

# Writing Sample - Biotechnology - User Guide

## Part 2

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### Background

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.

I developed the guide by myself, as a lead technical writer, while managing other writers tasks, schedules, and performances.

### Scope

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.

The guide is one of the many documents that I developed for the AB 3500/3500xL system.

The authoring tool for drafting this guide was Oxygen XML Author, and I used single-sourcing and content reuse techniques.

### Audience

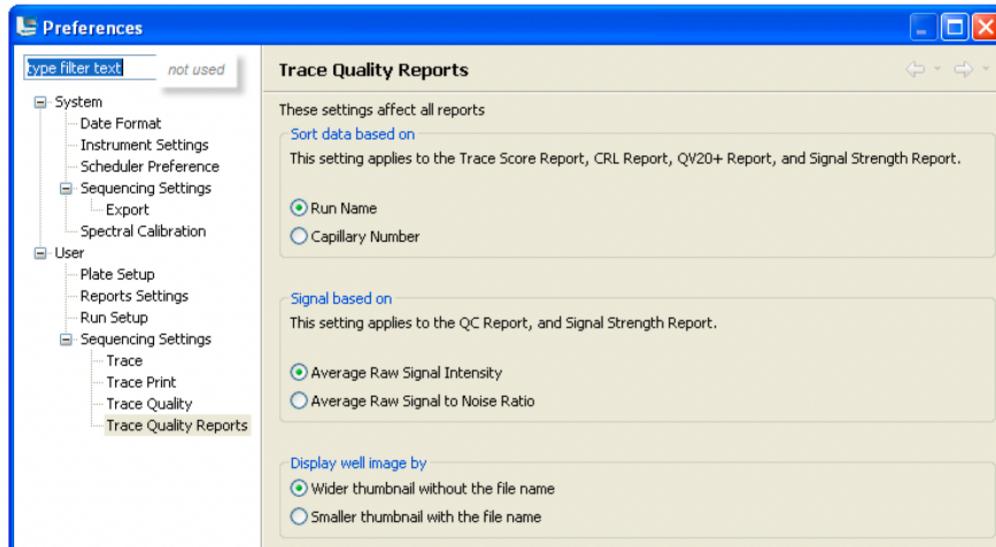
This user guide is written for principal investigators and laboratory staff who are planning to operate and maintain the Applied Biosystems 3500/3500xL Genetic Analyzers.

### About writing sample - Biotechnology - User Guide

The following writing sample contains parts of the actual user guide.

The user guide, in its entirety, is available in case it is needed.

I gathered the information required for this user guide by interviewing scientists, product managers, bioinformatics analysts, and lab technicians.



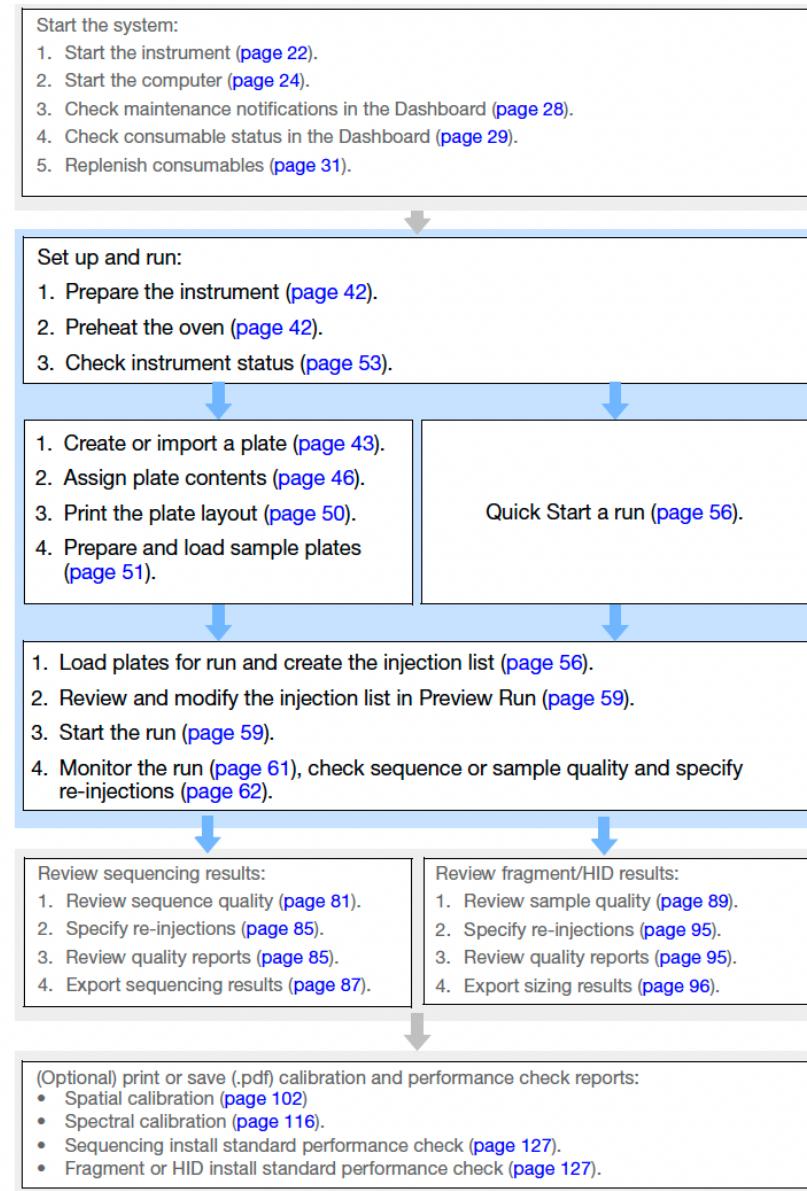
2. Specify the following settings.:

