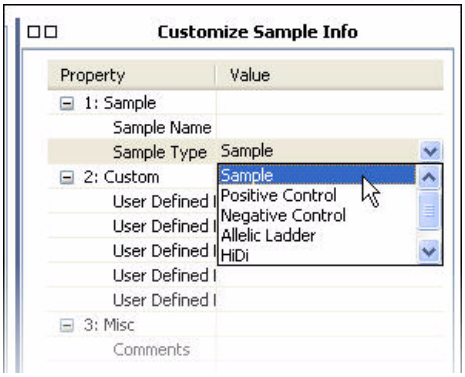


4. At the bottom right of the Assign Plate Contents screen, expand the Customize Sample Info pane.

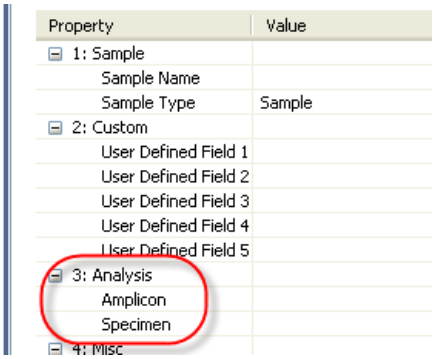
5. In the plate view, click-drag to select wells of interest.

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.



8. (Optional) 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 49.

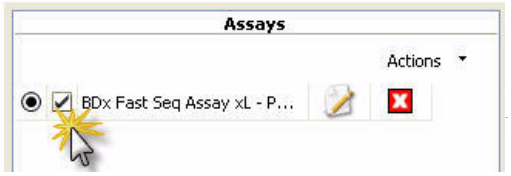


Note: For HID applications, include the well position in the allelic ladder sample names. Well position is needed to identify the position of allelic ladder samples during re-injection.

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

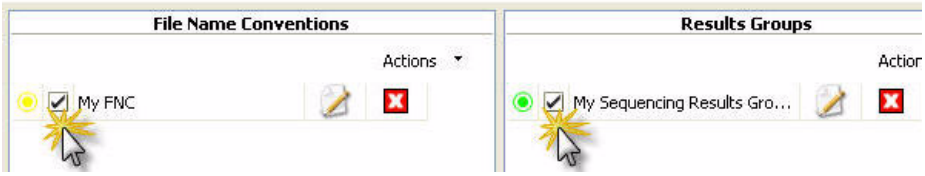
Note: If an assay, file name convention, or results group is not listed for the plate, go to "Add assays, file name conventions, and results groups to a plate" on page 73.

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.



Note: To normalize fragment analysis or HID data, select an assay that contains a sizecalling protocol or a QC protocol that specifies a normalization size standard.

3. (Optional) Repeat for file name conventions and results group.



4. Select **Save Plate**.
5. Go to "Print the plate layout" on page 50.

How file location in file name conventions and results groups work

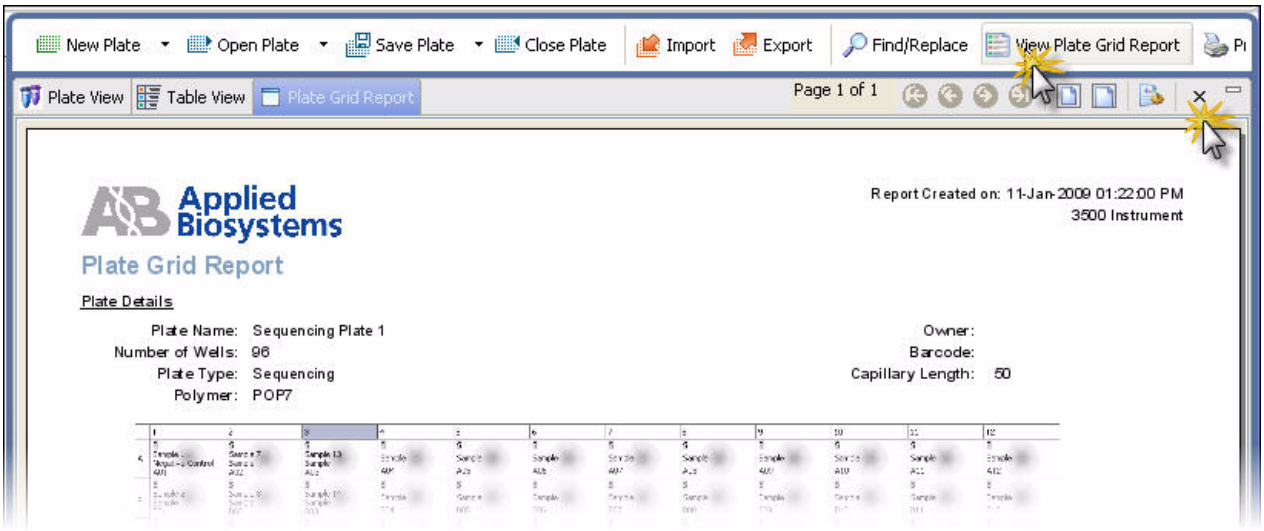
If you do not specify a file name convention, data files are named in this format: <sample name>_<well>.

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 34).

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

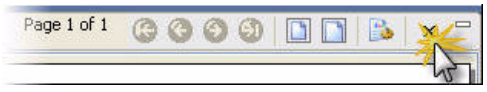
Print the plate layout

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



Note: A 384-well report displays the plate layout in four quadrants on four pages.

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 51.

Prepare and load sample plates

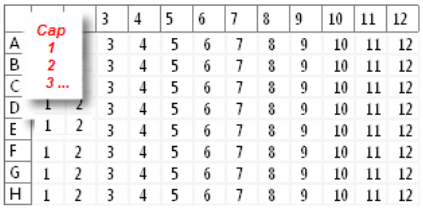
IMPORTANT! Do not use warped or damaged plates.

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.

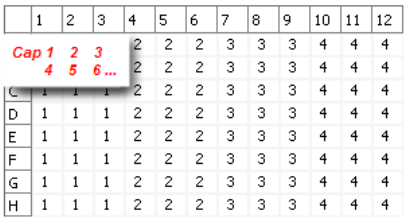
- **96 –** Supports 96-well standard reaction plate. 8-strip standard tubes are also supported with appropriate retainers.
- **96-Fast Tube –** Supports 96-well Fast reaction plate. 8-strip fast tubes are also supported with appropriate retainers.

8-capillary: 96-well plate



8-capillary: 384-well plate
Not supported on the 3500 Dx Genetic Analyzers (8-capillary)

24-capillary: 96-well plate



24-capillary: 384-well plate

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
B	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
C	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
D	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
E	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
F	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
G	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
H	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
I	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
J	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
K	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
L	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
M	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
N	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16
O	1	3	1	3	1	3	5	7	5	7	5	7	9	11	9	11	9	11	13	15	13	15	13	15
P	2	4	2	4	2	4	6	8	6	8	6	8	10	12	10	12	10	12	14	16	14	16	14	16

Allelic ladder run requirements

Applied Biosystems recommends 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. Applied Biosystems recommends 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.

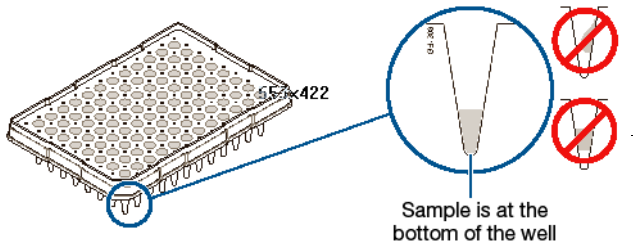
Results group for one allelic ladder per run folder

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 161.

Prepare sample plates

1. Pipette samples into the plate according to the plate layout (see "Print the plate layout" on page 50).
2. Briefly centrifuge the plate.
3. Verify that each sample is positioned correctly in the bottom of its well.



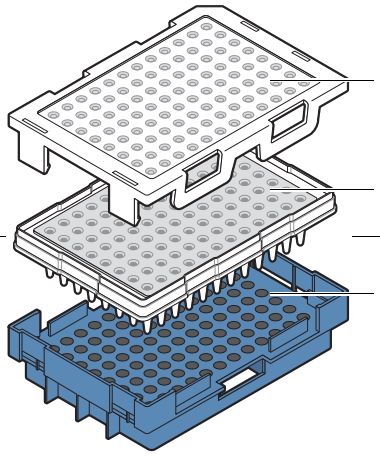
IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

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

Prepare the plate assembly

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

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
2. Place the sample plate into the plate base.



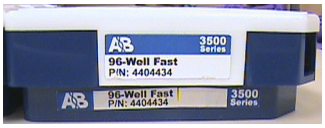
IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

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.

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

Load the plate in the instrument

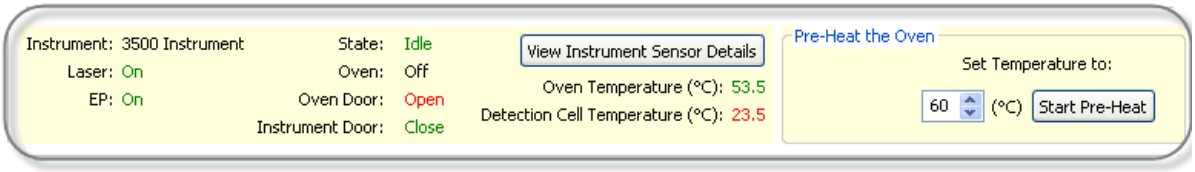
1. Place the plate in the autosampler with the labels facing you (or the instrument door) and the notched corner of the plate in the notched corner of the autosampler.
2. Close the instrument door to re-initialize the instrument.



Check instrument status

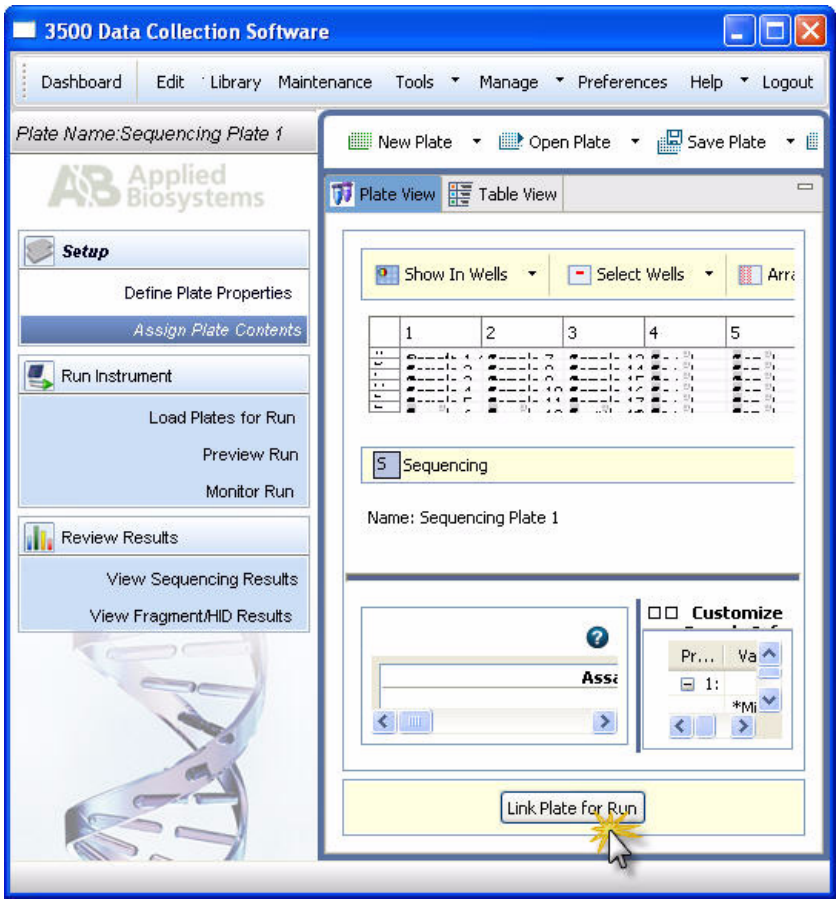
Check instrument status in the Dashboard. Temperatures are displayed in red as they warm to the set-points. When temperatures are at the set point they are displayed in green. Temperatures may fluctuate slightly when they reach the set point as they stabilize.

Applied Biosystems recommends 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 56.

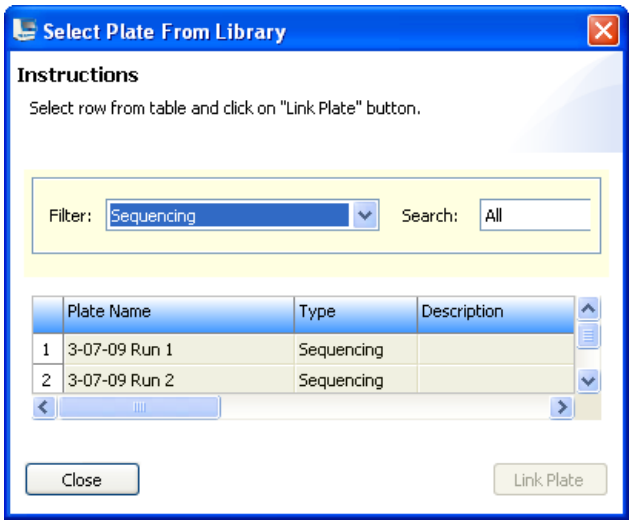
Note: By default, the plate in position A is selected.

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 53).

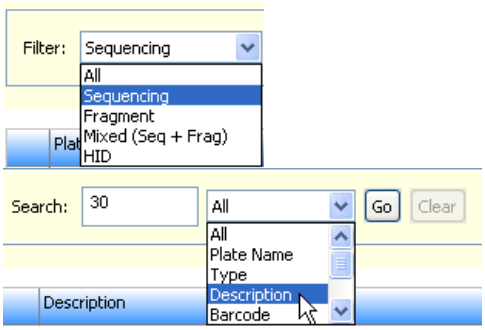
1. In the Dashboard, click **Quick Start Run** to display the Select Plate from Library dialog box.



2. (Optional) Filter the plates listed:

   a. Select a plate type (you can set the default plate type in Preferences, see "Specify the default plate type for the Open Plate dialog box" on page 76).

   b. Find a plate based on an attribute by selecting an attribute, entering the text to search for, then clicking **Go**. (Click **Clear** to clear the field and enter different search criteria).



3. Select a plate, then click **Load Plate**.

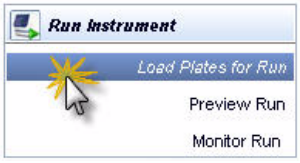
4. Click **Start Run** from the Load Plates on Run Screen.

IMPORTANT! It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.

Load plates for run and create the injection list

Load the plate in the instrument (see "Load the plate in the instrument" on page 53) and link the plate ("Link the plate" on page 54) before proceeding.

1. Access the Load Plates for Run screen (Figure 6 on page 56) 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.

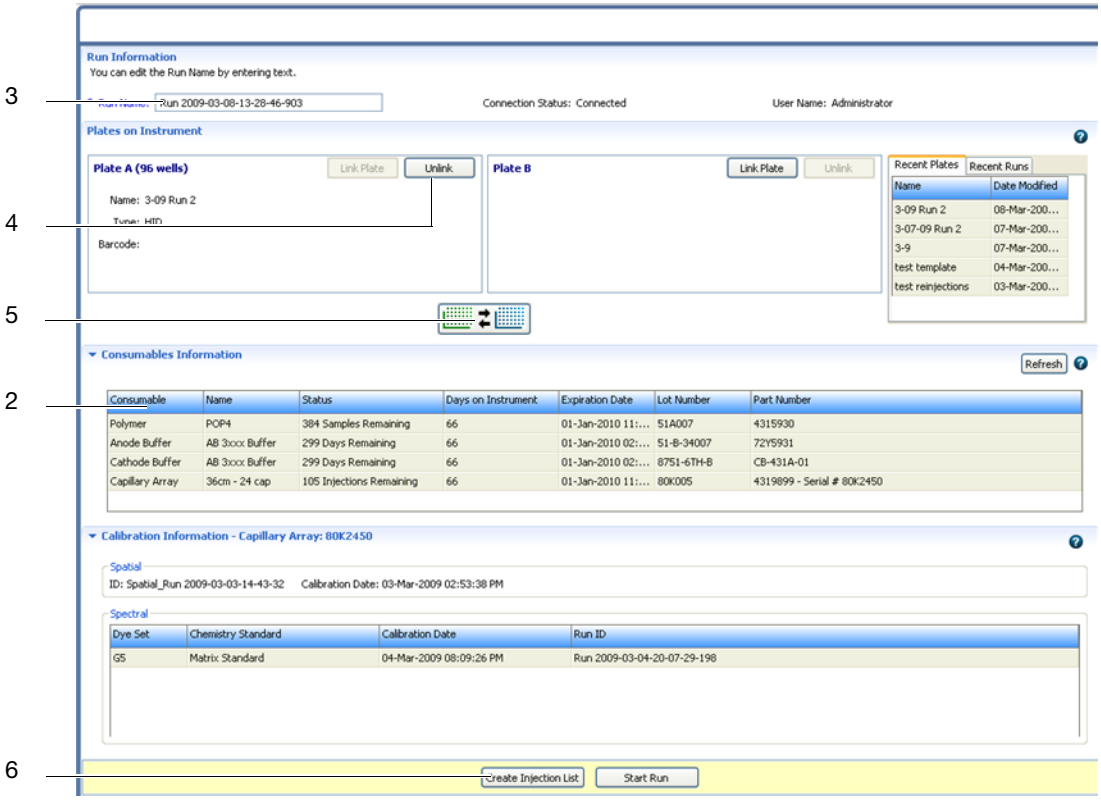


Figure 6 Load Plates for Run

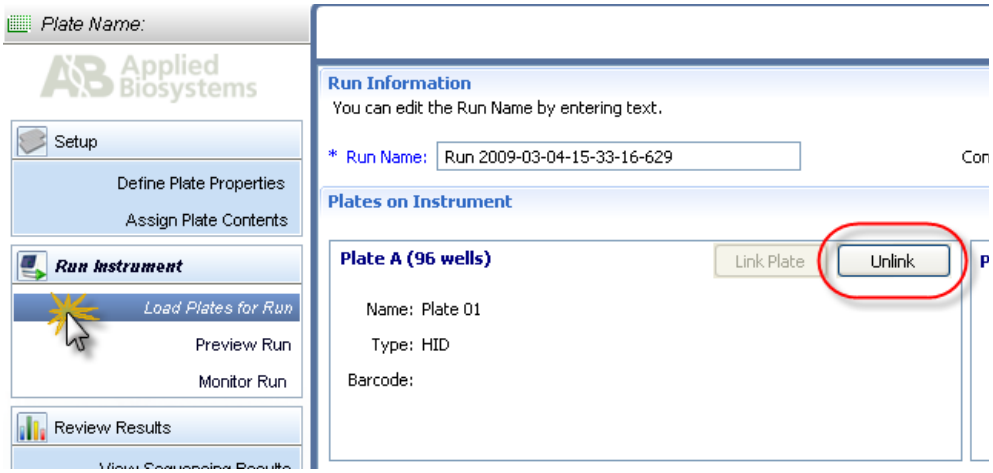
2. Review the consumables information and the calibration information and ensure the status is acceptable for a run.

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.

   IMPORTANT! It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.

   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).



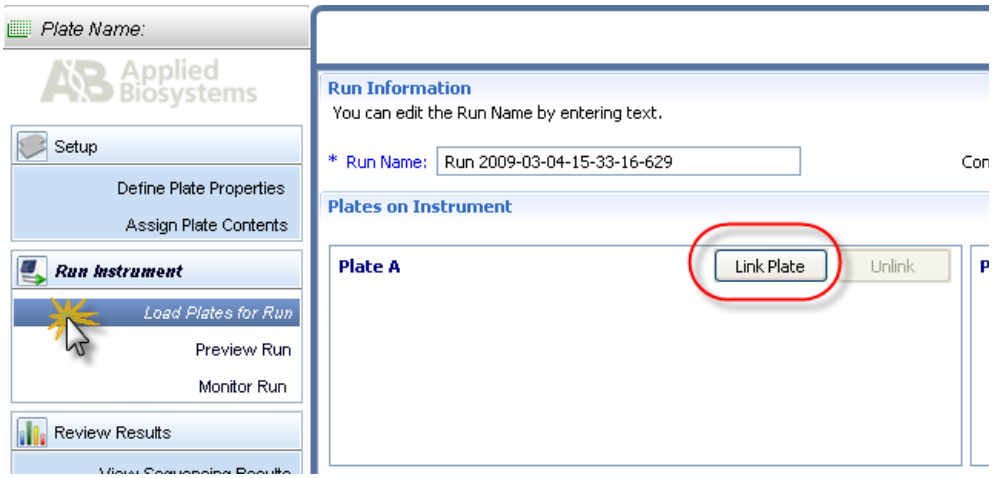
4. If needed, click **Unlink**, then follow the steps in “If a plate is not linked” below.

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 59.
   - **Start Run** – Displays the Monitor Run screen. Go to “Monitor the run” on page 61.

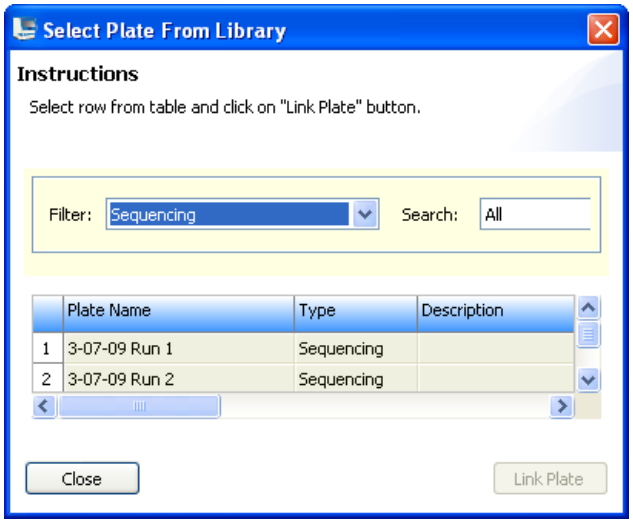
If a plate is not linked

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



To link a plate:

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



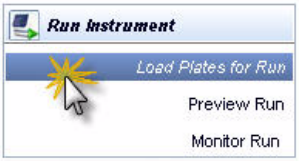
2. Select a plate, then click **Link Plate**.
3. Do either of the following:
   - Click **Create Injection List**, then go to "Review and modify the injection list in Preview Run" on page 59.

     or
   - Click **Start Run**, then go to "Monitor the run" on page 61.

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 7 on page 59) 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.

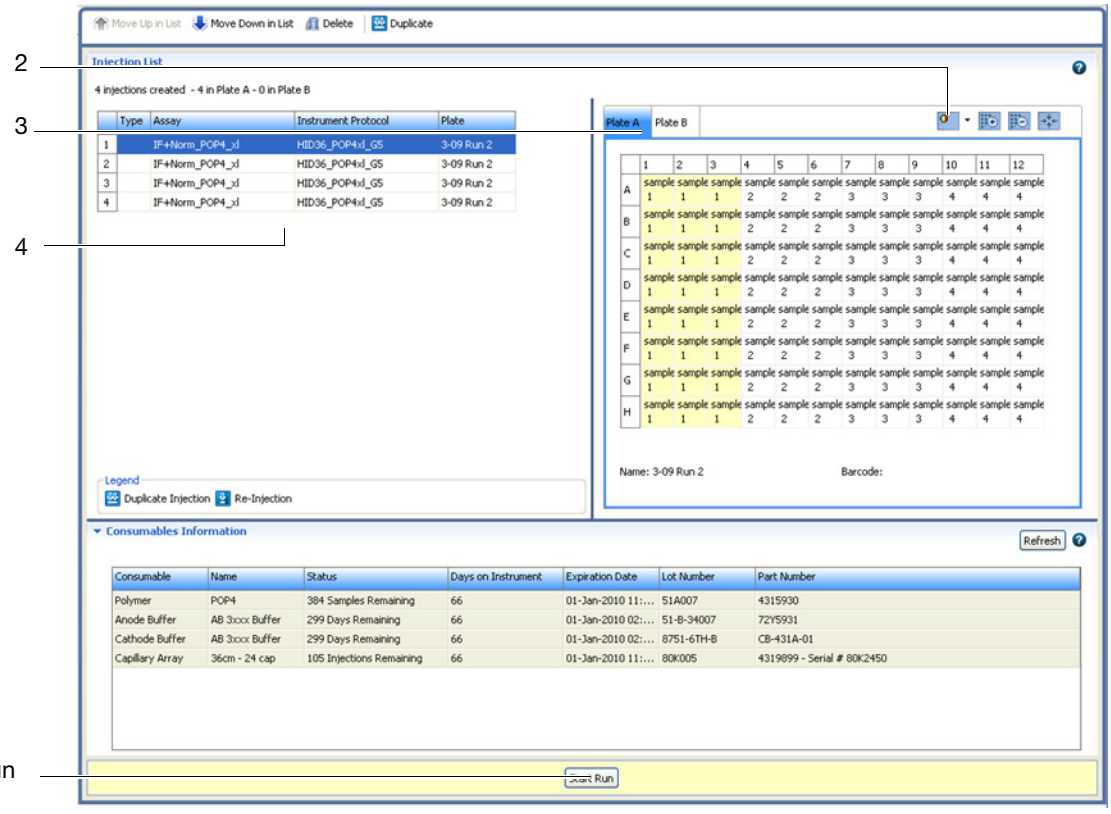


Figure 7 Preview Run screen

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.

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

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. For more information, see "Results group example 3: store re-injections in separate folders" on page 162.

   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.

IMPORTANT! You must specify re-injections before the run completes.

Note: It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.

Monitor the run

The Monitor Run screen (Figure 8 on page 61) 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.

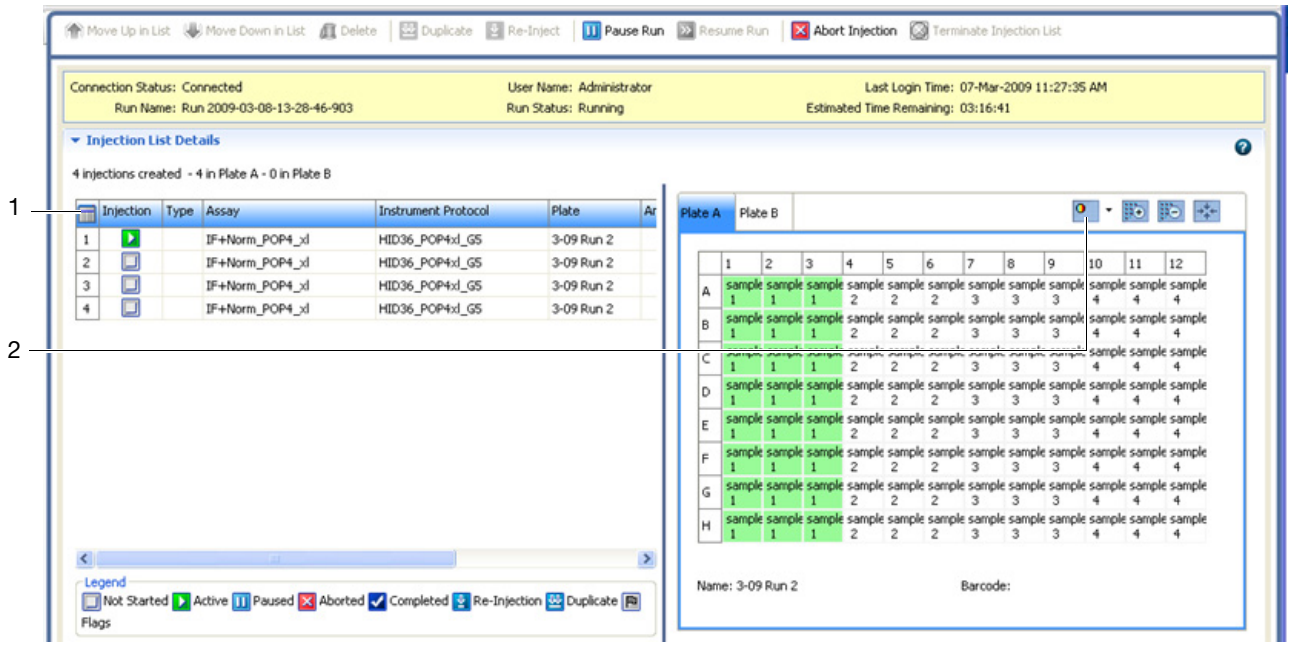
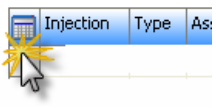


Figure 8 Monitor Run screen

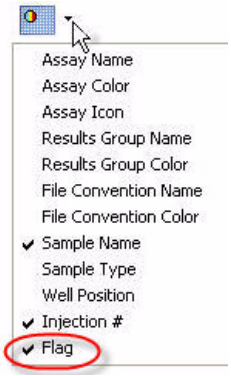
Note: Samples with assays that specify more than one instrument protocol are listed one time in the injection list for each instrument protocol.

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

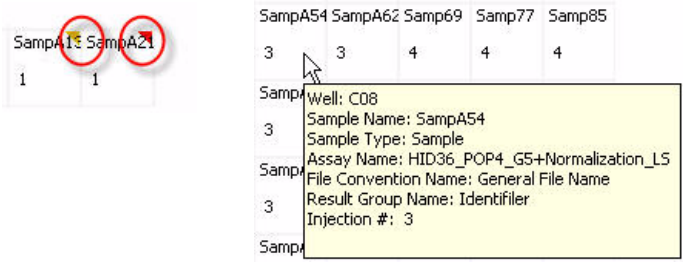


2. Optional:
   - Click the icon above the plate to specify the attributes to display in the plate view. In addition to the attributes available in Preview Run, a Flag attribute is available.

     If you select the Flags attribute, yellow or red marks are displayed for wells with an Average QV value (sequencing) or an SQ value (fragment/HID) in the Fail or Suspect range. Red marks are displayed for wells with offscale data.

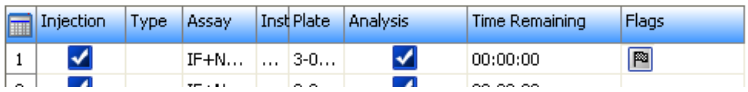


   - Place the mouse pointer over a well to display sample details.

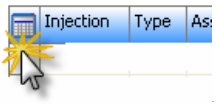


Check sequence or sample quality and specify re-injections

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 🏁.

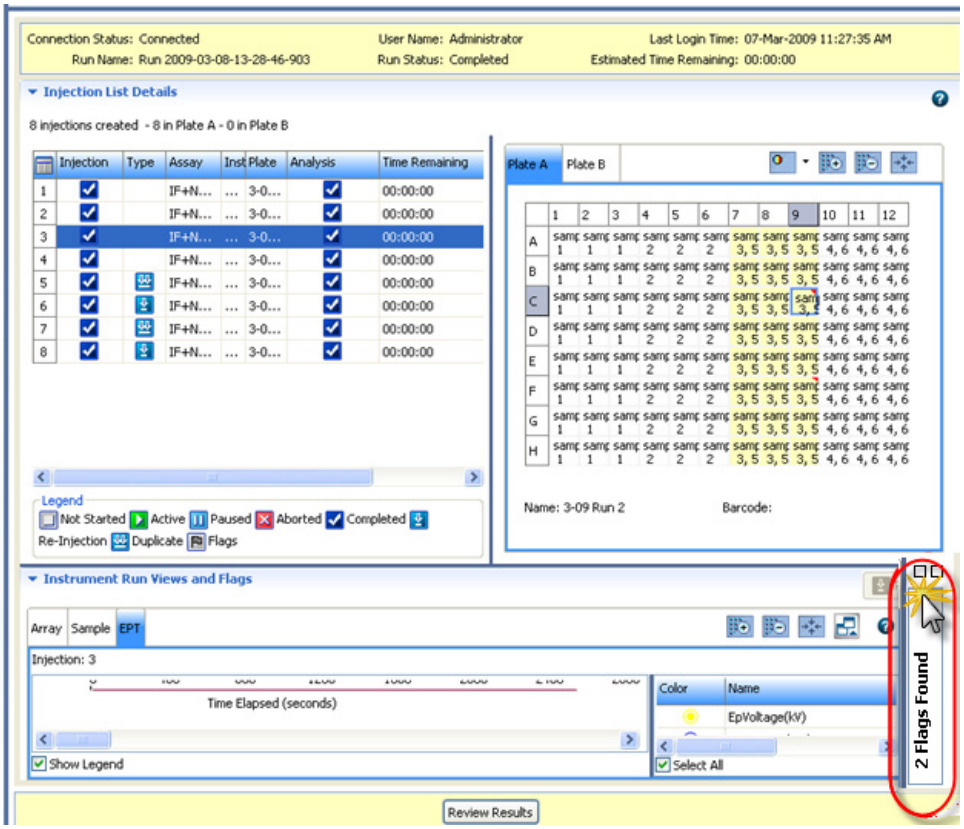


Note: If the Injection, Analysis, or Flags columns are not displayed, you can click the Table Settings button, then show them in the injection list.

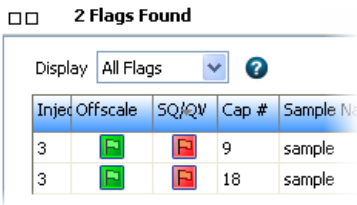


Check sequence or sample quality

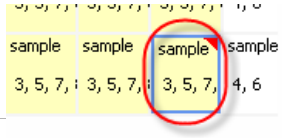
1. Expand the Flag pane at the bottom right of the screen.



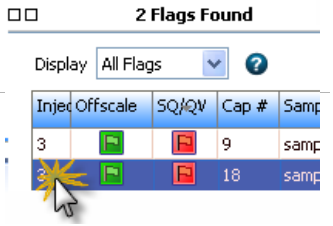
The flag table displays a quick preview of sample quality and identifies samples that may need investigation.



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

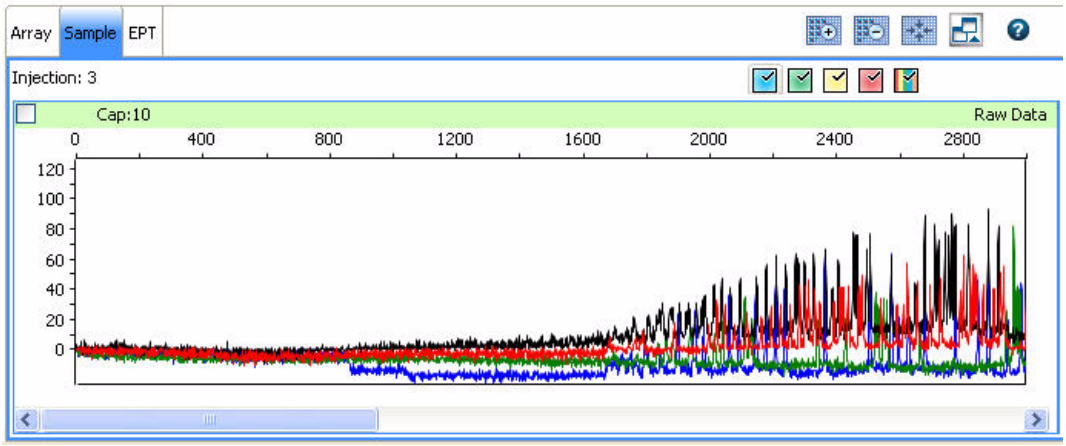
2. To filter the flag table, select a flag type. To display HID flags, select **All**.

   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 178.
Sizing Quality (fragment/HID)  (green, yellow, red)	(yellow) or (red) The Sizing Quality is in the Suspect or Fail range. For information, see, Table 15 on page 183 or Table 17 on page 188. **IMPORTANT!** Normalization is not applied to samples with (red) Sizing Quality.

3. 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

You can specify a re-injection before the run completes. A re-injection physically re-injects all samples in the capillary array. You can select a different instrument protocol than the original injection and can specify whether to collect data for all or only selected samples in the array.

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

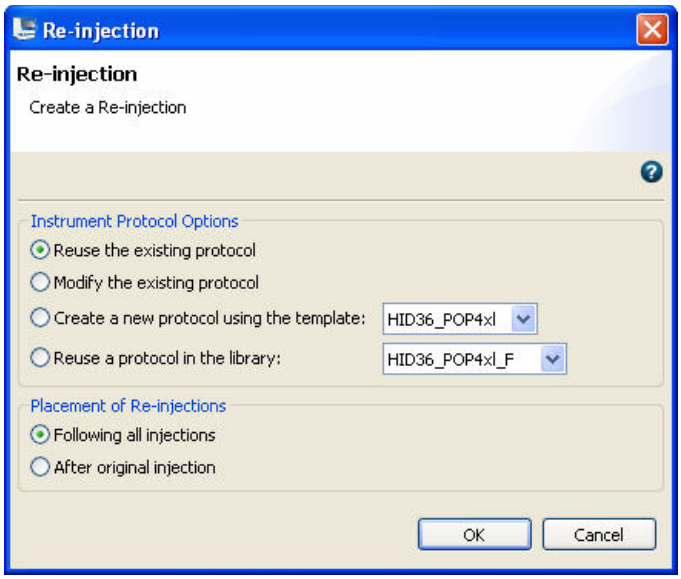
Note: Re-inject is grayed if you select an injection that contains more than one results group, or if you select flags in the flags table that correspond to samples with different results groups. To enable Re-inject, select samples that specify the same results group.

To collect data for all wells in an injection	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) **Note:** You can also specify re-injections for specific samples in Review Results.	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 77). 3. Click **Re-inject**.
To collect data for only samples that contain flags	1. Select the samples in the flag table (see "Check sequence or sample quality" on page 62). 2. Click **Re-inject**.

Note: If you are running an HID plate, see "Re-injections of HID allelic ladder samples" on page 67.

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. For more information, see "Results group example 3: store re-injections in separate folders" on page 162.



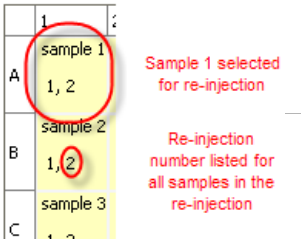
If you select a protocol other than the original

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.



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.

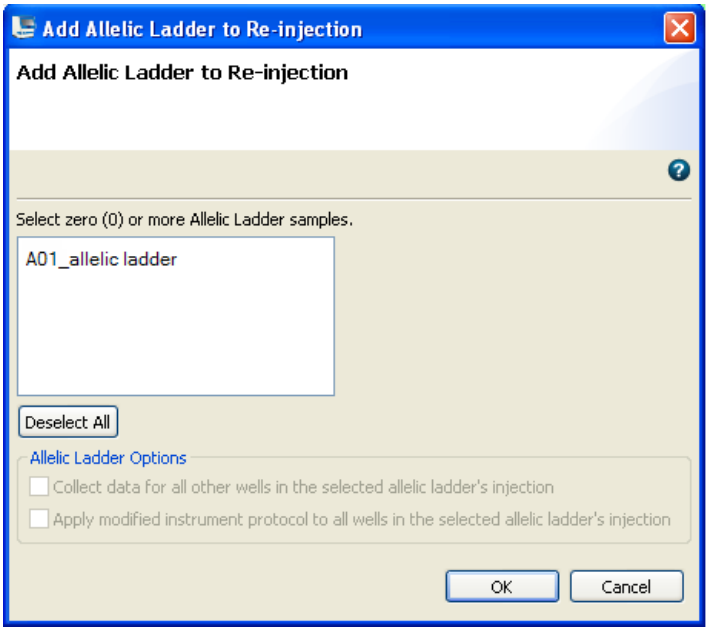
Re-injections of HID allelic ladder samples

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 161)
- 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 v1.2 or later).

4. Click **OK**.

5. Specify the remaining re-injection settings as described in "Specify re-injections" on page 65.

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 "Review Results" on page 79.

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 56).
- Preview Run screen (see "Start the run" on page 61).

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** – Stops the current injection. 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.

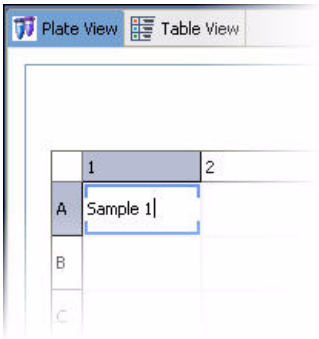
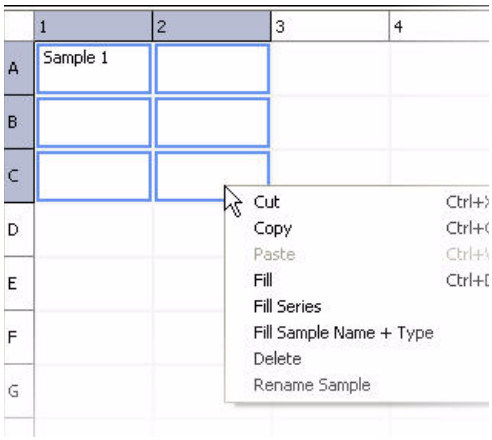
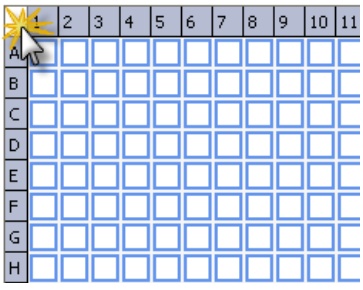
- **Terminate** – Stops the instrument run. Terminate is active only when a run is paused.

More features in Assign Plate Contents

Use the Plate View

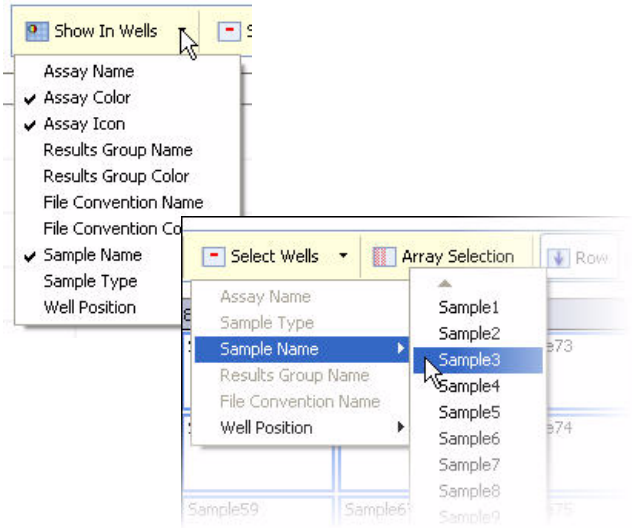
Name samples in the Plate View

To name samples in the Plate View:

To name one sample	• Click a well, then type a sample name directly into the field, then press **Enter**. *or* • Copy and paste a name from another well. To set the direction for the cursor when you press Enter: • 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	1. Click a named well. 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 also copy and paste sample names. 
To name all wells at one time	1. Select all wells. 2. Select assays, file name conventions, and results group for the plate. 3. Enter name and select sample type (in the Customize Sample Info pane) for the whole plate. 

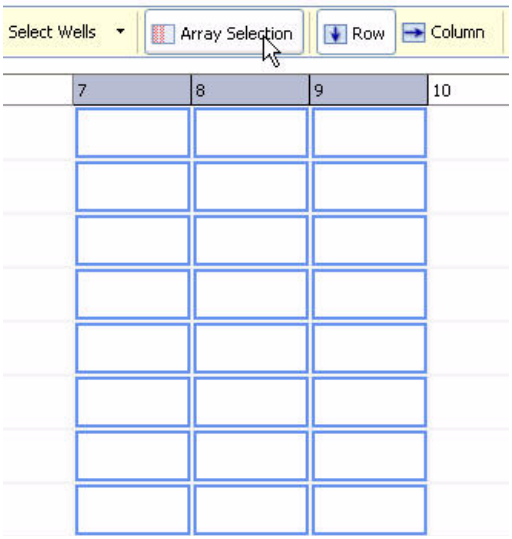
Customize the plate view

- 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.



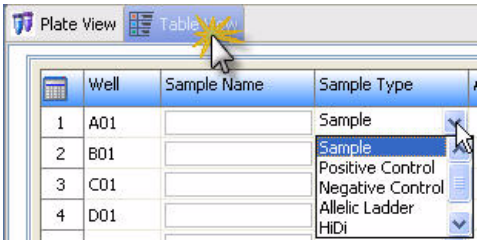
View the capillary/plate map

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

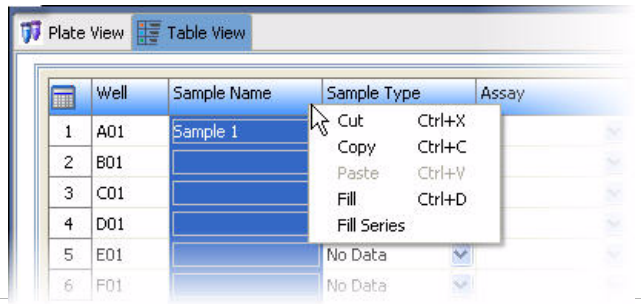


Use the Table View

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.

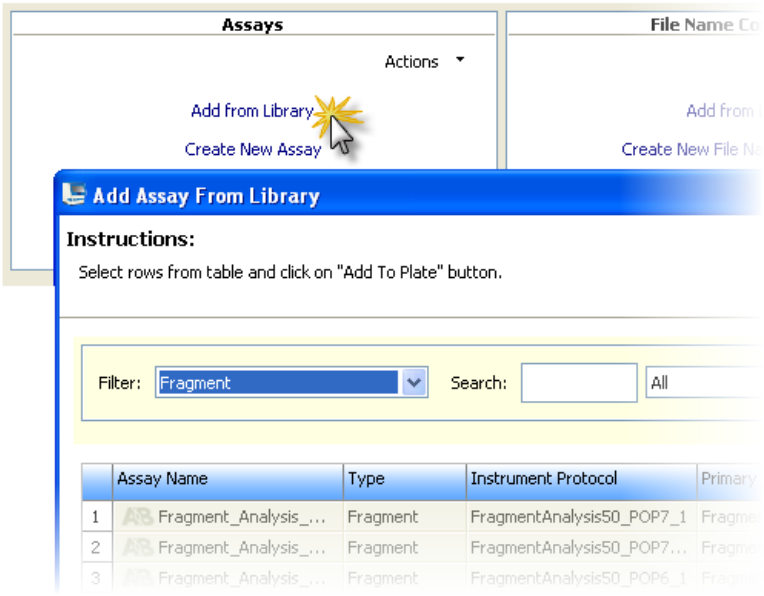
Numbers in the column headers reflect sort order.

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

1. If no assay is listed at the bottom of the Assign Plate Contents screen, add at least one assay. You can specify different assays for different wells.



2. (Optional) If no file name conventions or results groups are listed at the bottom of the Assign Plate Contents screen, add as needed. File name conventions and results groups are optional, but are very useful for naming and organizing data files.

Create a plate for importing

Create a plate import template

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

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

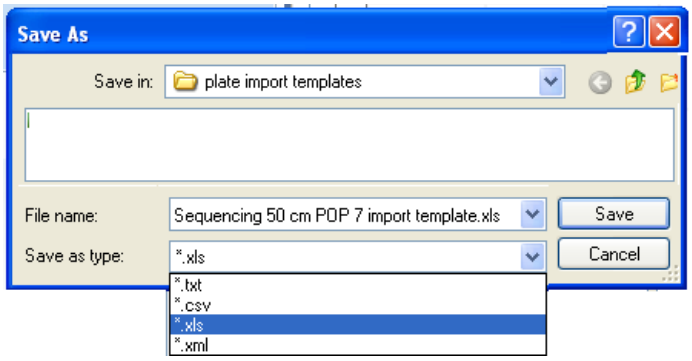
1. In the Dashboard, click **Create Plate from Template**.

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 46.

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 Defi
7	A01								
8	B01								
9	C01								

Create a plate import file

1. Open a plate import template (see "Create a plate import template" on page 73).
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 or 3500xL analyzer instrument. See "Import and export a library entry" on page 141.
- **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.

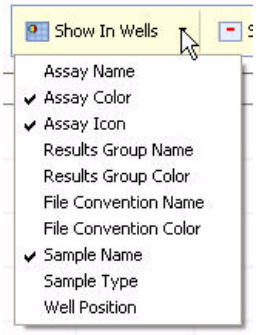
Create a plate template

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 144).
2. (Optional) Add sample names and sample types (see "Name samples and assign sample types in the plate view" on page 48).
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 73).

   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.

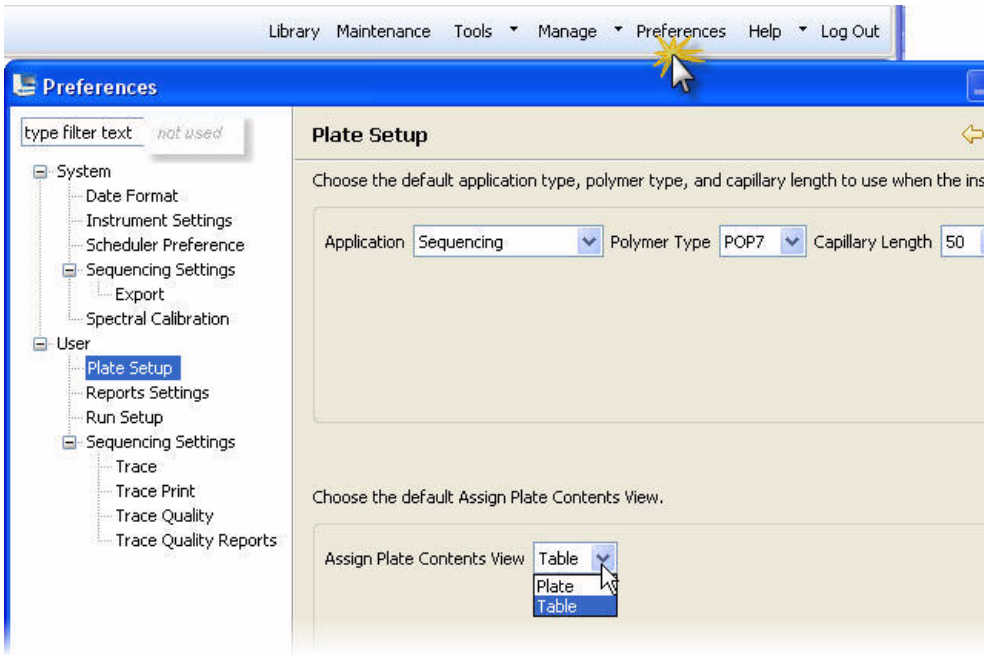
- (Optional) Click **Show In Wells** to specify the attributes to display in wells in the template.



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.



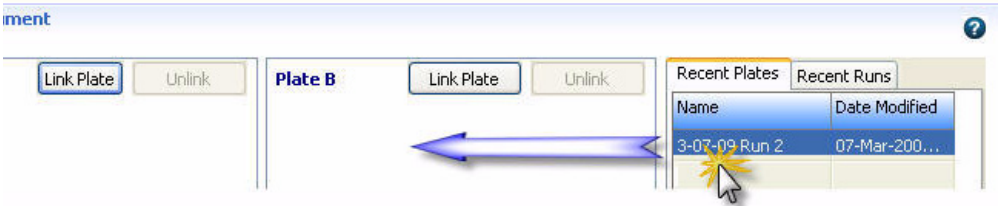
Save electronic version of reports

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).



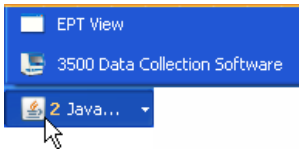
More features in Monitor Run

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.

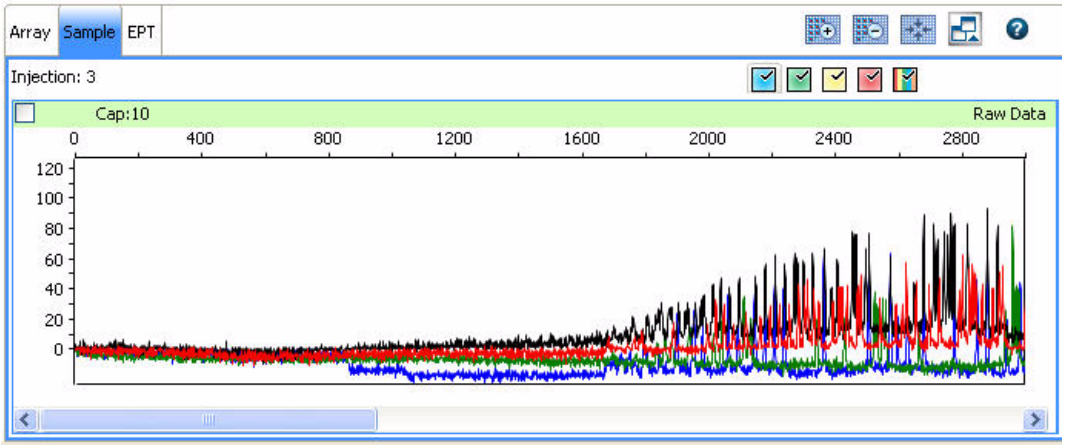


Array view

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.

