Reference Guide

Applied Biosystems

Peak Scanner™ Software

Version 1.0

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Contents

Preface

How to Use This Guide

Purpose of This Guide

The *Peak Scanner™ Software Version 1.0 Reference Guide* provides brief instructions for peak identification and fragment sizing for application-specific capillary electrophoresis assays.

Audience

This guide is intended for Peak Scanner™ Software users.

Assumptions

This guide assumes that:

- You have downloaded and installed *Peak Scanner™ Software Version 1.0.*
- You have a working knowledge of the Microsoft® Windows® 2000 or XP operating system.

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 analyzing, *always* prepare fresh matrix.
- 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.

Examples of the user attention words appear below:

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

How to Obtain More Information

Obtaining Information from the Help System

Peak Scanner™ Software 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 toolbar of the Peak Scanner window
- Select **Help ▸ Contents and Index**
- Press **F1**

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Find a specific topic (Ctrl-F)
- Searching though an alphabetized index

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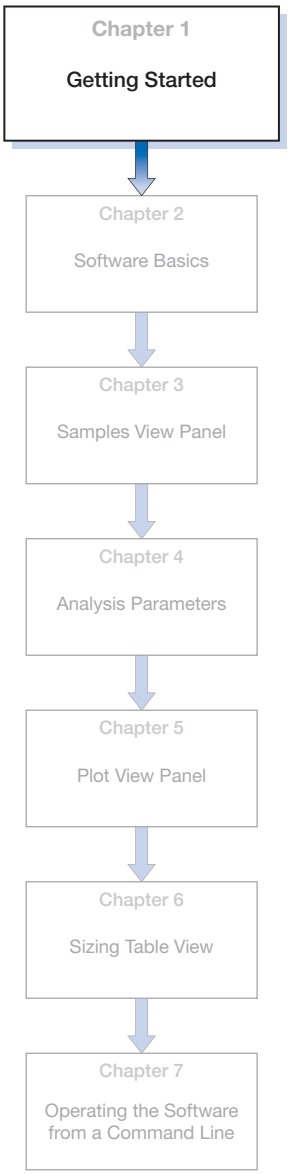
1 Getting Started



This chapter covers:

About Peak Scanner™ Software v1.0

Overview

Peak Scanner™ Software is a nucleic-acid-sizing software that identifies peaks and fragment sizes for application-specific capillary electrophoresis assays. This software allows you to annotate data with functions such as labeling, merging, and splitting peaks. The software stores all editing and analysis data in the original .fsa data files generated on Applied Biosystems genetic analysis instruments. Both GeneMapper® Software and Peak Scanner™ Software perform analysis on original .fsa files.

Features in the Peak Scanner™ Software

- Import and analyze fragment analysis sample files (.fsa) from all currently supported Applied Biosystems genetic analyzers
- Analyzed data (sizing information) is written back to the sample files (.fsa)
- Ability to organize the sample files in a project
- Simultaneous viewing of raw and analyzed data
- Large fragment sizing up to 1200 bp
- Ability to define the expected linear range in large fragment size standards where non-linearity might be expected
- Expanded feature set for editing peaks that includes labeling, merging, and splitting peaks
- Customizable sizing table
- Ability to overlay sizing curves on analyzed data
- Ability to display and print plots in thumbnail view.
- Lightweight software application with easy installation
- Ability to archive projects with sample files and associated reference data (analysis methods, size standards and so on) for data sharing purposes

GeneMapper® Software, another program offered by Applied Biosystems, offers a full array of fragment–analysis applications. For more information on the GeneMapper® Software, refer to "Appendix A".

Analysis

By using Peak Scanner™ Software to analyze files you can:

- Size (in base pairs) small and large nucleic acids fragments, such as PCR products, using an internal size standard
- Detect the presence of a peak

Sample Files

Analyzed data and associated information are written to the sample files. Raw data remains unaltered. You can export sizing information and perform downstream analysis. Using the sample files documentation available at **www.appliedbiosystems.com/softwarecommunity**, independent software vendors can write their own software to exploit the sizing accuracy results stored in the sample files.

Note: Peak Scanner™ Software treats a file analyzed by GeneScan® Software as a raw data file; it does not read analyzed data.

Note: In GeneMapper® Software, all edits and projects are saved in the GeneMapper® Software database. Peak Scanner™ Software v1.0 cannot retrieve any edits from the GeneMapper® Software, which processes raw data in the .fsa files before analysis. Similarly, any editing performed in Peak Scanner™ Software is not recognized by GeneMapper® Software, which processes raw data in the .fsa files before analysis.

About Example Data

Sample Files

Peak Scanner™ Software installs example sample files. Use these sample files to see the functionality of the software and the variety of application-based data that can be analyzed with the software.

Instrument and Size Standard

Sample files were generated by running fluorescently tagged fragments on an Applied Biosystems 3130*xl* or 310 Genetic Analyzer using the GeneScan™ LIZ® 600 Size Standard.

Peak Scanner™ Software Workflow

A typical workflow for using Peak Scanner™ Software requires that you:

- Launch the Peak Scanner™ Software.
- Select a new or existing project.
- Add sample files.
- Select
  - A size standard.
  - An analysis method.
- Click **Analyze**.
- Check the plot view and sizing view panels to evaluate the size quality.
- Perform additional tasks (if needed)
  - Edit peaks by merging, splitting, labeling, adding, removing.
  - Compare plots through the overlay function.
  - Generate sizing tables with labeled peaks.
- Export or print data.

Starting Peak Scanner™ Software

To start Peak Scanner™ Software Version 1.0,

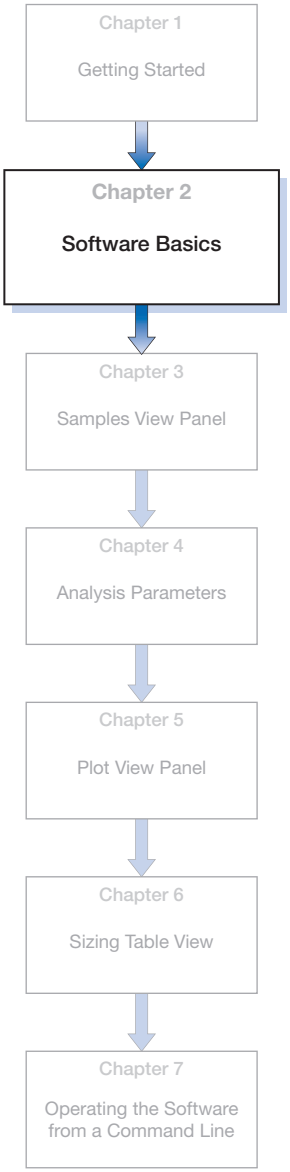
Click  (Peak Scanner™ Software)

or

Select **Start** ▸ **Programs** ▸ **Applied Biosystems** ▸ **Peak Scanner v1.0** ▸ **Peak Scanner v1.0**.

Chapter 1 Getting Started
Starting Peak Scanner™ Software

2 Software Basics



This chapter covers:

Application Layout

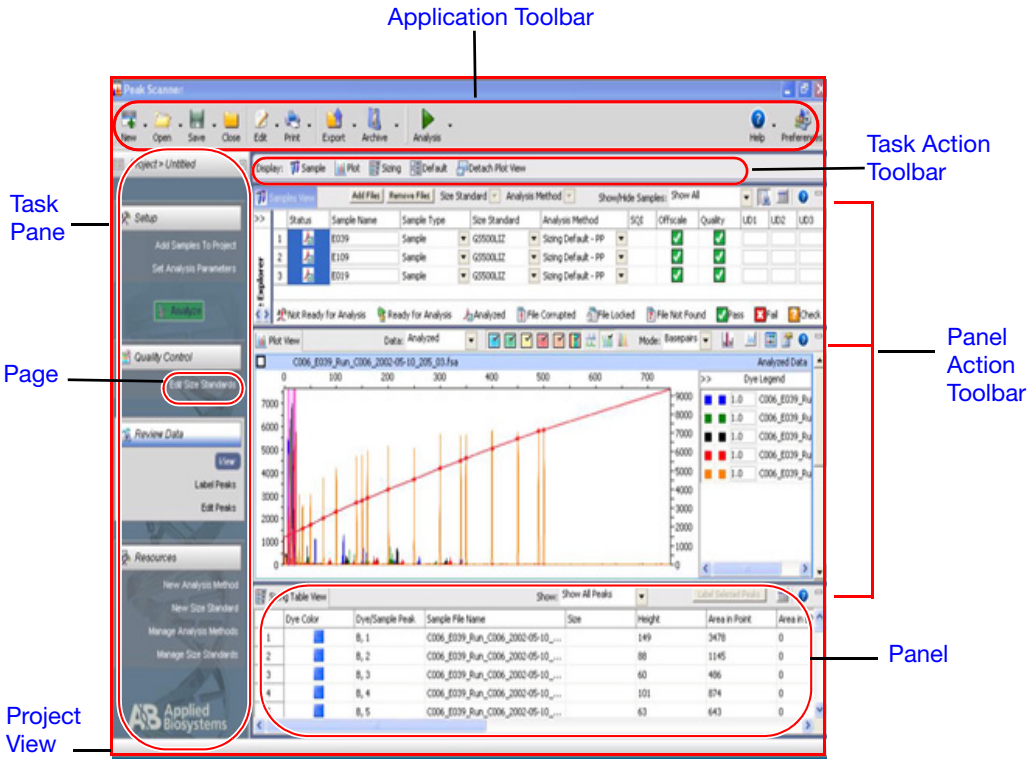


Figure 1 Application layout

Panels

Samples View This panel allows you to view all samples in a project and associated tags and parameters for analysis.