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

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

# Set Up and Run

## Workflow



## 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.

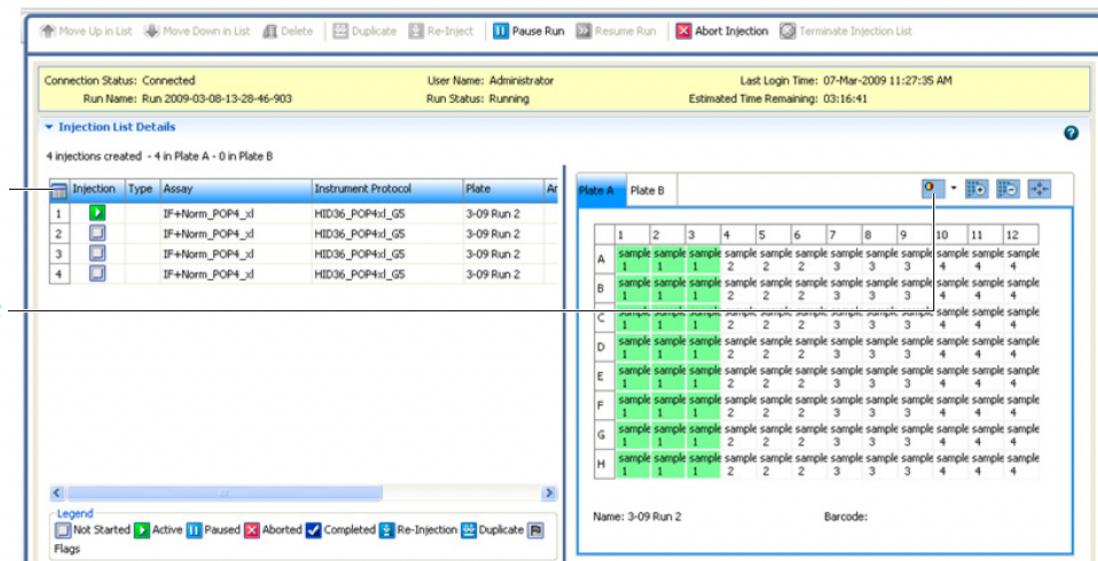
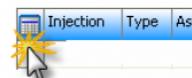


Figure 8 Monitor Run screen

**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.



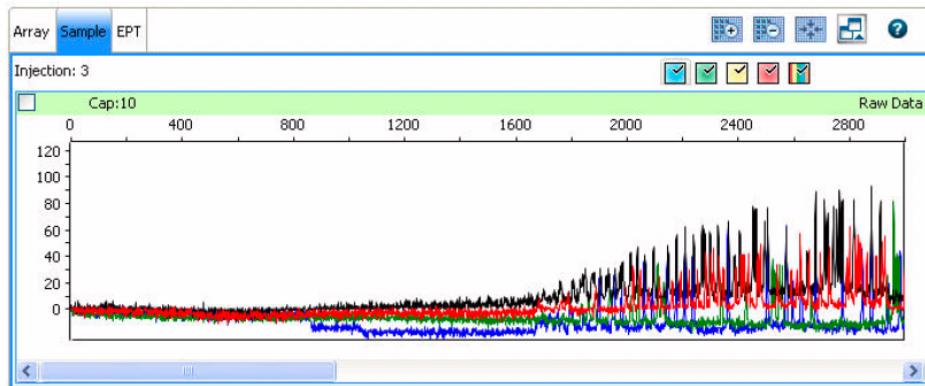
- To filter the flag table, select a flag type. To display HID flags, select All.

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. <b>IMPORTANT!</b> Normalization is not applied to samples with  (red) Sizing Quality.

- 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.



## More features in Assign Plate Contents

### Use the Plate View

#### Name samples in the Plate View

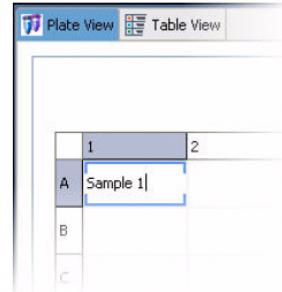
To name samples in the Plate View:

##### 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:

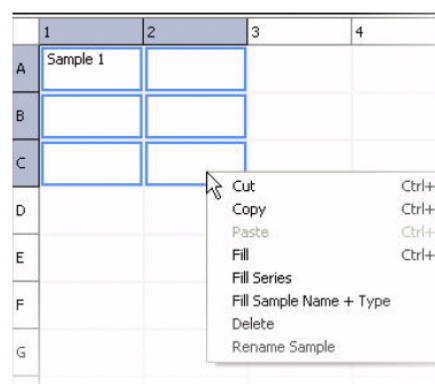
- Click Row to set the Enter key to move the cursor vertically to the next row.
- Click Column to set the Enter key to move the cursor horizontally to the next column.