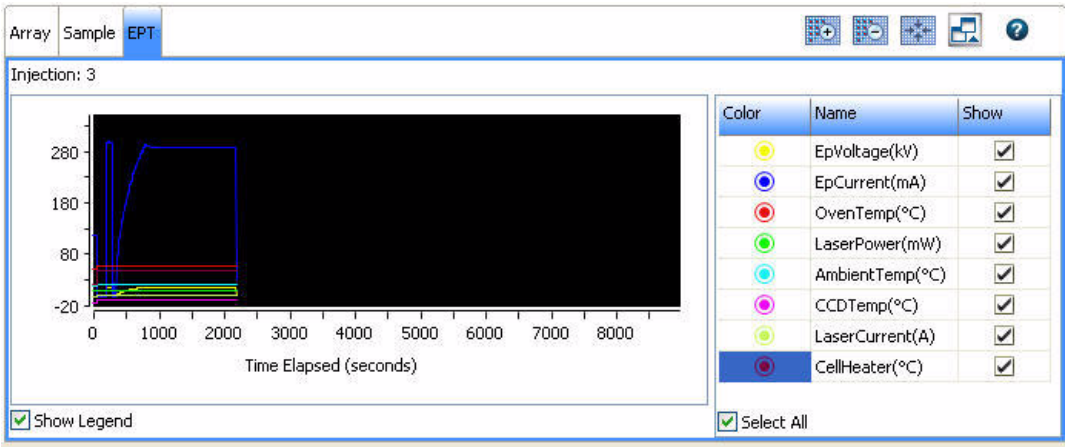
Sample view

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.



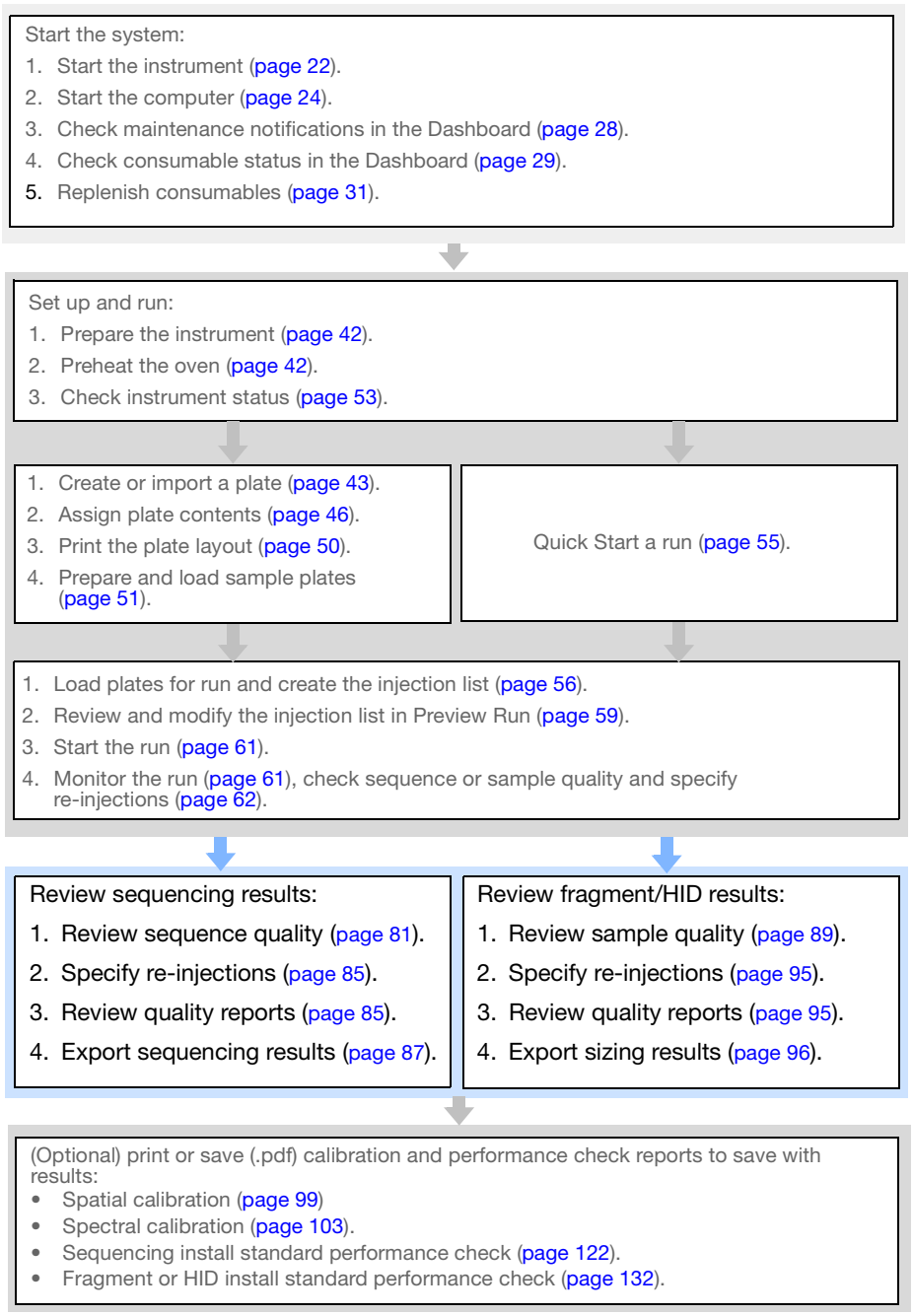
EPT view

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.



Review Results 4

Workflow



Review Sequencing Results

Access the View Sequencing Results screen

Access the View Sequencing Results screen from:

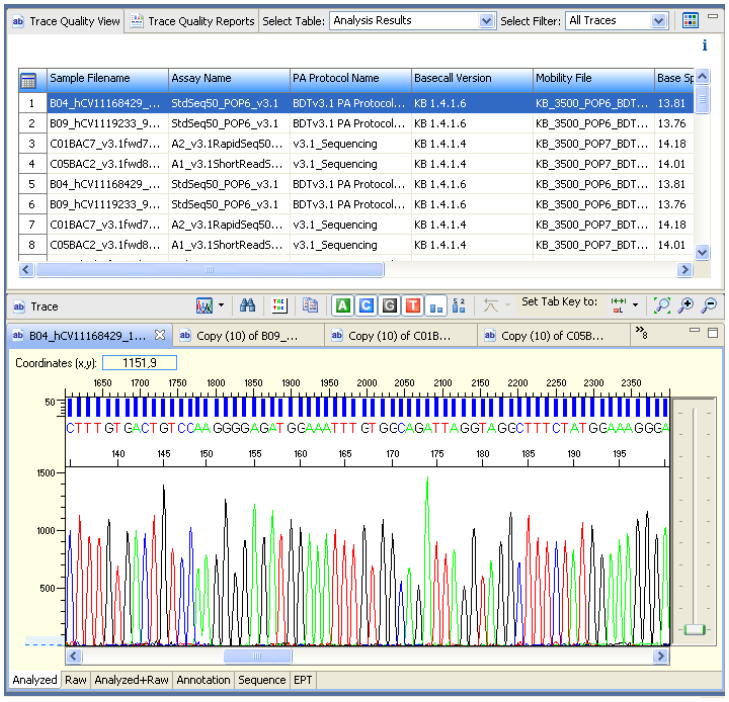
- The Monitor Run screen by clicking **Review Results**.
- The navigation pane by selecting **View Sequencing Results**.
- The Dashboard by clicking View Run Results.

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.



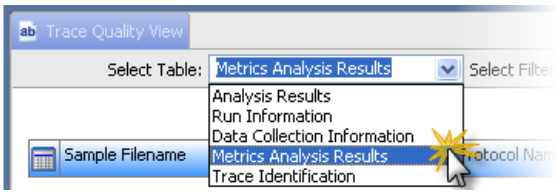
Review previously run samples

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.



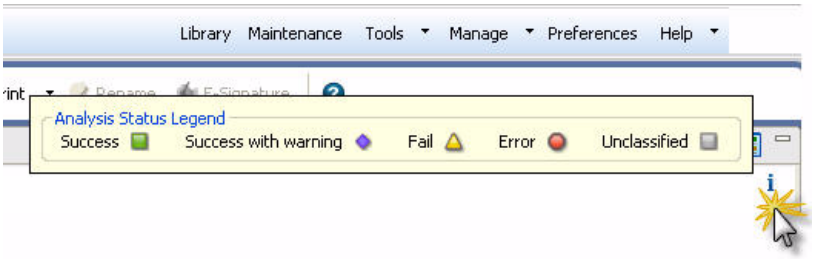
2. Click the Table Settings button, then specify the columns to show or hide.

3. Double-click column headers to sort columns. Multi-column sorting is supported (see "Sort" on page 97).

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:

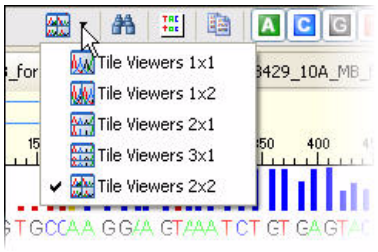
Result	Description
Success	Basecalling and trimming successful.
Success with warning	Basecalling successful, trimming not successful. Warning messages are listed in the Warning/Error Message column (default position is the last column in the table).
Fail	Basecalling and trimming failed, no results generated.
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.

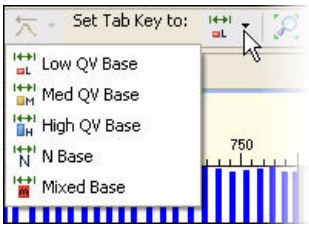
Review traces

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.

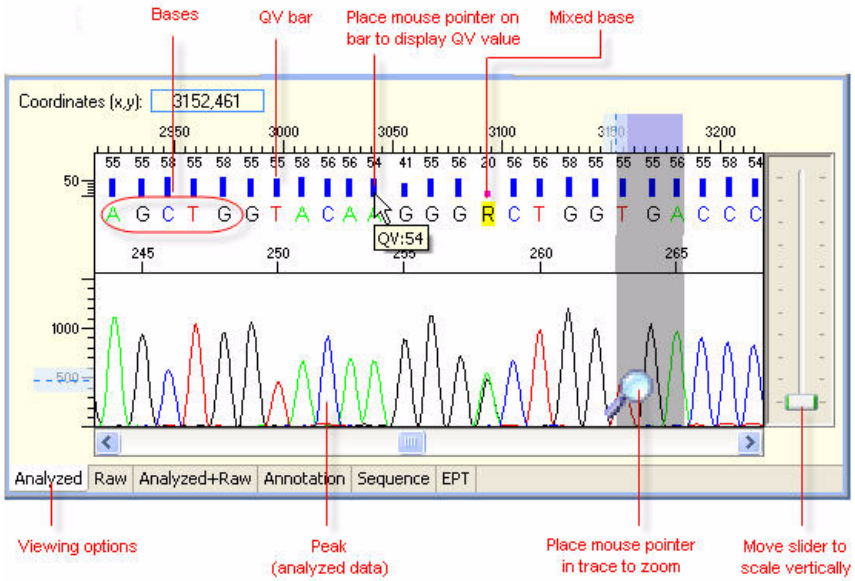
3. (Optional) Modify trace display:
   - Use the Tile Viewer options to display up to four traces at a time.
   - Set trace colors in Preferences (see "Set sequencing preferences" on page 36).



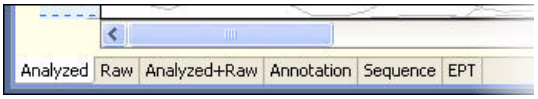
4. Set the category of base for the Tab key.



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

Applied Biosystems recommends the following ranges for QVs (set in Preferences, see "Set sequencing preferences" on page 36):

- **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)

Note: The predicted probability of error for a basecall is high QV > 10.

Note: You can set the software to trim (set the clear range) using quality values in the basecalling protocol (see "Basecalling protocols library (primary analysis – sequencing)" on page 174).

Pure base versus mixed base QVs

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

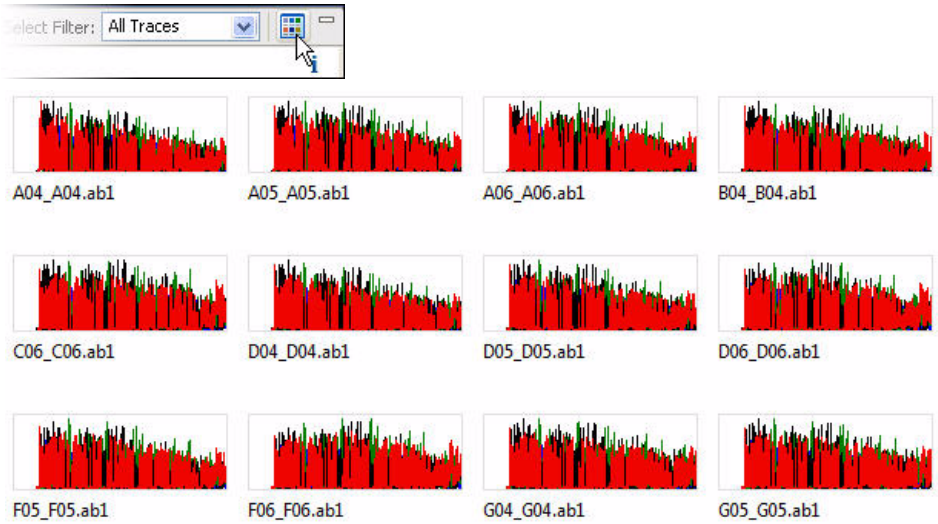
- High-quality pure bases typically have QVs of 20 or higher.
- The distribution of quality values for mixed bases differs dramatically from that of pure bases.
- For mixed bases, quality values greater than 30 are rare.
- Good mixed bases may be assigned quality values as low as 5, because the probability of error with mixed bases is higher. Review mixed bases with QVs between 5 and 10.

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

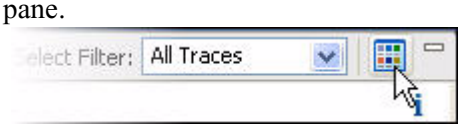
QV	Pe	QV	Pe
1	79.0%	30	0.10%
5	32.0%	35	0.032%
10	10.0%	40	0.010%
15	3.2%	45	0.0032%
20	1.0%	50	0.0010%
25	0.32%	60	0.00010%

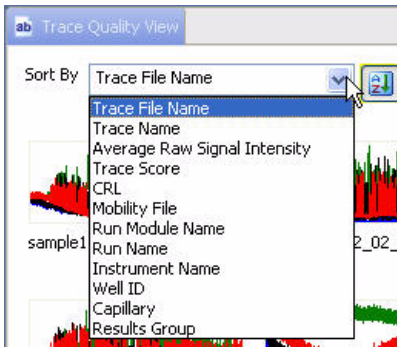
Display thumbnails

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.





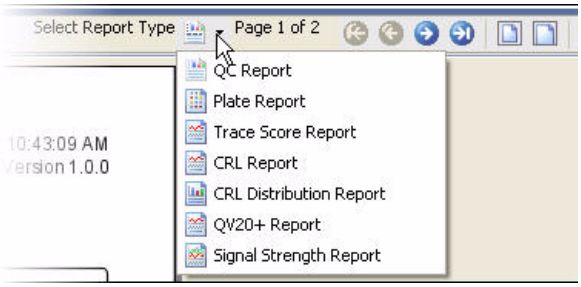
Specify re-injections

Before the run is complete, you can select a sample, then click Re-inject.

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

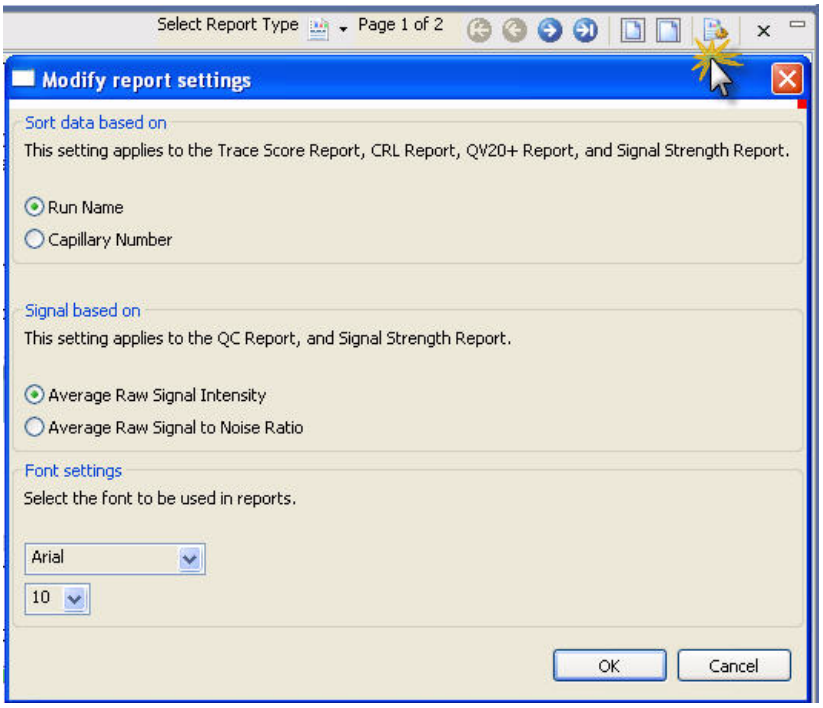
View Trace Reports

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 36).



2. Select the report type and review the content of each report. See "Report options" on page 86.

3. Modify report settings as needed. You can specify additional report settings in Preferences (see "Trace Print (user preference)" on page 38, "Trace Quality (user preference)" on page 38, and "Trace Quality Report (user preference)" on page 39).



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

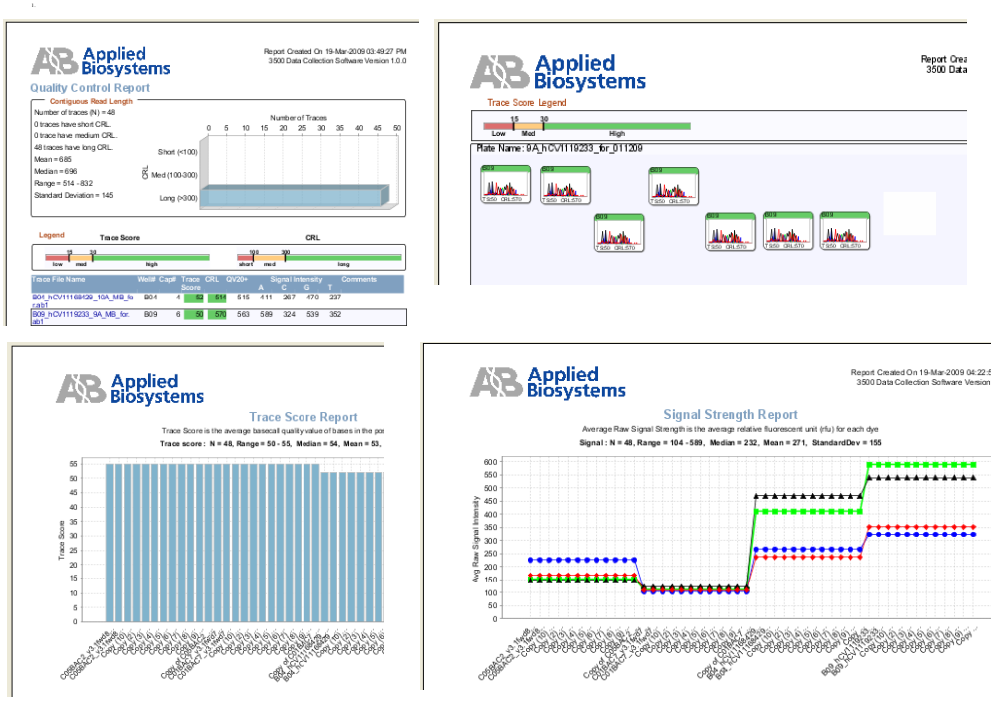
5. To print the report, click **Print**, then preview or print.

6. To save the report electronically (.pdf), print the report and select **CutePDF Writer** as the printer.

7. Close the report.

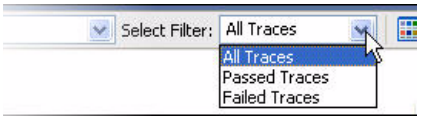
Report options

- **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

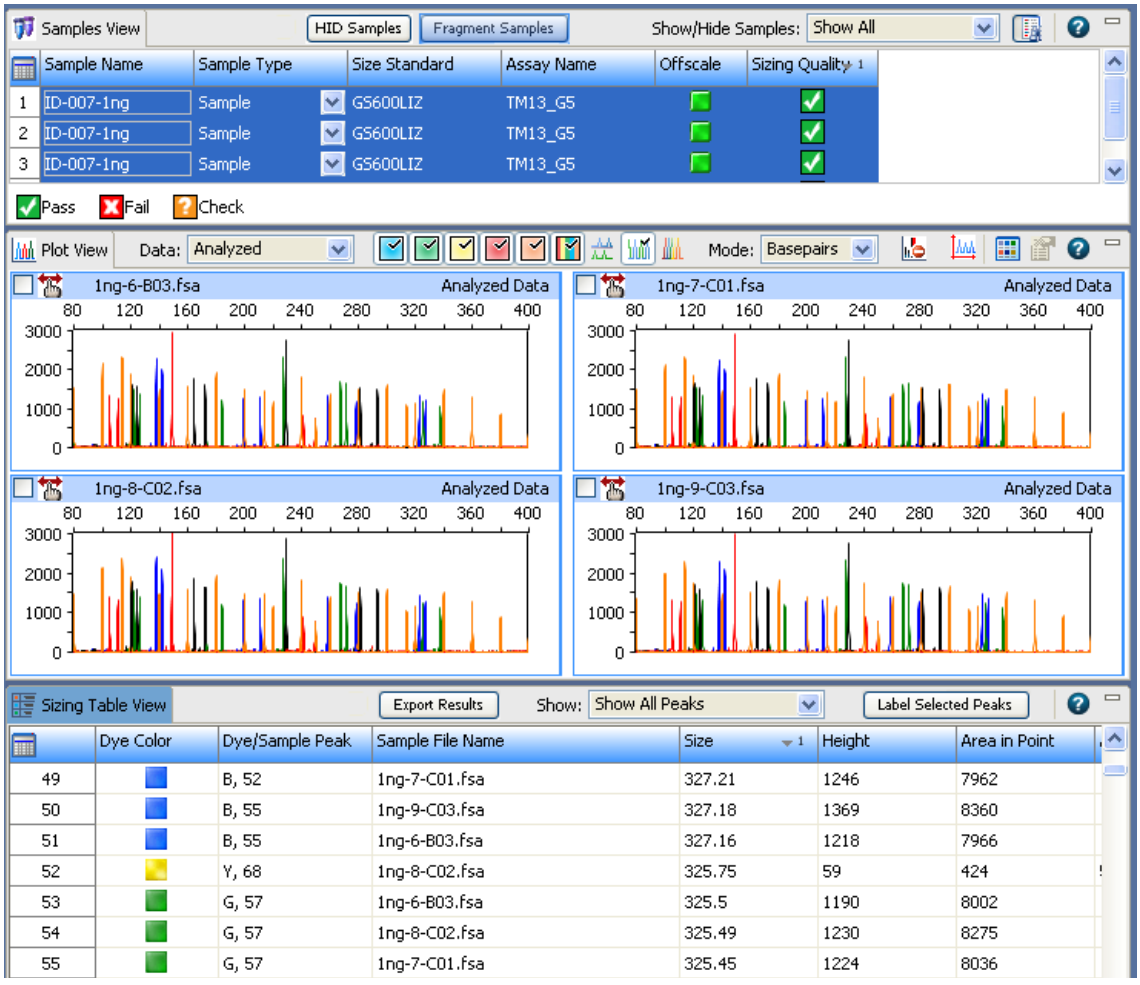
Access the View Fragment/HID Results screen from:

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

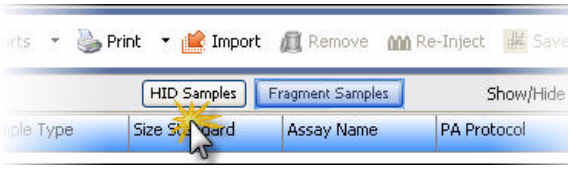


Review previously run samples

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**.

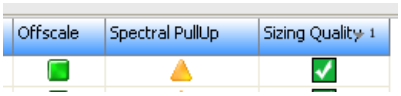


Review sample quality

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 97).

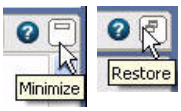


Flag/Symbols	Description
Offscale	At least one data point in the analysis range has saturated the CCD camera. **Note:** In the Monitor Run screen, an offscale sample is flagged with .
Spectral Pull-Up (fragment analysis only)	At least one peak contains a pull-up peak. A pull-up peak is identified when the peak height of the minor peak is ≤ X% of and within ±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	• – 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 or 3500xL analyzer instrument. For more information, see "Review normalized data" on page 90. **Note:** If the Sizing Quality is , normalization is not applied, even if the Normalization Factor is within the normalization range.

Flag/Symbols	Description
Sizing Quality  **Note:** If the Sizing Quality is  , normalization is not applied, even if the Normalization Factor is within the normalization range.	 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.

3. 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.

4. (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 171.

How normalization is applied

To normalize, the software:

1. Determines if the data was collected on the 3500 or 3500xL 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.

   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).
6. 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 Data Collection Software, the calculated Normalization factor is stored with the raw data and is applied to the raw data in the GeneMapper® *ID-X* Software v4.1 and the GeneMapper® *ID-X* Software Software v1.2 secondary analysis software. You can turn normalization off and on in the analysis method used in the GeneMapper® v4.1 and GeneMapper® *ID-X* Software v1.2 secondary analysis software. If normalization is not applied in the 3500 Series Data Collection Software (either a normalization size standard was not used, or Sizing failed), normalization cannot be applied in the secondary analysis software.

Review plots

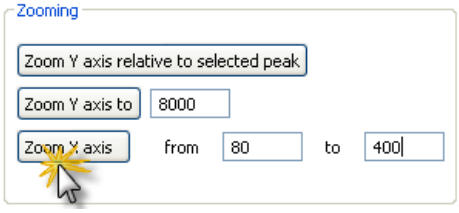
1. Select the samples of interest in the samples table.
2. Select items from the plot toolbar to manipulate the plot as needed. Place the mouse pointer over a button for the description of the button.

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:

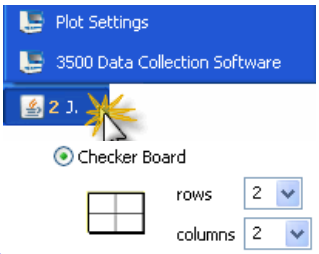
   Enter the range for Y axis and X axis, then click the Zoom buttons.

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.



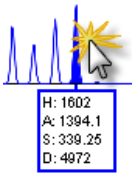
To apply scaling settings to all samples in the samples table, select all of the samples in the samples table to display them in the plot view, specify the scaling settings, click **Zoom**, then click **Page Up** and **Page Down** in the plot view to move through the samples.

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**.

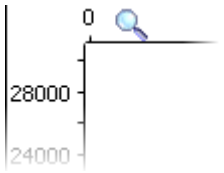
5. Click a peak to label it (to label all peaks, see "Label peaks" on page 93).



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:

a. Under Labels to Show, select the needed labels.

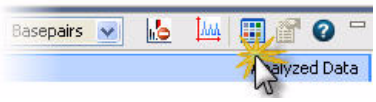
b. Under Labelling Options:
   - Enable **Show Peak Labels**.
   - To label all peaks with the selected labels, click **Label Peaks** (make sure **All** is selected).
   - To label selected peaks, select the category from the Label Peaks list (Height, Area, Size), specify the range to label for the selected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

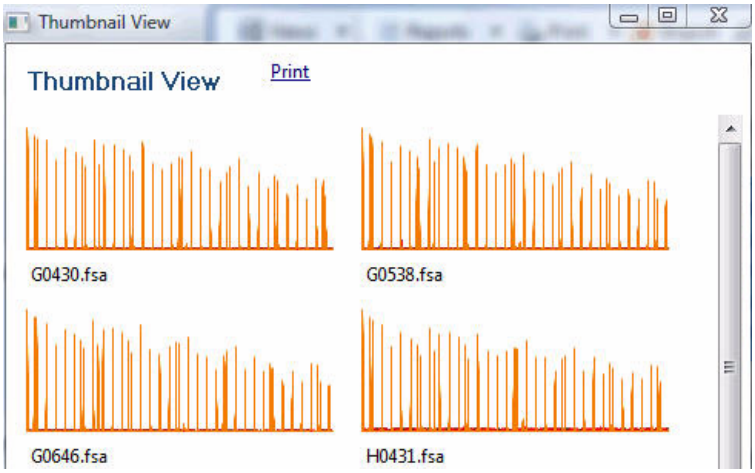
a. Click **Save to Preferences** to save these settings for future use. You can change preferences at any time.

b. 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.





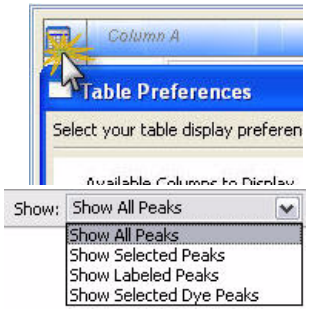
Review sizing

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 97).
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

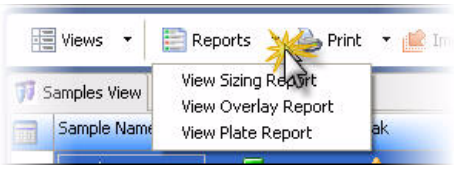
1. Click (Plot Settings) in the Plot View toolbar.
2. Select **Overlay Sizing Curve** in the Display tab.

Specify re-injections

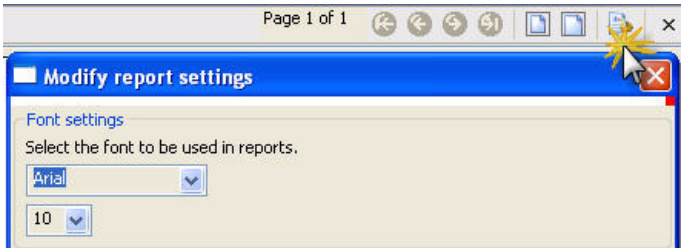
Before the run completes, select a sample with suspect or failing flags, then click **Re-inject**.

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.



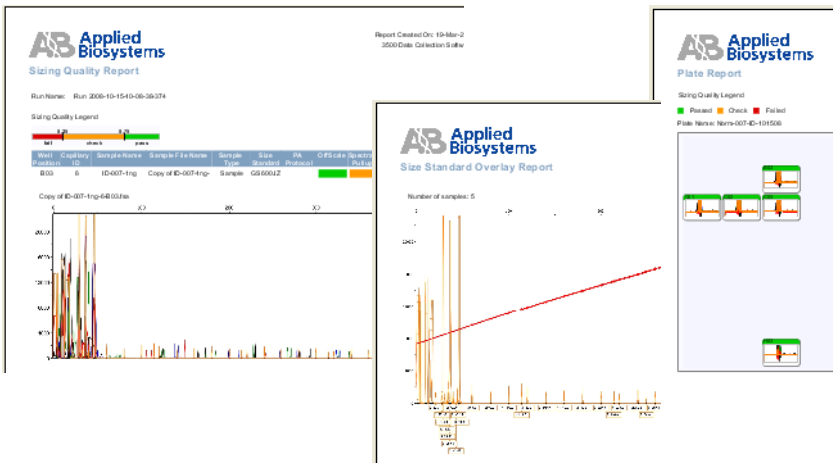
3. Select the report type. Reports are displayed in the Sizing Table View at the bottom of the screen.
4. Modify report settings as needed.



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 sizecalling 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 v2.7 (or later), MicroSeq® ID Analysis Software v2.2 (or later), Variant Reporter™ Software (v1.1 or later), and Sequence Analysis (SeqA) Software (v5.4 or later)
- **Fragment analysis** – GeneMapper® Software v4.1 (or later)
- **HID** – GeneMapper® *ID-X* Software v1.2 (or later)

Calibrate and Check Performance 5

Section 1 Calibration

Spatial calibration

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

When to perform a spatial calibration

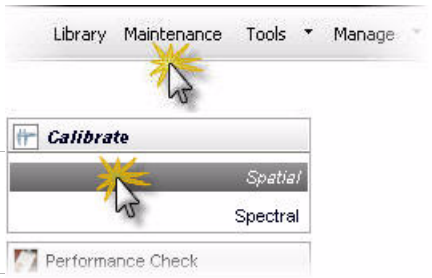
Perform a spatial calibration after you:

- Remove or replace the capillary array
- Open the detector door or move the detection cell
- Move the instrument

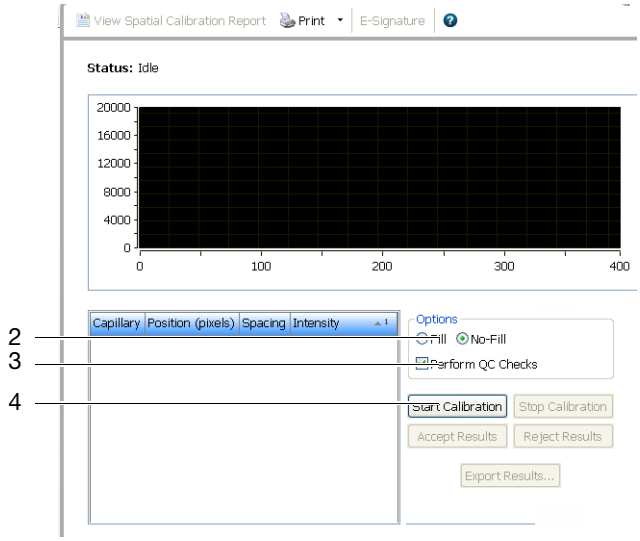
Perform a spatial calibration

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 Data Collection Software.

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.



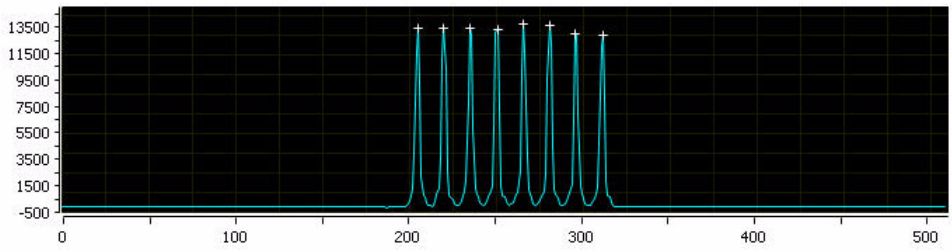
2. Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.

   (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	$\frac{\text{sum of all peak heights}}{\text{number of peaks}}$	• 8-cap: 6400 RFU • 24-cap: 3000 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.



   If the average of any of the QC values exceeds the threshold, a Spatial QC Check error message is displayed.

Evaluate the spatial calibration profile

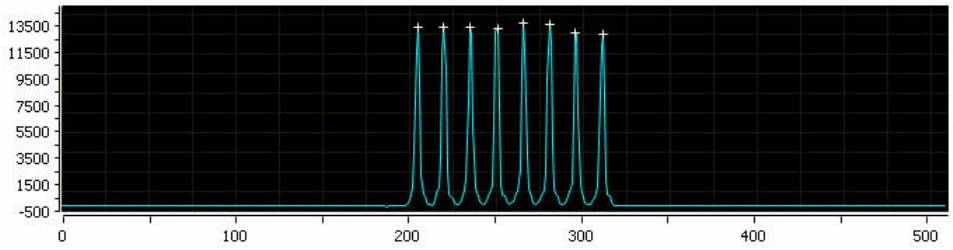
When the run is complete:

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**.

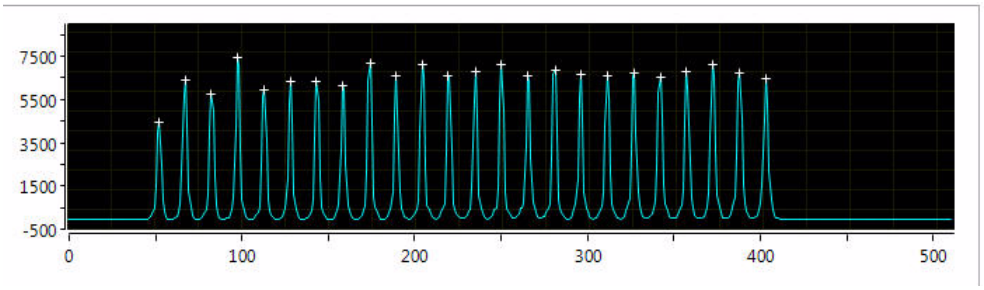
   If the results do not meet the criteria above, click **Reject Results**, then go to "Spatial calibration troubleshooting" on page 300.

Example spatial profiles

8-capillary



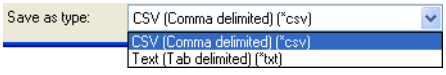
24-capillary



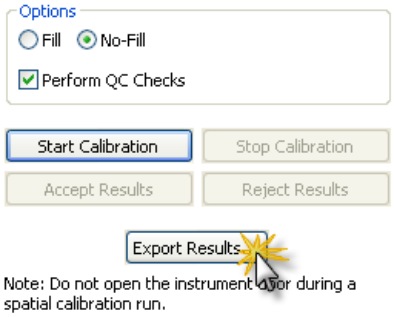
Export spatial calibration results

To export spatial calibration results:

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



4. Click **Save**.



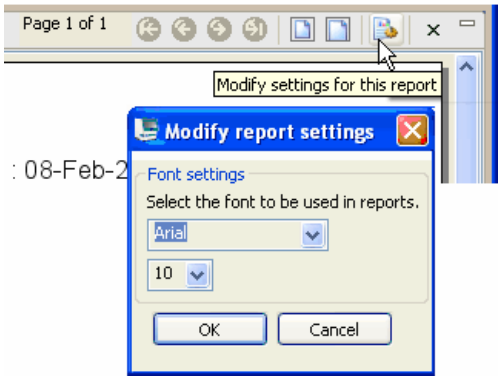
The export file contains the following results:

- 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 4-dye, 5-dye, 6-dye, or AnyDye data stored in each sample file.

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
- Change the capillary array
- Change the polymer type
- 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 "Run the sequencing install standard performance check" on page 119.

Estimated run times

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 99).
2. In the Dashboard, check consumable status (page 29). 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 31).
4. Set the oven temperature, then click **Start Pre-heat**:
   - **60** °C – POP-7™ polymer
   - **50** °C – POP-6™ polymer

   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.

   Applied Biosystems recommends 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 251).

Prepare the standard calibration plate

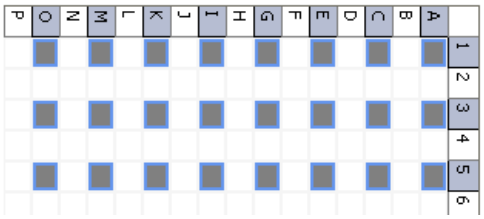
IMPORTANT! Do not use warped or damaged plates. 

1. Prepare the calibration standard as described in the standard product insert. See Table 28 on page 259 and Table 29 on page 260 for standard part numbers.

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

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

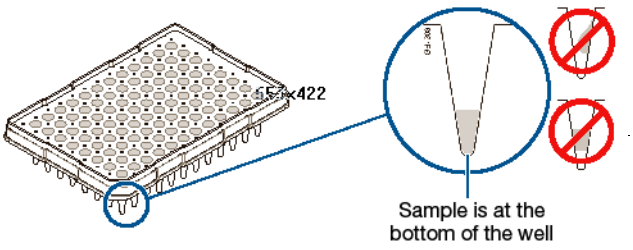
IMPORTANT! You do not create a plate for the calibration. The software uses predetermined positions for the calibration. You cannot specify standard location on the plate. If you do not place calibration standards in the positions indicated, the calibration will fail.

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.

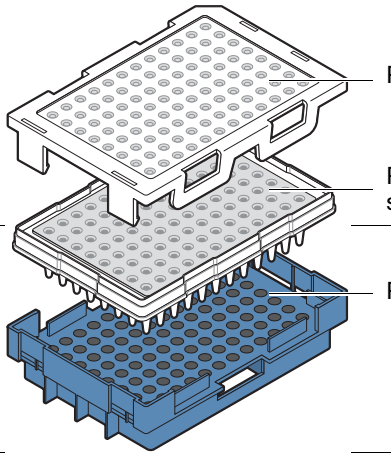
5. Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.

Prepare the plate assembly

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

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
2. Place the sample plate into the plate base.

IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

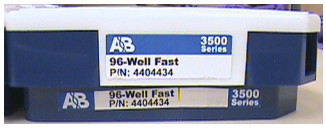


3. Snap the plate retainer (cover) onto the plate, septa, and plate base.
4. Verify that the holes of the plate retainer and the septa strip are aligned. If not aligned, re-assemble and then assemble the plate assembly.

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

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.

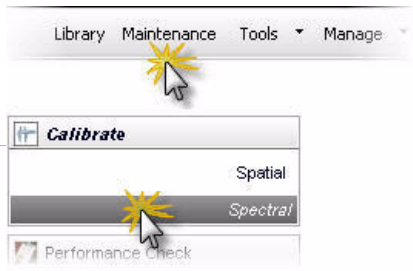


Perform a spectral calibration

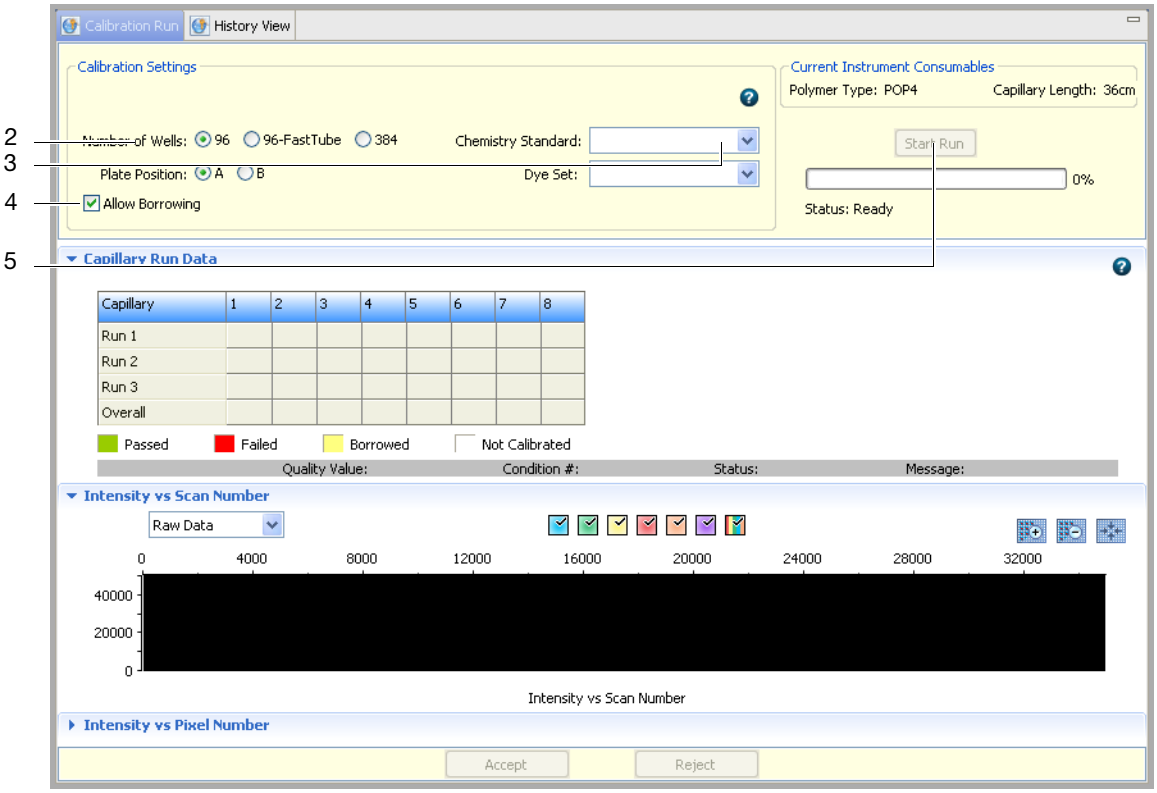
IMPORTANT! Do not change electronic signature settings during a spectral calibration.

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. Select the number of wells 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 168), 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. (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 112.

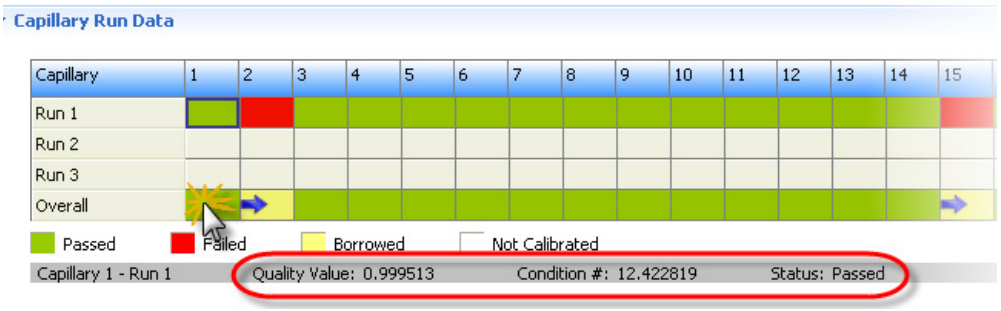
5. Click **Start Run**. The following occurs:
   - The system sets up three injections (see "What you see during a spectral calibration" on page 112 for information on the number of injections performed).
   - The Capillary Run Data display updates after each injection is complete.
   - The status bar 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 116.



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 110).

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

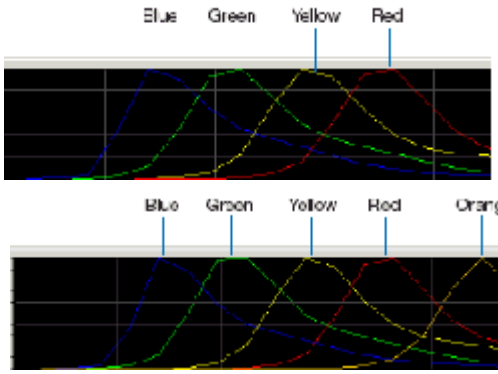
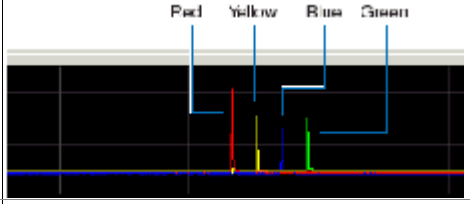
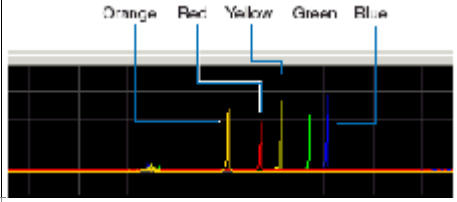
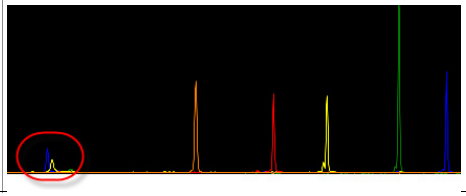
Evaluate the spectral calibration data

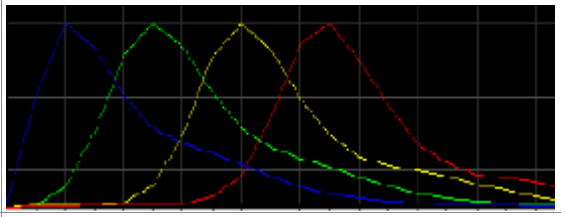
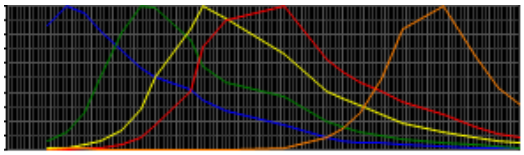
IMPORTANT! Do not accept a spectral calibration until you examine the data for all capillaries.

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

For each capillary:

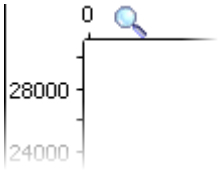
1. Click a capillary to display the spectral and raw data for a capillary.
2. Check that the data meet the following criteria:

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

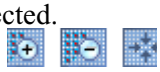
Attribute	Acceptance Criteria	Example
Peak morphology in the spectral profile	• No gross overlaps, dips, or other irregularities • Peaks separate and distinct	
	Note: The profiles of G5 (shown to the right), F, and J6 may not be as smooth as the profiles for other dye sets (shown above) due to the effect of variable binning (a feature that reduces signal variation between dyes of different fluorescent efficiencies).	

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

   a. 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  .

   b. 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 301.

What you see during a spectral calibration

A spectral calibration automatically sets up 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

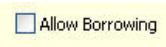
Note: The first time you perform a spectral calibration (for each dye set) after installing a new capillary array, you may notice pull-down peaks (or mirror image peaks). These pull-down peaks will eventually correct themselves once the run completes.

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 109, 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 109 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



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

Injection 1	• 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	• 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	• 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

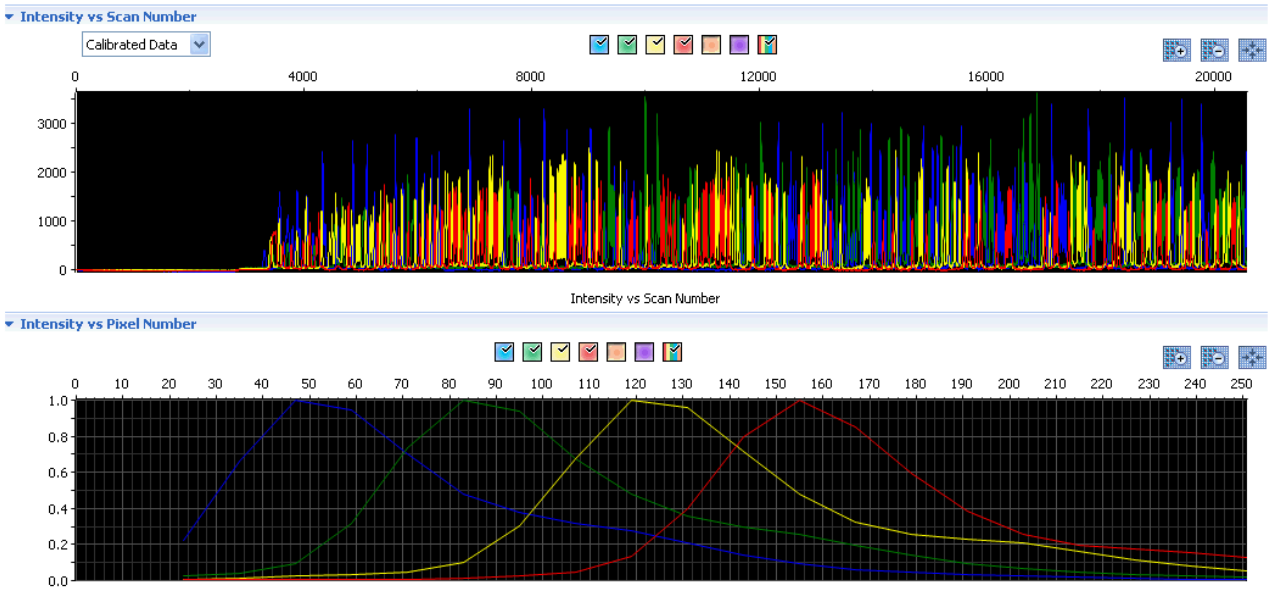
When Borrowing is *enabled*, all capillaries have to pass (meet the spectral Quality Value and Condition Number limits) within the borrowing limits:

- 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).