Plot View This panel allows you to view one or multiple sample file electropherograms. You can view and edit raw and analyzed peaks, peak labels, dye colors, and sizing curves.

Sizing Table View This panel allows you to view all dye-labeled peaks and their associated characteristics.

Start View

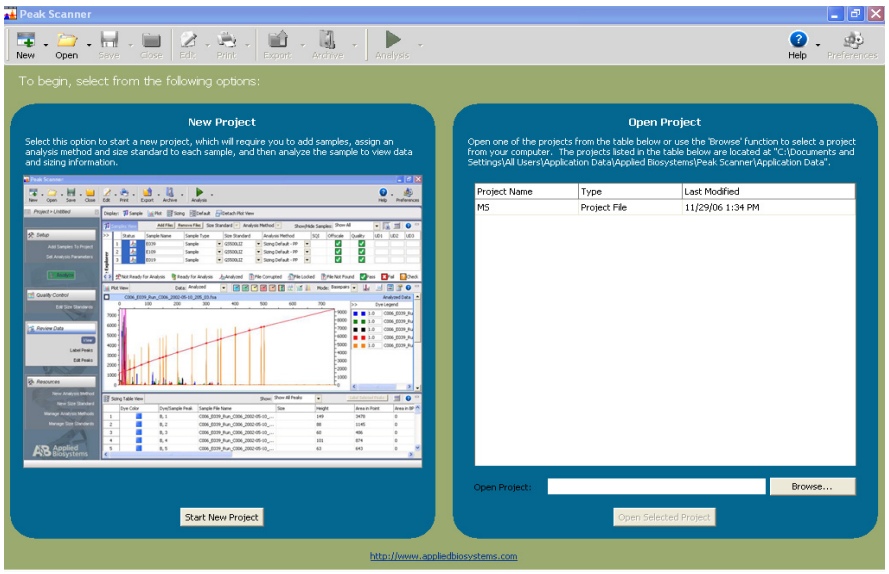


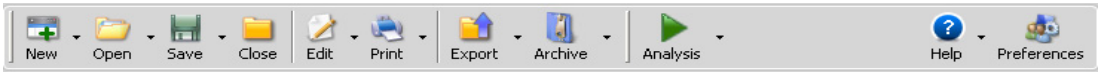
Figure 2 Start View

New Project To start a new project:

1. Click **Start New Project**.
2. Add samples, assign analysis methods and size standards to each sample.
3. Analyze the samples to view data and sizing information.

Open Project To open a project, select from the list of displayed projects in your Application data folder or browse to the project location.

Application Toolbar



New Creates a new project, analysis method, or size standard.

Open Opens an existing project, analysis method, or size standard.

Save Saves the current project under the same or a new name.

Note: The default directory for saving projects is **C:\Documents and Settings\All Users\Application Data\Applied Biosystems\Peak Scanner\Application Data**. Although you can save projects in any location, only projects saved in this default directory are available for selection in the Start View Open Project panel. Use the Browse button at the bottom of the Open Project panel to access projects stored in a directory other than the Peak Scanner™ Software default directory.

Close Closes the current project and returns to the Start View.

Edit Accesses the following commands:

- Undo (Ctrl+Z)
- Redo (Ctrl+Y)
- Cut (Ctrl+X)
- Copy (Ctrl+C)
- Paste (Ctrl+V)
- Fill Down (Ctrl+D)

Print Prints the following: Sample Table, Sizing Table, Plots, and Thumbnails. The application prints the panel that is highlighted. From the Plot View, you can print the Thumbnail view. (Users cannot print the Detached Plot View directly, but can print the Plot View from the main application window.)

Export

Exports the currently selected table data or a combined table (samples and sizing) in a tab−delimited format (*.txt) or as a comma- separated values file (*.csv).

To export the Sizing or the Samples Table, select the panel containing the table to be exported, click 

(Export) in the application toolbar, then select **Export**.

Note: The Export function is enabled only when viewing the Samples or the Sizing Table View panels.

To export the Combined Table, which contains information displayed in both the Sample table and the Sizing table, click 

(Export) in the application toolbar, then select **Export Combined Table**.

Archive

Archives or dearchives a project. You can choose the folder in which to archive or dearchive the project. Archived projects are compressed versions of the project files with all the associated .fsa files.

Analysis

You can:

- Analyze Selected − Analyzes the selected files
- Analyze All − Analyzes all files in the project
- Analyze − Analyzes “ready for analysis” files

Help

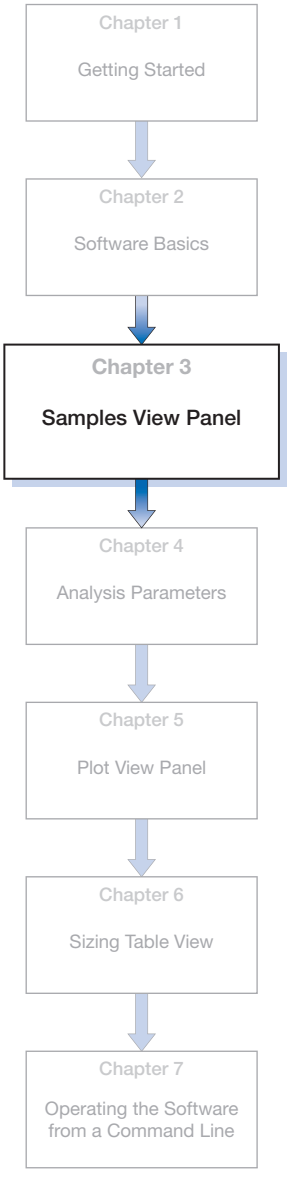
Opens the online Help file, which is a PDF file that you can view with Adobe® Reader version 6.0 or higher.

Preferences

Fragment Print Jobs − You can select if your print jobs are sent to the printer in 10-page sections or combined as one print job. If the printer has low memory, it is recommended that you fragment your print jobs.

Display Quality Values as Images − You can change between displaying quality values as numerical values or colored icons that indicate pass, fail, or check.

3 Samples View Panel



This chapter covers:

Overview

Use Samples View panel to:

- Add and remove samples from a project
- Select the sample type, size standard, and analysis method
- Enter open comments in user-defined fields

The Samples View panel appears in multiple pages that display the Sample Explorer, Samples Panel Action toolbar, and the Samples table.

Note: The tasks available in the Samples View panel vary among the pages and depend on the purpose of each page. However, the tasks on many pages overlap.

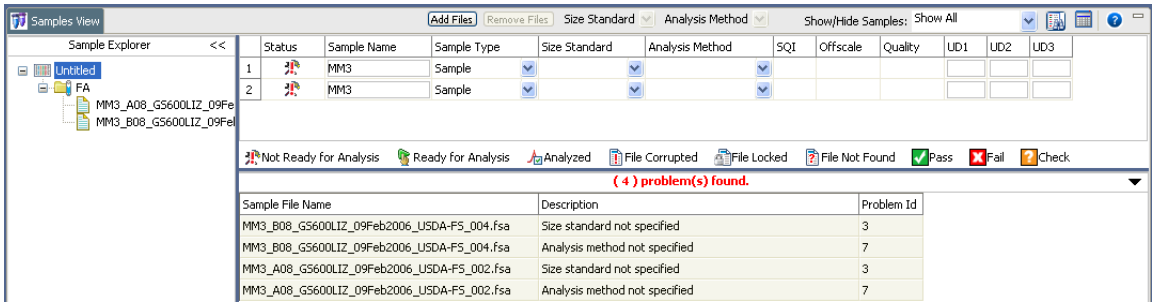


Figure 1 Samples View panel

The Sample Explorer

The Sample Explorer displays all the sample files that are included in a project in a folder structure based on the sample file location. The Sample Explorer can be minimized by clicking the double arrow <<.

The Samples Panel Action Toolbar

This toolbar contains page-specific actions such as:

Add Files

To add samples to the current project:

1. Select **Add Files** in the panel action toolbar.
2. Browse to the .fsa files you want to add to the project
3. Select either the folder containing the files or the individual files to be added
4. Click **Add selected files**, then click **OK**.