##### To name multiple samples

- Click a named well.
- Click-drag multiple wells.
- 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.



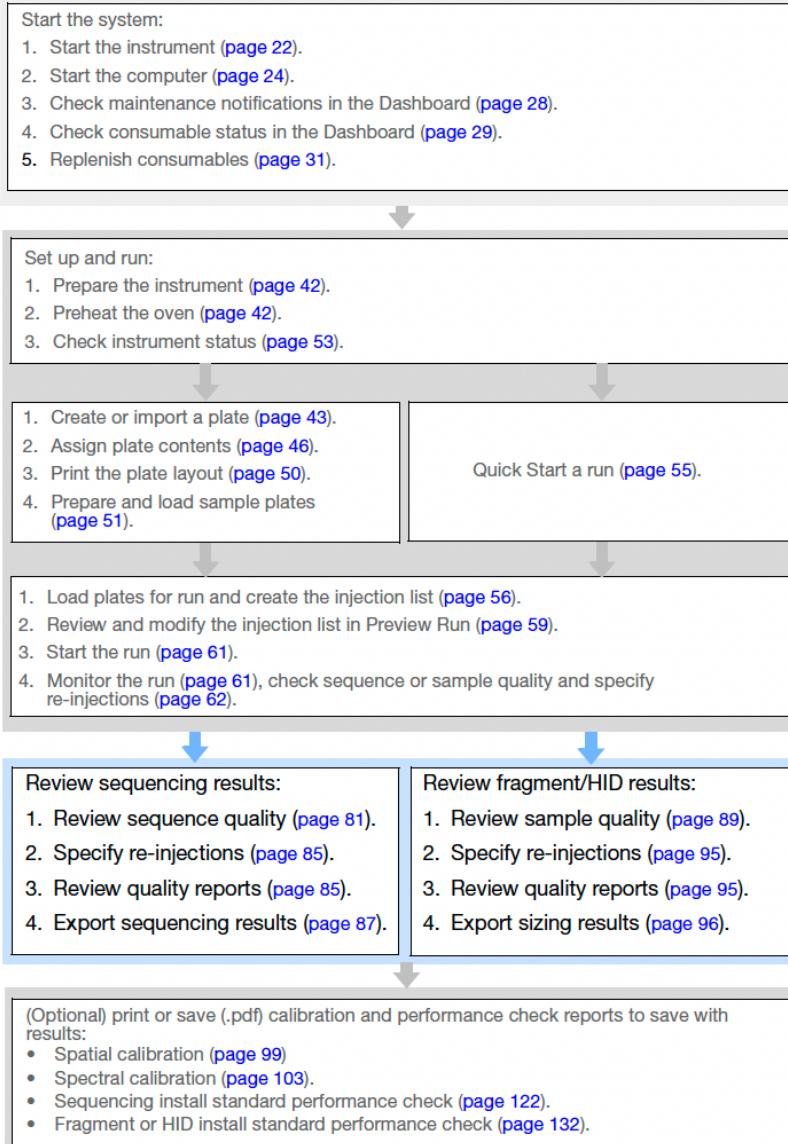
##### To name all wells at one time

- Select all wells.
- Select assays, file name conventions, and results group for the plate.
- Enter name and select sample type (in the Customize Sample Info pane) for the whole plate.

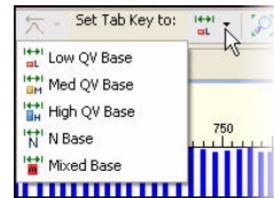


# Review Results

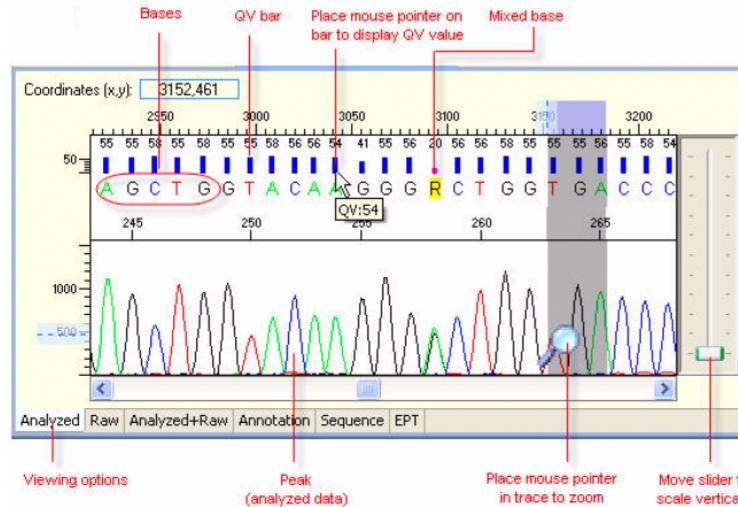
## Workflow



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.