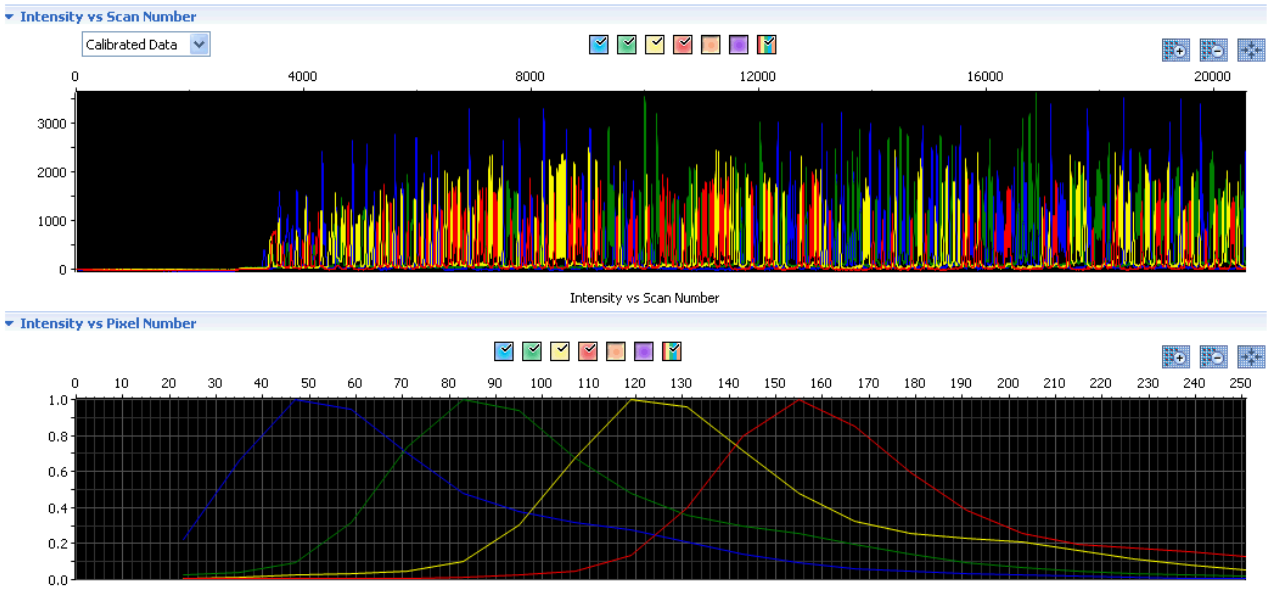
Injection 1	• The software evaluates the Quality Value and Condition Number of all capillaries. • If all capillaries pass, the calibration is complete, and injections 2 and 3 are not performed. • If any capillaries fail, the software borrows from an adjacent capillary. • If, after borrowing, >1 or > 3 capillaries fail, injection 2 is performed.
Injection 2	• 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	• 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

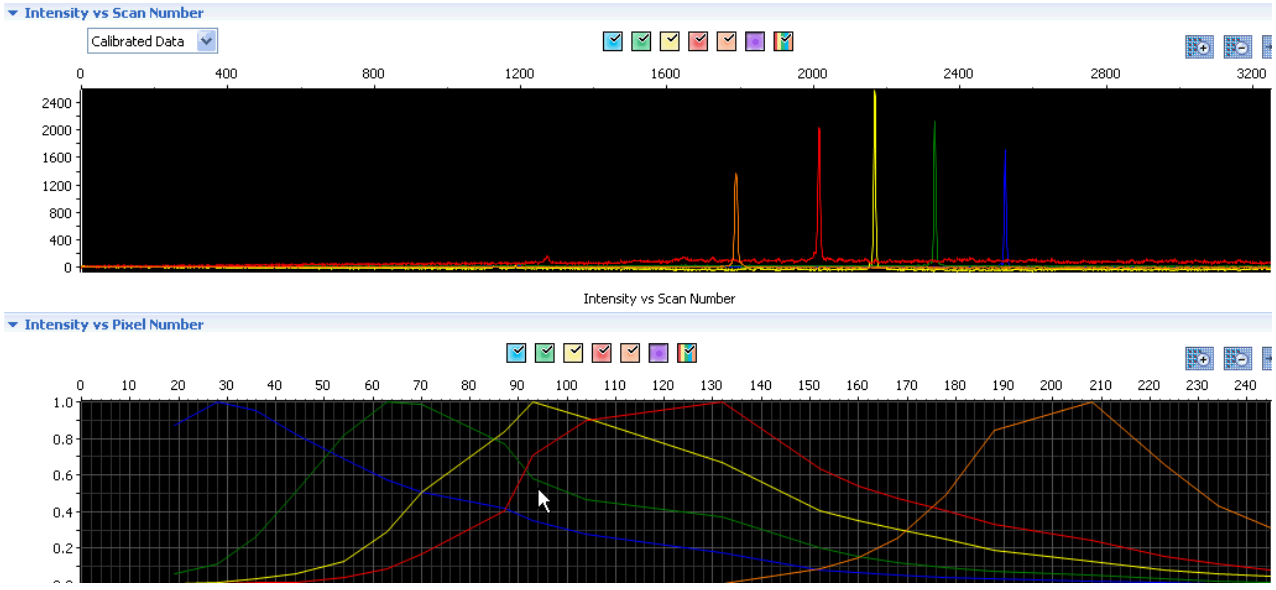
Dye Set E created from Sequencing Standard



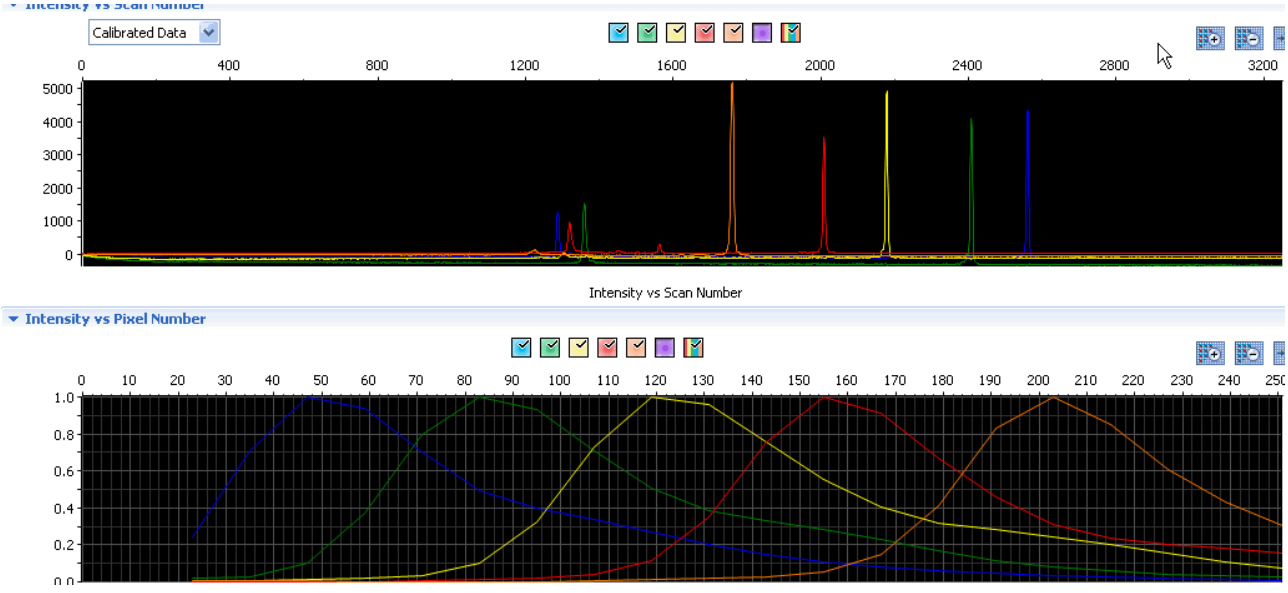
Dye Set Z created from Sequencing Standard



Dye Set G5 created from Matrix Standard Set DS-33



Dye Set E5 created from Matrix Standard Set DS-02



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**.

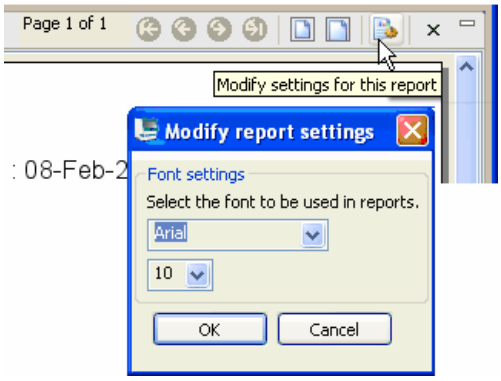
The export file contains the following results:

- 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.

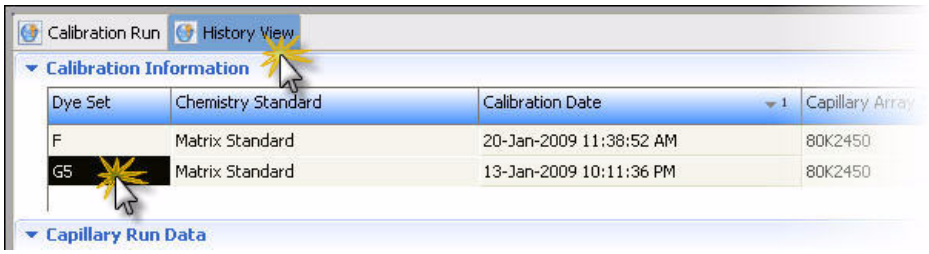
Save historical calibration reports (.pdf) for record keeping

IMPORTANT! After performing a calibration, save the calibration report electronically for record keeping. 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 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

Select **History View**, then select a dye set to view the associated calibration history.



Section 2 Performance check

The Performance check allows you to periodically self-check the instrument system using Applied Biosystems standard.

Run the sequencing install standard performance check

When to perform

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

Applied Biosystems recommends 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 103) for E and Z dye sets. You still need to run spectral calibrations for other dyes sets.

The performance check is application-specific. If you will run general sequencing applications with POP-7™ polymer and MicroSeq® ID applications with POP-6™ polymer, install the appropriate polymer and perform separate performance checks.

Estimated run times

- General sequencing – 45 minutes
- MicroSeq® ID – 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 29). 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 31).
3. Set the oven temperature, then click **Start Pre-heat**:
   - **60** °C – General sequencing POP-7™ polymer
   - **50** °C – MicroSeq® ID POP-6™ polymer

   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.

   Applied Biosystems recommends 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 page 251).

Prepare the installation standard plate

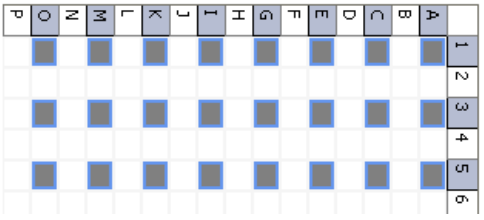
IMPORTANT! Do not use warped or damaged plates.

1. Prepare the sequencing install standard as described in the product insert. See Table 28 on page 259 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:

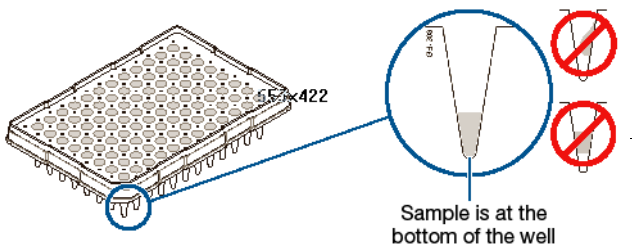
IMPORTANT! You do not create a plate for the performance check. The software uses predetermined positions for the performance check run. You cannot specify standard location on the plate. If you do not place standards in the positions indicated, the calibration will fail.

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 **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.



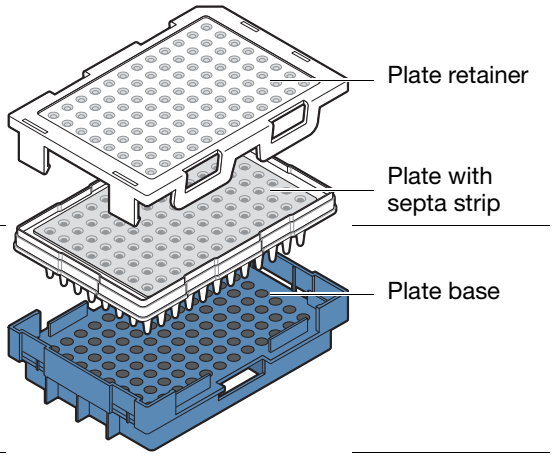
IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

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

Prepare the plate assembly

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

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
2. Place the sample plate into the plate base.



IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

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 not aligned, re-assemble and then assemble the plate assembly.

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

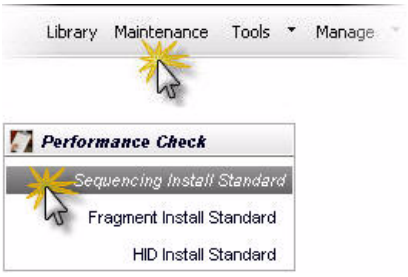
Load the plate in the instrument

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 sequencing install standard performance check

1. Access the sequencing install standard screen (Figure 9 on page 122): Select **Maintenance**, then select **Sequencing Install Standard** in the navigation pane.



2. Select the chemistry type: **General Sequencing** or **MicroSeq® ID**.

3. 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.

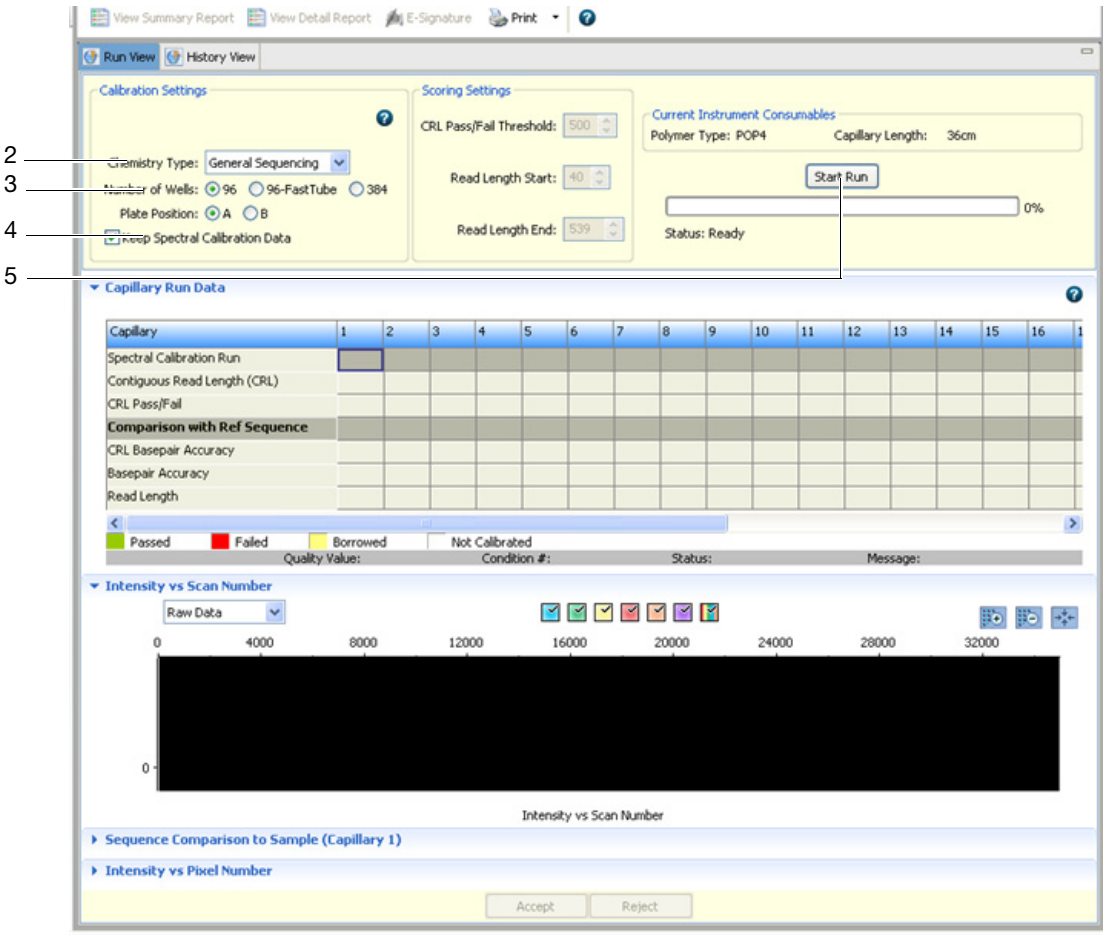


Figure 9 Sequencing Install Standard screen

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. General Sequencing with BDTv3.1 Install standard and POP-7™ polymer generates a Z dye set spectral calibration.

   Note: he 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.

   - MicroSeq® with BDTv1.1 Install Standard and POP-6™ polymer generates an E dye set spectral calibration.

5. Click **Start Run**.

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

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 10 on page 124) 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.

  Note: Clicking a borrowed capillary displays borrowed, not failed, data. For information on why a capillary failed, look in the Sequencing Install Standard Detail Report.

- 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.

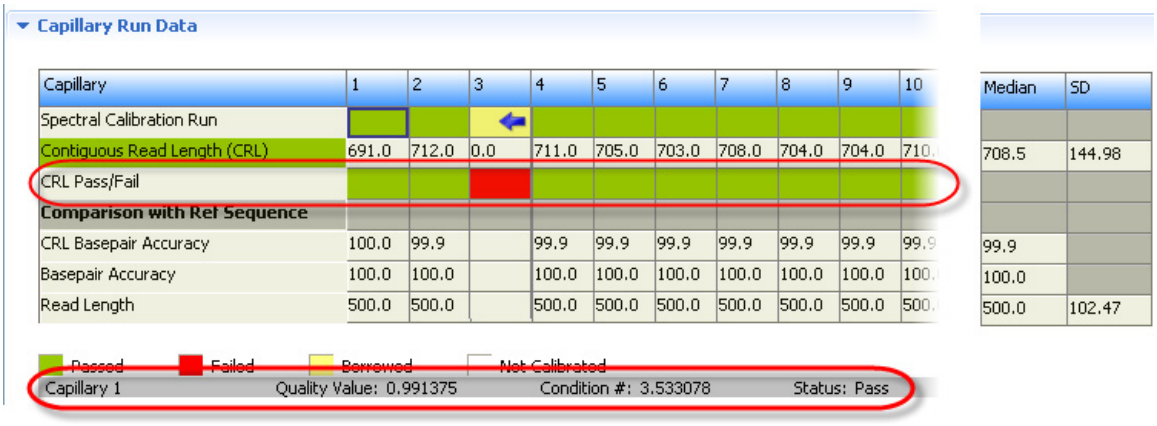


Figure 10 Sequencing install standard – capillary run data

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 109).

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 112. The number of borrowing events can be decreased – see "User preferences" on page 34.

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.

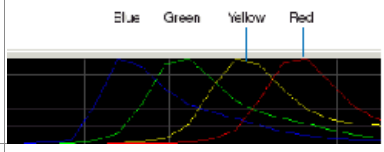
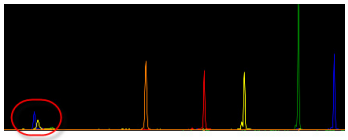
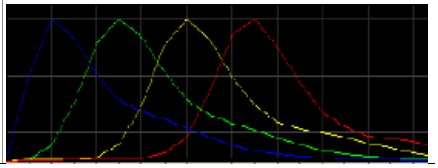
Result	Description
Contiguous Read Length (CRL)	The longest uninterrupted segment of bases with an average Quality Value (QV) ≥ 20. In addition to evaluating the QV of a base call, the software considers the QV of adjacent bases within a ± 20-bp moving average to determine a contiguous read length based 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	• **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 ≥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 ≥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)	• No gross overlaps, dips, or other irregularities • Peaks separate and distinct • Peak apexes are separate and distinct (the tails will overlap)	

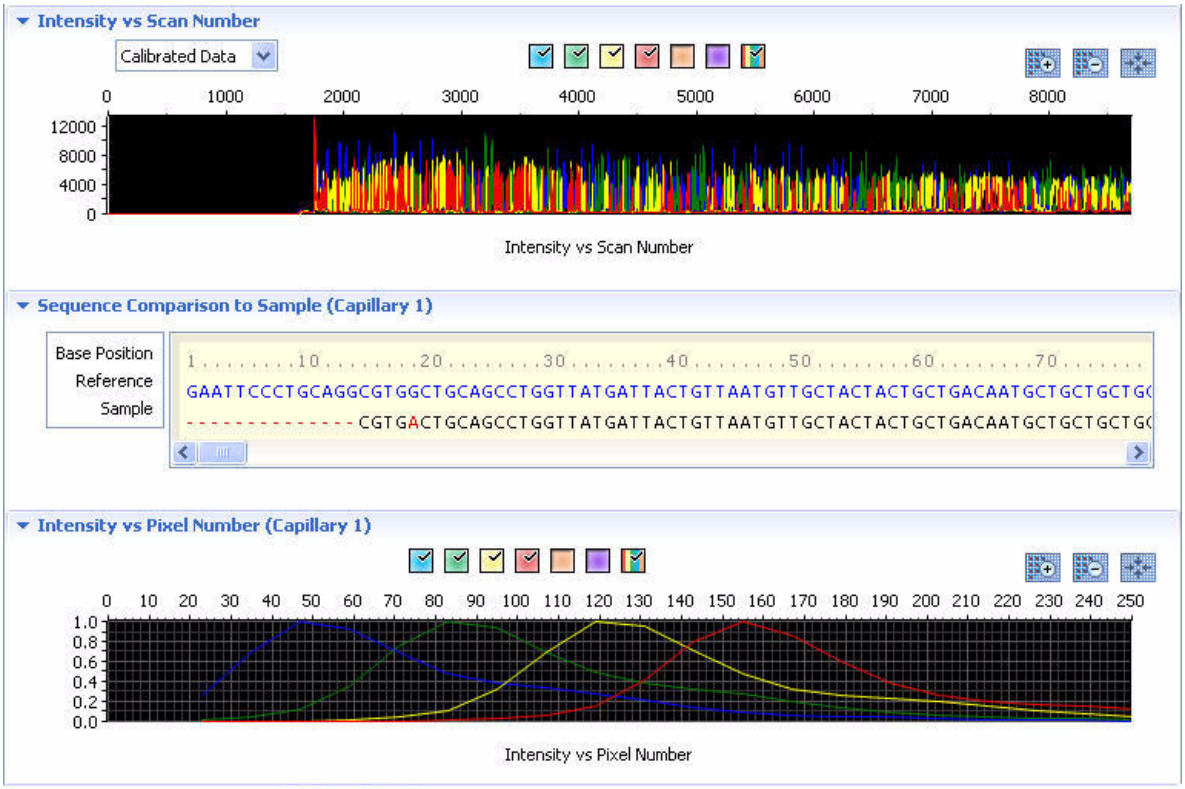
3. (Optional) Review the CRL accuracy to determine discrepancies from the reference sequence:
   - General sequencing: 40 to 539 bp
   - MicroSeq® ID: 20 to 619 bp

   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 251), then repeat the run as needed.
4. If the data for all capillaries meet the criteria above, click **Accept Results**.
5. If the data for the required number of capillaries do not meet the criteria above (7 capillaries for 8-capillary instruments, 21 capillaries for 24-capillary instruments):
   a. (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.

b. Click **Reject Results**. For troubleshooting information, see "Sequencing install standard troubleshooting" on page 302.

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

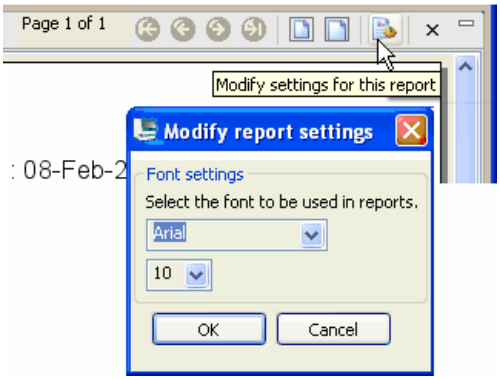
IMPORTANT! Ensure that all dyes are selected before viewing the report. The report may contain incomplete data if all dyes are not selected.

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

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 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.

Run the 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.

Applied Biosystems recommends that you 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 99).
2. In the Dashboard, check consumable status (page 29). 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 31).
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.

   Applied Biosystems recommends 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 251).

Prepare the installation standard plate

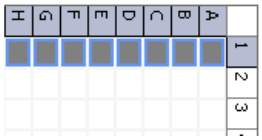
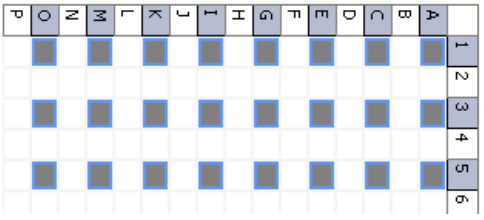
IMPORTANT! Do not use warped or damaged plates. 

1. Prepare the installation standard as described in the product insert. See Table 29 on page 260 for standard part numbers.

Application	Installation Standard
Fragment analysis (G5 dye set, POP-7™ polymer, 50 cm capillary)	GeneScan™ Installation Standard DS-33
HID (G5 dye set, POP-4™ polymer, 36 cm capillary)	AmpFℓSTR® Identifiler® Allelic Ladder

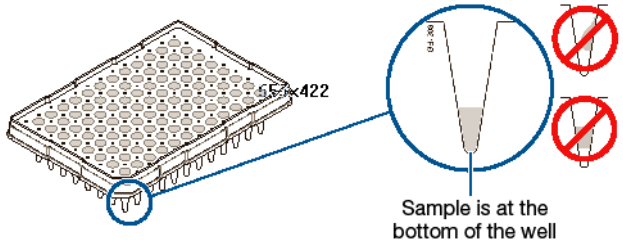
2. Load the standards in injection position 1 in the plate:

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 **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	

3. Briefly centrifuge the plate containing the standards.

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



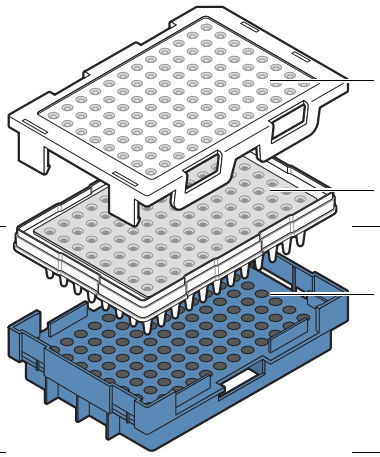
IMPORTANT! If the reagents of any well contain bubbles or are not located at the bottom of the well, briefly centrifuge the plate, remove the plate from the centrifuge, and verify that each sample is positioned correctly in the bottom of its well.

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

Prepare the plate assembly

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

1. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
2. Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
3. Place the sample plate into the plate base.



IMPORTANT! Make sure to use the correct plate base for standard plates versus 8-tube strips and fast plates. Using the wrong plate base may affect performance.

4. Snap the plate retainer (cover) onto the plate, septa, and plate base.
5. Verify that the holes of the plate retainer and the septa strip are aligned. If not aligned, re-assemble and then assemble the plate assembly.

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

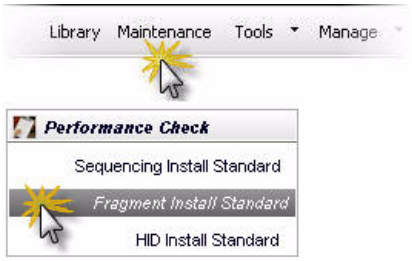
Load the plate in the instrument

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.

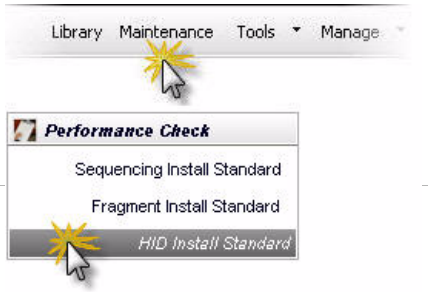
Run the fragment analysis or HID install standard performance check

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.

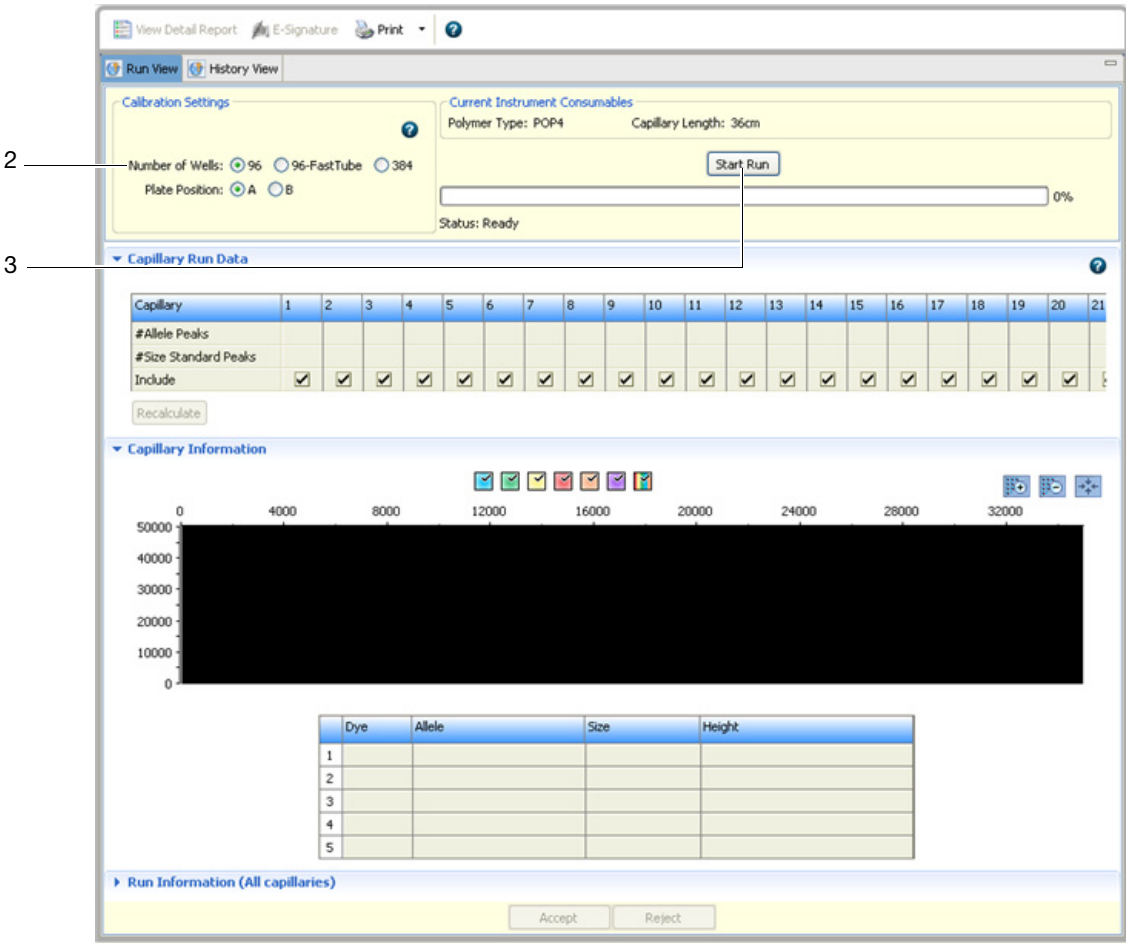


2. Select the plate type and plate position in the instrument.

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**.

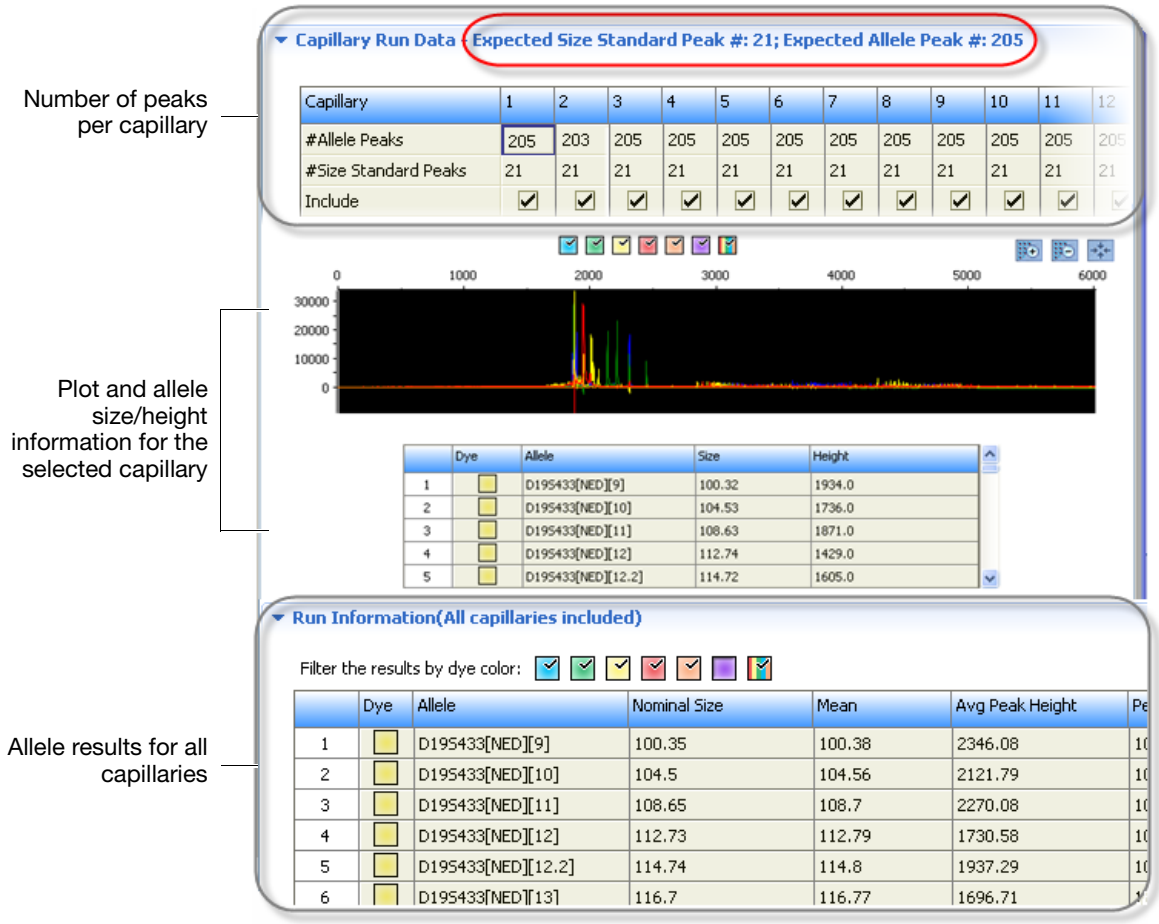

2
3
View Detail Report
E-Signature
Print
Run View
History View
Calibration Settings
Number of Wells: 96 96-FastTube 384
Plate Position: A B
Current Instrument Consumables
Polymer Type: POP4
Capillary Length: 36cm
Start Run
0%
Status: Ready
Capillary Run Data
Capillary
#Allele Peaks
#Size Standard Peaks
Include
Recalculate
Capillary Information
Dye
Allele
Size
Height
Run Information (All capillaries)
Accept
Reject

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):

- All markers except THO1: ± 0.7 bp of nominal size for the allele
- THO1: ± 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.

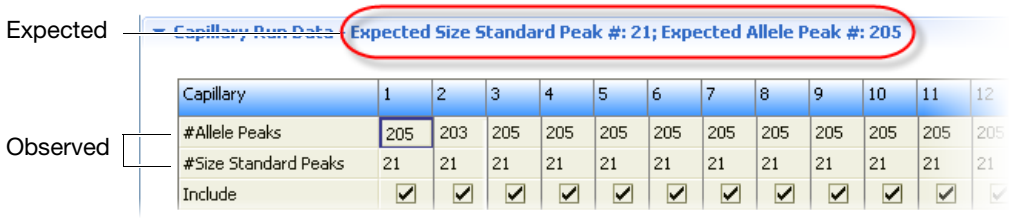
Result	Description	Threshold
Avg Peak Height	Average of peak heights for observed allele peaks of the included capillaries.	• Fragment: > 175 RFU • HID: > 400 RFU
Sizing Precision	Standard deviation of the observed allele fragment sizes.	≤0.15 for expected alleles
Pass/Fail	Alleles with a sizing precision and average peak height that do not meet thresholds fail. **Note:** 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

1. 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.

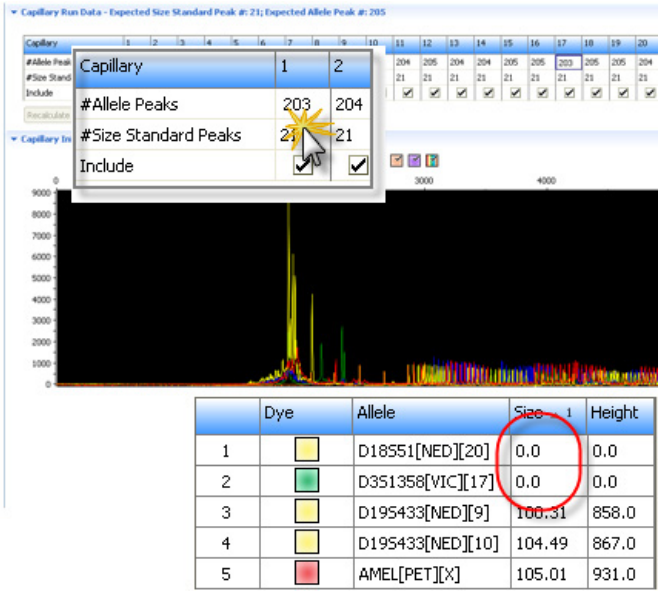


2. If the expected number of alleles and size standard peaks are found, click **Accept Results**.

   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 THO1).

3. Troubleshoot failing data:

   a. Analyze the install standard data files in your secondary analysis software (GeneMapper® Software v4.1 or later; GeneMapper® *ID-X* Software Software v1.2 or later using Identifiler® kit panels and bins).

   b. Evaluate the failed data and examine the alleles not found by the 3500 Series Data Collection Software.

   c. If the alleles are properly called in the secondary analysis software, you can:

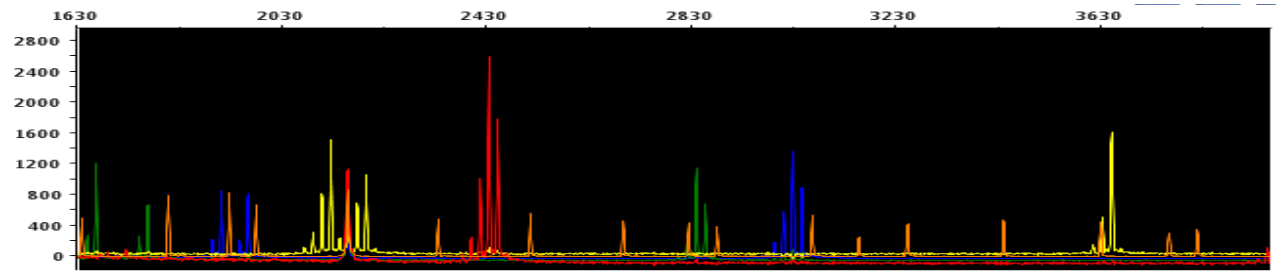
      – Deselect the Include checkmark for a capillary.
      – Click **Recalculate**.
      – Accept the install standard results.

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

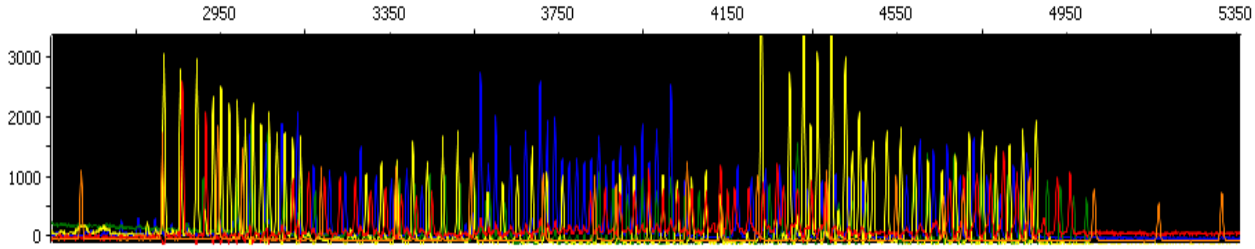
      If the alleles are not properly called:

– (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 138.
– 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 303.

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.

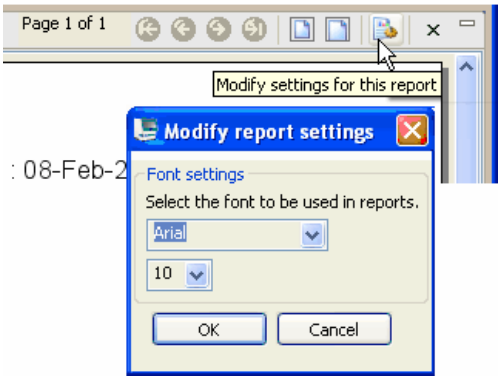
View and print a fragment or HID install standard detail report

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.

Manage Library Resources 6

Overview of libraries

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 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)
  - Optional secondary analysis protocols – Sequencing analysis, fragment analysis, and HID analysis
- Items you select when you create instrument sizecalling and QC protocols:
  - Dye sets
  - Size standards

Factory-provided, template, and locked items

The 3500 Series Data Collection Software 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). You can use the factory-provided items directly. If the factory-provided items do not suit your needs, you can modify the factory-provided items, or create new items.

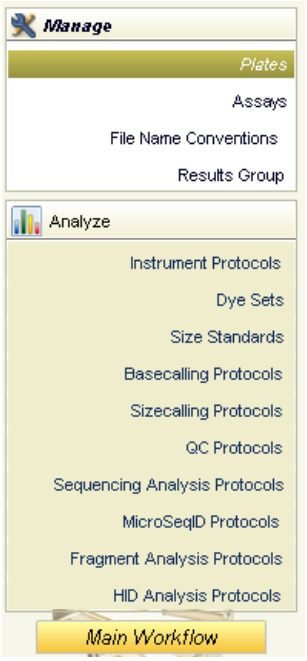
Entries in the library may be flagged with the following symbols:

- Factory-provided. Cannot be edited or deleted.
- Template.
- Locked. If your system includes the SAE module, can be unlocked and modified only 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 197.

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)
  - Optional secondary analysis protocols – Sequencing analysis, fragment analysis, and 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.

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 210.

1. Select the factory-provided entry in the library.
2. Click **Duplicate**. The software creates a "Copy of" the item you duplicated.
3. Select the "Copy of" item, then click **Edit**.
4. Enter a name for the item.
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 210.

Note: You cannot delete AB 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 210.

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 210.

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 or 3500xL 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.

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 197.

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 209.

Sort, filter, and search library entries

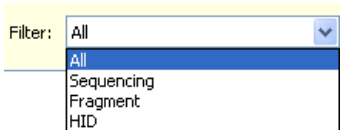
Sort

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.

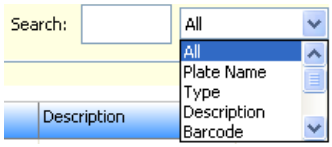
Numbers in the column headers reflect sort order.

Filter



You can select an application type from the Filter list to display only plates for the selected application.

Search

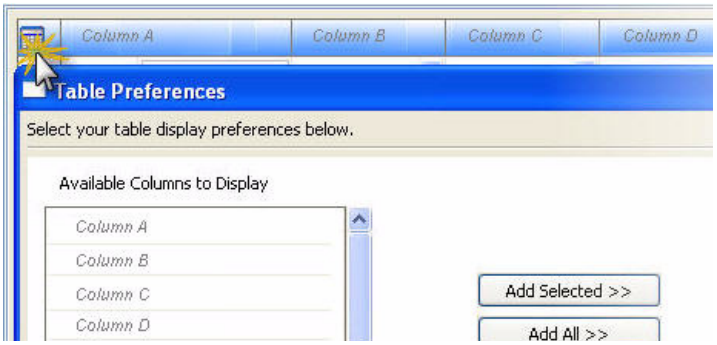


In each library, you can select a category to search, then enter the text to search for. The list of categories corresponds to the column headers in each library.

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 210.

Plate templates

The Plates library includes templates that are optimized for different applications (for example, plates defined with the appropriate polymer and capillary length) that you can use to create new plates.

Create a new plate

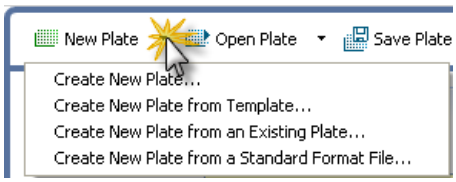
1. Access the Plates library.
2. Click **Create**.

   The software switches to the Main workflow and displays the Define Plate Properties screen (Figure 11 on page 145).

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 6 on page 145).

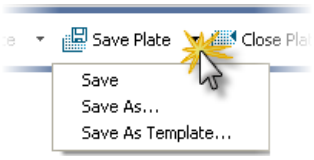
   To create a new plate based on an existing plate, click **New Plate**, then select an option. Select a plate, click **Open**, then specify settings.



   For information on other Create New Plate options, see:

   - "Create a plate from a template" on page 43.
   - "Create a plate for importing" on page 73 (for the Create New Plate from a Standard Format File option)

4. Select a Save option.



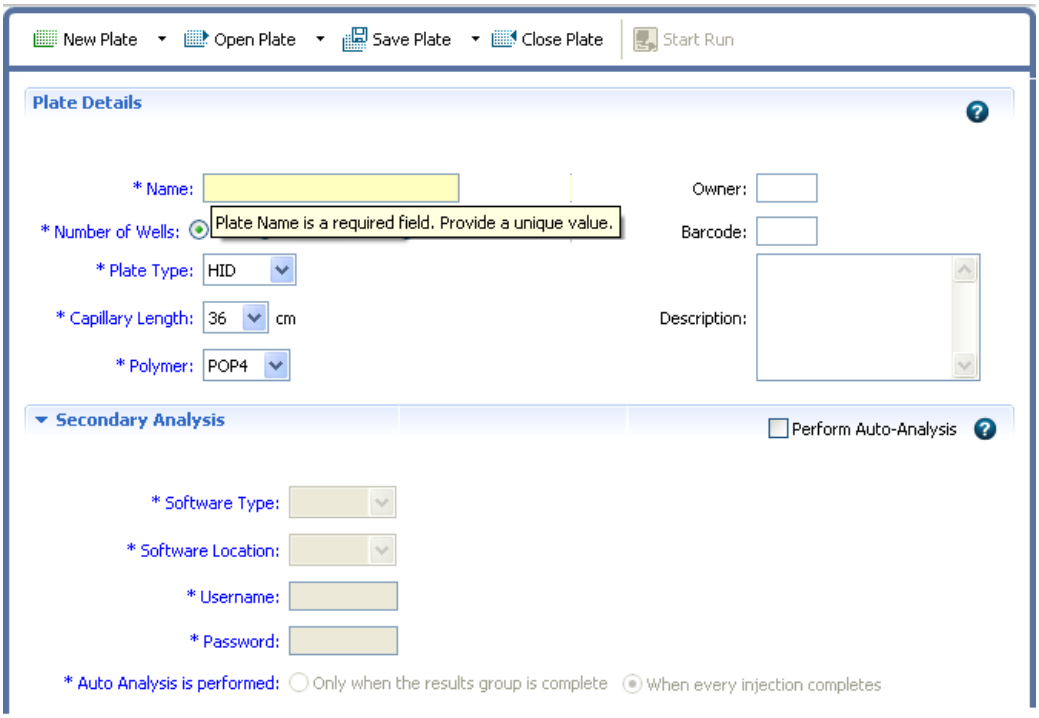


Figure 11 Define Plate Properties

Table 6 Define Plate Properties

Setting	Description
Plate Details	
Name	Plate name. Names must be unique.
Number of Wells	• **96 well** – For standard 96-well plates – 96–Supports 96-well standard reaction plate. 8-strip standard tubes are also supported with appropriate retainers. • **96 Fast tube** – For Fast 96-well plates and 8-strip tubes – 96-Fast Tube– Supports 96-well Fast reaction plate. 8-strip fast tubes are also supported with appropriate retainers. • **384 well** – 384-well plates
Plate Type	• 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).

Table 6 Define Plate Properties *(continued)*

Setting	Description
Secondary Analysis **Note:** The secondary analysis protocol settings you specify in an assay must match the auto-analysis settings for the plate. For more information, see: • "Sequencing analysis protocols library (secondary analysis)" on page 189 • "MicroSeq® ID protocols library (secondary analysis)" on page 191 • "Fragment analysis protocols library (secondary analysis)" on page 193 • "HID analysis protocols library (secondary analysis)" on page 195	
Perform Auto-Analysis	Enables the plate for use with auto-analysis with a supported secondary software
Software Type	Supported secondary software
Software Location	Computer on which the supported secondary software is installed
Username	User name and password required by the secondary analysis software
Password	
Auto-Analysis is performed (fragment/HID only)	Determines when data is sent to the secondary analysis software: • Only when the results group is complete • When every injection completes

Assays library

Assay overview

An assay contains the instrument protocol (dye set and run configuration) 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 protocol
- (Optional) A secondary analysis protocol that depends on your application:
  - **Sequencing –** SeqScape® Software v2.7 or later) or MicroSeq® ID Analysis Software v2.2 (or later)
  - **Fragment analysis –** GeneMapper® Software v4.1 (or later)
  - **HID –** GeneMapper® *ID-X* Software ID-X Software v1.2 (or later)

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.

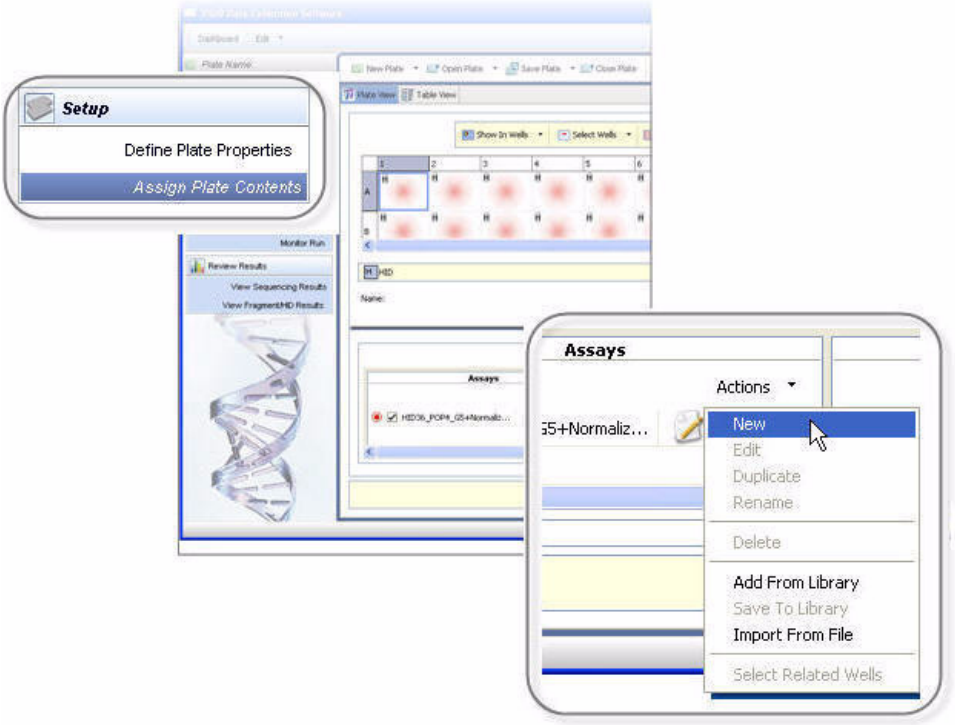
When you create an assay, you add one or more instrument protocols and a primary analysis protocol. If you add these items from the library, a *copy* of the items is added to the assay, and can be modified independently from the original items stored in the library. For information on how changes are tracked if auditing is enabled, see "Audit action" on page 210.

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.



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 12 on page 149 shows the sequencing screen).
4. Specify settings (see Table 7 on page 149).
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**.