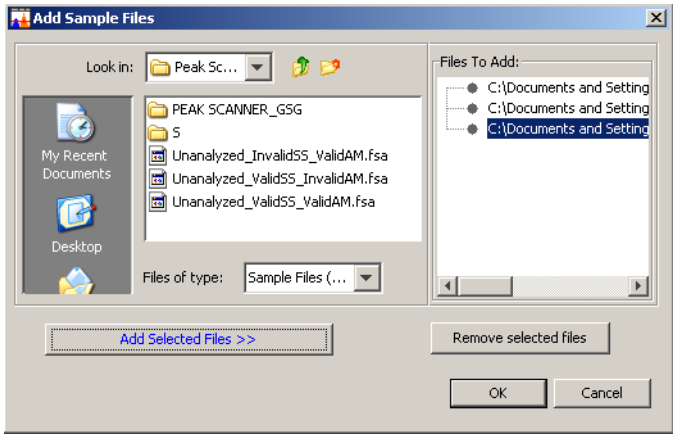


Figure 2 Add Sample Files dialog box

You can also add files to a project by dragging the files/folders directly into the open application.

Note: After you add files, the software assesses the state of each sample for analysis and indicates this through a status column (see below). A problems view also appears below the list of samples, prompting you to determine the appropriate parameters necessary for analysis, such as size standard and analysis method.

Remove Files

To remove samples from the current project, select the files to be removed, then click **Remove Files** in the panel action toolbar.

Samples Table

This table contains a list of all samples and their associated properties. Refer to the table below for a description of the available fields within the table.

Column	Description
Status	Indicates the status of the sample. The legend for the icons is below the table. Possible states include: • Not Ready for Analysis–The sample must be assigned the correct size standard and analysis method • Ready for Analysis–The sample is ready for analysis • Analyzed–The sample is already analyzed • File Corrupted–The sample file format or data is unrecognizable by the software • File Locked–The sample file is being used by another application, and therefore no data can be written to this file until the sample file is not being used by the other application. • File Not Found–The sample file cannot be located in its original folder. **Note:** The status legend at the bottom of the samples table can be turned on and off using the Hide/Show Legend icon within the panel action toolbar.

Column	Description
Sample Name	The name of the sample is derived from the sample name tag used within the sample file; user editable.
Sample Type	Indicates the Sample type: • Sample • Allelic Ladder • Positive Control • Negative Control This field is user editable.
Size Standard	Indicates the size standard name.
Analysis Method	Indicates the analysis method name.
Quality	The quality flag can be displayed numerically or with an icon. The icons are pass, fail, or check. The sizing quality is defined in the Quality Flags tab in the analysis method and is user editable.
Sizing Quality Invalidated (SQI)	A green check mark indicates that the Size Standard peaks definition for the particular sample has been manually edited by the user and, therefore, the Sizing Quality automatically generated by the software is invalid.
Offscale	Indicates if the sample contains offscale peaks. If a green check mark is displayed, the fluorescence signal was greater than the maximum readable signal on the Genetic Analyzer. To correct offscale data, adjust the amounts of labeled fragments. The software cannot correct for offscale data. Although offscale samples can still be analyzed, their peak sizes may not be accurate.

Column	Description
UD1	A user-defined field where you can make notes or comments about the samples.
UD2	A user-defined field where you can make notes or comments about the samples.
UD3	A user-defined field where you can make notes or comments about the samples.

After addition of samples to the current project, the software displays the sample type, the status of the sample, the quality, and any associated problems. Problem descriptions appear below the Status legend, if applicable.

Problem View

The Problem View is displayed only if a sample has a problem that prevents the sample from being analyzed. This view contains a list of all samples and their associated problems.

Sample file not found

The sample file has been moved from its original folder location, and the application can no longer find the link to the file.

Sample file in another open project

The sample file is a read-only file because it is already open in another application. The sample can be viewed, but no changes such as analysis or peak editing can be made to the file.

Note: Closing the open file in another application does not make the file available for editing. The only way to fix this problem is to remove the locked file from the project, close the open file, then re import the file into the project.

Size standard not specified

The sample file does not have an associated size standard. To resolve this issue, define the size standard for the file.

Assigned Size standard not found

The size standard name associated with the sample file cannot be found by the application. To resolve this issue, define a different size standard for the file.

Analysis method not specified

The sample file does not have an analysis method specified. To resolve this issue, define an analysis method for the file.

Assigned analysis method not found

The analysis method name associated with the sample file cannot be found by the application. To resolve this issue, define a different analysis method for the file.

Show/Hide Samples

You can show/hide samples in one of the following ways:

- Show all samples contained in the project by selecting **Show All** from the Show/Hide Samples drop-down list.
- Hide samples by selecting the samples you want to display in the Samples table of the Sample Explorer, then selecting **Show Only Selected** from the Show/Hide Samples drop-down list.

Note: Samples that are hidden remain in the project, but are not viewable in the samples table, plot view, and sizing table.

- Hide samples by selecting the samples in the Samples table, then selecting **Hide Selected** from the Show/Hide of the Show/Hide Samples settings in the Panel Action toolbar.

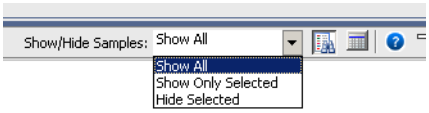


Figure 3 Show/Hide Samples

Note: This action is not available in the Set Analysis Parameters page.

Hide Status Legend

You can hide the sample status legend by clicking  (Hide Legend) in the Samples View Panel Action toolbar.

Edit Table Settings

Note: This action is not available in the Set Analysis Parameters page.

Users can display, hide, order, and sort columns in ascending or descending order. In addition to the fields described in the "Samples Table" on page 16, additional information generated from the Data Collection Software after samples are processed is indicated below.

Fields	Description
Panel	Panel name
Matrix	Matrix name
SNP Set	SNP Set name
Run Name	Run name
Instrument Type	Instrument type
Instrument ID	Instrument ID
Run Date	Run date
Sample File	Sample file name
Cap/Lane	Capillary number or lane number
Plate Name	Plate name
Well	Well location in plate
Auto Sampler tube	Auto sampler tube name
DC version	Data Collection software version
EP Current	Electrophoresis current value

Fields	Description
EP Power	Electrophoresis power value
EP Voltage	Electrophoresis voltage value
Injection Time	Injection time
Injection Voltage	Injection voltage
Instrument Name	Instrument name
Laser Power	Laser power
Module File	Run module file
Number Channel	Channel number
Number Avg Channel	Average channel number
Number Cap/Lane	Number of capillaries or lanes
Run Duration	Run duration
Total Data Points	Number of total data points
Temperature	Temperature
Well to Read distance	Well to read distance or capillary length

To change the Samples Table settings, click  (Edit Table Settings) in the Samples View Panel Action toolbar.

You can do the following:

Add Sample Fields to Display

In the Available Columns to Display section highlight the fields to add, then select **Add Selected** *OR* **Add All** to add every available field. Click **Apply** to apply the changes to the current project.

Remove Sample Fields from Display

In the Columns Selected section highlight the fields to remove, then select **Remove Selected** *OR* **Remove All** to remove every field. Click **Apply** to apply these changes to the current project.

Adjust the Order of Fields in the Table

Select the field whose order needs to be changed, then use the **Move Up** or **Move Down** buttons to adjust the display order.

Sorting

Adjust the sort order of a column from ascending to descending by selecting from the drop-down list in the Sort Order Field of the Columns Selected table.

You can sort fields within the application by **Alt clicking** the field header. An arrow appears indicating the sorting order followed by a number designating the column number. Repeating the Alt-click on the same header changes the sort order from ascending to descending and vice versa. Alt-Shift clicking in a second column provides secondary sorting, in a third column tertiary sorting, and so on.

Note: To apply any table setting to the current project, you must click **Apply**.

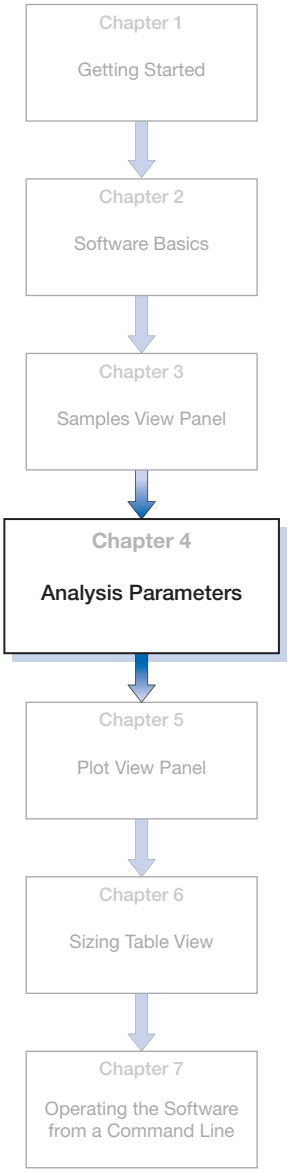
Saving a Sample Table Setting Template

You can save table setting templates to be used in a future project. To do this, adjust the Sample Table Settings, then click **Save** or **Save As**. The files are saved to: C:\Documents and Settings\All Users\Application Data\Applied Biosystems\Peak Scanner\Application Data\Sample Table Settings.

Apply a Saved Sample Table Settings

Select a previously saved Sample Table Setting from the Sample Table Settings drop-down list, then click **Apply**.