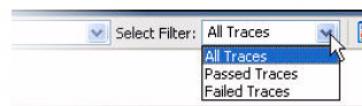


## Export sequencing results

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)

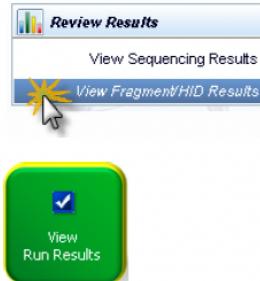


## Review Fragment/HID Analysis results

### Access the View Fragment/HID Results screen

Access the View Fragment/HID Results screen from:

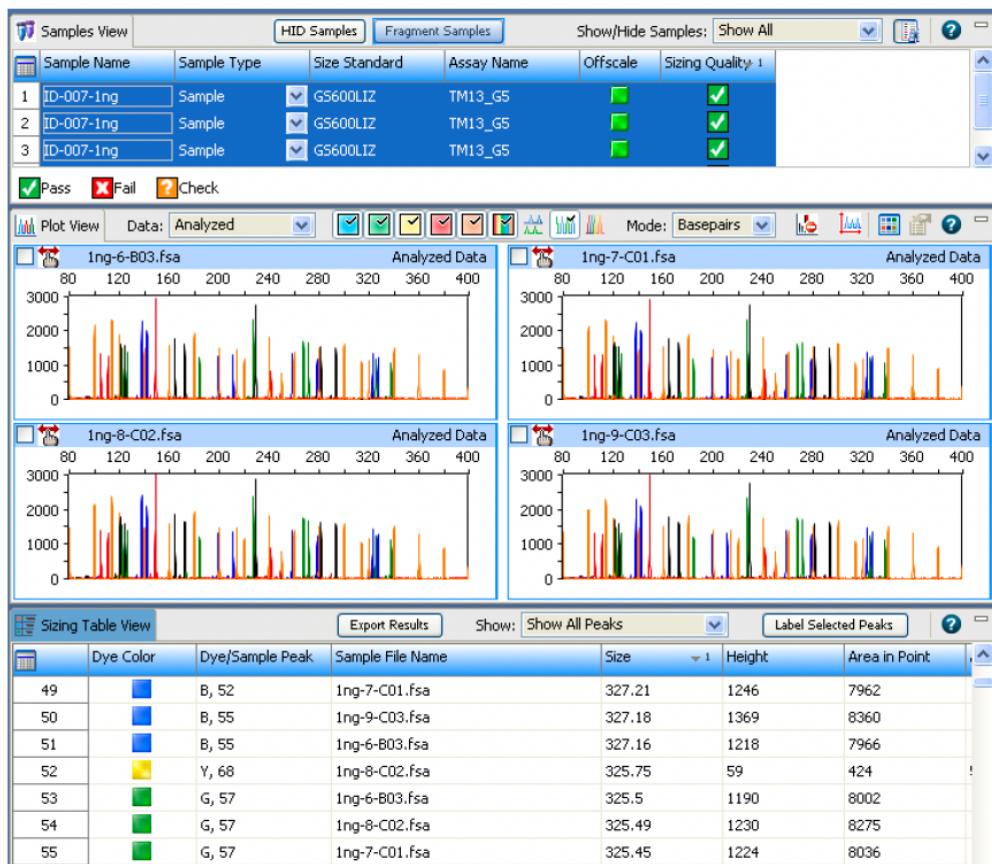
- The Monitor Run screen by clicking **Review Results**.
- The navigation pane by selecting **View Sequencing Results**.
- The Dashboard by clicking **View Run Results**.



#### 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.



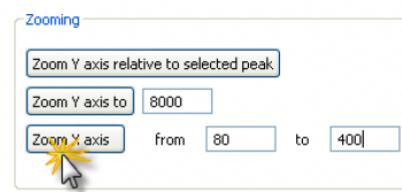
**IMPORTANT!** If you first view a 4-dye sample, then view a 5-dye sample, you must manually select the fifth dye. It is not automatically selected when you switch to a 5-dye sample.

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**.

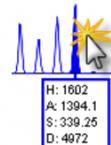
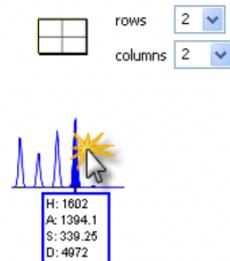


To apply scaling settings to all samples in the samples table, select all of the samples in the samples table to display them in the plot view, specify the scaling settings, click **Zoom**, then click **Page Up** and **Page Down** in the plot view to move through the samples.

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

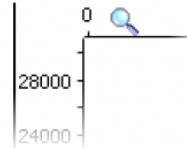


4. Display multiple plots as needed: in the Plot Settings Display tab, select **Checkerboard**.
5. Click a peak to label it (to label all peaks, see “[Label peaks](#)” on page 93).



### 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.

# Calibrate and Check Performance

## 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:

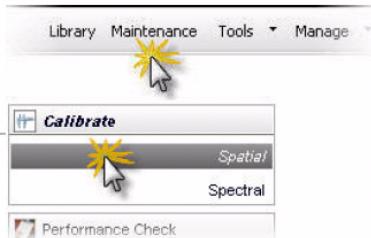
- Remove or replace the capillary array
- Open the detector door or move the detection cell
- Move the instrument

#### Perform a spatial calibration

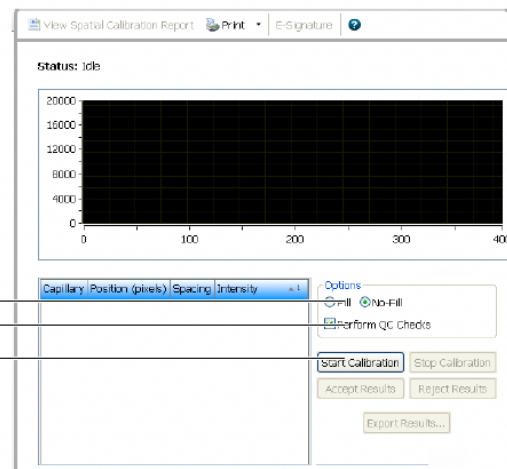
**IMPORTANT!** Do not open the instrument door during a spatial calibration run. Doing so will stop the run and require you to restart the 3500 Series Data Collection Software.