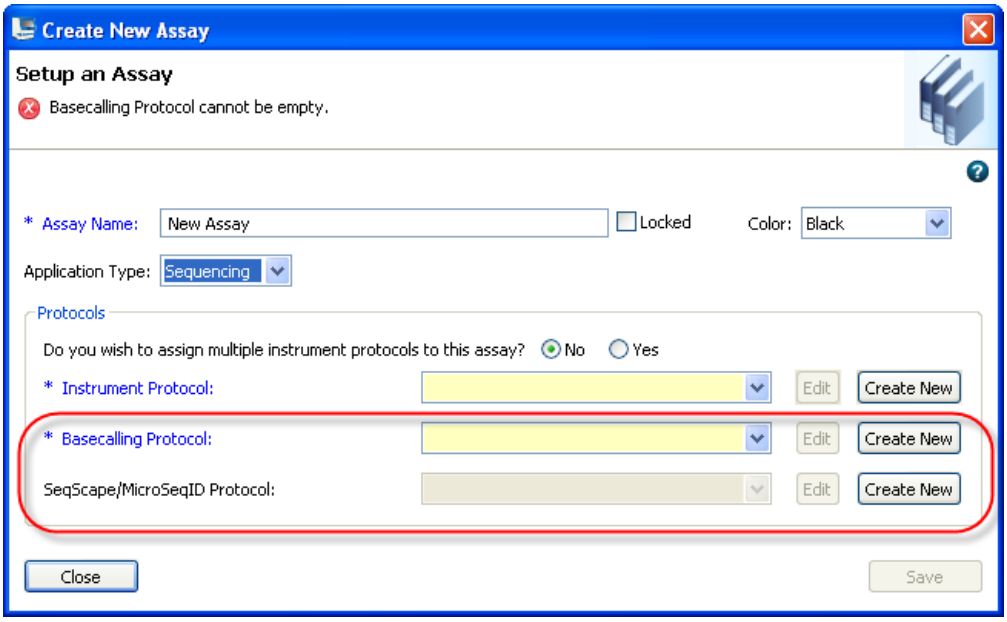


Figure 12 Create New Assay – sequencing – the highlighted area changes based on the Application Type

Table 7 Assay settings

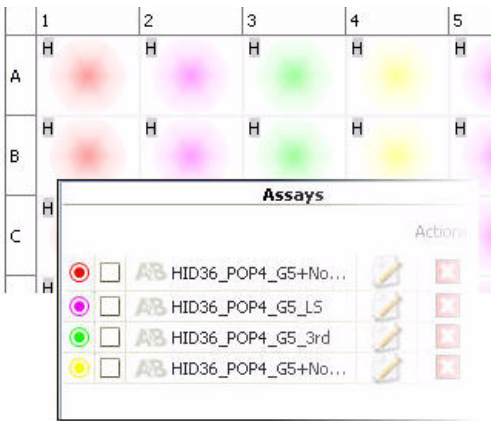
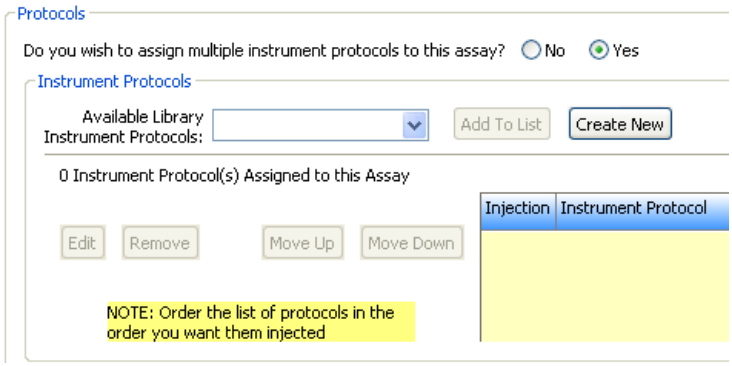
Setting	Description
Assay Name	Name of the assay. Names must be unique.
Locked	When enabled, allows the entry to be unlocked and modified only by the user who created it, the administrator, or another user with unlock permissions. Useful when your system includes the SAE module (described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 197.
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	• Sequencing • Fragment analysis • HID

Table 7 Assay settings *(continued)*

Setting	Description
Do you wish to assign multiple instrument protocols to this assay?	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. 
Instrument Protocol	Instrument protocol for data collection. For information, see "Instrument protocol library" on page 165.
Sequencing	
• Basecalling Protocol	Protocol for primary analysis (basecalling and trimming) and quality determination. For information, see "Basecalling protocols library (primary analysis – sequencing)" on page 174.
• SeqScape software /MicroSeq® software D Protocols	Optional protocol for secondary analysis (auto-analysis). For information, see: • "Sequencing analysis protocols library (secondary analysis)" on page 189. • "MicroSeq® ID protocols library (secondary analysis)" on page 191.
Fragment	
• Sizecalling Protocol	Protocol for primary analysis (peak detection and sizing) and quality determination. For information, see "Sizecalling protocols library (primary analysis – fragment)" on page 179.
• GeneMapper® software Protocol	Optional protocol for secondary analysis (auto-analysis). For information, see "Fragment analysis protocols library (secondary analysis)" on page 193.
HID	
• QC Protocol	Protocol for primary analysis (peak detection and sizing) and quality determination. For information, see "QC protocols library (primary analysis – HID)" on page 184.
• GeneMapper® ID-X Protocol	Optional protocol for secondary analysis (auto-analysis). For information, see "HID analysis protocols library (secondary analysis)" on page 195.

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 36.)
- **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.

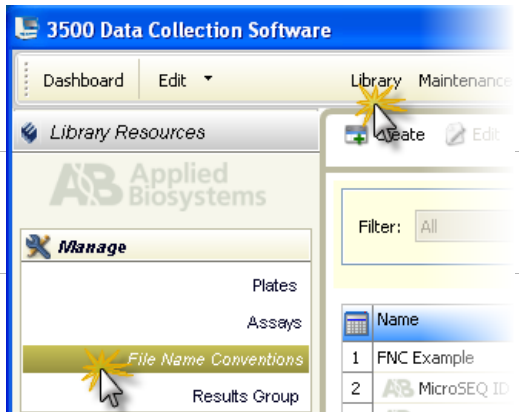
When you set up a plate for a run, you can optionally add file name conventions to the plate. If you add this item from the library, a *copy* of the item is added to the plate, and can be modified independently from the original item stored in the library. For information on how changes are tracked if auditing is enabled, see "Audit action" on page 210.

Create a new file name convention

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

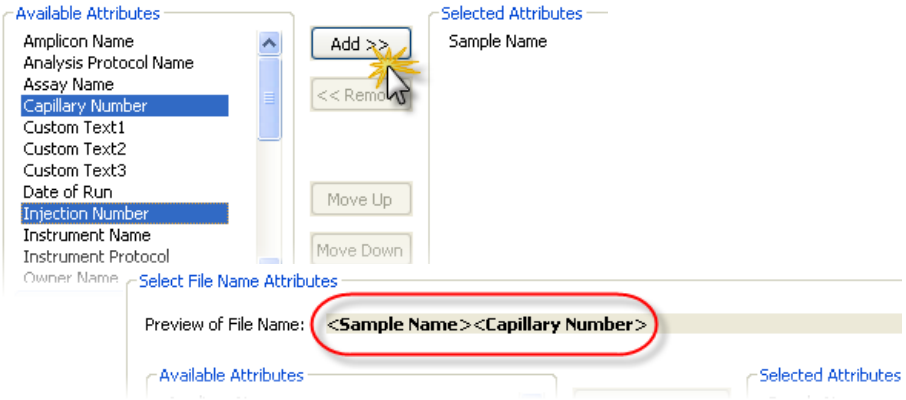
1. Access the File Name Conventions library.
2. Click **Create**.

   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 13 on page 153), select attributes and delimeters (see Table 8 on page 153).

   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 assay from the Assign Plate Contents screen, click **Apply to Plate** or **Save to Library**.

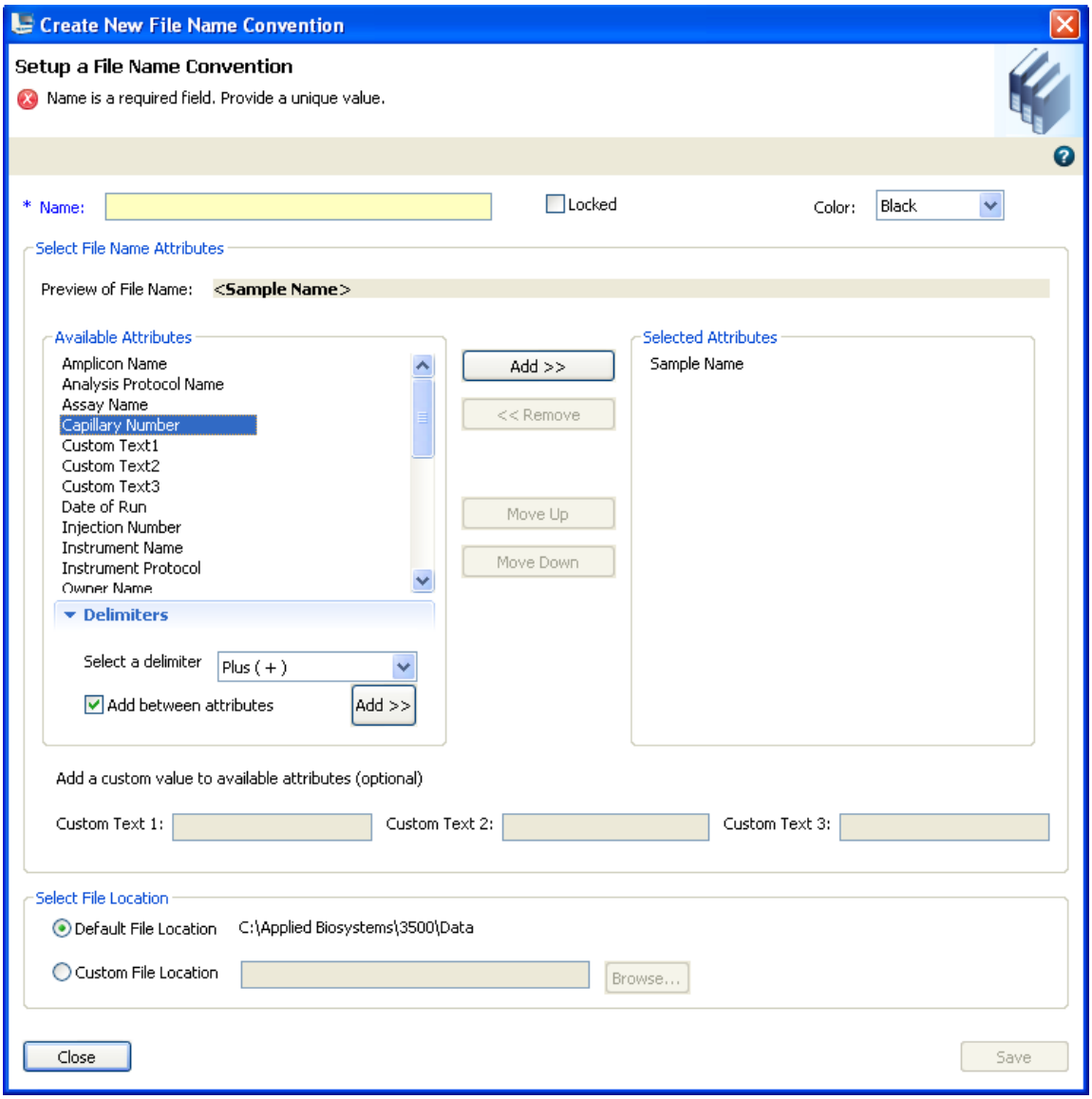
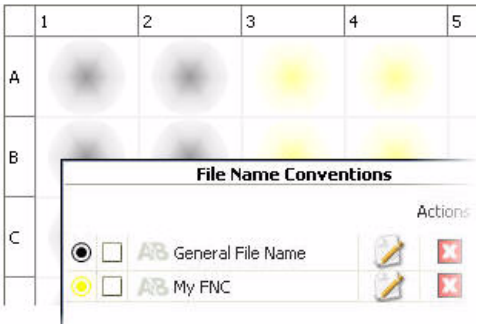


Figure 13 Create New File Name Convention

Table 8 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 your system includes the SAE module (described in Chapter 7, "Use Security, Audit, and E-Sig Functions (SAE Module)" on page 197.

Table 8 File name conventions settings *(continued)*

Setting	Description
Color	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). 
Preview of name	Interactively displays the attributes you select.
Available attributes	• 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 48) • User Name (available only when security is enabled in the SAE module) • Well Position **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.
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	The file location in the file name convention is used only if no results group is specified for a well.f The Results Group file location overrides the File Name Convention file location.

Result 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 210.

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.)

Applied Biosystems recommends 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 161 for a results group example that places three injections in each run folder for 8-capillary instruments.

Create a new results group

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

1. Access the Results Groups 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 14 on page 158), select attributes and delimeters (see Table 9 on page 158).

   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.

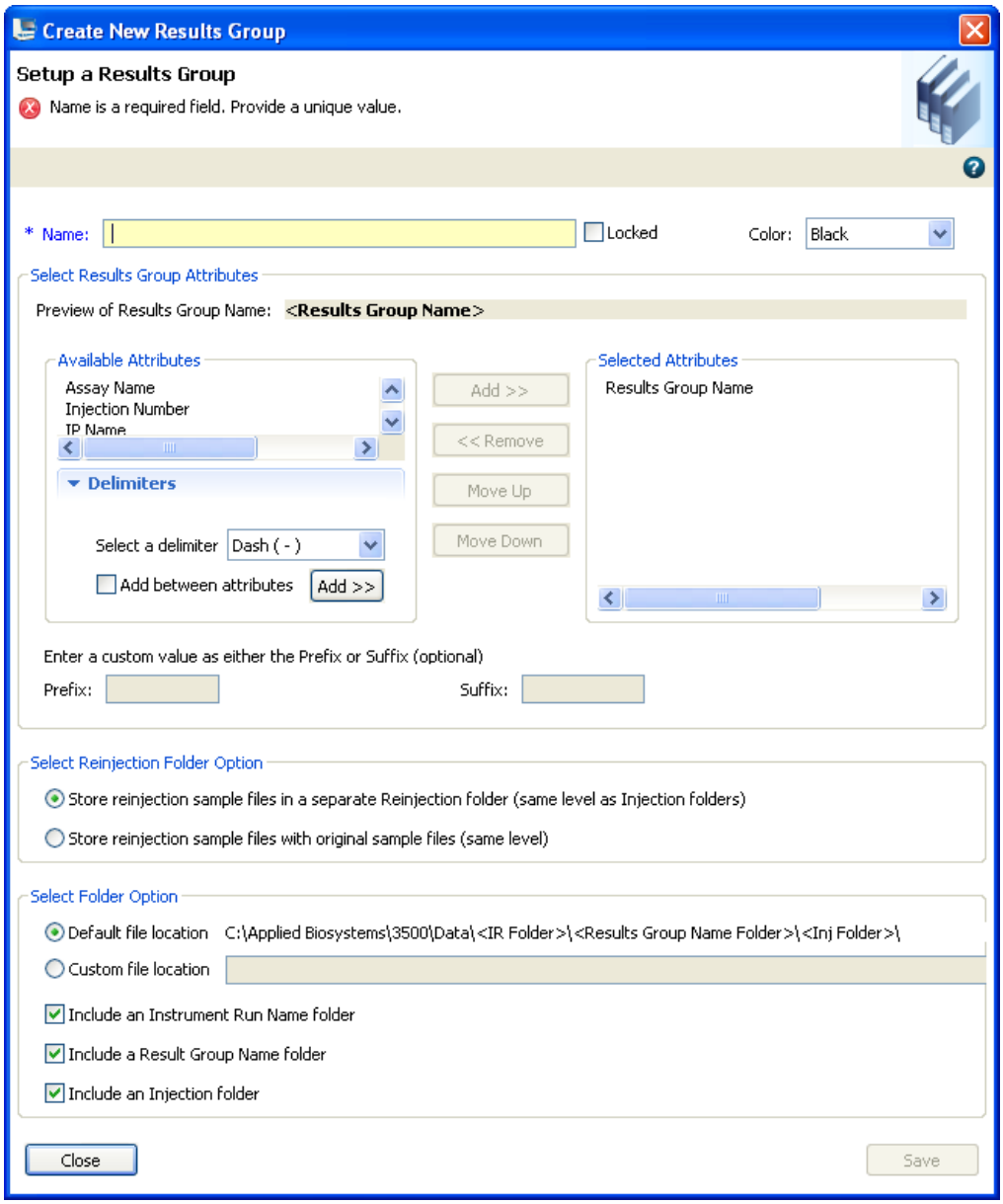
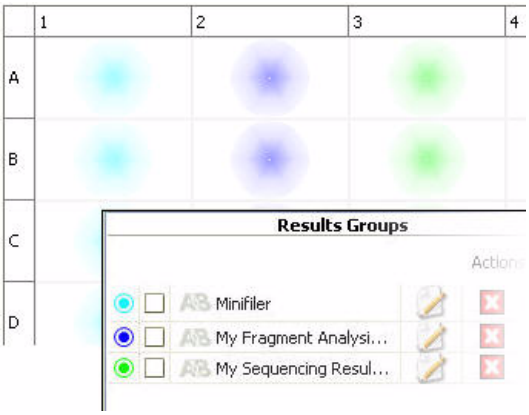


Figure 14 Create New Results Group

Table 9 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 your system includes the SAE module (described in Chapter 7, “Use Security, Audit, and E-Sig Functions (SAE Module)” on page 197.

Table 9 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	• 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) • 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	• 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	Location: • Default file location (specified in Preferences ▸ User ▸ Run Setup) • Custom location Sub-folder options: • 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 15 on page 160 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 16 on page 161 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.

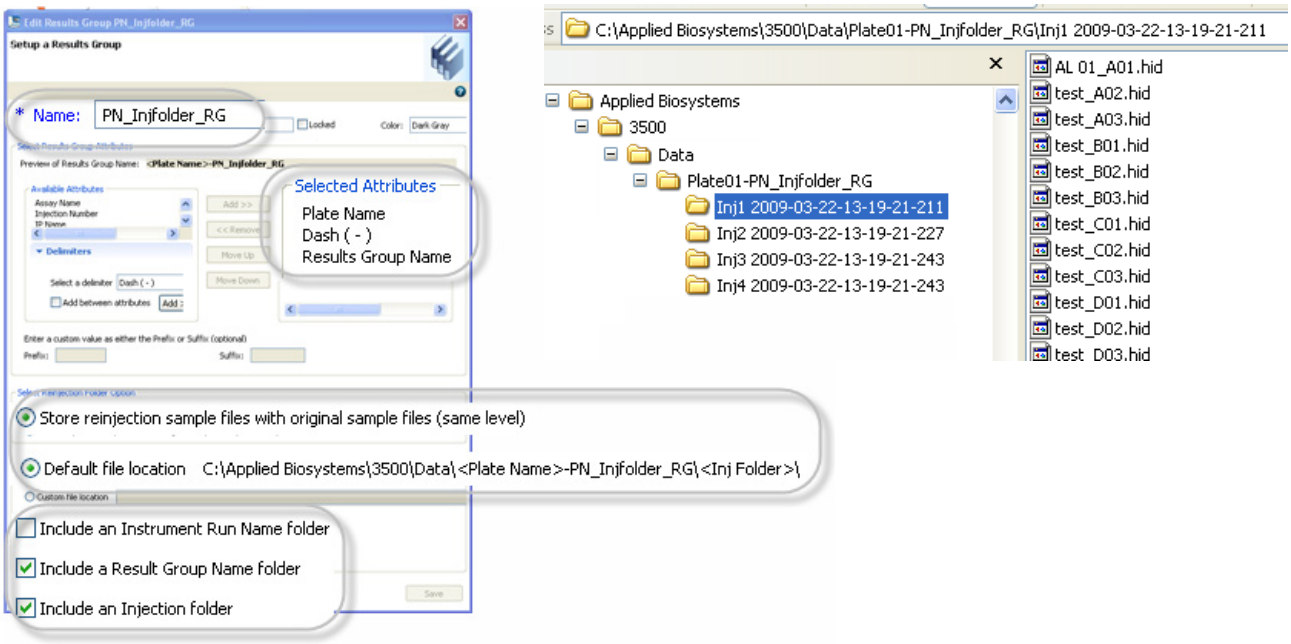


Figure 15 PN_Injfolder_RG results group

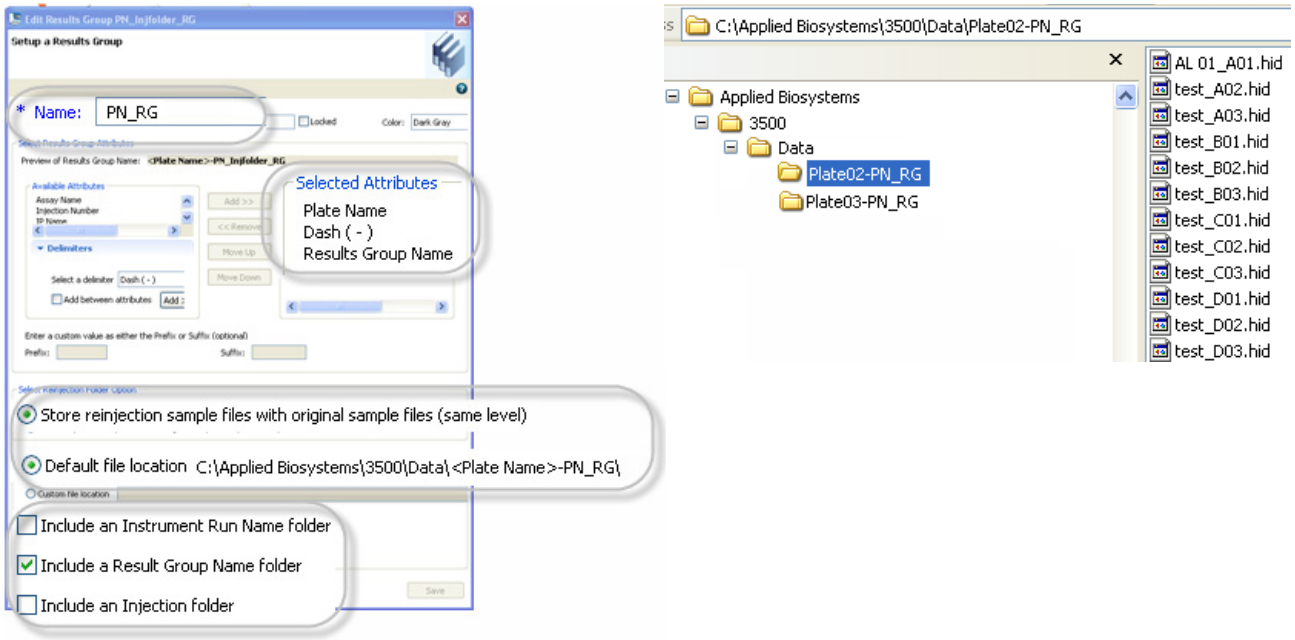


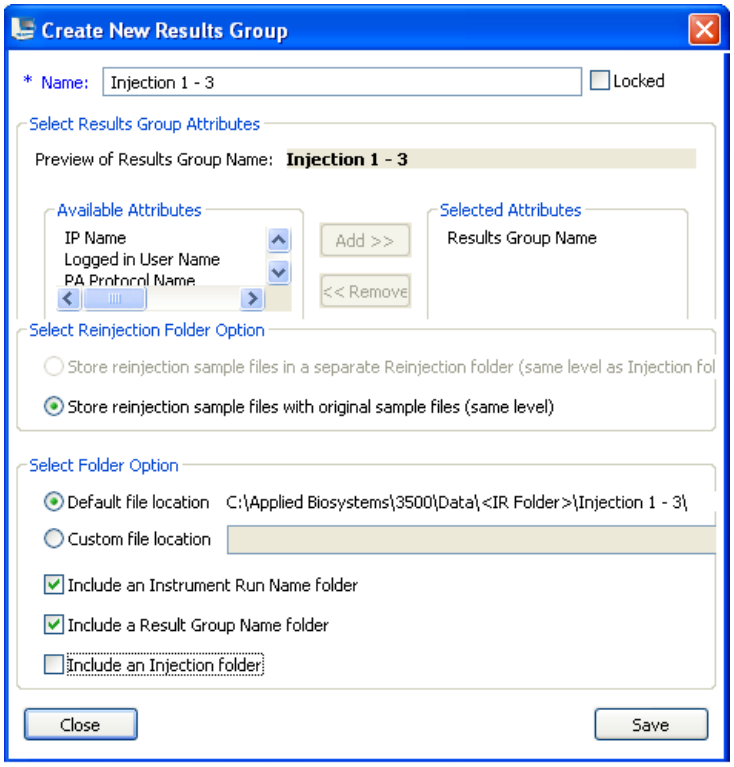
Figure 16 PN_RG results group

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

Applied Biosystems recommends that you run one allelic ladder for each set of 24 samples (see "Allelic ladder location (HID analysis)" on page 155).

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 17 on page 163 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 20 on page 164).

Figure 17 Results group example

Figure 18 on page 163 shows the injection list for a run that specifies duplicate and re-injections.

The numbers in the figure relate the elements in the injection list with the elements in the file hierarchy created by this run (Figure 20 on page 164).

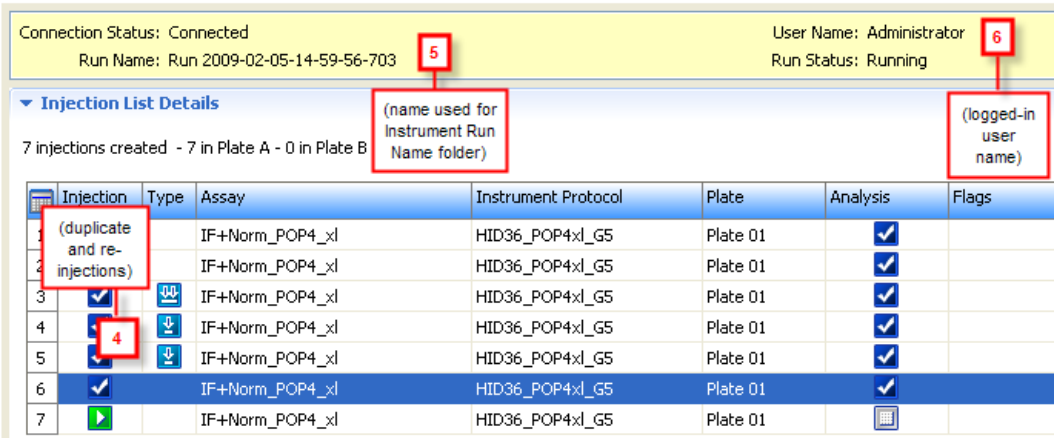


Figure 18 Injection list example

Figure 19 on page 164 shows an example file name convention that specifies a sample name syntax of:

sample name.(primary) analysis protocol name.unique time stamp integer

The numbers in the figure relate the elements in the file name convention with the files created by a run that uses file name convention (Figure 20 on page 164).

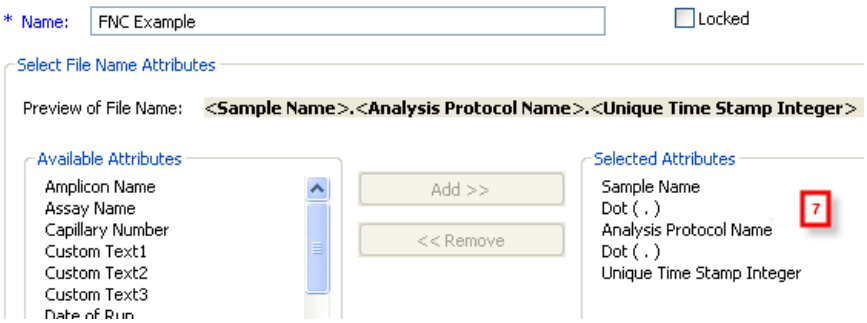


Figure 19 File name convention example

Figure 20 on page 164 shows the folders and files generated by the results group, file name convention, run name, and injections shown in Figure 17 on page 163, Figure 18 on page 163, and Figure 19 on page 164.

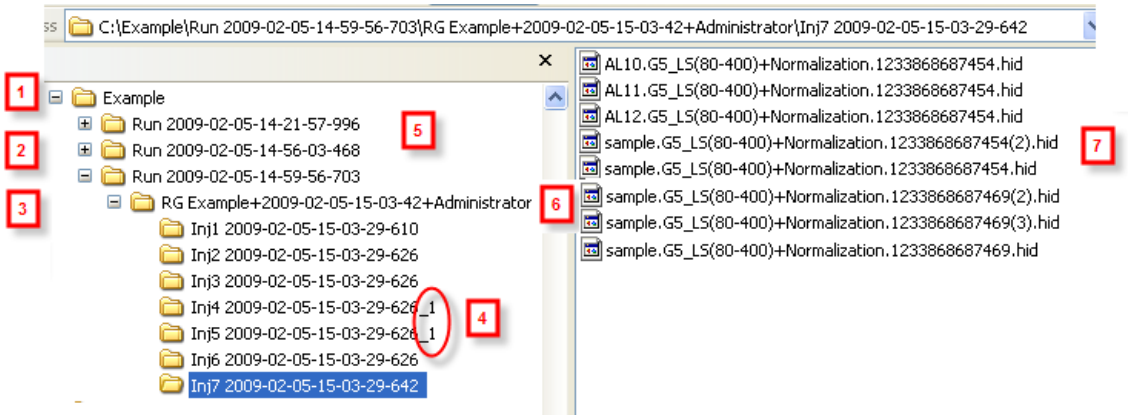
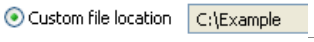
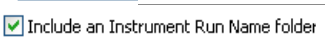
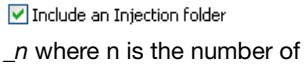


Figure 20 Folder hierarchy and file naming example

1	File location from results group 
2	Instrument Run Name folder from results group 
3	Results group Name folder from results group 
4	Injection folder from results group  Duplicate injections indicated with *_n* where n is the number of duplicates.
5	Run name (default or user-defined) from injection list
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

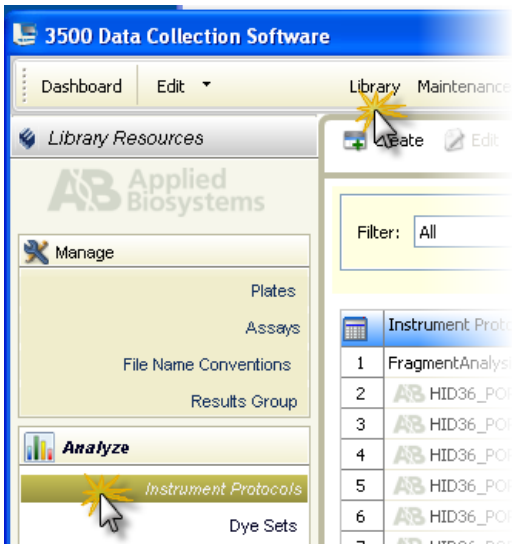
An instrument protocol contains the parameters that control the instrument during data acquisition. An instrument protocol is a required element of an assay for all applications.

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 210.

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 21 on page 166), 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 10 on page 166).
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**.



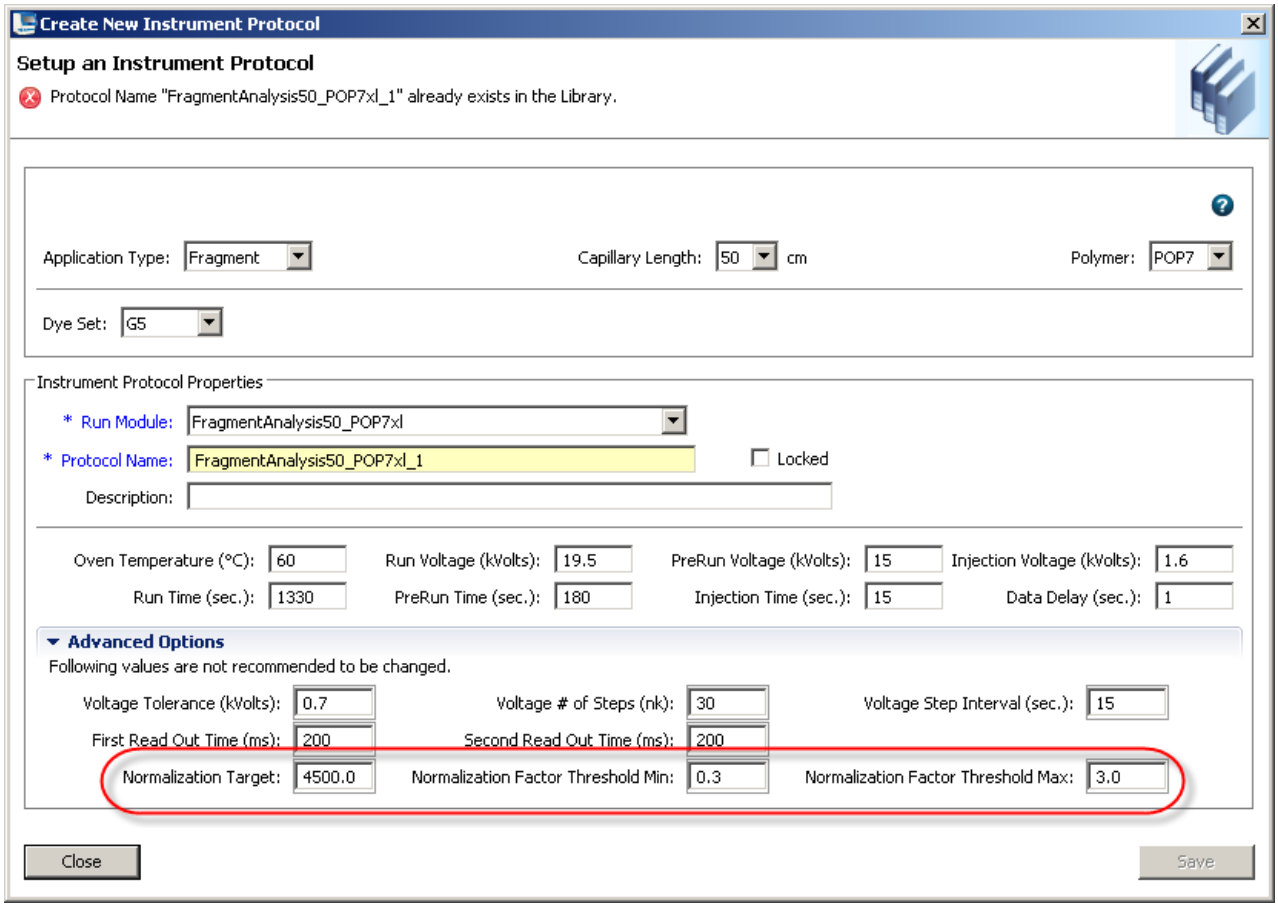


Figure 21 Create New Instrument Protocol – normalization parameters circled in red are displayed for fragment analysis and HID applications only

Instrument protocol settings

Table 10 Instrument protocol settings

Setting	Description
Application Type	• 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 263.
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 197.
Description	Optional text entry.

Table 10 Instrument protocol settings *(continued)*

Setting	Description
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.
Advanced options – *Do not change unless advised otherwise by Applied Biosystems support personnel*	
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 90).	
Normalization Target	The expected average RFU for the subset of peaks in the GS600 LIZ® v2 size standard used for normalization. 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. **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).
Normalization Factor Thresholds	The passing range for Normalization Factor (default range is 0.3 to 3.0). **IMPORTANT!** Increasing the factor threshold above 3.0 may cause amplification of noise. 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).
Normalization Factor	Average peak height of the subset of peaks in the GS600 LIZ® v2 size standard used for normalization divided by the Normalization Target. Samples are flagged with  in results if Normalization Factor is within threshold range, or with if it is out of threshold range.

Dye sets library

Dye set overview

A dye set defines the following for an instrument protocol:

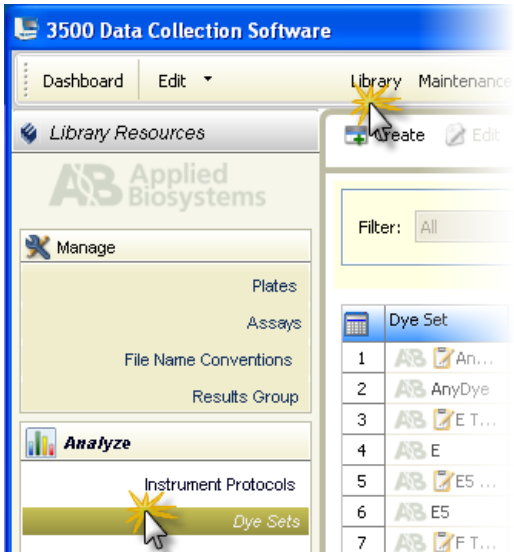
- Dye color(s)
- Order of the dye peaks in the standard
- Spectral analysis parameters

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 210.

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 22 on page 169).
4. Specify settings (Table 11 on page 169).
5. Click **Save**.



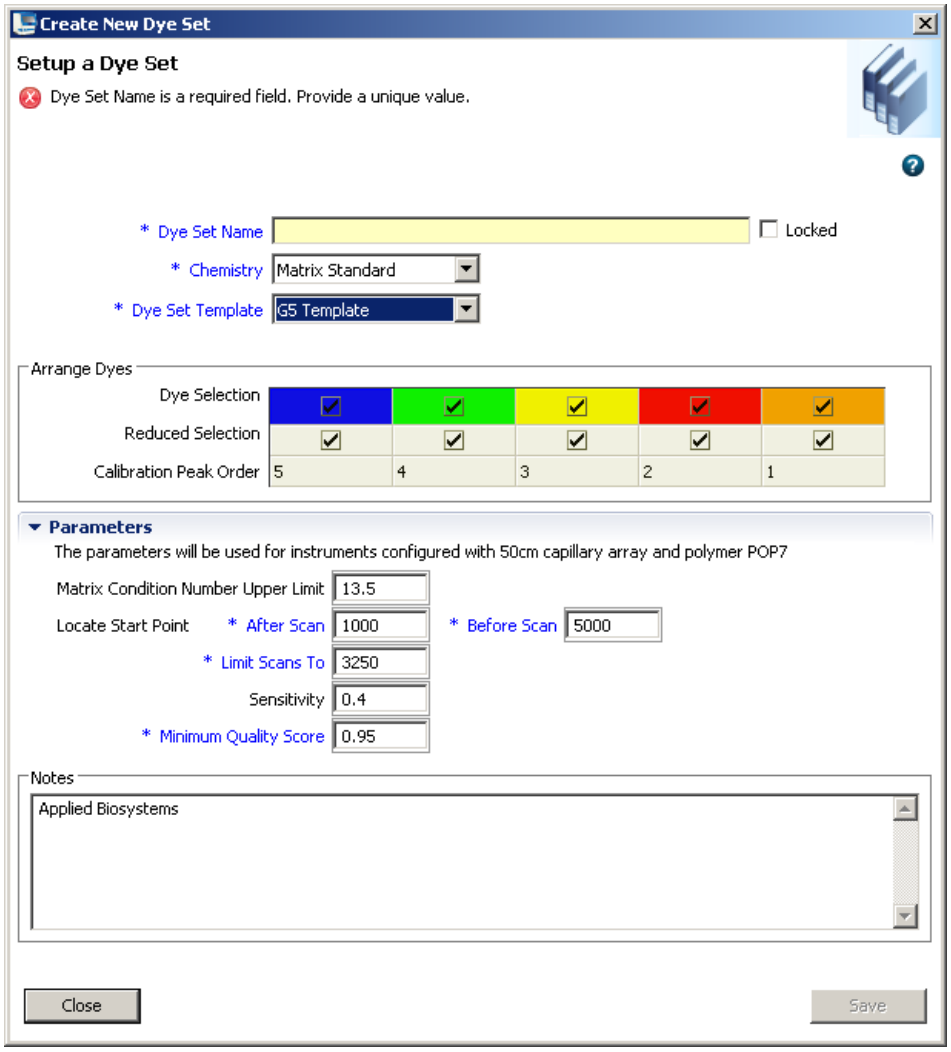


Figure 22 Create New Dye Set

Table 11 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 197.
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.

Table 11 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: • **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.

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 210.

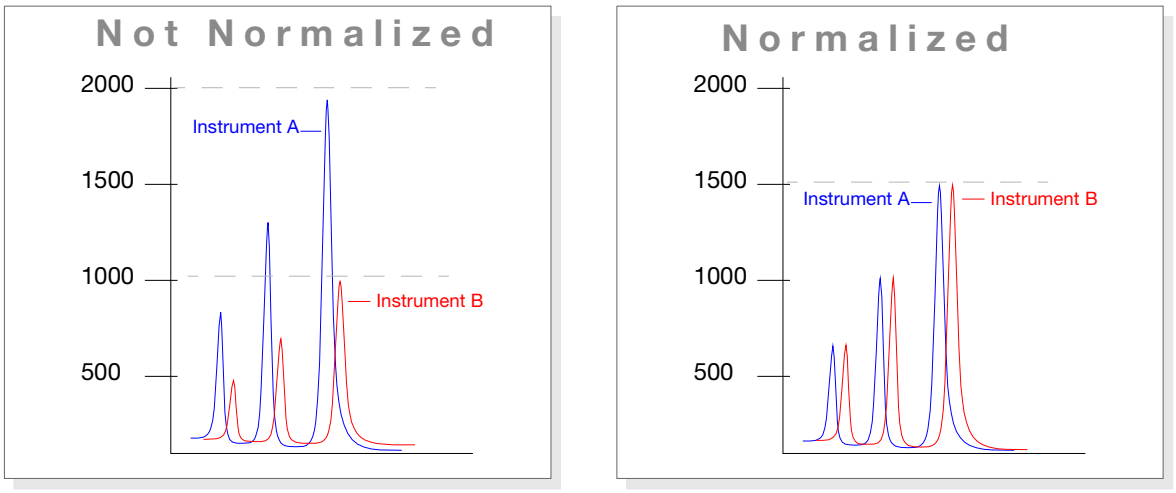
Normalization size standards provided

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

- Fragment analysis:
  - 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.
- HID:
  - 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.

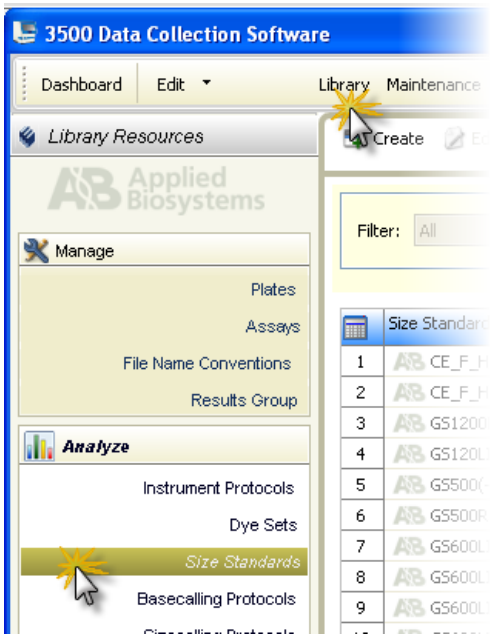


For more information, see "Review normalized data" on page 90.

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 22 on page 169), 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 197.
   - 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**.

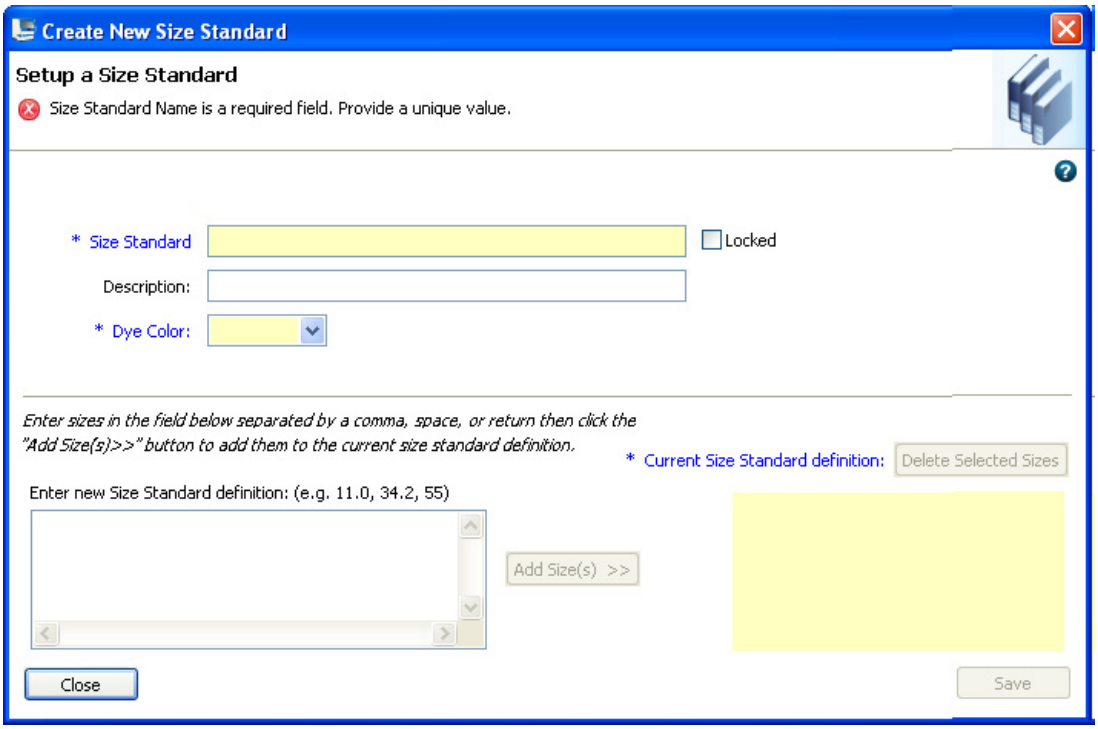


Figure 23 Create New Size Standard

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:

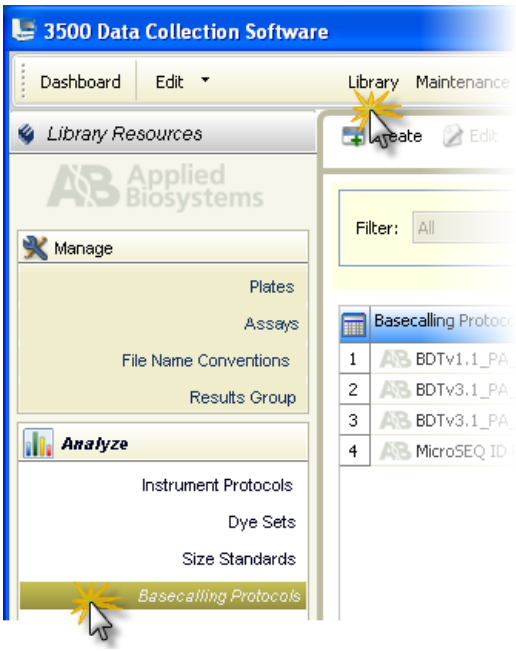
- Analysis settings
- Ranges for the sequencing quality flags displayed in View Results

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 210.

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 24 on page 175), specify settings (see Table 12 on page 175).
4. Click **QV Settings**. In the QV Settings tab of the Create New Basecalling Protocol dialog box (Figure 25 on page 177), then specify settings and Table 13 on page 178).
5. Click **Save**.



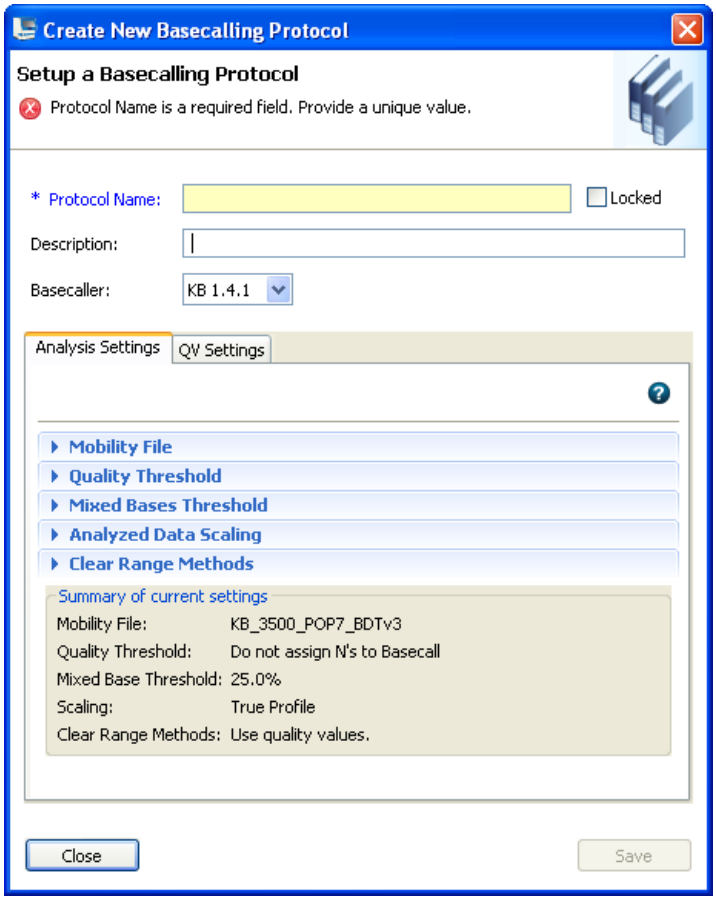
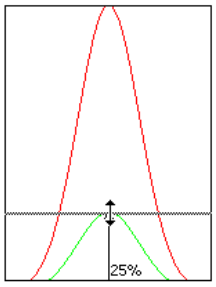


Figure 24 Create New Basecalling Protocol – Analysis Settings

Table 12 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 197.
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 12 Basecalling protocol – Analysis settings *(continued)*

Setting	Description
Quality Threshold	• Basecall Assignment (ambiguous bases): – 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: – At PCR Stop – After X number of Bases – After X number of Ns in X number of Bases – After X number of Ns **Note:** If you have short PCR products, select the At PCR Stop check box.
Mixed bases threshold	When enabled, allows the software to determine the secondary peak height where the base position is considered a potential mixed base. Adjust this parameter by dragging the bar in the display or typing in a numeric value. ☑ Use Mixed Base Identification Do not assign a mixed base when the secondary peak height is <= to 25 % 
Analyzed Data Scaling	Determines scaling of the processed traces. This parameter does not affect the accuracy of the basecalling. • **True Profile** – The processed traces are scaled uniformly so that the average height of peaks in the region of strongest signal is about equal to a fixed value. The profile of the processed traces will be very similar to that of the raw traces. • **Flat Profile** – The processed traces are scaled semi-locally so that the average height of peaks in any region is about equal to a fixed value. The profile of the processed traces will be flat on an intermediate scale (> about 40 bases).
Clear range methods	• **Use clear range minimum and maximum** – Specifies the first and last base in the range to consider, or trims the specified number of bases from the 3' end. • **Use quality values** – Sets a window with a specified number of allowed low-quality bases by removing bases until there are < X number of bases per Z number of bases with QV < Y. • **Use identification of N cells** – Sets a window with a specified number of allowed ambiguous base calls (Ns) by removing bases until there are < X number of Ns per Y number of bases.

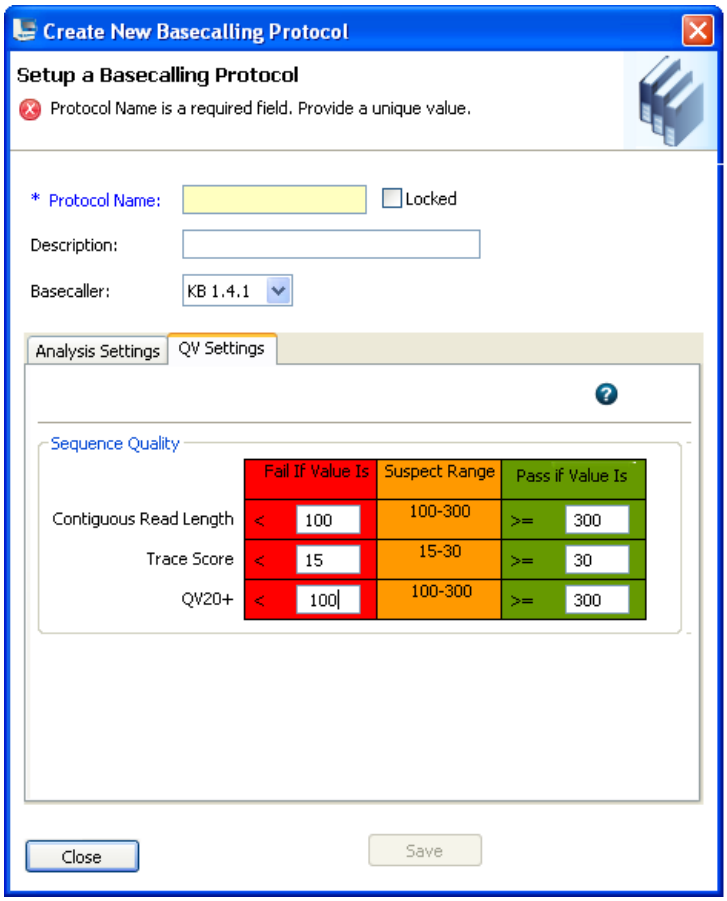


Figure 25 Create New Basecalling Protocol – QV Settings

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 value fails 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 13 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.

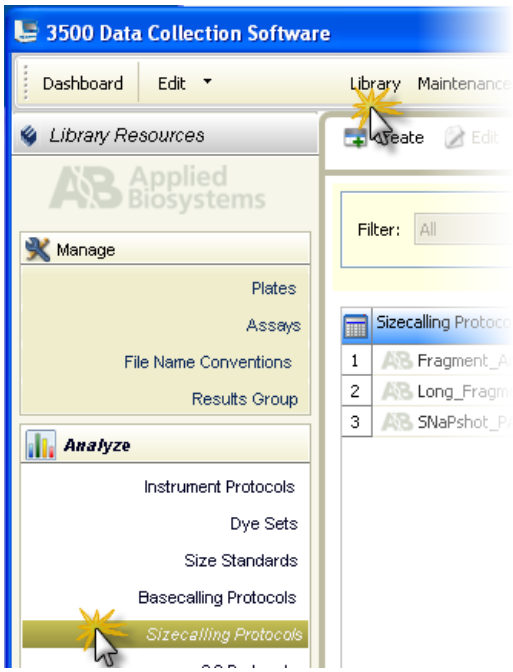
A sizecalling protocol defines peak detection, sizing, and quality values.

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 210.

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 26 on page 180), specify settings (see Table 14 on page 180).
4. Click **QC Settings**. In the QC Settings tab of the Create New Sizecalling Protocol dialog box (Figure 27 on page 183), then specify settings and Table 15 on page 183).
5. Click **Save**.