4 Analysis Parameters



This chapter covers:

Defining the Sample Type

In the Sample Type column, select a sample for which to specify sample type, then select one of the following:

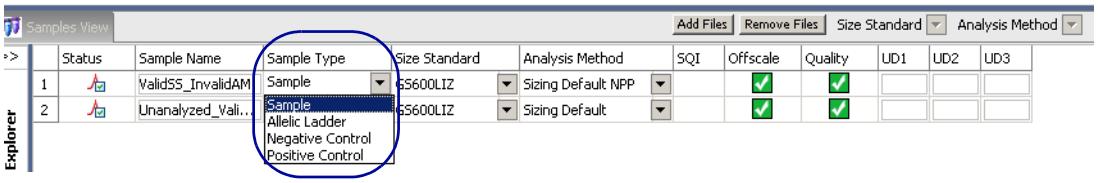


Figure 1 Sample Type

Sample Any DNA specimen or sample.

Negative Control A sample that contains no DNA but all other reagents used in the experiment.

Positive Control A sample that contains a known DNA sample and all appropriate reagents and that generates positive data if the experimental conditions are performed correctly.

Allelic Ladder A sample of DNA that contains a combination of the most common alleles for a specific marker or set of markers.

Note: The Sample Type field is displayed by default in the Set Analysis Parameters page, but can be added to the other pages using the Edit Table Settings function.

Note: To copy the same column value in a set of selected samples, highlight the cells of interest, then use the fill down function (**Ctrl+D**). The column value that is applied is copied from the top-most cell of the selected cells.

Note: Peak Scanner™ Software does not perform allele calling but GeneMapper® Software can.

Defining the Size Standard

Under the column heading Size Standard, select the drop-down list to choose the size standard used for each sample.

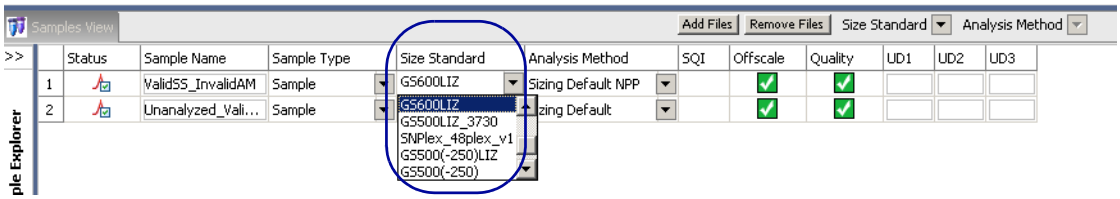


Figure 2 Size Standard

Size Standards Definition

A collection of DNA fragments of known lengths within a range (for example, 20 to 600 bp) all tagged with the same dye. The size standard is co-injected into the genetic analyzer capillary with the sample, then used to size the sample data. All Applied Biosystems size standards are labeled with a proprietary dye, Rox™ (red) or VIC® (orange) dyes.

Note: When the analysis method file name does not match any of the analysis method filenames in the folder AppliedBiosystems\PS\Appdata\AnalysisMethod, it is displayed in ***bold*** text. When the analysis method file name appears in the size standard folder AppliedBiosystems\PS\Appdata\AnalysisMethod, but its analysis method definition does not match the analysis method file definition in the folder, it is displayed in italic text.

Defining the Analysis Method

Under the column heading Analysis Parameter, select the drop-down menu to choose the analysis method.

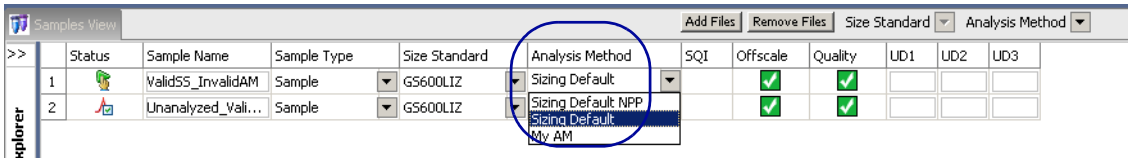


Figure 3 Analysis Method

The two default analysis methods provided with the Software are: Sizing Default and Sizing Default NPP.

Using the Sizing Default analysis method assumes that primers have not been removed from the sample. The Sizing Default_NPP (NPP= no primer peak(s)) should be used for samples that do not contain primers or have had primers removed.

Note: When the size standard file name does not match any of the size standard filenames in the folder AppliedBiosystems\PS\Appdata\SizeStandard, it is displayed in ***bold*** text. When the size standard file name appears in the size standard folder AppliedBiosystems\PS\Appdata\SizeStandard, but it's size standard definition does not match the size standard file definition in the folder, it is displayed in italic text.

Creating and Editing Size Standards

In the Set Analysis Parameters page, you can create and edit the size standards in individual sample files and save these changes for future use. You can be do this in the New Size Standard or the Manage Size Standard page.

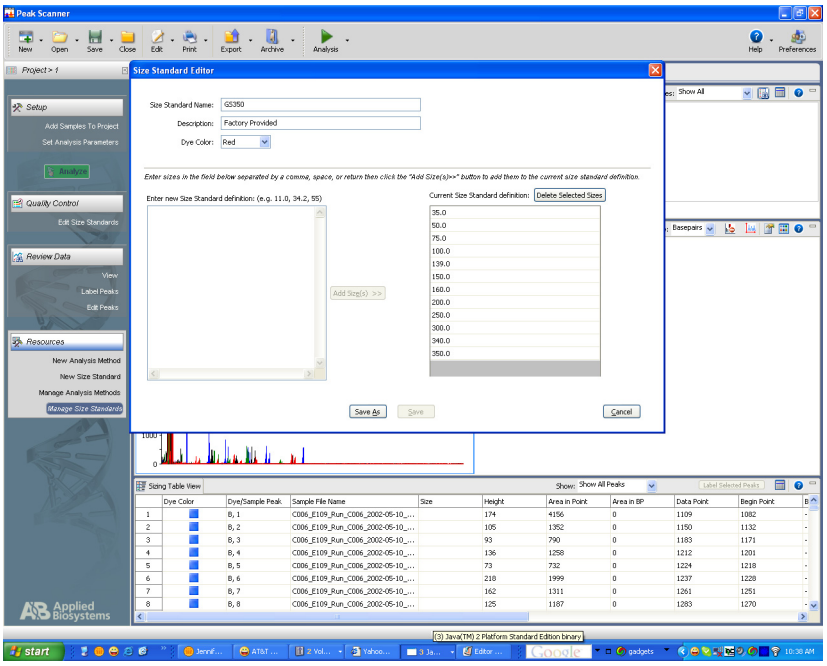


Figure 4 Create or edit new size standards

Create and Edit Size Standard in Sample File

You can create or make edits to individual sample files. These edits will not affect other samples.

Create and Edit Size Standard in Folder

You can create or make edits to the saved Size Standard file. These edits are applied to all samples using the size standard.

Extract to folder You can save the size standard for the selected sample file to the folder to be available in the drop-down list for the current and future projects.

Creating and Editing Analysis Method

In the Set Analysis Parameters page, you can create and edit individual file analysis methods and save the changes for future use. This can be done in the Analysis Method or Manage Analysis page.

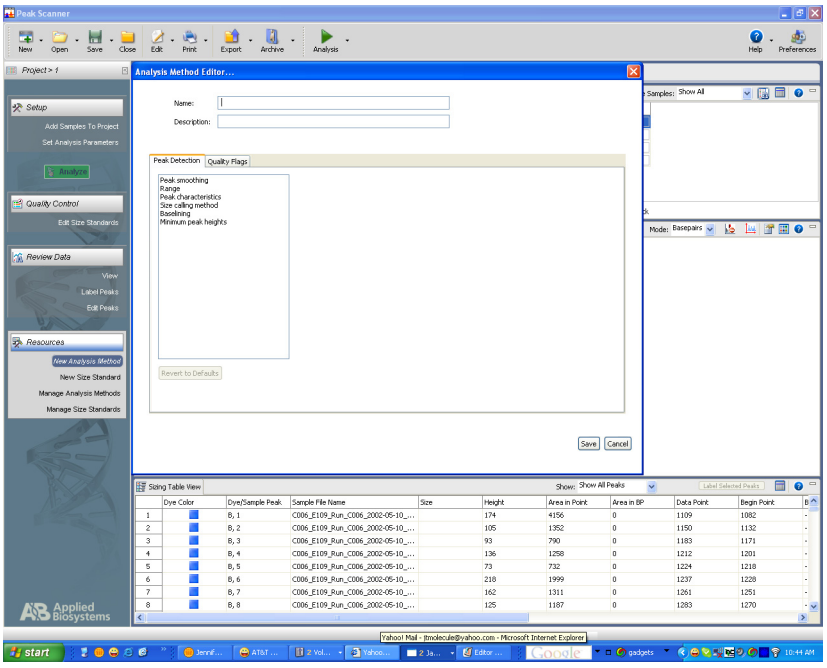


Figure 5 Create or edit new analysis method

Create and Edit Analysis Method in Sample File You can create or make edits to the individual sample file. These edits do not affect other samples.