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.



## Section 1 Calibration



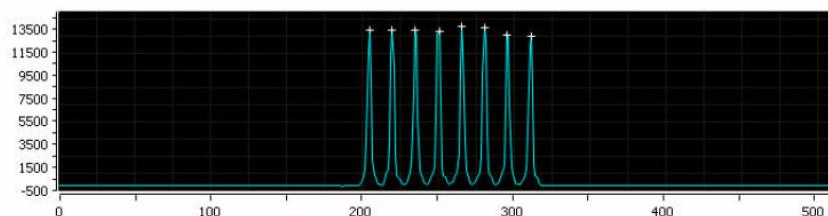
2. Select **No Fill**, or select **Fill** to fill the array with polymer before starting the calibration.

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

Attribute	Calculation	Threshold
Average peak height	$\frac{\text{sum of all peak heights}}{\text{number of peaks}}$	<ul style="list-style-type: none"><li>• 8-cap: 6400 RFU</li><li>• 24-cap: 3000 RFU</li></ul>
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.

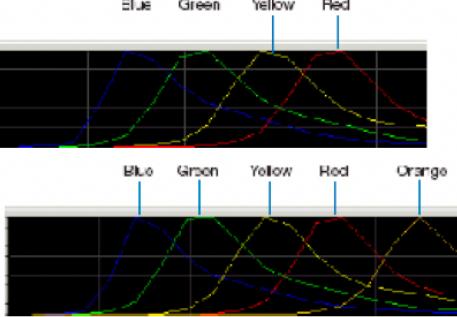
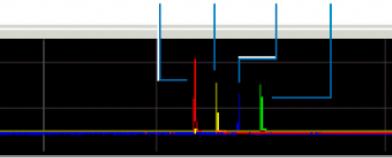
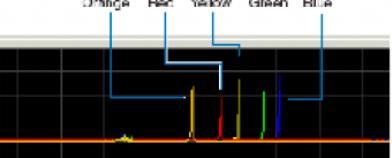
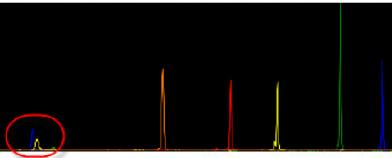
## Evaluate the spectral calibration data

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

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

For each capillary:

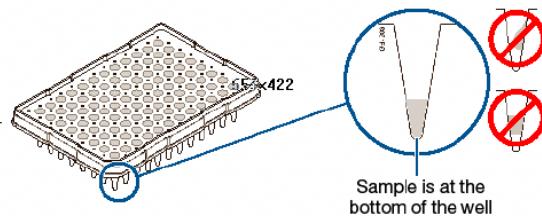
1. Click a capillary to display the spectral and raw data for a capillary.
2. Check that the data meet the following criteria:

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

- 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.



Sample is at the bottom of the well

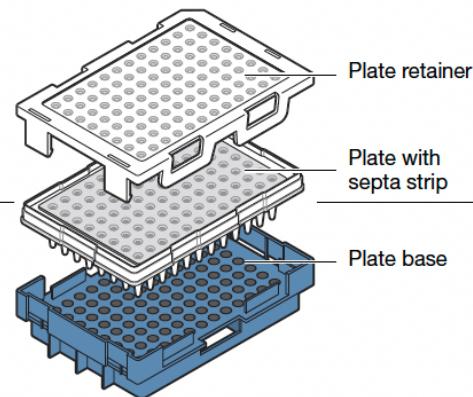
- Store the plate on ice until you prepare the plate assembly and load the plate in the instrument.

## Prepare the plate assembly

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

- Align the holes in the septa strip with the wells of the plate, then firmly press downward onto the plate.
- 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.



- Snap the plate retainer (cover) onto the plate, septa, and plate base.
- 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

- 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.



- Close the instrument door to re-initialize the instrument.

## 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.

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

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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.

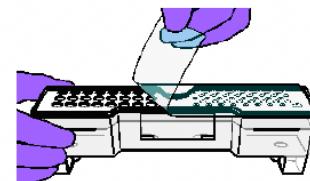


3. In the Create New Assays dialog box, select an application type: Sequencing, Fragment, or HID. The screen changes depending on the application type you select ([Figure 12 on page 149](#) shows the sequencing screen).
4. Specify settings (see [Table 7 on page 149](#)).
5. Save the assay:
  - If you 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**.

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

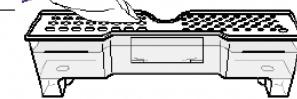
**Note:** If you do not tilt the CBC back and forth, the buffer sticks to the baffles, due to surface tension.

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.