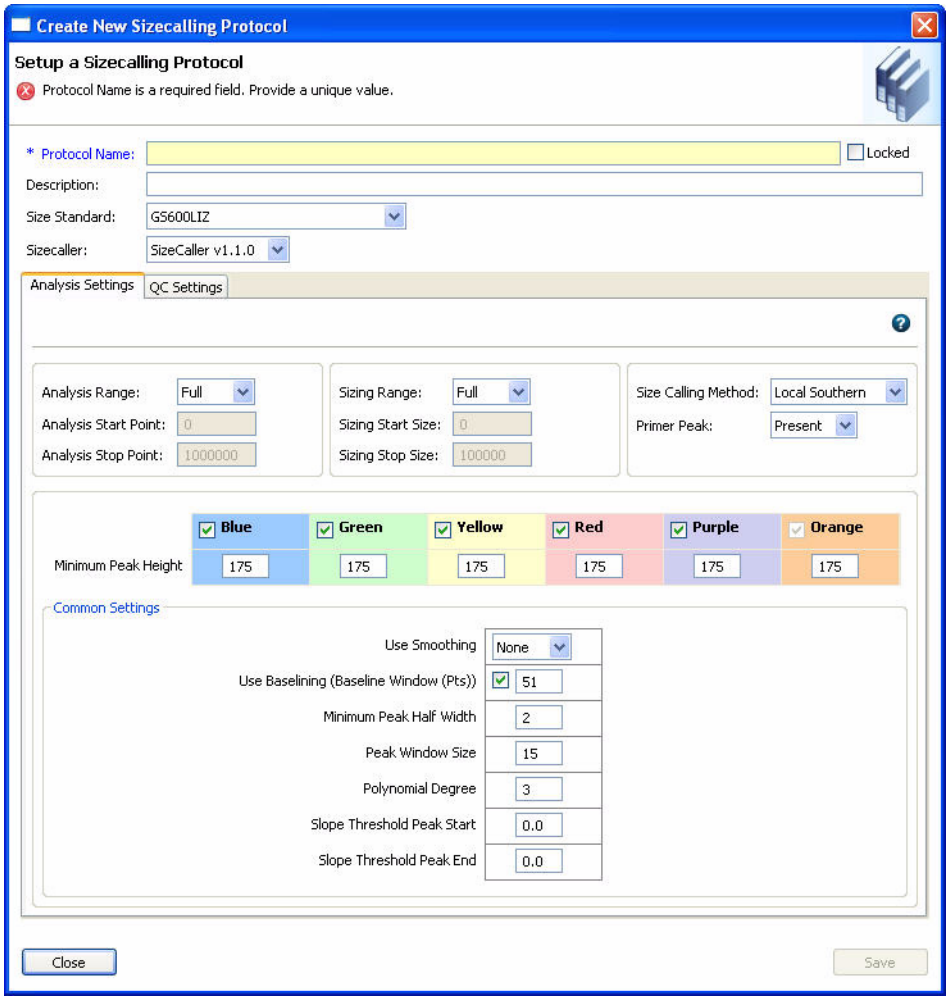


Figure 26 Create New Sizecalling Protocol – Analysis Settings

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 Data Collection Software does not support re-analyzing data with new settings.

Table 14 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 171).

Table 14 Sizecalling protocol – Analysis settings *(continued)*

Setting	Description
Analysis Range	Specify the range (in data points) to analyze: • **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. Data points outside the specified analysis range are ignored. **Note:** Ensure the Analysis Range contains all size standard fragments included in the Sizing Range specified below.
Sizing Range	Specify the size range (in base pairs) appropriate for the kit you are using: • 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	• **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	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. **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.
Peak Amplitude Thresholds	Specify the threshold (RFU) for peak detection for each dye color. Peaks below the threshold are not detected. 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. **Note:** Set the peak amplitude thresholds to 175 in your GeneMapper® Software analysis method.
Smoothing	Select an option to smooth the outline of peaks and reduce the number of false peaks detected: • **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.

Table 14 Sizecalling protocol – Analysis settings *(continued)*

Setting	Description
Baseline Window	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: • 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	Polynomial Degree cannot be greater than Peak Window Size. 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. 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. 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. For information on optimizing Polynomial Degree and Peak Window Size, see
Peak Window Size	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: • The maximum value is the number of data points between peaks. • The Peak Window Size setting is limited to odd numbers. To increase peak detection sensitivity: Increase polynomial degree, decrease peak window size. To decrease peak detection sensitivity: Decrease polynomial degree, increase peak window size.
Slope Thresholds Peak Start and End	• **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.

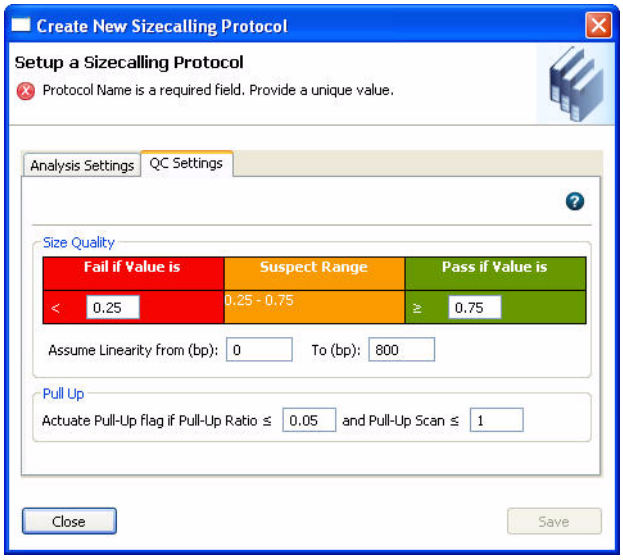


Figure 27 Sizecalling Protocol – QC Settings

IMPORTANT! Normalization is not applied to samples with Size Quality flags. The 3500 Series Data Collection Software does not support re-analyzing data with new settings.

Table 15 Sizecalling protocol – QC settings

Setting	Description
Size Quality	Enter the Pass Range and the Low Quality Range for the SQ flag displayed in View Fragment Results. 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). 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 . **How Size Quality is determined** 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).
Assume Linearity	Defines the expected linear range. Useful in large fragment size standards where non-linearity might be expected.
Pull-Up	Enter the pull-up ratio and tolerance for pull-up peak identification. A pull-up peak is identified when the peak height of the minor peak is: • ≤ X% (pull-up ratio) of the major peak *and* • Within ±Y data point (pull-up scan) of the major peak 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.

QC protocols library (primary analysis – HID)

QC protocol overview

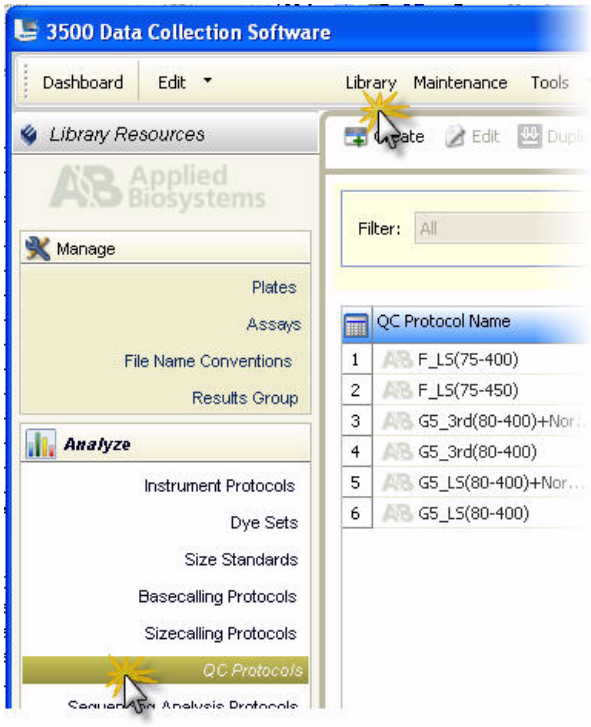
A QC protocol is the required primary analysis protocol for HID applications. A QC protocol defines peak detection, sizing, and quality values.

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 210.

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 28 on page 185), specify settings (see Table 16 on page 185).
4. Click **QC Settings**. In the QC Settings tab of the Create New QC Protocol dialog box (Figure 29 on page 188), specify settings (Table 17 on page 188).
5. Click **Save**.

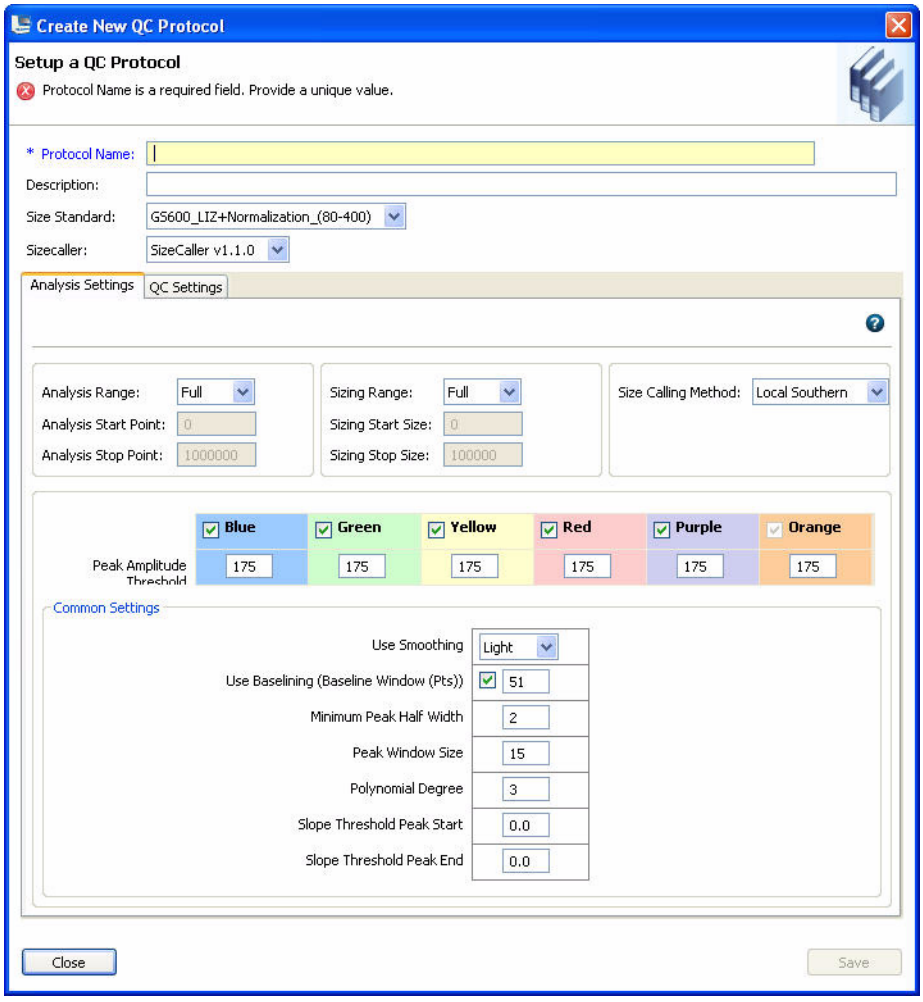


Figure 28 Create New QC Protocol – Analysis Settings

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 Data Collection Software does not support re-analyzing data with new settings.

Table 16 QC protocol – Analysis settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.

Table 16 QC protocol – Analysis settings *(continued)*

Setting	Description
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 171).
Analysis Range	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. Note: If you select Partial, ensure that the Analysis Range contains all size standard fragments included in the Sizing Range specified below.
Sizing Range	Select **Partial**, then specify **80** to **400** to limit the fragment sizes evaluated for the size standard. If you specify sizes outside this range, the Sizing Quality may fail.
Size Calling Method	Select the method to determine the molecular length of unknown fragments appropriate for the AmpFℓSTR® kit you use: • **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. – Identifiler® kit – SEfiler Plus™ kit – Sinofiler™ kit – Yfiler® kit – Profiler Plus® kit – COfiler® kit – Profiler® kit – SGM Plus® kit • **3rd Order Least Squares** - Uses regression analysis to build a best-fit size calling curve. – MiniFiler™ reagent. Size calling options for kits other than those listed above are: • **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).
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 Data Collection Software does not support re-analyzing data with new settings. For more information on peak detection parameters, see the GeneMapper® *ID-X* Software Reference Guide.	
Smoothing	Select an option to smooth the outline of peaks and reduce the number of false peaks detected: • **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	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: • 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 16 QC protocol – Analysis settings *(continued)*

Setting	Description
Peak Amplitude Thresholds	**IMPORTANT!** Optimize these thresholds during internal HID validation. Specify the threshold (RFU) for peak detection for each dye color. Peaks below the threshold are not detected. 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. **Note:** Ensure that the same peak amplitude thresholds are used in secondary analysis software such as GeneMapper® (v4.1 or later)
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	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. 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. 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.
Peak Window Size	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: • The maximum value is the number of data points between peaks. • The Peak Window Size setting is limited to odd numbers. To increase peak detection sensitivity: Increase polynomial degree, decrease peak window size. To decrease peak detection sensitivity: Decrease polynomial degree, increase peak window size.
Slope Thresholds Peak Start and End	Not recommended for use with AmpFℓSTR® kit data. • **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.

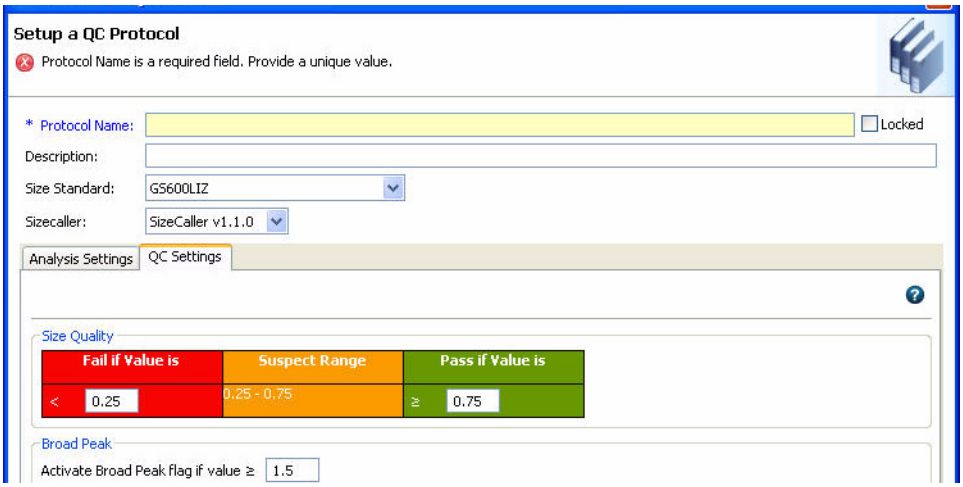


Figure 29 Create New QC Protocol – Analysis Settings

IMPORTANT! Normalization is not applied to samples with Size Quality flags. The 3500 Series Data Collection Software does not support re-analyzing data with new settings.

Table 17 QC Protocol – QC Settings

Setting	Description
Size Quality	Enter the Pass Range and the Low Quality Range for the SQ flag displayed in View HID Results. 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). 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 .
Size Quality	**How Size Quality is determined** 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). **Weighting** The Broad Peak (BD) threshold specified in the QC Protocol - QC Settings tab affects the SQ. To determine the final SQ value, the software: • 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) **Note:** The GeneMapper® *ID-X* Software allows you to set broad peak weighting. For more information, see the GeneMapper® *ID-X* Software Reference Guide.
Broad Peak	Enter the maximum peak width (in base pairs). 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.

Sequencing analysis protocols library (secondary analysis)

Sequencing analysis protocol overview

A sequencing protocol is the optional secondary analysis (auto-analysis) protocol for SeqScape® Software v2.7 or later sequencing applications.

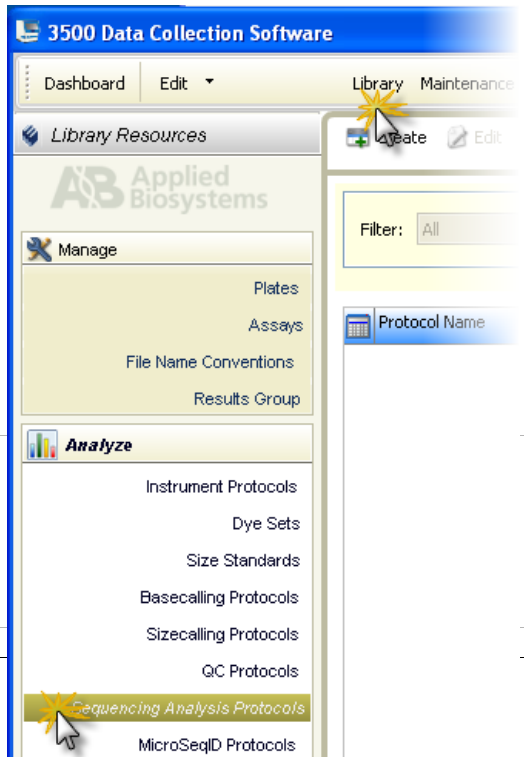
A sequencing analysis protocol defines the:

- Secondary analysis software (SeqScape® Software) location
- SeqScape® Software project, template, and specimen to use for auto-analysis

When you create a sequencing assay, you can optionally add a sequencing analysis 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 210.

Create a new sequencing analysis protocol

1. Access the Sequencing Analysis Protocols library.
2. Click **Create**.
3. In the Create New Sequencing Analysis Protocol dialog box (Figure 30 on page 190), specify settings (see Table 18 on page 190).
4. Select the remaining secondary analysis items, then click **Save**.



Note: If the project, project template, or specimen of interest is not displayed in a list, re-select the secondary analysis software instance to update the list.

IMPORTANT! The auto-analysis settings you specify for the plate to run with this protocol must contain the same secondary software and location settings. For more information, see "Create a new plate" on page 144.

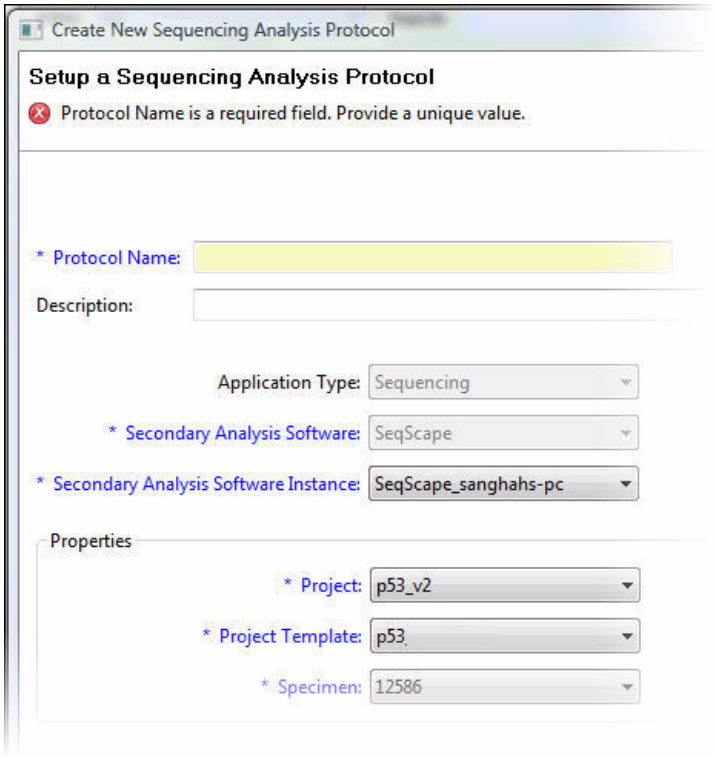


Figure 30 Create New Sequencing Analysis Protocol

Table 18 Create New Sequencing Analysis Protocol

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Lock	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 197.
Application Type	Automatically set to Sequencing.
Secondary Analysis Software	**IMPORTANT!** The secondary analysis software must be installed and properly configured with the 3500 Series Data Collection Software before it is listed as a selection in this screen. For information on setting up the SeqScape® Software for auto-analysis.
Secondary Analysis Software Instance	Computer on which the secondary analysis software is running.
Project	SeqScape software project to create.
Project Template	Project template to use.
Specimen	Specimen in which to save the sample data files. **Note:** For each specimen a sequencing analysis protocol is required.

MicroSeq® ID protocols library (secondary analysis)

MicroSeq® ID analysis protocol overview

A MicroSeq® ID protocol is the optional secondary analysis (auto-analysis) protocol for MicroSeq® *ID* Analysis Software v2.2 or later sequencing applications.

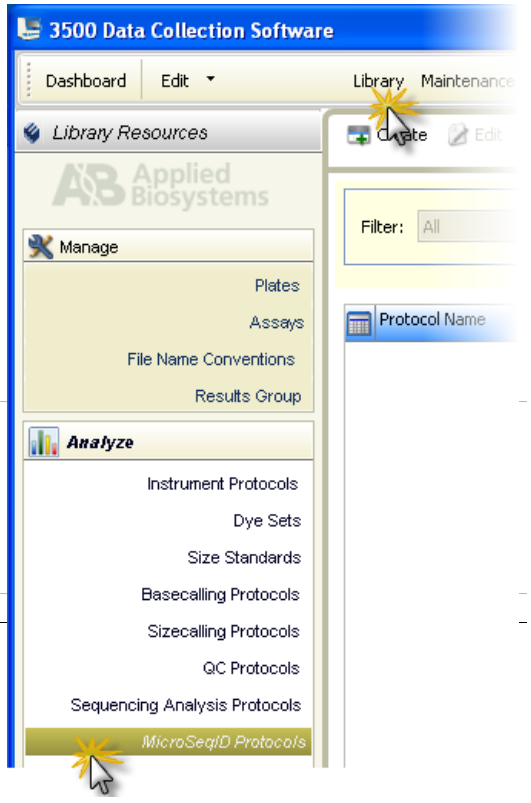
A MicroSeq® ID analysis protocol defines the:

- Secondary analysis software (MicroSeq® *ID* Analysis Software) location
- MicroSeq® *ID* Analysis Software project and specimen to use for auto analysis

When you create a sequencing assay, you can optionally add a MicroSeq® ID analysis 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 210.

Create a new MicroSeq® ID analysis protocol

1. Access the MicroSeq® ID Protocols library.
2. Click **Create**.
3. In the Create New MicroSeq® ID Protocol dialog box (Figure 31 on page 192), specify settings (see Table 19 on page 192).
4. Select the remaining secondary analysis items, then click **Save**.



Note: If the project or specimen of interest is not displayed in a list, re-select the secondary analysis software instance to update the list.

IMPORTANT! The auto-analysis settings you specify for the plate to run with this protocol must contain the same secondary software and location settings. For more information, see "Create a new plate" on page 144.

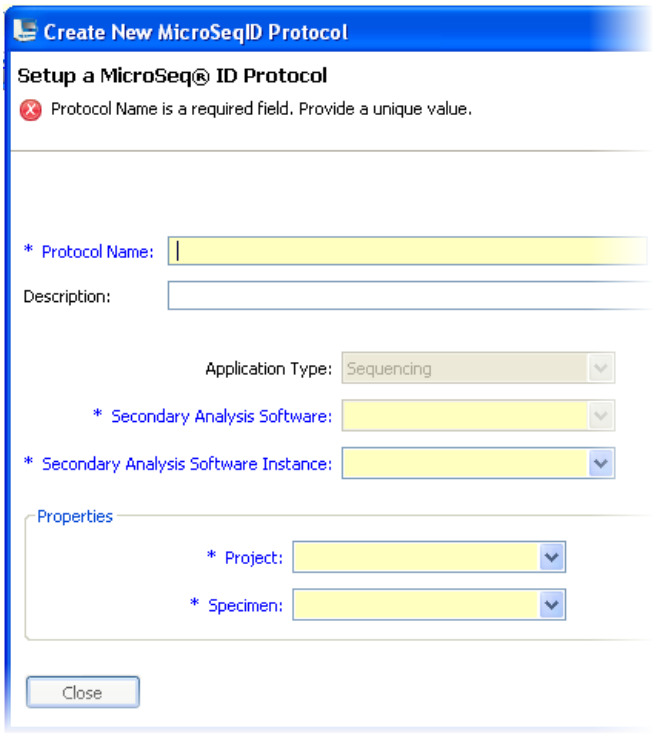


Figure 31 Create New MicroSeq® ID Protocol

Table 19 MicroSeq® ID Analysis protocol settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Lock	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 197.
Application Type	Automatically set to Sequencing.
Secondary Analysis Software	**IMPORTANT!** The secondary analysis software must be installed and properly configured with the 3500 Series Data Collection Software before it is listed as a selection in this screen. For information on setting up the MicroSeq® *ID* Analysis Software for auto-analysis, see the *MicroSeq® ID Analysis Software Getting Started Guide*.
Secondary Analysis Software Instance	Computer on which the secondary analysis software is running.
Project	MicroSeq® ID software project and specimen to create.
Project Template	Project template to use.
Specimen	Specimen in which to save the sample data files.

Fragment analysis protocols library (secondary analysis)

Fragment analysis protocol overview

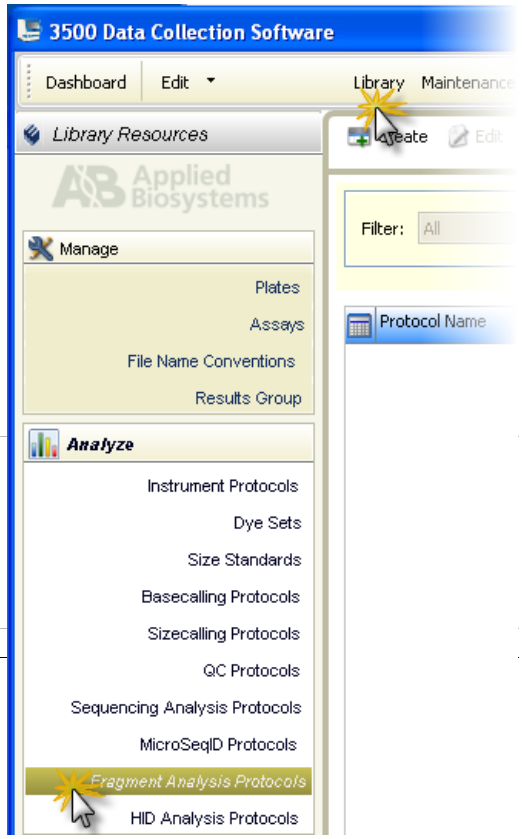
A fragment analysis protocol (GeneMapper® protocol) is the optional secondary analysis (auto-analysis) protocol for GeneMapper® Software v4.1 or later fragment applications.

A fragment analysis protocol defines the:

- Secondary analysis software (GeneMapper® Software) location
- GeneMapper® software analysis method, size standard, and panel that the GeneMapper® software will use during auto-analysis

Create a new fragment analysis protocol

1. Access the Fragment Analysis Protocols library.
2. Click **Create**.
3. In the Create New Fragment Analysis Protocol dialog box (Figure 32 on page 194), specify settings (see Table 20 on page 194).
4. Select the remaining secondary analysis items, then click **Save**.



Note: If the analysis method, size standard, or panel of interest is not displayed in a list, re-select the secondary analysis software instance to update the list.

IMPORTANT! The auto-analysis settings you specify for the plate to run with this protocol must contain the same secondary software and location settings. For more information, see "Create a new plate" on page 144.

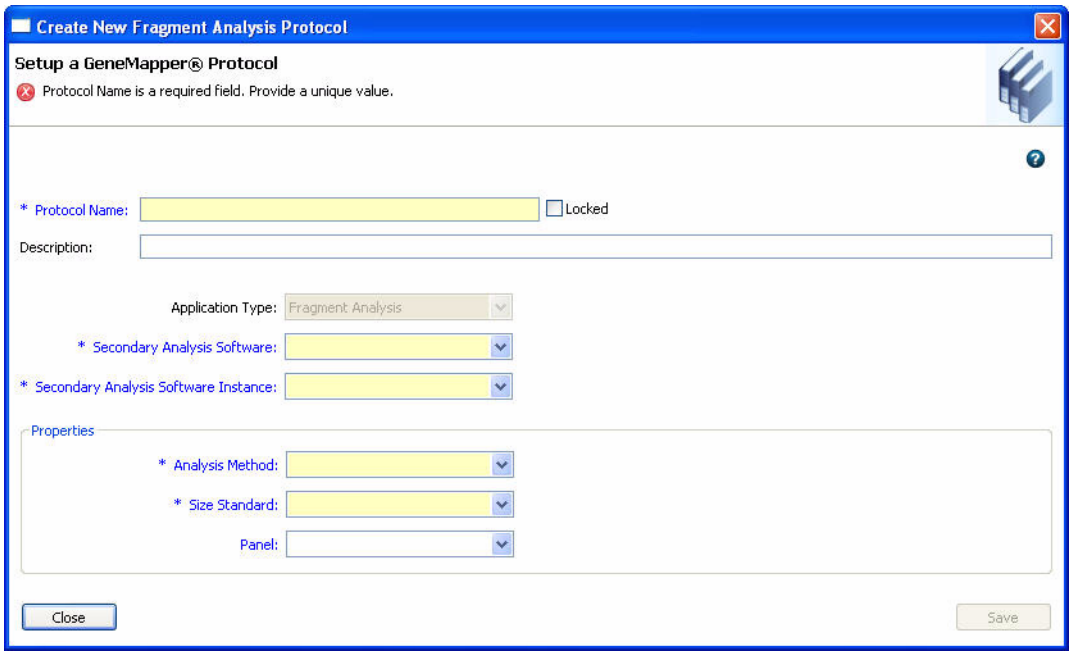


Figure 32 Create New Fragment Analysis Protocol

Table 20 Fragment Analysis protocol settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Lock	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 197.
Application Type	Automatically set to Fragment analysis
Secondary Analysis Software	**IMPORTANT!** The secondary analysis software must be installed and properly configured with the 3500 Series Data Collection Software before it is listed as a selection in this screen.
Secondary Analysis Software Instance	Computer on which the secondary analysis software is running
Properties	GeneMapper® software analysis method, size standard, and panel to use for auto-analysis.

HID analysis protocols library (secondary analysis)

HID analysis protocol overview

An HID analysis protocol (GeneMapper® ID-X protocol) is the optional secondary analysis (auto-analysis) protocol for GeneMapper® *ID-X* Software v1.2 or later for HID applications.

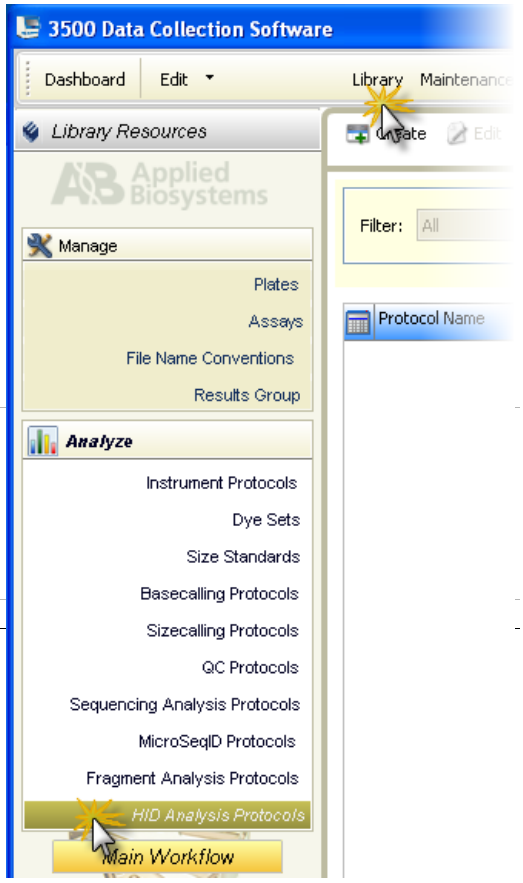
An HID analysis protocol defines the:

- Secondary analysis software (GeneMapper® *ID-X* Software) location
- GeneMapper® *ID-X* Software analysis method, size standard, and panel that the GeneMapper® *ID-X* Software will use during auto-analysis

When you create an HID assay, you can optionally add an HID analysis 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 210.

Create a new HID analysis protocol

1. Access the HID Analysis Protocols library.
2. Click **Create**.
3. In the Create New HID Analysis Protocol dialog box (Figure 33 on page 196), specify settings (see Table 21 on page 196).
4. Select the remaining secondary analysis items, then click **Save**.



Note: If the analysis method, size standard, or panel of interest is not displayed in a list, re-select the secondary analysis software instance to update the list.

IMPORTANT! The auto-analysis settings you specify for the plate to run with this protocol must contain the same secondary software and location settings. For more information, see "Create a new plate" on page 144.

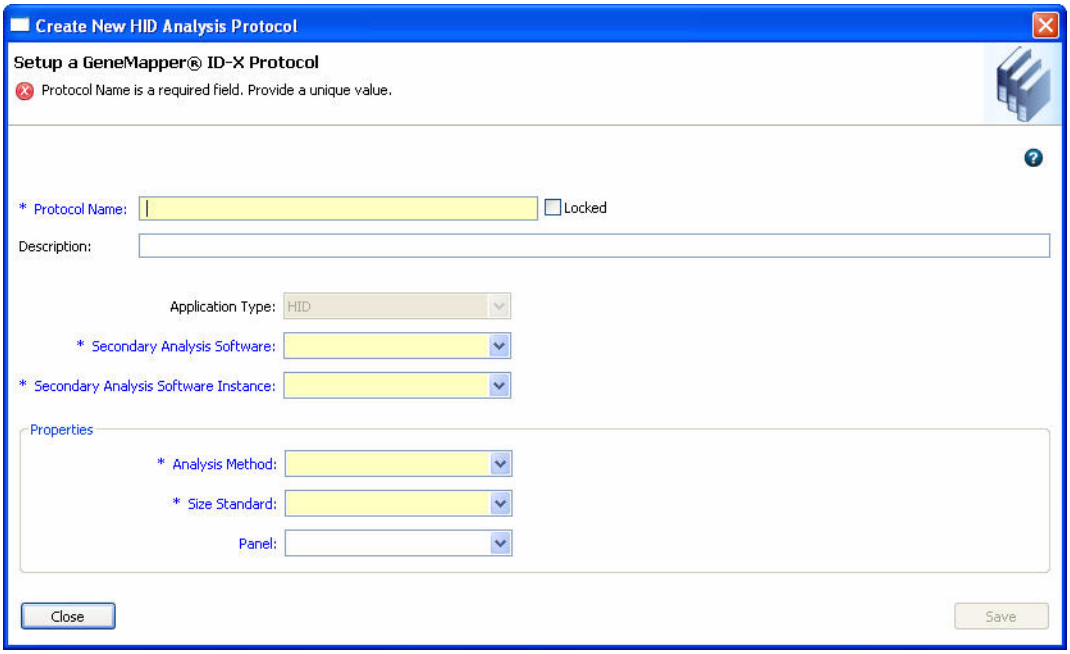


Figure 33 Create New HID Analysis Protocol

Table 21 HID Analysis protocol settings

Setting	Description
Protocol Name	Name of the protocol. Names must be unique.
Description	Optional text entry.
Lock	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 197.
Application Type	Automatically set to HID.
Secondary Analysis Software	**IMPORTANT!** The secondary analysis software must be installed and properly configured with the 3500 Series Data Collection Software before it is listed as a selection in this screen. For information on setting up the GeneMapper® *ID-X* Software for auto-analysis, see the GeneMapper® *ID-X* Software *v1.2 Installation Guide*.
Secondary Analysis Software Instance	Computer on which the secondary analysis software is running.
Properties	GeneMapper® *ID-X* analysis method, size standard, and panel to use for auto-analysis.

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

Use Security, Audit, and E-Sig Functions (SAE Module) 7

Section 1 Administrators

Administrators overview of system security, auditing, and electronic signature

The SAE (Security, Audit, E-Signature) module is an optional component of the 3500 Series Data Collection Software. The 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.

  System security can be enabled or disabled globally.
- **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.

  Auditing can be enabled or disabled globally and by record type. It is enabled globally by default.
- **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.

  Electronic signature can be enabled or disabled globally and by e-sig type. It is enabled globally by default.

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 create or modify protocols.
- Allow only certain users to approve reviewed samples.

Configure the security system

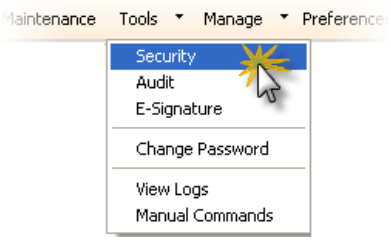
Access the Security screen and enable or disable security

The Security screen allows you to disable and enable security, control restrictions and security policies for all user accounts, and set up notifications when certain security events occur.

Security is enabled by default.

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions. However, when you disable security, no audit record is generated to indicate that audit and electronic signature functions are disabled.

1. Access the Security screen.



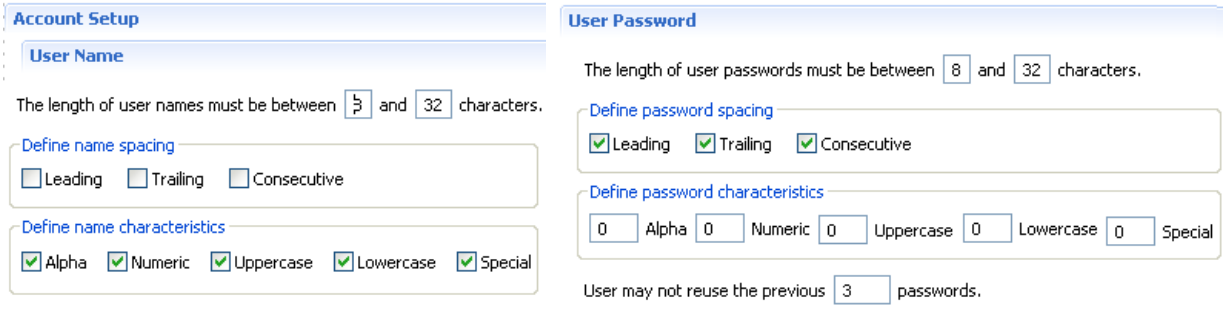
2. Click **Disable** or **Enable** (Figure 34 on page 198). Note the following:
   - Disabling Security inactivates Auditing and E-signature.
   - The Disable and Enable commands are grayed when a run is in process.
   - The software requires you to enter your user name and password when you enable security.
   - When security is disabled, the ? is not active in lower parts of the screen.

Figure 34 Security – disable or enable

Set account setup and security policies

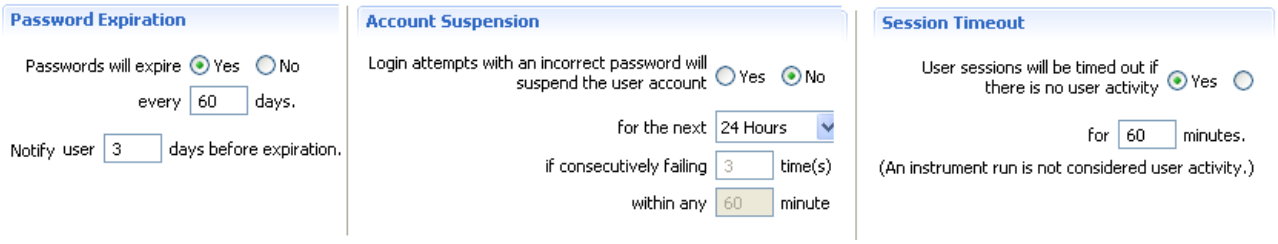
Security policies apply to all user accounts.

1. Under Account Setup, specify user name limits.



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 200.

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.



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 Settings** to specify when and how to notify the administrator of certain security events. For information, see "Set up messaging notifications" on page 200.
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

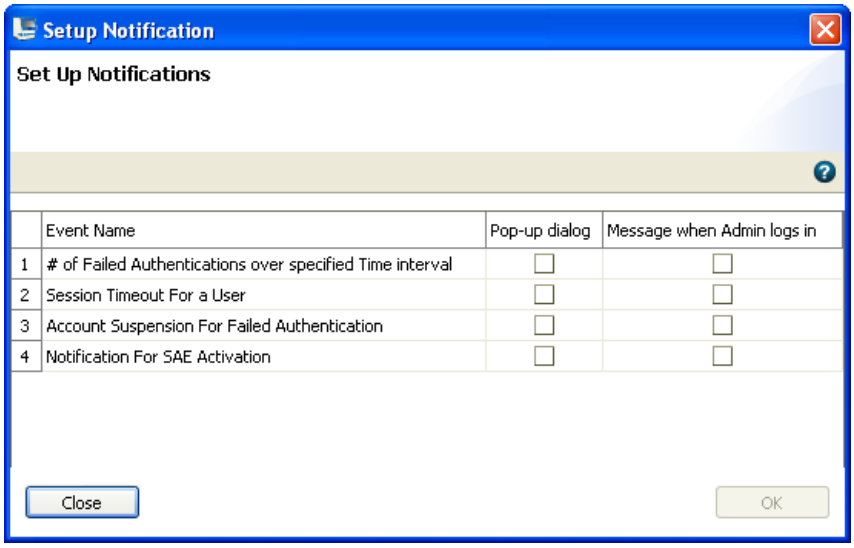
If you allow spaces in user names, be aware of the following issues:

- 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 34 on page 198), click **Messaging Notifications** to display the Setup Notifications dialog box.



2. Select the events for notification:
   - **# 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**.

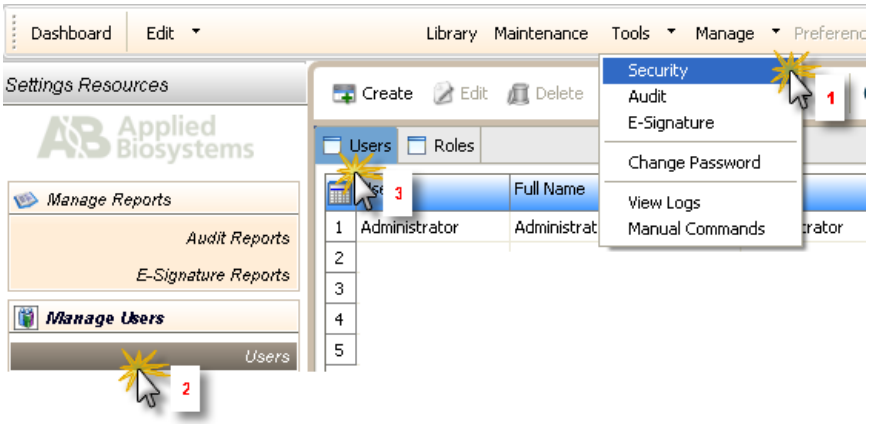
Manage user accounts

Create or edit a user account

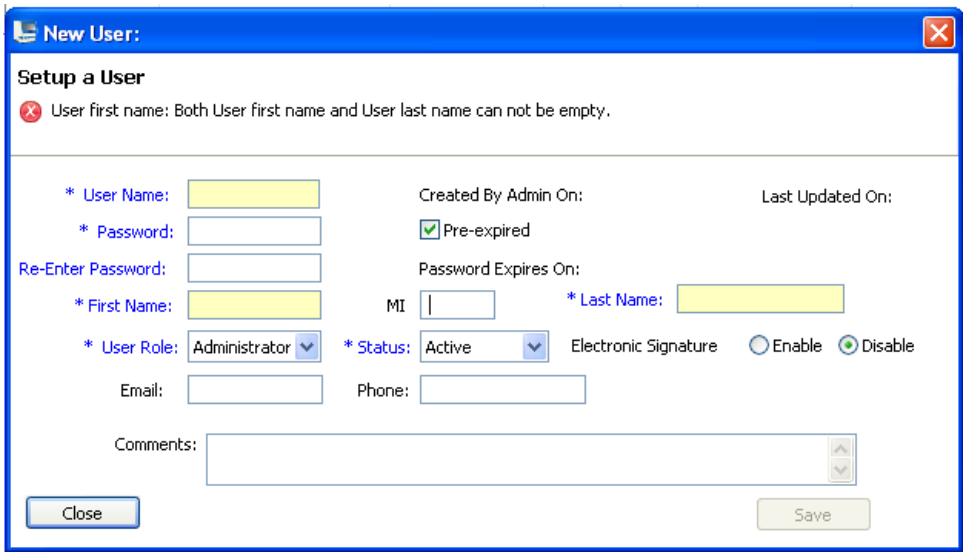
The software includes a default Administrator user account with permissions (defined by the account user role) to perform all functions in the software. You cannot modify this account.

Create a user account

1. Access the Users screen.



2. Click **Create** to display the New User dialog box.



3. Enter user name, password, first name, 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 203) 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.

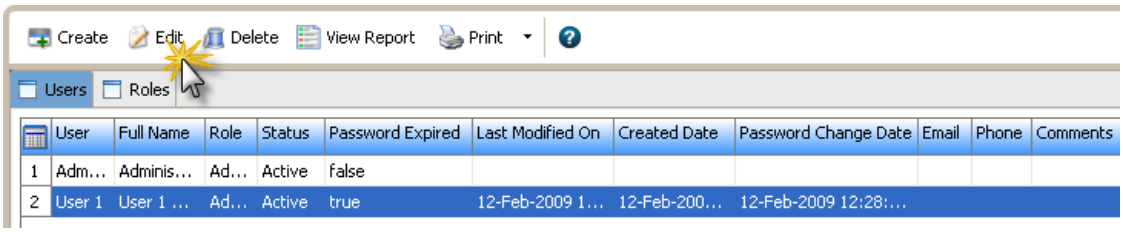
   The Users screen displays the following information for each user account:

   - Full Name
   - Role
   - Status
   - Password Expired (true=yes, false=no)
   - Last Modified On
   - 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.

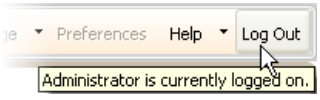
Delete (inactivate) a user account

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.

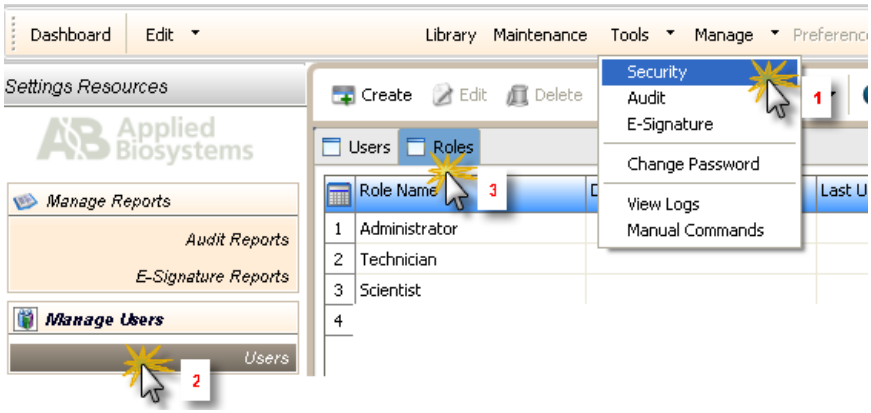
Three default user roles are included in the software. You can modify two of them, and can create your own roles with customized settings as needed:

- 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 22 on page 204). To select all permissions in a category, select the checkbox next to the category.
5. Click **Save Role**.

Table 22 User role permissions

Category	Permissions
Setup	Create plate/plate template
Run	• Edit default instrument name • Manage injection list • Duplicate injection • Re-inject
Primary Analysis	Edit sample names Export sequencing results
• Assay • File name convention • Results group • Instrument protocol • PA protocol (primary analysis: basecalling and sizecalling) • SA protocol (secondary analysis: sequencing, fragment analysis, HID analysis) • QC protocol (primary analysis: HID) • Size standard • Dye set	• Create • Edit • Delete • Import • Export

Table 22 User role permissions *(continued)*

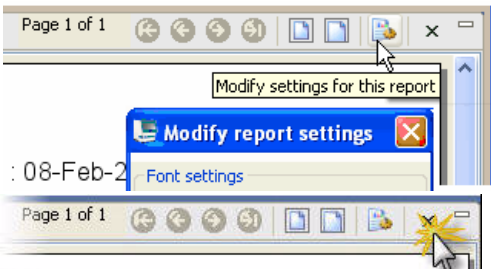
Category	Permissions
Plates and templates	• Edit • Delete • Import • Export
Locking/Unlocking	• Assay • File name convention • Results group • Instrument protocol • PA protocol • SA protocol • QC protocol • Size standard • Dye set
Preferences	• Edit system preferences • Export system preferences • Import system preferences • Export user preferences (all)
Calibrations	• Perform spatial calibration • Perform spectral calibration
Performance check	Run performance check install standards
Archiving	• Archive • Purge • Restore
SAE configuration	• 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.

Example reports

User Report

#	User	Full Name	Role	Email	Phone	Status	Created Date	Last Modified On	Password Change Date	Password Expired	Comments
1	Administrator	Administrator	Administrator			Active				false	
2	technician	First Name MI User2	Technician			Active	29-Jan-2009 10:13:57 AM	01-Feb-2009 10:10:49 AM	31-Jan-2009 10:12:48 AM	false	
3	scientist	FirstName MI User3	Scientist			Active	29-Jan-2009 11:30:12 AM	01-Feb-2009 10:10:22 AM	31-Jan-2009 03:36:58 PM	false	
4	Analyst1	First Name MI Analyst1	Log in to timed-out session			Active	01-Feb-2009 10:21:26 AM	01-Feb-2009 10:21:26 AM	01-Feb-2009 10:21:26 AM	true	

User Role Report

#	Role Name	Description	Last Updated Date	User Counts
1	Administrator			3
2	Technician			1
3	Scientist			1
4	Log in to timed-out session		01-Feb-2009 10:12:18 AM	1

Manage auditing

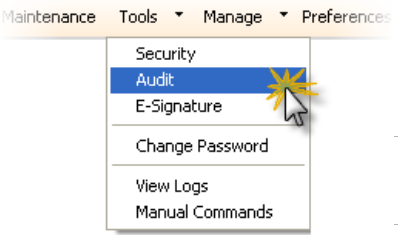
Access the Audit screen and enable or disable auditing

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** or **Enable** (Figure 35 on page 207).



Note: When auditing is disabled, the ? is not active in lower parts of the screen.

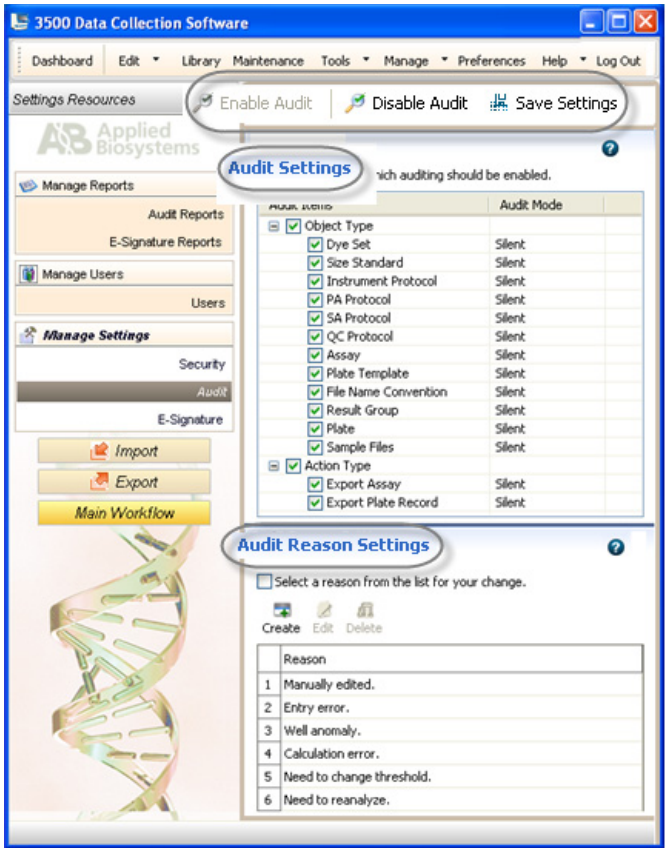


Figure 35 Audit – disable or enable

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)
• Dye set • Size standard • Instrument protocol • PA protocol (primary analysis) • SA protocol (secondary analysis) • QC protocol • Assay • Plate template • File name convention • Results group • Plate • Sample files	• Export assay • Export plate record

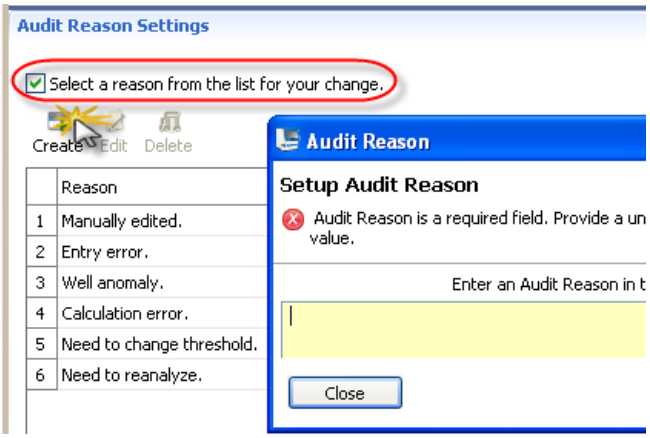
Note: For a list of items that the system audits silently in addition to the configurable items listed above, see "Generate audit reports" on page 209.

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 **User must select a reason** 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 142.