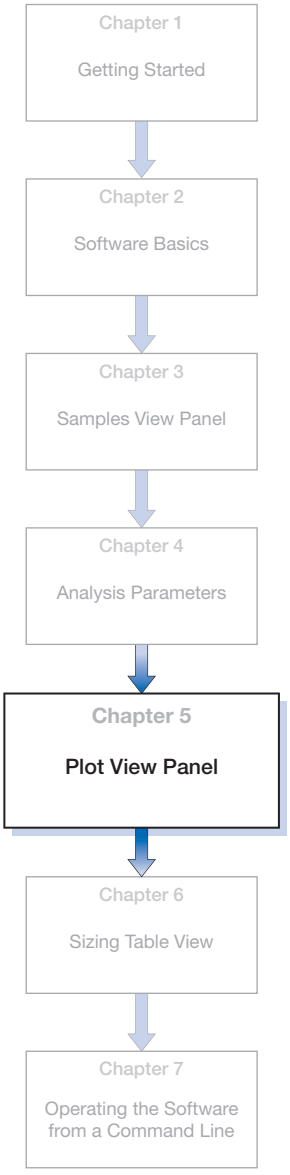
Create and Edit Analysis Method in Folder

You can create or make edits to the saved Analysis Method file. The edits are applied to all samples using this Analysis Method.

Extract to folder

You can save the Analysis Method for the selected sample file to the folder to be available in the drop-down list for the current and future projects.

5 Plot View Panel



This chapter covers:

Overview

The Plot View Panel allows you to view and review electropherograms and to annotate data. The Plot View Panel is activated immediately after analysis of sample files but also can be accessed using the task action toolbar from multiple pages. After data analysis, the plot panel can be independently launched in a new window to maximize the viewing experience. You can also minimize the Plot View Panel to gain more screen space for the Samples and Sizing Table View Panels.

The Plot View Panel displays one or more selected electropherograms of raw and/or analyzed data and their corresponding dye legends as well as a Plot View Panel Action Toolbar that varies slightly in functions based on the selected page.

Plot View Panel Action Toolbar

The four pages on the Task Pane are: Edit Size Standards, View, Label Peaks, and Edit Peaks.

Note: The tasks available in the Plot View Panel vary among the pages and depend on the purpose of each page. However the tasks on many pages overlap.

Scaling and Viewing

The axis scaling is determined by the viewing mode. You can select the viewing mode of the data in the Plot Panel Action Toolbar or in the Display tab of the Plot Setting window from the Data drop-down menu.

Data	X-Axis Scale	Y-Axis Scale
Raw	Scan number or data points	Relative fluorescence units
Analyzed	Size (base pairs)	Relative fluorescence units
Raw and Analyzed	Size (base pairs)	Relative fluorescence units

At the top of each electropherogram, the data type for each sample is coded by an information bar.

Data	Information bar color
Raw	Light green
Analyzed	Light blue
Raw and Analyzed	Light purple

Show/Hide Dyes

 Allows you to show or hide selected dye color data from the display using the plot panel action toolbar. Click a colored icon to show or hide peaks of that color. A check mark indicates that the color is displayed. This setting is applied to all samples selected for viewing. The data remain in the sample files. This option is also available in the display tab of the plot settings.

Show Peak Positions

 Displays brackets that define minimum peak detection limit, peak start and end points, and peak height.

Full View

 Allows you to restore the plot view to include all data and restore original view by clicking the full view icon.

Edit Plot Setting

 Displays a dialog box that allows allows you to edit, save, and apply plot settings. See below for more information.

Show Thumbnail View

 Creates a new window that displays all selected plot views in the format that is currently displayed (for example, if you turn off the blue color and view raw data, the new data are displayed in the thumbnail view). You can print the thumbnail views. The sizing curve is not displayed in the thumbnail view.

Electropherogram Features

The following functions are available in the Electropherogram plot.

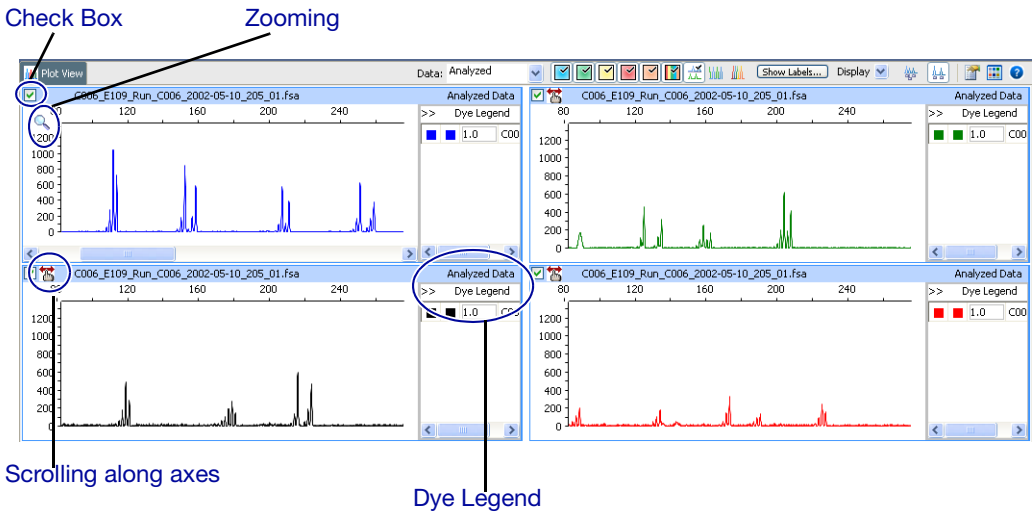


Figure 1 Electropherogram features

Check boxes

You can select the box in the upper left corner of each electropherogram to enable zooming of multiple sample electropherograms. After you select an electropherogram you can use CTRL - A to select all sample electropherograms.

Scrolling along axes

In some modes, the scrolling bar may temporarily be removed from view. To reactivate the scrolling function, click in the left portion of the information bar.

Zooming

You can change the magnification of the electropherogram by placing the pointer over the x- or y-axis until a magnifying glass icon appears. Drag the magnifying glass icon over the area on the axis where you want zooming (a highlighting box appears). You can return to the original view by clicking (Full view) in the Plot Panel Action Toolbar or by double-clicking the axis on which zooming was originally performed.

Alternatively, you can use tools in the Scaling and Zooming tab in the Plot Settings dialog box.

Dye Legend

The dye legend appears when the Show Color Legend box is checked under Plot Settings (See next section). To the right of each electropherogram, the Dye Legend indicates the displayed dye colors and their relative scale. The default color and scale are preset, but can be customized for ease of viewing or for normalization of peaks within or among samples. The dye legend view can also be minimized by clicking the double arrow >>.

To change a displayed dye color, double-click the right colored square to choose a different color. To modify the scale for a particular dye, click the scale indicator, then change the number. Custom colors and scaling can also be personalized through the Scaling and Zooming tabs in the Plot Settings dialog box.

Plot Settings

You can adjust a variety of plot settings. Collectively, a group of specific settings can be saved in a plot settings file to apply to specific projects. These plot setting files are saved in **C:\Documents and Settings\All Users\Application Data\Applied Biosystems\Peak Scanner\Application Data\Plot Settings**

The plot settings are categorized by their effects on display, labeling, and scaling and zooming.

Display Tab

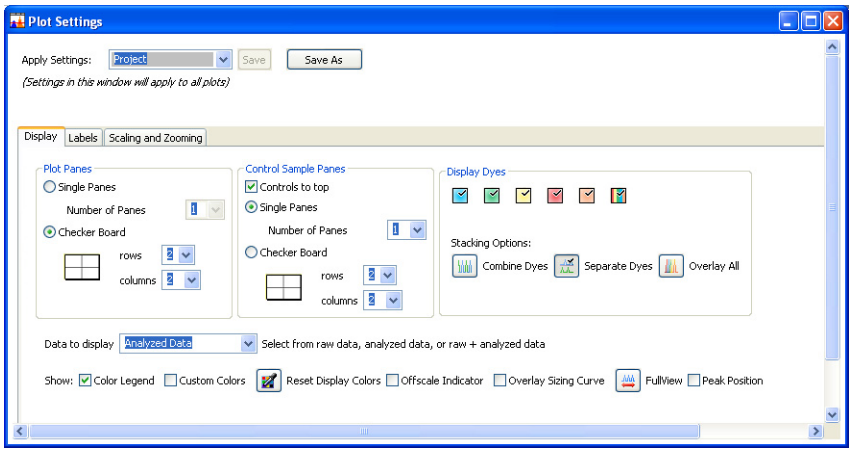


Figure 2 Plot Settings - Display tab

Control and Experimental Sample Plot Panes

Single Panes: Displays one or more electropherograms in a serial fashion.

Checkerboard: Displays more than one electropherogram in a grid layout by selecting the number plots shown per row and column.

Note: Electropherograms from control samples can be displayed in a similar manner and can precede electropherograms from experimental samples during viewing by selecting the “controls to top” check box.

Display Dyes

Combine Dyes: Displays one plot combining all dye color data sets. This setting is the user default.