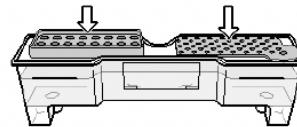


9. Wipe off any buffer on top of the CBC with a lint-free cloth. Ensure that the top of the container is dry.

**IMPORTANT!** Failure to perform this action may result in an arcing event and termination of the run.

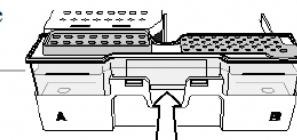


10. Place the appropriate septa on both sides of the CBC.
  - a. Align the buffer septa (the part that is symmetrical) over the 24 holes of the CBC.
  - b. Push the septa lightly into the holes to start and then push firmly to seat the septa.



11. Install the CBC on the autosampler.

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

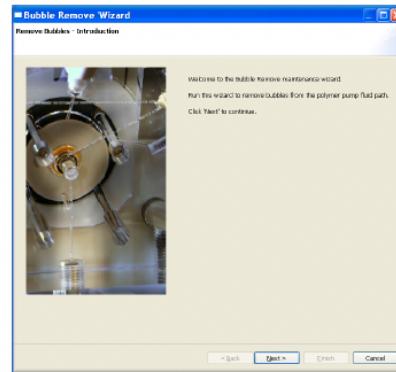


12. Close the instrument door to re-initialize.

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.

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.

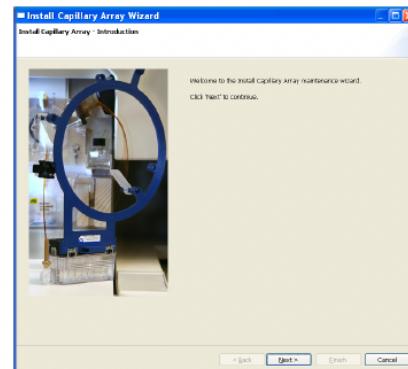
For details see "[Instrument reagents and consumables](#)" on page 9.

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.



# 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 33 Capillary array and polymer (sequencing analysis run modules) (continued)

Run Module Type & Run Module Name	Configuration		23 hours Throughput <sup>‡</sup>			Performance
	Capillary Length (cm)	Polymer Type	Run Time (min)	3500	3500xL	
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 <sup>‡</sup>			Performance		
	Capillary Length (cm)	Polymer Type	Run Time (min)	3500	3500xL	Range <sup>§</sup>	Sizing Precision <sup>#</sup>	
				50bp-400bp	401bp-600bp		601bp-1200bp	
Fragment analysis FragmentAnalysis50_POP7	50	POP-7™	≤40	≥280	≥840	≤40 to ≥520	<0.15	<0.30
Fragment analysis FragmentAnalysis50_POP6	50	POP-6™	≤100	≥112	≥336	≤20 to ≥550	<0.15	<0.30
Long fragment analysis LongFragAnalysis50_POP7	50	POP-7™	≤125	≥88	≥360	≤40 to ≥700	<0.15	<0.30
HID HID36_POP4	36	POP-4™	≤35	≥312	≥936	≤60 to ≥400	<0.15	NA <sup>#</sup>
HID HID36_POP7	36	POP-7™	≤26	≥424	≥1272	≤60 to ≥400	<0.15	NA <sup>#</sup>
SNaPshot® SNaPshot50_POP7	50	POP-7™	≤30	≥376	≥1104	≤40 to ≥120	<0.50	NA <sup>#</sup>

‡ 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  $\geq 1$ . The table shows the resolution range in  $\geq 90\%$  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  $\geq 90\%$  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:

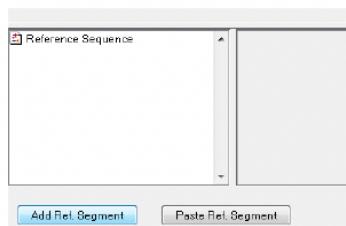
1. Create a RDC by importing a Reference Sequence
2. Define Analysis and Display Settings
3. Create a Project Template
4. Create an empty Project with blank Specimens

### Import a reference

1. Start the SeqScape® Software (SeqScape v2.7), 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**.



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.