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 72.
   - 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
- Size standard
- Instrument protocol
- PA protocol (primary analysis)
- SA protocol (secondary analysis)
- QC protocol
- Assay
- Plate template
- File name convention
- 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	The auditing of updates depends on whether an object is modified or overwritten: • **Modified** – A record is created when an object is modified. • **Updated** – A record is not created when an object is overwritten in the library. 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.
Create	A record is created when you: • 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).
Delete	The auditing of deletions depends on the item deleted: • **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 *delete* a size standard from the library, no audit record for the deletion is listed in the Object Audit History. • **Items within other items** – The deletion of an item from within another item is audited. For example, if you *change* the size standard in a QC protocol, an audit record for the change (considered a deletion) is listed in the Object Audit History.

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, the primary analysis protocol (and therefore dye set, and size standard), and the secondary analysis protocol 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 23 Audit – system configuration history

Record Type	Action	Corresponds To
Security settings	Update	• Enable security • Disable security • Modify security policies: • Session timeout settings
Account settings	Update	• Modify user name settings • Modify password settings • Modify security policies: • 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	Enable auditing Disable auditing
Audit type	Update	• Modify audit settings
Audit type	Update	• Modify audit settings • Create reasons for change • Delete reasons for change
E-Signature function	Update	• Modify the number of signatures or the authorities for a “prompt before” function • Modify the Enable state of either a “check before” or “prompt before” function
E-Signature settings	Update	• Enable e-signature • Disable e-signature
E-Signature type	Update	• Modify e-signature settings • Modify the enable state of an E-Signature Type

Table 23 Audit – system configuration history *(continued)*

Record Type *(continued)*	**Action**	**Corresponds To**
Role assignment	Create	• 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	• 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 24 Audit – action log

Category	**Action**
Assay	Assay exported successfully **Note:** Only one audit record is generated if you export multiple assays.
Log In	• User logged in • Login failed • User logged out
Maintenance Wizards	• Remove Bubbles Wizard started • Flush Array Port Wizard started • Change Polymer Type Wizard started • Change Array Wizard started • Replenish Polymer Wizard started • Perform Fill Polymer Wizard • Perform Water Wash Wizard
Plate	Plate exported successfully **Note:** Only one audit record is generated if you export multiple plates.

Table 24 Audit – action log *(continued)*

Category	Action
Run	• Start • Pause • Resume • Stop (Abort injection) • Terminate (injection list)
SAE Configuration	• Export
System Audit Records	• Archive • Purge • Restore
System Action Records	• Archive • Purge • Restore
User Profile	• 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

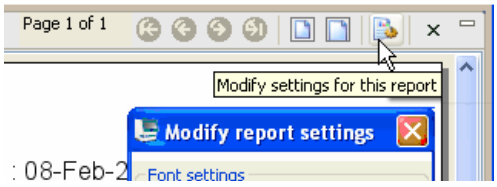
2 **Date :** 28-Jan-2009 05:00:57 PM **Action :** Update

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.

For information on archiving library items (datastore), see "Archive, purge, and restore data" on page 254.

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.

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 Data Collection Software.

1. Display the records of interest as described above.
2. Click **Export Audit Records**.
3. Specify a name and location for the export .txt file.
4. Click **Save**.

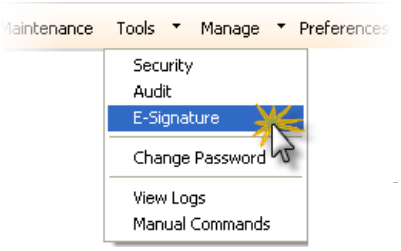
Note: If you export audit records for samples that are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

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 screen.
2. Click **Disable** or **Enable** (Figure 36 on page 216).



Note: When e-sig is disabled, the ? is not active in lower parts of the screen.

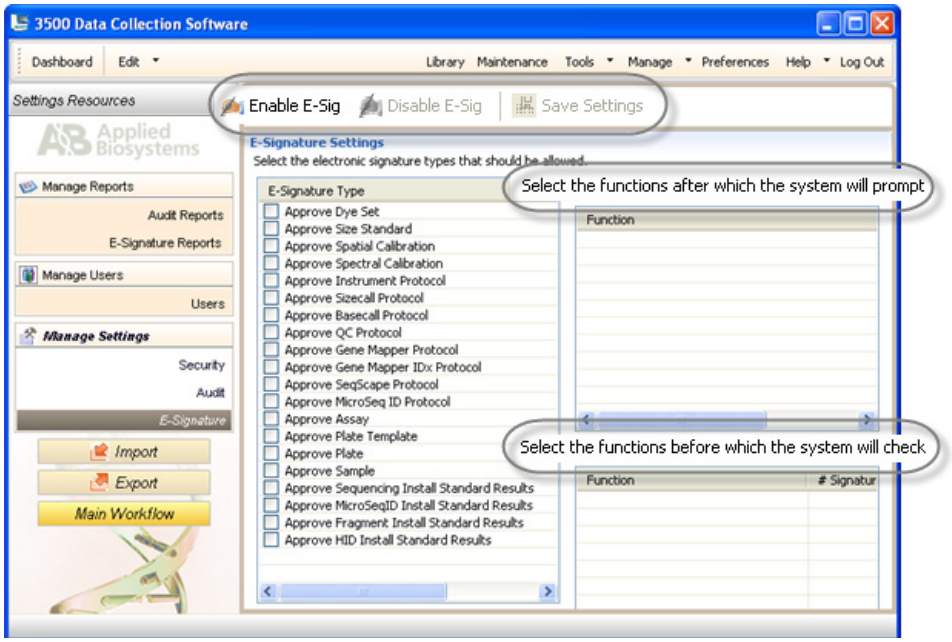
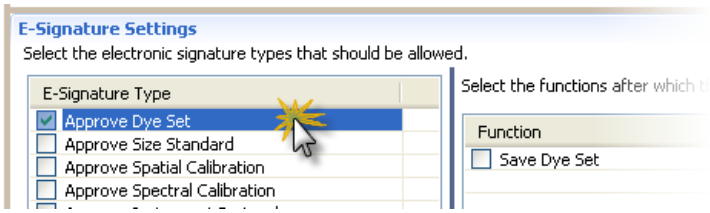


Figure 36 E-Sig – disable or enable

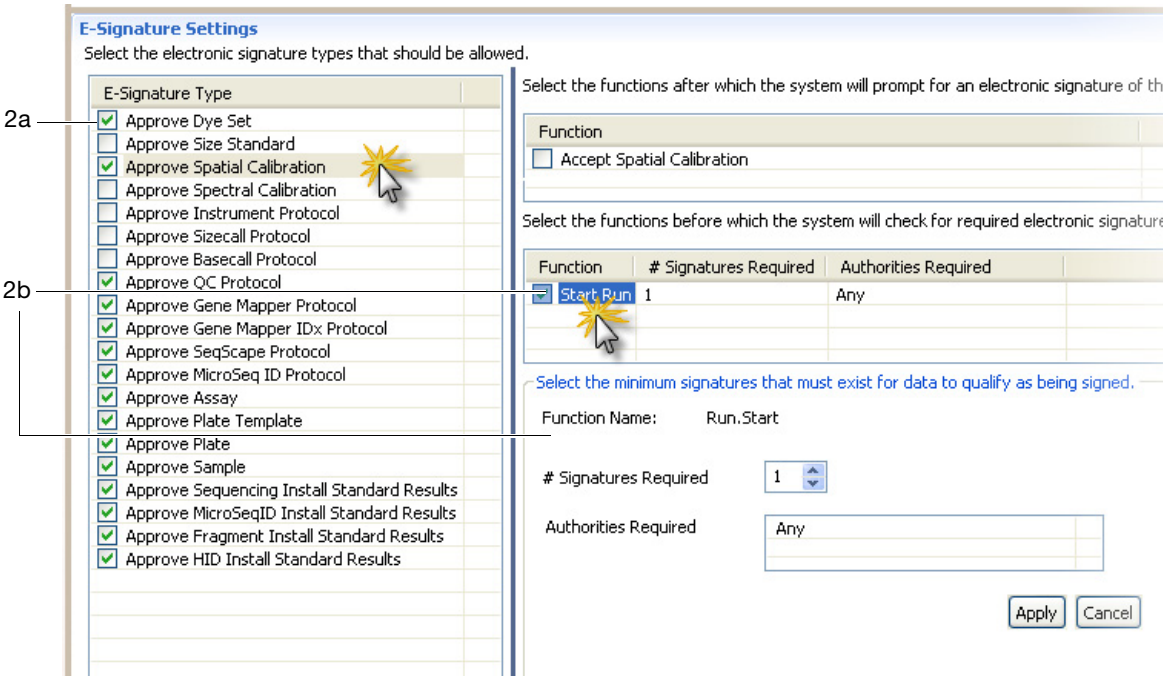
Select the actions that allow signature

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 25 on page 218). 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,:
   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 26 on page 219). 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**.



3. Click **Save Settings**.

By default, no E-Signature types are enabled.

Table 25 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 Spatial Calibration	Accept
Approve Spectral Calibration	Accept
Approve Instrument Protocol	Save
Approve Sizecall Protocol	Save
Approve Basecall Protocol	Save
Approve QC Protocol	Save
Approve GeneMapper Protocol	Save
Approve GeneMapper IDX Protocol	Save
Approve SeqScape Protocol	Save

Table 25 E-signature settings to prompt after *(continued)*

E-Signature Type	Function to Prompt After
Approve MicroSeq ID Protocol	Save
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 Fragment Install Standard Results	Accept
Approve HID Install Standard Results	Accept

Table 26 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 Spatial Calibration		
Approve Spectral Calibration		
Approve Plate		
Approve Sequencing Install Standard Results		
Approve MicroSeq ID Install Standard Results		
Approve Fragment Install Standard Results		
Approve HID Install Standard Results		

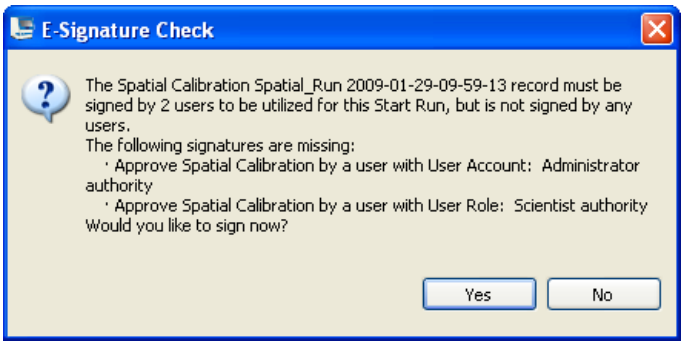
How the software prompts electronic signature before a run

If the system is configured to check that data is signed fore 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, this message is displayed again.

Generate e-signature reports

Display e-signature records

1. Access the E-Signature Reports screen.

2. (Optional):
   - Specify filters (date range, user name, action, 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 72).
   - Customize the table (see "Customize tables" on page 72).
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 Genemapper Protocol
   - Approve Genemapper ID-X Protocol
   - Approve Seqscape Protocol
   - Approve Microseq ID Protocol
   - Approve Assay
   - Approve Plate Template
   - Approve Plate
   - Approve Sample
   - Approve Sequencing Install Standard Results
   - Approve Microseq ID Install Standard Results
   - Approve HID 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**.

Applied Biosystems

Report created on : 04-May-2009 03:13:09 PM
3500 Data Collection Software Version 1.0.0

E-Signature Summary Report

#	Date	User Name	User Full Name	Object Type	Object Name	Comments
1	04-May-2009 03:11:47 PM	Administrator	Administrator	Approve Spatial Calibration	Spatial_Run 2009-05-04-15-10-51	Spatial calibration is acceptable

Applied Biosystems

Report created on : 04-May-2009 03:14:16 PM
3500 Data Collection Software Version 1.0.0

E-Signature Detailed Report

1 **User Name :** Administrator **User Full Name :** Administrator

Object Type : Approve Spatial Calibration **Object Name :** Spatial_Run 2009-05-04-15-10-51

Date : 04-May-2009 03:11:47 PM **Comments :** Spatial calibration is acceptable

Esignature Details

Esignature Type : Approve Spatial Calibration

Signed By : Administrator

Full Name : Administrator

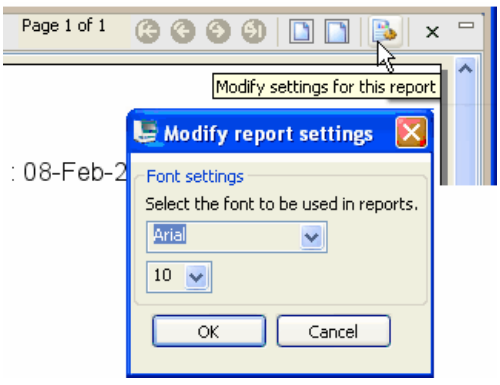
Signed On : 04-May-2009 03:11:47 PM

Authority : User Account: Administrator, User Role: Administrator

Object Details

5	Intensity	16 5 -5 5 13 2 8 9 12 -1 -1 7 3 6 -1 1 -7 4 4 8 5 -2 0 9 2 -3 0 -6 -6 -2 7 -4 -4 -9 -3 -8 -3 8 -7 4 2 1 -3 -5 -2 1 -3 -4 5 6 5 3 0 2 0 1 -1 2 5 1 3 2 3 0 1 3 0 1 -3 -4 0 -5 -7 -5 -7 -3 1 4
6	Run ID	Spatial_Run 2009-05-04-15-10-51
7	Number of Capillaries	24
8	Locked	false
9	Instrument	13527-029
10	Capillary Array	80K0850

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 Data Collection Software.

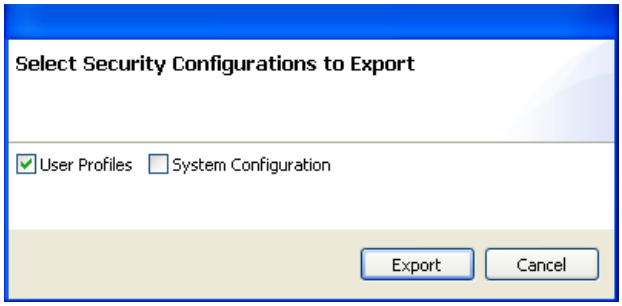
1. Display the records of interest as described above.
2. Click **Export E-Sig Records**.
3. Specify a name and location for the export .txt file.
4. Click **Save**.

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.

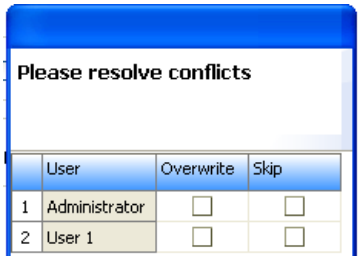
Import

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 2 Users

Users overview of System Security, Audit Trail and E-Signature

The Security, Audit, E-Signature (SAE) module is an optional component of the 3500 Series Data Collection Software. The SAE module provides the following functionality:

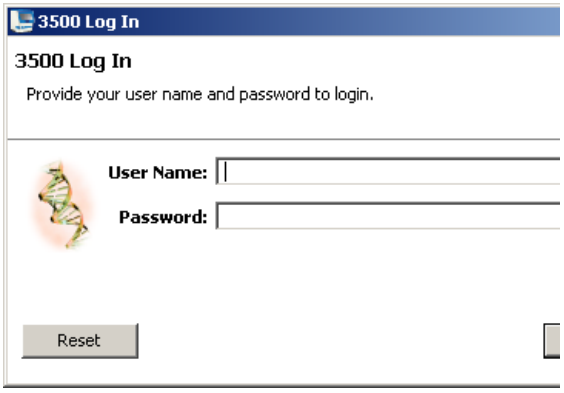
- **System security** – Controls user access to the software.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the SAE settings.
- **Electronic signature (e-sig)** – Requires users to provide a user name and password when performing certain functions.

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.

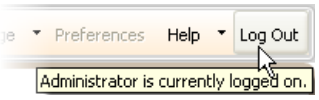
If your system is configured for password expiration, you will periodically be prompted to change your password. If your system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password for a specified number of times.

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.

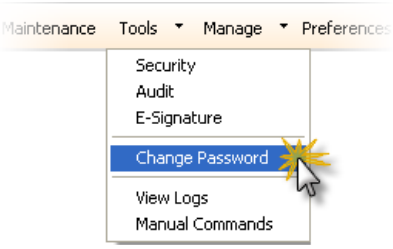


Change your password when it expires

When your password is about to expire, a message is displayed when you log in.

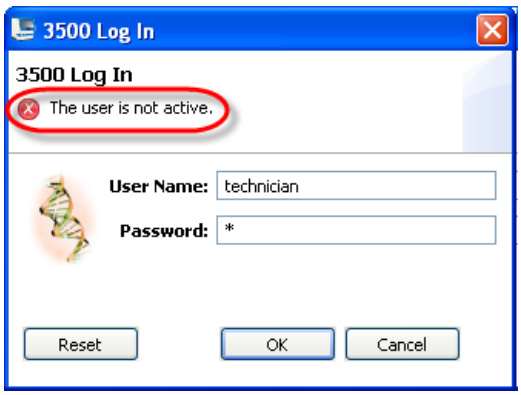
To change your password, select **Tools** ▸ **Change Password**.

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.



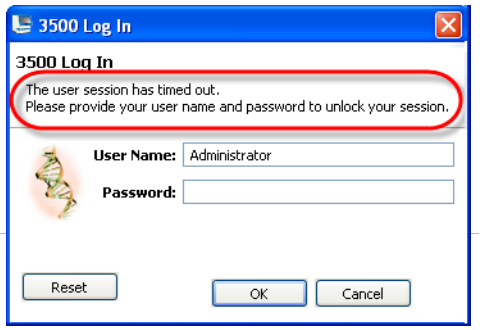
There are two ways to activate a suspended account:

- 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.

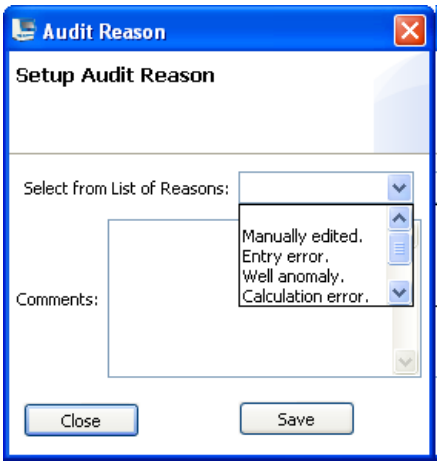


Note: The administrator or another user with permission to log in to timed-out sessions can click **Reset**, then log in.

Audit

If your system is configured for auditing, you may be prompted to specify a reason when you make certain changes in the software.

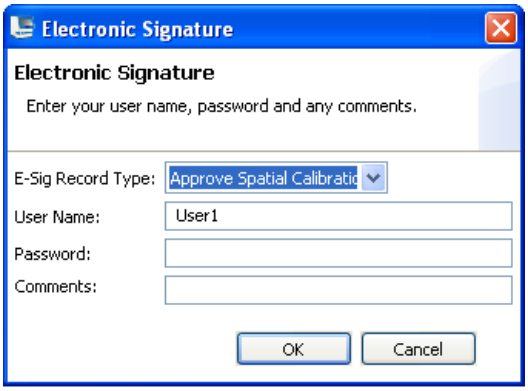
Based on your system configuration, you can either select a reason or enter a reason for change.



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.

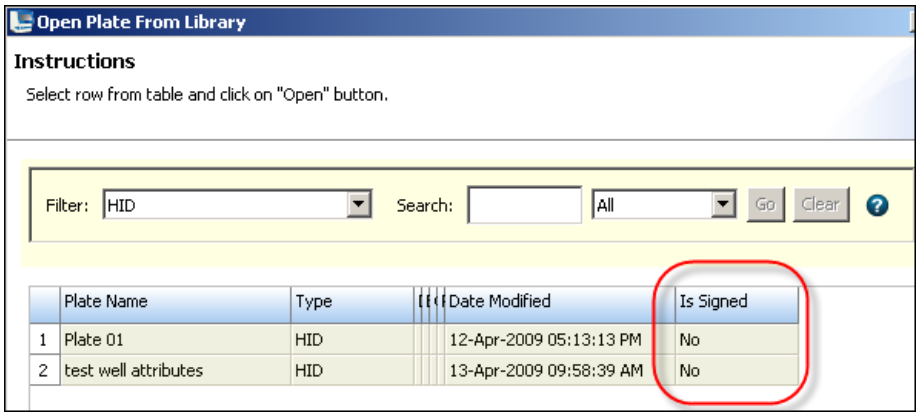
If an item is set to require two signatures, the signers are not required to sign at the same time. When the first signer signs, the E-Sig status is set to Partially Signed. When the second signer signs, the E-Sig status is set to Signed.



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 220.

- The Open Plates dialog box or the library displays an “Is signed” column that reflects the electronic signature status of an item.



Maintain the Instrument 8

Maintenance schedule

This section lists the common tasks required to maintain your Applied Biosystems 3500/3500xL Genetic Analyzers in good working condition.

The Dashboard, in conjunction with the data entered in the schedule section of the Planned Maintenance, provide a comprehensive outline of maintenance tasks.

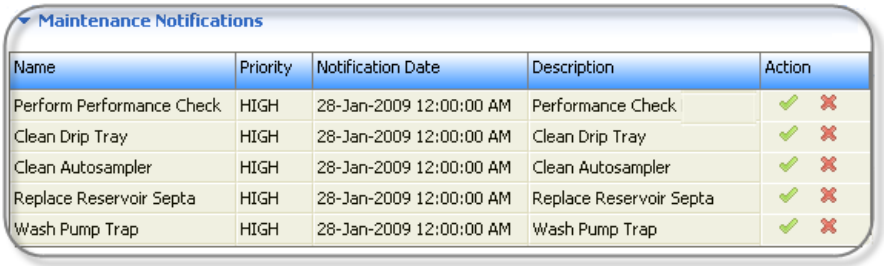
WARNING! Wear appropriate protection, including gloves, laboratory goggles, and coat whenever you work with the fluids used on this instrument, or parts that may come into contact with these fluids.

IMPORTANT! Use the cleaning agents as described in this manual, only. Use of cleaning agents not described in this manual can impair the instrument.

For the instrument troubleshooting issues, see Appendix E, “Troubleshoot” on page 299.

Review maintenance notifications

Review maintenance notifications list in the Dashboard daily, then perform the scheduled tasks.



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 257.

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 the cleaning agents as described in this manual, only. Use of cleaning agents not described in this manual can impair the instrument.

Task	Frequency	For information, see ...
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 236
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 cathode buffer container (CBC)" on page 238
Ensure that the plate assemblies are properly assembled. **IMPORTANT!** Align the holes in the plate retainer with the holes in the septa to avoid damaging capillary tips.		"Prepare the plate assembly" on page 53
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 53
Ensure the array locking lever on the capillary array is secured.		Chapter 1, Instrument and Software Description
Check for bubbles in the pump block and channels. **Note:** Use the Remove Bubble wizard to remove bubbles.	Daily or before each run	"Remove bubbles from the polymer pump" on page 251
Check the loading-end header to ensure that the capillary tips are not crushed or damaged.		"To change the capillary array" on page 252
Ensure that the pump block is in pushed back position.	Daily	Chapter 1, Instrument and Software Description
Clean the instrument surfaces of dried residue, spilled buffer, or dirt.		"Routine instrument cleaning" on page 242
Check for leaks and dried residue around the Buffer-Pin Valve, check valve, and array locking lever. **IMPORTANT!** If leaks persist, contact Applied Biosystems.		"Check maintenance notifications" on page 28

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 240
Run the Wash Pump and Channels wizard.		"Wash the pump chamber and channels" on page 249
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 314.

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 241
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 238
Run a performance check		Chapter 5, Calibrate and Check Performance
Clean the autosampler		"Routine instrument cleaning" on page 242
Clean the drip tray		
Check disk space		"Monitor disk space" on page 256
Defragment the hard drive	Monthly Before fragmentation reaches 10%.	"Defragment the computer hard drive" on page 257

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 Applied Biosystems 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 242
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

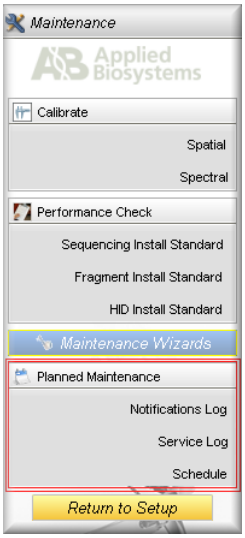
The Maintenance calendar is a monthly or daily view of the routine maintenance tasks scheduled for your instrument. When a task is due to be performed, it is listed in the Maintenance Notifications list in the Dashboard (see "Review maintenance notifications" on page 229).

View the calendar

To go to the Schedule from the Dashboard:

1. In the Dashboard, click **Maintain Instrument** toggle key.

   The Planned Maintenance options appear on the left-hand pane, highlighted below:



   The Dashboard provides you with a list of current maintenance notifications, as shown. Click ? for information.

2. From the Left-hand pane, under Planned Maintenance, click **Schedule**

3. Click ? on the top left-hand corner of the Schedule for more information.

   Additionally, Applied Biosystems suggests that you add the regular maintenance tasks listed below to the maintenance calender.

Default calendar entries

A set of Applied Biosystems-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 can change the priority of factory tasks, but you cannot remove them from the calendar or alter the frequency at which the notifications for the tasks are displayed.

Additionally, Applied Biosystems suggests that you add to the maintenance calendar:

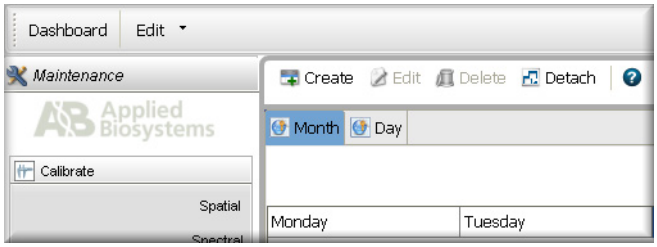
- The regular maintenance tasks.
- A maintenance task to replace a consumable based on its installation date (for example, create a task to replace the polymer for two days before the polymer will expire)

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 calender.

The Month and Day tabs allow you to view your schedule in different formats. Click **Detach** to move the calendar window.



Review the Maintenance Notifications Log

The Notifications Log is a history of all notifications messages and the action taken for the task (completed or dismissed). You can use this option to review a previous run information.

The Dashboard provides you with a list of current routine and maintenance notifications, as explained below.

Multi-column sorting is supported (see "Multi-column sorting" on page 72.

To go to the Notifications Log from the Dashboard:

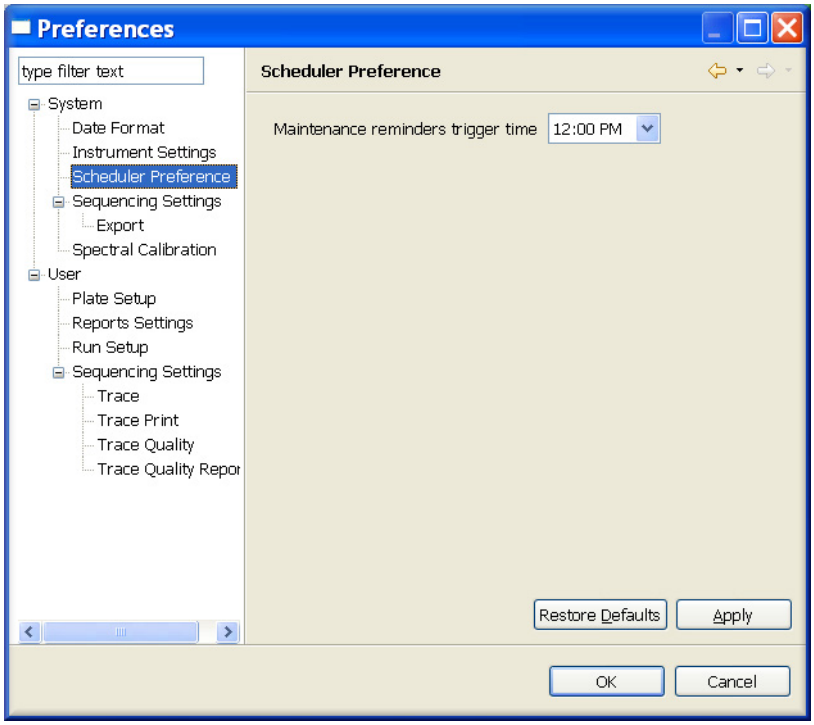
1. Click **Maintain Instrument**
2. From the Left-hand pane, under Planned Maintenance, click **Notifications Log**

Click  on the top left-hand corner of the Notification Log for more information.

The Notification Log provides the following information on each event:

Notification	Description
Name	The name of the event.
Priority	The event priority.
Notification Date	The date of notification.
Status	The current status of the event.
User	The name of the user.
Acknowledge Date/Time	The date and time when the event was acknowledged.
Description	The description for the event.

Notification time is determined in the Preferences. From the Dashboard, click Preferences, to open the Preferences dialog box, click Scheduler Preference, and follow the prompts.


Preferences
type filter text
System
Date Format
Instrument Settings
Scheduler Preference
Sequencing Settings
Export
Spectral Calibration
User
Plate Setup
Reports Settings
Run Setup
Sequencing Settings
Trace
Trace Print
Trace Quality
Trace Quality Repor
Scheduler Preference
Maintenance reminders trigger time
12:00 PM
Restore Defaults
Apply
OK
Cancel

Instrument operational procedures

The day-to-day operation of the instrument involves performing the following tasks.

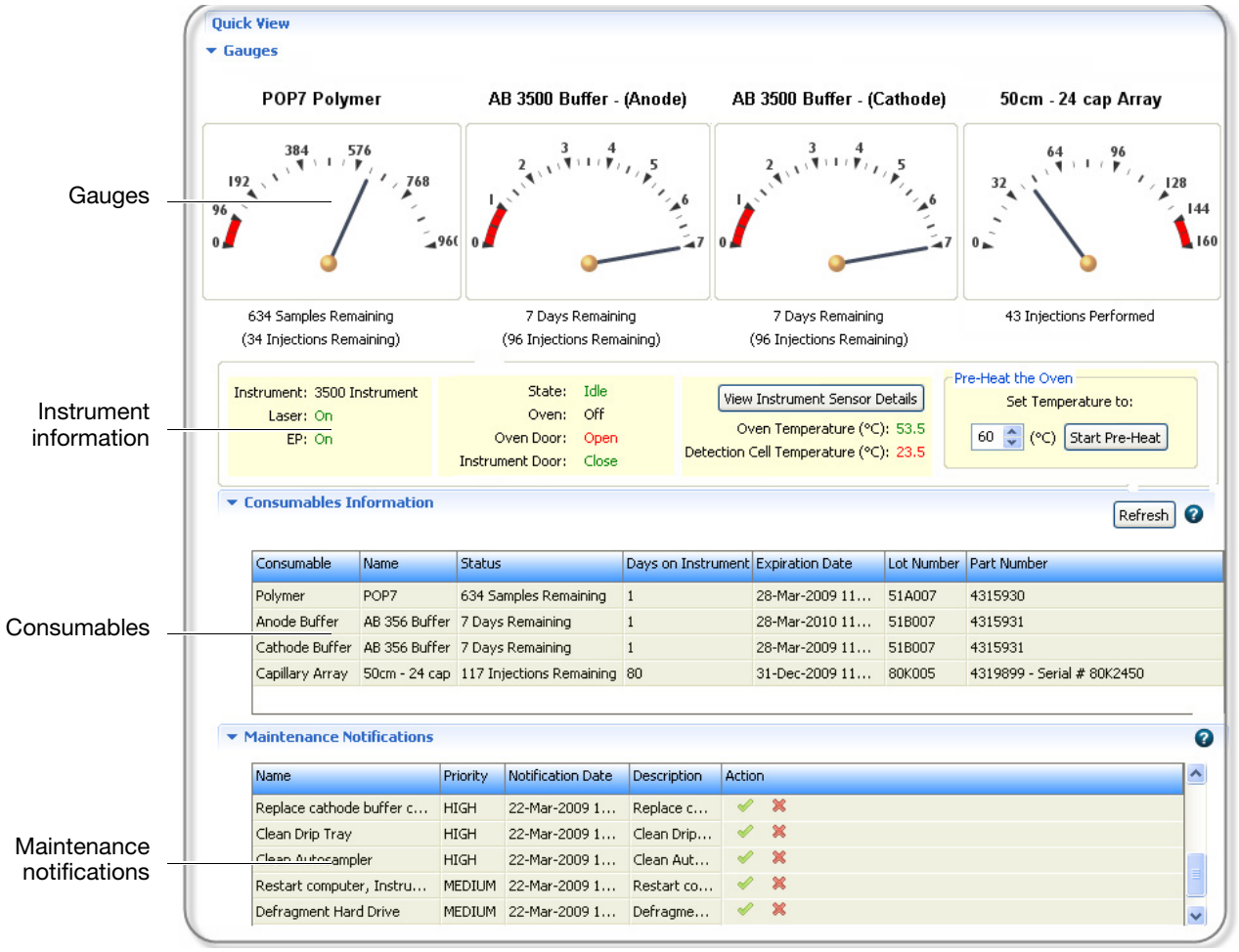
Check consumables on the Dashboard

- 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 the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. Anode Buffer Container (ABC).

For details see "Instrument reagents and consumables" on page 9.

Contamination might cause poor-quality data. To prevent the contamination, use genuine packaged polymer, anode buffer, cathode buffer and conditioning reagent.

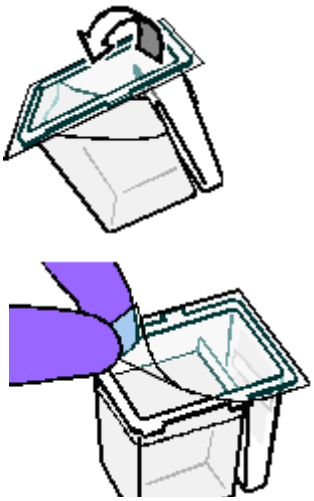
1. Remove the ABC from storage.
2. Check for expiration date on the ABC label to make sure it is not expired prior to or during intended use.
3. Allow refrigerated ABC to equilibrate to ambient temperature prior to first use. Do not remove the seal until you have completed step 5, below.

   IMPORTANT! Ensure that all the buffer is moved to the larger side of the ABC prior to removing the seal.

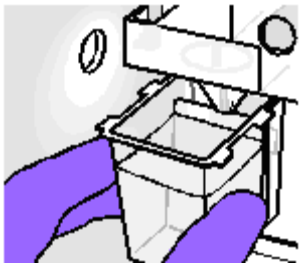
4. Verify that buffer level is at or above the fill line and check that seal is intact.

   IMPORTANT! Do not use if buffer level is too low or seal has been compromised. A fill tolerance of ± 1 mm is acceptable.

5. Tilt the ABC slightly (as shown in the figure below) to make sure most of 1X buffer is in the larger side of the container. There should be less than 1 ml of 1X 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 the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. Cathode Buffer Container (CBC).

For details see "Instrument reagents and consumables" on page 9.

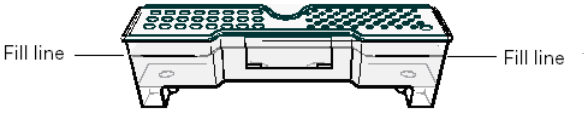
Contamination might cause poor-quality data. To prevent the contamination, use genuine packaged polymer, anode buffer, cathode buffer and conditioning reagent.

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 ± 0.5mm is acceptable.

Note: The meniscus must be at or above the fill line.

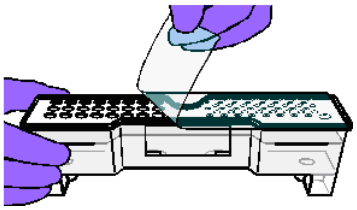


6. Tilt the CBC back and forth gently and carefully to ensure that the buffer is evenly distributed across the top of the baffles.

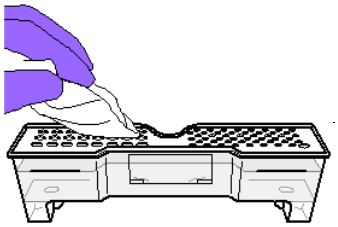
   Note: If you do not tilt the CBC back and forth, the buffer sticks to the baffles, due to surface tension.

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.

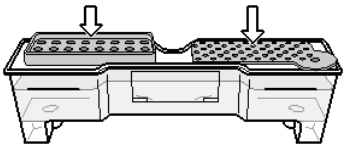


9. Wipe off any buffer on top of the CBC with a lint-free cloth. Ensure that the top of the container is dry.



   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.

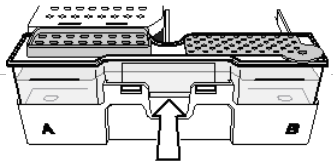


    a. Align the buffer septa (the part that is symmetrical) over the 24 holes of the CBC.

    b. Push the septa lightly into the holes to start and then push firmly to seat the septa.

11. Install the CBC on the autosampler.

    Note: When properly installed, it will click on the autosampler as the tabs are snapped in place.



12. Close the instrument door to re-initialize.

13. Click **Refresh** from the Dashboard to update the screen.

14. Check the Quick View section of the Dashboard for updated status after changing the CBC.

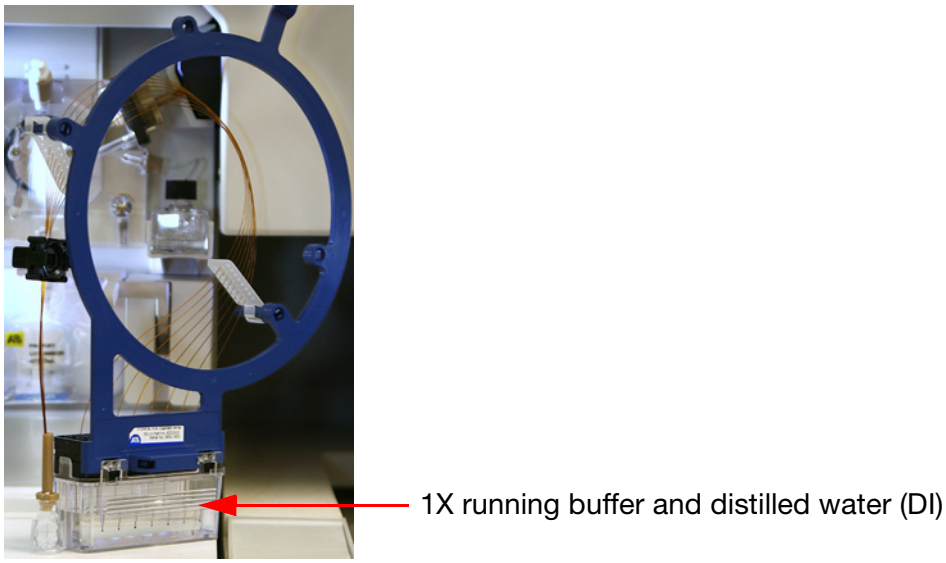
Check stored capillary arrays

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. Applied Biosystems recommends you keep the electrodes on the bottom in the tray with 1✕ running buffer. For details see "Instrument reagents and consumables" on page 9.

IMPORTANT! Keep the loading-end of the capillary array in 1✕ 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.



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.

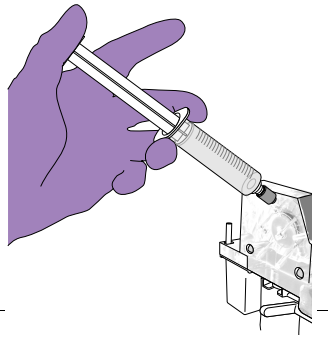
Flush with either distilled or deionized water and ensure that the water flows into the overflow container. Dispose the excess water (inside the overflow container). See "General chemical safety" on page 328.

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, 4359572) 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 µL, 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.

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.

   IMPORTANT! Use the cleaning agents as described in this manual, only. Use of cleaning agents not described in this manual can impair the instrument. Please contact your local Life Technologies sales office if you have any questions.

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 (absolute), and lint-free tissue.

   Note: The drip tray can be removed.

Move and level the instrument

CAUTION! 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 "To shutdown the instrument" on page 253.
   - 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)

Use the Maintenance Wizards to perform operations

About Maintenance Wizards

To activate the Maintenance Wizards from the Dashboard, click **Maintain Instrument** toggle key.

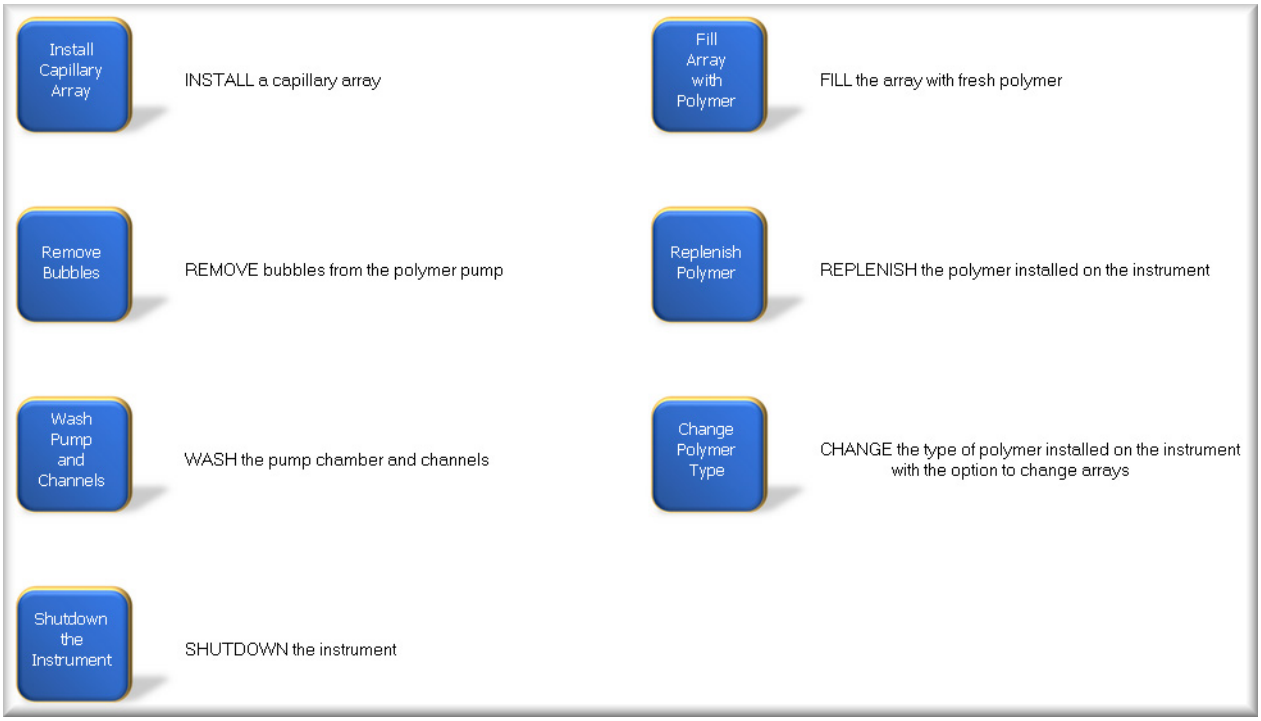
The Maintenance Wizards feature of the Data Collection software allows you to perform operations necessary for sustaining the instrument.

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 the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. POP-4™, POP-6™, and POP-7™ polymers.

For details see "Instrument reagents and consumables" on page 9.

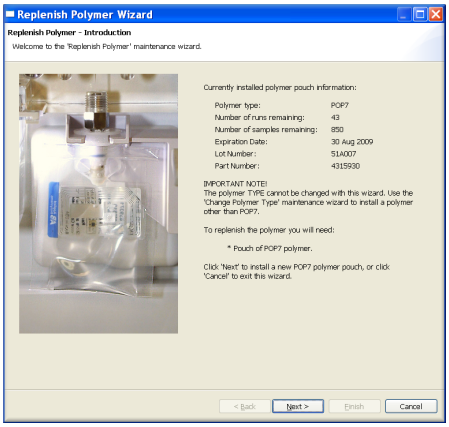
If you are replacing the same polymer type only, follow the procedures below:

IMPORTANT! If you remove a polymer pouch for storage, place a Pouch Cap (PN 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.

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.

For the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

WARNING! CHEMICAL HAZARD. POP-4™, POP-6™, and POP-7™ polymers.

IMPORTANT! If you remove a polymer pouch for storage, place a Pouch Cap (PN 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.

For details see "Instrument reagents and consumables" on page 9.

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 (PN 4427991).

Note: Expired pouches cannot be used 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.

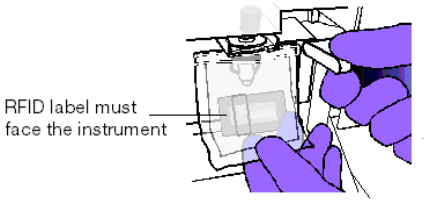
   IMPORTANT! Do not use if the pouch and/or the label is damaged or the top seal is missing.

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.

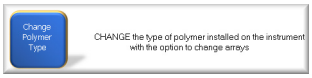
5. Slide the pouch fitment on to the slot of the lever assembly. Push the lever up to snap the pouch into the connector end of the instrument pump.

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**.

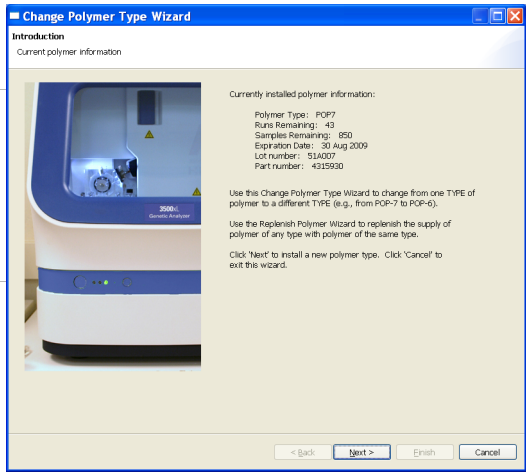


IMPORTANT! This feature allows you to change the type of polymer installed on the instrument with the option to change the Capillary Arrays.

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 "Use the conditioning reagent" on page 250.



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.

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 (4412619).

If you remove a polymer pouch for storage, place a Pouch Cap (PN 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. 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.

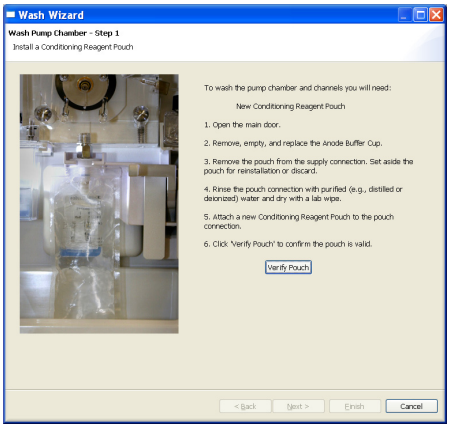
IMPORTANT! Follow the instructions in the wizard to ensure the proper installation and operation of the pouch and the instrument.

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 9.

IMPORTANT! Expired pouches cannot be used on the instrument. 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.

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 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

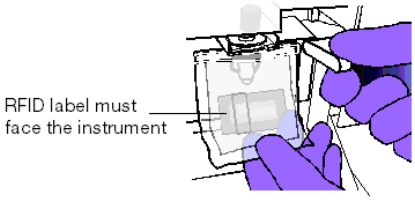
1. Check for expiration date on the label to make sure it is not expired prior to use.

   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 the following hazard(s), see the complete safety alert descriptions in "Specific chemical alerts" on page 333.

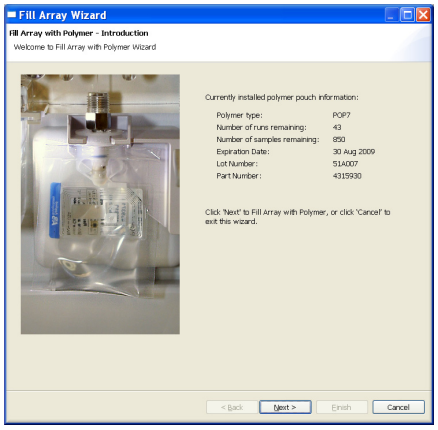
WARNING! CHEMICAL HAZARD. POP-4™, POP-6™, and POP-7™ polymers.

For details see "Instrument reagents and consumables" on page 9.

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

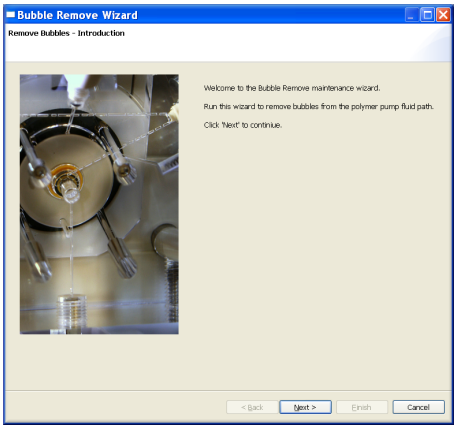
Remove bubbles from the polymer pump fluid path before each run. See "Daily instrument maintenance tasks" on page 230 for more information.

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.
3. Check the Quick View section of the Dashboard for updated status of the polymer pouch after removing bubbles from the polymer pump fluid path.



To change the capillary array

CAUTION! SHARP The load-end of the capillary array has small but blunt ends and it could lead to piercing injury.

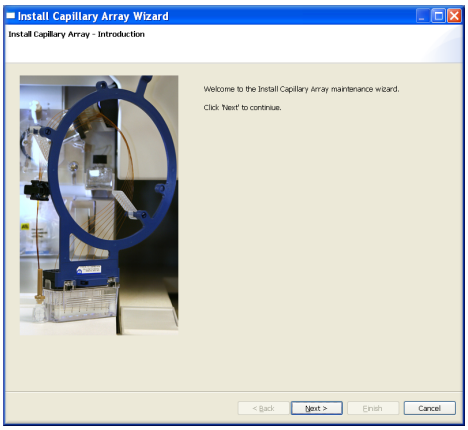
IMPORTANT! Check the loading-end header to ensure that the capillary tips are not crushed or damaged.

For details see "Instrument reagents and consumables" on page 9.

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.



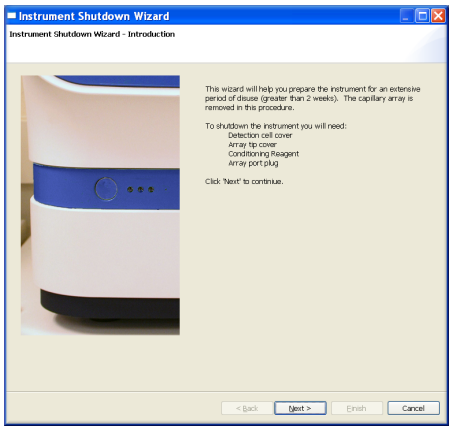
To shutdown the instrument

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	**IMPORTANT!** 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.
for more than 2 weeks	Long-term. See below for long-term instrument shutdown.

Computer maintenance

This section lists the common tasks required to maintain the computer for your 3500 or 3500xL analyzer in good working condition.

For the computer troubleshooting issues, see Appendix E, "Troubleshoot" on page 299.

Uninstall the software

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).

IMPORTANT! Do not back up the datastore to the installation directory. The installation directory is deleted during the uninstall.

Archive, purge, and restore data

- **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.

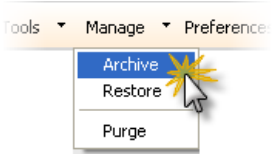
Frequency

Applied Biosystems recommends that you purge the library objects once every three months.

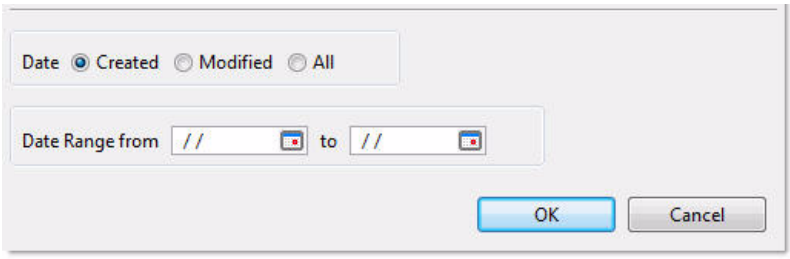
Archive library items

This function archives items stored in the library. To archive audit records, see "Archive, purge, and restore audit records" on page 214.

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 x:\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

There are two ways to archive the data files

1. Start ▸ Control Panel ▸ System and Maintenance ▸ Backup and Restore Center
   OR
   Programs ▸ Accessories ▸ System tools ▸ Backup
2. Use either Back up File folder or Back up Computer options.

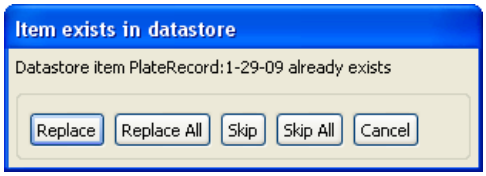
Note: If you export audit records for samples that are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

Restore

This function restores items stored in the library. To restore audit records, see "Archive, purge, and restore audit records" on page 214.

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.



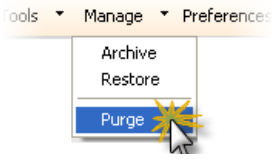
3. Select an option to continue.