Separate Dyes: Displays individual plots for each dye color data set for each sample.

Overlay All Dyes: Displays one plot with all color dye data superimposed from selected sample files.

Show Color Legend

Shows or hides dye legend.

Show Custom Colors

Preselects custom colors for each displayed dye if the box is checked. Unchecking the box returns all displayed dye colors to their original colors.

Reset Display Colors

Returns all custom colors, which are user-selected directly, from the dye legend to their original dye color.

Show Offscale Indicator

Highlights the offscale peaks in the plot view panel if the box is checked. Refer to “Offscale” on page 17 for a definition also available in the Panel Action toolbar on the “view” page under review data.

Overlay Sizing Curve

Allows the sizing curve to be overlaid directly on the electropherogram of an analyzed sample.

Show Full View

Refer to “Full View” on page 35.

Show Peak Position

Refer to “Show Peak Positions” on page 35.

Labels Tab

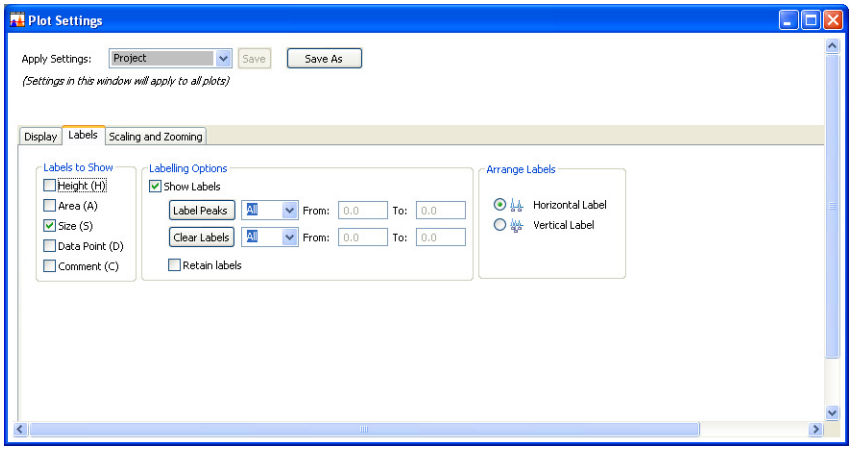


Figure 3 Plot Settings - Labels tab

Labels to Show

You can select peak information to be displayed through check boxes (peak height, peak area, size, data point, user comment)

Labeling Options

You can decide which peaks should or should not be labeled based on height, area, or size using the drop-down list. Click **Label Peaks** or **Clear Label** to perform selective actions.

IMPORTANT! The Show Labels check box must be selected for labeling to take effect.

Retain lables

OFF: When you click a peak, the labels are displayed. When you click a peak whose labels are displayed the labels disappear. You can click a peak once to see the labels, then click again to hide the labels.

ON: When you click a peak, the labels are displayed. When you click a peak whose labels are displayed the labels remain displayed.

Arrange labels

You can align peak labels horizontally or vertically on the x-axis of the electropherogram by selecting the appropriate icon.

The Scaling and Zooming Tab

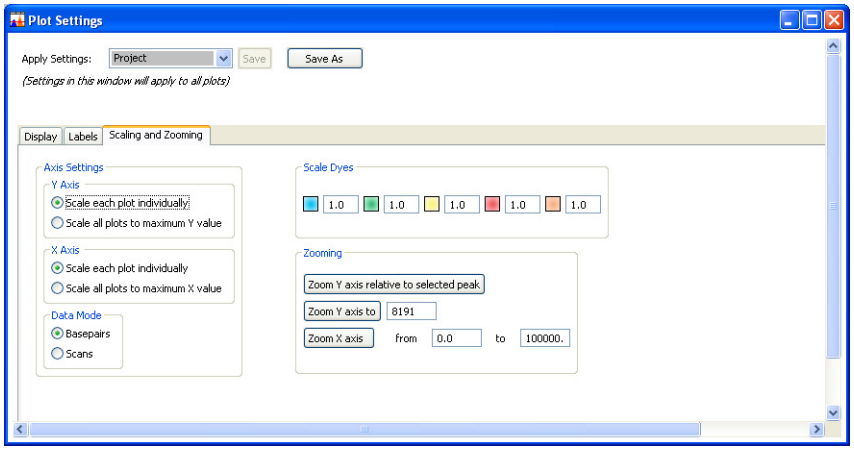


Figure 4 Plot Settings - Scaling and Zooming tab

Axis settings

You can scale electropherograms of selected samples individually or based on the maximum x or y value of a series of electropherograms. The data mode refers to the x-axis units for viewing, namely basepairs or scan number.

Scale Dyes

You can adjust the dye scale for normalization of dye intensities by entering a numerical value. The default for all dyes is set to 1.0

Zooming

You can zoom in on a region of the electropherogram based on one or more criteria.

- Selected peak – Rescaling the y-axis based on the peak height in relative fluorescent units of the peak.

- Defined y-axis value – Rescaling from 0 to a user-defined y-axis value in relative fluorescence units.
- Defined x-axis range – Rescaling based on user-defined x-axis range where values depend on the data mode (basepairs or scans) selected in the axis settings.

Plot Panel Associated Pages

Functionality of the Panel Action toolbar may change from page to page.

Add Samples to Project Page (Under Setup Section)

You can access the Plot View Panel by selecting (Plot) in the TaskAction toolbar for viewing raw data. The Plot Panel Action Toolbar allows for dye selection, stacking, plot setting options, and thumbnail views of selected electropherograms.

Edit Size Standard Page (Under Quality Control Section)

After samples are analyzed, you can select this page to view/alter the size-standard definition. The analyzed data in the electropherogram display peaks that are associated only with the internal size standard (e.g. GeneScan™ LIZ® 600 Size Standard) and the associated sizing curve of the selected sample. In this mode, you can select peaks to be included or excluded in the sizing curve by assigning their relative sizes based on the size-standard definition.

Peak selection is performed by clicking the peak of interest (in all pages where peak selection is appropriate). After the peak is selected, the area under the peak is filled in with the appropriate dye color. You can select multiple peaks simultaneously by pressing the CTRL or SHIFT key while making selections. You can deselect peaks by clicking on the peak again.

After you select a peak, peak information is displayed below the peak, and peak information is highlighted in the sizing table. You can perform one of the following actions:

Add label

Allows you to include a peak in the sizing curve and subsequently assign a size to that peak based on the size-standard definition.

Delete label

Removes the peak from the size-standard definition by assigning a size of 0 bp.

Replace label

Changes the peak size to another size listed in a drop-down list of the size-standard definition.

When these actions are performed, the Sizing table is updated with the changes simultaneously.

Changes to the size standard prompt you to reanalyze the sample, as indicated by the Ready for Analysis icon. Samples must be reanalyzed after editing the sizing curve.

View Page (Under Review Data):

The Plot View Panel is displayed after data analysis by clicking **Analyze**. This panel is also displayed on page selection. The Plot View Panel Action Toolbar allows you to change viewing mode, dye selection, stacking, plot setting options, offscale peak designation, and thumbnail views of selected electropherograms.

Detach Plot View

To maximize your viewing size, you can create a new window that displays only the electrophoretic views of selected sample files. Click the **Detach Plot View** of the Display mode preferences at the top of the Task Action Toolbar. Closing the window, returns you to the current project view. This option is available in all pages under the Review Data section. This page can be printed by selecting **Detached Plot View**, then selecting **Print** in the task panel.

Label Peaks Page (Under Review Data)

After data analysis the plot panel is available on page selection. The Plot Panel Action Toolbar allows for shortcuts to labeling preferences described in the plot settings/preferences, such as showing labels based on specific criteria, label display parameters (height, area, size, user comment), and label alignment (vertical or horizontal). When space is limited for full label display, the labels are minimized into square icons. You can obtain peak information by placing the pointer over the appropriate icon and reading the tooltip.

Each Peak can be labeled with the following :

H–Height

A–Area

S–Base Pair position

D–Scan number

C–Open comment field for users to enter text

You can select the appropriate parameters using the drop-down menu under "Display" in the Panel Action Toolbar. To start labeling ("Labels Tab" on page 40), click **Show labels**.

Edit Peaks (Under Review Data)

After data analysis, the plot panel is available on page selection. This page allows you to redefine, add, delete, merge, and split peaks, which may be necessary during cases of sample overloading, a typical sample migration due to contaminants, and suboptimal resolution.

You can place the pointer over a peak to select it. A bin defining the peak is shown in gray. The bin boundaries and peak apex are each indicated by vertical lines. Place the pointer over the peak, then right click to obtain the drop-down menu for peak editing. You can perform the following actions:

Range select:

Allows you to select one or more peaks for editing. The selected section will appear in yellow.

Add peaks:

Allows you to add and enter into the sizing table peak information that was not included in the original analysis.

Merge peaks:

Allows two individual peaks to be treated as a single peak. The new "bin" is automatically determined. Such a function is typically used for offscale peaks, where overloading of sample results in peak splitting.