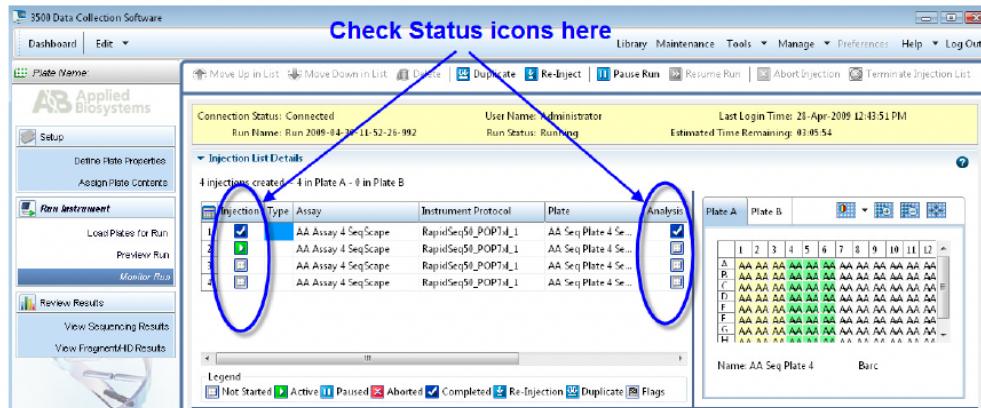


7. Click **Apply to Plate**, then **Save to Library** if you want to use this assay again.
8. Click **Close**.

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

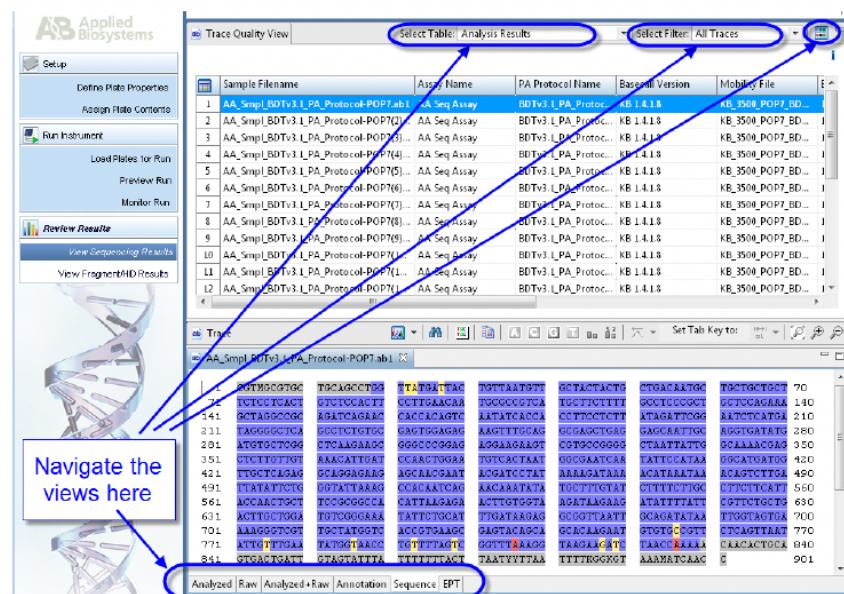
## Appendix B Secondary Analysis: Sequencing

**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.



## Secondary Analysis: Fragment

### 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® <i>ID-X</i>	A software for use in Human Identification testing (databasing, casework, and paternity applications) and used in conjunction with AmpF/STR 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.

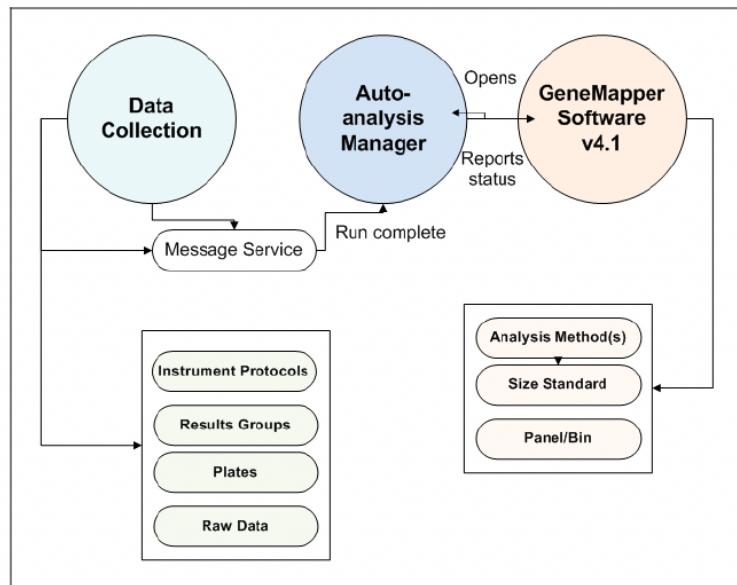
This procedure initially describes how to set up panels and bin sets in GeneMapper® Software v4.1 and then describes how to auto-analyze samples using the 3500 Series Data Collection Software. Once a run is complete, your data is seamlessly transferred into GeneMapper® for analyzing, processing and reporting.

**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.

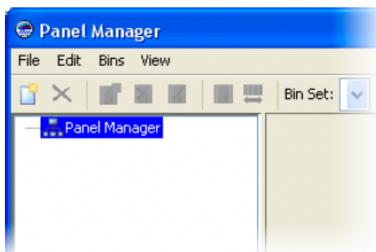
The fragment analysis workflow for auto-analysis is summarized in this flow chart.



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.

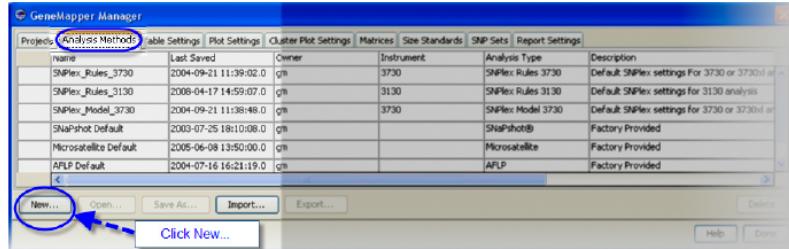
### Specify a kit, a panel, and a bin set for the project

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.