   A message is displayed when the restore is complete.

Purge

This function purges (deletes) items stored in the library. To purge audit records, see "Archive, purge, and restore audit records" on page 214.

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:

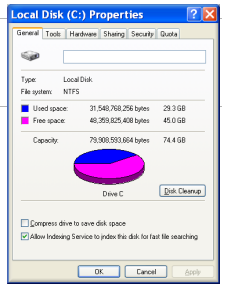
- Archiving data
- Deleting unneeded files
- Emptying the trash
- Defragmenting the drives

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.

Note: The Data Collection software will prompt you when it is 70-75% 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 E, “Troubleshoot” on page 299.

Also, check the status light in the bottom left-hand corner of the data collection window to see if it flashes red.

Review the Maintenance Notifications Log

The Notifications Log is a history of all notifications messages and the action taken for the task (completed or dismissed). You can use this option to review a previous run information.

The Dashboard provides you with a list of current routine and maintenance notifications, as explained below.

Multi-column sorting is supported (see “Multi-column sorting” on page 72).

To go to the Notifications Log from the Dashboard:

1. Click **Maintain Instrument**
2. From the Left-hand pane, under Planned Maintenance, click **Notifications Log**

Click on the top left-hand corner of the Notification Log for more information.

The Notification Log provides the following information on each event:

Notification	Description
Name	The name of the event.
Priority	The event priority.
Notification Date	The date of notification.
Status	The current status of the event.
User	The name of the user.
Acknowledge Date/Time	The date and time when the event was acknowledged.
Description	The description for the event.

Notification time is determined in the Preferences. From the Dashboard, click Preferences, to open the Preferences dialog box, click Scheduler Preference, and follow the prompts.

Service Log

The Service Log is a record of instrument service, and it is used and completed by the Applied Biosystems service engineer at the time of service.

To go to the Service Log from the Dashboard:

1. Click **Maintain Instrument**
2. From the Left-hand pane, under Planned Maintenance, click **Service Log**

Click  on the top left-hand corner of the Service Log for more information.

The Service Log screen contains a history of all the service events that have occurred on the system, starting with the most recent event, and provides the following information on each event:

Event	Description
Ticket Number	The number assigned to the event.
Service Type	The type of service requested.
Event Occur Date	The date that the event took place.
Service Start Date	The date that the service started.
Service End Date	The date that the service ended.
Service Engineer	The name of the service engineer.
Reason	The reason for logging the event.
Comments	Any additional comments.

Application Reagents and Run Modules

A

Sequencing analysis reagents

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

The following table shows all the reagents for sequencing analysis.

Table 27 Sequencing analysis reagents

Name	Part Number	Storage Conditions	On-instrument Shelf-life at Environmental Temperature
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 24 reactions	4337454	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 100 reactions	4337455	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 1000 reactions	4337456	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v3.1 Cycle Sequencing Kit 5000 reactions	4337457	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 24 reactions	4337449	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 100 reactions	4337450	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 1000 reactions	4337451	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v1.1 Cycle Sequencing Kit 5000 reactions	4337452	-15 °C to -25 °C	24 hours

Table 28 Sequencing standards

Name	Part Number	Storage Conditions	On-instrument Shelf-life at Environmental Temperature
BigDye® Terminator (BDT) v3.1 Sequencing Standard (long read)	4404312	-15 °C to -25 °C	24 hours

Table 28 Sequencing standards

Name	Part Number	Storage Conditions	On-instrument Shelf-life at Environmental Temperature
BigDye® Terminator (BDT) v1.1 Sequencing Standard (long read)	4404314	-15 °C to -25 °C	24 hours
BigDye® Terminator (BDT) v3.1 Matrix Standard	4336974	2 °C to 8 °C	24 hours
BigDye® Terminator (BDT) v1.1 Matrix Standard	4336824	2 °C to 8 °C	24 hours

Fragment and HID analysis reagents

Note: For reagent or consumable shelf-life expiration date, see the package label.

The following table shows all the reagents for fragment and HID analysis.

Table 29 Fragment analysis HID standards

Name	Part Number	Storage Conditions	On-instrument Shelf-life at Environmental Temperature
Fragment Analysis Matrix Standards (5-Dye) -DS-02	4323014	2 to 8°C	24 hours
Fragment Analysis Matrix Standards (4-dye) - DS-32	4345831	2 to 8°C	24 hours
Fragment Analysis Matrix Standards (5-Dye) -DS-33	4345833	2 to 8°C	24 hours
Fragment Analysis Installation kit (5-Dye) -DS-33	4376911	2 to 8°C	24 hours
GS120LIZ Size Standard	4322362	2 to 8°C	24 hours
GS500ROX Size Standard	401734	2 to 8°C	24 hours
GS600 LIZ Size Standard v2 (for Normalization)	4408399	2 to 8°C	24 hours
GS1200 LIZ Size Standard	4379950	2 to 8°C	24 hours

Sequencing analysis dye sets for all applications

Note: For reagent or consumable shelf-life expiration date, see the package label.

The following table shows all the dye sets for various applications.

Table 30 Dye Sets for various applications

Dye Set	Application Name
E (v1.1 BigDye® Terminator)	Rapid DNA sequencing
Z (3.1 BigDye® Terminator)	DNA sequencing

Fragment analysis dye sets for all applications

Note: For reagent or consumable shelf-life expiration date, see the package label.

The following table shows all the dye sets for fragment analysis.

Table 31 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 sets

Table 32 AmpFℓSTR Kit Table

AmpFℓSTR® Kits	Dye set (use with HID Fragment Analysis 36_POP4 run module)
4-dye: • COfiler® • Profiler Plus® • Profiler Plus® *ID* • SGM Plus® • Other 4-dye kits	F
5-dye: • Identifiler® • Minifiler™ • SEfiler™ Plus • SinoFiler™ • Yfiler® • Other 5-dye kits	G5

Run modules

Capillary array and polymer (sequencing analysis run modules)

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

Table 33 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	3500xL	Contiguous Read Length (CRL)§
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 ShortReadSeqPOP7	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
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

Table 33 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	3500xL	Contiguous Read Length (CRL)§
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 0.5 hour 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 34 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 ≥520	<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
HID HID36_POP4	36	POP-4™	≤35	≥312	≥936	≤60 to ≥400	<0.15	NA‡‡	NA‡‡
HID HID36_POP7	36	POP-7™	≤26	≥424	≥1272	≤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 GS600 or GS1200 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 GS600 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.

Secondary Analysis: Sequencing B

Perform secondary analysis on sequencing experiments

The Applied Biosystems 3500/3500xL Genetic Analyzers and 3500 Series Data Collection Software provide integration between the instrument and secondary sequencing analysis software applications— specifically SeqScape® Software v2.7 and MicroSeq® ID Software v2.2. Using auto-analysis, samples are loaded, sequencing data is generated, and basecalling along with secondary analysis is performed according to the protocols assigned to the plates prior to the run.

Software	Purpose
SeqScape®	A comprehensive resequencing tool designed to detect SNPs, profile mutations, perform medical sequencing, identify haplotypes, subtype pathogens, and confirm clone constructs.
MicroSeq® ID	A comparative sequencing tool for microbial identification of bacteria and fungi.

Auto-analyze projects in the sequencing analysis software

Auto-analysis can only be performed on the same computer that collects the sample files, therefore SeqScape® or MicroSeq® ID Software must be co-installed and configured with the 3500 Series Data Collection Software on a Windows Vista® operating system. Automated basecalling occurs with KB™ Basecaller v1.4.1 (calls pure or mixed bases with quality values) and secondary analysis occurs with SeqScape® or MicroSeq® ID Software.

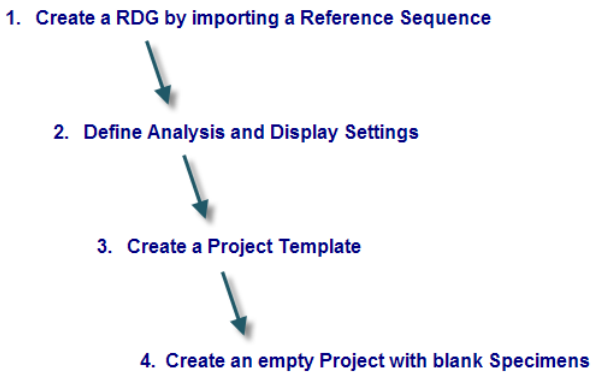
This procedure initially describes how to set up panels and bin sets in SeqScape® and then describes how to auto-analyze samples using the 3500 Series Data Collection Software. Once a run is complete, your data is seamlessly transferred into SeqScape® for analyzing, processing and reporting.

Note: For detailed information on setting up a MicroSeq® ID project to auto-analyze in the 3500 Series Data Collection Software, see the *MicroSeq® ID v2.2 Getting Started Guide*.

Set up an auto-analysis project in SeqScape®

IMPORTANT! When using SeqScape® Software to auto-analyze results data from the 3500/3500xL analyzer, you must have v2.7 installed on the *same* computer as the 3500 Series Data Collection Software.

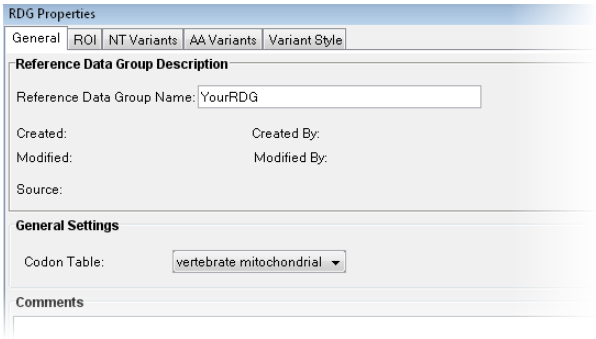
Set up a project in the secondary analysis software before starting a run on the 3500/3500xL analyzer. All analysis in SeqScape® occurs in a project. Create a project by following these steps:



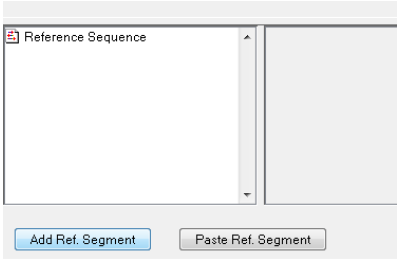
Import a reference

1. Start the SeqScape® Software (

), then select **Tools** ▸ **SeqScape Manager**.
2. Select the **Reference Data Group** tab, then click **New** and enter a name.



3. Select the **ROI** (Regions of Interest) tab, then click **Add Ref. Segment**.



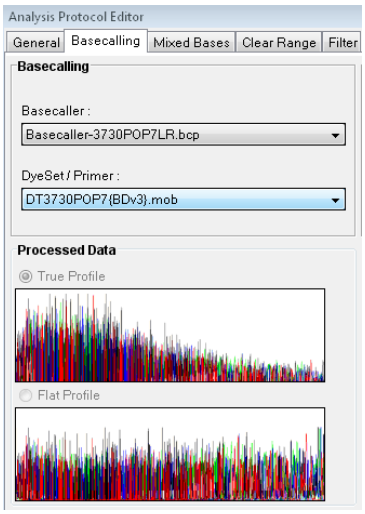
4. Select the file you want to use as your reference file, then click **Import**.
5. In the **NT Variants** tab (RDG Properties), select the NT Variants that you want to add to the reference sequence, then click **Import** to import a tab-delimited variants file or a multi-aligned sequence (.fsta) file.

   Note: When importing an amino acid variants file, use a tab-delimited format.

6. Click **OK**.

Define settings

1. Open **Tools** ▸ **SeqScape Manager**.
2. Select the **Analysis Protocols** tab, then click **New** to enter a name.
3. Select the **Basecalling** tab, then select your Basecaller and Dye/Primer files.



   Note: Unless a project requires a custom setting, keep the Processed Data, Ending Base and Quality Threshold settings at their default values.

4. In the Mixed Bases tab, specify the secondary analysis peak threshold for mixed base identification.
5. Keep the default settings for the other parameters listed in the Clear Range and Filter tabs, then click **OK**.
6. From the SeqScape Manager, select the **Analysis Defaults** tab, then click **New** and enter an Analysis Defaults Name.
7. Go to the **Sample** tab and select the Analysis Protocol you just created in the drop-down list.
8. Keep the default settings in the Project and Specimen tabs, then click **Save**.

   In most cases, you will want to keep the default Display Settings and continue with creating a project template in the SeqScape® Software.

Create a project template

1. Open **Tools** ▸ **SeqScape Manager**.
2. Select the **Project Templates** tab, then click **New** and enter a name for the template.
3. In the Reference Data Group and Analysis Defaults drop-down lists, select the RDG and Analysis Default that you previously created.
4. Keep the default Display Settings, then click **OK**.

With the project template created, continue with adding your sample files.

Create an empty project

1. In the SeqScape® Software, select **File** ▸ **New Project**.
2. Name the project.
3. In the Project Template list, select the project template that you previously created.
4. When the project opens, click **Add Specimen** (**Tools** ▸ **New Specimen**) to create as many blank specimens as you have in your project, then click **OK**.
5. Close the SeqScape® Software.

   You are now ready to set up a run in the 3500 Series Data Collection Software specifying a SeqScape Protocol as your secondary analysis method.

Set up a SeqScape plate in the 3500 Series Data Collection Software

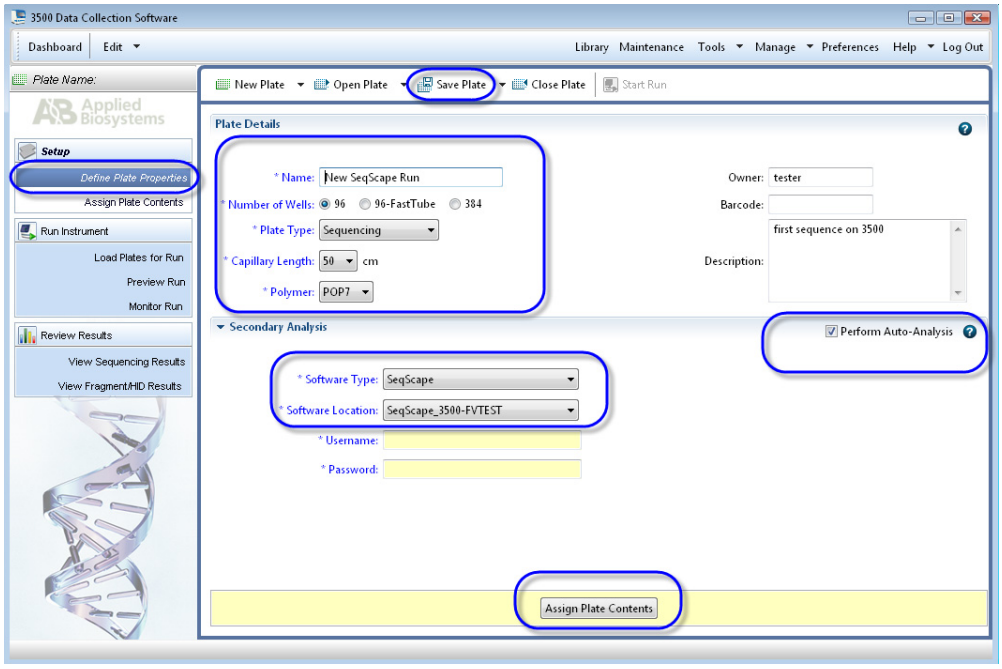
Start the 3500 Series Data Collection Software

1. Start the Auto-Analysis Manager before starting the 3500 Series Data Collection Software.
2. Start the 3500 Series Data Collection Software ▸ Dashboard ▸ 

3. Name your new plate.
4. Select the Number of Wells, Plate Type as Sequencing, Capillary Length and Polymer associated with this plate for the current run.
5. (Optional) Enter your name as Owner, a barcode and description for the plate.

Specify auto-analysis for secondary analysis

1. Check **Perform Auto-Analysis** (right side of the Plate Details section), to expand the Secondary Analysis section.



2. Confirm **SeqScape** auto-populates as the Software Type.

   Note: If SeqScape does *not* appear in the drop-down list under Software Type, check your installation. Secondary analysis software must be installed correctly before the 3500 Series Data Collection Software is automatically listed as a selection.

3. Confirm **Your computer name** auto-populates as the Software Location.

   IMPORTANT! For auto-analysis to be successful, the secondary analysis protocol must match the software location set here.

4. Enter your Username and Password for auto-analysis access to the secondary analysis software.

5. Click Save Plate ▸ **Save** to save your plate with these settings, then **Assign Plate Contents** to advance to the next screen.

Assign plate contents

When assigning plate contents, you are assigning assays, file name conventions and results group to be associated with your auto-analysis.

Set up an assay

1. In the Assign Plate Contents screen, go to the Assays box and select either **Create New Assay** or **Add From Library**.

2. Name your assay in the Setup an Assay dialog box.

Note: (Optional) Select a color for this assay to display with in the Plate View.

3. Select an Instrument Protocol to apply to the assay.

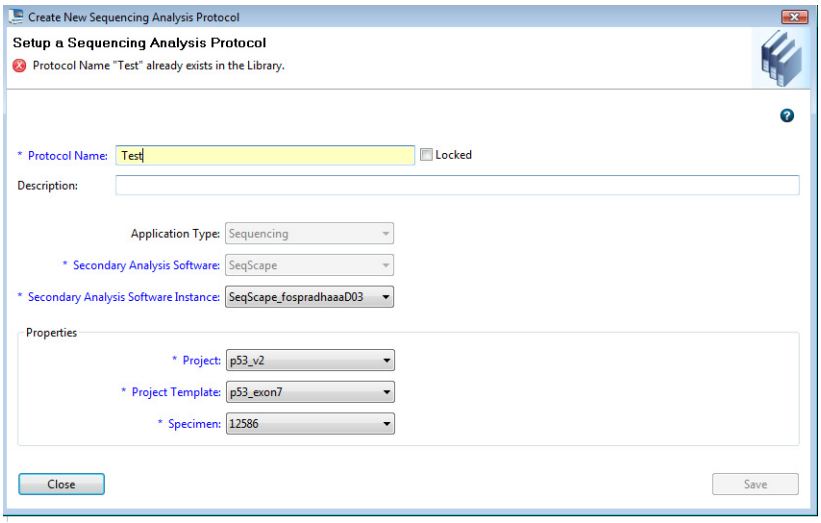
Note: For more instruction on setting up an instrument protocol, see "Create a new instrument protocol" on page 165.

4. Select a Basecalling Protocol to apply to the assay.

IMPORTANT! Make sure your basecalling settings match the Analysis Settings specified in SeqScape.

Note: For more instruction on setting up a Basecalling protocol, see "Create a new basecalling protocol" on page 174.

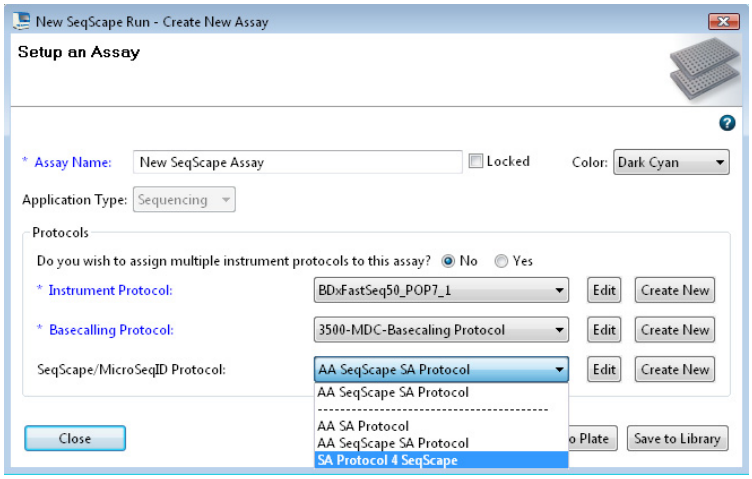
5. Create a new sequencing analysis protocol to apply to the samples by clicking **Create New**.



6. Name your new sequencing analysis protocol, then select your specimens one by one, clicking **Save** after each specimen.

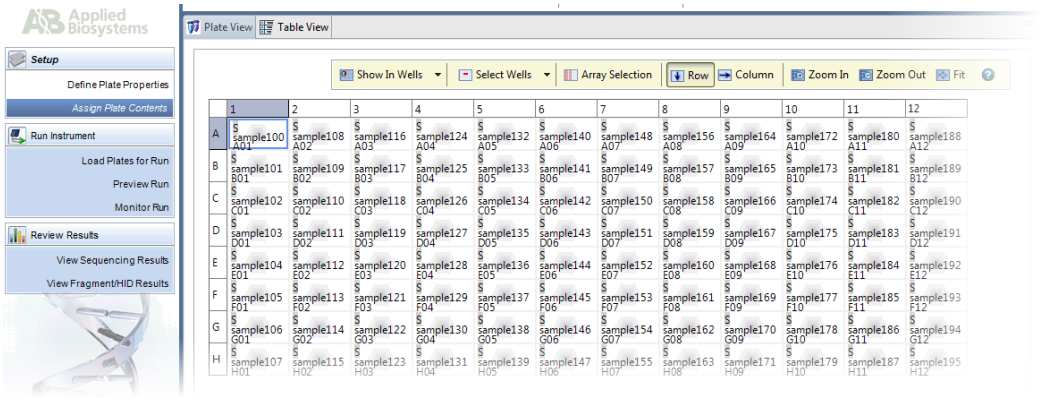
IMPORTANT! Each SeqScape protocol has one specimen, so you will need to create multiple protocols for multiple specimens. If you have multiple protocols, you will have multiple assays, as each assay is associated with one secondary analysis protocol.

Note: For more instruction on setting up a secondary analysis protocol, see "Create a new sequencing analysis protocol" on page 189.

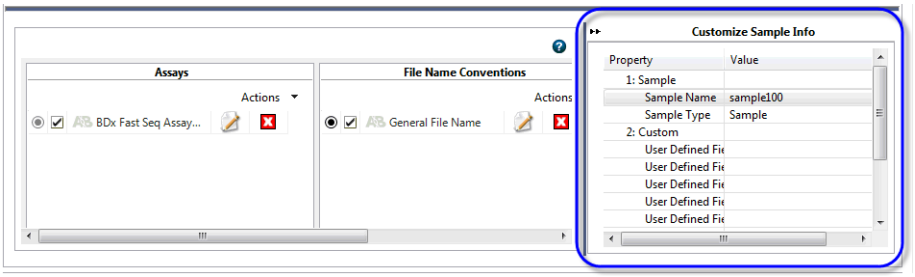


7. Click Apply to Plate, then **Save to Library** if you want to use this assay again.

8. Click **Close**.



9. Name your samples by highlighting the number of wells in your plate and naming the sample in Customize Sample Info box.



Note: For more information on naming samples, see "Name samples in the Plate View" on page 70.

Specify FNC and RG

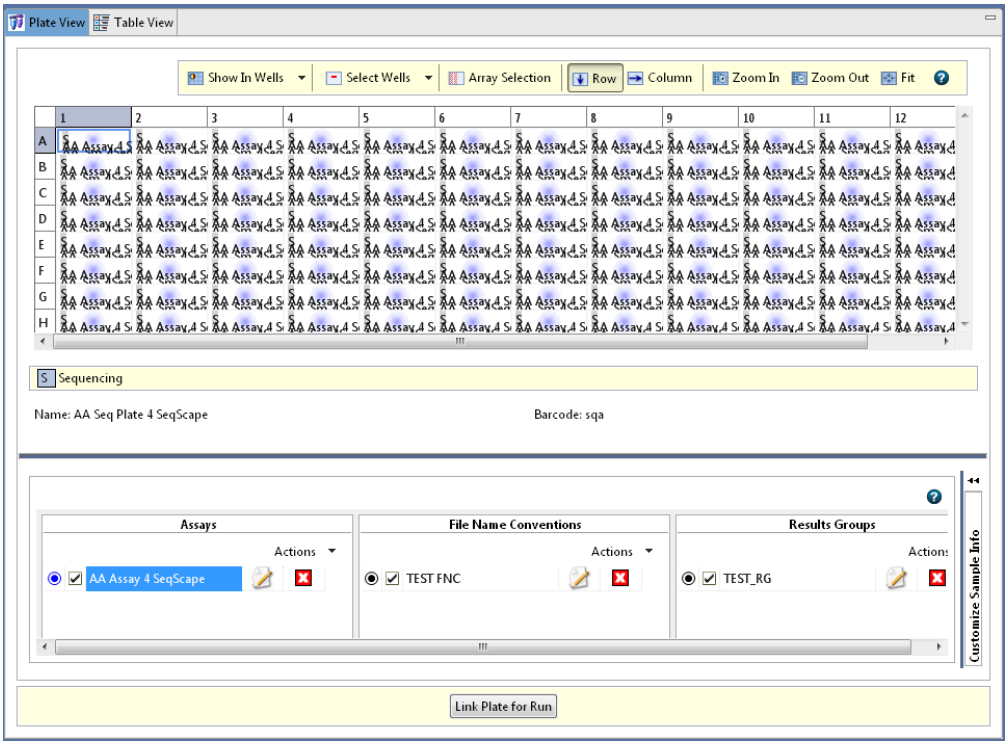
1. Specify a File Name Convention (FNC) and a Results Group (RG) to associate with your project.

   Note: You can create a FNC with the specimen name as a part of your sample file name.

2. Highlight the wells of your plate configuration (Plate View) and check the box next to the appropriate FNC to apply it to your project.
3. Repeat for the Results Group.

   Note: For more information on setting up a FNC see "Create a new file name convention" on page 151. For more information on setting up a RG, see "Create a new results group" on page 156.

4. Click Save Plate ▸ **Save**.



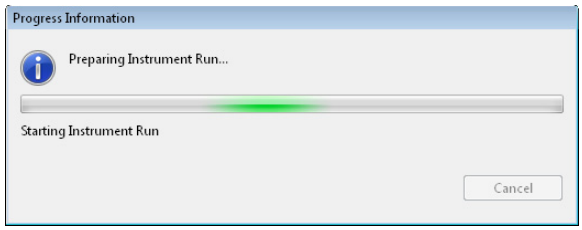
5. Click Link Plate for Run.

6. Click **Create Injection List**, then click **OK** after the instrument performs its validations.

Start the auto-analysis run

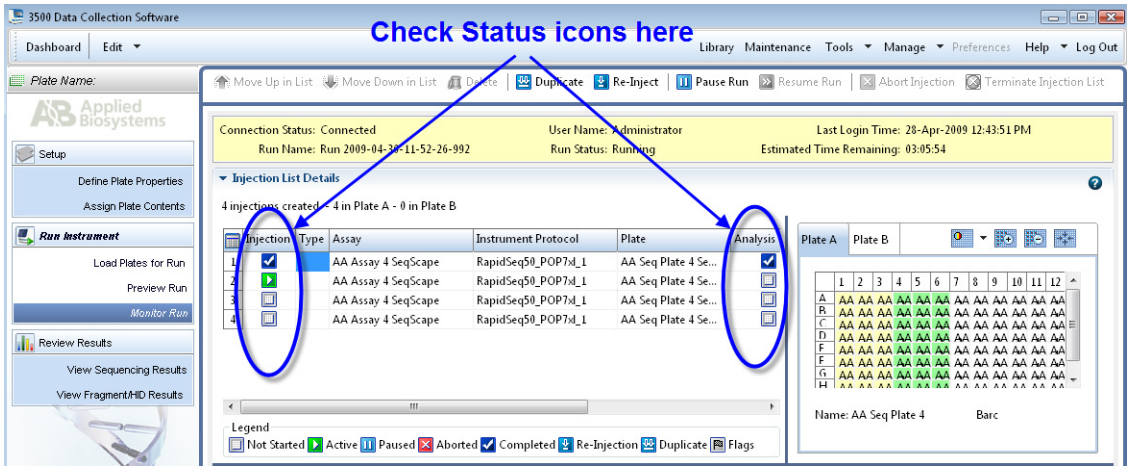
Click **Start Run** to begin your auto-analysis.

The 3500/3500xL analyzer display a progress indicator while it checks the level of consumables on the instrument.



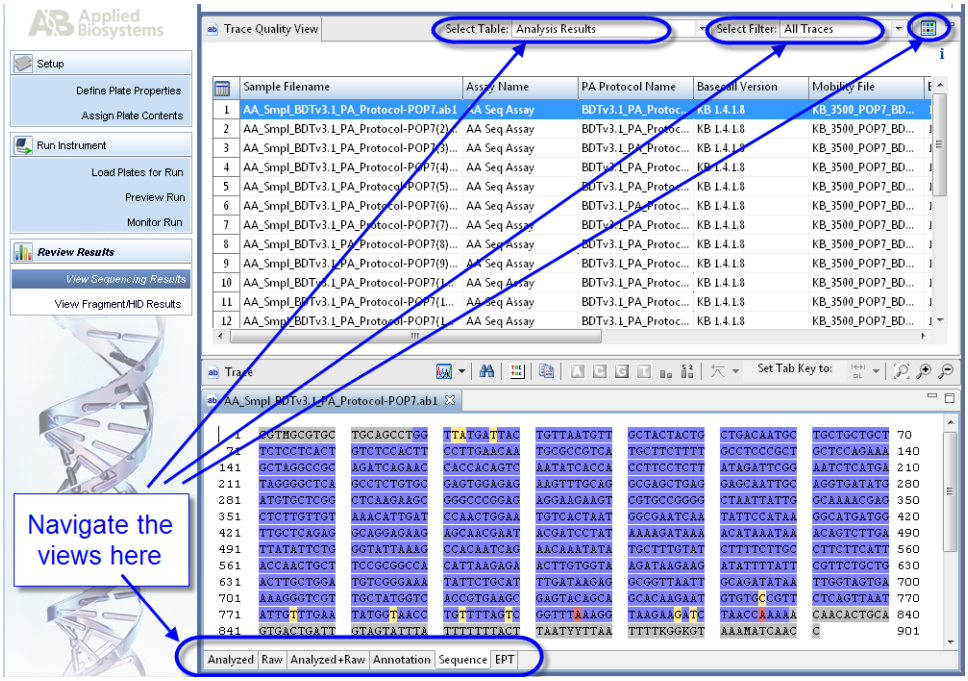
Monitor the run

Monitor the run by checking the status icons in the Injection Details section (Monitor Run screen).



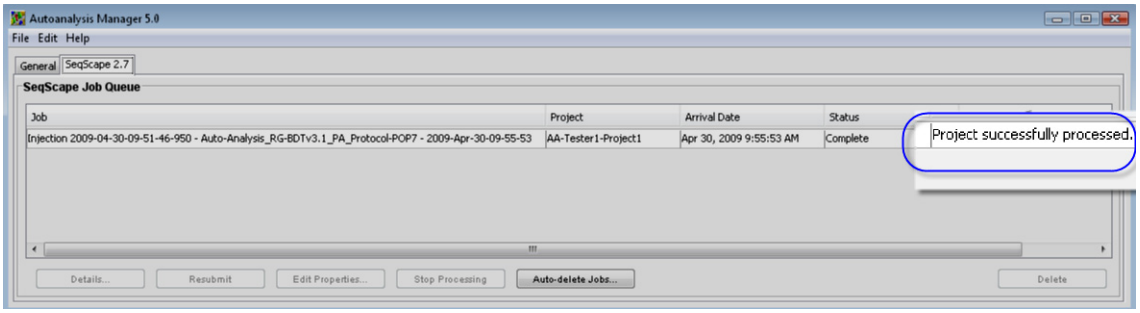
View sequencing results

You can view the Sequencing Results in the 3500 Series Data Collection Software by going to the View Sequencing Results screen and selecting the tab of interest.



Confirm run completion

When the run successfully transfers for downstream analysis, the Autoanalysis Manager displays the project as successfully processed.



You can now launch SeqScape® and review the analyzed project.

Note: For guidelines on reviewing data and results, see the *SeqScape® Software v2.7 Workflow Quick Reference Guide* (PN 4401740) or the *SeqScape® Software User Guide* (PN 4359442).

Auto-analysis with MicroSeq® ID

For instructions detailing how to set up a MicroSeq® ID analysis protocol, see "Create a new MicroSeq® ID analysis protocol" on page 191. For installation information on setting up the MicroSeq® ID Software to work with the 3500 Series Data Collection Software, see the *MicroSeq® ID v2.2 Getting Started Guide*.

Secondary Analysis: Fragment C

Perform secondary analysis on fragment experiments

The Applied Biosystems 3500/3500xL Genetic Analyzers and 3500 Series Data Collection Software provide integration between the instrument and secondary fragment analysis software applications — specifically GeneMapper® Software v4.1 and GeneMapper® *ID-X* Software v1.1. Using auto-analysis, samples are loaded, fragment data is generated, and allele calling is performed according to the protocols assigned to the plates prior to the run.

Software	Purpose
GeneMapper®	A high-performing and versatile software package for all fragment analysis and genotyping applications.
GeneMapper® *ID-X*	A software for use in Human Identification testing (databasing, casework, and paternity applications) and used in conjunction with AmpFℓSTR kit and the 3500/3500xL analyzer.

Auto-analyze projects in the fragment analysis software

Auto-analysis can only be performed on the same computer that collects the sample files, therefore GeneMapper® or GeneMapper® *ID-X* Software must be installed and configured with the 3500/3500xL analyzer on a Windows Vista® operating system. Secondary analysis occurs within the GeneMapper® or GeneMapper® *ID-X* Software.

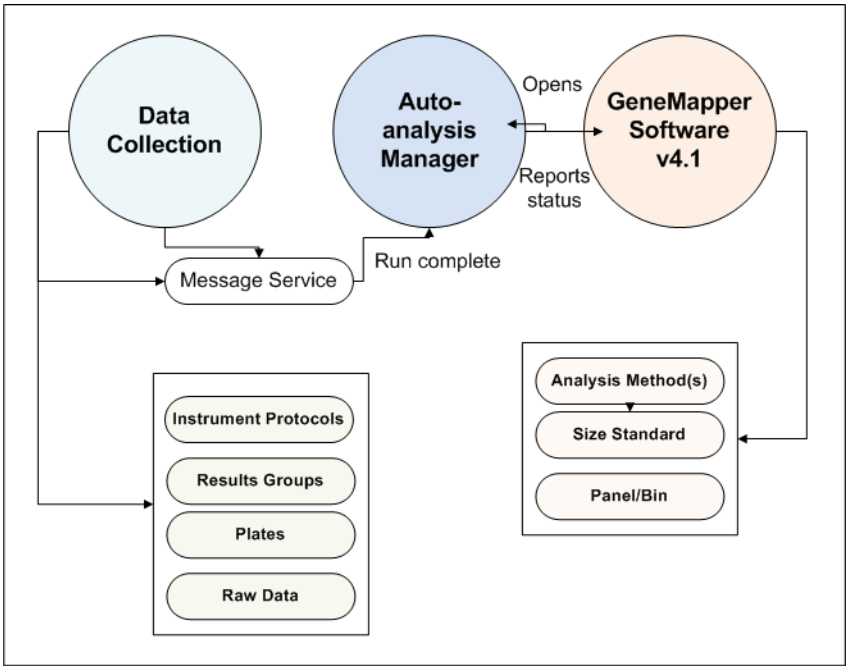
This procedure initially describes how to set up panels and bin sets in GeneMapper® Software v4.1 and then describes how to auto-analyze samples using the 3500 Series Data Collection Software. Once a run is complete, your data is seamlessly transferred into GeneMapper® for analyzing, processing and reporting.

Note: For detailed information on setting up a GeneMapper® *ID-X* analysis to auto-analyze in the 3500 Series Data Collection Software, see *GeneMapper® ID-X v 1.1 User Guide*.

Set up an auto-analysis project in GeneMapper®

IMPORTANT! When using GeneMapper® Software to auto-analyze results data from the 3500/3500xL analyzer, you must have v4.1 installed on the *same* computer as the 3500 Series Data Collection Software.

The fragment analysis workflow for auto-analysis is summarized in this flow chart.

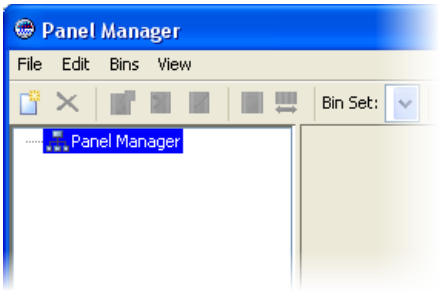


Set up a project in the secondary analysis software before starting a run on the 3500/3500xL analyzer. All analysis in GeneMapper® occurs in a project.

Specify a kit, a panel, and a bin set for the project

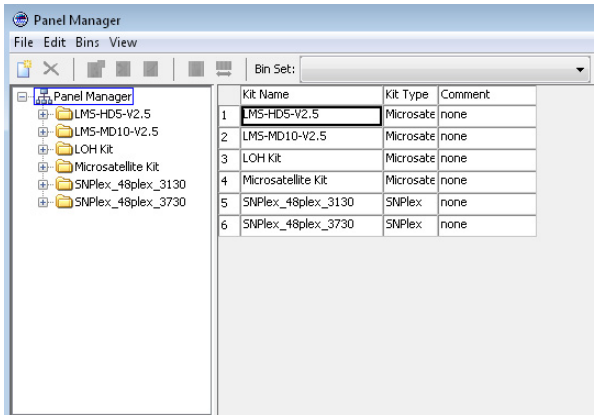
1. Open GeneMapper® v4.1 by double-clicking 

.
2. Click  to open the Panel Manager.
3. Select the Panel Manager node (in the Navigation pane) to highlight.



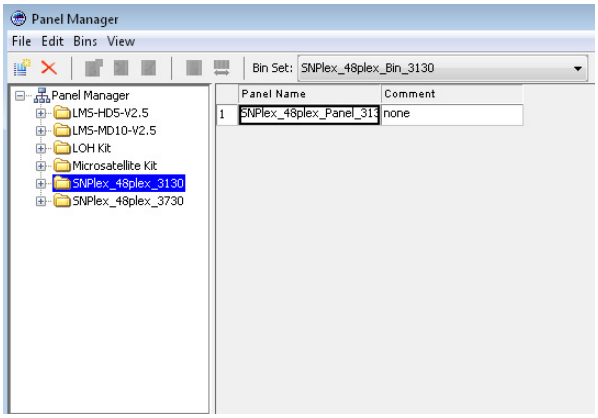
4. Select the panel, then **Import** to import a previously created kit folder with panel marker information.

Note: You have to import panels one by one; repeat this step for each panel.



5. Import the bin sets that are associated with the panels you just imported above. Click **Import** for each bin set.

Note: You have to associate a bin set to every panel that you imported.

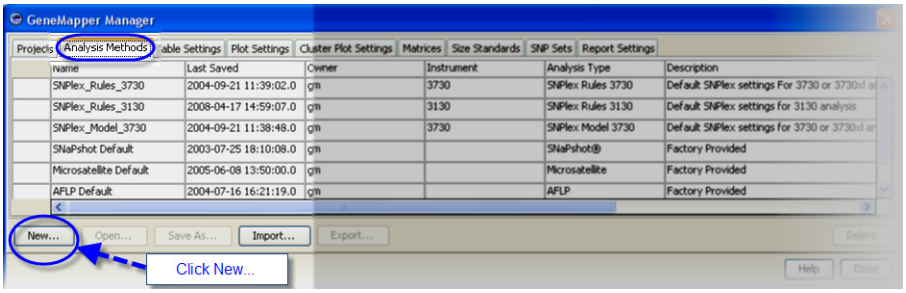


6. Click **OK** to save and close the Panel Manager.

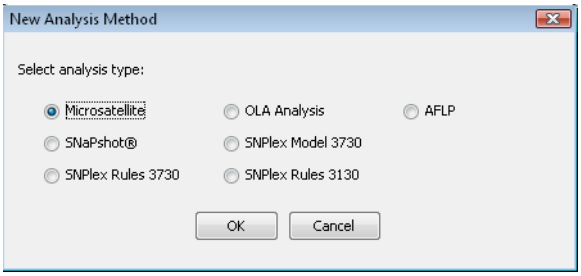
Note: For more information on how to create panels and bin sets, see the *GeneMapper® v4.1 Quick Reference Guide (PN 4362816)* or refer to the specific Getting Started Guide for your application.

Create a new project

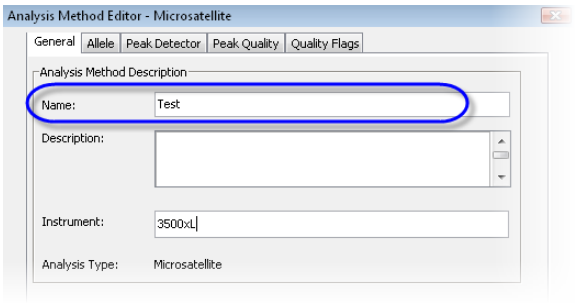
1. Click (GeneMapper Manager) to open the GeneMapper Manager.
2. Select the Analysis Method tab, then click **New**.



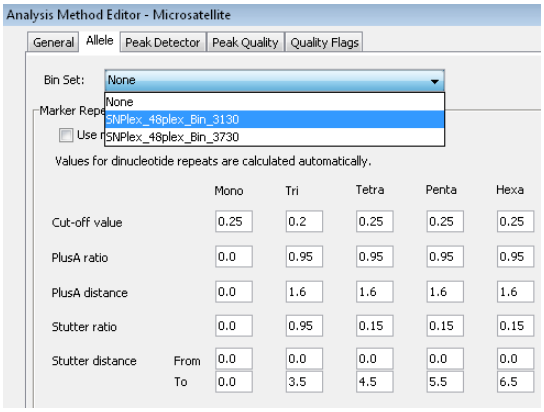
3. Select the Analysis Method Type you want, then click **OK**.



4. Name your Analysis Method (General tab).

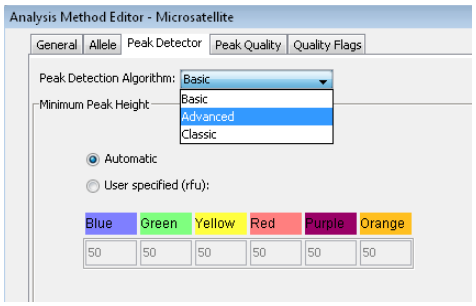


5. Select your Bin Set (Allele tab).

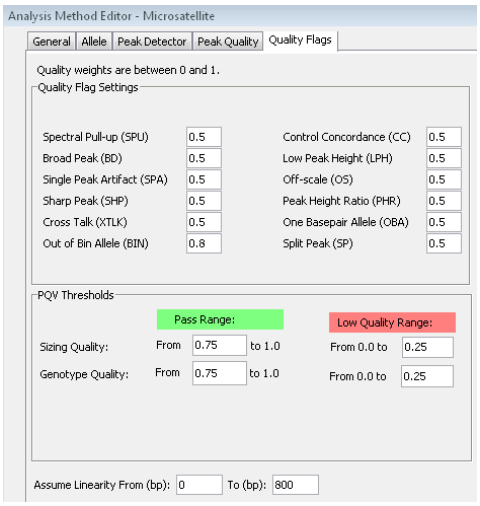


6. Select your Peak Detector Algorithm as Basic, Advanced or Classic (Peak Detector tab).

IMPORTANT! If you want to enable size standard normalization, you must select **Advanced**.



7. Customize your Peak Quality and/or Quality Flag settings in the appropriate tab, then close the Analysis Method Editor.



8. Click **OK** to save, then click **Done** to close GeneMapper®.

IMPORTANT! Close GeneMapper® v4.1 before performing the auto-analysis run on the 3500/3500xL analyzer.

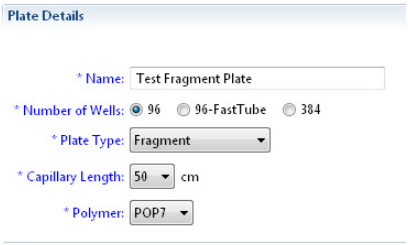
Set up a GeneMapper plate in the 3500 Series Data Collection Software

Set up a fragment analysis run in the 3500 Series Data Collection Software by assigning an Assay, a File Name Convention and a Results Group.

Start the 3500 Series Data Collection Software

1. Start the Auto-Analysis Manager before starting the 3500 Series Data Collection Software.
2. Start the 3500 Series Data Collection Software, then go Dashboard ▸ 

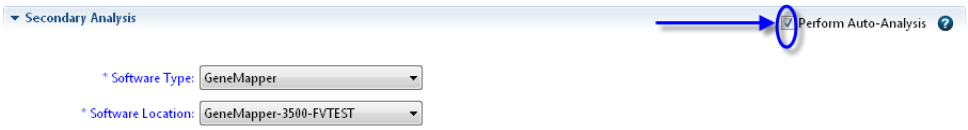
3. Name your new plate.



4. Select the Number of Wells, Plate Type as Sequencing, Capillary Length and Polymer associated with this plate for the current run.

Specify auto-analysis for secondary analysis

1. Check **Perform Auto-Analysis** (right side of the Plate Details section), to expand the Secondary Analysis section.



2. Confirm **GeneMapper** auto-populates as the Software Type.

Note: If GeneMapper does *not* appear in the drop-down list under Software Type, check your installation. Secondary analysis software must be installed correctly before GeneMapper is automatically listed as a selection.

3. Confirm **Your computer name** auto-populates as the Software Location.

IMPORTANT! For auto-analysis to be successful, the secondary analysis protocol must match the software location set here.

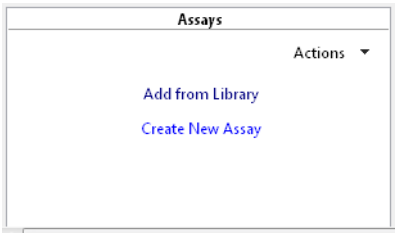
4. Enter your Username and Password for auto-analysis access to the secondary analysis software.
5. Click Save Plate ▸ **Save** to save your plate with these settings, then **Assign Plate Contents** to advance to the next screen.

Assign plate contents

When assigning plate contents, you are assigning assays, file name conventions and results group to be associated with your auto-analysis.

Set up an assay

1. In the Assign Plate Contents screen, go to the Assays box and select either **Create New Assay** or **Add From Library**.



2. Name your new assay in the Setup an Assay dialog box.

Note: (Optional) Select a color for this assay to display with in the Plate View.

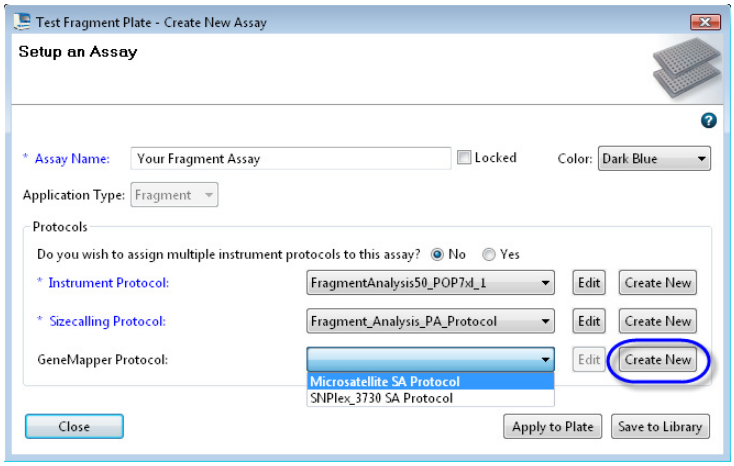
3. Select an Instrument Protocol to apply to the assay.

Note: For more instruction on setting up an instrument protocol, see "Create a new instrument protocol" on page 165.

4. Select a Sizecalling Protocol to apply to the assay.

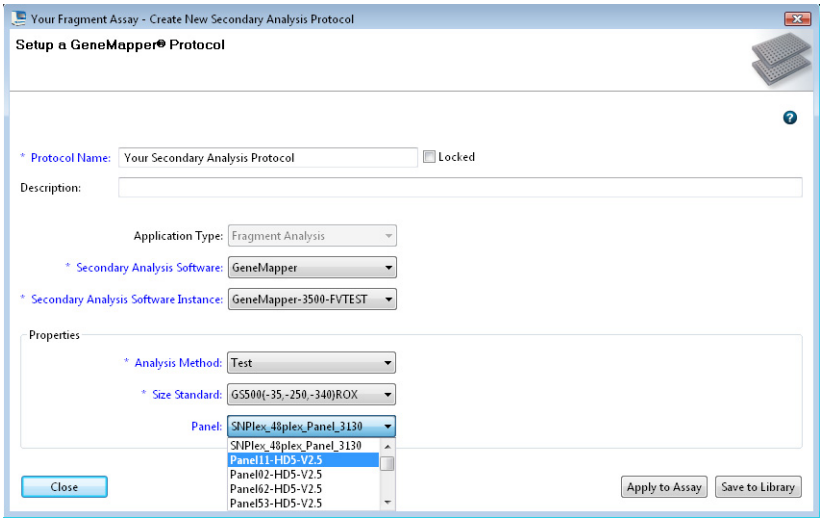
Note: For more instruction on setting up an instrument protocol, see "Create a new sizecalling protocol" on page 179.

5. Create a new fragment analysis protocol (GeneMapper Protocol) to apply to the samples by clicking **Create New**.



6. Name your new fragment analysis protocol and (optionally) enter a description.

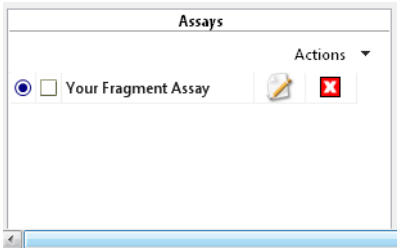
7. Select the panel(s) you previously created in GeneMapper®, then click Apply to Assay.



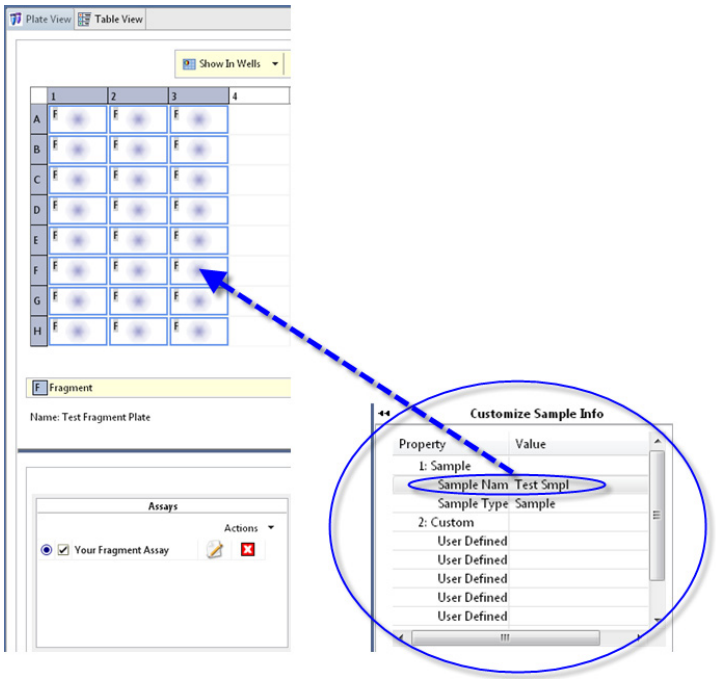
Note: For more instruction on setting up a secondary analysis protocol, see "Create a new fragment analysis protocol" on page 193.

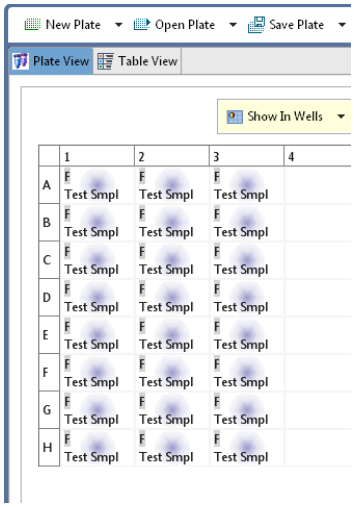
8. Click **Close** when you are finished applying all the panels to the assay.

9. Click Apply to Plate, then close the Setup an Assay dialog box.



10. Name your samples by highlighting the number of wells in your plate and naming the sample in Customize Sample Info box.





Note: For more information on naming samples, see "Name samples in the Plate View" on page 70.

Specify FNC and RG

1. Specify a File Name Convention (FNC) and a Results Group (RG) to associate with your project.

   Note: You can create a FNC with the specimen name as a part of your sample file name.

2. Highlight the wells of your plate configuration (Plate View) and check the box next to the appropriate FNC to apply it to your project.
3. Repeat for the Results Group.

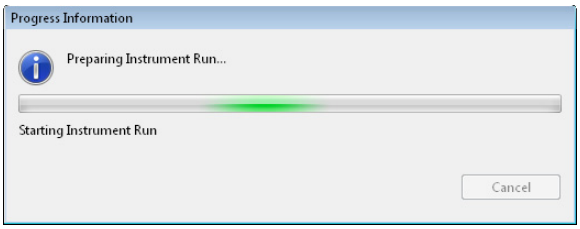
   Note: For more information on setting up a FNC see "Create a new file name convention" on page 151. For more information on setting up a RG, see "Create a new results group" on page 156.