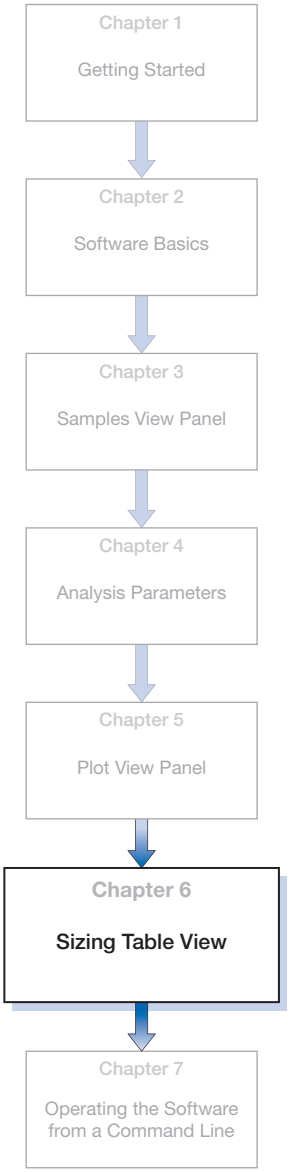
Split peaks:

Allows you to separate a single peak into two peaks. The new bins are automatically determined for both peaks by the software. This option is commonly used when two peaks are not completely resolved.

Remove peaks:

Allows you to remove extraneous peaks that may results from dye-labeled primers, contaminants, or nonspecific PCR amplification. Information associated with a particular peak is subsequently deleted from the sizing table, but the peak is still displayed in the plot view.

6 Sizing Table View



This chapter covers:

The Sizing Table

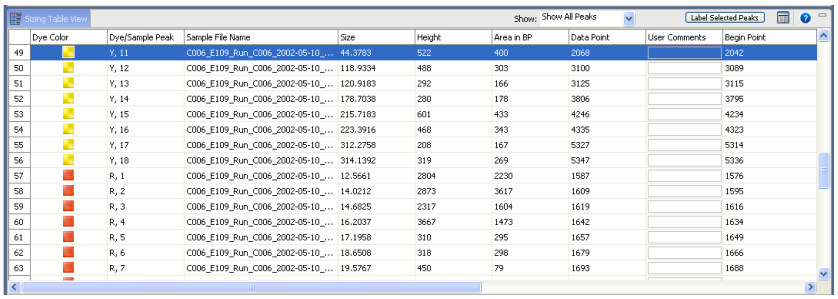


Figure 1 Sizing Table

The Sizing Table View panel allows you to view peak information for the selected samples within the Samples View panel. The Sizing table contains a list of all peaks for the selected samples from the Samples Table with properties that are associated with each peak.

Column	Description
Dye Color	Indicates the peak dye color. Possible states are blue, green, yellow, red, and orange.
Dye/Sample Peak	Assigns a name to each peak. The format is color, peak number.
Sample File Name	Indicates the Sample file name.
Size	Indicates the peak size based on base pairs.
Height	Indicates the peak height.
Area in Point	Indicates the peak area based on scan number.
Area in BP	Indicates the peak area based on base pairs.
Data Point	Indicates the peak data point or scan number.
Begin Point	Indicates the begin point of the peak based on the scan position.

Column	Description
Begin BP	Indicates the beginning of the peak based on basepairs.
End Point	Indicates the end point of the peak based on the scan position.
End BP	Indicates the end point of the peak based on basepairs.
Width in Point	Indicates peak width based on scan numbers.
Width in BP	Indicates peak width based on basepairs.
User Comments	Allows you to make notes or comments about the peaks.

The Sizing Table View Panel Toolbar

Show

You can change displayed data in the sizing table based on the filter you select.

You can hide sizing table peaks by selecting peaks to keep, and then selecting **Show Selected Peaks** from the drop-down list in Show. Peaks that are hidden remain in the project, but will not be viewable in the sizing table.

You can filter the sizing table to show only peaks that are labeled by selecting **Show Labeled Peaks** from the drop-down list in Show. Peaks that are hidden remain in the project, but are not viewable in the sizing table.

You can show all samples contained in the project by selecting **Show All** from the drop-down list in Show.

Label Selected Peaks

You can display a peak label from the Sizing Table. To label peaks in the plot view using the Sizing table, select the sample peaks from the table, then select **Label Selected Peaks**.

Note: To label peaks, you must select **Show labels** in the display tab of Plot Settings.

Edit Table Settings

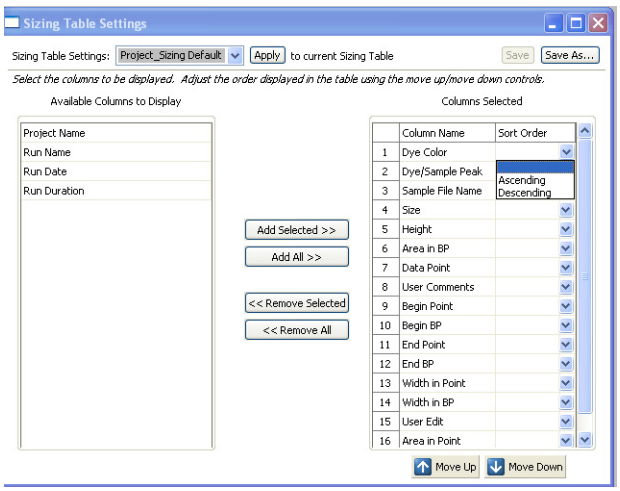


Figure 2 Edit Table Settings

You can adjust the column order and sorting to customize your data. Additional files that you can include or remove from the table are described in the table below.

Column	Description
User Edit	Indicates if the peak has been modified by the user. User-modified peaks are indicated as Edited. If a peak has not been modified, the cell is empty.
Project Name	Includes the name of the Project.
Run Name	Indicates the run name.
Run Date	Indicates the run date.
Run Duration	Shows the run time sample.

To change the Sizing Table Settings, select **Edit Table Settings** from the Sizing Table View panel action toolbar, then you can do the following:

Add Sizing Table fields to Display

Highlight the fields to add from the Available Columns to Display table, and select **Add Selected** or **Add All** (to add every available field), then click **Apply** to apply the changes to the current project.

Remove Sizing Table fields from Display

Highlight the fields to remove from the Columns Selected table, select **Remove Selected** or **Remove All** (to remove every field), then click **Apply** to apply the changes to the current project.

Adjust the Order of Fields in the Table

Select the column whose order needs to be changed, then use **Move Up** or **Move Down** to adjust the order.

Adjust the Sort Order

Adjust the sort order from ascending to descending by adjusting the drop-down list within the Sort Order field of the Columns Selected table.

Apply a Saved Sizing Table Settings

Select a previously saved Sizing Table Setting from the drop-down list, then click **Apply**. You can also save the table settings to be used in a future project. To do this, adjust the Sizing Table Settings, then click **Save** or **Save As**. These files are saved to: C:\Documents and Settings\All Users\Application Data\Applied Biosystems\Peak Scanner\Application Data\Sizing Table Settings.

Note: To apply these saved changes to the current project, you must click **Apply**.

Sorting Tables

All tables within the application can be sorted by Alt-clicking the field header. Repeating the Alt-click on the same header changes the sort order from ascending to descending and vice versa. Alt-shift clicking in a second column provides secondary sorting, in a third column tertiary sorting, and so on.

Export

You can export the displayed sizing table by clicking **Export** in the Application Toolbar, then selecting **Export**. This function allows you to export table data as either a Tab Delimited format (*.txt) or a comma separated values file (*.csv).

To export the Sizing or the Sample table, select the panel containing table to be exported, click 

(Export) from the application toolbar, then select **Export**.

Note: The Export selected table option is enabled only when the Samples or the Sizing Table View panels are displayed.

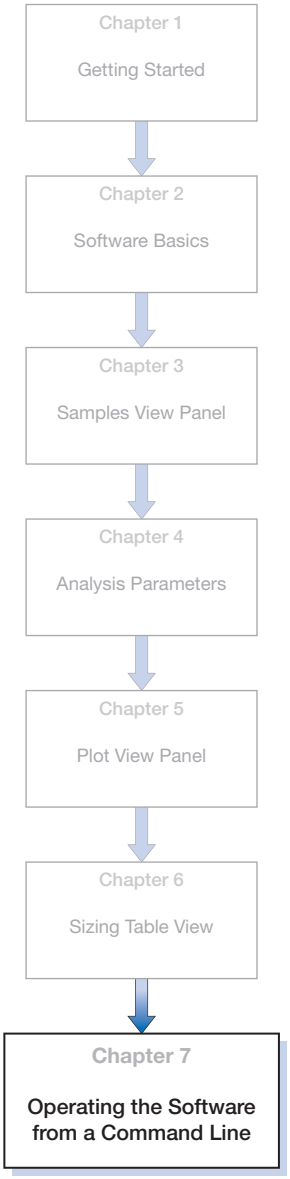
To export the Combined table, which contains information displayed in both the Sample and Sizing tables, click 

(Export) in the application toolbar, then click **Export Combined Table**.

Print

You can print the displayed sizing table by clicking **Print** in the Application Toolbar, then selecting **Print**.