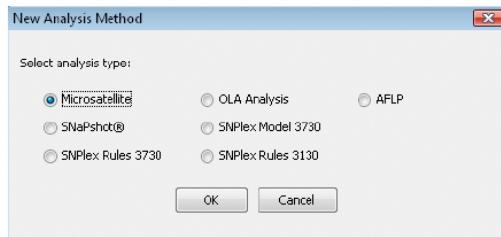


### Create a new project

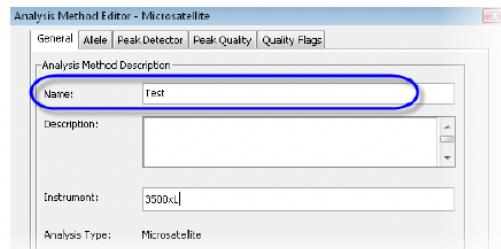
1. Click  (GeneMapper Manager) to open the GeneMapper Manager.
2. Select the Analysis Method tab, then click **New**.



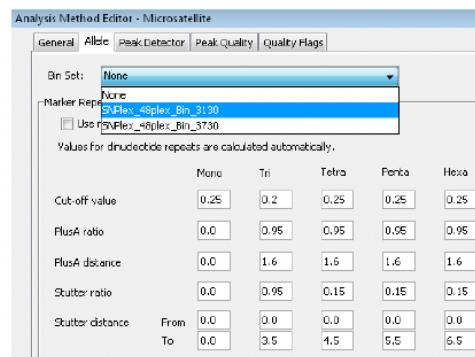
3. Select the Analysis Method Type you want, then click **OK**.



4. Name your Analysis Method (General tab).

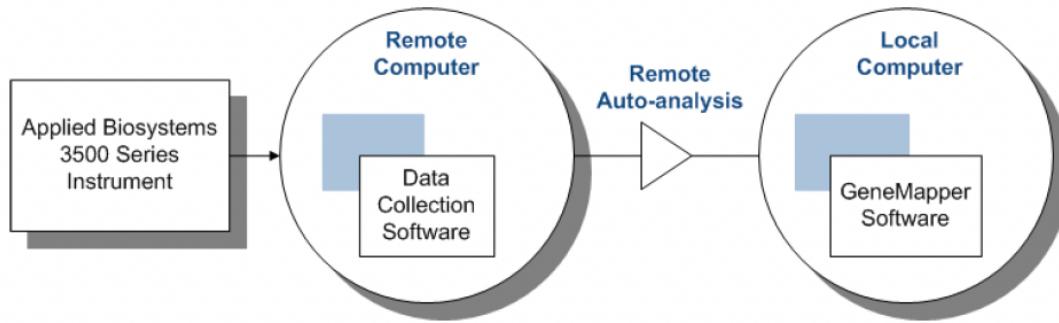


5. Select your Bin Set (Allele tab).



# Remote Auto-Analysis Setup

## 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.

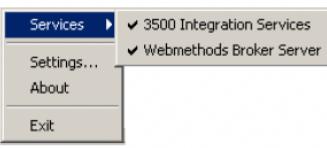
---

# Troubleshoot

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	<ol style="list-style-type: none"> <li>Power off the instrument.</li> <li>Power on the instrument.</li> <li>Restart the computer.</li> </ol>
"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.	<p>Right-click the status icon, then select <b>Services</b>. If any item does not display a checkmark, click the item to start the service.</p> 
		
"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.	<p>Replace the ABC.</p> <p>Ensure that the ABC is being replaced per 3500 Series Data Collection Software notifications.</p>

## 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 <ul style="list-style-type: none"><li>• If more than one failed capillary (for 8-capillary).</li><li>• If more than three failed capillary (for 24-capillary).</li></ul> Accept button is not active, but Reject button is active.	Blocked capillary  Incorrect chemistry file, dye set, and/or run module selected.  Insufficient filling of array.  Expired matrix standards or old reagents.  Expired polymer.  Bubbles in the polymer system.  Possible contaminant or crystal deposits in the polymer.	Refill the capillary array. You may have to install a fresh array or consider that capillary non-useable for purposes of planning your runs.  Correct the files and rerun the calibration.  Check for broken capillaries and refill the capillary array.  Check the expiration date and storage conditions of the matrix standards and/or reagents. If necessary, replace with a fresh lot.  Replace the polymer with a fresh lot using the Replenish Polymer Wizard.  Select the Bubble Remove Wizard to clear the bubbles.  Properly bring the polymer to room temperature; do not heat. Replace the polymer if it has expired.

# Safety

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## 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 <b>On</b> position of the circuit breaker.
	Indicates the <b>Off</b> position of the circuit breaker.
	Indicates a standby switch by which the instrument is switched on to the <b>Standby</b> condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the <b>On/Off</b> 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.



## 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:



## 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

**Magyar**

[Hungarian]

Alulírott, ART Technology Co., Ltd. nyilatkozom, hogy a ASI4000-98-BS1 megfelel a vonatkozó alapvető követelményeknek és az 1999/5/EC irányelv egyéb előírásainak.

**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]

Härmad 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. erklaerer herved at utstyret ASI4000-98-BS1 er i samsvar med de grunnleggende krav og øvrige relevante krav i direktiv 1999/5/EU.



**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.

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[www.appliedbiosystems.com/about/offices.cfm](http://www.appliedbiosystems.com/about/offices.cfm)