4. Click Save Plate ▸ **Save**.
5. Click Link Plate for Run.
6. Click Create Injection List, then click **OK** after the instrument performs its validations.

Start the auto-analysis run

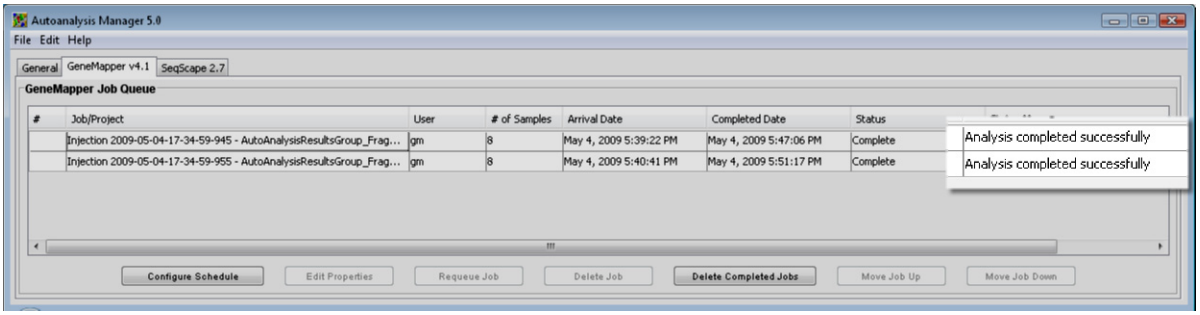
Click **Start Run** to begin your auto-analysis.

The 3500/3500xL analyzer displays a progress indicator while it checks the level of consumables on the instrument.



Confirm run completion

When the run successfully transfers for downstream analysis, the Autoanalysis Manager displays the project as successfully processed.



You can now launch GeneMapper® and review your analysis.

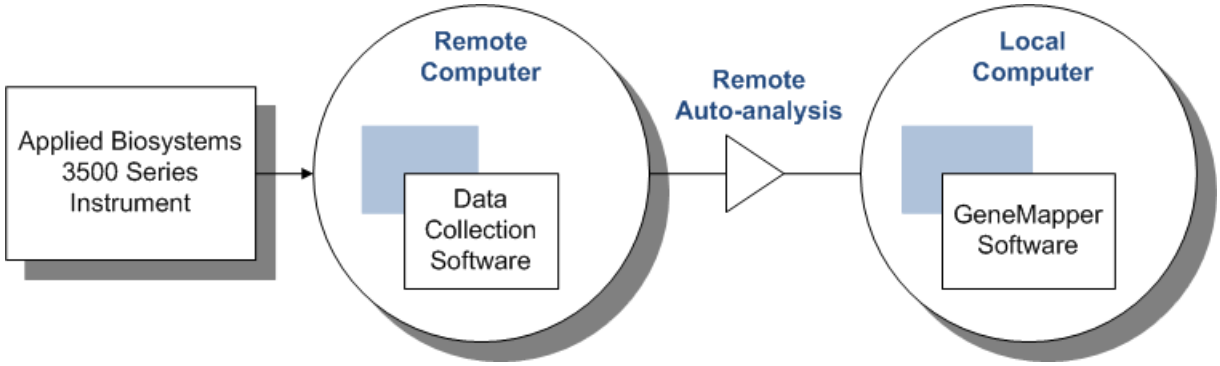
Note: For guidelines on reviewing fragment data and results, see the *GeneMapper® v4.1 Quick Reference Guide (PN 4362816)* or refer to the specific Getting Started Guide for your application.

Auto-Analysis with GeneMapper® *ID-X*

For instructions detailing how to set up a GeneMapper® *ID-X* analysis protocol, see "Create a new HID analysis protocol" on page 195. For installation information on setting up the GeneMapper® *ID-X* Software v1.1 to work with the 3500 Series Data Collection Software, see the *GeneMapper® ID-X Software v1.1 User Guide.*

Remote Auto-Analysis Setup D

Remote auto-analysis configuration



For remote auto-analysis, the 3500 Series Data Collection Software resides on the instrument computer and the GeneMapper® Software resides on a *different* computer.

In this configuration, you can set up both softwares so that GeneMapper®:

- connects to a remote computer running the 3500 Series Data Collection Software
- obtains sample files from the remote 3500 Series Data Collection Software database
- performs analysis of the generated sample files automatically

Remote auto-analysis installation

Install the remote auto-analysis configuration when you want to auto-analyze data and you plan to connect to a separate computer running the 3500 Series Data Collection Software.

Installing GeneMapper® Software as a remote auto-analysis configuration requires that you:

1. Start the Data Collection services on the remote Data Collection computer.
2. Install GeneMapper® Software v4.1 on the local computer.

IMPORTANT! Before installing GeneMapper®, start the Data Collection services on the remote Data Collection computer.

Start the 3500 data collection services

1. On the Data Collection computer, select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **Data Collection** ▸ **Run Data Collection version 1.0**.

   Note: If the services do not start automatically, click **Start All**.

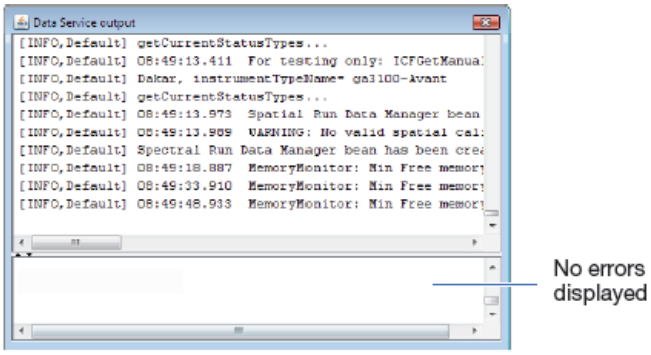
   STOPPING POINT. Wait until all services have changed to green before continuing.

2. If the 3500 Series Data Collection Software requires a password, type the login name and password, then click **OK**.

3. Verify that Data Service started without errors:

   a. In the Service Console, right-click the graphic next to each service listed and select **Show Console** to display the Data Service output window.

   b. Verify that no errors are displayed, then close the Data Service dialog box.



4. Obtain the host name (full computer name):

   a. Right-click **Computer** on the desktop, then select **Properties**.

   b. Locate the full computer name. (You will need to enter the name when you install the GeneMapper® Software).

Windows Vista

   c. Close the dialog box.

Install GeneMapper® v4.1 for remote auto-analysis

1. Insert the *GeneMapper® v4.1 Software Full Installation* DVD into the DVD drive to start the installer.

   If the installer does not start automatically:

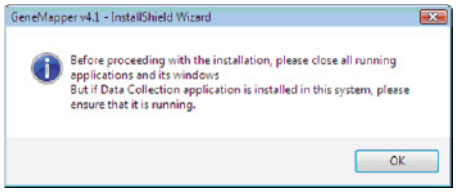
   a. Right-click **Computer**, then select **Explore**.

   b. Expand the DVD drive, then select the GeneMapper® v4.1 folder to display its contents.

   c. Double-click 

to start the installer.

2. Close all other applications and windows, then click **OK** to close this message:



3. In the Welcome window, click **Next**.

4. Review the installation requirements status, then click **Next**.

5. Select **Remote Analysis** for type of installation, then click **Next**.

6. In the GeneMapper Client setup window, type the server name (full computer name) for the Data Collection computer (see step 4 on page 292), select ABI 3500, then click **Next**.

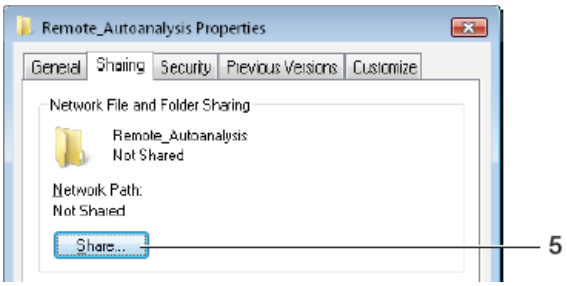
7. Read the release notes, then click **Next**.

Note: For other installation and configuration setup instructions, see Chapter 3 of the *GeneMapper® v4.1 Installation and Administration Guide*.

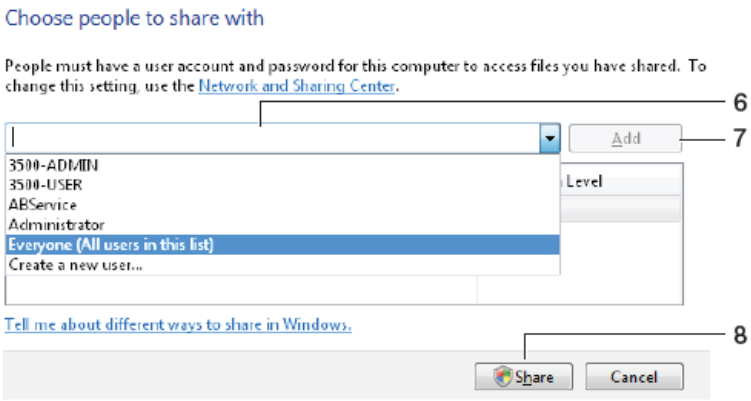
Create a shared folder

Create a shared folder (Windows Vista®)

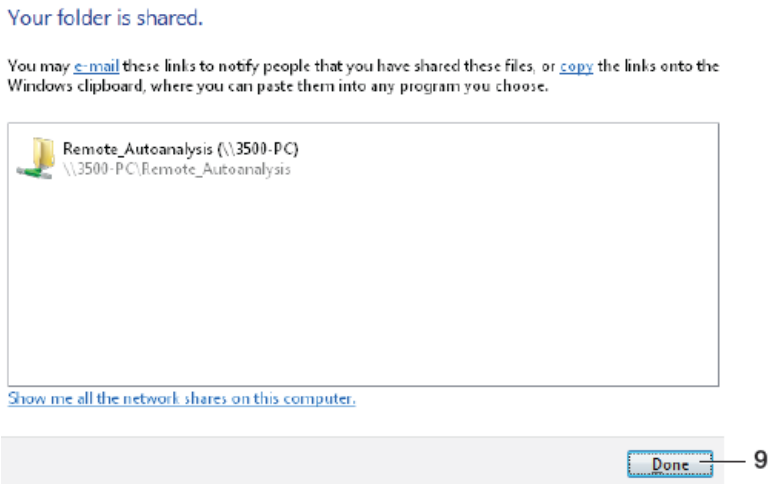
1. Select **Start** ▸ **Computer**, then double-click the drive on which you want the shared folder to reside.
2. Select **File** ▸ **New** ▸ **Folder**.
3. Name the folder (for example: Remote_Autoanalysis).
4. Right-click the new shared folder, then select **Properties**.
5. Select the Sharing tab, click **Share**.



6. In the Choose people to share with dialog box, click the drop-down and select **Everyone (All users in this list)**.



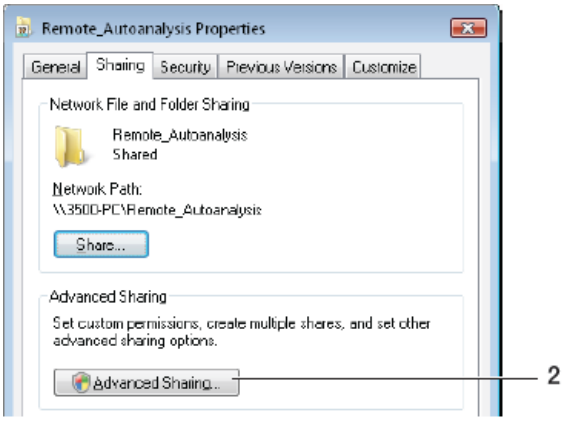
7. Click **Add**.
8. In the Permission Level column, change the value from Reader to Co-owner.
9. Click **Share**, then click **Done** and **Close**.



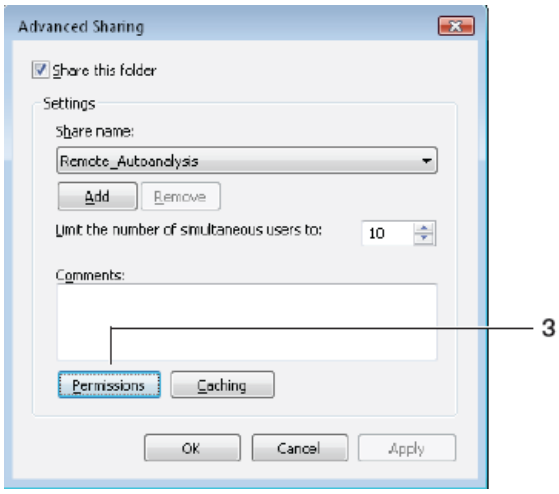
10. Click **OK**.

Set security preferences for the shared folder

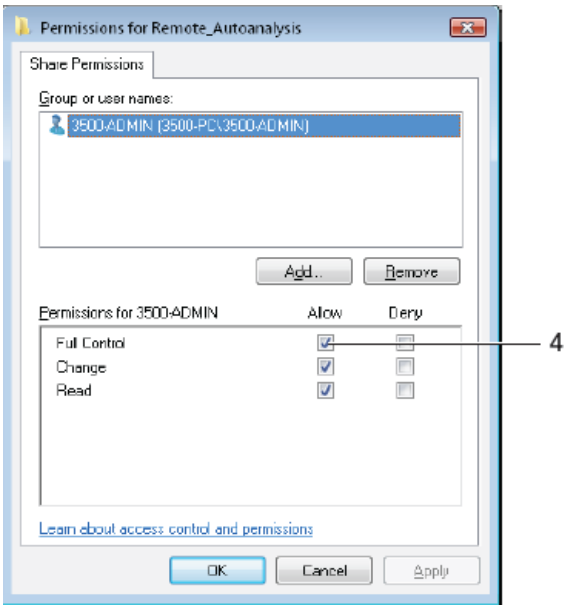
1. Right-click the shared folder, then select **Properties**.
2. Select the Sharing tab, then click **Advanced Sharing**.



3. On Advanced Sharing window, click **Permissions**.



4. In the Permissions for the <*shared folder name*> dialog box, select the checkbox for **Full Control** (in the **Allow** column).



5. Click **OK** twice.

6. Click **Close**.

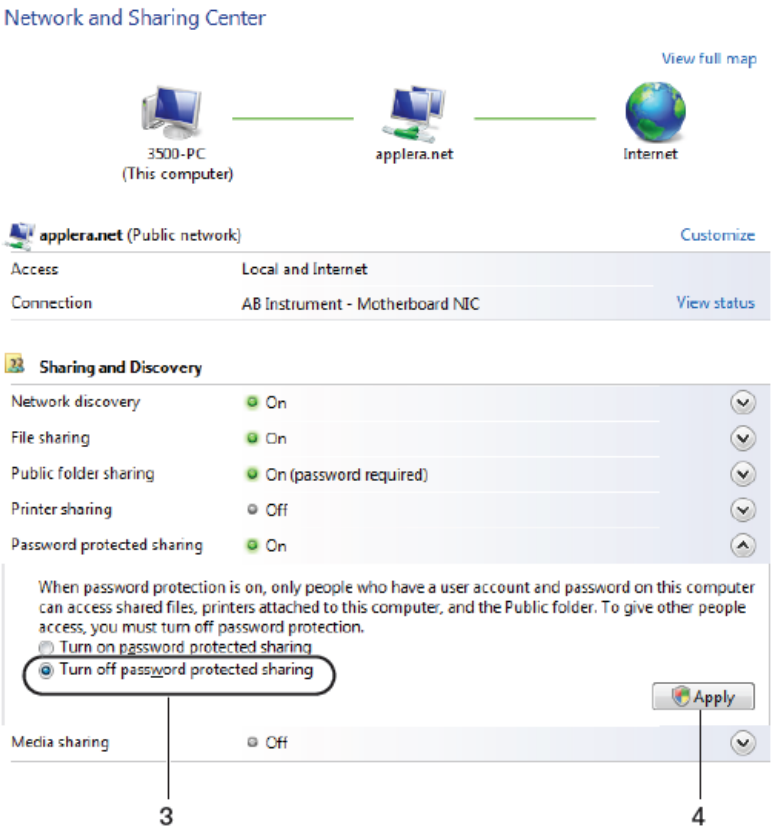
Turn off password protected sharing

IMPORTANT! Before starting Remote Auto-analysis, you must make sure that the password protected sharing settings on the Data Collection computer are turned off.

1. On the Data Collection computer, select **Start** ▸ **Control Panel** ▸ **Network and Sharing Center**.

2. Click the expand button ( ) for Password protected sharing,

3. Select **Turn off password protected sharing**.



4. Click **Apply**.

Set up the 3500 Series Data Collection Software v1.0

Complete auto-analysis setup on the Data Collection computer

To complete your remote auto-analysis setup, you must create a new Results Group.

1. Select the Results Group node in the navigation pane.
2. Click **New** to open the Results Group editor.
3. Complete the selections in the General tab by:
    a. Enter the new Results Group name.
    b. Enter the Results Group owner.
    c. (Optional) Enter the Results Group comment.
    d. Check **Results Group Entry Completed**.
4. Complete the selections in the Analysis tab by:
    a. Select the GeneMapper instance (GeneMapper + computer name) from the drop-down list.

b. Check **Do Autoanalysis**.

Note: If you plan to perform an auto-analysis for every Results Group Complete instead of each run individually, check **Results Group Entry Completed**.

c. Enter the GeneMapper Login ID and password.

5. Complete the selections in the Destination tab by:

a. Check **Use Custom Location**.

b. Enter the Destination using the format:

*Remote analysis computer name**Shared folder name*, for example:

myPC\Remote_Autoanalysis

c. (For remote auto-analysis specifically) Establish a connection with the remote analysis computer by:

- Select **Start** ▸ **Run**.
- Enter the destination path, then click **OK**.
- Click **Test** to test the Location path name connection.

STOPPING POINT. If the test **Passes**, the message displays "Path Name test successful." If the test **Fails**, the message displays "Could not make the connection. Please check that the Path Name is correct."
In this case, click **Browse**, then select the correct location.

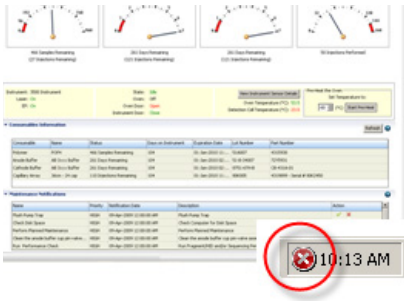
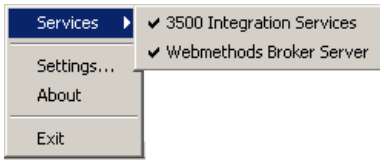
Troubleshoot

E

If you encounter any unforeseen and potentially hazardous event while operating the instrument, turn off the power switch, unplug the instrument, and call your Applied Biosystems representative.

IMPORTANT! See the Safety appendix for instrumentation and chemical safety information and guidelines.

Instrument troubleshooting

Symptom	Possible cause	Action
Amber light (blinking)	Run paused	Resume run
	Door open	Close the instrument door
	Run failure that doesn't require restart of instrument	Conduct another run
Instrument status light is blinking red	Instrument error	1. Power off the instrument. 2. Power on the instrument. 3. Restart the computer.
"An error has been detected from the instrument."	Instrument monitor circuit failure	Restart the computer
3500 Series Data Collection Software status icon is instead of  . 	One or more of the services are stopped.	Right-click the status icon, then select **Services**. If any item does not display a checkmark, click the item to start the service. 
"Unable to transmit measurement data. Internal data buffer overflow."	Communications error.	Restart instrument and computer.
Electric discharge message during runs.	The ABC buffer may be low.	Replace the ABC. Ensure that the ABC is being replaced per 3500 Series Data Collection Software notifications.

Spatial calibration troubleshooting

Symptom	Possible cause	Action
“Start” Spatial Calibration button is disabled.	Communication failure between the Data Collection Software and instrument	Restart instrument and computer. Check the NIC cable connection.
Unusual peaks or a flat line for the spatial calibration.	Improper installation of the detection cell: Detection cell on the array is not properly seated.	Unistall, then re-install the array: Reinstall the detection cell to reposition and make sure it fits in the proper position. If the calibration fails again: 1. Fill the capillaries with polymer. 2. Repeat the spatial calibration.
	The instrument may need more time to reach stability. An unstable instrument can cause a flat line with no peaks in the spatial view.	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 Wizard.
Persistently bad spatial calibration results.	Bad capillary array.	Replace the capillary array, and then repeat the calibration. Call your Applied Biosystems representative if the results do not improve.
"Spatial Calibration Error" message. The instrument cannot perform Spatial Calibration with Array fill.	Conditioning reagent is installed.	Replace the Conditioning reagent with an appropriate Polymer.

Spectral calibration troubleshooting

Symptom	Possible cause	Action
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 Applied Biosystems representative.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Call your Applied Biosystems representative.
If the spectral calibration fails, or if a message displays “No spectral files found.”	Blocked capillary	Refill the capillary array. You may have to install a fresh array or consider that capillary non-usable for purposes of planning your runs.
	Incorrect chemistry file, dye set, and/or run module selected.	Correct the files and rerun the calibration.
	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.
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, and if necessary, increase the amount of spectral standard added.
Spikes in the data or “Bad dye order detected” error message.	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.
Elevated baseline.	Poor spectral calibration.	Perform new spectral calibration.
Spectral calibration history does not display previously run calibration.	If you change polymer type, spectral calibrations for the original polymer type are not retained.	No action.
Pull-down (mirror image) peaks	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.

Sequencing install standard troubleshooting

Symptom	Possible cause	Action
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 Applied Biosystems representative.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Call your Applied Biosystems representative.
If the Sequencing install standard (Performance check) fails. Fail capillary • If more than one failed capillary (for 8-capillary). • If more than three failed capillary (for 24-capillary). Accept button is not active, but Reject button is active.	Blocked capillary	Refill the capillary array. You may have to install a fresh array or consider that capillary non-usable for purposes of planning your runs.
	Incorrect chemistry file, dye set, and/or run module selected.	Correct the files and rerun the calibration.
	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.

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 Applied Biosystems representative.
	The capillary tips may be hitting the bottom of the wells. Autosampler not correctly aligned.	Call your Applied Biosystems representative.
If the 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.

Anode buffer container troubleshooting

Also see "Data/electropherogram troubleshooting" on page 306.

Symptom	Possible cause	Action
Electrophoresis failure.	Buffer below fill line (inadequate amount of buffer).	Ensure that buffer level is at or above the fill line. Do not use if buffer level is too low or seal has been compromised.

Cathode buffer container troubleshooting

Also see "Data/electropherogram troubleshooting" on page 306.

Symptom	Possible cause	Action
Electrophoresis failure.	Buffer below fill line (inadequate amount of buffer).	Ensure that buffer level is at or above the fill line. Do not use if buffer level is too low or seal has been compromised.

RFID troubleshooting

Symptom	Possible cause	Action
Unable to read RFID information. "Failure to Read from RFID tag"	Consumable package is improperly installed or defective label. Polymer/Conditioning reagent pouch mis-oriented.	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 consumable, and click **Refresh** on the dashboard. If no results, restart the instrument and the computer. If no results, install a new consumable (if available), and call your Applied Biosystems representative for a replacement.

Link a plate troubleshooting

Symptom	Possible cause	Action
Plate does not link.	Spatial/Spectral calibration was not performed	1. Perform spatial calibration. 2. Relink the plate(s).
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). Follow the prompts.
"No plate detected" message	The plate is in position B.	Place the plate in position A.

How to search and use the log files

The 3500 Series Data Collection Software generates the following log files that you view using a text editor such as Wordpad:

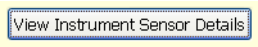
- **3500UsageStatistics.txt**–Provides a summary of the number of plates run, as well as number of run types (sequencing, fragment, and HID).

  Stored in: x:\Applied Biosystems\3500\UsageData
- **3500ConsumableUpdates.txt**–Provides a summary of consumables installation information and dates.

  Stored in: D:\Applied Biosystems\3500\LogFiles

View instrument sensor details

Click **View Instrument Sensor Details** in the Dashboard to display instrument information.



Run status of the instrument is displayed while a run is in progress.

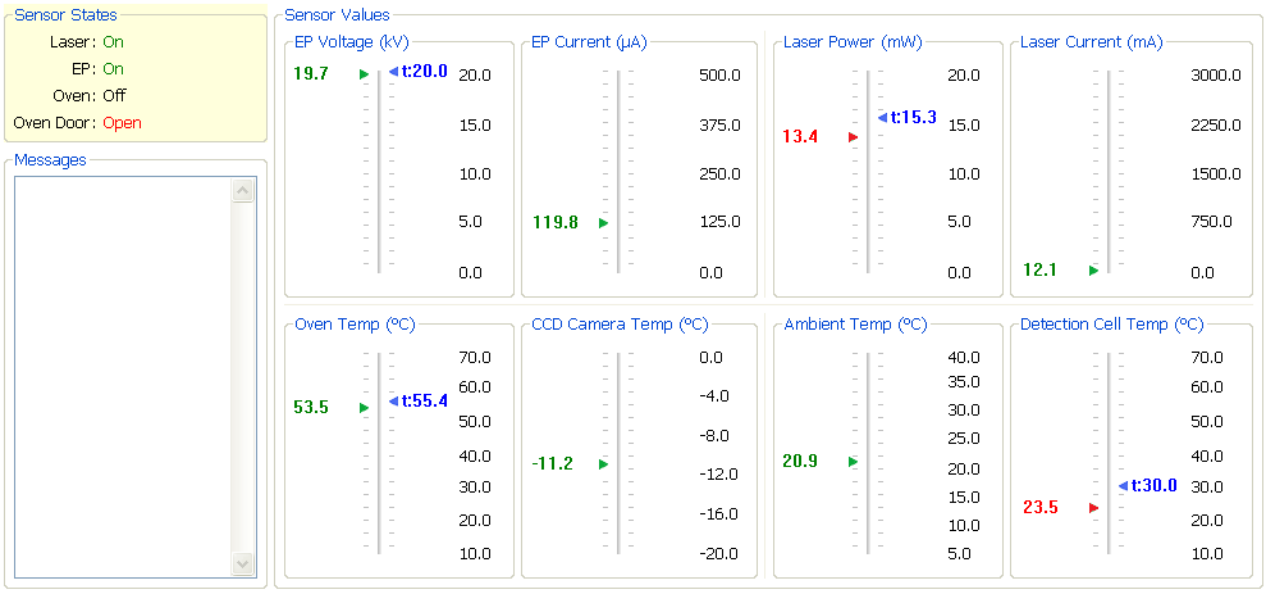


Figure 37 Instrument sensor details

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.	Failed reaction.	Repeat reaction.
	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.
	Bent capillary array tips.	Replace the capillary array.
	Cracked or broken capillary array.	Visually inspect the capillary array, including the detector window area for signs of breakage.
Low signal strength.	Degraded Formamide.	Use a fresh aliquot of Hi-Di™ Formamide.
	Not enough sample: Pipetting error.	Increase the amount of DNA added.
	Sample has high salt concentration.	Dilute with distilled or deionized water.
		Desalt using a column purification method.
	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.
	Autosampler out of calibration.	Check the volume of your samples. If still low signal strength, call your Applied Biosystems representative.
Elevated baseline.	Possible contaminant in the polymer path.	Use the conditioning reagent for washing the polymer pump.
	Possible contaminant or crystal deposits in the polymer.	Bring the polymer to room temperature.
		Replace the polymer if it has expired.
	Poor spectral calibration.	Perform new spectral calibration.
Loss of resolution.	Too much sample injected.	Dilute the sample and re-inject.
	Poor quality water.	Use distilled or deionized water.
	Degraded polymer.	Use a fresh supply of polymer.
	Capillary array used for more than 160 injections.	Replace with new capillary array.
	Degraded formamide.	Prepare fresh Hi-Di™ Formamide and re-prepare samples.
	High salt concentration in samples.	Use a recommended protocol for salt removal.
		Dilute salts with water.

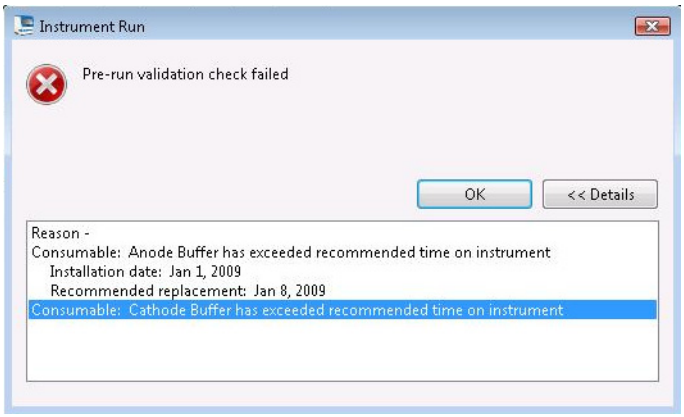
Symptom	Possible cause	Action
Poor resolution in some capillaries.	Insufficient filling of capillary array.	Refill the capillary array and look for polymer leakage. If problem persists, call your Applied Biosystems representative.
		Re-inject the same samples.
	Poor quality samples.	Check the sample preparation.
	Leak in system.	Tighten the connectors and array lever.
No current.	Not enough buffer in ABC.	Ensure that the buffer is filled up to the fill line.
	Bubble(s) present in the lower polymer block and/or the array and/or channels.	Pause the run and inspect for bubbles hidden in the tubing connectors. Select the Bubble Remove Wizard to remove the bubbles.
Elevated current.	Degraded polymer.	Open fresh supply of polymer and use Replenish Polymer Wizard.
	Arcing in the lower polymer block.	Inspect the lower polymer block for discoloration or damage. Replace the lower polymer block if necessary.
Fluctuating current.	Bubble in polymer block.	Pause run and inspect for bubbles hidden in the tubing connectors. Select Bubble Remove Wizard to remove the bubbles.
	A slow leak may be present in the system.	Check polymer blocks for leaks. Tighten all fittings.
	Not enough buffer in ABC.	Ensure that the buffer is filled up to the fill line.
	Arcing	Check for moisture in and around the septa, the CBC, the oven, and the autosampler.
Poor performance of capillary array used for fewer than 100 runs.	Poor quality samples, possible cleanup problems.	Desalt samples using a recommended purification protocol.
	Poor quality formamide.	Prepare fresh Hi-Di™ Formamide and re-prepare samples.
	Leak in system.	Tighten the connectors and array lever.
Migration time becomes progressively slower.	Leak in system.	Tighten the connectors and array lever.
	Improper filling of the system with polymer.	Polymer delivery pump may need to be serviced. If the issue persists, call your Applied Biosystems representative.
Migration time becomes progressively faster.	Water in polymer system, resulting in diluted polymer.	Use Bubble Remove Wizard to add polymer to system.
	Buffer valve leakage.	Check the Buffer-Pin Valve and see if it closes correctly.

Symptom	Possible cause	Action
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.
Peaks exhibit a shoulder in GeneMapper® *ID-X* Software applications.	Sample re-naturation.	Heat-denature the sample in good quality formamide and immediately place on ice.
Error messages: • "Leak detected during polymer delivery" • "Leak detected during bubble compression" The run aborts.	Bubbles in the polymer system.	Select the Bubble Remove Wizard to clear bubbles.
	Leak in the polymer system.	Check for evidence of leaks. If polymer leak occurred, conduct a water wash and wash the pump trap using the cleaning kit supplied.
	Buffer valve leakage.	Check the Buffer-Pin Valve and see if it closes correctly. Clean the Buffer-Pin Valve. Ensure that the maintenance schedule is followed per 3500 Series Data Collection Software notifications.
	Filling the array during install array.	Run Fill the Array with fresh Polymer wizard, or run Change Polymer Type wizard.
Detection cell stuck. It is difficult to remove when changing the capillary array.	Improperly placed detection cell.	To loosen the detection cell: 1. Undo the array lever and pull the polymer block towards you to first notch. 2. Hold both sides of the capillary array around the detection cell area, and apply gentle pressure equally on both sides. 3. Release.
Electrophoresis current is unstable	Bubbles in the polymer system.	Select the Bubble Remove Wizard to clear bubbles.
Electrophoresis failure.	Buffer below fill line.	Ensure that the buffer has not split into the overflow. If so, move the buffer back to main reservoir.

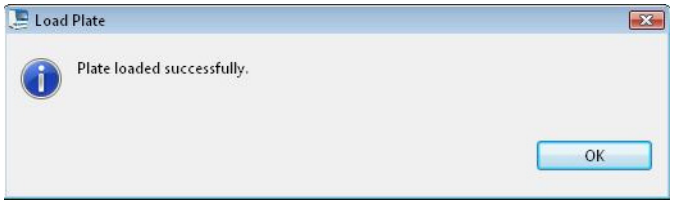
Dashboard troubleshooting

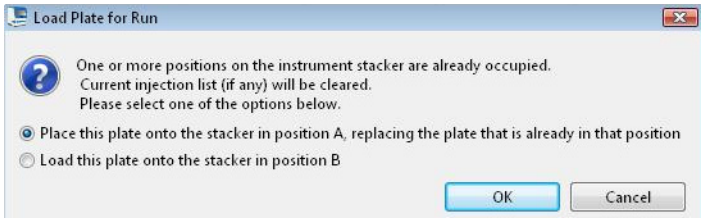
Symptom	Possible Cause	Action
The Days Remaining value for buffer/polymer does not automatically update.	The Days Remaining for buffers updates only when you click **Refresh** or **Start A Run**.	As part of daily startup, click **Refresh** to update buffer status.

Load plate troubleshooting

Symptom	Possible Cause	Action
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.	Read the details.	Click **Details** to determine the cause of the error. When the plate is successfully loaded, the Load Plates for Run screen is displayed.
		

Symptom	Possible Cause	Action
“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). Follow the prompts.
“No plate detected” message	The plate is in position B.	Place the plate in position A
"Fragment performance check is required" message.	Running fragment modules after loading the plate.	Change polymer to POP-7™. Run fragment analysis performance check.
"Sequencing performance check is required" message. After loading the plate.	Running sequencing modules (POP-6™) after loading the plate.	Change polymer to POP-7™. Run sequencing performance check.
Load plate or Load Plate for Run message.	Performance issues.	Click **OK** and follow the prompts.





Monitor run troubleshooting

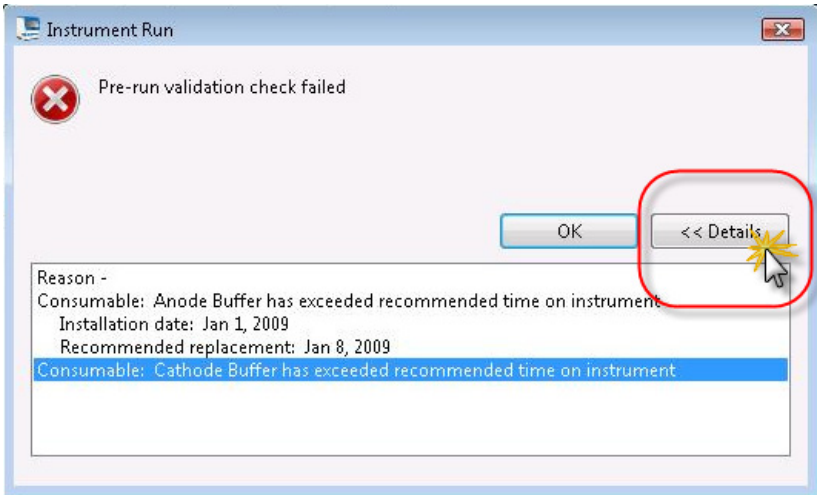
Symptom	Possible Cause	Action
Re-inject button is dimmed when you select an injection	Injection contains samples with assays that specify more than one instrument protocol.	Select in the injection list the injection with the instrument protocol of interest, select in the array view the capillary that corresponds to the well of interest, then click Re-inject.
The instrument run goes into pause state, unexpectedly.	RFID read/write process.	Check the Dashboard. Conduct an RFID refresh, if it does not refresh, restart both the computer and instrument.
Start run does not respond	The instrument has not initialized.	It takes, approximately, 10 seconds for the instrument to initialize after the instrument door is closed. Do not start a run until the instrument status light is green.
"Fragment performance check is required" message.	Running fragment modules after loading the plate.	Change polymer to POP-7™. Run fragment analysis performance check.
"Sequencing performance check is required" message. After loading the plate.	Running sequencing modules (POP-6™) after loading the plate.	Change polymer to POP-7™. Run sequencing performance check.

Review results troubleshooting

Symptom	Possible Cause	Action
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 93.
x and y scaling plot settings are not applied when you click Apply.	Scaling settings are applied only when you click Zoom.	Click **Zoom**.
The sizing quality result reported in the 3500 Series Data Collection Software 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 Data Collection Software *does not* consider the presence of broad peaks when determining sizing quality for fragment analysis data, therefore the sizing quality result reported in the 3500 Series Data Collection Software will differ from the sizing quality result reported in the GeneMapper® *ID-X* Software, which considers broad peaks in sizing quality.	No action.

Review error message details

Error messages in the 3500 Series Data Collection Software include a Details button. Click Details to display more information about an error message.



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
Electronic signature prompt is displayed when you edit sample comments.	Electronic signature prompt is displayed for sample comments, regardless of the electronic signature setting.	No action.

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.

Miscellaneous

Symptom	Possible Cause	Action
Polymer crystals on the Buffer-Pin Valve.	Buffer valve leakage.	Clean the Buffer-Pin Valve. Ensure that the maintenance schedule is followed per 3500 Series Data Collection Software notifications.
Fluid does not move through the pump and into the ABC from polymer or conditioning pouch.	Not applicable.	Call your Applied Biosystems representative.
Electric discharge message during runs.	ABC may be low.	Replace the ABC. Ensure that the ABC is being replaced per 3500 Series Data Collection Software notifications.
Leak detected during bubble compression during run or while filling the array.	Leak in system.	Run the Bubble Removal wizard. Ensure that there are no bubbles in the pump. If problem persists, use conditioning pouch for water wash. Use Replenish Polymer wizard to fill pump and array with polymer.
Only some injections, from a series of injections, are completed.	3500 Series Data Collection Software never moves on to the next injection.	Check connection between the instrument and computer and restart both the instrument and computer. Set up the injections again and started the runs.
"Injection failed" message. After some of the injections complete.	Capillary RFID cannot be read.	
When you click **Refresh** on the dashboard, and consumables information is listed as "Unknown."	Connection between the computer and instrument.	
“Instrument is not connected” message. After you start 3500 Series Data Collection Software.		
“Internal buffer data overflow” 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

Reset with the Reset button

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 by powering down

1. Shut down the computer.
2. Close the instrument doors.
3. Power off the instrument by pressing the on/off button on the front of the instrument.
4. Power on the instrument and wait until indicator light turns solid green.
5. Power on the computer.
6. Launch the Data Collection software (Service Console applications start automatically).

IMPORTANT! Wait until the computer has completely restarted before proceeding.

Safety F

Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

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

Symbol	Description
	Light switch.
	Indicates the **On** position of the circuit breaker.
	Indicates the **Off** position of the circuit breaker.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see "Safety labels on instruments" on page 317). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

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

Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

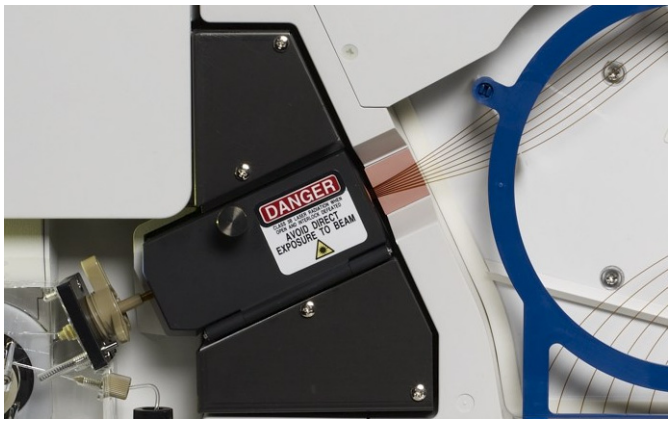
Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Safety labels on instruments

The Applied Biosystems 3500/3500xL Genetic Analyzers contain warnings at the locations shown below:

Locations of laser warnings

On the detection cell as shown below.



General instrument safety

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

WARNING! PHYSICAL INJURY HAZARD. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting the instrument

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

Moving and lifting stand-alone computers and monitors

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

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

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page 329.

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Solvents and pressurized fluids

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

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the 3500/3500xL analyzers without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

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

WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The 3500/3500xL analyzers system has an installation (overvoltage) category of II, and is classified as portable equipment.

Laser safety

Laser classification

The 3500 or 3500xL analyzer uses a a solid state laser. The laser specifications are:

- Wavelength 505nm
- Output power 20mW

The LED specifications are:

- Emitting color Natural White
- Luminous Intensity 250 Cd

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

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

Laser safety requirements

To ensure safe laser operation:

- The system must be installed and maintained by an Applied Biosystems Technical Representative.
- All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present and the instrument is Class I. If any panel is removed when the laser is operating (during service with safety interlocks disabled), you may be exposed to laser emissions in excess of the Class 3B rating.
- Do not remove safety labels or disable safety interlocks.

Additional laser safety information

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

Also, note the laser warnings provided in "Safety labels on instruments" on page 317.

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

Bar code scanner laser safety

Using a bar code scanner is optional.

Laser classification

The bar code scanner must be categorized as a Class 2 (II) laser.

Laser safety requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

WARNING! LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation safety

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

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

To minimize musculoskeletal and repetitive motion risks:

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

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- "U.S. and Canadian safety standards" on page 322
- "Canadian EMC standard" on page 323
- "European safety and EMC standards" on page 323
- "Australian EMC Standards" on page 328

U.S. and Canadian safety standards

The 3500 or 3500xL analyzer has been tested to and complies with standard:

UL 61010-1/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

The 3500 or 3500xL analyzer has been tested to and complies with the "21 CFR, 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007, as applicable."

For the Reader/Writer unit in the Applied Biosystems 3500/3500xL Genetic Analyzers

FCC WARNING

This device complies with Part 15 of FCC Rules. Operation is subject to the following two conditions:

1. This device may not cause interference, and
2. This device must accept any interference, including interference that may cause undesired operation of this device.

Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTICE

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation.

This equipment generates uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna

- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators." Cet appareil numerique de la classe B est conforme a la norme NMB-001 du Canada.

Canadian Department of Communications Industry Canada (IC) Notice

This device complies with RSS-Gen of IC Rules. Operation is subject to the following two conditions:

1. This device may not cause interference, and
2. This device must accept any interference, including interference that may cause undesired operation of this device.

European safety and EMC standards

CE

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EN 60825-1, "Radiation Safety of Laser Products, Equipment Classification, Requirements, and User's Guide.

EMC

The 3500 or 3500xL analyzer meets European requirements for emission and immunity (EMC Directive 2004/108/EC).

EN 61326-1:2006 "Electrical equipment for measurement, control and laboratory use- Part 1 General EMC requirements." (Group 1, Class B)

For the Reader/Writer unit in the Applied Biosystems 3500/3500xL Genetic Analyzers

CE Notice (European Union)

Marking by the symbol indicates compliance of this ASI4000-98-BS1 RFID R/W Module to the Electromagnetic Compatibility Directive and the Low Voltage Directive of the European Union. Such making is indicative that this RFID R/W Module meets the following technical standards:

- EN 300330 – "Electromagnetic compatibility and Radio spectrum Matters (ERM); Short Range Devices (SRD)."

- EN 301489 – “Electromagnetic compatibility and Radio spectrum Matters (ERM); ElectroMagnetic Compatibility (EMC) standard for radio equipment and services.”
- EN 60950 – “Safety of Information Technology Equipment.”

Europe – CE declaration of conformity (Reader/Writer)

EN 300 330-1 V1.5.1 (2006-04), EN 300 330-2 V1.3.1 (2006-04), EN 301 489-3 V1.4.1 (2002-08), EN 301 489-1 V1.6.1 (2005-09), EN 60950-1:2006

English Hereby, ART Technology Co., Ltd. declares that this ASI4000-98-BS1 is in compliance with the essential requirements and other relevant provisions of Directive 1999/5/EC.

Français

[French]

Par la présente ART Technology Co., Ltd. déclare que l'appareil ASI4000-98-BS1 est conforme aux exigences essentielles et aux autres dispositions pertinentes de la directive 1999/5/CE.

Deutsch

[German]

Hiermit erklärt ART Technology Co., Ltd. dass sich das Gerät ASI4000-98-BS1 in Übereinstimmung mit den grundlegenden Anforderungen und den übrigen einschlägigen Bestimmungen der Richtlinie 1999/5/EG befindet.

Italiano

[Italian]

Con la presente ART Technology Co., Ltd. dichiara che questo ASI4000-98-BS1 è conforme ai requisiti essenziali ed alle altre disposizioni pertinenti stabilite dalla direttiva 1999/5/CE.

Español

[Spanish]

Por medio de la presente ART Technology Co., Ltd. declara que el ASI4000-98-BS1 cumple con los requisitos esenciales y cualesquiera otras disposiciones aplicables o exigibles de la Directiva 1999/5/CE.

Português

[Portuguese]

ART Technology Co., Ltd. declara que este ASI4000-98-BS1 está conforme com os requisitos essenciais e outras disposições da Directiva 1999/5/CE.

Suomi

[Finnish]

ART Technology Co., Ltd. Vakuuttaa täten että ASI4000-98-BS1 tyyppinen laite on direktiivin 1999/5/EY oleellisten vaatimusten ja sitä koskevien direktiivin muiden ehtojen mukainen.

Nederlands

[Dutch]

Hierbij verklaart ART Technology Co., Ltd. dat het toestel ASI4000-98-BS1 in overeenstemming is met de essentiële eisen en de andere relevante

Česky

[Czech]

ART Technology Co., Ltd. tímto prohlašuje, že tento ASI4000-98-BS1 je ve shodě se základními požadavky a dalšími příslušnými ustanoveními směrnice 1999/5/ES.

Dansk

[Danish]

Undertegnede ART Technology Co., Ltd. erklærer herved, at følgende udstyr ASI4000-98-BS1 overholder de væsentlige krav og øvrige relevante krav i direktiv 1999/5/EF.

Eesti

[Estonian]

Käesolevaga kinnitab ART Technology Co., Ltd. seadme ASI4000-98-BS1 vastavust direktiivi 1999/5/EÜ põhinõuetele ja nimetatud direktiivist tulenevatele teistele asjakohastele sätetele.

Ελληνική

[Greek]

ΜΕ ΤΗΝ ΠΑΡΟΥΣΑART Technology Co., Ltd. ΔΗΛΩΝΕΙ ΟΤΙ ASI4000-98-BS1 ΣΥΜΜΟΡΦΩΝΕΤΑΙ ΠΡΟΣ ΤΙΣ ΟΥΣΙΩΔΕΙΣ ΑΠΑΙΤΗΣΕΙΣ ΚΑΙ ΤΙΣ ΛΟΙΠΕΣ ΣΧΕΤΙΚΕΣ ΔΙΑΤΑΞΕΙΣ ΤΗΣ ΟΔΗΠΑΣ 1999/5/ΕΚ.

Latviski

[Latvian]

Ar šo ART Technology Co., Ltd. deklarē, ka ASI4000-98-BS1 atbilst Direktīvas 1999/5/EK būtiskajām prasībām un citiem ar to saistītajiem noteikumiem.

Lietuvių

[Lithuanian]

Šiuo ART Technology Co., Ltd. deklaruoja, kad šis ASI4000-98-BS1 atitinka esminius reikalavimus ir kitas 1999/5/EB Direktyvos nuostatas.

Malti

[Maltese]

Hawnhekk, ART Technology Co., Ltd. jiddikjara li dan ASI4000-98-BS1 jikkonforma mal-
fid-Dirrettiva 1999/5/EC.

Magyar

[Hungarian]

Alulírott, ART Technology Co., Ltd. nyilatkozom, hogy a ASI4000-98-BS1 megfelel a vonatkozó alapvetõ követelményeknek és az 1999/5/EC irányelv egyéb elõírásainak.

Polski

[Polish]

Niniejszym ART Technology Co., Ltd. oświadcza, że ASI4000-98-BS1 jest zgodny z zasadniczymi wymogami oraz pozostałymi stosownymi postanowieniami Dyrektywy 1999/5/EC.

Slovensko

[Slovenian]

ART Technology Co., Ltd. izjavlja, da je ta ASI4000-98-BS1 v skladu z bistvenimi zahtevami in ostalimi relevantnimi določili direktive 1999/5/ES.

Slovensky

[Slovak]

ART Technology Co., Ltd. týmto vyhlasuje, že ASI4000-98-BS1 spĺňa základné požiadavky a všetky príslušné ustanovenia Smernice 1999/5/ES.

Svenska

[Swedish]

Härmed intygar ART Technology Co., Ltd. att denna ASI4000-98-BS1 står I överensstämmelse med de väsentliga egenskapskrav och övriga relevanta bestämmelser som framgår av direktiv 1999/5/EG.

Íslenska

[Icelandic]

Hér með lýsir ART Technology Co., Ltd. yfir því að ASI4000-98-BS1 er í samræmi við grunnkröfur og aðrar kröfur, sem gerðar eru í tilskipun 1999/5/EC.

Norsk

[Norwegian]

ART Technology Co., Ltd. erklærer herved at utstyret ASI4000-98-BS1 er i samsvar med de grunnleggende krav og øvrige relevante krav i direktiv 1999/5/EF.

Australian EMC Standards

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

Chemical safety

General chemical safety

Chemical hazard warning

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

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

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 329.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

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

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

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

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

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

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

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

Waste disposal

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

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

Biological hazard safety

General biohazard

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:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **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**

Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page xiii.

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page xiii.

General alerts for all chemicals

WARNING! 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 the cleaning agents as described in this manual, only. Use of cleaning agents not described in this manual can impair the instrument. Please contact your local Life Technologies sales office if you have any questions.

- Read the MSDS for this product, and follow the handling instructions.
- Avoid inhalation, contact with eyes, skin, clothing, and prolonged or repeated exposure.
- Consumables have a limited lifetime. Overusing the parts might result in poor quality data.

Specific chemical alerts

WARNING! CHEMICAL HAZARD. POP-4™ POLYMER. Causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation.

WARNING! CHEMICAL HAZARD. POP-6™ POLYMER. Causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation.

WARNING! CHEMICAL HAZARD. POP-7™ POLYMER. Harmful by inhalation and if swallowed. Causes eye, skin, and respiratory tract irritation. Do NOT taste or swallow. Avoid breathing vapor (or dust). Keep container tightly closed. Use only with adequate ventilation. Wash thoroughly after handling.

WARNING! CHEMICAL HAZARD. Hi-Di™ Formamide. Causes eye, skin, and respiratory tract irritation. Possible developmental and birth defect hazard. Avoid breathing vapor. Use with adequate ventilation.

WARNING! CHEMICAL HAZARD. Anode Buffer Container (ABC). May cause eye, skin and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation.

WARNING! CHEMICAL HAZARD. Cathode Buffer Container (CBC). May cause eye, skin and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation.

WARNING! CHEMICAL HAZARD. 1× GA Buffer/EDTA. May cause eye, skin and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation.

Instrumentation alerts

General instrumentation alerts

WARNING! 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.

Specific instrumentation alerts

WARNING! 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.

The instrument uses a Solid-state laser. Under normal operating conditions, the instrument is categorized as a Class I laser / LED product. When safety interlocks are disabled during certain servicing procedures, the laser can cause permanent eye damage, and, therefore, is classified under those conditions as a Class 3B laser.

The instrument has been tested to and complies with 21 CFR, 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated June 24, 2007, as applicable.

The instrument has been tested and complies with standard EN60825-1: 2001, “Radiation Safety of Laser Products, Equipment Classification, Requirements, and User’s Guide.’

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

Laser Parameter

Wave length 505nm, Output power 20mW

LED Parameter

Emitting color Natural White, Luminous Intensity 250 Cd.

Documentation

Related documentation

The following related documents are shipped with the system:

Document	Part number	Description
Applied Biosystems 3500/3500xL Genetic Analyzers Quick Reference Card	4401662	• Provides a flowchart on how to run your samples and instrument • Provides a table of maintenance tasks and • Contains Data Collection Software reference guide
Applied Biosystems 3500 Series Genetic Analyzer Site Preparation Guide (4401689) **Note:** The purpose of the Site Prep Guide is to help you prepare your site for installation of the 3500 or 3500xL analyzer. For specific details about your system, please refer to this user guide.	4401663	Provides information about the space, environmental, and electrical requirements needed to support the Applied Biosystems 3500/3500xL Genetic Analyzers.

Portable document format (PDF) versions of this guide as well as the Quick Reference Card and the Warranty statement are also available on the Applied Biosystems 3500/3500xL Genetic Analyzers the software installation CD, which will be shipped with the system.

Note: For additional documentation, see "How to obtain support" on page xvii.

Obtaining information from the Help system

The 3500 Series Data Collection Software 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 or 3500xL 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 Data Collection Software window.
- Select **Help ▸ Help Contents**.

You can use the Help system to find topics of interest by:

- Reviewing the contents
- Searching for a specific topic
- Searching an alphabetized index

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see "How to obtain support" on page xvii.

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