7 Operating the Software from the Command Line



This chapter covers:

Overview

This appendix explains how to load, analyze and export data from the command line interface of the Peak Scanner™ Software.

IMPORTANT! Applied Biosystems supports the use of the command line interface only as it is explained in this manual.

Note: If you are unfamiliar with Microsoft® DOS, Applied Biosystems recommends running the application from the user interface.

About the Interface

The primary advantage of the Peak Scanner™ Software command line interface is that it can automate most software operations without using the graphical user interface. If incorporated as part of a batch file or a scripted sequence, the commands can eliminate most of the repetitive, data-entry tasks associated with project analysis. Use of the command line interface is intended for advanced users (such as systems administrators, bioinformaticians, and network administrators) who choose to operate the application using a scripting language.

Command Syntax

Commands are issued to the Peak Scanner™ Software command line interface via the MS-DOS shell of the Windows® operating system.

The basic format for all commands is:

`<PeakScanner.exe> -commandline <arguments>` where:

- < PeakScanner.exe> is the path and filename of the executable file for the Peak Scanner™ Software.
- -commandline is the argument that placed the software into command line mode. -cmd can also be used.

- <arguments> is the series of arguments that specify the operation(s) to be performed.

Creating a Batch File to Run the Peak Scanner™ Software

This section explains how to use the command line interface of the Peak Scanner™ Software by creating a batch file for the Windows® operating system. The use of batch files is a convenient method to author and submit command line commands to the Peak Scanner™ Software; however, use of the command line interface is not limited to the method demonstrated here.

To create the batch file

1. In the desktop, open the Windows Notepad accessory (**Start** ▸ **All Programs** ▸ **Accessories** ▸ **Notepad**.
2. Press **Enter** to create a new line.
3. In the Notepad window, enter the following:

"PeakScanner" -commandline -project "project"

where:

- PeakScanner is the directory path for the Peak Scanner™ Software executable
- "project" is the name of the project to create or analyze (enclosed in double quotes).

For example

```
cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline
-project "c:\temp\Microsat.pjc" -folder "C:\Test
Data FSA\temp"
```

4. After the -project argument, enter any additional arguments to instruct the Peak Scanner™ Software to perform the desired functions.

IMPORTANT! Follow the guidelines below when entering commands

- Type all arguments on the same line of text (the command cannot contain hard or soft returns)
- Enclose the user-defined component of arguments in double quotes (for example: -project “my project”)
- Separate all arguments using a space (ASCII character 32).

5. (Optional) After typing the last argument in the command, repeat steps 3 through 4 to enter additional commands.

Note: The operating system executes the commands in the order that they appear in the batch file (from top to bottom).

Example batch file

```
cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline
-project "c:\temp\MS.pjc" -folder "C:\Test Data
FSA\temp" -analysis -analysismethod
"C:\Data\Analysis Methods\Sizing Default.met"
-sizestandard "C:\Data\Size Standards\
GS500LIZ.sts"
```

6. When finished, carefully review the text of the batch file for any errors or typos.

7. When satisfied with the content of the file, save the text as a batch file:

   a. Select **File** ▸ **Save**.

b. In the File name field, enter a name for the batch file that terminates in the .bat file extension. For example: mybatchfile.bat or PeakScanneranalysis.bat

c. Click **Save**.

8. Select **File ▸ Exit** to close the batch file.

To run the batch file, double-click the file icon and wait for the computer to run the scripted command.

Note: Depending on the speed of your computer and the number of commands included in your batch file, the operating system may take several minutes to a number of hours to process the batch file.

Example Commands

Example Commands in This Section

Example #1: Create or Edit a Project with Sample files

Example #2: Basic Sample File Analysis with Analysis Method & Size Standard

Example #3: Export of Sample data, Sizing Data & Combined Data

Example #1: Basic Sample File Analysis

Purpose: This command was written to create a project with sample files. This batch file creates a project called “Micorsat.pjc” and adds the sample files in the C:\Test Data FSA\temp folder.

Example File

```
cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline
-project
"c:\temp\Microsat.pjc" -folder "C:\Test Data
FSA\temp"
```

Example #2: Basic Sample File Analysis with Analysis Method & Size Standard

Purpose: This command was written to analyze sample files by setting the analysis method and size standard.

Example File

```
cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline
-project "c:\temp\MS.pjc" -folder "C:\Test Data
FSA\temp" -analysis
-analysismethod "C:\Data\Analysis Methods\Sizing
Default.met"
-sizestandard "C:\Data\Size Standards\
GS500LIZ.sts"
```

Example #3: Export of Sample Data, Sizing Data & Combined Data

Purpose: This command was written to export the sample data, sizing data, and the combined data for the sample files of an existing project.

```
Example File

@echo off

#Export Sample Data as TXT file

cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline
-project "c:\temp\Microsat.pjc"
-exportsampledata "C:\Temp\
sampleCommandlline.txt;tab"

#Export Sizing Data as CSV file

cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline-
project "c:\temp\Microsat.pjc" -exportsizingdata
"C:\Temp\SizingCommandlline.csv;CSV"

#Export Combined Data as CSV file

cd C:\Program Files\Applied Biosystems\Peak
Scanner\app
"C:\Program Files\Applied Biosystems\Peak
Scanner\app\PeakScanner.exe" -commandline -
project "c:\temp\Microsat.pjc"
-exportcombineddata"C:\Temp\
SampleSizingCommandlline.csv;CSV"
```

Tips & Suggestions

- When you copy from a Microsoft® Word® document to a text file, some characters are decoded incorrectly. Therefore, you must manually enter the text document..

  For example quotes [“] are decoded as ô.
- Turn on Word Wrap in the text pad. Select **Format ▸ Word Wrap**.
- Do not use the Enter key to go to the next line. This happens automatically with Word Wrap on.

Argument	Action/Definition	Usage
-commandline	Configures the Peak Scanner™ Software to operate in command line mode.	-commandline
-project	Specifies the project to be created or opened. The argument of this option is the path of a valid Peak Scanner project. Both absolute and relative paths are supported. If the project file exists, the project is open. Otherwise, a new project is created.	-project “projectpath”
-folder	Specifies the path of the folder from where sample files can be added. This command when used with a project loads the samples in the folder into the project	-project “projectpath” –folder “c:\\ temp”
-file	Specifies the path of the sample file. This command when used with project loads the samples in the folder into the project	-project “projectpath” –file “file1, file2” Can specify more than one file
-analysis	Analysis is performed on the samples in the specified project. Optional parameters with this are -analysismethod and -sizestandard	-project “projectpath” -analysis OR -project “projectpath” –analysis -analysismethod “ampath” –sizestandard “sizestdpath”

Argument	Action/Definition	Usage
-analysismethod	Specifies the full path of the analysis method to be used for analysis. This is an optional parameter.	-project “projectpath” –analysis -analysismethod “ampath”
-sizestandard	Specifies the full path of the size standard to be used for analysis. This is an optional parameter.	-project “projectpath” –analysis -sizestandard“sizestdpath”
-exportsampledata	Exports the sample table information from the specified project. Supported export formats are CSV and TAB.	TAB -project “projectpath” - exportsampledata “sampletable.txt;tab” CSV -project “projectpath” -exportsampledata “sampletable.csv;csv”
-exportsizingdata	Exports the sizing table information from the specified project. Supported export formats are CSV and TAB.	TAB -project “projectpath” -exportsizingdata “sizingtable.txt;tab” CSV -project “projectpath” -exportsizingdata “sizingtable.csv;csv”
-exportcombineddata	Exports the sample table and sizing table information. Supported export formats are CSV and TAB.	TAB -project “projectpath” - exportcombineddata “samplessizingtable.txt;tab” CSV -project “projectpath” -exportcombineddata “samplessizingtable.csv;csv”

Appendix A A

Features of GeneMapper® Software

- Sizing Algorithm
- Multiple Algorithm Allele Determination for Microsatellite-Based genotyping
- Remote auto-analysis and distributed computing
- Multi-user, client server deployment
- Security and audit features which assist with 21CFR11 requirements
- Process Quality Values (PQVs) for high-throughput genotyping
- Applications include amplified fragment length polymorphism (AFLP), loss of heterozygosity (LOH), microsatellite, and SNP genotyping (SNPlex ™ Genotyping System) analysis
- Automatic Bin Assignment
- Automatic Bin Builder (ABB)
- SNP Allele Caller: Auto-Panelizer
- Cluster plot analysis and display for SNPlex™ assay genotyping provides easy-to interpret visualization of allele calls
- Complete automation with ABI PRISM® 3100 or ABI PRISM® 3100-*Avant* Genetic Analyzers, Applied Biosystems 3130 and 3130*xl* Genetic Analyzers, and Applied Biosystems 3730 and 3730*xl* DNA Analyzers allow data collection and analysis on one computer
- Database Manager helps you organize and manage your data
- OLA–Analysis method for certain oligo ligation assay
- (OLA)–based mutation analysis
- Plot and Printing views

Chapter A Appendix A
Features of GeneMapper® Software

Index

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com**.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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